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## **LIGHT AND ELECTRON MICROSCOPICAL OBSERVATIONS ON THE VON EBNER'S SALIVARY GLANDS IN THE DONKEY (EQUUS ASINUS)**

(With 24 Figures)

By

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(Received at 31/12/2001)

### **مشاهدات بالمجهر الضوئي والإلكتروني على غدد فن إبنرز اللعابية في الحمار أحمد عمر سالم**

أجري هذا البحث على ستة حمير بالغة من كلا الجنسين بغرض دراسة غدد فن إبنرز باستخدام المجهر الضوئي والنافذ الإلكتروني. أظهرت الدراسة وجود غدد فن إبنرز تحت الحلمات ذو الوريقات والحلمات قذحية الشكل، وهي عبارة عن غدد من النوع المختلط المكون من وحدات إفرازية انبوبية سنخية مركبة وكذلك جهاز إخراجي. وقد لوحظ أن الوحدات الإفرازية تتكون من عنبيات مصلية تكون معظم الغدد، ومصليه هلالية وكذلك أسناخ تحتوي على كل من الخلايا المصلية والمخاطية. أوضحت الدراسة أن الخلايا المصلية تعكس خصائص الخلايا المفرزة للبروتين مثل إحتوائها على كثير من الحبيبات الإفرازية القائمة الكترونيا المحتوية على أجسام أكثر عتامة، جهاز جولجي وشبكة اندوبلازمية خشنة متطورة. أما الخلايا المفرزة للميوسين فتميزت بإحتوائها على العديد من القطيرات الإفرازية اللامعة الكترونيا والتي يحتوي البعض منها على جسم قاتم الكترونيا، وكذلك على جهاز جولجي المتسع التطور وعلى كمية متوسطة من الشبكة الإندوبلازمية الخشنة. هذا وأظهرت الدراسة أن كل من الخلايا الإفرازية تحتوي على العديد من الريبوزومات والمتقدرات والخيوط السيتوبلازمية والقطيرات الدهنية وكذلك السنتربول. أوضحت الدراسة أيضا أن كل من الخلايا المصلية والمخاطية تفتقر إلى الأنشاءات القاعدية، أما الفراغات البين خلوية الجانبية فكانت واسعة بين الخلايا المصلية عنها في الخلايا المخاطية. القنيات بين خلوية لوحظ وجودها بين الخلايا المصلية الهلالية. أظهرت الدراسة أن الجهاز الإخراجي لغدد فن إبنرز في الحمار يتكون من الأقنية العنبيية والقنوات الإخراجية وقناة رئيسية تفتح أما في متراس الحلمات ذو الوريقات أو أخدود الحلمات قذحية الشكل. أما الأقنية المخططة لم تلاحظ في هذه الغدد. أظهرت الدراسة أن الخلايا العضلية الطلائية تحيط بالوحدات الإفرازية ولها خصائص الخلايا القلوصية. كما أن لهذه الخلايا العضلية الطلائية علاقات غير مالوفة بالوحدات الإفرازية حيث لوحظ أن خلية واحدة تحيط بوحدين متجاورين أو أكثر في آن واحد. هذا ونوقشت نتائج البحث بمثلتها في غدد فن إبنرز في الثدييات.

## SUMMARY

The von Ebner's glands of six adult apparently healthy donkeys of both sexes were examined using the light and transmission electron microscope. These glands were present under the foliate and vallate papillae. The von Ebner's glands were of the mixed type mainly serous, consisting of compound tubulo-alveolar secretory units and an excretory duct system. The secretory units were formed of pure serous end-pieces (majority of the glands), serous demilunes capping the mucous tubules as well as those consisting of admixture of serous and mucous cells. The serous cells reflected the characteristics of protein secreting cells as numerous electron-dense granules with more electron-dense bodies, well-developed Golgi-apparatus and infranuclearly stacked RER-cisternae. The mucous producing cells were recognized by their abundant electron-lucent mucous droplets, some with electron-dense body, extensively developed Golgi-apparatus and moderate amount of RER. Both the secretory cells contained numerous ribosomes, mitochondria, cytoplasmic filaments, lipid droplets and centrioles. The serous and mucous cells lacked basal folds, while the lateral intercellular spaces were shown to be wider between the adjacent serous cells than the mucous variety. Intercellular canaliculi, containing secretory materials, were observed between the cells of the serous demilunes. The duct system of the von Ebner's glands of the donkey was consisted of intercalated ducts, excretory ducts and a main duct, which open in the vallum of the vallate and in the groove of the foliate papillae. Striated ducts were not observed. Myoepithelial cells with common features of contractile elements were observed surrounding the secretory end-pieces. Unique relations of myoepithelial cells to the secretory end-pieces were demonstrated, where one cell was observed surrounding two or three secretory units.

