Korean J. Zool. 39:400-409, 1996

Ultrastructural Changes of the Vas Deferens Epithelium by Season in a Slug *Incilaria fruhstorferi*

Nam-Sub Chang, Kye-Heon Jeong†, and Jong-Min Han*

Department of Biology, College of Science and Engineering, Mokwon University, Taejon 301-729;
†Department of Biology, College of Natural Science, Soonchunhyang University, Asan 336-745, Korea

A study on the ultrastructural changes in the epithelium of the vas deferens by season was conducted for the spring and summer specimens of a slug *Incilaria fruhstorferi*. The vas deferens of the spring specimen was muscular tube about 0.4 mm in diameter. Its lumen was divided into three flat grooves and the each groove was subdivided into two subbranches. The luminal epithelial cells of the Vas deferens which were irregular in shape showed strong methylenophilia in a double stain of methylene blue and basic fuchsin. The lumen of the vas deferens was filled with components strongly stained by methylene blue. The circular muscle layers surrounding the luminal epithelium of the vas deferens contained numerous granules arranged at regular intervals. The vas deferens of the summer specimen also was a thick muscular tube showing 0.4 mm in diameter. Its lumen was divided into four grooves but, the each of the grooves was not subdivided to form certain branches unlikely to the spring specimen. The lining epithelium of the lumen was consisted of simple ciliated columnar cells, irregular columnar cells and conical cells. The histological features were quiet different from those of the spring specimen which showed irregular cell arrangement. According to electron microscopy the epithelium of the vas deferens in the spring specimen was composed of irregular columnar cells which had irregular shaped nuclei. The nuclei of the epithelial cells were relatively large in comparison to their cytoplasm. The overall electric density of the cytoplasm was relatively high. The lumen of the vas deferens in the summer specimen was lined by a epithelium with tall ciliated columnar cells and irregular cells. The nuclei of the epithelial cells were long ellipsoid or irregular in shape. Both of the cytoplasm and the nuclei were showed low electric density. In consideration with the observable cell organelles were only endoplasmic reticulum, lysosomes and microtutules, the cell organelles were poorly developed. The apical surfaces of the epithelial cells possessed brush borders with numerous microvilli and cilia with 9+2 arrangement of microtubules. The circular muscle layers surrounding the epithelium are usually thick and the degree of development of the circular muscle layers seems to be even in the both of the spring and summer specimens.

KEY WORDS: *Incilaria fruhstorferi*, Vas Deferens, Ultrastructure, Seasonal Change

The reproductive systems of the pulmonate snails have long been the subjects of studies since Semper (1857), Meisenheimer (1907) and Ikeda (1929). On the atrial gland, an accessory gland connected to the hermaphrodite duct, Beeman (1970), and Arch *et al.* (1980) and Beard *et al.*

*To whom correspondence should be addressed.

(1982) reported results of morphological and physiological observations that the gland was an apocrine gland engaged in one of major roles in the reproductive system. Besides them, Heller *et al.* (1980), Schlesinger *et al.* (1981) and Rothman *et al.* (1982, 1984) stated that the atrial gland contained some materials including several peptides which have an effect on egg-laying activity. Otherwise, the atrial gland of *Aplysia* was not active in peptide control of egg laying by copulatory behavior (Blankenship *et al.*, 1983a, b). Studies on the spermatheca were conducted by several authors (Rigby, 1963; Bayne, 1970, 1973; Németh and Kovàcs, 1972; Reeder and Rogers, 1979; Jeong, 1993). But, there have been few light microscopic studies so far performed on the male genital organs (Horm, 1946; Duncan, 1960; Stears, 1974; Rudolph, 1983, Lee *et al.*, 1992; Chang *et al.*, 1995). Present studies was planned to find out the ultrastructural changes of the vas deferens by season according to a meaningful previous report on the hermaphrodite duct (Chang and Jeong, 1996).

Material and Methods

Animals

The material was the slug, *Incilaria fruhstorferi*, collected from the humid oak woods valley near to the Tonghak buddhist temple in the mountain Kyeryong, Kongju, Choongnam, Korea. They were collected in the spring (March to April) and in the summer (July to August) of 1995.

Light and Transmission electron microscopy

The slugs were anesthetized with 30% ethyl alcohol before dissection. After that the vas deferens was isolated from the snail body and was cut into sections right before fixation. The specimens were fixed with 2.5% paraformaldehyde-3% glutaraldehyde for 1 1/2 hours and postfixed with phosphate buffered OsO_4 for 2 hours. The fixed specimens were washed three times with 0.2 M phosphate buffer (pH 7.3), dehydrated in a graded series of ethyl alcohol, embedded in Epon 812 mixture, and were incubated for 40 hours at 60°C Thick sections in 1 μm thick obtained from the Epon blocks were stained with methylene blue-basic fuchsin for light microscopic observations. Ultrathin sections were double stained with uranyl acetate and lead citrate and were observed under the transmission electron microscope (JEM 100CX-II, 80KV).

