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ULTRASTRUCTURE OF THE ESOPHAGEAL EPITHELIUM OF THE ONE-HUMPED CAMEL (CAMELUS DROMEDARIUS)

(With 17 Figures)

By

YOUSRIA A. ABDEL-RAHMAN

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التركيب الدقيق لطلائية المريء في الجمل وحيد السنام

يسريه عبد الغني عبد الرحمن

أجـرى هـذا الـبحث لدراسة التركيب الهستولوجي لطلائية المريء في الجمل وحيد السنام بواسـطة المجهر الضوئي والألكتروني الماسح والنافذ وقد وجد أن هذه الطلائية في أجزاء المـريء المختلفة لها نفس التركيب ومن النوع الحرشفي المطبق الغير مقرن. وقد أوضح المجهر الألكتروني الماسح للغشاء المخاطي المبطن للمريء وجود طيات طولية تنفصل عن بعضـها الـبعض بواسطة حفر طولية متوازية. كما لوحظت مرتفعات قبية الشكل ومتوازية الـترتيب أيضاً. وقد أظهر كل مرتفع فتحة مركزية للأنبوبة الإخراجية لغدد المريء. كانت حـدود الخلايـا واضحة وسميكة والثنايات الدقيقة موزعة بكثافة على السطح الطلائي . أما المجهر الألكتروني النافذ فقد أظهر أن طلائية المريء في الجمل تتكون من الطبقة القاعدية، الطـبقة الشـوكية والطبقة السطحية . تميزت خلايا الطبقة السطحية بإحتوائها على الحبيبات المغـلفة الداكنة والرائقة (الفاتحة) الكترونيا وكذلك القطيرات الدهنية. كما لوحظت محتويات هـذه الحبيبات في سيتوبلازم الخلايا وكذلك في التجويف البين خلوي. أما في طبقاتها العليا فقـد ازدادت خلاياهـا تفـلطحا واحتفظت بأنويتها، بينما أظهرت عضياتها تغيرات هدامة واستبدلت غالبا بالخيوط السيتوبلازمية التي ملأت معظم الخلية. خلايا الطبقة الشوكية وكذلك القاعديـة تماثل ماوصف في الطلائية الحرشفية المطبقة غير المقرنة بوجه عام . أوضحت الخلايـا القاعديـة وجـود الإنقسـام الميتوزي ، كما لوحظ وجود الخلايا الليمفاوية وخلايا لنجرهانز في التجويف البين خلوي للطبقة القاعدية.

SUMMARY

The histological characteristics of the esophageal epithelium of the camel were investigated by light, scanning and transmission electron microscope. The epithelium of the different portions of the camel esophagus was thick stratified squamous nonkeratinized type. Scanning electron microscopy revealed that the mucosal surface formed of somewhat parallel longitudinal folds, which were separated from one another by sulci. Dome-shaped elevations appeared parallely arranged on the mucosal surface. Each elevation showed a central opening of the excretory duct of the underlying esophageal glands. The cell boundaries were distinct and the microridges were densely distributed on the epithelial surface. The transmission electron microscopy revealed that the stratified squamous epithelium of the camel esophagus consisted of Stratum basale, Stratum spinosum and Stratum superficiale. The cells of the latter stratum contained both electron-dense and electron-lucent membrane-coating granules as well as some fat droplets. The contents of these granules were observed free in the cytoplasm and in the intercellular space. The cells of the upper most layers of this stratum became slightly flat, retained pyknotic nucleus and their organelles undergo degenerative changes and replaced by tonofilaments which fill most of the cell. The basal cells showed mitotic figures. Lymphocytes and Langerhans cells were observed in the intercellular space of the basal cell layer.

Keywords: Ultrastructure, stratified squamous epithelium, membrane-coating granules, Langerhans cells, esophagus, camel.

INTRODUCTION

It is true that the esophagus transports food and drink from the oral cavity and pharynx to the stomach. The mucosa however, must be resistant to physical trauma from the components of food and drink and must remain largely impermeable to the fluid environment to which it is exposed (Toner, Carr and Wyburn, 1971). The stratified squamous epithelium meet these requirements either by thickening, keratinization,

numerous glands (Goetsch, 1910, Long and Orlando, 1999) or microplications (Cleoton-Jones, 1975; Telford and Bridgman, 1995).

