

LIPID COMPOSITION OF FAT BODY AND ITS CONTRIBUTION TO THE MATURING OÖCYTES IN *PYRRHOCORIS APTERUS*

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Abstract—The fat body of adult, female *Pyrrhocoris apterus* undergoes cyclic growth and diminution correlated with cycles of oöcyte maturation. At all stages lipid accounts for 80 per cent of the dry weight of the fat body and the content of lipid, protein, and glycogen change in proportion to the changes in dry weight. The fatty acid composition of the lipid in fat body, ovaries, the whole insect, and the diet was examined by gas-liquid chromatography. *Pyrrhocoris* is similar to other insects in having triglyceride as the predominant class of lipid in the fat body but is distinctive in having an unusually high (more than 50 per cent) proportion of linoleate among its fatty acids, presumably owing to its diet. Reasons are presented for considering that little, if any, fatty acid is synthesized by the fat body and the ovaries during the reproductive cycle.

During the first 5 days after the larval-adult ecdysis the *Pyrrhocoris* fat body first accumulates lipid and then releases it for utilization by the ovary. This temporary storage by the fat body compensates for the apparent inability of the insect to ingest a sufficient amount of lipid during vitellogenesis. The ovary normally utilizes dietary lipid as well during vitellogenesis; when the dietary supply was experimentally interrupted the amount of lipid released by the fat body increased. Storage of lipid in the fat body represents an important physiological adaptation since it frees the insect from dependence on a constant food supply during vitellogenesis.

INTRODUCTION

THIS STUDY is an investigation of the source of lipid yolk in the bug *Pyrrhocoris apterus*. *P. apterus* produces eggs which are one-third lipid (by dry weight) and which develop simultaneously, thus permitting examination of the events occurring during vitellogenesis. Oögenesis requires a rapid accumulation of reserves which, in insect eggs, take the form of protein yolk spheres and lipid yolk spheres as well as glycogen deposits. The possible sources of these yolk components are *de novo* synthesis by the ovary, accumulation from digested food, or accumulation from some other organ in the insect's body. In his recent review, TELFER (1965) discussed the extensive contribution made by extra-ovarian sources to the protein yolk spheres and noted that experimental information on the source of lipid yolk is entirely lacking. No information on this subject has appeared since Telfer's review.

In this study all three possible sources of lipid yolk were examined. Since the most likely extra-ovarian source of lipid in the body is the fat body, the amount and composition of the lipids in the fat body and the ovaries were examined in both reproducing and non-reproducing females during the time of the first two reproductive cycles. The amount of lipid ingested at this time was measured and the effect of interruption of feeding was examined in relation to both oöcyte production and the lipid content of the fat body. A subsequent communication will examine the processes related to lipid incorporation and mobilization by the fat body and the regulatory effect on these processes exerted by the developing ovary, as well as the transport of lipid in the haemolymph.

MATERIALS AND METHODS*

Experimental insects

P. apterus L. has five larval instars, each lasting about 6 days except for the last instar, which lasts about 10 days. The adults normally spend the winter in diapause, during which respiratory metabolism is greatly reduced and reproductive activity is suspended (SLÁMA, 1964). Feeding continues if food is available, but at a reduced rate. Under normal conditions *Pyrrhocoris* terminates diapause during the spring, returning to a state of normal activity and reproduction. The adult life expectancy of active females in captivity is 6 to 8 weeks during which time eggs are laid in batches every 3 to 5 days.

Adult female *P. apterus* were used in the present experiments; their age was measured from the day of the larval-adult ecdysis, that day being considered as day 0. Females were usually housed with males. There is some variation among individuals in the length of the reproductive cycle. The first batch of eggs is laid by most females (regardless of copulation) on day 5 but occasional bugs will

TABLE 1—OÖCYTE SIZE IN RELATION TO AGE OF ACTIVE, ADULT FEMALES

Age of female (day)	Oöcyte length (mm)
1	0.3
2	0.3-0.4
3	0.4-0.8
4	0.8-1.1
5*	1.1-1.4
6	0.4-0.5
7	0.6-0.9
8*	1.1-1.3
9	0.3-0.5

* Chorion observed in mature oöcytes on this day, the day on which eggs are laid.

