Journal of Optoelectronics and Advanced Materials Vol. 2, No. 3, September 2000, p. 281-291

HORNET (HYMENOPTERA, VESPINAE) SILK AS COMPLEX ELECTRIC CIRCUIT: THE ANALOGY TO VERTEBRATE MYELINATED NERVE FIBERS

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The silk weave spun by pupating larvae of the Oriental hornet is endowed with typical features, which find their expression both in the structure and the electric properties of their silk. Thus the silk weave is comprised of a dome or cap that protrudes from the larval cell and contains most of the silk fibers and a few flat surfaces of amorphous silk as well as of a sleeve-like casing, which extends along the inner walls of the cell and contains an increasingly smaller number of silk fibers, with the amorphous flats interconnecting the fibers comprising the major part of the sleeve. The entire weave is in fact constructed of a single continuous fiber that is approximated as an infinitely long cylinder whose diameter varies between 1-10 μm and which is made of a core of fibroin and a coat of sericin. In the majority of fibers, this basic makeup is retained but there are some segments of fibers in which the sericin coating is interrupted by missing circles of various width. From an electric standpoint the fibrous weave seems to be comprised of two groups, to wit: a majority of discrete fibers whose coating is continuous and which act as capacitors and a minority of fiber segments whose coating is interrupted at intervals and they therefore leak, from an electrical standpoint. The paper discusses the effect which the entire structure of the silk weave, including its various parts, has on the thermoregulation of the single pupa within its cell as well as of the entire nest. Also discussed is the analogy between structure of the silk fibers and that of peripheral nerves in vertebrates that are coated with myelin.

(Received June 7, 2000; accepted September 6, 2000)

Keywords: Cocoon, Hornets, Conductive fibers, Thermoelectric properties

1. Introduction

In hornets, being holometabolous insects, there is a stage between the larval stage and the adult stage, which is called the pupal stage. At the onset of the pupal stage, the full-term larva commences spinning a silk weave around its entire body. The greater part of the pupating larva is encased in the comb cell housing it, but its head which can be seen protruding to the outside becomes coated in a silk cap [1]. The silk of pupating hornet larvae has drawn the attention of our laboratory, particularly in the last decade. Accordingly our investigations of vespan silk have extended in several different directions, yielding a series of papers describing various phenomena and properties and associated with this silk, to wit: luminescence [2], morphology [3], capacitance [4, 5], photovoltaic effects [6], electric effects such as voltage, current and resistance which are changeable under varying illumination, relative humidity and temperature [7, 8], photodetector properties [9], the silk as a material that maintains a "clean room" for the cuticle during the formation by the pupating larva [10], thermoelectric properties during the various stages of pupation [11], the silk as a biodegradable polymer [12], electric charge transfer in hornet silk [13] and Fourier transform infrared (FTIR) spectroscopy [14].

The thermoelectric properties of male silk caps were measured in the dark at a relative humidity of 90% or more, and at a temperature ranging between 20-32 °C. A typical picture of such assessment is given in Fig. 1, which shows the appearance of electric currents of up to 50 nano Amperes (nA) with increase of the temperature and a drop of the current down to OnA as the temperature is decreased to the starting level (20 °C). This phenomenon is reversible over a long period and the electric energy stored in the silk is retainable for many days, provided it is not discharged. Such measurements were published earlier as data recorded from worker larvae silk caps [11].

