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RAPID COMMUNICATION

A New Mutation Involving the Sublingual Gland in NFS/N Mice

Partially Arrested Mucous Cell Differentiation

YOSHIO HAYASHI, DDS, D Med Sci, AKINORI KOJIMA, MD, MAMI HATA, BP, and KATSUIKU HIROKAWA, MD

From the Department of Pathology, Tokyo Metropolitan Institute of Gerontology, Tokyo, and the Laboratory of Experimental Pathology, Aichi Cancer Center Research Institute, Aichi, Japan

A new mutation in mice affecting the mucous cell differentiation of the sublingual glands is described. The normal mouse sublingual glands are mucus-secreting and virtually all the acinar cells differentiate to mucus-rich cells by the day of birth. In contrast, all endpieces of newborn mutant mice consisted of acini of immature cuboidal cells. However, normal mucous cells, staining intensively with mucin-specific stains such as Alcian blue at pH 2.5 or mucicarmine, appeared in the mutant mice from an early age singly or in groups in a small number of acini, and their number apparently increased with age to occupy over 30% of the total acinar cells. Ultrastructurally, irregular secretion granules of varying electron-density, distinct from ordinary sublingual mucin granules, were frequently observed in the cytoplasm of the immature acinar cells in the mutant phenotype. The genetic analysis showed that a single autosomal recessive gene determined the observed abnormality. This is the first salivary gland mutation and will provide a critical model for the study of salivary mucous cell differentiation. (Am J Pathol 1988, 132:187–191)

IT HAS BEEN SHOWN that the salivary acini are composed of highly differentiated serous and mucous cells. In the sublingual glands of many species, the mucus-secreting cells are the predominant component, although there are scattered, purely serous acini occurring as demilunes capping otherwise normal mucous tubulo-acini. A well-known difference between serous and mucous cells is that the former contain secretory granules rich in various proteolytic enzymes, whereas the latter do not.[1-3] Therefore, the distinction between the serous and mucus glands is based on the nature of the secretion products. Conventionally, the morphologically defined mucus-secreting cells can be stained with various mucin-specific stains.[1-3]

A new spontaneous autosomal recessive mutation occurring in the inbred mouse strain NFS/N severely inhibits the acinar cells of the sublingual gland from differentiating to the mucus-secreting cells. This new mutation has provisionally been named sublingual gland differentiation arrest, with the gene symbol *sld*.[4] This study reports data on its natural course and basic histopathology.

Materials and Methods

Animals

The NFS/N strain mice carrying the mutant gene *sld* were obtained as breeding pairs from Dr. T. Nomura of the Central Institute for Experimental Animals, Kawasaki, Japan, and propagated for experimental use. A congenic normal strain, NFS/N("+/

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Address reprint requests to Y. Hayashi, DDS, Department of Pathology, Tokyo Metropolitan Institute of Gerontology, Sakaecho, Itabashi-ku, Tokyo 173, Japan.

