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Ultrastructural Study on the Salivary Gland of a Korean Freshwater Pulmonate, *Radix auricularia coreana*

Kye-Heon Jeong and Yong-Hwan An[1)]

Division of Life Sciences, College of Natural Sciences, Soonchunhyang University, [1)]Research Institute for Basic Science, Soonchunhyang University, P.O. Box #97 Asan, Chungnam, 336-600 Republic of Korea

ABSTRACT

A histochemical and ultrastructural study on the salivary gland of a freshwater snail *Radix auricularia coreana* was conducted. The epithelial cells of the salivary gland are composed of 9 types of cells. Two types out of them work as frame cells supporting the epithelium and the secretory cells embedded within the epithelium. Seven types of secretory cells are classifiable depend on their histochemical reactions and ultrastructures. The materials secreted by the secretory cells are neutral mucopolysaccharide, acid mucopolysaccharide, and glycogen.

Key words: Gastropod, Salivary gland, Ultrastructure, Snail

INTRODUCTION

In general in the pulmonates most of the extracellular enzymes are supplied by the salivary glands. Lobulated shapes, clean color and arrangement figures of the salivary glands is often impressive to the dissecting malacologist. Morphological studies on the salivary glands of the gastropods had been carried out mainly in coordination with overall studies on the alimentary canals (Fretter, 1937; Graham, 1967, Campbell, 1965). Ultrastructural studies have been performed by Bouillon (1960), Walker (1970), Quarterini (1967), Ponder (1970, 1972), Morgen *et al.* (1982) and so on. Recently, Seerband *et al.* (1996) and Clang and Han (1996) reported Ultrastructural observations.

Two types of salivary glands are so far known in the gastropods. The one is tubular gland and the another one is acinous gland.

Present study observed the acinous salivary gland, of a Korean freshwater pulmonate snail *Radix auricularia coreana*, which discharge secretions into the roof of the buccal cavity to aid lubrication in food swallowing. The object of the present study is to characterize the cell types, and substances secreted in the freshwater pulmonate.

MATERIALS AND METHODS

1. Materials

The freshwater snail was *Radix auricularia coreana* (Planorbidae), a Korean freshwater snail widely distributed throughout the country, The specimens were collected from a creek at Bongsan-myeon, Yesan-gun, Chungnam, Korea.

2. Methods

The collected snails were cultured in the laboratory aquarium in good food air supply. For dissection of the snail, they were anesthetized in the finger bowl by menthol crystals melted slowly in the finger bowl.

The anaesthetized snails were dissected carefully to remove the tiny salivary gland. The salivary gland specimens were fixed with 10% neutral formalin for

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Corresponding author: Jeong, Kye-Heon
Tel: (82) 418-530-1249: e-mail: jngkh@asan.sch.ac.kr
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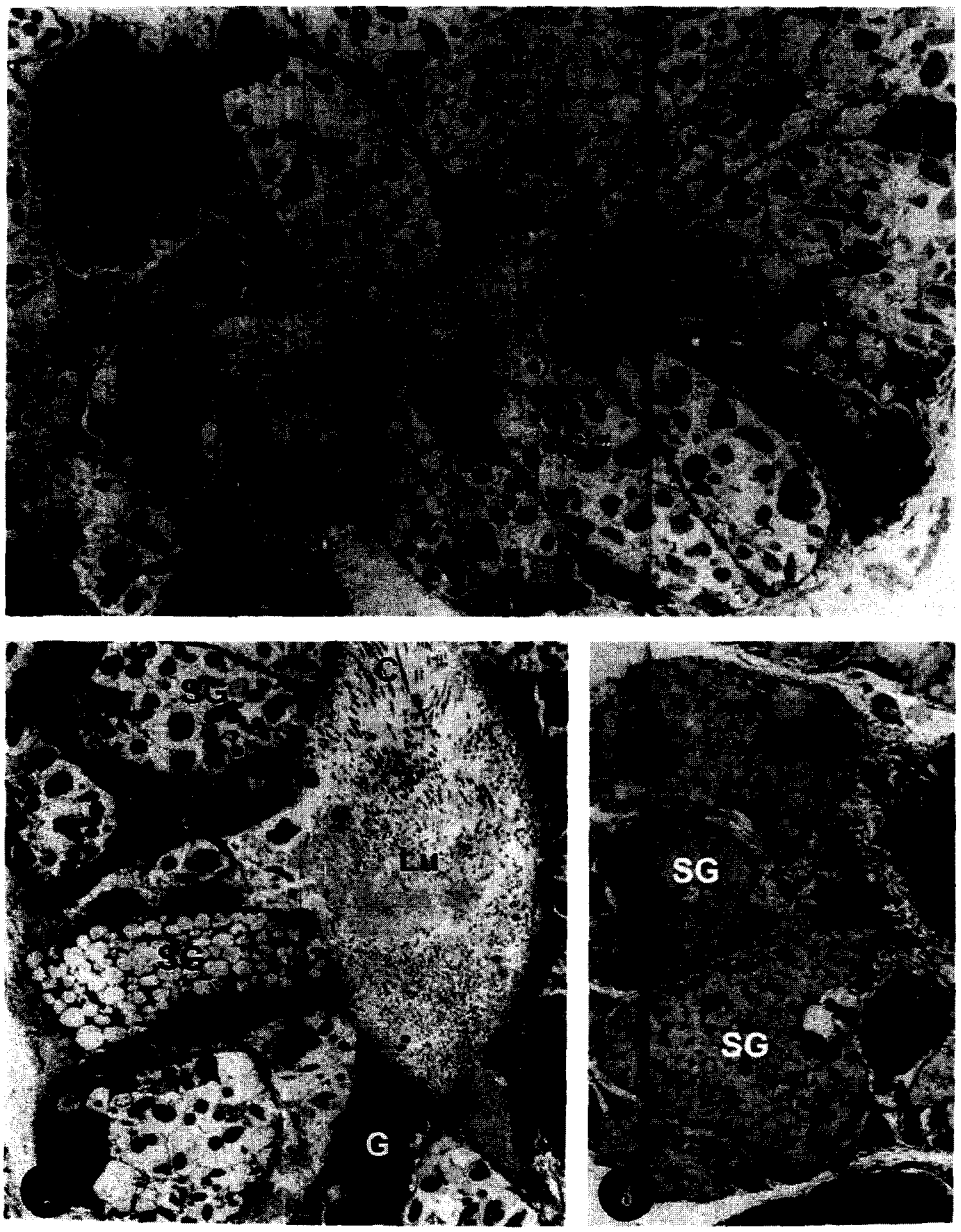
3 hours and embed in paraffin blocks in routine methods. The Paraffin blocks were sectioned with rotary microtome.

