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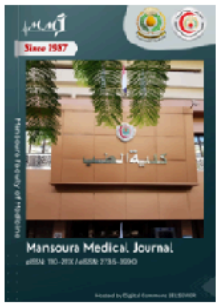

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MYOEPITHELIAL CELLS AFTER DUCT LIGATION AND DE-LIGATION OF RAT SUBMANDIBULAR GLAND, LIGHT AND ELECTRON MICROSCOPIC STUDY

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By

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ABSTRACT

Myoepithelial cells are observed in several exocrine glands. They are star-shaped cells that lie in between the basal lamina and the acinar and ductal cells (Ogawa et al., 1999)[30]. These cells have the structural features and function of both smooth muscle cells and epithelium (Franke et al., 1980)[31]. They contract when the gland is stimulated to secrete. They aid expulsion of glandular secretion through compressing or reinforcing the underlying parenchymal cells. It was suggested that the major function of myoepithelial cells in salivary glands is to support the glandular structure through isometric contraction (Segawa et al., 1995)[36]. They also display the characteristics of epithelium in that they are situated within the glandular epithelium between secretory cells and the basement membrane (Tandler, 1965; Harrop, 1968; Leeson and Leeson, 1971)[45,19,26].

Myoepithelial cells have speculated to play an important role in histogenesis of some salivary gland tumors, such as pleomorphic adenoma, myoepithelioma, adenoid cystic carcinoma and certain other tumors (Batsakis et al., 1983; Dardick and Buford-Mason, 1993; Redman, 1994) [2,9,33].

Studies of the responses of SMG to ligation of the main excretory duct have established that both acinar cells and cells of the granular ducts are markedly altered morphologically

and functionally (Kasai, et al., 1993; Kern, 1993) [24,25]. Moreover, ligation of the main excretory duct of the rat submandibular gland (SMG) produces a pronounced atrophy that is reversed upon ligature removal. The altered morphology and function of ligated salivary glands recover toward the normal state after removal of ligature (Junqueira and Rabinovitch, 1954; Harrison and Garrett, 1976; Randriamampita and Tsien, 1993; Ahn et al., 2000)[23,17,32,1]. However, it is not established whether myoepithelial cells are able to proliferate in atrophic rat submandibular glands, which differ histologically from parotid glands. Burgess et al. (1996)[6] observed that atrophy of the rat parotid glands which induced by duct ligation was associated with proliferation of myoepithelial cells. However, Bataskis et al. (1989)[3] found low proliferation in myoepithelial cells in atrophied rat salivary glands.

Therefore, the present study was undertaken to clarify the effects of duct ligation and ligature removal on the myoepithelial cells of rat SMG. Also, it is an attempt to determine the crucial role of myoepithelial cells upon the intergrity of acinar cells in both ligated and de-ligated ducts of SMG of rat.

MATERIAL AND METHODS

Animals :

Twenty adult male rats were used in this study. The rats were divided into 3 main groups:

* **Group I :** contained 5 animals were used as control (non operated).
* **Group II:** contained 9 animals, which were exposed to ligation of the duct of the submandibular glands (SMG). The operated animals were sacrificed 1, 2 and 4 weeks after duct ligation (three animals were used each time).
* **Group III:** contained 6 animals, in which the submandibular gland was ligated for only 2 weeks, then the ligature was removed and the animals allowed to survive for another 2 weeks and were sacrificed.

Anesthesia :

The rats were anaesthetized using pentobarbitone sodium (60 mg/ml)

given by intraperitoneal injection at a dose of 40 mg/kg body weight (Takahasi et al., 2001)[41].

Surgical Procedure :

Submadibular gland duct ligation: the main right SMG excretory duct was performed as described by Turner et al. (1997)[46]. The main excretory duct of SMG on the right side of the neck was surgically exposed through a ventral incision in the neck under a surgical microscope. The duct was doubly ligated with metal clips designed specifically to allow for ease of removal after periods of duct ligature at a point 5mm distal to the hilum of the gland, with particular care taken to avoid ligation of the accompanying nerves and blood vessels. The skin incision was closed with surgical clamps.

Post-operative care :

The rats were given 0.1 ml penicillin G benzathine intramuscular to prevent infection. They were given food and water ad libitum until the date of scarification.

Histological methods :

At the assigned time, the operated and control animals were anaesthetized (as before), were perfused with 2% paraformaldehyde-1.25% glutaraldhyde buffered at pH 7.4 with 0.05 M sodium cacodylate and the right submandibular glands were thoroughly dissected out and removed. Specimen of the gland was placed in 10% buffered formalin and processed by conventional methods for embedding in paraffin wax. Sections were cut at 8-10 ∘m thickness and stained with haematoxylin and eosin for light microscopy.

