93. The Control of Growth and Development in *Bombyx mori*. X

Effect of the Suboesophageal Ganglion Hormone on the Incorporation of ^{14}C -Glucose into the Lipids of the Fat Body, Haemolymph and Ovary

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Although many papers have been published dealing with the effect of the corpus allatum hormone on the lipid metabolism of insects, studies of effect of the suboesophageal ganglion (SG) hormone on lipid metabolism are relatively few. Hasegawa and Yamashita (1967) reported that the SG hormone is responsible for the induction of diapause eggs and for the accelerated accumulation of carbohydrate and lipid by the ovaries of silkworm pupae. Carbohydrates and lipids which are source of energy for diapause eggs are stored in order for the eggs to survive the winter. The present paper reports a study of the incorporation of ^{14}C -glucose into the fat body, haemolymph, and ovary lipids during pupal development.

Materials and methods. Female pupae which were F_1 hybrids between the Daizo and Japanese No. 106 races were used. Their eggs were incubated at 16°C in the dark or 25°C in the light. The former eggs are symbolized as LD and the latter as HL. The female moths of the LD series laid non-diapause eggs only and the female moths of the HL series laid diapause eggs only (see Table I). When three

Table I. Alteration of voltinism by transplantation (+) or extirpation (–) of the SG

Host	Operation	No. of pupae operated	No. of moths that laid diapause eggs	No. of moths that laid mixed eggs	No. of moths that laid non-diapause eggs
Insects destined to lay diapause eggs	Sham	15	15	0	0
	–SG	16	0	0	16
	+SG(3)	18	18	0	0
Insects destined to lay non-diapause eggs	Sham	18	0	0	18
	–SG	15	0	0	15
	+SG(3)	16	12	4	0

suboesophageal ganglia were transplanted into 1-day-old female pupae of the LD series, the resulting adults produced batches of diapause and mixed eggs. When the SG was extirpated from 1-day-old female pupae of the HL series, the ♀♀ subsequently produced only non-diapause eggs.

1-¹⁴C-glucose (S.P. 20 mCi/mM) was purchased from Dai-ichi Pure Chemical Co. Ltd. of Tokyo, Japan. The extirpations or transplantations of glands were done a few hours after pupation. Then, after maintaining the pupae for 7 hours at 25°C, 0.2 μCi (0.01 ml/pupa) of radioactive compound was injected into the pupae with a micro-injector. The methods described by Folch (1957) were modified for lipid extraction and analyses. The fat body or ovary was homogenized in a glass homogenizer containing chloroform-methanol (2 : 1). After filtration, the residue was re-extracted, and final extracts were washed with 0.2 vol of distilled water. One ml of haemolymph was extracted with 20 vol of a 2 : 1 mixture of chloroform-methanol. The collected extracts were dried under a stream of nitrogen, and the residues were dissolved in 20 ml chloroform.

The dried lipid fractions were dissolved in 15 ml scintillation fluid (PPO-5 g, POPOP-0.25 g in 1 l toluene), according to Pachard liquid scintillator formulation. An automatic external standard was utilized to determine the efficiency of the instrument.

Results. The influence of extirpation or transplantation of the SG on the total lipid content in the ovary, fat body, and haemolymph of pupae is shown in Fig. 1 (LD series) and Fig. 2 (HL series).

From these two figures we see that total lipid content was higher in the HL series than in the LD series. The total lipid content in the ovary was higher following transplantation of the SG and lower following extirpation of the SG in both the LD and HL series. On the other hand, total lipid content in the fat body was higher after extirpation of the SG and lower after transplantation of the SG. In the haemolymph no effect of the treatment on the total lipid content was observed.

In general, the difference of total lipid content between the experiments and sham-controls was larger when voltinism was altered by operation. That is, in the LD series (Fig. 1), the difference of total lipid content between the +SG and sham-controls was larger than that between the -SG and sham-controls. Also in the HL series (Fig. 2), the difference of total lipid content between the -SG and sham-controls was larger than that between the +SG and sham-controls.

