Korean J Biol Sci 6: 327-333, 2002

Fine Structure of the Glandular Epithelium during Secretory Silk Production in the Black Widow Spider *Latrodectus mactans*

Myung-Jin Moon* and Edward K. Tillinghast[1]

Department of Biological Sciences, College of Natural Sciences, Dankook University, Cheonan 330-714, Korea; [1]Department of Zoology, University of New Hampshire, Durham, NH 03824, USA

Key Words:
Fine structure
Silk
Epithelium
Spider
Latrodectus mactans

Among the silk glands in the black widow spider *Latrodectus mactans*, the ampullate one is the most predominant gland in both sexes, and is composed of three functional parts - excretory duct, storage ampulla and convoluted tail regions. This experiment was performed using mechanical pulling stimulation with electric motor equipment to reveal a correlation between silk usage and silk producing system in this poisonous spider. The mature secretory products in glandular epithelium are closely packed and appear as electron-opaque spherical vesicles. A part of the vesicles with fine fibrillar paracrystalline texture seems to store some proteins which will function at the time of final assembly into fibrils. Most of the secretory silk products which originated from the rough endoplasmic reticula of the glandular epithelial cells are grown by fusion with surrounding small vesicles. However, the Golgi complex does not seem to play an important role in this process of secretion. According to progressive maturation of secretory silk product, these granules are progressively filled with a fine fibrillar material, and thus appear much more electron-dense than those of earlier states. When the secretory product is extruded from the glandular cavity, the epithelium is rapidly changed to a thinner layer of tall columnar cells with less definitive cell membranes. After extruding there are a few secretory droplets within these cells, thus causing this region to stain much lighter.

Proteins can be divided into two major classes, globular and fibrous. While the globular proteins generate complex conformations, the fibrous proteins are characterized by a single repetitive structure (Kaplan, 1998). The spider silks, as one group of fibrous proteins, are exceptional structural materials. Especially in terms of strength, toughness and energy absorption prior to break, spider silks are unmatched in the world of synthetic or natural fibers (Gosline et al., 1984).

It has been reported that most of Araneidae spiders (Peters, 1987; Tillinghast and Townley, 1987) as well as black widow spiders (Kovoor, 1977; Coddington, 1986) use silk from two kinds of ampullate glands for web building. However, exact functions are still not known sufficiently. Cytologic aspects of spider silk elaboration have been studied only in the case of the ampullate glands of *Araneus* (Peakall, 1965; Bell and Peakall, 1969; Tillinghast and Townley, 1986) and *Nephila* (Tillinghast and Christenson, 1984; Moon et al., 1988).

* To whom correspondence should be addressed.
Tel: 82-41-550-3445, 3474, Fax: 82-41-551-9229
E-mail: moonmj@dankook.ac.kr

Although Bücherl (1971) mentioned only about 100 spider species as actually dangerous to humans, all spiders with venom apparatus may be considered as toxic animals. Among these poisonous spiders, *Latrodectus* spiders were considered as the most toxic to human beings (Maretic, 1987). The black widow spiders of genus *Latrodectus* include six species and they are all poisonous to humans (Levi, 1959; Bettini and Maroli, 1978). They spin irregular webs in crevices and other dark, protected spots.

Because of its high toxicity, the venoms of *Latrodectus* spiders have received the most attention (Frontali et al., 1972; Maretic, 1987; Moon and Tillinghast, 1996), while the silk of this spider has been nearly neglected except some of the histologic and fine structural works by Kovoor (1977) and Moon et al. (1998, 1999). To begin filling this gap, we have conducted this experiment to establish a correlation between the silk usage and silk producing system in poisonous spiders. We describe here fine structural analysis of glandular epithelium during silk production in the black widow spider *Latrodectus mactans*, which is one of the most poisonous spiders.

Materials and Methods

Specimens of *Latrodectus mactans,* the black widow spider, were collected at southern California and reared in the laboratory of Zoological Department, University of New Hampshire, NH, USA. All spiders were maintained under ambient conditions with natural lighting in wooden frames (20×20×4 inches) with glass plates front and back, and fed insects and water daily.

Mechanical pulling stimulation was performed using electric motor driven glass rod after attachment of the ampullate silks on it. The speed of pulling silk was adjusted at 3-5 cm/sec and it was maintained for 60 min. After this pulling stimulation, both groups of treated and untreated female spiders were used in this experiment.

The specimens were dissected under a dissecting light microscope in a drop of spider Ringer's solution consisting of 160 mM NaCl, 7.5 mM KCl, 4 mM $CaCl_2$, 1 mM $MgCl_2$, 4 mM $NaHCO_3$, and 20 mM glucose, pH 7.4 (Groome et al., 1991). For light microscopic observation, the tissues were fixed with buffered, neutral formalin solution, and were dehydrated using ethanol and embedded in Paraplast plus medium (Polyscience) via xylene.

