Morphologcal and Ultrastructural Study on the Prostate of a Land Snail *Nesiohelix samarangae*, a Stylommatophoran Pulmonate

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ABSTRACT

A morphological and ultrastructural study on the prostate of a land snail *Nesiohelix samarangae* was conducted. The prostate of *Nesiohelix samarangae* is a tubular gland connected with the large hermaphrodite duct. The lining of the prostate tubules possesses two distinct types of epithelial cells, one secretory and the other non-secretory. The secretory cells contained numerous secretory granules in various sizes and electron density. Most of the secretory granules showed light electron density but some of them showed heavy density. The ciliated cells were non-secreting cells situated only toward the lumen of the tubules and appeared as ordinary epithelial lining cells. The ciliated cells of the epithelium extensively interdigitate with each other and their apical surfaces had numerous cilia and microvilli. The bases of the ciliated cells did not reach the basal region of the secretory cells.

Key Words: land snail, Nesiohelix, prostate

INTRODUCTION

The prostate gland or prostate of pulmonate snails is consisting of tubular glands connected to the proximal part of the vas deferens.

Hubendick (1948) proposed a concept that the prostate gland might be summarized as a morphological unite with exclusively glandular structures that are developed from that part of the vas deferens situated proximal to its passage though the musclature of the body wall.

The prostate gland extends the whole lengh of the large hermaphroditic duct is well developed and consists of closely packed rows of branching secretory tubules which are radially arranged and converge to a number of channels. These converge further to form the single prostatic duct that opens into the seminal duct which in turn opens to vas deferens (Egonmwan, 1996).

Holm (1946), Abdel-Malek (1952), Kugler (1965), Stears (1974), Rudolph (1983), Tompa (1984), and Lee *et al*. (1992) examined the molluscan prostate glands in connection with morphological or histochemical studies on the reproductive tracks. Ultrastructural studies on the prostate glands were carried out by Quattrini (1967), West (1978), and Egonmwan (1996). The present study was conducted to understand morphology and ultrastructures of the prostate gland of a snail, *Nesiohelix samarangae*.

MATERIAL AND METHOD

All the snail material for this study were collected from a small island, Gaeuido, located in the West Sea of Taean-gun, Chungnam, Korea. The snails were kept in plastic containers at 25℃ and fed lettuce, carrots and calcium carbonate powder.

For light microscopic observations, the prostate

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gland and the large hermaphrodite duct were dissected from the snails, then fixed with 10% neutrally buffered formalin for 24 hours and washed. The specimens were dehydrated in alcohol series, embedded in paraffin, and sectioned at 7 μm in thickness with a rotary microtome (Histocut, Reichert-Jung 820). The tissue sections were stained with the methods of hematoxylin-eosin, PAS, Acian blue, PAS-Alcian blue, and silver impregnation. The stained tissue sections were examined with a light microscope, Optiphot-Ⅱ (Nikon).

For transmission electron microscopic observations, the specimens dissected from the snails were prefixed with 2% glutaraldehyde for 2 hours, and postfixed with 2% OsO_4 for 2 hours. The fixed specimens were washed three times with 0.1M phosphate buffer (pH 7.2), dehydrated in alcohol-acetone series, embedded in Spurr mixture, and sectioned at 70 nm in thickness with a ultramicrotome (Reichert supernova ultramicrotome). The thin tissue sections were double stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope (JEM-100CX Ⅱ).

RESULTS

1. light microscopic observations

The prostate seemed to be a compound tubular gland. In cross section each prostate tubule consists of a single layer of several prismatic cells arranged around a central lumen.

The epithelium was of two cell types : elongated gland cells and small ciliated non-secretory cells wedged between the larger secretory cells.

The larger secretory cells, which may reach about 45 μm in height, formed the largest part by mass of the prostate. Their cytoplasm was nearly always full of secretory material in the form of fine granules distributed more or less evenly. In the ordinary stain with hematoxylin-eosin stain, cytoplasm of the secretory cells were stained pink and their nuclei were stained blue.

In the stain with PAS, the cytoplasm of the secretory cells reddish purple and their nuclei were stained blue. In the single stain with Alcian blue, the cytoplasm of secretory cells stained pink and their nuclei were stained red. In a double stain with PAS and Alcian blue, the cytoplasm containing secretory granules were stained reddish purple, otherwise their nuclei were stained blue. The rest of the wall of the single tubules is made up of a connective tissue layer, thinner that epithelium and containing muscle fibers, haemolymph spaces and nerve fibers. In the silver impregnation stain, the connective tissue reacted black. The secretory tubules opened into prostatic duct which was connected spermatic duct, a sperm channel or sulucus that is really a continuation of the large hermaphrodite duct.

