Scientific paper

ULTRASTRUCTURAL CHANGES IN THE SILK GLAND DURING SECRETORY AND POST-SECRETORY PHASES IN THE TROPICAL TASAR SILKWORM, *ANTHERAEA MYLITTA* (D.) (LEPIDOPTERA: SATURNIIDAE)

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The electron microscopic studies reveal the presence of greatly lobulated nuclei and well-equipped secretory apparatus consisting of rough endoplasmic reticulum, Golgi bodies and microtubular radial channels in the cells of medial and posterior regions of the silk gland (MSG and PSG respectively) during the secretory phase in ***Antheraea mylitta.*** *The protein droplets are secreted continuously by the Golgi bodies which are intermingled with the microtubular radial channels. Prior to spinning, the formation of intracuticular spaces takes place through which the protein droplets are transported from the cells to the gland lumen.*

With the onset of the regression phase, a large number of lysosomal bodies are evident in the cells of the MSG and PSG which first attack the cytoplasmic organelles and later on, the nuclei causing degeneration of the MSG and PSG during the prepupal period of metamorphosis.

Keywords: *Antheraea mylitta,* silk gland, ultrastructure.

INTRODUCTION

Ultrastructure of the silk gland in *Bombyx mori* and some temperate species of silkworms revealed distinctly the secretory characteristics of the epithelial cells in MSG and PSG consisting of polyploid and highly ramified nuclei, large number of nucleoli, rough endoplasmic reticulum, Golgi bodies and microtubular radial channels forming a well-equipped secretory apparatus for the synthesis of silk proteins, sericin and fibroin respectively (Prudhomme *et al.,* 1985; Sehnal and Akai, 1990).

Although some histological (Barsagade and Tembhare, 2000) and ultrastructural (Akai *et al.,* 1993,1994) observations were made on the silk gland in the tropical tasar silkworm, *A. mylitta,* further investigation is needed to elucidate thoroughly the subcellular mechanism of silk secretion and silk gland degeneration. The present ultrastructural studies were, therefore, undertaken to explore subcellular secretory and degenerative changes in the silk secretory cells of MSG and PSG.

MATERIAL AND METHODS

During July 1997 to November 2000, about 20 to 30 fifth instar larvae of the tropical tasar silkworm, *Antheraea mylitta,* trivoltine Dabha race, were brought several times to the laboratory from the rearing center and acclimatized (Barsagade and Tembhare, 2000). The silk glands were dissected gently from the 3, 6, 9, 12, 15,18, 21 and 24 day-old last instar larvae. The MSG and PSG were separated and fixed in the Karnovsky's Fixative. The ultra-thin sections were cut on the LKB's Reickert Ultracut-S-1 Ultramicrotome and observed under the'JEOL, 1200 EX- II'(Japan)

transmission electron microscope at desirable magnifications at the Regional Sophisticated Instrumentation Centre (RSIC), Nagpur University, Nagpur.

OBSERVATIONS

The histological observations on the silk glands in *Antheraea mylitta* elucidated the MSG and PSG as the silk protein secretory regions. With the process of cocoon construction, the silk glands undergo regression and degeneration (Barsagade and Tembhare, 2000). The ultrastructural changes occurring in the silk secreting cells of MSG and PSG during the silk protein secretory and post-secretory phases are described below:

