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The Effect of Cholesterol on the Adult Development of Diapausing Pupae of the Giant Silkmoth, Philosamia cynthia cynthia

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Abstract

Philosama cynthia cynthia is one of those insects whose pupal diapause is determined by the endocrine activity of the neurosecretory cells of the brain.

Two substances were extracted from the brain of *Bombyx mori* independently, and they were identified with the "brain" hormone of insects concerned with their metamorphosis. One of them was reported as cholesterol.

This paper presents the result of the experiments carried out to examine the hormonal action of cholesterol on the development of the diapausing pupa in *Philosamia cynthia cynthia.*

In one group of the experiments, various doses of cholesterol suspended in 0.02ml of 9% ethyl alcohol were injected into the fourth or fifth abdominal segment of the diapausing pupa, then they were incubated at 25°C for about 250 days. Cholesterol by Nutritional Biochemical Co. (N.B.C.) U.S.A. and by Tokyo Kasei Kogyo Co. Tokyo was used. In the other group of experiments, cholesterol was injected to the decerebrated pupa.

Any evidence suggesting the hormonal action on the development of the diapausing pupa was not obtained from the experiments in which cholesterol was injected to the pupa, since none out of the 20 pupae developed to imago in all cases when they were incubated at 25°C for about 250 days.

Therefore, as far as present experiments were concerned, it seems to be improbable that pure cholesterol has some effect on the pupal diapause in *Philosamia cynthia cynthia.*

Introduction

Philosamia cynthia cynthia is the one of those insects that the beginning and

ending of their pupal diapause are highly concerned with the change of the endocrine activity of their brain.[2] The role of the brain on the pupal diapause of this insect is consistent with that of another giant silkworm, *Platysamia cecropia*, in which the role of the endocrine activity of the brain on the beginning and ending of pupal diapause has been clearly shown by C. M. WILLIAMS.[9-11]

Two different substances were extracted from the brain of silkworm, *Bombyx mori*, independently, and reported as the "brain" hormone of the insects concerned with metamorphosis respectively.[3,4,6-8]

According to KOBAYASHI et al., the "brain" hormone extracted from the silkworm is identical with pure cholesterol.[8]

According to the studies on the diapause of cecropia silkworm by WILLIAMS,[9-11] the "brain" hormone does not show a species-nor genus-specificity, but it shows a common effect on metamorphosis of different lepidopterous genera or species. Moreover, it was reported by FUKUDA[2] that the brains of silkworm, *Bombyx mori*, were effective on the adult development of the diapausing pupae of giant silkmoth, *Ph. c. cynthia*, when the former organs were implanted into the latter. Therefore, if the effective result on the development is obtained from the experiment in which the substance concerned is injected to the diapausing pupae of *Ph. c. cynthia*, it becomes highly probable that the substance is identical with the true "brain" hormone.

In this paper, there are some results from the experiments attempted to test the effect of cholesterol on the adult development of the diapausing pupae of *Ph. c. cynthia*.

Material

Pupae of *Ph. c. cynthia* used for experiments were collected near Matsumoto city early in September. The ages of the pupae at the injection of cholesterol were supposed 2 to 14 days after pupation. The pupae were divided into two groups, the one for experiments, and the other for control, where the pupae were kept in a cotton bag hanging outdoor without direct sunshine. In latter, 28 out of 35 pupae developed into imagos between next June 10. and July 2., and the maximal emergence to moth occurred on June 18..

When the intact pupae were incubated at 25°C, none out of 80 pupae developed into imago throughout the period of the observation during about 250 days. But they were survived throughout the period.

Experiments

a) *The effect of cholesterol injected to the diapausing pupae of Philosamia cyntiha cynthia.*

Various doses of cholesterol suspended in 0.02ml of 9% ethyl alcohol were injected into the fourth or fifth abdominal segment of the diapausing 20 pupae in each group, and the effect on the adult development of them incubating at

25°C was observed during about 250 days. The doses of cholesterol injected to each pupa were 2, 10, 20, 50 and 100$^{\mu g}$ respectively. In the case of 100$^{\mu g}$, cholesterol was injected to each pupa with 0.04^{ml} of 9% ethyl alcohol containing the dose of cholesterol.

Two kinds of cholesterol were tested, the one was prepared by Nutritional Biochemical Co. (N.B.C.) U.S.A. and the other by Tokyo Kasei Kogyo Co. Tokyo.