Other specimen of the gland was taken and prepared for electron microscopy study. Small pieces of tissue about 1 mm^3 were immersed in a solution formed of 2% paraformaldehyde-1.25% glutaraldhyde for 1 hour, then immersed in 1% osmium tetroxide for 1-2 hours at 4^oC, dehydrated with acetone, stained en bloc with 4% Uranyl acetate and embedded in Epon 812 (Structure Probe, Westchester, PA, USA). Semi-thin sections (1 μm) were cut and stained by toluidine blue. Ultra-thin sections were cut on copper grids and stained with Uranyl acetate and lead citrate and examined under a Phillips

EM208s transmission electron microscope (Luft, 1961; Millonig, 1961; Hayat, 1970)[28,29,20].

RESULTS

A- Group I : (Control submandibular glands)

Haematoxylin and eosin stained sections revealed a compact lobular gland filled with seromucous acini, intercalated, striated and granular convoluted ducts with supporting connective tissues (Fig. 1). The acini formed of pyramidal cells. The striated ducts were lined by tall columnar cells, and the interlobular ducts were lined by pseudostratified columnar cells. Many flat myoepithelial cells were seen surrounding the acini between the acinar cells and their basement membrane (Fig. 2).

Semi-thin sections of Control submandibular glands stained with toluidine blue : showed presence of seromucous acini, striated ducts with dark blue granules in the apical cytoplasm and flat myoepithelial cells that lied in the basal lamina between the acinar and ductal cells (Fig. 3).

Electron microscopy of Control glands : the myoepithelial cells could be detected surrounding the acini. In their cytoplasm, myo-microfilaments, scattered mitochondria as well as rough endoplasmic reticulum and dense bodies were observed (Fig. 4).

B- Group II : (Duct-ligated submandibular glands)

1. one week after submandibular duct-ligation :

Haematoxylin and eosin stained sections showed a loss of normal glandular structure. The individual lobules were shrunken, with more interlobular and interlobar connective tissue. The connective tissue increased around the atrophied acini and ducts. The lobules appeared to contain numerous structures resembling transversely sectioned ducts (duct-like structures) lined by cuboidal cells, with little cytoplasm and the ducts had dilated lumina (Fig. 5). The atrophied acini were shrunken, appeared smaller and lined by indistinct cells with non granular vacuolated cytoplasm. Persistent flat myoepithelial cells were observed in the basement membrane (Fig. 6).

Toluidine blue stained sections revealed less dark blue granules in the apical cytoplasm of atrophied ducts. Intact flat myoepithelial cells surrounded the acini and ducts (Fig. 7).

Electron microscopy of submandibular gland one week after duct ligation showed remarkable atrophic changes in the myoepithelial cells. It contained a small nucleus with nuclear chromatin aggregated into dens mass, degenerated mitochondria, degenerated myo-microfilaments with presence of vacuoles in the thick basement membrane (Figs. 8, 9).

2. Two weeks after submandibular duct-ligation :

Haematoxylin and eosin stained sections showed more atrophic changes. The lobules were more shrunken, with increased the intervening connective tissue. The cells lining the duct-like structures were small, cuboidal with a centrally placed large vesicular nucleus. Many of the duct-like structures had multi-layered walls. Both intralobular and interlobular ducts were markedly dilated (Fig. 10). The atrophic acini were seen lined by small cells with non granular vacuolated cytoplasm. Most of striated ducts lost their characteristic basal striations. Flat myoepithelial cells were seen surrounding the acini and the ducts (Fig. 11).

Toluidine blue stained sections : showed few persisting flat myoepithelial cells between the acinar and atrophied ductal cells and their basement membrane (Fig. 12).

Electron microscopy revealed thick basement membrane of atrophied acinar cells which were arranged in many irregular folds and contained degenerated myoepithelial cells formed of irregular nucleus with chromatin aggregated into dens mass and the basement membrane was thrown into folds (Fig. 13). Other myoepithelial cells showed irregular nucleus with loss of nuclear of chromatin condensation, degenerated myo-microfilaments and presence of large vacuoles in thick basement membrane (Fig.14).

3. Four weeks after submandibular duct-ligation :

Haematoxylin and eosin stained sections showed much less glandular

tissue. The lobules were more shrunken, atrophic and contained more connective tissue with a few scattered dilated duct-like structures. The individual parenchymal elements were more widely spaced. Dilatation of atrophic intra- and inter- lobular ducts was still present (Fig.15). Few flat myoepithelial cells were still present between the acinar cells and their basement membrane (Fig.16).

Toluidine blue stained sections : few myoepithelial cells surrounded the acini, between the acinar and ductal cells and their basement membrane (Fig.17).

Electron microscopic studies : showed degenerated myoepithelial cells contained irregular nucleus with nuclear chromatin aggregated into a dense mass, degenerated myomicrofilaments, electron density of its cytoplasm and thick basement membrane (Fig. 18).

C- Group III : De-ligated glands

The haematoxylin and eosin stained sections revealed that the architecture of the gland resembled that of the control ones. However, the lobules were smaller than the normal and the acini were loosely packed. The interlobular connective tissue was more conspicuous than in control ones. The Intercalated and striated ducts were identifiable, the interlobular ducts remained dilated giving appearance of duct-like structures for the lobules (Fig. 19). Flat myoepithelial cells were present surrounding the ducts and the acini (Fig. 20).

