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Ultrastructural Changes of the Hermaphrodite Duct Epithelium by Season in a Korean Slug *Incilaria fruhstorferi*

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A study on the ultrastructural changes of the hermaprodite duct epithelium in spring and summer specimens of a Korean slug *Incilaria fruhstorferi* was conducted. In spring specimen, the hermaphrodite duct was 0.3 × 0.2 mm in diameter and seemed to be a little bit abnormal feature, due to shrinkage. The epithelium of the duct was composed of cells containing various-sized vacuoles and their cytoplasm showed high electron density. In summer specimen, the hermaphrodite duct was 0.4 × 0.23 mm in diameter and seemed to be more voluminous than that of the spring specimen. The lumen of the duct in the summer specimen was lined by a epithelium composed of several cell types such as ciliated cuboidal, pseudostratified cuboidal, ciliated columnar and irregular cells. Phagocytotic activities of the duct epithelium are observed only in the summer specimen. Both of the duct epithelia in the spring and summer specimens were surrounded by connective tissues at their bases. The results obtained suggest that the duct epithelia of the slugs experience seasonal variation in relation to their reproductive cycles.

KEY WORDS: Slug, Hermaphrodite Duct, Ultrastructural Changes

Studies on the reproductive organs in slugs (Philomycidae, Stylommatophora, Mollusca) were performed relatively early by several investigator (Semper, 1857; Baudelot, 1863; Meisenheimer, 1907; Baeker, 1932).

Other investigators were Pennypacker (1930) on the albumen gland, Pullet and Watts (1951), and Lariolette (1950) on the dart sac. Ancel (1903) and Pennypacker (1930) studied on the hermaphrodite ducts of *Helix pomatia* and *Polygra appressa*. Duncan (1958) in *Physa fontialis*, Sirgel (1973) in *Agriomax caruanae* and Els (1974) in *Milax gagates* stated that the hermaphrodite ducts of the species had ciliary luminal surfaces and these were common in the morphology of the pulmonates.

Stears (1974) mentioned that the hermaphrodite duct in *Limax valentianus* was divisible into three distinct regions such as two extentionally visible regions and the third region embedded in the albumen gland. The first two regions of the duct not embedded in the albumen gland were pigmented. Pigmentation of the hermaphrodite duct was also reported in (Duncan, 1958), *Limax tenellus* (Quick, 1960) and *Succinea pfeifferi* (Duncan, 1961). But the pigmentation was not observed in *Limax Flavus* (Quick, 1960). A short distance anterior to the point where the hermaphrodite duct emerges from between the lobes of the ovotestis it became

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dilated to form the vesicular seminalis. The diameter of the vesicular seminalis was two to three times that of the first part of the hermaphrodite duct (Stears, 1974; Duncan, 1961; Rigby, 1963). Stears (1974) stated that the vesicular seminalis was packed with spermatozoa and it was lined by cuboidal epithelium in about two thirds of the periphery and by ciliated columnar epithelium in the remaining part.

The present study was conducted to find out the morphological changes of the hermaphrodite duct epithelium depending on the seasons such as spring and summer.

Material and Methods

Material

The material was the Korean slug, *Incilaria fruhstorferi*, collected from the humid oak woods valley near to the Tonghak buddist 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.

Methods

The slugs were anesthetized with 30% ethyl alcohol before dissection. The hermaphrodite ducts isolated from the snail body were cut into sections and 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 and were embedded in Epon 812 mixture. Semithin sections were obtained with LKB-V ultramicrotome and stained with methylene blue. 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 hermaphrodite ducts between the spring and summer specimens of Korean slug *Incilaria fruhstorferi* was confirmed by the light and electron microscopic observations.

Light microscopic finding

Spring specimen: The hermaphrodite duct is a convoluted tiny duct which passes from the ovotestis distally to the albumen gland and fertilization chamber. Its lumen seemed to be shrunk with a diameter of 0.3×0.3 mm and was filled with spermatozoa. The epithelial cells of the duct neither had regular cell shapes nor ordinary cell organelles but various-sized vacuoles throughout all the epithelium (Fig. 1 and 2).

