Assiut Vet. Med. J. Vol. 37 No. 73, April 1997

Dept. of Anatomy & Histology
Fac. Vet. Med., Assiut University
Head of Dept. Prof. Dr. G. Kamel

ELECTRON MICROSCOPICAL STUDIES ON THE LINING EPITHELIUM OF THE GLANDULAR STOMACH OF ADULT FOWL WITH SPECIAL REFERENCE TO PROGRAMMED CELL DEATH, APOPTOSIS

(With 26 Figures)

By
A.O. SALEM

(Received at 14/1/1997)

دراسات بالمجهر الإلكتروني على الطلائية المبطنه للمعدة الغدية في الفراخ البالغة مع إشارة خاصة لموت الخلايا المبرمج

أحمد عمر سالم

تمت دراسة الطلائيـة المبطنـة للمعدة الغدية بإستخدام الميكروسكوب الضوئي والميكروسكوب الإلكتروني الماسح والنافذ على عدد عشرة طيور بالغة من كلا الجنسين من سلالة الدندراوي . أوضحت الدراسة أن الغشاء المخاطي يتكـون من طيات مخاطية مبطنة بخلايا طلائية عمادية طويلة التي تظهرمكعبة على الجزء السفلي لهذه الطيات. وأظهرت هذه الخلايا تفاعلاً موجباً قوياً مع معامل شيف ومتوسطاً مع الألسين الأزرق وبذلك تعتبر خلايا منتجة للميوسين بسيادة النوع المتعادل منه. وأوضح الميكروسكوب الإلكتروني الماسح أن الغشـاء المخاطى يتكون من طيات مخاطية مرتبة دائرياً وكذلك طيات أصبعية الشكل. هذا وظهرت الأسطح اللمعية للخلايا المبطنه سداسية الشكل مزودة بزغيبات عديدة. وأظهرت هذه الخلايا بإستخدام الميكروسكوب الألكتروني النافذ خصائص الخلايا المفرزة النشطة من حيث إحتوائها على شبكة أندوبلازمية خشنة متطورة وجهاز جولجى واضح وعديد مـن الحبيبـات الإفرازيـة التـي تحتوي علـى قـالب ليفـى دقيق فـاتح إلكترونيا. وقد ظهرت الشبكة الأندوبلازميـة الخشنة فـي صـورة مميزة غير معتادة عبارة عن صهاريج أنبوبية طويلة متسعة ترى أيضاً بوضوح بإستخدام الميكروسكوب الإلكتروني الماسح. وقد لوحظت أيضا المتقدرات والخيـوط السيتوبلازمية ، والريبوزومـات والجسيمات الجالـة والفجوات البلعمية. وقد تميز موت الخلايا المبرمج بإنكماش هذه الخلايا مـع إحتوائها علـى نـواة صغيرة غامقه وزيـادة فـي كثافـة السيتوبلازم وكذلك بـروز قممهـا. وبإستخدام الميكروسـكوب الإكتروني الماسح ظهرت هذه الخلايا عـادة علـى طـرف الطيـات المخاطيـة مميزة بتقبب وعدم إنتظام أسطحها اللمعية مـع نقص فـي عدد الزغيبات. بعد ذلك تتفتت هذه الخلايا إلـى أجسام مستديرة ترى على الأسطح المخاطية . وعلى مستوى الميكروسكوب الإلكتروني النافذ فقد تميزت

هذه الخلايا بتكاثف الكروماتين النووي وحفظ سلامة الأغشية وكذلك تجزوء النواة بعد ذلك. وقد تم تمييز أربع أنواع من الخلايا الصماء طبقاً لشكل وحجم حبيباتها الإفرازية وكذلك خلايا لمفاوية في الجزء السفلي للطيات المخاطية.

SUMMARY

Light, transmission and scanning electron microscopical investigations were conducted on the lining epithelial cells of the glandular stomach of ten healthy adult fowl of both sexes (Dandarawi strain). The lining epithelial cells appeared tall columnar, that became cuboidal type in the basal region of the mucosal folds. These cells revealed strong and moderate reactivity with PAS and alcian blue respectively and consequently, they were considered as mucin producing cells with predominance of the neutral type. Scanning electron microscope revealed that the mucous membrane showed both concentrically and finger-like longitudinally arranged mucosal folds. The lining cells of these folds possessed hexagonal-shaped luminal surface with numerous microvilli. These cells revealed the characteristic features of highly active secretory cells. They contained well-developed infranuclearly located rough endoplasmic reticulum, conspicuous Golgi-apparatus and numerous secretory granules in the cell apex. The later possessed electron-lucent fine filamentous matrix. The rough endoplasmic reticulum was characterized by very long and dilated tubular shaped cisternae, that were easily demonstrable in scanning electron microscope. Mitochondria, cytoplasmic filaments, ribosomes, lysosomes and phagocytic vacuoles were also observed. The apoptotic cells appeared as shrinkaged cells, characterized by small darkly stained nuclei, increased cytoplasmic density and protruded cell apices. By scanning electron microscope, these cells were frequently observed at the tip of the mucosal folds. They were characterized by bulged irregular luminal surfaces and reduction in the number of microvilli. Later on, these apoptotic cells disintegrated into spherical apoptotic bodies seen upon the mucosal surfaces. At the transmission electron microscopical level, the apoptotic cells were characterized by condensation of the nuclear chromatin, preserved membranous integrity. Later on, fragmentation of the nucleus occur. Four types of endocrine cell were distinguishable on the basis of the size and shape of their secretory granules. Lymphocytes were also demonstrated in the basal region of the mucosal folds.

Key words: *Lining epithelium, apoptosis, PCD, endocrine cells, glandular stomach, electron microscope, fowl.*

INTRODUCTION

Considerable interest has been focusd on the structure of the gastric mucosa for both theortical and clinical reasons. The gastric mucous cells produce mucus gel (Talley et al., 1992) that forms a continous intra-luminal layer covering the surface epithelium (Suprasert and Fujioka, 1990) and plays an important role in gastric cytoprotection (Ho et al., 1995) by maintaining a favourable pH gradient and preventing autodigestion by acid, pepsin or ingested agents (Playford et al., 1995; Shekels et al., 1995). Maintenance of gastric mucosal structure depends also on a dynamic balance between cell loss, through a process called programmed cell death, apoptosis, and cell birth (Stachura et al., 1993). This apoptosis or programmed cell death (PCD) occurs through a series of morphological distinct alterations including condensation, fragmentation and phagocytosis. During this process the membranous integrity is maintained and the inflammatory signs are lacking (Bursch et al., 1992). It is mostly associated with embryogenesis, morphogenesis and with discrete cell death in every tissue of the body (Anilkumar et al., 1992). Concerning the gastric mucosa, apoptosis was observed in mouse (Pipan and Sterle 1986; Karam, 1993; Karam and Leblond, 1993b&c) and in rat stomach (Stachura et al., 1993).

The fine structure of the gastric mucous cells has been studied in mouse (Helander, 1962); rat (Corpron, 1966; Wattel et al., 1977); man (Rubin et al., 1968) and in african game ruminants (Weyrauch and Saber, 1985).

The stomach of fowl consists of glandular stomach (proventriculus) and muscular stomach (gizzard). The glandular stomach recieved extensive histological (Michel, 1971; Hodges, 1974; Banks, 1993) and histochemical studies (Suprasert and Fujioka, 1990; Salem, 1991). However, the ultrastructure of the lining epithelial cells of the glandular stomach of adult fowl was described only during the embryonic stages (Avila et al., 1986), the scanning electron microscopy is completly lacking in the avilable literature. Moreover, both transmission and scanning electron microscopy of the lining epithelium of the glandular stomach of adult fowl present a paucity of knowledge. Therefore, the present study has been performed to clarify the ultrastructural features of this epithelium as well as the existance of programmed cell death (PCD), apoptosis.

MATERIAL and METHODS

This study was carried out on 10 adult healthy fowls, 18 months, (Dandarawi strain) of both sexes, kept under normal (ad libitum) feeding conditions. The females were at the end of laying period. They were obtained from the experimental poultry farm, Faculty of Agriculture, Assuit University. The glandular stomach (proventriculus) was obtained immediately upon sacrifice, opened and washed rapidly with saline solution for removal of its content.

For light microscopical investigation pieces from the proventriculus were taken, fixed in Bouin's fluid, processed for paraffin embedding, sectioned (5µm thick) and stained with H&E, alcian blue, PAS and combination of alcian blue and PAS (Mowry, 1956).

For transmission electron microscopy, samples from the gastric mucosa were fixed in a mixture of 2.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3 for 4 hours at 4°C, washed in the same buffer and then post-fixed in 1% osmium teteroxide in 0.1 M phosphate buffer for further 2 hours at 4°C. The samples were then dehydrated in alcohol and embedded in Araldite-Epon mixture (Anderson and Andre, 1968). Semithin sections were cut and stained with toluidine blue. The ultrathin sections were obtained, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined under JOEL 100 CX II transmission electron microscope.

