STUDIES ON THE VENOMOUS SPICULES AND SPINES OF
MOTH CATERPILLARS

I. FINE STRUCTURE AND DEVELOPMENT OF THE VENOMOUS
SPICULES OF THE EUPROCTIS CATERPILLARS

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(Received: December 27, 1977)

SUMMARY: Scanning and transmission electron microscopy (SEM and TEM)
revealed unique structures and development of the venomous spicules of tussock
moth caterpillars of the genus Euproctis: (1) Flower-like structure at the distal end
and a longitudinal minute depression on the proximal subapical wall of these
spicules were observed by SEM. This depression was revealed to be a small hole
by TEM. (2) During molting, observed were cytoplasmic processes of several
trichogen cells penetrating the cytoplasm of a tormogen cell to form the spicules
with the holes at their subapical portions. A papilla was formed by a tormogen
and several epidermal cells. (3) After the molting, the cytoplasmic process in a spicule
disappeared and the spicule cavity was replaced by electron-dense materials secreted
apparently from the trichogen cell. (4) It was considered that the electron-dense
materials were the main toxic or precursory substances in the Euproctis spicules.

INTRODUCTION

Of the medically important larvae of the urticating moths in Japan, three
species belonging to the genus Euproctis, namely, the oriental tussock moth
E. subflava (Bremer), the yellow-tail moth E. similis (Fuessly) and the tea tussock
moth E. pseudoconspersa (Strand), are known to be very harmful (Ogata,
1958a, b). Particularly, E. subflava does harm very frequently to the residents
in many parts of Japan. To cite an example (Asahina and Ogata, 1956), no less
than 200,000 people suffered from dermatitis caused by the contact with this
moth in 1955, and thereafter its subperiodic outbreaks have occurred at intervals
of about 8 years, arousing the attention of both the inhabitants and public
health officials. Moreover, there are tendencies towards enlarged infested areas
and increased dermatitis victims with the progress of land exploitation towards
the suburban areas.

It is well known that the venomous spicules of the above moths measure
about 150 μm in length and about 5 μm in maximal diameter, and that derma-
titides are usually caused by the pricks made by these spicules, which are easily detached and penetrate the skin with the pointed proximal ends at the head. With regard to the morphology of the venomous spicules of the genus *Euproctis*, many investigators (Tyzzer, 1907; Kephart, 1914; Gilmer, 1925; Pawlowsky and Stein, 1927; Tonkes, 1933; Morishita, 1957; Tsutsumi, 1958; Muroga, 1963) have given various descriptions since the early part of this century. However, most of these reports were based on observations by light microscopy (LM), and there has been no definite explanation of the mechanism of spicule formation and of the toxin(s) involved.

We have made a series of studies on the ultrastructure of these spicules by scanning and transmission electron microscopy (SEM and TEM).

**Materials and Methods**

Larvae of *E. subflava* and *E. similis* were collected in nature in Nagoya City. For SEM, they were fixed in 2.5% glutaraldehyde, dehydrated with ethanol series, coated with gold and carbon, and examined with a JEOL JEM-S1 scanning electron microscope. To prepare for TEM, tissues were fixed with 2.5% glutaraldehyde and 2% osmium tetroxide, dehydrated with ethanol series and embedded in Spurr's medium (Spurr, 1969). Sections were stained with uranyl acetate and lead citrate, and examined with a Hitachi HU-11-D electron microscope.

**Results**

The venomous spicules of *Euproctis* larvae at an early stage are found on the first pair of subdorsal tubercles of the first abdominal segment. At later stages, these spicules appear on the subdorsal and lateral tubercles of the 2nd to 8th abdominal segments. Figure 1a shows a scanning electron micrograph of the spicules of both *E. subflava* and *E. similis*.
Fig. 1.
Fig. 2, 3.
Fig. 4.
of a cluster of spicules and several sensillae on the first subdorsal tubercles. At higher magnifications, flower-like structures are seen at the tops of the spicules of *E. subflava*, each of which forks into three to six petal-like barbs (Figs. 1b and 1c). In *E. similis*, most of the spicules have three barbs, and each of them is more sharp and slender than that of *E. subflava* (Fig. 1d). No opening was observed at the distal ends of these barbs.

Each spicule is buried singly in its own socket of the cap-shaped papillae (Fig. 1e). The number of these sockets, or that of the spicules, on a papilla ranged from three to 12 with an average of seven. When the spicule was removed from these sockets, a small longitudinal depression was found in the side view at 1 to 3 μm away from the proximal end (Figs. 1f and 1g). No opening was observed at this end of the spicule either (Fig. 1g, inset).

Figure 2 is a series of micrographs indicating the spicule formation during ecdysis of the larva of *E. subflava*. At an early stage, when the newly formed spicules and papillae are protruding into the molting fluid under the old cuticle, observed are cytoplasmic processes of several trichogen cells penetrating through the cytoplasm of a large tormogen cell. Abundant microtubules and microfibrils are also seen in the cytoplasm of the both cells (Fig. 2a). By this time, a protruding part of the spicule has been covered with the outer epicuticle (Fig. 2b). As the spicule extrudes gradually, the inner epicuticle is deposited, but the cytoplasmic process within the spicule is still connected with the trichogen cell through a hole that is not sealed with the cuticle (Fig. 2c, arrow). This hole is, as observed by SEM, located on the lateral wall of the spicule at about 1 to 3 μm above the pointed base. Absence of openings at the both ends of the spicule is evidenced by these figures.