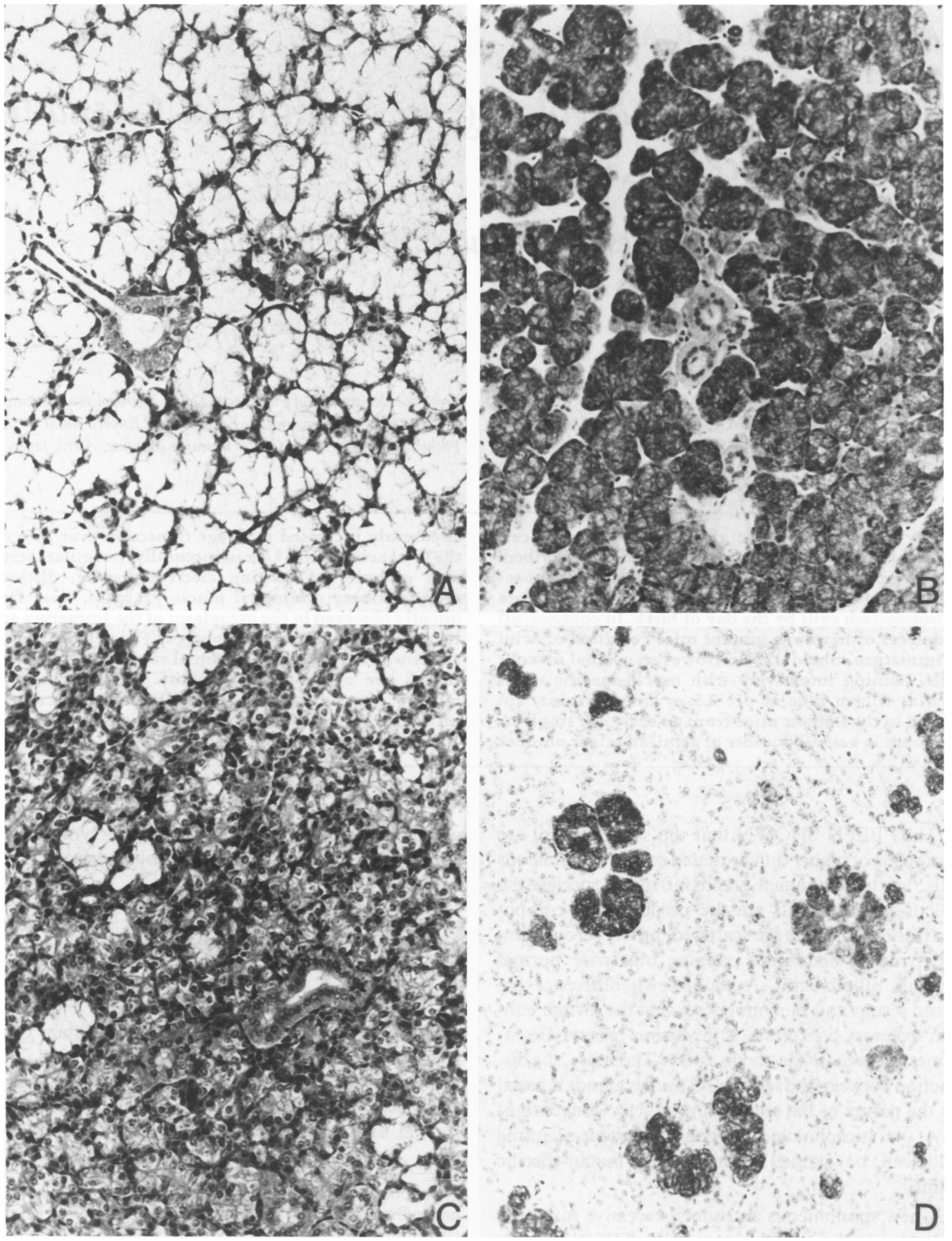

Figure 1—Histologic appearances and mucin-specific stains of the sublingual glands in mice. Photomicrographs of a normal (+/+) mouse at 6 weeks of age composed of predominantly mucous acini. (**A**—H & E, ×220, **B**—Mucicarmine stain, ×220) Photomicrographs of the mutant (*sld*/*sld*) phenotype at the same age demonstrating a small number of mucous acini positively stained with mucin-specific stain. (**C**—H & E, ×220, **D**—Mucicarmine stain, ×220)

+"), was established by introducing the normal wild gene "+" from a BALB/c mouse into the NFS/N strain and was used as a control after 8 successive backcrosses.[4] None of the 19 biochemical markers examined (Idh-1^a, Pep-3^b, Akp-1^b, Hc^1, Car-2^b, Mup-1^a, Gpd-1^b, Pgm-1^a, Ldr-1^b, Gpi-1^b, Hbb^d, Es-1^b, Es-2^b, Thy-1^b, Mod-1^a, Trf^b, Es-3^a, H-$2K^s$, H-$2D^q$) differed between congenic normal NFS/N and original mutant NFS/N-*sld/sld* mice. (Katoh H, personal communication). Mice were reared with commercial pellet diet and provided with tap water *ad libitum*.

Genetic Analysis

The mode of inheritance of the sublingual gland abnormality was studied by crossing NFS/N mice of the mutant phenotype to BALB/c mice of normal phenotype. When the offspring were 1 month old, their sublingual glands were examined histologically and the normal and mutant phenotypes were determined.

