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Ultrastructural Anatomy of Silkgland
I. Anterior Part

Somboon SrungBoonmee
Kowit Chaisiwamongkol
Pipatphong Kanla

Department of Anatormy
Faculty of Medicine
Khon Kaen University

บทคัดย่อ

เราได้ชำแหละเอาต่อมสร้างเส้นไหมออกมาจากตัวหนอนไหมวัยห้า การชำแหละนั้นได้กระทำโดยใช้กล้องจุลทรรศน์ stereo ช่วย ต่อมสร้างเส้นไหมนั้นประกอบด้วย 3 ส่วน ส่วนหัวมีลักษณะเป็นท่อตรง ส่วนกลางมีลักษณะเป็นท่อหักพับแบบกิ๊บติดผม ส่วนหางมีลักษณะขดงอ เราแยกเอาส่วนหัวของต่อมสร้างเส้นไหมออกมาผ่านขบวนการเตรียมเนื้อเยื่อเพื่อตรวจศึกษาด้วยกล้องจุลทรรศน์อิเล็คตรอน เราพบว่าเซลล์ของต่อมส่วนนี้เป็นแบบทรงสูง ภายในเซลล์มี เซลล์ออร์กะเนลล์ที่เรียกว่า rough endoplasmic reticulum บรรจุอยู่เต็ม cytoplasm และยังมีไมโตคอนเดรียเป็นปริมาณมาก สิ่งที่เราได้พบนี้จึงทำให้เราเสนอว่าต่อมสร้างเส้นไหมส่วนนี้ทำหน้าที่สังเคราะห์โปรตีนด้วยเหมือนกัน เราได้ค้นพบด้วยว่า ท่อทางเดินอากาศจะไปสิ้นสุดภายในเซลล์ของต่อมสร้างเส้นไหมด้วย จึงเป็นการพิสูจน์ได้ว่าไม่ว่าจะเป็นก๊าซออกซิเจน และสิ่งอันตรายต่าง ๆ ที่ปนอยู่ในอากาศ จะสามารถเข้าถึงภายในเซลล์ของต่อมสร้างเส้นไหมได้อย่างง่ายดาย

Abstract

The silkglands of the 5th instar larvae of silkworms were dissected under the stereomicroscope. There are 3 parts to the gland : anterior, straight part; middle, loop-shaped part; and the posterior, coiled part. The anterior part of the silkglands were processed through the standard technique for the transmission electron microscope for this study. We found in the columnar cells of this simple tubular gland that it possessed the rough endoplasmic reticulum and a great number of mitochondria. This finding led us to propose that the anterior part of the gland also synthesized proteins. We also disclosed that the respiratory tract (air tubes) sent its terminal branches to the gland cell cytoplasm. This gave proof through our study that both oxygen and harmful things in the air could reach the cytoplasm of silkgland cells quite readily.

Key words : silk gland, *Bombyx mori,* function, ultrastructure.

Background

Silkglands are bilateral structures localizing lateral to the straight, digestive tract. Each is made up of 3 different parts : anterior, straight part; middle, loop-shaped part; and the posterior, coiled part (Tazima, 1978). The microanatomy of the anterior part is rather simple, making up of one-cell-thick wall surrounding a lumen (SrungBoonmee, unpublished data). The lumen is filled with the material like that of the mammalian colloid substance in the thyroid follicle in H & E technique of preparation. SrungBoonmee et al (1991) found that the gland is readily innervated as the nerve has been seen running from the ganglion to the silkgland. What structure and function relationship exists here is hard to interprete at this stage. It is established that the cross section of silk consists of two triangular fibroin in the form of fibers surrounded by sericin in the form of a coat (Tazima, 1978). According to the same source of information, sericin is synthesized and secreted in the middle part of the silkgland whereas the fibroin is synthesized and secreted in the posterior part of the gland. The function of the anterior part of the gland has not been studied. As we believe that structure determines function, we proposed to study its anatomy at the molecular level.