*Key words: Von Ebner's glands, serous & mucous cells, ultrastructure, donkey.*

## INTRODUCTION

Von Ebner's glands (gustatory glands) were firstly described in man by Ebner (1873). They are made up of small groups of tubulo-alveolar salivary glands located under the foliate and vallate papillae that open into the trough at the base of the papillae.

The von Ebner's glands have been examined ultrastructurally in rats (Hand, 1970), rabbits (Toyoshima and Tandler, 1986), bats (Azzali, Gatti, Bucci and Orlandini, 1989b), bovines (Gargiulo, Ceccarelli, Dall's Aglio and Pedini, 1995), camels (Salem, 1996), horses (Gargiulo, Ceccarelli, Parillo and Pedini, 1993) and man (Testa Riva, Cossu, Lantini and Riva, 1985; Azzali, Bucci, Gatti, Orlandini and Ferrari, 1989a).

Data regarding the ultrastructure of the von Ebner's glands of the donkey could not be found in the available literature. Therefore, the aim of this investigation is to elucidate mainly the ultrastructural features of the von Ebner's glands of the donkey and to compare them with those of other mammals.

## **MATERIAL and METHODS**

The foliate and vallate lingual papillae with the underlying glands were obtained from tongues of six adult clinically healthy donkeys of both sexes.

For light microscopy, the specimens were fixed in Bouin's fluid and processed for paraffin embedding. 5 µm thick sections were stained with haematoxylin and eosin.

For electron microscopy, small pieces including the von Ebner's glands were taken and fixed by immersion in a cold mixture of 2.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2 for 4 hours at 4°C. After washing in the same buffer used, the pieces were then post-fixed in 1% buffered osmic acid (pH 7.2) for further 2 hours. After dehydration in ethanol, the pieces were embedded in a mixture of Epon-araldite (Anderson and Andre, 1968). Semithin sections were stained with toluidine blue, while the ultrathin ones were double stained with uranyl acetate and lead citrate. They were examined and photographed in JEOL 100 CX II transmission electron microscope.

## **RESULTS**

### **Light microscopy:**

The von Ebner's glands of the donkey (Figs. 1-5), consisting of small lobules of compound tubulo-alveolar secretory portions and an excretory duct system, were located in the connective tissue under the foliate and vallate papillae as well as between the lingual skeletal muscle bundles.

The secretory portions were formed of pure serous alveoli, mucous tubules capped with serous demilunes, in addition to secretory

units consisting of admixture of mucous and serous cells. The pure serous acini constituted the majority of the gland lobules, while the mixed varieties were localized as a single lobule or sometimes as groups among the serous masses (Figs. 1 & 2). The duct system was organized as intercalated ducts, excretory ducts and a main duct. The intercalated ducts, representing the beginning of the duct system, were lined with cuboidal-shaped cells with rounded nucleus. The striated ducts were not observed. The epithelium lining of excretory ducts was formed by a cellular bilayer, an outer layer of basal cells and an inner layer of cuboidal or columnar ones. The lining epithelium of the main excretory duct was consisted of stratified columnar epithelium, which changed into stratified squamous epithelium at its opening in the vallum of the vallate or in the groove of the foliat papillae (Fig. 3).

In toluidine blue stained semithin sections, the serous end-pieces were consisted of pyramidal-shaped cells with narrow central lumen and somewhat rounded basally located nuclei. Their darkly stained granules characterized these cells. In the mixed varieties, the mucous tubules were lined with truncated pyramidal-shaped cells with flattened or ovoid basally located nuclei. These cells had a large amount of faintly stained mucous droplets (Figs. 4 & 5).