The size of the secretory granules in the endocrine cells was established by measuring the diameters of twenty profiles in four cells of each type in electron micrographs (13400 times magnification) and the corresponding standard deviation was calculated. The measurements were carried out on Leica Q 500 MC lmage analyser.

For scanning electron microscopical examination, the fixed tissue samples (0.5 X 0.5 cm^2) were dehydrated in ethanol, processed in amyl acetate and critically-point dried with Co_2. They were sputterd with gold and observed at JEOL 5400 LV scanning electron microscope.

RESULTS

Light microscopical observations:

The mucous membrane of the glandular stomach of adult fowl (Dandarawi strain) was thrown into irregular longitudinal mucosal folds. These folds were lined with tall columnar epithelial cells which became low

columnar or cuboidal at the basal region of the folds. These cells possessed oval vesicular basally located nuclei and numerous secretory granules occupying their supranuclear region. These granules were fewer in cells lining the basal region of the mucosal folds (Fig. 1). The infra-and/or paranuclear region contained RER-cisternae, which were appeared as pale stained long tubules (Fig. 2). Pyknotic cells most probably *apoptotic cells* were singly recognized among the neighbouring viable lining mucous epithelial cells. They were appeared as shrinkaged cells, characterized by small darkly stained nuclei, increased cytoplasmic density and protuberance of their apices (Fig. 3).

The histochemical observations: The secretory products filling the apical portions of the lining epithelial cells of the glandular stomach of fowl as well as the intra-luminal mucus coat were strongly stained with PAS (Fig. 4), however moderate reactivity with alcian blue was observed and gradually decreased toward the base of the mucosal folds (Fig. 5). With a combined AB-PAS method, the cell apices showed a regional distribution of the positively reacted secretory granules, where the PAS positive granules were demonstrated above the alcianophilic ones. The intra-luminal mucus coat also showed AB-PAS positive staining appearance (Fig.6).

Scanning electron microscopical observations:

The luminal surface of the glandular stomach of adult fowl exhibited concentrically arranged mucosal folds (Fig. 7,8) around the openings of the deep compound proventricular glands forming the macroscopically visible mucosal papillae (Fig. 7). Between these papillae, numerous finger-like mucosal folds were observed (Fig. 9). The lining epithelial cells were tall columnar-shaped with hexagonal-like boundary of their luminal surfaces (Fig. 10), that were covered with numerous microvilli (Fig. 11).

The apoptotic cells were frequently observed at the tip of the mucosal folds. They were recognized from the neighbouring healthy epithelial cells by bulged irregular luminal surface and reduction in the number of microvilli (Fig. 12). Later on, these cells exhibited a cauliflower appearance with ruffled cell surface (blebs) and constricted neck separating it from the neighbouring healthy epithelial cells and seems to be in its way out into the proventricular lumen (Fig. 13). Apoptotic bodies were observed protruding upon the mucosal surface. They appeared as smooth surfaced spherical bodies separated from the apoptotic cells of different sizes with few small microvilli on its membrane (Fig. 14).

Transmission electron microscopical observations:

The electron microscopical investigation revealed that the mucosal folds of the glandular stomach of adult fowl consisted of surface epithelial cells (including those undergoing apoptosis), endocrine cells and migratory lymphocytes.

The lining (surface) epithelial cells of the glandular stomach of adult fowl showed nearly similar ultrastructural appearance along the mucosal folds. The luminal surface of these cells was provided with variable number of microvilli. The adjacent cells were attached apically together with well developed zonula occludens, (1µm in length), followed by zonula adherens and desmosomes (Fig. 15 a,b). The wavy lateral cell membranes of the adjacent cells formed strong interdigitations and richely endowed with desmosomes (Fig. 16). The basal plasma membrane of these cells run irregularly following the thick basal lamina to which they were attached with hemidesmosomes.

The lining epithelial cells showed a polarity concerning the distribution of their cytoplasmic contents, where the apical region was filled with secretory granules, the basal region was occupied with RER-cisternae and the nucleus was somewhat basaly located.

The infra- and/or paranuclearly located well-developed rough endoplasmic reticlum was consisted of numerous cisternae. It possessed a characteristic long (12.29µm length) and dilated (approximatly 0.429µm diamter) tubular form (Fig. 17 & 18 a,b). These cisternae contained fine filamentous materials, which arranged parallel to their longitudinal axis (Fig. 18c) while in cross sections they appeared as fine electron-dense granules (Fig. 18d). The conspicuous supranuclearly located Gogi-apparatus was consisted of 3-4 complexes. Each complex was formed of 4-5 flattened closely packed saccules and associated vesicles (Fig. 19). A moderate number of mitochondria and cytoplasmic filaments were observed mostly in the cell apex in relation to the secretory granules (Fig. 20). Large membrane bounded phagocytic vacuoles containing heterogenous electron-dense materials (Fig. 21a,b) as well as remnant of a nucleus of an apoptotic body (Fig. 21c) were demonstrated. Numerous ribosomes and lysosomes were also observed. The secretory granules were the most distinctive feature of these cells, which sometimes occupied most of the supranuclear region. They appeared as membrane bounded rounded or oval-shaped granules consisting of electron-lucent fine fibrillar matrix. During the process of exocytosis, fusion of these granules at their limiting membranes as well as with the apical cell membrane was observed (Fig. 22).

The nucleus was basally located contained prominant nucleolus, marginal heterochromatin and chromatin islands scattered in the karyolymph.

The apoptotic cells were characterized firstly by aggregation of the nuclear chromatin forming uniformaly dense masse giving the nucleus more electron dense appearance (Fig. 23). These changes were followed by further condensation of the nuclear chromatin while the cytoplasmic organelles remained well preserved. At the stage of nuclear fragmentation (Fig. 24), it was observed that, the cell exhibited smooth slightly bulged luminal surface without microvilli and vacuolated apical region.

The endocrine cells (Fig. 25a-d) were scattered between the lining epithelial cells only in the deep portions of the mucosal folds. They were ovoid with broad base directly rest on the basal lamina to which they were attached with hemidesmosomes. These cells did not reach the luminal surface and were joined to the lateral portion of the neighbouring surface epithelial cells with desmosomes. These endocrine cells were characterized by large ovoid nucleus and an electron-lucent cytoplasm containing numerous basally located electron-dense homogenous granules variable in size and shape. The cytoplasm contained also moderate amount of rough endoplasmic reticulum cisternae, few mitochondria, Golgi-apparatus, lipid droplets and cytoplasmic filaments.

Four endocrine cell types were distinguishable according to the size and shape of their secretory granules into: a) cells with rounded small-sized granules of mean diameter of 132.08 nm ±7.48 (Fig. 25a); b) cells with rounded medium-sized granules had mean diameter of 200.75 nm ± 44.78 (Fig. 25b); c) cells with rounded large-sized granules had mean diameter of 302.23 nm ± 26.87 (Fig. 25c) and d) cells with polymorphic granules (rounded, ovoid, biconcave and curved in shaped) with mean orthogonal diameter of 138.75 nm ± 15.89 (Fig. 25d).

Migiratory lymphocytes were also demonstrated in the intercellular spaces between the lining epithelial cells of the mucosal folds. They contained the characteristic nucleus and electron-lucent cytoplasm with few organelles (Fig. 26).

DISCUSSION

The present investigation revealed that the mucosal folds of the glandular stomach of adult fowl (Dandarawi strain) were lined with tall columnar epithelial cells which became cuboidal type toward the basal region of these folds similar to that observed by Aitken (1958), Michel (1971), Hodges (1974), Suprasert and Fujioka (1990) and Banks (1993).

The histochemical observations revealed that the secretory products of the lining epithelial cells of the glandular stomach stained intensely with PAS and moderatly with alcian blue methods. These results indicate that, these mucin producing cells secret both acidic and neutral mucopolysaccharides with predominance of the later type. These histochemical findings are consistant with that reported by Hodges (1974), Altamirano et al. (1984) and Salem (1985) in fowl as well as Salem (1982) in duck. However, Prasad and Kakade (1990) demonstrated strong acidic mucosubstance in the lining epithelium of duck proventriculus.

The neutral and acidic mucopolysaccharides were demonstrated in the gastric epithelium of rat (Scheahan and Jervis, 1976; Wattel et al. 1977; Van Huis and Kramer, 1979) as well as of mouse, dog and baboon (Scheahan and Jervis, 1976). On the other hand, only neutral mucopolysaccharide was observed in the gastric epithelium of guinae pig, rabbit and cat (Scheahan and Jervis, 1976).

The predominance of the neutral in relation to acidic mucosubstance within the lining epithelium of the glandular stomach of fowl was explained on the basis of an age-dependent deletion of the sulfate and neuraminic sialytransferase that introduce terminal acidic residues into the secretory mucosubstance (Spicer et al., 1978).

The observed difference in the distribution of the mucosubstance within the lining epithelial cells of the glandular stomach, after stains with AB-PAS method applied, might be due to different functional and maturation stages of this epithelium (Suprasert and Fujioka, 1990).