For transmission electron microscopic examination, each silk gland was gently removed and fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer at pH 7.4. Postfixation was performed with 1% osmium tetroxide in the same buffer. Subsequently, the tissue pieces were dehydrated in ascending concentrations of ethanol and embedded in Poly/Bed 812-Araldite medium (Polysciences, Inc.) via propylene oxide.

Semithin sections stained with 1% toluidine blue (dissolved in 1% borax) were used to study the gross morphology. Ultrathin sections were obtained from a LKB-V ultramicrotome, and were double stained with uranyl acetate, followed by lead citrate. The sections were examined with a JEM 100 CX-II electron microscope (JEOL) at 80 kV.

Results

There are two pairs of ampullate silk glands in *Latrodectus mactans*. These silk glands are the most prominent of all silk glands observed in this spider. One pair of major ampullate glands send secretory ductules to the anterior spinnerets, and another pair of minor ampullate glands supply the median spinnerets. Each ampullate gland is characterized by a protein-synthesizing tail region, expanded storage ampulla, and a

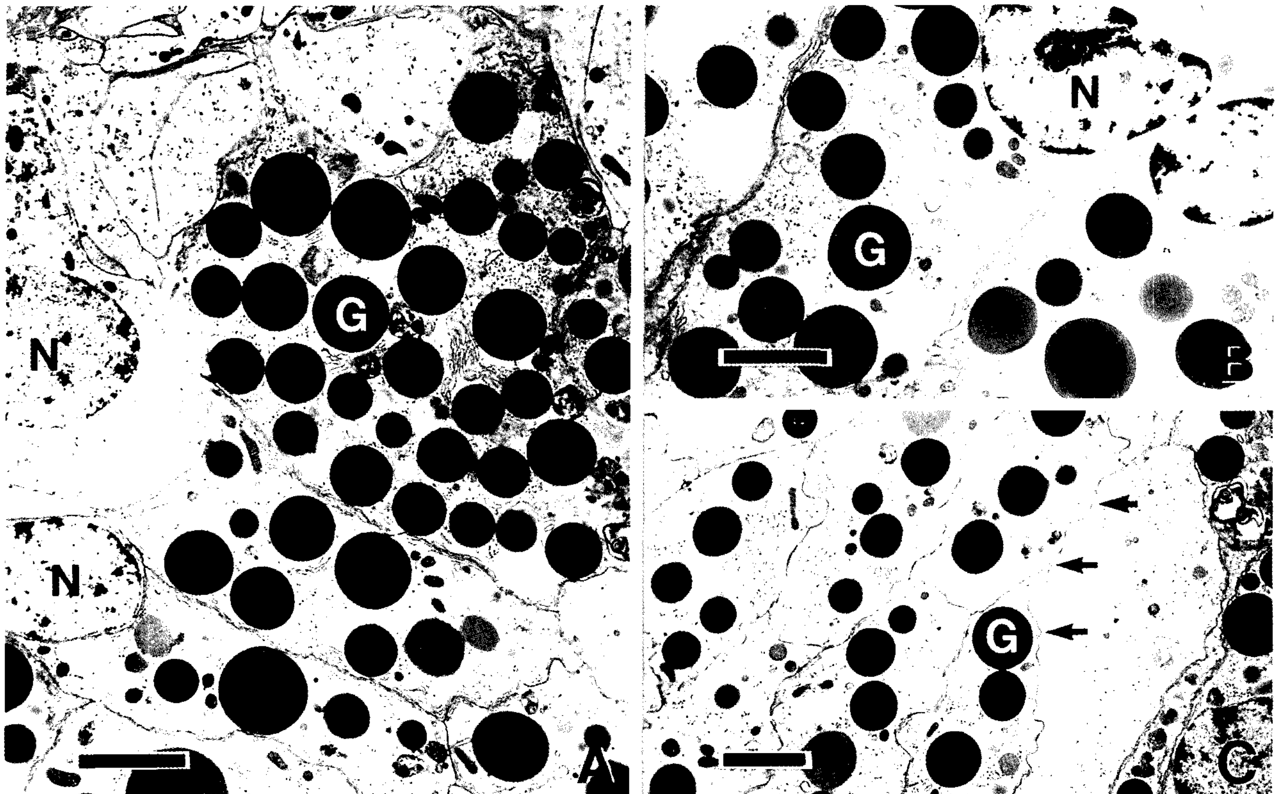

Fig. 1. A, The protein-synthesizing epithelial cells of ampullate silk gland is composed of tall columnar cells with large, irregularly ellipsoidal nuclei (N). The electron dense secretory granules (G) are densely packed, and remain close to each other without fusion. B, The mature secretory product in glandular epithelium appears almost spherical granules. C, The appearance of secretory granules differs from cell to cell and can be representative of the secretory cycle. The limiting membranes (arrows) of the glandular epithelial cells can be easily distinguished. Scale bars = 2 μm.