#### **Electron microscopy:**

##### **I- The secretory cells:**

Morphologically, two different cell types were observed composing the von Ebner's glands of the donkey, viz., the serous and mucous cells. The pyramidal-shaped serous cells constituted the pure serous acini (Fig. 6) and serous demiluens, that capping the mucous tubules (Fig. 7). They assembled also with the mucous cells mixed secretory units (Fig. 8).

The luminal surface of both the secretory cells had microvilli, which were numerous in the serous cells (Figs. 9 & 10). Apically, the serous cells were joined either together (Fig. 9) or with the mucous ones (Fig. 10) by zona occludens, zonula adherens and desmosomes. The lateral plasmalemma was thrown into cytoplasmic folds, extending into wide or narrow intercellular spaces between the serous and mucous cells, respectively, forming extensive interdigitations with the adjacent cells (Figs. 11 & 12). The basal plasmalemma of the serous and mucous cells lacked nearly basal folds (Figs. 11 & 12) and attached to the basal lamina with hemidesmosomes or to the myoepithelial cell with desmosomes. Intercellular canaliculi, lined with microvilli and contained

secretory material, were present between the demilune cells. They were separated from the intercellular space by junction complex (Figs. 12 & 16).

Within the serous cells, the organelles had a fairly constant arrangement. They were characterized by abundant electron-dense secretory granules filling the cell apical portion, supranuclear Golgi-apparatus and well-developed rough endoplasmic reticulum. The latter was arranged infranuclearly in the form of parallel cisternae (Fig. 13). In addition, short cisternae were interspersed among the secretory granules throughout the cytoplasm. Some RER-cisternae were observed distended with fine electron-dense materials (Fig. 13 inset). The well-developed Golgi-apparatus was represented by 2-3 complexes. Each one was consisted of 4-5 dictyosomes and associated vesicles (Fig. 14). Mitochondria of crista-type were scattered all over the cytoplasm. The membrane bounded secretory granules were the most distinctive feature of the serous cells. They were composed of electron-dense matrix containing more electron-dense bodies (Figs. 6-10 & 14-17). The process of formation of the serous secretory granules began inside the RER-cisternae as fine electron-dense materials (Fig. 13 inset). Within the Golgi area, these granules appeared in various stages of development (Figs. 14-16). In the earlier stage, stage<sub>1</sub> (S<sub>1</sub>), the granular content was electron-lucent fine fibrillar. Later on, moderate electron-dense material was observed in stage<sub>2</sub> (S<sub>2</sub>), which increased in amount until the granular content as a whole became of moderate electron density in stage<sub>3</sub> (S<sub>3</sub>). An increase in electron density of the granule was observed, giving it entirely a homogenous electron-dense appearance in stage<sub>4</sub> (S<sub>4</sub>). In the mature, stage<sub>5</sub> (S<sub>5</sub>) deposition of extremely electron-dense bodies was observed. In some cells, these bodies appeared as large patches (Fig. 16). The granular content was released by exocytosis in the form of fine fibrillar electron-lucent materials, resembling the prospective granule, with electron-dense bodies (substructures) into the acinar lumen and the intercellular canaliculi (Figs. 16, 17).

Within the mucous cells, the rough endoplasmic reticulum did not take the form of parallel arrays, rather long and short segments were observed throughout the cytoplasm (Fig. 18). The supranuclear Golgi-apparatus was extensively-developed, where it was consisted of 3-5 complexes. Each one was formed of 6-14 closely packed semicircularly arranged saccules and associated vesicles. Mitochondria with densely packed cristae were scattered between the mucous droplets. These droplets were the most characteristic feature of the mucous cells, where

they occupied sometimes most of the cytoplasm pushing the flattened nucleus towards the cell base.

These mucous droplets (Figs. 7, 8, 10, 12 & 18-20) were easily distinguished from the serous granules by their electron-lucent matrix. They appeared as skein of lucent threads, some with electron-dense body. These droplets showed a propensity for fusion with one another specially before discharging their content by exocytosis into the lumen. The latter contained occasionally intact mucous droplet (Fig. 20). The formation of the mucous droplets was observed in the vicinity of the Golgi-complex. They appeared firstly as pale structurless droplets in stage<sub>1</sub> (S<sub>1</sub>). Their matrix increased slightly in density with appearance of small electron-dense foci in stage<sub>2</sub> (S<sub>2</sub>). The mature droplets, stage<sub>3</sub> (S<sub>3</sub>) had skein of lucent threads with or without electron-dense body (Figs. 10, 18 & 20).

