**A HISTOLOGICAL STUDY OF THE DEVELOPMENT OF THE
URTICATING SPICULES OF THE FAR EASTERN URTI-
CATING MOTH, *EUPROCTIS FLAVA* BREMER
(LEPIDOPTERA : LYMANTRIIDAE* **)**

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In the summer of 1955, about two hundred thousand inhabitants in the vicinity of Nagoya City were affected with an accidental dermatitis caused by contact with the yellowish moths, which were attracted by light and invaded into house rooms. These moths were identified as the Far Eastern urticating moth, *Euproctis flava* Bremer, which is very common from southern Hokkaido to Kyushu, and is known for some fifty years as a medical pest mainly in Tohoku and Hokuriku District. This rash has been ascribed to the minute barbed spicules attached mainly on the anal tuft of the female moth. Observations have affirmed that these urticating spicules are carried by the adult moth from the cocoon into which the caterpillar deposited them. The spicules are, therefore, originally a product of the larval insects. Clinical studies have shown the possibility that the dermatitis is caused by a poisonous substance originated from the urticating spicules. Therefore it is most interesting to know the fine structure of the subcutaneous tissue of the larva and the detailed process of the development of the urticating spicules.

Histological investigations were made in the past by a number of authors, such as Tyzzer (1907), Eltringham (1913), Kephart (1914), Gilmer (1925), and Pawlowsky and Stein (1927). Kephart (1914) was the first to show two kinds of specialized hypodermal cells, *viz.*, the poison-secreting cells and the hair formative cells, in the subdorsal tubercles of a larva of the brown tail moth. Gilmer (1925), on the contrary, had the opinion that poisonous substance was secreted through small chitinous intracellular ducts of one poison gland cell just beneath each papilla, in spite of the fact that both authors used the same material and the same slide specimens. Pawlowsky and Stein (1927) illustrated the strongly pigmented hypodermal cells, the large gland cells and small trichogen cells from the thickened hypodermal tissue of the brown tail moth. It was in 1933 that the developmental process was first described in detail by Tonkes, who observed three kinds of cells ;

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the small papilla formative cells, the large gland cells and the small trichogen cells. Tonkes' conclusion is entirely different from Gilmer's, which is quoted in several text books. Weidner (1937) compared the tissue of the larvae of the urticating moth of the genus *Euproctis* with that of the procession caterpillar, *Thaumetopoea pityocampa* Schiff, and hypothesized that the large gland cell of the brown tail moth might be a membrane cell. Recently Morishita (1957) stated that the large cells of the uppermost layer secrete the urticating spicules. Various interpretations have thus been presented, but it appears still worthy to add another investigation of the fine structure of the larval epidermis and a histological observation concerning the development of the spicule formative epidermis.

MATERIALS AND METHODS

The middle instar larvae were proved to be excellent for the purpose of sectioning. This is because the spicule formative epidermis of an older instar larva is too complicated to be exactly sectioned. But, in addition to the middle aged larvae the first instar and overwintered larvae were also used for comparison. As a rule, four larvae were fixed every day during the period shortly after a molting to the next. But three larvae were fixed every three hours during the resting stage before molting, as it was found that the greatest change in the epidermis occurred during that period. When only a limited portion of the body was fixed, it shrank very often helplessly during the later treatment. Therefore fixing reagent was first injected into the body of a larva, and then the whole material was preserved in the same reagent for definite hours. In most cases, Bouin's picro-formol or Carnoy's acetic-ethanol reagent was used. Sometimes formol-acetic-ethanol and picro-chlor-acetic fixative were used. All these fixatives gave similar results. The fixed material was dehydrated through ethanol or iso-propanol, cleared in toluol and embedded in paraffin. Transverse and horizontal sections in a thickness of 5μ were successively made. For staining the eosin alone or Delafield's haematoxylin with a counterstain of eosin was employed. Sections were mounted in xylol-balsam. In order to confirm the location of nuclei, the Feulgen reaction and the related treatment were utilized in several stages.