Toluidine blue stained sections: few flat myoepithelial cells were seen in between the acini and ductal cells and their basal membranes, in basket shape. Dark blue granules reappeared in the apical cytoplasm of the cells of the ducts (Fig. 21).

Ultra-thin sections: showed few myoepithelial cells were identified at the periphery of the acini within the thick basement membrane. The myoepithelial cells were recognizable by their normal shape and contents and looked like control ones (Fig. 22).

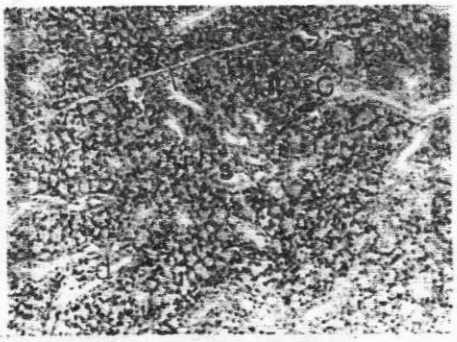

Fig. (1) : A photomicrograph of a section of control rat submandibular gland, showing seromucous acini (A), granular convoluted tubules (G), striated (S) and interlobular (d) ducts and interlobular connective tissue (arrows). (HX. & E.; X 100)

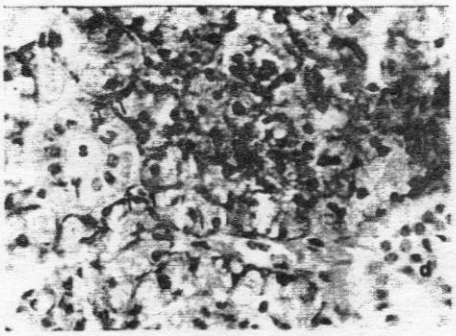

Fig. (2) : A photomicrograph of a section of control rat submandibular gland, showing the serous acini (A) is formed of large pyramidal cells, the striated duct (S) is lined by tall columnar cells and interlobular duct (d) lined by pseudostratified columnar cells. Flat myoepithelial cells (arrows) are seen in the basement membrane around the acini and ducts.
(HX. & E.; X 400)

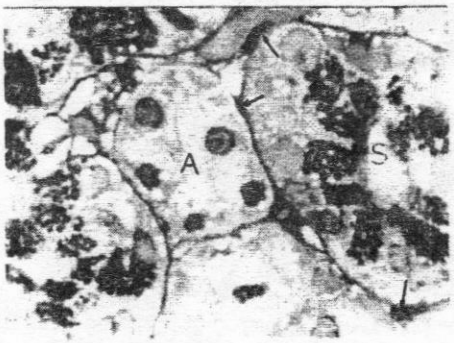

Fig. (3) : A photomicrograph of semi-thin section of control rat submandibular gland, showing acinar cells (A), striated duct (S) containing dark blue granules in the supranuclear cytoplasm and flat myoepithelial cells (arrows) in the outer border of basement membranes of ducts and between it and acini . (Toluidine blue; X 1000)

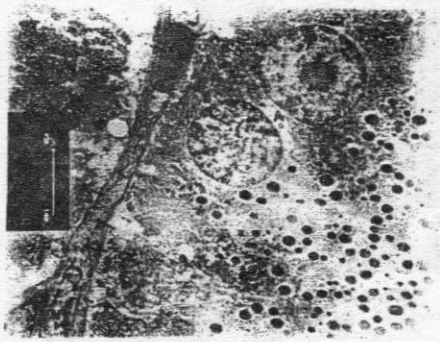

Fig. (4) : An electron photograph of control rat submandibular gland, showing flat myoepithelial cell surrounding the basement membrane of acinar cell (A). It contains myo-microfilaments (crossed arrows), dens bodies (arrows), mitochondria (m) and nucleus (N) with chromatin condensation. (X 6000)

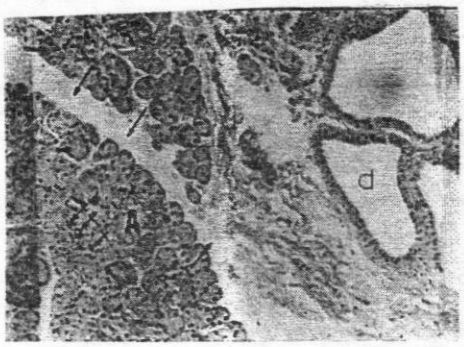

Fig. (5) : A photomicrograph in a section of rat submandibular gland one week after duct-ligature, showing atrophic small acini (A), the connective tissue (arrows) is increased between the lobules, dilated atrophied interlobular ducts (d) and atrophied striated ducts (s). Note: the degenerated ducts gave the appearance of duct-like structure.
(HX. & E.; X 100)