The sectioned tissues were stained with PAS-Alcian blue, H-E, methylene blue-basic fuchsin, Alcian blue and toluidine blue for the histochemical observations.

For the electron microscopic observations, the salivary tissues were prefixed with 2.5% glutaraldehyde phosphate buffered (pH 7.4) at 4℃ and postfixed with 1% OsO_4 after washing with the buffer solution and dehydrated in series of graded acetone concentrations. The fully fixed specimens were embedded in Epon-812 mixture, sectioned with ultramicrotome, stained with uranyl acetate and lead citrate, and observed with the JSM CX I electron microscope.



Figs. 1-3. Low magnification of the thin sectioned salivary gland showing the acinar cells and lumen (Lu) of the intralobular duct.

RESULTS

In a Korean freshwater pulmonate, *Radix auricularia coreana*, investigated in the present study, two lobulated salivary glands were located on either side of the esophagus. Each one of the glands was at the post end of the salivary ducts which run forward

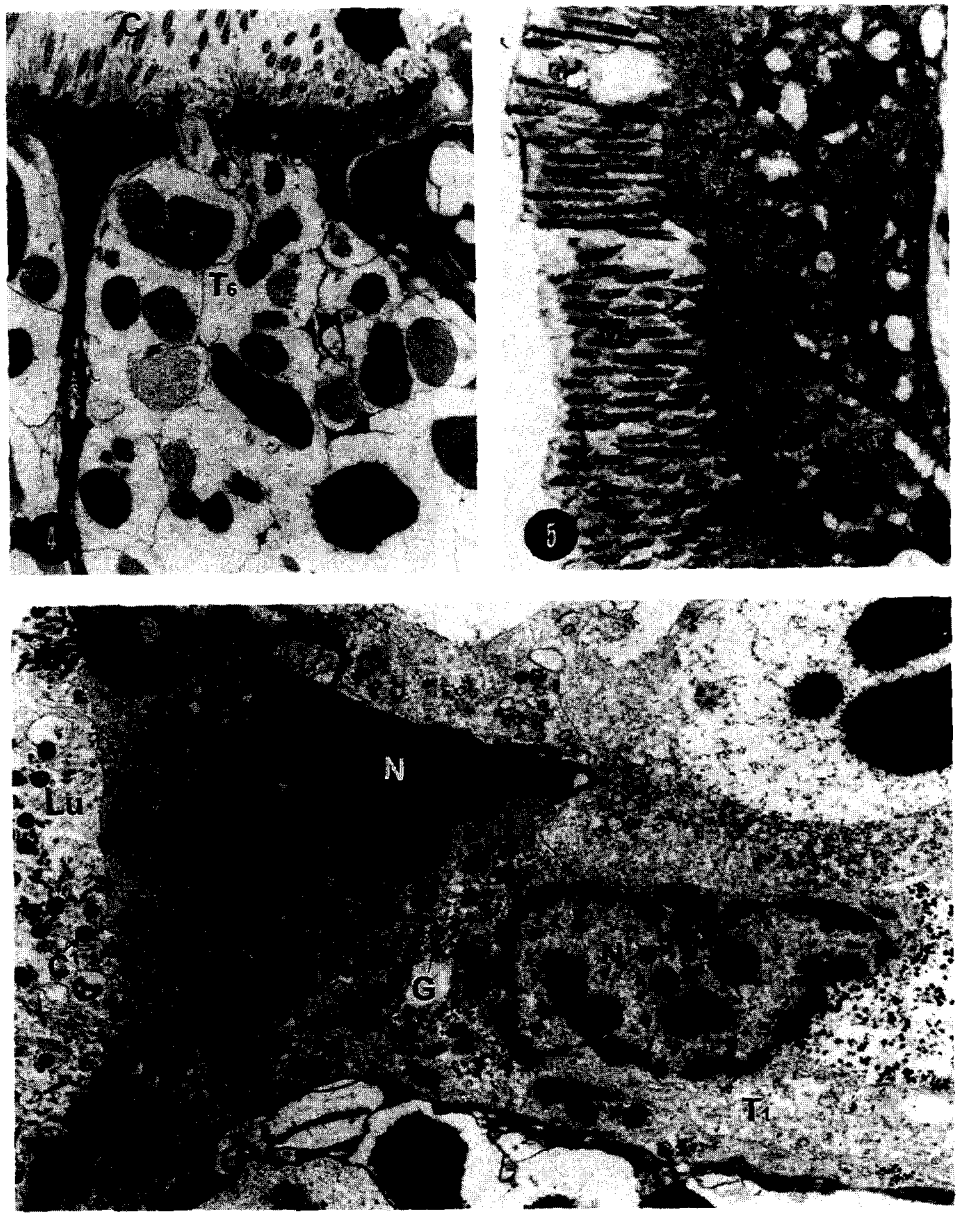


Fig. 4. T_1 cell showing the frame work function and microvilli (Mv) and cilia (C) on the apical surface. × 10,000

Fig. 5. T_2 cell showing only microvilli (Mv) on the free surface. × 16,000

