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Studies on the Insect Metamorphosis

IV. Prothoracic Glands of *Ephestia cautella*¹⁾

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In recent years the interest of insect physiologists has been focused on the study of the causal analysis of ecdysis and metamorphosis, with the resulting discovery that these phenomena are under the control of hormones coming from various organs in different insects. In lepidopterans, for instance, the prothoracic gland and corpus allatum are shown to be such endocrine organs. The former releases a hormone that can induce the pupal and imaginal metamorphosis when it acts alone, while the latter secretes another hormone that causes the larval moulting when it acts not alone but co-operatively with the hormone from the prothoracic gland. Moreover, the brain has more recently been found to secrete a different hormone that can stimulate the prothoracic gland so as to produce the metamorphosis-promoting hormone just mentioned (Williams, '47, Ichikawa and Nishiitsutsuji, '51, 52, Ichikawa, Yashika and Nishiitsutsuji, '53). Based on the several experimental data, we have proposed the following formula for the process of metamorphosis: Brain hormone \rightarrow prothoracic gland hormone \rightarrow pupation or emergence.

On the other hand, however, Kühn and Piepho (1936) have reported that in *Ephestia kühniella* the brain is involved directly in metamorphosis as the unique source of the metamorphosis hormone, and Plagge (1938) confirmed Kühn and Piepho's result in *Deilephila elpenor*, *Sphinx ligustri* and *S. pinastri*. Piepho (1938) insisted again that the brain is the organ releasing the pupation hormone in *Galleria mellonella* and *Achroea grisella*. Afterwards he (1948) stressed once more his argument and said that the organ where the pupation hormone is produced may be different among the large and small lepidopterans. If Piepho's

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argument is correct, the validity of our formula elucidating the process of pupation and adult development must be decreased apparently in the very same order of Lepidoptera.

From the theoretical view point the senior author (1950) has already suggested the existence of the prothoracic gland in a small lepidopterans like *Ephestia*. Indeed, Rehm (1951) has reported the occurrence of the prothoracic gland in *Ephestia kühniella*. We have also succeeded independently in finding out the organ in Japanese mealmoth *Ephestia cautella* and ascertained experimentally that the organ is really the prothoracic gland. The anatomical and histological structure of the organ, from the mature larva to the newly emerged adult, will be described in this paper.

Material and Method

When the larvae of *Ephestia cautella* become mature, they cease taking food and begin to creep about in the container. Such caterpillars were used as the "young" specimens. The "old" specimens were obtained from the cocoons. These young and old caterpillars were arranged into 8 stages, according to the degree of withdrawal of pigment from the ocelli, after Kühn and Piepho's classification (Fig. 1). In determining the stages of pupae, the colouration of the

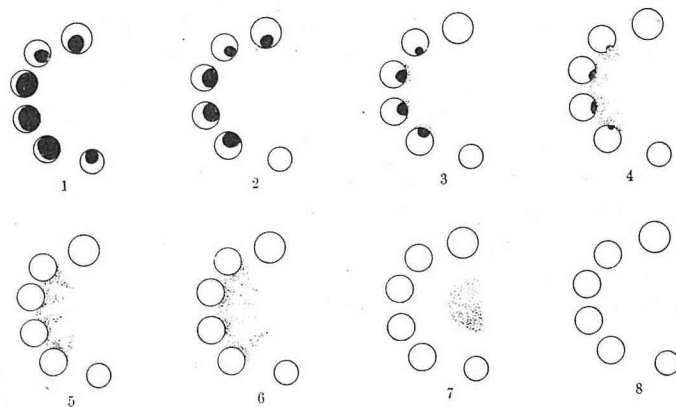


Fig. 1. Diagrammatic representation of the stages established by the degrees of pigment-withdrawal from the larval ocelli.

compound eyes developing under the pupal skin was taken into consideration.

For permanent preparations, etherized larvae were cut transversely at the middle of the body, and their anterior halves were fixed in Bouin's mixture or in Carnoy's fluid. Paraffin sections 5 micra thick were stained with Delafield's hematoxylin and eosin.