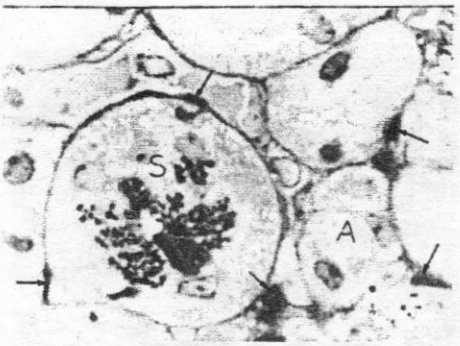

Fig. (7) : A photomicrograph in a section of rat submandibular gland one week after duct-ligature, showing atrophic acini (A), atrophic striated duct (S) containing few dark blue granules in the supranuclear cytoplasm and few flat myoepithelial cells (arrows) lying between the basal lamina and the atrophied acinar and ductal cells.
(Toluidine blue; X 1000)

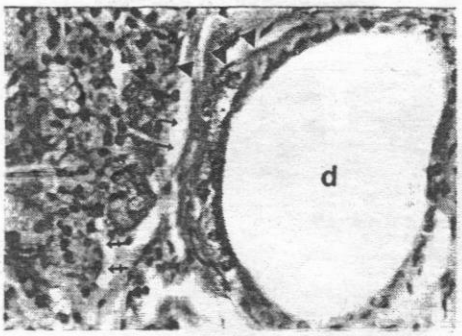

Fig. (6) : A photomicrograph in a section of rat submandibular gland one week after duct-ligature, showing atrophic small acini (A) lined by cells with non-granular vacuolated cytoplasm, the connective tissue (arrows) is increased around the ducts and acini (crossed arrows) and dilated atrophic interlobular duct (d). The flat myoepithelial cells (arrow heads) are seen in the basal membrane in between the ducts and the acini.
(HX. & E.; X 400)

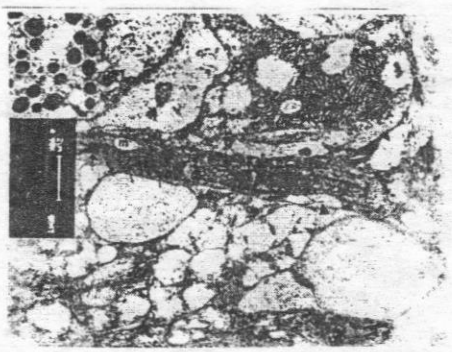

Fig. (8) : An electron photograph of rat submandibular gland one week after duct-ligature. Note: presence of a flat myoepithelial cell in the basement membrane of atrophied acinar cells. It contained small flat nucleus (N) with nuclear chromatin aggregates into dens mass, increased electron density (crossed arrows), degenerated mitochondria (m) and caveolae (arrow head). (X 6000)

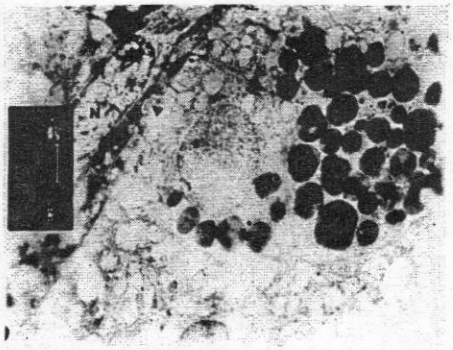

Fig. (9) : An electron photograph of rat submandibular gland one week after duct-ligature. Degenerated flat myoepithelial cell is seenin the basement membrane of atrophied acinar cell (A). It contains irregular nucleus (N) with loss of chromatin condensation, degenerated microfilaments (arrows), degenerated mitochondria (arrow head), condensation of dens bodies (crossed arrows) in thick basement membrane. (X 6000)

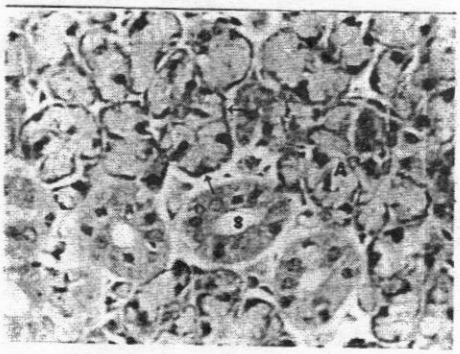

Fig. (11) : A photomicrograph in a section of rat submandibular gland two weeks after duct-ligature, showing atrophic acini (A) lined by cells with non-granular vacuolated cytoplasm and the striated ducts (S) lost the basal. Few flat myoepithelial cells (arrows) are seen surrounding the acini in the basement membrane. (HX. & E.; X 400)

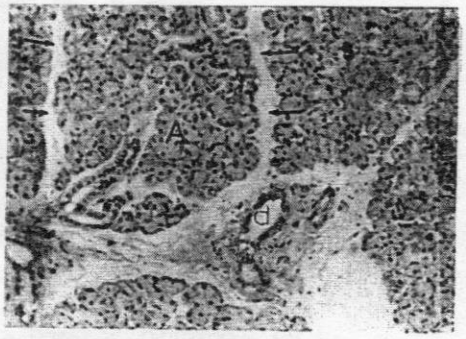

Fig (10) : A photomicrograph in a section of rat submandibular gland 2 weeks after duct-ligature, showing shrunken lobules which contained atrophic loosely packed seromucous acini (A), separated by wide areas of connective tissue (arrows). The interlobular ducts (d) were dilated and the striated ducts (S) lost the basal striation giving the appearance of duct-like structures.
(Hx,& E.; X100)

