

**IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY
OF THE STRIATED DUCTS OF THE SUBMANDIBULAR
AND SUBLINGUAL SALIVARY GLANDS OF ALBINO RAT**

By

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INTRODUCTION

The striated ducts of the salivary glands of many mammalian species engage in secretion of organic products in addition to their role in electrolyte homeostasis (Tandler and Phillip, 2000). They are the major site of modification of the electrolyte content of the primary saliva. Na^+ and Cl^- are reabsorbed and K^+ and HCO_3^- are secreted, with a net reduction in tonicity of the saliva (Hand, 1979; Roussa et al., 1998). The organic products include Kallikrein and may be some growth factors, vasoactive substances and digestive enzymes (Tandler and Phillip, 2000).

The resorption and secretion in exocrine glands have been related to S100 proteins (Lauboeck and Egerbacher, 1997). S-100 is a group of closely related, small, acidic Ca^{2+} -binding proteins (S-100a0, S-100a and S-100b). It is structurally related to calmodulin and other Ca^{2+} -binding proteins (Donato, 1986). S100 proteins have no known enzymatic activity and exert their intracellular effects via interaction with and regulation of the activity of other proteins, termed target proteins, in both a Ca^{2+} -dependent and Ca^{2+} -independent manner (Zimmer et al., 2003). A unique feature of these proteins is that individual members are localized in specific cellular compartments from which some are able to relocate upon Ca^{2+} activation, transducing the Ca^{2+} signal in a temporal and spacial manner by interacting with different targets specific for each S100 protein (Heizmann et al., 2002). Within cells, S-100 proteins have been reported to regulate protein phosphorylation, ATPase, adenylate cyclase, and aldolase activities, the dynamics of cytoskeleton components, transcription factors, Ca^{2+} homeostasis, and cell proliferation and differentiation (Donato, 1991, 1999, 2001).

The cells composing the striated ducts are heterogeneous. They consist of principal (light cell types I and II), dark, tuft and basal cells (Sato and Miyoshi, 1998 a,b). Some structural variations among species were observed in both the submandibular and the sublingual salivary glands in rat (Sato and Miyoshi, 1998-a,b), in ferret (Jacob and Poddar, 1987), in hare (Menghi et al., 1984), in white winged vampire bat (Tandler and Phillips,

2002), in mouse (Kurabuchi and Gresik, 2001), in cat (Tandler and Poulsen, 1976) and in African multimammate rodent (Toyoshima and Tandler, 1991). This study is carried out to find the distribution of S-100 protein immunoreactivity in the striated ducts of the submandibular and sublingual salivary glands of male albino rats. The ultrastructural morphology and functional correlation was looked for.

MATERIALS AND METHODS

Five albino male rats aged two months were used in this study. They were anaesthetized with ether and the submandibular and sublingual salivary glands were dissected out. For light microscopy, the right glands were fixed in 10% buffered neutral formalin for 24hs, cleared, embedded in paraffin and cut at 5 μ m thickness. They were stained with S-100 protein immunohistochemistry. The left glands, on the other hand, were prepared for electron microscopy.

Immunohistochemistry: The sections were deparaffinized, hydrated and incubated with blocking solution TBT (Tris Base Saline (TBS) 0.5M, pH 7.4, containing 3% (w/v) bovine serum albumin (BSA) and 0.05% (v/v) Triton X-100) for 30 min at room temperature to reduce non specific binding. The tissue sections were subjected to a preliminary heat induced antigen retrieval step. Sections were then incubated overnight at 4 °C in a humidified chamber with the anti-S-100 mouse antibody (Dako) at a 1:50 dilution. The slides were washed for 5 min in TBS. Immunodetection was performed using biotinylated anti-mouse immunoglobulins followed by incubation with 3, 3'-diaminobenzidine (DAB) chromogen (Dako) in hydrogen peroxide for 5-10 minutes for brown staining. The sections were lightly counterstained with Mayer's hematoxylin, dehydrated and mounted.