Light and Electron Microscopy

Major organs as well as submandibular, parotid, and sublingual glands were removed from the sacrificed mice, fixed with 10% phosphate-buffered formaldehyde (pH 7.2), and prepared for histologic examination. All the sections were stained routinely with hematoxylin and eosin (H & E) and some were stained with periodic acid-Schiff (PAS), Alcian blue at pH 2.5, or mucicarmine staining. To quantify the degree of partially arrested differentiation of the sublingual mucous cells in mutant mice, the numbers of mucicarmine-stained and unstained acinar cells were counted microscopically in cross-sections at the middle part of the gland after mucicarmine staining at × 100 magnification. The percentage was calculated as the proportion of the mucicarmine-positive cells in the total number of acinar cells. Values are the averages of 3 samples.

For transmission electron microscopy, the sublingual gland tissues were fixed for 2 hours in 2.5% glutaraldehyde buffered with 0.1 M phosphate (pH 7.2) and dissected into small pieces of about 2 cu mm. After being washed in phosphate buffer, these materials were postfixed with 1% phosphate buffered osmium tetroxide for 1 hour, dehydrated step-by-step with ethanol, and embedded in Quetol-812. Ultrathin sections were cut on a LKB ultramicrotome and stained with uranyl acetate and lead hydroxide. The finished preparations were observed under a Hitachi electron microscope, Model H-600.

Table 1—Percentage of Mucous Cells to the Total Number of Sublingual Acinar Cells in NFS/N-*sld/sld* Mice

Weeks of age	Mucous cells* in sublingual gland	
	Male	Female
1	2.08 ± 0.45%†	2.43 ± 0.51%
2	3.46 ± 0.59%	3.72 ± 0.60%
3	4.26 ± 0.34%	4.18 ± 0.47%
4	4.82 ± 0.56%	4.54 ± 0.82%
6	6.98 ± 1.02%	7.12 ± 0.89%
8	9.24 ± 1.17%	10.08 ± 1.32%
12	14.06 ± 1.78%	13.25 ± 1.66%
16	17.88 ± 1.98%	16.12 ± 1.82%
24	21.45 ± 2.17%	23.07 ± 2.28%
36	30.14 ± 2.44%	28.76 ± 2.59%

* The number of mucicarmine-stained mucous cells was microscopically counted in cross-sections at the middle part of the gland under × 100 magnification. More than 500 acinar cells were counted for each sample.

† The percentage was calculated as the proportion of the mucicarmine-positive cells to the total number of acinar cells. Values are the average of 3 samples ± standard deviation.

Results and Discussion

Macroscopically, the sublingual glands of the mutant NFS/N-*sld/sld* mice were similar in their position, size, and color to those of normal congenic NFS/N-"+/+" mice. In normal mice, the sublingual glands were predominantly mucus-secreting and composed of acini containing tall pyramidal cells with basally located nuclei and pale-to blue-staining cytoplasm with H & E (Figure 1A). They stained intensely with PAS, Alcian blue at pH 2.5, and mucicarmine stains (Figure 1B). In contrast, the sublingual glands of mutant phenotype were characterized by severely inhibited acinar cell differentiation to the mucus-secreting cells in both sexes, their cytoplasm being acidophilic with H & E (Figure 1C). In the mutant phenotype, the mucus-rich cells did not exist in newborn mice, but appeared in neonatal mice singly or in groups in a small number of acini and gradually increased in number with age to occupy over 30% of the total acinar cells (Figure 1D). These mucous cells were indistinguishable from those of normal phenotype in their morphology and stainability with mucin-specific stains including Alcian blue, which at pH 2.5 selectively stains the acidic mucosubstances of mucus-secreting cells.[1-3] Table 1 summarizes the proportion of mucicarmine-positive mucous cells to the total number of acinar cells in the mutant mice aged from 1 to 36 weeks.