The camel esophagus has been studied developmentally (Berg, Kassem, Hemmoda and Amin, 1984), anatomically (Hegazi, 1945; Omar, 1980 and Ibrahim, 1983) as well as anatomically and histologically (Anis, Bareedy, Ammar and Ewais, 1981). The available literature had nothing about the ultrastructure of the camel esophagus. The purpose of the present work was to investigate the lining epithelium of the esophagus of camel by light, scanning and transmission electron microscope.

MATERIAL and METHODS

Samples from the cervical, thoracic and abdominal regions of the esophagus were obtained from 10 adult apparently healthy camels of both sexes. They were gently washed with saline solution and immediately fixed in solution containing 2.5% glutaraldehyde and 2.5% paraformaldehyde in cacodylate buffer (pH 7.2) for 2 hours (Karnovsky, 1965). After rinsing in 0.1M cacodylate buffer, the samples were post-fixed for further 2 hours in 1% osmium tetroxide, dehydrated in ethanol and embedded in Epon-Araldite. Semithin sections were cut and stained with toluidine blue. Ultrathin sections were contrasted with uranyl acetate followed by lead nitrate and examined with JOEL 100 CXII transmission electron microscope.

For scanning electron microscopy, the fixed tissue samples were dehydrated in ethanol and processed in amyl acetate and critical-point dried using Co_2, mounted on copper stubs and coated with gold. They were examined with JOEL 5400 LV scanning electron microscope.

RESULTS

Light microscopy:

The Lamina epithelialis of the esophagus of the camel had the same structure in the cervical, thoracic and abdominal portions. It was consisted of thick nonkeratinized stratified squamous epithelium measuring about 419. 62 µm ± 5.18 in thickness and formed of about 50-60 layers. The nuclei of the superficial cell layer were appeared darkly stained, while those of Stratum basale showed mitotic division. The papillary bodies were variable in depth (Fig. 1).

Scanning electron microscopy:

The esophageal mucosa was arranged into longitudinal folds, separated by sulci, running parallel to each other (Figs. 2-4). Dome-shaped elevations were seen distributing along the whole surface of the esophagus. They were arranged in rows and contained the openings of the excretory duct of esophageal glands (Figs. 2 & 3). Each elevation was formed by fusing of 2-4 mucosal longitudinal folds and separated from the other one by 4-6 folds.

At higher magnification, the epithelial cell surfaces appeared irregular polygonal in shape with prominent cell boundaries (Fig. 5) and showed densely distributed well-developed microplicae, which anastomosed together (Fig. 6). Desquamating cells were also seen (Fig.7).

Transmission electron microscopy:

The non-cornified stratified squamous epithelium of the esophagus of the camel was composed of Stratum basale, Stratum spinosum and Stratum superficiale.

Stratum basale:

The Stratum basale of the esophageal epithelium consisted of elongated columnar-shaped cells. Most of the cell was filled with large oval nucleus with few indentations. The nuclear membrane and their pores were distinct.

The cells had short cytoplasmic processes extended into wide intercellular space and connected with each other by interdigitations and few desmosomes (Figs. 9&10). The basal lamina was somewhat irregular, accompanied the basal plasma membranes of the basal cells and connected to them by hemidesmosomes. Different stages of mitotic division were seen only in this layer (Figs.8a-b).

The most characteristic features of these cells were the numerous bundles of tonofilaments, which run in different directions in the cytoplasm or attached to the desmosomes and hemidesmosomes. Numerous free ribosomes, polysomes, mitochondria, long cisternae of rough endoplasmic reticulum and supranuclear Golgi-complex were observed (Figs. 9&11). The latter was seen also infranuclear in some cells (Fig. 11).

Stratum spinosum:

The Stratum spinosum (Figs. 12 & 13) consisted of polyhedral-shaped cells with centrally located nuclei. Distinct peripherally located nucleolei were observed. This Stratum was characterized by the numerous cell processes, which attached to each other with numerous desmosomes and interdigitations.

The cytoplasm of the deep spinous cell layers had nearly the same features as those of the Stratum basale. It contained numerous bundles of tonofilaments either distributed all over the cytoplasm or associated with the desmosomes. Large amounts of polysomes, numerous rounded, oval and elongated mitochondria, moderate rough endoplasmic reticulum and extensive Golgi-complex were also observed. In the upper spinous layers these organelles became fewer.

