50. The Control of Growth and Development in *Bombyx mori*. VII

Incorporation of ^{14}C -Leucine into the Fat Body, Haemolymph and Ovary Proteins in Allatectomized Pupae

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It has been observed that the corpus allatum hormone of insects influences protein metabolism (Coles, 1965; Engelmann and Penny, 1966; Wilkens, 1968; Morohoshi *et al.*, 1969; Cummings and King, 1970). In earlier studies on nitrogen metabolism in the silkworm, *Bombyx mori*, we identified and determined (1969) the quantity of total proteins present in the haemolymph and ovaries by the micro-Kejeldahl method. In this paper we will describe the results of a study of the influence of the corpus allatum hormone on protein synthesis measured in terms of incorporation of ^{14}C -leucine into the fat body, haemolymph and ovary proteins in allatectomized insects.

Materials and methods. Eggs of the tetramolting and bivoltine strain, Japanese No. 106×Daizo, were incubated under two different temperature regimes in order to obtain both diapause and non-diapause insects. Diapause insects were produced by eggs exposed to a long day photoperiod at 25°C (H) and non-diapause ones by eggs exposed to a short day photoperiod at 17°C (L). Larval and pupal periods were shorter by 2 days in the latter than in the former.

Removal of the two corpora allata (CA) was carried out on the 1st day of the 5th instar. The control groups were also operated upon, to check if the loss of haemolymph during extirpation might have an effect. Allatectomized female larvae spent about half a day less in the 5th instar, but took 1 or 2 days longer in the pupal period than did the control animals.

Larvae and pupae injected with ^{14}C -leucine (Sp. activity 25.0 mCi/mM) were dissected after a specified period, and their haemolymph, fat body and ovaries were collected in sample bottles. The haemolymph was transferred into a centrifuge tube with 2 vol. of cold distilled water. After centrifugation at 1000 rpm for 5 min, the precipitated blood cells were discarded. In order to make the acid in the supernatants 10% concentration 20% trichloroacetic acid (TCA) was added to the solution. The resulting precipitates were

collected by centrifugation at 3000 rpm for 7 min, washed first with a 10% TCA solution, next with a 80% ethanol solution, and next for three times with an ethanol-ether (1:3 v/v) mixture. This fraction, consisting mainly of acid-insoluble protein was then dried.

Fat body and ovary extracts were homogenized with a 10% TCA solution in a motor. They were then washed with an ethanol solution and finally with an ethanol : ether (1:3 v/v) mixture as same way as haemolymph. After the precipitations were washed with a 5% TCA solution for 20 min at 70°C, the nucleic acids were removed by centrifugation. The precipitates were again washed with ethanol : ether to remove the TCA, and then were dried. The dried precipitates were dissolved in formic acid at the room temperature, and after centrifugation the radioactivity of the supernatant was measured with a gas-flow counter.

Results. *I. Incorporation of ^{14}C -leucine into the fat body, haemolymph and ovary proteins immediately after injection.* ^{14}C -leucine (0.2 μCi /0.01 ml per insect) was injected into the abdomen of full grown larvae or pupae. Incorporation of ^{14}C -leucine into the fat body, haemolymph and ovary proteins was determined 1 and 4 hr after injection. The incorporation of ^{14}C -leucine into tissue proteins was higher in the L series than in the H series and also higher at 4 hr than at 1 hr after injection.

The development of the ovaries accelerates with age during pupal life. The ovary weight of the 5-day-old pupa and of the 7-day-old pupa is 7 times or 20 times as much as that of the 2-day-old pupa respectively (Table I). In 5 pupal ovaries incorporation of ^{14}C -leucine into protein was the lowest in the 2-day-old pupa and the highest in the 7-day-old pupa. When compared with the specific radioactivity per 100 mg ovary weight, it was the highest in the 2-day-old pupa and the lowest in the 7-day-old pupa. The difference of the radioactivity between 1 and 4 hr after injection was larger in the 2-day-old pupa, but smaller in the 5-day-old pupa as shown in Table I.

Effect of allatecomy on incorporation of ^{14}C -leucine into proteins was clear cut in the fat body, but was negligible in the haemolymph and ovaries (see Figs. 2, 3 and 4).

