家蚕の休眠ホルモンの作用機構に関する研究

誌名	日本蠶絲學雜誌
ISSN	00372455
著者	一政, 祐輔
巻/号	44巻2号
掲載ページ	p. 137-145
発行年月	1975年4月

日蚕雑 44 (2), 137～145, 1975
(J. Sericult. Sci. Japan)

Studies on the Mode of Action of the Diapause Hormone with Special Reference to Lipid Metabolism in the Silkworm, *Bombyx mori* L.

II. Lipid Component and Fatty Acid Composition of Glyceride in Pupal Ovary, Fat Body and Haemolymph[1)]

YUSUKE ICHIMASA

Department of Biology, Faculty of Science,
Ibaraki University, Bunkyo, Mito, Japan

(Received June 17, 1974)

During metamorphosis of endopterygota, histogenesis goes on at the expense of materials originated from histolized larval tissues because of the cleidoicity in pupae. According to THOMAS and GILBERT (1969) working with *Hyalophora gloveri* pupae, the fat body synthesized lipid and protein to form lipoprotein, and one specific class of synthesized lipoprotein was selectively acquired by maturing oocytes from haemolymph after the material was released into haemolymph. In *Bombyx mori*, a number of workers conducted on lipid analysis (see ICHIMASA and HASEGAWA, 1973), but few studies have been made regarding the lipid analysis from the standpoint of oocyte development.

In the previous paper (ICHIMASA and HASEGAWA, 1973), lipid content and lipid composition in silkworm pupal ovaries were analysed to see the effect of the diapause hormone on lipid metabolism. The consequent result was that the hormone acted mainly for triglyceride to accumulate in ovaries at middle pupal stage. To understand further the mode of action of the diapause hormone on lipid metabolism in silkworm pupae, lipid analyses of fat body and haemolymph were essential.

In the present paper the lipid component and fatty acid composition of glyceride of fat body, haemolymph and ovary of silkworm pupae were analysed, and the effect of the diapause hormone on them was inferred from the extirpation experiments of the suboesophageal ganglion of diapause egg producers on the day of pupation.

MATERIALS AND METHODS

Animals

Silkworms used were diapause egg producers of two hybrid races of Kinshu×Showa and Shungetsu×Hosho. To change diapause egg producers into non-diapause

1) This research was supported in part by a research grant from the Ministry of Education, Japan.

egg producers, the suboesophageal ganglion (SG) was extirpated on the day of pupation. The diapause hormone action was inferred by comparing the data obtained in pupae with the SG removed (−SG) with those of pupae with the intact SG (+SG). Ovaries, fat body and midgut were dissected out in cold saline (0.75% NaCl) from 5-50 pupae according to pupal development, isolated from other tissues and washed once with cold saline solution. The tissues blotted on filter paper were immediately weighed gravimetrically.

About 5-8 ml of haemolymph was collected from 10 to 15 pupae at desired stages in a test tube containing a small amount of phenyl thiourea and was centrifuged at 2,500 rpm for 5 min to remove haemocytes and tissue fragments.

Lipid extraction and fatty acid analysis

Extraction and fractionation of lipid were described previously (ICHIMASA and HASEGAWA, 1973). Fatty acids of the glyceride fractions were methylated according to the method of MCGINNIS and DUGAN (1964). Gas chromatographic separation of these methyl esters was carried out with a JGC model 1100 flame ionization gas chromatograph equipped with a stainless steel column (2000×3 mm) with 12% DEGS on Anakrom (60-80 mesh): column temperature of 169°C with nitrogen as the carrier gas, detector temperature of 250°C, and injection temperature of 235°C. Identification of fatty acids in the samples was carried out by comparing the retention times with those of authentic fatty acids which were developed in the same condition.

RESULTS

Lipid content of fat body, midgut, haemolymph and ovary

To see the effect of the diapause hormone on lipid content in some tissues, lipid content of each tissue of "+SG" and "−SG" pupae was determined as previously reported (ICHIMASA and HASEGAWA, 1973). The results are indicated in Fig. 1, where lipid content is expressed in terms of mg per g wet weight of tissues except ml in haemolymph.

