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Morphological and Histochemical Studies on the Hermaphroditic and Male Reproductive Organs of a Korean Slug *Incilaria fruhstorferi*

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= 국문초록 =

한국산 산민달팽이(*Incilaria fruhstorferi*)의 자웅공통생식기관과 웅성생식기관의 형태 및 조직화학적 연구

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한국산 산민달팽이(*Incilaria frushtorferi*)의 웅성생식기관 및 자웅공통생식기관에 대하여 조직화학적방법을 이용하여 염색하고 광학현미경으로 관찰한 결과 다음과 같은 결론을 얻었다.

1. 자웅동체관은 가늘고 꾸불꾸불하고 긴 관으로 대부분 성숙한 정자로 가득차 있었다. 이 관의 내강상피는 단층섬모상피와 단층섬모원주상피 그리고 위중층 원주상피 등 다양한 세포로 구성되어 있었다.
2. 대자웅동체관은 위로 소자웅동체관과 알부민선이 있으며, 그 밑으로는 수란관과 연결되어 있었다. 이들의 내강상피는 불규칙한 단층섬모원주상피로 구성되어 있으며 결합조직내 선세포로부터 형성된 산성 및 중성 점액성 과립들이 기막을 통과하여 내강 속으로 분비되었다.
3. 전립선의 내강은 키 큰 단층원주섬모세포로 구성되었으며, 결합조직 내의 분비과립세포에서 형성된 중성 점액과립을 상피세포를 통해 내강으로 분비하였다.
4. 정관은 직경 0.5 x 0.25 mm 정도인 타원형의 관상구조로 이루어져 있고, 이 관을 0.1 mm 정도의 매우 두터운 근육층이 둘러싸고 있었다.
또한 정관주위에는 다양한 형태의 공포로 가득찬 세포의 집단이 관찰되었다.
5. 수정관의 내강은 결합조직성 돌기에 의해 4부분으로 분지되어 있으며 내강은 키 큰 단층섬모원주상피세포로 구성되어 있었다. 또한 수정관 주위에는 두터운 환상근층이 둘러싸고 있었는데, 이들 사이에서 2종의 분비성 과립이 확인되었다.
6. 상음경은 내강이 十字로 열려있으며, 키 큰 단층섬모원주상피와 단층입방상피세포로 구성되어있었다. 내강을 구성하는 근육들은 매우 두터웁고 환상근층과 종주근층이 교대로 둘러싸고 있었다.
7. 음경은 상음경이 짐점 굵어지서 형성된 큰 생식기관으로 내강은 주름 형태인 많은 돌기들을 가지고 있었다. 내강상피 세포는 키가 큰 단층원주상피세포와 단층입방상피가 부위에 따라 다르게 분포하고 있으며, 상피세포 밑 결합조직에는 두터운 근육층이 있어 음경의 강한 운동성이 감지 되었다.

INTRODUCTION

The high degree of organization of land snails is reflected in their complex reproductive systems. But, it is far behind our understanding of reproductive system in the two most closely related groups, the Basommatophora and the Opisthobranchia. Furthermore most of the informations on their reproduction available are relatively old ones. Early works were usually emphasized on the anatomy, morphology and physiology of the land snail.

The usual molluscan specimens used as subject materials by early workers were belonged to families Arionidae (Semper, 1857; Mesenheimer, 1907; Pullet and Watts, 1951; Laviolette, 1954; Lusis, 1961; Quick, 1960; Webb, 1961), Helicidae (Baecker, 1932; May, 1934; Mesenheimer, 1907; Ancel, 1903), and Limacidae (Quick, 1960; Stears, 1974).

Morphological studies on the hermaphrodite duct were carried out in *Helix pomatia* (Aucel, 1903) and in *Polygyra appressa* (Pennypacker, 1903).

Morphological and histochemical studies were undertaken in four species of genus *Sonorella* (Reader and Roger, 1979), in *Helix pomatia* (Ancel, 1903; Neméth and Kovăcs, 1972), in *Agriolimax caruanae,* (Sirgel, 1973) and in *Nesiohelix samarangae* (Jeong, 1993).

The studies on the ovotestis were more rarely conducted such as in *Arion circumscriptus* (Luchtel, 1972). Recently, an anatomical and morphological study on the several parts of the male genital organs was carried out in a Korean land snail, Nesiohelix samarangae (Lee *et al.*, 1992).

Present study was undertaken to find out the morphological and histochemical features of the hermaphroditic and the male organs in a Korean slug *Incilaria fruhstorferi.*

MATERIALS AND METHODS

1. Materials

The specimens used for present study were the slug of *Incilaria fruhstorferi,* belonging to the families Philomycidae in class Gastropoda.

This species is one of the common slugs which inhabits around humid woods of the mountain in Korea.

This slug has a long mantle which covers its entire back. A large shell sac is located inside the mantle, but it has no outer shell. The mantle and back has a longitudinal dark brown band on each side and a pale band at the center of the back.

The slugs were collected from the humid oak woods in mountain Kye Ryong, Tae Jeon, Korea and maintained in terraria.