Results

Morphological differences in the epithelia of the vas deferens between the spring and summer specimens of a slug *Incilaria fruhstorferi* was confirmed by the light and electron microscopic observations.

Light Microscopic Findings

Spring Specimen The vas deferens of the spring specimen was muscular tube about 0.4 mm in diameter. Its lumen was divided into three flat grooves and the each groove was subdivided into two subbranches. Thus, the cross sectioned view of the lumen of the vas deferens apparently looked like wings of an electric fan. This feature was because of the three big protrusions of the wall toward the center of the lumen (Fig. 1). The epithelial cells of the wall which were irregular in shape showed strong methylenophilia in a double stain of methylene blue and basic fuchsin (m-b).

The lumen of the vas deferens was filled with components strongly stained by methylene blue.

The circular muscle layers surrounding the luminal epithelium of the vas deferens contained numerous granules arranged at regular intervals. These granules did not reacted any to the above m-b double stain (Fig. 1, arrowed).

Summer Specimen The vas deferens of the summer specimen also was a thick muscular tube showing 0.4 mm in diameter. Its lumen was divided into four grooves but, the each of the grooves was not subdivided to form certain branches unlikely to the spring specimen (Fig. 7). The lining of the lumen was consisted of simple ciliated columnar cells, irregular columnar cells and conical cells. The histological features were quiet different from those of the spring specimen which showed irregular cell arrangement. Either in m-b

