한국전자현미경학회지 제 35권 제 2호 2005
Korean J. Electron Microscopy 35(2), 57~64(2005)

Ultrastructure of the Submandibular Gland in the Big White-Toothed Shrew, *Crocidura lasiura*

Soon-Jeong Jeong, Do-Seun Lim[1], Joo-Cheol Park,
Heung-Joong Kim[2], Je-O Jeong, Baik-Dong Choi,
Myung-Hee Yoon[3] and Moon-Jin Jeong*
Department of Oral Histology, [2]Department of Oral Anatomy, College of Dentistry,
Chosun University, Gwangju, 501-759, Korea;
[1]Department of Dental Hygiene, Seoul Health College, Sungnam, Korea;
[3]Division of Natural Sciences, College of Sciences, Kyungsung University,
Busan, 608-736, Korea
(Received April 2, 2005; Accepted May 20, 2005)

우수리땃쥐 *Crocidura lasiura* 악하선의 미세구조

정순정, 임도선[1], 박주철, 김흥중[2], 정제오, 최백동, 윤명희[3], 정문진*
조선대학교 치과대학 구강조직학교실, [1]서울보건대학 치위생과,
[2]조선대학교 치과대학 구강해부학교실, [3]경성대학교 자연과학대학 자연과학과

ABSTRACT

The ultrastructure and histochemical characteristics of the submandibular gland was examined in the big white toothed shrew, *Crocidura lasiura*.

A submandibular gland of *Crocidura lasiura* was a mixed gland composed of serous and mucous acinar cells. Secretory granules from the acini were discharged through the intercalated duct, the granular duct and the striated duct into the oral cavity.

Serous and mucous acinar cells and granular duct cells had large amount of rough endoplasmic reticulum, free ribosome and prominent Golgi apparatus at the basal cytoplasm of the cell, and many granules at the apical cytoplasm. Oval type serous granules had a homogeneously pale round shape of bead at the center. Mucous granules were distinct from those of the other mammalian species having variety patterns with several dense bands into homogeneous pale matrix. A serous like secretory granules and myelin like body were observed in the cytoplasm and the lumen of granular duct cells. The myelin like body is a characteristic structure only reported in the salivary glands of two shrews, *Suncus murinus* and *C. dsinezumi*. Striated duct cell had numerous well developed mitochondria but secretory granule was not shown at all.

Key words : *Crocidura lasiura*, Mucous acinar granule, Myelin like body, Submandibular gland, Ultrastructure

This work was supported by research funds from Chosun University, 2004.
*Correspondence should be addressed to Dr. Moon-Jin Jeong, Department of Oral Histology, College of Dentistry, Chosun University, Gwangju, 501-759, Korea. Ph.: 062-230-6895, FAX: 062-224-3706, E-mail: mjjeong@chosun.ac.kr

INTRODUCTION

Mammalian salivary glands are highly complex organ associated with the mucosa of the oral cavity. Because of the potential value of inter specific ultrastructural differences with possible evolutionary significance at the cellular level, this organ has been examined recently in a variety of species (Hand, 1980; Phillips & Tandler, 1987; Phillips et al., 1987a,b, 1993; Tandler & Phillips, 1993; Tandler et al., 1990, 1994).

Soricidae belong to the Order Insectivora are a primitive mammalian group, thought to be ancestral to many groups of mammals, exhibiting characteristics of specific interest for the study of mammalian evolution (Eisenberg, 1981; Tsuchiya, 1985; Churchfield, 1990; Carson & Rose, 1993). Studies of the salivary glands, especially the submandibular gland has been reported for a few members of Soricidae, European water shrew, *Neomys Fodiens* (Schaffer, 1908), the house shrew *Crocidura russula* (Raynaud, 1964), the black and Rufous elefant shrew, *Rhynchocyon chrysopygus* (Mineda, 1981) and the musk shrew, *Suncus murinus* (Mineda, 1985). However, most of these studies showed only simple structural results at the light microscopic level and a few ultrastructural results of submandibular gland cells and secretory granules.

By studying the histochemistry and ultrastructure of the submandibular salivary gland in the big white-toothed shrew, *Crocidura lasiura*, we determined the ultrastructure of submandibular gland cells and characteristics and ultrastructural patterns of secretory granules, and compared these features to the other small mammalian species.

