Acta anat. *117*: 15–20 (1983)

0001-5180/83/1171-0015 $ 2.75/0

Freeze-Fracture Surface of Salivary Glands of Rat Observed by Scanning Electron Microscopy

E. G. Espinal, A. M. Ubios, R. L. Cabrini

Department of Pathology, Faculty of Dentistry, University of Buenos Aires and Department of Radiobiology, National Atomic Energy Commissión, Conicet, Buenos Aires, Argentina

Key Words. Scanning electron microscopy · Salivary glands · Secretory granules · Cryofracture · Submandibular gland

Abstract. The structure of parenchymal components of rat submandibular glands observed with scanning electron microscopy is presented. The glands were fixed, submerged in liquid nitrogen, cryofractured, dehydrated and critical-point-dried. The fracture surface displayed the acini, granular ducts and striated ducts. Acini exhibit a typical sponge-like pattern of irregular and empty cavities. Granular ducts are lined by bulging cells laden with numerous secretory granules. Their diameter ranged from 0.2 to 3.2 μm, with a mean value of 1.240.4 μm. Measurement of the actual granules was rendered possible by direct observation. Striated ducts exhibited a cribiform pattern near the basal cell region which corresponds to infoldings of the basal cell membrane.

Introduction

The cytological characteristics of salivary glands have been described at light and electron microscope levels [1–3].

Only few studies with the scanning electron microscope are available on salivary glands or related structures. They are concerned with the inner structure of the submandibular duct [4], the myoepithelial cells of rat sublingual gland [5] and the topography of the parenchymal components of the submandibular gland [6].

The present sutdy involves the scanning electron microscopy of submandibular glands after freeze-fracture. This method allows the observation of inner structures which have not been described by scanning electron microscopy before.

Materials and Methods

Adult male Wistar rats, weighing approximately 250 g, were anesthesized with ether from 8 to 10 a.m. The submandibular glands (SBM) were removed and

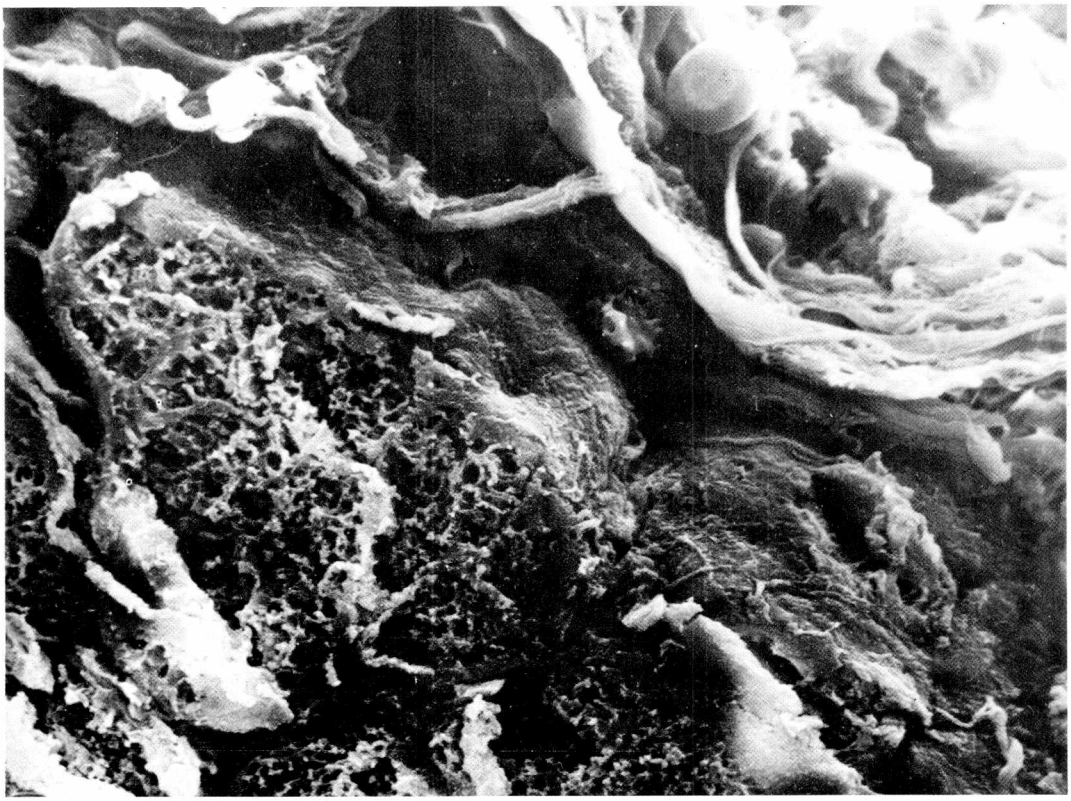

Fig. 1. Acini showing a sponge-like pattern. Cavities are surrounded by anfractuous walls. Their external rough surface is lobulated. Collagen fibers of the capsule can also be observed. ×2,500.

sublingual glands were dissected subsequently. 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 transferred to chilled 100% acetone and critical-point-dried [7].

