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LIGHT AND ELECTRON MICROSCOPICAL STUDIES ON THE GLAND ASSOCIATED WITH THE THIRD-EYELID IN WATER BUFFALO

(with 18 Figures)

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دراسات بالميكروسكوب الضوئى والأليكترونى لغدة الجفن الثالث فى الجاموس

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يتميز الجاموس بوجود غدة كبيرة مرتبطة بالجفن الثالث للعين فى زاويتها الأنسية. تتكون هذة الغدة من فصوص مكونة من نهيات مفرزة على شكل أنابيب حويصلية. تتبطن هذة النهيات المفرزة بخلايا عمادية أو هرمية الشكل والتى تظهر اما قاتمة واما فاتحة بأستخدام صبغة الهيماتوكسيلين و الأيوسين. عند أستخدام صبغة الألسيان الزرقاء مع طريقة حمض البير أيودك أسيد شيف (بى أيه أس) تميزت الخلايا المبطنة الى نوعين: خلايا النوع الأول أعطت تفاعلا موجبا مع صبغة شيف لأحتوائها على الميوسين المتعادل وخلايا النوع الثانى أظهرت تفاعلا موجبا مع صبغة شيف والألسيان الأزرق معا لأحتوائها على خليط من الميوسين المتعادل و الحامضى. بأستخدام الميكروسكوب الأليكترونى أظهرت خلايا النوع الأول وجود حبيبات مفرزة مملوءة بمادة متجانسة وقاتمة اليكترونيا متجمعة فى الجزء العلوى من السيتوبلازم. أما خلايا النوع الثانى فيوجد بها حبيبات تحتوى على خليط مكون من المادة المتجانسة القاتمة اليكترونيا على هيئة مساحات مختلفة الحجم و الشكل وأخرى على شكل حبيبات متناهية فى الصغر. كذلك يوجد بالخلايا محتويات دهنية مرتبطة بأجسام ليسوسومية. كما تم التعرف على أختلافات بين الخلايا مرتبطة بالجنس ، فقد شوهدت تركيبات على هيئةتجمع من الأسطوانات الدقيقة داخل خلايا الغدد التى تنتمى الى الذكور فقط. وتقترح الدراسة أن غدة الجفن الثالث للعين تفرز مادة مخاطية مصلية. من خلال السطح العلوى عن طريق اندماج غشاء الخلية مع الغشاء المغلف للحبيبات المفرزة. كما لوحظ وجود خلايا طلائية عضلية لها نتوءات متصلة بجسم الخلية موجودة بين الجدار القاعدى و الحافة القاعدية للخلايا الغدية. بالأضافة الى ذلك فقد لوحظ وجود نهايات عصبية محورية عارية فى المسافات البين خلوية و على اتصال بكل من الخلايا الطلائية و الغدية. ولم يلاحظ وجود أى اختلاف بين النهايات العصبية المتصلة بالخلايا الطلائية العضلية والآخرى المتصلة بالخلايا الغدية وهذا قد يوضح أن كلا من الخلايا الطلائية العضلية و الغدية تتغذى عصبيا بنوع واحد من النهايات العصبية.

SUMMARY

The water buffalo was characterized by a well-developed gland associated with the third-eyelid in the medial canthus of the eye. It was formed of glandular lobules of tubuloalveolar secretory endpieces. The endpieces were lined by columnar or pyramidal cells. They were divided into light and dark secretory cells with haematoxylin and eosin stain. With application of alcian blue (pH 2.5) - PAS technique, two types of cells were demonstrated. The first type displayed secretory substance of neutral mucin reacting positively with PAS technique. The second type was filled with secretory material formed of a mixture of neutral and acidic mucins, and stained positively with both alcian blue and PAS. The fine structure of type I -cells showed that, the apical accumulated secretory granules were filled with electron dense homogenous material, while in cells of type II, granules containing a mixture of electron dense homogenous patches and fine granular material were observed. In addition, both secretory cells revealed frequently lipid inclusions in close contact with lysosomal material. Ultrastructural sexual dimorphism between male and female glands had been observed. Clusters of cylindrical tubules were usually seen only in male secretory cells. The present findings suggest that the gland associated with the third-eyelid in water buffalo produces seromucous substance by merocrine mode of secretion. Well-developed myoepithelial cells formed of cell-body and cytoplasmic processes occupying a position between the basal lamina and basal borders of secretory cells. Naked nerve terminals were located in the intercellular spaces and formed frequently synaptic contacts with both secretory and myoepithelial cells of glandular endpieces. No remarkable morphological differences between the contents of nerve terminals in either regions of neuroglandular or neuromuscular synapses could be detected. Our observations may indicate that the secretory and myoepithelial cells were innervated by one type of nerve terminals.

Key words: *Water buffalo - Third-eyelid gland - Nerve terminals - Myoepithelial cells - Morphology.*

INTRODUCTION

The eyeball of mammals is associated with two major glands, the harderian gland and the lacrimal gland. The harderian gland is found in most terrestrial vertebrate associated with the third eyelid (Sakai, 1981 and Payne, 1994). In mammals, the harderian gland may lose its sebaceous properties which characterize the harderian glands of logomorphs and rodents (Paule et

al., 1955; Paule and Hayes, 1958; Sakai, 1981). Franz (1934) and Sakai (1981) reported that the gland associated with the third eyelid and secrete lipid by a merocrine mechanism is considered as harderian gland to differentiate it from the nictatins gland which is located within the third eyelid. Additionally, some authors called the deep gland associated with the third eyelid a harderian gland (cited by Dellmann and Collier, 1993). Recent morphological, physiological and biochemical investigations on the harderian glands reported that the gland may act as a source of saliva, a part of retinal-pineal-axis, a site of immune response, a source of pheromones and a photoprotective organ (Puig-Domingo et al., 1988; Chieffi-Baccari et al., 1992; Meusy-Dessolle et al., 1992; Baccari et al., 1993; Djeridane, 1994; Payne, 1994; Buzzell et al., 1995; Cameron et al., 1995; Di Matteo et al., 1995). However, the morphological data concerning histochemistry, histology and fine structure of the gland associated with the third-eyelid in domestic animals, particularly in water buffalo lack in the available literature. Ibrahim (1990) reported that the gland associated with the third eyelid in water buffalo was relatively well-developed in comparison with the gland associated with the third eyelid , harderian gland, in camel and donkey. Therefore, the present investigation was done to describe the light and electron microscopic features of the secretory endpieces of the gland associated with the third eyelid in Egyptian water buffalo with special reference to their innervation.