MATERIALS AND METHODS

1. Sampling

Seven adult males of *C. lasiura* were collected at June, October and November 1999 and March and April 2000 from Mt. Jiri using the Sherman live traps.

2. Histochemistry

In other to observe histochemistry of the submandibular gland, one submandibular gland from the sacrificed specimen was dissected 0.74% saline (physiological saline solution), and fixed in the 4% formaldehyde (phosphate buffer, pH 7.2) for 12 hr. Samples were dehydrated with a series of the graded ethyl alcohol and embedded in Paraplast (Sherwood Medical Industries, St. Louis). The blocks were cut 6~7 μm sections. Sections were stained with Hematoxylin-Eosin, Periodic acid-Schiff (PAS) to determine vicinal hydroxyl group near the glycoprotein, Alcian blue (AB, pH2.5) to determine distribution of acidic glycoprotein and simultaneously both of Alcian blue and Periodic acid-Shiff (AB-PAS) (Mowry, 1956). The observation according to Spicer (1963) and Sun's method (Spicer & Sun, 1967) was applied to interpret the intensity of the stained tissue: the negative reaction was −, and the symbols according to the intensity were represented as ±, +, + +, +++.

3. Electron microscopy

To observe ultrastructure of the submandibular gland, the other submandibular gland was fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in Millonig's phosphate buffer (pH 7.4) for 1 hr. Samples were post-fixed with 1.3% osmium tetroxide in the same buffer for 2 hr, dehydrated with a series of the graded ethyl alcohol and acetone, and embedded in epoxy resin. Thick sections (0.5~1 μm) were stained with 5% toluidine blue for light microscopy. Thin sections (60~90 nm) were double stained with uranyl acetate and lead citrate. All of the thin sections were examined with a JEOL 100S transmission electron microscope.

RESULTS

The ultrastructure and histochemical characteristics of

Table 1. Histochemistry of the submandibular gland of *Crocidura lasiura*.

Stain	Serous cell	Mucous cell
AB, pH 2.5	+	++
PAS	+	+++
AB-PAS	++	+++

AB, Alcian blue; PAS, Periodic acid-Schiff; AB-PAS, simultaneously both of Alcian blue and Periodic acid-Shiff.

the submandibular gland was examined in the big white-toothed shrew, *C. lasiura*.

From the observation of the microscopic specimens, a submandibular gland of *C. lasiura* was composed of acini and salivary ducts (Fig. 1). A submandibular acinus was a mixed gland composed of serous and mucous cells, secreted granules from the acinus were discharged through the intercalated duct, the granular duct and the striated duct into the oral cavity. From the histochemistry of submandibular gland, Serous cells had large amount of serous granules that were stained a little intensity in AB and PAS separated stains and much intensity in AB-PAS simultaneous stain, also mucous cells had large amount of mucous granules that were stained much intensity in AB stain and strong intensity in the other stains, PAS and AB-PAS stains (Table 1).

From the observation of ultrastructure of acinar cells in the submandibular gland, serous demilune cells had large amount of rER (rough endoplasmic reticulum), free ribosome and prominent Golgi apparatus at the basal cytoplasm of the cell and large amount of oval type serous granules delimiting by a single membrane at the apical cytoplasm (Fig. 2). An immature serous granule had only minute little dense specks and according as granules progress, a matured granule had a homogeneously pale round shape of bead at the center (Fig. 3). Mucous cells also had large amount of rER, free ribosome and prominent Golgi apparatus at the basal cytoplasm and large amount of serous granules delimiting by a single membrane at the apical cytoplasm (Fig. 4). Mucous granules were distinguished from those of the other mammalian species, having a complex substructure. Mucous granules had variety patterns with several dense bands into the homogeneous pale matrix (Fig. 5). Granular duct cells had large amount of rER, free ribosome and prominent Golgi apparatus at the basal cytoplasm and large amount of round secretory granules delimiting by a single membrane at the apical cytoplasm like acinar cells, serous and mucous cells (Fig. 6). A secretory granule of the granular duct cell was a serous-like granule, an immature granule had dense specks but a mature granule had a homogeneous dense matrix (Fig. 7). Mature secretory granules of granular duct cell, droplets were discharged from secretory cells into lumen by the manner of exocytosis that was already well investigated. Thus droplets moved to the luminal border of the cell and the outermost membrane of such droplet fused with the plasma membrane, these fused membranes had disappeared and the contents of the droplet flowed into lumen (Fig. 9). Also Myelin-like bodies were observed in lumen and cytoplasm of the granular duct cells of *C. lasiura* (Figs. 8, 9). Myelin-like body was a manifold form of membranes having small pale dense granular sacks at the center and mostly observed near the well-developed rER and Golgi apparatus. Striated duct cell had numerous well-developed mitochondria and free ribosome, but very little rER and Golgi apparatus, and secretory granule was not at all shown, and the apical surface of striated duct epithelium was covered with short microvilli (Fig. 10).