As shown in this figure, lipid accumulation in ovary was observed up to 2 days before adult emergence and then decreased thereafter. On the other hand, lipid content in fat body appeared to run oppositely to that of ovary. In pupae with the SG removed (−SG), the lipid content was lower in both fat body and ovaries than that of these tissues of non-operated pupae (+SG). But the lipid content in midgut and haemolymph was not affected by the SG-removal.

Lipid component of fat body, haemolymph and ovary

Analyses of lipid components of fat body, haemolymph and ovary were conducted on pupae of Shungetsu×Hosho hybrid. As already reported (ICHIMASA and HASEGAWA, 1973), the relative amount of triglyceride and that of phospholipid were almost the same, each occupying more than 40% of the ovarian lipid at 3-day old pupal age,

but as the adult development proceeded, the amount of triglyceride exceeded that of phospholipid, the former becoming the major component and the latter taking the second place. In this experiment the anthor examined the lipid content in haemolymph and fat body of 3-, 5-, 6-, and 7-day old pupae. The relative amounts of lipid components of these two tissues showed each characteristic profile and were almost constant at pupal ages examined. Then, one example of haemolymph and fat body of 7-day old pupae is shown in Table 1 together with ovarian lipid components.

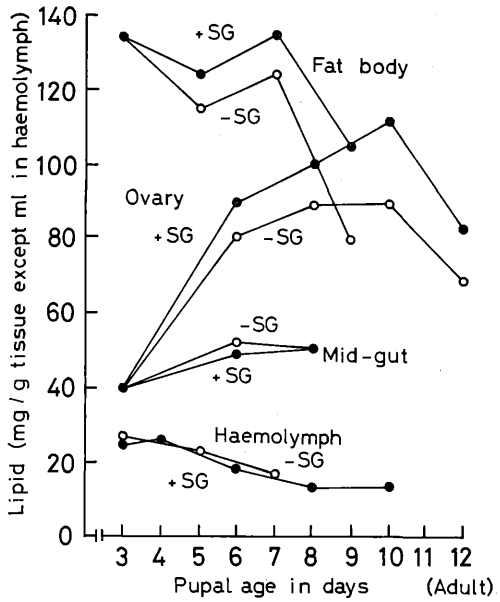

Fig. 1. Effect of SG-removal on lipid content in several tissues of silkworms during adult development (silkworm variety: Kinshu×Showa). Ovary, fat body, midgut and haemolymph used for each experiment were collected from 5–50 pupae. Closed circle indicates "+SG" and open circle "−SG".

In fat body lipid, the main component was triglyceride which occupied about 80% of total lipid. Phospholipid took the second place, occupying 12% and the other components were below 8% in all. Predominance of triglyceride over phospholipid which came to the second place in insect fat body lipid was already reported by GILBERT (1967) in female *Leucophaea maderae* adults and by BEENAKKERS and GILBERT (1968) in male *Hyalophora cecropia* diapausing pupae and pupae one day before emergence.

Instead of triglyceride which was the major component in fat body lipid, diglyceride was the major component in haemolymph lipid of silkworm pupae as observed in male *H. cecropia* pupae (CHINO and GILBERT, 1965). High relative amount of sterol

Table 1. Lipid composition of fat body, haemolymph and ovary of 7-day old pupae

Tissue	Per cent of lipid component							
	Hydro-carbon	Sterol ester	Tri-glyceride	Free fatty acid	Di-glyceride	Sterol	Mono-glyceride	Phospho-lipid
Fat body	1.0	0.7	80.5	1.8	0.8	2.7	0.5	12.0
Haemolymph	t	6.8	t	10.0	61.8	7.0	2.4	12.0
Ovary	4.3	4.4	66.0	5.0	3.7	1.7	1.9	13.0

Tissues used were collected from 10–15 pupae of Shungestu × Hosho. Lipids were extracted with ethanol-ether (3:1) and subjected to thin layer chromatography. t: trace amount (the same applies to the following tables).