Stratum superficiale:

The Stratum superficiale (Figs. 14 – 16) was formed of spindle- or flat-shaped cells containing large oval nuclei which, became pyknotic at the superficial ones. The cytoplasm of the deep cell layers contained numerous free ribosomes, tonofilaments, mitochondria, membrane-coating granules as well as some fat droplets. While, the cytoplasm of the most superficial layers was flocculent, often lacked organelles and occupied mostly with meshwork of tonofilaments. The membrane-coating granules were of two types electron-dense and electron-lucent. They were present in association with one another as well as with the tonofilaments. The content of the electron-dense granules was observed in the cytoplasm as well as in the intercellular space (Figs. 14 & 15). However, the membrane of the electron–lucent type was seen fusing with plasma membrane and discharged their content into the intercellular space (Fig. 14). Interdigitations and numerous desmosomes joined the adjacent cells. The desmosomes became few superficially and disappeared totally when the cells undergo desquamation. The luminal surface of the superficial cell layer was studded with numerous microridges (Fig. 16).

Migratory cells:

Langerhans cells were recorded either singly or in association with lymphocytes in the intercellular space of the basal layer (Fig. 17).

Langerhans cells were identified by the indented nucleus and faint cytoplasm. The latter contained Langerhans cell granules, numerous RER-cisternae and moderate mitochondria. They lacked tonofilaments and desmosomes.

DISCUSSION

The present work revealed that the mucous membrane of the esophagus of the camel was lined with nonkeratinized stratified squamous epithelium. These findings simulate that mentioned in dog (Goetsch, 1910; Henk, Hoskins, Abdelbaki, 1986; Stinson and Calhoun, 1993) and man (Smell, 1984; Leeson, Leeson and Paparo, 1988; Fawcett, 1994). In the contrary, Anis et al. (1981) observed that the esophageal epithelium of the camel was of keratinized stratified squamous type as that of ruminants and horse (Goetsch, 1910; Slocombe, Todhunter and Stick, 1982; Negm, El-Fiky, Aidaros and Mehalb, 1989; Stinson and Calhoun, 1993).

Scanning electron microscopical observations of all examined regions of the esophageal mucosa of the camel revealed numerous longitudinal folds and regular distribution of the esophageal gland openings. In agree with Trautmann and Fiebiger (1957) and Smell (1984), these folds probably accommodate the mechanical expansion of the esophageal lumen during the passage of bolus.

The observed microplicae over the entire surface of the cells were similar to that described on the dorsal surface of the human tongue by Kullaa-Mikkonen and Sorvari (1985) and Telford and Bridgman (1995). These microplicae appear as stem from plasmalemmal folds (Andrews, 1975). Sperry and Wassersug (1976) added that the microplicae are stable features on the cell surface and their appearance is unchanged in stretched and unstretched conditions. Moreover, Telford and Bridgman (1995) mentioned that the microplicae are displayed on the exposed outer surface of certain moist stratified squamous epithelial cells. When the moist epithelium is repeatedly exposed to air, it loses its mucin coating, becomes dehydrated keratinized, and finally deprived of all microplicae. On the other hand, the keratinized cells of human and rat oral as well as horse esophageal mucosa have a pitted or knobby-appearance (Cleaton-Jones and Fleish, 1973; Cleaton-Jones, 1975; McMillan, 1979; Slocombe et al., 1982). Microplicae appear to provide

a structure to facilitate the even spreading and gross retention of a protecting mucus coat to the epithelium (Andrews, 1975; Sperry and Wassersug, 1976; Kullaa-Mikkonen and Sorvari, 1985). The latter authors added that these microplicae reduce the surface area available for environmental contact, so that, in the keratinized epithelium where the mechanical stress is great enough and there is no protective mucus, the surface microplication is absent. It could be suggested also that, the functional importance of microridges lie in the distribution as well as retention of minute amounts of mucus on the esophageal surface to facilitate the passage of the hard spiny food and to protect the epithelium.