The specimens were mounted on aluminium stubs with silver conductive paint, and vacuum-coated with carbon and gold paladium. The observations were made in a JEOL S 25 scanning electron microscope at 15 kV.

In one case a large portion of the gland was photographed at 1,000×, and the prints used as a photomontage. After its observation, the same sample was embedded in paraffin and the first section obtained was mounted and stained with hematoxylin-eosin. A projection of this section was superimposed on the photomontage for comparison of both images.

The size of secretory granules was obtained by measuring their diameter on projections of negatives taken with the same magnification and tilt. Measurements were made of 628 granules from 10 different granular ducts.

Results

The specimens revealed the structure of the different components of the gland on the fractured surface. Spaces tend to open up between them.

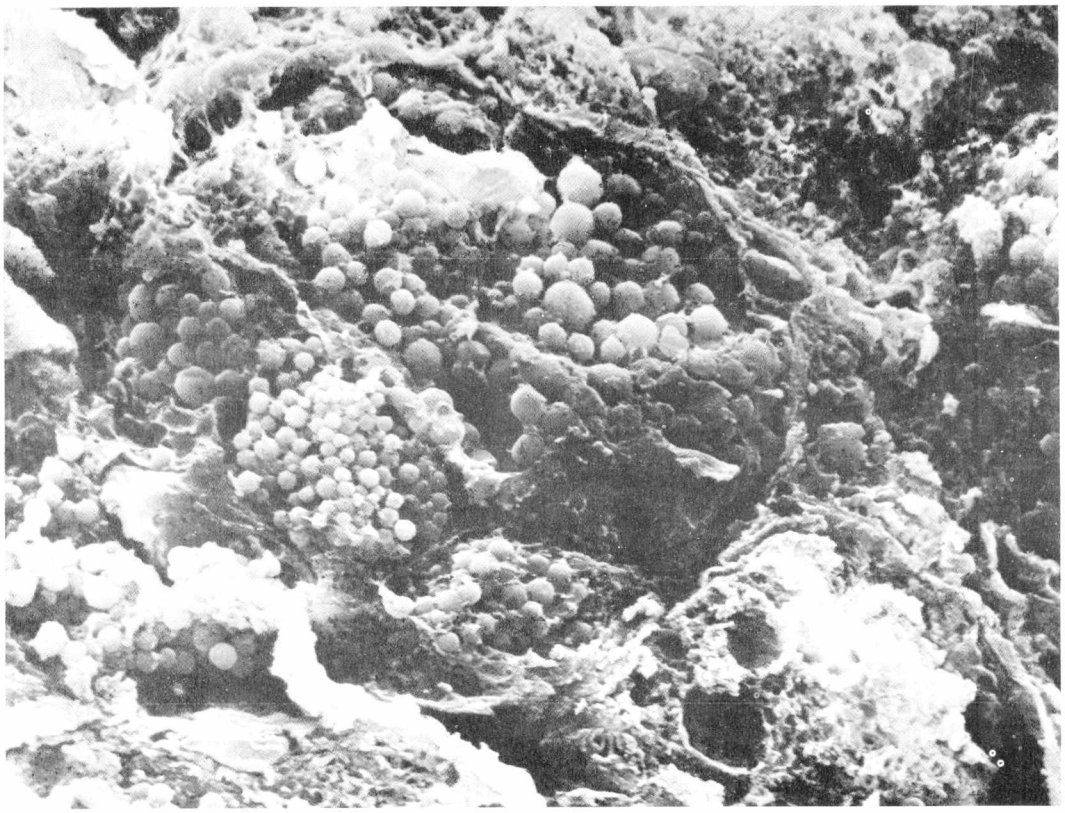

Fig. 2. Transverse section of a granular duct showing secretory granules. Imprints left by their loss are observed. Some acini surround the duct. ×2,500.

When the projection of the paraffin section was superimposed on the scanning electron microscope photomontage, a clear coincidence of the glandular structures was observed.

Collagen bundles appeared dividing the gland into lobules and surrounding the acinar structure of an irregular ellipsoidal shape. The inner structure of the acini presented a cribiform pattern that resembled a sponge (fig. 1). Anfractuous walls limited cavities of different shapes. On occasions, the fusion of two or more cavities was observed. A few rounded wider cavities which seemed to extend inwards were taken to represent the lumen. Spherical bodies of the shape and size of nuclei were seen close to the basal membrane of the cell.

