Morphological, histochemical and immunohistochemical characterization of secretory production of the ciliary glands in the porcine eyelid

T. Yasui,[1] A. Tsukise,[1] T. Nara,[1] Y. Kuwahara,[1] W. Meyer[2]

[1]Department of Veterinary Anatomy, College of Bioresource Sciences, Nihon University, Kameino, Fujisawa, Kanagawa, Japan; [2]Anatomical Institute, University of Veterinary Medicine Foundation, Bischofsholer Damm, Hannover, Germany

In addition to performing general histology and cytology of the ciliary glands of the miniature pig, we studied the localization of glycoconjugates and β-defensins in these glands with the use of carbohydrate histochemical and immunohistochemical methods. The secretory cells of the glands were equipped with non-homogeneous secretory granules, a well-developed Golgi apparatus and rough endoplasmic reticulum. The secretory epithelium and luminal secretion of the glands contained large amounts of neutral and acidic glycoconjugates with various saccharide residues (α-L-Fuc, β-D-Gal, α-D-GalNAc and sialic acid). The sebaceous glands and tarsal glands also exhibited positive reactions to most of the histochemical methods. Additionally, the antimicrobial peptide group of β-defensins was demonstrated to be products of the ciliary glands, as well as the sebaceous glands and tarsal glands. The results obtained are discussed with regard to the specific function of the ciliary glandular secretions. These secretory products may be related to the moistening and general protection of the skin surface of the eyelid and ocular surface.

Key words: ciliary glands, histochemistry, glycoconjugate, β-defensins, eyelid, miniature pig.

Correspondence: A. Tsukise,
Department of Veterinary Anatomy, College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-8510, Japan
Tel/Fax: +81.0466.843629.
E-mail: tsukise@brs.nihon-u.ac.jp

In mammals, the upper and lower eyelids protect the eyeball and support the maintenance of a moist surface. The eyelids are covered internally with the palpebral conjunctiva, and externally with the skin. The marginal part of the eyelids contains eyelashes, sebaceous glands (*glands of Zeis*), tarsal glands (*glands of Meibom*) and specialized apocrine glands (referred to as *glands of Moll* or *ciliary glands*) (Bloom and Fawcett, 1962; Vaughan and Asbury, 1980; Calhoun and Stinson, 1981). Previous studies have only examined the ultrastructure and secretory products of the ciliary glands in humans and primate species (Stoeckel-huber *et al.,* 2003, 2004). These apocrine glandular secretions seem to function as agents of host defense in the eyelid shaft and on the ocular surface.

According to Ikeda (1953), the ciliary glands in the eyelids of non-primate mammals (e.g. pig, cow, horse, dog and cat) are abundant as compared to those of primates, e.g. humans and monkeys. The porcine ciliary glands are known to be particularly well developed within the domesticated mammalian species (Bacha and Bacha, 2000). In the non-primate mammals, however, the function of the secretion of this gland type has not been elucidated.

The secretory glycoconjugates produced by other modified apocrine glands, such as the ceruminous glands, may have functional significance, such as protection against microbial activity and water retention on the skin surface of the external auditory canal (Yasui *et al.,* 2003, 2004b, 2005). Additionally, in certain mammals, recent studies have demonstrated that the apocrine glandular secretions contain antimicrobial substances such as lysozyme and defensins (Meyer *et al.,* 2003; Yasui *et al.,* 2005). Among such substances, defensins are one of the main antimicrobial peptide families and are widely distributed in mammalian cells and tissues which are involved with host defense against microbial contamination (Ganz *et al.,* 1985; Ganz,

T. Yasui et al.

2003, 2004; Meyer and Seegers 2004).

Based on these findings, the present study on the ciliary glands of the miniature pig examined the general histology and cytology, the characterization and distribution of glycoconjugates and the expression of defensins, using conventional techniques, carbohydrate histochemical and immunohistochemical methods. The results obtained provide basic data on the functions of this gland type.

Materials and Methods

Experiments were performed in accordance with the guidelines for the care and use of laboratory animals at the Institute of Experimental Animal Science, College of Bioresource Sciences, Nihon University. Four adult male miniature pigs (potbelly, 1-2 years, 40-50 kg) were deeply anesthetized and then exsanguinated from the common carotid arteries. After bloodletting, skin samples were taken from the upper eyelid, because eyelashes are absent in the porcine lower eyelid.

General histology and cytology

The skin specimens for general histological observation were fixed in Bouin's solution, and then washed and dehydrated in a graded series of ethanol. The materials were embedded in paraffin wax and cut at a thickness of 5 μm. The paraffin sections were stained with hematoxylin and eosin (H-E) for histological purposes.

For general cytological observation, the skin samples were fixed in a mixture of 2% glutaraldehyde in 0.1M phosphate-buffered saline (PBS) (pH 7.4) for 2 h at 4°C, post-fixed in 2% osmium tetroxide solution for 2 h, and then dehydrated in a graded series of ethanol. The skin specimens were routinely embedded in Epon 812 (Luft, 1961). From these tissue blocks, ultrathin sections were cut using an ultramicrotome, mounted on copper grids and stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963). The ultrathin sections prepared were examined under an electron microscope (JEM 1010; JEOL Ltd., Tokyo, Japan).

Carbohydrate histochemistry

For carbohydrate histochemistry, the paraffin sections were prepared in the same way as for general histology. For the demonstration of glycoconjugates, the following histochemical staining methods were employed: the periodic acid-Schiff (PAS) method for the identification of glycans and glycoconjugates containing hydroxyl groups occurring in glycogen and neutral or weakly acidic glycoproteins (Pearse, 1985); alcian blue 8GX (AB) at pH 1.0 for the characterization of sulfated glycoconjugates, and AB at pH 2.5 for simultaneously testing for carboxyl groups and some sulfate esters in glycoconjugates. Simultaneous demonstration of neutral and acidic glycoconjugates was accomplished using the AB pH 2.5-PAS method (Spicer and Schulte, 1992). Neutral glycoconjugates were detected using the periodic acid-thiocarbohydrazide-silver proteinate-physical development (PA-TCH-SP-PD) (Nakamura *et al.,* 1985; Kitamura *et al.,* 1988; Yamada, 1993). Sensitized high iron diamine (S-HID) and sensitized low iron diamine (S-LID) (Hirabayashi 1992) techniques were used for the detection of small amounts of acidic glycoconjugates.