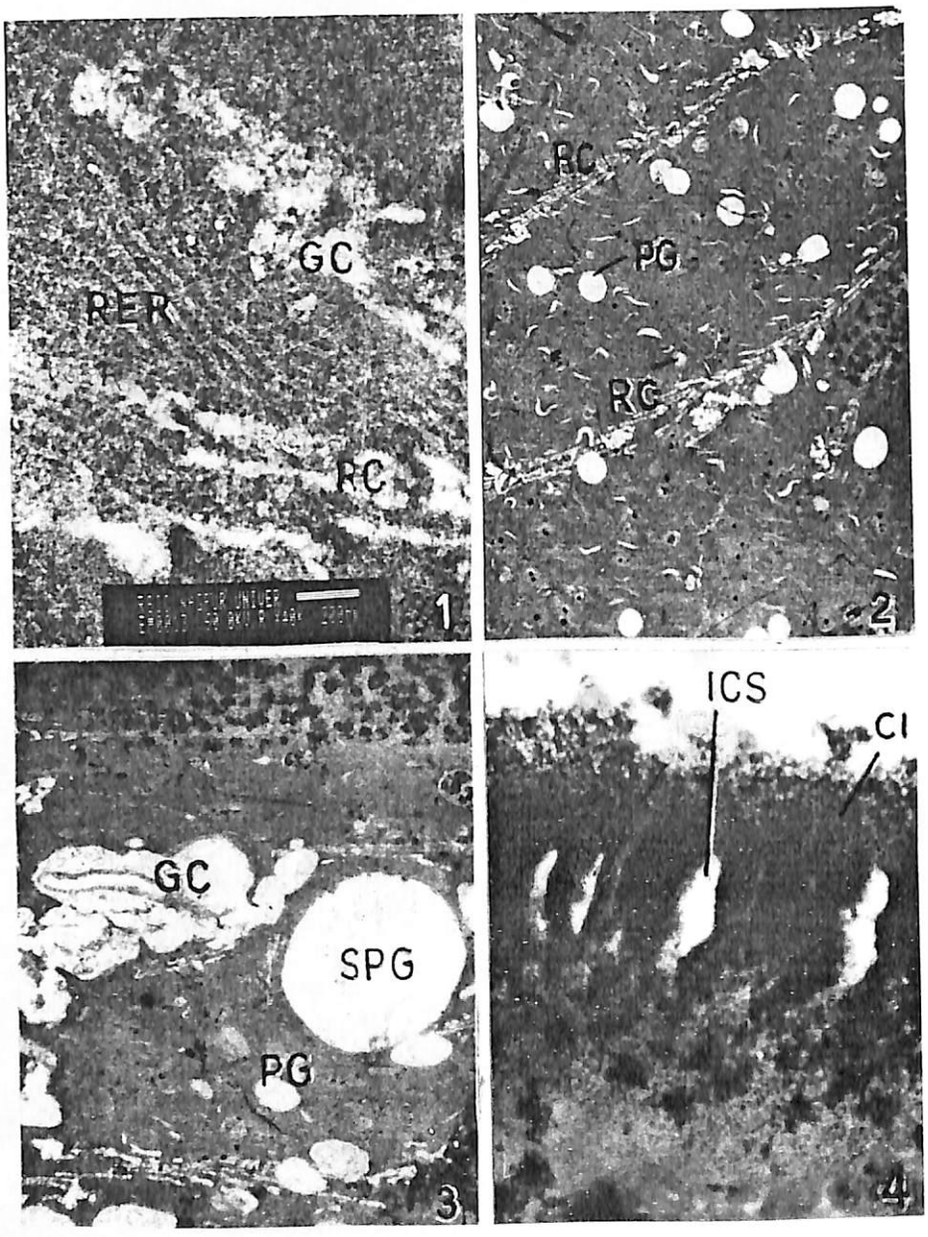
Secretory Phase: (Figs. 1-4)

In the 6- to 15-day old larvae, the nuclei of the secretory cells of MSG and PSG are giant and spindle-shaped or lobulated respectively and are compactly filled with large chromatin bodies and some nucleoli. The entire cell body of the cells in both, the MSG and PSG is occupied with the rough endoplasmic reticulum (RER) forming a sort of carpet-like appearance. The radial channels are formed by the bundles of long microtubules which run from the membranous infoldings of apical basement membrane to the distal microfilament bundles adjacent to the microvilli of the cell wall. Large number of Golgi bodies are lying along the radial channels. In the cells of MSG and PSG, the Golgi bodies are numerous and large in size while the mitochondria are indistinct. The primary fine spherical protein droplets are discharged from the Golgi bodies which later on, fuse with each other forming the large secondary protein globules. The protein globules are larger in the cells of PSG than those in MSG. The well-defined spaces in the cuticular intima are formed in the 15-18 day old larvae. The transport of protein globules from cell membrane microvilli to the lumen of MSG and through spaces in cuticular intima to the lumen of the PSG is well-evident. In the 15 day-old last instar larvae, the lumen of MSG and PSG is filled with their own spherical and elongated protein droplets respectively.