2. transmission electron microscopic observations

The epithelium was about 50 μm in height and was composed of columnar secretory cells) which rest on a thin basal lamina and ciliated cells(Figs. 1-6). The secretory cells (Type 1 cells) possessed one nucleus per each always at the bases of the cell, and the nuclei were usually round in shape. The secretory cells contained numerous secretory granules in various sizes and electron density. Most of the secretory granules showed light electron density but some of them showed heavy density (Figs. 7,8, 10-12).

Golgi bodies were abundant, especially toward the basal region of the secretory cells, and mainly consisted of tight packets of double smooth membranes. Mitochondria were randomly found among the secretory granules in moderate electron density (Figs. 11-12).

The ciliated cells (Type 2 cells) were non-secreting cells situated only toward the lumen of the tubules and appeared as ordinary epithelial lining cells.

The ciliated cells of the epithelium extensively interdigitate with each other and their apical surfaces had numerous cilia and microvilli. The bases of the ciliated cells did not reach the basal region of the secretory cells.

The nuclei of the ciliated cells were lobed and their cytoplasm, showing relatively high electron density, contained mitochondria and rich glycogen particles (Figs. 8-9)

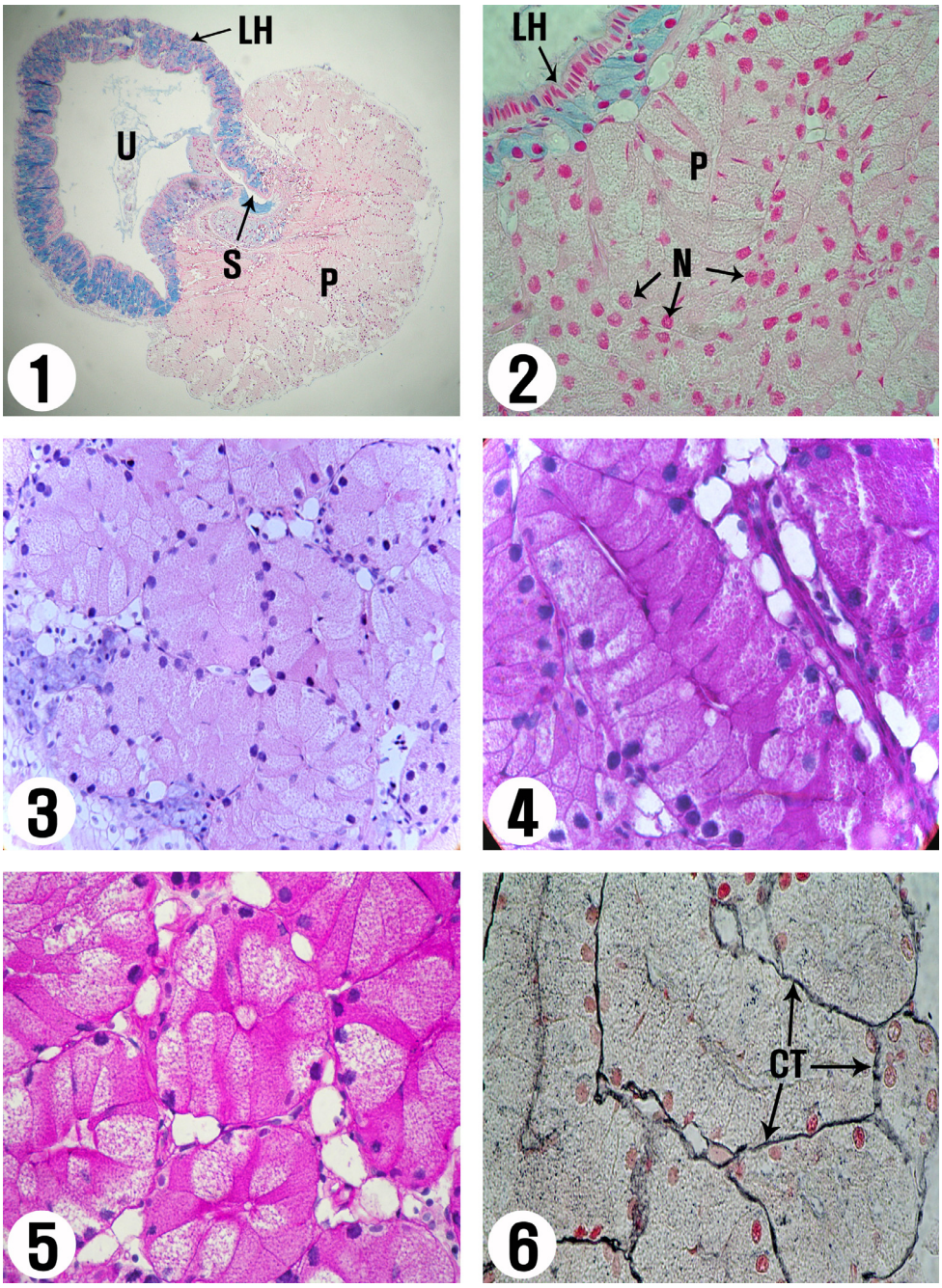

Fig. 1-6 Light micrographs.