Fig. 6. T_1 cell showing internal structures. The amorphous nucleus (N) is located in the middle or slightly upper part of the cell and numerous mitochondria(M) and glycogen (G) particles are positioned in the upper part of the cell. The apical cell surface is covered with microvilli (Mv) and cilia (C). × 12,000

along the esophagus and finally open into the buccal cavity.

The composite layers of the salivary gland were a luminal epithelium, fibromuscular layers, submuscular layers, and a capsule. The epithelium of the salivary gland was covered with columnar cells which were roughly classified into supporting cells and secretory cells (Figs. 1-3).

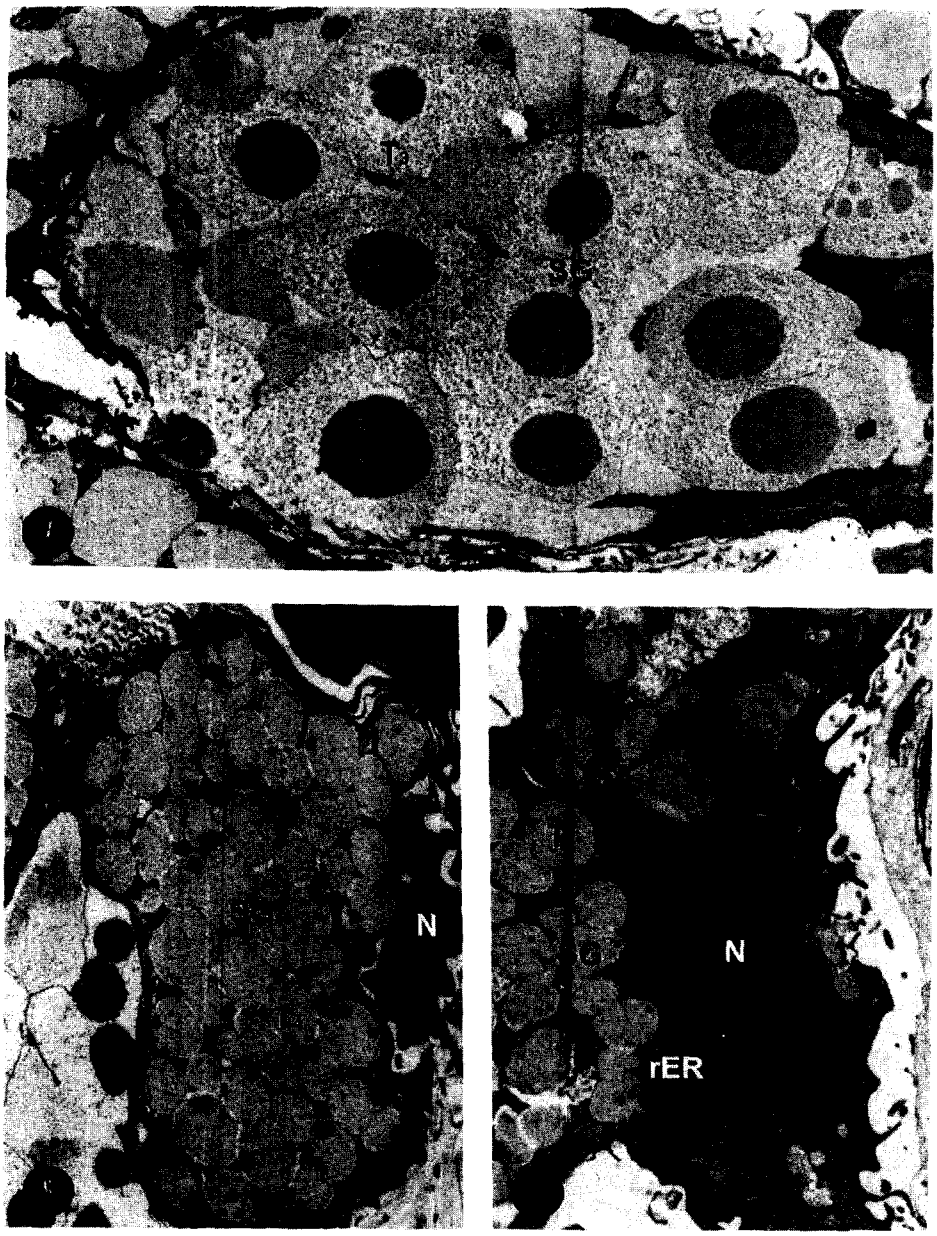


Fig. 7. T_3 cell showing the secretory granules (SG) in different stages of maturity and well developed rER. × 8,400

Fig. 8. The upper part of T_4 cell filled with secretory granules (SG) in different size and maturation stages. × 8,400

Fig. 9. The lower part of T_4 cell containing nucleus(N) basally located. The rER is well developed in the surrounding cytoplasm of the nucleus(N) and the secretory granules (SG).× 8,400

In detail, the epithelial cell of the salivary gland were classified into nine types based on their ultrastructures.

