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THE FINE STRUCTURE OF THE PAROTID SALIVARY GLAND OF THE DONKEY

(With 15 Fig.)

By

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التركيب الدقيق للغده اللعابيه النكفيه في الحوـــار

أجرى هذا البحث بغرض دراسة التركيب المجهرى الدقيق للغده اللعابيه النكفيه في عدد ستة

أظهرت الدراسه أن الغده اللعابيه النكفيه في الحمار تتكون من عنيبات مصليه بحته ، مبطنه بخلايا مفرزه هرمية الشكل .

وأوضحت الدراسه أن الخلايا المفرزه تفتقر إلى الأنثناءات القاعديه تتميز باحتوائها على العديد من الحبيبات الافرازيه التى تظهر عباره عن ماده ذو عتامه الكترونيه متجانسه محاطه بماده محببه دقيقه .

هذا ووجدت الشبكه الاندوبلازميه الخشنه أما فى صورة صهاريج متراصه متوازيه تحت النواه فى الخلايا المحتويه على قليل من الحبيبات الافرازيه أو فى صورة صهاريج طويله وقصيره موزعه بين العديد من الحبيبات الافرازيه . وكذلك وجد العديد من المتقدرات وجهاز جولجي والريبوزمات وكذلك الخيوط السيتوبلازميه .

هذا وشوهدت خطوات تكوين وتطور الحبيبات الافرازيه فى الشبكه الاندوبلازميه الخشنه وكذلك جهاز جولجى .

وأظهرت الدراسه أيضًا أن الأقنية العنبية مبطنه بخلايا هرميه الشكل تحتوى على عديد من الخيوط الستوبلازميه والمتقدرات وكذلك الحبيبات الافرازيه .

أما الأقنية المخططه فخلاياها تفتقر الى الأنثناءات القاعديه المميزه ولكنها تحتوى على متقدرات مستديرة أو بيضاوية الشكل موزعه أساساً حول النواه بالاضافه الى صهاريج الشبكه الاندوبلازميه الخشنه والريبوزومات والنقط الدهنيه.

أما الخلايا العضليه الطلائيه فتحيط بالعنيبات المفرزه وكذلك الأقنية العنبية وتظهر خصائص الخلايا القلوميه .

SUMMARY

The parotid salivary gland of the donkey was composed of pure serous acini. They were lined by pyramidal secretory cells, which were reacted negatively with PAS and alcian blue. The secretory cells which lacked the basal folds, possessed numerous granules which were the distinctive feature of the acinar cells. They appeared as homogenous electron dense substance surrounded by a rim of fine granular material. Abundant RER cisternae, either in the form of parallel stacked cisternae infranuclearly located in cells containing few secretory granules or short and long cisternae among the numerous secretory granules, well developed Golgi-apparatus, numerous mitochondria, ribosomes and cytoplasmic filaments were observed. Steps of formation and development of these granules within the RER and Golgi-apparatus were recorded. The nuclei were large, slightly rounded somewhat basally located. The intercalated duct cells were pyramidal in shape. They were poor in organelles except of the numerous cytoplasmic filaments and mitochendria as well as few secretory granules. The striated duct cells did not possess the characteristic deep basal infoldings. They contained numerous rounded or oval shaped mitochondria mainly scattered around the nucleus in addition to the short RER cisternae, ribsomes and lipid droplets. The myoepithelial cells were found around the secretory acini as well as the intercalated duct, demonstrating the common feature of contractile cells.

Keywords: Fine structure, parotid salivary gland, donkey.

INTRODUCTION

The main secretory products of salivary glands are water, electrolytes and proteins (Van LENNEP et al., 1977). In herbivores, the parotid glands are large; their secretion with high water content moistens the food (MANDEL, 1987). Also, the parotid saliva contains proline-rich glucoprotein which has lubricant effect (HATTON et al., 1985) and histidine-rich peptides which have a growth inhibitory and bactericidal effect on the oral bacteria (MACKAY et al., 1984). They perform buffering effect once they are diffused into the bacterial plaque (VRATSANSO and MANDEL, 1985).