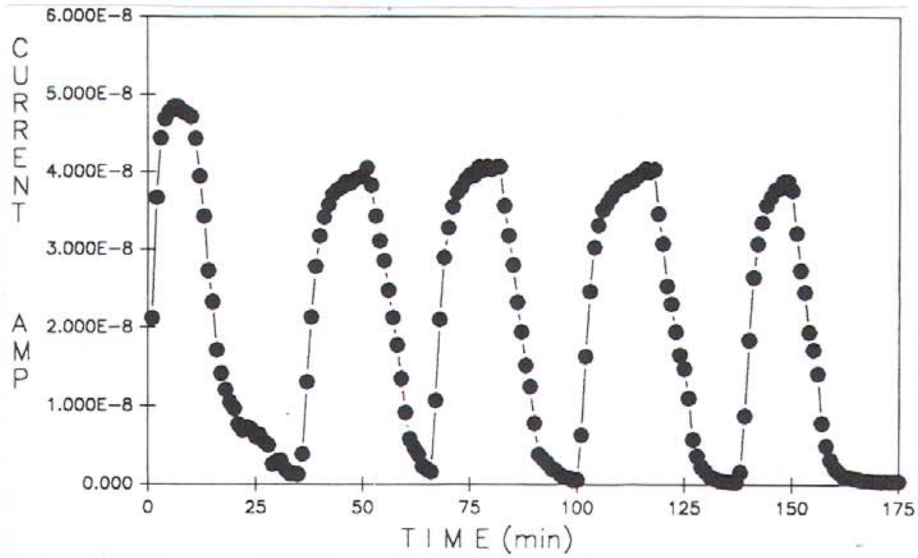

Fig. 1. Typical recording of electrical current measured on silk caps. The duration of a cycle is about 30-35 min. When warming, the current obtained increases to about 40 nano Amperes (nA) (= 40×10^{-8}A) while at cooling it returns to about zero nA. The first cycle reaches higher values. Current production by silk caps can be repeated many times.

The distribution of the silk fibers in the cocoon is not uniform. In fact, the greatest amount of silk occurs in the center of the silk cap, and the farther we descend from the cap into the interior of the comb cell, the fewer silk fibers are detected, which together with the amorphous material glued among them, form a thin coating. Similarly, in measuring the temperature distribution in the silk cap via a thermographic method we found that the center of the cap is the hottest and the farther we proceed toward the margins, the lower the temperature. This would seem to indicate that the silk picks up heat from the environment within the dark nest, and converts it into electric energy that is then stored in the silk fibers which, as already mentioned, are composed of a central fibril of fibroin and an outer coating of sericin [15]. Both fibroin and sericin are high molecular weight proteins [16, 17, 18]. Yet the manner in which heat is transferred from the silk cap to other parts of the silk weave is still undetermined, albeit in the present paper we suggest a tentative mechanism for such transfer.

2. Materials and methods

Nests of the Oriental hornet *Vespa orientalis* (Hymenoptera, Vespinae) containing combs with sealed brood (pupae) were collected in the field as previously described [19] and this at the end of the summer when the nests are at peak development and activity. From the combs we removed the pupal casings (cocoons), starting with the protruding portion, which we have named the silk cap and ending with the weave that encases the pupating larva up to its abdominal tip. The silk enwrapping the various bodily parts was prepared for observation and visualization through both scanning (SEM) and transmission (TEM) electron microscopy by methods described elsewhere [20]. In all, we examined 22 silk caps: 6 from queen pupae, 8 from worker pupae and 8 from drone pupae. The pupae of the three castes are easily distinguished. Thus, the cells of the worker pupae are smaller and their silk cap is prominent; the drone pupae have larger cells, but their cap is almost flattened, while the queen pupae have the largest cells and their caps are quite protrusible.

3. Results

SEM observations taken from the exterior of the silk caps revealed that at the center of the cap: 1) the silk fibers criss-cross to form at least 10 layers; 2) the fibers are of different diameter, the most common diameter being 7-8 μm but with some fibers that are thicker and others that are only 2-3 μm in diameter; also, between the fibers there are flats that fill the gaps between them (Fig. 2, Fig. 2 A); the flats are more numerous on the exterior of the cap and fewer or entirely absent on the inner side of the cap (Fig. 2, Fig. 2 B); 3) the fibers which connect to the flats bear granules of about 0.5 μm in diameter which cling to their entire length (Fig. 2, Fig. 2 C,D). These granules or spherical bodies represent symbiotic bacteria identified as *Staphylococcus xylosus* and *S. gallinarum* and are usually found in a small pocket made by flats in the cocoon cap.

