Silk formation mechanisms in the larval salivary glands of *Apis mellifera* (Hymenoptera: Apidae)

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The mechanism of silk formation in *Apis mellifera* salivary glands, during the 5th instar, was studied. Larval salivary glands were dissected and prepared for light and polarized light microscopy, as well as for scanning and transmission electron microscopy. The results showed that silk formation starts at the middle of the 5th instar and finishes at the end of the same instar. This process begins in the distal secretory portion of the gland, going towards the proximal secretory portion; and from the periphery to the center of the gland lumen. The silk proteins are released from the secretory cells as a homogeneous substance that polymerizes in the lumen to form compact birefringent tactoids. Secondly, the water absorption from the lumen secretion, carried out by secretory and duct cells, promotes aggregation of the tactoids that form a spiral-shape filament with a zigzag pattern. This pattern is also the results of the silk compression in the gland lumen and represents a high concentration of macromolecularly well-oriented silk proteins.

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1. Introduction

Silks produced by insects and spiders are composed of fibrous proteins, forming water-insoluble fibers that exhibit high strength and toughness. Silk production in spider, generally used for prey capture, occurs within specialized glands, such as the major ampullate gland, which synthesize large fibrous proteins and process them to form an insoluble fiber (Casem *et al* 2002). The fibrous proteins, constituting the silk, are produced in different glands (colleterial, salivary, dermal glands, and Malpighian tubules) by almost all insect taxa – in both adult insects and larvae (Rudall and Kenchington 1971).

In larvae of many insects, silk is often produced by the labial or larval salivary glands (also known as silk glands) just before pupation in Hymenoptera and immediately after hatching in Lepidoptera and Trichoptera (Chapman 1998).

Both spider and insect adults, and insect larvae can produce silks whose proteins have similar conformation of the fibroin structures (Rudall and Kenchington 1971). Special attention has been focused on the silk secreted by hymenopteran larvae that exhibit a distinctive conformation of the fibroin structures. While the term fibroin is used to describe the protein of the solid fibers of lepidopteran cocoons, the cement coating the fibers and frequently sticking them together is another protein called sericin. Many researchers use the term fibroin for any of the silken fibers of arthropods.

While silk synthesis in the silkworm, *Bombyx mori* (Lepidoptera), occurs in the posterior and middle region

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Abbreviations used: MFFs, Mass of fibroin fibers; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

of the secretory portion of the gland (Sehnal and Akai 1990; Akai 1983; Akai *et al* 1987), the anterior region of the gland is the silk outlet (Sehnal and Akai 1990). The posterior region secretes fibroin and additional proteins. In the middle region, the cells secrete the inner, the middle and the outer sericin layers around the fibroin column. While Hymenoptera order, most of the silk proteins of ants, bees and wasps are phylogenetically conserved and have few glycine residues and a high content of acidic residues (Rudal 1962; Flower and Kenchington 1967; Rudal and Kenchington 1971); the classical lepidopteran silk present a high content of glycine residues. Thus, a great variety of amino acid sequences in insect silk may occur.

Craig *et al* (1999) made a comparison of the composition of silk proteins produced by spiders and insects. They concluded: (i) that the silks produced by Lepidoptera have 60% or more of their composition consisting of two or three amino acids: alanine, glycine and serine; (ii) that the silks produced by Embiidina, larval Symphyta and spiders (Oribiculariae) have 60% of their composition consisting of a combination of two of the amino acids: alanine, glycine and serine plus either proline or glutamine; (iii) that in Diptera, Trichoptera and Coleoptera larvae, adult Mantoidea, adult Hymenoptera and the spiders (Mygalomorphae) the silk composition is more varied; and (iv) that the amino acid composition of silks produced by arthropods is highly variable and, at least across orders, shows no overarching trends, suggesting phylogenetic constraint.

Although the composition of silk has been well studied, the degree of orientation and folding of silk proteins and the level of proteins macromolecular organization are poorly understood. Polarization-optical methods are useful to determine these features of the silk, since filamentous structural proteins display birefringence (this birefringence is a physical property observed in biopolymers that are arranged orderly in a typical pattern).

The morphological alignment of proteins to form silk has been well studied in Lepidoptera and in Hymenoptera. In silkworm the silk is composed by elementary fibroin fibers that form spherical mass of fibroin fibers (MFFs) in the gland lumen (Akai *et al* 1987) and in Apidae the silk is composed by molecules that are orderly arranged to form spindle-shaped tactoids, with 3–4 μm long, showing strong positive birefringence under polarized light and a regular axial repeated cross striation (Flower and Kenchington 1967). In Formicidae, tactoids were also observed in the silk glands of *Pachycondyla* (= *Neoponera*) *villosa* (Zara and Caetano 2002). In Bombini, non-striated tactoids are produced sparsely in the anterior region of the silk gland, while fibrous bars have been described in other glandular regions. The silk secretion of Vespidae is stored only as fibrous bars in the gland lumen (Flower and Kenchington 1967).

Tactoids and fibrous bars of all hymenopteran insects are birefringent due to their macromolecular array. It is not a satisfactory explanation about the meaning of the two types of arrangement of stored silk molecules (tactoids and fibrous bars) in the gland lumen of the Hymenoptera larvae. Flower and Kenchington (1967) suggest that the tactoids are more easily and efficiently spun into a well-oriented silken thread than the masses of fibrous bars.

Although the arrangement of the Hymenoptera silk has been described (Rudall and Kenchington 1971), synthesis of silk and its processing in silk glands remains unknown. The silk gland of *Apis mellifera* provides a good model to investigate the formation and processing mechanisms of the silk – since the glandular morphology is well characterized (Silva de Moraes and Cruz-Landim 1975; Silva 1999; Silva and Silva de Moraes 2002).

Studies on the mechanism of silk processing in glands are necessary. Jin and Kaplan (2003) emphasized that it is the lack of understanding of this mechanism which has prevented *in vitro* synthesis of the silk proteins and/or the genetically engineered silks, both or either of which can be used in the silk-manufacture industry.

This paper analyses silk-formation mechanism of the *A. mellifera* in the lumen of the salivary glands (or silk glands) of 5th instar, using morphological, histochemical and histophysical analyses.

2. Materials and methods

2.1 *Material*

Larvae of 5th instar *A. mellifera* workers were collected from the Apiary of the Biology Department, Institute of Bioscience, UNESP, Rio Claro, Brazil. The instar was identified by larval cephalic capsule size (according to Goitein 1989).

