Studies on the Role and Sexual Dimorphism of Lipid during Metamorphosis of the Silkworm, *Bombyx mori* L. (Lepidoptera : Bombycidae)¹

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The role of lipid during the metamorphosis of the silkworm, *Bombyx mori* was studied. Male pupae retained a higher lipid content in the whole body and the fat body through the pupal period. In females, lipid content of the whole body decreased remarkably at the late pupal period and the fat body lipid also decreased suggesting that lipid is transported into the developing ovary *via* the blood. Ovariectomy immediately after pupation resulted in remarkable lipid deposition in the whole body, fat body and blood. These results indicate that the sexual dimorphism observed in the whole body and fat body lipid content of *B. mori* pupae is closely connected with the ovarian development.

INTRODUCTION

Lipids are of vital importance to insects as an energy substrate for embryogenesis, metamorphosis and flight as well as being an important cell component (cf. FAST, 1964; GILBY, 1965; GILBERT, 1967). In the past, a number of workers have analyzed the lipid composition of eggs, larvae, pupae and adults of the silkworm, *Bombyx mori*. Recently, attention has been paid to lipid synthesis (SRIDHARA and BHAT, 1964; SRIDHARA et al., 1966) and fatty acid composition (SRIDHARA and BHAT, 1965; NAKASONE and ITO, 1967; NAKASONE and HORIE, 1969; SUZUKI et al., 1970) from a biochemical point of view. However, lipid metabolism of *B. mori* during pupal-adult development has not been studied extensively with reference to both the histolysis and the histogenesis.

In *Hyalophora cecropia*, GILBERT and SCHNEIDERMAN (1961) observed sexual dimorphism in the lipid content of pupae; females consumed a considerable amount of lipid, while males possessed a higher lipid content during pupal-adult development. DOMROESE and GILBERT (1964) also reported that male pupae have a lipid-sparing metabolism, resulting in sexual dimorphism of the cecropia pupae. The present paper deals with the role of lipid during metamorphosis of *B. mori*, in relation to sexual dimorphism of lipid content.

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MATERIALS AND METHODS

Silkworms used were the hybrids of Hozan $\times$ Ginko and Shungetsu $\times$ Hosho. Only those pupae within a narrow range of body weight were used in the experiment; selected females weighed 1.6 to 1.7 g and males were 1.2 to 1.3 g in the Hozan $\times$ Ginko and females were 1.7 to 1.8 g in the Shungetsu $\times$ Hosho on the day of pupation. Uni- and bilateral ovariectomies were conducted on anesthetized females with ether immediately after pupation.

To determine the lipid content of pupal blood at each pupal age, about 2 to 4 ml of blood was collected from 20 pupae into a chilled test tube and centrifuged for 5 min at 3,000 rev/min to remove hemocytes and other tissue fragments. The supernatant was used as the blood sample. Bled pupae were dissected out in 0.75% saline and the tissues such as the fat body, midgut and gonad were used for lipid analysis.

One pupa and the above tissues with exception of blood were homogenized with 5 vol of ethanol-ether (3 : 1, V/V), while the blood sample was thoroughly mixed with 20 vol of ethanol-ether. These homogenates were centrifuged for 10 min at 3,000 rev/min and the supernatant was removed. Thereafter, the precipitate was suspended in ethanol-ether and recentrifuged. This lipid extraction procedure was repeated four times, and the supernatant was combined and evaporated *in vacuo*. The resultant residue was dissolved in chloroform-methanol (2 : 1) and washed with 1/5 vol of 0.25 M NaCl. The chloroform fraction was then evaporated *in vacuo* and lipid was gravimetrically determined.

RESULTS

Lipid content in whole pupae

To cast some light upon lipid metabolism in pupae of both sexes during pupal-adult development, the lipid content of each whole pupa (Hozan $\times$ Ginko) was analyzed. As shown in Fig. 1, the lipid content per individual male decreased slightly throughout development and only 15% during pupal period. The lipid content per individual females decreased remarkably at late pupal ages accounting about a 40% decrease during pupal period.

When the lipid content was expressed on a fresh weight basis (Table 1), the content in the males was almost constant throughout pupal-adult development, and at emergence about 80% increase was noticed, which will be discussed later. The females showed an almost constant level of lipid content except at 8 days of age. When comparing both sexes, the lipid content on a fresh weight basis in males was higher by more than 30% that of females at each pupal age.

Lipid content in pupal tissues

Lipid content in the pupal tissues of both sexes (Hozan $\times$ Ginko) was analyzed through pupal-adult development. In Fig. 2, lipid content in the fat body, midgut and gonad is expressed as per individual and the blood lipid content per ml.