Table 2. Fatty acid composition of glycerides of 7-day old pupal fat body

Lipid fraction	Relative percentage of fatty acids					
	C16 : 0	C16 : 1	C18 : 0	C18 : 1	C18 : 2	C18 : 3
Triglyceride	28	t	7	27	5	31
Diglyceride	30	t	6	22	8	34

Gas chromatographic analysis was carried on a 2000×3 mm in DEGS column at 169°C, detector temp. 250°C, injection temp. 235°C. Fat body used was collected from 50 pupae of Shungestu×Hosho.

and its ester as well as phospholipid in silkworm pupal haemolymph lipid is comparable to that of the neutral lipid components from *H. gloveri* pupae (THOMAS and GILBERT, 1969). Among tissue lipids examined the highest relative amount of free fatty acid in haemolymph lipid was worth noting in respect to the fact that *Cecropia* pupal fat body released free fatty acid into haemolymph (BEENAKKERS and GILBERT, 1968).

As for ovarian lipid of 7-day old pupae, triglyceride was expectedly predominant in the lipid components, and phospholipid took the second place. This pattern was almost similar to that reported previously in ovaries of Kinshu×Showa hybrid 6-day and older after pupation (ICHIMASA and HASEGAWA, 1973).

In short, lipids of fat body and ovary contained similar constituents which differ from those of haemolymph. The former contained high relative amount of triglyceride while the latter diglyceride. Although not indicated here, the lipid composition of fat body of "−SG" pupa was similar to that of "+SG" pupa.

Fatty acid composition of fat body, haemolymph and ovarian lipids

Analysis of fatty acid composition of glycerides in fat body, haemolymph and ovarian lipids was conducted on 4-, 5-, 7-, and 9-day old pupae.

1) Fatty acids in fat body glycerides.

As fat body contained only very low amount of monoglyceride (Table 1), fatty acid composition of fat body monoglyceride was not analyzed. Relative percentages of fatty acids of fat body glycerides did not greatly change during adult development (unpublished data). Then, the fatty acid composition of glycerides of 7-day old pupae was indicated in Table 2. As shown in this table, triglyceride, the main component of fat body lipid, and diglyceride consisted mainly of C16 : 0, C18 : 1 and C18 : 3 with a small portion of C18 : 0 and C18 : 2. The profiles of fatty acid composition in both triglyceride and diglyceride are comparable to that of total fat body lipid from silkworm 5th instar larvae (NAKASONE and ITO, 1967) and of fat body diglyceride fraction from *Philosamia cynthia* (CHINO *et al.*, 1969).

2) Fatty acids in haemolymph glycerides.

Table 3 shows the relative percentages of fatty acids in pupal haemolymph, 5-

Table 3. Fatty acid composition of glycerides of pupal haemolymph

Lipid fraction	Pupal age in days	Relative percentage of fatty acids					
		C16: 0	C16: 1	C18: 0	C18: 1	C18: 2	C18: 3
Triglyceride	5	42	4	11	18	7	19
	7	54	10	14	15	3	5
Diglyceride	5	33	t	7	30	7	23
	7	33	2	8	32	7	19
Monoglyceride	5	48	15	12	12	5	7
	7	48	13	11	8	10	10

Gas chromatographic conditions are the same as in Table 2. Haemolymph used for each experiment was collected from 50 pupae of Shungestu×Hosho.

and 7-day old. In this case, the fatty acid composition was different according to pupal ages and glyceride fractions, especially in the latter. A property common to all glycerides was that C16: 0 fatty acid was the main component of haemolymph glycerides, and C18: 3 was relatively low comparing with that of fat body glycerides. The diglyceride, one of the major components of haemolymph lipid, was different from triglyceride and monoglyceride in three points that only the trace of C16: 1 and a higher percentage of C18: 1 and C18: 3 were found.

The highest relative amount of C16: 0 in haemolymph glyceride seemed to be a characteristic in silkworm pupal haemolymph. In the 5th instar silkworm larvae C18: 3 takes the first place, occupying about 40%, followed by C16: 0, about 25% of total haemolymph lipid (NAKASONE and ITO, 1967). Furthermore, in *H. cecropia* (BEENAKKERS and GILBERT, 1968) and *H. gloveri* (THOMAS and GILBERT, 1969), C18: 1 and C18: 3 are predominant occupying about 30% of each of haemolymph glycerides, and C16: 0 is far less, taking the third place in fatty acid composition of the glycerides.

3) Fatty acids in ovarian glycerides.