DISCUSSION

Serous granule of the big white-toothed shrew, *C. lasiura* belongs to Soricidae (Tsuchiya, 1985; Churchfield, 1990) was an oval type delimiting by a single membrane (Jamieson & Palade, 1967), similar to those of the other mammalian species. An immature serous granule had only minute little dense specks, but a mature serous granule had homogeneously pale central portion and minute little dense specks at the outer. It was already

known a dense degree of components in the mature serous granules indicates amount of enzymes (Phillips et al., 1987a, b). According to literatures about systematically well-investigated salivary gland of bats, in case of serous granules, scarce electron-dense granules of frugivorous bats have extremely low enzyme and largely electron-dense granules of insectivorous bats have rich enzymes (Junqueira et al., 1973; Tandler et al., 1990). Moderately dense serous granules of *C. lasiura* compared with insectivorous bats indicate low enzymes, those of *C. lasiura* is similar to the already reported other shrews. The nature and electron dense of the salivary glands and their secretory products must relate to these various diets (Tandler et al., 1990). Although we cannot say precisely how the serous granules in *C. lasiura* correlate with their diet; eating earthworm in the soil, insects and carcass of the mammals (Churchfield, 1990), it is clear an ability to digestive protein by serous products of submadibular gland is not powerful.

Mucous acinar granule of *C. lasiura* was a characteristic type that is distinct from the other mammalian species, including bats that were systematically well investigated. It also delimited by single membrane but had a complex substructure into the homogeneous pale matrix with several dense bands, producing a variety of pattern. Secretory granules contain a mixture of proteins both enzymatic and nonenzymatic, and glycoconjugates, lipid, certain vitamins and electrolytes (Tandler & Phillips, 1993). These granule components sort themselves out according to chemical affinities and interactions, electrical charge and molecular configuration to yield particular designs based on the relative electron densities of these constituents (Tandler et al., 1994). Because the secretory process appears to be widely conserved ranging from yeast to many in mammals (Rothman & Orci, 1992), there can be considerable variation among secretory products exported by homologous salivary gland secretory cells in different species (Ball, 1993; Tandler et al., 1994). The striking variation in secretory granular ultrastructure implies specific differences in post-translational modification of relatively small number of basic products. Finally, specific differences in granule ultrastructure might be indicative of functional differences in saliva (Levine et al., 1987; Tandler et al., 1990). Also these inter-specific variations are not random but might be correlated with genetic history (Tandler et al., 1986; Phillips et al., 1987a), diet (Phillips et al., 1987b; Tandler et al., 1990) and species isolation (Nagato et al., 1984). Thus, Ultrasructure of mucous acinar granules in *C. lasiura* might be used with a key, which is classified from the other mammalian species.

Myelin-like body, only reported in the salivary glands of two shrews, *Suncus murinus* and *C. dsinezumi* (Mineda, 1985), was observed in the cytoplasm and lumen of the granular duct cell of *C. lasiura*. Myelin-like body is a manifold form of membranes having small pale dense granular sacks at the center, origin of rER near Golgi complex. It is considered formation at the basal cytoplasm of the granular duct cell and discharge into lumen by the secretory manner of granules, exocytosis (Mineda, 1985). Myelin-like body had few report and is not at all unknown in the other mammalian salivary gland, these construct and discharge of much components of membrane is very interesting.

ACKNOWLEDGEMENT

This work was supported by research funds from Chosun University, 2004.

