한국패류학회지 : 제 9 권 2 호
Korean J. Malacol.
Vol. 9(2) : 94-102, 1993

The Ultrastructure of the Spermatheca of the Pulmonate Snail *Nesiohelix samarangae*

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=국문요약=

동양달팽이 정자낭 (Spermatheca)의 미세구조

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정 계 헌

동양달팽이(*Nesiohelix samarangae*)의 자성생식기 중 정자낭(spermatheca)의 미세구조를 투과전자현미경을 이용하여 관찰하였다.

성체의 정자낭의 내강에서는 정자와 이와 관련된 물질들 또는 상피로부터의 분비물질들이 관찰되었다. 정자낭의 내강을 둘러싸고 있는 벽의 상피는 단층원주상피로서 내강과 연한 세포의 원형질막은 미세융모를 가지고 있었으며 섬모는 가지고 있지 않았다. 이 미세융모들의 기부에서는 고배율 하에서 pinocytotic vesicle들이 관찰되었고 때로 세포의 분비물을 세포 밖으로 방출하는 모습도 관찰되었다. 세포들의 상부세포질에는 많은 수의 mitochondria가 있었고, 때로 분비과립과 lysosome들이 많이 존재하기도 하였다. 이들보다 아래에는 잘 발달한 골지체와 과립소포체(RER)들이 있었다. 이 부위에서 분비과립들이 많이 형성되어 있는 모습이 세포에 따라 관찰되었고, 그 아래에 즉, 각 세포의 하반부 세포질에 상하로 긴 핵이 관찰되었다. 세포의 기부 원형질막은 상부 세포질 내로 깊게 그리고 수없이 많이 주름을 이루고 있어 인접한 세포와의 경계를 구분할 수 없을 정도였다. 기저막의 아래에는 근육다발과 색소세포들이 결합조직 내에서 관찰되었고, 정자낭의 외막은 큰 액포로 인하여 세포질들이 원형질막 주변으로 밀려나 있는 특이한 세포들로 이루어져 있었다.

정자낭의 기능은 정자를 일시 저장하며 정자낭 상피에서 분비되는 소화액들로 정자를 일차적으로 세포외 소화하고 소화되지 않은 물질들을 흡수하여 이차적으로 세포내소화하는 것으로 생각된다.

INTRODUCTION

The spermatheca, also referred to as seminal receptacle, is a small accessory organ that is one of the characteristic parts of the genital system of the pulmonates. It is a pear-shaped blind sac originating from the vagina through a narrow stem, called spermathecal duct. Its wall is composed of simple columnar epithelium and a thin connective tissue sheath. The postulated functions of the spermatheca which have been suggested were various such as a sperm storage function (Kilas, 1960), a sperm activation function (Els, 1973; Sirgel, 1973; Els, 1974; Stear, 1974) and a sperm degradation-reabsorption function (Jong-Brink, 1969; Thomson and Bebbington, 1969; Németh and Kovács, 1972; Lind, 1973; Hryhiewiecka-Szyfter and Redzinkiak, 1976; Reeder and Rogers, 1979; Rogers *et al.*, 1980; Kitajima and Paraense, 1983).

The purpose of present study was to investigate the ultrastructures of the spermatheca of *Nesiohelix samarangae* in regard to its function.

