

# Morphological and Histochemical Study on the Female Reproductive Organs in a Korean Slug, *Incilaria fruhstorferi*

Nam-Sub Chang and Kye-Heon Jeong\*

Department of Biology, College of Science and Engineering, Mokwon University  
24 Mok-dong, Chung-gu, Taejeon, 301 720 Korea

\*Department of Biology, College of Natural Science, Soonchunhyang University  
P.O. Box 97 Asan, Chungnam, 330 600 Korea

=국문초록=

## 한국산 산민달팽이 (*Incilaria fruhstorferi*)의 자성 생식기관의 형태 및 조직화학적 연구

북원대학교 이공대학 생물학과 · 순천향대학교 사인과학대학 생물학과\*

장남섭 · 정계현\*

한국산 산민달팽이 (*Incilaria fruhstorferi*)의 자성 생식기관을 조직화학적 방법을 이용, 광학 현미경으로 관찰한 결과는 다음과 같다.

알부민선 (albumen gland)은 대자용동체관 위에 있으며, 산성 점액다당류와 중성 점액다당류 그리고 단백질성 분비물을 형성하는 세포들로 구성되어 있었다.

수정실 (fertilization chamber)을 구성하는 내강 상피는 불규칙한 단층섬모원주상피와 위중층섬모원주상피세포가 3 대 1의 비율로 구성되어 있으며, 길항조직에서 형성된 산성 점액다당류성 과립들이 상피세포를 거쳐 내강 속으로 분비되었다. 또한 수정실의 외측 표면은 기능을 알 수 없으나 산성 점액다당류를 분비하는 대형 세포들로 둘러싸여 있었다.

수관관 (oviduct)의 내강은 크고 작은 물기로 인해 병모양을 나타내었다. 내강은 위중층원주상피세포로 구성되어있으며 그들 사이에서 분비세포의 존재는 확인되지 않았다.

질 (vagina)의 내강은 넓고 많은 주름으로 이루어져 있고, 이들 주름들은 둥근 형태의 나무 숲처럼 보였다. 질의 내강을 덮은 세포는 해변조직 형태의 세포와 단층섬모원주상피세포 그리고 위중층섬모원주상피세포 등 다양한 종류로 구성되어 있었으며, 잘 발달된 두터운 근육층은 질의 신축성을 높여 주었다.

정자낭 (spermatheca)은 그 형태와 크기, 색깔 등이 종에 따라 다양하고, 길과 연결되는 자성생식기관으로 알려져 있다. 이들의 내강상피는 기가 큰 단층섬모원주상피세포로서 PAS에

organs of the pulmonate snails have been conducted by several authors such as AnceI (1903), Meisenheimer (1907), Baecker (1932), Els (1973, 1974) and Sirgel (1973). Especially on the female

### INTRODUCTION

Since Semper (1857), studies on the reproductive

reproductive organs, May (1934), Pelluet and Watts (1951), Laviolette (1954), Webb (1961), Kugler (1965), Noyce (1973), Stears (1974), Reeder and Rogers (1979), Rogers *et al.* (1980), Kitajima and Paraense (1983) and Jeong (1993) performed the morphological, histological or ultrastructural studies. Present study was carried out to find out the morphological and histochemical characteristics of the female reproductive organs in a Korean slug, *Incilaria fruhstorferi*.

## MATERIAL AND METHODS

### 1. Material

Adult specimens of the Korean slug *Incilaria fruhstorferi*, belonging to Philomycidae, Stylomatophora, Pulmonata, Gastropoda were collected from their habitats, the humid oak woods of the 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 genital organs under the stereoscope.

The whole genital organs isolated from the body were cut into sections 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, the sections (7  $\mu$ m in thickness) were stained with periodic acid Schiff (PAS) and Alcian-blue (pH 2.5) to identify the acid and neutral mucopolysaccharide components in the tissue and were double stained with methylene blue and basic fuchsin to identify the basophilic components.

After the staining procedures, the tissue sections showing histochemical reactions were observed under the light microscope (BHS Olympus microscope).