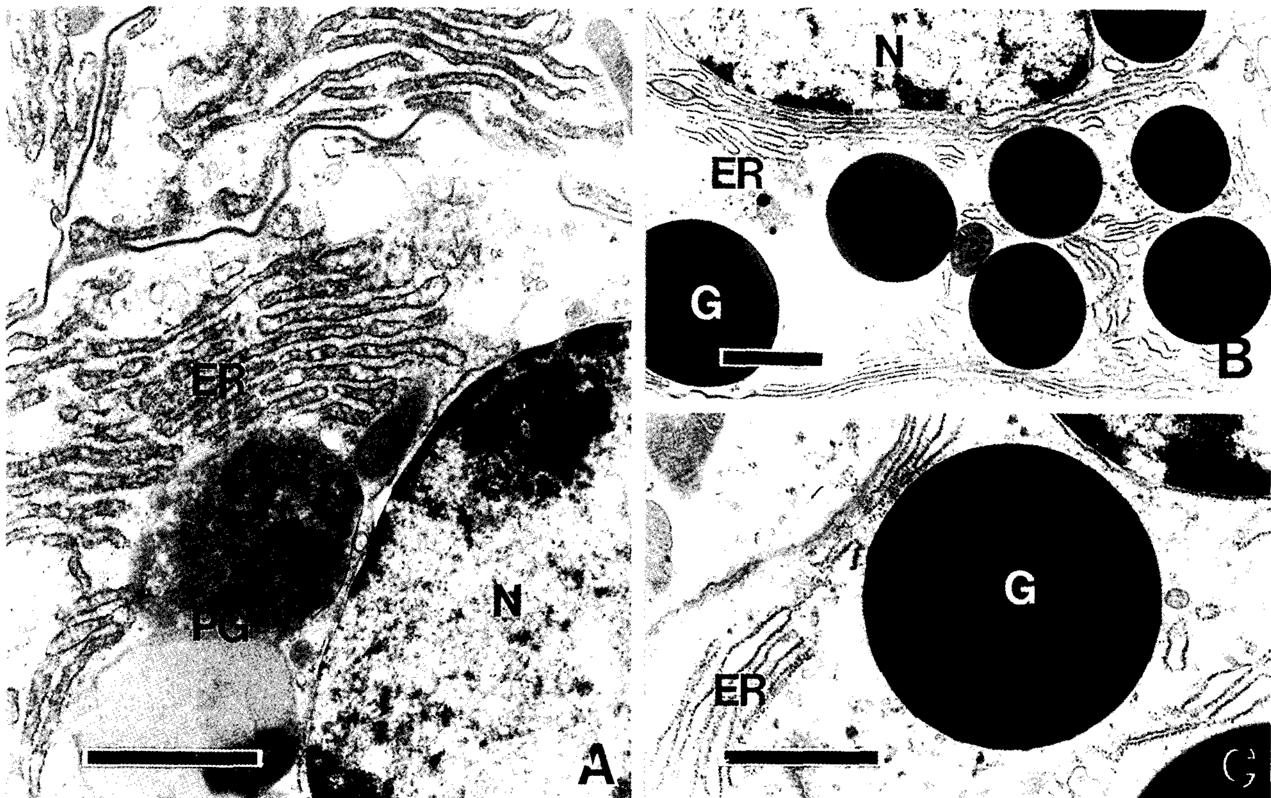

Fig. 2. Electron micrographs of the ampullate silk gland in the black widow spider, *Latrodectus mactans*. A, In the cytoplasm of the glandular epithelial cell, broad cisternae of the rough endoplasmic reticulum (ER) which contain fine granulated material are found near the nucleus (N). Presumptive precursors (PG) of the secretory granules appeared near nucleus. B, C, The extensive rER occupies the whole remaining space of the epithelium, however no definitive Golgi apparatus appeared except for occasional small agranular cisternae. Scale bars = 1 μm.

long duct which passes via a spigot of each spinneret.

The protein-synthesizing epithelial cells of ampullate silk gland are composed of tall columnar cells with large, irregularly ellipsoidal nuclei. The mature secretory product in glandular epithelium appears as almost spherical granules (Fig. 1A). These electron dense granules are densely packed, and remain close to each other without fusion. The appearance of secretory granules differs from cell to cell and can be representative of the secretory cycle (Fig. 1B). However, the number of the granules per cell is relatively lower than those of web-building spiders. So the limiting membranes of the glandular epithelial cells can be easily distinguished (Fig. 1C).