The present ultrastructural study of lining epithelial cells of the glandular stomach revealed a distinct polarity in the distribution of their contents, where the well-developed RER was infranuclearly located, the Golgi-Apparatus was supranuclearly located and the secretory granules were apically placed.

In the present investigation the RER showed unusual pale staining appearance because, they were formed of long and dilated tubular shaped cisternae, as revealed by scanning and transmission electron microscope. These cisternae might reflect the efficiency of RER in the secretory process and subsequentely reflect the condition of massive secretion.

The secretory granules consisted of electron-lucent fine fibrillar matrix, which was considered as mucin molecules as reported by Shackleford and Wilborn (1970b). The ultrastructural appearance of these secretory granules as well as the histochemical findings consequently support the notion that the surface epithelial cells might be considered as mucin

producing cells (Hodges, 1974; Vial and Garrido, 1979; Suprasert and Fujioka, 1990 and Banks, 1993).

The mucus secretion into the proventricular lumen occured through the common way, exocytosis, as mentioned by Suprasert and Fujioka (1990). In addition, Zalewsky and Moody (1979) observed apical expulsion and cell exfoliation as another mechanisms of mucus release in the canine gastric mucosa.

It is generally accepted that this mucus plays a significant role not only in the defense of the mucosa against injury and ulceration (Suprasert and Fujioka, 1990), but also in preventing further damage after the initial insult, by forming a cap or gelatinous layer together with the exfoliated surface epithelial cells. This acts as barrier to substance in the lumen and traps an alkaline fluid next to the healing surface (Lacy, 1985). The protective mechanism of gastric mucus is also explained in the way that, it delays proton permeation into the gastric surface cells, enabling secreted bicarbonate to neutralize the luminal acid (Engel et al., 1995) or to trap the bicarbonate ions secreted by gastric epithelium into it. This established a gradient from pH 1-2 at the lumen to pH 6-7 at the cell surface (Bhaskar et al., 1992).

Both scanning and transmission electron microscopical examination confirmed that the pyknotic cells observed by the light microscope showed the typical morphological criteria of apoptotic cells. They appeared as scattered single cells charactarized by reduced cell volume, condensed nucleus, darkely stained cytoplasm and protruded cell apices. Similar results were recorded by Walker et al. (1988) and Anilkumar et al. (1992). The reduction of the cell volume is due to loss of water and ions that leads to increased cell density (Campana and Cleveland, 1996). The mechanism responsible for extrusion of water is not known (Anilkumar et al., 1992).

In accordance with Pipan and Sterle (1986), the first sign of identification of apoptosis in the gastric epithelium at scanning electron microscopical level was the reduction in the number of microvilli upon the slightly bulged luminal surface. The cell finally disintegrated into spherical bodies (apoptotic bodies) of different sizes seen at the mucosal surface.

The present ultrastructural investigation revealed that the apoptotic cells were characterized by condensation of the nuclear chromatin and absence of luminal microvilli, while the integrity of the cell membranes and cytoplasmic organoids was largly retained. Later on, fragmentation of the nucleus and vacuolation of the cell apical region were observed. Similar findings were recorded in the mucoid gastric epithelium of mouse (Pipan and

Sterle, 1986) and rat (Stachura et al., 1993) as well as in pit and zymogenic cells of mouse stomach (Karam and Leblond, 1993 b,c). The preserved integrity of the internal and external membranes of the apoptotic cells is due to the increase in tissue transglutaminase activity during apoptosis. This enzyme is believed to result in cross-linkage of proteins to prevent cell distintegration and subsequent release of harmful intracellular molecules (endogenous endonucleases) before the apoptotic cell can be phagocytized (Piacentini et al., 1991).

These apoptotic cells can be either extruded into the lumen concerned (Karam and Leblond, 1993 c) as revealed by scanning electron microscope in the present investigation or undergo phagocytosis (Wyllie, 1987; Anilkumar et al., 1992; Bursch et al., 1992). In the present study, the presence of large phagocytic vacuoles containing heterogenous electron-dense materials as well as remnant of a nucleus may indicate phagocytic function of these lining epithelial cells and similar to those reported by Morris and Harding (1979) and Pipan and Sterle (1986) in rat and mouse gastric epithelium respectively.

Apoptosis is considered as a benevolent physiological reaction in the body instituted for removal of unwanted cells and its defective execution can cause developmental abnormalities or development of tumors (Anilkumar et al., 1992).

The present ultrastructural investigation revealed the existance of four variable types of endocrine cells distributed within the surface epithelial lining the basal region of the mucosal folds. In agree with Martinez et al. (1991) they were considered as "closed" type as they had no contact with the luminal surface.

Based on previously reported ultrastructural and immuno-cytochemical investigations on the glandular stomach of fowl (Alumets et al., 1977; Usellini et al., 1983; Martinez et al., 1991), it can be speculated that the observed endocrine cells containing rounded small-, medium- and large sized granules as well as that containing the polymorphic granules are identical to bombesin, neurotensin, somatostatin and serotonin cells respectively. However, further investigations using immunocytochemical techniques are required to identify each type of those endocrine cells according to their hormonal content.

In the present investigation, interepithelial lymphocytes were demonstrated specially in the basal region of the mucosal folds, that may provide the glandular stomach an immunologic defense.

In conclusion, the lining of the mucosal folds of the glandular stomach of adult fowl consisted of mucin producing surface epithelial cells (including those undergoing apoptosis), four distinct endocrine cell types and migratory lymphocytes.

ACKNOWLEDGEMENT

I would like to thank Prof. Dr. **H.M. Badawi** and Prof. Dr. **A. Hassan,** Department of Anatomy and Histology, Faculty of Veterinary Medicine, Assiut University, for valuable comment concerning apoptosis.

REFERENCES

Aitken, R.N.C. (1958): A histochemical study of the stomach and intestine of the chicken. J. Anat., 92: 453-468.

Altamirano, F.; Avila, R.; Maria, E.; Samar, E. and De Fabro, P. (1984): Cytochemical characterization of mucosubstances in the chick glandular stomach during embryonary and postnatal development. Folia Histochem. Cytochem., 22: 105-111.

Alumets, J.; Sundler, F.; Hakanson, R. (1977): Distribution, ontology and ultrastructure of somatostatin immunoreactive cells in the pancreas and gut. Cell Tissue Res., 185: 465-479.

Anderson, W.A. and Andre, J. (1968): The extraction of some cell components with pronase and pepsin from thin sections of tissue embedded in an Epon-Araldite mixture. J. de Microscopie, 7: 343.

Anilkumar, T.V.; Sarraf, C.E. and Alison, M.R. (1992): Scientific reviews: The Biology and pathology of programmed cell death (Apoptosis). Vet. Hum. Toxicol., 34: 251-254.

Avila, R.E.; Samar, M.E. and De Fabro, P. (1986): Ultrastructural differentiation of the glandular stomach (proventriculus) in chick embryo. Folia Histochem. Cytobiol., 24: 227-231.

Banks, W.J. (1993): Applied veterinary histology, 3rd Ed. Mosby Year Book. St. Louis. Baltimore. Boston. Chicago. London. Philadelphia. Sydney. Toronto.

Bhaskar, K.R.; Garik, P.; Turner, B.S.; Bradley, J.D.; Bansil, R.; Stanley, H.E. and LaMont, J.T. (1992): Viscous fingering of Hcl through gastric mucin. Nature, 360(6403): 458-461.

Bursch, W.; Oberhammer, F. and Schulte-Hermann, R.(1992): Cell death by apoptosis and it's protective role against disease. Tips, 13: 245-250.

Campana, D. and Cleveland, J.L. (1996): Regulation of apoptosis in normal hemopoiesis and hematological disease: In Brenner, M.K. and Hoffbrand, A.V. Recent advances in haematology. Vol. 8: 1-19. Churchill Livingstone, Edinburgh.

Corpron, R.E. (1966): The ultrastructure of the gastric mucosa in normal and hypophysectomized rats. Am. J. Anat., 118: 53-90.

Engel, E.; Guth, P.H.; Nishizaki, Y. and Kaunitz, J.D. (1995): Barrier function of the gastric mucus gel. Am. J. Physiol., 269: G994-999.

Helander, H.F. (1962): Ultrastructure of fundus glands of the mouse gastric mucosa. J. Ultrastruct. Res. Suppl., 4: 1-15.

Ho, S.B.; Roberton, A.M.; Shekels, L.L.; Lyftogt, C.T.; Niehans, G.A. and Toribara, N.W. (1995): Expression cloning of gastric mucin complementary DNA and localization of mucin gene expression. Gastroentero., 109: 735-747.

Hodges, R.D. (1974): The histology of the fowl. Academic Press, London. New York. San Francisco.

Karam, S.M.(1993): Dynamics of epithelial cells in the corpus of the mouse stomach. IV- Bidirectional migration of parietal cells ending in their gradual degeneration and loss. Anat. Rec., 236: 314-332.

Karam, S.M. and Leblond, C.P. (1993b): Dynamics of epithelial cells in the corpus of the mouse stomach. II- Outward migration of pit cells. Anat. Rec., 236: 280-296.

