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Histomorphology of the Harderian Gland in the Angora Rabbit

A. G. Bayraktaroğlu[1]* and E. Ergün[2]

Addresses of authors: [1] Department of Histology and Embryology, Faculty of Veterinary Medicine, University of Ankara, 06110 Diskapi, Ankara, Turkey;
[2] Department of Histology and Embryology, Faculty of Veterinary Medicine, Kirikkale University, 71451 Yahsihan, Kirikkale, Turkey

***Correspondence:**
Tel.: +90 312 3170315; fax: +90 312 316 44 72; e-mail: dralevgurol@gmail.com

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Summary

This study was aimed to demonstrate the morphological and histochemical properties of the Harderian gland in the Angora rabbit. Ten healthy adult Angora rabbits obtained from private breeders constituted the material of the study. The Harderian gland, which is composed of the pink and white lobes, consists of cells that produce a secretion of lipid character. The pink lobe contained type I cells with large lipid vacuoles. Cells with small lipid vacuoles (type II) were found in the white lobe. Type III cells containing both large and small lipid vacuoles were not observed. While type I cells reacted strongly to staining with Oil red O, type II cells reacted weakly to this stain. The number of plasma cells was greater in the white lobe when compared to the pink lobe. The apical granules within the epithelial cells lining the intralobular and inter-lobular excretory ducts of the gland were positive for periodic acid-Schiff (PAS), periodic acid-Schiff/alcian blue (PAS/AB), alcian blue (AB) and performic acid/alcian blue (PA/AB). Electron microscopic examination revealed that type I cells contain large electron-light lipid vacuoles and an eccentric heterochromatic nucleus, due to the presence of these vacuoles. The cells, which were connected by tight junctions, possessed apically located microfolds. The nucleus of type II cells was situated basally and had an oval shape. Type II cells had apical microvilli-like cytoplasmic protrusions, longer than those of type I cells. Oval shaped myoepithelial cells were observed between the glandular epithelial cells and their basal lamina. The epithelium lining the excretory ducts of the gland contained two types of granules, which were dark and lightly coloured. Histochemical and ultrastructural examinations revealed no difference in the structure of the Harderian gland between female and male Angora rabbits.

Introduction

The Harderian gland (Glandula profunda palpebrae tertiae) is an exocrine gland with a secretion of varying character in mammals, birds, rodents, amphibians and reptiles. Amongst mammalian species, the Harderian gland is reported to be absent in bats and terrestrial carnivores and is known to be rudimentary in monkeys (Baccari et al., 1996). Three distinct types of the gland, which vary by the morphology of the epithelial cells and the lobulation pattern, have been reported in avian species (Burns, 1992). In reptiles, the gland is composed of three lobes, namely the medial, intermediate and lateral lobes (Baccari et al., 1990). In rabbits, the Harderian gland is an intra-orbitally located tubuloalveolar gland composed of two distinct parts, viz. a pink lobe and a white lobe (Eglitis, 1964; Payne, 1994; Janssens et al., 1999).

Based on the variety of its secretory products, the Harderian gland has numerous functions, including the

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lubrication of the eye, immune response, pheromone production, osmoregulation, thermoregulation and photoprotection in different animal species. The major secretory products of the gland include lipids, porphyrins and indoles (Olcese and Wesche, 1989; Payne, 1994).

In the rabbit, the pink lobe contains 80% lipid (Olcese and Wesche, 1989). The primary function of the secretion is to facilitate the movement of the eyelids (Payne, 1994). Although the mechanism of lipid release has been claimed to be apocrine by some researchers (e.g. Tsutsumi et al., 1966), the Harderian gland is unusual in releasing lipids by an exocytotic mechanism (Winterhager and Kühnel, 1983; Lopez et al., 1993; Payne, 1994).

Hormones of the gonads (Payne et al., 1982; Mcmasters and Hoffman, 1984; Seyama et al., 1996), thyroid (Hoffman et al., 1989) and epiphyseal gland (Baccari et al., 1996) are reported to lead to gender differences of the Harderian gland. Secretory cells of the gland in the golden hamster (*Mesocricetus auratus*) and the Black Sea bottlenose dolphin (*Tursiops truncatus ponticus*) also display sexual dimorphism (Hoffman, 1971; Bodyak and Stepanova, 1994).