In the cytoplasm of the glandular epithelial cell, broad cisternae of the rough endoplasmic reticulum (rER) which contain fine granulated material are found near the nucleus. Numerous multivesicular bodies, which are presumed to be the precursors of silk granules, also appear at the base of this epithelium. Apparently these small vesicles are synthesized from the rER, and the secretory granules are formed by fusion with these small vesicles (Fig. 2A). This extensive rER occupies the whole remaining space of the epithelium, however no definitive Golgi apparatus appeared except for occasional small agranular cisternae. So, the Golgi complex does not seem to play an important role in the secretory process of silk production in this silk glands (Figs. 2B, C).

The mature secretory product in glandular epithelium appears as almost spherical granules. These electron dense granules are densely packed and remain close to each other without fusion. The final electron-dense granules of the secretory product originated from the distended cisternae are observed near the microvilli of the glandular epithelium. The quantity of fine granular material gradually increases the release of secretory silk (Fig. 3A). These granules after releasing from the epithelial cells frequently appear to aggregate with several others, forming amorphous electron dense deposits (Fig. 3B). These fine granular materials, possibly proteins, seem to be transported across the apical membrane by exocytotic activities, and are added to the luminal secretory products as precursors of the ampullate silk (Fig. 3C).

After the mechanical pulling stimulation, secretory granules accumulated in the apical area are rapidly released to the luminal cavity. In contrast to that of non-treated specimen, the epithelium of the treated ampullate gland has a thinner layer of tall columnar