Summer specimen: The lumen of the hermaphrodite duct was become voluminous ellipsoid with a diameter of 0.4×0.23 mm, and apparently larger than that of spring specimen. The epithelium of the duct showed strong methylenophilia in the double stain of methylene blue and basic fuchsin like those of the spring specimen. Their internal structures were quite different by showing the epithelium composed of simple columnar epithelial cells, and pseudo-stratified epithelial cells irregularly mixed and certain part were covered with only irregular columnar cells (Fig. 3, 4 and 5).

Electron microscopic finding

Spring specimen: The epithelium of the hermaphrodite duct in the spring specimen was morphologically irregular in comparison with that of the summer specimen. The epithelium was 13.0 μm in thickness and the epithelial cells were filled with various-sized vacuoles ranged 0.6 μm~6.0 μm in diameter and the cytoplasm of the epithelial cell was dark, due to high electron density (Fig. 6, 7 and 8). On the luminal surface, the cells were covered with microvilli 0.9 μm in height and equipped with irregularly distributed cilia 4.0 μm long (Fig. 7). Certain part of the epithelial cells possessed numerous vacuoles and finally seemed to fall off into the lumen (Fig. 8). The lower part of the luminal epithelium was surrounded by 0.8 μm thick connective tissue with collagenous fibers and muscle tissue. The muscle cells possessed ellipsoid nuclei 8.0×2.0 μm in size (Fig. 7). There were well developed several circular muscle layers in the connective tissue (Fig. 8).