Numerous ribosomes, cytoplasmic filaments (Figs. 9, 11, 14, 15 & 18), lipid droplets and globules (Figs. 11 & 19) as well as centerioles (Fig. 11) were observed in both the serous and mucous cells. The cytoplasmic filaments were scattered all over the cytoplasm and were easily distinguished at the cell apex and in the vicinity of the secretory granules of the serous cells (Figs. 9, 11, 14 & 15).

The nucleus of both the secretory cells (Figs. 6-8, 11, 18, 19, 22 & 23) possessed a prominent nucleolus and large amounts of euochromatin. In addition marginal heterochromatin and chromatin islands were observed.

## **II- Myoepithelial cells:**

The myoepithelial cells (Figs. 21-23) were located between the basal lamina and the basal border of the secretory cells, where they possessed an elongated cell body with flattened nucleus and many cytoplasmic processes. They contained abundant myofilaments, (with dense areas), mitochondria, small profiles of RER, ribosomes and lipid droplets. The myoepithelial cells were attached to the basal lamina with hemidesmosomes and to the secretory cells with desmosomes.

A single myoepithelial cell was observed embracing with their processes either two (Fig. 22) or three (Fig. 23) closely located secretory end-pieces. In these localities, the myoepithelial cell and the secretory end-pieces were enclosed within a continuous basal lamina.

## **III- Lymphocytes:**

Lymphocytes were occasionally seen in the intercellular spaces between the secretory cells. They possessed the general characteristics as

large dense nucleus surrounded by a thin rim of cytoplasm poor in organelles (Fig. 24).

## DISCUSSION

The present study revealed that the von Ebner's glands of the donkey were present beneath the foliate and vallate papillae. Similar to that of rats (Hand, 1970), rabbits (Toyoshima and Tandler, 1986), bats (Azzali *et al.*, 1989b), bovines (Gargiulo *et al.*, 1995), camels (Salem, 1996), horses (Gargiulo *et al.*, 1993) and man (Testa Riva *et al.*, 1985; Azzali *et al.*, 1989a), the von Ebner's glands of the donkey were consisted of compound tubulo-alveolar secretory portions and an excretory duct system.

Unlike the aforementioned mammals, the von Ebner's glands of the donkey were of the mixed type mainly serous, consisting of pure serous acini (majority), serous demilunes and admixture of serous and mucous cells side by side within one acinus. On the other hand, Junqueira, Carneiro and Contopoul (1971) stated that, the von Ebner's glands in man contain mucous and serous acini.

The present ultrastructural findings of the serous cells of the von Ebner's glands of the donkey simulate more or less those observed in von Ebner's glands of bats (Azzali *et al.*, 1989b), bovines (Gargiulo *et al.*, 1995), horses (Gargiulo *et al.*, 1993), camels (Salem, 1996) and man (Testa Riva *et al.*, 1985; Azzali *et al.*, 1989a) as they presented well-developed RER-cisternae at the basal pole, supranuclear Golgi-apparatus and numerous electron-dense secretory granules within the apical cytoplasm. In accordance with Testa Riva *et al.* (1985) and Azzali *et al.* (1989a), this indicates an important secretory activity and characterizes a gland with protein synthesis. It correlates well with the lipolytic activity (Hamosh and Burns, 1977) as well as IgA and lactoferrin secretion of these glands in man (Moro, Umemura, Crago and Mestecky, 1984). This was confirmed by Blaker, Kock, Ahlers, Buck and Schmale (1993); Spielman, D'Abundo, field and Schmale (1993); Kock, Ahlers and Schmale (1994a) and Garibotti, Christiansen, Schmale and Pelosi (1995) who mentioned that, the von Ebner's glands produce a lipophilic ligand carrier protein called von Ebner's gland protein (VEGP), which may be essential for the regulation of taste sensation. The VEGP binds and presents lipophilic gustatory molecules to the taste receptors and it proves essential for the effective cleaning the furrows of the vallate and foliate papillae of lipophilic tastants which otherwise will cause

long-lasting taste sensation (Schmale, Holtgreve and Christiansen, 1990; Kock, Blaker and Schmale, 1992). This protein protects also the taste epithelium (Koch *et al.*, 1994a) and has a pheromone transporting function (Schmale, Ahlers, Blaker, Koch and Spielman, 1993).