2. Methods

The specimens were anesthetized with 8% ethanol for 15 minutes and dissected in 0.2 M phosphate buffer (pH 7.3) to isolate the whole genital organs under the stereoscope (Diag. 1).

The whole genital organs taken out were separated by part and fixed with 10% buffered neutral formalin for 3 hours and washed three times with the buffer. The specimens were dehydrated in a graded series of alcohol concentrations and were embedded in hard paraffin wax.

For histochemistry, sections(7 μm) were stained with periodic acid-Schiff (PAS : neutral polysaccharides) and Alcian blue (pH 2.5) to confirm acid, and neutral mucopolysaccharide components in the tissue and were double stained with methylene blue - basic fuchsin to confirm the basophilic com-

ponents.

After staining procedures. the slides showing histochemical reactions were observed and taken pictures with the light microscope (BHS Olympus microscope).

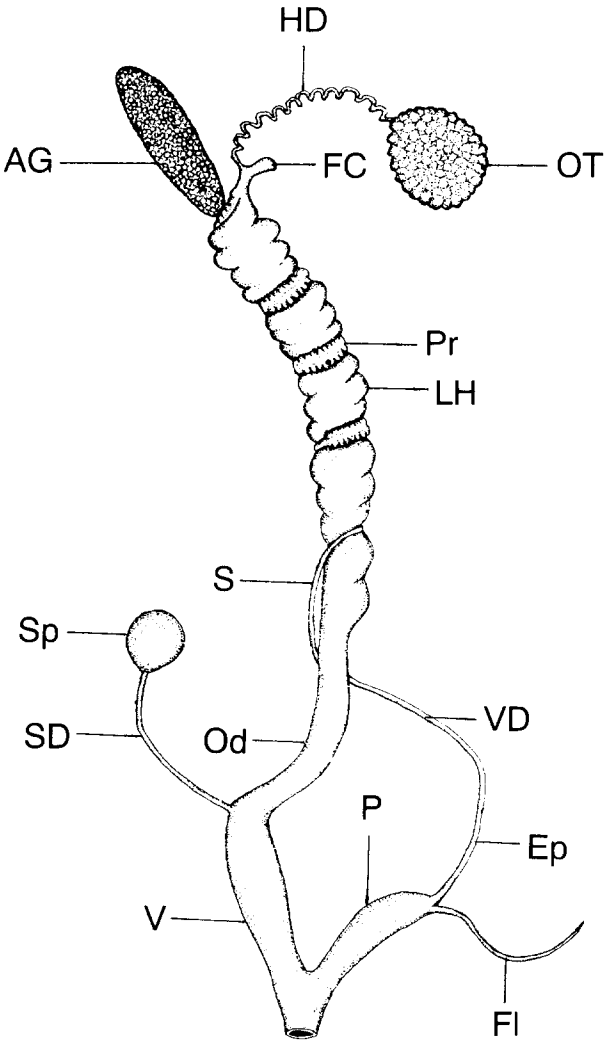

Diagram 1. Schematic diagram of the genital organs of *Incilaria fruhstorferi.*
AG, albumen gland; Ep, epiphallus; FC, fertilization chamber; Fl, flagellum; HD, hermaphrodite duct; LH, large hermaphrodite duct; Od, oviduct; OT, ovotestis; P, penis; Pr, prostate gland; S, sperm duct; SD, spermathecal duct; Sp, spermatheca; V, vagina; VD, vas deferens

RESULTS AND DISCUSSION

1. Hermaphroditic organs

1) Hermaphrodite duct

The hermaphrodite duct also called spermoviduct or gonoduct is a long coiled tiny duct which passes both male and female gametes through from the ovotestis to the fertilization chamber and vas deferens.

The cross sectioned view was ellipsoid (0.5 × 0.8 mm in diameter) and filled with mature sperms (Fig. 1) as reported in *Nesiohelix samarangae* (Lee *et al.*, 1992) and in *Philomycus carolinianus* (Kugler, 1965).

The cytoplasms of the epithelial cells of the hermaphrodite duct were methylenophilic in double stain of methylene blue-basic fuchsin.

The lumen was lined by a simple ciliated cuboidal or simple ciliated columnar or pseudostratified cuboidal epithelium by part (Figs. 2, 3).

The arrangement patterns of the epithelium were different from those of *Nesiohelix samarangae* (Lee *et al.*, 1992) with only ciliated columnar epithelium and of Limax valentianus (Stears, 1974) with two types of epithelia such as ciliated cuboidal or ciliated columnar epithelia. The thin circular muscle layer and a connective tissue with vesicular cells were observed around the hermaphrodite duct (Figs. 2, 3).

2) Large hermaphrodite duct

This organ is also referred to as spermoviduct (Kugler, 1965; Stears, 1974) or oviduct (Barnes, 1987; Engemann, 1987).