Anatomical Observation

Etherized caterpillars were cut along the dorsal body wall from the second or third abdominal segment to the anterior end of the head and opened to show their internal structure. The glandular structures that we prefer to call the prothoracic glands are thus exposed to view. But they were very difficult, if not impossible, to see on account of their transparency and resemblance to the neighbouring tissues. When acetocarmine is applied, the identification of the structures becomes comparatively easy. They are paired, band-like organs located under the prothoracic spiracles in close proximity to a large tracheal trunk running longitudinally through the body, and they consist of some thirty cells each. Their anterior end extends to or beyond the level of the posterior margin of the brain, while their posterior end lies within the prothorax. From the mere topographical point of view, the structure seems equivalent to the prothoracic glands of silkworm and other lepidopterans. There can remain little doubt that their function revealed by means of implantation was to secrete the pupation hormone.

When the structure is brought onto a slide glass and stained *in toto* by Delafield's hematoxylin or other stains, it manifests itself more clearly, i. e., the shape of the individual cell differs considerably from stage to stage. In young stages, the cells are rather angular and arranged closely, whereas as the development advances, they become distended and touch each other more loosely. Finally, however, they take angular shapes again and are brought closely together in the last prepupal as well as in the pupal stage. This change of shape seems to parallel the functional activity of the cells: that is, when their activity is high, the cells become distended, and when it is low, they are angular.

A small trachea, which branches away from the main trunk at a point a little anterior to the spiracle, arrives at a point one-third of the distance from the caudal end of the gland. Here it ramifies into two tracheoles, one of which runs posteriorly, while the other extends anteriorly, either along the surface of the gland or through the intercellular spaces. No nerves can be found to enter the unstained gland, but some innervation would probably be disclosed by means of methylene blue staining. Since no ducts are found in connection with this gland, the secretory products, as in the case of the silkworm, may be released directly into the blood.

Histological Observation

Histological examination reveals that all of the glandular cells, irrespective of their shape and arrangement, contain large nuclei deeply stained. The shape of the nuclei is irregular. Generally speaking, the nuclei in young stages are packed with chromatin-like granules of medium size and uniform distribution

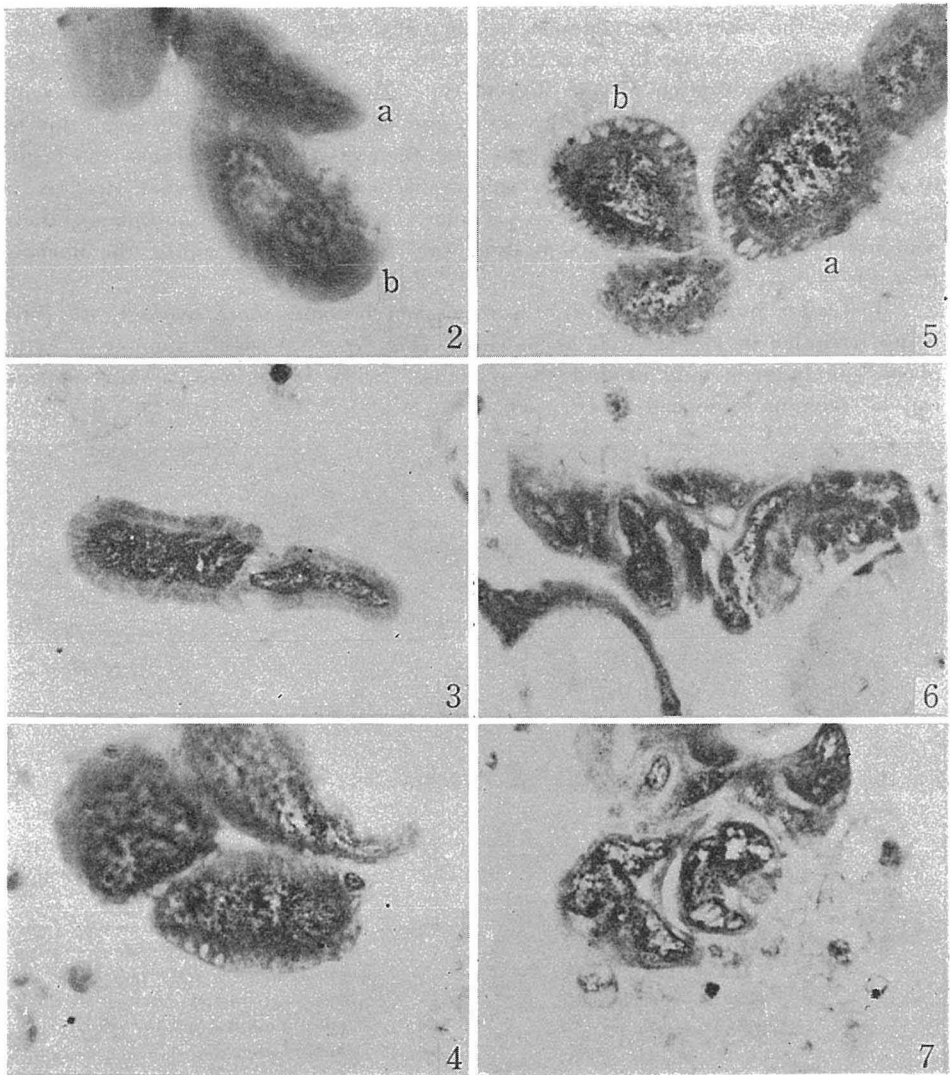
(Fig. 2, a). But in some specimens the granules are densely located in the peripheral region of the nucleus, while in others they seem to flow out from the nucleus into the cytoplasm (Fig. 2, b). Even in this case a distinct nucleolus is present. In young stage the cytoplasm is scanty, existing only around the nuclei. But in stage 2, the cytoplasm becomes more abundant, and it is possible to observe in it many fine striae that run radially (Fig. 3). We surmise that this striated structure of the plasm is related to secretion.