MATERIALS and METHODS

Third-eyelids (Fig. 1) of normal healthy 7 male and 6 female buffaloes were collected immediately after slaughtering in the early morning. The glandular portions were separated from the membranous and fatty portions of the third eyelids. Small pieces of glandular tissue representing the different areas of the glandular portions were cut and immersed in a fixative solution.

Fixation was performed in Bouin's solution for light microscopic examination, and in paraformaldehyde - glutaraldehyde fixative as described by Karnovsky (1965) for transmission electron microscopy. For identification of the nature of the secretory material using light microscopic examination, paraplast sections of about 5-7 µm in thickness were cut and stained with haematoxylin and eosin, alcian blue (pH 2.5), alcian blue (pH 2.5) - PAS techniques. For lipid detection in the gland associated with the third-eyelid, tissue samples were fixed in 10% formol calcium were used. Cryostat

sections of about 15 - 20 µm in thickness were cut and stained with sudan black B as described by Bancroft et al. (1996).

For transmission electron microscopy, small pieces were washed in 0.1 M Phosphate buffer (pH 7.4) several times after immersion fixation for 4 hours. The tissue blocks were postfixed for 2 hours in 1% osmium tetroxide. After washing several times in phosphate buffer (pH 7.4), tissue sample were dehydrated in graded ethanol. Then the materials were embedded in a mixture composed of Epon and Araldite as described by Mollenhauer (1964). Semithin sections were cut and stained with tuloidine blue. Ultrathin sections were mounted on uncoated copper grids, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined by electron microscope in the Unit of Electron Microscopy, Assiut University.

RESULTS

I- Light microscopical observations.

A well-developed gland associated with the third eyelid was demonstrated macroscopically in water buffalo (Fig.1). It was formed of glandular lobules of variable sizes. In both male and female water buffalo, each lobule was composed of tubuloalveolar secretory endpieces (Figs. 2 & 3) lined by pyramidal or columnar secretory cells with oval or slightly flattened basely located and deeply stained nuclei. Flattened myoepithelial cells were also observed at the peripheral borders of the secretory end-pieces (Fig. 2). The secretory cells forming the glandular endpieces were differentiated into light and dark cells after using haematoxylin and eosin stain (Figs. 2 & 3). The majority of the glandular endpieces were formed of both light and dark cells, while endpieces composed only of light or dark-cells were also observed. By application of combined alcian blue (pH 2.5) - PAS technique (Fig. 4), the endpieces lining cells were divided into two types of cells. The type I - cells contained variable amounts of PAS -positive secretory granules of neutral mucins. The secretory granules of type II -cells reacted positively with both alcian blue (pH 2.5) and PAS - techniques. It means that the secretory granules contain a mixture of neutral and acidic mucins. Some granules within type II -cells reacted only with PAS. The type II -cells were found in groups forming secretory endpieces or scattering also inbetween the type I cells in the glandular endpieces. The secretory endpieces contained few homogenous sudanophilic material (Fig. 5) distributed through the glandular lobules as well as fine lipid droplets located in infranuclear position were observed, but large sudanophilic droplets or globules could not be seen.

II- Electron microscopical observations.

The fine structure of the secretory cells in both male and female water buffalo revealed the typical ultrastructural features of the exocrine secretory cells. They were characterized by a variable amount of secretory granules accumulated in the apical cytoplasm, Golgi-apparatus located in supranuclear area, and rough endoplasmic reticulum (RER) and oval nuclei occupied usually the basal cytoplasm. The apical secretory surface (Fig. 6) was covered regularly by slender shaped microvilli of variable lengths projecting into the lumen of the secretory end-pieces. The lateral plasma membranes of the adjacent cells appeared smooth and attached together with apical junctional complexes (Fig. 6) and many lateral desmosomes (Fig. 7). Sometimes, the adjacent glandular cells were interdigitated with lateral cytoplasmic processes (Fig.10). On the basal border, many hemidesmosomes attaching the plasma membrane to the relatively thick basal lamina were usually observed. The RER (Fig. 10) was composed of parallel flattened lamellae which were heavily dotted with ribosomes. They occupied usually the basal cytoplasm and the perinuclear area. Few short flattened lamellae of RER appeared usually scattering in the apical cytoplasm and in close association with the secretory granules. In the supranuclear region semicircular arranged dilated parallel cisternae of Golgi-apparatus (Fig. 7) were usually seen. The peripheral membranes of Golgi-cisternae appeared in close relation with lamellae of RER. In the central zone of Golgi-region short lamellae of RER and many secretory vesicles at different degrees of development were demonstrated. These secretory vesicles were variable in size and contained homogenous secretory material of different electron densities. The basely located oval nuclei (Fig. 6) revealed a typical distribution of heterochromatin within the nucleus. It appeared as masses of variable sizes attaching to the inner nuclear membrane. Also, chromatin islands were observed scattering within the karyolymph. Some nuclei possessed irregular outer surface and showed invaginations of the nuclear envelope. The nucleoli were usually found in excentrical position within the nuclei of the glandular cells. In addition, free and polyribosomes, mitochondria (Fig. 10) and microfilaments (Fig. 11) could be seen. Merocrine exocytosis of the secretory substance through the apical surface of the glandular cells was demonstrated in Fig. 9.

The fine structure of the PAS-positive granules (Fig. 6 & 9) in type I - cells appeared round in shape and variable in size. They were filled with a homogenous electron dense substance, while the alcian blue (pH 2.5) - PAS - positive granules (Fig. 8) in type II -cells were filled with patches of electron

dense homogenous substance and fine granular material. The patches of electron dense homogenous substance appeared in different forms; round, oval, or irregular in shape occupying central or excentrical positions. Sometimes, they appeared as isolated small pieces scattering within the secretory granules, or as small pieces still in contact with each other by fine bridges.