Material and Method

Silkgland of the 5thinstar larvae of the silkworm, *Bombyx mori,* were dissected out and processed through the standard preparation of the tissue for the transmission electron microscopy as follow :

1. Primary fixation for 2 hrs. at 4 ℃ in 2% paraformaldehyde + 2.5% glutaraldehyde in 0.1 M. phosphate buffer, pH.72
2. Wash in 0.1 M. phosphate buffer, pH.7.2
3. Post fixation for 2 hrs. at room temperature in 1% osmium tetroxide in 0.1 M. phosphate buffer, pH. 7.2
4. Wash in 0.1 M. phosphate buffer, pH. 7.2
5. Dehydration in graded series of ethanol.
6. Infiltration and embedding in Epon 812
7. Ultrathin sections were cut at 60-90 nm.
8. Staining with uranyl acetate and lead citrate.
9. Examined under HITACHI H-600 Transmission Electron Microscope.

Results and Discussion

The anterior part of the silkgland is a simple tubular gland by the microanatomical classification. Its lumen serves both as a duct and a storage. Its cells are columnar type. The following are its ultrastructural anatomy. The plasma membrane on the basal side is thick and is in a continuity with the basement membrane while on the luminal side it is hard to discern (Plate 1). We observed the abundance of intracellular canaliculi in the basal part of the cells. The cell nuclei were localized at some distance from the basement membrane. They possessed different sizes of electron densed heterochromation (Plate 2).

The rough endoplasmic reticulum filled the whole cytoplasm and the great number of mitochondria were found (Plate 3). The lumen of the gland and the apical portions of the gland cells contained both the masses of different sizes and fibrillar materials of which the latter may have come from the former (Plate 4). This finding led us to postulate the synthesis and secretion function for this anterior part of the silkgland, although we can not suggest the chemical substance of such secretion other than silk fibrils at the moment.

As one would expect, since the silkworm is a simple form of animal with a poorly developed system of circulation and a no-lung respiratory system; it somehow must have ready supply of oxygen to the cells of the body. We have studied the 3rd instar larvae at the microscopic level and found that branches of air tubes reach the silkgland and muscles (SrungBoonmee, *et al* 1991 unpublished data). The transmission electron microspic study revealed that the cross sections of the air tubes could be found both intercellularly and intracellularly (Plate 5). This ultrastructural anatomy is but physiologically

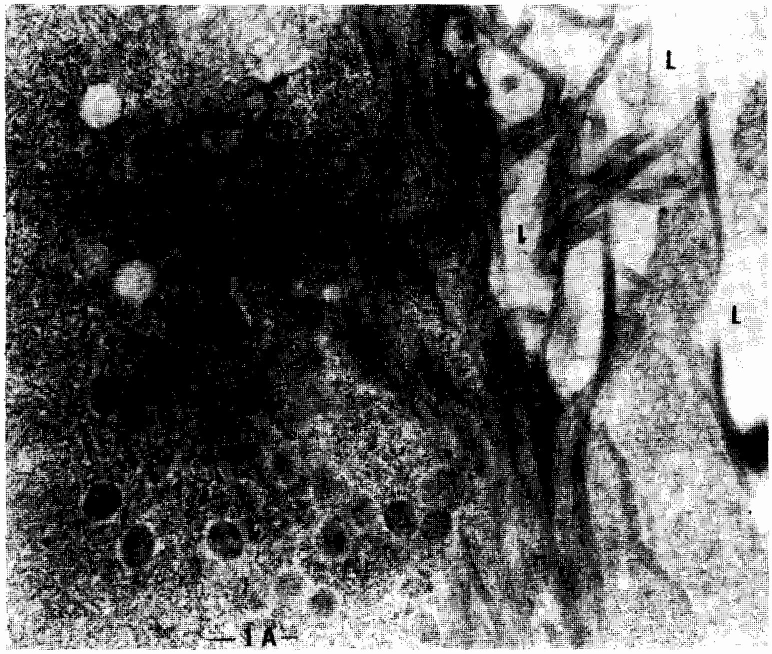

Plate 1: A shows the apical portion of silkgland cell. Observe the microvilli projecting into the lumen (L). X15,000 SS.KC.PK.