2.2 *Light microscopy and polarized light microscopy*

The 5th instar larvae silk glands were dissected in buffered saline solution and immediately fixed in 4% paraformaldehyde in 0·1 M sodium phosphate buffer (pH 7·4). After fixation, the glands were rinsed in the same buffer (0·1 M, pH 7·4) and dehydrated in a standard ethanol series ranging from 70% to 100% ethanol solution for 10 min in each of the solutions. These glands were embedded in historesin JB4 (Polyscience), and 5μm thick sections (obtained in a microtome) were stained with Xylidine Ponceau (pH 2·5) for estimation of total protein (Mello and Vidal 1980). After staining, the sections were rinsed in 2% acetic acid solution for 30 min followed by a brief rinse in distilled water. The stained sections were mounted on Permount and analysed under a light micro-

scope and polarized light microscope. Following the same procedure, some of the glands were also fixed and dehydrated and were used in whole mounts (i.e. placed on glass slides and mounted in Permount). For the histophysical studies both the gland sections and the whole mounts were analysed with polarized light microscopy to detect the macromolecular array of the secretion. They were also analysed with a polarized light microscopy using a first order red compensator filter to establish the optical direction of the secretion components secreted along the lumen.

The macromolecular array of the silk is reflected by its anisotropy proprieties, i.e. by its birefringence under polarized light. When biopolymers are orderly arranged they alter the polarized light propagation, displaying a distinct brightness. After using a compensator filter, it is possible to detect two colours (yellow and blue) that represent two optical directions in the cluster of birefringent biopolymers, indicating the direction of the fibers in the cluster (Mello and Vidal 1980).

2.3 *Scanning and transmission electron microscopy*

Fifth instar larvae silk glands were dissected and fixed in Karnovsky fixative (2% glutaraldehyde, 4% paraformaldehyde, 0·1 M sodium cacodylate buffer pH 7·4) and rinsed in 4% phosphate buffer. For scanning electron microscopy (SEM), the glands were dehydrated in a standard acetone series, from 70% to 100% acetone solutions for 15 min in each of the solutions. The glands were subjected to a BALZERS/CPD 030 machine, subsequently placed on stubs and gold-coated in a BALZERS SCD 050 sputter coater. These glands were examined using a JEOL JSM-P15 scanning electron microscopy. For transmission electron microscopy (TEM), the glands were post-fixed in a solution of 0·5% osmium tetroxide and 0·8% potassium ferrocyanide, in 0·1 M sodium cacodylate buffer (pH 7·4). The glands were then rinsed in 0·1 M sodium cacodylate buffer pH 7·4, incubated in aqueous solution of 0·15% tannic acid, treated with uranyl acetate, dehydrated in a standard acetone series and embedded in Epon-Araldite resin. Ultra-thin sections were examined with a Philips CM 100 Electron Microscope.

3. Results

The luminal secretion of silk glands stained by Xylidine Ponceau showed different aspects during the 5th instar. We characterized three different physiological periods: namely, the beginning, the middle, and the end of the 5th instar.

In the beginning of the 5th instar, the luminal secretion is homogeneous, and is weakly stained by the Xylidine Ponceau, just as in the case of proximal secretory portion as in the duct (figures 1A and 8B). However, in the

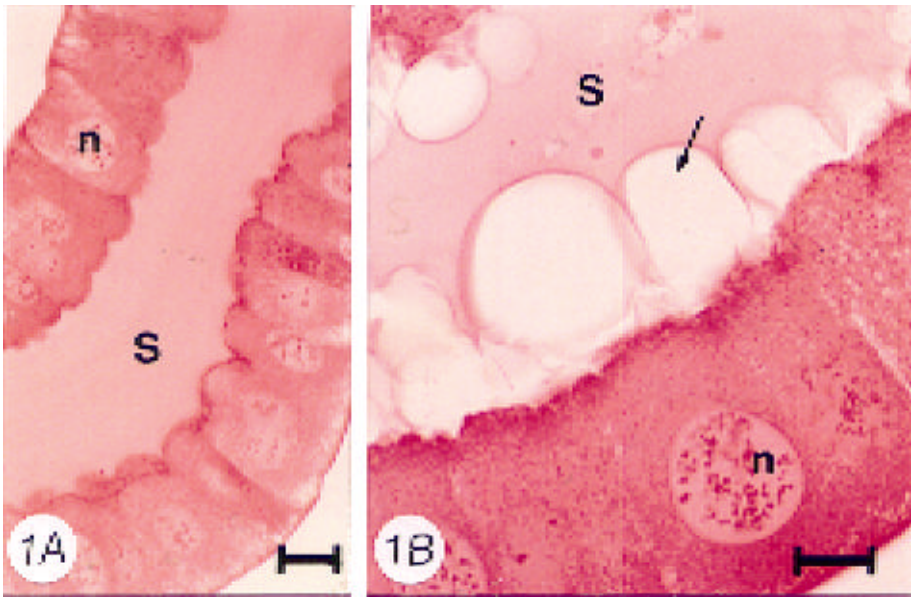

Figure 1. Silk glands histological sections stained with Xylidine Ponceau, at the beginning of the 5th instar. Light microscopy. (**A**) Secretory portion showing the secretory cells and a homogeneous secretion (S) in the lumen both stained by Xylidine Ponceau. Scale bar: 40 μm. (**B**) Secretory portion, where areas with no secretion (arrow) can be seen in the lumen in contact with the apex of the secretory cells. Scale bar: 20 μm. n, nucleus; S, secretion.

posterior region, there are unstained and apparently empty areas at the lumen periphery like bubbles in contact with the apical surface of the ephitelium (figures 1B and 8B).

At the middle of the 5th instar, the homogeneous secretion is still seen in the lumen of the duct, but in the secretory portion of the silk gland the two types of secretion are observed (figures 2, 8C, D, E): namely, the homogeneous secretion, which is weakly stained by the Xylidine Ponceau; and a peripheral secretion which is strongly stained by the Xylidine Ponceau. The homogeneous secretion is centrally located in the lumen of the glandular secretory portion and its homogeneous aspect does not vary along the gland, but the morphological aspect of the peripheral secretion varies from homogeneous to fibrilar according to the region of the secretory portion. In the proximal region, this peripheral secretion is dense and homogeneous (figures 2A and 8E). While the secretion in the middle secretory region, presents a fibrilar portion (figures 2B and 8E); the secretion becomes completely fibrilar in the distal region (figures 3A and 8E).

At the end of the 5th instar, the silk gland shows the whole glandular lumen filled with fibrilar secretion, which forms highly compact structures strongly stained by Xylidine Ponceau and which are immersed in a homogeneous weakly stained material (figures 3C, 8F, G).