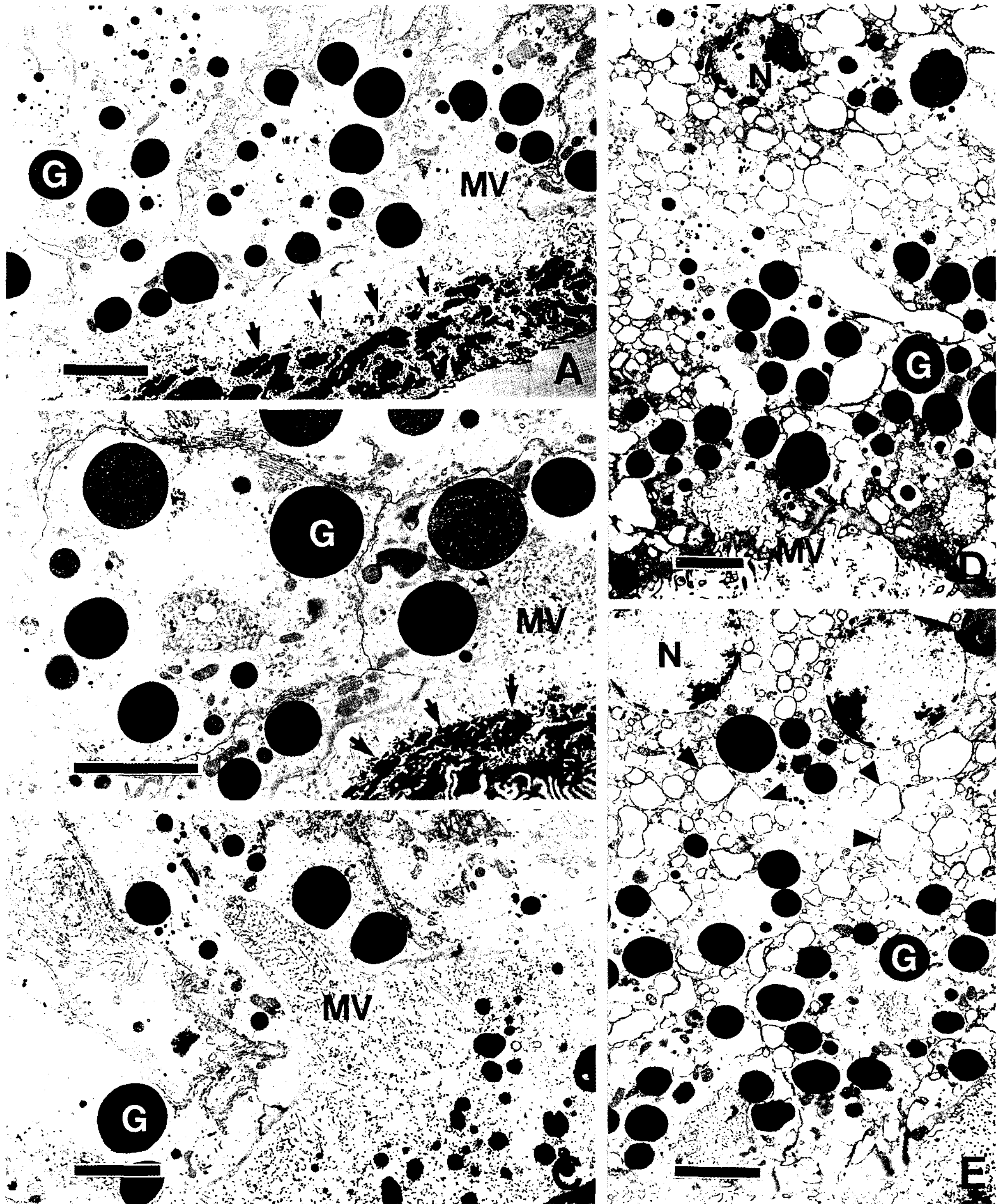

Fig. 3. A, B, C, Before stimulation, the final electron-dense granules of the secretory product originated from the distended cisternae where they appear attached to the microvilli (MV) of the glandular epithelium. The granules (G) after releasing from the epithelial cells frequently appear to aggregate with several others, forming amorphous electron dense deposits (arrows). They are added to the luminal secretory products as precursors of the ampullate silk. D, E, After treatment of the mechanical pulling stimulation, secretory granules accumulated in the apical area are rapidly released to the luminal cavity. There are a few secretory droplets (arrow heads) within this cells, thus causing this region to stain much lighter. N: nucleus. Scale bars = 2 μm.