In males, the lipid content of the fat body decreased with initiation of adult development and increased at late pupal ages; fat body at 5 and 6 days of age contained minimum amount of lipid and again increased strikingly. Lipid content in the blood of males increased abruptly at the early phase of adult development, and the midgut

Fig. 1. Changes in lipid content of whole pupal body during pupal period (Hozan×Ginko). Female and male individuals emerged as adults in 11 and 10 days, respectively. Open and closed circles represent the lipid content of female and male pupae, respectively. Each value is the mean of 5 replications and the vertical lines show the standard errors.

Table 1. CHANGES IN LIPID CONTENT OF PUPAE AND ADULTS^a

Stage	Lipid content in	
	Male	Female
Pupa (2-day old)	104.1±5.3	70.7±3.7
Pupa (4-day old)	105.5±2.7	68.1±1.4
Pupa (6-day old)	104.0±4.3	69.6±1.4
Pupa (8-day old)	104.6±3.4	52.4±2.2
Adult	189.2±5.0	70.1±2.4

^a Adult emergence occurred 11 days after pupation in females and 10 days in males. Silkworm strain, Hozan×Ginko. Measurements are mean values and their standard errors, and they are expressed in mg/g fresh body weight.

and testis contained little lipid through the pupal period.

In females, lipid content of the fat body decreased rapidly without any increase thereafter and 8-day pupae contained only 1/5 the lipid of the 1-day pupae. Contrary to the pattern observed for the fat body, ovarian lipid increased greatly at the late pupal ages. The blood and midgut of the females showed similar fluctuation in lipid content as that seen in males.

When the lipid content of the fat body is expressed on a fresh weight basis (Table 2), the male fat body was found to contain about 80, 90 and 130% more lipid than that of the female pupae at 1, 4 and 8 days of age, respectively. In 8 day-old males, the fat body contained about 60% more lipid than those 1 day of age.

Lipid content in ovariectomized females

To understand the role of lipid in relation to ovarian development, uni- and

Fig. 2. Changes in lipid content of various tissues during the pupal period (Hozan $\times$ Ginko). Female and male individuals emerged as adults in 11 and 10 days, respectively. Each point represents the result of each measurement. $\circ$, fat body; $\bullet$, blood; $\times$, midgut; $\square$, ovary; $\triangle$, testis.

Table 2. CHANGES IN LIPID CONTENT OF THE FAT BODY DURING PUPAL PERIOD^a

Pupal age (days)	Lipid content in	
	Male	Female
1	221.8 $\pm$ 8.1	123.9 $\pm$ 7.2
4	251.0 $\pm$ 10.5	130.2 $\pm$ 3.8
8	347.7 $\pm$ 12.0	149.3 $\pm$ 3.9

^a Adult emergence occurred 11 days after pupation in females and 10 days in males. Silkworm strain, Hozan $\times$ Ginko. Measurements are mean values and their standard errors, and they are expressed in mg/g fresh weight.

bilateral ovariectomy was performed on newly pupated females (Shungetsu $\times$ Hosho). Whole body lipid content of ovariectomized females is shown in Table 3. Unilaterally-castrated females contained almost the same amount of lipid and bilaterally-castrated ones contained more than non-operated ones at each pupal age: The difference in the content between bilaterally-castrated and non-operated pupae increased after development, and those which were bilaterally-castrated contained about 40% more lipid per individual at emergence and about 50% more lipid on a fresh weight basis than those which had not undergone operation.

Lipid content in the fat body and blood of ovariectomized females is shown in Table 4. No differences were observed in the lipid content of the fat body and the blood between non-operated and ovariectomized females in 5 day-old pupae. At 9

Table 3. EFFECT OF OVARIECTOMY ON THE LIPID CONTENT OF WHOLE BODY OF FEMALES^a

Stage	Ovariectomy	Lipid content per	
		Insect	Gram fresh weight
Pupa (5-day old)	Non-operated	125.6±5.9	70.5±2.0
	Unilateral	127.1±2.8	75.2±1.6
	Bilateral	136.1±2.6	80.5±1.5
Pupa (9-day old)	Non-operated	91.8±3.7	53.8±3.6
	Unilateral	97.1±4.0	60.4±3.0
	Bilateral	105.8±2.3	71.3±3.1
Adult	Non-operated	72.0±3.7	71.9±3.1
	Unilateral	83.6±5.0	78.4±1.3
	Bilateral	100.6±2.2	110.3±1.9

^a Uni- and bilateral ovariectomies were made on the day of pupation. Adult emergence of females occurred 12 days after pupation. Silkworm strain, Shungetsu×Hosho. Measurements are mean values and their standard errors, and they are expressed in mg.

Table 4. EFFECT OF OVARIECTOMY ON THE LIPID CONTENT IN THE FAT BODY AND BLOOD OF FEMALE PUPA^a

Pupal age (day)	Ovariectomy	Lipid content in		
		Fat body per		Blood per ml
		Pupa	Gram fresh weight	
5	Non-operated	30.99	133.2	15.90
	Unilateral	30.07	134.4	15.30
	Bilateral	31.69	138.5	15.55
9	Non-operated	13.10	171.6	13.68
	Unilateral	18.15	172.0	16.38
	Bilateral	46.74	170.3	31.84

^a Uni- and bilateral ovariectomies were made on the day of pupation. Adult emergence of females occurred 12 days after pupation. Silkworm strain, Shungetsu×Hosho. Measurements are mean values, and they are expressed in mg.