MATERIALS AND METHODS

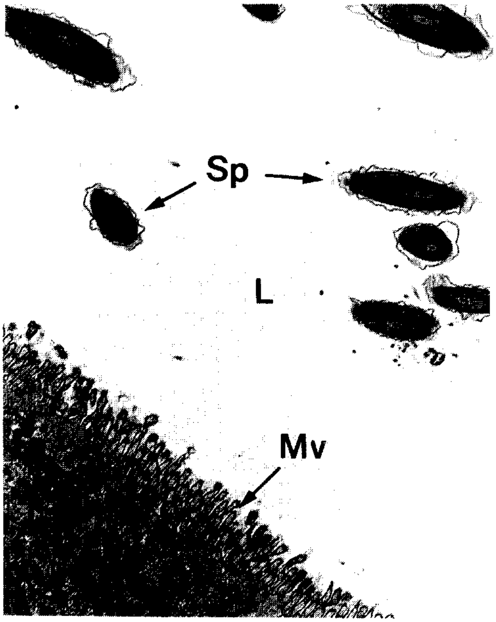
Material was a land snail, *Nesiohelix samarangae* (Pulmonata: Bradybaenidae), the only Korean species belonging to genus *Nesiohelix*. Adult individuals of the land snails were collected at the Kaeui-do, a small island located in the West Sea of Chungnam province in the late summer of 1992 and reared under laboratory conditions. They were maintained in a colony and allowed to mate freely for this experiment. The snails were anesthetized with a mixture of 2% magnesium chloride, 0.01% succinylcholine chloride and 0.005% streptomycin sulfate and dissected under the sterescope after having their shells broken. The spermatheca was removed and immediately fixed with 1% glutaraldehyde-1% paraformaldehyde mixture buffered with phosphate buffer, postfixed in 1% OsO4 also buffered, dehydrated in a graded series of alcohol-acetone mixture, and embedded in Epon 812 mixture. The ultrathin sections were double satined with uranyl acetate and lead citrate, and observed with JEM CX I transmission electron microscope.

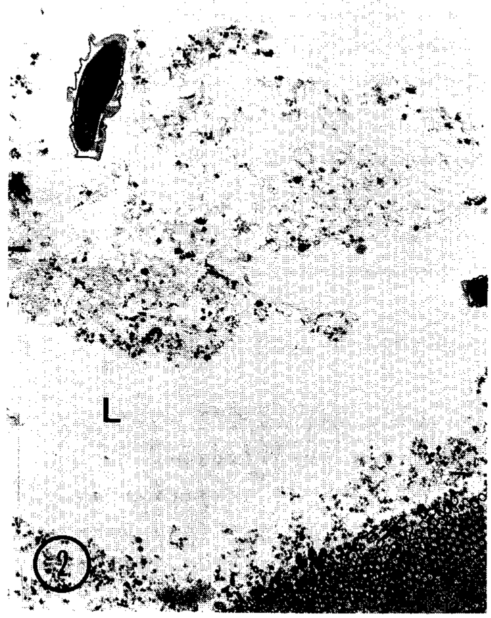
OBSERVATIONS AND DISCUSSION

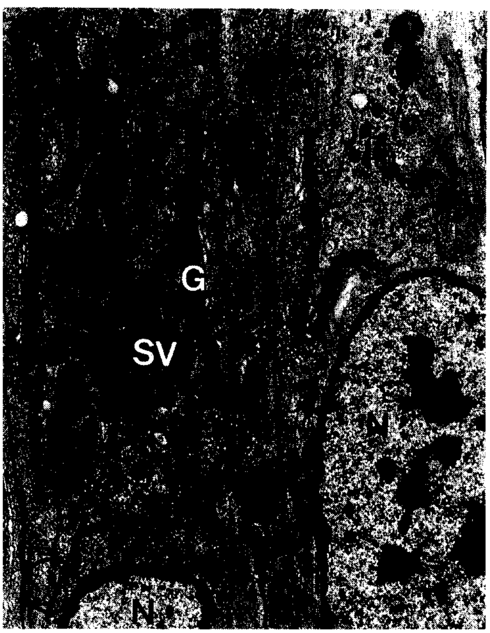
The spermatheca of *Nesiohelix samarangae* is thin-walled structure with a large lumen which is filled with digested or partially digested sperm and associated materials. Sections of sperm tails are found throughout the luminal contents of the spermatheca and spermathecal duct (Figs. 1, 2).