Additionally, the sections were incubated with different biotinylated lectins (Seikagaku Kogyo Co., Tokyo, Japan) at concentrations of 10-20 μg/mL in 0.05M PBS (pH 7.2) for 90 min at room temperature, following treatment with 0.1% avidin solution and 0.01% biotin solution for inactivation of endogenous biotin and then 0.3% hydrogen peroxide for the suppression of endogenous peroxidase

Table 1. The lectins used and their sugar binding specificities.

	Lectins	Sugar binding specificity	Inhibitory sugar
Con A	concanavalin A	α-D-Man, α-D-Glc	Man, α-methyl-D-glucoside
UEA-I	*Ulex europaeus agglutinin-I*	α-L-Fuc	α-L-Fuc
DBA	*Dolichos biflorus agglutinin*	GalNAcα1-3GalNAc	α-D-GalNAc
RCA-120	*Ricinus communis* agglutinin-120	Galβ1-4GlcNAc	β-D-Gal
PNA	peanut agglutinin	Galβ1-3GalNAc	β-D-Gal, α-lactose
WGA	wheat germ agglutinin	β-D-GlcNAc	β-D-GlcNAc
SSA	*Sambucus sieboldiana agglutinin*	Siaα2-6Gal/GalNAc	α2-6sialyllactose
MAM (MAL)	*Maackia amurensis agglutinin*	Siaα2-3Gal1-4GlcNAc	α2-3sialyllactose

Abbreviations: Gal: galactose, GalNAc: N-acetyl-galactosamine, Glc: glucose, GlcNAc: N-acetyl-glucosamine, Fuc: fucose, Man: mannose, Sia: sialic acid.

activity. The lectins used, their specific sugar residues and inhibitory sugars are listed in Table 1 (for lectin specificities see Debray *et al.,* 1981; Spicer and Schulte, 1992; Danguy, 1995). After rinsing with PBS, the sections were incubated in peroxidase-labeled streptavidin (SAB-PO) (Nichirei Co., Tokyo, Japan; 100 μg/mL) for 30 min at room temperature. The activity of the peroxidase employed for labeling was revealed by a solution containing 0.05M Tris-HCl buffer (pH 7.6), diaminobenzidine (DAB) and hydrogen peroxide.

Lectin controls were performed by the addition of inhibitory sugars at a final concentration of 0.01M to the respective lectin solutions, by the substitution of unconjugated lectins for biotinylated lectin-conjugates, and by the exposure of sections to the SAB-PO-DAB system without lectin. In addition, enzyme digestion with sialidase (from *Arthrobacter ureafaciens,* Nacalai Tesque Inc., Kyoto, Japan; 1.0U/mL in 0.1M PBS, pH 5.3, at 37°C for 18 h) (Spicer *et al.,* 1967) after saponification with 0.5% potassium hydroxide-70% ethanol (Spicer, 1960), was performed before the incubation with SSA and MAM solutions.

Immunohistochemistry

The paraffin sections were treated for the blocking of endogenous biotin and peroxidase activities, respectively, following incubation with proteinase K solution (Dako Co. Ltd., Glostrup, Denmark) or 0.1% trypsin for 30 min at 37°C (Hautzer *et al.,* 1980). Then, these sections were stained immuno-histochemically for the demonstration of β-defensin-1 (dil. 1:250, 1:500; anti-human, from rabbit, Biologo, Kronshagen, Germany) and β-defensin-2 (dil. 1:250, 1:500; anti-human/anti-mouse, from rabbit, Biologo) (Meyer *et al.* 2004). Following incubation for 12 h at 4°C, the sections were incubated with biotinylated secondary antibody (dil. 1:200; anti-rabbit, from goat, Zymed Laboratories Inc., South San Francisco, CA, USA) and then with SAB-PO. The activity of the peroxidase employed for labeling was revealed by a DAB-hydrogen peroxide system.

Controls for immunohistochemical methods were performed by the replacement of the primary antibodies with normal (non-immune) rabbit immunoglobulin (Dako Co. Ltd.) diluted to the same extent as the specific antibodies, the incubation with appropriate buffer without primary antibodies or the exposure of sections to the SAB-PO-DAB system without primary or secondary antibodies.

Results

General histology and cytology

In the upper eyelid of the miniature pig, the well-developed apocrine ciliary glands and sebaceous glands were located near the eyelash follicles (Figure 1a). The height of the secretory cells of the ciliary glands varied from flattened cuboidal to tall columnar, depending on the secretory status of the cells (Figure 1a, b). Many of the latter cells formed an apical protrusion indicating an apocrine secretory mechanism toward the glandular lumen. The amount of secretion differed greatly with respective lumina. These secretory materials appeared as irregular granular masses, which were the most common, or spherical drops spread between the granular masses. The ducts of these glands were connected with the hair follicle of the eyelash. Additionally, the less-abundant tarsal glands were observed at the inner side of the eyelids (Figure 1a).