Analyses of the histological sections and whole mounts of the silk glands under polarized light microscope revealed that the homogeneous secretion of the glands is always isotropic. However, the fibrilar secretion that appears at the middle (figure 3A) and at the end (figure 3C) of the 5th instar is birefringent (figure 3B, D). Histological sections of glandular secretory portion, at the end of the 5th instar, show structures strongly stained by the Xylidine Ponceau (figure 3C); and are highly birefringent (figure 3D). These structures are more packed in the central region than at the periphery of the lumen (figures 3C, D). This birefringence is also confirmed with polarized light microscopy in whole mounts of silk glands.

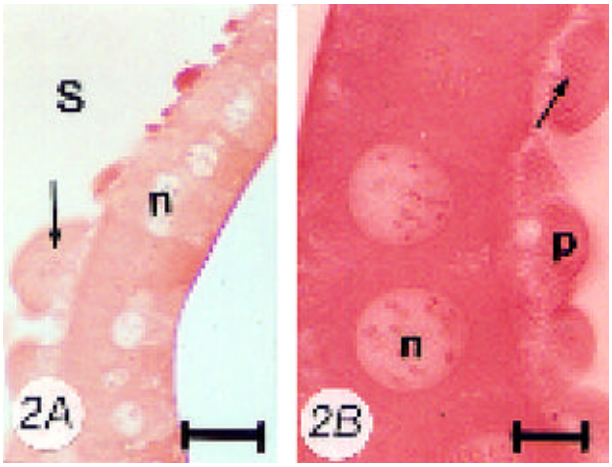

Figure 2. Silk glands histological sections stained with Xylidine Ponceau, at the middle of the 5th instar. Light microscopy. (**A**) The gland secretory portion has two types of secretion in the lumen: a homogeneous peripheral secretion (arrow); and a secretion located in the center (S). Scale bar: 25 µm. (**B**) Peripheral secretion (p) strongly stained with fibrils (arrow) immersed in the homogeneous material. Scale bar: 20 µm. n, nucleus; S, secretion.

At the end of the 5th instar, analysis of the whole mounts of the silk glands with polarized light microscopy using a first order red compensator filter revealed a pattern of two optical directions (zigzag pattern of the silk filament) (figure 4A). This pattern varies from gland to gland. The zigzag pattern is less clear when there is little birefringent secretion in the lumen (figure 4D), becomes clearer when the amount of birefringent secretion is high [i.e. as in the duct (figure 4A), or as in the secretory portion (figure 4C)]. In this last region, transverse commissures in the secretion (figure 4B) corresponding to the point of optical direction change can be seen.

TEM confirmed the presence of homogeneous and less dense secretion filling the lumen of the glands in the beginning of the 5th instar (figure 5A). TEM also showed the presence of less dense central secretion and a dense peripheral secretion in the gland lumen at the middle of the 5th instar (figure 5B). In the distal region of this same gland, very dense structures were seen in the peripheral secretion (figure 5C). At the end of the 5th instar, the whole secretion which filled the lumen contained dense and compact structures immersed in a granular material (figure 5D).

The SEM images showed a flocculated secretion in the lumen of the gland in the beginning of the 5th instar (figure 6A). In the end of the 5th instar, the secretion presented an organization in fibrils (figure 6B) that forms a fiber cluster in the lumen. The lumen secretion appears closely in contact with the epithelium of the secretory portion (figures 6A, B and 8), but no contact is established between the secretion and duct epithelium (figures 6C and 8).

A transition region occurs between the duct and the secretory portion (figure 7A). The figures 7B and 8G show a large cluster of compact fibrillar secretion in a spiral shape.

4. Discussion

The results showed morphological, histochemical and histophysical differences of the secretion during the 5th instar in the larval salivary gland of *A. mellifera*.

At the beginning of the 5th instar, the silk gland's lumen is filled by a homogeneous and isotropic protein secretion which has a flocculated aspect by SEM. Empty areas