The control experiments, the effect of the mere alcohol injection without cholesterol was observed in the two cases of 0.02^{ml} and 0.04^{ml} of 9% ethyl alcohol injection respectively.

Results were present in Table 1. In this table, it was clearly shown that none of the diapausing pupae injected cholesterol was developed into imago in all cases.

Though the internal structure of the pupae incubated at 25°C for 250 days after the treatments indicates slight development, the hormonal action of cholesterol is not reliable (figures 1 to 9).

Table 1 Effect of cholesterol injected into the diapausing pupae of *Philosamia cynthia cynthia.*

Dose of cholesterol	No. of Pupae injected	No. of Pupae developed	No. of Pupae survived with 250 days	No. of Pupae dead within 250 days
100$^{\mu g}$*	20	0	18	2
50 *	20	0	18	2
20 *	20	0	17	3
10 *	20	0	19	1
2 *	20	0	19	1
100 **	20	0	17	3
50 **	20	0	19	1
20 **	20	0	17	3
0 ***	20	0	14	6

(Note)

* Cholesterol was prepared by N. B. C. U.S.A.

** Cholesterol was prepared by Tokyo Kasei Kogyo, Tokyo.

*** 0.02^{ml} of 9% ethyl alcohol without cholesterol was injected to each pupa.

b) The effect of cholesterol injected to the decerebrated diapausing pupae of Philosamia cynthia cynthia.

Various doses of cholesterol suspended to 9% ethyl alcohol were injected to the decerebrated diapausing pupae. Each pupa was injected 0.02^{ml} of 9% ethyl alcohol containing a given dose of cholesterol into the fourth or fifth abdominal

segment, and it was incubated at 25°C. The doses of cholesterol tested were 10γ and 20γ. In control, the decerebrated pupa was injected 0.02ml of 9% ethyl alcohol only and they were incubated at 25°C.

When the brain, supraoesophageal ganglion, of the diapausing pupa was removed, the spontaneous movement of the pupa became active and continuous.

Results were present in Table 2. It is clear that none of the decerebrated puae injected cholesterol developed into the imago during the long observed period of about 250 days.

Figures 10 to 12, show the internal structure of the decerebrated pupa incubated for 250 days. No suggestive appearance of the adult development of the pupa was present in these figures.

Table 2 Effect of cholesterol injected into the decerebrated pupae of *Philosamia cynthia cynthia.*

Dose of cholesterol	No. of pupae injected	No. of pupae developed	No. of pupae survived	No. of pupae dead within 250 day
20 *	20	0	12	8
10 *	20	0	18	2
20 **	20	0	18	2
0 ***	20	0	17	3

(Note)

* Cholesterol was prepared by N. B. C. U. S. A.

** Cholesterol was prepared by Tokyo Kasei Kogyo, Tokyo.

*** 0.02ml of 9% ethyl alcohol was injected without cholesterol.

Discussion

According to WILLIAMS,[11] the pupal diapause of the giant silkworm, *Platysamia cecropia* is controlled by the pupal brain endocrinologically in the following way that the pupal dormancy is caused by the decrease of the "growth and differentiation" hormone secreted from the prothoracic gland, and this decrease of the hormone is caused with the decrease of the "brain" hormone secreted from the brain of the insect, and the ending of the pupal dormancy is caused with the incaease of the "growth and differentiation" hormone secreted from the prothoracic gland triggered by the "brain" hormone secreted to recover its endocrine activity by the long exposure to low temperatures.

He showed also that the "brain" hormone was not species nor genus-specific at least in Lepidoptera, for the same result was optained by the implantation of the brain of other lepidopterous species or genera.