Type 1 (T_1) cell, the ciliated cell, formed a basic frame of the overall epithelium and contained several types of secretory gland cells between them. This type cell located its nucleus in the middle or upper portion of the cell and contained numerous mitochondria in the apical cytoplasm. The T_1 cell did not contain any secretory materials in the cytoplasm. The internal structure of the cilia showed the 9+2 microtubular arrangement (Figs. 4, 6).

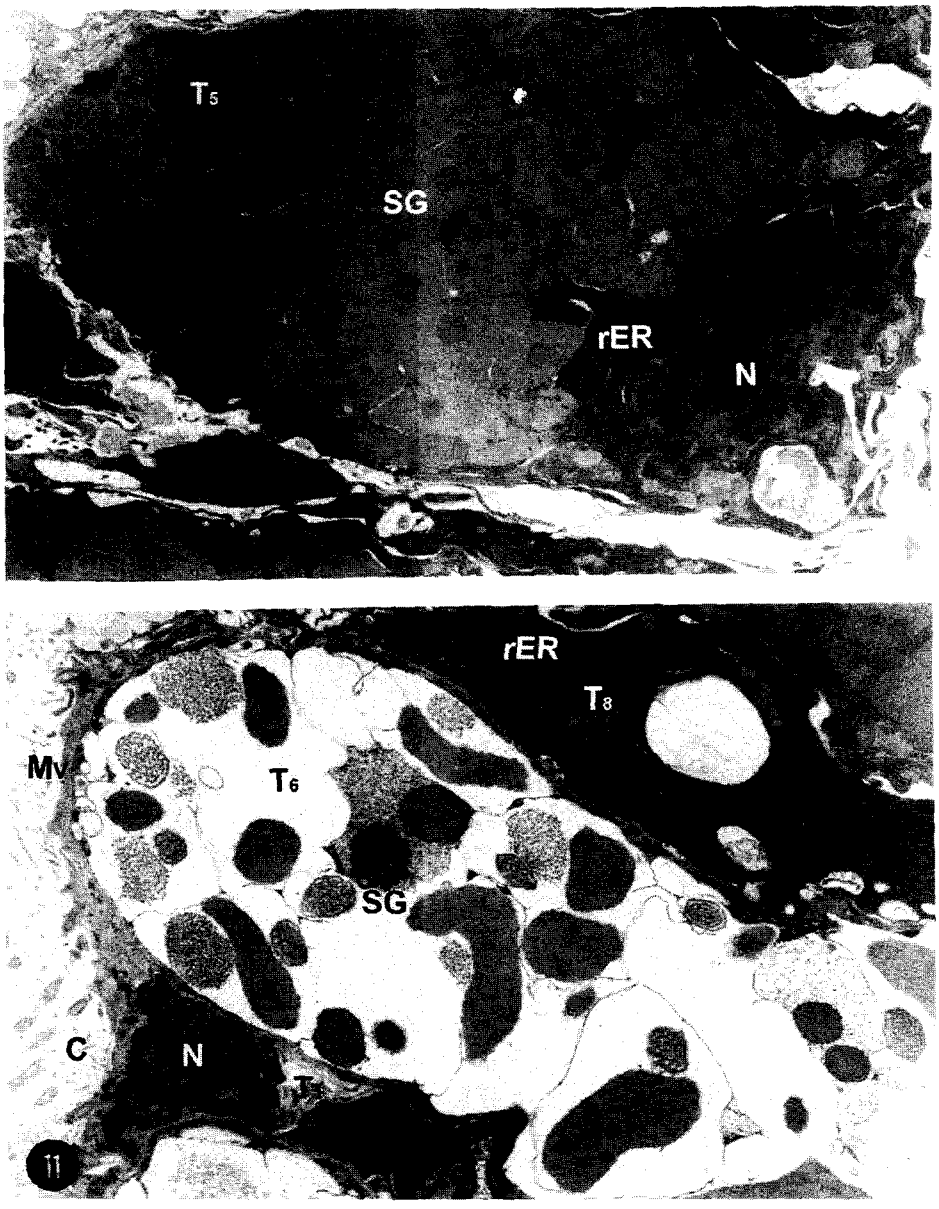
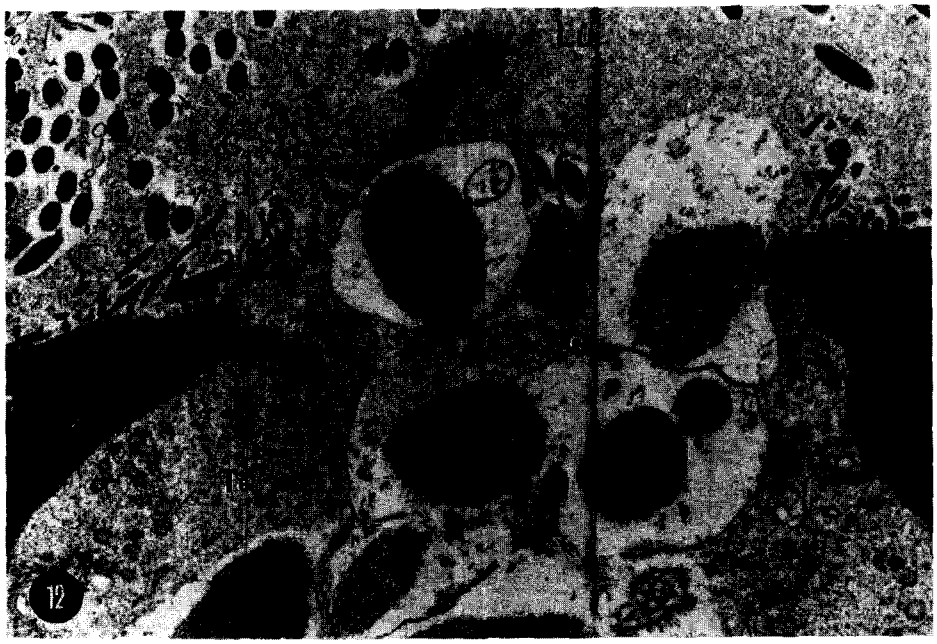