## RESULTS AND DISCUSSION

### 1. Albumen gland

The albumen gland surrounds the anterior part of the hermaphrodite duct, the fertilization chamber complex and the posterior part of the spermoviduct. It is composed of numerous closely-packed tubule, each enclosed in a thin layer of connective tissue which is continuous with the connective tissue sheath covering the gland externally. The lumina of groups of tubules join and open into a centrally situated lumen which runs the length of the gland (Fig. 1).

Histologically a tubule consisted of a layer of large wedge shaped glandular cells in two types. Both types of the cells possessed extremely large nuclei and relatively large nucleoli compared to their cytoplasm. Each of the nuclei was filled with metachromatic granules (Fig. 2, 3). The intracellular granules responded in three different ways according to the stains such as alcianophilia in PAS-Alcian blue (pH 2.5), PAS positive and Millon positive.

This suggests that the secretory granules of the albumen gland are acid mucopolysaccharides (alcianophilia), neutral mucopolysaccharides (PAS positive) and mucoprotein (Millon positive) products. Kugler (1965) stated that the secretory tubules of the albumen gland were positively stained in PAS and Best's carmine techniques. He also stated that the epithelium of the albumen canal gave a positive stain for glycogen and strong alkaline-phosphatase activity.

### 2. Fertilization chamber

The fertilization chamber is U shaped small duct situated between the almost portion of the

hermaphrodite duct and the spermooviduct. The fertilization chamber appeared to be one of common organs in the pulmonates. Furthermore, it seems that different authors applied the terms to different organs because of a lack of uniformity in terminology employed. Quick (1960) called this organ in the pulmonate slugs a vesicular seminalis. Lusi (1961) in *Arion ater rufus*, Duncan (1961) in *Succinea pfeifferi* and Morton (1955) in pulmonates in Ellobiidae called it seminal vesicle. Baecker (1932) called this organ a fertilization chamber in *Limax cinereus* and *Arion empiricerum* as stated by present authors in *Incilaria fruhstorferi*.

Stears (1974) mentioned this organ in *Limax valentianus* embedded in the basal portion of the albumen gland, and Minato (1977) reported that the fertilization chamber was not found in *Nesiohelix*.

In *Incilaria fruhstorferi*, the fertilization chamber was situated in U shape on the basal surface of the albumen gland as reported in *Nesiohelix samarangae* (Lee *et al.*, 1992). The fertilization chamber was surrounded by thick muscle layers and certain large round cells secreting acid mucopolysaccharide granules surrounded the outer surface of the fertilization chamber (Fig. 5).

Its lumen was lined by irregular simple ciliated columnar cells and pseudostratified columnar cells in the 3:1 ratio (Figs. 4, 5).

It seemed that the acid mucopolysaccharide granules secreted from the secretory cell embedded in the connective tissue pass to the lumen of the fertilization chamber through the basement membrane and the epithelium.

Stears (1974) stated that the lumen of the fertilization chamber was lined by a ciliated cuboidal or very low columnar epithelium in almost same proportion in *Limax valentianus*.

### 3. Oviduct

The oviduct situated between the hermaphrodite duct and the vagina. The lumen of the oviduct was lined by pseudostratified columnar epithelium and was subdivided into several grooves due to some

leaf-like protrusions of the internal wall of the duct (Fig. 8). No secretory cells were observed in the epithelium and the epithelium was surrounded by the circular muscle layers (Fig. 7)

Stears (1974) described in *Limax valentianus* that the spermooviduct emerged from the lateral surface of the albumen gland as a continuation of the fertilization chamber complex and ran anteriorly to the point where it bifurcates into the free oviduct and vas deferens. He also mentioned that the luminal surface of the anterior part was lined by ciliated columnar cells similarly to the hermaphrodite duct. Its posterior part of the duct joined the vagina and the epithelia of the anterior and posterior parts were similar to each other. In *Incilaria fruhstorferi* the epithelia of the oviduct and vagina also were partly identical.