The excretory duct system of the Harderian gland is well developed in avian species and can be subdivided in primary, secondary and central ducts (Shirama et al., 1996). The intralobular primary and secondary ducts discharge into a central duct (Olah et al., 1992). The most peculiar characteristic of the Harderian gland in the turkey, duck and chicken is the presence of periodic acid-Schiff (PAS)-positive glycogen granules in the cytoplasm of the duct epithelium. Numerous goblet cells are scattered among these cells in the turkey (Maxwell and Burns, 1979). In the gerbil, the wall of the single excretory duct is lined by three different types of cells, including surface epithelial cells, serous cells and goblet cells (Sakai and Yohro, 1981). In a study carried out in rats, Djeridane (1994) reported the presence of serous and a few mucous cells in the duct epithelium.

The Harderian gland of the Angora rabbit, which is a species endemic to Turkey, has not been studied previously. This study was aimed to demonstrate the difference of the Harderian gland and its excretory ducts in the Angora rabbit, compared with other rabbit breeds, at light and electron microscopic levels.

Materials and Methods

Ten healthy adult Angora rabbits of both sexes were included in the study after the local ethics committee of the Veterinary Medicine of Kirikkale University approved the protocol (d.d. 30 April 2007). Animals were obtained from private breeders. Tissue samples of the Harderian gland were taken after the animals were euthanized by intravenous administration of sodium pentobarbital (150 mg/kg).

For light microscopic examination, part of the tissue samples was fixed in a buffered neutral formaldehyde solution of 3.5%, subjected to routine tissue processing for light microscopic examination, embedded in paraplast and sectioned into 5 μm thick slides (Culling et al., 1985). The sections were stained by the Mallory's triple staining technique modified by Crossmon (1937) for general histological examination, PAS reaction for demonstrating neutral mucosubstance, alcian blue (AB) pH 2.5 reaction for acidic mucosubstance, PAS/AB pH 2.5 reaction for the demonstration of both neutral and acidic mucosubstance, the performic acid-alcian blue method for the demonstration of proteins and methyl green pyronin for the demonstration of plasma cells (Culling et al., 1985). After fixation in Baker's formol-calcium, cryostat sections were prepared from the remaining part of the tissue samples taken, and these sections were stained with Oil red O (Bancroft and Gamble, 2002).

For electron microscopic examination, the tissue samples were pre-fixed in glutaraldehyde-paraformaldehyde (pH 7.4) as described by Karnovsky (1965) and subsequently fixed in 1% osmic acid solution for 2 h. Following the second fixation, tissue samples were maintained in 1% uranyl acetate for 2 h, dehydrated through an ascending series of graded alcohols and propylene oxide and embedded in Araldite M (Fluka, Steinheim, Germany). The semi-thin 1-μm thick sections cut from these blocks were stained with toluidine blue and, following the marking of the targeted area, sections of 30–40 nm thickness were cut. These sections were contrast stained as described by Veneable and Coggeshall (1965) and were then examined under a Carl Zeiss EM 9 S-2 transmission electron microscope (Zeiss, Oberkochen, Germany).

Results

Macroscopic observations

In the Angora rabbit, the large Harderian gland was localized in the lower anterior region of the orbit and was composed of two lobes, pink and white in colour (Fig. 1a). The pink lobe constituted the major part of the gland.

Light microscopic observations

Light microscopic examination revealed that the cells of the pink lobe (type I cells) were stained lightly, whereas the cells of the white lobe (type II cells) were stained darkly (Fig. 1b). Staining of serial sections revealed the absence of mixed lobes (type III cells). Type I cells were cuboidal and contained large lipid vacuoles (Fig. 2a). As a