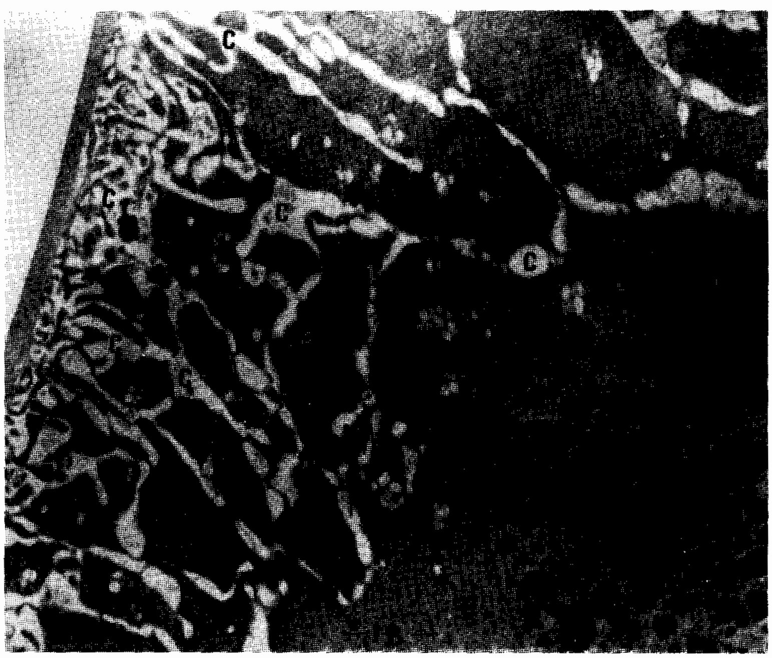

Plate 1: B shows the canaliculi (C) in the basal portion of silkgland cell. Note the nucleus on the lower right corner X5,000 SS.KC.PK.

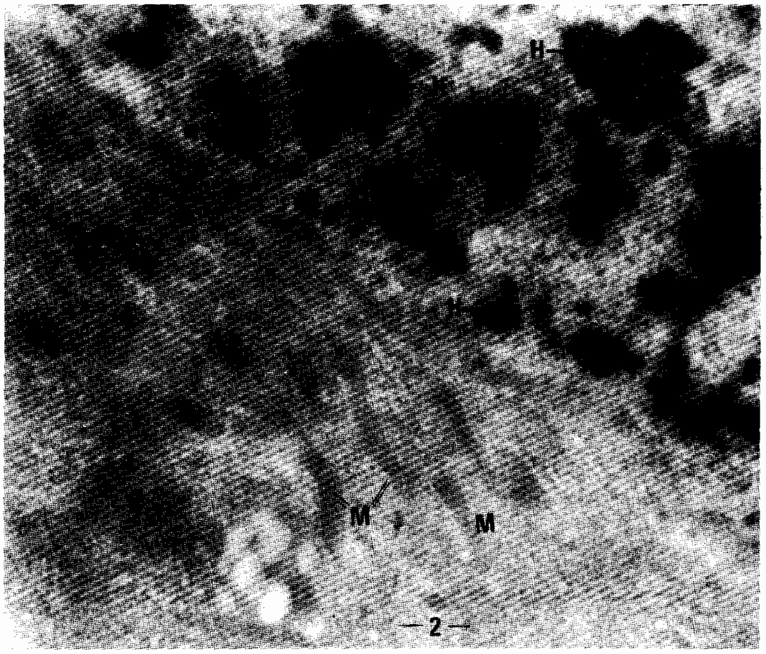

Plate 2: Shows different sizes of the electrondensed heterochromatins (H). Also note the mitochondria (M) adjacent to the nucleus.
X17,000 SS.KC.PK.