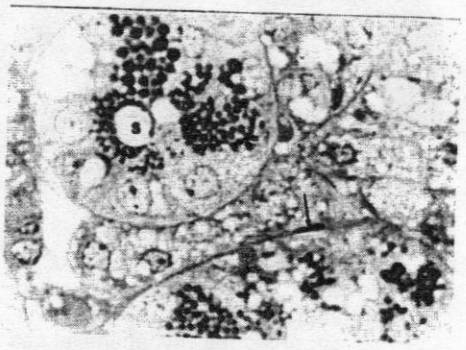

Fig. (12) : A photomicrograph in a section of rat submandibular gland 2 weeks after duct-ligature. Note: atrophied striated ducts (S) containing little dark blue granules and few flat myoepithelial cells (arrow) are present in the outer border of basal membrane of the ducts.
(Toluidine blue; X 1000)

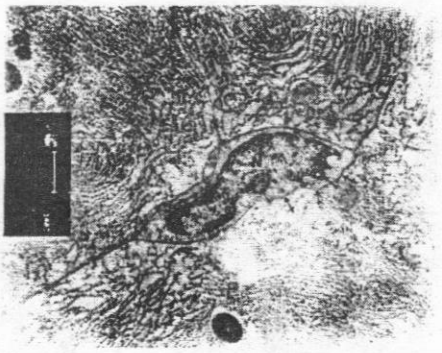

Fig. (13) : An electron photograph of rat submandibular gland 2 weeks after duct-ligature. Note: atrophied myoepithelial cell in the thick basement membrane surrounding acinar cell which contains irregular nucleus (N) with more nuclear chromatin aggregates into dens mass (arrows) and thick basement membrane that arranged in folds (crossed arrows). (X 6000)

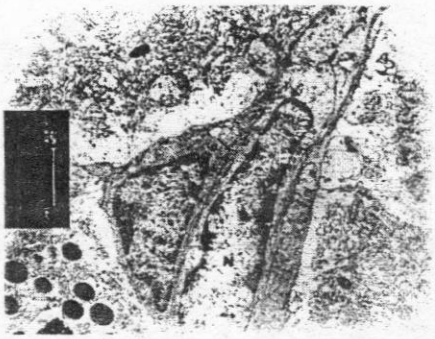

Fig. (14) : An electron photograph of rat submandibular gland 2 weeks after duct-ligature, showing, atrophied flat myoepithelial cell in the thick basement membrane surrounding atrophic acinar cell (A) and contains a nucleus (N) with loss of nuclear chromatin condensation, degenerated microfilaments (arrows) and presence of large vacuoles (V) in the basement membrane. (X 6000)

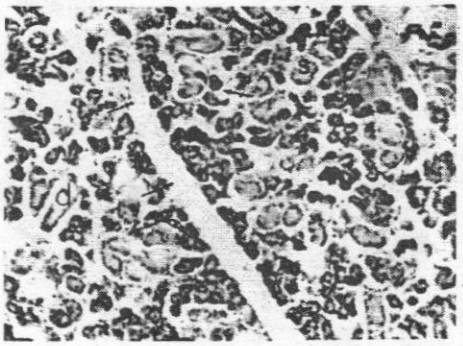

Fig (15) : A photomicrograph in a section of rat submandibular gland 4 weeks after duct-ligature. The lobules contained small atrophic seromucous acini (A) and increase of connective tissue (arrows) around the acini, ducts and between the lobules, the striated ducts (S) are seen with lost basal striations and dilated interlobular ducts (d). Note: scattered dilated duct-like structures of the lobules. (HX. & E.; X 100)

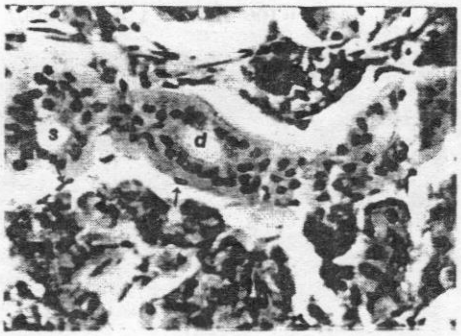

Fig. (16) : A photomicrograph in a section of rat submandibular gland 4 weeks after duct-ligature, showing atrophic acini (A) lined by cells with non-granular vacuolated cytoplasm, the striated ducts (S) lost the basal striation and dilated atrophic interlobular ducts (d). Flat myoepithelial cells (arrows) are seen surrounding the ducts and the acini in the basement membrane. (HX. & E.; X 400)