In the 18 to 21 day-old larvae, the protein globules are transported from the lumen of PSG to that of MSG and as a result, the PSG protein globules are often observed at the center while the MSG protein droplets at the periphery in the lumen of MSG. Both, MSG and PSG protein secretions are, subsequently transported to the ASG and finally to the spinneret. The vacuoles in the cells as well as in the lumen intermingled with the protein droplets are not seen in either MSG or PSG during the present study.

Post-secretory Phase: (Figs 5-10)

At the onset of spinning, the 18 day-old last instar larva stops feeding and the large concentric whorls of RER are formed. The cells of MSG and PSG become devoid of secretory droplets. The lysosomes occur in large quantity in the cells of MSG and PSG. The lysosomes attack the cytoplasmic organelles including the microtubular radial channels. The cytoplasmic content is almost digested. Large phagosomes embodying variable size of vacuoles increase in number in the cells of MSG and PSG. The secretory apparatus is almost destroyed so that in the place of microtubular radial channels, Golgi bodies, RER and cytoplasmic content, the cells are packed with the lysosomal bodies, cytoplasmic filaments, membranous vesicles and tubules.


GC
RER
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1
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PG
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2
GC
SPG
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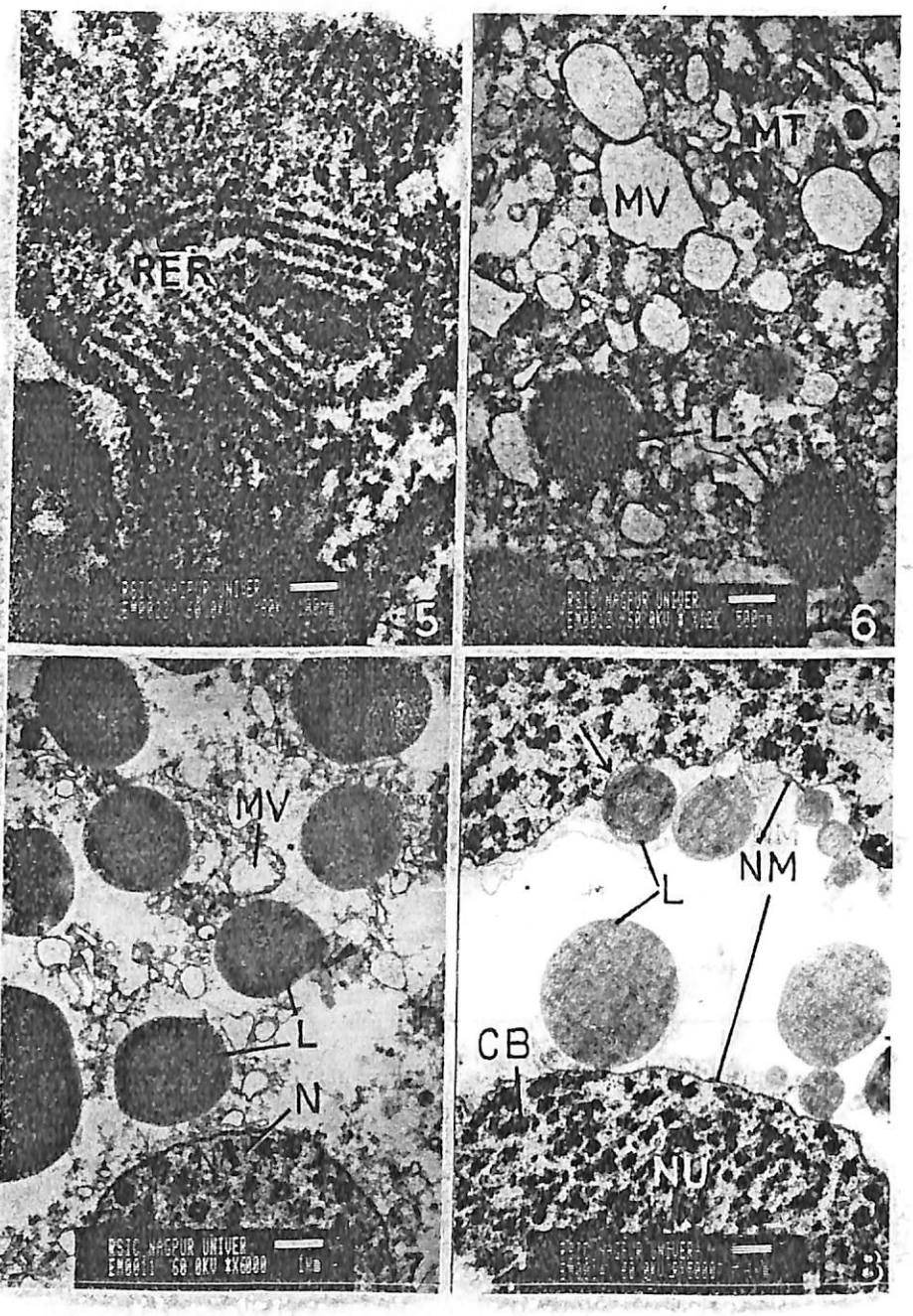
3
ICS
CI
4