Fig. 1. Overall view of the prostate and the large hermaphrodite duct stained with alcian blue. P: prostate; LH: large hermaphrodite duct; S: seminal furrow (of the large hermaphrodite duct); U: uterine part of the large hermaphrodite duct.

Fig. 2. Epitheria of the prostate lobules and the large hermaphrodite duct stained with alcian blue.

Fig. 3. Epitheria of the prostate lobules stained with hematoxylin and eosin.

Fig. 4. Epithlial cells double stained with PAS-alcian blue.

Fig. 5. Epithelial cells stained with PAS.

Fig. 6. Prostate lobules stained with silver impregnation method. The connective tissue (CT) shows strongly positive reaction.

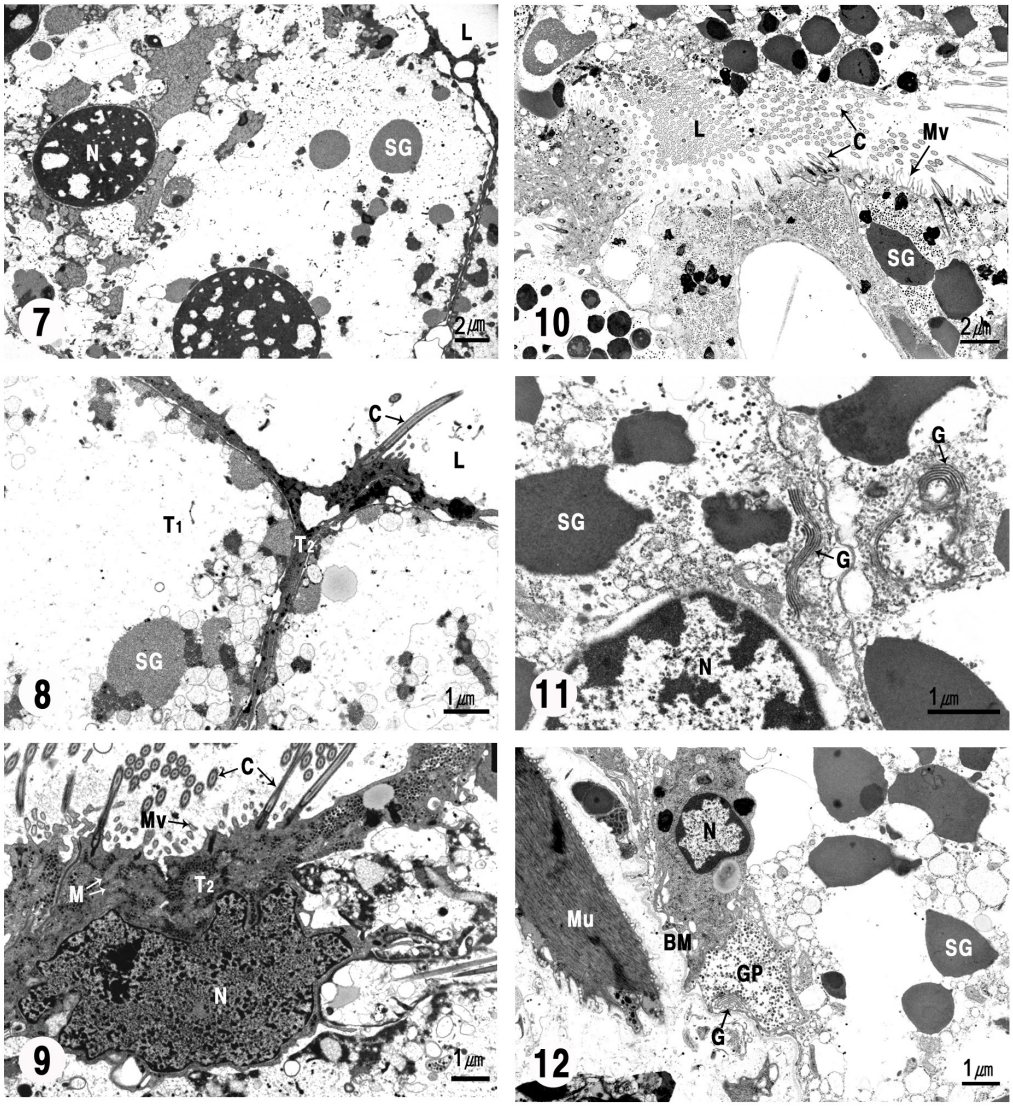

Fig. 7-12 Transmission electron micrographs.
Fig. 7. Secretory cells (T1) shows nucleus (N) and secretory granules (SG) in various stages.
Fig. 8. Secretory cells (T1) and a ciliated cell showing a cilium and microvilli.
Fig. 9. Ciliated cell (T2) showing the nucleus (N), mitochondria (M), microvilli, and numerous glycogen particles in the cytoplasm.
Fig. 10. Luminal portions (L) of the prostate showing neighboring ciliated cells and secretory cells. C: cilia; Mv: microvilli; SG: secretory granules.
Fig. 11. Secretory cell containing secretory granules (SG) with high electron density and developed Golgi apparatus (G) and glycogen particles.
Fig. 12. Basal portion of the secretory cells showing the basement membrane (BM) and developed muscle fibers (Mu) in the connective tissue.

DISCUSSION

The prostate seems to be a compound tubular gland. In cross section each prostate tubule consists of a single layer of several prismatic cells arranged around a central lumen. The epithelium is of two cell types: elongated gland cells and small ciliated non-secretory cells wedged between the larger

secretory cells. None of the ciliated cells was seen to extend more than a small fraction to the distance to the base of the larger cells. Therefore, Holm (1946) stated that the epithelium of the prostate might be a pseudostratified type. The larger secretory cells formed the largest part by mass of the prostate. Their cytoplasm is nearly always full of secretory material in the form of fine granules distributed more or less evenly. Runham and Laryea (1968) reported three cells types in the prostate of *Agriolimax reticulatus*.