Karam, S.M. and Leblond, C.P. (1993c): Dynamics of epithelial cells in the corpus of the mouse stomach. III- Inward migration of neck cells followed by progressive transformation into zymogenic cells. Anat. Rec., 236: 297-313.

Lacy, E.R. (1985): Prostaglandins and histological changes in the gastric mucosa. Dig. Dis. Sci., 30: 83S-94S.

Martinez, A.; Lopez, J.; Barrenechea, N.A. and Sesma, P. (1991): Immunocytochemical and ultrastructural characterization of endocrine cells in chicken proventriculus. Cell Tissue Res., 263: 541-548.

Michel, V.G. (1971): Zur Histologie und Histochemie der Schleimhaut des Druesen-und Muskelmagens von Huhn und Ente. Monatsh. Vet. Med. Jena, 26: 207-911.

Morris, G.P. and Harding, R.K. (1979): Phagocytosis of cells in the gastric surface epithelium of the rat. Cell Tissue Res., 196: 449-454.

Mowry, R.W. (1956): Observations on the use of sulphuric ether for the sulphation of hydroxyl groups in tissue sections. J. Histoch. Cytoch., 4: 407.

Piacentini, M.; Autuori, F. and Dini, L. (1991): "Tissue" transglutaminase is specifically expressed in neonatal rat liver cells undergoing apoptosis upon epidermal growth factor-stimulation. Cell Tissue Res., 263: 227-235.

Pipan, N. and Sterle, M. (1986): Cytochemical and scanning electronmicroscopic analysis of apoptotic cells and their phagocytosis in mucoid epithelium of the mouse stomach. Cell Tissue Res., 246: 647-652.

Playford, R.J.; Marchbank, T.; Chinery, R.; Evison, R.; Pignatelli, M.; Boulton, R.A.; Thim, L. and Hanby, A.M. (1995): Human spasmolytic polypeptide is a cytoprotective agent that stimulates cell migration. Gastroentero., 108: 108-116.

Prasad, R.V. and Kakade, K. (1990): Histology and histochemistry of proventriculus of domestic duck (Anas platyrhynchos linnaeus). Mysore Journal of Agricultural Sciences, 25: 506-511.

Reynolds, E.G. (1963): The use of lead citrate at high pH as elecron-opaque stain in electron microscopy. J. Cell. Biol., 17: 208-212.

Rubin, W.; Ross, L.L.; Sleisenger, M.H. and Jeffries, G.H. (1968): The normal human gastric epithelia. A fine structural study. Lab. Invest., 19: 598-626.

Salem, A.O. (1991): Licht-und electronenmikroskopischer Nachweis der Ca^{2+}- ATPase in Oberflaechen- und Druesenepithel des Vorderdarms beim Huhn. Diss. Med. Vet. Giessen.

Salem, H.F. (1982): Micromorphological studies on the oesophagus and stomach of growing sudanese ducks. M. Sc. Thesis. Fac. Vet. Med., Zagazig University.

Salem, H.F. (1985): Histological and histochemical studies on stomach and intestine of Fayoumi fowl with special reference to age and ration variations. Ph. D. Thesis. Fac. Vet. Med., Zagazig University.

Scheahan, D.G. and Jervis, H.R. (1976): Comparative Histochemistry of gastrointestinal mucosubstances. Am. J. Anat., 146: 103-132.

Shackleford, J.M. and Wilborn, W.H. (1970b): Ultrastructural aspects of calf submandibular glands. Am. J. Anat., 127: 259-280.

Shekels, L.L.; Lyftogt, C.; Kieliszewski, M.; Filie, J.D.; Kozak, C.A. and Ho, S.B. (1995): Mouse gastric mucin: Cloning and chromosomal localization. Biochem. J., 311: 775-785.

Spicer, S.S.; Katsuyama, T. and Sannes, P.L. (1978): Ultrastructural carbohydrate cytochemistry of gastric epithelium. Histochem. J., 10: 309-331.

Stachura, J.; Tarnawski, A. and Dabros, W. (1993): Apoptosis: Genetically programmed physiologic cell loss in normal gastric mucosa and in mucosa of grossly healed gastric ulcer. J. Clin. Gastroentero. Suppl., 1: S70-S77.

Suprasert, A. and Fujioka, T. (1990): Use of lectins for detection of glycoconjugate changes in mucous epithelium of the chicken proventriculus. Anat. Anz. Jena, 170: 91-98.

Talley, N.J.; Ormand, J.E.; Frie, C.A. and Zinsmeister, A.R. (1992): Stability of pH gradients *in vivo* across the stomach in Helicobacter pylori gastritis, dyspepsia and health. Am. J. Gastroentero., 87: 590-594.

Usellini, L.; Tenti, P.; Fiocca, R.; Capella, C.; Buffa, R.; Terenghi, C.; Polak, J.M. and Solcia, E. (1983): The endocrine cells of the chicken proventriculus. Bas. Appl. Histochem., 27: 87-102.

Van Huis, G.A. and Kramer, M.F. (1979): Glycoprotein synthesis in the mucous cells of the vascularly perfused rat stomach. I. surface mucous cells. Am. J. Anat., 156: 301-312.

*Vial, J.D. and Garrido, J. (1979):*Comparative cytology of hydrochloric acid secreting cells. Arch. Biol. Med. Exper., 12: 39-48.

Walker, N.T.; Harmon, B.V.; Gobe, G.C. and Kerr, J.F.R. (1988): Patterns of cell death. Meth. Archiev. Exp. Pathol., 13: 18-54.

Wattel, W.; Geuze, J.J. and de Rooij, I.G. (1977): Ultrastructural and carbohydrate Histochemical studies on the differentiation and renewal of mucous cells in the rat gastric fundus. Cell Tissue Res., 176: 445-462.

Weyrauch, K.D. and Saber, A.S. (1985): Die Feinstruktur des Epithels der Fundusdruesenzone einiger ostafrikanischer Wildwiederkauer. Anat. Anz. Jena, 158: 437-451.

Wyllie, A.H. (1987): Apoptosis: Cell death under Homeostatic control. Arch. Toxicol. Suppl., 11: 3-10.

Zalewsky, C.A. and Moody, F.G. (1979): Mechanisms of mucus release in exposed canine gastric mucoa. Gastroentero., 77: 719-729.

LEGENDS

Fig. 1: Photomicrograph of toluidine blue stained semithin section showing the mucosal folds of the glandular stomach of fowl. Secretory granules (arrowheads), base of the folds (asterisks), lumen (L) contains secretory products, lamina propria (Lp). X 160.

Fig. 2: High magnification of toluidine blue stained semithin section of a part of mucosal folds showing the pale stained tubular shaped RER-cisternae (arrows). Secretory granules within the cell apex (arrowheads), secretory materials between the folds (asterisk), lamina propria (Lp). X 1000.

Fig. 3: Toluidine blue stained semithin section showing apoptotic cell (arrow) with small darkly stained nucleus and protruded cell apex (arrowhead). Nucleus of healthy epithelial cells (double arrow). X1000.

Fig. 4,5: Photomicrographs of paraffin sections showing strong PAS positive reaction (4) and moderate alcainophilia (5) that decreases toward the base of the mucosal folds (asterisks). Lumen (L) with positively reacted secretory products, lamina propria (Lp). X 250.

Fig. 6: Photomicrograph of combined AB-PAS technique showing PAS +ve granules (arrows) and alcainophilic ones (arrowheads) within the cell apex. Lumen (L) with positively stained secretory products, lamina propria (Lp). X 400.

Fig. 7: Low magnification scanning electron micrograph of the mucosal surface of the glandular stomach showing a quarter of mucosal papilla and their concentrically arranged mucosal folds. Opening of the deep compound proventricular gland (asterisk), parts of mucus between the folds (arrows).

Fig. 8,9: Scanning electron micrographs of the concentrically arrangend (8) and finger-like mucosal folds (9) showing hexagonal shaped appearance of their surfaces.

Fig. 10: Scanning electron micrograph of cracked mucosa showing the tall columnar-shaped surface mucous cells with hexagonal shaped boundaries of their luminal surfaces (asterisks).

Fig. 11: Scanning electron micrograph showing numerous microvilli covering the luminal surfaces of the columnar mucous cells.

Fig. 12: Scanning electron micrograph showing apoptotic cells (asterisks) with bulged luminal surfaces and few small microvilli (arrowheads). Viable epithelial cells (arrows).

Fig. 13: Scanning electron micrograph showing cauliflower appearance of the apoptotic cell with ruffled surface (asterisk) and constricted neck (arrowhead). Viable epithelial cells (arrows) with numerous microvilli.

Fig. 14: Scanning electron micrograph showing spherical apoptotic bodies (arrows) of different sizes with few small microvilli (arrowheads).

Fig. 15a: Electron micrograph of the apical portions of two surface mucous cells showing well-developed zonula occludens (Zo), zonula adherens (Za) and desmosome (D). Microvilli (arrow), lumen (L), secretory granules (Sg), intercellular space (arrowheads). X 44000.