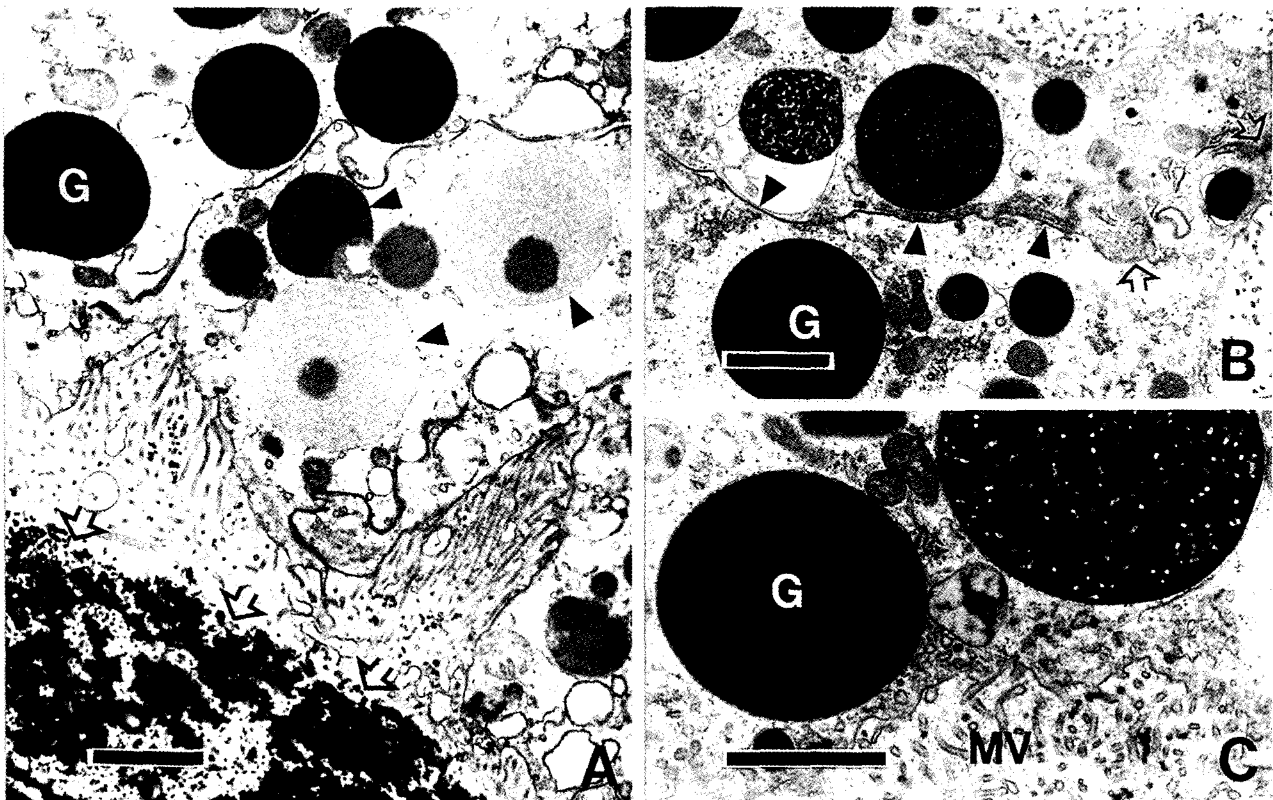

Fig. 4. A, Several types of silk precursors (arrow heads) appear at the secretory region of the ampullate silk gland after mechanical pulling stimulation. The lumen (arrows) contains crystals, concretions and vesicles filled with granules and cell debris. B, Diverse electron densities among the secretory granules reflect the maturity of the secretory granules (G). Specialized septate junctions (arrow heads) and desmosomes (arrows) are clearly observed along the plasma membranes of the epithelial cells. C, The lumen or apical border of the cells comprises a series of microvilli (MV). Scale bars = 1 μm.

cells with less definitive cell membranes (Fig. 3D). There are a few secretory droplets within these cells, thus causing this region to stain much lighter. It has been observed that the secretory products located in the apical cytoplasm are rapidly released through the digitated surface of glandular epithelial cells. It is obvious that the cell seldom loses part of its cytoplasm in this rapid process (Fig. 3E).

Several types of silk precursors appear at the secretory region of the ampullate silk gland. These secretory silk products accumulate in the luminal cytoplasm of the glandular epithelial cells as diverse forms of secretory granules. The lumen contains crystals, concretions and vesicles filled with granules and cell debris. Apparently more than two types of secretory granules and secretory deposits are seen at both of cells and lumen. Gradually, the deposits from electron lucent granules are located along the borders of the cells, whereas electron dense granules are at the core of the lumen (Fig. 4A).

It is obvious that the difference in electron density among the secretory granules reflects maturation process of secretory granules within the cell. So we could observe a variety of intermediate forms of granules within one cell. Specialized septate junctions and desmosomes are clearly observed along the plasma membranes of epithelial cells (Fig. 4B). The lumen or apical border of cells comprises a series of microvilli. The apices of the glandular cells are fringed with short and irregular microvilli. Vigorous exocytotic actions appear at the base of these microvilli, and disorganization of the secretory product also occur when it is extruded from the cells (Fig. 4C).

Discussion

Like the other araneid spiders, the highly poisonous spiders also have silk-producing apparatus and use the silk throughout their life (Coddington, 1986). The silk glands of *Latrodectus mactans* are arranged in seven groups, each group feeding silk into one of the three spinnerets pairs. Our previous work has revealed that the spinning apparatus of *Latrodectus mactans* is located on the spinnerets including each pair of major and minor ampullates, 3 pairs of tubuliforms, 1 pair of flagelliforms, 2 pairs of aggregates, about 50 pairs of pyriforms and over 250 pairs of aciniform glands respectively (Moon et al., 1998).

At least six types of silk glands have been described in araneid spiders (Foelix, 1982; Kovoor and Peters,

1988). Among these major silk glands, the ampullate gland is present in all spiders (Foelix, 1982), and are responsible for the production of dry silk used in dragline (Nentwig and Heimer, 1987). Following the observations by Peakall (1964) on silk synthesis in *Araneus diadematus*, regulation of secretory protein release in the ampullate silk glands has been frequently considered (Peakall, 1965; Bell and Peakall, 1969; Tillinghast and Townley, 1986).

Changes within the glandular epithelium of araneid silk glands have been revealed using various experimental techniques (Tillinghast and Townley, 1986; Kovoor, 1987; Moon et al., 1999). Synthesis of protein by the major ampullate silk glands can be stimulated by either cholinergic stimulation (Peakall, 1964, 1965) or a mechanical pulling process (Tillinghast and Townley, 1986, 1987; Moon et al., 1998).

As it has been already reported that the synthesis of each type of secretion granule does not necessarily take place in a uniform manner throughout the length of gland (Kovoor and Peters, 1988), and the individual gland secretion granules may themselves be heterogeneous (Moon et al., 1988), our trial using mechanical pulling stimulation could lead to epithelial changes during silk production more effectively.

It has been reported that the spider silk is a crystalline protein composed of strands of disordered amino acid chains (Vollrath, 1992; Vollrath and Knight, 2001). Each type of construction has its characteristic fiber composition, and any construction of these spiders comprises several types of silk fibers (Andersen, 1970; Candelas and Cintron, 1981; Tillinghast and Townley, 1987). Silk is secreted into the lumen as protein droplets that can be stored from extrusion via the gland duct. Unlike poison glands, no muscles are associated with the ducts to facilitate movement of the proteinaceous secretion (Foelix, 1982).

