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Neurosecretory Cells of Pars Intercerebralis in the Developing Pupa of Samia cynthia pryeri

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Abstract

Neurosecretory cells in the pars intercerebralis of the brains of the diapausing and developing pupae of *Samia cynthia pryeri* were observed. The brains obtained from the various states of the pupae were fixed with neutralized formalin solution and stained with GOMORI's chrome-haematoxyline-phloxin or with paraldehyde-fuchsin. Structural changes of the cells corresponding to the state of the diapausing or developing pupae were observed in the phloxinophilic medial neurosecretory cells of the pars intercerebralis. This fact seems to make more probable the possibility of the "B"-type medial neurosecretory cells in the pars intercerebralis of the pupal brain for the source of brain hormone at the activation of the diapausing pupa.

Introduction

The mechanism of the pupal diapause of the cecropia silkworm, *Hyalophora cecropia*, which is a closely related species with the cynthia silkworm, *Samia cynthia pryeri*, has been clarified by C. M. WILLIAMS ('46, '47, '48, '52), and that the mechanism of the pupal diapause of the cynthia silkworm closely resembles to that of the cecropia silkworm has been reported by S. FUKUDA('59). In both cases, the secretion of the pupal brain plays an important role in the termination of the pupal diapause.

It has been known that there are some groups of the neurosecretory cells in the insect brain, especially in the location of the pars intercerebralis. (SCHARRER, B. '52) These cells have been able to be recognized as the large cells containing the substance in the perikaryon which is stainable with GOMORI's chrome-haematoxyline-phloxin or with paraldehyde-fuchsin. They have been classified mainly according to their histochemical properties, shape and size, and the most common types of them are the so called A-type cell whose granules are stainable

dark purple with paraldehyde-fuchsin and deep blue with chrome-haematoxyline-phloxin, and the B-type cells whose granules are phloxinophilic and stainable red with chrome-haematoxyline-phloxin and green or bluishgreen with paraldehyde-fuchsin.

VAN DER KLOOT regarded the intercerebral neurosecretory cells of the cecropia silkworm as the source of the brain hormone which activated the prothoracic glands to secrete the hormone for the termination of the pupal diapause, although he did not show the histochemical data which correlated directly to the development of the pupa. ('55) In another giant silkworm, *Philosamia cynthia ricini*, M. ICHIKAWA reported the cyclic change of the granules in the intercerebral neurosecretory cells during the development of the pupa, but this pupa was non-diapausing. ('56) TAKEDA reported the disappearance of the granules in the "B-type" intercerebral neurosecretory cells corresponding to the breaking of diapause in the prepupa of a lepidoptrous insect, *Monema Flavescens*. ('71)

It has been reported that there is a critical period of the transplantation of the brain of the chilled pupa for the termination of the diapause of the recipient pupa in a giant silkworm, *Samia cynthia pryeri*. (KOENUMA, A. '68, '72, '73)

In this study, the histological change of the intercerebral neurosecretory cells was observed in the diapausing and the developing pupae of the giant silkworm, *Samia cynthia pryeri*.

Material and Methods

The pupae used in this study were those collected near Matsumoto city in late September, and kept in 26°C until the beginning of the chilling in 5°C. The duration of the chilling was over 10 weeks. After the chilling, they were transferred into an incubator kept 26°C.

The brain was isolated from the pupa at a proper duration of the incubation, and was fixed with neutralized folmalin. After the fixation, it was prepared in a paraffinized section of 10μ thick. The samples were stained with GOMORI's chrome-haemotoxyline-phloxin staining method chiefly, and some portion of them were stained with GOMORI's paraldehyde-fuchsin staining method.

Results

A transverse section of the pars intercerebralis of the brain of the diapausing pupa without chilling is presented in figure 1. In this figure, several neurosecretory cells in the median neurosecretory cell group were able to discriminate. These cells were about 20μ in diameter, and they had the nucleus stained strongly with phloxin. The cells, however, had not the stainable granules in the perikaryon. With the aldehyde-fuchsin staining, it was difficult to discriminate

such cells.

In figure 2, there are the medial neurosecretory cells of the pars intercerebralis of the brain of the pupa kept in prolonged chilling. In this condition, three cells with over 25μ in diameter were discriminated in each side of the pars intercerebralis. Of these cells, the medial cell was filled with the granules which were stained with phloxin remarkablly in the perikaryon, while the rests had the vacuolized cytoplasm without the stainable matter and the nucleus which was stained slightly with phloxin. This feature was different remarkablly from that of the brain of the non-chilled pupa.

