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Studies on the Insect Metamorphosis
VII. Effect of the Brain Hormone to the Isolated Abdomen of the Eri-Silkworm, *Philosamia cynthia ricini*

By

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In the previous papers we stated repeatedly that the brain hormone is possessed of a prothoracotropic action, and further that it is equally effective in its action regardless of whether it is released directly from the brain or from the corpus allatum after it has been stored in it (ICHIKAWA and NISHIITSUTSUJI-UWO, 1959; ICHIKAWA and TAKAHASHI, 1959). In one series of these experiments we tried the implantation of the brains, brain-cardiaca-allata complexes, or corpora allata, either alone or together with the prothoracic glands, into the isolated abdomen. The former experiment was performed as the control to the latter with an anticipation that the isolates would remain unchanged. To our surprise, however, the controls yielded a quite similar result to that of the aimed experiment, namely, for example, the isolates which had received 3 adult brains metamorphosed into adult forms and those implanted with 3 brain-cardiaca-allata complexes underwent generally an additional pupal molting. The difference between the aimed and control experiments was a slight prolongation in the latter of the time that was required for the respective re-pupation or adult formation. These phenomena were incomprehensible in the light of our knowledge of the mechanism involved in the insect metamorphosis so far obtained, because of the fact that the isolated abdomens were all cut apart from the thorax prior to the critical period of the prothoracic gland hormone for adult differentiation. The finding tempted us to repeat the same experiment again. This paper is concerned with the results of these repetitions during the past three years.

Material and Method

The isolated pupal abdomens of the Eri-silkworm, *Philosamia cynthia ricini*, were used as the test animals. After deep etherization, the anterior half of the pupal body was generally cut off with a sharp razor blade through the level between the first and second abdominal segments within 4 to 50 hours after pupation, since the critical period of the prothoracic gland hormone for emergence had been proved to be at 75–85 hours after pupation at 27°C. The cut wound of the posterior half was capped with a slip of paraffin plate and completely sealed by a hot needle. In the case of younger pupae less than 3 hours after pupation, the transection was rather difficult, so the ligation was applied to the same level of the abdomen before cutting. Following a storage of more than 2 weeks at about 25°C, the isolates were provided each with 3 brain-cardiaca-allata complexes, 3 brains or 6 corpora allata. The isolates which had received 2 prothoracic glands plus 1 brain, 2 prothoracic glands alone or 3 suboesophageal ganglia served as the control.

The organs to be tested were excised from the following animals: 1) mid- or late-fourth instar larvae, 2) fifth instar larvae which were in the spinning stage, 3) pupae which were ready to initiate the adult formation and 4) adults one day before or after emergence.

The procedures of implantation were the same as described in our previous communications (ICHIKAWA, NISHIITSUTSUJI and YASHIKA, 1955; ICHIKAWA and NISHIITSUTSUJI-UWO, 1959). After implantation, the specimens were placed in the glass containers and kept again in the incubator at about 25°C.

Results

1. *Implantation of the brain-cardiaca-allata complexes.*

Three complexes were implanted into each isolated abdomen. The results are different according to the source of implants, as is seen in Table 1. That is to say, the complexes removed from the pupae can cause the realization of

Table 1. Implantation of brain-cardiaca-allata complexes into isolated pupal abdomens.

Stage of donors	Number of implanted complexes	Number of experimental specimens	Number of death or non-development	Number of adult formation	Percentage of adult formation	Number of repupation	Percentage of repupation	Average days required for repupation or adult formation
Adult	3	24	19	0	—	5	20.8	29.0
Pupa	3	24	15	9*	37.5	0	—	40.9
5th instar larva	3	26	15	0	—	11	42.3	30.6
4th instar larva	3	17	9	0	—	8	47.1	29.4

* One is a mosaic of adult and pupal characters.

adult characters (37.5%), while those coming from the adults and larvae can bring about an additional pupal molting. This difference of the developmental pattern is obviously due to the conjoint action of the juvenile hormone released from the implanted corpora allata of the adults and larvae. The percentage of re-pupation differs much due to variety of the source of implants whence they come; i.e., the complexes derived from the fourth instar larvae are the most effective in the event (47.1%), those of the fifth instar larvae come next (42.3%) and the adult complexes are the last (20.8%). But we are rather sceptical to believe this order to reflect the actual endocrine activity of the complexes, because, as is seen in the last column of the table, the days required for re-pupation are nearly the same, regardless of the stage difference of the implants. It will be significant, however, that the time required for adult formation is longer than that of re-pupation, for it accords with the time-table of the normal development of this moth.