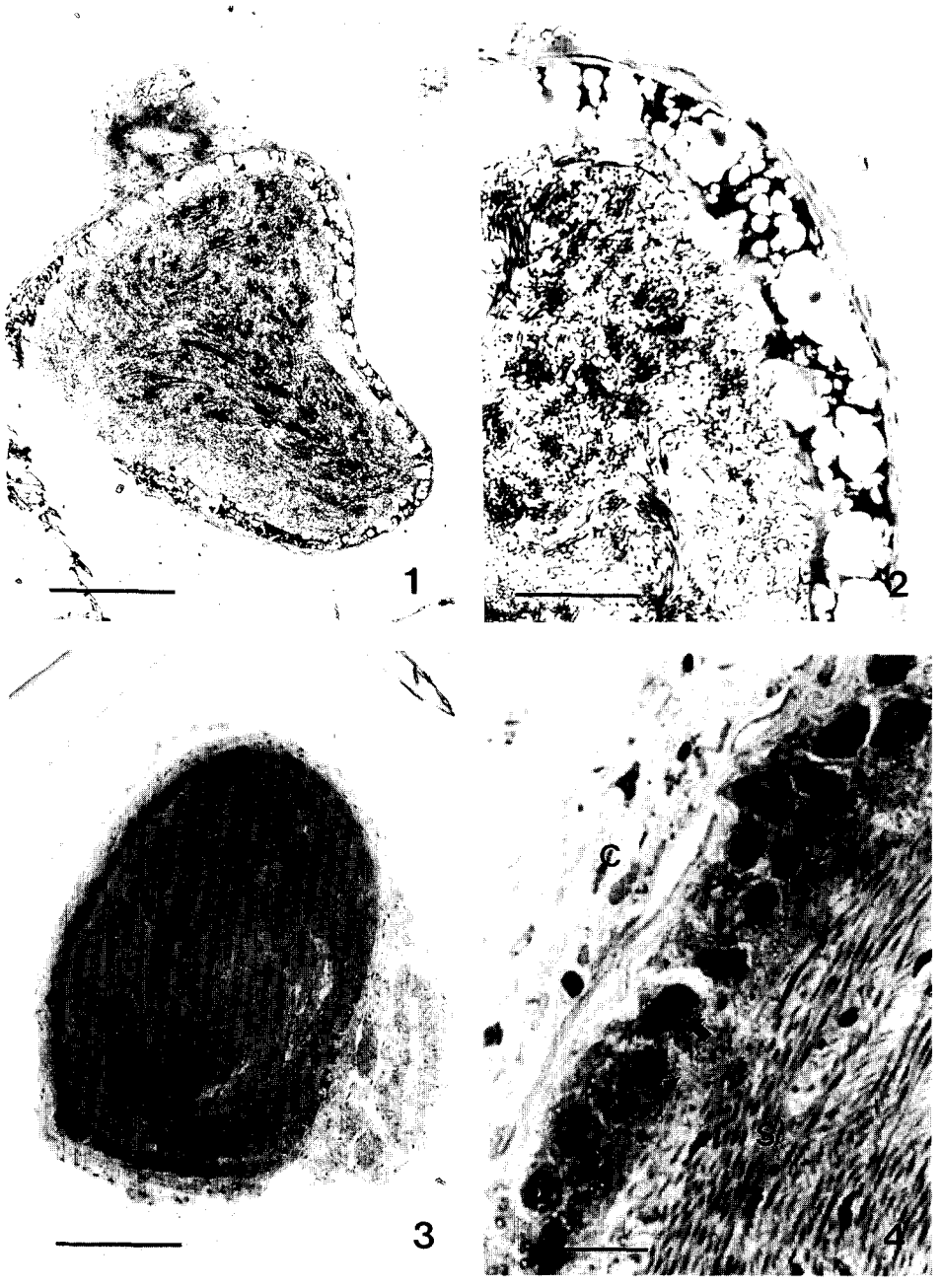

Fig. 1. Light micrograph showing the hermaphrodite duct (Spring specimen). Methylene blue-basic fuchsin double stain. Scale bar= 100 μm.

Fig. 2. Higher magnification of Fig. 1. Arrow, vacuole; arrowhead, spermatozoa. Methylene blue-basic fuchsin double stain. Scale bar= 50 μm.

Fig. 3. Light micrograph showing the hermaphrodite duct (Summer specimen). Methylene blue-basic fuchsin double stain. Scale bar = 100 μm.

Fig. 4. Higher magnification of Fig. 3. Arrow, simple cuboidal cell; arrowhead, simple columnar cell; two arrow, pseudostratified cuboidal cell; C, connective tissue; S, spermatozoon. Scale bar = 20 μm.