The sections of the sperm tails indicate that the sperm of *Nesiohelix samarangae* has a tail with a single set of the microtubular system as a locomotor organelle(Fig. 1). The wall of the spermatheca is composed of three layers such as inner most epithelium, median muscle layer and outermost layer as reported in *Sonorella santaritana*(Rogers *et al.*, 1980).

All of the cells consisting of the epithelium of the spermatheca has a brush border with microvilli on the apical part facing to the lumen(Figs. 1, 2, 6~8). Unlike to the epithelial cells of the spermatheca of *Biomphalaria glabrata* (Kitajima and Parasense, 1983), the epithelial cells of the spermatheca of *Nesiohelix samarangae* do not have ciliary border on their luminal surfaces. The lateral protoplasmic membranes of the epithelial cells contact with each other by septate desmosomes at the upper part of the cells (Figs. 1, 6). At the basal part, the cell membrane deeply infolded so many times into the above cytoplasm. Because of the numerous infoldings of the basal lamina into the cytoplasm, the structure of the basal part by cell is not distinguishable (Figs. 11, 12). Therefore, it is a possible postulation that the epithelial cells may exist in a syncytial arrangement as suggested in *Sonorella santariana* (Rogers *et al.*, 1980). The spermatheca may digest the remnant sperm and sperm fluid after copulation. The epithelium may secrete digestive enzyme to the lumen, or alternally, the sperm material may be phagocytosed and digested intracellularly in the phagosomes. These processes may take place simultaneously. These kinds of postulations on the basis of histochemical and ultrastructural evidences were pre-







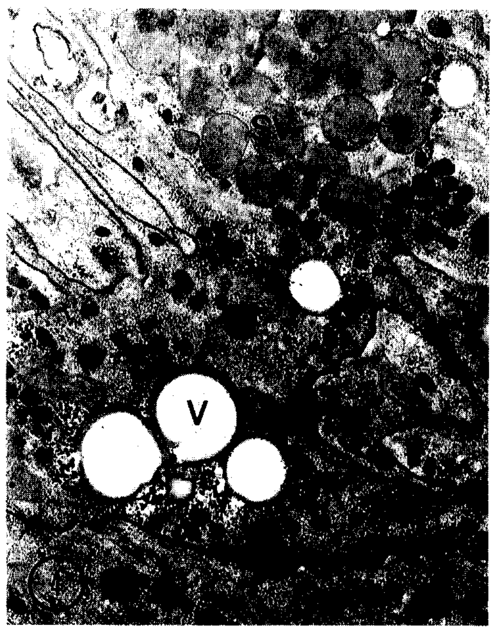
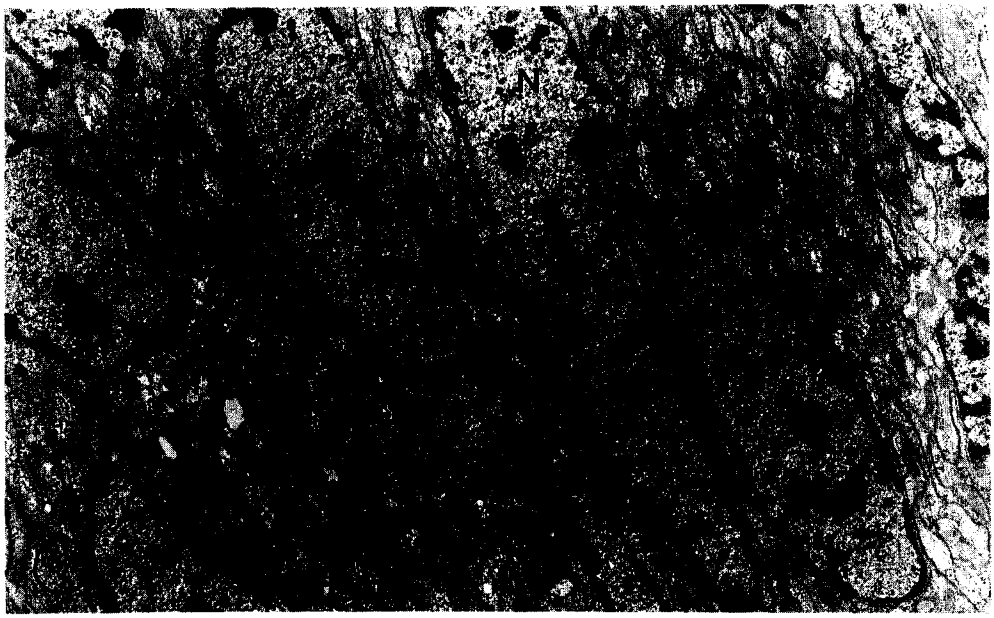