REFERENCES

Ball WD: Cell restricted secretory proteins as markers of cellular phenotype in salivary glands. In Dobrosielski Vergona K (ed) Biology of the salivary glands. CRC Press Boca Raton pp. 355 396, 1993.

Carson KA, Rose RK: Fine structure of the submandibular salivary gland of the Venomous short tailed shrew,

Blarina brevicauda (Insectivora: Soricidae). European J Morph 31 : 111 128, 1993.

Churchfield S: The natural history of Shrews. A & C black Ltd London, 1990.

Eisenberg JF: The mammalian radiations an analysis of trends in evolution, adaptation and behavior. University of Chicago Press Chicago IL 1981.

Hand AR: Salivary glands. In Bhaskar SN (ed) Orban's oral histology and embryology. Mosby St Louis pp. 336 370, 1980.

Jamieson JD, Palade GE: Intracellular transport of secretory proteins in the pancreatic exocrine cell II. Transport to condensing vacuole and zymogen granules. J cell Biol 34 : 597 615, 1967.

Junqueira LCU, Toledo AMS, Doine AI: Digestive enzymes in the parotid and submandibular glands of mammals. Anais da Academia Brasileira de Ciencias 45 : 629 643, 1973.

Levine MJ, Reddy MS, Tabak LA, Loomis RE, Bergey EJ, Jones PC, Choen RE, Stinson MW, Al Hashimi I: Structural aspect of salivary glycoproteins. J Dental Res 66 : 436 441, 1987.

Mineda T: Histological and histochemical investigations on the salivary glands of the black and refous elephant shrew (Rhynchocyon chrysopygus). J Dent Aichi Gakuin univ 19 : 78 85, 1981.

Mineda T: Fine structure of salivary gland and cytomegalovirus of the House musk shrew, Suncus murinus. In Oda S, Kitoh J, Ota K and Isomura G (eds.) Suncus murinus Biology of the laboratory shrew. Japan scientific societies press (JSSP) pp. 370 388, 1985.

Mowry RW: Alcian blue techniques for the histochemical study of acidic carbohydrates. J Histochem 4 : 407, 1956.

Nagato T, Tandler B, Phillips CJ: Unusual smooth endoplasmic reticulum in submandibular acinar cells of the male round eared bat, Tonatia sylvicola. J ultrastruct Res 87 : 275 284, 1984.

Phillips CJ, Tandler B: Mammalian evolution at the cellular level. Current mammalogy 1 : 1 66, 1987.

Phillips CJ, Nagato T, Tandler B: Comparative ultrastructure and evolutionary patterns of acinar secretory product of parotid salivary glands in Neotropical bats. Zoology 39 : 213 229, 1987a.

Phillips CJ, Tandler B, Pinkstaff CA: Uniques salivary glands in two genera of tropical microchiropteran bats An example of evolutionary convergence in histology and histochemistry. J Mammal 68 : 235 242, 1987b.

Phillips CJ, Tandler B, Nagato T: evolutionary diversity of salivary gland acinar cells a format for understanding molecular evolution. In Dobrosielski Vergona K (ed) The biology of the salivary glands. CRC Press Boca Raton pp. 41 80, 1993.

Raynaud J: Dimorphism sexuel de la gland sous maillarie chez la Musaraigne (Crocidura). Compte Rendu soc biol 158 : 942 947, 1964.

Rothman JE, Orci L: Molecular dissection of the secretory pathway. Nature 355 : 409 415, 1992.

Schaffer J: Zur histologie der unterkiefersperchеldrusen ber Insectivoren. Zeit Wissenschaft Zoo l89 : 1 27, 1908.

Spicer SS: Histochemical differentiation of mammalian mucopolysaccharides. Ann NY Acad Sci 106 : 379 388, 1963.

Spicer SS, Sun DCH: Carbohydrate histochemistry of gastric epithelial secretions in dog. Ann NY Acad Sci 140 : 762 783, 1967.

Tandler B, Phillips CJ: Structure of serous cells in salivary glands. Microscopy Res Tech 26 : 32 48, 1993.

Tandler B, Phillips CJ, Nagato T, Toyoshima K: Ultrastructural diversity in chiropteran salivary glands. In Riva A and Motta PM (eds) Ultrastructure of the Extrapariental glands of the Digestive tract. Kluwer academic publishers pp. 31 52, 1990.

