Kasetsart J. (Nat. Sci.) 36 : 285 - 290 (2002)

Ultrastructure of Connective Tissue Cells of Giant African Snails *Achatina fulica* (Bowdich)

Viyada Seehabutr

ABSTRACT

The connective tissue sheath of cerebral ganglion of giant African snails *Achatina fulica* (Bowdich) contain many types of cells such as pore cells, granular cells, vesicular connective tissue cells, amoebocytes, fibroblasts and muscle cells. Pore cells are characterized by numerous invagination of the cell membrane. Granular cells contain numerous granules. The vesicular connective tissue cells contain round nuclei, located in the centre of the cells. Amoebocytes have pseudopodia. Fibroblasts are spindle-shaped or elongate cells. They contain ovoid or elongate nuclei. Muscle cells have ovoid nuclei located in the centre of the cells.

Key words: connective tissue cells, pore cell, vesicular connective tissue cell, amoebocyte, fibroblast, muscle cell

INTRODUCTION

Achatina fulica (Bowdich) belongs to Phylum Mollusca, Class Gastropoda, Subclass Pulmonata, Order Stylommatophora, Family Achatinidae. The nervous system of this snail consists of 11 ganglia. Each ganglion is covered with connective tissue. There are two layers, a thick outer layer and a thin inner layer.The connective tissue of *Helix aspersa* the pulmonate snail, has a large number of globular cells that contain glycogen, muscle cells and fibroblasts (Kerkut and Walker, 1975).

In *Archachatina marginata* (Pulmonata) all ganglia are enclosed in a thick and loose connective tissue which contain many cell types and muscle fibers (Nisbet, 1961). In *Lymnaea stagnalis* (Pulmonata), the main cellular constituents of connective tissue surrounded the ganglia are fibrocytes, pigment cells, amoebocytes, granular cells and smooth muscle fibers (Wendelaar Bonga, 1970). Sminia (1972) reported that there are 8 different cell types in the connective tissue of *Lymnaea stagnalis.* These are the pore cells, the granular cells, the vesicular connective tissue cells, the amoebocytes, the fibroblasts, the undifferentiated cells, the pigment cells and the muscle cells. At the ultrastructural levels, Wendelaar Bonga (1970) found that granular cells of *Lymnaea stagnalis* contain large granules of variable sizes (0.5-4 μm) whereas the muscle cells contain thick filaments (ϕ300-600 A°). In addition, Sminia (1972) reported that the pore cells of this snail are the most conspicuous cell types in the connective tissue. These cells have many invaginations which are bridged by cytoplasmic tounges (Sminia *et al.*, 1972; Sminia and Boer, 1973). Sminia and Boer(1973) suggested that the pore cells in the connective tissue of *Lymnaea stagnalis* are protein producing cells, as they contain very extensive granular endoplasmic reticulum. Fernandez and Fernandez (1972) found that the ganglionic sheath

Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.

of *Helix aspersa* contains pigment cells which have melanosomes (0.5-1 μm in length). They concluded that these cells are involved in melanin synthesis. In *Lymnaea stagnalis*, the amoebocytes contain round or oval nuclei, many vacuoles and a few granules in the cytoplasm. In addition, the cells possess many long pseudopodia (Sminia, 1972). Sminia (1972) suggested that these cells are involved in endocytosis. Moreover,Sminia(1972) found the vesicular connective tissue cells,the fibroblasts and the muscle cells in *L. stagnalis*. The vesicular connective tissue cells are very large (up to 60 μm). The cytoplasm of this cell is present as a thin rim against the cell membrane. The space in the cell is the storage for reserve material (Sminia, 1972). The fibroblasts are spindle-shaped or elongate and have several branching processes. Moreover, there are collagen fibrils in the cytoplasm (Sminia, 1972). The muscle cells contain the thin (ϕ 50-100 A°) actin and the thick (ϕ 300-600 A°) paramyosin filament in the cytoplasm (Sminia, 1972).

MATERIALS AND METHODS

Preparation of the specimen

The mature snails, *Achatina fulica* were collected from the wild during the rainy season. The snails with the shell length of 6.7 cm or the approximate weight of 25-30 g were used in the experiment. After the snails were anesthetized with 1 % nembutal for 30 min, they were sacrificed and the cerebral ganglia together with surrounding connective tissue sheath were dissected out.