RER
5
MT
MV
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6
MV
L
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7
CM
L
NM
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8

Fig. 1. TEM of PSG showing rough endoplasmic reticulum (RER), Golgi complex(GC) and radial channels (RC) at the onset of secretory phase in 6-day old larvae. × (scale).

Fig. 1. Microscopie électronique à transmission de la glande sericigène postérieure montrant le réticulum endoplasmique rugueux (RER), le complexe golgien (GC) et les canaux radiaux (RC) au début de la phase sécrétrice chez les larves de 6 jours. × (échelle).

Fig. 2. TEM of PSG showing radial channels (RC) secreting protein globules (PG) during secretory phase in 12-day old larvae. × 1800.

Fig. 2. Microscopie électronique à transmission de la glande sericigène postérieure montrant les canaux radiaux (RC) sécrétant les globules de protéines (PG) pendant la phase sécrétrice chez des larves de 12 jours. × 1800.

Fig. 3. TEM of PSG showing large GC and PG fusing with each other and forming large secondary protein globules (SPG) during secretory phase in 15-day old larvae. × 2500.

Fig. 3. Microscopie électronique à transmission de la glande sericigène postérieure présentant de grands complexe golgien et globules de protéines fusionnant ensemble et formant de grands globules de protéines secondaires (SPG) pendant la phase sécrétrice chez des larves de 15 jours. × 2500.

Fig. 4. TEM of PSG showing formation of intracuticular spaces (ICS) in the internal cuticle intima (CI) in the 15-day old larvae. × 37500.

Fig. 4. Microscopie électronique à transmission de la glande sericigène postérieure présentant une formation d'espaces intracuticulaires (ICS) dans les intima cuticulaires internes (CI) chez des larves de 15 jours. × 37500.

Fig. 5. TEM of PSG showing formation of concentric whorls of RER in 18 day old larvae. × (scale).

Fig. 5. Microscopie électronique à transmission de la glande sericigène postérieure montrant la formation de volutes concentriques du reticulum endoplasmique rugueux chez des larves de 18 jours. × (échelle).

Fig. 6. TEM of PSG showing lysosomes (L) causing destruction of cytoplasmic organelles and formation of membranous vesicles (MV) and tubules (MT) in 21-day old larvae. N – nucleus. × (scale).

Fig. 6. Microscopie électronique à transmission de la glande sericigène postérieure présentant des lysosomes (L) provoquant la destruction des organelles cytoplasmiques et la formation de vesicules (MV) et de tubes membranaires chez les larves de 21 jours. N – noyau. × (échelle).

Fig. 7. TEM of PSG showing cells containing a large number of lysosomes (L), membranous vesicles (MV), scanty cytoplasm and nucleus (N). × (scale).