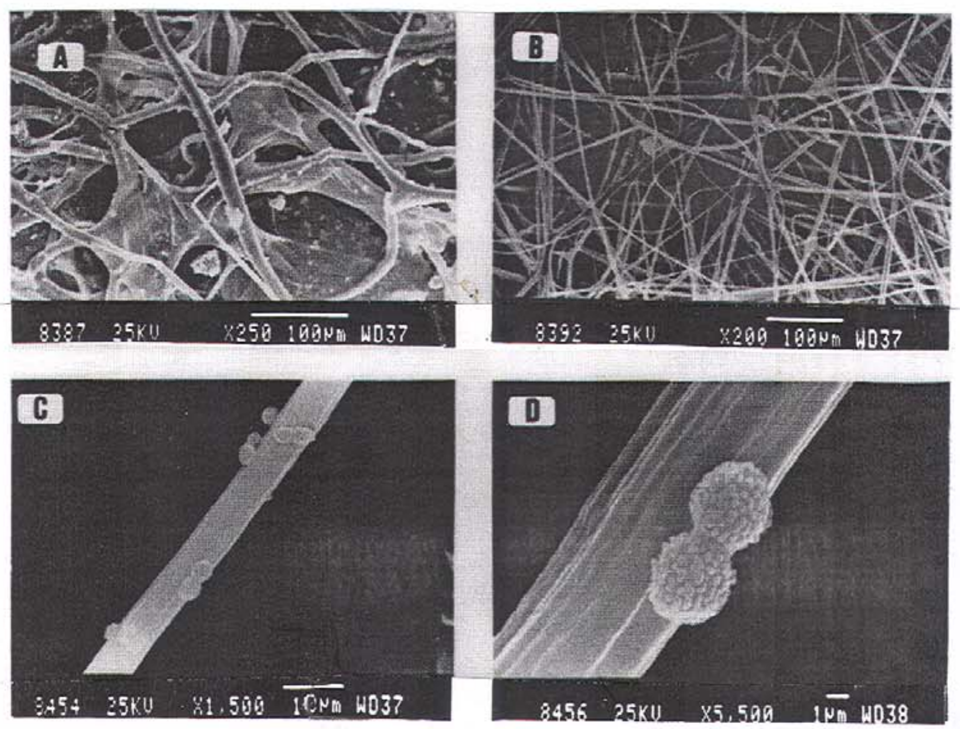

Fig. 2. Fibers and flats in the outer part of the silk cap (A) (bar = 100 μm) and on the inner side (B) (bar = 100 μm) seen by SEM. Several bacterial spores are seen on one fiber (C) (bar = 10 μm) and two of them, are shown enlarged (C) (bar = 1 μm).

