

## STUDIES ON THE MODE OF ACTION OF THE DIAPAUSE HORMONE IN THE SILKWORM, *BOMBYX MORI* L.

### VI. THE TARGET ORGAN OF THE DIAPAUSE HORMONE

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#### INTRODUCTION

In general, glycogen and crude fat contents in insects increase before the onset of the diapause. In silkworms it is known that diapause eggs and the ovaries of diapause egg producers (in short 'diapause' ovaries) have more glycogen than those of non-diapause type (Chino, 1957; Yamashita & Hasegawa, 1964*b*). As already reported, the diapause hormone released from the suboesophageal ganglion (SG) of the silkworm is responsible for the induction of diapause in this insect (Hasegawa, 1952, 1957).

Our recent experiments have shown that the removal of the SG from 'diapause' pupae just after pupation not only causes the pupae to produce non-diapause eggs but also raises the carbohydrate content of the blood and fat body and lowers that of the ovaries to the same level as in natural 'non-diapause' ovaries, without any detectable changes in the carbohydrate content in the Malpighian tubules, integument, midgut or the whole pupa. On the contrary, the injection of extracts of diapause hormone into 'non-diapause' pupae lowers the fat body glycogen and the blood sugar level, but raises the glycogen content in the ovaries proportionally to the amount of the hormone dosage. From these results, it has been inferred that the diapause hormone facilitates the penetration of blood sugars into pupal ovaries and/or glycogenesis therein, resulting in lessening the blood sugar level and raising ovary glycogen content, a hypoglycaemic effect which is similar to the action of insulin in mammals (Yamashita & Hasegawa, 1964*b*).

The SG in male pupae is scarcely concerned with carbohydrate metabolism, for SG-removal from male pupae induced no significant changes in carbohydrate content in the pupal tissues (Yamashita & Hasegawa, 1965). From this one may conjecture that the SG in males functions differently from that in females or that the SG does not secrete the diapause hormone. Nevertheless, male pupae are able to produce diapause eggs when ovaries have been transplanted into them (Hasegawa, 1952), and, in fact, diapause hormone can be extracted from the heads of male moths (Hasegawa, 1963, 1964).

The present experiments were undertaken to elucidate the role of pupal ovaries in the carbohydrate metabolism of silkworm pupae and the effect of the diapause hormone on this metabolism. For this purpose, ovariectomy and ovary transplantation into

males were carried out during the larval stages, the SG being subsequently removed on the day of the pupation. From the results obtained it is clear that the SG in males, as well as in females, is concerned with carbohydrate metabolism so far as ovaries have been implanted and that, from the standpoint of carbohydrate metabolism also, the ovary is the target organ of the diapause hormone.

#### MATERIALS AND METHODS

The silkworms used were diapause egg producers of the bivoltine race (Daizo) which had experienced a high temperature and long photoperiod during embryonic life. Ovariectomy, unilateral or bilateral, was carried out on the third day of the fifth larval stage. Ovary transplantation into male larvae was performed at the same stage in the same race; immediately after unilateral gonadectomy of the male larvae, each operated male received an ovary implanted through the incision of the hemi-orchotomy and the male pupae, each with a developing ovarian implant, were used for further experiments. SG-removal was carried out on the day of the pupation of both the operated and non-operated pupae of both sexes.

Of the carbohydrates in the pupal tissues, reducing sugars and trehalose were determined by the anthrone method (see Wyatt & Kalf, 1957), and glycogen by the method of Kemp & van Heijningen (1954). In each experiment about fifteen pupae were sacrificed. Blood collected in cold tubes was used after centrifugation at 2500 r.p.m. for one minute, and fat body, ovaries and whole pupae were used after homogenation with a glass homogenizer in an ice bath.