Electron microscopic observation of the pig ciliary glands revealed the glandular acinus to consist of a single layer of columnar secretory cells. The secretory cells with associated myoepithelial cells rested on a basement membrane and their oval nuclei were located in the central or basal region of the cytoplasm (Figure 2a). The plasma membrane of the secretory cells facing the glandular lumen projected into microvilli (Figure 2a), whereas there were very few microvilli on the surface of the tall apical protrusions. Additionally, some secretory cells, which appeared to pinch off the apical protrusion according to the apocrine secretion mode, also had no microvilli at their luminal surface. In the supranuclear cytoplasm of the secretory cells, a well-developed Golgi apparatus with some associated immature granules was detected. The supranuclear and apical regions of these cells contained a varying number of non-homogeneous secretory granules possessing medium electron density and sometimes containing distinct dense substances (Figure 2b). Additionally, small vesicles were found below the luminal surface. These granules and vesicles frequently showed the morphological features suggestive of exocytosis. Another prominent feature noted in the secretory cells was rough endoplasmic reticulum, mainly in the perinuclear cell region (Figure 2c). Relatively numerous mitochondria with varying morphologies were scattered throughout the cytoplasm of the secretory cells among the ultrastructures described above.
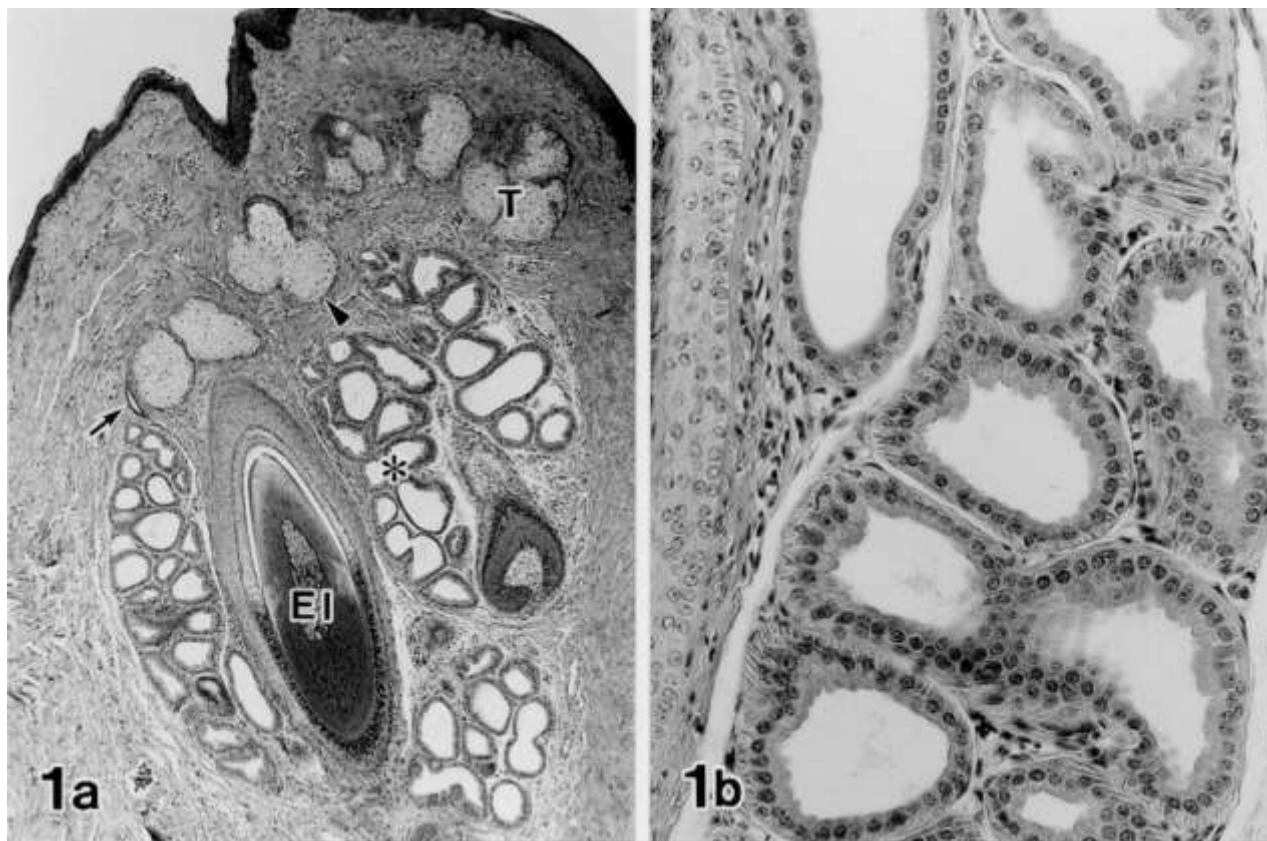
T. Yasui et al.

Figure 1. Light microscopical structure of the upper eyelid of the miniature pig stained with H-E. a) The ciliary glands are observed near the eyelash follicles. ×50; b) Higher magnification of the apocrine ciliary glands. ×250. El: eyelash, T: tarsal gland, arrow: excretory duct, arrowhead: sebaceous gland, asterisk: ciliary gland.

Table 2. Glycoconjugate histochemical reactions of the ciliary glands in the miniature pig.

| | Ciliary glands | | | | |
	Secretory cells	Luminal secretion	Excret. duct cells	Sebaceous glands	Tarsal glands
PAS	2	2	1-2	1	1
AB pH 1.0	2	2-3	1	1	1
AB pH 2.5	1-2	2	1	1	1
AB pH 2.5-PAS	2	2-3	2	1-2	1-2
PA-TCH-SP-PD	3-4	4	3	2	2
S-HID	4	4	1-2	2	2
S-LID	3-4	4	1-2	2-3	2-3
Con A	3	3-4	3	3	3
UEA-I	4	3-4	3	3	3
DBA	3-4	3-4	3	2-3	3
RCA-120	3	3-4	2	3	3
PNA	4	4	3-4	3-4	3
WGA	2-3	2	1-2	2-3	2-3
SSA	4	4	1-2	3	3
MAM (MAL)	3-4	3-4	2	3-4	3-4

Reaction intensities: 0: negative; 1: very weak; 2: weak; 3: moderate; 4: strong.

The excretory ducts consisted of a bistratified epithelium, apical and basal cells being relatively rich in mitochondria (Figure 2d). In the duct epithelial cells, microvilli were not prominent at the apical plasma membrane, and their nuclei were situated in the central or basal cytoplasm.

Carbohydrate histochemistry

The structural components of the ciliary glands showed various reactions to the different carbohydrate histochemical methods applied, the histochemical results of which are summarized in Table 2.