Figure 3 indicates the section of the pars intercerebralis of the chilled pupa incubated for 3 days. The medial neurosecretory cells of this pupa were different from those of the unincubated chilled pupa. The phloxinophilic granules decreased from the medial cell, while the change in the rests of the neurosecretory cells was insignificant.

In figure 4, a section of the brain of the 6-day incubated chilled pupa is presented. In this stage of incubation, the phloxinophilic granules of the perikaryon of the neurosecretory cells of the pars intercerebralis seem to be almost disappeared. The phloxinophilic substance in the nucleus seems to be almost disappeared too.

A section of the brain of the 8-day incubated chilled pupa is presened in figure 5. In this figure, the medial neurosecretory cells of the pars intercerebralis contains a large amount of the granules stained strongly with phloxin in the perikaryon. In this case, the increase of the phloxinophilic granules in perikaryon seems to occur in the cells which contains a small amount of granules than the cell which cantains a large amount of the phloxinophilic granules previously.

In figure 6, a section of the pars intercerebralis of the 11-day incubated chilled pupa is indicated. In this case, there are no neurosecretory cells which are stainable with either phloxin or paraldehyde-fuchsin in the pars intercerebralis but there are quite large vacuoles. These vacuoles seem to be derived from the breaking down of the medial neurosecretory cells in the pars intercerebralis.

Such changes in the medial neurosecretory cells in the pars intercerebralis as those described previously were not able to be found in the preparations stained with paraldehyde-fuchsin.

In figure 7, there is another section of the brain of the non-chilled pupa. In this section, a small amount of the granules stainable with phloxin were found in the nerve fibres.

In figure 8, there is another preparation of the brain of the 8-day incubated chilled pupa. A large amount of the phloxinophilic material was found in the

nerve fibres in this preparation. This increase of the phloxinophilic material in the nerve fibres seems to correspond to the decrease of the phloxinophilic granules in the neurosecretory cell of the pars intercerebralis.

In the brains of the chilled pupae which were incubated over 6 days, a large amount of the haematoxylinophilic granular substance was found in the interstitial space.

Discussion

C. M. WILLIAMS discovered the role of the each component of the brain–prothoracic glands system upon the mechanism of the pupal diapause in the cecropia silkworm, *Hyalophora cecropia*. ('47, '52) The prolonged exposure of the diapausing pupa to the low temperatures before the incubation is required for the initiation of the adult development in this insect. A similar mechanism of the pupal diapause was found in another closely related species, *Samia cynthia pryeri*, by S. FUKUDA. ('59)

There is a critical period in which the pupal brain is indispensable to the termination of diapause. (WILLIAMS '47, '52, VAN DER KLOOT '56, KOENUMA '68) When the diapausing pupa initiates the imaginal development, it has been known that a hormone is secreted from the pupal brain at first, then another hormone is secreted from the prothoracic glands of the pupa in response to the brain hormone. (WILLIAMS '47, '52, FUKUDA '59) According to C. M. WILLIAMS, the diapausing non-chilled pupa implanted the precritical brain of the developing chilled pupa initiated the imaginal development, while the one implanted the postcritical brain of the developing chilled pupa did not initiate the development. ('47, '52) Similar results were obtained from cynthia pupae (KOENUMA '68, '73). WILLIAMS regarded the difference of the effect of the pupal brain on the recipient pupa in the transplantation experiments between the precritical brain and the postcritical brain as the difference between the endocrinological properties of the brains, for there was an evidence that the brain initiated the secretion of the hormone shortly before the critical period and it failed to secrete the hormone soon after the critical period.

In insect brain, it has been well known that there were some neurosecretory cells in the pars intercerebralis. (B. SCHARRER '52) In regard to the source of this brain hormone, VAN DER KLOOT noticed the neurosecretory cells in the pars intercerebralis of the pupal brain, but he did not observe the change in the neurosecretory cells with the process of the termination of the diapause('55). In the present study, it was shown that the medial neurosecretory cells in the pars intercerebralis of the cynthia silkworm changed its structure corresponding to the various states of the diapausing or developing pupae. This structural change

of the neurosecretory cells is not contradictory to the KOENUMA's previous results ('68, '73). Therefore, this seems to confirm the view that the intercerebral neurosecretory cells may be the source of the brain hormone for the termination of the pupal diapause in this cynthia silkworm.