Fig. 15b: Higher magnification of a well-developed zonula occludens (Zo). Microvilli (arrow), secretory granules (Sg). X 80000.

Fig. 16: Electron micrograph showing strong cytoplasmic interdigitations along the lateral cell membranes (arrowheads) and desmosomes (D). Secretory granules (Sg). X 80000.

Fig. 17: Scanning electron micrograph of cracked mucosa showing the tubular -shaped RER-cisternae in the basal region of the lining cells (arrows) and secretory granules in the cell apex (arrowheads). Luminal surface (Ls).

Fig. 18a: Electron micrograph of the well-developed infranuclearly located RER- cisternae (arrows). Nucleus (N), secretory granules (Sg), basal lamina (Bl). X 13400.

Fig. 18b: low magnification electron micrograph showing RER-cisternae in L.S. (arrows). Nucleus (N), basal lamina (BL). X 5400.

Fig. 18c: High magnification of a part of RER-cisterna revealed filamentous materials arranged parallel to it's longitudinal axis (arrowhead). X60857.

Fig. 18d: Electron micrograph of C.S. of RER-cisternae showing fine electron-dense granular materials. X 28000.

Fig. 19: Electron micrograph of the supranuclearly located Golgi-apparatus showing 4-complexes (arrows). Vesicles (arrowheads). X 40000.

Fig. 20: Electron micrograph of the cell apex showing bundle of cytoplasmic filaments (arrow). Secretory granules (Sg), nucleus (N), lumen (L). X 40000.

Fig. 21a: Low magnification electron micrograph showing the phagocytic vacuoles (asterisks) within the lining surface epithelial cells. Secretory granules (Sg), nucleus (N), RER-cisternae (arrow), lumen (L) X 8000.

Fig. 21b: High magnification electron micrograph of a phagocytic vacuole (asterisk) with heterogenous content. Nucleus (N), secretory granules (Sg), RER-cisterna (arrow). X 28000.

Fig. 21c: High magnification electron micrograph of a phagocytic vacuole (asterisk) containing remnant of a nucleus of an apoptotic body (arrowhead). RER-cisternae (arrows). X 28000.

Fig. 22: Electron micrograph of the secretory granules (Sg) in the cell apex showing their filamentous structure. These granules fuse with each other (arrowheads) or with the apical plasma membrane during exocytosis (arrow). Nucleus (N), lumen (L) with secreted filamentous materials. X 54000.

Fig. 23: Electron micrograph of an apoptotic cell showing its nucleus (N_1) with condensed heterochromatin. Nucleus (N_2) of the neighbouring viable epithelial cell. X 13400.

Fig. 24: Electron micrograph of an apoptotic cell (arrow) located between viable epithelial cells (double arrows). Vacuolated apical region of the apoptotic cell (asterisk), fragmented nucleus of apoptotic cell (N_1), nucleus of healthy epithelial cells (N_2). Lumen (L). X 15000.

Fig. 25a-d: Electron micrographs of interepithelial endocrine cells showing nucleus (N), lipid droplet (L), RER-cisternae (arrowheads), mitochondria (M). Desmosome (D), hemidesmosome (Hd), basal lamina (Bl), lining epithelial cells (asterisk).

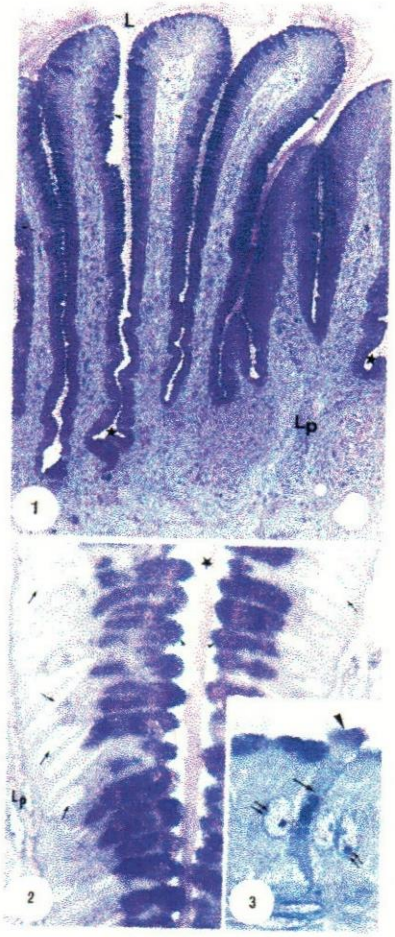
a- Cell with rounded small-sized granules. X 13400.

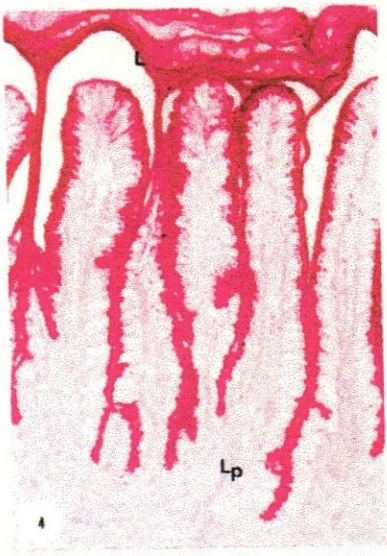
b- Cell with rounded medium-sized granules. X 13400.

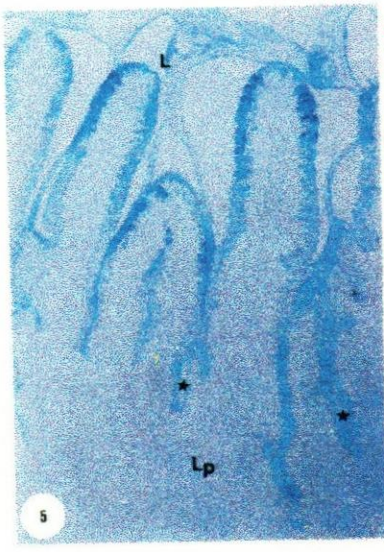
c- Cell with rounded large-sized granules. X 13400.

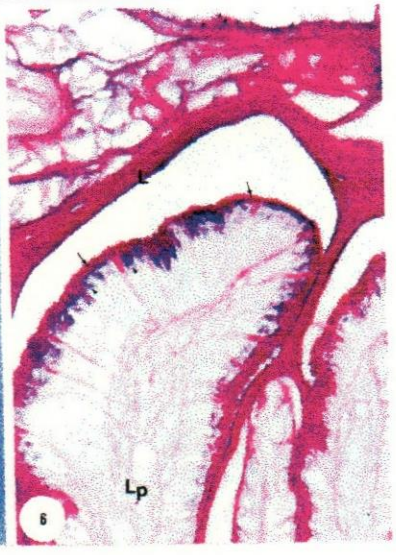
d- Cell with polymorphic granules. X 28000.

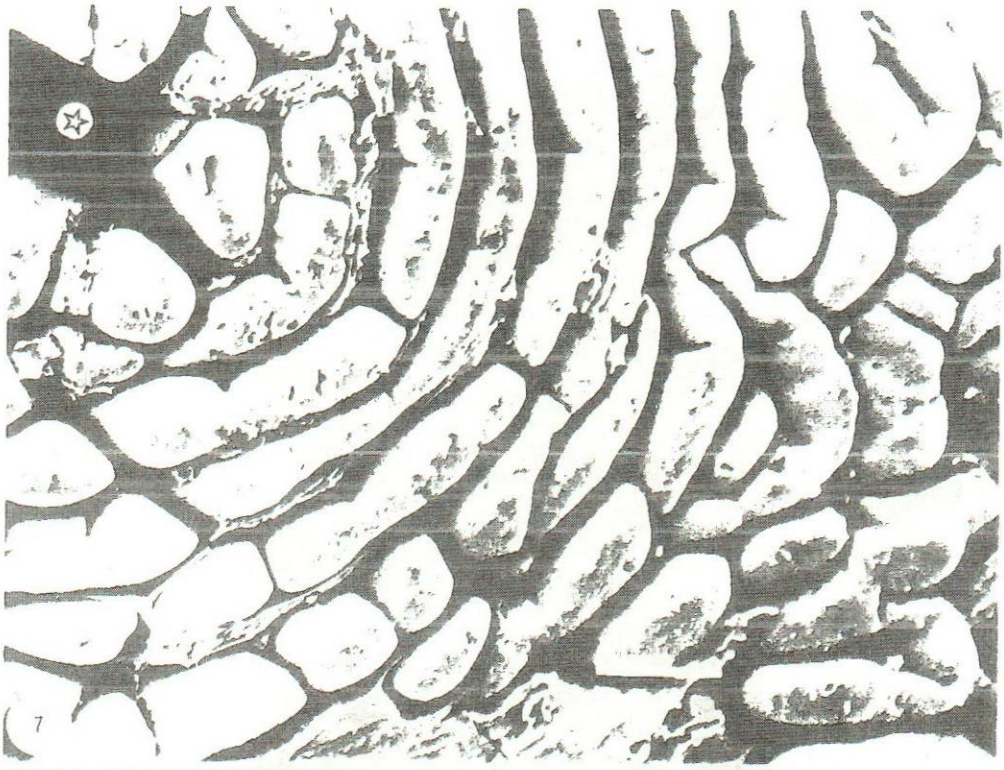
Fig. 26: Electron micrograph of lymphocyte (Ly) in the intercellular space between the epithelial cells (asterisk). Basal lamina (Bl). X 10000.