At any rate, it will be a magnificent event that the isolated abdomen, remaining otherwise unchanged, can proceed to the differentiation for a moth or second pupa without the help of the prothoracic gland hormone. The finding leads us to surmise that the principal factor responsible for the development of the isolated abdomen will be the brain hormone, produced in the neurosecretory cells in the brain and stored in the corpora allata. In order to get some evidence for this assumption we excised and tested the brains and corpora allata separately.

2. *Implantation of the brains.*

The brains to be tested were derived from the individuals in various stages of development, but they yielded substantially the same result of imaginal differentiation of the recipients, as in the previous experiment. In this experiment, as can be seen in Table 2, the brains coming from the fifth instar larvae are

Table 2. Implatnation of brains into isolated pupal abdomens.

Stage of donors	Number of implanted brains	Number of experimental specimens	Number of death or non-development	Number of adult formation	Percentage of adult formation	Average days required for adult formation
Adult	3	28	20	8	28.6	44.3
Pupa	3	29	21	8	27.6	37.0
5th instar larva	3	20	12	8	40.0	29.9
4th instar larva	2, 3	37	26	11	29.7	47.6

proved to be the most effective in terms not only of the percentage of the positive cases but also of the time that was required for the adult development, and also to be equally effective when compared with the brain-cardiaca-allata complexes of the same fifth instar larvae in the previous experiment. The brains coming from other 3 stages are approximately the same in effectiveness.

In short, a total of 35 out of 114 preparations indicates the adult formation, and the remaining 79 specimens show no effect of the implants or died.

3. *Implantation of the corpora allata.*

Since our previous experiments using the brainless pupae as the test animals have demonstrated that the corpus allatum substitutes for the corpus cardiacum in the storage function of the brain hormone in such Lepidopterous insects as *Philosamia* and *Bombyx*, next the corpora allata were tested in the isolated abdomens. The results are recorded in Table 3.

Table 3. Implantation of corpora allata into isolated pupal abdomens.

Stage of donors	Number of implanted corpora allata	Number of experimental specimens	Number of death or non-development	Number of adult formation	Number of re-pupation	Percentage of re-pupation	Average days required for re-pupation
Adult	6	28	24	0	4	14.3	20.0
Pupa	6	21	21	0	0	—	—
5th instar larva	6	24	23	0	1	4.2	27.0
4th instar larva	4, 6	36	33	0	3	8.3	25.0

As is clear from the table, the implantation is proved to give the same result as anticipated, but effect of it is very slight beyond expectation, even the adult corpora allata, though being most effectual in this series of experiment, show only 14.3 per cent of the positive cases. The pupal corpora allata give the negative tests in all 21 isolates, due presumably to their unhealthy condition to react to the molt inducing factor coming from the implants.

4. *Implantation of the prothoracic glands together with or without the brain into the isolated abdomens.*

The prothoracic glands were excised from the fifth instar larvae and tested alone or in conjunction with the brain in the isolated abdomens with the aim of comparing the sequence of event caused by the regular growth and differentiation hormone coming from the prothoracic glands with that induced by the possible brain hormone so far described. The results are arranged in Table 4.

Table 4. Implantation of prothoracic glands with or without brain into isolated pupal abdomens.

Implanted organs	Number of experimental specimens	Number of death or non-development	Number of adult formation	Percentage of adult formation	Average days required for adult formation
2 prothoracic glands plus 1 brain	24	11	13	54.2	40.8
2 prothoracic glands	36	21	15	41.7	40.5

The table indicates that when implanted with two glands together with one brain, 54.2 per cent and, when with two glands alone, 41.7 per cent of the test preparations underwent the adult formation. These percentages are higher, while the days required for the adult differentiation are not necessarily shorter than in the case of the brain implantation. Strictly speaking, however, it is impossible to compare the results of the heterogeneous experiments, because of the lack of measure of how many prothoracic glands being equivalent to how many brains in the present phenomena. By increasing the number of the prothoracic glands, the percentage of the positive cases will be expected to become higher within a certain limit.