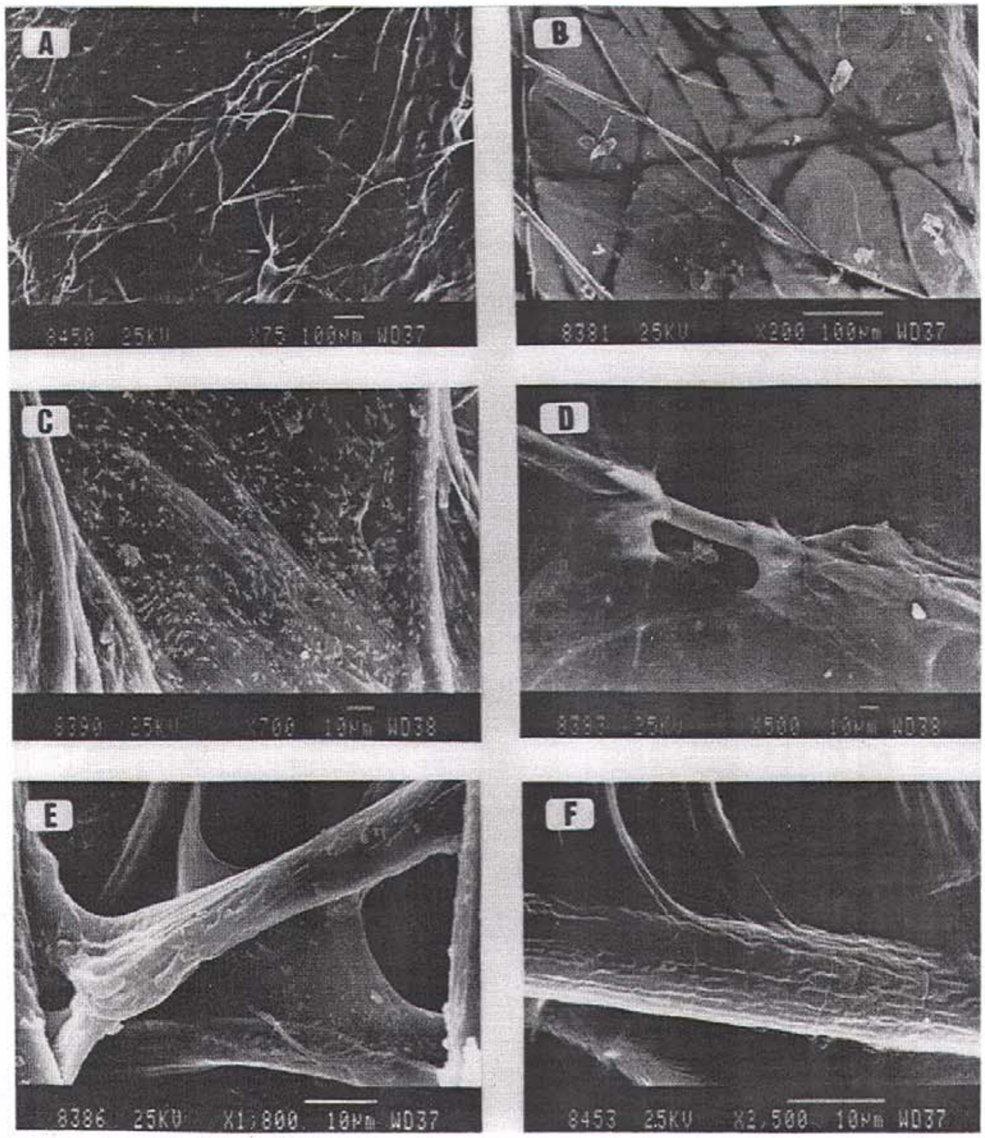

Fig. 3. Part of the silk cocoon alongside the cell walls of the pupating larva seen by SEM. The fibers are sparse. Most of them are embedded in the amorphous material of the flats (A, bar = 100 μm, B, bar = 100 μm). The flat material appears transparent (C, bar = 10 μm, D, bar = 10 μm). Only small segments of fibers are outside the flats (D, E) (E, bar = 10 μm). In F (bar = 10 μm) strips of amorphous material (flats) are connected tightly to a fiber on which transverse delicate grooves are seen.

As one proceeds from the cap to the silk weave lining the cell wall, several changes are noticeable, to wit: 1) the layers of silk fibers begin diminishing in number at the lip of the cell and dwindle to a single layer a few millimetres deeper into the cell (Fig. 3 A,B); 2) the fibers themselves become fewer and farther apart (Fig. 2 C,D), so that each fiber is embedded within a flat which encases it almost fully, leaving but a tiny part free (Fig. 3 C,D,E); 3) the fibers extend lengthwise in the silk weave (as the venation in the leaf of a plant), but in certain areas they are grooved crosswise (Fig. 3 F); in other words, the overlying layer, which is made of the protein sericin is missing, i.e. a gap is produced forming rather complete or partial 'gap rings' and in these parts the fibroin fibrils are exposed; 4) the number of fiber segments showing this transverse grooving is greater on the inner portion of the silk weave (i.e., that within the cell) than on the regions of the cap; 5) these transverse grooves are in no way uniform (Fig. 4 A-F), not in the intervals between them (Fig. 3 A), not in the width of the portion from which the sericin envelope detached (i.e., the gap) (Fig. 4 A-C), not in continuity of the groove around the inner fibril of fibroin (Figs. 4 D,E) and not even in their diameter, in that some are 2 μm in diameter (Fig. 4 F) and others attain 10 μm in diameter (Fig. 4 E), while all the rest range between these two limits.