Preparation for transmission electron microscopy

The specimens were fixed in a mixture of 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4 at 4°c for 1.5 hrs and washed three times with cacodylate buffer. They were post-fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 2 hrs and washed three times with distilled water. After tertiary fixation in 1% uranyl acetate for 0.5 hr, the specimens were washed in distilled water and dehydrated in graded series of ethanol (50% to 100%) and propylene oxide. Then the specimens were embedded in araldite. Semi-thin and ultra-thin sections were cut with MT-2 ultramicrotome using glass knives.The semi-thin sections were stained with 1% methylene blue in 1% borax and examined with light microscope. In addition, the ultra-thin sections were stained with saturated uranyl acetate in 70% alcohol (Watson, 1985) and lead citrate (Reynold, 1963), for 7-12 min each. Sections were then examined under a Hitachi h-300 TEM operating at 75 Kv.

RESULTS AND DISCUSSION

There are 6 different cell types found in the connective tissue sheath of cerebral ganglia (Figure 1). These are pore cells, granular cells, vesicular connective tissue cells, amoebocytes, fibroblasts,and

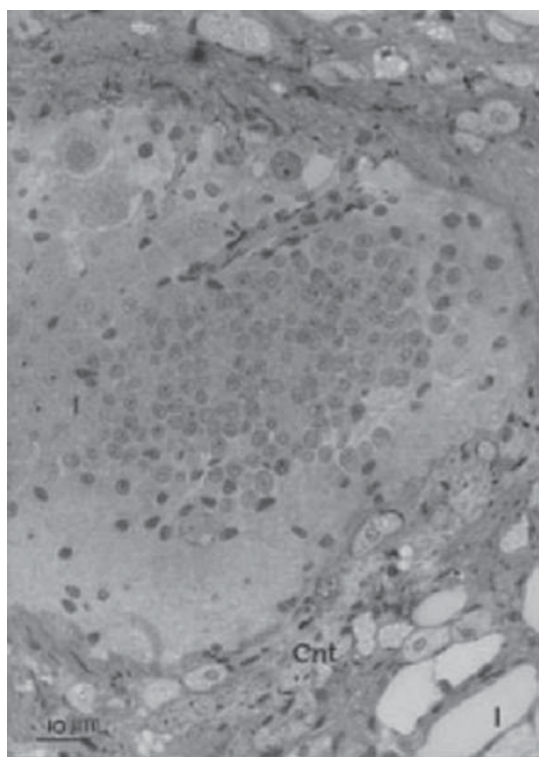


Figure 1 Photomicrograph of cerebral ganglion. Cnt = connective tissue

muscle cells.

Pore cell. There are many tubular and vesicular invaginations of the cell membrane in the pore cell. Moreover, there is many pores at the periphery of the cell, oval nucleus and many granules in the cell (Figure 2). These cells are similar to those found in *Lymnaea stagnalis* (Sminia, 1972).

Granular cell. It can be indicated that the Golgi apparatus is involved in the formation of granules in the granular cell(Figure 3) because the Golgi apparatus is rather extensive. The stacked lamellae are swollen. There are numerous round heterogeneous granules (∅2-3 μm) in the cell. The rough endoplasmic reticulums are well developed. They are located between the granules. The granular cells are commonly found in the connective tissue of gastropod (Sminia, 1972).

Vesicular connective tissue cells. These cells are called vesicular cells because they have spaces in the cell after preparing with routine fixation and staining techniques. There are round nuclei, mitochondria, small vesicles and tubules in the cell (Figure 4). These cells are abundant in the digestive gland and the gonad of gastropods (Sminia, 1972).

Amoebocyte. They are the round in shape and possess many pseudopodia (Figure 5-6).In addition,they contain nuclei, mitochondria, small vesicles and many vacuoles. These cells are also found in the blood of gastropoda (Sminia, 1972).