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Although the fine structure of the parotid gland has been extensivly described in man (FERNER and GANSLER, 1961; RIVA et al., 1969; RIVA and RIVA-TESTA, 1973); Spider monkey (LEESON, 1969); Pig (BOSHELL and WILBORN, 1978); goat (TAKANO et al., 1977); goat and dog (SUZUKI et al., 1975); Ovine (PATTERSON et al., 1976; Van LENNEP et al., 1977); horse (SUZUKI and OTSUKA, 1977) and bovine (SHACKLEFORD and WILBORN, 1969; TAKANO et al., 1978), no information is available about the ultrastructure of the parotid gland in donkey. Therefore the present investigation was undertaken to study the fine structure of the Gl. parotis of donkey.

MATERIAL and METHODS

Small specimens of the parotid gland were obtained from 6 adult donkeys of both sexes after killing them by cutting their common carotid arteries under chloroform inhalation anesthesia.

For histochemical study, Bouin's-fixed paraffin- embedded samples were employed. Sections of about 3 μm in thickness were cut and stained with alcian blue- PAS (MOWRY, 1956).

For ultrastructural study, the specimens were fixed in solution formed of 2,5% glutaraldehyde and 2,5% paraformaldehye in 0.1 M phosphat buffer (pH 7.3) for 2-3 hours. They were washed several times in the same buffer and postfixed in 1% buffered osmium tetroxide for 2 hours.

After dehydration in ascending grades of ethanol, the specimens were embedded in ERL after SPURR (1969) and sectioned on a LKB ultramicrotome. Semithin sections were stained with toluidine blue and PAS-Alcian blue (BOECK, 1984). Thin sections underwent contrastation by using uranyl acetate and lead citrate and examined with JEOL 100 CXII electron microscope.

RESULTS

Histologically, the parotid salivary gland of the donkey was composed of pure serous acini, which were closely packed together. These acini have narrrow central lumina which were surrounded by pyramidal shaped secretory cells and basally located myoepithelial cells. The secretory cells, contained numerous secretory granules and somewhat basally located rounded nuclei. These secretory acini were drained by intercalated ducts which lead to the striated ducts and consequently to a larger interlobular duct (Fig. 1 & 2).

Histochemically, the secretory acinar cells exhibited negative reaction for either PAS or alcian blue but few striated duct cells contained fine deposits with positive PAS

reaction in their cytoplasma (Fig. 3).

Ultrastructurally, the pyramidal acinar secretory cells appeared filled with secretory granules (Fig. 4). Their apical portions provided with short microvilli, that were seen within the narrow central lumen and joined together with tight junction (Fig. 5). Laterally they were attached together with many desmosomes and cytoplasmic interdigitations through short processes which were extended into a somewhat straight narrow intercellular space. In addition, lateral intercellular canaliculi (Fig. 6) were also observed between the secretory cells. They were filled with numerous microvilli and bounded apically and distally by tight junctions. The basal border of the secretory cells, which appeared smooth, attached to the basal lamina with hemidesmosomes and to the myoepithelial cells by desmosomes.

Intracellulary, the secretory cells were characterized by regional distribution of their content; they contained numerous secretory granules in the apical portion, well developed rough endoplasmic reticulum in the basal portion and a supranuclearly active Golgi-apparatus. The rough endoplasmic reticulum, that contained fine granular material, exhibited a range of variation in its distribution. It consisted of numerous parallel stacked cisternae infranuclearaly (Fig. 7) with some degree of continuity and formation of vesicles at the free ends when the secretory granules were few, or a mixture of long and short cisternae that distributed inbetween the numerous secretory granules (Fig. 8). A rough endoplasmic reticulum-mitochcndrial complex was also observed, where the RER cisternae were seen enclosing the mitochendria (Fig. 9). The latter organelle was numerous, appeared rounded or elongated and randomly distributed in the cytoplasm among the secretory granules. The Golgi-apparatus was well-developed and represented by 1-2 semicricularly arranged complexes supranuclearly located. Each complex was consisted of 3-4 cisternae and their associated vesicles and vacuoles. Numerous free ribosomes and polysomes were scattered in the cytoplasm.

The more distinctive feature of the secretory cells was the membrane bounded secretory granules, which were rounded in shape and of varying size occupying most of the cell cytoplasm. The process of formation of these secretory granules in the RER and Golgi-complex was observed. They firstly appeared as fine granular material inside the RER cisternae and the vesicles at the free ends (Fig. 10a). The developing secretory granules appeared at the Golgi zone as homogenous electron dense substance surrounded by fine granular material (Fig. 10b). Then the centrally located electron dense substance

increased in size and occupied the major part of the secretory granules. At higher magnification (Fig. 10c) these secretory granules appeared as a large homogenously electron dense substance surrounded by a rim of fine granular or filamentous material. In the apical portion of the cell these granules were fused together at the fine granular or filamentous zone to form a secretory mass (Fig. 11a). Discharge of their content occured by exocytosis into the acinar lumen (Fig. 11b). Cytoplasmic filaments were observed in the form of bundles in the apical portion of the cell (Fig. 12a) as well as in association with the secretory granules (Fig. 12b).