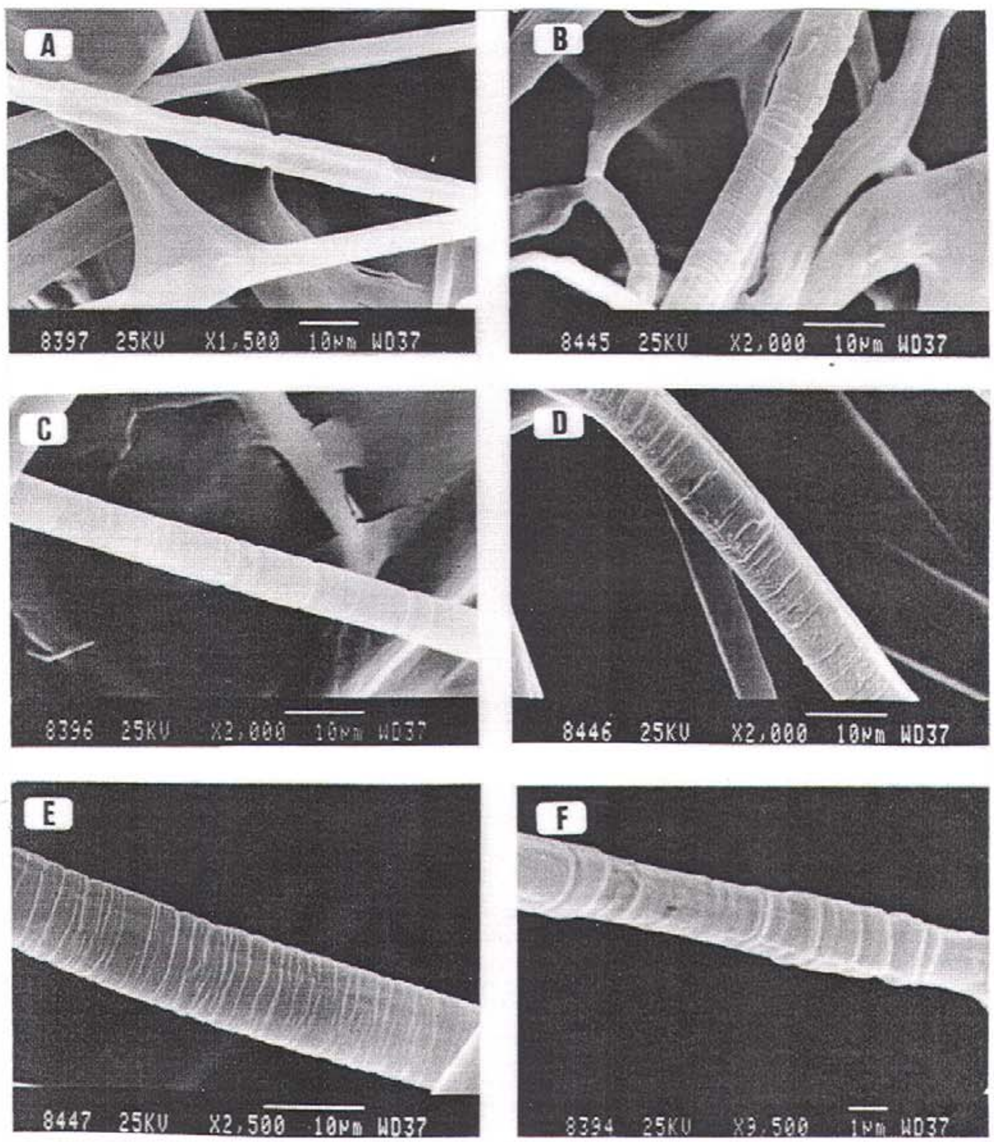

Fig. 4. Single fibers grooved lengthwise seen by SEM. For more details consult text.
Bars = A-E = 10 μm, F = 1 μm.