Present authors cited the term large hermaphrodite duct recommended by The Conchological Society of Great Britain and Ireland (Block, 1980). It was reported that the epithelium of the large hermaphrodite duct was strongly positive for both glycogen and alkaline phosphatase. The large hermaphrodite duct is lined by ciliated columnar epithelial cells. The nidamental gland cells situated in the large hermaphrodite duct were PAS-positive and amylase fast. The glands were composed of mucous cells containing neutral and acid mucopolysaccharide (Kugler, 1965). The large hermaphrodite duct was at first embedded in the albumen gland and it soon emerged from the gland and coursed in the body as a large whitish, spirally twisted cylindrical tube having numerous folds (Fig. 4). The large hermaphrodite duct had glandular epithelium. The cytoplasms of the epithelial cells were strongly methylenophilic or non-reactive in the combination of dyes, methylene blue and basic fuchsin. The above methylenophilic cytoplasms seemed to be contain acid mucopolysaccharide because they were alcianophilic in PAS-alcian blue (pH 2.5), and the non-reactive cytoplasm seemed to contain neutral mucopolysaccharide since they were PAS-positive. These results are different from the report of Kugler (1965) who suggested that epithelial gland seemed to secrete only neutral mucopolysaccharide. Each of the cells consisting of the acini of the gland had relatively rich cytoplasm and small nuclease. Their cytoplasm contained discoidal or ellipsoidal granules in various sizes (Fig. 5).

The luminal surface of the epithelium was composed of thick irregular simple ciliated cuboidal cells as observed by Stears (1974) (Fig. 6).

The two types of granules, the acid and neutral mucopolysaccharide granules, which were well developed in the connective tissues were observed to be secreted to the lumen through the epithelium (Fig. 6).

2. Male genital organs

1) Prostate gland

The prostate gland is a ribbon-like structure winding round the large hermaphrodite duct and vas deferens as it follows the male tract along its spiral path. Externally it is distinguished from the large hermaphrodite duct by its more compact appearance.

As reported by Stears (1974), the cross-sectioned view of a tubule of the prostate gland consisted of large glandular cell around a central lumen which was lined by a layer of cuboidal cells bearing long, fine cilia which point into the lumen. The epithelial cells lining the lumen of tubules seemed to be engaged in an apocrine secretion. Similar conditions were observed in Limax valentianus (Stears, 1974) and in *Agriolimax caruanae* (Sirgel, 1973).

The epithelial cells lining the lumen contained strong methylenophilic granules which were large oval or ellipsoid in shape. The nuclei of the cells irregular or long ellipsoidal shape due to the neighboring granules (Figs. 7, 8).

The secretory granules were produced in the connective tissues under the epithelium and secreted to the lumen through the basal lamina (Fig. 8). And the granules showed PAS-positive reaction indicating that their components were neutral mucopolysaccharides.

Around the glandular cells in the connective tissues, many long cells with large and oval metachromatic nucleus were also observed.

The cells contained numerous compact spherical granules showing medium level of methylenophilia. The methylenophilic granules were weak PAS-positive in PAS-alcian blue (pH 2.5) reaction. This suggests that they are neutral mucopolysaccharides (Fig. 9).

2) Sperm duct

A part of genital tract situated between the prostate gland and the vas deferens was named sperm duct. Externally it was not easily distinguished from the vas deferens.

For this reason, this male genital part was subdivided into the prostate gland and vas deferens (Block, 1980; Lee *et al.*, 1992) or into the sperm duct and vas deferens (Barnes, 1987; Engemann, 1987).

Histologically the sperm duct was distinguished by some differences.

In present study, this part was subdivided into three parts such as the prostate gland, sperm duct and vas deferens based on histological differences by part. The sperm duct was 0.5 × 0.25 mm in diameter. The muscle layer of the sperm duct was 0.1 mm thick (Fig. 10). In the lumen of the duct, numerous sperms were observed in the lumen (Fig. 11). There were many cells filled with various sized vacuoles which were not confirmed in their nature (Figs. 10, 11).

3) Vas deferens

The vas deferens is a part of the male genital tract which is situated between the prostate gland and the epiphallus.

This tubule surrounded by thick circular muscles passes the sperms from the prostate gland to the epiphallus (Fig. 12).

The lumen was lined by tall simple ciliated columnar or cuboidal cells. The nuclei of the cells were spherical or ellipsoidal in shape and were situated near basal lamina (Fig. 13).

The arrangement of patterns of epithelial cells were not similar to the results of the report of Noyce (1973) and Stears (1974). The lumen was almost subdivided into four grooves due to the pronounced folds of the internal wall of the vas deferens.

The duct possessed well-developed inner longitudinal muscle layer and outer circular muscle layer as reported in *Lymnaea stagnalis* (Holm, 1946), *Agriolimax caruanae* (Noyce, 1973), *Limax valentianus* (Stears, 1974) and in

Nesiohelix samarangae (Lee *et al*., 1992). As commented by Stears (1974), the posterior part of the vas deferens which was near to the prostate gland, differs histologically from the anterior part. The lumen was lined by different epithelial cell types by duct. The anterior, middle and posterior parts were lined by ciliated cuboidal cells, small ciliated columnar cells and by ciliated columnar cells, respectively.