Fig. 10. T_5 cell showing overall shape such as basally located nucleus (N), rER and fully compacted secretory granules (SG) in the cell × 6,000

Fig. 11. T_6 cell neighbouring with the T_1 and T_8 cells. The numerous secretory granules (SG) with substrate in electron lucent have electron dense matrices in various shapes. The matrices form certain kinds of cores of the granules. × 8,400

Type 2 (T_2) cell was the another type of the endothelial cell with only microvilli on the luminal surface. This type of cell was located at non secretory portion or on the shoulder of the secretory cells of the salivary gland (Fig. 5).

The all types of the secretory cells embedded in



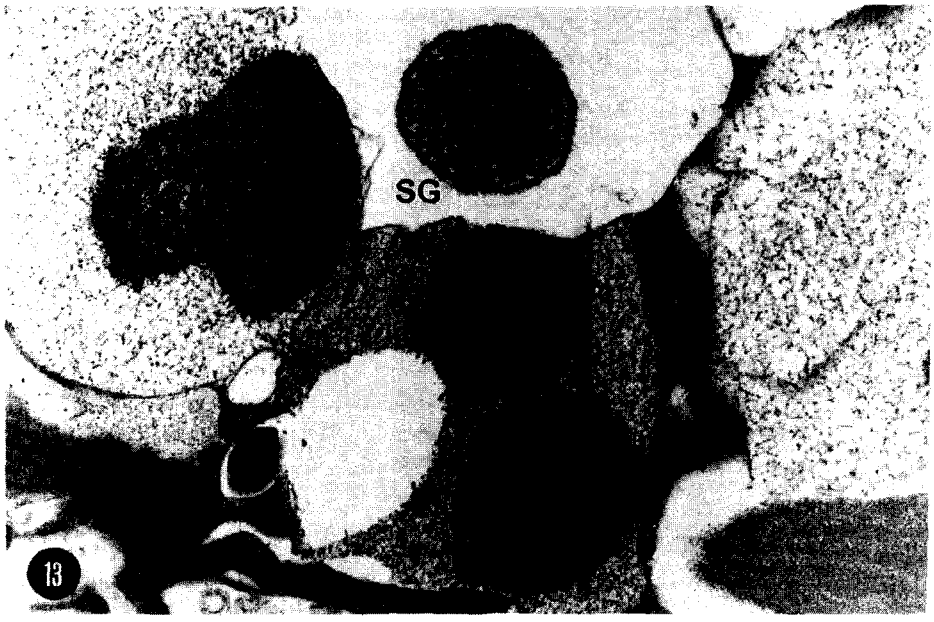
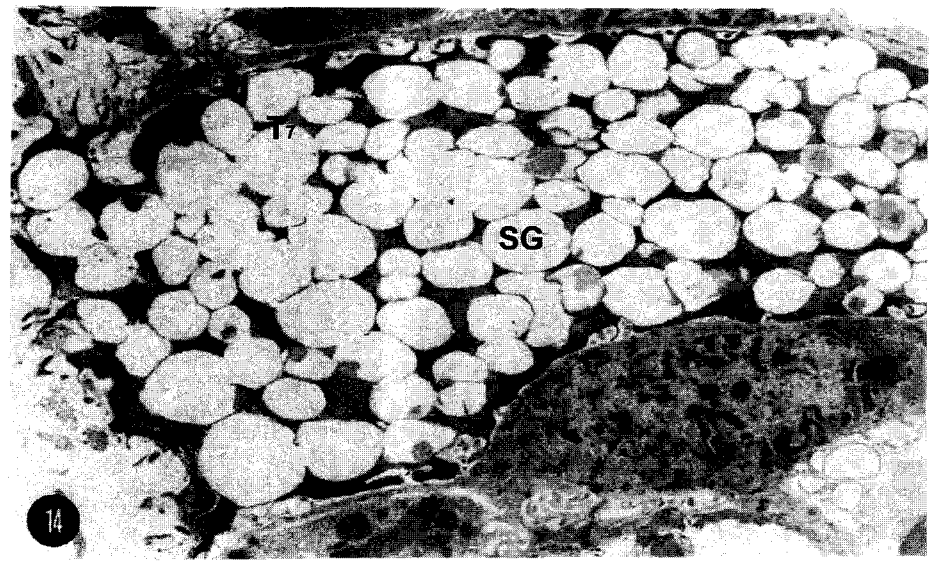