Lipid inclusions (Figs. 10 & 11) of different sizes, which appear to be lysosomal structures, were also seen in the basal cytoplasm of the secretory cells. The lipid inclusions were limited by a clear unit membrane and occupied a basolateral or infranuclear position. They were frequently surrounded by the flattened lamellae of RER (Fig. 11) or appeared in close contact with the peripheral ends of the lamellae of RER (Fig. 10). The content of the lipid inclusions was mostly homogenous in appearance. The fat substance of some inclusions was formed of irregular shaped areas of variable densities separated from each other by clear borders (Fig. 11). At their periphery and in close contact with them, an area of homogenous dense substance was frequently observed. It was seen connecting two lipid droplets or forming an excentrically located peripheral area. Ultrastructural variations between male and female glands associated with the third eyelid of water buffalo have been observed. In the male gland, the glandular cells demonstrated clusters of tubular structures of variable sizes. They were usually found in positions near the Golgi-area or in close relation to the Golgi-cisternae (Fig. 7). These tubular structures have been not recorded in the secretory cells of the female gland associated with the third-eyelid.

Myoepithelial cells (Fig.12) were ultrastructurally demonstrated in position between the basal lamina and the basal borders of the secretory cells. They were easily identified by their electron dense myofilaments filling most of their cytoplasm. The attachment sites of myofilaments appeared as electron dense line on the outer borders of the myoepithelial cells (Fig. 12). Also, electron dense attachment sites within the cytoplasm were demonstrated (Fig.12). Each one of myoepithelial cells was differentiated into cell body and cytoplasmic processes. The cell body region (Fig. 13) appeared as a large oval or triangular-shaped area of cytoplasm containing oval or slightly elongated nucleus. The cytoplasmic processes of myoepithelial cell (Fig. 14) appeared slender in shape. The few cell organelles such as small mitochondria, short lamellae of RER, free and polyribosomes were restricted to the few areas of peripheral cytoplasm free of myofilaments. The plasma membranes of the cell body region and the cytoplasmic processes were firmly attached not only by hemidesmosomes to the relatively thick basal lamina,

but also by desmosomes to the plasma membrane of the secretory cells (Fig. 14). In addition, micropinocytosis (Fig. 14) was frequently seen along the borders of the cytoplasmic processes.

Unmylinated and without neurolemmal sheath nerve terminals (Fig. 15) were observed frequently in the intercellular spaces between the secretory cells of the glandular end-pieces. These naked nerve terminals penetrated the basal lamina of the glandular end-pieces and located mostly in a position between the secretory cells or inbetween the secretory and myoepithelial cells in the basal intercellular spaces. In longitudinal sections (Fig. 15), areas containing mitochondria, a few number of electron dense granules, and many small clear vesicles were observed along the course of the intercellular nerve terminals. Profiles of nerve terminals (Figs. 16, 17& 18) in cross sections were frequently seen in the intercellular space not only in close contact with secretory cells but also with myoepithelial cells. They formed synaptic contacts with both secretory and myoepithelial cells (Figs. 17 & 18). In the synaptic areas of nerve terminals, there were no remarkable morphological differences between the secretory vesicles in the neuroglandular and neuromuscular synapses could be seen. These nerve terminals usually contained accumulations of few electron dense granules and many small agranular vesicles, as well as large clear vesicles were observed (Fig. 17 & 18). Additionally, few small round mitochondria, neurofilament and neurotubules were demonstrated

DISCUSSION

The results of the present investigation revealed a well-developed gland associated with the third eyelid in both male and female water buffalo. It is composed of glandular lobules of tubulo-alveolar secretory endpieces. These observations confirm the previous findings reported by Ibrahim (1990) about the third eyelid associated glands in buffaloes, camels and donkeys. Although, Das (1979) reported in his light microscopic study about the third eyelid that the harderian gland is absent in Indian buffalo, and he considered the gland associated with third eyelid as a nictatins gland, our morphological findings about the gland associated with the third eyelid in Egyptian buffalo are in consistence with that reported by Franz (1934) and Sakai (1981). The gland can be consider as a harderian gland for the following reasons:

(i)- The gland is well-developed in water buffalo and nearly similar in its position and structure to the harderian gland of camel as mentioned by Ibrahim (1990).

(ii)- The gland has a unique histological structure and is associated with the third-eyelid.

(iii)- The gland is deeply located in the medial canthus of the eye, while the nictatins gland is an accessory lacrimal gland within the third-eyelid as reported by Franz (1934) and Saki (1981).

Moreover, some authors considered the deep gland associated with the third-eyelid in mammals a harderian gland (Dellmann and Collier, 1993). Ultrastructural sexual dimorphism has been also found between male and female glands associated with the third-eyelid in water buffalo. Similar observations were described in the hamster harderian glands (Bucana and Nadakavukaren, 1972a; Nadakavukaren and Lin,1983; Lopéz et al., 1992). Clusters of cylindrical tubules were only found in the male gland, while female gland showed a membranous structure arranged in concentric lamellae. Experimental studies indicated that the occurrence of the tubular structure in the male or female harderian glands depends on the maintenance of elevated level of testosterone in blood (Lin and Nadakavukaren, 1979). These morphological observations clarify the relation between the gland associated with the third-eyelid in water buffalo and the sexual hormones and strongly suggest that the gland associated with the third eyelid in water buffalo can be consider as a harderian gland.

The secretory cells of the third-eyelid associated gland in water buffalo were differentiated into light and dark cells. Similar observations have been also reported in other mammalian harderian glands (Woodhouse and Rhodin, 1963; Tsutsumi et al., 1966; Brownscheidle and Niewenhuis, 1978; Sayed, 1988; Abou-Elmagd et al., 1990). Some authors suggested that the light and dark cells are probably different forms of metabolic or physiological stages of activity of one secretory cell type (Keleny and Orban, 1965; Bucana and Nadakavukaren, 1972a; Rothwell et al., 1972; Brownscheidle and Niewenhuis, 1978). In addition, ultrastructural observations which probably support the previous suggestion were reported by Tsutsumi et al. (1966) and Bucana and Nadakavukaren (1972a). They described variations in the number of free and polyribsomes, mitochondria and secretory granules between light and dark cells. With respect to the previous findings, our ultrastructural observations displayed variations in the form and content of the secretory granules of the light and dark cells. Moreover, the histochemical examination of these secretory granules, after application of combined alcian blue (pH 2.5) - PAS technique, revealed two types of secretory cells; one contains neutral mucins and the other is filled with a mixture of neutral and acidic mucins. These results suggest that the differentiation of the secretory cells

into light and dark cells may be due to the fact that the cells contain different secretory substance at least in water buffalo.