4) Epiphallus

Epiphallus proceeds anteriorly to the penis as a continuation of the vas deferens. It was histologically very similar to the vas deferens. The lumen of the epiphallus also was subdivided into four grooves but its luminal capacity was relatively larger than that of the vas deferens (Fig. 14).

The lumen was lined by simple ciliated columnar cells in the pronounced part and by simple cuboidal cells in the thin part of the wall (Figs. 15, 16). This result was different from that of *Nesiohelix samarangae* (Lee *et al*., 1992) mentioned that the lumen was lined by non-ciliated columnar cells.

Stears (1974) suggested that the epiphallus might be a modified organ of the vas deferens. The present results are agreed with his suggestion, based on the histological characteristics in common between the species.

Lee *et al*. (1992) stated that the epiphallus was surrounded by connective tissue and thick circular muscle layers but in present species the muscle layers surrounding the lumen were composed of thick circular and longitudinal muscles. Especially two thin layers of circular muscles were intermediarily located in the longitudinal muscle layers (Fig. 16). These structures may suggest that the epiphallus supports the penial function of contraction and extension.

5) Penis

The penis is a muscular organ which is situated anterior to the epiphallus and opened into the genital atrium and the genital aperture. The penial lumen was composed of numerous grooves derived by leaf-like protrusions of internal wall. The lumen was lined mostly by simple columnar epithelium and partially by pseudostratified columnar epithelium and the cells were methylenophilic or transparent with methylene blue-basic fuchsin combination (Fig. 17).

The luminal folds which possessed well-developed muscle bundles were deep and irregular in shape and arrangement. Especially the muscular bundles in the folds were relatively short in length and were dense. Otherwise, the muscle bundles in the basal portions of the folds looked like broad leaves with light and dark regions alternatively (Figs. 17, 18).

The leaf-like cells shown in Fig. 20 appeared to the spongy cells mentioned in *Agriolimax caruanae* (Noyce, 1973).

The internal structures of the penis observed in the present study were greatly different from those in *Limax valentianus* (Stears, 1974) mentioned that its lumen was surrounded by thick and rugose walls as a result from numbers of longitudinal ridges running through the lumen, and various types of epithelia lined the lumen such as unciliated digitiform cells with basally situated nuclei and unciliated cuboidal cells in between.

Stears (1974) and Kugler (1965) stated that all of the penial lumen was lined by unciliated columnar or unciliated cuboidal epithelium, but present authors found that most part of the penial lumen like thick ridge were lined by simple columnar epithelium and only the basal portions of the ridges forming thin penial walls were lined by pseudocolumnar epithelia (Fig. 19).

SUMMARY

A morphological and histochemical study on the hermaphroditic and male organs on a Korean slug *Incilaria fruhstorferi* was conducted.

The followings are summarized results obtained from the study.

1. The hermaphrodite duct was a long coiled tiny duct filled with mature sperm. Its lumen was lined by various epithelia such as simple cuboidal, simple columnar and pseudostratified ciliated cuboidal epithelia by part.

2. The large hermaphrodite duct was at first embedded in the albumen gland and it soon emerged from the gland and coursed in the body as a large whitish, spirally twisted cylindrical tube with numerous folds. The large hermaphrodite duct had glandular epithelium.

The lumen of the large hermaphrodite duct was lined by irregular simple ciliated columnar epithelium. From the gland cells situated in the connective tissue, both of acid and neutral mucopolysaccharide granules were secreted to the lumen through the basal lamina.

3. The lumen of the prostate gland was lined by simple ciliated columnar epithelium. The mucopolysaccharide granules secreted by the secretory cells in the connective tissue transported to the lumen through the epithelium.

4. The oval sperm duct was 0.5 x 0.25 mm in diameter. This duct was surrounded by thick muscular layer (0.1 mm) and cells were with various shaped vacuoles in their cytoplasms.

5. The lumen of the vas deferens was subdivided into four grooves by protrusions of internal wall and was lined by tall ciliated columnar cells. This duct was surrounded by thick circular muscle layer.

6. The lumen of the epiphallus was also subdivided into four grooves and it was lined by tall simple columnar epithelium and simple cuboidal epithelia. Its lumen was surrounded by circular and longitudinal muscle layers.

7. The penis was a muscular organ which is situated anterior to the epiphallus and opened into the genital atrium and the genital aperture. The penial lumen was composed of numerous grooves derived by leaf-like protrusions of internal wall. The lumen was lined mostly by simple columnar epithelium and partially by pseudostratified columnar epithelium and the cells were methylenophilic or transparent with methylene blue-basic fuchsin combination.

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Explanations of Figures

Fig. 1. Light micrograph showing the hermaphrodite duct.
E. epithelium; Sp. spermatozoa. ×100.

Fig. 2. Simple columnar epithelial cell of the hermaphrodite duct.
Vs. vascular cell; N. nucleus; Sp. spermatozoa. ×1000.

Fig. 3. Wall of the hermaphrodite duct.
arrow, simple cuboid epithelial cell; arrowhead, pseudostratified cuboidal epithelial cell. ×200.