Table 4 indicates fatty acid composition of ovarian glycerides of pupae at 4-, 5- and 9-day old ages. The main component of ovarian lipid, triglyceride, consisted mainly of C16: 0, C18: 1 and C18: 3 with a small proportion of C16: 1, C18: 0 and C18: 2, throughout development similar to fat body triglyceride and diglyceride. On the other hand, diglyceride and monoglyceride consisted of extremely high relative amount of C16: 0 with trace amount of C18: 1 and C18: 3 which were the major components of ovarian triglyceride. Another interesting point was that unknown compounds (X_1 and X_2) were detected.

Relative retention time of X_1 and X_2 as well as other fatty acids of ovarian glycerides from pupae is indicated in Table 5 together with authentic fatty acid methyl esters by gas chromatography. From this table, nothing can be concluded concerning X_1 and X_2 in mono- and diglyceride fractions. The identification of these

Table 4. Fatty acid composition of glycerides of pupal ovary

Lipid fraction	Pupal age in days	Relative percentage of fatty acids							
		C16 : 0	C16 : 1	C18 : 0	C18 : 1	C18 : 2	X_1	C18 : 3	X_2
Triglyceride	4	27	2	4	31	8	—	29	—
	5	37	2	5	37	5	—	13	—
	9	29	1	3	29	6	—	31	—
Diglyceride	4	51	t	25	t	9	16	—	—
	5	55	t	13	t	8	8	1	13
	9	74	t	14	t	11	—	—	—
Monoglyceride	4	61	t	23	t	4	12	—	—
	5	46	t	18	t	19	18	—	—
	9	74	t	19	t	4	4	—	—

Gas chromatographic conditions are the same as in Table 2. Ovaries used for each experiment were collected from 50 pupae of Shungestu × Hosho. X_1 and X_2 are unidentified compounds.

Table 5. Comparison of the relative retention time between fatty acid methyl esters from ovarian glycerides and authentic fatty acid methyl esters by means of gas chromatography

Fatty acid and unidentified compound	Relative retention time			
	Authentic fatty acid methyl ester	Fatty acid methyl ester from ovarian glycerides		
		Monoglyceride	Diglyceride	Triglyceride
C16 : 0	1.00	1.00	1.00	1.00
C16 : 1	1.23	1.25	—	1.14
C18 : 0	1.88	1.91	2.00	1.93
C18 : 1	2.19	—	2.32	2.25
C18 : 2	2.89	3.07	3.00	2.93
X_1		3.53	3.48	—
C18 : 3	3.94	—	4.12	4.07
X_2		5.35	5.29	—

X_1 and X_2 are unidentified compounds in gas chromatographical analysis as in Table 2. Relative retention times of fatty acids and unidentified compounds are expressed relatively to C16 : 0 retention time.

substances is now in progress.

4) Effect of SG-removal on fatty acid composition of ovarian glycerides.

In the previous paper (ICHIMASA and HASEGAWA, 1973), the existence of SG or the diapause hormone was reported to facilitate triglyceride accumulation in ovaries at middle pupal stage. An attempt was made to see whether or not the diapause hormone affected also the fatty acid composition of ovarian glycerides. To this end, diapause egg producers received the SG-removal on the day of pupation, and the fatty

acid composition of ovarian glycerides of these pupae was analyzed during adult development together with that of non-operated animals. These parallel experiments disclosed that the presence or the absence of the SG did not significantly affect the relative amounts of fatty acid in ovarian glycerides. In other words, the SG or the diapause hormone did not seem to affect the fatty acid composition in pupal ovaries.

DISCUSSION

During the adult development of silkworms, lipid content was different from tissue to tissue. Among them midgut and haemolymph showed a somewhat constant level but fat body represented a precipitous decline which corresponded roughly to an increment in ovaries (Fig. 1). Lipids are known to be utilized as an energy source in insects as well as other organisms. In fact, *Cecropia* female pupae were reported to utilize lipid as an energy source for adult development while in male pupae carbohydrates were predominantly utilized instead of lipid (DOMROESE and GILBERT, 1964). In *B. mori* the decline of fat body lipid seemed not to be caused by that the lipid was utilized but itw as released into haemolymph according to its histolysis, regarding that the haemolymph of silkworm pupae also contained diglyceride as a main lipid class (Table 1), and it seems to function in transport in the form of lipoprotein as demonstrated in some insects (THOMAS and GILBERT, 1968; 1969; CHINO *et al.*, 1969). Ovaries grew vigorously by incorporating much nutrients from haemolymph during adult development. As for lipids, ovaries continued to uptake lipids or their precursors from haemolymph throughout development. Therefore, the ovarian lipids were mainly supplied from fat body via haemolymph throughout development. Such utilization of fat body lipids by developing ovaries was demonstrated in several insects (DUTKOWSKI and ZIAJKA, 1972; DUTKOWSKI and SARZALA-DRABIKOWSKA, 1973; DUTKOWSKI, 1973).