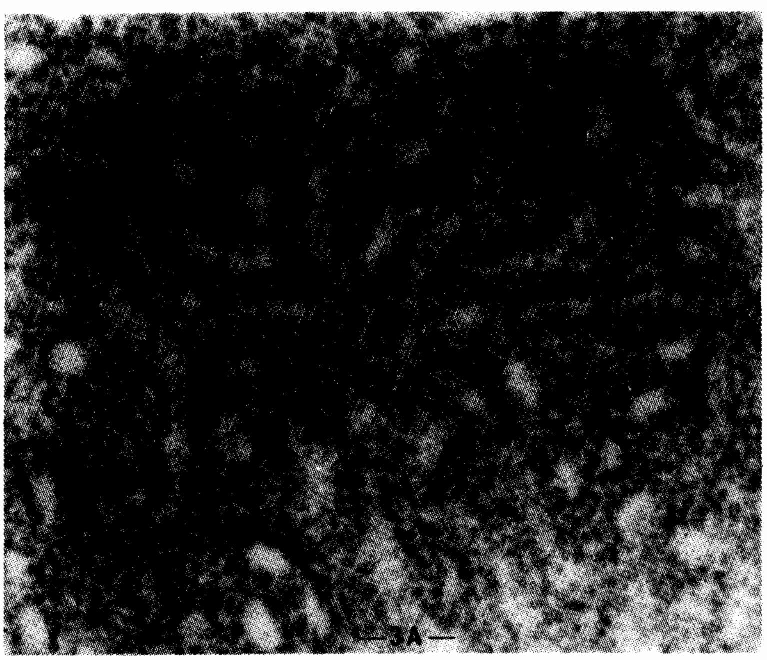

Plate 3: A shows the rough endoplasmic reticulum.
X60,000 SS.KC.PK.

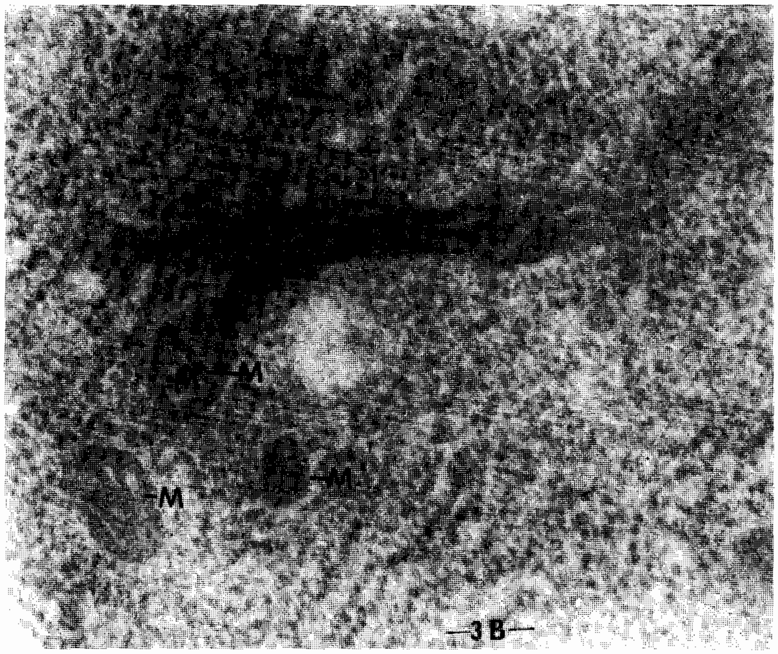

Plate 3: B shows the mitochondria X60,000

SS.KC.PK.