It was clearly shown by FUKUDA,[2] that the difference of the pupal fate between two nearly related silkmoths, *Ph. c. ricini* and *Ph. c. cynthia* was based

upon the difference of their cerebral chracterisics. In the former, there is no pupal diapause, and the pupa develops to imago promptly, whereas in the latter, the pupation is followed by a long pupal diapause. In ricini, the facts that the removal of the pupal brain soon after the pupation is followed by its dormancy, and the cerebral implantation to the diapausing pupa is followed the end of the diapause, clearly show that in ricini the pupal brain keeps its endocrine activity for the further development of the pupa. In cynthia, the adult development of the pupa occurs when the pupa is implanted the brain from the pupa chilled or from that of non-diapausing species. The facts that the adult develepment of the decerebrated pupa of *Ph. c. ricini* occurred when the brain of the chilled pupa of *Ph. c. cynthia* was implanted, but the development did not occur when the brain of the diapausing pupa of *Ph. c. cynthia*, suggest that the cerebral endocrine activity decreases in the diapause, but the chilling increases the activity. From these facts, it is highly probable that the mechanism of the pupal diapause in *Ph. c. cynthia* is almost consistent with that of the cecropia silkworm.[9, 11]

It is reasonable to estimate that the pupae used were in normal condition, for they developed into imagos after they were kept through winter, but none out of 80 pupae developed into imago after incubation at 25°C since earler stage of the pupa.

From the brains of the pupae of the silkworm, KOBAYASHI et al.[8] succeeded to extract an effective substance for the decerebrated pupa of the silkworm to develop to the adult. According to them, this substance was the "brain" hormone of the insect, and it was identical with cholesterol. If the "brain" hormone is identical with cholesterol, the adult development will occur, when pure cholesterol is injected to the diapausing pupa. Therefore, when pure cholesterol is injected to the diapausing pupa of *Ph. c. cynthia* kept at such high temperatures as the diapause does not end, the pupa may be expected to develop into an imago. As far as present experiments were concerned, no diapausing pupa developed into an imago, when cholesterol was injected to the diapausing pupa of *Ph. c. cynthia* incubating at 25°C. This result was inconsistent with that of the experiments of KOBAYASHI et al. in which the positive effect occurred on the development of the decerebrated pupae of silkworms with injection of 0.02μg cholesterol isolated from their brains. A possibility that the pupae did not develop into imagos by the injury in the treatment may be excluded by the following facts that the remarkable increase of death rate of treated pupae did not occur for 250 days, and that the pupae not treated also did not develop into imagos when they were kept at 25°C.

Any suggestive appearance for the internal structure of the pupa incubated at 25°C for about 250 days after the injection of cholesterol to develop to adult could not be found clearly. There was, however, a slight sign which suggested the fact that cholesterol was more effective on the intact pupa than on the decerebrated pupa. From this fact, it may be shown a possibility that the

injection of a more abundant dosage of cholesterol may produce the adult development of the diapausing pupa of *Ph. c. cynthia*. From the result of present experiments, it may be probable to conclude that the "brain" hormone concerned with the pupal diapause of this insect is not the pure cholesterol, for the fact that no ending of diapause occurred in spite of the injection of such large dosage of cholesterol exceeding a hundred times as much as the dosage of KOBYASHI et al. in *Bombyx mori*, whereas the ending occurred with the implantation of only a pupal brain chilled in FUKUDA's experiment.[2]

Another much effective preparation of "brain" hormone in a crystalline form has been obtained from brains of *Bombyx mori* by ICHIKAWA and ISHIZAKI.[3] According to them[4] the substance has protein nature.

One of the active substances on the insect metamorphosis has been isolated in crystalline form from the pupae of *Bombyx mori* by BUTENADT and KARLSON.[1] It was named ecdyson after its action on the ecdysis of insect and was identified with the "growth and differentiation" hormone secreted from the prothoracic gland of lepidopteran insects.

An evidence in which cholesterol is a precursory substance of ecdyson has been obtained from Calliphora larva by KARLSON, P. and H. HOFFMEISTER.[5] It seems to be very interesting fact that the "brain" hormone obtained from *Bombyx mori* by KOBAYASHI et al. was identical with cholesterol being a precursory substance of ecdyson, since "brain" hormone plays a very important role for the initiation of the adult development of the diapausing pupae of cecropia silkworm or its nearly related insects by the prothoracotropic action. This fact does not, however, imply that no substance besides cholesterol is the brain hormone of insects. In this regard, it seems to have significance that an effective substance quite different from that of KOBAYASHI et al., has been obtained from the brains of *Bombyx mori* by ICHIKAWA.

