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Scanning Electron Microscopy of Human Submandibular Gland

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Abstract. The fracture surface of human submandibular gland analyzed by scanning electron microscopy is studied here. Acini showed spherical granules of 0.7 ± 0.28 μm diameter, their most distinctive feature. Some empty, septate cavities found contiguous to serous acini were considered to be mucous acini. Striated ducts had a circular lumen, with microvilli forming prominences. Blebs, some intact and others ruptured, were interpreted as apocrine secretion. The 'separating zone' of the striated cells was distinguishable from the rest of the cell because the structure of the cell was granular whereas the 'separating zone' was fibrillar.

Introduction

Extensive studies exist of the histology and submicroscopic structure of the human submandibular gland [1–6]. However, recent studies report some information obtained by scanning electron microscopy (SEM). *Testa Riva* et al. [7] correlated SEM and TEM (transmission electron microscopy) analysis of the excretory duct.

In rat and seal the SEM provided information on the apocrine secretion of striated ducts [8–10]. In rats, correlated SEM-TEM studies have been more complete, as *Shackleford and Schneyer* [11] studied the excretory duct, and *Brocco and Tamarin* [12] the topography of their parenchymal components. Analysis of freeze-fractured surfaces by SEM also yielded interesting information on their internal structure [13].

As the observation of the inner structure of human salivary glands by SEM is not complete, the aim of this study has been to add to present knowledge of the intralobular arrangement of the human submandibular gland, and also of the morphological aspects of its secretions and its correlation with other animal salivary glands.

Materials and Methods

Specimens of submandibular glands were obtained in the operating rooms of the Head and Neck Department of the Angel Roffo Oncology Hospital. Specimens were taken from 12 patients of both sexes ranging in age from 32 to 67 years, who underwent surgery of the neck region for removal of primary tumors of the neck or metastatic lymph nodes in the cervical region, where submandibular gland had to be ablated. All the patients were operated on under general anesthesia with Narcayne and all had been treated previously with atropine. Analeptics and a muscle relaxant (succinylcholine) were also administered as required.

A portion of each specimen was fixed in 10% formalin, embedded in paraffin and stained with HE and Gomori's trichrome staining as controls.

For SEM observations, portions of the glands were fixed in 2.5% glutaraldehyde for 24 h, washed in multiple changes of cacodylate buffer (pH 7.4) and dehydrated in graded acetone. Following dehydration, the specimens were submerged in liquid nitrogen, and fractured with the aid of a chilled scalpel. The samples were then transferred to chilled 100% acetone and critical-point dried [14].

The specimens were mounted in aluminum stubs with silver conductive paint, and vacuum-coated with carbon and gold palladium. The observations were made in a Jeol S-25 Scanning Electron Microscope accelerated at 15 kV.

Results

All the submandibular glands were normal under light microscopy. As it is classically described, the submandibular gland is a mixed gland composed of seromucous acini (traditionally termed serous) and mucous acini. Serous acini greatly outnumber the mucous portion.

In the observation of the fractured surface of the serous acini by SEM, the secretory granules were the

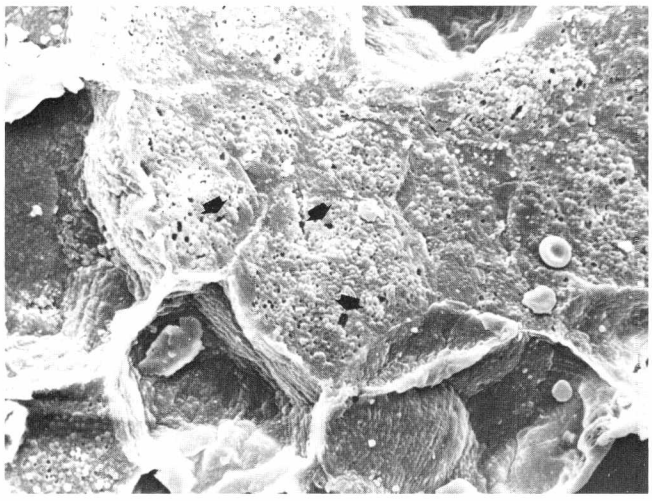

Fig. 1. Acini in a sector of a submandibular gland, some clearly defined with secretory granules and some lumina (arrows). Empty spaces were possibly left by fat lost in the procedure. ×3,000.