The secretion of the third eyelid associated gland in vertebrates is variable in nature from one species to another. Paule and Hayes (1958), Wight et al. (1971) and Brobby (1972) reported that the mucoid secretion is characteristic of birds, while the secretion is lipoidal in mammals and serous or seromucous in reptiles. In the present investigation, the secretion of the gland of buffalo appeared to be composed of secretory granules formed mainly of neutral and acidic mucins. A mixture of neutral and acidic mucins has been also described in the harderian glands of pigs (Kühnel, 1974) and camel (Fahmy et al., 1979 and Sayed, 1988). The mucoid secretion may function, in addition to lubrication, as a protective media which maintains the antibodies and increases their concentration within the eye.

The close contact and formation of synapses between the nerve terminals and both myoepithelial cells and secretory cells in water buffalo suggest the presence of a very organized and well-developed functional relationship between nerve terminals and both, myoepithelial and secretory cells. The pattern of distribution of nerve terminals and their relation to the myoepithelial and secretory cells was previously described in harderian glands of rabbits and pigs (Kühnel, 1971 & 1974), hamsters (Bucana and Nadakavukaren, 1972b), rats (Leeson, 1960; Leeson and Leeson, 1971; Brownschiedle and Niewenhuis, 1978), mice (Watanabe, 1980), mongolian gerbils (Sakai and Yohro, 1981) and camel (Abou-Elmagd, 1992). After acetylcholine injection in rats, Tashiro et al. (1940) recorded an increase in the secretion of the harderian gland, and suggested that the cholinergic innervation of myoepithelial cells whose contraction causes expulsion of the secretory product. This suggestion has been recently confirmed by morphological experimental observations in ginea pigs by Satoh et al. (1993) and in frogs by Di Matteo et al. (1995). They observed a reduction in the cell height and dilatation of the alveolar lumina containing secretory granules discharge after injection of cholinergic secretagogue substance and concluded that acetylcholine elicits exocytosis in glandular cells and contraction of the myoepithelial cells. Huhtala et al. (1977) added that the harderian gland is regulated by both cholinergic and adrenergic nerves, and concluded that the secretory process seems to be indirectly under the dual control of both cholinergic and adrenergic fibers which innervate the blood vessels of the gland. In water buffalo, there are no remarkable morphological differences between the components of the nerve terminals in regions of neuromuscular and neuroglandular synapses. In consistence with the previous

conclusion, our observations indicate that both myoepithelial and secretory cells are probably supplied by one type of nerve terminals which seem to be cholinergic nerve terminals. In addition, the present data suggest strongly that the direct contact of nerve terminals through formation of synapses may help in the process of synchronization between the role of neuroglandular synapse in enhancing the cell activity to increase synthesis and exocytosis of the secretory product,and the role of myoepithelial cell contraction for extrusion of the secretory product outside the lumen of the end-pieces.

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LEGENDS

Fig. 1: Third eyelid of water buffalo shows membranous portion (M), gland associated with the third eyelid (G) and fatty portion (F).

Fig. 2: Endpieces showing light cells (thin arrow) and dark cells (arrow head), and myoepithelial cell (thick arrow) in gland associated with the third eyelid in male water buffalo. Paraplast section, haematoxylin & eosin stain. x 400.

Fig. 3: Tubuloalveolar secretory endpieces lined by light cells (thin arrow) and dark cells (arrowhead) in the gland associated with the third eyelid in female water buffalo. Paraplast section, haematoxylin & eosin stain. x400.

Fig. 4: Secretoy cells are differentiated into cells stained only with PAS (arrowhead) and others stained with both alcian blue and PAS (arrow). Paraplast section, alcian blue (pH 2.5)-PAS stain. x 400.

Fig. 5: Homogenous sudanophilic substance in the acini of the gland associated with the third eyelid in buffalo. Cryostat - section stained with sudan black B. x200

Fig. 6: Electron micrograph of the type I -cells of male water buffalo shows the general ultrastructural features: Basely located lamellae of RER and nucleus (N). Golgi-apparatus (Ga)is located in the supranuclear area, and secretory granules (Sg) containing electron dense homogenous material in the apical cytoplasm. Apical junctional complex (thin arrow) and lumen of the secretory endpiece (Lu) filled with microvilli were demonstrated. Basal lamina (arrow) and cytoplasmic processes of myoepithelial cell (arrowhead). x 5 300.

Fig. 7: Golgi-areas (arrowheads) in the type I -cells of male water buffalo are formed of diluted cisternae, and in the centre many developing secretory vesicle containing homogenous secretory substance of variable densities. Tubular structures (arrows) in close relation to the Golgi-cisternae and lamellae of RER. Desmosome (empty arrow). x6300

Fig. 8: Secretory granules (Sg) in type II cell containing different forms of mixture of electron dense homogenous patches and fine granular material. x 10 000.

Fig. 9: Merocrine exocytosis (arrows) at the apical surface of the secretory cells. The secretory granules (Sg). x 27000.

Fig. 10: Lipid inclusions (L) in the basaolateral cytoplasm appear in close association with lysosomal homogenous dense substance and lamellae of RER. Cytoplasmic processes interdigitations (arrow) and mitochondria (M). x 8 000.

Fig. 11: Lipid inclusions appear in the basal cytoplasm in close contact with lamellae of RER. The fatty substance (empty arrow) is formed of areas of different electron densities with clear borders. Basal lamina (thick arrow) and microfilament (thin arrow). x 10 000.