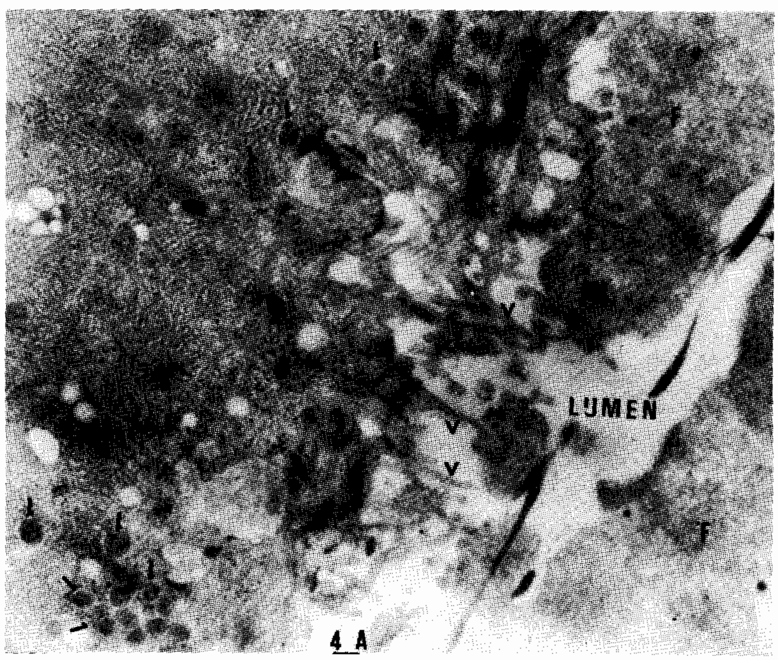

Plate 4: A shows the apical portion of anterior silk gland cell (left) with villi (V) and the lumen. Also notice the secretory granules (pointers). X8,000

SS.KC.PK.

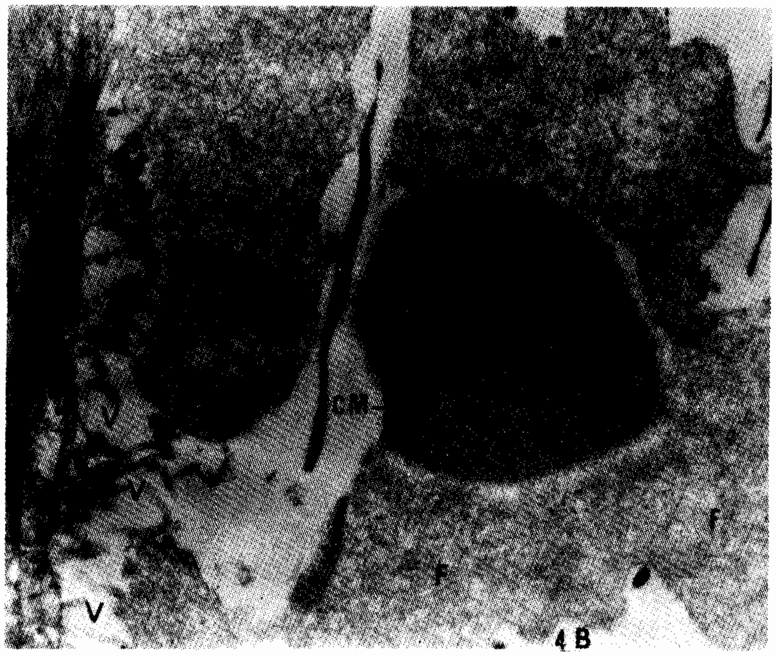

Plate 4: B illustrates the clumps of material (C.M) and fibrils (F) X10,000 SS.KC.PK.

Plate 4: C demonstrates higher magnification of clumps of material (left) and the fibrils (right). X100,000 SS.KC.PK.