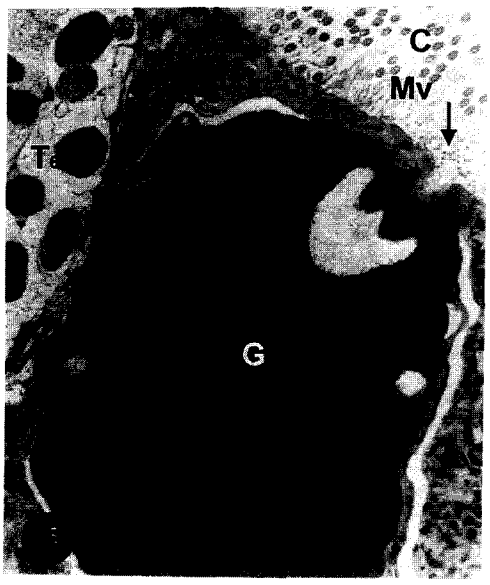
Fig. 12. Secretory granules (SG) of the T6 cell passing to the lobular duct lumen (Lu) through the apical opening (arrowed). × 1,600

Fig. 13. High magnification of the secretory granules (SG) produced by the T_6 cell. The substrate of the secretory granules in various electron densities, and the fibrous reticular structures in high electron density are located within the granules in various arrangement. × 24,000

the ciliated endothelial cells were based on the columnar cells in shape but were modified much by the types. They did not possess either of the microvilli or the ciliates on their apical surfaces. Most of the apical surfaces of the secretory cells, except secreting portions, were covered with the extended upper cytoplasm of the ciliated cells.

Type 3 (T_3) cell with a nucleus mostly at center, distributed periphery of the lobules, contained many oval or amorphous secretory granules 2-3 μm in diameter. The granules contained within the cell showed methylenephilia in double stain with





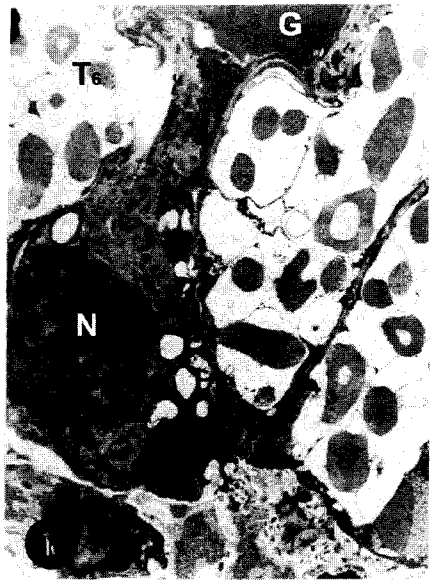


Fig. 14. T_7 cell containing numerous secretory granules (SG) in various sizes and shapes. × 6,000

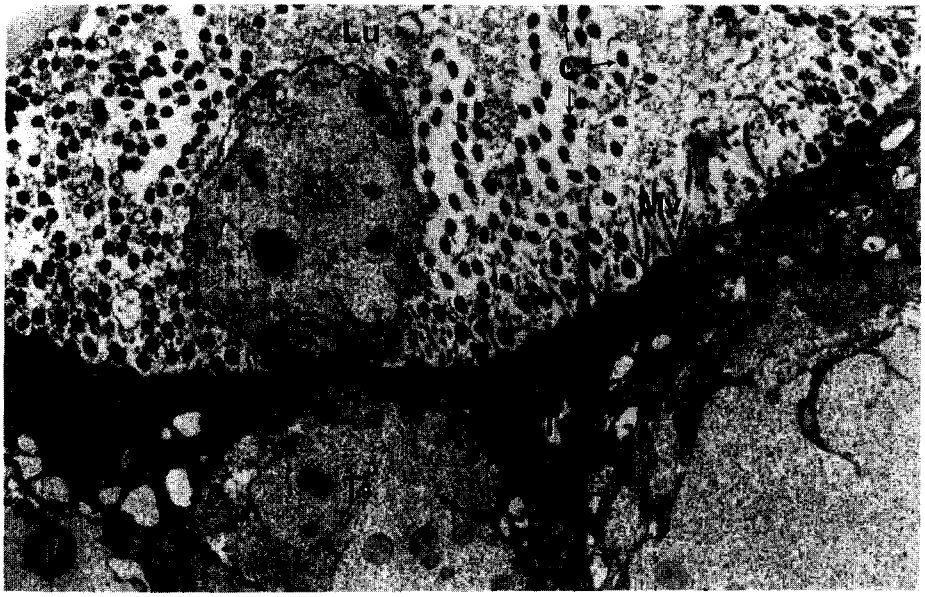
Fig. 15. T_8 cell so called glycogen cell. A big glycogen (G) mass is partly being dissolved and passed into the lumen of the duct (arrowed). × 6,000

Fig. 16. T_6 cell body. The nucleus (N) is located in the lower cytoplasm of the cell, and the glycogen (G) particles forming a big mass is located in the apical cytoplasm. × 4,000

methylene blue and basic fuchsin. The nucleus reacted PAS positive. Thus, the cells seemed to produce acid mucopolysaccharide.

The matrix of the granules in electron lucid with numerous dust-like electron dense materials scattered all over the granules. The secretory granules of this cell were not uniform in shape and size (Fig. 7).