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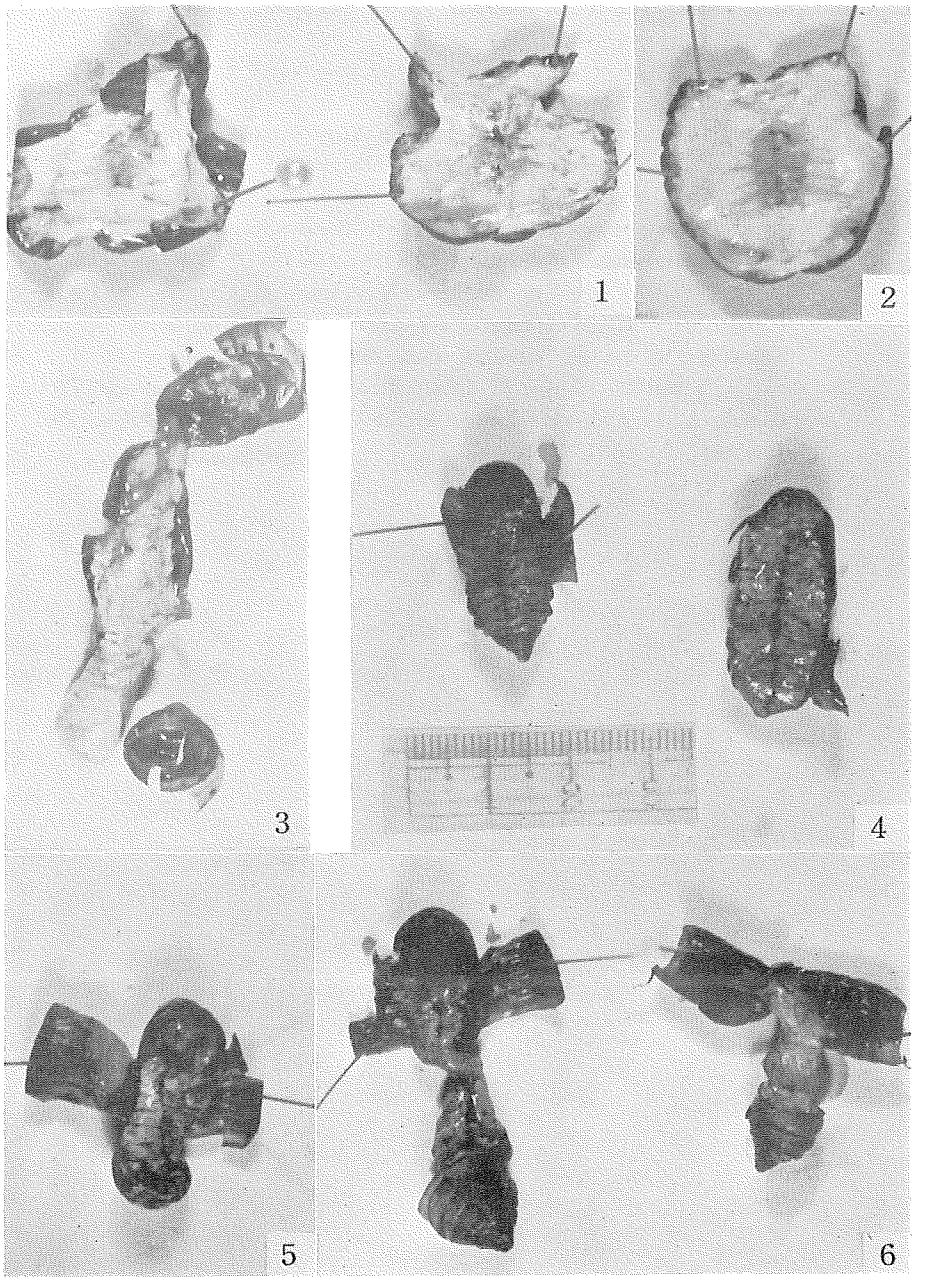
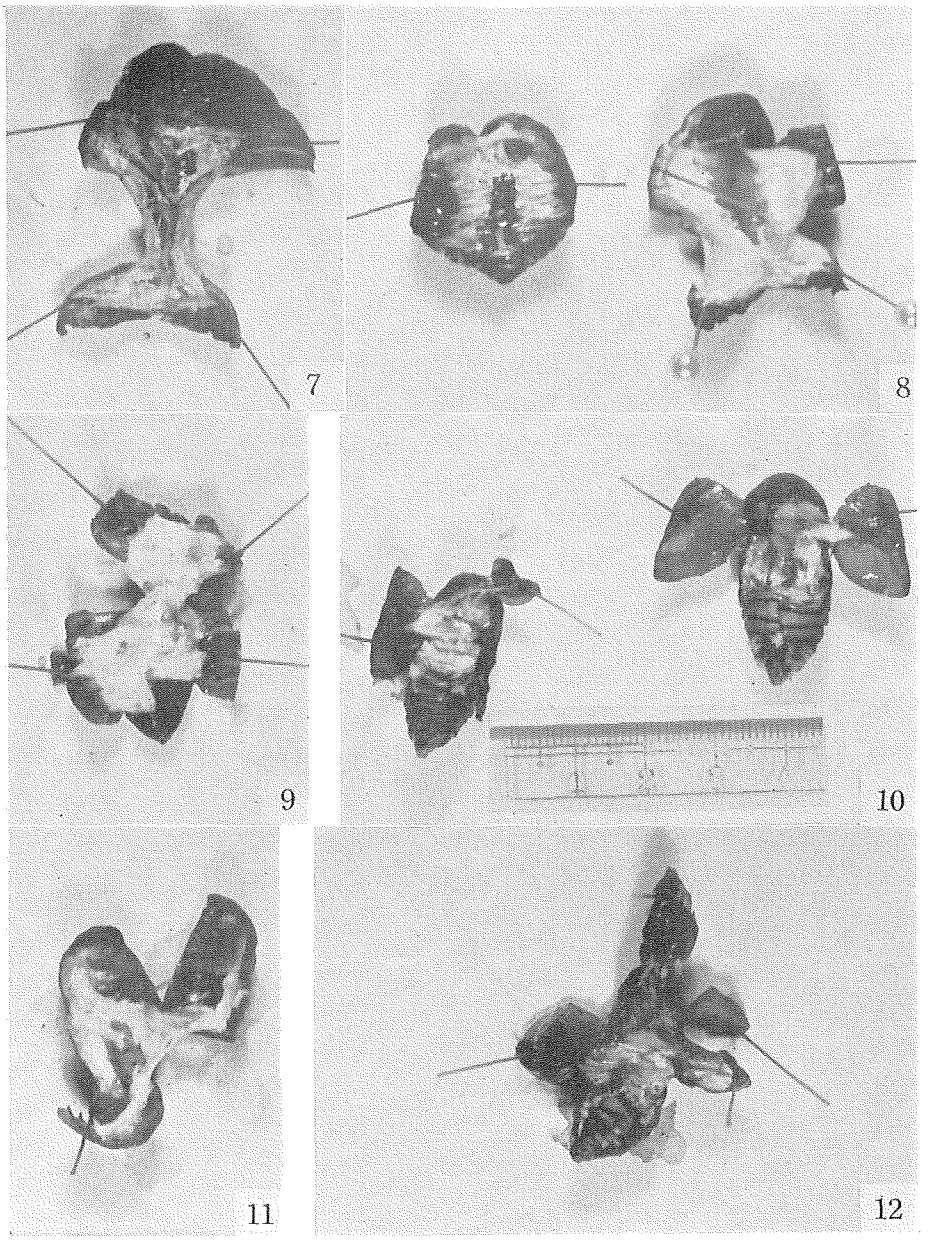


Figs. 1~6 Internal structure of the pupae incubated at 25°C for about 250 days. (*Ph. c. cynthia*)
1: intact pupae, 2: pupa injected 9% ethyl alcohol, 3: pupa injected 2μg of cholesterol (NBC.), 4: pupae injected 10μg of cholesterol (NBC.), 5: pupa injected 20μg of cholesterol (NBC.), 6: pupae injected 20μg of cholesterol (Japan).



Figs. 7~9 Internal structure of the pupae incubated at 25°C for about 250 days. (*Ph. c. cynthia*)
7: pupa injeted 50㎍ of cholesterol (NBC.), 8: pupae injected 50㎍ of cholesterol (Japan), 9: pupa injected 100㎍ of cholesterol (Japan).

Figs. 10~12 Internal structure of the decerebrated pupae incubated at 25°C for about 250 days. (*Ph. c. cynthia*) 10: pupae injected 9% ethyl alcohol, 11: pupa injected 10㎍ of cholesterol (NBC.), 12: pupa injected 20㎍ of cholesterol (NBC.).