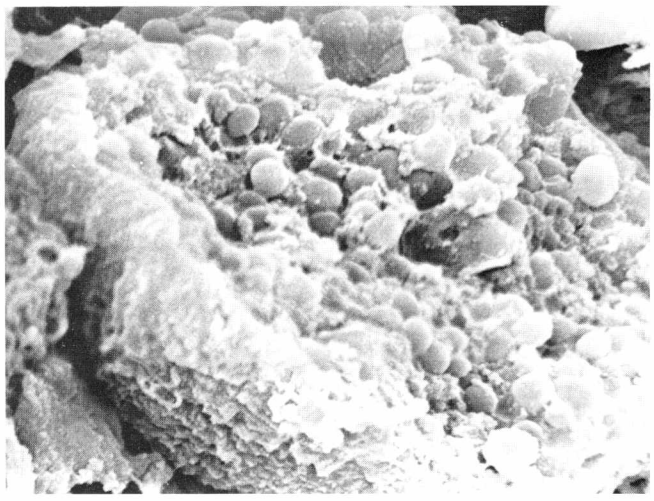

Fig. 2. External surface and cytoplasm of an acinus; note secretory granules. ×6,800.

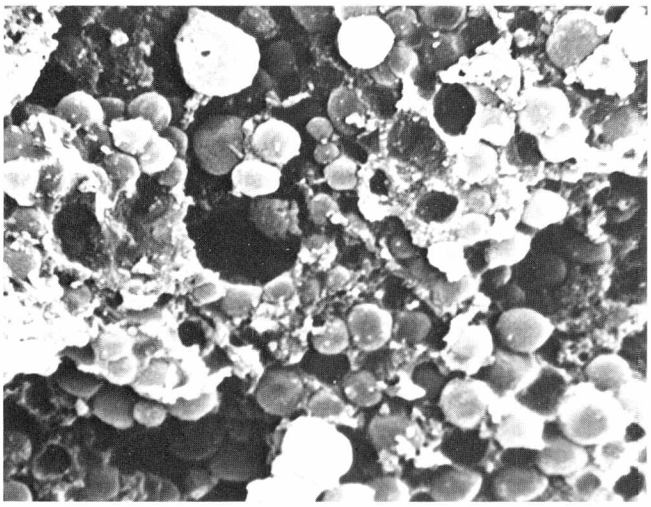

Fig. 3. Secretory granules in the acinar cytoplasm. Diameter: 0.7 ± 0.28 μm. ×9,000.

most striking feature. They were round and their mean diameter was 0.70 ± 0.28 μm (fig. 1–3). Where these granules were absent, a circular cavity with the shape of the implantation of the granule was observed. The lumen was not often seen. When it was, it had an irregular shape and was always empty, although some granules protruded in the lumen. The acini sometimes contained cavities, not centrally located, thought to be accesory lumina (fig. 1).

Several large empty cavities surrounding the acini (fig. 1) were frequently observed, and were considered to be lipid material. This partial replacement of the salivary parenchyma was also observed by light microscopy. With SEM, empty, rounded cavities septated by laminae forming smaller spaces (possibly left by secretion lost by technical artifacts), and contiguous with serous acini, were seen. The characteristics and disposition of these structures closely resembled mucous acini. Intercalated ducts were observed. The lumen was circular, with no structure within. The wall of the lumen was rough but without bleb formation. The cells were lower than those found in striated ducts. Striated ducts had a circular lumen with a thick wall. In it, two different zones were distinguishable. The sector limiting the lumen had a smoother structure than the rest of the wall. In the outer portion, the cytoplasmic material was condensed into small granules, thus producing a granular appearance, occupying 2/3 or more of the cell. Some nuclei or material derived from them could be observed when the cleavage plane passed through these structures. The nuclei had a granular material within them (fig. 4). Numerous microvilli protruded from the lumen, and a few blebs were also seen, some of which had ruptured membranes. In addition, extensive longitudinal and perpendicular folds were observed (fig. 5).