Fig. 1. Lumen(L) of the spermatheca containing the sperms(Sp). The epithelium of the spermatheca brushbordered with microvilli(Mv) has numerous mitochondria(M) in the apical cytoplasm. D: desmosome(× 5,600)

Fig. 2. Spermathecal duct with a sperm and debris. (× 5,600)

Fig. 3. Upper part of the cytoplasm with Golgi apparatus(G) and secretory vesicles(SV). N: nucleus (× 11,000)

Fig. 4. Cytoplasm showing some vacuoles and secretory vesicles. (× 11,000)



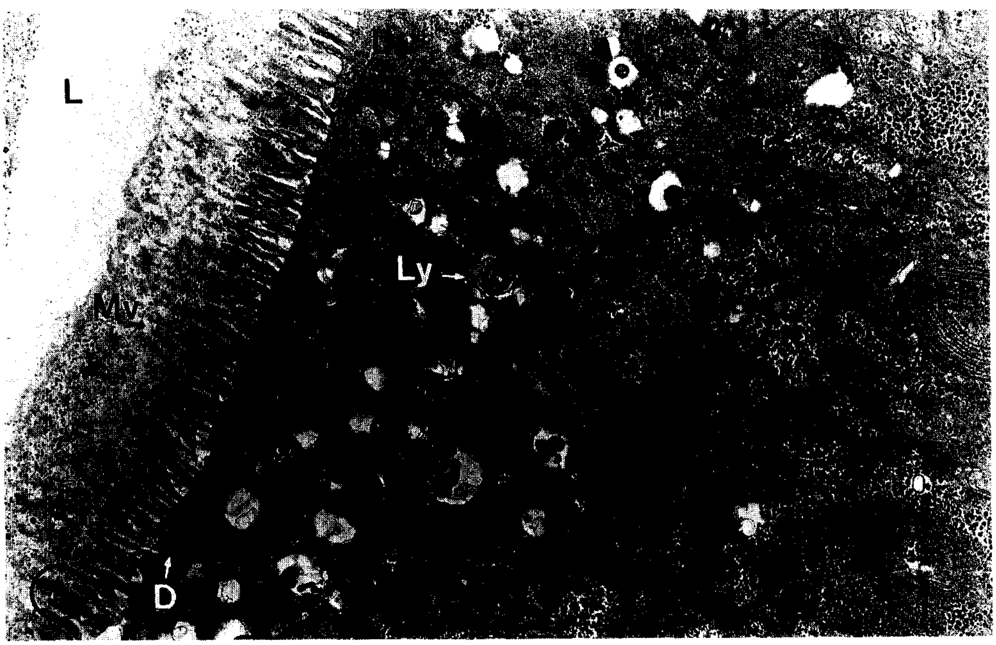


Fig. 5. Cells with a oblong nucleus(N) for each in the lower cytoplasm. Numerous infoldings of the plasma membranes form a labyrinth by intertwining. (×8,000)

Fig. 6. Cells showing rich lysosomes(Ly) in the cytoplasm indicate active intracellular digestion. L: lumen, M: microvilli, D: septate desmosome, GP: glycogen particle, RER: rough surfaced endoplasmic reticulum (×8,000)