II. The distribution of radioactivity. The incorporation of ^{14}C -leucine was observed at regular intervals for 24 hr after it was injected into the hemocoel of the fully grown larvae. The fat body radioactivity was about 3000 cpm per pupa just after pupation. It then decreased day by day and fell below 1000 cpm per pupa by the 5th day after pupation. The haemolymph radioactivity was about 2000 cpm per pupa up to the 3rd day after pupation. It decreased to

Table I. Incorporation of ^{14}C -leucine into pupal ovary proteins
1 hr and 4 hr after injection

Stage	Temperature incubated	Treatment	Wet pupal ovary weight of 5 individuals		Total activity (10^3 cpm)		Sp. Activity (10^3 cpm/100mg)	
			1 hr	4 hr	1 hr	4 hr	1 hr	4 hr
2-day-old pupa	25°C	C	0.0711	0.0666	12.3	27.1	17.4	40.6
	25°C	-CA	0.0683	0.0711	12.7	23.6	18.8	33.3
	17°C	C	0.0625	0.0625	24.3	40.1	38.8	64.1
	17°C	-CA	0.0688	0.0685	30.3	44.4	44.1	64.7
5-day-old pupa	25°C	C	0.4869	0.6452	46.0	72.7	9.4	11.3
	25°C	-CA	0.4820	0.5927	40.5	59.8	9.2	10.1
	17°C	C	0.5925	0.4662	60.0	60.6	9.8	13.7
	17°C	-CA	0.5801	0.7256	32.0	52.3	5.5	7.2
7-day-old pupa	25°C	C	1.5623	1.5800	32.1	80.3	1.6	5.1
	25°C	-CA	1.2348	1.2851	41.2	73.8	3.3	5.7
	17°C	C	1.5796	1.0898	57.8	71.6	3.5	5.4
	17°C	-CA	1.2098	1.1189	54.0	70.1	4.4	6.3

C: Control pupae, -CA: Allatectomized pupae.

Fig. 1

Fig. 2

Fig. 1. Radioactive distribution in the fat body, haemolymph and ovary proteins of the pupal stage after ^{14}C -leucine was injected in the full grown larvae.

Ordinate: 10^3 cpm/fat body, haemolymph, ovaries/pupa, abscissa: Days in pupal stage, -●-: Fat body, -○-: Haemolymph, -□-: Ovaries.

Fig. 2. Radioactive distribution in the ovary proteins of the pupal stage after ^{14}C -leucine was injected in the full grown larvae.

Ordinate: 10^3 cpm/100 mg dried ovaries, abscissa: Days in pupal stages and adult, —: Pupae treated to lay diapause eggs, ----: Pupae treated to lay non-diapause eggs, -○-: Allatectomized pupae, -●-: Control pupae.

Fig. 3

Fig. 4

Fig. 3. Radioactive distribution in the fat body of the pupal stage after ^{14}C -leucine was injected in the full grown larvae.

Ordinate: 10^3 cpm/100 mg dried fat body, abscissa: Days in pupal stage. —: Pupae treated to lay diapause eggs, ---: Pupae treated to lay non-diapause eggs, -○-: Allatectomized pupae, -●-: Control pupae.

Fig. 4. Radioactive distribution in the haemolymph proteins of the pupal stage after ^{14}C -leucine was injected in the full grown larvae.

Ordinate: 10^3 cpm/ml haemolymph, abscissa: Days in pupal stage, —: Pupae treated to lay diapause eggs, ---: Pupae treated to lay non-diapause eggs, -○-: Allatectomized pupae, -●-: Control pupae.

about 1000 cpm per pupa in the 5th day after pupation (Fig. 1). In the ovaries the radioactivity gradually increased up to the 5th day after pupation and after that decreased (Fig. 1).

Moreover, the radioactivity per 100 mg ovary weight gradually decreased with pupal age (Fig. 2). Incorporation of ^{14}C -leucine into ovary proteins was higher in the L series than in the H series, and also was higher in the allatectomized pupae than in the control ones. These tendencies were striking in the fat body (Fig. 3) and less clear in the haemolymph (Fig. 4).

Discussion. The prepupal and pupal stages in the silkworm are characterized by the breakdown of larval tissues and the synthesis of adult tissues accompanying metamorphosis. Incorporation of ^{14}C -leucine into the fat body proteins during 1 or 4 hr after injection showed higher values in the fully grown larvae, and gradually decreased with the age in pupae. On the other hand, the radioactivity in the ovary increased with the age in pupae. Observing the distribution of radioactivity in various tissues during the pupal stage after ^{14}C -leucine was injected in the full grown larvae, it is presumed that the migration of radioactivity from the fat body and haemolymph to the ovary went on with the lapse of time. This result accords with the results by Tojo (1968).