OBSERVATIONS

A) *The Development of the Urticating Spicules*

Upon a subdorsal tubercle of the first abdominal segment of a middle instar larva are found a number of minute papilla-like structures,* each of which has two to twelve sockets. Each papilla holds several urticating spicules, which are hollow tubes, pointed at the base, inserted in the sockets. The length of the spicules is from 60μ to 150μ . Each one is covered with small barbs throughout the entire length and diverges into three to five barbs at the apex. Usually the urticating spicules of the central part of a tubercle are longer than those of the peripheral part. Prior to the resting stage somewhat before molting, the endocuticle is laid down under the epicuticle through which a number of fine tubes or pore canals run to the papillae. The glandular epidermis of a subdorsal tubercle consists of a number of cells and that of the central portion is fifteen times as thick as the ordinary epidermis. These epidermal cells were divided into three groups according to the shape, size and distribution of their nuclei.

* This term will subsequently be abbreviated to "papillae".

*Period 1**: (Textfig. 1; Textfig. 2 A, B; Plate-Fig. 1).

In a transverse section, it was recognized in the outermost part of the epidermis that there were cells with oval nuclei, which were in average 4.5μ in length, 4μ in diameter; in the middle layer, there were cells with large nuclei, 17μ in length, 7.5μ in diameter. They were situated directly under the papillae. In the innermost part, numerous cells with long fusiform nuclei, 17μ in length, 4μ in diameter, were crowded. Cytoplasmic strands of the innermost layer cells irregularly encircled the single middle layer cell and proceeded outwards through the deposited endocuticle, uniting into a single tube until branching off again to reach the sockets of each papilla.

Textfig. 1. A transverse section of a subcutaneous tissue of the subdorsal tubercle of a larva during feeding stage. The urticating spicules are inserted within the sockets of papilla-like structure. Beneath the deposited endocuticle, there are three kinds of cells, the nuclei of which are arranged in three layers.

Period 2: (Textfig. 2 C, D; Plate-Figs. 2-4).

After the thickness of the endocuticle of larval skin reached its maximum, which were shortly before entering the resting stage of the larva, the following changes were observed in transverse sections:

1. Elongation of the nuclei of the outermost layer cells.
2. Inward retreat of the middle layer cells.
3. Gradual inward invasion of the intercellular cavities towards the outer sides of the middle layer cells.
4. Increase in volume of the cytoplasmic strands and the innermost layer cells.
5. Loosening of the old cuticle from the underlying epidermal cell layer and inward retraction of the whole epidermal cells.

* This period corresponded to the feeding stage of the larva.

In about the middle of this stage, it was usually recognizable that the nuclei of the middle layer cells dwindled down to the size of those of the innermost layer cells. The cytoplasmic strands, stretching into the cavities, became clearer as if the cords or processes, while the middle layer cells retreated more and more inwards and the cavities extended further inwards.

Period 3: (Textfig. 2 E—G; Plate-Figs. 5 and 6).

When the cytoplasmic processes produced in the cavities reached the maximum in volume, they developed into the urticating spicules. In this earlier stage of urticating spicule formation, it was difficult to recognize four or five barbs which are to be seen on the apex of each urticating spicule which still seemed to

Textfig. 2. Diagrams showing changes of the epidermal cells under a single papilla-like structure during the formation of the urticating spicules.

ps: papilla-like structure, ep: epicuticle, en: endocuticle, hc: ordinary epidermal cell, sc: tormogen cell, tc: trichogen cell, us: urticating spicule.

be elastic. Similarly as the apical barbs the minute barbs on the shaft did not develop any more and remained the irregular folds. The papillae began to appear at the bases of the newly formed urticating spicules, slightly projecting from the outermost layer cells. The thickened portion of the spicule formative cytoplasm just beneath the papilla disappeared altogether. (Plate-Fig. 5)