Transmission electron microscopy denoted that the cytoplasm of the most cell layers of the Stratum superficiale was characterized by the presence of membrane-coating granules. This agree with that reported in the nonkeratinized epithelium of the human buccal mucosa (Hayward and Hackemann, 1973; Landay and Schroeder, 1977). The membrane-coating granules probably produce a glycoprotein surface coat (Hayward and Hackemann, 1973) as well as contribute to the thickening of the plasma membrane of the epithelial cells and form the protective barrier (Matoltsy and Parakkal, 1965). In agree with Stinson and Calhoun (1993) in the epidermis, this barrier prevents both the penetration of substances from the environment and the loss of body fluids.

The content of the membrane-coating granules was also observed in the cell cytoplasm as seen by Matlotsy (1969) in chick skin and in the intercellular space of the deepest cell layers of the Stratum superficiale as described in human oral epithelium by Hayward and Hackemann (1973) and Cleaton-Jones and Fleisch (1973) as well as in mice esophageal epithelium by Parakkal (1975).

The present study revealed that the cells of the Stratum superficiale contained meshwork of filaments filling most of the cytoplasm as stated by Landay and Schroeder (1977) in the human buccal mucosa and Milstone (1981) in bovine esophageal epithelium. The former authors suggested that these filaments serve as the functional matrix for epithelial distensibility.

In mammals, Goetsch (1910) stated that there is a very evident relation between the presence of glands and the degree of cornification. The epithelium of the esophagus of the dog is nonkeratinized and the

glands were present throughout its length. Pig has a moderate number of glands and the epithelium shows slight cornification. In the contrary, the glands were found only at the pharyngoesophageal junction in sheep, goat, ox and horse, whereas the epithelium was highly cornified. The opinion of Goetsch (1910) and Wilson (1984) was acceptable. Where, the epithelial lining of the esophagus in camel was nonkeratinized and its protection against the coarse and spiny food comes from the numerous esophageal glands, extending along the whole length of the esophagus and the thickened epithelium.

Only the basal cells of this epithelium showed mitotic division. The daughter cells replace the desquamated superficial ones that undergo physiological degenerative changes by the mechanical stress caused by food and drink during swallowing and rumination.

In accordance with Cormack (1987) in man and Bykov (1997) in mice and rats, in wet stratified squamous epithelia lining the oral cavity, esophagus, vagina and rectum as well as in the epidermis, the present investigation revealed the occurrence of Langerhans cells in the intercellular space of the basal cells either singly or associated with the lymphocytes. However, Krause and Cutts, (1981), Banks (1993) and Stinson and Calhoun (1993) mentioned that, these cells were found in the upper spinous layer of man and domestic animals epidermis. The ultrastructural features of these cells simulated that described by Krause and Cutts, (1981) and Stinson and Calhoun (1993). Langerhans cells are motile antigen-presenting cells that promote cutaneous delayed hypersensitivty reactions (Cormack, 1987; Stinson and Calhoun, 1993). Krause and Cutts (1981) and Banks (1993) stated that the Langerhans cells represented epidermal phagocytes.

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LEGENDS

Fig. 1: Light micrograph of Tunica mucosa of the esophagus of the camel showing thick stratified squamous epithelium, lamina propria (Lp) and mitotic figures (arrow). X 250.

Figs. 2 & 3: Scanning electron micrographs of the mucosa of the esophagus of the camel showing longitudinal folds (arrow) separated by grooves (arrowhead). Mucosal elevations (asterisk) with openings of the esophageal glands (double arrow).

Fig. 4: Higher magnification scanning electron micrograph of the mucosa of the esophagus of the camel showing longitudinal folds separated by sulci (arrowhead). Desquamated cells (arrow).

Fig. 5: Scanning electron micrograph of the mucosa of the esophagus of the camel showing irregular polygonal-shaped epithelial cells with prominent cell boundaries (arrow).

Fig. 6: Scanning electron micrograph of the mucosa of the esophagus of the camel showing junction between the polygonal cells with anastomosing microplicae.

Fig. 7: Scanning electron micrograph of the mucosa of the esophagus of the camel showing desquamated cells.

Figs. 8a & b: Electron micrographs of the basal layer of the esophageal epithelium of the camel showing intercellular space (Is), desmosomes (arrow), basal lamina (arrowhead) and nucleus (N). Basal cells undergo mitosis: Prophase in Figs. 8a (asterisk) X 6857 and metaphase in Figs. 8b (thick arrow) X 5833.