Our electron microscopical results also suggest that the numerous electron-lucent vesicles which are presumed to be the precursors of secretory silk could be formed by vesicular fusion. Moreover, the secretory silk is produced in a form which is ready for secretion, and undergoes no further concentration. Candelas and Cintron (1981) and Candelas and Lopez (1983) also reported that the secretory product of the large ampullate glands of *Nephila clavipes* migrates as one homogeneous band in denaturing electrophoresis, and that the luminal contents comprise the liquid silk.

In spite of our observation that the secretory products of the ampullate silk glands in this spider are accumulated in luminal cytoplasm of the glandular epithelium as a form of electron dense granules, it is still uncertain whether a series of granular modifications shows progressive maturation of the secretory product. However, it is certain that several kinds of secretory granules with fine fibrillar structure are produced after our mechanical pulling stimulation, and electron densities of the granules are gradually increased coincident with their maturation. So, when the secretory product is extruded from the glandular cavity, the epithelium is rapidly changed to a thinner layer of tall columnar cells with less definitive cell membranes. After extruding, there are a few secretory droplets within these cells thus causing this region to stain much lighter.

The mechanism of transport of secretions from the gland cell to lumen has been frequently considered, especially with relation to disorganization of its secretory product (Peakall, 1965; Bell and Peakall, 1969; Tillinghast and Townley, 1986; Moon et al., 1998). In this experiment, electron micrograph of stimulated epithelium shows a thinner layer of tall columnar cells with a few secretory droplets. Obviously, the glandular epithelial cell only loses the secretory granules from its cytoplasm during the process of rapid silk production. So, it could be concluded that the silk proteins produced within epithelial cells of the ampullate silk glands are released by the typical mechanism of merocrine secretion.

Structural analysis using high magnification electron micrographs on the ampullate silk glands have revealed that the secretory silks are produced by way of rough endoplasmic reticulum of each glandular epithelial cell with no relationship with Golgi apparatus. Unlike the typical silk production in *Bombyx mori* (Sasaki and Tashiro, 1976; Sasaki et al., 1981) via both rER and Golgi apparatus, the ampullate silk glands of this spider seem to bypass either the concentrating or packaging steps by Golgi apparatus. Thus, Golgi apparatus does not seem to play an important role in the secretory process of silk production. This unique phenomenon was first recognized by Bell and Peakall (1969) in the ampullate gland of the spider *Araneus sericatus*. Our results also suggest that the secretory silk is rapidly produced via rER in a form which is ready for release and undergoes no further steps for concentration or remodeling via Golgi apparatus.

With regards to silk production within the gland cells, we could observe more than two types of secretory granules in the glandular epithelium, and more diverse secretory deposits in both of cells and lumen of the ampullate gland. While deposits from electron lucent granules are located along the cell borders, the electron dense granules are only seen at the core of the lumen. Recently, Knight and Vollrath (1999) presented evidence from transmission electron microscopy of the major ampullate gland cells in *Nephila edulis*. They observed two transverse zones which produce the core of silk and a thin coat enveloping the core. In spite of distinct cytochemical differences (Kovoor, 1987), both of two zones are constructed from tall columnar epithelial cells containing numerous spherical secretory vesicles. This finding confirms that the spider silk gland not only synthesizes the silk protein itself but also stores some proteins which will function at the time of final assembly into fibrils (Vollrath and Knight, 2001).

References

Andersen SO (1970) Amino acidic composition of spider silks. *Comp Biochem Physiol* 35: 705-713.

Bell AL and Peakall DB (1969) Changes in fine structure during silk protein production in the ampullate gland of the spider *Araneus sericatus*. *J Cell Biol* 42: 284-295.

Bettini S and Maroli M (1978) Venoms of Theridiidae, genus *Latrodectus*. In: Bettini S (ed), Arthropod Venoms. Springer-Verlag, Berlin, pp 149-185.

Bücherl W (1971) Spiders. In: Bücherl W and Buckley EE (eds), Venomous Animals and Their Venoms. Academic Press, New York, pp 197-277.

Candelas GC and Cintron J (1981) A spider (*Nephila clavipes*) fibroin and its synthesis. *J Exp Zool* 216: 1-6.

Candelas GC and Lopez F (1983) Synthesis of fibroin in the cultured glands of *Nephila clavipes*. *Comp Biochem Physiol B* 74: 637-642.

Coddington J (1986) The monophyletic origin of the orb web. In: Shear WA (ed), Spider Webs and Spider Behavior. Stanford University Press, pp 319-363.

Foelix RF (1982) Biology of Spiders. Harvard University Press, Cambridge, pp 109-147.