Electron microscopy: The left glands were immersed in 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.2) and the capsule was then removed. The glands were kept in the fixative for 30 minutes. Then each gland was held with forceps and was softly tapped with a second forceps with rounded tips then shaken in fixative. The procedure was repeated several times till most of the secretory end pieces were removed and intact ductal tree was obtained (Sato and Miyoshi, 1999). The ductal tree of both glands were kept for additional 2 hours in the same fixative, postfixed in 1% osmium tetroxide in the same buffer, dehydrated with ethanol, cleared in toluene and embedded in Araldite. Semithin and ultrathin sections were cut with an LKB Ultratome III. Semithin sections were stained with toluidine blue while ultra thin sections were stained with uranyl acetate and lead citrate and examined in a JEOL 100S electron microscope.

RESULTS

S-100 immunohistochemistry: Most of the cells in the striated ducts of both the submandibular and sublingual glands showed strong reaction in their cytoplasm. The reaction was usually basolateral but some cells showed apical reaction as well. Few cells showed negative or a weak basal reaction (Figs. 1, 2).

Electron microscopy: Different types of cells: light (types I, II), dark and basal cells were recognized in the striated ducts of the submandibular and sublingual glands. The light cells dominated while the basal cells were the least encountered.

Light cells:

Type I cells:

They were columnar cells extended from the lumen to the basal lamina. Their lateral borders showed cellular projections in the basal two third of the cell which interdigitated with those of the neighbor cells. While the apical one-third of the cells was smooth where opposite cell membranes were joined by junctional complex. The intercellular spaces were narrow in the apical one third and widened basally. The free apical border showed short projections or microvilli. Basal plasma membrane infoldings were well developed and extended from the basal lamina into the basal one third of the cell (Figs. 3, 5).

The euchromatic nucleus occupied the middle third of the cell. It was usually oval and sometimes indented (Fig. 3).

The mitochondria were numerous and were present in the supranuclear and infranuclear regions and extended into the basal infoldings. They were ovoid or elongated and their flattened cristae were oriented in all directions. The Golgi apparatus was small and supranuclear. Short segments of rough endoplasmic reticulum and free ribosomes were scattered in the cytoplasm. Multivesicular bodies and peroxisomes were present in the cytoplasm (Figs. 3, 4).

Vesicles containing granular material were present in the apical cytoplasm. Some of the vesicles were seen fused with the apical plasma lemma (Fig. 4). An irregular protrusion from the cellular apex with constricted base or with a row of vesicles at its base was occasionally found. It contained some secretory vesicles and free ribosomes with no other organelles (Figs. 3, 7).

Tonofibrils were dispersed in the cytoplasm especially in its apical part and converging at the sites of desmosomes (Fig. 8).

Type II cells:

They resembled type I cells except for a bulging apical cytoplasm and less vesicles (Fig. 6).

Dark cells:

They were more numerous in the sublingual than the submandibular striated ducts. They had more electron dense cytoplasm and nucleus than the light cells (Fig. 7). The vesicles in the apical part of the cells were smaller. Some of the vesicles were electron lucent and occasionally elongated like tubules (Fig. 8).

Basal cells:

They lied on the basal lamina and occupied the basal half of the epithelium. They had moderately electron dense, irregular nuclei occupying most of the cell. The basal plasma lemma was smooth and without infoldings. The lateral and apical cell membrane was irregular and showed many projections interdigitating with those of other types of cells. They were connected to other cells by desmosomes and to the basal lamina with hemidesmosomes.

They contained few mitochondria dispersed in the cell. They had also sparse amount of other organelles (Figs. 3, 9).