The serous secretory granules of the von Ebner's glands of the donkey consisted of an electron-dense matrix with more electron-dense bodies, which simulate those of human parotid (Riva and Riva-Testa, 1973) and major salivary glands (Riva, Motta and Riva-Testa, 1974). However, they differ from those of the von Ebner's glands of rats (Hand, 1970), bats (Azzali *et al.*, 1989b), camels (Salem, 1996) and man (Testa-Riva *et al.*, 1985; Azzali *et al.*, 1989a) as they formed of homogenous electron-dense matrix. In horses (Gargiulo *et al.*, 1993) and bovines (Gargiulo *et al.*, 1995), the secretory granules consisted of homogenous electron-dense matrix, which may possess a complex of a substructure. The substructure may depend on the presence of biologically active materials stored in specific areas of the granules (Pinkstaff, 1993). Although, Gargiulo *et al.* (1995) stated that it is very difficult to correlate the different structures of the secretory granules with their chemical diversity, it could be suggested that the variance in electron densities of the serous secretory granules represents stages in their maturation as revealed in the present study and may reflect qualitative differences in chemical content and molecular organization. Jacob and Paddar (1987) supported this suggestion, as they found two types of acidic mucosubstances representing granules with different electron densities.

The ultrastructure of mucous cells of the von Ebner's glands in the donkey alludes to typical mucin-secreting cells as reported by Pinkstaff (1980) in some salivary glands of other species.

Ultrastructurally, the mucous droplets of the von Ebner's glands of the donkey resembled those found in the cat submandibular (Shackleford and Wilborn, 1970a) and sublingual glands (Tandler and Poulsen, 1977). The structure of the mucous droplets depends upon the choice of fixative and the histochemical nature of the mucus (Jacob and Paddar, 1987). The filamentous structure of the mucous droplets has been interpreted as being mucin molecules (Shackleford and Wilborn, 1970a), while the electron-dense core as concentrated protein (Lavker, 1969).

It is acceptable that the large amount of mucous droplets within the mucous cells could be related to the spontaneous secretion of the gland, a process that occurs without any secretory stimuli (Harrison,



1974). This could be supported by high propensity of fusion between the mucous droplets, which makes easy discharge of their content by exocytosis as reported by Tandler and Poulsen (1977). This mucin with high viscosity probably plays an important role in defense mechanism of taste buds and prevents food fermentation within the papillary furrows as well as it protects the non-cornified epithelium of the papillae from dehydration and injury.

The present investigation revealed that the lateral intercellular spaces were wider between adjacent serous cells in comparison with the mucous variety. Frederiksen and Rostgaard (1974) stated that, the increase in the size of the intercellular space might be artifactual produced during tissue processing, what is not accepted in the present study. The lateral intercellular space plays a major role in transepithelial transport of water (Pinkstaff, 1993), where the size of the lateral intercellular space increases when fluid transport by such cells increases (Spring and Hope, 1978). This is greatly accepted, where wider intercellular spaces were observed between the serous cells, which produce more watery secretion.

Similar to that observed in the von Ebner's glands of rats (Hand, 1970), bovines (Gargiulo *et al.*, 1995), horses (Gargiulo *et al.*, 1993), camels (Salem, 1996) and man (Testa-Riva *et al.*, 1985; Azzali *et al.*, 1989a), as well as human labial glands (Tandler, Denning, Mandel and Kutscher, 1969) and parotid gland of donkey (Salem, Abd El-Rahman and Abou El-Maged, 1995), all the secretory cells of the von Ebner's glands of the donkey, lacked basal folds, indicating a low salivary flow (Azzali *et al.*, 1989b). The relatively low salivary secretion of the von Ebner's glands in comparison with other major salivary glands is probably a nature of this gland, where both of the serous and mucous secretory products perform a restricted function within the papilla. The serous secretion serves only the tasting process, while the relatively low mucin is required for covering and protecting the non-cornified epithelium of the papillae.