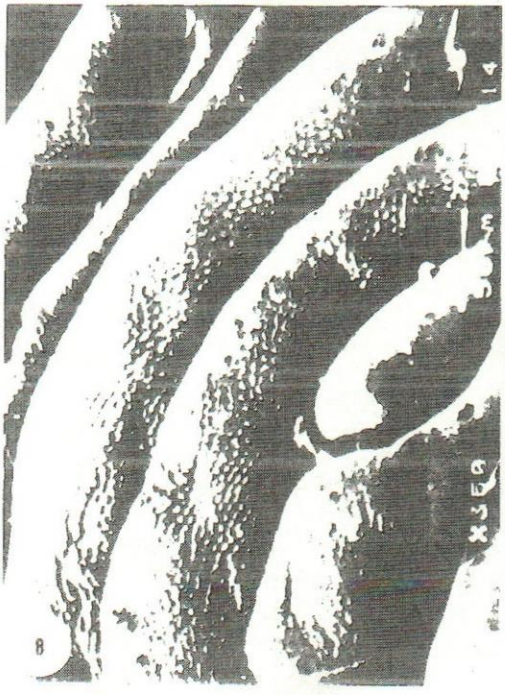

L
Lp
1
Lp
2
3

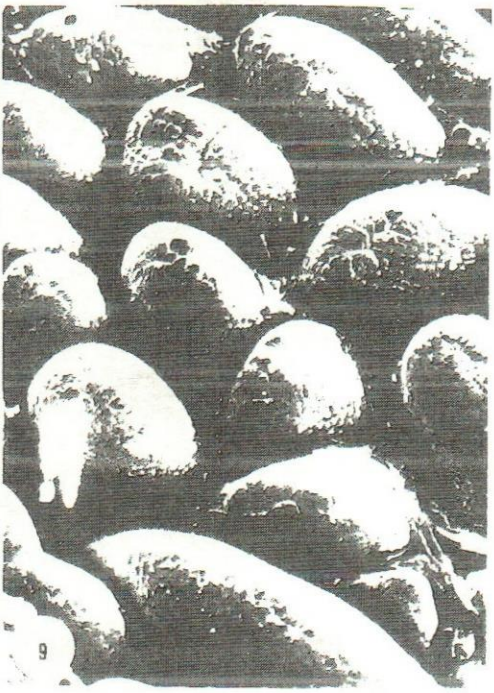

L
Lp
4

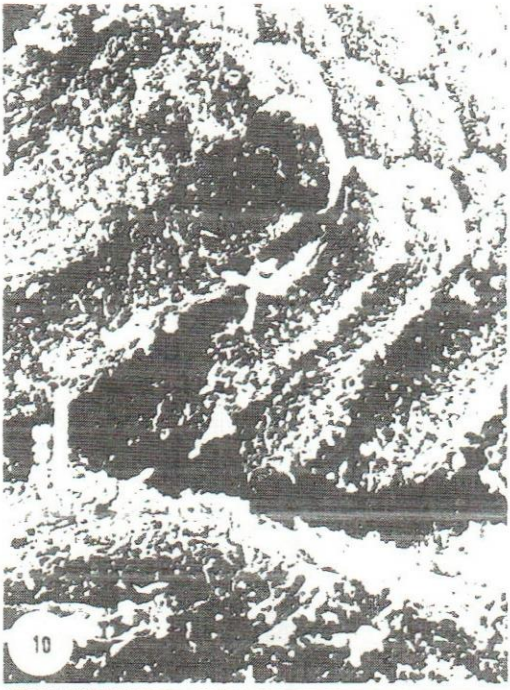

L
Lp
5

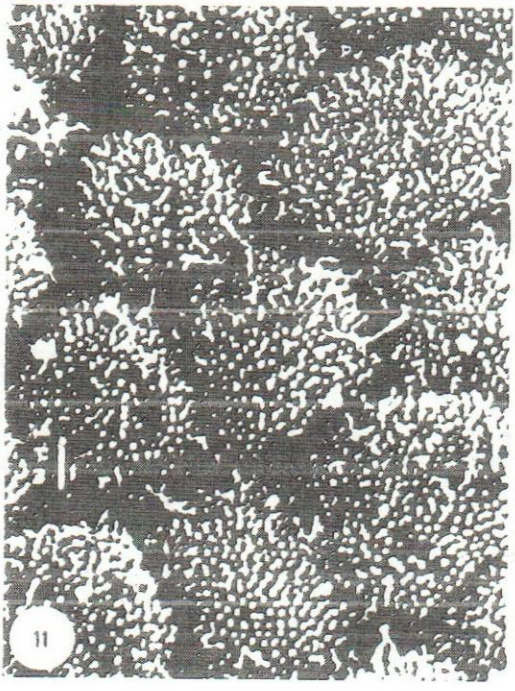

L
Lp
6

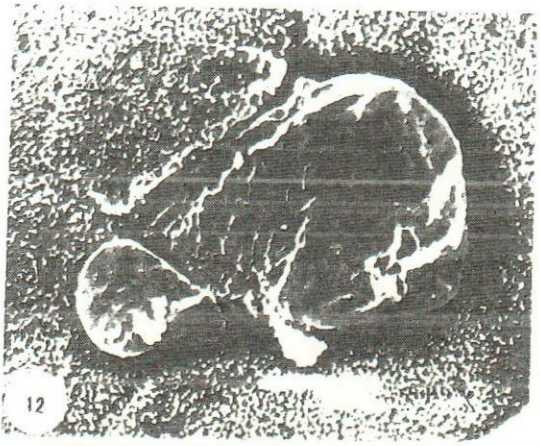


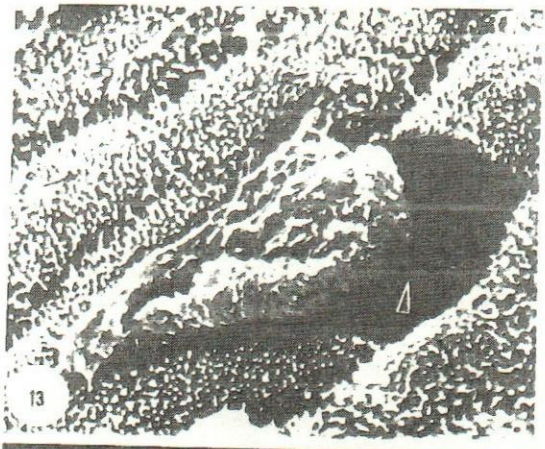


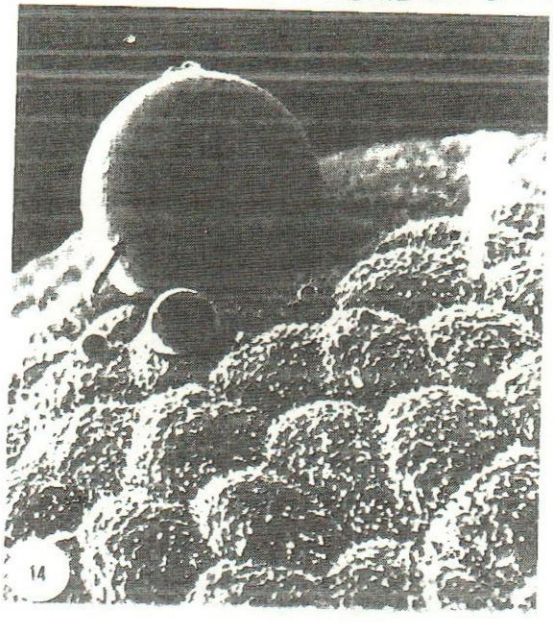



10