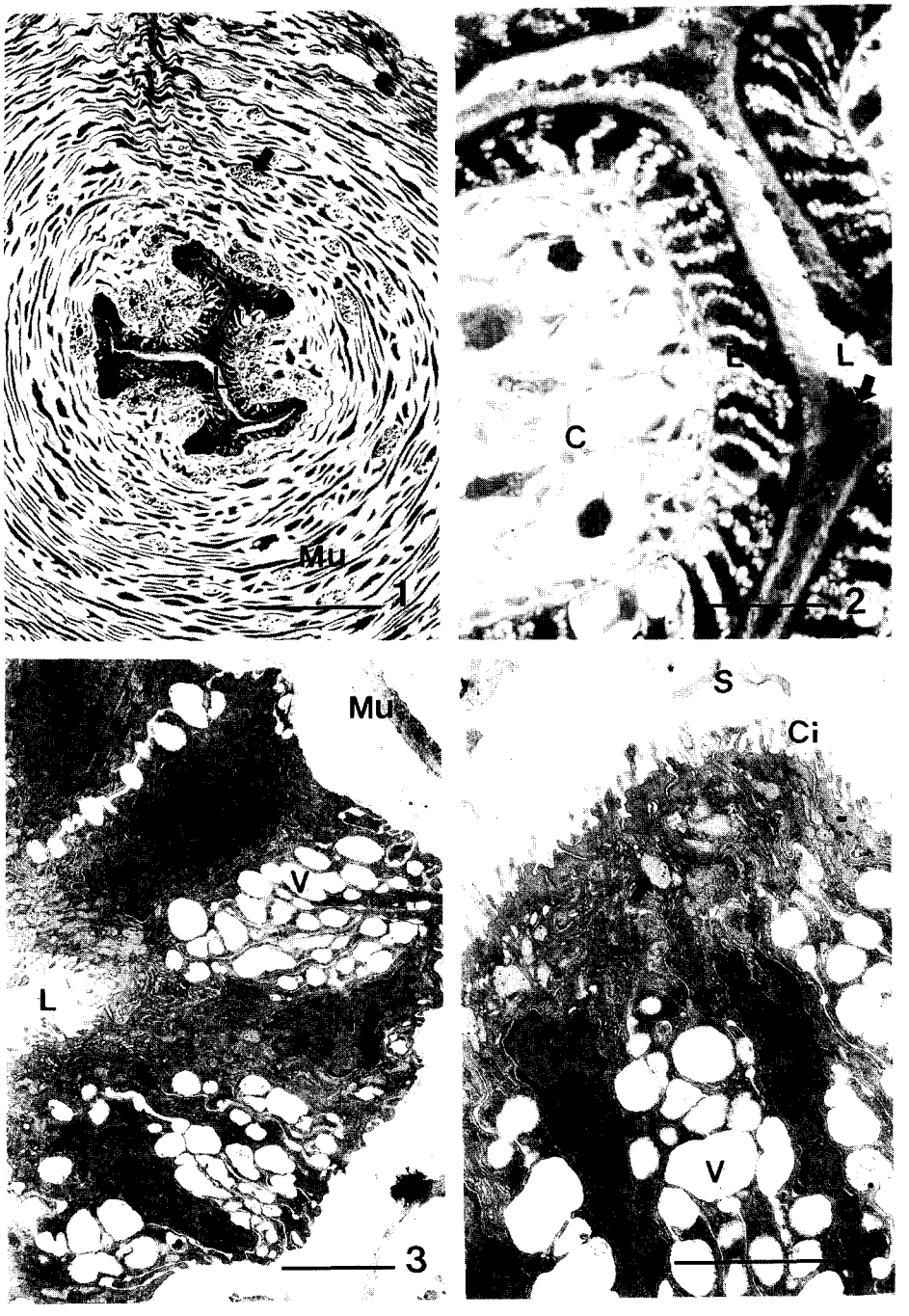


Fig. 1. Cross-section through the vas deferens of the spring specimen. Arrow, granule; L, lumen; Mu, circular muscle layer. methylene blue-basic fuchsin double stain. Scale bar = 200 μm.
Fig. 2. Magnification of Fig. 1. Arrow, cilia; L, lumen; E, epithelium; C, connective tissue. Scale bar = 20 μm.
Fig. 3. Electron micrograph showing the luminal epithelium of the spring specimen. N, nucleus; V, vacuole; L, lumen; Mu, muscle. methylene blue-basic fuchsin double stain. Scale bar = 2 μm.
Fig. 4. Magnification of Fig. 3. Arrow, granule; S, spermatozoon; Ci, cilia; N, nucleus; V, vacuole. Scale bar = 2 μm.

double stain, the epithelial cells did not show strong methylenophilia unlikely to the spring specimen.

Electron Microscopic Findings

Spring Specimen The epithelium of the vas deferens was composed of irregular columnar cells which had irregular shaped nuclei. The nuclei of the epithelial cells were relatively large in comparison to their cytoplasm. The overall electric density of the cytoplasm was relatively high. The serrated nuclear membrane evenly surrounded granular heterochromatin. The free surfaces of the luminal epithelial cells were covered with microvilli and the apical cytoplasm of the cells contained ovale or irregular granules in moderate electron density. The lateral protoplasmic membranes of the epithelial cells were very irregular and the cytoplasm of the cells contained many vacuoles ovale or ellipsoid in shape. The vacuoles situated mostly in the lateral cytoplasm of the cells (Figs. 3 and 4). The luminal epithelium of the vas deferens was surrounded by circular muscle layers up to 400 μm in thickness and the each of the muscle cells possessed ellipsoid nucleus 7 $\times$ 3 μm in size.

Between the muscular layers there were many vesicular structures containing numerous granules about 2 μm in diameter and small crystals in various shapes. The crystaloid granules had concentric patterns within (Figs. 5 and 6).

Summer Specimen: The lumen of the vas deferens in the summer specimen was lined by a epithelium with tall ciliated columnar cells and irregular cells. The nuclei of the epithelial cells were long ellipsoid or irregular in shape. Both of the cytoplasm and the nuclei were showed low electric density. In consideration with the observable cell organelles were only endoplasmic reticulum, lysosomes and microtubules, the cell organelles were poorly developed (Figs. 11 and 12).

The apical surfaces of the epithelial cells possessed brush borders with numerous microvilli and cilia with 9+2 arrangement of microtubules and rootlets deeply inserted into the apical cytoplasm of the cells. In the lumen of the vas deferens, some mature spermatozoa were observed (Fig. 14).