The duct system of the von Ebner's glands of the donkey like that in other mammals (Hand, 1970; Testa-Riva *et al.*, 1985; Azzali *et al.*, 1989a & b; Gargiulo *et al.*, 1993 & 1995; Salem, 1996), consisted of intercalated ducts, excretory ducts and a main duct, without interposition of striated ducts. The latter was also absent in other salivary glands as human labial glands (Tandler, Denning, Mandel and Kutscher, 1970), palatine glands of man (Black, 1977) and rats (Pinkstaff, 1980) and lingual glands of cats and horses (Tandler and Poulsen, 1977; Gargiulo,

Ceccarelli, Parillo and Pedini, 1993a). The absence of striated ducts is responsible for production of isotonic saliva (Testa-Riva *et al.*, 1985; Azzali *et al.*, 1989a). It could be suggested that, This isotonic saliva acts as good milieu for the sapid molecules within the groove and the taste cells, which intern not affect the taste cell depolarization and consequently the tasting process as a whole.

The present ultrastructural study revealed unique arrangement of the myoepithelial cells. Similar relations were observed in von Ebner's glands (Salem, 1996) and parotid gland (Salem and Abd El-Rahman, 2000) of the camels, where the myoepithelial cell was demonstrated embracing more than one secretory end-pieces. It may be suggested that this myoepithelial cell perform its contractile action on the secretory units at the same time in order to ensure a simultaneous release of a copious secretory product from these units.

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## LEGENDS

- Fig. 1:** Photomicrograph of the foliate papilla of the donkey, showing papillary groove (g), excretory duct (arrow), a main duct (double arrow), serous lobules (Sl), mixed lobule (Ml) and epithelial covering (Ep). Hx & E. X 25.
- Fig. 2:** Higher magnification of figure 1, showing serous lobules (Sl), mixed lobule (Ml), excretory duct (arrow), a main duct (double arrow), taste buds (arrowhead) and papillary groove (g). Hx & E. X 50.
- Fig. 3:** Photomicrograph of the distal part of the papillary groove showing, a main duct (M) of the von Ebner's glands with its stratified columnar epithelium (arrow), which changed into stratified squamous epithelium (double arrow). Mucous tubule opens into the duct (arrow head), serous end-pieces (S). Hx & E. X 200.
- Fig. 4:** Photomicrograph of the serous units of the von Ebner's glands of the donkey with their darkly stained granules and rounded nuclei (arrow). Toluidine blue. X 200.
- Fig. 5:** Photomicrograph of the mixed variety of the von Ebner's glands of the donkey, showing the mucous tubules with their faintly stained mucous droplets. Cells of the serous demilune (thin arrow), admixture of serous and mucous cells within a secretory unit (thick arrow). Toluidine blue. X 250.
- Fig. 6:** Electron micrograph of a serous secretory unit of the von Ebner's glands of the donkey, showing the pyramidal-shaped cells (Sc) with somewhat rounded nuclei (N). Nucleolus (Nu), lumen (L), wide intercellular space (Ic) with cytoplasmic interdigitations. X 3780.
- Fig. 7:** Electron micrograph of a mixed secretory unit of the von Ebner's glands of the donkey, showing cells of the serous demilune (Sc) with their electron-dense granules that capping the mucous cells (Mc) with their electron-lucent mucous droplets. X 3000.
- Fig. 8:** Electron micrograph showing admixture of serous (Sc) and mucous cells (Mc) in one acinus. Serous cells with electron-dense granules and rounded nucleus (N<sub>1</sub>), mucous cell with

electron-lucent droplets and flattened nucleus (N<sub>2</sub>). Lumen (L), part of a myoepithelial cell (My). X 3510.

**Figs. 9 & 10:** Electron micrographs of the apical portions of the secretory cells, showing the junctions between the adjacent serous cells with each other (9) and with the mucous cells (10). Zonula occludens (Zo), zonula adherens (Za), desmosomes (D), intercellular space (arrow), lumen (L), microvilli (Mv), secretory material with electron-dense bodies (arrowhead), serous granules (Sg), mucous droplets (Md), cytoplasmic filaments (f). (9) X 19833; (10) X 28571.

**Fig. 11:** Electron micrograph showing the wide lateral intercellular space (Ic) between two serous cells. Folds (arrowhead), desmosome (double arrowhead), centrioles (arrow), mitochondria (M), rough endoplasmic reticulum (RER), lipid (Ld), cytoplasmic filaments (f), nucleus (N), basal lamina (Bl). X 10667.