Incorporation of ^{14}C -leucine into the fat body, haemolymph and ovary proteins during the later larval and pupal stages showed higher values in the larvae or pupae determined to lay non-diapause eggs than in those determined to lay diapause eggs. This finding agrees with our previous results (1969). This difference was especially clear in the ovaries in the 2-day-old pupa, but it gradually decreased with the age of pupa (Fig. 2). The effect of allatectomy on incorporation of ^{14}C -leucine into proteins was clearly observed in pupae of both egg types. Allatectomized pupae showed a higher incorporation of ^{14}C -leucine into ovary proteins than the controls (Fig. 2) contrary to our previous results (Morohoshi *et al.*, 1969). From these results we must conclude that the corpus allatum hormone inhibits protein synthesis in the ovary. That is, reduced secretion of the corpus allatum hormone is accompanied by increased synthesis of proteins.

The corpus allatum in the insect is a very small organ, and its capacity for secretions may be limited throughout the life cycle. When a large amount of corpus allatum hormone is released during an early developmental stage, it may secrete less during a later developmental stage. Accordingly, we must conclude that the activity of the corpus allatum in the insect determined to lay non-diapause eggs is greater in the early developmental stages, but weaker in the

Fig. 5. A diagram illustrating the effect of the modification of maturing genes with incubating temperatures on molting and voltine characteristics.

L: Low incubating temperature, H: High incubating temperature, $+L_m$: Intermediate maturing gene, L_m^e : Early maturing gene, L_m : Late maturing gene, $\Rightarrow$: Strong stimulus, $\rightarrow$: Weak stimulus, CA: Corpus allatum hormone, SG: Suboesophageal ganglion hormone, ----: Modification by brain stimulus, M: Early stage, V: Late stage. Compare with Fig. 2 in Vol. 45, No. 10 of this series.

later developmental stages compared to that of the insect determined to lay diapause eggs. A diagram (Fig. 2) drawn in a report of Vol. 45, No. 10 (Morohoshi, 1969) of this series must be revised as shown in Fig. 5. These results very well accord with the results (Fig. 1 and Table II) in a report of Vol. 45, No. 9 (Morohoshi, 1969) of this series. The fact that the effects of environmental factors on molting and voltine characteristics differ quite distinctly at the boundary of early and late instars of larval development seems to be due to the rise and fall of the corpus allatum hormone. The greater the secretion of the corpus allatum hormone, the longer the larval instar, and the less the corpus allatum hormone secreted, the shorter the larval instar becomes.

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References

- Coles, G. C. (1965): Haemolymph proteins and yolk formation in *Rhodnius prolixus* Stahl. J. Exp. Biol., **43**, 425-431.
- Cummings, M. R., and King, R. C. (1970): The cytology of the vitellogenic stages of oogenesis in *Drosophila melanogaster*. II. Ultrastructural investigations on the origin of protein yolk spheres. J. Morph., **130**, 467-478.
- Engelmann, F., and Penny, D. (1966): Studies on the endocrine control of metabolism in *Leucophaea maderae* (Blattaria). I. The haemolymph proteins during egg maturation. Gen. Comp. Endocr., **7**, 314-325.
- Morohoshi, S., Iijima, T., Kikuchi, S., and Ikeda, S. (1969): Effect of the corpus allatum hormone on carbohydrate and nitrogen metabolism in *Bombyx mori*. Proc. Japan Acad., **45**, 328-333.
- Morohoshi, S. (1969): The control of growth and development in *Bombyx mori*. IV. Relationship between environments and molting or voltine characteristics. Proc. Japan Acad., **45**, 797-802.
- (1969): The control of growth and development in *Bombyx mori*. V. Racial differences in molting and voltine characteristics. Proc. Japan Acad., **45**, 937-942.
- Wilkens, J. L. (1968): The endocrine and nutritional control of egg maturation in the fleshfly *Sarcophaga Bullata*. J. Insect Physiol., **14**, 927-945.
- Tojo, S. (1968): Catabolism of nitrogen compounds in insects, with special reference to uric metabolism in a closed system (in Japanese). Science of Biology, **20**, 102-112.