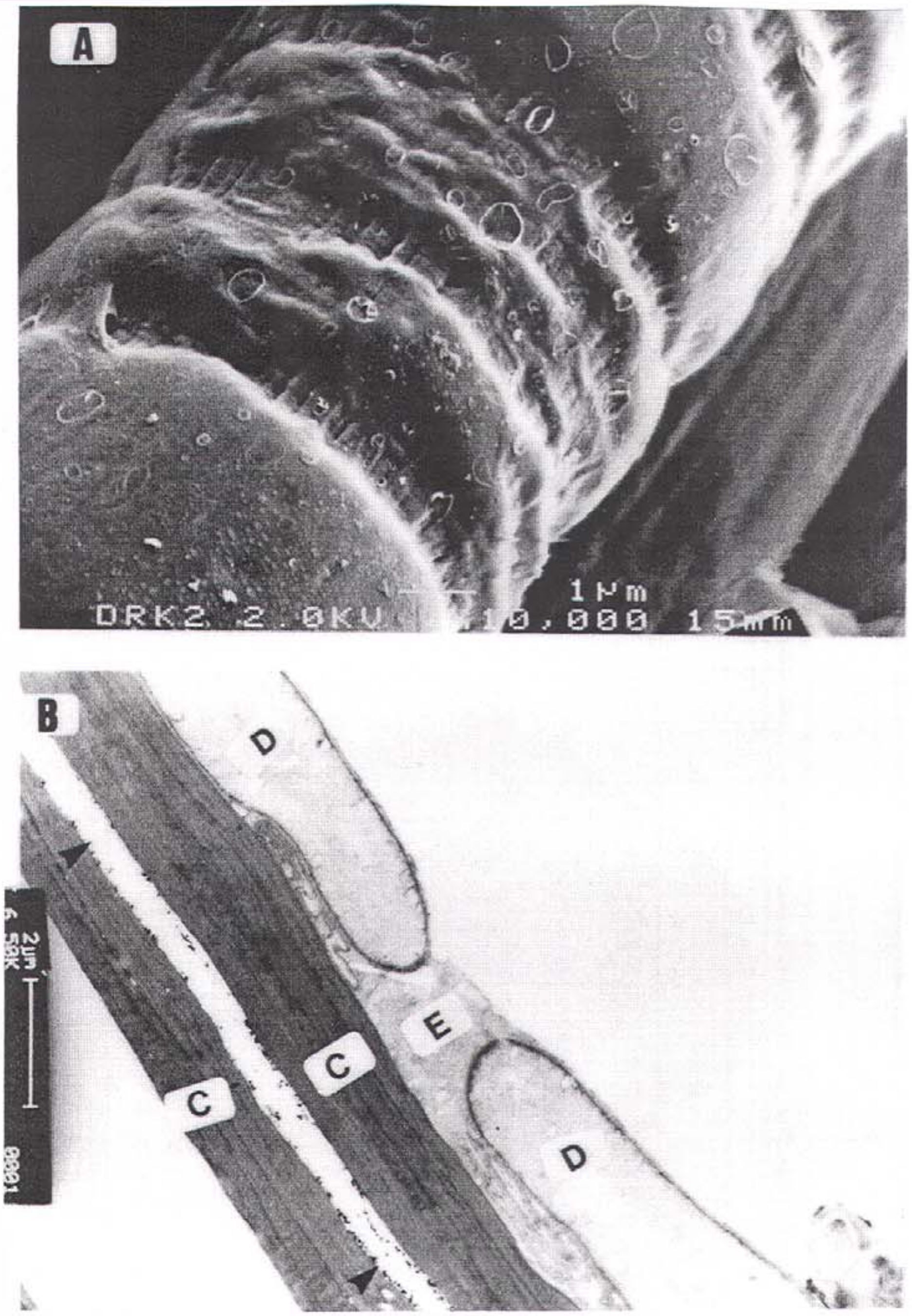

Fig. 5. A – A segment of a grooved fiber seen by TEM. One can discern a cross-sectioned cylinder-like configuration with a core of fibroin (C) in the middle of which there is an electron dense canal (arrows). Outside the core one can see the coating of sericin (D) and at the area of coat interruption one sees the filling material (E) coming in contact with the exposed fibroin and also under the sericin. It is worth mentioning that the sericin coat appears as an amorphous material enwrapped by a thin shell of electron dense material while the fibroin appears as organized in fibrils running lengthwise within the silk fiber.

The grooved portions of the fiber are brief segments extending only 100-200 μm; before and behind them, the fibers are intact, that is, the sericin outer coat fully covers the inner fibroin fibril. Around the grooved fibers, the majority of surrounding fibers are not grooved (A-B). A short segment of grooved fibers is shown in Fig. 5 A at a magnification of ×10,000. At this magnification, one can see numerous fibrils running along the inner part of the fibers (about 20 fibrils visible on the surface exposed to the naked eye), whereas the interruptions in the outer envelope are transverse rather than longitudinal and occur at the periphery of the fiber. The phenomenon of interruptions in the fiber's covering occurs in all the vespan pupae, be they of queens, workers or drones.

In a longitudinal section visualized via TEM, one can discern in the fiber the following parts: 1) a region of fiber extending longitudinally (C); this is probably the region seen in Fig. 5 A, underneath the interrupted envelope (of sericin); 2) a region in the core of the fiber which is electron dense (see arrows), where 1+2 represent the fibroin, while the sericin is seen in D. These two regions (i.e., fibroin and sericin) are separated one from the other. Around the sericin envelope one sees, as an envelope enwrapping them, a narrow region which is electron dense, while between the two ends of the sericin envelope is visible an amorphous material which fills the gaps (E).

4. Discussion

In all our previous assessments of hornet silk we found that if the silk caps are kept at conditions similar to those prevailing in the vespan nest (i.e., relative humidity exceeding 90%, darkness or very low illumination and temperatures alternating within the range of 20-32°C), we get a rise in the cap's electric current upon increase in the temperature, and vice versa [11](see Fig. 1). Under illumination, however, there is rise in the voltage upon rise in temperature, which means that we obtain the classic thermoelectric phenomenon, namely, the Seebeck effect [21] which previously we had already recorded from hornet cuticle [22].