The earlier experiments, in which the effect of SG-removal and the effect of diapause hormone injections on the carbohydrate content of pupal tissues was investigated, showed that these procedures affected chiefly the major carbohydrate components in the fat body, blood and ovaries (Yamashita & Hasegawa, 1964*b*). In the present paper, therefore, only the major components in each tissue are indicated in the accompanying tables, i.e. glycogen in fat body and ovaries, and trehalose in blood, and these are expressed in terms of glucose equivalent.

#### EXPERIMENTAL RESULTS

##### 1. *Effects of SG-removal and ovariectomy on carbohydrate content of the tissues of female pupae*

For the determination of tissue carbohydrate content both normal female pupae and hemi-castrated or wholly castrated female pupae (with removal of SG) were sacrificed on the eighth day after pupation or 2 days before adult emergence. The major components in the fat body, blood and ovaries are tabulated in Table 1.

*Fat-body glycogen.* Although SG-removal from female pupae raised the glycogen level in the fat body as expected from the previous papers (Yamashita & Hasegawa, 1964*b*), the effect of SG-removal is different owing to the castration. Ovariectomy in the larval stage resulted in a high level of glycogen in the fat body; i.e. the glycogen content of hemi-castrated and wholly castrated pupae is about three times and more than seven times, respectively, that of pupae with the ovaries intact. Another interesting point is that, although SG-removal raised the fat-body glycogen content in both non-castrated and hemi-castrated pupae, this effect vanished after com-

plete castration. In other words, when ovaries are absent the fat-body glycogen which has been accumulated during the early pupal stage (Yamashita & Hasegawa, 1965) is not mobilized so readily as it is in normal female pupae; it remains at the same high level irrespective of the presence or absence of the SG.

*Blood trehalose.* As already reported (Yamashita & Hasegawa, 1964*b*), blood sugars, especially trehalose, increase when the SG is excised from female pupae. It is of interest that unilateral castration causes the pupae to store more blood trehalose than normal pupae, but the removal of both ovaries maintains the blood trehalose at about the same level as in non-operated pupae. Combining SG-removal with castration is also interesting; removal in hemi-castration raises the blood trehalose level higher than that of pupae with two intact ovaries, whereas this effect does not appear in total castration.

Table 1. *Effect of SG-removal and castration on major components of carbohydrates in some tissues of female pupae*

Presence of		Glycogen in fat body (mg./g.)	Trehalose in blood (mg./100 ml.)	Glycogen in ovary (mg./g.)	Total carbohydrate in whole pupae (mg./g.)	Remarks
SG	Ovary					
+	++	5.1	461	20.1	14.2	Non-operated or normal pupae
-	++	8.2	627	14.5	13.4	Pupae with SG removed
+	+	17.7	590	19.8	13.6	Pupae hemi-castrated
-	+	24.5	696	15.3	12.8	Pupae hemi-castrated but with SG removed
+	-	38.5	483	.	13.4	Pupae completely castrated
-	-	37.8	493	.	12.5	Pupae completely castrated but with SG removed

Silkworms used, Daizo race (diapause type); SG-removal, on the day of pupation; hemi-castration and complete castration, on the third day of 5th-instar larval stage; pupal age at sacrifice, on the eighth day after pupation or 2 days before adult emergence.

*Ovary glycogen and total carbohydrate in whole pupae.* Even when pupae suffered hemi-castration at the larval stage the glycogen content (per wet weight) of the remaining ovary was identical with that in non-castrated pupae; and the content was decreased by SG-removal in the same way as observed on non-castrated pupae. Neither SG-removal nor castration could alter the total carbohydrate content of the whole pupae.

## 2. *Effects of SG-removal and ovary transplantation on the carbohydrate content of the tissues of male pupae*

The effect of SG function in male pupae seemed to be different from that in female pupae, for SG-removal from males on the day of the pupation, in contrast to its effect in female pupae, induced little change in the carbohydrate content of the pupal tissues (Yamashita & Hasegawa, 1965). Male pupae, however, are able to produce diapause eggs if ovaries have been transplanted into them (Hasegawa, 1952). For an analysis of the function of the SG in males each male larva was grafted with one ovary in lieu of a testis after hemi-castration. On the seventh and ninth day after pupation the male pupae were sacrificed and used as material for carbohydrate analysis of the tissues in those specimens in which the ovary implants had developed similarly to the

ovaries in female pupae at the corresponding stage. The results obtained are summarized in Table 2.