### 4. Vagina

The vagina leads from the anterior end of the spermooviduct to open anteriorly into the genital atrium just ventral and anterior to the spermatheca into the genital atrium. The lumen of the vagina was relatively spacious but subdivided into many grooves due to the strong folds of the vaginal wall when relaxed (Figs. 9, 10). The epithelium covering the luminal surface was composed of various cell types by part. The upper part was covered by spongy like cells (Fig. 12), the middle part by simple ciliated columnar cells (Fig. 13) and the lower part by pseudostratified columnar cells (Fig. 11). The epithelium of the vagina was supported by a thick layer of connective tissues interspersed with circular and longitudinal muscle fibers (Fig. 9). The structures mentioned above seem to suggest that the vagina has a great pliability. These results are different from the reports of Stears (1974) and Kugler (1965). Stears (1974) stated that the lumen of the vagina in *Limax valentianus* was lined by an unciliated epithelial and the epithelial cells varied from cuboidal to columnar with basally situated nuclei, and in *Philomyces carolinianus* Kugler (1965) mentioned that the vaginal lumen was lined by

relatively low columnar cells and its wall contained muscle and elastic fibers.

### 5. Spermatheca

The spermatheca is an oval or ellipsoidal structure that opens into the vagina by means of a muscular spermathecal duct. The spermatheca itself is a voluminous organ varying in size. It has been known that it achieves its greatest size in specimen which is considered sexually mature. Spermathecal lumen is usually filled with fluid secreted presumably by its surrounding epithelium.

The materials in lumen was basophilic in combination dye of methylene blue-basic fuchsin employed. It also positively reacted in PAS-Alcian blue (pH 2.5). The materials in the lumen might be mucopolysaccharides.

Internal epithelium of the spermatheca was composed of tall simple ciliated columnar cells having brush border with microvilli on their luminal surfaces unlike that of *Nesiohelix samarangae* (Fig. 15). Jeong (1993) who studied ultrastructures on the spermatheca in a Korean land snail *Nesiohelix samarangae* stated that the lumen of the spermatheca was lined by unciliated columnar cells with brush border of microvilli. Even though we could not confirm concrete evidence in the present study, there has been a strong suggestion that the spermatheca may digest the remnant sperm and sperm fluid after copulation. The epithelium may secrete digestive enzyme to the lumen, or alternatively, 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 presented by Jong-Brink (1969) in *Biomphalaria glabrata*, Németh and Kovács (1972) in *Helix pomatia*, Reeder and Rogers (1979) in four species of *Sonorella*, Rogers *et al.* (1980) in *Sonorella santaritana*, Kitajima and Paraense (1983) in *Biomphalaria glabrata*, and Jeong (1993) in *Nesiohelix samarangae*.

### 6. Spermathecal Duct

The spermathecal duct varying in size by species connects the spermatheca to the vagina. This duct in *Incilaria fruhstorferi* was relatively short and its lumen lined by pseudostratified columnar epithelium was subdivided into several grooves due to the pronounced wall of the duct (Figs. 17, 18).

The luminal epithelium showed strong methylenophilia in double stain of methylene blue and basic fuchsin employed, and was surrounded by connective tissue and muscular layers (Figs. 17, 19).

This epithelium may be engaged in apocrine secretion as mentioned in a American slug *Philomycus carolinianus* (Kugler, 1965).

### SUMMARY

The results obtained from a morphological and histochemical study on the female reproductive organs in a Korean slug *Incilaria fruhstorferi*, can be summarized as follows.

The albumen gland surrounds the anterior part of the hermaphrodite duct, the fertilization chamber complex and the posterior part of the sperm oviduct. The secretory granules of the albumen gland are involved in acid mucopolysaccharides, neutral mucopolysaccharides and mucoprotein.

The fertilization chamber is U-shaped small duct situated between the almost portion of the hermaphrodite duct and the spermoviduct.

This organ is surrounded by thick muscle layers and its lumen was lined by irregular simple ciliated columnar cells and pseudostratified columnar cells in the 3 : 1 ratio. It seemed that the acid mucopolysaccharide granules secreted from the secretory cell embedded in the connective tissue pass to the lumen of the fertilization chamber through the basement membrane and the epithelium.

The oviduct situated between the hermaphrodite duct and the vagina. The lumen of the oviduct was lined by pseudostratified columnar epithelium and was subdivided into several grooves due to some

leaf-like protrusions of the internal wall of the duct. No secretory cell was observed in the epithelium, and the epithelium was surrounded by the circular muscle layers.

The vagina leads from the anterior end of the spermooviduct to open anteriorly into the genital atrium just ventral and anterior to the spermatheca into the genital atrium. The lumen of the vagina was relatively spacious but subdivided of into many grooves due to the strong folds of the vaginal wall when relaxed. The epithelium covering the luminal surface was composed of various cell types by part.