Type 4 (T_4) cell contained secretory granules in moderate electron density up to 1-2 μm in diameter. The nuclei of these cells were located on the bases or lower lateral sides of the cells beside the



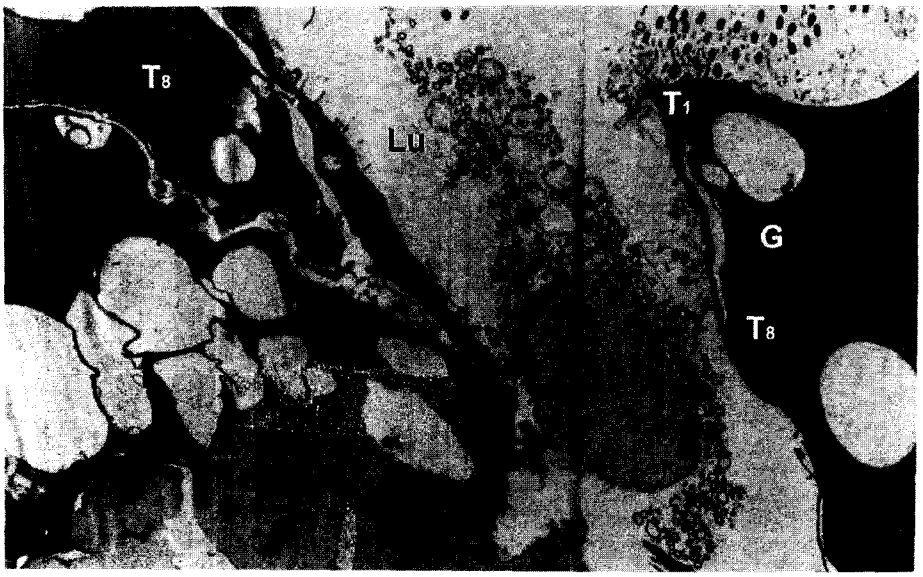


Fig. 17. T_9 cell is passing its secretory granules (SG) into the lobular duct lumen (Lu) through its apical portion. This cell also is supported by the T_1 cells neighbored. × 8,000

Fig. 18. Parts of secretory granules of T_9 cell already passed into the duct lumen (Lu). On the other side of the duct lumen, parts of a T_1 cell and a T_8 cell with the glycogen (G) mass are observed. × 5,000

secretory granules and the rER in high electron density were full of the cytoplasm (Figs. 8-9).

Type 5 (T_5) cell contained nucleus in the basal part of the cell and its secretory granules (-3.5 μm) in relatively high electron density were full of the cytoplasm beside the rich rough endoplasmic reticulum (Fig. 10).

Type 6 (T_6) cell was the largest one among the epithelial cells of the salivary gland and contained well developed electron dense rER and secretory granules with electron lucid granule substrate and electron matrices in various shapes and electron density. Most of the matrices show fibrous figures in various arrangement and some of them show figures of amorphous particles in different arrangement or density. In many secretory granules, the internal matrices exist in multiple number and fuse together sometimes.

The secretory granules of this type cells were stained purple in a double stain with methylene blue and basic fuchsin. They did not reacted to Alcian-blue, but to PAS (Figs. 11-13).

Type 7 (T_7) cell was columnar cell containing numerous electron lucid secretory granules and well developed rER. This cell was rarely found in the epithelium of the salivary gland and duct. This cell did not reacted to the H-E double stain, but to the M-B double stain. This type cell was supposed to contain secretory granules of acid mucopolysaccharide (Fig. 14).

Type 8 (T_8) cell was columnar cell with electron dense cytoplasm. The nucleus was located in the basal cytoplasm and well developed rER situated in the middle part of the cell. In the apical cytoplasm of this type cell contained a large mass of certain material. This material was electron dense and partly dissolved into small particles and finally secreted into the lumen(Figs. 15-16). This mass was not stained with Alcian blue, but stained red with PAS. In another double stain with methylene blue and basic fuchsin this mass was stained purple. The cytoplasm of this type cell was stained blue and purple with the both of the stains.

Type 9 (T_9) cell contained numerous large and irregular shaped secretory granules in light electron density. The matrices of the granules were electron lucid and dust-like fine particles were homogeneously distributed and some moderate dense structures were embedded in each of the granules. This type cell had outwardly protruded cytoplasm like cap and sometimes parts of the secretory granules were secreted through the apical surfaces(Figs. 17-18).

DISCUSSION

There have been great discrepancies of the literatures on the number of cell types of the salivary gland epithelium.

The opinions on the cell types of the salivary gland range from those of authors such as Pacaut and Vigier (1906) in *Helix pomatia* Linnaeus, Carriker and Bilstad (1946) in *Lymnaea stagnalis*, Morton (1955) in *Otina*, and Pan (1958) in *Australorbis glabratus*, who reported only one cell type or Fretter (1943) in *Onchidella*, Ghose (1963) in *Achatina* and Quatterini (1967) in *Helix aspersa*, and Das *et al.* (1989) *Viviparus benalensis*, *Acrostoma variable*, *Indoplanorbis exustus* and *Macrochlamys indica*, who reported two cell types, to those of authors who reported several cell types. Gabe and Prenant (1948) reported six types in *Lymnaea stagnalis* which was previously reported one cell type by Carriker and Bilstad (1946). Chang and Han (1995, 1996) reported eight cell types in a land slug *Incilaria fruhstorferi*. Walker (1970) classified the cell types into ten in *Deroceras reticulatum* (Muller).

