32. The Control of Growth and Development in *Bombyx mori*. XI

Effect of the Corpus Allatum, Suboesophageal Ganglion, Brain and Ecdysterone on the Incorporation of ^{14}C -U-Glucose into Pupal Fat Body and Ovary Lipids

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Lipids serve as the major source of energy in insects. They are also needed essentially for some of biosynthetic process associated with metamorphosis and reproduction (Gilbert and Schneidermann, 1961; Gilbert, 1967). Many studies on the hormonal control of the lipid metabolism have been done in insects. In previous paper (X in this series), we reported that the total lipid content in the ovary is increased by transplantation of the suboesophageal ganglion (SG) and decreased by extirpation of the SG. The present paper reports the effect of the SG-hormone, corpus allatum hormone (CA), ecdysterone (Ec) and brain (Br) on the incorporation of ^{14}C -U-glucose into the fat body and ovary lipids during pupal development in the silk-worm, *Bombyx mori*.

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 17°C in the dark or 24°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 as seen in Table I.

Although the extirpation of the CA was performed in the female larvae just before spinning, the change of voltinism was not found in this time. The extirpation of the SG or Br was done in the 5- to 10-hr-old pupae. The transplantation of the SG was performed in the female larvae just after the 4th ecdysis. The injection of the Ec (purified by Prof. T. Takemoto) was done in the 15- to 20-hr-old female pupae. Sham-controls were done in the same way except that glands were left intact.

When the SG was extirpated from the young female pupae of the HL series, the voltinism altered to the non-diapause direction. The extirpation of the Br, in both the LD and HL series, produced batches of diapause, non-diapause and mixed eggs. The percentages

Table I. Alteration of voltinism by transplantation (+), extirpation (—) of endocrine organs or injection of ecdysterone (Ec)

Host	Operation	No. of operation	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 (HL)	Sham	15	15	0	0
	—SG	15	0	0	15
	—Br	20	4	7	4
	—CA	15	15	0	0
	Ec (1 γ)	15	11	0	0
	Ec (10 γ)	15	8	0	0
Insects destined to lay non-diapause eggs (LD)	Sham	15	0	0	15
	+SG(3)	15	14	1	0
	—Br	20	7	5	8
	—CA	15	0	0	15
	Ec (1 γ)	15	0	0	15
	Ec (10 γ)	15	0	0	9

Fig. 1. Incorporation rate of ¹⁴C-U-glucose per mg dry weight into the fat body lipids in the HL series.

of alteration of voltinism caused by extirpating the Br were about 75% in both series, and pupal development was lengthened for 2 days. When three subesophageal ganglia were transplanted into last instar larvae of the LD series, the resulting adults laid batches of diapause

Fig. 2. Incorporation rate of ^{14}C -U-glucose per mg dry weight into the ovary lipids in the HL series.

Fig. 3. Incorporation rate of ^{14}C -U-glucose per mg dry weight into the fat body lipids in the LD series.

Fig. 4. Incorporation rate of ^{14}C -U-glucose per mg dry weight into the ovary lipids in the LD series.

and mixed eggs. The injection of the Ec had no effect on the change of voltinism.

^{14}C -U-glucose was purchased from Dai-ichi Pure Chemical Co. Ltd. of Tokyo, Japan. After maintaining the pupae for 24 hours at 25°C , $0.2 \mu\text{Ci}/0.01 \text{ ml/pupa}$ of radioactive compound was injected into the 1-day-old female pupae with a micro-syringe. The total lipids were extracted from homogenates of the fat body or ovary by Folch's method. The fat body or ovary was homogenated 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. The collected extracts were dried under a stream of nitrogen, and 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 the Pakard liquid scintillator formulation. The radioactivity was measured by Pakard scintillation spectrorometer Model 3320. An automatic external standard was utilized to determine the efficiency of the instrument.

Results. In previous paper, we reported that total lipid content in the ovary was increased after transplantation of the SG and decreased after extirpation of the SG, whereas the total lipid content in the fat body was decreased following transplantation of the SG and increased following extirpation of the SG unlike the case of the ovary.

In the present experiments we found that the incorporation of ^{14}C -U-glucose into the fat body lipid gradually decreased with the age of pupa. On the other hand, the incorporation of ^{14}C -U-glucose into the ovary lipid gradually increased with the age of pupa. These results were similar to the results obtained in previous experiments.

Comparing the results in the HL series with those in the LD series, the incorporation of ^{14}C -U-glucose into the fat body and ovary lipids was slightly higher in the HL series than in the LD series.

When the SG or brain was extirpated from the newly pupated silkworms, the incorporation of radioactive compound into the fat body lipid fractions was higher as compared with the sham-controls in both the LD and HL series but the incorporation into the ovary lipid fractions was lower. Allatectomy showed a similar tendency to the results obtained by extirpation of the brain or SG. The incorporation of ^{14}C -U-glucose into the fat body lipid was the highest in the debrained series.

Following the injection of 1 γ Ec per pupa, no significant difference was found between the experimentals and controls in terms of the pupal duration and the incorporation of ^{14}C -U-glucose into the fat body or ovary lipid fractions. However, after injecting 10 γ Ec, the pupal developmental stages were shortened by 1 day as compared to the controls, and the incorporation of the radioactive compound was decreased in the fat body and increased in the ovary over the control values.

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, 1964a, b; Odhiambo, 1966) have since reported similar results after extirpating the CA from insects.

These reports agree with our results that total lipid content in the fat body was higher following extirpation of the CA. The extirpation of the brain or SG raised up the accumulation of lipids in the fat body more than the results of allatectomy. Allatectomy just before spinning lengthened pupal development for about 1 day in females and increased finally the incorporation of radioactive compound into the ovary as compared with the controls in both the LD and HL series.

From these results, we conclude that the presence of factors from the CA, SG and Br or ecdysone secretion during pupal development promote the accumulation of lipids into the ovary accompanying the decrease of accumulation of lipids into the fat body.

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