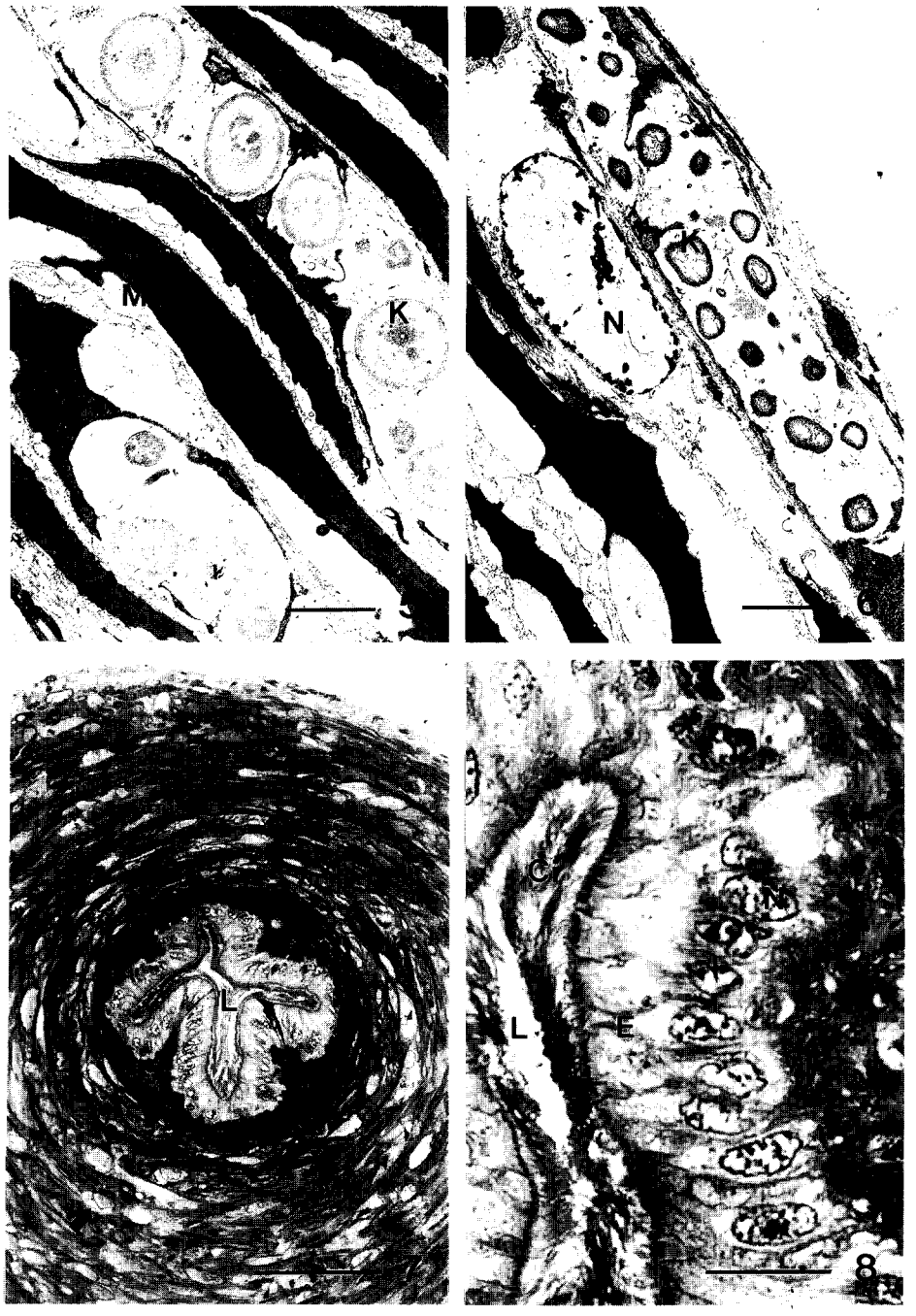
The lateral protoplasmic membranes of the upper parts of the cells infolded into each other of the neighbouring cells, but the membranes of the lower part of the cells neighboured ran relatively straight and parallel.

The luminal epithelium of the vas deferens also was surrounded by a layer of connective tissue fibers and a heavy layer of circular muscle fibers.

Discussion

The vas deferens follows a tortuous path connecting the prostate gland to the epiphallus or penis when the epiphallus is absent. This duct, in some species, is surrounded by the prostate gland up to three quarters of its whole length. In Basommatophorans such as *Physa fortinalis*, *Lymnaea peregra* and *Planorbis corneus*, the positions of the vas deferens are different from each other. The vas deferens positioned anteriorly to the prostate gland is usually longer than that in the other cases (Duncan, 1960).