The nuclei were large, rounded and contained an eccentric nucleolei, marginal heterochromatin as well as chromatin islands. Their nuclear envelopes were distinct and interrupted with many nuclear pores. The outer nuclear membrane was seen to be studded with ribosomes.

The epithelium lining the intercalated ducts (Fig. 13 a&b) consisted of regularly arranged pyramidal shaped cells arround a somewhat wider lumen. The luminal surface was provided with few short microvilli. The basal border was attached to the basal lamina with hemidesmosomes and to the myoepithelial cell and their processes with desmosomes. At the luminal surface these cells were joined with zonula occludens and zonula adherens.

Intracellularly, the intercalary ductal cells were rich in cytoplasmic filaments, which were concentrated supranuclear region and attached to the desmosomal plaque (Fig. 13c). Mitochondria, polysomes, few lipid droplets and short cisternae of RER were also observed within these cells. Some of these cells were observed containing secretory granules similar to those of the secretory acinar cells (Fig. 13b). Their basally located nuclei were relatively larger in size and appeared slightly rounded or ovoid in shape, occupying most of the cytoplasm. They contained eccentric nucleolei and relatively abundant heterochromatin.

The cells that lining the striated ducts (Fig. 14 a&b) had not the typical deep basal infoldings. These cells were occupied with numerous rounded or oval mitochondria, which were scattered around the nucleus. Some mitochondria were seen between the few ill-developed basal infoldings. Few short cisternae of RER, ribosomes, cytoplasmic filaments as well as lipid droplets were also seen. The striated duct cells contained large somewhat centrally located nuclei.

The myoepithelial cells were composed of cell body and cytoplasmic processes. They have been observed inbetween the basal lamina and cells forming the secretory acini (Fig. 15a)

and those of the intercalated duct (Fig. 13a & 15b). They possessed the common general feature of the myoepithelial cells as numerous myofilaments. mitochondria and lipid droplets. They contained flattened nuclei and attached to the basal lamina with numerous hemidesmosomes and to the adjacent lining cells with desmosomes.

DISCUSSION

Our investigation reveald that the secretory cells of the parotid gland of the donkey reacted negatively for either PAS or alcian blue, this indicates that the secretory product dose not contain carbohydrates or the carbohydrate content being insufficient to produce a discrnible stain with this technique, these results are in accordance with Von LENNEP et al. (1977) in sheep . On the contrary, MORI (1978) and HARRISON et al. (1987) have demonstrated neutral mucin in the secretory cells of cow and human parotid gland respectively. The failure to demonstrate acid mucin in human parotid secretion (MANDEL, 1977) is due in fact to the majorty of the carbohydrate centent is present as a glycoprotein which contains sialic acid but nevertheless is cationic and so would not be expected to be stained by the histochemical method for acid mucin. SHACKLEFORD and WILBORN (1968), classified the mammalian salivary glands on the basis of their carbohydrate content into four types; mucous, serous, seromucous and special serous gland, which was not accepted by HARRISON et al. (1987) who stated that it is convenient to continue to define salivary glands acinar cells as serous and mucous base on the histological preparations stained by haematoxylin and eosin. In spite of our respect to HARRISON et al's opinion (1987), the histochemical classification seems more acceptable as it is based on the nature of the secretion.

The present ultrastructural study revealed that the acini of donkey parotid gland similar to those of horse (SUZUKI and OTSUKA, 1977). They provided with a narrow central lumen and intercellular canaliculi, that were lined with short microvilli. However, SUZUKI et al. (1981 a,b) noted that the lumina of the bovine and goat parotid acini were notably spacious and considered such characteristics to be closely related to the continuous salivation in these animals.

The secretory cells of the parotid acini of the donkey lack the basal folds. These results, were also observed in baboon parotid gland (TANDLER and ERLADSON, 1975), human labial (TANDLER et al., 1969) and Ebner's glands (RIVA - TESTA et al., 1985), indicating a low salivary flow (AZZALI et al., 1989). Although, intercellular canliculi are present in the parotid

gland of donkey, the absence of the basal folds may lead to the decrease of the salivary flow in this organ. Similar results have been stated by TANDLER and ERLANDSON (1975) in baboon

parotid gland.