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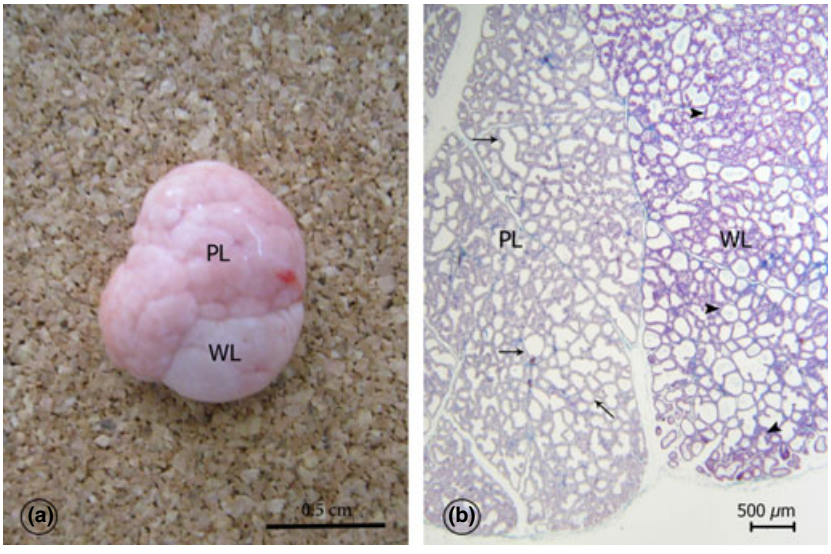


Fig. 1. (a) Anatomical structure of the Harderian gland. Pink lobe (PL), white lobe (WL). Bar: 0.5 cm. (b) Light microscopic appearance of the gland. Pink lobe (arrows), white lobe (arrowheads). Paraffin section, Mallory's triple staining. Bar: 500 μm.

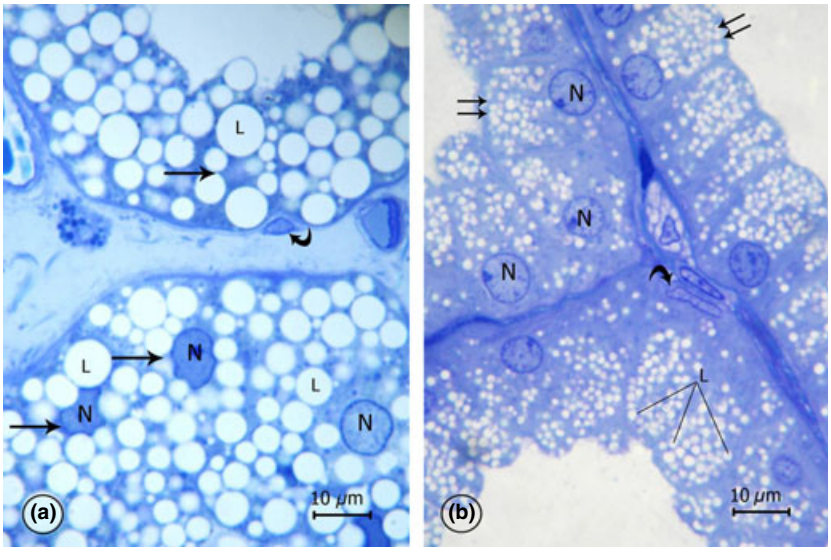


Fig. 2. Light microscopic appearance of semi-thin sections of the Harderian gland. (a) Type I cells (arrows). (b) Type II cells (double arrows), lipid vacuoles (L), nucleus (N), myoepithelial cell (curved arrows). Semi-thin section, toluidine blue. Bar: 10 μm.

result of the presence of these vacuoles, the nucleus of the cells was localized at different levels. Furthermore, nuclear fragments were also observed within the glandular lumen. Type II cells were columnar and contained small vacuoles. The basal cytoplasm of these cells stained dark and their nuclei were localized at this level (Fig. 2b).

When Oil red O lipid staining was applied, type I cells displayed a strong reaction, while type II cells reacted weakly (Fig. 3). In both lobes, oval shaped myoepithelial cells were observed below the epithelial cells of the gland (Fig. 2b). The number of plasma cells was greater in the white lobe compared with the pink lobe (Fig. 4).

The excretory ducts of the gland were situated intralobularly and inter-lobularly (Fig. 5). The apical cytoplasm of the epithelium lining both duct types contained PAS (+), PAS/AB (+), AB pH 2.5 (+) and performic acid/alcian blue (PA/AB) (+) granules (Fig. 6). The histochemical staining properties of the Harderian gland showed no gender differences.