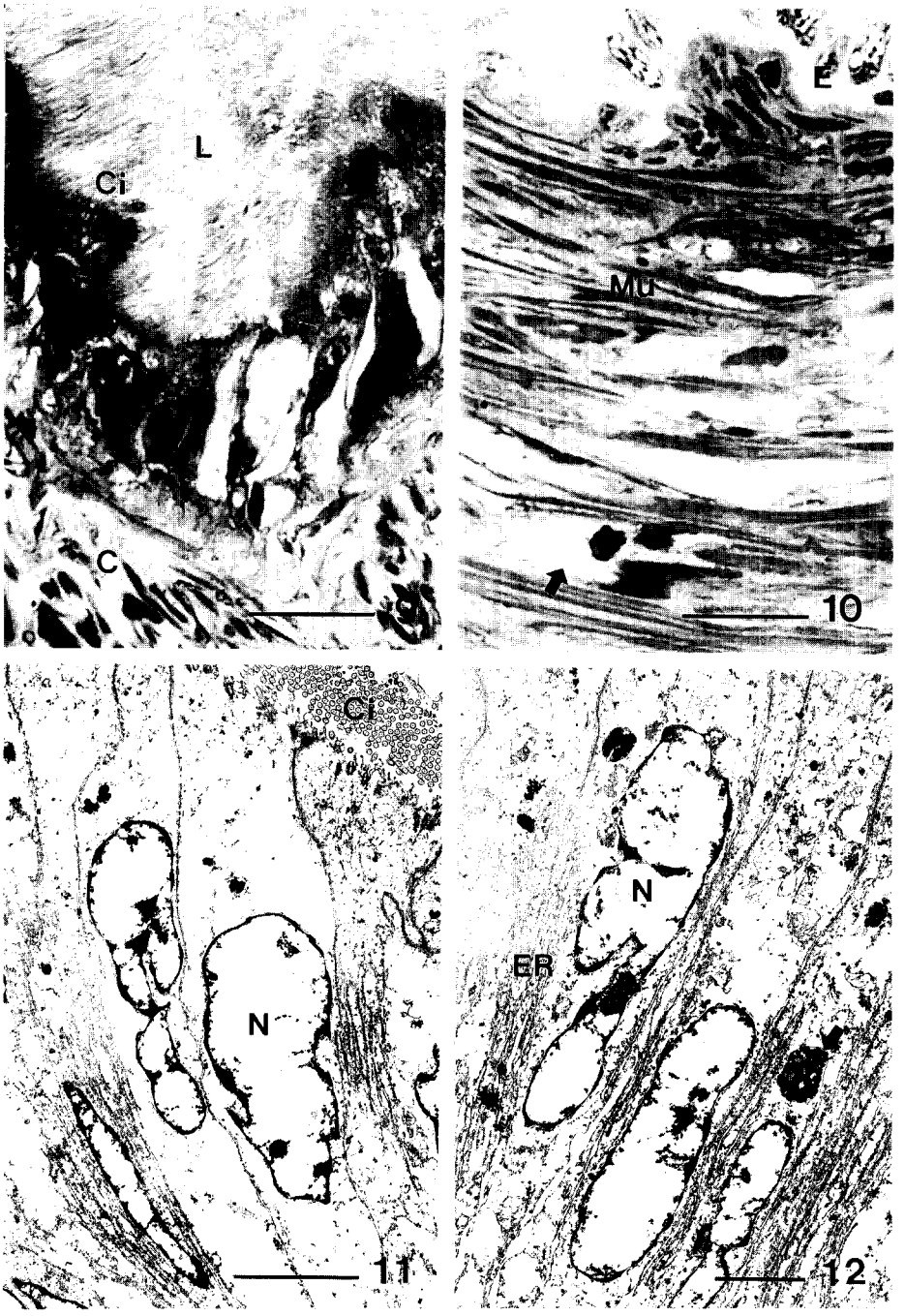
According to Pabst (1914) and Lusis (1961) in *Arion* the anterior part of the vas deferens is differentiated into an epiphallus for the formation of spermatophores. Otherwise, according to Quick (1960) in *Limax valentianus* neither has epiphallus nor forms spermatophores. Stears (1974) in *Limax valentianus* mentioned that the vas deferens proceeds anteriorly from the bifurcation of the spermoviduct as a continuation of the spermatic groove. It is completely surrounded by the prostate gland for approximately three quarters of its length and it opens into the penis. The posterior part of the vas deferens, surrounded by the prostate gland, differs histologically from the anterior part. The lumen of the posterior part is lined by a strongly ciliated, columnar epithelium. As the vas deferens proceeds anteriorly, the epithelial cells change to a cuboidal type except for a narrow strip which consists of high columnar cells forming a ridge which protrudes into the lumen. In *Incilaria fruhstorferi* the vas deferens is thin long tube which is not so convoluted as usual and likely to that of *L. valentianus* (Stears, 1974), it is stretched out of the posterior part of the prostate



Figs. 5, 6. Electron micrographs showing the many crystal granules between the circular muscle layers of the spring specimen. N, nucleus; K, crystal granule; Mu, muscle. Scale bars = 2 μm.

Fig. 7. Cross-section through the vas deferens of the summer specimen. Arrow, granule; L, lumen; Mu, circular muscle layer. methylene blue-basic fuchsin double stain. Scale bar = 200 μm.

Figs. 8. Magnification of Fig. 7. L, lumen; Ci, cilia; E, epithelium; N, nucleus. methylene blue-basic fuchsin double stain. Scale bars = 20 μm.



Figs. 9, 10. Magnification of Fig. 7. Arrow, vesicular cell; L, lumen; Ci, cilia; E, epithelium; Mu, circular muscle layer; N, nucleus; C, connective tissue. methylene blue-basic fuchsin double stain. Scale bars = 20 μm.

Figs. 11, 12. Electron micrographs showing the luminal epithelial cells of the summer specimen. Arrow, phagosome; Ci, cilia; N, nucleus; ER, endoplasmic reticulum. Scale bars = 4 μm, 2 μm.