Fig. 4. Nidamental gland and spermoviduct of the large hermaphrodite duct.
E. endothelium of spermoviduct; L. lumen; arrowhead, PAS-positive secretory granule cell; arrow, alcianophilia secretory granule cell. ×200.

Fig. 5. Light micrograph showing the nidamental gland cell.
N. nucleus; arrow, PAS-positive secretory granule cell; arrowhead, alcianophilia secretory granule cell. ×1000.

Fig. 6. Magnification of Fig. 4. The cross sectioned view of the oviduct.
L. lumen; Ci. cilia; N. nucleus; arrow, PAS-positive secretory granule; arrowhead, alcianophilia secretory granule. ×1000.

Fig. 7. Light micrograph showing the prostate gland.
L. lumen; E. endothelium; arrow, PAS-positive secretory granule; arrowhead PAS-weak positive secretory granule. ×200.

Figs. 8, 9. Magnification of Fig. 7. Cross section through the endothelium and the connective tissue.
L. lumen; Mv. microvilli; N. nucleus; arrow, PAS-positive secretory granule; arrowhead, PAS-weak positive secretory granule cell. ×1000, ×1000.

Fig. 10. Cross-sectioned view of the sperm duct. L. lumen; Mu. muscle; arrow, vacuolar cell. ×200.

Fig. 11. Magnification of Fig. 10.
Mu. muscle; arrow, sperm head. ×400.

Fig. 12. Cross-sectioned view of the vas deferens.
L.lumen; E. endothelium; Mu. muscle. ×200.

Fig. 13. Magnification of Fig. 12. Light micrograph showing the simple ciliated columnar epithelial cell.
L. lumen; N. nucleus; Mu1, longitudinal muscle layer; Mu2, circular muscle layer; Mv, microvilli. ×1000.

Fig. 14. Cross-section through the epiphallus.
L. lumen; E. endothelium; Mu. muscle. ×200.

Figs. 15, 16. Magnification of Fig. 14. Light micrograph showing the simple ciliated columnar epithelium and the simple ciliated cuboidal epithelium.
L. lumen; Mv. microvilli; N. nucleus; Mu1, longitudinal muscle layer; Mu2, circular muscle layer. ×1000.

Figs. 17, 18. Cross-section of the penis.
L. lumen; E. endothelium; Mu. muscle; Mu1, longitudinal muscle layer; Mu2, circular muscle layer; arrow, spongy-like cell. ×100, ×1000.

Figs. 19, 20. Magnification of Figs.17 and 18. Light micrograph showing the pronounced folds and lower connective tissue.
L. lumen; E. endothelium; Mu. muscle; Mu2, circular muscle layer; arrow, spongy-like cell. ×200, ×400.

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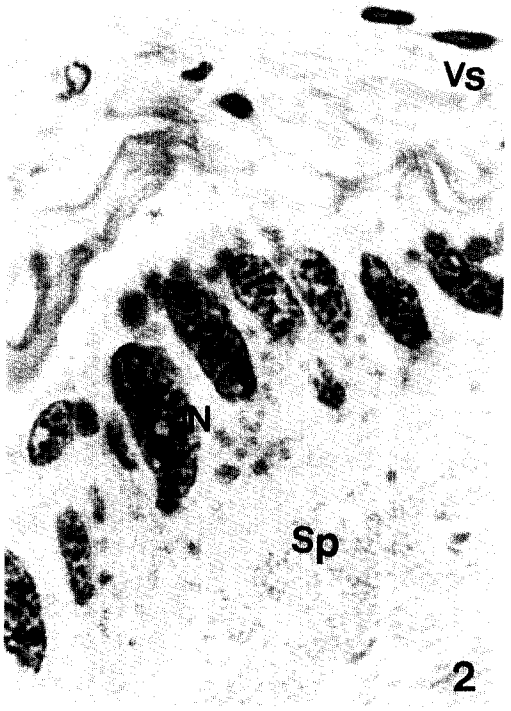

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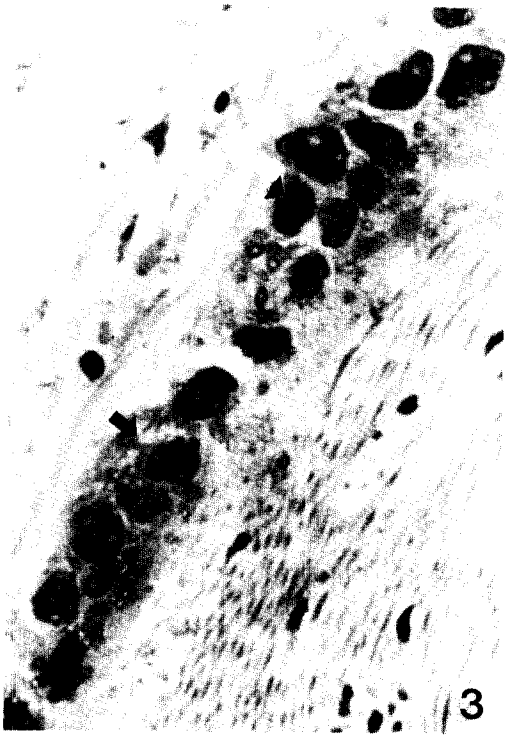