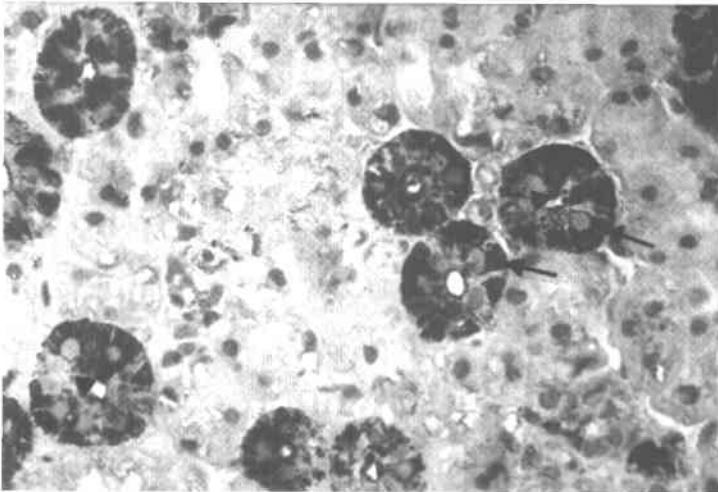


Fig. (1): A photomicrograph of striated ducts of the submandibular gland showing intense baso-lateral S-100 immunoreactivity (arrows) in the cytoplasm of most of the cells with apical reaction (arrowheads) in some cells as well. (S-100 immunoreactivity; X400)

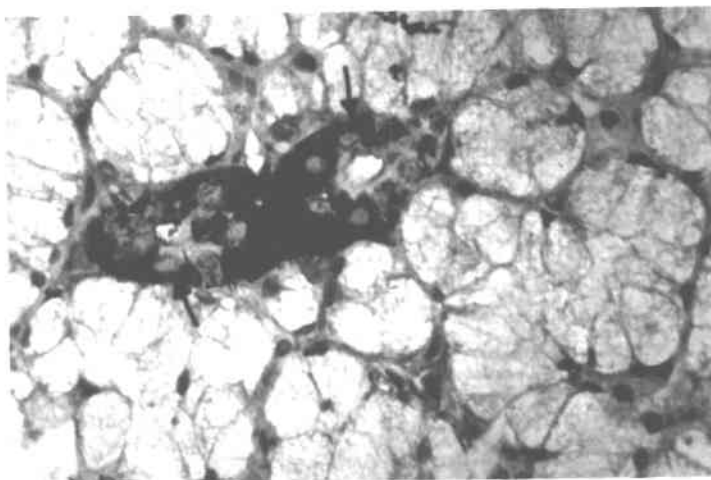


Fig. (2): A photomicrograph of two striated ducts of the sublingual gland showing intense baso-lateral S-100 immunoreactivity (arrows) in the cytoplasm of most of the cells with apical reaction (arrowhead) in some cells as well. (S-100 immunoreactivity; X400)

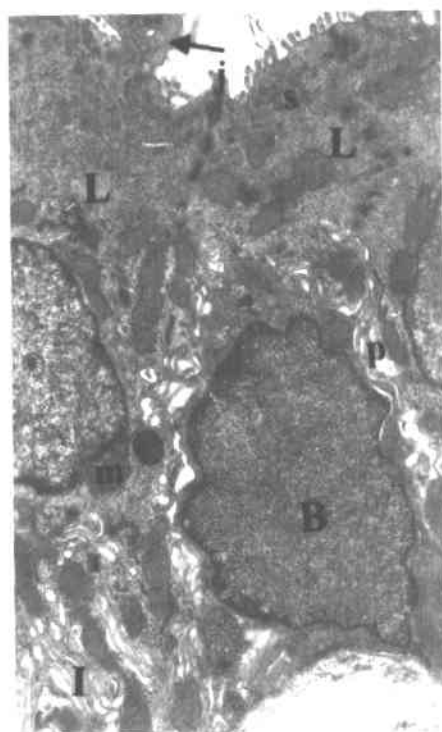


Fig. (3): Electron micrograph showing light cells type I (L) and one basal cell (B) of the submandibular gland. The apical plasma membrane of the light cells has microvilli. A protrusion (arrow) with constricted base is seen. The lateral plasma membrane has junctional complex (j) at its upper part and projections (p) in the lower two-thirds. The basal plasma membrane infoldings (l) are well developed with many mitochondria (m) present in them. Secretory granules (s) are present in the apical part of the cytoplasm. (X7,500)