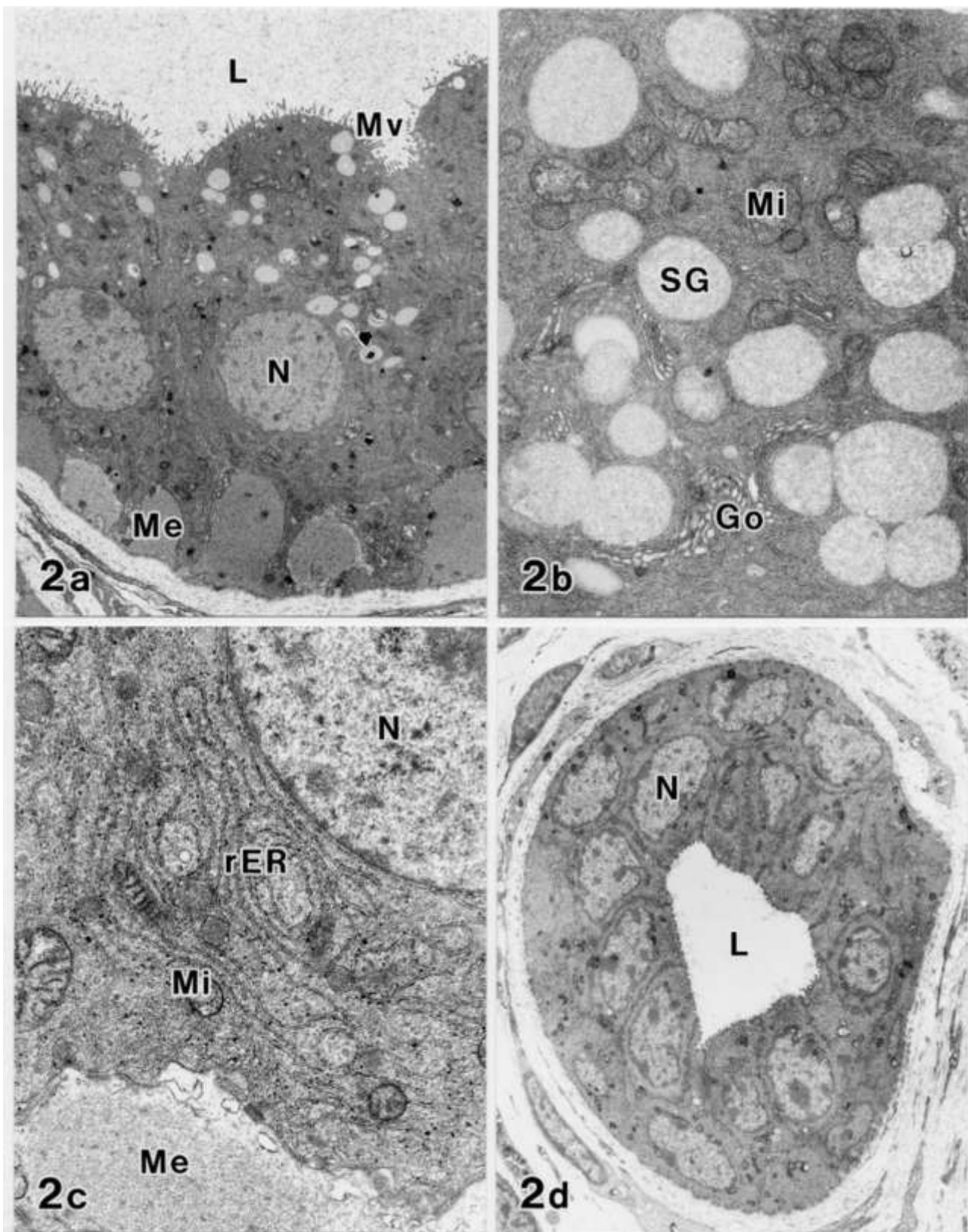In the section stained with PAS, AB pH 1.0, AB

Figure 2. Ultrastructure of the ciliary glands in the miniature pig stained with uranyl acetate and lead citrate. a) The secretory portion consists of a single layer of columnar secretory cells with myoepithelial cells. ×4,500; b) Part of the supranuclear cytoplasm of secretory cells. ×16,000; c) Part of the infranuclear cytoplasm of secretory cells. ×17,000; d) The excretory ducts consist of typical luminal and peripheral cells. ×3,000. Go: Golgi apparatus, L: lumen, Me: myoepithelial cell, Mi: mitochondria, Mv: microvilli, N: nucleus, rER: rough endoplasmic reticulum, SG: secretory granule.
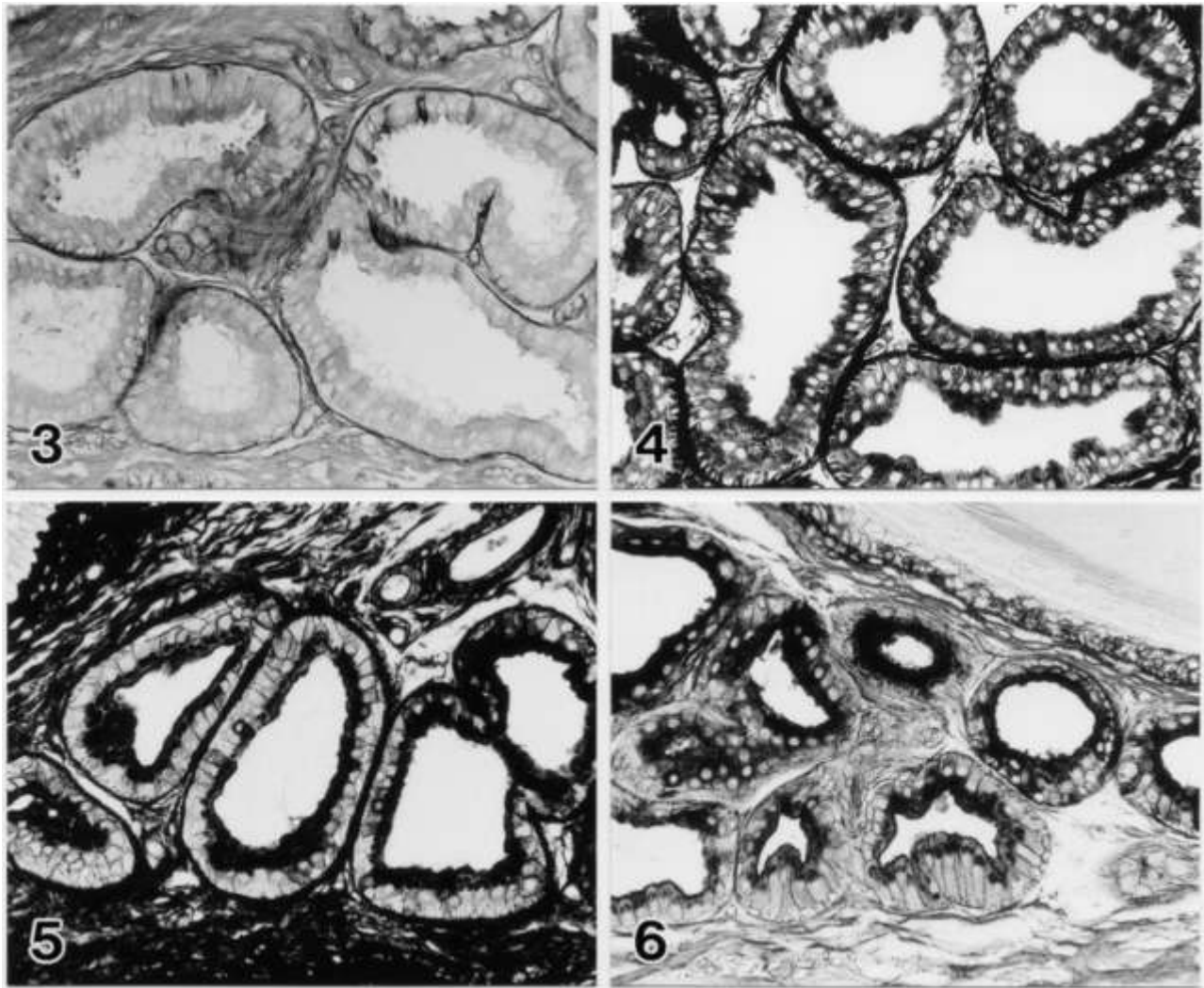
T. Yasui et al.