Fig. 9: Electron micrograph of a basal portion of the basal cell showing mitochondria (M), ribosomes (R), rough endoplasmic reticulum (rER), Golgi-complex (G) and tonofilaments (Tf). Nucleus (N), intercellular space (Is), desmosome (arrow), basal lamina (Bl), X 13333.

Fig. 10: Higher magnification of the marked area in Figs. 9 showing well- developed bundles of tonofilaments (Tf), ribosomes (R), intercellular space (Is) and desmosomes (arrow). X 40000.

Fig. 11: Electron micrograph of supra- and infranuclear portions of two adjacent basal cells showing mitochondria (M), ribosomes (R), Golgi-complex (G), bundles of tonofilaments (Tf) and rough endoplasmic reticulum (rER). Nucleus (N), intercellular space (Is), desmosome (arrow). X 20000.

Fig. 12: Electron micrograph of the Stratum spinosum showing mitochondria (M) and bundles of tonofilaments (Tf). Nucleus (N), intercellular space (Is), desmosomes (arrow). X 3915.

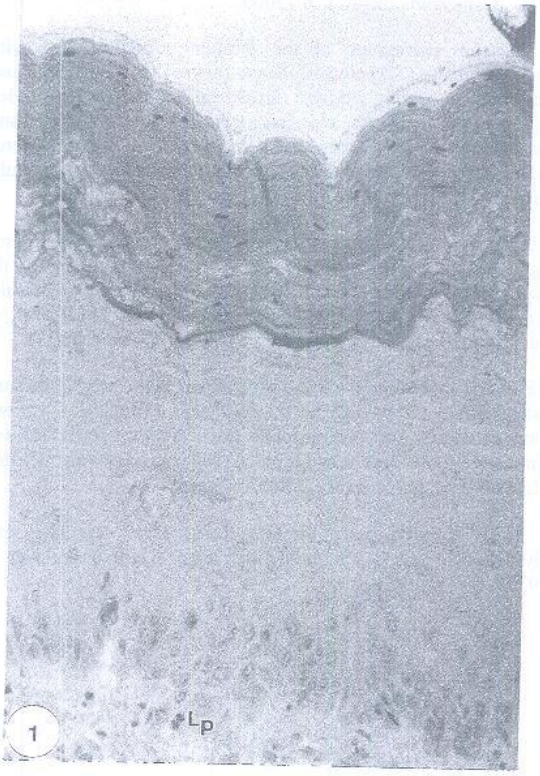
Fig. 13: Higher magnification of a cell of Stratum spinosum showing bundles of tonofilaments (Tf), ribosomes (R), Golgi-complex (G) and mitochondria (M). Nucleus (N), intercellular space (Is), desmosome (arrow). X 12000.

Fig. 14: Electron micrograph of the Stratum superficiale showing tonofilaments (Tf), electron-dense (arrow) and electron-lucent membrane- coating granules (arrowhead) as well as fat droplets (Fd). Fusion of the electron-lucent granules with plasma membrane (double arrowhead), content of the electron-dense ones in the cytoplasm (double arrow), nucleus (N), intercellular space (Is). X 7000.

Fig. 15: Higher magnification showing the content of the electron-dense granules in the intercellular space (thick arrow) and in the cytoplasm (double arrow). Electron-lucent granules (arrowhead), electron-dense ones (thin arrow), meshwork of tonofilaments (Tf), fat droplets (Fd). X 16666.

Fig. 16: Electron micrograph of the superficial cells of the Stratum superficiale showing microridges (double arrow), tonofilaments (Tf), fat droplets (Fd), electron-dense (arrow) and electron-lucent membrane-coating granules (arrowhead). Lumen (Lu), nucleus (N). X 8333.