According to HERMAN and GILBERT ('65), there are two types of neurosecretory cells in the pars intercerebralis of the cecropia brain, the A-type cells contains the granules stainable with haematoxyline and B-type cells contains the granules stainable with phloxin. In this study, it seems to be possible that the brain hormone which activates the prothoracic glands at the first step of the termination of the pupal diapause is secreted from the B-type cell in the medial neurosecretory cells in the pars intercerebralis of the insect brain, for the cells which changed corresponding to the change of the state of the pupal diapause were those cells contained the phloxinophilic granules in perikaryon. KOENUMA reported previously the inhibitory effect of the postcritical brain on the termination of the pupal diapause of the precritical pupa in the brain transplantation experiment ('73). In this study, the facts that the accumulation of the phloxinophilic granules in the cells was diphasic and occurred in different cells at each period seems to be favorable to interprete the KOENUMA's previous results.

TAKEDA, N. reported that the "B" cells of the medial neurosecretory cells in the pars intercerebralis changed their structure at diapause break of the prepupa of a lepidopterous insect, *Monema flavescens* ('72). In the present study, it was the "B" type cells of the medial neurosecretory cells in the pars intercerebralis that the structural change was observed at the termination of the diapause in the cynthia silkworm, *Samia cynthia pryeri*. It is quite interesting that a consistency was observed in the structural change of the medial neurosecretory cells in the pars intercerebralis at the termination of the diapause between the two different species.

In the brain of a giant silkworm, *Philosamia cynthia ricini*, which is a closely related species with *Samia cynthia pryeri* and which has not diapausing state during pupal stage, ICHIKAWA, M. reported the appearance of the large vacuoles in the intercerebral neurosecretory cells during imaginal development ('56). These vacuoles in the neurosecretory cells seems to be equivalent to those vacuoles in the medial neurosecretory cells in the pars intercerebralis of the pupal brain of the pryeri silkworm at the late stage of the incubation. The large vacuoles appearing in the intercerebral portion of the pupal brain of the incubated pupa at the postcritical period for the termination of the diapause seem to be the vacuole which drived from the collapse of the intercerebral neurosecretory cells, for no neurosecretory cell except the large vacuoles found in the pars intercerebralis of the pupal brain. This fact also seems to be well interpretable

to the effect of the postcritical brain of the incubating chilled pupa that the inhibition of the termination of the diapause of the precritical recipient pupa resulted from the transplantation of such brain.

Thus it may be concluded from the previous discussion that at least one of the brain hormone may be secreted from the "B-type" cells in the medial neurosecretory cells located in the pars intercerebralis at the termination of the pupal diapause in the cynthia silkworm, and the hormone or the precurcor of the hormone may be accumulated during the exposure of the diapausing pupa to the low temperatures, then it may be released shortly before the critical period of the termination of the diapause during the incubation, finally the cells may collapse.

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Plate.

Fig. 1 A transverse section of the pars intercerebralis of the non-chilled diapausing pupa of *Samia cynthia pryeri*. Arrows indicate the medial neurosecretory cells. (chrome-haematoxyline-phloxin staining)

Fig. 2 Medial neurosecretory cells in the pars intercerebralis of the chilling pupa. (chrome-haematoxyline phloxin staining)

Fig. 3 Medial neurosecretory cells in the pars intercerebralis of the 3-day incubated chilled pupa. (chrome-haematoxyline phloxin staining)