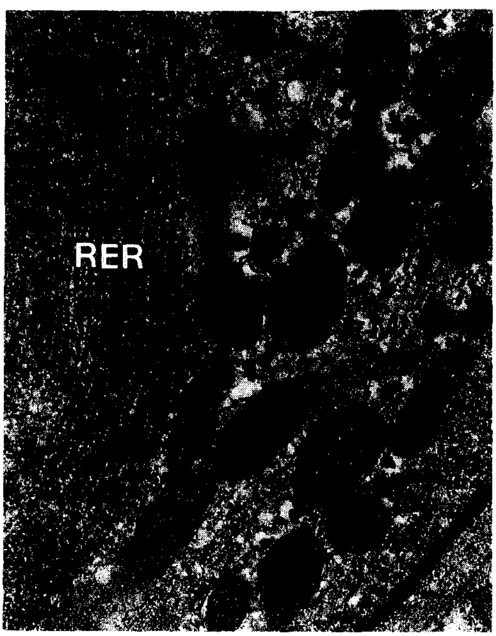


Fig. 7. Epithelial cell showing a extrusion phase. Numerous mitochondria(M) are located in the apical cytoplasm. L: lumen, Mv: microvilli (×5,600)

Fig. 8. A cell of the spermathecal duct near to the spermatheca is extrud-ing the cellular products. It seems to be a apocrine secretion. SV: secretory vesicle, Ly: lysosome (×5,600)

Fig. 9. Well developed rough surfaced endoplsmic reticulum(RER) and mitochondria(M) found in the middle part of the cytoplasm. (×30,000)

Fig. 10. Apical cytoplasm showing the pinocytic vesicle(PV) beneath the microvilli, the multivesicular bodies(VB) and the glycogen particles(GP). Mv: microvilli (×30,000)