Fig. 12: Myoepithelial cell (Me) between the basal lamina and the secretory cells (Sc). Arrowhead indicates attachment site of myofilament on the

periphery of myoepithelial cell. Areas of cyoplasm free of myofilaments contain mitochondria (thin arrow). Electron dense attachment sites of myofilaments (thick arrow) within the cytoplasm. Lumen (Lu) of secretory endpiece. x 5 100.

Fig.13: Cell body region of myoepithelial cell (Cb) demonstrates centrally located nucleus, and peripheral cytoplasm containing few mitochondria, free and poly- ribosomes (thick arrow). Nerve terminal (empty arrow) and secretory cell (Sc).
x 13 400

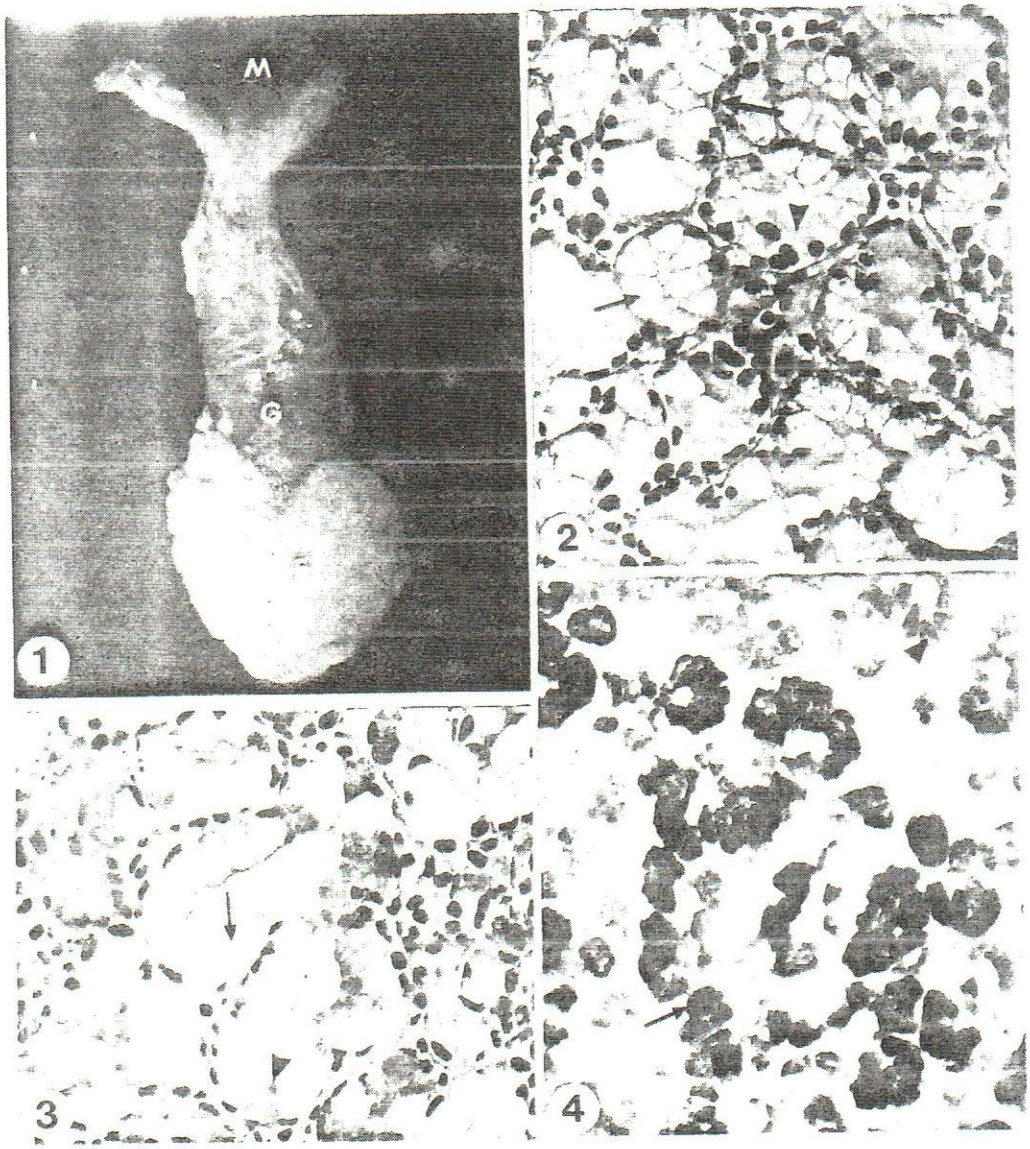
Fig.14: Longitudinal section of cytoplasmic process (Cp) of myoepithelial cell appears filled with myofilaments and attached to the secretory cell by desmosome (thin arrow). Micropinocytosis (thick arrow) and mitochondria (arrowhead) in peripheral cytoplasm. x 15 000.

Fig.15: Longitudinal section in nerve terminal (Nt) in the basal intercellular space showing many small clear vesicles (thick arrow) and few large dense granules (thin arrow) and neurotubules (arrowhead). It forms direct contact with both myoepithelial cell (Me) and secretory cell (Sc). Basal lamina (Bl). x 10 000.

Fig.16: Nerve terminal (empty arrow) forming direct contact with myoepithelial cell (Me). It contains mitochondria (M), dense granules (thin arrow) and small clear vesicles (thick arrow). Secretory cell (Sc). x 14 000.

Fig.17: Nerve terminal (empty arrow) in intercellular space forms a synaptic contact (arrowhead) with the glandular cell. It contains dense granules (thin arrow) and large clear vesicle (thick arrow). x 15 000.

Fig.18: Nerve terminal (empty arrow) forms a synaptic contact (arrowhead) with the myoepithelial cell (Me) and contains accumulated clear vesicles (thick arrow), few dense granules (thin arrows), large clear vesicle (empty arrow) and mitochondria (M). Basal lamina (Bl). x14000.