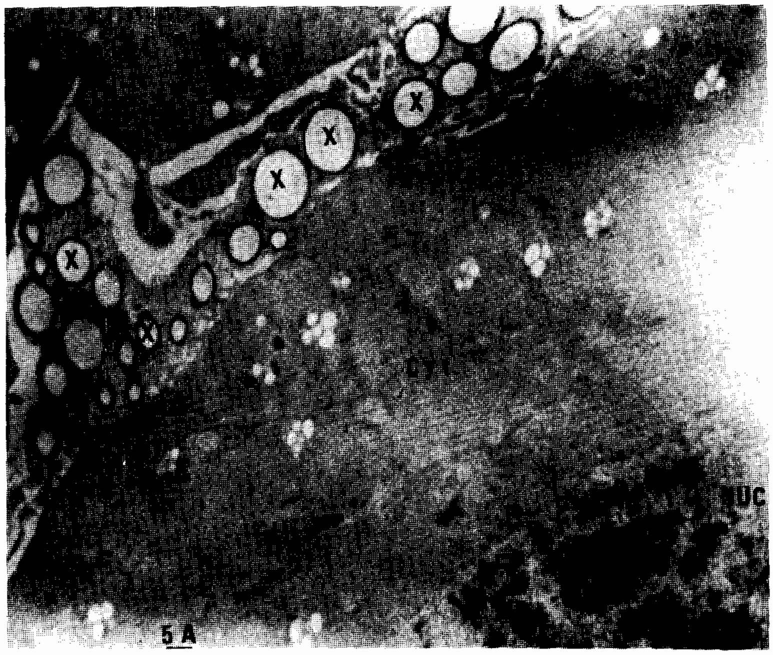

Plate 5: A Electron-microphotograph showing cross sections of intercellular air tracts of different sizes (X). X4,000 NUC. = nucleus Cyt. = cytoplasm SS.KC.PK.

Plate 5: B. longitudinal sections of intracellular air tracts (I). X25,000 SS.KC.PK.

Plate 5: C showing high magnification of the X-section of the intracellular air tract. X75,000 SS.KC.PK.

sound since it has no lung and well-developed cardio-vascular system as that of the higher species. Its poorly evolved hemolymph system must have functioned mainly in mobilizing the nutrients and waste products. Such ultrastructural anatomy of the silkworm would be convenient for the air-born agents or substances to have an access to the silkgland cells as well. We can conclude that the oxygen consumption of the silkgland cells is as high as other tissues. Our micro-anatomical studies have presented us the evidence of contacts between the respiratory tract (air tube) and the digestive tract wall. The latter prompts the electron microscopic studies to reveal whether the tubes also end in the cells.

It is known that a silkworm larva consumes about 50 grams of mulberry leaves from which 60% of nitrogen is used for silk protein synthesis (Hiratsuka, 1917). One larva can produce 0.6 g. of silk protein, estimated as the cocoonshell weight. The silk protein consists of about 80% fibroin and 20% sericin. The length of the fibroin thread per cocoon is about 1,000-1,500 metres (Tazima, 1978).

An experiment using radioactive alanine and glycine, given to the 5th instar larvae before the 3rd day, showed that the amino acids were absorbed by other tissues rather than the silkgland. If given after the 4th day of the 5th instar, however, amino acids entered fibroin (Fukuda and Florkin, 1959; Fukuda, 1960).

Conclusion

The anterior part of the silkgland is a simple tubular type with the columnar epithelium and one-cell-thick wall on the basement membrane. Our transmission electron microscopic study revealed that the cells of the anterior part of the silkgland were equipped with the cell organelles suitable for protein synthesis and secretion.

The cytoplasm is filled with the rough endoplsmic reticulum and the mitochondria in a great quantity. The appearance of DNA in the form of heterochromation typical of the inactive phase of synthesis and secretion was observed. This is not necessarily be in a permanent static stage. Since we found the ultrastructures of the cytoplasm both as clumps of fibrils and fibrillar forms in the apical portion of the cells of the silkgland; we proposed to assign the functions of synthesis and secretion to the anterior part of the silkgland, though we do not know its exact chemical substances other than the silk fibrils. In the literatures we have surveyed, the function of this part was not mentioned.

We have found the branches of the respiratory tubes at the ultrastructural level both in the cytoplasm of cells and between cells of the silkgland. This provides a direct oxygen supply to the cells for further use in metaboism. On the other hand such respiratory tracts could be the path of entry for the air-born pathogens-- be it micro-organisms or toxic substances.

We are not certain whether what we called the intracellular canaliculi are parts of the respiratory tracts or a system by itself. Further investigations on the subject should settle the question.

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