The cell type classification in certain organ is usually based on their shapes or functions. Considering this basic conception, the cell types previously mentioned by former researchers make us confused because some of them are too simple and some others are too complex to be understood. Even in studies on the same species as in *Lymnaea stagnalis*, Carriker and Bilstad (1946) mentioned only cell type with various functional phases, otherwise Gabe and Prenant (1948) mentioned six cell types such as basophil cell, pseudochromosome cell, alveolar cell, granular cell, acinophil cell and mucous cell. In *Agriolimax reticulatus*, Walker (1972) reported 10 cell types based on the light microscopic observations, but only eight types base on transmission electron microscopic observations. This kind difference of results between LM and TEM observations occurred in the present study. Recently, Chang and Han (1995, 1996) reported six cell types

of salivary gland in *Incilaria fruhstorferi*. Their classification seemed to be based on the cell type classification by Serrno *et al.* (1996) who reported the salivary gland cell types in six species of Helicoidea. In the present study, the author tried to refer the above precious reports so far possible. In *Radix auricularia coreana*, nine cell types are identifiable from each other depend on their ultrastructures. Two of them are supposed to be supporting cells because they do not show any secreting activities but stand formly between the numerous gland cells classified into seven types. The supporting cells can be classified into two types, T_1 and T_2.

The T_1 cell, apparently showing letter T in shape, contained a little dense cytoplasm and numerous mitochondria and glycogen particles in the upper part of the cell. Referring to the above cell inclusions and additional existence of microvilli and cilia protruded from the apical surface of the cell, this type might carry out active duty in liquid saliva transportation in the lumen of the acina and ducts of the salivary gland. The widely extended apical cytoplasm of T_1 cells make the apical portions the gland cells narrowed. Thus, all of the gland cells have narrowed necks. The T_2 cells which also seemed to contribute to supporting the frame work of T_1 cells, are small in number and size compare to T_1 cells. This type cells are usually located on the shoulder of gland cells in moderate dense cytoplasm and possess only microvilli on the free surfaces. The microvilli existing on the apical surfaces of two supporting cells types suggest that they contribute to liquid and ion transportation though intra- or extralobular ducts of the salivary gland as mentioned early (Pease 1956; Doyle 1960; Diamond and Tormey 1966).

Serrano *et al.* (1996) classified the cell types of the salivary gland in Helioids based mainly on the secretory vesicle and shapes of rough endoplasmic reticulum. Moreno *et al.* (1982) classified the cell types of salivary gland in *Helix aspersa* depend on the histochemical natures of the cell components and ultra structures. They matched the results obtained from the both techniques well. Chang and Han (1995) also tried to have accuracy in interpreting the epithelial cells of salivary gland in *Incilaria fruhstorferi*.

In *Radix auricularia coreana*, the gland cells that composing the salivary gland can be classified into several groups according to chemical natures of their secretory material and their shapes.

The T_1 and T_2 cells are supposed to be frame cells supporting the epithelium and the various secretory cells neighboured. These two types are firstly reported in detail in the present study.

A total of seven cell types is classifiable beside the supporting cells mentioned above. The T_3 cell contained secretory granules showing methylenophilia in double stain with methylene blue and basic fuchsin. The nucleus reacted PAS positive. Thus, this type seems to produce acid mucopolysaccharide.

It has been known that the results obtained from the histochemical work under the light microscope using thick sections from the paraffin blocks and the ultrastructural work using thin sections from the Epon block often do not match well. This problem is also experienced by the present authors throughout the process.

In the present study, the chemical natures of cytoplasmic components of the T_4, T_5, and T_9 cells are not clearly identified as yet. The T_6 cells are supposed to contain neutral mucopolysaccharide granules depend on the double stains applied. The secretory granules of this type cells were stained purple in a double stain with methylene blue-basic fuchsin, and they did not reacted to Alcian-blue, but to PAS.

The T_7 cell seems to contain secretory granules of acid mucopolysaccharide considering that this cell is not stained with the H-E double stain, but stained red with the M-B double stain. The T_8 cell contains an electron dense mass in the apical cytoplasm. According to a series of histochemical works and ultrastructural observations undertaken, the electron dense material must be a glycogen mass which has not been reported by previous reports. Each of these granules shows different internal structures from each other due to many variations in internal ultrastructures. Most of the matrices show fibrous figures in various arrangement, and some of them show figures of amorphous particles in different arrangement or density. In many secretory granules, the internal matrices exist in multiple number and fuse together sometimes. Due to the more or less reticular figures of the matrices in the secretory

granules of the gland cell, Moreno *et al.* (1982) even called this cell pseudochromosome cell. The nomenclature of the pseudochromsome cell they used was maintained by tradition.

The secretory granules, observed throughout the present study, show homogeneous matrices in some, and some others show heterogeneous matrices by having certain electron dense matrices centrally or periphery in the granules. According to the report of Moreno *et al.* (1982), the notable fact was that there was no ciliated epithelial cells in the duct, but in the present study on the salivary gland and duct of *Radix auricularia coreana* the ciliated cells exists in the epithelial cells of the both. The different figures in the internal structures of the secretory granules seem to show different stages of maturity as mentioned by Moreno *et al.* (1982). As stated once by Blain (1957) some of the cells out of the various types of the secretory cells may be under the transformation to certain types. The epithelial cells of the salivary gland and the salivary duct showed almost similar pattern in their types.

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