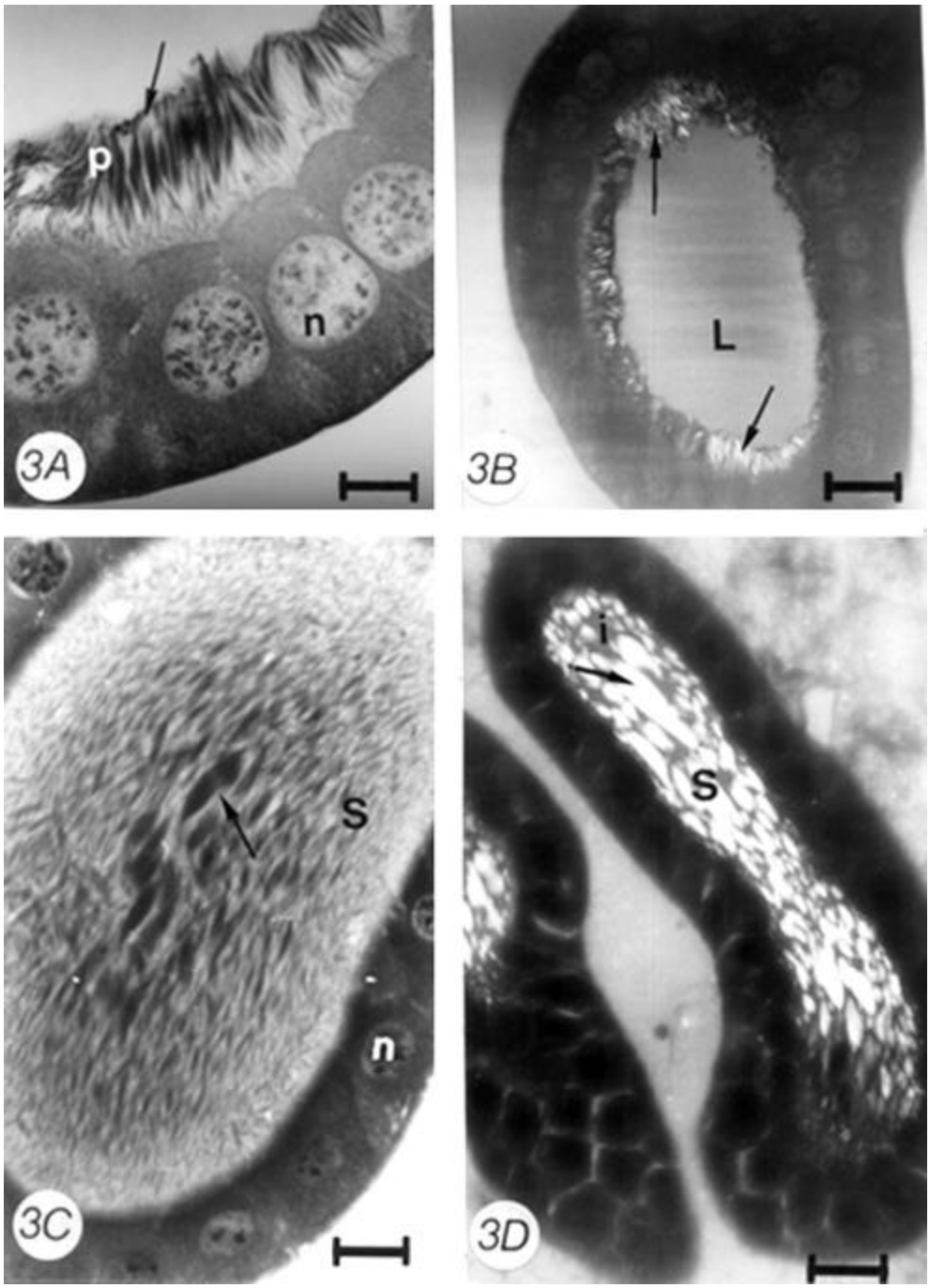

Figure 3. Silk glands histological sections stained with Xylidine Ponceau, in different physiological periods of the 5th instar larvae. (**A**) Light microscopy. The gland at the middle of the 5th instar shows a fibrilar peripheral secretion (p), which is strongly stained by Xylidine Ponceau. Scale bar: 20 μm. (**B**) Polarized light microscopy. The gland at the middle of the 5th instar displays birefringence in the fibrilar peripheral secretion (arrow) while the secretion located centrally in the lumen is isotropic (L). Scale bar: 30 μm. (**C**) Ordinary light microscopy. The gland at the end of the 5th instar shows a fibrilar secretion (S) organized in strongly stained tactoids (arrow), which fills the whole lumen of the secretory portion. Scale Bar: 50 μm. (**D**) Polarized light microscopy. The gland at the end of the 5th instar presents secretion (S) composed by anisotropic tactoids (arrow) immersed in an isotropic substance (i). Scale bar: 65 μm. n, nucleus.

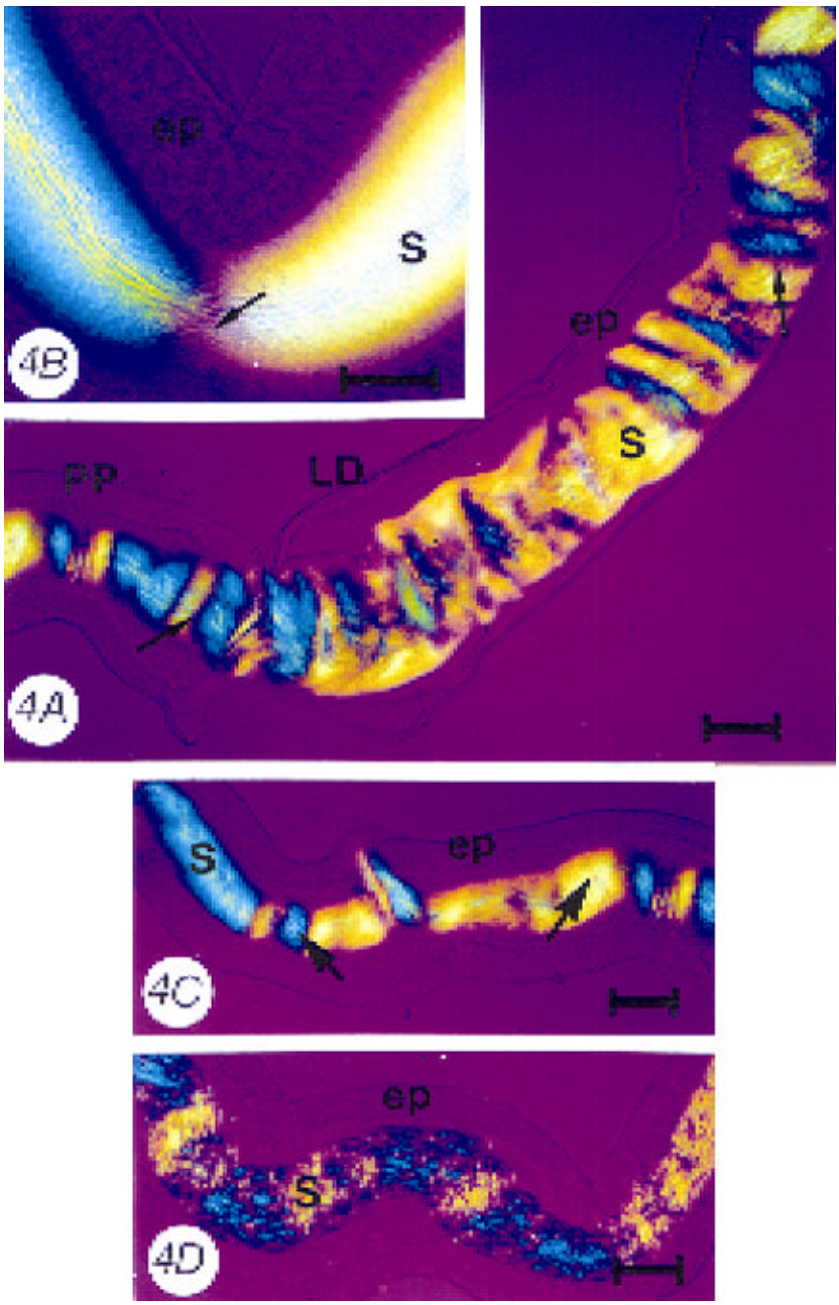

Figure 4. Unstained silk glands whole mounts at the end of the 5th larval instar analysed with polarization microscopy after using the first order red compensator filter. (**A**) Glandular proximal secretory portion (PP) and lateral duct (LD) displaying a large amount of anisotropic secretion (S), highly compacted and with the maximum clearness of the optical direction pattern (arrow). Scale bar: 50 µm. (**B**) Detail of the highly compacted, anisotropic secretion (S) found in the glandular lumen, showing the transverse commissure (arrow) at which change in optical direction takes place. Scale bar: 25 µm. (**C**) Glandular secretory portion with a large amount of compact, anisotropic secretion (S), with the pattern of two optical directions (arrows). Scale bar: 50 µm. (**D**) Glandular secretory portion showing large amount of anisotropic secretion (S), but slightly compacted and with little clearness of the optical direction pattern. Scale bar: 50 µm. ep, epithelium.

found in the lumen periphery in contact with the epithelium suggest that this secretion is synthesized and released before silk production. According to Silva and Silva de Moraes (1996), the secretion detected at the beginning of the 5th instar is composed of acid glycoproteins, neutral glycoproteins and proteins, which could have enzymatic activity during the larval nutrition period. In the larval salivary gland of *Solenopsis invicta* (Petralia *et al* 1980) and in *Pachycondyla* (= *Neoponera*) *villosa* (Zara 2002), the existence of protease activity suggests enzymatic activity for the gland secretion in these Hymenoptera larvae.