The spermatheca is an oval or ellipsoidal structure that opens into the vagina by means of a muscular spermathecal duct. The spermathecal lumen is usually filled with fluid secreted presumably by its surrounding epithelium. The materials in lumen was basophilic in combination dye of methylene blue-basic fuchsin employed.

It also positively reacted in PAS-Alcian blue (pH 2.5). The materials in the lumen might be mucopolysaccharides. Internal epithelium of the spermatheca was composed of tall simple ciliated columnar cells having brush border on microvilli of their luminal surfaces.

The spermathecal duct in *Incilaria fruhstorferi* was relatively short, and its lumen lined by pseudostratified columnar epithelium was subdivided into several grooves due to the pronounced wall of the duct. The luminal epithelium showed strong methylenophilia in double stain of methylene blue and basic fuchsin employed, and was surrounded by connective tissue and muscular layers.

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## EXPLANATIONS OF FIGURES

**Fig. 1.** Light micrograph showing the albumen gland. x200.

**Figs. 2, 3.** Magnification of Fig. 1.

a, alcianophilia secretory granular cell; b, PAS-positive secretory granule cell; c, Millon positive secretory granule cell; Nu, nucleolus; N, nucleus. x1000.

**Fig. 4.** Cross section through the fertilization chamber.

E, epithelium; Mu, muscle; arrow, secretory granular cell. x1000.

**Fig. 5.** Simple and pseudostratified ciliated columnar epithelial cell of the fertilization chamber.

Ci, cilia; Gr, secretory granule; N, nucleus. x1000.

**Fig. 6.** Light micrograph showing the outer wall of the fertilization chamber.

N, nucleus; Gr, secretory granular cell. x 1000.

**Fig. 7.** Cross-section through the oviduct.

L, lumen; Mu, muscle; arrow, processing fold. x100.

**Fig. 8.** Magnification of Fig. 7. Arrow, the endothelial cell of processing fold.

N, nucleus; L, lumen. x1000.

**Fig. 9.** Cross-section through the vagina.

L, lumen; Mu, muscle; arrow, processing fold. x80.

**Fig. 10.** Magnification of Fig. 9. Upper part of the processing fold. L, lumen; arrow, secreting mucus. x200.

**Fig. 11.** Magnification of Fig. 9. Middle part of the processing fold. Light micrograph showing the pseudostratified ciliated columnar epithelium.

N, nucleus. x1000.

**Fig. 12.** Magnification of Fig. 10. Spongy like epithelium.

N, nucleus; Va, vacuole; arrow, microvilli. x1000.

**Fig. 13.** Magnification of Fig. 9. Lower part of the processing fold showing the simple columnar epithelium.

N, nucleus. x1000.

**Figs. 14, 15.** Cross-section through the spermatheca.

L, lumen; E, endothelium; N, nucleus; arrow, microvilli. x40, x200.