Fig. 7. Microscopie électronique à transmission de la glande sericigène postérieure présentant des cellules contenant un grand nombre de lysosomes (L), de vésicules membranaires (MV), et un cytoplasme et un noyau peu abondants (N). × (échelle).

Fig. 8. TEM of PSG showing lysosomes (L) attacking nuclear membrane (NM) of the nuclei (arrow) after digesting cytoplasmic contents and cell wall in the 24-day old larvae. CB - chromatin bodies. × (scale).

Fig. 8. Microscopie électronique à transmission de la glande sericigène postérieure présentant les lysosomes (L) attaquant la membrane nucléaire (NM) des noyaux (flèche) après la digestion du contenu cytoplasmique et de la paroi cellulaire chez les larves de 24 jours. CB – corps chromatiniens. × (échelle).

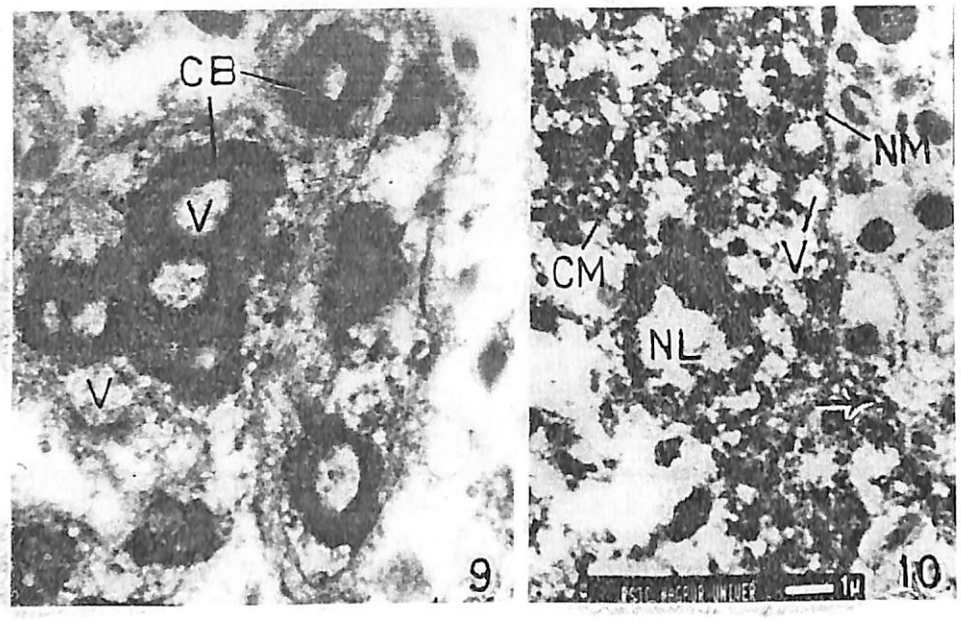

Fig. 9. TEM of PSG showing formation of vacuole (V) at the center of chromatin bodies (CB) in 24-day old larvae. × 18750.

Fig. 9. Microscopie électronique à transmission de la glande sericigène postérieure présentant la formation de vacuoles (V) au centre des corps chromatiniens (CB) chez des larves de 24 jours. × 18750.

Fig. 10.TEM of PSG showing colloidal chromatin material (CM) due to destruction of chromatin bodies, breaking down of nucleolus (NL), vacuoles (V) in nucleoplasm and release of nuclear contents from nucleus into cell body after breaking down of nuclear membrane (arrow). X (scale).

Fig. 10. Microscopie électronique à transmission de la glande sericigène postérieure présentant un matériau chromatinien colloïdal (CM) dû à la destruction des corps chromatiniens, la rupture du nucléole (NL), les vacuoles (V) dans le nucléoplasme et la libération du contenu nucléaire dans le corps cellulaire après la rupture de la membrane nucléaire (flèche). × (échelle).

After digesting the cytoplasmic contents, the lysosomes attack the nuclei. They rupture first the nuclear membrane and thereafter destroy the chromatin material. The vacuolization and disintegration of chromatin bodies and nucleoplasm causing fragmentation of the nuclei and discharge of the amorphous nuclear material in the cell body of MSG and PSG cells is well-evident.