5. *Implantation of the suboesophageal ganglia into the isolated abdomens.*

The suboesophageal ganglia were dissected off from the adults and the fifth instar larvae and planted into the isolated abdomen to see whether the presumably indifferent organ to the present matter could cause the development of the recipient. These isolates indicate, as expected, no developmental response before as long as 100–180 days, as is recorded in Table 5. In addition, this means that the mere wound infliction can not be the cause for the initiation of development of the isolated abdomen.

Table 5. Implantation of suboesophageal ganglia into isolated pupal abdomens.

Stage of donors	Number of implanted ganglia	Number of experimental specimens	Number of death or non-development	Number of adult formation	Number of re-pupation
Adult	3	29	29	0	0
5th instar larva	3	9	9	0	0

Discussion

The present experiments with the Eri-silkworm, *Philosamia cynthia ricini*, have revealed that the isolated pupal abdomen, when provided with the brains, corpora allata or brain-cardiaca-allata complexes, can give rise to either the adult form or the second pupal form according to the absence or presence of the juvenile hormone released from the corpora allata. These results are substantially identical to those in our previous experiments using the brainless diapausing pupae as the test animals (ICHIKAWA and NISHIITSUTSUJI-UWO, 1959; ICHIKAWA and TAKAHASHI, 1959). But here it must be emphasized that the percentage of the positive response to the implants is far less in the present experiment than in the previous, but contrarily, the time required for the re-pupal or imaginal development is much longer in the isolated abdomen. This is due apparently to the fact that the isolated abdomen lacks the prothoracic gland that is the center of production and release of the growth and differentiation hormone. According to our recent knowledge of metamorphosis in lepidopterans, the pupae are caused to re-pupate by the conjoint action of this growth and differentiation hormone and the juvenile hormone from the corpus allatum, while, when the former hormone is acting alone, they can undergo the normal course of development for adult; the brain hormone from the neurosecretory cells in the brain participating in these phenomena of metamorphosis only by virtue of its stimulating action to the prothoracic glands. In this line of thought we can elucidate the results of our previous experiments, only assuming the corpus allatum as a storage and release center of the brain hormone, although we believe this assumption is a true state of affair.

However, how can we explain the present finding? The explanation will become possible only when the brain hormone is extracted as a pure form. Until that time a tentative elucidation will be possible. It is that the brain hormone will be a precursor of the growth and differentiation hormone of the prothoracic glands. In the intact worm the brain hormone is soaked in the prothoracic glands to be modified or converted effectively into their own hormone. This process will be seeming prothoracotropic action of the brain hormone. But in the absence of this exchange store, it is likely that the brain hormone would turn less effectively over the growth and differentiation hormone, or other substance equivalent to it in action, in some unknown agency like the peritracheal glands. It is, however, impossible to state whether the peritracheal glands function as such in the normal development or so do they under the special condition where the prothoracic glands are lacking, because of the technical difficulty to remove the peritracheal glands completely from the intact worm.

Summary

1. Tests of the brain-cardiaca-allata complexes, brains or corpora allata in the isolated abdomens have yielded the adult or pupal differentiation without the conjoint action of the growth and differentiation hormone from the prothoracic gland.

2. When the isolated abdomens were supplied with the brains of all stages of life history or with brain-cardiaca-allata complexes of pupae, they underwent always the adult differentiation, while, when provided with the complexes or corpora allata excised from adults and larvae, they performed an extra pupal molting.

3. From these facts, we are tempted to assume that the brain hormone released from the implanted brains or corpora allata is a principal factor responsible for the differentiation of the recipient abdomens, and our tentative view is discussed concerning the intricate problem of how the brain hormone can work as the molt-promoting agent in the isolated abdomen.

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