**Fig. 16.** Magnification of Fig. 15. Light micrograph showing the simple ciliated columnar epithelium.

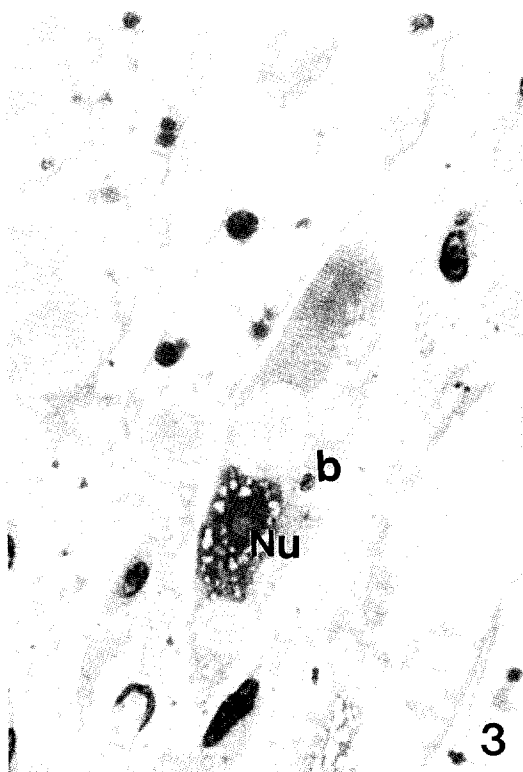
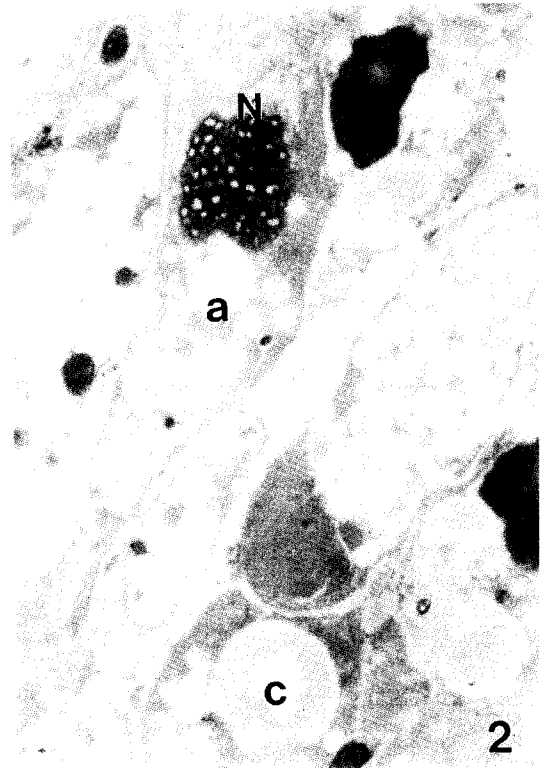
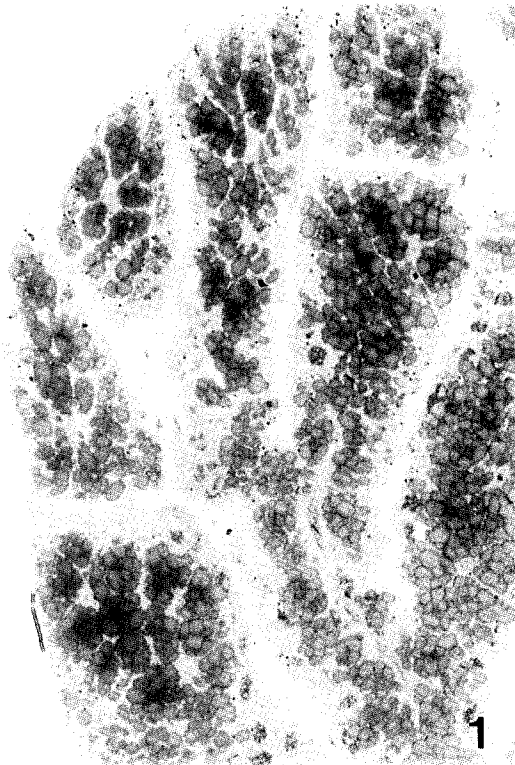
Va, vacuole; N, nucleus. x1000.

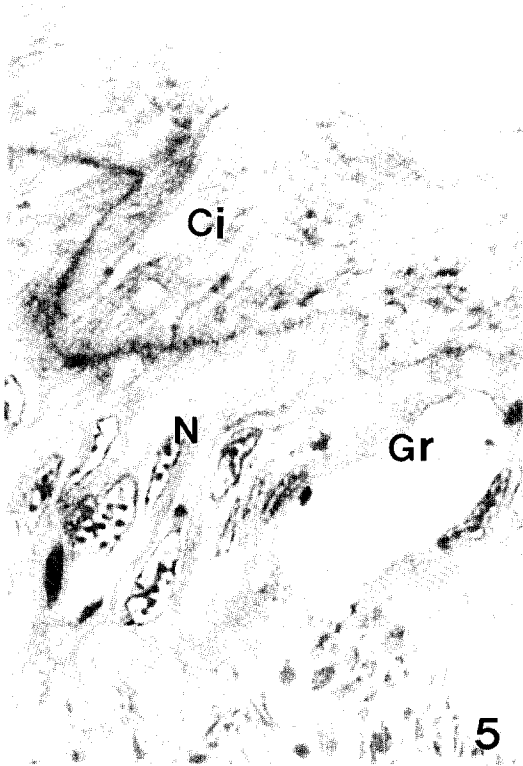
**Figs. 17, 18.** Cross-section of the spermatheca duct.