At the middle of the 5th instar, the silk gland produces a second type of secretion, which is discharged in the lumen of the gland, representing the depolymerized silk that had initially accumulated in the empty areas. The polymerization of this secretion (in the gland lumen) forms structures similar to the tactoids described by Flower and Kenchington (1967). These tactoids are present in most silks of Hymenoptera-Aculeata. The high birefringence under polarized light and the high electron density under TEM of these protein structures is the strongest evidence supporting the suggestion that these structures are tactoids.

At the end of the 5th instar, the secretion is comprised of birefringent dense tactoids immersed in a homogeneous material. The water absorption by the epithelial cells of the *Apis* silk glands promotes aggregation of these tactoids. This conclusion is supported by the ultra-

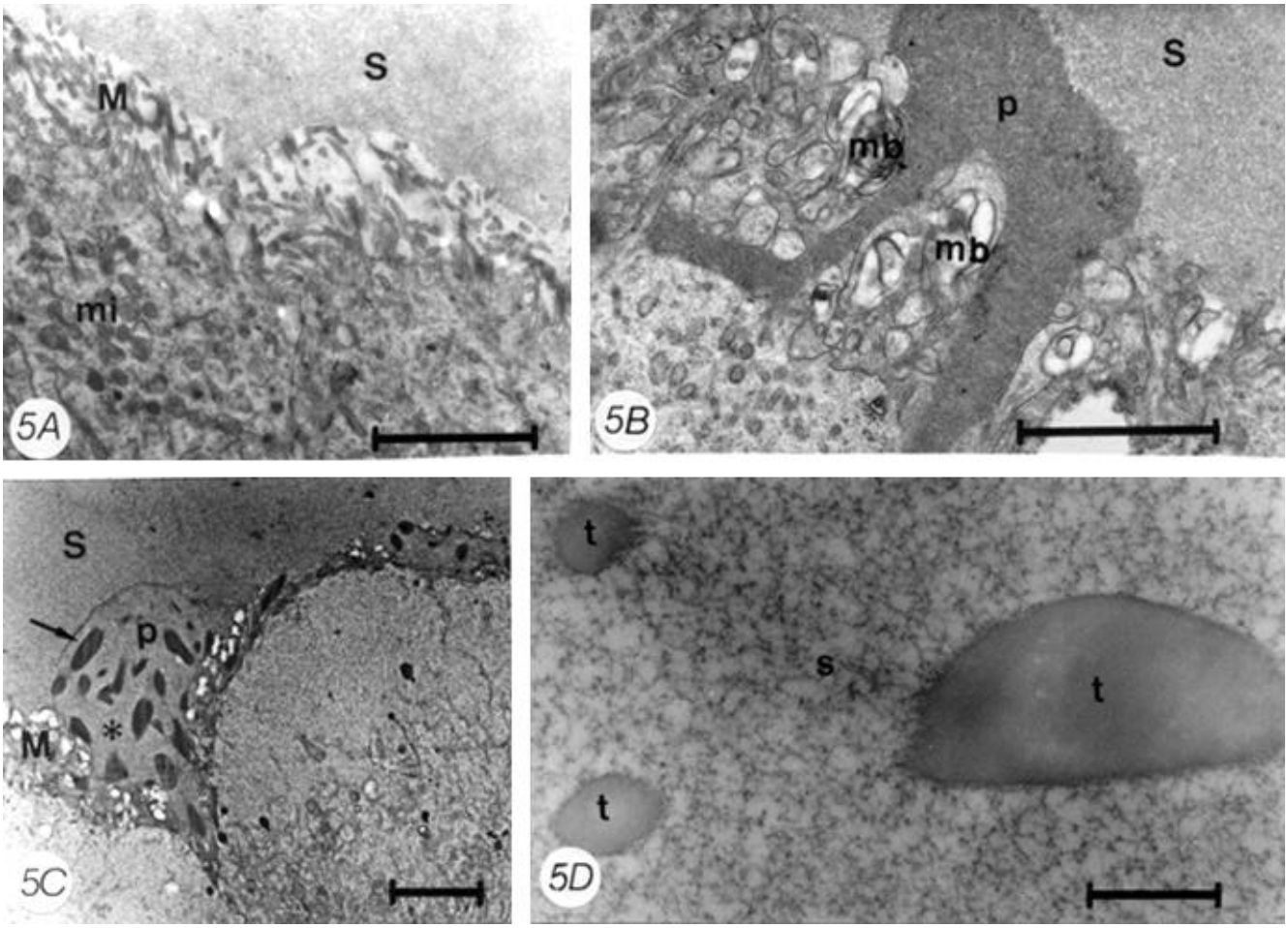

Figure 5. TEM micrography of silk glands in the different physiological periods of the 5th instar larvae. (**A**) Gland in the beginning of the 5th instar showing a less dense secretion (S) in the lumen. The secretory cells have microvilli (M) at their apex and many mitochondria (mi) in their apical region. Scale bar: 0·5 µm. (**B**) Detail of apical region of gland at the middle of the 5th instar showing the membranous bodies (mb), resulting from high secretory activity of these cells. Note two types of secretion in the lumen: the dense peripheral secretion (p) and the less dense secretion (S), both with homogeneous aspect. Scale bar: 0·5 µm. (**C**) Gland at the middle of the 5th instar showing the two types of lumen secretion: the less dense secretion (S) and the peripheral secretion (p), which is composed by dense material (arrow) immersed in a less dense material (*). Note the dilated microvilli (M) at the apex of the cell. Scale bar: 5 µm. (**D**) Detail of lumen of the gland at the end of the 5th instar that is filled with silk. This secretion (s) is composed of dense tactoids (t) immersed in a less dense material. Scale bar: 0·5 µm.

structural features of the secretory cells which show microvilli associated to the mitochondria, related to water reabsorption, according to Oschman and Berridge (1970). This dehydration process occurs while the polymerized secretion moves through the lumen of the gland, thereby promoting aggregation of tactoids and, consequently, their compaction. Microvilli involved in the reabsorption of water and subsequent dehydration of the silk have also been described in the duct of the major ampullate gland of the black widow spider by Casem *et al* (2002).

