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► **To cite this version:**

Anna Maria Gargiulo, Paola Coliolo, Piero Ceccarelli, Vera Pedini. Ultrastructural study of sheep lacrimal glands. *Veterinary Research*, 1999, 30 (4), pp.345-351. hal-00902573

**HAL Id: hal-00902573**

**<https://hal.science/hal-00902573>**

Submitted on 11 May 2020

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## Ultrastructural study of sheep lacrimal glands

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(Received 22 October 1998; accepted 26 March 1999)

**Abstract** – Sheep lacrimal glands are mixed glands, consisting of tubulo-acinar units succeeded by ducts of simple morphology. The secretory portions consist of three cell types: mucous, seromucous and serous, which may be intermingled in the same acinus or may form acini wholly made of only serous or mucous cells. Mucous cells show a rough endoplasmic reticulum that is reduced to a few cisternae located near the cell base and among the interstices of the secretory droplets. Mucous granules appear uniformly electron-lucent. Serous cells display a typical structure; serous granules can be uniformly electron-dense or composed of dense inclusions dispersed in an electron-lucent matrix. The seromucous granules have a bizonal substructure: a dense core is embedded in a lighter matrix. Secretory acini are succeeded by intercalated ducts; the epithelium of these ducts gradually increases in height to form a kind of excretory duct, without the intervention of striated ducts. © Inra/Elsevier, Paris.

**sheep / lacrimal gland / ultrastructure / mucous cell / serous cell**

**Résumé** – **Étude ultrastructurale des glandes lacrymales chez le mouton.** Les glandes lacrymales des ovins sont des glandes mixtes constituées par des unités tubulo-acineuses suivies de canaux excréteurs de morphologie simple. Les acini glandulaires sont constitués par trois types de cellules : des cellules muqueuses, des cellules séromuqueuses et des cellules séreuses qui peuvent être mélangées dans le même acinus ou qui peuvent former des acini entièrement constitués seulement par des cellules muqueuses ou séreuses. Le réticulum endoplasmique rugueux des cellules muqueuses se réduit toujours à quelques citernes situées près de la base de la cellule et entre les interstices des grains de sécrétion qui ont un aspect uniformément transparent aux électrons. Les cellules séreuses ont une structure typique ; les granules séreux peuvent être uniformément denses aux électrons ou peuvent être constitués d'inclusions denses dispersées dans une matrice transparente aux électrons. Les granules séromuqueux ont une substructure à deux zones : un noyau dense est encastré dans une matrice moins foncée. Les acini sécrétoires sont suivis par des canaux excréteurs qui ressemblent à des canaux intercalaires et qui se continuent en des canaux intralobulaires revêtus d'un épithélium cylindrique simple sans l'intervention des canaux striés. © Inra/Elsevier, Paris.

**ovin / glande lacrymale / ultrastructure / cellule muqueuse / cellule séreuse**

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## 1. INTRODUCTION

In all mammals the surface of the eye is protected, nourished and lubricated by the aqueous tears that are synthesized and secreted by the lacrimal gland. Lacrimal gland anatomy has been studied in various laboratory and domestic animals [1, 7, 8, 10, 12, 13, 17, 21]. These studies demonstrated that lacrimal glands are similar in various species, having a compound tubulo-acinar structure. However, on the basis of histochemical testing and ultrastructural features, species variability has been reported. The main differences detected regard secretory granules.

For some time many authors [15, 18] have put forward the problem of an exact classification of the secretion of several exocrine glands, especially of the salivary glands, examining morphologic and histochemical criteria. In a previous study [2], we classified the glandular cells of the sheep lacrimal gland as mucous, seromucous and serous cells on a histochemical basis.

Young and van Lennep [34] advocated a classification based on ultrastructure. Cells whose granules appeared to have an electron-dense matrix were considered to be serous. Moreover, these authors classified as seromucous the secretory granules that showed a moderate electron-dense matrix often with one or more dense inclusions. In contrast, the typical mucous granules have a lucent matrix in which a faint fibrillogranular material is suspended [24].

Because there have been no previous specific ultrastructural descriptions of sheep lacrimal glands, the purpose of this study was to assess the ultrastructural morphology of sheep lacrimal glands, with particular attention to the secretory process, in an attempt to confirm the previously proposed classification and to make a comparison with data obtained in other mammalian species.

## 2. MATERIALS AND METHODS

### 2.1 Animals

Lacrimal glands were obtained from ten adult sheep of both sexes that were free of ocular disease and had normal tear production, killed at the local slaughterhouse. The lacrimal gland was a flattened, oval, pale red gland situated within a special division of the periorbital between the dorsolateral aspect of the eyeball and the supraorbital process of the frontal bone.

