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Study on the Endocrine Activity of *Bombyx-Allatum*
in the Diapausing *Philosamia-Pupa*¹⁾

By

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In the previous paper we have revealed that in *Philosamia cynthia ricini* the implants of corpora allata isolated from pupae can cause realization of adult characters of the host pupae in which the diapause has been artificially induced by the removal of the brain, while the implants from the adults and the fourth instar larvae can bring about an extra pupal moult of the recipient pupae (ICHIKAWA and NISHITSUTSUJI-UWO, 1959). This finding indicates that the implanted corpus allatum of *Philosamia* can furnish, in addition to the juvenile hormone, the principle which terminates the diapause of pupa deprived of its brain. We are of the opinion that this principle is the brain hormone stored in it, because, on one hand, of the existence of a neurosecretory pathway connecting the neurosecretory cells in the brain with the corpora-cardiaca-allata, and on the other, of the evidence that the diapausing pupae can not develop for more than one year, unless the brain hormone is supplied by the implantation of the active brains.

In order to gain more information in favour of our opinion, the present experiments were performed, using the domestic silkworm, *Bombyx mori*, as the donor species of corpora allata.

Materials and Methods

Surgical procedures were quite the same as in our previous experiments. Under etherization, pupae of *Philosamia cynthia ricini* were deprived of their brains not later than 19 hours after pupation. Brainless pupae thus operated upon had continued to diapause for 224-269 days before they were used as the test animals. Corpora allata to be tested were removed from the fifth instar larvae and adults of the various races of *Bombyx mori* with sharply pointed forceps of watchmaker under the dissecting binocular microscope. The racial difference seemed not to alter the results.

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Six to ten corpora allata were implanted into each test animal through a small hole inflicted in the dorsal integument of the second or the third abdominal segment. After implantation, the cut piece of integument was put upon the hole and sealed up with melted paraffin. Operated specimens were kept at room temperatures of about 25°C.

Results

1) *Implantation of corpora allata from larval donors.*

As the donors of corpora allata, the larvae in two different stages were used; i.e., those in a mature stage and those in a stage two days before maturation. Despite the difference of stage, the results were essentially the same, as is shown in Table 1.

Table 1. Tests of larval corpora allata of *Bombyx* in brainless diapausing pupae of *Philosamia*.

Donors	Number of implanted organs	Number of experimental specimens	Number of deaths or undeveloped cases	Number of adults	Number of mixed forms
Mature larvae	10	20	0	3	17
				(100%)	
Premature larvae (2 days before maturation)	8	29	7	14	8
				(75%)	

From our previous experience, a lot of re-pupation in the recipients were expected, but there were none which underwent the complete re-pupation.

A half of the recipients developed into pupal-adult mixtures within 15–17 days after implantation. Generally speaking, the anterior part of the animal was imaginal, while the posterior part, re-pupal. But, the vertex of head was exceptional which was equipped invariably with a small patch of the second pupal cuticle. Some of the thoracic and abdominal segments were also a mosaic of pupal and adult characters. In the adult part there were many scales and hairs. Caudal end of the body was complex in structure and different from either pupal or adult one. It retained rather pupal characters in many points. Detailed description of these mixed forms will be given on later occasion.

Within 15–28 days, 17 recipients transformed into moths, though they could not shed their old pupal cuticle by themselves. Some of them discharged the fluidal meconium, as did the normal moths when they emerged from the cocoon.

Five out of 7 residual specimens showed no effect of the implants and remained unchanged. Two died without differentiation.

2) *Implantation of corpora allata from adult donors.*

The heads of female or male moths whose adult age was 1–3 days were cut

off with scissors in the insect Ringer solution. A pair of corpora allata, locating side by side on the dorsal wall of the gut, revealed itself in the cut surface with a little practice in decapitation, so that it was easily picked up with forceps. Six corpora allata were implanted, as before, into each diapausing pupa. The results are summarized in Table 2.

Table 2. Tests of adult corpora allata of *Bombyx* in brainless diapausing pupae of *Philosamia*.

Number of implanted organs	Number of experimental specimens	Number of undeveloped cases	Number of second pupae	Number of mixed forms
6	17	3	0	14 (82%)
Control*	10	0	0	0

* Diapausing pupae without implantation of any organs.

In this case the second pupae were also anticipated to occur after moulting, since the adult corpora allata in *Philosamia* had been demonstrated to have a great activity of producing the juvenile hormone. But as is shown in the table, all the test specimens developed into pupal-adult mixtures. This is probably due to low concentration of the juvenile hormone from *Bombyx* implants. There remains, therefore, a possibility that the second pupae will be produced by augmenting the implanting corpora allata.

In the present experiments we have lacked the specimens which could demonstrate the endocrine activity of corpora allata in the pupal stage. However, we can easily surmise it from the result of experiment with the organs from the fifth instar larvae. Namely, the fact that some of the specimens in this group developed into adults indicates that the implanted corpora allata were inactive or at least not so active as to produce an appreciable amount of the juvenile hormone, only that they furnished the brain hormone which evoked the development in the brainless pupae. Pupal corpora allata are also considered to be inactive in respect to the release of the juvenile hormone. Consequently, the implants from the pupal donors would be easily expected to induce the adult development in recipients.

Discussion

In our previous experiments, the diapausing brainless pupae of *Philosamia* which had received 4 to 6 corpora allata from the larval or adult donors of the same species moulted again into the second pupae. But in the present experiments the similar brainless host animals which had received 6 to 10 *Bombyx* corpora allata transformed in a high percentage into mixtures of pupal and adult characters. This may be due to low concentration of the juvenile hormone released by the

Bombyx corpora allata which are smaller in size or due to their subnormal activity in the heteroplastic environment of *Philosamia*.

Recently, WILLIAMS (1959) has reported quite similar "bizarre creatures" obtained by implanting one to three pairs of adult corpora allata into the brainless pupae. In his case implantation was carried out among the individuals belonging to the same species of *Hyalophora cecropia*. Therefore, our elucidation for the formation of a monster mixed with pupal and adult characters may or may not be right. At any rate, it will be right for one to expect the second pupa from a test animal in the presence of higher concentration of the juvenile hormone.

By far the important matter in the present experiments is, however, neither the formation of bizarre creatures nor the production of the second pupae, but it is the fact that implants of *Bombyx corpora allata* have induced the moulting in the brainless *Philosamia* pupae, which otherwise would continue to diapause. In other words, *Bombyx corpora allata* can furnish a factor responsible for the termination of diapause. WILLIAMS (l. c.) has assumed with reserve that the factor secreted from the corpora allata can mimic the brain hormone. We believe, however, as stated in our previous paper, that the factor is nothing but a brain hormone transported, via axons, from the neurosecretory cells and stored in them.

Summary

Implants of *Bombyx corpora allata* derived from adult, mature and premature larvae were able to induce the moulting in the brainless, diapausing *Philosamia* hosts, which otherwise would have remained unchanged. The moult induced was or was not coupled with adult development depending presumably upon the amount of the juvenile hormone. The most frequent occurrence was the pupal-adult mixtures.

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