On electron microscopy, large, homogenously electron lucent secretion droplets occupied most of the mucous cells in the normal phenotype. In contrast, several unusual ultrastructural features were observed in the immature acinar cells of the mutant mice (Figure 2). Their cytoplasm contained irregular secretion granules of varying electron density, distinct from or-

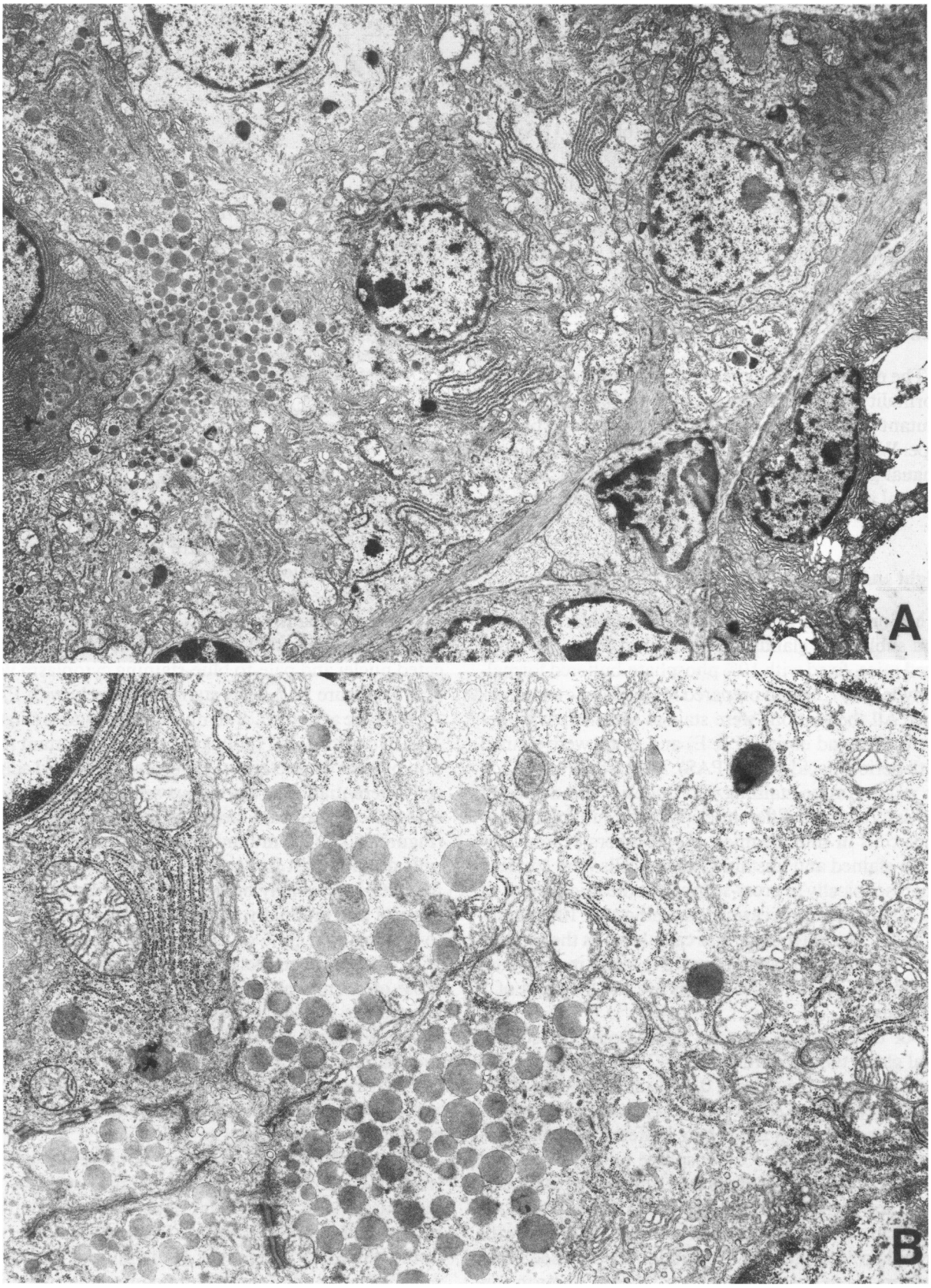

Figure 2—Electron micrographs of the sublingual gland of the mutant (*sld/sld*) phenotype at 6 weeks of age. **A**—Immature acinar cells, adjacent to the mucous cells (right, lower side), contain irregular secretion granules of varying electron density and dilated rough endoplasmic reticulum. (×3200) **B**—Higher power view of A showing the secretion granules in the apical cytoplasm. (×7200)