DISCUSSION

In *B. mori* the silk producing secretory cells of MSG and PSG under electron microscope showed presence of extensively lobulated nuclei with numerous nucleoli, a bulk of chromatin bodies and cytoplasmic organelles consisting of RER, the Golgi bodies and microtubular radial channels (Sasaki

and Tashiro, 1976; Sasaki and Nakagari, 1980; Couble *et al.*, 1983; Couble *et al.*, 1984). The secretory globules are first released from the Golgi bodies of radial channels into the cytoplasm as the opaque elementary globules which adhere to each other and form the large globules and pass through the intracuticular spaces of the degenerated intima and accumulate in the gland lumen (Sasaki *et al.*, 1982). The accumulation of opaque spherical globules in the lumen of MSG and radially arranged filamentous globules in the lumen of PSG is well-evident even in the histological preparations in *Antheraea mylitta* (Barsagade and Tembhare, 2000) similar to that in other silkworms (Akai and Kataoka, 1978). Formation of intracuticular spaces in the degenerated cuticle- intima of MSG and PSG is noticed quite distinctly in *Antheraea mylitta* (Akai *et al.*, 1993).

In *A. mylitta*, ultrastructure of posterior region of the silk gland has been studied earlier (Akai *et al.*, 1993, 1994). These workers have reported numerous vacuoles within the silk liquid accumulated in the gland cavity and because of this reason in their opinion the silk filaments become porous rather than the compact one as found in *B. mori* (Akai, 2000). During the present study, however, vacuoles could not be recognized within the liquid secretion in the lumen of MSG or PSG.

In *B. mori* the middle region of the silk gland is known to be differentiated into three divisions- posterior, middle and anterior showing difference in ultrastructure, tinctorial properties and x-ray spectra of the sericin molecules they produce (Akai, 1984). The present study, moreover, does not show such differentiation of MSG into various divisions in *A. mylitta*. Couble *et al.*, (1987) noticed secretion of different sericin molecules by individual divisions of MSG in *B. mori* forming three layers- inner, middle and outer of sericin, around the central fibroin fibers. In *A. mylitta*, moreover, the central core of fibrous secretion transported from PSG is encircled by a thick homogenous layer of sericin secretion in the lumen of MSG and ASG. In *B. mori*, the MSG lumen serves as a silk reservoir (Prudhomme *et al.*, 1985), while in *A. mylitta* the silk secretion is stored independently in the lumen of MSG and PSG prior to spinning.

The *A. mylitta* silkworm stops feeding in order to initiate spinning the cocoon and the process of silk secretion is regressed. The electron microscopic observations showed that the RER, Golgi bodies and secretory globules reduce greatly and at the same time, myelin bodies, lysosomes, autophagic bodies, whorls of rough endoplasmic reticulum and vacuoles appear in the cytoplasm. The cytoplasmic organelles are rapidly digested by the lysosomes so that the entire cell body is filled with empty membranous vesicles and tubules. The lysosomes thereafter, attack the nuclear membrane and destroy chromatin bodies and nucleoli autophagically similar to that in *B. mori* (Sehnal and Akai, 1990). Matsuura *et al.*, (1976a,b) also reported sudden and rapid formation of lysosomes in MSG and PSG causing regression and degeneration in *B. mori*. The MSG and PSG degeneration mechanism in *A. mylitta*, however, resembles with that in *B. mori*. The lysosomes and the autophagic vacuoles are known to be heavily loaded with acid phosphatase (Matsuura *et al.*, 1976a,b). During the prepupal period, the secretory apparatus is destroyed completely by lysosomes, autophagic vacuoles and hunger spheres through cytolytic enzymes in *B. mori* (Matsuura *et al.*, 1976b). Both MSG and PSG are completely degenerated while ASG are reconstructed into imaginal labial glands which secrete the special enzyme, cocoonase that dissolves the labial opening of the cocoon so that, the adult silk moth could easily emerge out (Kafatos, 1972). The present study demonstrates the similar mechanism of degeneration of the silk gland in *A. mylitta* also.

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