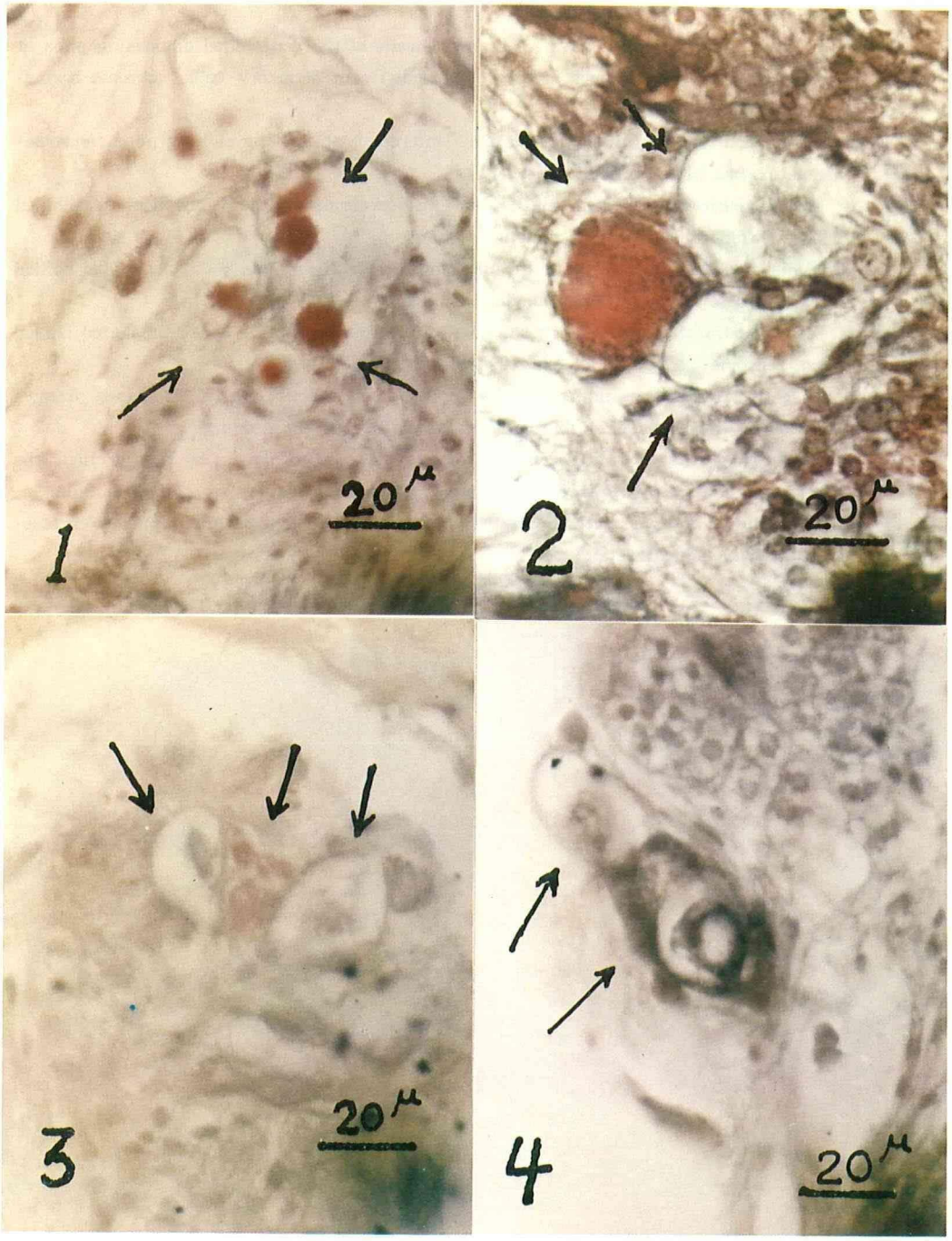
Fig. 4 Medial neurosecretory cells in the pars intercerebralis of the 6-day incubated chilled pupa. (chrome-haematoxyline phloxin staining)

Fig. 5 Medial neurosecretory cells in the pars intercerebralis of the 8-day incubated chilled pupa. (chrome-haematoxyline phloxin staining)

Fig. 6 A section of the pars intercerebralis of the 11-day incubated chilled pupa. Arrows indicate the large vacuoles. (chrome-haematoxyline-phloxin staining)

Fig. 7 A section of the pars intercerebralis of the non-chilled pupa. In this section, the portion distributed nerve fibres is indicated. (chrome-haematoxyline-phloxin staining)