### 2.2 Tissue preparation

Small tissue blocks were fixed in phosphate-buffered Karnovsky's fixative pH 7.2 for 3 h at room temperature and then postfixed in a 2 % osmium tetroxide solution for 2 h. Dehydration of specimens in graded concentration of ethanol was followed by embedding in Epon 812.

#### 2.2.1. Histology

Semi-thin sections (0.5–1  $\mu\text{m}$  thick) were stained with methylene blue and Azur blue II [6] for light microscopy.

#### 2.2.2. Electron microscopy

Ultra-thin sections, stained with uranyl acetate followed by lead citrate [28], were examined under a Philips 208 electron microscope.

## 3. RESULTS

### 3.1. Histology

The sheep lacrimal gland was a compound tubulo-acinar gland, distinctly lobulated and highly vascularized. In semi-thin sections, lacrimal glands consisted of secretory endpieces lined by cells filled with variably stained granules (*figure 1*).

### 3.2. Electron microscopy

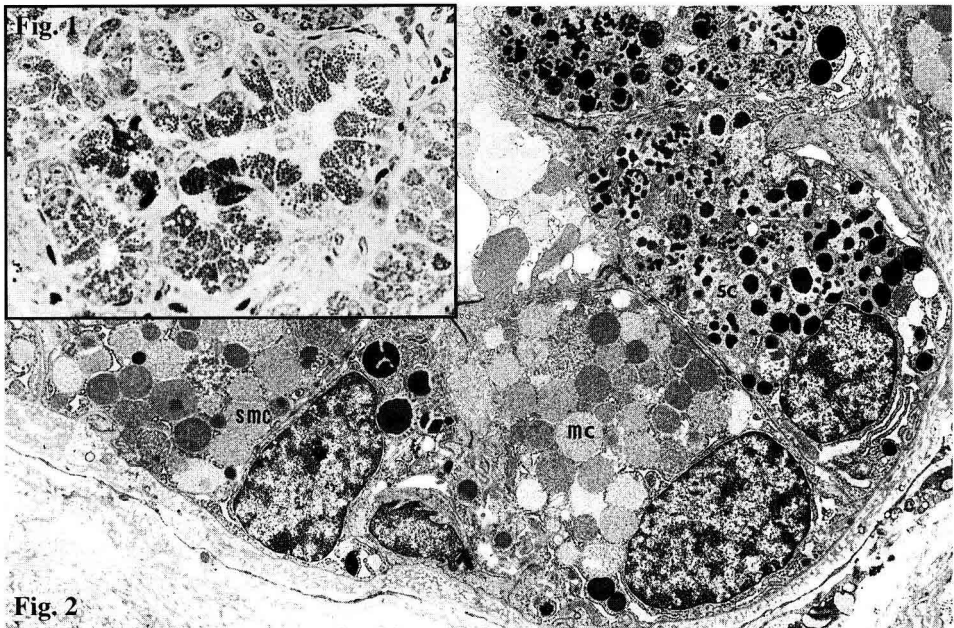
At the ultrastructural level, the secretory endpieces were tubulo-acinar units, with

several acini arranged around a tubular segment. Each acinus was composed of pyramidal or columnar cells, two thirds of which were filled with morphologically heterogeneous secretory granules (*figure 2*). On the basis of their morphological appearance mucous, seromucous and serous cells could be distinguished. The apical surface of all acinar cells had multiple, short microvilli projecting into the acinar lumen; microvilli were more abundant in the serous cells. Lateral cell borders were straight with a junctional complex, consisting of a zonula occludens, a zonula adherens and a macula adherens (*figure 3*). A small number of lateral folds was also present, especially pronounced in the serous cells. The basal surface in both cell types was smooth and surrounded by a thin basal lamina. Typical

myoepithelial cells incompletely encircled the acini.