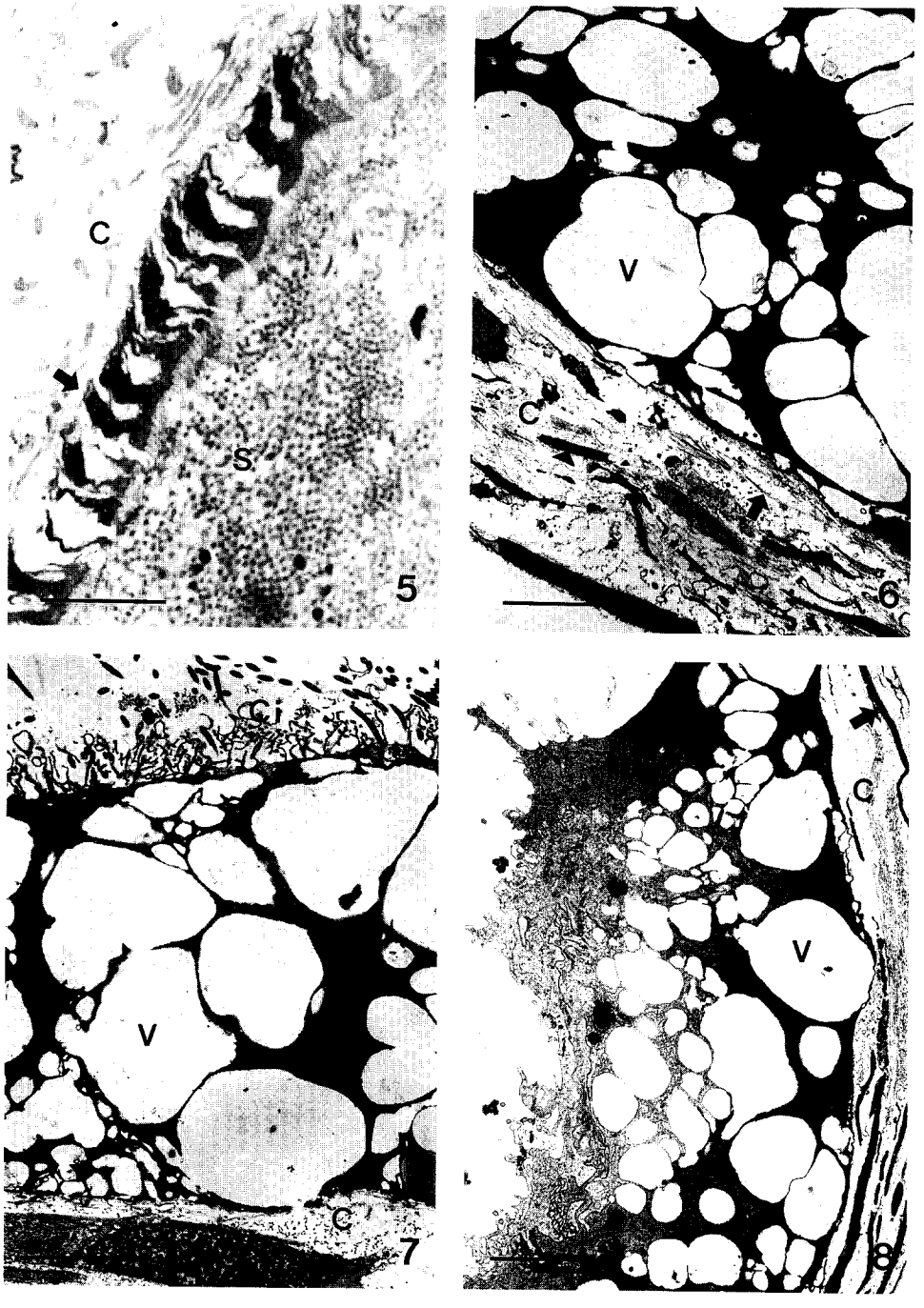

Fig. 5. Higher magnification of Fig. 3. Arrow, simple irregular columnar cell; S, spermatozoon; C, connective tissue. Scale bar = 20 μm.

Figs. 6, 7. Electron micrographs showing the epithelial cell of hermaphrodite duct (Spring specimen). Arrow, collagenous fiber; arrowhead, muscle cell; V, vacuole; C, connective tissue; Ci, cilia; N, nucleus. Scale bar=4 μm.

Fig. 8. Eletron micrograph showing the degenerating epithelium of hermaphrodite duct(Spring specimen). Arrow, circular muscle layer; V, vacuole; C, connective tissue. Scale bar = 4 μm.