The acinar cells contained a large number of serous secretory granules. They appeared as a homogenous electron dense substance surrounded by a zone of fine granular material. While, in sheep (Van LENNEP et al., 1977), bovine and goat parotid glands (SUZUKI et al., 1981 a,b) two to three types of secretory granules according to their electron density were observed. Although RIVA and RIVA-TESTA (1973) have reported that the secretory granules in the human parotid gland contain an electron dense core of uncertain nature. The electron dense substance of the secretory granules in the parotid gland of the donkey appeared to be of proteineous substance which did not react with PAS.

In the present study, the RER was demonstrated either as stacked parallel cisternae in the cell basal portion, when the secretory granules were few or as long and short cisternae inbetween them. Such phenomenon was also observed in human parotid (RIVA and RIVA-TESTA, 1973). The latter authors suggested that this dual behaviour of RER could imply it's functional differentiation.

Ultrastructrally, the intercalated duct cells of the parotid gland of the donkey were similar to those of other salivary glands as reported by SHACKLEFORD and WILBORN (1970b), TANDLER and POULSEN (1976), Van LENNEP et al., 1977) and TOYOSHIMA and TANDLER (1986). In addition to the myoepithelial cells, the tonofilaments of the intercalated duct cells as stated by Van LENNEP et al. (1977) in sheep parotid gland form the means of passive and active support preventing excessive distension and allowing the duct epithelium to resume it's normal dimension.

Our observations reaveld, in accordance with TANDLER and ERLANDSON (1975) in baboon parotid, DOREY and BHOOLA (1972 b) in mammalian submaxillary glands and QWARNSTROEM and HAND (1983) in rat submandibular gland, that some intercalated duct cells contained secretory granules of the same apperance as those of the serous cells. QWARNSTROEM and HAND (1983) suggested that these cells represent progenitor cells for acinar or intercalated duct cells or both, this suggestion was not accepted by TOYOSHIMA and TANDLER (1986), who stated that these cells appear to be fully differentiated cell types which contribute through their unique organic secretory products to the developing saliva.

In the present study the myoepithelial cells have the common general features which resemble those of the other salivary glands. They surround the secretory acini as well as the intercalated but not the striated ducts. TANDLER and ERLANDSON (1975) and SUZUKI et al. (1981 a,b) in baboon, bovine and goat parotid glands respectively could not observ such cells surrounding the intercalated duct. On the other hand by immunohistochemical technique DARDICK et al. (1988) in human parotid gland confirmed the observation of Van LENNEP et al. (1977) that the striated ducts were also surrounded by myoepithelial cells.

It is generally accepted that these myoepithelial cells must be able to excert a pressure on the acini and intercalated duct cells in order to favour a faster expulsion of secretory granules from the apical cytoplasm (AZZALI et al., 1989). Particularly secretory cells have been observed in the intercalated ducts of parotid gland in donkey.

In conclusion, the lack of the basal folds of acinar secretory cells, the narrowness of the acinar lumen and the ill-developed deep basal infoldings of the striated ducts indicate a low rate of parotid saliva in donkey than that of ruminant animals which need a large amount of saliva during the process of rumination.

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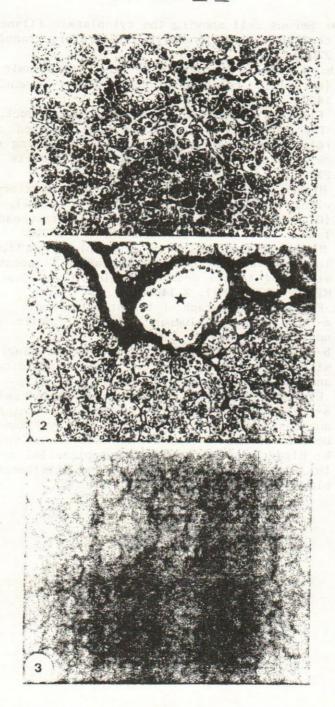
LEGENDS