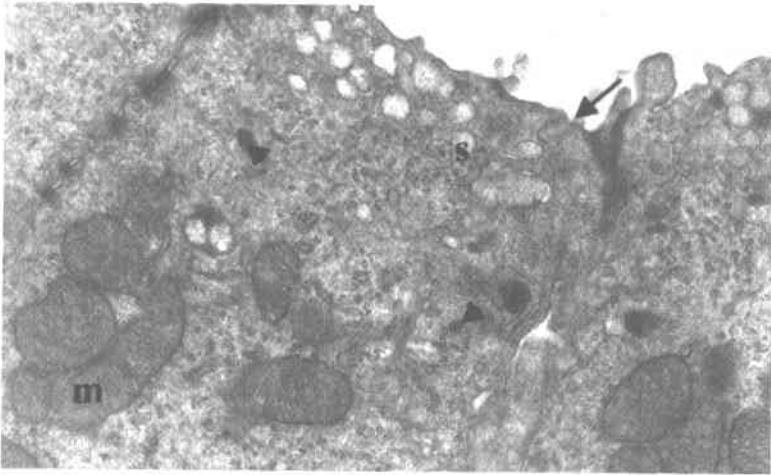


Fig. (4): Electron micrograph showing apical parts of light cells type I of the submandibular gland with secretory granules (s) containing granular material. Some are fusing with the apical plasma membrane (arrows). Peroxisomes are also seen (arrowheads). Flattened cristae are oriented in all directions in the mitochondria (m). (X15,000)

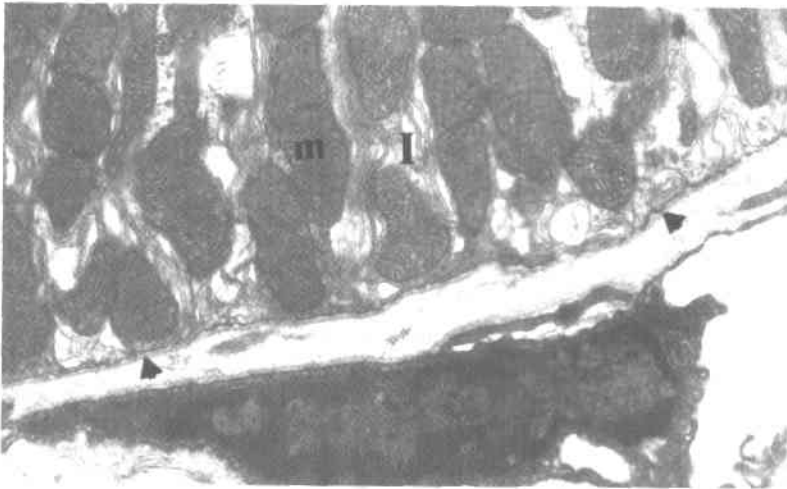


Fig. (5): Electron micrograph showing basal infoldings (l) and related mitochondria (m) in light cell of the sublingual salivary gland. The flattened cristae of the mitochondria are running in different directions. The plasma membrane is attached to the basal lamina with hemidesmosomes (arrowheads). Note: a blood capillary with its endothelial cell lies in close relation to basal infoldings. (X10,000)

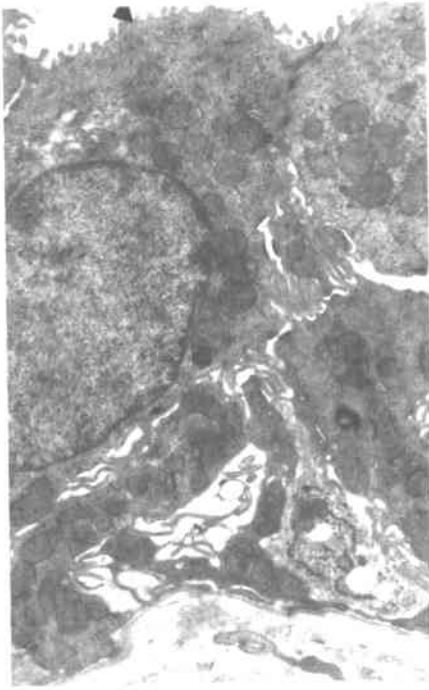


Fig. (6): Electron micrograph showing light cell type II with a bulging apical cytoplasm (arrowhead). (X7,500)