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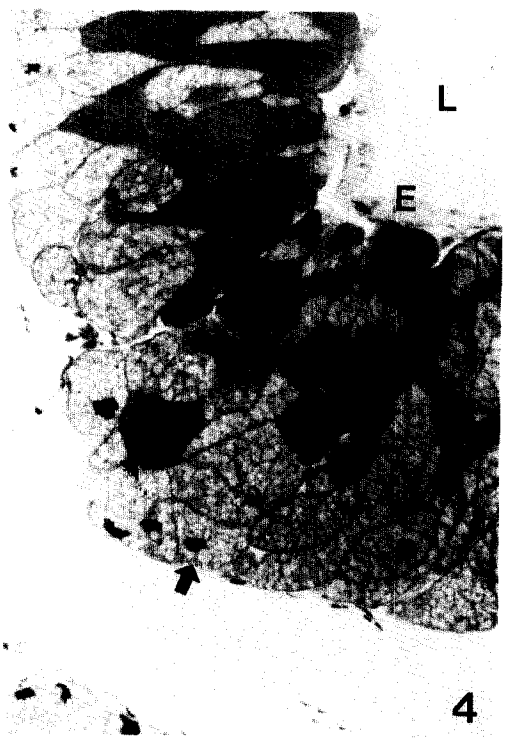

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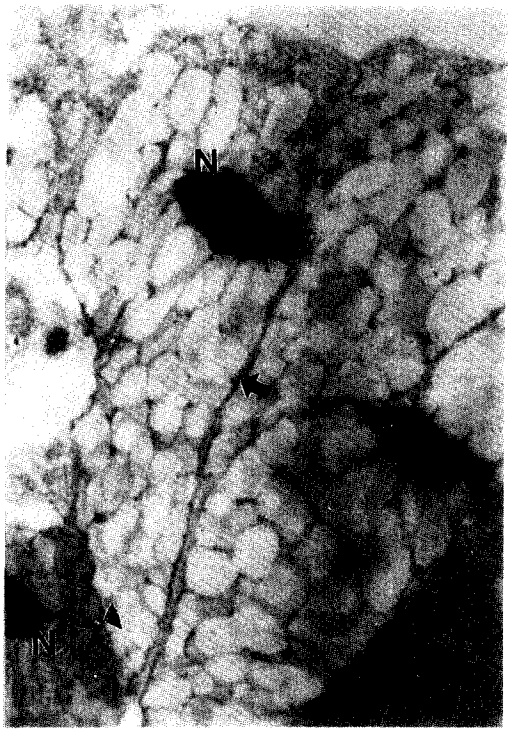
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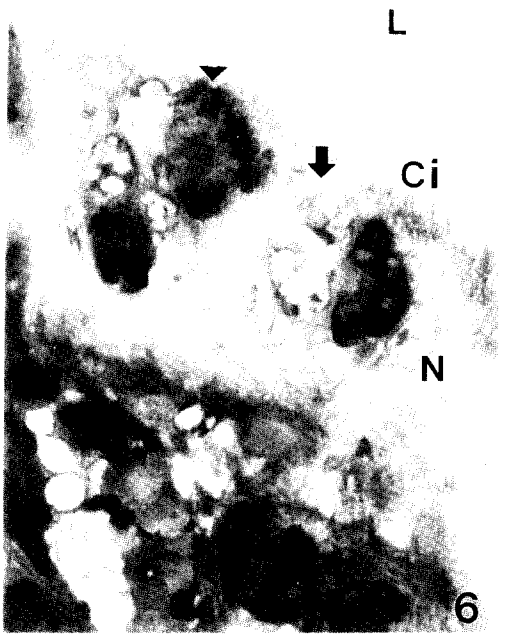
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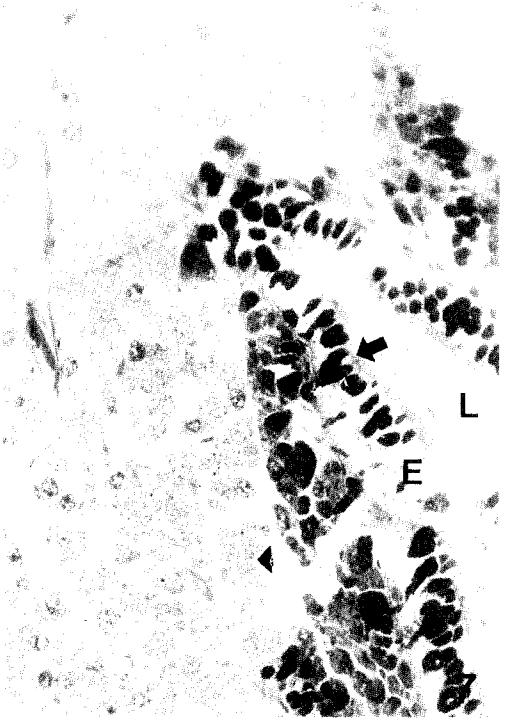
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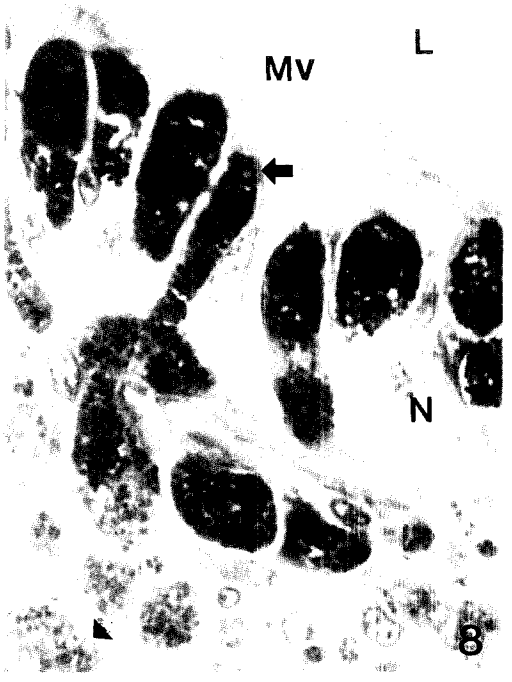
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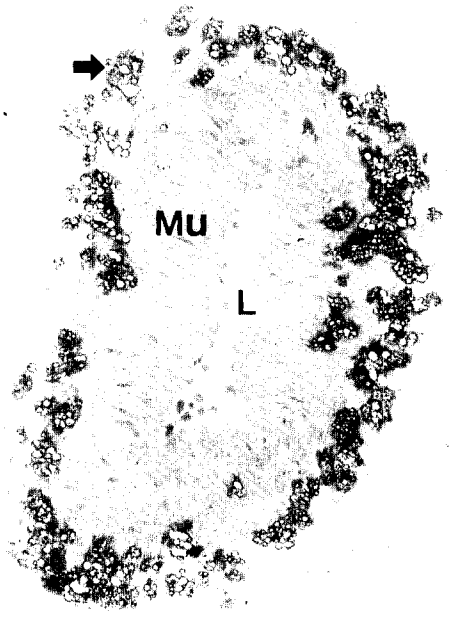