Fibroblasts.These cells contain elongate nuclei, rough endoplasmic reticulum, electron dense elementary granules, mitochondria, lysosome-like structures and connective tissue fibrils (Figure 7). This type of cell can be found in connective tissue of *Lymnaea stagnalis* (Sminia, 1972).

Muscle cell. They have ovoid nuclei located in the centre of the cells. The cytoplasm is occupied by filaments. Moreover, there are many pinocytic and small vesicles in the peripheral cytoplasm of the cells (Figure 8). These cells are similar to those of muscle cells of *Lymnaea stagnalis* (Sminia, 1972).

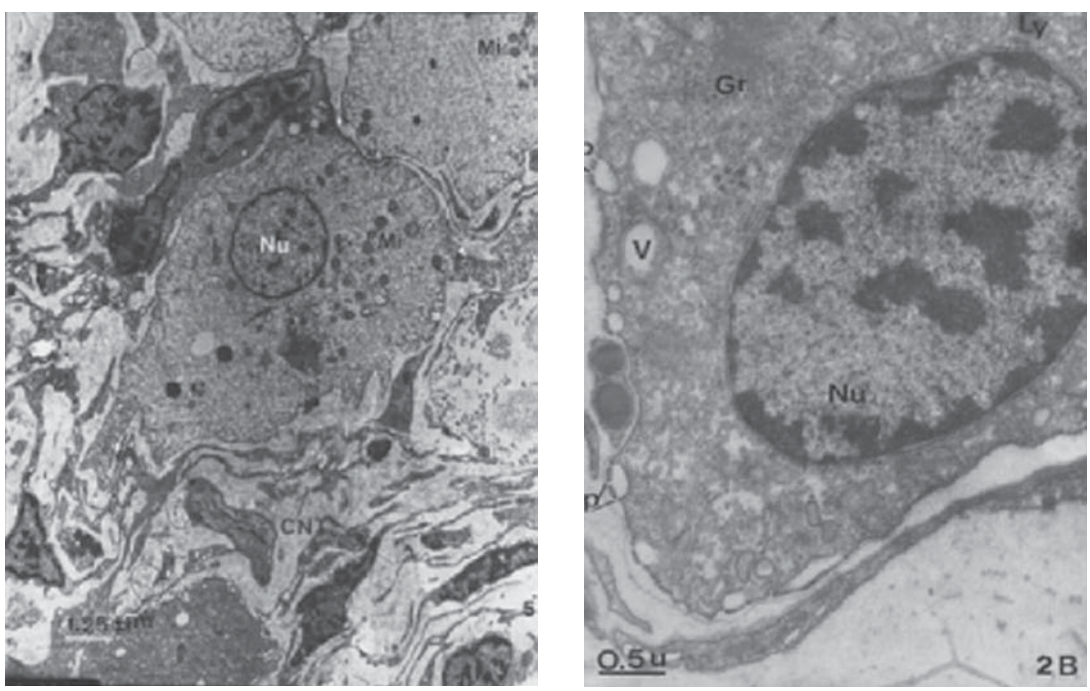


Figure 2 (A-B) Transmission electron micrographs of pore cells. (Lower left in Figure 2A) Nu = nucleus, P = pore, V = vesicular, Gr = granule

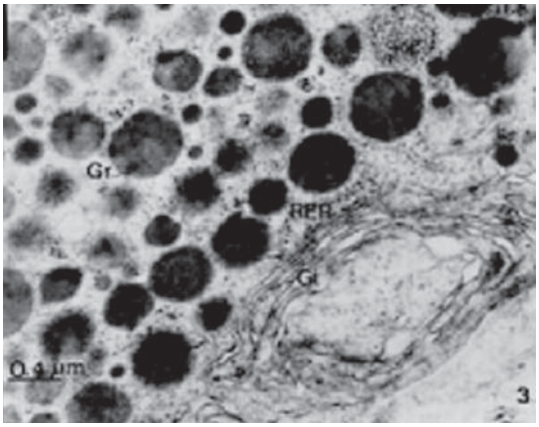


Figure 3 Transmission electron micrographs of granular cells.
Gr = granule, RER = rough endoplasmic reticulum
Gi = Golgi apparatus