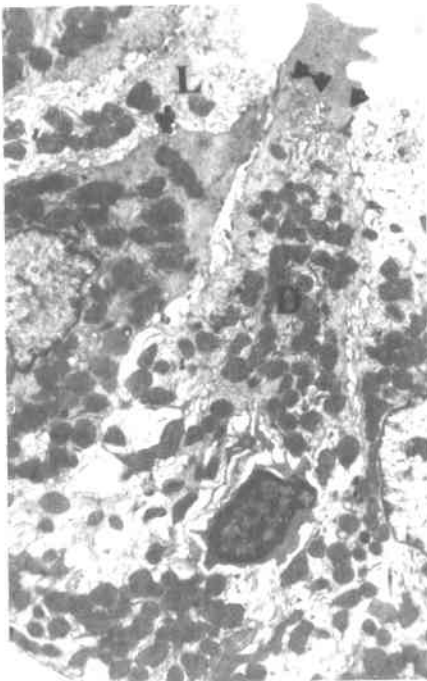


Fig. (7): Electron micrograph showing light (L) and dark (D) cells of the sublingual salivary gland. Apical protrusion from a dark cell with small vesicles (arrowheads) at its base is seen. It contains only free ribosomes. (X4,000)

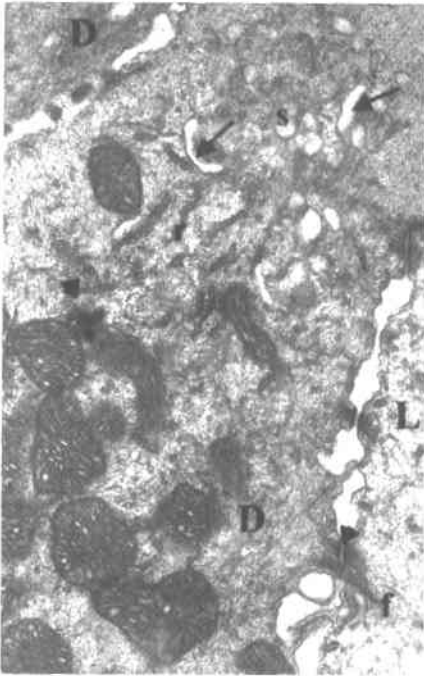


Fig. (8): Electron micrograph showing higher magnification of the vesicles (s) containing granular material in the apical part of the dark cell (D) while some of the vesicles are electron lucent and occasionally elongated like tubules (arrows). Small segments of rough endoplasmic reticulum (r) and multivesicular bodies (arrowhead) are present. Tonofibrils (f) are dispersed in the cytoplasm of the light (L) and dark cells especially in their apical part and converging at the sites of desmosomes (arrowhead). (X13,000)

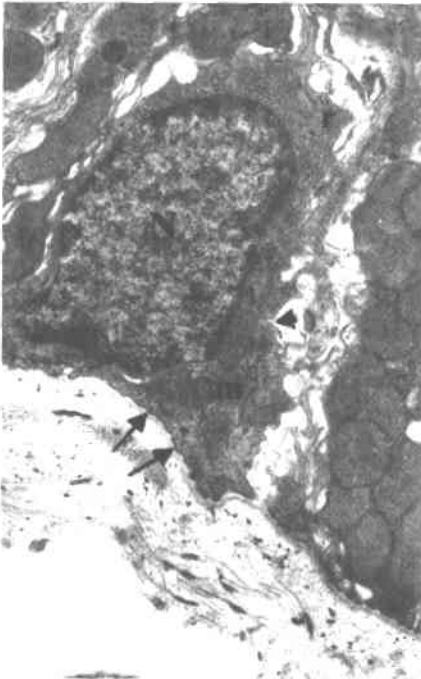


Fig. (9): Electron micrograph showing a basal cell of the sublingual gland. The nucleus (N) occupies most of the cell. The basal plasma membrane shows no basal infoldings and is attached to the basal lamina with hemidesmosomes (arrows). Pinocytotic-like vesicle can be seen at the lateral plasma membrane (arrowhead). Few mitochondria (m) and rough endoplasmic reticulum (r) are seen. (X10,000)