Fig. 17: Electron micrograph showing Langerhans cell (Lc) and lymphocyte (Ly) in the intercellular space of the basal layer. X 9375.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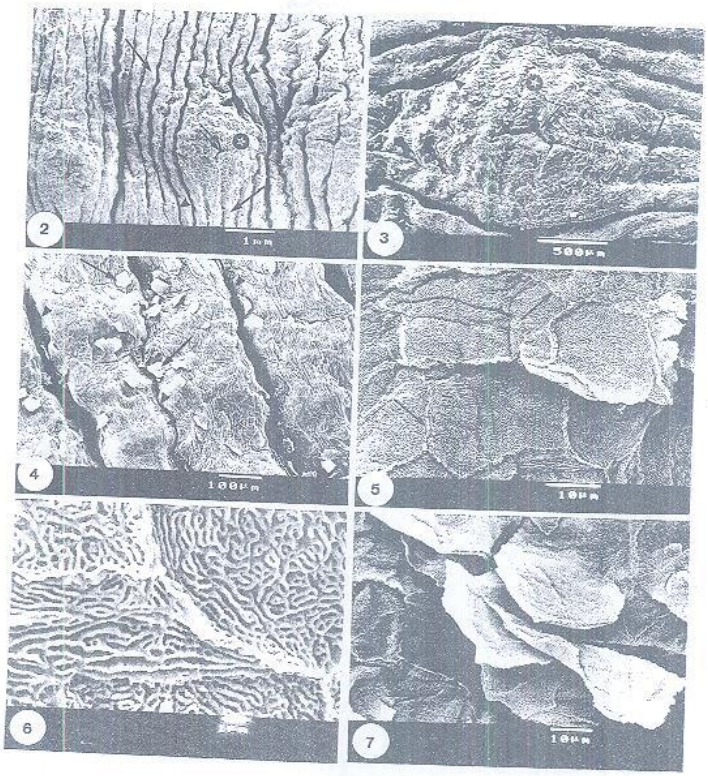
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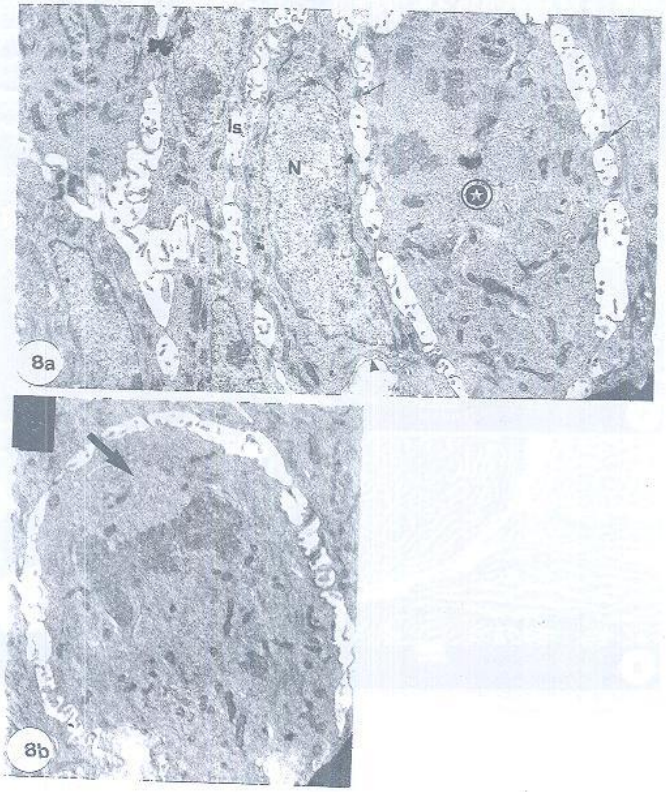
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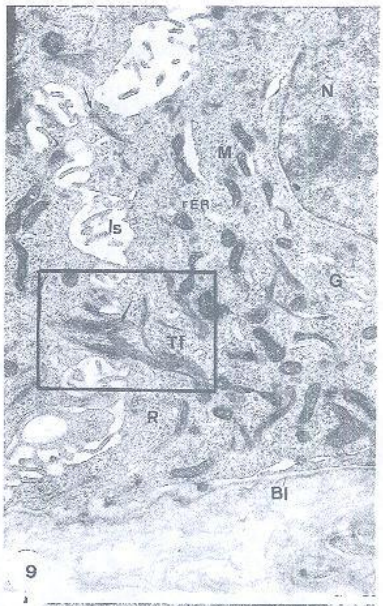

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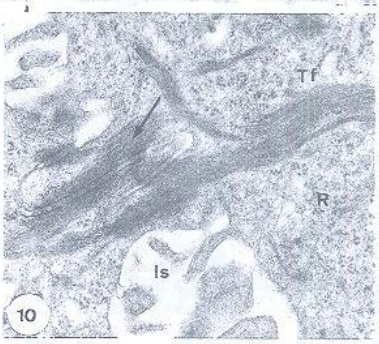