Electron microscopic observations

The type I cells of the pink lobe contained large, electron-light lipid vacuoles of varying size in their cytoplasm (Fig. 7a). Due to the presence of these lipid vacuoles, the heterochromatic nucleus displayed an eccentric localization. Microfolds were present in the apical region of the cells, which were connected by tight junctions. A large number of mitochondria were present just beneath the apical cell membrane.

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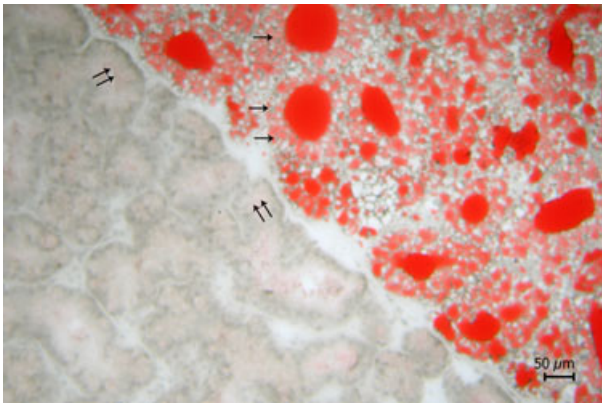


Fig. 3. Light microscopic picture of the Harderian gland. Type I cells (arrows), Type II cells (double arrows). Cryostat section, Oil red O. Bar: 50 μm.

The white lobe was composed of columnar type II cells with electron-light lipid vacuoles of smaller size (Fig. 7b). The oval nuclei were localized in the basal region of the cells. Mitochondria displayed a homogeneous distribution. In addition to being connected by tight junctions, the cells also possessed lateral cytoplasmic protrusions. Microvilli longer than those of type I cells were present in the apical region of type II cells.

In both lobes, the lumen of the some glandular lobules was observed to contain nuclear and cytoplasmic particles (Fig. 8). Damaged cells were recognized by their cytoplasm having a lighter colour. Oval shaped myoepithelial cells were observed between the glandular epithelial cells and their basal lamina (Fig. 9).

The apical cytoplasm of the cells lining the intralobular and inter-lobular excretory ducts contained two types of granules, which were dark and lightly coloured, respectively (Fig. 10). These granules were also observed within the lumen of the ducts. Lipid vacuoles were not observed in the cells lining the ducts. The indented nucleus was situated basally, just beneath the granules. Furthermore, the duct cells contained many mitochondria, a well developed endoplasmic reticulum and a Golgi apparatus with juxtanuclear localization. The epithelial cells were connected by tight junctions.

Discussion

The secretion of the Harderian gland is mucous in avian species and amphibians, serous or seromucous in reptiles and of lipid character in mammals (Sakai, 1981). In the armadillo, the gland is composed of two parts, which produce mucous and lipid secretions (Weaker, 1981). It is reported that the secretion of the gland can be different in varying breeds of the same species. For example, amongst amphibians, the bull frog (*Rana catesbeiana*) and leopard frog (*Rana pipiens*) are known to produce a mucous secretion, while the toad (*Bufo viridis*) is reported to have a secretion of lipid character (Payne, 1994). In this study, it was demonstrated that the Harderian gland produces a secretion of lipid character.

The lobulation and the cellular composition of the lobes of the Harderian gland vary between animal species (Payne, 1994). The Harderian gland of avian species is classified in three distinct groups, according to epithelial cell composition and lobulation (Burns, 1992). The gland is composed of three lobes in reptiles, namely the medial, intermediate and lateral lobes (Baccari et al., 1990). The gland contains two types of secretory cells in the male hamster, mouse and rat, whereas the female hamster and