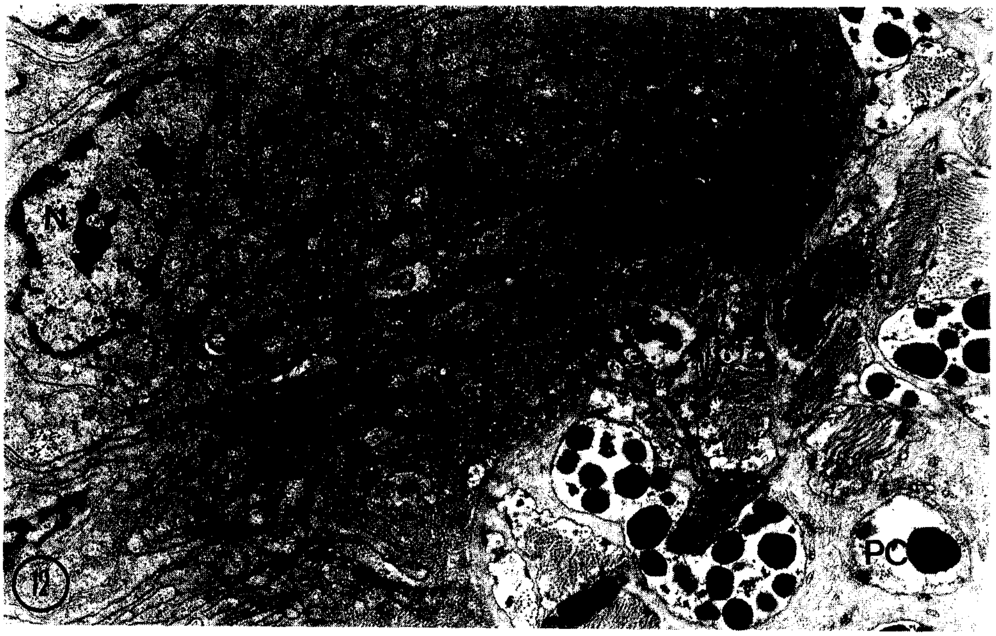
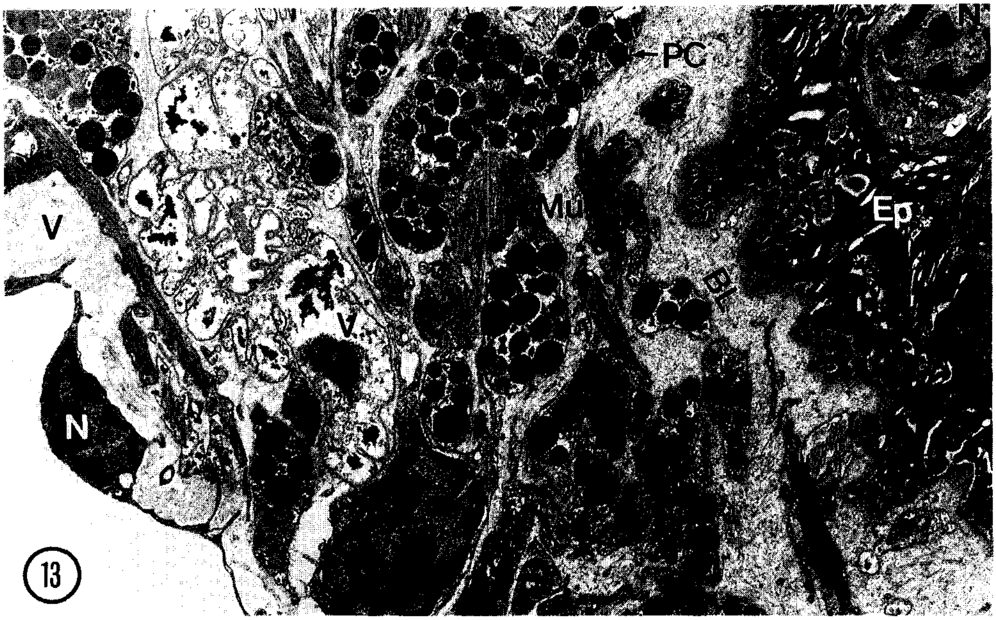


Fig. 11. Micrograph showing the labyrinth of intertwining basal plasma membrane(PL), basal lamina(BL), and a layer consisted of muscle bundles(Mu) and pigment cells(PC). (×8,000)

Fig. 12. Micrograph of a region taken near to that of Fig. 11. (×8,000)



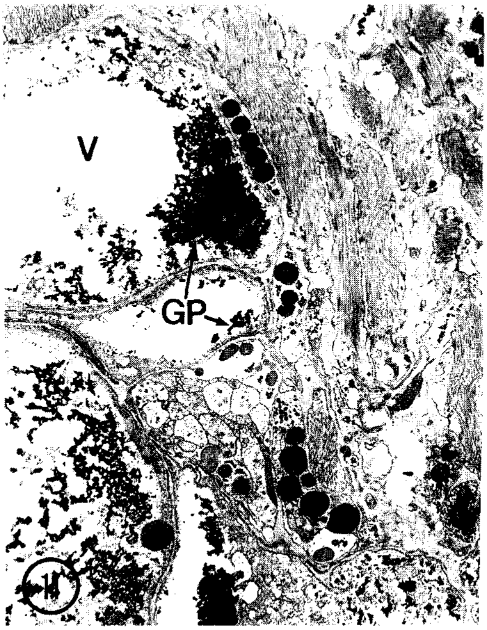


Fig. 13. Micrograph showing a overall internal view of the spermatheca except the upper part of the epithelium(Ep). The epithelium(Ep), basal lamina(BL), medial layer with the muscle(Mu) bundles and pigment cell(PC), and the adventitia consisted of cells with large vacuoles(V) are arranged in order. N: nucleus (×5,600)

Fig. 14. Adventitia consisted of the cells with large vacuoles(V) and peripherally located cytoplasm. G: glycogen particles (×5,600)

Fig. 15. A medial layer of the spermathecal wall. BL: basal lamina, PC: pigment cell (×5,600)

sented by Jong-Brink (1969) in *Biomphalaria glabrata*, Németh and Kovács (1972) in *Helix pompatia*, Reeder and Rogers (1979) in four species of *Sonorella*, Rogers *et al.* (1980) in *Sonorella santaritana*, and by Kitajima and Paraense (1983) in *Biomphalaria glabrata*.