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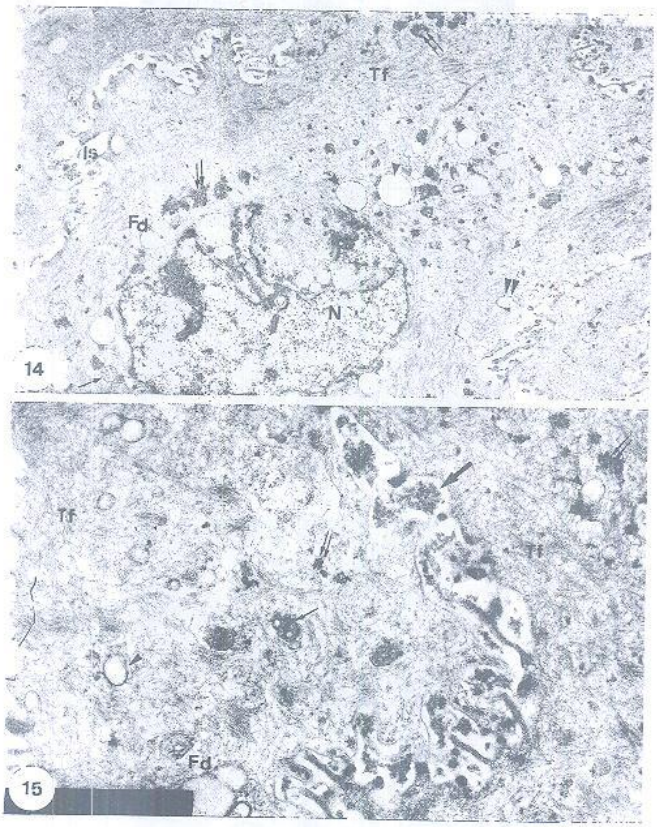
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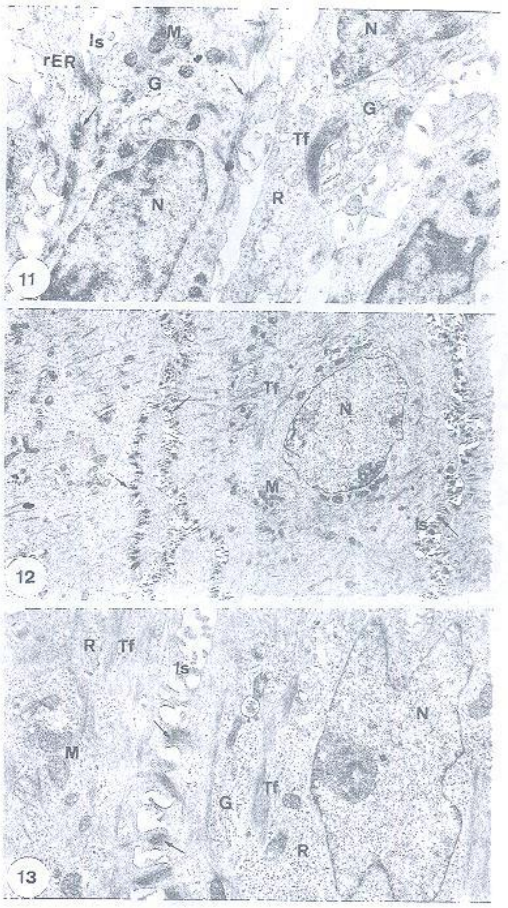
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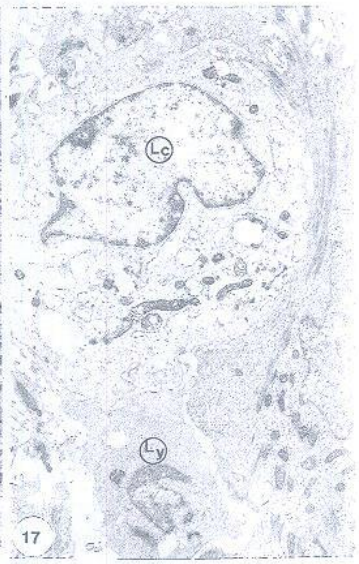

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