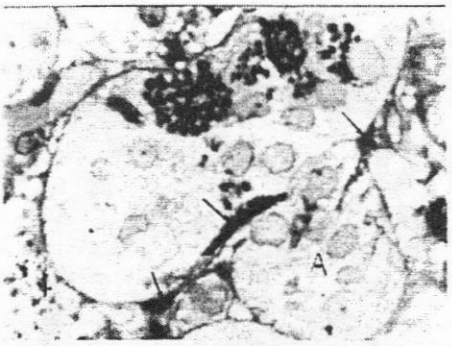

Fig. (17) : A photomicrograph in a section of rat submandibular gland 4 weeks after duct-ligature, showing few flat myoepithelial cells (arrows) surrounded the basal membrane of acini (A). (Toluidine blue; X 1000)

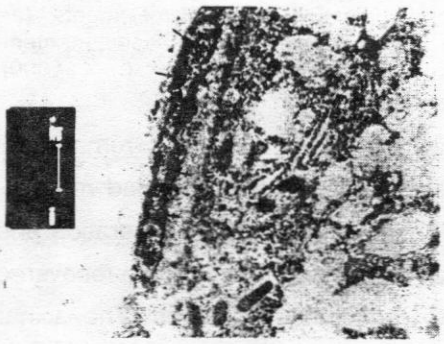

Fig. (18) : An electron photograph of rat submandibular gland 4 weeks after duct-ligature, showing atrophic myoepithelial cell in the basement membrane of atrophied acinar cell (A). It contains irregular nucleus (N) with nuclear chromatin aggregates into dens mass (arrows), degenerated microfilaments (crossed arrows), increased electron density in the cytoplasm and thick basement membrane. (X 6000)

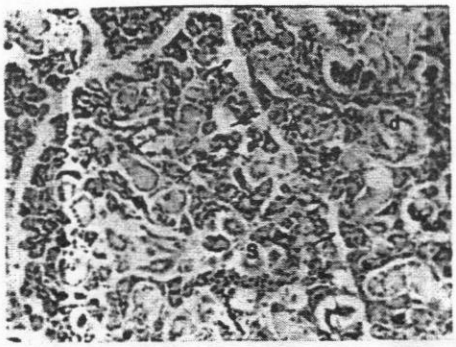

Fig (19) : A photomicrograph in a section of rat submandibular gland 2 weeks after duct de-ligature. The lobules are small and contain loosely packed seromucous acini (A) similar to control ones. The connective tissue (arrows) is still thick and the striated (S) and dilated interlobular (d) ducts are seen. Note: scattered dilated duct-like structures of the lobules. (HX. & E.; X 100)

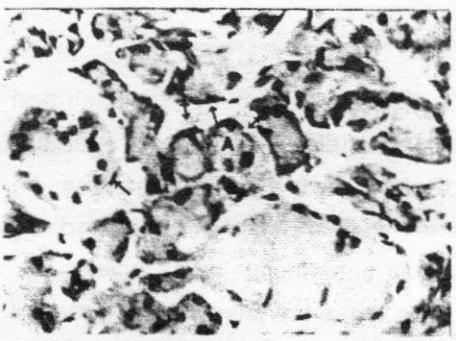

Fig. (20) : A photomicrograph in a section of rat submandibular gland 2 week after duct de-ligature, showing the acini (A) looking like the control, increased connective tissue (crossed arrows) around the acini and presence of flat myoepithelial cells (arrows) in the basal lamina in between the acini and the dilated duct. (HX. & E.; X 400)

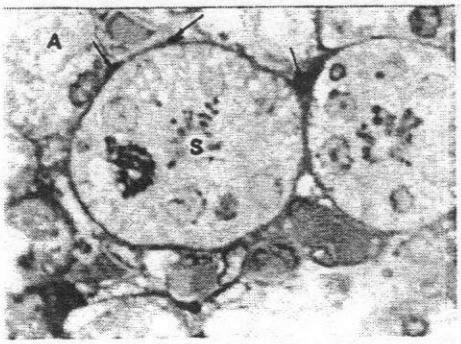

Fig. (21) : A photomicrograph in a section of rat submandibular gland 2 weeks after duct de-ligature, showing flat myoepithelial cell (arrow) surrounding the basement membrane between the ducts (S) and the acini (A), and appearance of dark blue granules in the apical cytoplasm of cells lining the ducts. (Toluidine blue X 1000)

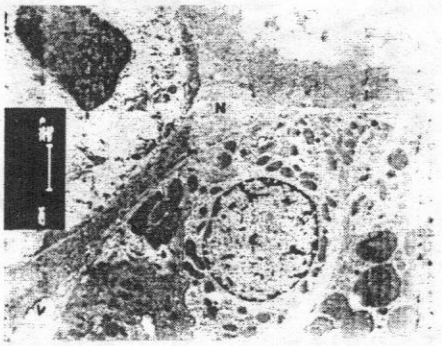

Fig. (22) : An electron photograph of rat submandibular gland 2 weeks after duct de-ligature. Showing flat myoepithelial cell in the basement membrane of the adjacent acinar cells. It contains a nucleus (N) with normal nuclear chromatin condensation, myo-microfilaments (arrows) and the basement membrane is still thick. (X 6000)

DISCUSSION

The present study demonstrated that, after duct ligation of the submandibular gland, there was progressive atrophic changes of the acini and dilated interlobular and intralobular ducts that were lined with degenerated vacuolated cuboidal to flat cells. The interlobular connective tissue increased in thickness.