In fact, some of the epithelial cells of the spermatheca and spermathecal duct observed throughout this study show the cellular extrusion activity which may be concerned with the extracellular digestion (Figs. 7, 8). The many of the secretory vesicles formed by the Golgi apparatus may fuse together before being extruded and the type of secretion seems to be apocrine secretion (Fig. 7, 8).

Some multivesicular bodies encircled by a single membrane are also found in the apical portion of the epithelial cells (Fig. 10). Friend (1969) and Turner *et al.* (1975) stated that the multicellular bodies are formed by vesicles originating from the Golgi apparatus. They are supposed to be involved in the digestive process in the cell as suggested by Friend (1969) and Cantin *et al.* (1975). Kitajima and Paraense (1983) mentioned their view that the multicellular bodies, arising from the activity of the Golgi apparatus would contain predonmінantly proteolytic enzymes as well as DNAase, and possibly lipase, for extracellular digestion of the sperm material. Reeder and Rogers (1979) demonstrated DNAase and protease activity in the spermathecal lumen in four species of *Sonorella*.

Actually, the apical surface of the epithelial cell seems to absorb certain material from the lumen for intracellular digestion. The presence of the microvilli on the free cell surface, formation of pinocytic vesicles at the base of microvilli (Fig. 10), and the rich lysosomes in the cytoplasm support this idea (Fig. 6).

The most of the epithelial cells contain numerous mitochondria tightly packed in the apical region (Figs. 1, 7~9). The next region distal to the spermathecal lumen is an area with well developed Golgi apparatus, vacuoles, concentrations of RER, and glycogen particles (Figs. 3, 4, 9, 10). The presence of the above cell organelles also would be the evidence for a digestive function of the cells. The next to the above region is the nuclear zone. The nuclei are oblong or lobate. Cellular inclusions in this region are again varied with RER, glycogen particles deposited around the nuclei (Figs. 3, 5, 6, 8). Numerous infoldings of the basal lamina of the cell reach the surrounding area of the nucleus in some cells (Fig. 5).

The basal region of the columnar epithelial cells rests on the basal lamina distal to which is the second major layer of the spermathecal wall. The second layer that is the medial layer of the spermathecal wall encircles the spermatheca completely just distal to the basal lamina (Figs. 11, 12). The medial layer consists of two cell types such as nonstriated muscle cells and probable pigment cells situated themselves above or under the muscle bundles. The latter contain many darkly stained globules which are not membrane bounded (Figs. 11~15). Rogers *et al.* (1980) suggested that these cells might be pigment cells embedded in the connective tissue.

The outermost layer, the adventitia, of the spermathecal wall which is adjacent to the above second layer, is consisted of a single type of cells. As reported in *Sonorella santaritana* (Rogers *et al.*, 1980), each of the cells has cytoplasm peripherally to a large vacuole fully or partially filled with electron dence fine granules (Fig. 13, 14). The nucleus of the cell is located in the peripherally concentrated cytoplasm and the remainder of the cytoplasm is distributed in a thin layer around the large vacuole (Figs. 13, 14). Rogers *et al.* (1980) suggested that these granules might be glycogen granules only stored in the connective tissue.

In summary, the ultrastructural evidences obtained from present observations strongly support the presumptions so far appeared that the spermatheca is an organ where sperms are digested extracellularly, and certain materials absorbed into the epithelial cells of the spermatheca are further digested intracellularly.

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