DISCUSSION

S100 proteins are implicated in the immune response, differentiation, cytoskeleton dynamics, enzyme activity, Ca²⁺ homeostasis and growth (**Emberley et al., 2004**). In this study, most of the cells in the striated ducts showed strong basolateral S-100 immunostain, but occasionally apical as well. Some cells were negatively stained or showed weak basal reaction. **Molin et al. (1984)** reported also that S-100 alpha subunit was present in the striated ducts of salivary glands. The alpha subunit of S-100 was related to cells that are highly involved in pH, electrolyte, and water regulation (**Molin et al., 1985**). It has been suggested that S100-alpha is related to resorption and secretion in exocrine glands (**Lauboeck and Egerbacher, 1997**). The variable intensity of the reaction seen in this study may indicate different degrees of activity of the duct cells or may indicate different types of cells. **Roussa and Thevenod (1998)** found that the vacuolar type H⁺-ATPase (V-ATPase) shows diffuse intracellular distribution in both rat submandibular and sublingual salivary glands. Apical V-ATPase distribution was also detected in 5-6% of striated duct cells. It has been suggested that V-ATPase could be involved in the regulation of acid-base homeostasis by allowing a vectorial H⁺ transport from the cell into the lumen of the duct system (**Roussa et al., 1998**). As S-100 proteins have been reported to regulate enzyme activity like ATPase (**Donato, 1991, 1999, 2001**). Thus the distribution of S-100 protein immunoreactivity in most cells of the striated ducts with apical reactivity in some cells may be related to V-ATPase activity in these cells.

In the present study, the epithelium of the striated ducts was mainly of the light cells with lesser number of dark and basal cells. In the sublingual gland, the dark cells were more numerous than in the submandibular gland. The principal cells had vesicles, containing granular material, in the apical cytoplasm. The dark cells were characterized by regular microvilli, and many clear tubules and vesicles confined to the apical cytoplasm. This in agree with earlier reports by **Sato and Miyoshi (1998-a,b)**. The apical secretory granules have been found to contain Kallikrein in the parotid gland striated ducts (**Simson and Chao, 1994**). The kallikrein-containing granules undergo exocytosis at the cell apex (**Yahiro and Nagato, 2002**). However, **Penschow and Coghlan (1993)** stated that renal/pancreatic kallikrein (which has local vasoactive effects) was apparently secreted constitutively from the basolateral surface of striated duct cells whereas apical secretion occurred via the regulated pathway.

Yamamoto-Hino et al. (1998), using immunoelectron microscopy, identified small apical vesicles bearing inositol 1,4,5-trisphosphate receptors (IP₃Rs) in the apical pole of some cells of the submandibular gland ducts. This suggested that the apical vesicles might function as an intracellular Ca²⁺ pool. Certain "dark" and "principal" cells had a large amount of IP₃R2 (**Segawa et al., 1996**). **Yamamoto-Hino et al. (1998)** noticed that

after carbachol stimulation, an initial Ca^{2+} spike occurred in the apical region. Subsequently, repetitive Ca^{2+} spikes spread from the apical to the middle cytoplasm. These apical Ca^{2+} initiation sites were found only in some "pioneer cells," rather than in all duct cells. So, they suggested that any type of cell that expresses large amount of IP_3R_2 may function as a pioneer cell. In this study, some cells of the striated ducts showed intense S-100 reaction in their cytoplasm including the apical part. As S-100 protein regulates Ca^{2+} homeostasis, so it is possible that these cells may correlate with the cells that express large amount of IP_3R_2 .

Hand et al. (1987) reported that striated ducts may be involved in reabsorption of organic material from primary saliva. **Matsuoka et al. (2000)** demonstrated uptake of cationized ferritin by the small vesicles and multivesicular bodies of the light (types I and II) and dark cells in the epithelium of the main excretory duct of the rat submandibular gland. Almost all of the internalized proteins appeared to be processed by the lysosomal system, and some proteins were released into the basal extracellular spaces. They noticed uneven distribution of ferritin-containing vesicles, and suggested that the ferritin-free vesicles may belong to the secretory system.

Apical protrusions or blebs observed here and in earlier reports (**Testa-Riva et al., 1981; Messelt, 1982**) seem to detach from the main cell body by fusion of the vesicles at their base. They contained some secretory vesicles and free ribosomes with no other organelles. They were considered as manifestation of apocrine secretion (**Testa-Riva et al., 1981; Messelt, 1982**).