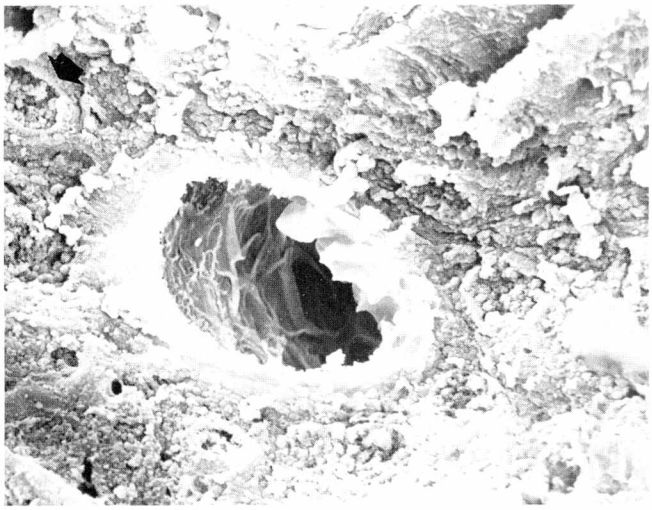

Fig. 4. Striated duct. Observe a nucleus (arrow), the granular appearance of the cytoplasm, and a fibrillar portion, possibly the 'separating zone'. ×2,900.

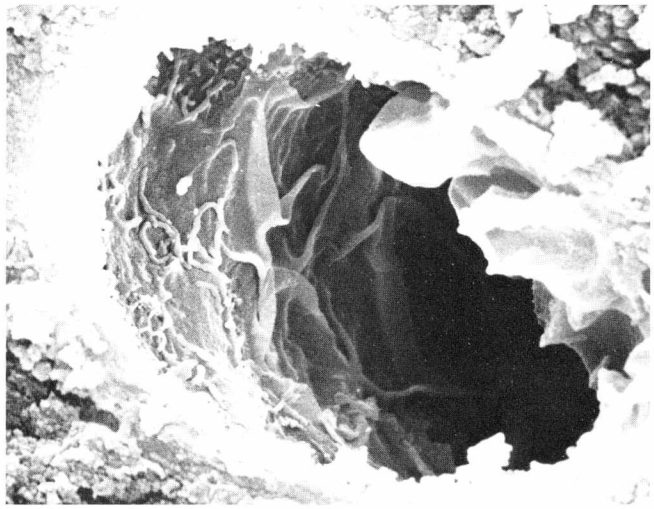

Fig. 5. Lumen of the striated duct shown in figure 4. Observe the microvilli, some blebs, and a rupture in one of them. Foldings result from continuous modification of this wall, due to cellular membranes left from former blebs. ×10,200.

Discussion

In this study, SEM observations of human SBM gland have been described. SEM was especially useful for the observation of shape and size of secretion, because serous granules could be measured. Although TEM studies of human SBM glands have accurately described the detailed morphological appearance of the secretory granules [6], observations by SEM provided a view of the whole granules, thus adding knowledge of their actual shape, size, and distribution. Although fixation modifies the structure of secretory granules [15], studies with SEM applied to rat salivary glands showed that serous acini (parotid gland) were loaded with spherical granules [16], just as was observed in the human submandibular glands. The method we used demonstrated that it is useful for the observation of serous material. In contrast, mucous material seemed to be lost, as we observed in preliminary studies of the sublingual gland of rats. Consequently, empty and septate cavities in contact with serous acini, and similar in diameter to the mucous acini measured by light microscopy, were interpreted as mucous acini.