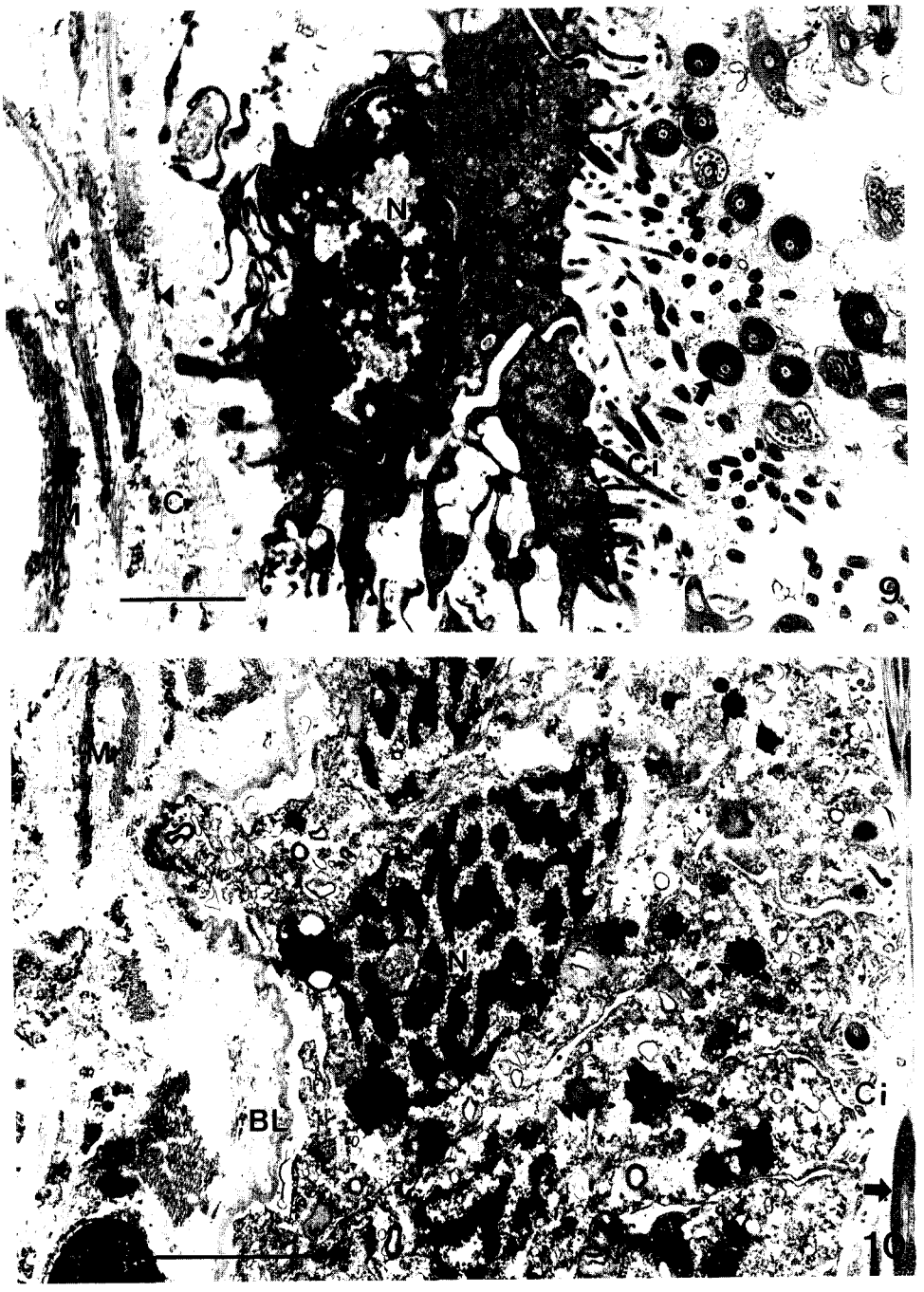

Fig. 9. Electron micrograph showing the irregular ciliated epithelial cell of hermaphrodite duct (Summer specimen). Arrow, spermatozoon; arrowhead, collagenous fiber; Ci, cilia; N, nucleus; M, circular muscle layer; C, connective tissue. Scale bar = 2 μm.

Fig. 10. Electron micrograph showing the simple cuboidal epithelial cell of hermaphrodite duct (Summer specimen). Arrow, spermatozoon; Ci, cilia; N, nucleus; M, circular muscle layer; C, connective tissue; L, lysosome. Scale bar= 2 μm.

Summer specimen: The epithelium of the hermaphrodite duct in the summer specimen was more developed than that of spring specimen. The epithelium was composed of various cell types such as cuboid, columnar, pseudostratified and irregular columnar cells by part (Fig. 9 and 10). Especially cytoplasm of the irregular columnar cells showed high electron density. Unlike the dark cells in the spring specimen their cytoplasm had well developed nuclei showing metachromatic reaction. This type of cells possessed microvilli and cilia on the luminal surface and the rootlets of the cilia also were observable in the deep cytoplasm (Fig. 9). In the lumen of the hermaphrodite duct, numerous spermatozoa appeared near to the epithelial cell surface. And the epithelium of the hermaphrodite duct was surrounded by a connective tissue (7.0~10.0 μm in thickness) with collagenous and muscle fibers (Fig. 9). Beside the epithelial cells of the hermaphrodite duct mentioned above, some groups of irregular small columnar cells with poor microvilli and cilia were observed. These cells possessed cone-shaped nuclei with granular heterochromatin 1.0×0.6 μm in size. A nucleolus with moderate electron density was situated in the heterochromatin of each nucleus.

These special cells possessed many active lysosomes seemingly digesting the spermatozoa in the upper part of cytoplasm. Particular to these regions having the above phagocytic cells, the basal part of the epithelium seemed to be thiner than those of other regions (Fig. 10).

Discussion

The hermaphrodite duct of the stylommatophora snail, *Incilaria fruhstorferi*, is a convoluted tiny duct emerged from the ovotestis and reached to the albumen gland and the fertilization chamber. This duct was filled with numerous mature spermatozoa like in the other species previously reported (Kugler, 1965 ; Lee *et al.*, 1992). The duct was oval in shape and was 0.4×0.2 mm in diameter. The present study was conducted in expectation of the morphological and functional changes of the hermaphrodite duct epithelia depending on the season. Actually some evident morphological changes were observed between the spring and summer specimens.