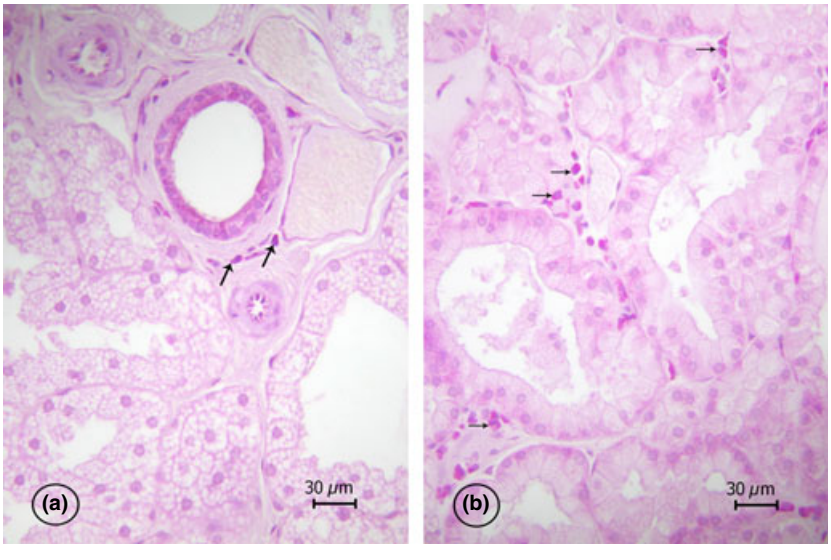


Fig. 4. Distribution of plasma cells in the pink and white lobes. (a) Pink lobe (PL). (b) White lobe, plasma cells (arrows). Paraffin section, methyl green pyronin. Bar: 30 μm.

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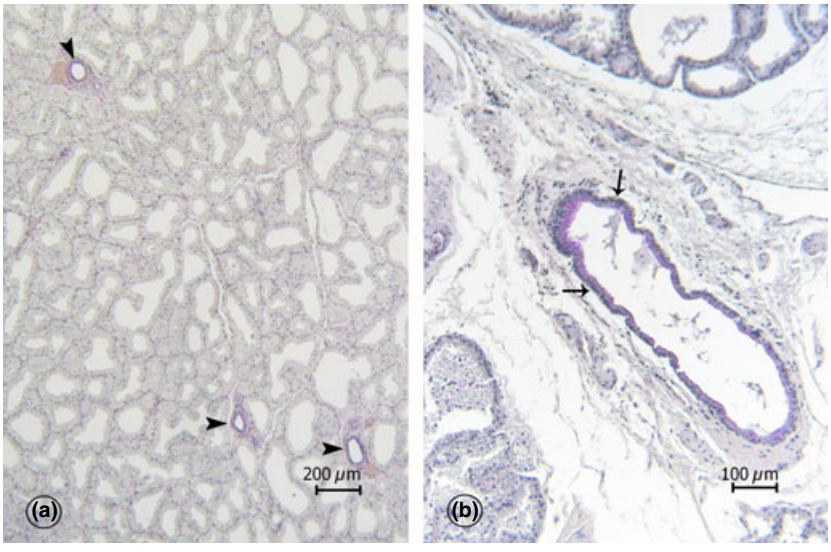


Fig. 5. Light microscopic picture of the excretory duct of the Harderian gland. (a) Intralobular ducts (arrowheads). Paraffin section, PAS. Bar: 200 μm. (b) Inter-lobular duct (arrows). Paraffin section, PAS. Bar: 100 μm.