In the lumina of the striated ducts, microvilli were easily observed. In several of them, some blebs of secretion protruded from the wall, sometimes with ruptures of their membrane, allowing secretion to escape. Foldings were consistently observed in the lumen, and were thought to be membranes that cover the blebs after they empty. Some cellular or membrane debris were also seen. Because of these characteristics, this surface would seem to undergo continuous modification. The blebs were not numerous, but these glands were not stimulated. Our findings in human striated ducts by SEM agree with *Messelt* [8, 9] and *Messelt and Dahl* [10] in their analysis of striated ducts of seal and rat submandibular glands. *Testa Riva* [7] also described apocrine secretion in the human excretory duct.

In some striated ducts, two different zones in their wall might be distinguished. The most extended appeared as a granular structure and occupied almost the entire cytoplasm. However, the area surrounding the lumen had a fibrillar or smoother surface, possibly taking up the 'separating zone'.

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References

1 Zimmermann, K.W.: Die Speicheldrüsen der Mundhöhle und die Bauchspeicheldrüse; in von Möllendorf, Handbuch der mikroskopischen Anatomie des Menschen, vol. 5, pp. 61–244 (Springer, Berlin 1927).

2 Babkin, B.P.: Secretory mechanism of the digestive glands (Hoeber, New York 1950).

3 Rauch, S.: Die Speicheldrüsen der Mensehen (Thieme, Stuttgart 1959).

4 Tandler, B.: Ultrastructure of the human submaxillary gland. I. Architecture and histological relationships of the secretory cells. Am. J. Anat. *111:* 287–308 (1962).

5 Tandler, B.; Erlandson, R.A.: Ultrastructure of the human submaxillary gland. IV. Serous granules. Am. J. Anat. *135:* 419–434 (1972).

6 Testa Riva, F.: Ultrastructure of human submandibular gland. J. submicrosc. Cytol. *9:* 251–266 (1977).

7 Testa Riva, F.; Puxeddu, P.; Riva, H.; Diaz, G.: The epithelium of the excretory duct of the human submandibular gland: a transmission and scanning electron microscopic study. Am. J. Anat *160:* 381–399 (1981).

8 Messelt, E.B.: Ultrastructural studies on the bleb formation in seal and rat submandibular gland striated ducts. Acta odont. scand. *40:* 25–33 (1982).

9 Messelt, E.B.: Ultrastructural studies on apical blebs of striated ducts in the rat submandibular gland. Acta odont. scand. *40:* 103–111 (1982).

10 Messelt, E.B.; Dahl, E.: Ultrastructure of the 'separating zone' of rat submandibular gland. Acta odont scand. *40:* 249–256 (1982).

11 Shackleford, J.M.; Schneyer, L.H.: Ultrastructural aspects of the main excretory duct of rat submandibular gland. Anat. Rec. *169:* 679–696 (1971).

12 Brocco, S.L.; Tamarin, A.: The topography of submandibular gland parenchyma as observed by SEM. Anat. Rec. *194:* 445–460 (1979).

13 Espinal, E.G.; Ubios, A.M.; Cabrini, R.L.: Freeze-fracture surface of salivary glands of rat as observed by scanning electron microscopy. Acta anat. *117:* 15–20 (1983).

14 Anderson, T.F.: Techniques for the presentation of three-dimensional structure in preparing specimens for electron microscope. Trans. N.Y. Acad. Sci. *13:* 130–134 (1951).

15 Simson, J.A.V.; Dom, R.M.; Saunes, P.L.; Spicer, S.S.: Morphology and cytochemistry of acinar secretory granules in normal and isoproterenol-treated rat submandibular gland. J. Microscopy *113:* 185–203 (1978).

16 Sodicoff, M.; Pratt, N.E.; Trepper, P.; Sholley, M.M.; Hoffenberg, S.: Effects of X-irradiation and the resultant inanition on amylase content of the rat parotid gland. Archs oral Biol. *22:* 261–267 (1977).

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