As in *Apis*, the occurrence of silk dehydration was also observed in *B. mori* silk glands (Akai *et al* 1987), where

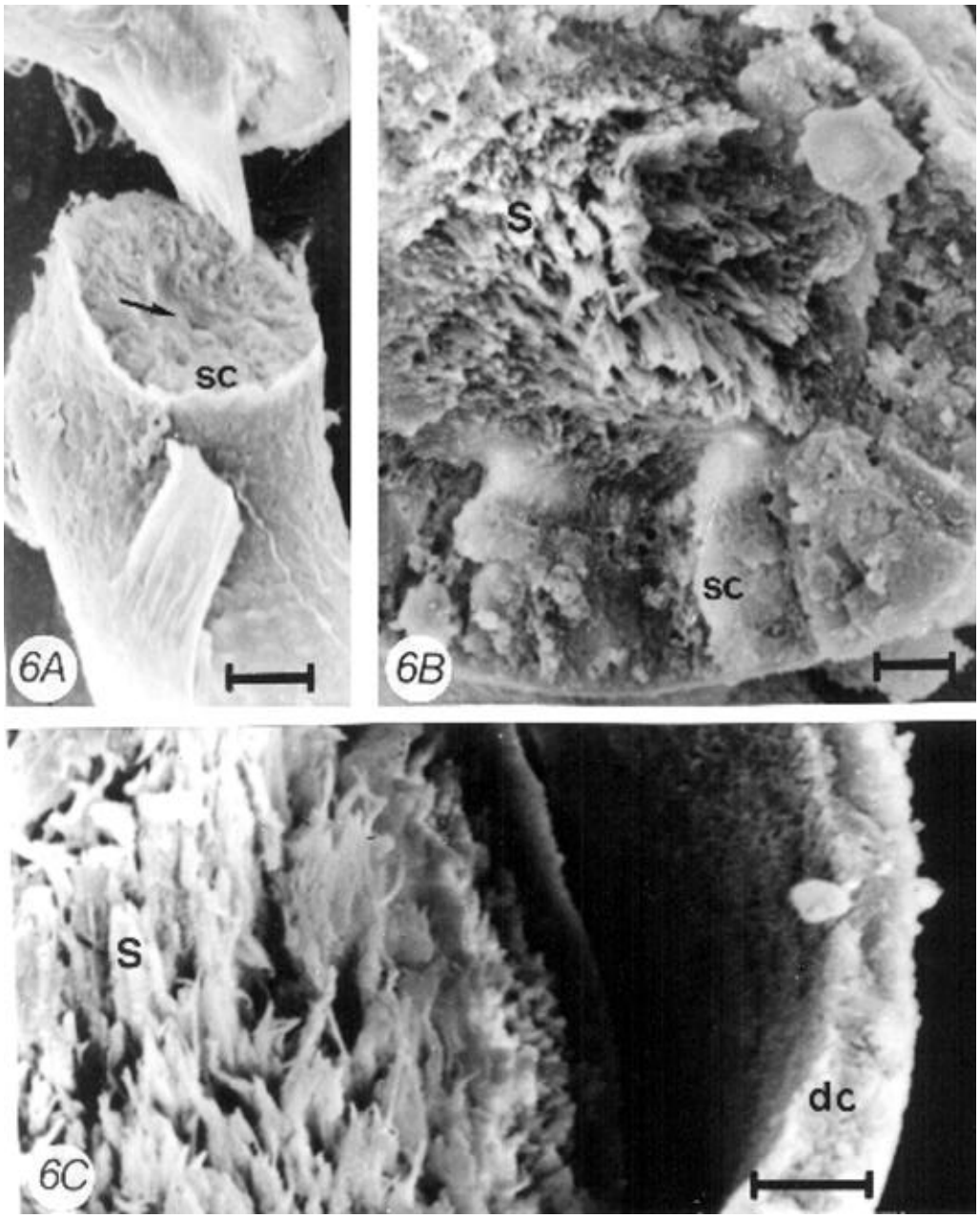

Figure 6. SEM micrography of silk glands in the different physiological periods of the 5th instar larvae. (**A**) General view of secretory portion at gland in the beginning of the 5th instar showing a secretion in the lumen with a flocculated aspect (arrow). Scale bar: 20 μm. (**B**) Secretory portion of gland at the end of the 5th instar whose lumen is filled with silk. The secretion (S) is organized in fibrils forming a fiber cluster closely to the epithelium. Scale bar: 10 μm. (**C**) Lateral duct of gland at the end of the 5th instar showing the fiber cluster located far from the epithelium. Scale bar: 10 μm. sc, secretory cells; dc, duct cells.

the silk is comprised of elementary fibroin fibers that form spherical MFFs in the gland lumen. According to Akai *et al* (1987), the secretory cells absorb the water from the spaces among these MFFs which are responsible for decrease of water content of silk from the posterior to middle region of the glandular secretory portion. This process promotes progressive structural changes in the fibroin column during the passage of the silk through the gland lumen (figure 9B).

Additionally, the secretion displacement results in a re-organization of the tactoids inside the lumen, which appear under polarized light microscopy as a pattern of two optical