Larvae removed from the cocoons (stage 3) show fairly large, glandular cells, on account of the increase of cytoplasm. In this stage, the cytoplasm of the glandular cells may be vacuolized or not. This difference is found not only between individuals, but also between cells of the same individual.

In such non-vacuolized cells, medium-sized granules can be found in the plasm. They are very similar to those in the nucleus in the preceding phase. We are inclined to believe that these granules are released from the nucleus at a certain point of the secretory phase, because figures showing the flowing out of these granules are often encountered in the sections (Fig. 2, b). The nuclear membrane is indistinct in some cells. In such nuclei, a nucleolus is always present. Therefore, the disappearance of the nuclear membrane in this case may be controlled by a different mechanism from that operating in the case of cell division, where simultaneous disappearance of both structures occurs without exception.

In stages 4 and 5, no prominent changes worth describing here are discernible. The nuclear membrane is distinct in some cells and indistinct in others. In the former, the nuclear granules occupy mostly the marginal portion of the nucleus, while in the latter, they are scattered rather uniformly throughout the nucleus. Similar granules are distributed even through the cytoplasm of these cells. Spindle-shaped vacuoles are discernible in some of the cells of the latter type.

In the vacuolized cells, small vacuoles of ellipsoidal or spindle-shape occur in such a manner that their longest axis lies parallel to the striae of the cytoplasm (Fig. 4). This characteristic feature permits us to surmise that such definite vacuoles would be found to contain the secretory products if an appropriate fixative were used. Namely, they may account for the secretory droplets. At any rate vacuolization of this type occurs more often as the stages advance, and becomes maximum in the caterpillars in stage 6 (Fig. 5, a). In this stage, however, vacuolization of the second type makes its appearance also in some glandular cells (Fig. 5, b). The vacuoles of the latter type are round or irregular and markedly larger than those of the first type. The nuclei of such large vacuolized cells contain a small number of medium-sized granules, and also other granules of irregular shape and size, located in the peripheral part near the nuclear membrane. This distribution of the granules produces a transparent appearance in the central part of the nuclei.



- Fig. 2. Photomicrograph, showing the medium-sized granules packed in the nucleus (a) and their outflow from the nucleus into the cytoplasm (b) (st. 2).
- Fig. 3. Photomicrograph, showing the radial striation of the cytoplasm, (st. 2).
- Fig. 4. Photomicrograph, showing the dispersion of the medium-sized granules throughout the nucleus and cytoplasm; nuclear membrane being indistinct, but nucleolus distinct (st. 6).
- Fig. 5. Photomicrograph, showing the first type (a) and the second type (b) of vacuoles (st. 6).
- Fig. 6. Photomicrograph, showing the shrinking of glandular cells just prior to pupation (st. 8).
- Fig. 7. Photomicrograph, showing the second type of vacuoles in the nuclei as well as in the cytoplasm of the degenerating cells (st. 8).