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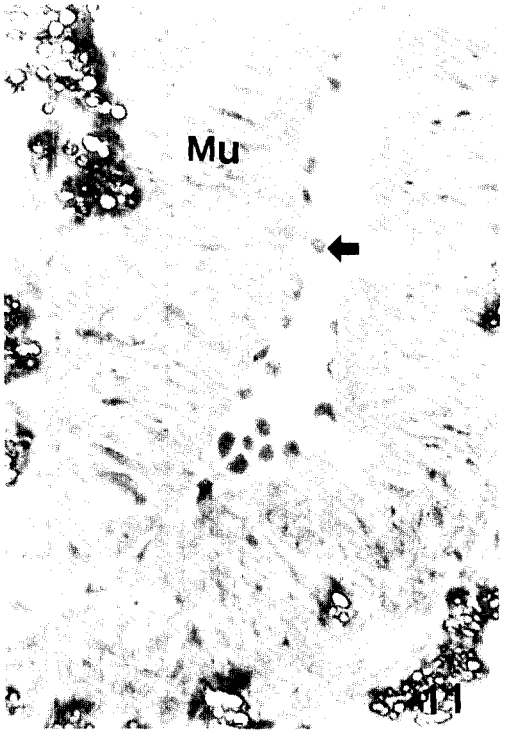

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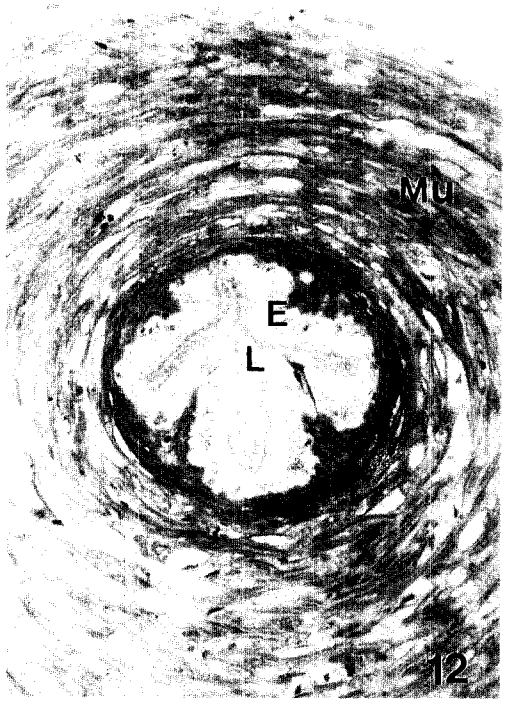