M
G
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2
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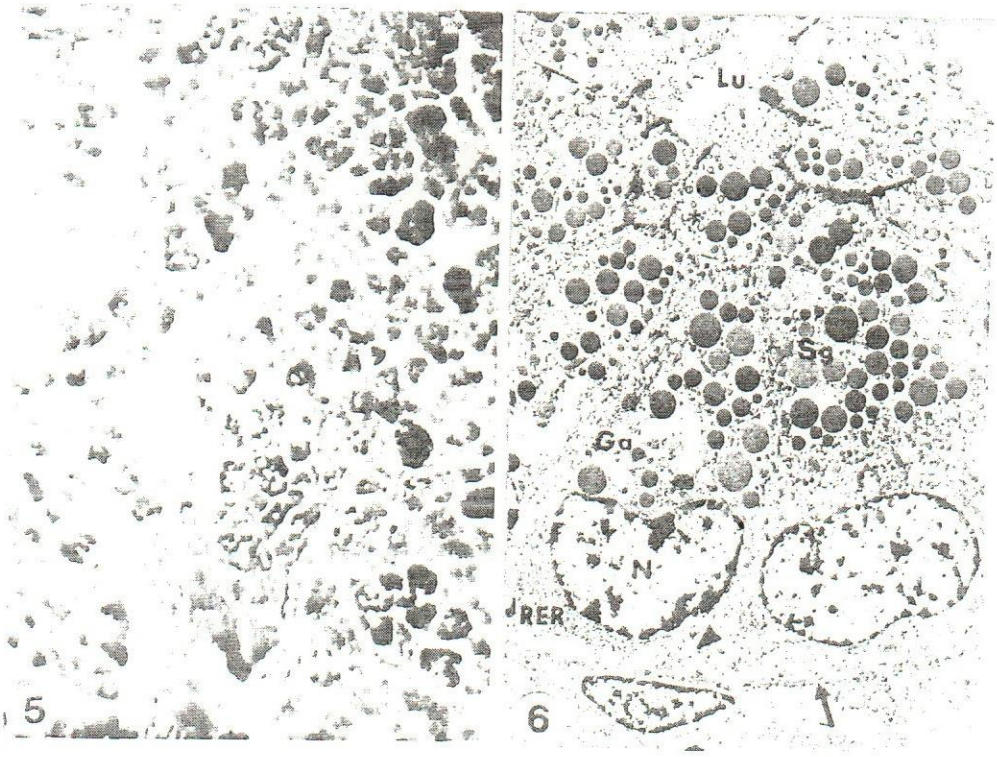
4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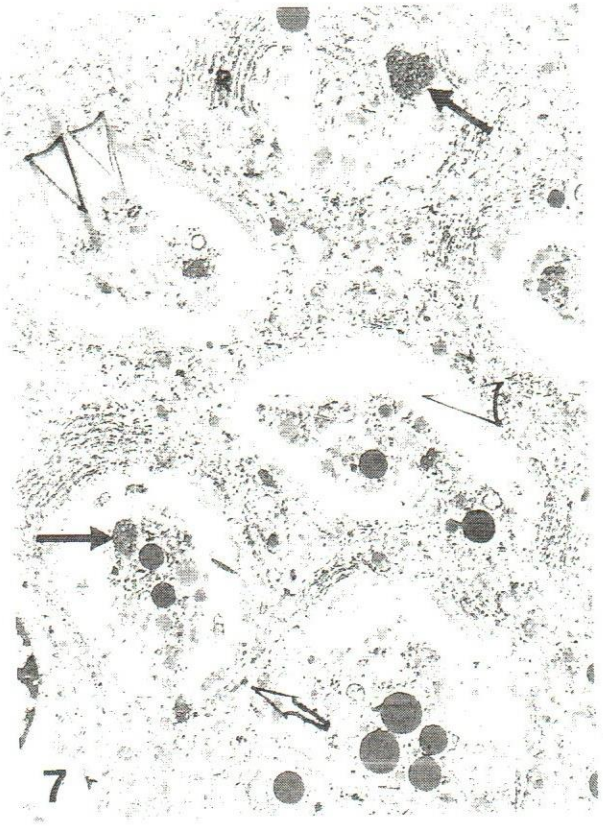
1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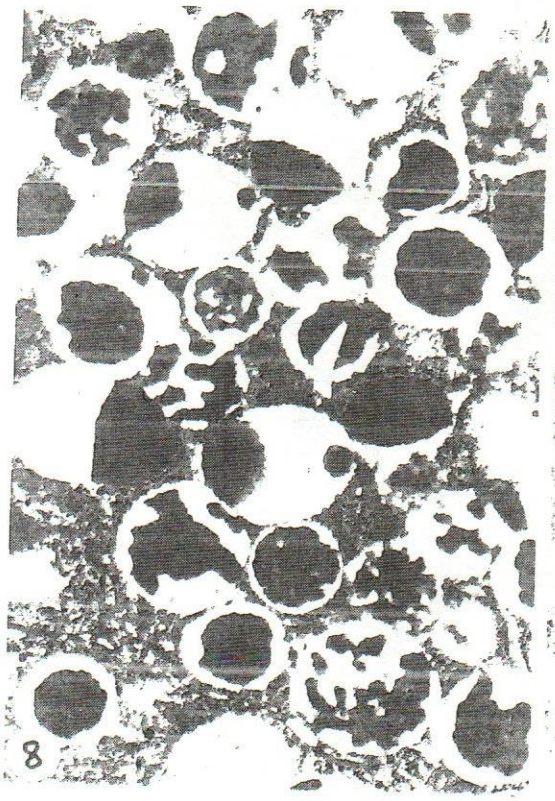

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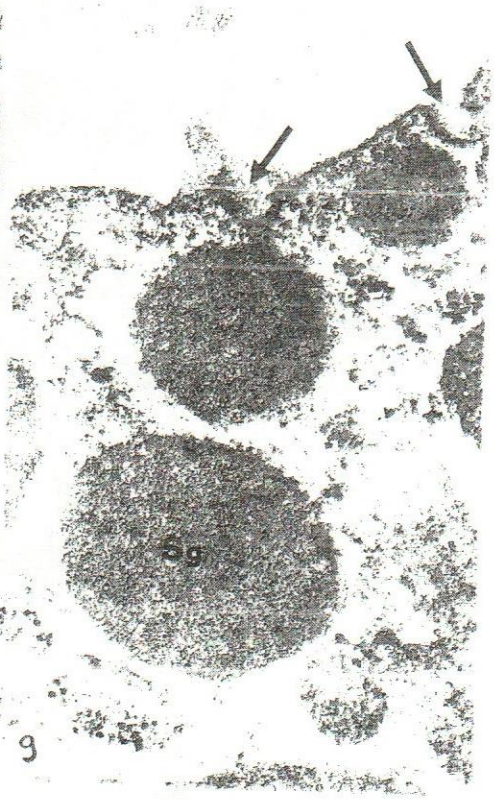