As a result of the spicule formation, intercellular spaces of all the epidermal cells became loosened, particularly in the inner part of the glandular tubercle. Even when the urticating spicules were fully extended, they still showed neither darkening nor hardening. At this stage the urticating spicules were tightly con-

nected with the papillae. The cytoplasm of the innermost layer cells was continuous with the content in the hollow of the urticating spicules. (Plate-Fig. 6) In the following stage, the barbs of the urticating spicules and the papillae were completed. In the majority of caterpillars, the urticating spicules were fully formed under the old cuticle in the beginning of the resting stage. Just before molting, they became readily breakable at the base in the papilla.

The deposition of the endocuticle under the epicuticle was, in the stadium immediately after molting, hardly recognizable. The innermost layer cells which formed the spicules, further decreased their volume until they became like waved strands.

On the horizontal section of the larva of the period 2, two to twelve dots were recognizable in a single intercellular cavity surrounded by the cells of the outermost layer. These dots were proved to be the cytoplasmic strands, in the cross-section, of the innermost layer cells. In the middle layer, the strands encircled irregularly the large nucleus of a single middle layer cell, and finally in the innermost part, they merged into the main bodies of the innermost layer cells. (Plate-Fig. 8)

B) The Origin and Development of the Spicule Formative Tissue

First instar larvae do not carry their own urticating spicules, although they usually carry irregularly scattered urticating spicules mainly on the seventh and eighth abdominal segments derived from the egg covering. In the second instar, newly formed urticating spicules are recognizable on the dorsal parts of the first abdominal segment.

Histologically, the subdorsal epidermis of the first abdominal segment was, soon after hatching, irregularly crowded with small cells in cluster, the nuclei of the cells were approximately 3μ in length. (Plate-Fig. 10) All the cells increased in volume by gradual elongation of the thread-like processes of cytoplasm until to a stadium when the larvae appeared opaque as a result of the deposition of the cuticle prior to the resting stage. In this stadium it was recognizable in transverse sections that, as observed in middle instar larvae, there are cells of small nuclei of 4.5μ in length in the outermost part, large nuclei of 15μ in the middle layer, and long nuclei of 15μ in the innermost part of the epidermis. Thus, a structure was then prepared in these first instar larvae to produce the urticating spicules, which were formed in the same way in the middle instar larva.

As the larval instar advanced, the innermost layer cells of the epidermis increased in length. But the main portion of the middle layer cells did not increase in length at the same rate.

Therefore, the middle layer cells of the last instar larva was only slightly longer than those of the first instar larva. The length of the innermost layer cells in the last instar was approximately 110μ and was about three to four times as long as that of the first instar larva.

The smaller epidermal cells which originated new spicule formative tissue could be recognized adjacent to the periphery of the established urticating tissue irrespective of the larval instar.

DISCUSSION

It has been generally recognized that an unit of the epidermal hair, or the seta, is formed by a trichogenous cell, or trichogen, and, there is, usually associated with it, the socket-forming cell, or tormogen. The trichogen cell is directly concerned with the development of a process which subsequently turns into a hair. The tormogen cell forms the setal membrane which is penetrated by the process above mentioned.

The homology of the structure of the poisonous cells has not sufficiently been discussed by Tyzzer or Eltringham. Kephart's diagram is quite insufficient from the view point of homology, as also pointed out by Tonkes. Gilmer seems not to have recognized that the urticating spicule is a sort of hair, much less that it is formed from a trichogen cell. Tonkes' illustration, being quite different from Gilmer's, is not the same with that of Pawlowsky *et al.* Tonkes interpreted that "les grandes cellules" (the present author's large middle layer cells) combined tightly with several buds of urticating spicules which were developed from "les petites cellules inférieures", and "les cellules a pigment" (the present author's outermost layer cells) formed the papillae. Tonkes said "Dans les cellules qui ont formé les papilles les noyaux ont complètement dégénéré, il ne reste de ces cellules qu'un peu de protoplasme, qui plus tard se transforme encore en chitine". If "les cellule a pigment" do degenerate and disappear as Tonkes claimed, how can the papillae repeatedly be built after each molting? If Tonkes' materials were exclusively ultimate instar larvae, this seems quite possible.