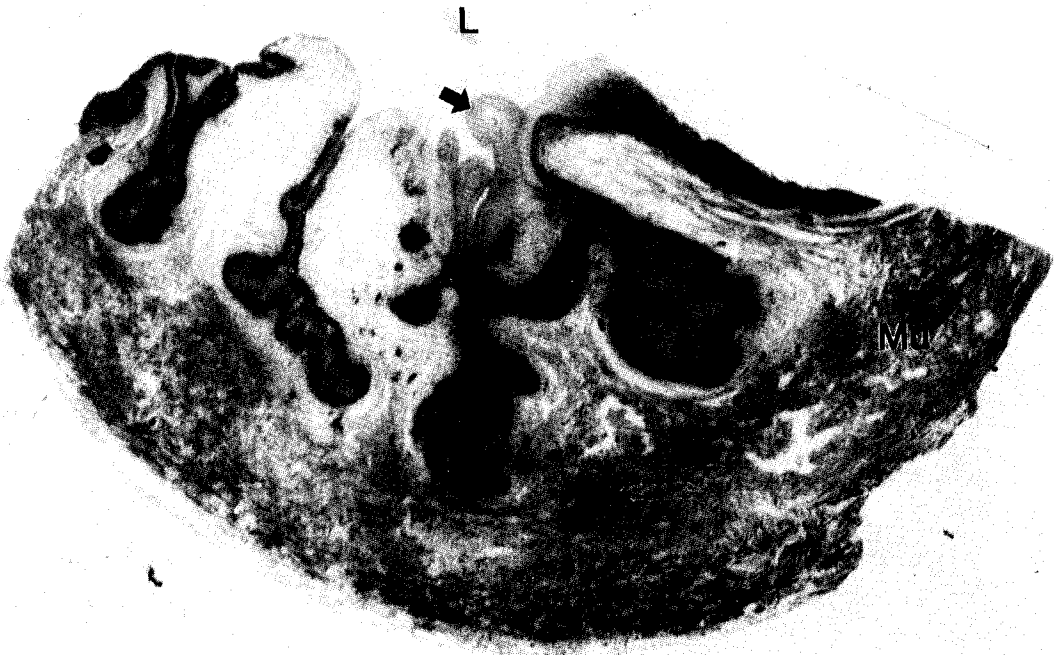
L, lumen; N, nucleus; E, endothelium; Mu1, longitudinal muscle layer; Mu2, circular muscle layer. x200, x400.

**Fig. 19.** Magnification of Fig. 18. Light micrograph showing the simple ciliated pseudostratified columnar epithelial cell.

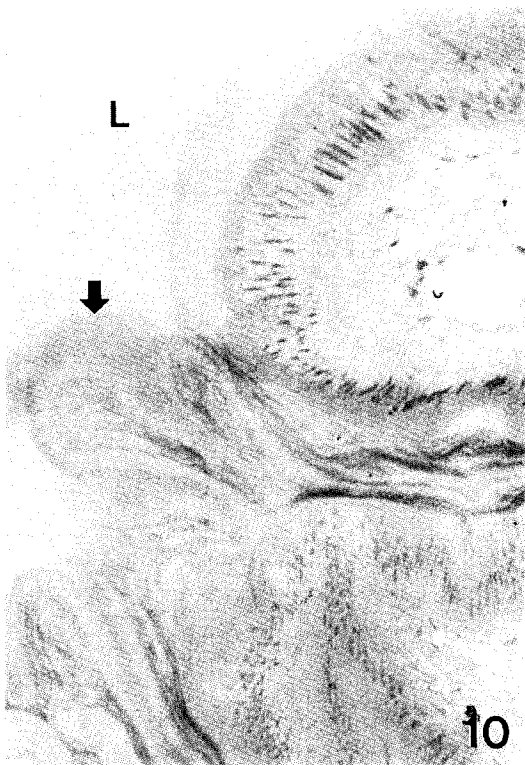
L, lumen; N, nucleus; arrow, microvilli. x1000.







9



10



11