When the caterpillars advance to the next stage (7), a sign of involution begins to appear in some of the grandular cells; i. e., their nuclei show an atrophic appearance, vacuolization of the second type develops highly in the plasm and the cellular contours become foamy here and there. In stage 8, just prior to the pupal moulting, the grandular cells lose their distended appearance and begin to shrink (Figs. 6 and 7). The cytoplasm becomes scanty and when present, it is occupied by many large scattered vacuoles. The nuclei are amorphous, their contours being obscure. Vacuolization can be seen even within the nucleus (Fig. 7).

Just after pupation (stage 9), the regression becomes more marked, the cells being irregular in size as well as in shape (Fig. 8). A small amount of cytoplasm can be seen only around the nucleus. The breakdown of the nucleus can be seen in some cells.

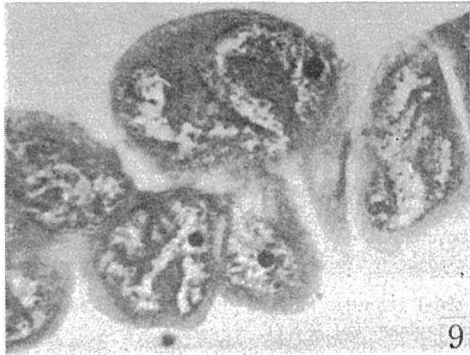
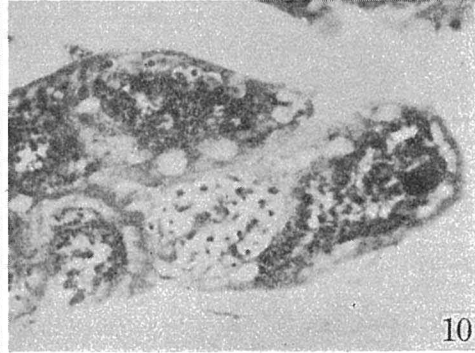


Fig. 8. Photomicrograph, showing the regression of cells just after pupation (st 9).

Fig. 9. Photomicrograph, showing some young cells among the degenerating cells (18 hours after pupation). Nuclei are lobular, nucleoli distinct, medium-sized granules scanty.

Fig. 10. Photomicrograph, showing the foamy appearance of the degenerating cells just after emergence.

These degenerating processes of the grandular cells progress continuously throughout the pupal life. We can find several types of degeneration according to different individuals. In one, the vacuolization takes place in both plasm and nucleus, so that the cell appears foamy. In another, the nucleus becomes lobular and its breakdown ensues; in this case nuclear fragments are scattered

throughout the coarse fibrous plasm. In still others, the nucleus is pycnotic and located in one side of the cell that is empty, leaving only the cell boundary. It is noteworthy that young glandular cells are often found among the degenerating cells in the young pupae (Fig. 9).

In the freshly metamorphosed moth, the degeneration of the gland is more severe and more conspicuous than in the pupa (Fig. 10). Whether the glands disappear completely or not could not be determined by the present observation.