Figure 3. General carbohydrate histochemical appearance of the ciliary glands. The apical cytoplasm and supranuclear region of the secretory epithelium show weak reactions. AB pH 2.5-PAS, ×250. Figure 4. PA-TCH-SP-PD reaction in the ciliary glands. The secretory epithelium exhibits a strong to moderate reaction, especially in the apical cytoplasm and supranuclear region of most of the secretory cells. ×250. Figure 5. S-HID reaction in the ciliary glands. Strong reactions are observed in the apical cytoplasm and supranuclear region of most of the secretory cells. ×250. Figure 6. S-LID reaction in the ciliary glands. The apical cytoplasm and supranuclear region of the secretory cells show relatively strong reactions. ×250.

pH 2.5 and AB pH 2.5-PAS, the reaction intensities of the secretory epithelium and luminal secretion were generally weak (Figure 3). Relatively weak reactions were observed in the excretory duct cells, sebaceous glands and tarsal glands after these histochemical procedures. The PA-TCH-SP-PD procedure produced a strong to moderate reaction in the secretory cells and a strong reaction in the luminal secretion (Figure 4), while the cells of the excretory ducts exhibited a moderate reaction. In the tissue sections reacted for the S-HID and S-LID procedures, the secretory cells and luminal secretion showed distinctly positive reactions (Figures 5, 6), whereas the excretory duct cells were stained weakly. The three histochemical techniques mentioned above resulted in moderate to weak reactions in the sebaceous glands and tarsal glands.

When the sections were subjected to the lectin histochemical procedures, the secretory epithelium and luminal secretion showed for the most part generally strong to moderate reactions (e.g. Con A, Figure 7a; RCA-120, Figure 7c; MAM, Figure 7f). In particular, distinct reaction intensities of the glandular acini were obtained after the application of UEA-I (Figure 7b), PNA and SSA (Figure 7e). On the other hand, the intensity of the WGA reactions was moderate to weak (Figure 7d). The excre-
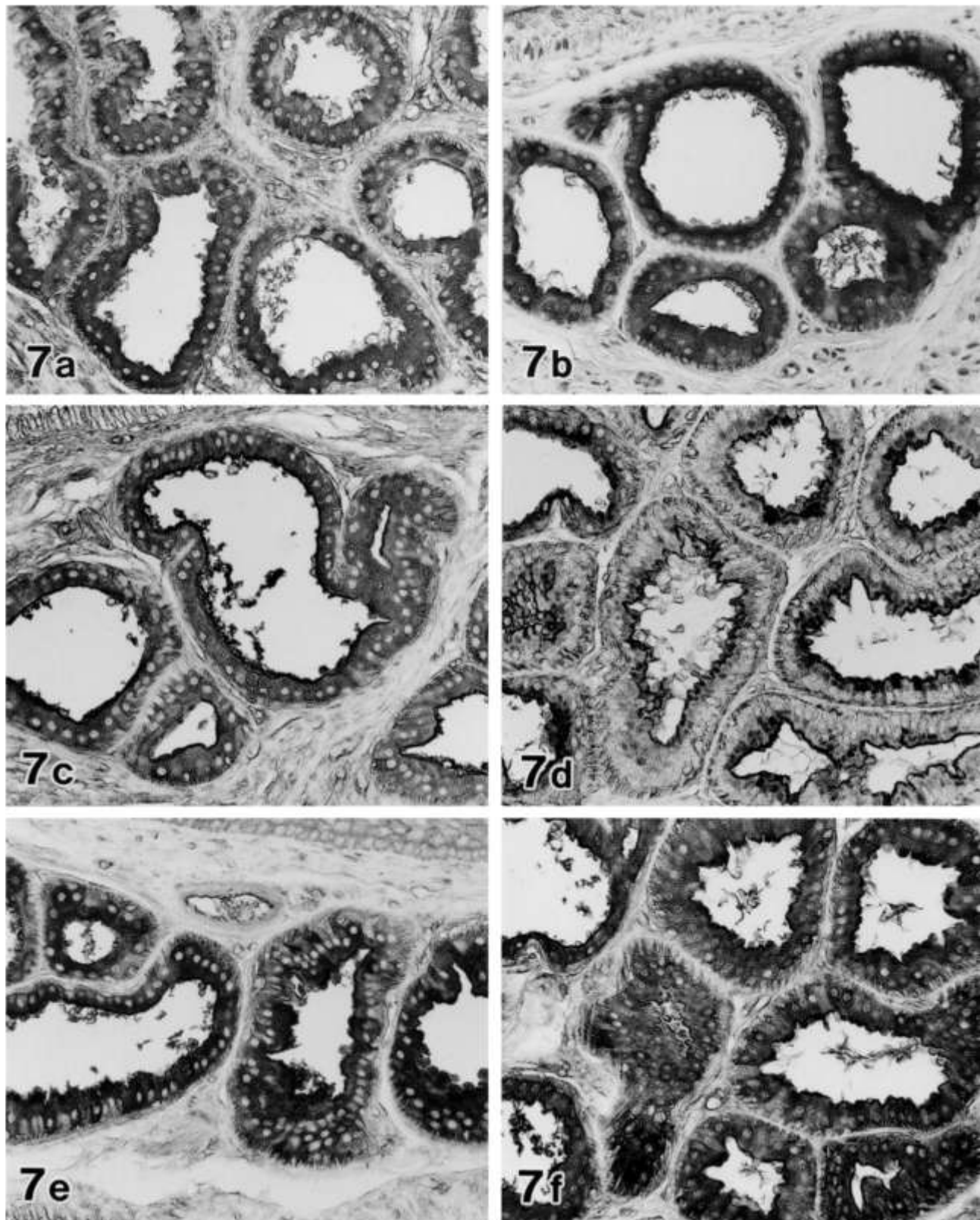
Figure 7. Lectin histochemical staining in the ciliary glands. a) The secretory epithelium reacts moderately. Con A, ×250; b) The reaction intensity of the secretory epithelium is strong. UEA-I, ×250; c) The secretory epithelium exhibits a moderate reaction, while relatively stronger staining is confined to the luminal surface. RCA-120, ×250; d) The secretory cells generally shows a weak reaction, whereas stronger reactions are often seen in the substance at the apical part and luminal surface of them. WGA, ×250; e) The secretory epithelium reveals a strong reaction. SSA, ×250; f) The secretory epithelium is found to exhibit a strong reaction. MAM, ×250.
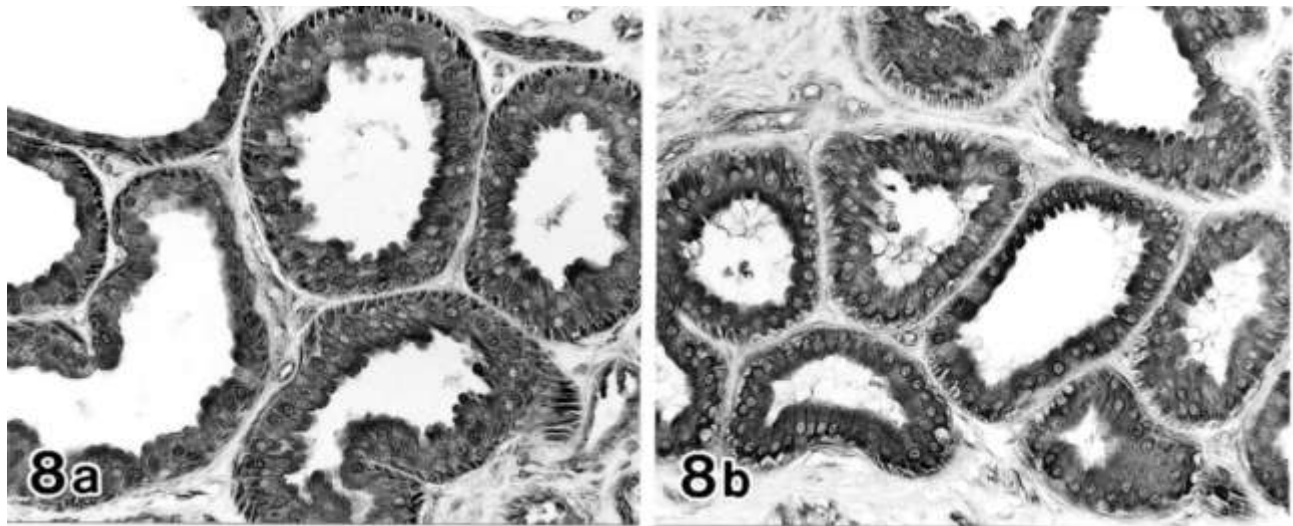
T. Yasui et al.