Tandler B, Phillips CH, Pinkstaff CA: Mucous droplets with multiple membranes in the accessory submandibular glands of Long winged bats. Anat Rec 240 : 178 188, 1994.

Tsuchiya K: The chromosomes of Insectivora. In Oda S, Kitoh J, Ota K, Isomura G (eds) Suncus murinus Biology of the laboratory shrew. JSSP pp. 51 6, 1985.

<국문초록>

악하선의 미세구조와 조직화학적 특성을 우수리땃쥐 *Crocidura lasiura*에서 연구하였다. 악하선은 장액선세포와 점액선세포로 구성된 혼합샘이었다. 이 샘포에서 분비된 과립들은 사이관, 과립관 그리고 줄무늬관을 거쳐 구강으로 분비되었다.

장액선세포, 점액선세포 그리고 과립관세포들은 많은 양의 조면소포체, 유리 리보솜 그리고 현저히 발달된 골

지체를 기저부에, 많은 양의 과립을 정점부에 가지고 있었다. 타원형의 장액선 과립은 균질하게 밝은 구형 구슬 모양을 중앙에 가지고 있었다. 점액선 과립은 균질하게 밝은 기질 내에 몇 개의 전자밀도가 있는 띠를 가짐으로서 다양한 문양을 가져 다른 포유류의 것과 구분되었다. 장액성의 분비과립과 땃쥐류, *Suncus murinus*와 *C. dsinezumi*에서 보고된 특징적 구조인 미엘린소체가 과립관세포의 세포질과 내강에서 관찰되었다. 줄무늬관세포는 많은 잘 발달된 미토콘드리아를 가지고 있었지만 분비과립은 전혀 관찰되지 않았다.

FIGURE LEGENDS

Fig. 1. Light micrograph of the submandibular gland. Acinus (A) composing serous (Sc) and mucous (Mc) cells contain numerous secretory granules. Gd, granular duct; Id, intercalated duct; Sd, striated duct. Scale bar = 1 μm.

Fig. 2. Electron micrograph of the serous demilune in the submandibular gland. The basal cytoplasm contains large amount of rER, free ribosome and prominent Golgi complex. The apical cytoplasm contains large amount of dense oval type granules (Sg) with various stages of the maturing process. M, mitochondria; N, nucleus. Scale bar = 0.1 μm.

Fig. 3. Higher magnification of the mature serous granules (Sg) in the serous acinar cell. Central portion of granules is occupied by homogeneously pale material. The other portion is dense and contains minute dense specks. M, mitochondria. Scale bar = 0.05 μm.

Fig. 4. Electron micrograph of the mucous acinar cell in submandibular gland. Large amount of electron dense mucous granules (Mg) concentrate in its apical cytoplasm. N, nucleus; Sg, serous granule. Scale bar = 0.1 μm.

Fig. 5. Higher magnification of the mature mucous granules (Mg) in mucous acinar cell. These granules have a complex substructure with several dense bands, into the homogeneous pale matrix, producing a variety of patterns. Ic, intercellular space. Scale bar = 0.05 μm.

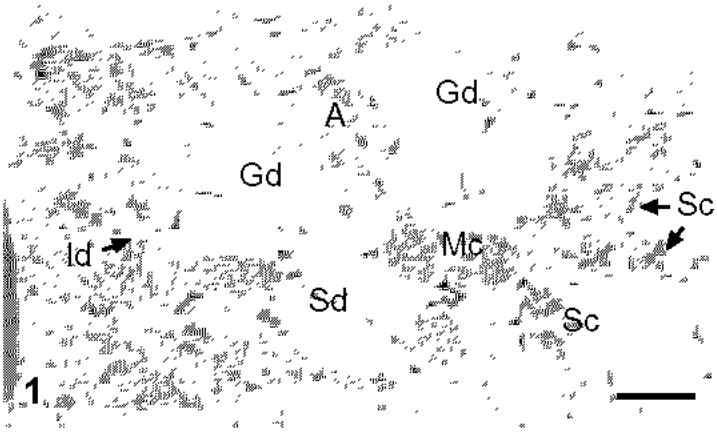
Fig. 6. Electron micrograph of the granular duct cells. The basal cytoplasm contains large amount of rER, free ribosome and prominent Golgi complex. The apical cytoplasm is filled with numerous forming secretory granules (G). M, mitochondria; N, nucleus. Scale bar = 0.1 μm.