The SG or the diapause hormone seemed to affect the lipid distribution between tissues; SG disturbed partially lipid release from fat body and enhanced clearly lipid accumulation in ovaries. As the diapause hormone acted on ovaries as the target organ with respect to carbohydrate metabolism (HASEGAWA and YAMASHITA, 1965), the hormone functioned in lipid metabolism of fat body as well as ovaries (Fig. 1). Further correlations between both actions of the hormone, however, remain to be resolved.

In silkworm pupae as in some insects, haemolymph lipids consisted mainly of diglyceride, and fat body and ovaries of triglyceride (Table 1). If almost all lipids of silkworm ovaries were transported from fat body without any modifications, fatty acid composition of ovarian and fat body lipids should not be dissimilar. But, the fatty acid composition of triglyceride was clearly different between fat body and ovaries (Tables, 2 and 4). These results suggested that bulk of ovarian lipid was

synthesized from non-lipoidal substrates and/or consisted of modified fatty acids which were originally derived from fat body lipids.

SHRIDHARA and BHAT (1965) showed that silkworms could incorporate acetate into palmitic, stearic and oleic acids except linoleic and linolenic acids after injection of ^{14}C-acetate. The similar results were demonstrated in *Cecropia* silkmoths (STEPHEN and GILBERT, 1969) and in cabbage looper (NELSON and SUKKESTAD, 1968). However, in these insects, synthesis of linoleic and linolenic acids from acetate was not observed and this inability appeared to be characteristic of all insects studied thus far (cf. GILBERT, 1967). Contrary to the above, the homogenates of larvae and pharate adults of *Ceratitis capitata* could desaturate and elongate the fatty acids *in vitro* (MUNICIO *et al.*, 1972). Considering these facts, silkworm ovaries could in part synthesize or modify fatty acids *in situ* in addition to the direct transport from haemolymph.

Several investigators (BUDOWSKI *et al.*, 1961; SCHJEIDE *et al.*, 1963) have demonstrated that the lipid moieties of plasma lipoproteins were modified extensively in the process of transport to egg york in hens. Recently, GORNALL *et al.* (1972) have shown a novel evidence incompatible with the previous proposals. However, the uptake mechanism of lipid in tissues from haemolymph remains as an interesting point to be investigated in insect lipid metabolism. In this sense the comparison of fatty acid composition presented in this paper will throw a light to this problem.

SUMMARY

The lipid and fatty acid compositions of glycerides in several tissues of silkworms, *Bombyx mori* L., were investigated during adult development and the effects of the diapause hormone on them were inferred from the SG extirpation experiments of diapause egg producers on the day of pupation.

1. Triglyceride was the main lipid component of fat body and ovary, while the main component of haemolymph lipid was diglyceride.

2. The fatty acid composition of each glyceride of fat body and haemolymph was similar with each other and each glyceride contained mainly palmitic, stearic, oleic and linolenic acids.

3. The main fatty acid components of ovary triglyceride were palmitic, oleic and linolenic acids, accounting for about 90% of the total fatty acid, whereas the main fatty acids of mono- and diglycerides were palmitic, stearic and linoleic acids except two unidentified fatty acids (X_1 and X_2).

4. The SG or the diapause hormone facilitated the lipid accumulation in ovaries at the middle pupal stage without any effect on midgut and haemolymph lipids. However, the hormone did not affect the fatty acid composition of ovarian lipids.

ACKNOWLEDGMENTS

The auther wishes to express his sincere gratitude to professor K. HASEGAWA and Dr. O. YAMASHITA of Nagoya University, for their helpful advices, encouragements and critical readings of the manuscript.