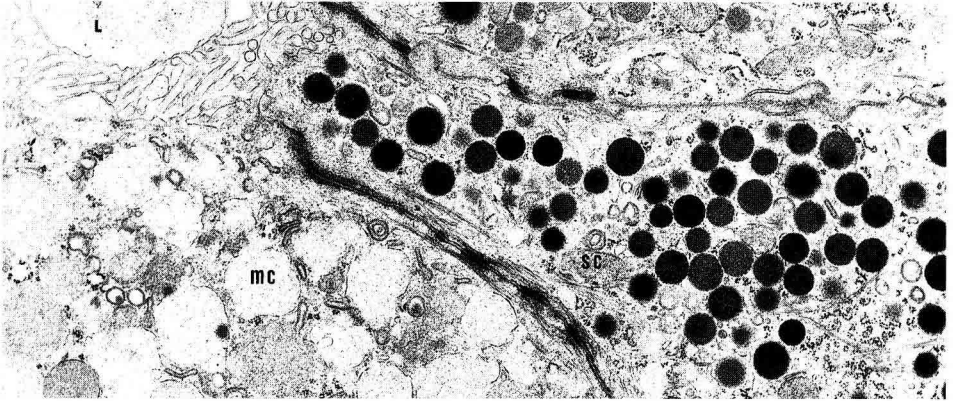
Mucous cells had basally located nuclei with marginated heterochromatin. Because the cells were filled with mucous droplets the rough endoplasmic reticulum (RER) was reduced to a few cisternae in the cell base and in the interstices of secretory droplets (*figures 2–4*). The Golgi apparatus was similarly obscured by abundant secretory droplets. Mucous granules, delimited by a single unit membrane were round to oval in shape, with a finely fibrillar homogeneous matrix, and showed a spectrum of electron densities ranging from 'empty appearing' to quite dense (*figure 4*).

Cytoplasmic organelles of seromucous cells were similar to mucous cells. Seromucous granules contained two distinct

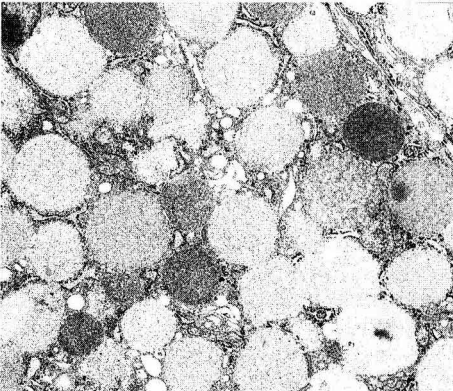


**Figure 1.** Photomicrograph of a semi-thin epoxy section of the lacrimal gland. Secretory endpieces are filled with variably stained granules. Methylene blue and Azur blue II;  $\times 500$ .

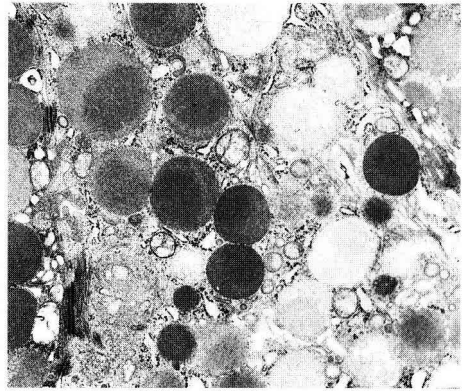
**Figure 2.** Electron micrograph of a secretory endpiece. The morphological variability of secretory granules is evident. Mucous, seromucous and serous cells are found side by side. mc, mucous cell; sc, serous cell; smc, seromucous cell;  $\times 4\ 800$ .



**Figure 3.** The apices of two disparate cell types within the same acinus: a mucous cell (mc) is flanked by a serous cell (sc). The serous granules are uniformly electron-dense; note the junctional complexes. L, lumen;  $\times 14\ 000$ .



**Figure 4.** Mucous granules characterized by a faint, fine fibrillar matrix. Rough endoplasmic reticulum, reduced to a few cisternae, is evident in the interstices of the secretory droplets;  $\times 10\ 000$ .



**Figure 5.** Seromucous granules showing their characteristic bizonal structure;  $\times 12\ 000$ .

areas: one was moderately dense and consisted of a fibrillogranular material; the other consisted of denser material confined in a dense, eccentrically located spherule (*figure 5*).

Serous cells had all the structural hallmarks of protein-secreting cells, e.g. basally located heterochromatic nuclei, an extensive rough endoplasmic reticulum (RER) made up of numerous parallel cisternae, mainly

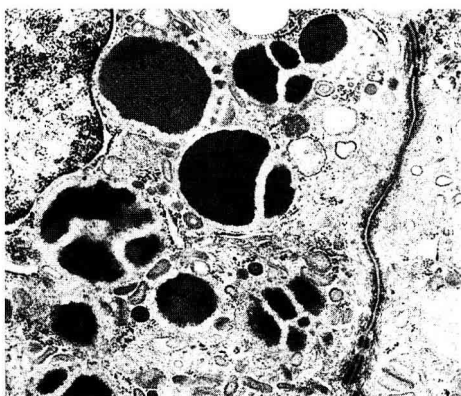
located basally and laterally to the nucleus, and a well-developed Golgi apparatus consisting of stacks of saccules or cisternae, vesicles and condensing vacuoles in the supranuclear cytoplasm. Serous granules were delimited by a single unit membrane, the simplest were uniformly electron-dense without a trace of substructure (*figure 3*); the more complex ones consisted of a number of dense inclusions dispersed in an electron-lucent matrix (*figure 6*).