In this table we can see that, as pupal development proceeds, fat-body glycogen and total carbohydrate in the whole male pupae decrease, and blood trehalose increases, which confirms the results of the previous experiments (Yamashita & Hasegawa, 1965). The following additional points are worth mentioning.

*Fat-body glycogen.* It is of interest that the transplantation of ovaries into males diminishes the glycogen content in the fat body. Where male pupae had not been grafted with ovaries, fat-body glycogen is not altered by SG-removal, as expected from the previous paper (Yamashita & Hasegawa, 1965). On the other hand, the glycogen content of pupae which had received ovary transplantation during the larval

Table 2. *Effect of SG-removal and ovary transplantation on major components of carbohydrates in some tissues of male pupae*

Pupal age at sacrifice	Presence of		Glycogen in fat body (mg./g.)	Trehalose in blood (mg./100 ml.)	Glycogen in ovary graft (mg./g.)	Total carbohydrate in whole pupae (mg./g.)	Remarks
	SG	Ovary graft					
Seventh day after pupation	+	-	12.2	472	.	8.7	Non-operated or normal pupae
	-	-	11.9	494	.	8.8	Pupae with SG removed
	+	+	7.9	403	24.3	8.7	Pupae with ovary graft
	-	+	10.1	455	15.6	8.5	Pupae with ovary graft but with SG removed
Ninth day after pupation	+	-	6.5	547	.	4.9	Non-operated or normal pupae
	-	-	6.6	561	.	4.8	Pupae with SG removed
	+	+	3.1	460	27.0	5.1	Pupae with ovary graft
	-	+	4.7	529	19.3	5.5	Pupae with ovary graft but with SG removed

Silkworms used, the same race as in Table 1; SG-removal, on the day of pupation; ovary transplantation into males, on the third day of 5th-instar larval stage; donors of ovaries, the same race at the same larval stage.

stage varied according to the presence or absence of the SG; i.e. SG-removal from these pupae induced a rise in fat-body glycogen above the level in pupae without SG-removal. In other words, the SG of male pupae, into which ovaries had been implanted, functions as well as that of female pupae in decreasing the glycogen content of the fat body.

*Blood trehalose.* The trehalose level in male pupae is scarcely affected by SG-removal, but is markedly different when ovaries have been transplanted; ovary transplantation lowers blood trehalose, but if this is combined with SG-removal the blood trehalose tends to resume its original level.

*Glycogen content of transplanted ovaries and total carbohydrate content in whole pupae.* As expected, the glycogen level in ovary implants developing under the influence of the SG or the diapause hormone is higher than that of ovaries of pupae deprived of the SG. The total carbohydrate content in whole male pupae, however, is not affected by SG-removal, and it is of interest that the total carbohydrate content of male pupae is almost the same in those with or without ovary implants.

## DISCUSSION

1. *Pupal ovaries and carbohydrate metabolism in the silkworm*

As already reported, the glycogen content of pupal ovaries (glycogen is the major component of ovary carbohydrates) is different according to the nature of the ovaries—'diapause' or 'non-diapause'. The glycogen content in 'diapause' ovaries, induced by higher temperature and increased photoperiod during their embryonic life, or by injecting diapause hormone extracts into 'non-diapause' pupae, exceeds that of 'non-diapause' ovaries induced by lower temperature and darkness during their embryonic life, or by SG-removal from 'diapause' pupae. In the latter group the fat-body glycogen content and blood trehalose level (the major components of carbohydrate in each tissue) are raised. The diapause hormone has been thought to regulate carbohydrate metabolism in silkworm pupae by stimulating glycogen accumulation in the ovaries (Yamashita & Hasegawa, 1964*b*, 1965).