The granular ducts are lined by bulging cells. In longitudinal sections the sinuous course of the ducts was easily observed. The lumen was not evident in some sections, but in others it was seen as an irregular channel.

Secretory granules deformed the membrane, protruding into the duct lumen (fig. 2).

As shown in figure 3, secretory granules, spherical in shape and of different sizes, fil-

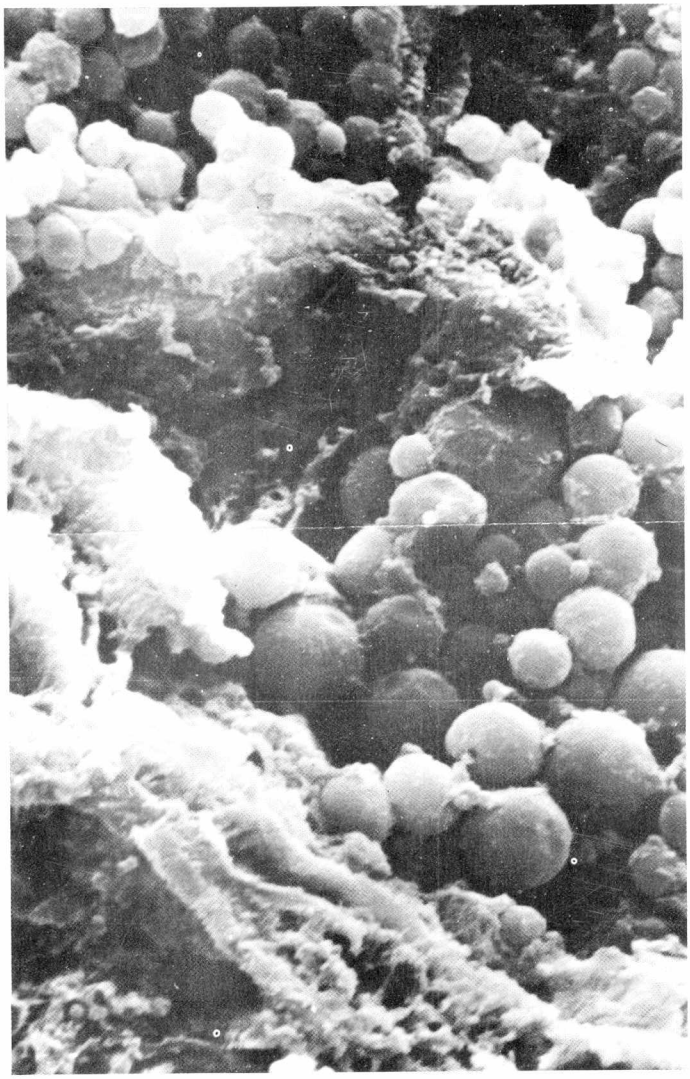

Fig. 3. Secretory granules are spherical in shape and of different sizes. ×8,000.

led the granular ducts. Diameter values were in the range of 0.2–3.2 μm, mean =1.24±° 0.4 μm. Their size distribution is shown in figure 4. When observed in the duct lumen, the structure of the external surface of the granules remained intact. When the granules were lost, a dark and spherical imprint was left in the cell (fig. 2).

In those cells laden with secretory granules, nuclei were confined to the basal membrane area. Those cells where the inner basal region could be observed exhibited a cribiform pattern with cavities surrounded by membranes. Round or elongated bodies about 1 μm in diameter were present both in these structures and in striated ducts.

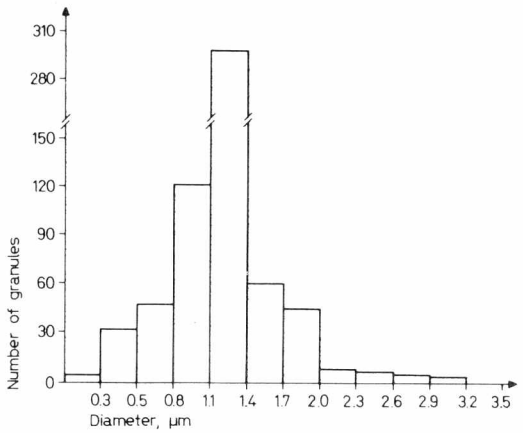

Fig. 4. Histogram showing the granular size distribution. There is a large population of granules of median size, between 1.1 and 1.4 µm, coincident with the mean = 1.24 µm.

Striated ducts could be observed in transverse as well as in longitudinal sections. They were composed of high cells with a cribiform pattern in their basal third and containing the small bodies described for the granular ducts. The nucleus was clearly observed in most of these cells. It was round and centrally located, presenting a rough surface. Where the nucleus was sectioned by the cleaving plane, dense, irregular, tightly-packed masses of chromatin were seen. The lumen was wide and generally contained a laminar structure (fig. 5).