Figure 8. Immunohistochemical staining for ,-defensins in the ciliary glands. a) The secretory epithelium reacts moderately, while the apical region of the epithelium shows a more or less stronger reaction. Anti-β-defensin-1, ×250; b) The secretory epithelium exhibits a moderate reaction. Anti-β-defensin-2, ×250.

tory duct cells showed generally moderate reactions with Con A, UEA-I, DBA and PNA, while the other lectins produced weak reactions. With regard to all the lectins employed, the sebaceous glands and tarsal glands were generally found to exhibit moderate reactions. In the carbohydrate histochemcial methods including lectin histochemistry, relatively distinct reactions generally occurred in the surpranuclear and/or apical regions of the secretory cells, and, in some cases, in the entire cell.

In the control tissue sections incubated with the lectin solutions containing inhibitory monosaccharides, the positive lectin reactions were abolished in the glandular structures. Additionally, no staining was evident in the lectin control procedures after the exposure of sections to a solution substituting unconjugated lectins or to the SAB-PO-DAB system without lectins. The SSA and MAM reaction intensities were almost negative with sialidase digestion after saponification.

Immunohistochemistry

The results obtained from the immunohistochemical staining are summarized in Table 3. Positive moderate reactions for β-defensin-1 were detectable in the secretory epithelium and luminal secretion, whereas the excretory duct cells, sebaceous glands and tarsal glands were seen to exhibit moderate to weak reactions (Figure 8a). Incubation with antibodies to β-defensin-2 led to the same staining images as those obtained with β-

defensin-1 (Figure 8b).

In the control experiments for immunohistochemical staining performed by the replacement of the primary antibodies with normal rabbit immunoglobulin, the incubation with appropriate buffer without primary antibodies or the exposure of sections to the SAB-PO-DAB system without primary or secondary antibodies, no skin structures exhibited any positive reactions.

Discussion

The present study revealed the general histology and cytology, and the characterization and localization of glycoconjugates and β-defensins in the ciliary glands of the miniature pig. The porcine upper eyelid contained prominent apocrine ciliary glands, sebaceous glands and less-abundant tarsal glands. In humans and primate species, the secretory cells of the ciliary glands have a cytoplasm containing dark secretory granules, well-developed rough endoplasmic reticulum and numerous mitochondria (Stoeckelhuber *et al.,* 2003, 2004). Furthermore, the lipid droplets and non-homogeneous granules were also observed in the secretory cells of the monkey (Stoeckelhuber *et al.,* 2004). In the secretory cells of these glands of the miniature pig, the secretory granules were similar to the non-homogeneous granules of the monkey species, although the dark granules and lipid droplets could not be found in the cytoplasm. Additionally, in the present study,

Table 3. Immunohistochemical reactions of the ciliary glands in the miniature pig.

	Ciliary glands				
	Secretory cells	*Luminal secretion*	*Excretary duct cells*	*Sebaceous glands*	*Tarsal glands*
β-Defensin-1	3	3	2-3	2-3	2-3
β-Defensin-2	3	3	2-3	2-3	2-3

Reaction intensities: 0: negative; 1: very weak; 2: weak; 3: moderate; 4: strong.

the columnar secretory cells, representing an active form of the gland, abounded in the ciliary glands as compared with the apocrine glands found in the common integument and scrotal skin (Tsukise and Yamada, 1981; Tsukise and Meyer, 1983).