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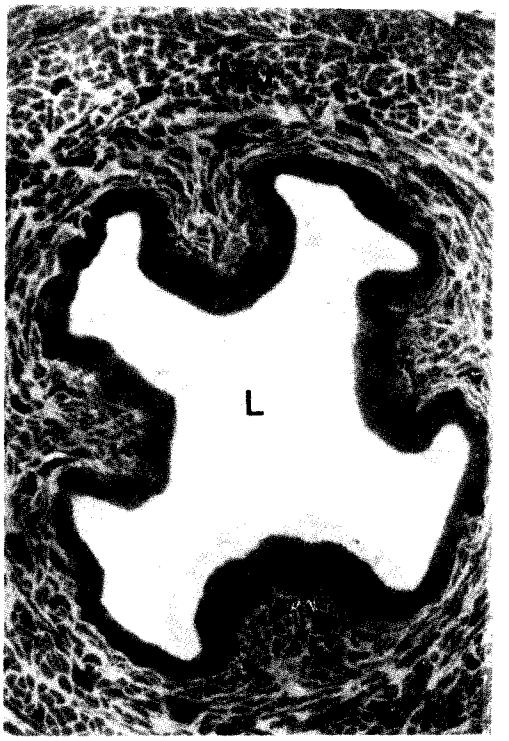
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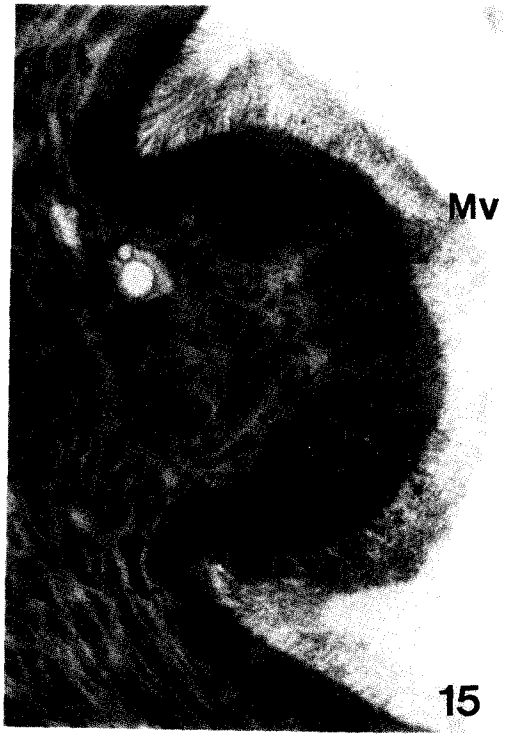
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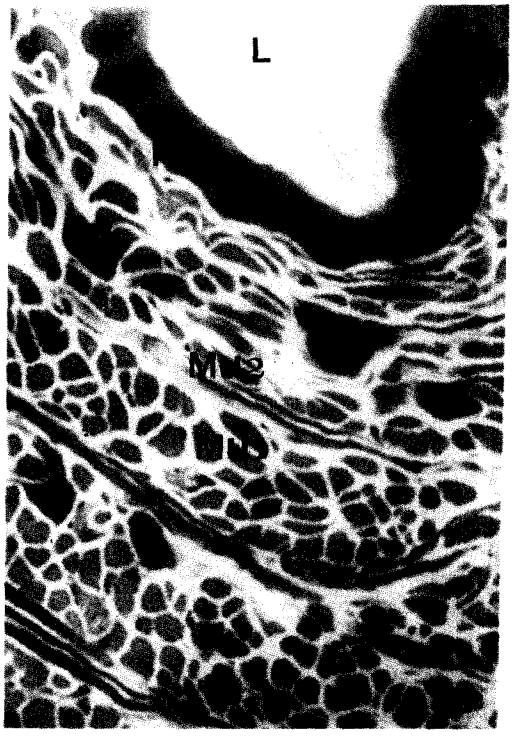
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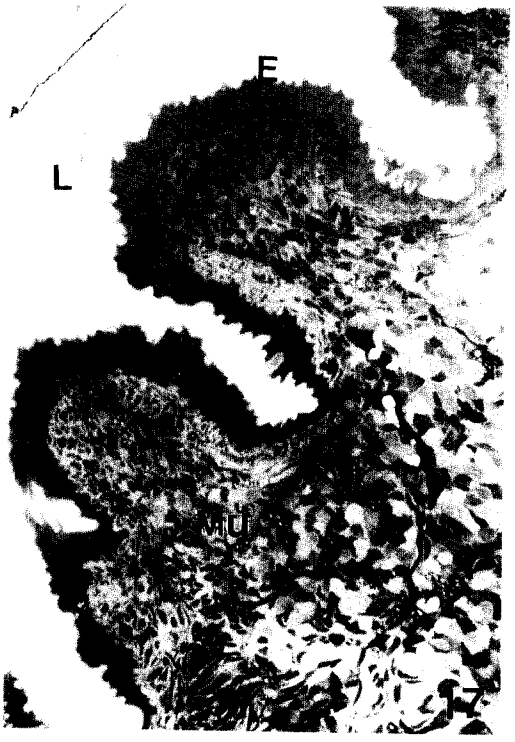

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