Discussion

Data available in the literature about the scanning electron microscope image of salivary glands are concerned with their outer structure. This provides only partial information. The study of the internal structure of such glands is of greater biological significance. The freeze-fracture techniques described for other organs made such a study possible [8].

The sponge-like array of irregular and empty cavities of acini were seen to be lined by rough walls. The water content or chemical composition of secretory granules would cause their dissapearance during specimen preparation, leaving the aforementioned cavities.

The most striking observations on the

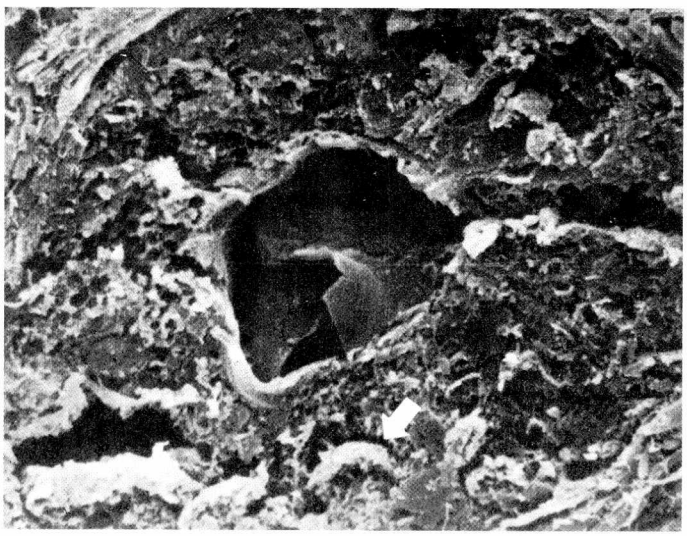

Fig. 5. Striated ducts exhibit a cribiform pattern. The lumen is wide and presents a laminar structure within. The rough external surface of some nuclei and others which are fractured arrow can also be observed. ×4,000.

fractured surface were the granular ducts and their content. The morphology observed by scanning electron microscopy is closely comparable to that described by *Tamarin and Sreebny* [1], and others by transmission electron microscopy [2, 3]. The apical cytoplasm of the cells tended to bulge. The cell cytoplasm was laden with secretory granules whereas the nuclei were located basally.

The measurement of the granules with transmission electron microscopy is altered by the incidence of the section. Because the whole structure of the secretory granule is present, scanning electron microscopy permitted the direct observation of the granular shape and the measurement of its true diameter. The measurement of 628 granules revealed that their diameters are in the range of 0.2–3.2 μm. Most of the granules are of medium size, with a diameter between 0.9 and 1.4 μm, coincident with the media. Their distribution showed a deviation to the left which could represent different secretory stages of the same granular type as proposed by *Scott and Pease* [2].

In our observations, those cells in which smaller and intermediate granules predominated seemed to be more numerous than those containing the larger ones.

Differential conservation of secretory granular ducts and acini may be due to varying chemical composition and water content.

The striated ducts exhibited a cribiform pattern in the basal third of their cells, which could correspond to their characteristic infoldings and interdigitations observed by transmission electron microscopy [1, 3].

Cryofracture offers the possibility of a different morphological visualization of salivary glands under physiological and pathological conditions.

Acknowledgments

The observations were made in the scanning electron microscope of the Faculty of Dentistry, University of Buenos Aires (UMIEBFO). The authors wish to acknowledge the technical assistance of *Dante Giménez* and *Guillermo Garbino*. Critical-point drying and vacuum coating were performed in the Instituto de Neurobiología, CONICET, Buenos Aires.

References

1 Tamarin, A.; Sreebny, L. M.: The rat submandibular salivary gland. A correlative study by light and electron microscopy. J. Morph. *117:* 295–352 (1965).

2 Scott, B. L.; Pease, D. C.: Electron microscopy of salivary and lacrimal glands of the rat. J. Anat. *104:* 115–161 (1959).

3 Leeson, C. R.; Jacoby, F.: An electron microscopic study of the rat submaxillary gland during its postnatal development and in the adult. J. Anat. *93:* 287–295 (1959).

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

5 Nagato, T.: Scanning electron microscopical image of myoepithelial cells. J. electron Microsc. *27:* 235–236 (1978).

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

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

8 Brooks, S. E. H.; Haggis, G. H.: Scanning electron microscopy of rat's liver. Application of freeze-fracture drying techniques. Lab. Invest. *29:* 60–64 (1973).

Received: November 30, 1982
Accepted: January 15, 1983

Dra. Elena G. Espinal,
Comisión Nacional de Energía Atómica,
Laboratorio de Radiobiología,
Adva. del Libertador 8250,
1429 Buenos Aires (Argentina)