Indeed the source of the pre-pupal silk is in glands resembling those that produce vespan cuticle, albeit the silk, as we know, is composed of amino acids making up the proteins of the inner fibril of the fiber (fibroin) and of the outer coating (sericin). Regarding these proteins, Ochiai [15] has determined their ratio and found it to be 4:1 in favor of fibroin, whereas vespan cuticle is made up primarily of N-acetylglucosamine, with some added proteins and other substances [23]. In an early study assessing the thermoregulation in the nest of the Oriental hornet [25] we found that there are factors in the nest, including the silk, which contribute to creation of heat and the maintenance of thermoregulation even several days after removal of the entire adult population from the nest.

As for the transformation from heat to electricity and vice versa, our suggestion is: The silk fibers most of which are concentrated in the silk cap, pick up thermal (solar) energy during the day, give rise to thermoelectric currents, which determines the electrical charging of silk. Then the slow electric discharge during night produces heat. It stands to reason that the accumulation of an electric charge is especially high at the center of the silk cap, simply because the charge is stored along the length of the fibers and the current flow is regulated by the interconnections between the fibers [11] and the number of fibers in the cap is the highest. Upon thermographic examination (Litinetsky et al., in preparation) we indeed found that the center of the cap is the hottest while the margins have a temperature which is lower by 3-5°C.

Bearing in mind that the vespan comb cell is 'inverted', with its outlet facing down, we can visualize the pupal silk weave as a vessel equipped with pipes that directs electric current from the warm cap below up to the tip of the silk weave and this according to the degree of its cooling. In this sense, we can find a corollary to what industry does when it is interested in maintaining a steady temperature within a fixture, namely, equips it with heat pipes [25,26] or uses ceramic fibers [27], or rare earth fibers [28]. In our case, and in nature, the silk weave is intended to enfold the pupa so as to maintain a constant temperature of its body throughout the pupation.

If indeed the silk weave is to be viewed as a network of isolated fibers, which conducts current throughout its length we can imagine the silk as a complex electric circuit. A schema of the infrastructure of a vespan silk fibers is given in Fig. 6a, which draws an array of capacitors and resistors that could possibly apply to the known structure of the silk fiber, with its inner fibril and outer coating. A similar theoretical model of electrical properties of the Oriental hornet's cuticle was presented earlier [29]. The schema presented now for hornet silk fibers is based on the following suppositions:

1) the inner fibril and the outer coating conduct current more efficiently along their length than from one to the other; in other words, the resistance along the length of the fiber is lower than that perpendicular to it, albeit we are dealing with a specific resistance and not with the overall resistance of the fiber.
2) there is a capacitance between the inner fibril and the outer coating.

In the schema basing on these two assumptions, the indicator designated as R-in represents the element of resistance (i.e., resistance per unit of length) along the length of the inner fibril while R-out designates the element of resistance along the outer coating. Furthermore, Cap designates the element of capacitance (per unit of length) between the inner fibril and the outer coating. The electric energy is in fact stored in the elements of capacitance extending down the length of the fiber. When the resistances R-in and R-out are sufficiently low, the capacitors behave as capacitors in parallel, so that when one of them is discharged, all the rest follow suit. In this situation, the entire fiber releases the energy. Regarding the break point in the outer coating of the fiber, we conjecture that this discontinuity (gap) of the outer coating prevents passage of charge between the detached segments and thereby the charge of each segment is retained, so that any discharge is only of the capacitors between one detached area and the next (see Fig. 6 B).

There are, of course, other possibilities (Fig. 6 C) as, for example, the possibility that the outer coating of the fiber is itself composed of two layers – an outer and inner - between which an electric charge could perhaps be stored, or, as another possibility – that between the inner fibril and the outer coating there exists another material which could conduct electricity. In both cases, we are dealing with an inner fibril (a core) and two encircling layers, somewhat like a tri-axial configuration as opposed to the co-axial configuration presumed in the two earlier models. The tri-axial model would necessitate the following suppositions:

1) that the inner fibril and the inner layer of the outer coating are better conductors of electric current along their length than between each other;
2) that the outer layer of the outer coating is relatively insulated; and
3) that there is capacitance between the inner fibril and the inner layer of the outer coating (or it and the hypothetical intermediary material).

On the base of the above suppositions, our designations need to undergo some alterations. Thus R-core now represents the element of resistance (per unit of length) along the inner fibril, R-in is the element of resistance along the inner layer of the outer coating, R-out is the resistance (relatively large) of the outer layer of the outer coating, and Cap Core-in indicates the element of capacitance (per unit of length) between the inner fibril and the inner layer of the outer coating. In this model, as well, the electric energy is stored in the elements of capacitance along the length of the fiber. Furthermore, in this model, provided the resistances R-core and R-in are low enough, the capacitors behave like capacitors in parallel, but the external resistors, namely, R-out, prevent escape of the energy except when there is a disruption in the outer coating. As suggested in Fig. 6 D, the energy is released only in the areas of disruption in the outer coating.