The glandular parenchyma consisted of a majority of serous acini composed of only

serous cells, and a minority of mucous acini, made up of only mucous cells. These last acini were preferentially located at the periphery of the lobules. In addition numerous mixed acini were present, in which a mixture of mucous, seromucous and serous cells was always found side by side.

The secretory endpieces were followed by a simple duct system. The most proximal component of the duct system had the appearance of intercalated ducts. These ducts, closely approximated to the secretory endpieces, were lined with a simple, cuboidal epithelium. Epithelial cells contained several small, moderately electron-dense granules in their apical portions, a single, centrally located nucleus and tightly interdigitated lateral membranes. Terminal ducts gave way to larger intralobular excretory ducts; the major difference between these last ducts and their predecessor ducts was that the epithelial cells became taller and secretory granules progressively decreased in number and finally disappeared.

The interstitium between adjacent secretory units varied from small spaces containing only a few collagenous fibres to larger spaces containing numerous small



**Figure 6.** More complex serous granules consisting of dense spherules immersed in an electron-lucent matrix;  $\times 16\ 000$ .

blood vessels and nerves, scattered plasma cells, mast cells and a few fibroblasts.

#### 4. DISCUSSION

The sheep lacrimal gland is a compound tubulo-acinar gland that shows morphological features similar to those of lacrimal glands of various laboratory and domestic mammals [5, 8–10, 13, 19].

Whereas all reports describe the lacrimal gland as compound tubulo-acinar, numerous differences are reported among species in the types of secretory granules, raising the question as to whether one, two or more kinds of cells are found in the acini. In pigs, goats, horses, dogs and guinea pigs the lacrimal glands have been described as mixed and mucoserous in secretion on the basis of histochemical findings [1, 21] and the ultrastructural features of their secretory granules [5, 8, 13]. In contrast, canine lacrimal glands are reported as being purely mucus-secreting glands [4] and rat lacrimal glands as serous [7].

The exact classification of secretory granules poses a very difficult and, as yet, unresolved problem, which has had to be dealt with by many authors, especially in relation to salivary glands. The classification used in this report follows that proposed by Tandler and Phillips [25] based on the granule ultrastructure: mucous granules have a lucent matrix in which a faint fibrillogranular material is suspended [24]. Seromucous granules are relatively large with a moderately electron-dense matrix, often with one dense inclusion; serous granules generally have an electron-dense matrix [25, 30], but they can show a variegated appearance as happens in the parotid of different mammalian species [14, 28].

According to these parameters, three different cell types occur in the sheep lacrimal gland: mucous, seromucous and serous. An entire acinus may be made up of a predominant cellular type (serous or mucous acini),

but often the acini contain a mixture of mucous, seromucous and serous cells (mixed acini). The relative proportion of serous, mucous and mixed acini is variable, depending on the position within the lobule. Mucous cells have a modicum of RER and the mucous granules, filling the supranuclear cytoplasm, vary in density from one mucous cell to another, echoing the situation in human labial glands [26]; in contrast, seromucous granules generally have a bipartite appearance resembling the seromucous granules of the lingual glands [29, 30]. Serous cells possess all the structural features of protein-secreting cells and serous granules are either homogeneously electron-dense, or display some dense spherules dispersed in a lucent matrix; the granule substructure is similar to that present in the parotid salivary gland [25], but has not been reported in any lacrimal glands of other species, emphasizing the many ultrastructural differences in granule structure which exist among the various mammalian species.

Lacrimal gland secretions constitute the main components of tears; the function of the mucus is to protect and lubricate the ocular surface. The lacrimal proteins released by the gland into tears include agents such as the major tear proteins, lactoferrin and lysozyme [3], and growth factors that promote corneal re-epithelization [11, 31, 33] which participates in the maintenance of a healthy corneal epithelium.

The excretory duct system of the sheep lacrimal gland is essentially similar to the lacrimal gland excretory systems of other mammals [22]. It is made up of intralobular and interlobular ducts; striated ducts are lacking. Terminal ducts have few uniformly and moderately dense secretory granules, showing a similar morphology to the intercalated ducts of the major salivary glands [20, 23, 27].

In conclusion, our study agrees with the observation that, also in sheep, lacrimal glands are mixed in secretion, but that the ultrastructural morphology of secretory gran-

ules is very particular, confirming the hypothesis that the secretion greatly varies among the various mammalian species.

## ACKNOWLEDGEMENTS

We are grateful to M.G. Mancini for her technical assistance. This work was supported by a grant from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (cod. 0101).

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