The general morphological features of the prostate may similar to those of other mollusks reported earlier (Holm, 1946; Hubendick, 1948; Abdel-Malek, 1954; Kugler, 1965; Egonmwan, 1996). In the series of histochemical investigation undertaken throughout the present study, the chemical nature of the secretory granules seem to be neutral mucopolysaccharide because the cytoplasm of the secretory cells stained reddish purple with PAS, pink with Alcian blue, and reddish purple with a double stain of PAS and Alcian blue. On the chemical nature of the secretory materials in *Colus stimpsoni*, West (1978) reported they might be sulfonated acidic mucopolysacchaarides. He classified the secretory cells into two types. On the cell types of the prostate, histological studies of the pulmonate prostate often report the presence of more than one type secretory cell (Abdel-Malek, 1954 ; Kugler, 1965). Rudolf (1983) describes as many as seven types of secretory cells in a freshwater snail *Stagnicola elodes*, and described that a certain cell type contains small particles which are composed of slightly sulfonated acidic mucopolysaccharide. But we could not identify the secretory cells of the prostate of *Nesiohelix samarangae* in such detail with the histological methods that we conducted. This suggests that the secretion of the prostate is not simple, but contains a variety of chemically distinct components as mentioned by Bayne (1967). A thin connective tissue layer with few connective tissue components surrounds the epithelial layers as mentioned by Holm(1946). Considering that connective tissue stained black with a technique of silver impregnation, it contains rich fine reticular fibers.

Even though we did not compare the sizes of the prostate of the snails in detail by reproductive cycles their sizes seem to be stable. Egonmwan (1996) reported that the prostate was not reduced during egg laying in *Limicolaria flammea*. Similar observations were made in *Achatina fulica* (Ghose, 1962), in Arion ater (Lüsis, 1961) and in *Agriolimax reticulatus* (Bayne, 1967). Bayne (1967) observed in the Agriolimax reticulatus the prostate secrets continuously during development of male and female gametes. The function of the prostate in mollusks has been controversy. It has been suggested that the prostatic secretion could function in egg production (Mead, 1950; Ghose, 1960; Bayne, 1967). Otherwise it has been described as a male secretion (Duncan, 1958; Smith, 1965; Thomson and Bebbington, 1969).

Lüsis (1961) suggested that the prostatic secretion of pulmonates may be a source of nutritive material for the spermatozoa. Rigby (1965) suggested that it helps the arrival of the spermatozoa from the seminal vesicle into vas deferens.

The reproductive physiology of the land snail seems to be a subject that should be studied and reviewed all again.

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