Later, Weidner suggested that "die grossen Zellen" (Tonkes' les grandes cellules) were membrane cells, comparing the structure of the spicule formative cells illustrated by Tonkes with that of an ordinary hair formative cell.

Tonkes believes that the papillae are produced by his "les cellules a pigment", but the present author's assumption as regards the papilla formation is as follows. In the stage D, the papillae are not recognizable yet, but just above a middle layer cell and at the bottom of the spicule cavity, there begins the formation of tube-like cuticular substance. In the stage E, when the prospective spicules proceed above the level of the outermost layer cells (or the layer of the outermost layer cells retreats inward), the tube-like cuticular structures are projected above the level of the outermost layer cells. At this stage, the tube-like structures are no longer recognizable around the middle layer cells. It seems probable that they gradually turn into papillae with deposition of the cuticle around them. Consequently, it can be concluded that the large middle layer cells mainly participate in the formation of the papillae. Tonkes hesitatingly concluded "A mon avis les ébauches des poils d'une papille traversent le protoplasme de la grande cellule; on ne saurait pourtant l'affirmer, et il est également possible, qu'elles en fassent le tour en s'y accollant étroitement". In fact, it is possible to interpret that these processes penetrate the cytoplasm of a middle layer cell, and at the same time it appears that the cytoplasmic processes of the future urticating spicules stick to the large middle layer cells. As this feature is somewhat different from that of the ordinary hairs, a speculation was induced that this contact or joining may be related to the poisonous activity of the urticating spicules. If this would be true, the large middle layer cells function as poison-

secreting cells. At the stage before the larva enters the resting period, the outermost layer cells, if observed on a transverse section, fill up the spaces among the bundles of cytoplasmic strands of innermost layer cells. They are situated near the body surface and are believed to secrete the endocuticle. Accordingly the outermost layer cells have a function similar to the ordinary epidermal cells; they participate partly in forming the outer surface of the papillae, by filling up the spaces among the papillae, but do not form any sort of microtrichia.

With regard to the possible function of the innermost layer cells, the evidences gained by the author's observation completely supported Tonkes' views. Without doubt the urticating spicules are formed by the innermost layer cells, although there are different opinions of Gilmer (1925), Weve (1931),* and Morisita (1957). Kephart interpreted the innermost layer cells as trichogen cells. Pawlowsky seems to have also supported this opinion without confirming the presence of the thread-like connection between an innermost layer cell and a socket within a papilla.

As mentioned above, a single unit of the spicule formative cells always consists of certain number of cells, usually seven, which are closely crowded and are very long like a thread.

It will be of great interest to trace the development and differentiation of the specialized spicule formative epidermal cells from their origin. Gilmer hypothesized that the whole papilla with its urticating spicules was derived from a single hair which has plum-like structure. According to his assumption, a single hair develops by extreme suppression of its main shaft, accompanied by a shortening and narrowing of the branches, finally becomes urticating spicules set in a papilla. The present observation did not support this hypothesis at all. As described above, it has been histologically demonstrated that the development of the spicule formative epidermis always begins by the division of small cells, which are hardly bounded each other. These "Anlagen" develop, increasing their volume and length, to differentiate into three kinds of cells. The outermost layer cells are homologous to the ordinary epidermal cells, secreting the epicuticle and endocuticle among the papillae. The middle layer cells function as the tormogen cell and form the sockets in the papillae. The innermost layer cells are trichogen cells which form the urticating spicules. About three epidermal cells, a single tormogen cell and in average seven trichogen cells cooperate together to form a set of the urticating structure and produce externally one papilla and average seven urticating spicules inserted in it.