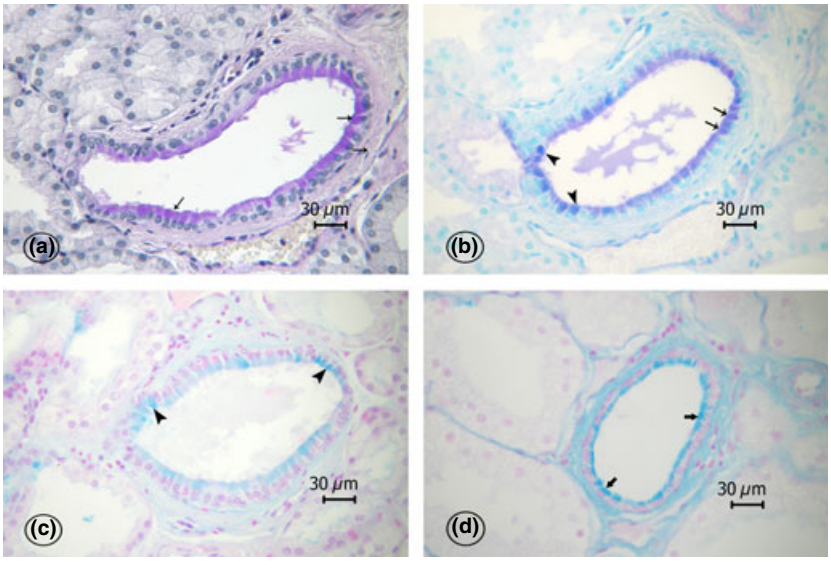


Fig. 6. Staining reactions of the excretory ducts. (a) PAS (+) cells (thin arrows), PAS. (b) PAS (+) cells (thin arrows), AB (+) cells (arrowheads), PAS/AB. (c) AB (+) cells (arrowheads), AB. (d) PA/AB (+) cells (thick arrows), PA/AB. Paraffin section. Bar: 30 μm.

gerbil have a single cell type (Sakai and Yohro, 1981). The Harderian gland in the rabbit is bilobated, one lobe being pink and composed of cubic epithelium with large vacuoles, and the other being the white lobe composed of columnar cells with small vacuoles. The two types of cells display a separate localization in the lobes, which is in agreement with the findings of other authors (Björkman et al., 1960; Payne, 1994; Shirama et al., 1997), but contrasts to the fully mixed distribution in other rodents (Shirama et al., 1997). Some researchers, however, have reported the presence of a mixed lobe at the border between the two lobes in rabbits (Björkman et al., 1960; Winterhager and Kühnel, 1983; Shirama et al., 1997; Janssens et al., 1999). Such a mixed lobe was not present in the Angora rabbit, based on the examination of the stained serial sections of the Harderian gland.

This study demonstrated that lipids are the major secretory product in both lobes. Type I cells of the pink lobe contained large lipid vacuoles, whereas type II cells of the white lobe possessed smaller vacuoles. As Type II cells gave a weaker reaction to Oil red O staining, the colour differences in the lobes were thought to be related to the size and chemical composition of the oil droplets. The lipid vacuoles of the gland are reported to vary in chemical composition, size and ultrastructural appearance (Winterhager and Kühnel, 1983). For instance, in the woodmouse (*Apodemus sylvaticus*), type II cells possess large and darkly stained lipid vacuoles, whereas type I

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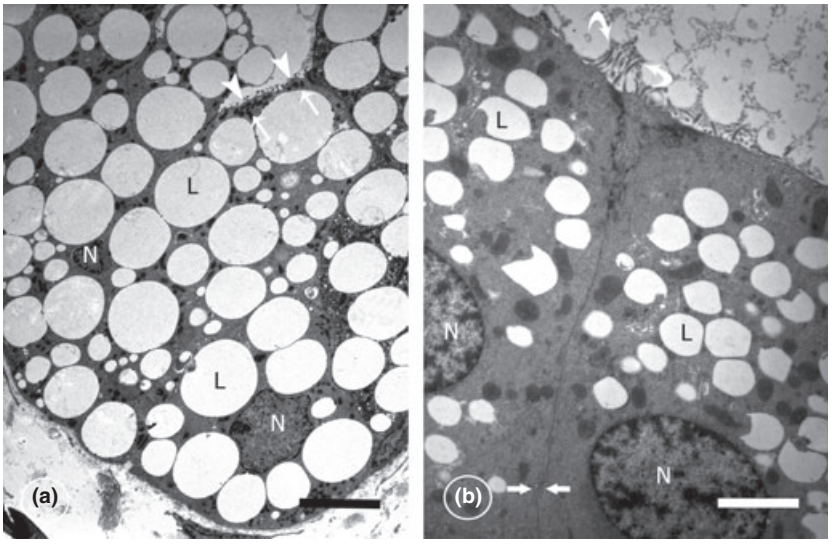


Fig. 7. Electron micrograph of the Harderian gland. (a) Type I cells with lipid vacuoles (L), nucleus (N), microfolds (arrowheads) and mitochondria (thin arrows). Bar: 0.8 μm. (b) Type II cells with lateral cytoplasmic protrusions (thick arrows) and microvilli (curved arrows). Bar: 2 μm.