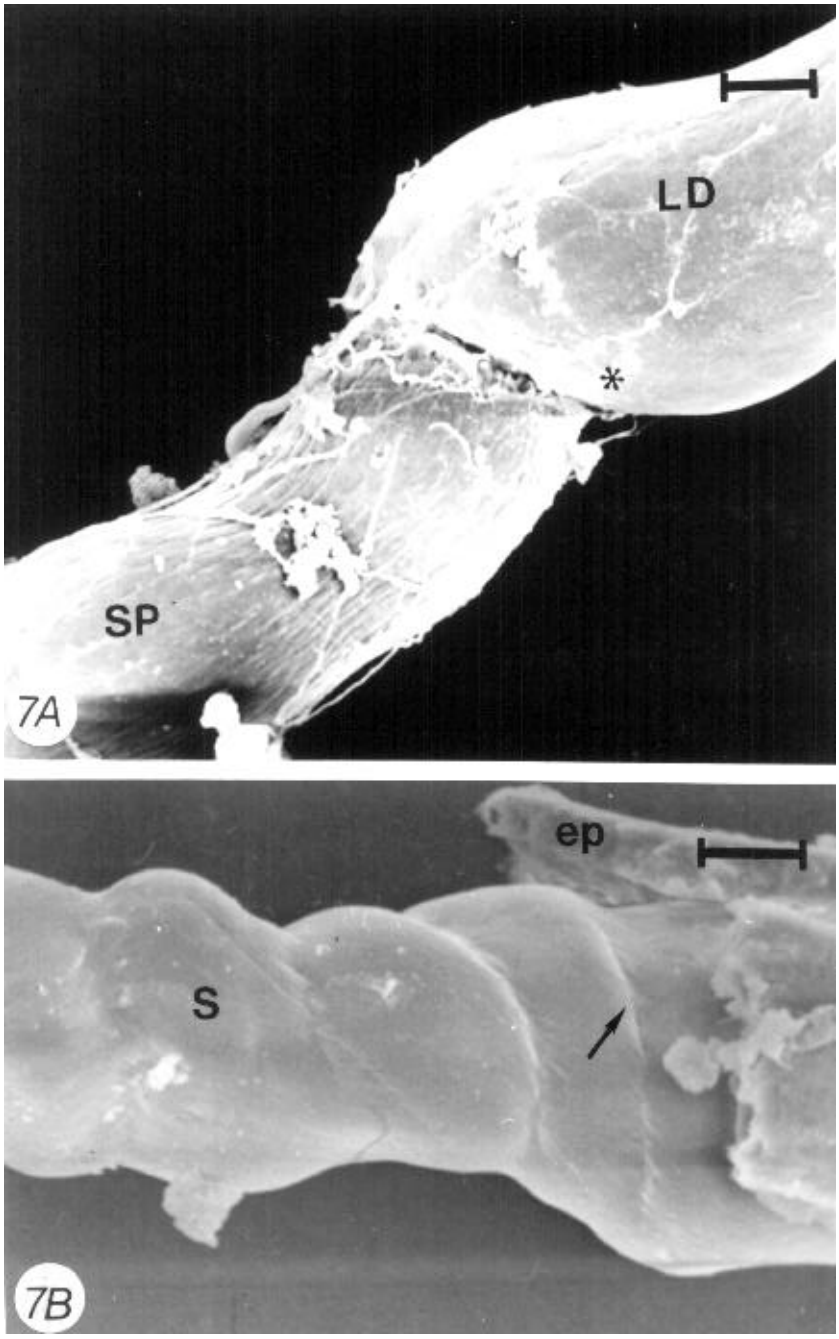

Figure 7. SEM micrography of silk glands at the end of the 5th instar. (**A**) General view of gland showing a convex transition area (*) in the lateral duct (LD) near to the limit with the secretory portion (SP). Scale bar: 20 μm. (**B**) Detachment of the duct epithelium (ep) where a compact cluster of fibrous secretion (S), with transverse commissures (arrow), display a spiral shape. Scale bar: 20 μm.

directions. According to Rudall (1962), during the displacement of secretion in the glandular lumen, new interfilamentary bonding occurs when the filaments touch each other. There is no functionally satisfactory explanation for the organization of the silk molecules, neither in birefringent tactoids nor for the optic direction pattern. Flower and Kenchington (1967) suggest that the macromolecular organization of silk in *Bombus* and *Vespa* in the glandular proximal portion (mainly in the lateral duct) promotes a re-orientation of the silk in parallel with the direction of spinneret flow. The re-orientation of the silk fiber facilitates its elimination from the spinneret during the cocoon spinning. In *Pachycondyla* (= *Neoponera*) villosa, the macromolecular organization of the silk thread, forming a two optical direction pattern, occurs only from the lateral ducts (Zara 2002).

In the present paper the re-orientation of the silk is shown to be more intense in the lateral duct. The changes in optical direction occur at shorter intervals in the duct than in the secretory of the larval salivary gland, due to the higher compaction and compression of secretion in the duct of the larval salivary gland. Thus, maximum degree