Similar changes has been reported by Tamarin (1971a), Shiba et al. (1972), Emmelin et al. (1974) as well as Garrett and Parsons (1979)[43,37,12,15]. After removal of ligature, the gland revealed morphological evidence of regeneration and all contents appeared to be recovered to the control condition. The acinar atrophy occurring after ligating the submandibular duct can be regarded as an evolved defense mechanism which protects the acinar cells during the period of potential damage from back pressure such that the acinar cell reverts to resting state with reduced expenditure of energy and lack of synthesizing or secretory activity, as suggested by Junqueira (1951)[22].

Subsequently, when obstruction is removed, the cycle of synthesis and secretion can be re-established, and the acinar cells reverted to their normal volume.

Numerous animal models have been utilized for the study of atrophy and recovery in different salivary glands of several species, based on the technique of main duct ligation and de-ligation. In general, such studies indicated that complete obstruction of the outflow of saliva causes atrophy of the individual acini together with inflammatory changes and shrinkage of the whole gland. The process of atrophy is variable in extent and intensity between glands and species (Bhaskar et al., 1966; Shiba et al., 1972; Donat et al., 1973; Harrison and Carrett, 1976)[4,37,11,17]. Moreover, following removal of ligatures, it has been shown that, in the rat parotid, the acini are capable of sustained morphological recovery (Burford-Mason et al., 1993)[7], although this does not occur in all cases (Shimizu et al., 1994)[38].

There is wealth of evidence indicating that the damage induced by rat SMG ligation is fully reversible after ligature removal (Bhaskar et al., 1966; Tamarin, 1971a, 1971b) [4,43,44].

The present study showed that: 1) Ligation of the submandibular gland duct produced atrophic changes in the gland, with acini and myoepithelial cells being predominantly affected, and 2) after removal of the ligature, the glands revealed evidence of regeneration of myoepithelial cells. It also has been shown that apoptosis is involved in acinar atrophy after ligation of excretory duct of pancreas (Walker, 1987)[47] and parotid gland (Walker and Gobe, 1987)[48].

In atrophy of the parotid gland (Emmelin et al., 1974; Garrett and Emmelin, 1979)[12,14] and involution of mammary glands (Radnor, 1972; Walker et al., 1989)[31,49], myoepithelial cells appeared with protruding cellular processes and irregular folds within thick basement membrane, probably due to new synthesis by myoepithelial cells (Emmelin et al., 1974; Garrett and Emmelin, 1979)[12,14]. The myoepithelial cells in this study also often showed similar

changes, and this finding demonstrates that atrophy was induced in the submandibular gland. This atrophy was induced both by obstruction of the excretory duct or damage to the chorda tympani (Harrison and Garrett, 1972; Harrison et al., 2001)[16,18].

It has been shown that the myoepithelial cells are able to proliferate in cultured and transplanted human mammary glands (Joshi et al., 1986)[21] and during development of hamster Harderian (Lopez et al. 1992)[27], rat parotid (Taga and Sesso, 1979; Redman et al., 1980)[39,34] and rat submandibular glands (Cutler and Chaudhry, 1973)[8]. In contrast, mature myoepithelial cells have been considered unable to proliferate (Cutler and Chaudhry, 1973; Joshi et al., 1986; Batsakis et al., 1989)[8,21,3] because they were end-differential cells (Batsakis et al., 1989)[3]. However, mitosis of myoepithelial cells was observed in developing rat parotid glands (Redman, 1994)[33], and cycling myoepithelial cells were identified in mouse mammary glands stimulated by hormone (Sapino et al., 1990)[35] and in atrophic rat parotid glands (Burgess et al., 1996)[6].

In the present study, persistent myoepithelial cells were found in atrophic rat submandibular glands. This suggests that mature myoepithelial cells in the rat submandibular gland possess proliferative ability that is especially active under some experimental conditions. Takahashi et al. (2004)[42] had shown that myoepithelial cells decreased in number 3 days after duct ligation and suggested that both apoptosis and proliferation of myoepithelial cells occur in early phase of atrophy of rat submandibular gland. Moreover, Takahashi et al (2000)[40] added that duct ligation of rat submandibular induced marked depletion of acinar cells. Burgess et al. (1996)[6] reported that the maximum of the proliferative cell nuclear antigen (PCNA) index of myoepithelial cells in atrophic submandibular gland is much lower than that in atrophic parotid gland. This may arise from differences in the myoepithelial cell distribution between submandibular and parotid glands in the rat. Myoepithelial cells are situated at the periphery of both acini and intercalated ducts in rat submandibular glands