dinary electron lucent mucus granules.[5-8] Because these abnormal secretion granules were morphologically similar in some portion to the serous secretory granules but also similar to the noncoalescent mucus granules of fetal mouse sublingual acinar cells, more detailed comparisons are needed and now in progress. Additional abnormal organelles were the irregularly dilated rough endoplasmic reticulum and the branched cisternae. Moreover, various unusual alterations such as vacuolar changes or decrease in the proportion of free ribosomes were frequently observed in the cytoplasm of the mutant phenotype. Golgi complexes were present mainly in the supranculear area, and the mitochondria with lamellar cristae were distributed in the entire cytoplasm with a slight preference for the peripheral areas. No distinct alterations were observed in the ductural and myoepithelial cell components compared with the normal structure.[9-11]

The 2 other major salivary glands, parotid and submandibular, appeared to be normal on conventional histologic examinations. Other mucous cells in organs such as the trachea, lung, stomach, and intestine had no specific morphologic abnormality.

Genetic analysis showed all the (NFS/N × BALB/c)F1-*sld*/+ mice, irrespective of sex, to have a normal phenotype, indicating the recessiveness of the mutant gene. In the F2 and backcross (to NFS/N) generations, the ratios of the mutant to normal phenotype were 21:55 (expected, 1:3) and 43:65 (expected, 1:1), respectively. In the subsequent backcross generations (N3-N8) studied to establish a congenic normal line, the mutant to normal ratio was 130:131, close to the expected 1:1 ratio. These data clearly show that a single autosomal recessive gene, *sld,* determines the abnormal phenotype of the sublingual glands in the NFS/N subline.[4] The gene effect of *sld* is obscure, but the fact that a considerable number of mutant acinar cells recover the normal phenotype with aging indicates that *sld* is a mutation of a regulatory gene and its inhibitory effect decreases with age. The new mutant phenotype involving the sublingual gland is confined so far to this NFS/N subline from a number of inbred strains of mice studied. In fact, another NFS/N subline available in Japan, possessing the same biochemical markers as the mutant line in all the 19 loci so far examined, had the normal sublingual gland. This suggests that the *sld* mutation occurred in an NFS/N colony after this strain was established, not introduced by genetic contamination of other strain of mice.

This study describes the characteristics of a new mutant phenotype affecting the sublingual mucous cell differentiation in NFS/N strain mice, together with the genetic data. This autosomal recessive mutation induces the partial arrest of the acinar cells of the sublingual glands to differentiate to the mucus-secreting cells. This is the first morphologically detectable salivary gland mutation in the mouse and will provide a critical model for the study of salivary mucous cell differentiation.

References

1. Shackleford JM, Klapper CE: Structure and carbohydrate histochemistry of mammalian salivary glands. Am J Anat 1962, 111:25–48
2. Young JA, Vanlennep EW: Microscopic anatomy, The Morphology of Salivary Glands. New York, Academic Press, 1978, pp 22–48
3. Munger BL: Histochemical studies on seromucous and mucous secreting cells of human salivary glands. Am J Anat 1964, 115:411–430
4. Kojima A, Hata M: Sublingual gland differentiation arrest in the NFS/N subline. Mouse News Letter 80, in press
5. Leeson CR, Booth WG: Histological, histochemical, and electron-microscopic observations on the postnatal development of the major sublingual gland of the rat. J Dent Res 1961, 40:838–845
6. Caramia F: Ultrastructure of mouse submaxillary gland. I. Sex differences. J Ultrastruct Res 1966, 16: 505–523
7. Tandler B, Poulsen JH: Ultrastructure of the cat sublingual gland. Anat Res 1977, 187:153–159
8. Dawes C, Wood CM: The composition of human lip mucous gland secretions. Archs Oral Biol 1973, 18: 343–349
9. Young JA, Vanlennep EW: Ultrastructure: The Morphology of Salivary glands. New York, Academic Press, 1978, pp 80–98
10. Tamarin A: Myoepithelium of the rat submaxillary glands. J Ultrastruct Res 1966, 16:320–338
11. Redman R, Sweney LR, Mclaughlin ST: Differentiation of myoepithelial cells in the developing rat parotid gland. Am J Anat 1980, 158:299–320