The incorporation of ¹⁴C-glucose per pupa into the fat body and ovary lipids is shown in Fig. 3.

Fig. 1. Total lipid content in the pupal ovary, fat body, and haemolymph in the LD series.

Ordinate: Lipid content (mg)/pupa. Abscissa: Days after pupation.

—●—: Sham-control, ---●---: -SG, -.-●-.-: +SG.

Fig. 2. Total lipid content in the pupal ovary, fat body, and haemolymph in the HL series.

Ordinate: Lipid content (mg)/pupa. Abscissa: Days after pupation.

—●—: Sham-control, ---●---: -SG, -.-●-.-: +SG.

The results of incorporation of radioactivity into the lipid fractions as shown in Fig. 3 agreed with the results from measurement

Fig. 3. Incorporation of ^{14}C -glucose per pupa into the fat body and ovary lipids.

Ordinate: 10^3 c.p.m./animal. Abscissa: Days after pupation.

—●—: Sham-control, ---●---: -SG, -.-●-.-: +SG.

of the total lipid content in the same fractions, as shown in Figs. 1 and 2. The incorporation of ^{14}C -glucose into the fat body lipid gradually decreased with the age of pupa. On the other hand, the incorporation of radioactivity into the ovary lipid gradually increased with the age of pupa. This increase was especially abrupt 4th days after pupation.

The incorporation of ^{14}C -glucose per dry weight (mg) into the fat body and ovary lipids is shown in Table II and Fig. 4. The incorporation of radioactivity into the lipid fractions is similar to the results shown in Figs. 1, 2 and 3.

Discussion. Pfeiffer (1945) demonstrated that allatectomy of the grass-hopper *Melanoplus* leads to the massive accumulation of lipids and glycogen in the fat body. Other workers (e.g. Vogt, 1949; Thomsen, 1952; Bodenstein, 1953; Doane, 1960, 1961; Orr, 1964 a, b; Odhiambo, 1966) have since reported similar results after extirpating the corpora allata from other insects. However, less is known of the

Table II. Incorporation of ^{14}C -glucose into the fat body and ovary lipids in female pupae of a hybrid, Daizo $\times$ Japanese No. 106 (counts/mg/min)

Organ	Days after pupation	HL series		LD series	
		Sham control	-SG	Sham control	+SG
Fat body	2nd	1198	1294	779	673
	4th	986	1153	554	448
	6th	505	843	438	402
Ovary	2nd	791	683	423	582
	4th	1096	893	601	693
	6th	1241	1074	663	751

Fig. 4. Incorporation rate of ^{14}C -glucose per mg dry weight into the fat body and ovary lipids.

Ordinate: 10^3 c.p.m./mg dry weight. Abscissa: Days after pupation.

—●—: Sham-control, ---●---: -SG, ---●---: +SG.

effects of removal of the suboesophageal ganglion hormone on lipid metabolism. Hasegawa and Yamashita (1967) reported that the SG hormone accelerates glycogen and lipid deposition into the ovary of the silkworm pupa. However, interactions between the lipid metabolism of the fat body, haemolymph, and ovary have not been studied.

We have demonstrated that the pupae destined to lay diapause eggs contain a higher lipid concentration than those destined to lay non-diapause eggs. The total lipid content in the ovary is increased by transplantation of the SG and decreased by extirpation of the SG. The rate of increase and decrease in the lipid content was larger when voltinism alters from the diapause to non-diapause direction or from the non-diapause to diapause direction. Total lipid content in the fat body is decreased by transplantation of the SG and increased by the extirpation of the SG unlike the case of the ovary. Accordingly, we

propose that the SG hormone accelerates the transport of lipids from the fat body to the ovary.

According to the studies of Hasegawa and Yamashita (1967), the SG hormone accelerates the transport of glycogen from the fat body to the ovary. From these results, the SG hormone seems to act similarly for lipid metabolism as well as carbohydrate metabolism.

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