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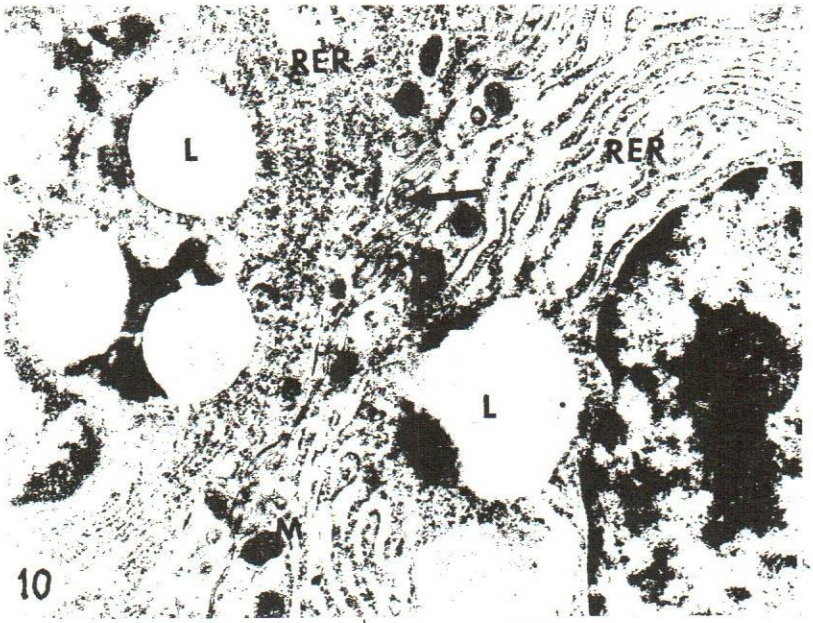

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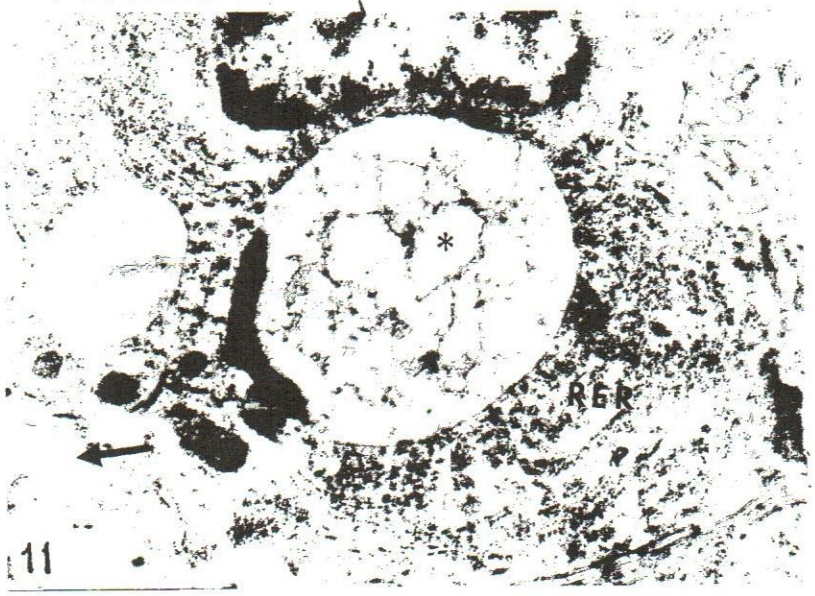

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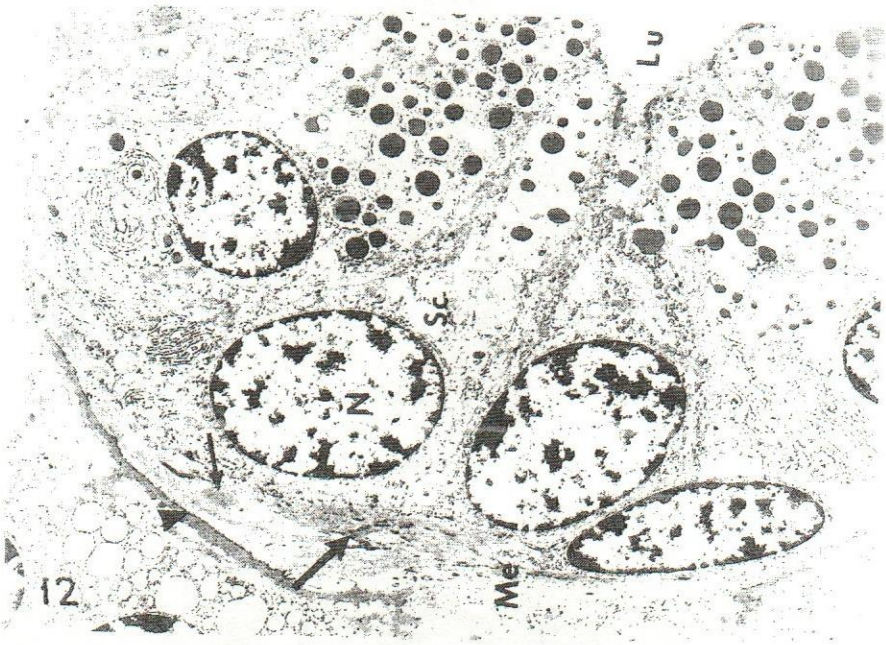