The carbohydrate histochemical methods applied demonstrated positive reactions of varying intensities for different glycoconjugates in the structures of the ciliary glands of the miniature pig. In view of the basic staining properties of the PAS and PA-TCH-SP-PD procedures, these results indicate a preponderance of neutral glycoconjugates (Pearse, 1985; Yamada, 1993). Additionally, the secretions produced were also found to contain acidic glycoconjugates with sulfate ester and/or carboxyl groups, the findings of which are supported by the staining results of the AB pH 1.0, AB pH 2.5, S-HID and S-LID methods (Hirabayashi, 1992; Spicer and Schulte, 1992).

The more sensitive lectin histochemical staining procedure revealed a spectrum of particular saccharide residues in the tissues examined. Thus, it became obvious that the porcine ciliary glands had a higher concentration of α-L-Fuc and sialyl residues as well as the Galβ1-3GalNAc sequence than the apocrine glands in the common integument (Tsukise and Meyer, 1983). In addition, our results are similar to the lectin binding patterns of the human ciliary glands (Stoeckelhuber *et al.,* 2003). According to the histological and ultrastructural observation of this study, the secretory mechanism in the porcine ciliary glands seems to be a combination of apocrine secretion and exocytosis. Furthermore, the histochemical staining patterns of the secretory cells may also demonstrate a secretory process of these secretory modes (Atoji *et al.,* 1987). The intercellular inclusion of α-L-Fuc, β-D-Gal, α-D-GalNAc and sialic acid represents the existence of mucin-type glycoproteins, which have the capability of water retention (Campbell, 1999). The secretory glycoconjugates of the ciliary glands also had a high sulfate group content, which seems

to be the regulatory factor of mucus viscosity with α-L-Fuc and sialic acid (Kasinathan *et al.,* 1991; Slomiany *et al.,* 1991; Majima *et al.,* 1999). In this regard, sulfation and sialylation have been considered to increase the resistance of mucus to bacterial degradation (Rhodes *et al.,* 1985). The tear film, which maintains a smooth and refractive surface of optical quality, is stabilized by the mucins, including the carbohydrate compositions (Argüeso and Gipson, 2001). It is conceivable that the glycoconjugate production of the ciliary glands supplements the ocular mucins.

The lectin histochemical results also disclosed that α-D-Man and β-D-GlcNAc residues were present in the secretions of the ciliary glands. Most of the terminal sugars secreted may show certain antimicrobial functions on the skin surface, because they have the ability to prevent the adherence of different bacteria and fungi to the epidermal cells (Meyer *et al.,* 2000, 2001; Yasui *et al.,* 2004a).

The present study demonstrated the expression of β-defensins in the ciliary glands, as well as the sebaceous glands and tarsal glands. Defensins have a role in the innate immune response of the skin against microbial invaders. They lead to the disruption and subsequent death of the microorganisms such as bacteria, fungi and some enveloped viruses by their ability to insert themselves into these cell membranes (Bos *et al.,* 2001). It has been shown that β-defensins are expressed in the ciliary glands of primates (Stoeckelhuber *et al.,* 2004), while porcine β-defensins are known to be produced by the epithelium of different organs (Zhang *et al.,* 1998; Shi *et al.,* 1999). Additionally, this peptide group may hold therapeutic potential in ocular infection as they have a broad spectrum of antimicrobial activity (Haynes *et al.,* 1999).

In conclusion, the results of the present study showed the localization of glycoconjugates and β-defensins in the ciliary glands of the miniature pig. Several other glandular structures, for example the lacrimal glands, goblet cells of the conjunctiva and

tarsal glands have been considered to contribute to the tear film (Argüeso and Gipson, 2001; Bron *et al.,* 2004). In addition to these secretory products, it is suggested from our findings that the glandular secretions of the porcine ciliary glands may play an essential role in the preservation of humidity and the protection against environmental pathogens not only in the eyelid skin, but also on the ocular surface.

References

Argüeso P, Gipson IK. Epithelial mucins of the ocular surface: structure, biosynthesis and function. Exp Eye Res 2001; 73: 281-9.

Atoji Y, Hori Y, Sugimura M, Suzuki Y. Lectin histochemical study on the infraorbital gland of the Japanese serow (Capricornis crispus). Acta Morphol Neerl-Scand 1987; 25: 201-13.

Bacha WJ Jr., Bacha LM. The eye. In: Color atlas of Veterinary Histology, 2nd edn. Lippincott Williams and Wilkins, Bartimore, Philadelphia, 2000, pp. 245-60.

Bloom W, Fawcett DW. The eye. In: A Textbook of Histology, 8th edn. Sauders, Philadelphia 1962, pp. 635-66.

Bos JD, Pasch MC, Asghar SS. Defensins and complement systems from the perspective of skin immunity and autoimmunity. Clin Dermatol 2001; 19: 563-72.

Bron AJ, Tiffany JM, Gouveia SM, Yokoi N, Voon LW. Functional aspects of the tear film lipid layer. Exp Eye Res 2004; 78: 347-360.

Calhoun ML, Stinson AW. Integument. In: Dellman HD, Brown EM, eds. Textbook of Veterinary Histology. 2nd edn. Lea and Febiger, Philadelphia, 1981, pp. 378-411.

Campbell BJ. Biochemical and functional aspects of mucus and mucin-type glycoproteins. In: Mathiowitz E, Chickering DE, Lehr CM, eds. Bioadhesive Drug Delivery Systems, vol. 98. Drugs and the pharmaceutical sciences. Marcel Dekker, New York, 1999, pp. 85-130.

Danguy A. Perspectives in modern glycohistochemistry. Eur J Histochem 1995; 39: 5-14.

Debray H, Decout D, Strecker G, Spik G, Montreuil J. Specificity of twelve lectins towards oligosaccharides and glycopeptides related to N-glycosylproteins. Eur J Biochem 1981; 117: 41-55.

Ganz T, Selsted ME, Szklarek D, Harwig SS, Daher K, Bainton DF, Lehrer RI. Defensins. Natural peptide antibiotics of human neutrophils. J Clin Invest 1985; 76: 1427-35.