In this study, and in correspondence with earlier reports (**Hand, 1979; Sato and Miyoshi, 1998-a,b**) the basal and lateral plasma membrane of the light and dark cells exhibited extensive infoldings and interdigitated with processes from adjacent cells. Many mitochondria were present in them. Na,K-ATPase has been demonstrated on basolateral cell membranes (**Simson and Chao, 1994**). These characteristics indicate that they are involved in fluid and electrolyte transport with the result of hypo-osmolarity of the final saliva (**Hand, 1979**).

The basal cells had indented nuclei occupying most of the cell. They showed fewer mitochondria and no basal infoldings. These cells were regarded as reserve cells. Strong immunoreactivity for carbonic anhydrase III (CA-III) isozyme has been demonstrated in basal cells. **Asari et al. (1993)** suggested that they may play a special physiological role, and that they do not only represent undifferentiated lining cells. CA III may function as an oxyradical scavenger and thus protects cells from oxidative stress (**Christie et al., 1997**), which may play a role as a common mediator of apoptosis. Thus CA III may be an important protective mechanism against oxidative damage and reactive oxygen species-induced cell death (**Räisänen et al., 1999**).

SUMMARY

In this study, the submandibular and sublingual salivary glands from five albino male rats aged two months were subjected to S-100 immunohistochemistry and electron microscopy. Most of the cells in the striated ducts of both the submandibular and sublingual salivary glands showed strong S-100 immunoreactivity in their cytoplasm. The reaction was usually basolateral but some cells showed apical reaction as well. Light (types I, II), dark and basal cells were recognized in the striated ducts of the glands with electron microscope. The dark cells had more electron dense cytoplasm and nucleus than the light cells. Both cells had secretory granules in their apical cytoplasm. The dark cells, however, showed some electron lucent vesicles that were occasionally elongated like tubules. The basal plasma membrane showed extensive infoldings with many mitochondria extended into them. The lateral plasma membrane showed projections that interdigitated with the adjacent cells. The basal cells occupied the basal half of the epithelium. They had moderately electron dense, irregular nuclei occupying most of the cell. The basal plasma lemma was smooth.

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الملخص العربي

دراسة هستوكيميائية مناعية للقنوات المخططة و تركيبها الدقيق

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منى عبد الرحيم الشحات

قسم التشرييح ، كلية الطب ، جامعة المنصورة

فى هذه الدراسة تم استخدام خمسة ذكور من الفأر الأبيض بعمر شهرين. وقد تم تشريح الغدتين تحت الفكبة وتحت اللسان بعد التضحية بالفئران. وقد تم استخدام الغدد اليمنى فى كل فأر فى عمل شرائح صبغت ب اس ١٠٠ الهستوكيميائية المناعية. أما الغدد اليسرى فقد حضرت للفحص بالميكروسكوب الالكترونى. وقد وجد أن معظم خلايا القنوات المخططة فى الغدتين أظهرت تفاعل قوى مع الاس ١٠٠. وقد تركز هذا التفاعل فى المناطق القاعدية والجانبية من الخلايا مع وجود تفاعل فى قسم بعض الخلايا. أما الفحص بالميكروسكوب الالكترونى فقد أظهر وجود عدة أنواع من الخلايا: الخلايا الفاتحة (النوع الأول والثانى) والخلايا الغامقة والخلايا القاعدية. وكان السيتوبلازم والنواة أكثر كثافة الكترونية فى الخلايا الغامقة عنه فى الخلايا الفاتحة. وقد تميز كلا النوعين بوجود حويصلات تحتوى على مواد محببة فى السيتوبلازم عند قسم الخلايا. ولكن الخلايا الغامقة تميزت بوجود حويصلات شفافة الكترونية والتي كانت فى بعض الأحيان تمتد كأنابيب. وقد أظهر الجدار الخلوى القاعدى كثير من التثنيات التى تحتوى على الميتوكوندريا. أما الجدار الخلوى الجانبى فقد برزت منه زوائد تداخلت مع غيرها من الخلايا المجاورة. وقد تميزت الخلايا القاعدية بوجود نواة متوسطة الكثافة تحتل معظم الخلية وجدار خلوى قاعدى بدون تثنيات.

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