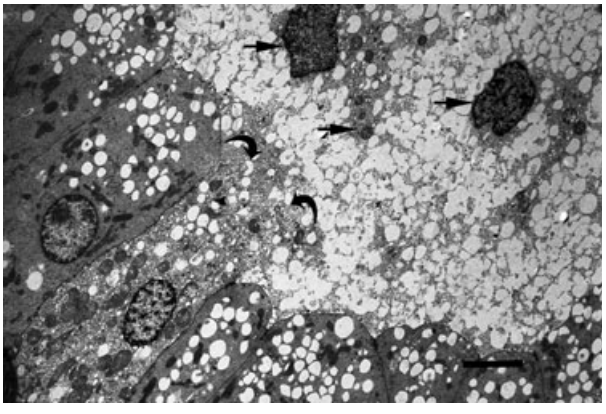


Fig. 8. Electron micrograph of type II cells. Particles of the cytoplasm and nucleus of type II cells (arrows) are present in the lumen of a glandular unit of the white lobe. Notice the degenerating cell with pale cytoplasm emptying its contents into the lumen (curved arrows). Bar: 0.9 μm.

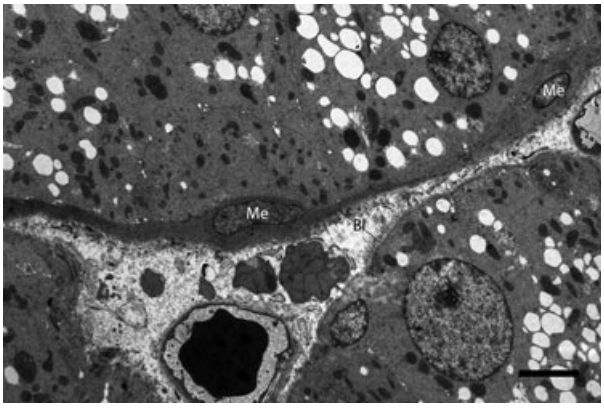


Fig. 9. Electron micrograph of the basal border of the Harderian gland. Myoepithelial cells (Me) lie between epithelial cells and the basal lamina (Bl). Bar: 1.2 μm.

cells contain small and more lightly stained vacuoles (Johnston et al., 1987). The presence of dense, ring-shaped material surrounding lipid vacuoles has been reported in the Mexican volcano mouse (*Neotomodon alstoni alstoni*) (Villapando et al., 2005) and Wistar rat (Djeridane, 1994). A similar dark coloured ring, which has been reported to surround small lipid vacuoles in the rabbit by Shirama et al. (1997), was not observed in this study.

While electron-dense secretory vesicles are described in the apical and basal regions of the epithelial cells of the gland in the ostrich (Altunay and Kozlu, 2004), only basally situated electron-light vesicles exist in the turkey (Maxwell et al., 1986). In this study, type I and type II cells of the Harderian gland in the Angora rabbit were noted to contain electron-light lipid vacuoles. The lipid release mechanism of the epithelium of the Harderian gland is controversially reported to be apocrine in rats (Tsutsumi et al., 1966), or to occur by an exocytotic mechanism (Winterhager and Kühnel, 1983; Lopez et al., 1993; Satoh et al., 1996). Furthermore, Björkman et al. (1960) mentioned the presence of a degenerative epithelium particularly in the pink lobe and rarely in the white lobe. In this study, the nuclear and cytoplasmic particles observed in the lumen of the glandular lobules in the Angora rabbit were thought to belong to degenerative cells. The Harderian gland is reported to display sexual dimorphism in the golden hamster (Hoffman, 1971; Payne et al., 1979; Mcmasters and Hoffman, 1984; Seyama et al., 1996) and Black Sea bottlenose dolphin