Frontali N, Granata F, and Parisi P (1972) Effects of black widow spider venom on acetylcholine release from rat cerebral cortex slices *in vitro*. *Biochem Pharmacol* 21: 969-974.

Gosline JM, Denny MW, and DeMont ME (1984) Spider silk as rubber. *Nature* 309: 551-552.

Groome JR, Townley MA, de Tschaschell M, and Tillinghast EK (1991) Detection and isolation of proctolin-like immunoreactivity in arachnids: possible cardioregulatory role for proctolin in the orb-weaving spiders *Argiope* and *Araneus*. *J Insect Physiol* 37: 9-19.

Kaplan D (1998) Fibrous proteins: silk as a model system. *Polymer Degrad Stab* 59: 25-32.

Knight DP and Vollrath F (1999) Hexagonal columnar liquid crystal in the cells secreting spider silk. *Tissue Cell* 31: 617-620.

Kovoor J (1977) Donnees histochimiques sur les glandes sericigenes de la veuve noire *Latrodectus mactans* Fabr. (Araneae: Theridiidae). *Ann Sci Nat Zool 12 Ser* 19: 63-87.

Kovoor J (1987) *Comparative structure and histochemistry of* silk-producing organs in Arachnids. In: Nentwig W (ed), Ecobiology of Spiders. Springer-Verlag, Berlin, pp 159-186.

Kovoor J and Peters HM (1988) The spinning apparatus of *Polenecia producta* (Araneae: Uloboridae): structure and histochemistry. *Zoomorphology* 108: 47-59.

Levi HW (1959) The spider genus *Latrodectus* (Araneae: Theridiidae). *Trans Am Microsc Soc* 78: 7-43.

Maretic Z (1987) Spider venom and their effect. In: Nentwig W (ed), Ecophysiology of Spiders. Springer-Verlag, Berlin, pp 142-159.

Moon MJ, Kim CS, and Kim WK (1988) Ultrastructure of the ampullate gland in the orb web spider, *Nephila clavata* L. Koc 2. Sac and tail portion of the large ampullate gland. *Korean J Electr Microsc* 18: 91-101.

Moon MJ and Tillinghast EK (1996) Fine structural aspects of the venom production in the black widow spider, *Latrodectus mactans*. *Korean J Electr Microsc* 26: 17-31.

Moon, MJ, Townley MA, and Tillinghast EK (1998) Fine structural analysis of secretory silk production in the black widow spider, *Latrodectus mactans*. *Korean J Biol Sci* 2: 145-152.

Moon MJ, Kim TH, Townley MA, and Tillinghast EK (1999) Fine structural analysis of the capture thread production in the black widow spider, *Latrodectus mactans*. *Korean J Entomol* 29: 225-232.

Nentwig W and Heimer S (1987) Ecological aspects of spider webs. In: Nentwig W (ed), Ecobiology of Spiders. Springer-Verlag, Berlin, pp 211-225.

Peakall DB (1964) Effects of cholinergic and anticholinergic drugs on the synthesis of silk fibroins of spiders. *Comp Biochem Physiol* 12: 465-470.

Peakall DB (1965) Regulation of the synthesis of silk fibroins of spiders at the glandular level. *Comp Biochem Physiol* 15: 509-515.

Peters HM (1987) Fine structure and function of capture threads. In: Nentwig W (ed), Ecobiology of Spiders. Springer-Verlag, Berlin, pp 187-202.

Sasaki S and Tashiro Y (1976) Studies on the posterior silk gland of the silkworm *Bombyx mori*. 5. Electron microscope localization of fibroin in the posterior silk gland a the later stage of the fifth instar. *J Cell Biol* 70: 648-659.

Sasaki S, Nakajima E, Fujii-Kuriyama Y, and Tashiro Y (1981) Intracellular transport and secretion of fibroin in the posterior silk gland of the silkworm *Bombyx mori*. *J Cell Sci* 50: 19-44.

Tillinghast EK and Christenson T (1984) Observations on the chemical composition of the web of *Nephila clavipes* (Araneae: Araneidae). *J Arachnol* 12: 69-74.

Tillinghast EK and Townley M (1986) The independent regulation of protein synthesis in the major ampullate glands of *Araneus cavaticus* Keyserling. *J Insect Physiol* 32: 117-123.

Tillinghast EK and Townley M (1987) Chemistry, physical properties, and synthesis of Araneidae orb webs. In: Nentwig W (ed), Ecobiology of Spiders. Springer-Verlag, Berlin, pp 203-210.

Vollrath F (1992) Spider web and silk. *Sci Am* 266: 70-76.

Vollrath F and Knight DP (2001) Liquid crystalline spinning of spider silk. *Nature* 410: 541-548.

[Received October 1, 2002; accepted November 5, 2002]