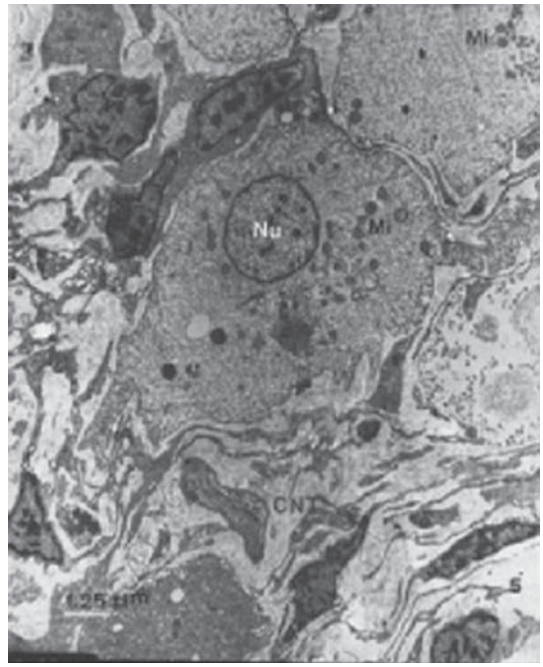
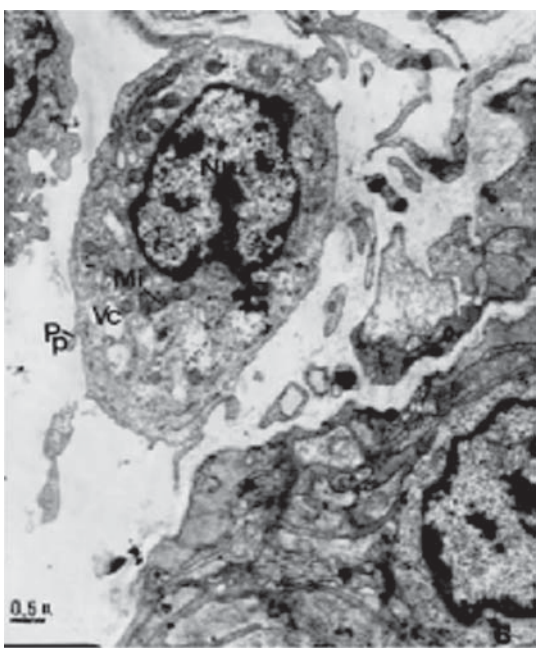


Figure 4 Transmission electron micrographs of vesicular connective tissue cell.
Nu = nucleus, Mi = mitochondria



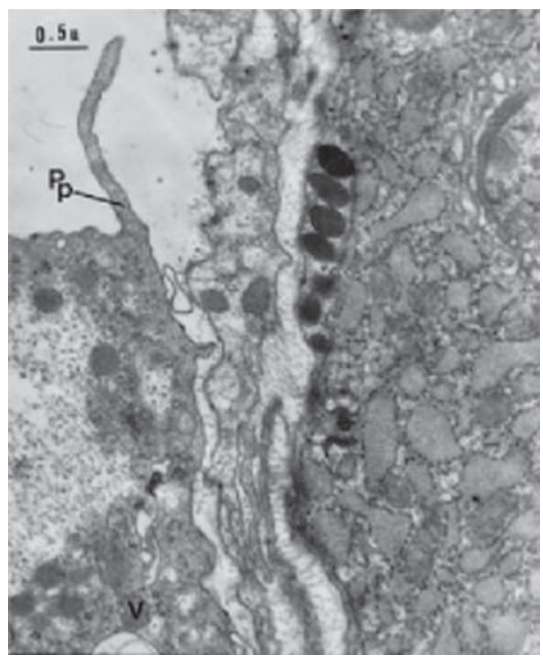


Figure 5-6 Transmission electron micrographs of amoebocytes.
Nu = nucleus, Vc = vacuole, Pp = pseudopodium, Mi = mitochondria
V = small vesicle