**Fig. 12:** Electron micrograph showing the intercellular canaliculi (thick arrow) between the serous cells of the demilune (Sc) containing secretory material. Narrow intercellular spaces (thin arrow) between mucous cells (Mc), basal lamina (Bl). X 4000.

**Fig. 13:** Electron micrograph of an infranuclear region of a serous cell, showing RER-cisternae (RER). Nucleus (N), basal lamina (Bl), hemidesmosomes (arrow), wide intercellular space (Ic). X 26444. **Inset:** showing distended RER-cisternae with electron-dense material (arrow) and stage<sub>3</sub> (S<sub>3</sub>). X 27000.

**Figs. 14-15:** Electron micrographs showing the stages of development of the serous granules:

**14:** Stage<sub>1</sub> (S<sub>1</sub>): Electron-lucent fine fibrillar matrix, stage<sub>2</sub> (S<sub>2</sub>): Appearance of moderate electron-dense material, stage<sub>3</sub> (S<sub>3</sub>): The granular content of moderate electron density, stage<sub>4</sub> (S<sub>4</sub>): Homogenous electron-dense matrix.

Well-developed Golgi-apparatus (GA) consisting of 3 complexes (arrow), nucleus (N), cytoplasmic filaments (f), RER-cisternae (arrowhead). X 16667.

**15:** Stage<sub>5</sub> (S<sub>5</sub>): Homogenous electron-dense matrix with deposition of extremely electron-dense bodies (arrow), cytoplasmic filaments (f), mitochondria (M). X 23333.

**Fig. 16:** Electron micrograph showing serous granules with electron-dense patches (arrowhead). Intercellular canaliculi (long thick arrow) bounded in both sides by junction complex (thick

arrow) and contained secretory material with electron-dense patches. Intercellular space (thin arrow), mucous droplets (Md) within the mucous cell with electron-dense body (double thin arrow). X 17500.

- Fig. 17:** Electron micrograph showing exocytosis of the serous granules (Sg) into the acinar lumen (L) as fine fibrillar electron-lucent material with electron-dense bodies (arrow). Microvilli (Mv), zonula occludens (Zo), zonula adherens (Za), desmosome (D). X 20222.
- Fig. 18:** Electron micrograph of a supranuclear region of a mucous cell of the von Ebner's glands of the donkey, showing extensively-developed Golgi-complex (GA) with stage<sub>1</sub> (S<sub>1</sub>), stage<sub>2</sub> (S<sub>2</sub>) and stage<sub>3</sub> (S<sub>3</sub>) mucous droplets. Short and long cisternae of RER (arrow), mitochondria (M), cytoplasmic filaments (arrowhead), mucous droplets of skein-lucent appearance (Md) with electron-dense body (double arrow), nucleus (N). X 15000.
- Fig. 19:** Electron micrograph of the basal region of a mucous cells, showing nucleus (N), mucous droplets (Md), RER-cisternae (arrow), mitochondria (M), lipid droplds (Ld), narrow intercellular space (arrowhead), myoepithelial cell (My), basal lamina (double arrow). X 12800.
- Fig. 20:** Electron micrograph showing exocytosis (arrow) of mucous droplets into the lumen (L). Fused mucous droplets (arrowhead), intact mucous droplet (Md). X 22909.
- Fig. 21:** Electron micrograph of a myoepithelial cell (My) surrounding a serous cell (Sc). Nucleus (N), myofilaments (arrow), dense-areas (arrowhead), desmosome (D), lipid (Ld), basal lamina (Bl). X 6429.
- Fig. 22:** Electron micrograph showing a myoepithelial cell (My) surrounding two closely located mixed acini (A<sub>1</sub>, A<sub>2</sub>). Continuous basal lamina (arrow). X 3090. **Inset:** Higher magnification of the marked area, showing a basal lamina reflected upon the two acini (arrow). X 9380.
- Fig. 23:** Electron micrograph showing a myoepithelial cell (My) surrounding three acini (A<sub>1</sub>, A<sub>2</sub> & A<sub>3</sub>) with continuous basal lamina (arrow). X 3960.
- Fig. 24:** Electron micrograph showing a lymphocyte (Lc) in the intercellular space between a mucous (Mc) and serous cell (Sc). Basal lamina (Bl), hemidesmosome (arrow). X 7593.