It has also been shown that, in 'diapause' pupae, ovary glycogen accumulating day by day after the onset of histogenesis is delayed by SG-removal, while fat-body glycogen and trehalose in the blood increase; whereas both trehalose and the level of reducing sugar in male blood become high after the middle pupal age with no relation to the SG (Yamashita & Hasegawa, 1964*b*, 1965).

As shown in the present experiments, the trehalose level in the blood of female pupae is increased by hemi-castration, but, after total castration, trehalose approximates to its original level. Fat-body glycogen, however, is always increased by castration; i.e. the glycogen content of pupae castrated unilaterally and bilaterally becomes, respectively, about three times and more than seven times as much as that of normal females (Table 1). On the other hand ovary transplantation into males decreases their fat-body glycogen and blood trehalose level (Table 2). Blood trehalose, however, is altered by these treatments only when ovaries are present in the body cavity, suggesting that the blood trehalose is the form in which fat-body glycogen is transferred to ovary glycogen. The carbohydrate content of whole pupae is not affected in either sex by ovariectomy and ovary transplantation (Tables 1, 2).

Considering these points it is highly probable that the increase in glycogen in the fat body after ovariectomy is the result of feedback control and that the accumulation or mobilization of fat-body glycogen during the early pupal period is dependent on the presence or absence of ovaries. This indicates that the pupal ovary plays an important part in carbohydrate metabolism in the silkworm; it synthesizes glycogen from carbohydrate which is carried via the blood from the fat body.

2. *The target organ of the diapause hormone*

In the first series of the studies on the mode of action of the diapause hormone (Hasegawa, 1963) it was suggested that the oocyte is the sole target organ of this hormone. This suggestion gained partial support from the accumulation of 3-hydroxy-kynurenine in 'diapause' ovaries (Yamashita & Hasegawa, 1964*a*). The experimental results of SG-removal and injections of diapause hormone, however, did not entirely support this suggestion, because of accompanying changes in the carbohydrate content of the fat body and blood as stated above. The possibility that the ovaries are the target organ of the diapause hormone therefore could not gain complete acceptance

from the standpoint of carbohydrate metabolism. The uncertainty on this point, however, has been removed by the present experiments.

As indicated in Table 1 the effect of removal of the SG from female pupae, on the carbohydrate content of the fat body and blood, occurred in non-castrated and hemi-castrated females, but there was no effect after total castration, indicating that the SG-removal does not primarily affect the carbohydrate content of fat body and blood, but that the effect is secondary, and that, for its realization, the presence of ovaries is necessary.

That the presence of ovaries is required if the SG is to influence the carbohydrate content of the fat body and blood was further substantiated by another experiment (Table 2). In this experiment SG-removal from male pupae did not induce any significant increase of carbohydrate content in fat body and blood, but SG-removal from male pupae carrying ovary implants brought about just the same effect as manifested in female pupae with two intact ovaries or in hemi-castrated pupae.

From these results it is clearly demonstrated that the SG or the diapause hormone regulates the carbohydrate metabolism through the pupal ovaries and that, without ovaries, the effect does not appear, indicating that the target organ of the diapause hormone is the ovary itself.

#### SUMMARY

1. Glycogen synthesis in the ovary of *Bombyx mori* is accelerated by the diapause hormone.
2. Such glycogen is derived, by way of the trehalose of the haemolymph, from carbohydrate in the fat body.
3. Extirpation of the suboesophageal ganglion has the converse effect: a fall in glycogen in the ovary and an increase in blood trehalose and fat-body glycogen.
4. Mobilization of fat-body glycogen is dependent on the presence of the ovaries, and of the suboesophageal ganglion.
5. The same results can be obtained in male pupae after the implantation of ovaries.
6. These observations on carbohydrate metabolism confirm the belief that the 'target organ' of the diapause hormone is the ovary itself. The changes in other tissues are due to feedback.

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