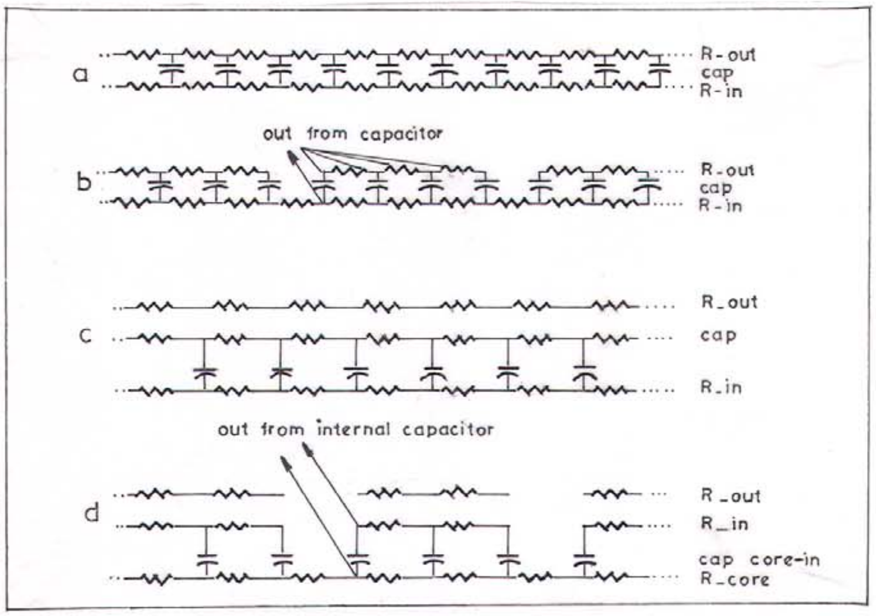

Fig. 6. A schema of the proposed electrical circuit for vespan silk fibers. R-in is the resistance along the inner fibril. R-out is the resistance along the outer coating. Cap is the capacitance between inner fibril and outer coating. Cap core-in is the capacitance between inner fibril and inner layer of the outer coating.

Such manner of annular gaps around the core of the silk fiber is somewhat reminiscent of the discontinuity in the myelin coating of peripheral nerves (vertebrate myelinated fibers). Such disrupted regions are known as the nodes of Ranvier [30], and in these regions permeability changes occur, thus causing a rapidly progressing type of ion jumping, or saltatory conduction.

Indeed there are several points of similarity between our silk fibers and the myelinated nerve fibers of vertebrates, to wit:

1) the diameter - which in the silk fiber ranges between 2-10 μm, while in myelinated nerve fibers is 1-22 μm [31];
2) the silk fibers have an outer coating and so do the peripheral nerve fibers, some of which are myelinated;
3) both types of fiber are composed of protein;
4) both types of fiber are conductors, albeit in the silk fibers it is electronic conduction whereas in the conductive nerve fibers it is ionic conduction.

That said, there are also points of difference or contrast between the two. For instance, a nerve is an extension of a living cell, while our silk is a secretion from a gland. Again, in nerve the interruptions in the nodes of Ranvier are of a uniform nature in terms of distance apart, size of the interruption, etc., whereas in the silk, as seen from Plate 3, the interruptions are non-uniform, whether in size of the annuli, their distance apart or even in their integrity, with some of the rings showing only partial detachment. However, under high magnification (Fig. 3, ×10,000) one can see that between the inner fibril and the area of disruption there is an amorphous material, actually the same material encountered in the flats between the fibers in the upper part of the silk weave and also occupying most of the area in the lower portions of the silk weave.

It seems reasonable, therefore, to suppose that emergence of the current from the silk fibers takes place between the core and the outer coating, in the interruptions between parts of the coating, and in these regions the amorphous material infiltrates and it is on this material that the current breaks up and converts to heat. As to how more heat is produced at times of need, when the nest temperature drops, this point is still unclarified but the subject is now in an advanced stage of investigation.

5. Conclusion

The structure and configuration of hornet silk fibers can be viewed as a complex electrical circuit endowed with properties that ensure thermo-regulation of the nest. It was shown that between the silk fibers and the myelinated nerve fibers of vertebrates there are several points of similarity.

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