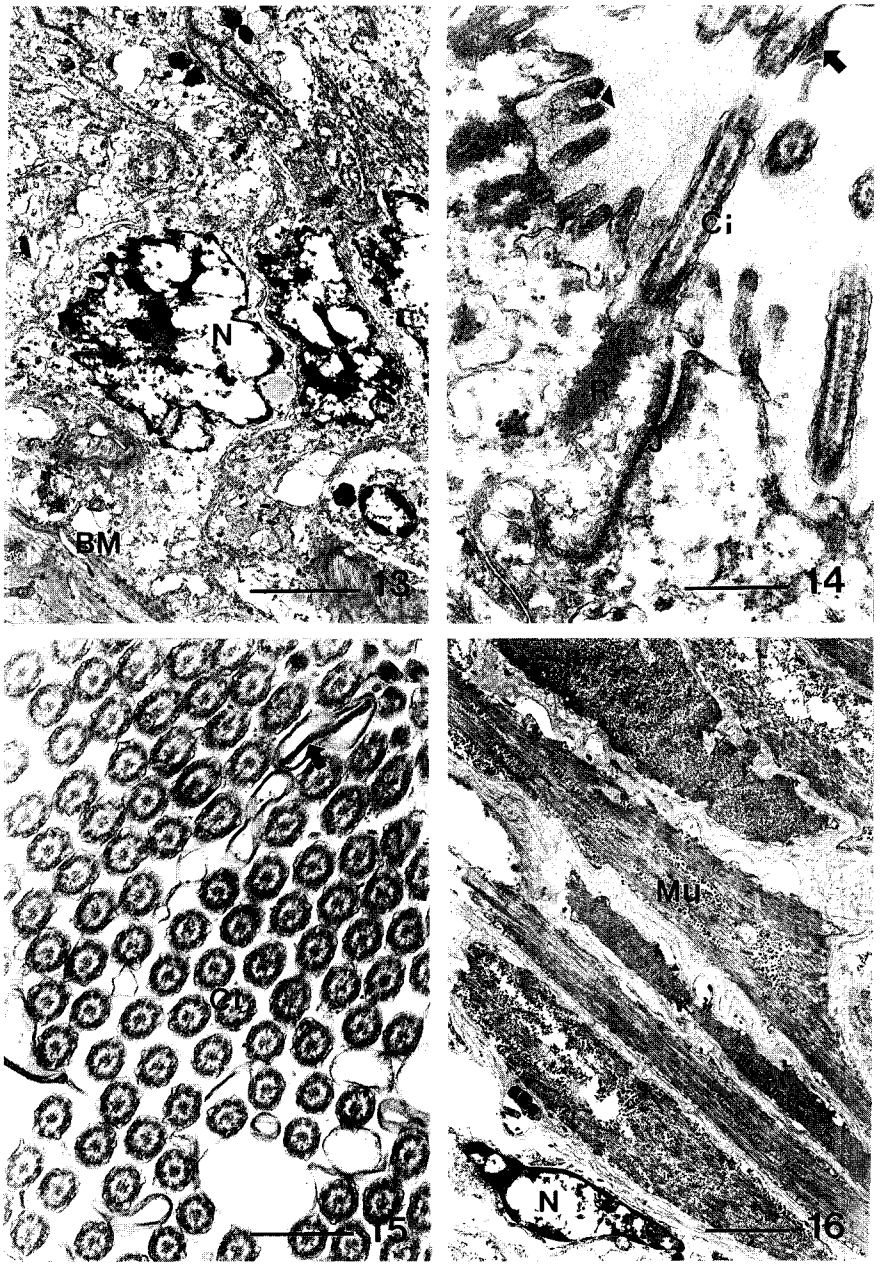


Fig. 13. Electron micrograph showing the irregular epithelial cells of the summer specimen. N, nucleus; BM, basement membrane. Scale bar = 2 μm.

Fig. 14. Electron micrograph showing the cilia and microvilli of the luminal epithelial cell of the summer specimen. Arrow, spermatozoon; arrowhead, branched microvillus; Ci, cilia; R, rootlet; J, junctional complex. Scale bar = 0.5 μm.

Fig. 15. Cross-section through the cilia of the luminal epithelium (summer specimen). Arrow, spermatozoon; Ci, cilia. Scale bar = 0.5 μm.

Fig. 16. Electron micrograph showing the circular muscle layer surrounded the luminal epithelium of the summer specimen. N, nucleus; Mu, muscle. Scale bar = 2 μm.

gland. The epithelial lining of the vas deferens composed of the ciliated columnar, ciliated conical, and irregular cells in the summer specimen is different from that of other species reported by Stears (1974), Duncan (1960) and Holm (1946).

According to Duncan (1960) in *Physa fortinalis*, *Lymnaea peregra* and *Planorbis corneus* and Kugler (1965) in *Philomycus carolinianus*, at the peak of activity of the vas deferens or at the time when the spermatozoa pass through the vas deferens small spherical granules are secreted into the lumen of the vas deferens. In *Incilaria fruhstorferi* some irregular granules are found only in the epithelial cells of the spring specimens. The epithelial cells of the vas deferens has poorly developed cell organelles such as endoplasmic reticulum, lysosomes and microtubules.

The existence of the lysosome phagocyting the spermatozoa in the epithelial cells of the vas deferens is one of new findings in the present study. Duncan (1960) stated that the epithelium of the vas deferens is formed of columnar cells which are filled apically with globules, stained blue after azan, whilst basally they are packed with purple granules and vacuoles are often present in this lower portion of the cells. He also stated that the whole cell is filled with blue-staining globules at the peak of activity. Duncan (1960) reported existence of some small ciliated interstitial cells at the apical region of the luminal epithelium.