After the molting and before hardening of the cuticle, a “cavity” becomes observable in the spicule (Figs. 2d and 2e). This cavity seems to be derived from cytoplasmic disintegration in the spicule after it has been separated from the trichogen cell.

Figures 3 and 4 indicate fine structures at a more advanced stage after the molting, when toxic activity has become detectable in the spicule. Passing through the subapical hole mentioned above, the electron-dense and osmiophilic materials are seen to enter the cavity of the spicule (Figs. 3a, arrow and 3b). These materials seem to be produced within the endoplasmic reticulum (ER) in the trichogen cell (Fig. 4c) and released into the microvillated area of the cytoplasmic process of the trichogen cell that is located beneath the papilla (Figs. 4a and 4b). After being released into the microvillated area, they are transferred and accumulated under the spicule socket and then enter the spicule cavity (Figs. 3 and 4a). They appear to be composed of very fine fibrous substances that are stained well with toluidin blue.

**DISCUSSION**

Many investigators have reported on the morphology of venomous spicules
in the *Euproctis* larvae; most of them stated that two openings at the both ends of the spicule were observable (Morishita, 1957; Ogata, 1958a; Muroga, 1963; Jong et al., 1976). They have concluded from their results that aqueous fluid, or venom, flows in from the base toward the tip of the spicule. On the other hand, Tyzzer (1907) reported that a dye (methylene-blue), in which a spicule was placed, first penetrated at a pointed end of the spicule and, in several hours, gradually spread throughout its inner cavity; he suggested that there was a pore at the pointed end of the spicule. Our unpublished data only partly accept Tyzzer’s statement; a dye (eosin) gradually entered a cavity of the spicule from its basal part, but it did so through the “subapical” hole. When the spicule was pressed to break down on a slide glass, the dye infiltrated rapidly. From these observations and from our data with SEM and TEM, we conclude that an opening exists only at the subapical portion of the spicule. We consider that the flower-like structure of the spicule is very fragile and that an aqueous fluid can enter rapidly the spicule as reported by the above authors, if the spicule is broken or cracked near this structure.

Recent studies by Jong et al. (1976) also showed by SEM the presence of dark spots at both the proximal and distal ends of spicules of *E. chrysorrhoea*, and they interpreted these spots as openings. However, we consider that their interpretation might be related to some artifacts during SEM, because these kinds of dark spots are frequently seen as the results of adverse charging effects. We have never observed any opening at either end of the spicule as evidenced by our electron micrographs.

This opening, or the longitudinal depression observed by SEM, seems to play very important roles in spicule formation (Fig. 2) and transfer of the electrondense materials into the cavity of the spicule (Figs. 3 and 4a). We recognized a similar depression at the similar part of *E. chrysorrhoea* spicule in Fig. 3 of Sellier et al. (1975), though they did not give any notice about it.

The process of spicule formation reported in this paper appears to be very similar to the formation of insect scales (Paweletz and Schlote, 1964; Greenstein, 1971) which are more easily detachable than common hairs of insect. As to the formation mechanism of venomous spicules in the genus *Euproctis*, the results of the previous studies by LM cited above seem to be conflicting as to which cell contributes in forming the spicule and papilla. We have given evidences that a spicule was formed by a trichogen cell and that a papilla was made from both a tormogen and several epidermal cells (Fig. 2). These results seem to agree well with the report by Tsutsumi (1958).

Regarding the secretion of the venom, many investigators have presented opinions roughly classified into two kinds; one laying stress on the trichogen cell (Tsutsumi, 1958; Muroga, 1963) and the other on the tormogen cell, or the large gland cell (Kephart, 1914; Gilmer, 1925; Tonkes, 1933). Kephart (1914), in the study of *E. chrysorrhoea* by LM, first reported a connection of the tormogen cell with the spicule and suggested that threads of this cell would secrete the venom. However, it seems to be misleading into a wrong inter-
pretation that the electron-dense materials in the intercellular spaces within the papilla (Figs. 3a and 4a) are cytoplasmic processes or threads of tormogen cell, because these materials are apparently secreted from trichogen cells, which lie in the innermost layer under the papilla, and transferred to the cavity of the spicule through the intercellular spaces and the subapical hole (Fig. 4).

It is probable that the electron-dense materials are the principal part of toxic or precursory substances contained in *Euproctis* spicules. We also found very similar materials in the spicules of *E. similis* and *E. pseudoconspersa* at the same stage as observed in *E. subflava* (unpublished). We estimate that they are composed of proteinaceous substances produced in ER of trichogen cells.

Details of the spicule formation will be published elsewhere.

**ACKNOWLEDGEMENTS**

The authors wish to thank Prof. Dr. R. Kano, Department of Medical Zoology, Tokyo Medical and Dental University, Dr. S. Asahina, Department of Medical Entomology, the National Institute of Health, for their suggestions and encouragement. We also thank Drs. J. Asai and H. Nagura, Department of Pathology, and Drs. J. Kito and Y. Sugiuira, Department of Anatomy, Nagoya University, for their kind advice during this study.

**REFERENCES**