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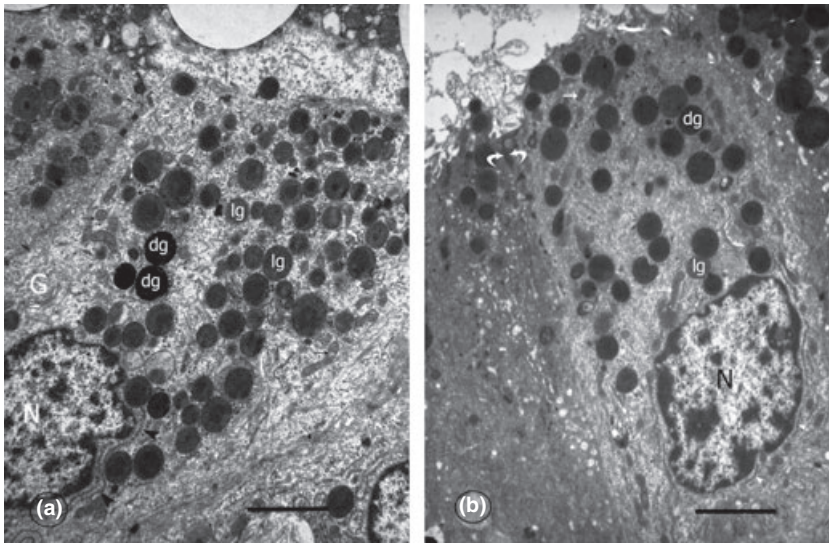


Fig. 10. Electron micrograph of a section of the excretory duct. (a) Light granules (lg), dark granules (dg), nucleus (N), mitochondria (arrows), Golgi apparatus (G). Bar: 3 μm. (b) Tight junction (curved arrows), rough endoplasmic reticulum (arrowheads). Bar: 2.1 μm.

(Bodyak and Stepanova, 1994). The secretion of the gland is of protein and carbohydrate character in the male dolphin and of lipid nature in the female dolphin (Bodyak and Stepanova, 1994). In contrast, sexual dimorphism has not been reported for the mouse (Woodhouse and Rhodin, 1963), rat (Kelenyi and Orban, 1965; Tsutsumi et al., 1966), rabbit (Björkman et al., 1960), guinea pig and Chinese hamster (Bucana and Nadakavukaren, 1972). Similarly, the histochemical and ultrastructural properties of the Harderian gland in the Angora rabbit were observed not to differ between males and females.

The ducts of the Harderian gland in the armadillo are situated intralobularly and inter-lobularly (Weaker, 1981; Aldana Marcos and Affanni, 2005). The duct is reported not to display any branching within the gland in the Mexican volcano mouse (*Neotomodon alstoni alstoni*) (Villapando et al., 2005) and Wistar rat (Djeridane, 1994). In this study, the ducts of the gland were determined to display both an intralobular and inter-lobular localization.

The duct epithelium is reported to contain lipid vacuoles in the woodmouse (Johnston et al., 1987) and serous and mucous granules in the Wistar rat (Djeridane, 1994). In studies conducted in the turkey, chicken and duck, the duct epithelium was determined to be PAS (+) and PAS/AB (+) (Burns and Maxwell, 1979; Maxwell and Burns, 1979). Secretion granules containing glucoconjugate were determined in the intralobular and inter-lobular ducts of the gland in the armadillo (Aldana Marcos and Affanni, 2005). To the best of our knowledge, no previous study exists on the histochemical and ultrastructural properties of the ducts of the Harderian gland in the rabbit. The excretory duct epithelium of the Harderian gland in the Angora rabbit displayed a similarity to that of avian species and the armadillo in containing PAS (+), PAS/AB (+), AB pH: 2.5 (+) and PA/AB (+) granules. Therefore, it was concluded that in addition to the lipid secretion of the gland, neutral and acidic mucopolysaccharides and proteins were secreted in the ducts. According to Djeridane (1994), these secretions may have a pheromonal function. The lipid vacuoles reported to exist in the duct epithelium of the woodmouse were not observed in the Angora rabbit.

Buzzle (1996) has drawn attention to the major role of the Harderian gland in the immune response in avian species. In studies carried out in the chicken (Wight et al., 1971), ostrich (Altunay and Kozlu, 2004) and turkey (Maxwell et al., 1986), numerous plasma cells were observed in the Harderian gland. In this study, plasma cells were abundant, particularly in the white lobe of the gland in the Angora rabbit. Therefore, it was considered that the white lobe may play a more significant role in the local immune protection of the eye.

In conclusion, the Harderian gland in the Angora rabbit was composed of two lobes only. Based on histochemical stainings, the Harderian gland secretion was determined to contain proteins and carbohydrates in addition to lipids. Because of the production of a variety of secretions, it was concluded that the Harderian gland may have a role in pheromonal functions in addition to being involved in the lubrication and local immune protection of the eye and in the movement of the eyelids.

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