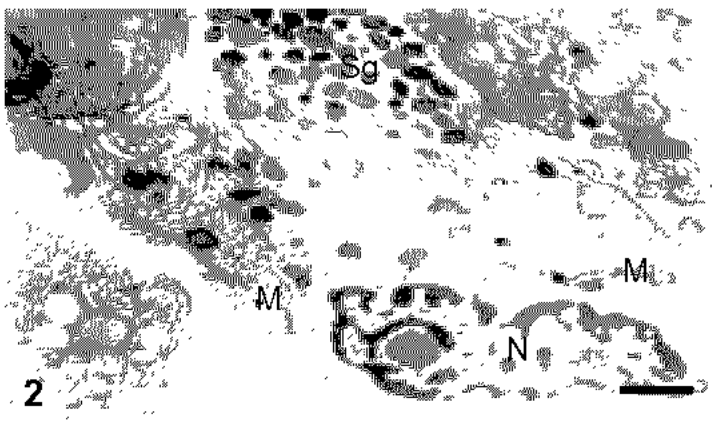
Fig. 7. Higher magnification of the secretory granules in the granular duct cell. Immature granules (Ing) contain minute dense specks on the border of the limiting membrane. Mature granules (Mag) are occupied by homogeneous dense materials. Scale bar = 0.05 μm.

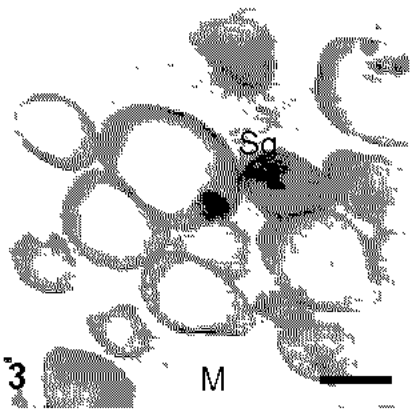
Fig. 8. Higher magnification of the myelin-like body (Mb) in the granular duct cell. M, mitochondria; N, nucleus; Rb, lysosomal residual body. Scale bar = 0.05 μm.

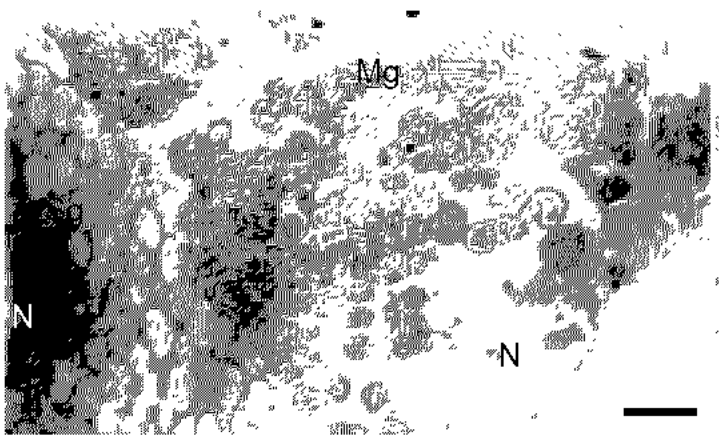
Fig. 9. Higher magnification of serous-like secretory granules (G) showing the various stages in the process of exocytosis in the granular duct. The lumen (L) contains droplets and a myelin-like body (Mb). Scale bar = 0.05 μm.

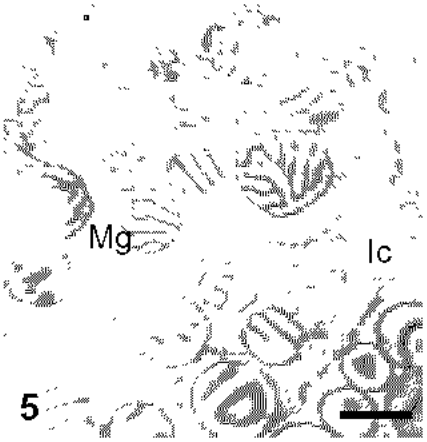
Fig. 10. Electron micrograph of striated duct cell having numerous well developed mitochondria (M) and short microvilli at the luminal surface. L, lumen; N, nucleus. Scale bar = 0.1 μm.