Discussion

From the above description it is certain that the glandular organs situated in the prothorax of *Ephestia cautella* are the prothoracic glands or their homologues. There is a slight difference among their secretory phases in various individuals that belong even to the same stage. This may indicate that our arbitrary classification of stages according to the pigment recession of the larval eyes does not tell exactly the internal physiology of the insects. But looking through all the stages from mature larvae to newly metamorphosed adults, the following findings become evident: 1) In the early phase of their functional activity, the glandular cells consist of the large nucleus laden uniformly with many granules of medium size, and a small amount of cytoplasm. 2) As the stage advances, they grow larger, mainly because of an increased amount of cytoplasm. Nuclear granules seem to flow out through the ruptured part of the nuclear membrane into this cytoplasm. It cannot be said that these are the secretory granules; however, it may be permissible to say that they seem to bear some connection with the formation of secretory products. 3) Next there appear a number of fine striae running radially from the nucleus to the cell periphery, followed by the small spindle-shaped or ellipsoidal vacuoles that we prefer to call the secretory droplets, although we lack good evidence that the above granules grow into these droplets. 4) These droplets occur most abundantly in the cells around stage 6. This figure, we may surmise, represents the most active phase of secretory function. 5) As these droplets decrease, the large vacuoles make their appearance in their place. The vacuolization of this type may indicate the first sign of the involutionary process of the cell. 6) Just after pupation some of the glandular cells are highly vacuolized and cell boundaries become obscure. This figure apparently represents the degenerating phase of the cell. Can these glandular cells recover once again their activity for their imaginal differentiation? The answer to this question is negative, so far as our observations are concerned, although Rehm (1951) has found a rejuvenescent phase of the cells in *Ephestia kühniella*. We could find often some distended glandular cells among the degenerating cells of pupae. These glandular cells, we may assume, have grown from the latent ones in the larval stages, and they are involved in the production of emergence hormone. But it may be possible that some hormone released from the glands on the

occasion of the pupation remains to be utilized as the emergence hormone in such non-diapausing pupae as *Ephestia*-pupae.

At any rate, the contradictory ideas concerning the source of the metamorphosis hormone in lepidopterans can be abandoned, and our formula respecting the mechanism of lepidopteran metamorphosis can safely be applied to the small lepidopterans as well as to the large ones, although Piepho (1948) suggested the different source of metamorphosis hormone for the large and small lepidopterans; i. e., the hormone is secreted from the prothoracic glands in larger forms, while it comes from the brain in smaller ones. Our opinion is that the brain hormone is, at least in lepidopterans, the activating agent of the prothoracic gland, from which the metamorphosis hormone is given off. The data favourable to our opinion have been increasingly reported in insects belonging to other orders. Scharrer (1948) obtained the prothoracic gland in the orthopteran, *Leucophaea maderae*, and recently Wigglesworth (1951) discovered the existence of a similar gland in the hemipteran, *Rhodnius prolixus*, in which formerly he ascribed the source of the same hormone to the neurosecretory cells found in the protocerebrum of the brain. Possompès (1950) demonstrated that the lateral cells of the ring gland in the dipteran, *Calliphora erythrocephala*, which are considered to be homologous to the lepidopteran prothoracic gland, are induced to secrete the metamorphosis hormone by a factor liberated from the brain. Under these considerations, our formula of brain hormone \rightarrow prothoracic gland hormone \rightarrow metamorphosis, based on the experimental data in lepidopterans, can be extended to include some insects of other orders.

Summary

1. The prothoracic glands of *Ephestia cautella* are paired band-like organs situated in close proximity to the first spiracle of the prothorax. Each ordinarily consists of some thirty large glandular cells, with enormously large nuclei. The organ is not provided with any efferent ducts, but has a small trachea that comes from a main tracheal trunk at a point a little anterior to the first spiracle.

2. In the early stages of the mature larvae, the glandular cells are angular and rather small, with enormously large nuclei and scanty cytoplasm. The nucleus is densely packed with medium-sized granules. As, however, the developmental stages advance, the cells become distended and larger, mainly as the result of an increase of cytoplasm. A characteristic radial striation is discernible in such cytoplasm from the nucleus to the cell periphery. Ellipsoidal or spindle-shaped secretory droplets appear along these fine striae of the cytoplasm.

3. The cells in stage 6 seem to represent the most active phase of secretory function, as judged by the abundant occurrence of these droplets in the entire margin of the cells.

4. When the larvae are ready for, or have just finished pupation, involution

begins to take place in some glandular cells. This manifests itself by an atrophic appearance of the nuclei, a foamy contour of cell boundaries and the abundant appearance of large vacuoles in the cytoplasm. But there are some cells mixed with them that seem to be juvenile with respect to their secretory function. They will be related to the production of the emergence hormone.

5. When the pupae become aged, the prothoracic glands can hardly be found by dissection, although they are present in sections. In the freshly metamorphosed moth, the glandular cells show more severe degeneration than in the pupae.

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