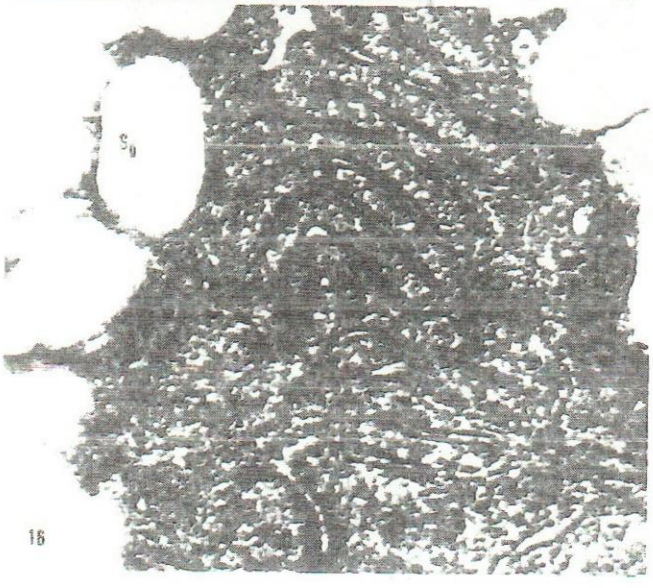

11

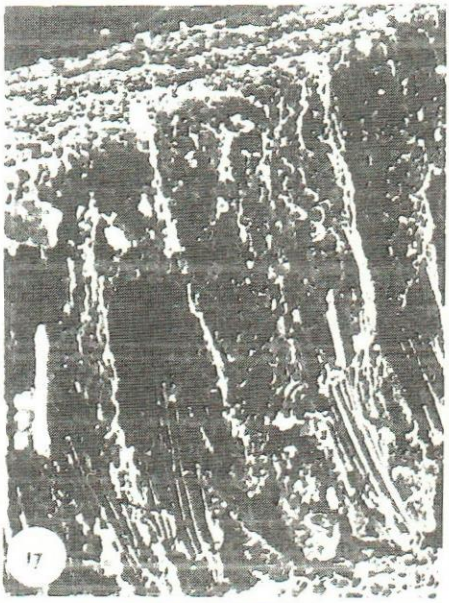

12


13

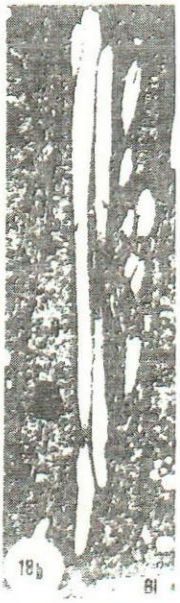

14


L
Sg
Sg
Sg
15a
15b

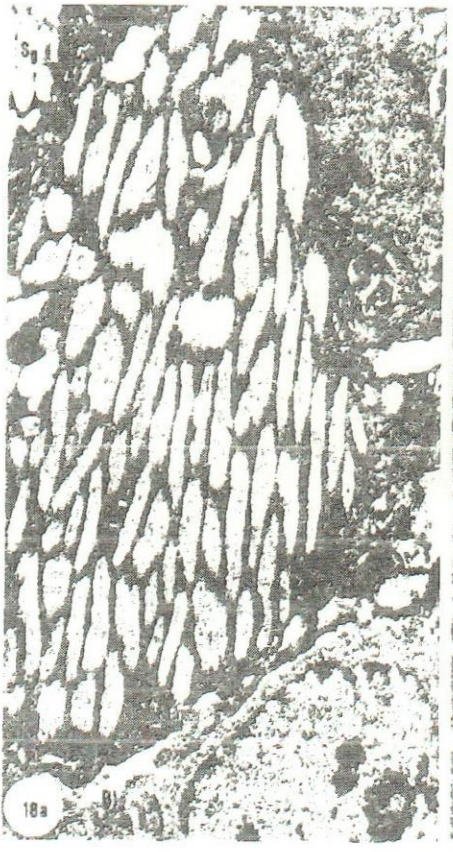

Sg
16



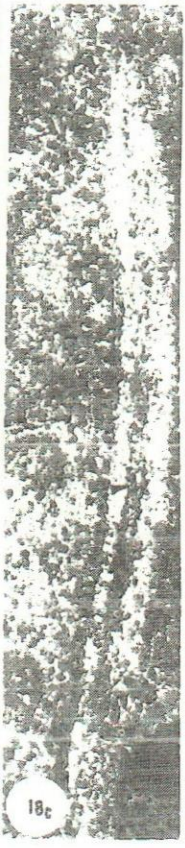
17



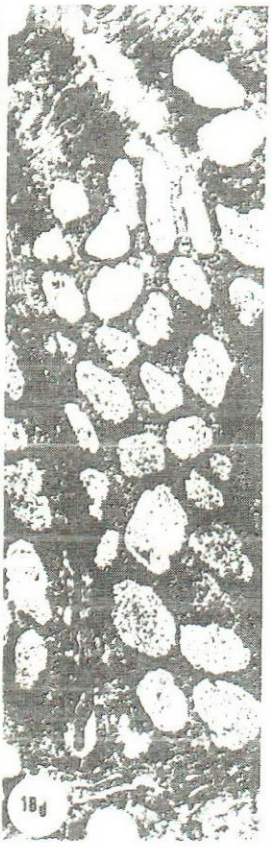
18b



18a



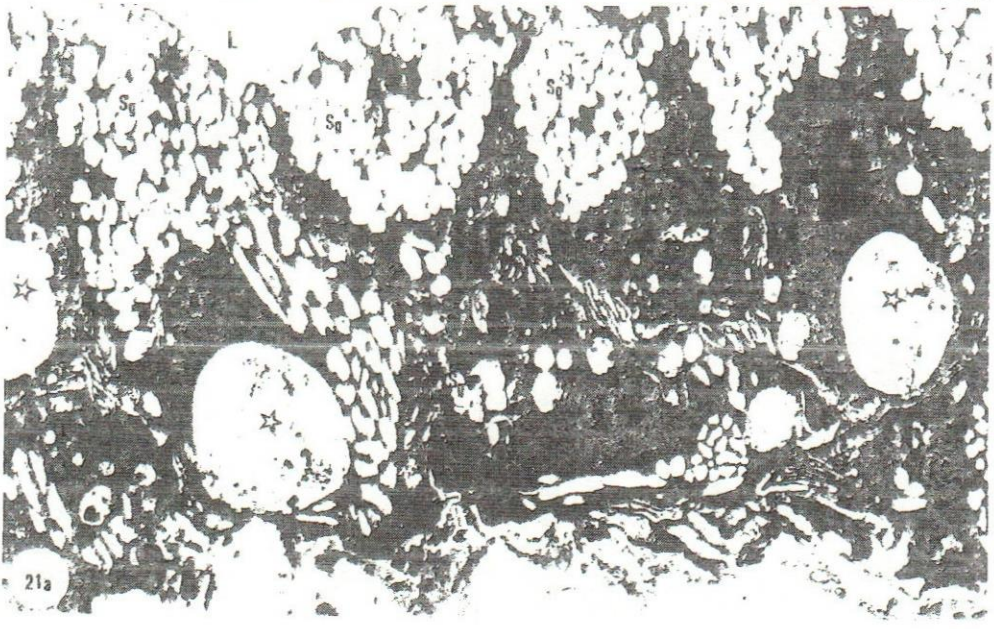
18c