REFERENCES

BEENAKKERS, A. M. TH. and L. I. GILBERT (1968): J. Insect Physiol., **14**, 481-494.
BUDOWSKI, P., N. R. BOTTINO and R. REISER (1961): Arch. Biochem. Biophys., **93**, 483-490.
CHINO, H. and L. I. GILBERT (1965): Biochim. Biophys. Acta, **98**, 94-110.
CHINO, H., S. MURAKAMI and K. HARASHIMA (1969): Biochim. Biophys. Acta, **176**, 1-26.
DOMROESE, K. A. and L. I. GILBERT (1964): J. Exp. Biol., **41**, 573-590.
DUTKOWSKI, A. B. (1973): J. Insect Physiol., **19**, 1721-1726.
DUTKOWSKI, A. B. and M. G. SARZALA-DRABIKOWSKA (1973): J. Insect Physiol., **19**, 1341-1350.
DUTKOWSKI, A. B. and B. ZIAJKA (1972): J. Insect Physiol., **18**, 1351-1367.
GILBERT, L. I. (1967): Comp. Biochem. Physiol., **21**, 237-257.
GORNALL, D. A., A. KUKSIS and N. MORLEY (1972): Biochim. Biophys. Acta, **280**, 225-230.
HASEGAWA, K. and O. YAMASHITA (1965): J. Exp. Biol., **43**, 271-277.
ICHIMASA, Y. and K. HASEGAWA (1973): J. Seric. Sci. Jap., **42**, 12-24.
MCGINNIS, G. W. and L. R. DUGAN (1964): J. Am. Oil Chem. Soc., **42**, 305-307.
MUNICIO, A. M., J. M. ODRIOZOLA, A. PINEIRO and A. RIBERA (1972): Biochim. Biophys. Acta, **280**, 248-257.
NAKASONE, S. and T. ITO (1967): J. Insect Physiol., **13**, 1237-1246.
NELSON, D. R. and D. R. SUKKESTAD (1968): J. Insect Physiol., **14**, 293-300.
SCHJEIDE, O. A., M. WILKENS, R. G. MCCANDLESS, R. MUNN, M. PETERSON and E. CARLSEN (1963): Am. Zool., **3**, 167-184.
SRIDHARA, S. and J. V. BHAT (1965): J. Insect Physiol., **11**, 449-462.
STEPHEN, W. F. and L. I. GILBERT (1969): J. Insect Physiol., **15**, 1833-1854.
THOMAS, K. K. and L. I. GILBERT (1968): Arch. Biochem. Biophys., **127**, 512-521.
THOMAS, K. K. and L. I. GILBERT (1969): Physiol. Chem. Physics, **1**, 293-311.

摘　要

家蚕の休眠ホルモンの作用機構に関する研究特に脂質代謝との関連について

Ⅱ．蛹期卵巣，脂肪体，血液の脂質成分及びグリセリドの脂肪酸組成

一政祐輔

家蚕蛹2-3組織の脂質含量と脂質組成及びグリセリドの脂肪酸組成を調べ，それらに及ぼす休眠ホルモンの作用を食道下神経節摘出実験から推定した。

1．家蚕蛹の脂肪体と卵巣の主なる脂質成分はトリグリセリドであり，血液脂質の主成分はジグリセリドであった。

2．脂肪体と血液の各グリセリドの脂肪酸組成は互いに類似していて，各グリセリドは主に，パルミチン酸，ステアリン酸，オレイン酸，及びリノレン酸からなっていた。

3．卵巣トリグリセリドの主な脂肪酸組成は，パルミチン酸，オレイン酸及びリノレン酸であって全脂肪酸の約90%をしめていた。それに対して，モノグリセリドとジグリセリドの主な脂肪酸はパルミチン酸，ステアリン酸，リノール酸，及び未同定の X_1 と X_2 であった。

4．食道下神経節の摘出は，蛹の発育中期の脂肪体と卵巣の脂質含量を低下させたが，中腸と血液の脂質含量には影響がみられなかった。これは休眠ホルモンが化蛹中期の脂肪体と卵巣の脂質代謝に影響することを推定させるものである。しかし休眠ホルモンが卵巣のグリセリドの脂肪酸組成に影響する結果は，SG摘出実験からは得られなかった。

（水戸市・茨城大学理学部）