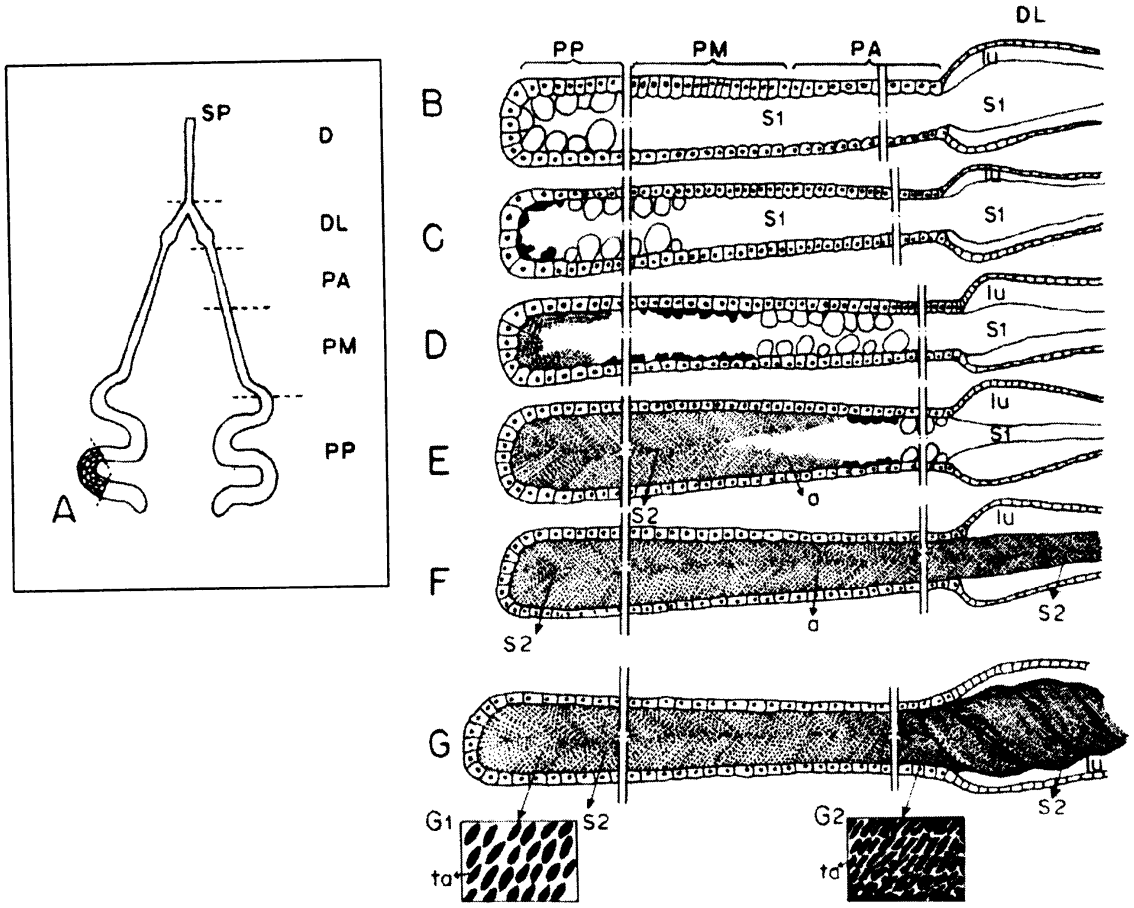

Figure 8. (**A**) Schematic drawing of the silk gland in *A. mellifera* (Hymenoptera) larva. SP, spinneret; D, duct; DL, lateral duct; PA, anterior portion of silk gland; PM, middle portion of silk gland; PP, posterior portion of silk gland. Adapted from Silva de Moraes and Cruz-Landim (1975). (**B–G**) Schematic drawing of the silk processing in glands of *A. mellifera* (Hymenoptera) larva during the 5th instar. (**B**) Beginning of the 5th instar. The luminal secretion is homogeneous (S1) and the empty areas are observed at the lumen periphery in the posterior portion (PP). (**C**) Middle of the 5th instar. Observe two types of secretion in the lumen: the homogeneous secretion (S1), along the lumen, and a dense peripheral secretion, in the posterior portion (PP). (**D**, **E**) Middle of the 5th instar. Successive stages of polymerization of the dense peripheral secretion whose morphological aspects varies from homogeneous to fibrilar, according to the region of the secretory portion. S2, fibrilar secretion. (**F**, **G**) End of the 5th instar. The glandular lumen is filled with fibrilar secretion (S2) composed by tactoids (ta) immersed in a homogeneous material. (**G1**, **G2**). Absorption of water from the secretion, when it moves through the lumen, promoting aggregation of the tactoids (ta) to form a spiral-shape silk filament. (a, macromolecular array of the silk; lu, lumen; SP, spinneret; D, duct; DL, lateral duct; PA, anterior portion of silk gland; PM, middle portion of silk gland; PP, posterior portion of silk gland.)

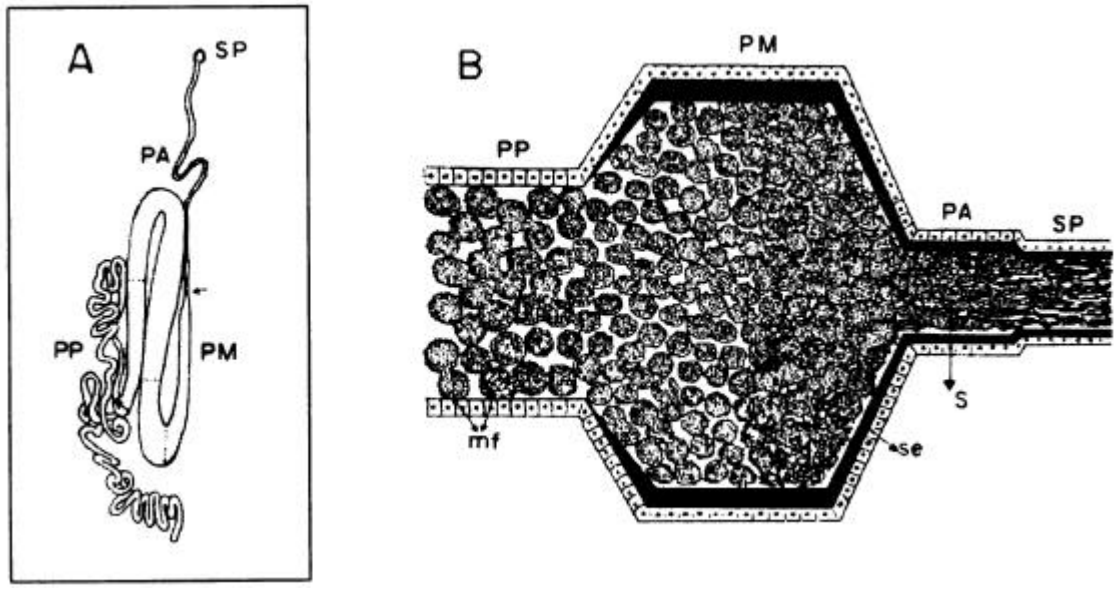

Figure 9. (**A**) Schematic drawing of a single tubule of the silk gland of a *B. mori* (Lepidoptera) larva, during the spinning stage. SP, spinneret; PA, anterior portion of silk gland; PM, middle portion of silk gland; PP, posterior portion of silk gland; small arrows, delimitation of portions. (**B**) Schematic drawing of the fine-structural changes that take place in the spherical masses of fibroin fibers (mf) as they pass through the lumen from the posterior to anterior silk gland. There is absorption of water from the lumen along the gland to form a well-organized and insoluble silk filament. SP, spinneret; PA, anterior portion of silk gland; PM, middle portion of silk gland; PP, posterior portion of silk gland; se, sericin layer; S, filament formed by a cluster of the spherical masses of fibroin fibers (mf). Adapted from Akai *et al* (1987).

of compaction in the duct results from an efficient absorption of luminal water, carried out by the duct cells whose ultrastructural features, such as microvilli associated to mitochondria (Silva de Moraes and Cruz-Landim 1975) suggest/or corroborate this function. Compression is also promoted by the accumulating secretion in the lateral duct. This probably results in a higher ordering of the tactoids and of the amount of inter-filamentary bonding (Rudall 1962), which leads to a distinct zigzag pattern. High concentration of secretion in the glandular duct also occurs in labial gland of Trichoptera (Engster 1976) and in the major ampullate gland of the black widow spider (Casem *et al* 2002).

The hypothesis relating to secretion compaction together with optical direction is reinforced by the alteration of the optical direction pattern in the silk glands during the end of the 5th instar which is characterized by an increased clarity in this pattern. When secretion is released to spin the cocoon, the secretion that remains in the lumen loses its compaction and, consequently, the clarity of the optical direction pattern. However, the silk secretion remains birefringent due to the silk biopolymers.

Furthermore, a convex transition area in the lateral ducts near to the limit with the secretory portion of the silk gland, observed by SEM, could indicate that the space between the secretion and the epithelium is necessary to complete the silk formation. The distance between the duct epithelium and secretion could facilitate the disposition in spiral of the fibrilar secretion, forming the transversal comissures in the silk filament, which corresponds to the change in optical direction.

Although the silk secreted by Hymenoptera and Lepidoptera silk glands differs both in its amino acid composition and in the conformation of its proteins (Rudall and Kenchington 1971), this paper shows that the main steps involved in the silk formation are similar in both the cases: namely, (i) polymerization of protein in the lumen, forming typical structures (tactoids in *A. mellifera* and MFFs in *B. mori*); and (ii) absorption of water from these structures along the gland to form a well-organized and insoluble silk filament. The comparative analyses of the figures 8 and 9 reflect the similarities in the silk processing in Hymenoptera and Lepidoptera glands.

Water absorption and polymerization in *A. mellifera* promote both compaction of the silk and its macromolecular array. This is reflected in the birefringence properties of the silk proteins forming the zigzag pattern. This pattern also results of the silk compression in the lumen and represents the high concentration of macromolecularly well-oriented silk proteins.

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