and mainly of intercalated ducts in rat parotid glands. Thus, there are more myoepithelial cells in submandibular glands than in parotid glands (Dewey, 1958; Bogart, 1971; Garrett and Emmelin, 1979; Redman, 1994)[10,5,14,33]. Therefore, it may be unnecessary for myoepithelial cells in submandibular glands to proliferate as actively as in parotid glands during atrophy (Takahashi et al., 2001)[41]. Recently, using digoxigenin-labelled P2Y2 receptor, one of the receptors of extracellular nucleotides (P2 receptors), was reported to be an important component of the response to injury in submandibular gland from the fact that P2Y2 receptor activity and mRNA levels were increased in duct ligated submandibular gland (Turner et al., 1997; Ahen et al., 2000)[46,1]. In the duct ligated submandibular gland, P2Y2 receptor mRNA was mainly detected in acini and intercalated ducts by in situ hybridization (Ahen et al., 2000)[1]. However, it is still not known whether P2Y2 receptor mRNA of myoepithelial cells surrounding acini and in tercalated ducts is upregulated in atrophic submandibular gland.

SUMMARY

The duct of the right submandibular gland was doubly ligated with metal clips to study the effects of duct ligation and ligature removal in rat SMG to elucidate the relationship between duct ligation and removal of the ligature and to determine whether proliferation of myoepithelial cells occur in atrophic rat submandibular glands.

Twenty adult male rats were used in this study. Rats were divided randomly into 3 main groups: a submandibular gland atrophy group (Ligated Group) in which the main submandibular duct was ligated for 1, 2 and 4 weeks; a recovery group in which duct ligation was in place for 2 weeks and then removed and the 3rd group was not subjected to operation and used as control group. At the assigned time, the animals were sacrificed. The right submandibular glands were thoroughly dissected and removed. Specimens of the gland were cut at 8-10 μm thickness and stained with haematoxylin and eosin. Other specimens of the gland were taken and prepared for electron microscopy study.

Duct ligation of SMG was accompanied by progressive atrophy of the gland. After 4 weeks of duct-ligation, light microscopy showed that the acini had atrophied and contained few secretory granules. There was dilated interlobular and intralobular ducts that lined by cuboidal flat cells. The interlobular connective tissue was increased in thickness. Persistent myoepithelial cells were also found in atrophic rat SMG in semi-thin and ultra-thin sections.

After removal of the ligature, the gland revealed histological evidence of regeneration. All cellular components appeared recovered to the control condition.

The present observations also suggest that ligation of the main duct of the rat SMG produces a pronounced atrophy that is reversed upon ligature removal.

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الخلايا العضلية - الطلائية بعد ربط وفك ربط قناة الغدة اللعابية تحت الفكية للجرذ - دراسة بالميكروسكوب الضوئى والألكترونى

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تم ربط قناة الغدة اللعابية تحت الفكية فى الجرذ باستعمال مشبك معدنى وذلك لدراسة تأثير عملية الربط والفك للغدة لدراسة مدى العلاقة بين ربط القناة وفكها لتحديد إذا ماحدث تكاثر للخلايا العضلية- الطلائية أثناء ضمور الغدة.

واستخدم فى هذه الدراسة عشرون جرذاً من الذكور البالغة، وتم تقسيمها إلى ٣ مجموعات: مجموعة تم ربط قناة الغدة فيها لمدة ١، ٢، ٤ أسابيع (٣ جرذان فى كل مدة)، ومجموعة ربطت لها القناة لمدة أسبوعين ثم فكت لها القناة وتركت لمدة أسبوعين آخرين (٦ جرذان)، والمجموعة الثالثة (٦ جرذان) استخدمت كمجموعة ضابطة. وفى الأوقات المحددة تم التضحية بها وتم التشريح الدقيق لها واستخراج الغدة تحت الفكية اليمنى، وتم أخذ بعض العينات لتمريرها فى البرافين وعمل الشرائح وصباغتها بالهيماتوكسيلين والأيوسين، بينما أخذت بعض العينات الأخرى وتم تجهيزها لعمل شرائح الميكروسكوب الألكترونى.

كان لتأثير ربط القناة على الغدة تأثير ضمورى متزايد، فكان تأثيرها بعد ٤ أسابيع وجود حويصلات ضامرة مع قليل من الحبيبات المفرزة مع تمدد فى القنوات بين الفصوص وداخل الفصوص وكانت خلاياها مكعبة الشكل وزاد سمك الغشاء الضام داخل الغدة.

واستمر وجود الخلايا العضلية-الطلائية حول الحويصلات والقنوات المتمددة الضامرة وذلك فى الشرائح المصبوغة بالتولودين الأزرق وكذلك شرائح الميكروسكوب الألكترونى .

وبعد فك ربط القناة أظهر الفحص بالميكروسكوب الألكترونى عودة التركيب الهستولوجى للخلية المصلية وكذلك الخلايا العضلية-الطلانية التى عادت الى حالتها الطبيعية وشفائها وظهور مكوناتها مثل الخلايا الضابطة..

تلك الملاحظات بينت أن لربط قناة الغدة تأثير ضمورى متزايد على تركيب خلايا الغدة بما فيها الخلايا العضلية-الطلائية مع عودتها لحالتها بعد فك ربط قناة الغدة .