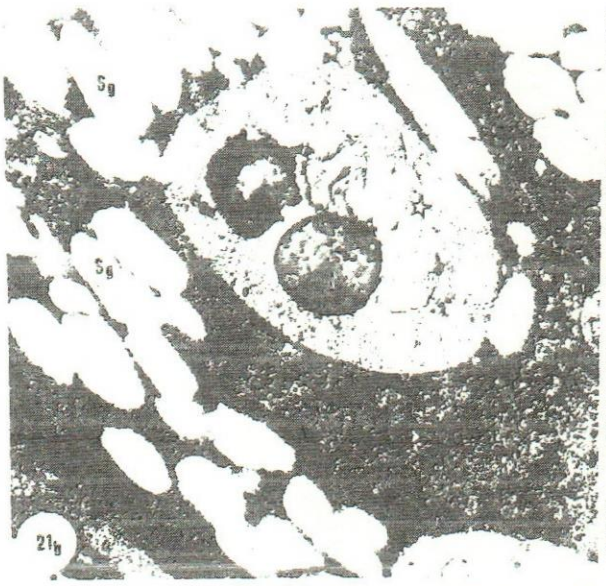
18d

19

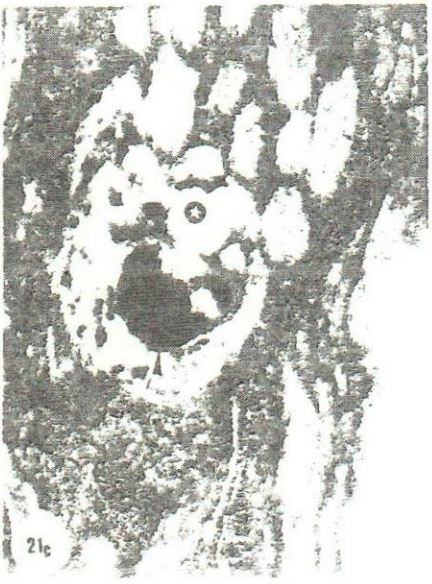
20



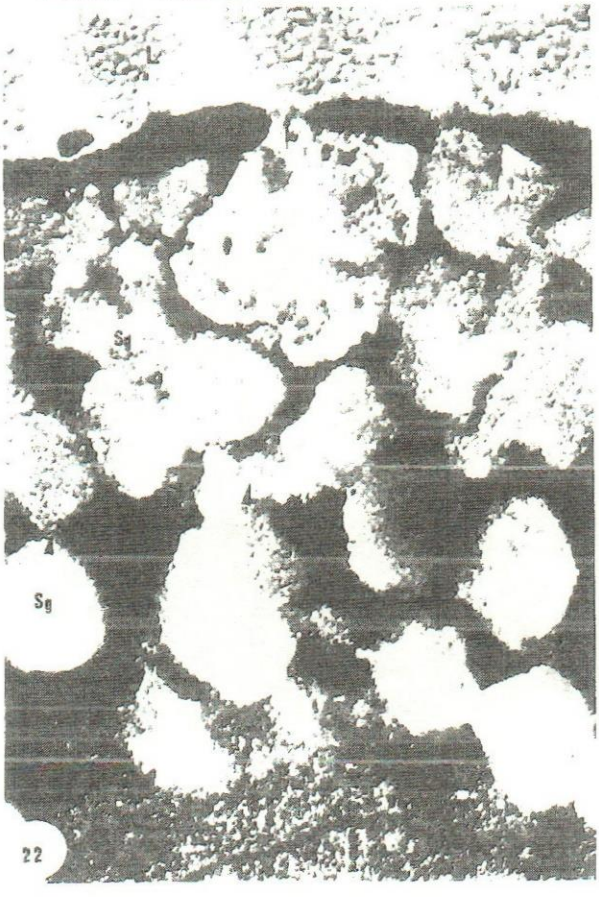
21a



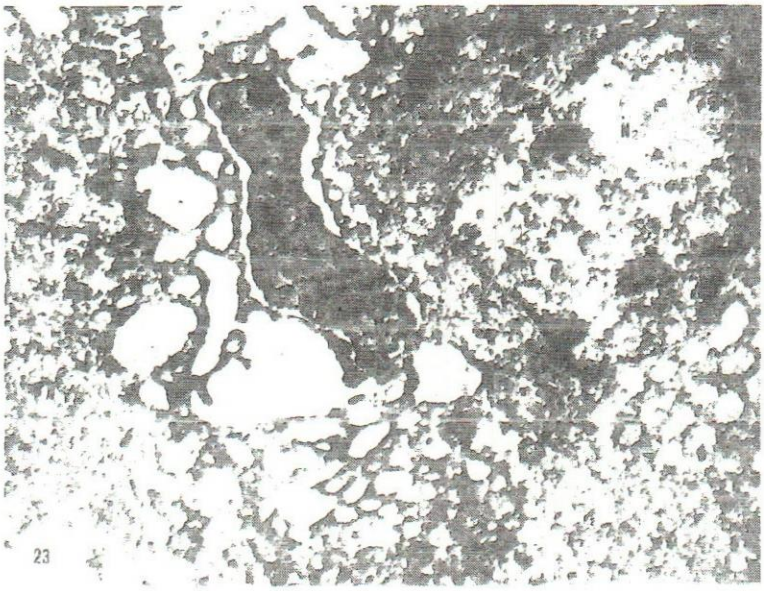
21b



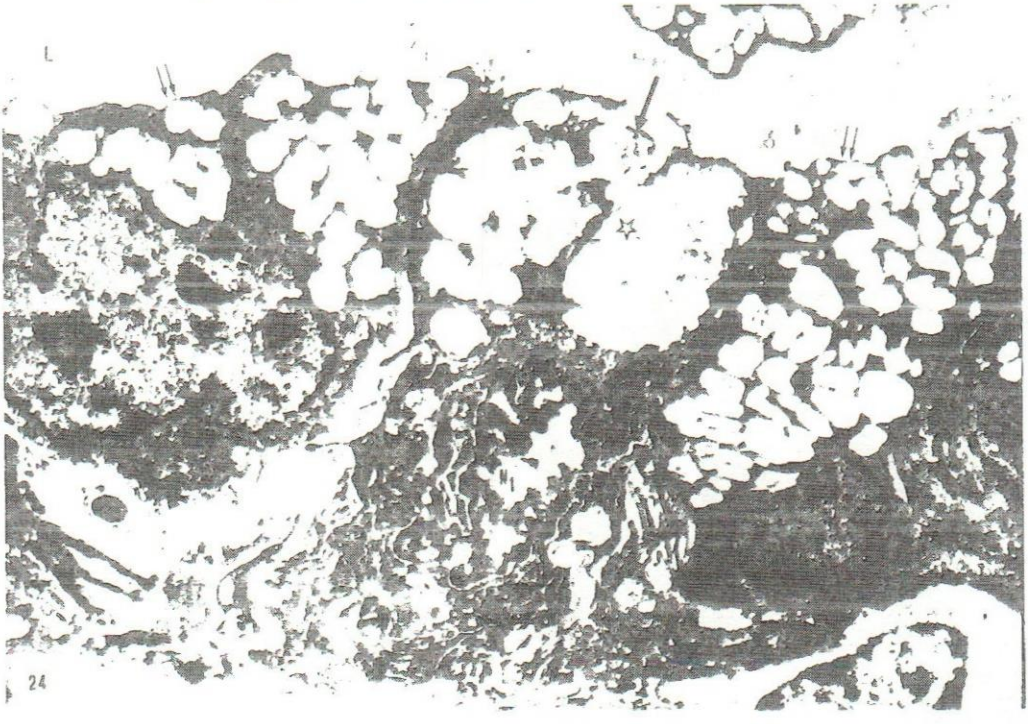
21c



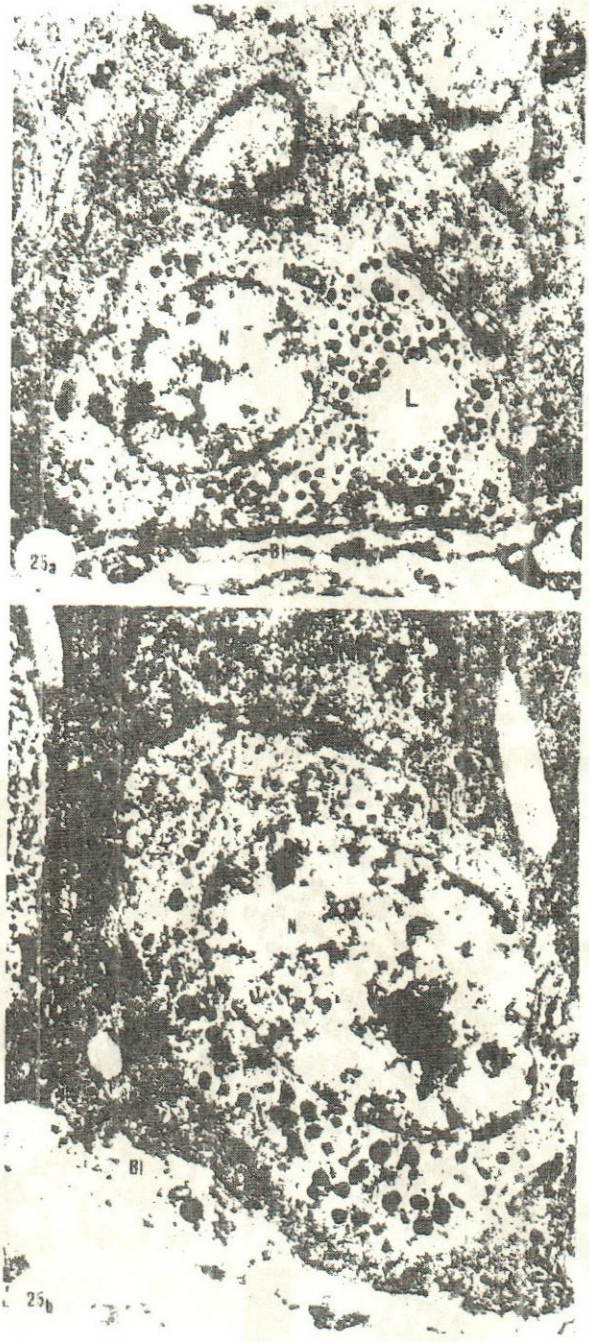
22

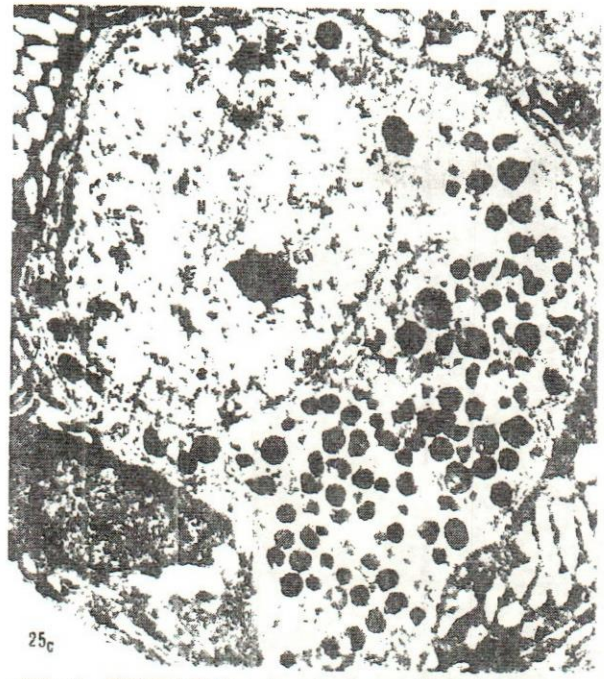


23



24


N
L
BI
25a
N
BI
25b



25c

25d

26