- Fig. 1 & 2: Semithin section of the parotid gland of the donkey showing serous acini with numerous secretory granules, intercalated duct (arrow), striated duct (arrowhead) and inter-lobular duct (asterisk) Toluidine blue. X 25.
- Fig. 3: Semithin section of the parotid gland of the donkey showing negative PAS-Alcian blue reaction within the acini. X 25.
- Fig. 4: Electron micrograph of a serous acinus of the parotid gland showing numerous secretory granules filling the cytoplasm. X 2700
- Fig. 5: Apical portion of two secretory cells demonstrate microvilli within the lumen (arrow), zonula occludens (Zo), zonula adherens (Za) and desmosomes (D). X 27000.
- Fig. 6: Electron micrograph of an intercellular canaliculus (arrow) filled with microvilli. X 10000.
- Fig. 7: Infranuclear region shows the numerous parallel stacked cisternae with some vesicles formation (arrow). Nucleus (N), basal lamina (BL). X 10000.
- Fig. 8: Short and long cisternae of RER (arrows) inbetween the numerous secretory granules (arrowheads). Nucleus (N). X 5000.
- Fig. 9: Electron micrograph of serous cell showing rough endoplasmic reticulum- Mitochondrial complex. Mitochondria (M). X 14000.
- Fig. 10: Electron micrographs showing the steps of formation of the secretory granules.
 - a- Accumulated fine granular material inside the RER cisterna. X 27000.
 - b- Apperance of the developing secretory granule (arrow) with homogenous electron dense substance surrounded by a rim of fine granular material at the well developed Golgi- complex. X 10000.
 - c- Higher magnification of the mature secretory granule at the apical portion of the cell formed of large electron dense substance surrounded by fine granular material. X 27000.
- Fig. 11a: Electron micrograph showing fusion of the secretory granules at their filamentous material (arrowheads). X 14000.
- Fig. 11b: Apical portions of the serous cells demonstrate exocytosis of secretory granules within the lumen. X 10000.

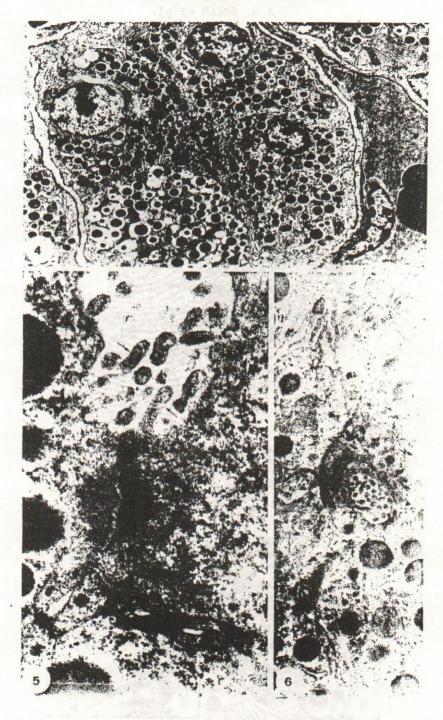
- Fig. 12a: Serous cell showing the cytoplasmic filaments in the cell apical portion (arrow). Secretory granules (Sg). X 27000.
- Fig. 12b: Electron micrograph of the cytoplasmic filaments (arrow) in relation to the secretory granule (Sg). X 14000.
- Fig. 13: Electron micrograph of an intercalated duct:
 - a- The regularly arranged pyramidal shaped cells with relatively large rounded nucleus occupying most of th cell cytoplasm. Myoepithelial cell process (arrow). X 2700.
 - b- Higher magnification demonstrates secretory granules within the intercalated duct cell (arrow), desmosomes (D) and few apical microvilli (arrowheads). Basal lamina (B1), hemidesmosomes (HD). X 10000.
 - c- Higher magnification of the apical portions of the intercalated duct cells. Cytoplasmic filaments (arrows) attaching to the desmosomes (D). Tight junction (Tj), Micro- villi (Mv). X 14000.
- Fig. 14: Electron micrograph of the striated duct:

 a- The lining columnar epithelial cells with their nuclei. X 2000.
 - b- Higher magnification demonstrating numerous mitochondria, ill-developed basal infoldings (arrow) and lipid droplets (arrowheads). X 27000.
- Fig. 15: Electron micrograph of the myoepithelial cells:

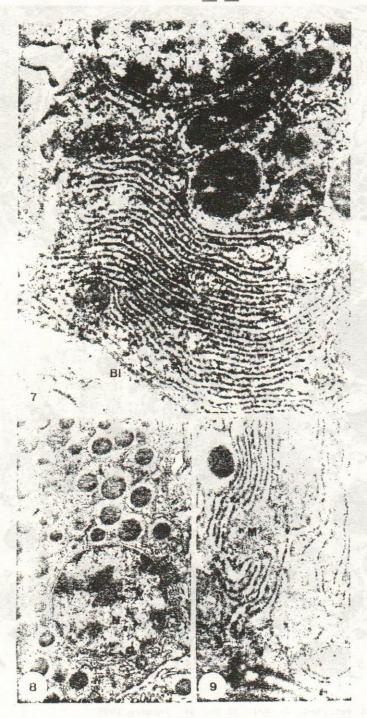
 a- Cell body of myoepithelial cell containing flattened nucleus (N) and bundles of myofilaments (F). Hemidesmosomes (arrowheads), basal lamina (B1). X 4000 b- Higher magnification of myoepithelial cell process in (Fig. 13a) showing many dense attachment areas at plasma membrane (arrowheads). X 27000.



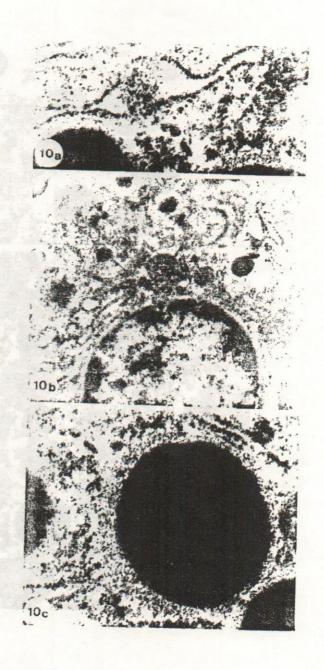
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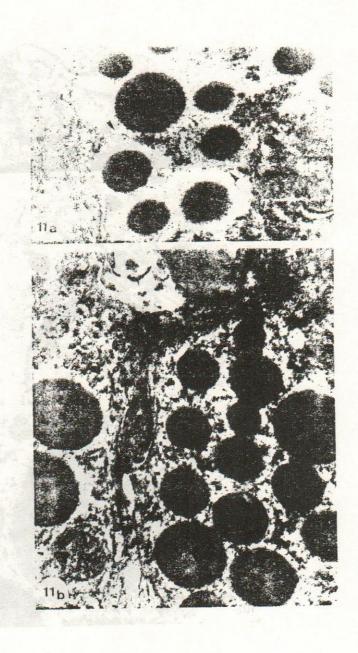
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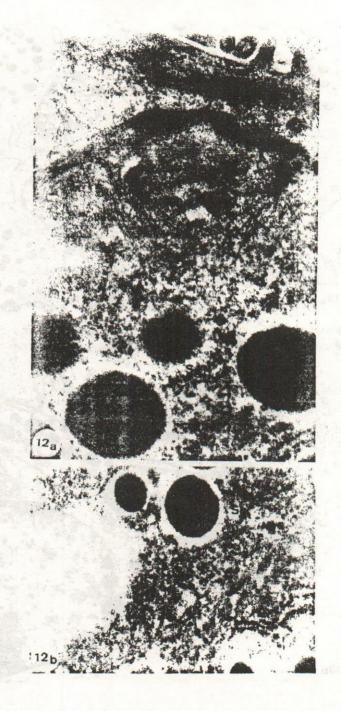


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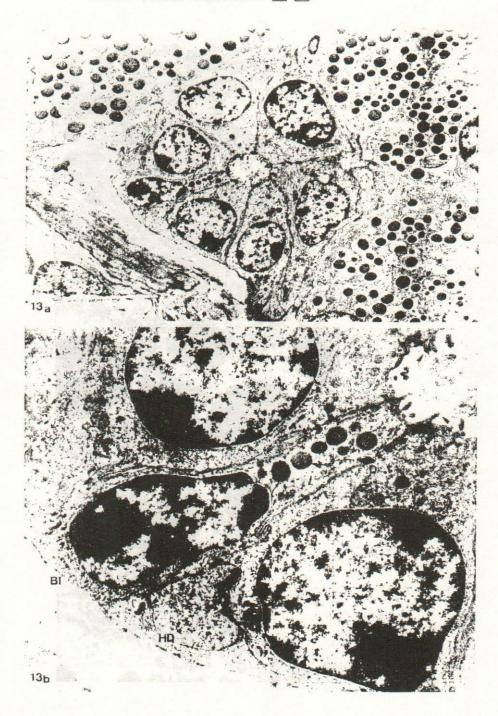


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