Fig. 8 A section of the pars intercerebralis of the 8-day incubated chilled pupa. (chrome-haematoxyline-phloxin staining)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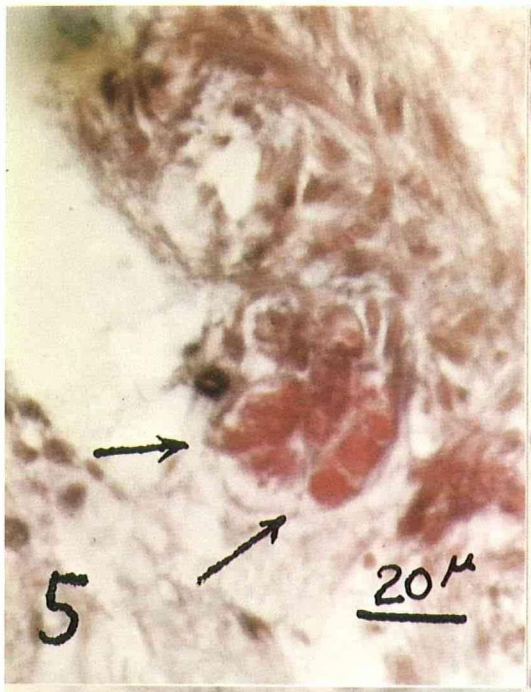
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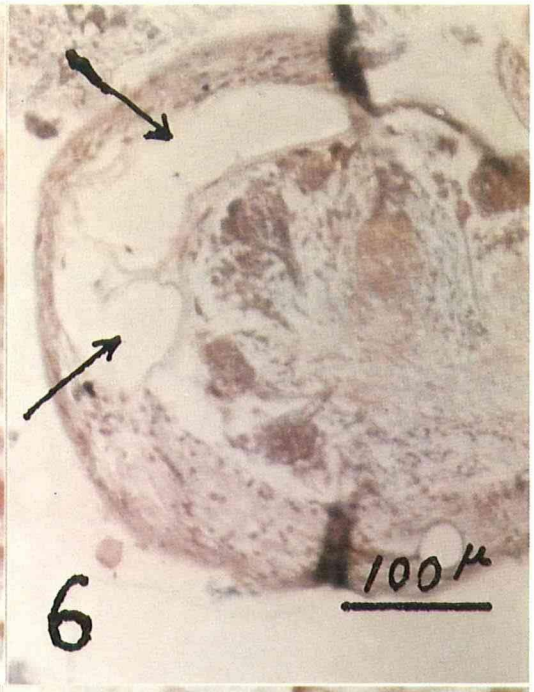
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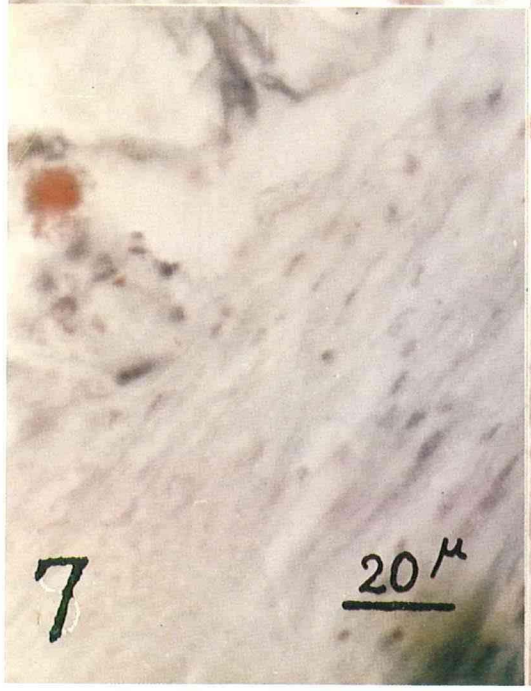
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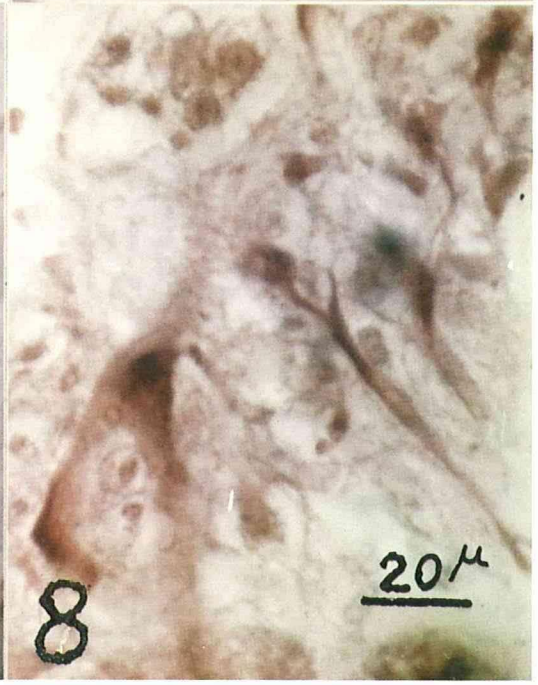
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