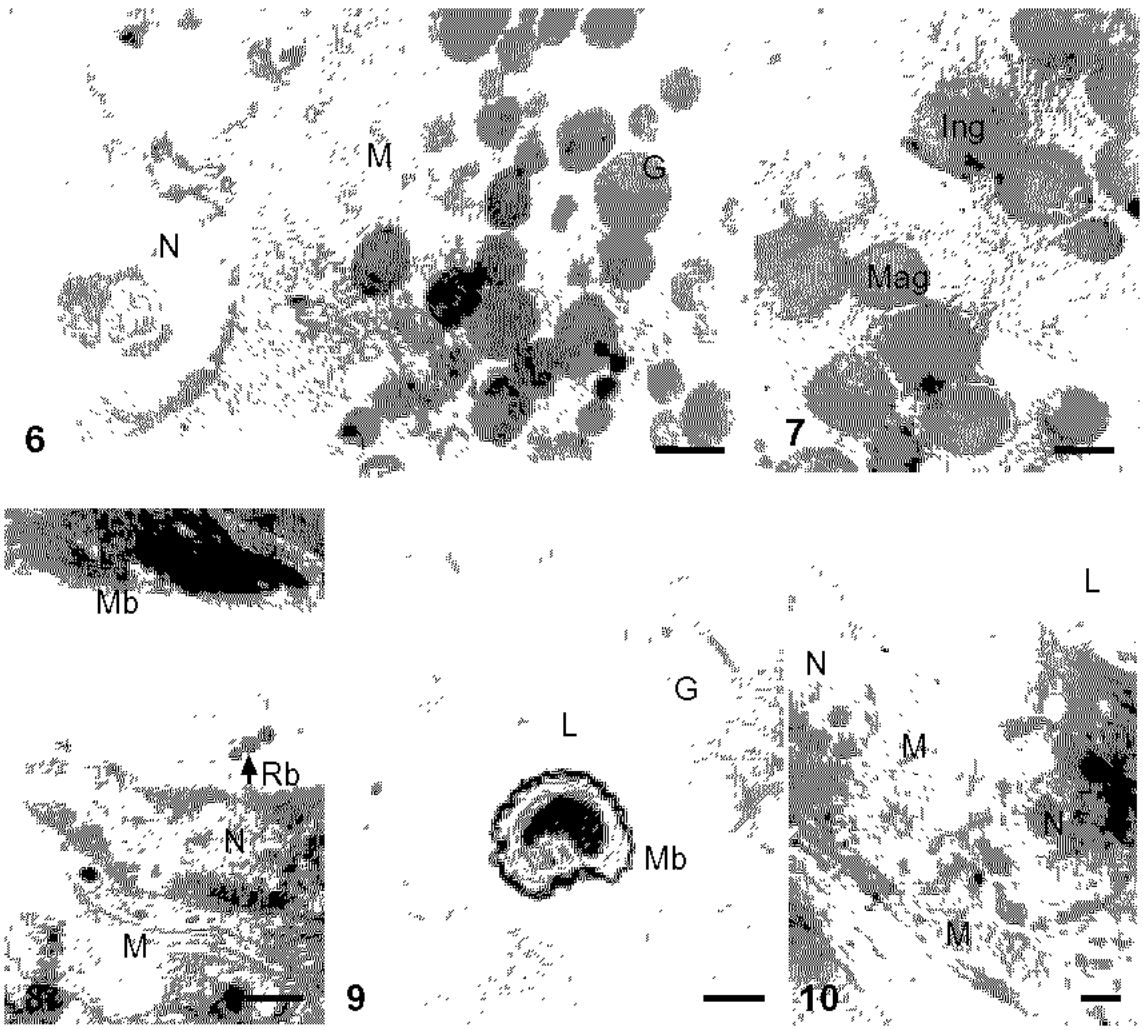

A
Gd
Gd
Sc
Mc
Id
Sd
Sc
1


Sg
M
M
N
2


Sg
3
M


Mg
N
N


Mg
Ic
5


M
G
N
6
Ing
Mag
7
Mb
Rb
N
M
9
G
L
Mb
L
N
M
N
M
10