The lumen of the hermaphrodite duct in the summer specimen was more voluminous than that of the spring specimen, because of well- developed duct epithelium and rich spermatozoa within the duct. In the spring specimen, the duct epithelium possessed numerous vacuoles and electron dense cytoplasm but the summer specimen possessed well-developed epithelium consisted of ciliated cuboidal cells, ciliated columnar cells and pseudostratified cuboidal cells by part. These features are quite different from that of *Nesiohelix samarange* (Lee *et al.*, 1992) and *Limax valentianus* (Stears, 1974). The morphological differences among the above three species are probably due to the season of collection or species specific features.

Duncan (1958) observed changes in the epithelium of the vesicular seminalis and he stated that the epithelium detached itself and formed a coat around the spermatozoa. This result was a resorption of spermatozoa and epithelium, leaving the canal open for ova to pass down. According to report on *Oxychilus cellarius* (Rigby, 1963), number of spermatozoa in the lumen remarkably decreased because of absorption by the syncytial epithelium during the egg-laying period. But in *Limax valentianus* (Stears, 1974) neither traces of absorption nor decrease of number of spermatozoa were found. In *Incilaria fruhstorferi*, reduction of sperm count was not recognisable even in the lumen of the duct covered with epithelial cells in active phagocytosis activity. Among the epithelial cells of the hermaphrodite duct, the cells which were irregular in shape and possessed relatively well-developed cilia were supposed to play important role in transporting the productives from the ovotestis to the distal parts. Stears (1974) stated that a possible explanation for the different histological appearance of the epithelium that the columnar epithelium of the unciliated part is stretched to have a cuboidal appearance in a distended part. But, in *Incilaria fruhstorferi*, the morphological appearance of the duct epithelium showing the various types of cells such as the cuboidal,

pseudostratified cuboidal, columnar and irregular columnar cells did not correspond to the results of stears (1974) because of neither contraction nor stretching of duct epithelium. A type of cell called vesicular cell found in the connective tissue surrounding the epithelia in *Theba pisana* (Noyce, 1973), *Physa fontinalis* (Duncan, 1958) and *Achatina fulica* (Ghose, 1962) was not found in *Incilaria fruhstorferi*, but only muscular cells with ellipsoid nucleus. In *Philomycus caralinianus*, Kugler (1965) observed flask-shaped mucous cells in the connective tissues surrounding the hermaphrodite duct. This type of cells was not observed in that of *Incilaria fruhstorferi*.

The result obtained from the present study strongly suggest that one of major functions of the hermaphrodite duct epithelia is sperm phagocytosis beside pathway of sperm from the ovotestis to the fertilization chamber. It is certain that the evident histological difference in the hermaphrodite duct epithelium between the spring and summer specimens may suggest histological changes occur in relation to their reproductive cycles.

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한국산 산민달팽이(*Incilaria fruhstorferi*) 자웅동체관 상피의 계절에 따른 미세구조의 변화

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한국산 산민달팽이(*Incilaria fruhstorferi*) 자웅동체관 상피조직의 미세구조 변화를 봄형과 여름형별로 관찰하였다. 봄형인 경우 자웅동체관의 직경은 0.3 × 0.2 mm정도였고, 수축으로 인해 정상형태보다 약간 작게 보였다. 내강상피조직은 다양한 크기의 공포들로 구성되어 있었으며 전자밀도가 높게 관찰되었다. 여름형인 경우는 자웅동체관의 직경이 0.4 × 0.23 mm로 관찰되고 봄형보다 팽윤되어 있었으며 내강상피조직은 섬모입방상피, 위중층입방상피, 섬모원주상피 그리고 불규칙한 세포들로 구성되어 있었다. 특히 여름형인 경우 내강상피조직에서 식작용이 관찰되었으며, 봄형과 여름형 공히 그 기저부가 결합조직으로 둘러싸여 있었다. 이상과같은 결과는 민달팽이의 관상피조직이 생식주기에 관계있는 계절변화에 영향을 받는다는 것을 암시한다 하겠다.