Ganz T. Defensins: antimicrobial peptides of innate immunity. Nat Rev Immunol 2003; 3: 710-20.

Ganz T. Defensins: antimicrobial peptides of vertebrates. C R Biol 2004; 327: 539-49.

Hautzer NW, Wittkuhn JF, Caughey WTE. Trypsin digestion in immunoperoxidase staining. J Histochem Cytochem 1980; 28: 52-3.

Haynes RJ, Tighe PJ, Dua HS. Antimicrobial defensin peptides of the human ocular surface. Br J Ophthalmol 1999; 83: 737-41.

Hirabayashi Y. Light-microscopic detection of acidic glycoconjugates with sensitized diamine procedures. Histochem J 1992; 24: 409-18.

Ikeda M. Über die Ciliardrüsen der Säugetiere. Okajimas Folia Anat Jpn 1953; 25: 163-168.

Kasinathan C, Liau YH, Murty VLN, Slomiany BL, Slomiany A. Identification of gastric mucosal mucus glycoprotein sulfotransferase. Biochem Int 1991; 24: 43-9.

Kitamura H, Nakamura M, Yamada K. The utility of two physical developers for certain histochemical methods for glycoconjugates in light and electron microscopy. In: Glycoconjugates in Medicine (Proceedings of an International Symposium, Kagoshima, Japan). Professional Postgraduate Services, Tokyo, 1988, pp. 24-8.

Luft JH. Improvements in epoxy resin embedding methods. J Biophys Biochem Cytol 1961; 9: 40914.

Majima Y, Harada T, Shimizu T, Takeuchi K, Sakakura Y, Yasuoka S, Yoshinaga S. Effect of biochemical components on rheologic properties of nasal mucus in chronic sinusitis. Am J Respir Crit Care Med 1999; 160: 421-426.

Meyer W, Bollhorn M, Stede M. Aspects of general antimicrobial properties of skin secretions in the common seal Phoca vitulina. Dis Aquat Organ 2000; 41: 77-9.

Meyer W, Neurand K, Tanyola? A. General anti-microbial properties of the integument in fleece producing sheep and goats. Small Rumin Res 2001; 41: 181-90.

Meyer W, Seegers U, Herrmann J, Schnapper A. Further aspects of the general antimicrobial properties of pinniped skin secretions. Dis Aquat Org 2003; 53: 177-9.

Meyer W, Seegers U. A preliminary approach to epidermal antimicrobial defense in the Delphinidae. Marine Biol 2004; 144: 841-4.

Nakamura M, Kitamura H, Yamada K. A sensitive method for histochemical demonstration of vicinal diols of carbohydrates. Histochem J 1985; 17: 477-85.

Pearse AGE. Carbohydrates and mucosubstances. In: Histochemistry, Theoretical and Applied, 4th edn. vol. 2. Churchill Livingstone, Edinburgh, 1985, pp. 675-753.

Reynolds ES. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J Cell Biol 1963; 17: 208-12.

Rhodes JM, Black RR, Gallimore R, Savage A. Histochemical demonstration of desialation and desulphation of normal and inflammatory bowel disease rectal mucus by faecal extracts. Gut 1985; 26: 1312-8.

Shi J, Zhang G, Wu H, Ross C, Blecha F, Ganz T. Porcine epithelial b-defensin 1 is expressed in the dorsal tongue at antimicrobial concentrations. Infect Immun 1999; 67: 3121-7.

Slomiany BL, Liau YH, Li Q, Fekete Z, Slomiany A. Role of sulphation in post-translational processing of rat salivary mucins. Arch Oral Boil 1991; 36: 785-90.

Spicer SS. A correlative study of the histochemical properties of rodent acid mucopolysaccharides. J Histochem Cytochem 1960; 8: 18-35.

Spicer SS, Horn RG, Leppi TJ. Histochemistry of connective tissue mucopolysaccharides. In: Wagner BM, Smith DE eds. The Connective Tissue. Williams and Wilkens, Baltimore, 1967, pp. 251-303.

Spicer SS, Schulte BA. Diversity of cell glycoconjugates shown histochemically: a perspective. J Histochem Cytochem 1992; 40: 1-38.

Stoeckelhuber M, Stoeckelhuber BM, Welsch U. Human glands of Moll: histochemical and ultrastructural characterization of the glands of Moll in the human eyelid. J Invest Dermatol 2003; 121: 28-26.

Stoeckelhuber M, Stoeckelhuber BM, Welsch U. Apocrine glands in the eyelid of primate contribute to the ocular host defense. Cells Tissues Organs 2004; 176: 187-94.

Tsukise A, Yamada K. The histochemistry of complex carbohydrates in the scrotum of the boar. Histochemistry 1981; 72: 511-21.

Tsukise A, Meyer W. Histochemistry of complex carbohydrates in the hairy skin of the domestic pig. Histochem J 1983; 15: 845-60.

Vaughan D, Asbury T. The lids. In: General Ophthalmology, 9th edn. Lange Medical Publications, Los Altos, 1980, pp. 45-6.

Watson ML. Staining of tissue sections for electron microscopy with heavy metals. J Biophys Biochem Cytol 1958; 4: 475-85.

Yamada K. Histochemistry of carbohydrates as performed by physical development procedures. Histochem J 1993; 25: 95-106.

Yasui T, Tsukise A, Meyer W. Histochemical analysis of glycoconjugates in the ceruminous glands of the North American raccoon (Procyon lotor). Ann Anat 2003; 185: 223-31.

Yasui T, Tsukise A, Meyer W. Histochemical analysis if glycoconjugates in eccrine glands of the raccoon digital pads. Eur J Histochem 2004a; 48: 393-402.

Yasui T, Tsukise A, Nara T, Habata I, Meyer W. Histochemistry of complex carbohydrates in the ceruminous glands of the goat. Arch Dermatol Res 2004b; 296: 12-20.

Yasui T, Tsukise A, Fukui K, Kuwahara K, Meyer W. Aspects of glycoconjugate production and lysozyme- and defensin-expression of the ceruminous glands of the horse (Equus prezewalskii f. dom.). Eur J Morphol 2005; 42: 127-34.

Zhang G, Wu H, Shi J, Ganz T, Ross CR, Blecha F. Molecular cloning and tissue expression of porcine b-defensin-1. FEBS Lett 1998; 424: 37-40.