SUMMARY

1. The present paper deals with a histological observation on the development of the urticating spicules and the spicule formative epidermis of the larva of the Far Eastern urticating moth, *Euproctis flava* Bremer.

2. Three kinds of cells, the nuclei of which are arranged in three layers respectively, are recognizable in the epidermis of the subdorsal tubercle of the first abdominal segment of a middle instar larva.

* Cited by Tonkes, but not referred by the present author.

3. Each middle layer cell has contact with a bundle of two to twelve (usually seven) innermost layer cells, which later proceed and traverse together through both the outermost layer cells and the endocuticle, and are reaching the base of the urticating spicules located within a papilla-like structure.

4. From serial observations on the shape and possible function of these cells in various stages of the development, it can be concluded that the outermost layer cells are the epidermal cells, the middle layer function as tormogen cells, whereas the innermost layer represents the trichogen cells which produce urticating spicules.

5. The first instar larva does not possess its own spicules. It has, immediately after hatching, a crowded mass of small cells, or "Anlagen", in the subdorsal epidermis of the first abdominal segment. In the larvae of the later instar, these small cells are also recognizable adjacent to the periphery of the established urticating tissue.

6. The development of the spicule formative epidermis always begins with the division of small cells, or "Anlagen", which then increase the volume of cells until to differentiate into three kinds of cells to produce the urticating spicules and the papilla-like structure of the following instar larva.

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EXPLANATION OF PLATES

Photomicrographs of sections of the subdorsal tubercles of larvae. All sections were stained with Delafield's haematoxylin except Fig. 9. Fig. 1-10: Sections of the first abdominal segment of the larva. Fig. 11: A section of the eighth abdominal segment. Fig. 12: A section of the third abdominal segment. Fig. 1-6, 9, 11, 12: Transverse sections. Fig. 7, 8: Horizontal sections. Fig. 10: A longitudinal section. Magnification *ca.* $\times 400$.

Fig. 1. A middle instar larva of four days after ecdysis. All the urticating spicules have been lost from the papilla-like structure, beneath which are recognizable the deposited endocuticle and three kinds of nuclei. (Refer to Textfig. 2 A & B)

Fig. 2. A middle instar larva before the resting stage. The cavities invade slightly into the outer (upper) side of all the middle layer cells. (Refer to Textfig. 2 C)

Fig. 3. A slightly later stage of Fig. 2.

Fig. 4. A stage just before the development of the urticating spicules. Several cytoplasmic processes can be seen in the cavities, which extend inwards (downwards). At the bottom of the cavities are recognizable tube-like cuticular structures. (Refer to Textfig. 2 D)

Fig. 5. A slightly later stage of Fig. 4. Developing urticating spicules and their underlying epidermis, *viz.* three kinds of cells, can be seen. The cavities and tube-like structures are no longer to be recognizable. (Refer to Textfig. 2 E)

Fig. 6. A middle instar larva of the resting stage. The urticating spicules are formed under the old cuticle.

Fig. 7. A middle instar larva of a stage shortly after molting. Two to twelve dots can be seen in a large intercellular cavity surrounded by the cells of the outermost layer. (Refer to Textfig. 2 G')

Fig. 8. A middle instar larva of a stage before the resting stage. In the cavities are two to twelve dots of cytoplasmic processes of the innermost layer cells, several of which encircle each middle layer cell. (Refer to Textfig. 2 D)

Fig. 9. A middle instar larva of a stage after molting. (Feulgen reaction)

Fig. 10. A larva immediately after hatching. A number of small cells, the "Anlagen", are closely crowded like a cluster. The large tadpole shaped cell at the center of the cell cluster is an ordinary hair formative cell.

Fig. 11. A larva two days after molting. Small cells are also recognizable as in a larva immediately after hatching.

Fig. 12. A section at the periphery of the subdorsal tubercle of an old instar larva three days after molting. Small cells develop, increasing their volume to differentiate into three kinds of cells.