Holm (1946) in *Lymnaea stagnalis* and Stears (1974) in *L. valentianus* reported existence of vesicular cells, in the connective tissue, which is large ellipsoid form with transparent cytoplasm and relatively small nuclei. But, in the spring specimens of *Incilaria fruhstorferi* the interstitial cells are absent in the epithelium and spherical crystals are present in the circular muscle layers and in the summer specimens some cells in the connective tissue under the epithelium are similar to the vesicular cells reported by Holm (1945) and Stears (1974).

The circular muscle layers surrounding the epithelium are usually thick as reported by earlier workers such as Duncan (1960), Kugler (1965) and Stears (1974). The degree of development of the circular muscle layers seems to be even in the both of the spring and summer specimens.

References

Arch, S., J. Lupatkin, T. Smock, and M. Beard, 1980. Evidence for an exocrine function of the Aplysia atrial gland. *J. Comp. Physiol.* **141A:** 131-137.

Bayne, C.J., 1970. Organization of the spermatozoan of *Agriolimax reticulatus*, the grey field slug (Pulmonata, Stylommatophora). *Z. Zellforsch.* **103:** 75-89.

Bayne, C.J., 1973. Physiology of the pulmonate reproductive tract: Location of spermatozoa in isolated self-fertilizing succinid snails. *Veliger* **16:** 169-175.

Beard, M., L. Millecchia, C. Masuoka, and S. Arch, 1982. Ultrastructure of secretion in the atrial gland of mollusc (*Aplysia*). *Tissue cell.* **14:** 297-308.

Beeman, R.D., 1970. The anatomy and functional morphology of the reproductive system in the opisthobranch mollusk *Phyllaplysia taylori* Dall, 1900. *Veliger* **13:** 1-31.

Blankenship, J.E., S.D. Painter, G.T. Nagle, and K.L. Kelner, 1983a. Immunological Approaches Towards Understanding the Role of Endogenous Atrial Gland Peptides in *Aplysia* egg Laying, In: Molluscan NeuroEndocrinology (Lever, J. and H.H. Boer, eds.). North Holland Publishing Co., New york, pp. 28-31.

Blankenship, J.E., M.K. Rock, L.C. Robbins, C.A. Livingston, and H.K. Lehman, 1983b. Aspects of copulatory behaviour and peptide control of egg laying in *Aplysia*. *Fed. Proc.* **42:** 96-100.

Chang, N.S., K.H. Jeong, and Y.U. Kim, 1995. Morphological and histochemical studies on the hermaphroditic and male reproductive organs of a korean slug *Incilaria fruhstorferi*. *Korean J. Malacol.* **11:** 78-91.

Chang, N.S. and K.H. Jeong, 1996. Ultrastructural changes of the hermaphrodite duct epithelium by season in a korean slug *Incilaria fruhstorferi*. *Korean J. Zool.* **39:** 139-146.

Duncan, C.J., 1960. The genital system of the freshwater Basommatophora. *Proc. Zool. Soc. Lond.* **135:** 339-355.

Heller, E., L. Kaczmarek, M. Hunkapiller, L. Hood, and F. Strumwasser, 1980. Purification and primary structure of two neuroactive peptides that cause bag cell after discharge and egg-laying in *Aplysia*. *Proc. Natl. Acad. Sci. USA* **77:** 2328-2332.

Holm, L.W., 1946. Histological and functional studies on the genital tract of *Lymnaea stagnalis* appressa Say. *Trans. Am. Microsc. Soc.* **65:** 45-68.

Ikeda, K., 1929. The spermatozoa of *Philomycus bilineatus*, with special reference to their metamorphosis in the receptaculum seminis. *An. Nat. Zool. Jpn.* **12:** 295.

Jeong, K.H., 1993. The ultrastructure of the spermatheca of the pulmonate snail *Nesiohelix samarangae*. *Korean J. Malacol.* **9:** 94-102.

Kugler, O., 1965. A morphological and histological study of the reproductive system of the slug *Philomycus caroliniacus* (Bosc.). *J. Morphol.* **116:** 117-131.

Lee, H.S., K.H. Jeong, and J.A. Park, 1992. A morphological study on the male genital organs of a land snail, *Nesiohelix samarangae*. *Korean J. Malacol.* **8:** 61-71.

Lusis, O., 1961. Postembryonic changes in reproductive system of the slug *Arion ater rufus* L. *Proc. Zool. Soc. Lond.* **137:** 433-468.

Meisenheimer, J., 1907. Biologie, Morphologie, und Physiologie des Begattungsvorgangs und der Eiablage von Helix pomatia. *Zool. Jahrb. Abt. 1* **25:** 465.

Németh, A. and J. Kovács, 1972. The ultrastructure of the epithelial cells of seminal receptacle in the snail *Helix pomatia* with special reference to the lysosomal system. *Acta Biol. Acad. Sci. Hung.* **23:** 299-308.