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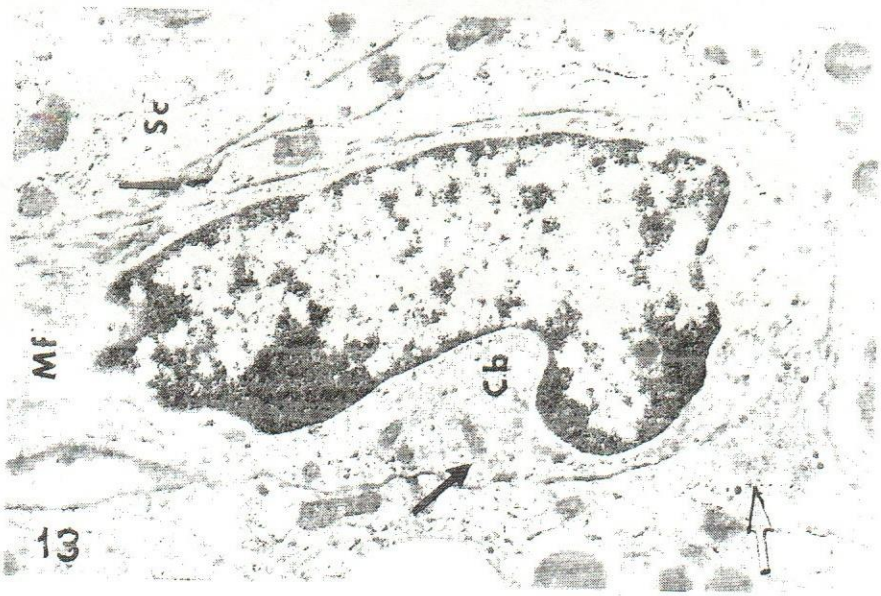

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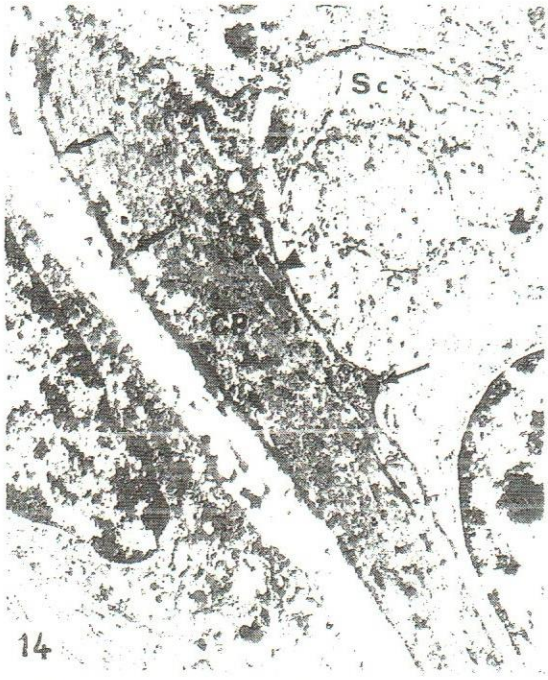
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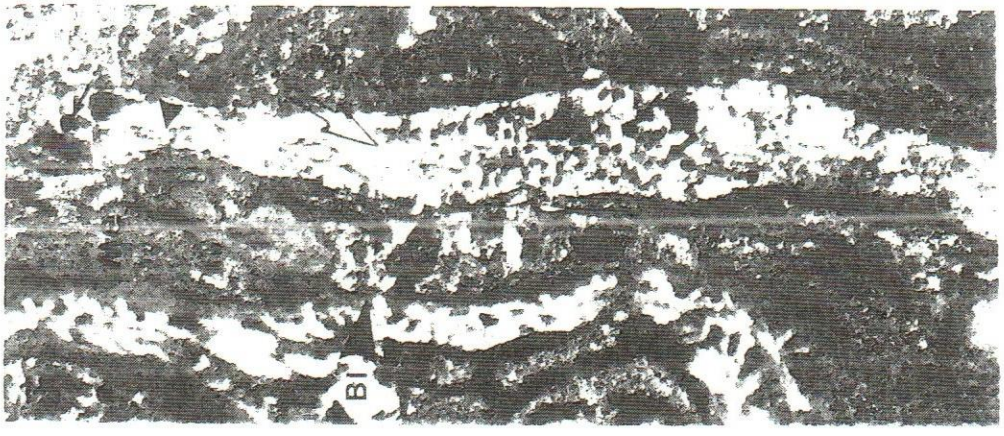
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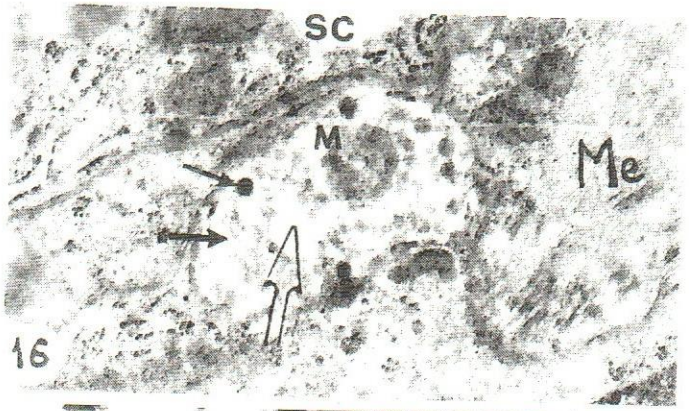
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Me
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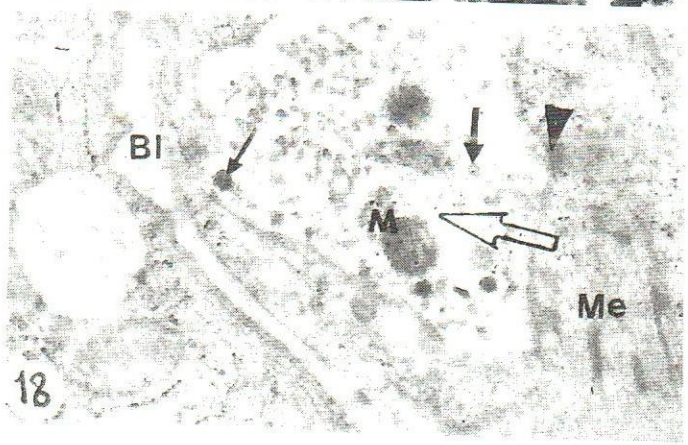
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Me
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