Table 5. EFFECT OF UNILATERAL OVARIECTOMY ON THE LIPID CONTENT OF THE REMAINING OVARY^a

Stage	Operation	Lipid content per	
		One ovary	Gram fresh weight
Pupa (9-day old)	Non-operated	23.41±0.51	93.57±0.95
	Unilateral	25.32±0.49	96.61±1.68
Adult	Non-operated	25.78±1.87	87.74±0.49
	Unilateral	32.94±0.48	93.69±1.24

^a Unilateral ovariectomy was made on the day of pupation. Adult emergence of females occurred 12 days after pupation. Silkworm strain, Shungetsu×Hosho. Measurements are mean values and their standard errors, and they are expressed in mg.

days of age, bilaterally-castrated females contained 3 times more lipid in the fat body per pupa and 2 times more lipid in the blood than non-operated individuals.

Lipid content in the remaining ovary of unilaterally-castrated females is shown in Table 5. At 9 days of age, unilateral-castration seems to slightly elevate the lipid content of the remaining ovary in comparison with the non-operated ovary. This was realized at emergence; unilaterally-castrated females contained about 30% more lipid per ovary and about 10% more lipid on a fresh weight basis than non-operated individuals.

DISCUSSION

Silkworm males retained a higher whole body lipid content through pupal-adult development (Fig. 1), which on a fresh weight basis was remarkably higher than that of the females (Table 1). As a consequence, sexual dimorphism in the lipid content of *B. mori* pupae is observed and coincides with the tendency reported in *Hyalophora cecropia* pupae (GILBERT and SCHNEIDERMAN, 1961; DOMROESE and GILBERT, 1964). The conspicuous decrease in lipid content observed in female pupae would suggest an active utilization of lipid as an energy source for ovarian development. The marked increase in lipid content per unit fresh weight basis noted in male adults is related to the striking decrease in fresh weight of males at emergence, contrary to the slight lipid increase seen in female adults; males excrete more meconium than females at eclosion (KAWANAMI, 1950).

As to the lipid content of pupal tissues, fat body lipid decreased remarkably in contrast to the conspicuous increase in blood lipid seen at the early pupal period in both sexes; the ovarian lipid increased intensively at the late pupal period in the females (Fig. 2). This pattern of tissue lipid distribution in pupae suggests that the fat body lipid of both sexes is released actively into the blood with initiation of pupal development and incorporated into the developing ovary *via* the blood in the female pupae. The remarkably high lipid concentration observed in the fat body of male pupae (Table 2) clearly demonstrates sexual dimorphism in the fat body lipid. It is understood that high lipid content in the male whole body reflects an abundance of lipid in the fat body. Also, the remarkable increase in lipid content observed in the male fat body at the late pupal period may indicate the occurrence of active lipid synthesis. Considering the report on the complete exhaustion of glycogen in the male fat body during the pupal period (YAMASHITA and HASEGAWA, 1965), lipid synthesis in the male fat body may be performed through the conversion of glycogen as stated by CHINO and GILBERT (1965) in *H. cecropia* whole pupae.

In bilaterally-ovariectomized females, conspicuous lipid deposition was observed in the whole body at the adult age (Table 3), indicating a close relation between ovarian development and lipid decrease in normal female pupae. Also, lipid deposits observed in the fat body and in the blood of bilaterally-ovariectomized females (Table 4) would signify the accumulation of lipid in the pupal tissues induced by the lack of oogenesis. In unilaterally-ovariectomized females, considerable lipid increase was noticed in the ovary compared with the non-operated individuals (Table 5), indicating an oversupply of the fat body lipid to the remaining ovary. The above results substantiate the aforementioned suggestion that the fat body lipid is transported into the developing ovary *via* the blood. Since the ovarian development of isolated pupal abdomens of *B. mori*

run parallel to molting hormone doses (SAKURAI and HASEGAWA, 1969), lipid transport from the fat body into the ovary may be facilitated by ecdysone. Taking into consideration the results seen in castrated females, it is concluded that the high lipid content in the whole body and in the fat body of male pupae is a tendency reflecting the lack of oogenesis.

In the present study, sexual dimorphism in the lipid content was observed in the whole body as well as in the fat body of *B. mori* pupae, which seems to be a phenomenon reflecting physiological characteristic of each sex : In female pupae, the fat body lipid is utilized as a substrate for oogenesis and as an energy source for ovarian development, while in males the fat body lipid serves mainly as an energy source for active mating behavior of adults.

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