Pabst, H., 1914. Entwicklung des Genital-Apparates von *Arion empiricorum*. *Zool. Anat. Ontog.* **38:** 465-508.

Quick, H.E., 1960. British slugs (Pulmonata: Testacellidae, Arionidae, Limacidae). *Bull. Br. Mus. (Nat. Hist.). Zool.* **6:** 103-226.

Reeder, R.L. and S.H. Rogers, 1979. The histochemistry of the spermatheca in four species of *sonorella* (Gastropoda: Pulmonata). *Trans. Am. Microsc. Soc.* **98(2):** 267-271.

Rigby, J.E., 1963. Alimentary and reproductive systems of *Oxychilus cellarius* (Müller), Stylommatophora. *Proc. Zool. Soc. Lond.* **141:** 311-359.

Rothman, B.S., R.O. Brown, E. Mayeri, and J.E. Shively, 1982. Isolation of novel, neuroactive, Elh-like peptides from the atrial gland of *Aplysia*. *Soc. Neurosci. Abstr.* **8:** 14.

Rothman, B.S., J. Shively, D. Hawkes, R.O. Brown, and E. Mayeri, 1984. Two neuroactive peptides from a common precursor in *Aplysia* atrial gland. *Trans. Am. Soc. Neurochem.* **15:** 119.

Rudolph, P.H., 1983. Histochemistry of the reproductive tract of *stagnicola elodes* (Basommatophora: Lymnaeidae). *Malacol. Rev.* **16:** 43-57.

Schlesinger, D.H., S.B. Babirak, and J.E. Blankenship, 1981. Primary structure of an egg laying peptide from the atrial gland of *Aplysia californica*, In: Symposium on Neurohypophyseal Peptide Hormones and Other Biologically Active Peptides (Schlesinger, D.H., ed.). New York, Elsevier, pp. 137-150.

Semper, C., 1857. Beitr ge zur Anatomie und Physiologie der Pulmonaten. *Z. Wiss. Zool.* **8:** 340-399.

Stears, M., 1974. Contributions to the morphology and histology of the genital system of *Limax valentianus* (Pulmonata, Limacidae). *Ann. Univ. Stellenbosch.* **49(A3):** 1-46.

(Accepted October 4, 1996)

산민달팽이(*Incilaria fruhstorferi*) 수정관 내강 상피조직의 계절에 따른 미세구조적 변화

장남섭 · 정계헌† · 한종민(목원대학교 이공대학 생물학과,
†순천향대학교 자연과학대학 생명과학부)

산민달팽이(*Incilaria fruhstorferi*)의 웅성생식기관인 수정관을 봄과 여름 등 계절별로 나누어 관찰한 결과는 다음과 같았다. 산민달팽이 봄개체의 수정관은 0.4 mm 정도의 근육질로 구성된 관상구조물로, 내강은 3갈래로 갈라져 있고, 전체 모습은 팔랑개비형태와 같았다. 그러나 여름개체인 경우에도 역시 근육질의 관상구조물로 구성되어 있었으나 내강은 4갈래로 갈라져 있었다. 광학현미경 관찰에서 수정관의 내강상피세포는 불규칙한 형태로서, 세포들은 m-b 이중염색에서 강한 methylenophilia를 보이거나, 반응 없는 밝은 부분이 교대로 배열되어 나타나는 현상을 보였다. 그러나 여름개체의 수정관 내강상피세포는 비교적 규칙적인 세포들로 배열되어 있었으며 m-b 이중염색에서 세포들은 강한 methylenophilia를 나타낸 봄개체와는 달리 밝게 관찰되어 서로 다른 양상을 보였다. 전자현미경 관찰에서도 봄개체의 내강상피세포는 불규칙한 섬모원주상피세포로서 전자밀도는 매우 높아 검게 관찰되고 이들이 소지한 핵도 그 형태가 불규칙하였으며, 세포들은 다양한 크기의 원형 또는 타원형의 공포들로 가득차 있었다. 반면, 여름개체의 내강상피세포는 키가 큰 섬모원주 상피세포와 섬모원추형 상피세포 그리고 불규칙형 세포들로 이루어져 있었는데 세포들은 전자밀도가 낮아서 모두 밝게 관찰되고 포식작용이 활발한 몇 개의 용해소체만이 관찰될 뿐이었다. 또한 수정관의 내강은 계절에 관계없이 모두 각각 400 μm 두께의 두터운 환상근육층으로 둘러싸여 있었는데 특히 여름개체의 환상근육층 사이에서는 비교적 큰 타원형의 vesicular cell이 관찰되고, 이들은 m-b 이중염색에서 약한 basophilia를 나타내었다. 그러나 봄개체에서는 vesicular cell 대신 원형의 결정과립들만이 관찰되어 두 개체 사이에 서로 다른 양상을 보였다.