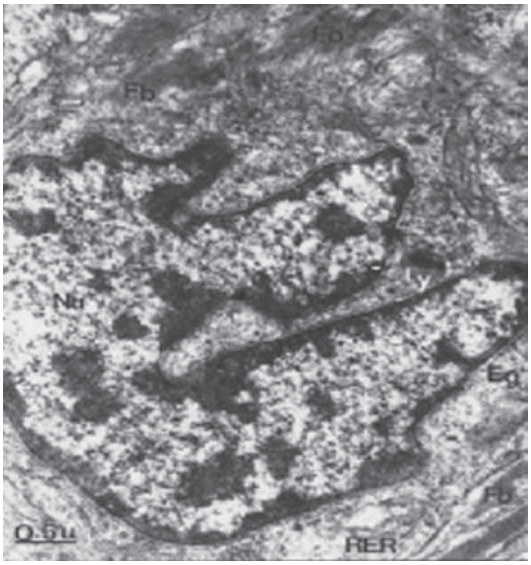


Figure 7 Transmission electron micrographs of fibroblast, Nu = nucleus, Ly = lysosome like structure, RER = rough endoplasmic reticulum, Eg = elementary granule, Fb = fibril

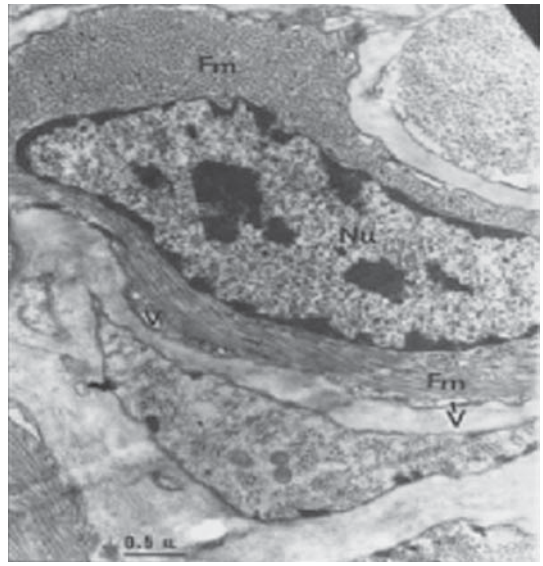


Figure 8 Transmission electron micrographs of muscle cell, Nu = nucleus, Fm = myofilaments, V = vesicle

CONCLUSION

The connective tissue sheath of the cerebral ganglion of *Achatina fulica* is composed of several cell types: pore cells, granular cells, vesicular connective tissue cells, amoebocytes,fibroblasts,and muscle cells.

ACKNOWLEDGEMENTS

The author would like to acknowledge the Kasetsart University Research and Development Institute for providing research fund.

LITERATURE CITED

Fernandez, J. and M. S. Fernandez. 1972. Nervous system of the snail *Helix aspersa*. Z. Zellforsch. 135 : 173-482.

Kerkut,G.A.and R.J Walker. 1975. Pulmonates. Nervous system, eye and statocyst. London Academic Press. 1 : 165-245.

Nisbet, R.H. 1961. Some aspects of the structure and function of the nervous system of *Archachatina marginata* (Swainson). Proc. Roy. Soc. B, 15 : 267-287.

Renold, E.S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17 : 208-212.

Sminia, T. 1972. Structure and function of blood and connective tissue cells of the fresh water pulmonate *Lymnaea stagnalis* studied by electron microscopy and enzyme histochemistry. Z. Zellforsch 130 : 497-526.

Sminia, T. and H. H. Boer. 1973. Haemocyanin producing in pore cells of the freshwater snail *Lymnaea stagnalis.* Z. Zellforsch 145 : 443-445.

Sminia, T., H.H.Boer, and A. Niemantsverdriet. 1972. Haemoglobin producing cells in freshwater snails. Z. Zellforsch 13 : 563-568.

Watson, M.L. 1985. Staining of tissue sections for electron microscopy with heavy metal. J. Biophys. Biochem. Cytol. 4 : 475-478.

Wendelaar Bonga, S.E. 1970. Ultrastructure and histochemistry of neurosecretory cells and neurohaemal areas in the pond snail *Lymnaea stagnalis* (L.). Z. Zellforsch 108 : 190-224.

Received date : 31/07/02
Accepted date : 30/09/02