* *Abbreviations*: TLC, thin-layer chromatography; GLC, gas-liquid chromatography; TG, triglyceride; DG, diglyceride; MG, monoglyceride; FFA, free fatty acid.

not oviposit until day 6 or 7. The second batch of eggs is usually laid after an additional 3 or 4 days. In this study, only those females which laid eggs on days 5 and 8 were used, unless stated otherwise. Table 1 shows the size range of oöcytes developing at a rate which results in mature eggs on days 5 and 8. Females were used for experiments only if their oöcytes were in the appropriate morphological condition for their age, as measured by the size range shown in Table 1. To minimize differences in physiological age even further for the study of the growth of the normal fat body and ovaries, tissues were taken only from females which had moulted between 9 a.m. and 5 p.m., in addition to the other criteria.

Maintenance of the colony

Insects were reared and maintained on a long-day photoperiod [17 hr light alternating with 7 hr dark (17 : 7)] at 25°C. They were given seeds of the linden tree *Tilia cordata* and water *ad libitum*. The insects serving as breeding stock for the colony were caused to undergo diapause as adults before breeding. Diapause was induced by maintenance of larvae on a short-day photoperiod (8 : 16) continuously after the bugs reached the second instar. Adults were kept in diapause at 25°C for a minimum of 4 weeks and at 5°C for a minimum of 4 additional weeks. Diapause terminated after the insects were returned to long-day conditions at 25°C; a full, non-resting level of activity was resumed by the end of 2 to 3 weeks.

Measurement of daily food consumption

The daily feeding rate was estimated on individual bugs by measuring the loss in seed weight/24 hr. This estimate was corrected for fluctuations in seed weight resulting from humidity changes on other causes by following the daily weight change in five control batches of seeds incubated in parallel without insects.

Surgical procedures

A. *Ovariectomy*. Ovaries were removed from female larvae 12 to 24 hr after ecdysis into the fifth instar. The larvae, anaesthetized by submersion in tap water, were placed under *Pyrrhocoris* Ringer* and each ovary was removed through a small, ventral incision in the second abdominal segment. Complete removal of both ovaries was confirmed when the abdomen was dissected for use in an experiment.

B. *Allatectomy*. The corpus allatum was removed from females which had been deprived of food for 1 day immediately after ecdysis into adults. The procedure followed was the 'neck membrane' technique used by SLÁMA (1964). Females were examined by dissection on the day of analysis to confirm that the corpora cardiaca were intact. Only those females with intact corpora cardiaca and undeveloped ovaries were used. The eggs do not mature in females allatectomized at this time.

* The *Pyrrhocoris* Ringer used throughout this study has the following composition (Sláma, personal communication): 0.128 M sodium chloride, 0.0013 M potassium chloride, 0.0024 M sodium bicarbonate, and 0.0018 M calcium chloride. The pH was 7.8.

Tissue preparation

The abdominal fat body was isolated under chilled Ringer (6°C) by carefully severing tracheal connexions and removing adhering pieces of Malpighian tubules. The tissue was gently teased free from the cuticle, removed in one piece, and placed directly into a tared container. Since the cephalo-thoracic fat body is relatively small in amount and difficult to isolate, only abdominal fat body was examined.

Ovaries were freed from tracheal connexions under chilled Ringer, were severed at their connexion to the genital duct, and were placed directly into a tared container.

Tissue analysis

A. *Dry weight and lipid content.* Tissues were weighed after drying to constant weight at 60°C overnight. The total lipid content was determined by measuring the decrease in weight following removal of the lipid from the dried tissue by extraction in three 2 ml portions of diethyl ether. Ether extraction yielded the same results on fat body and immature ovaries as those obtained by the chloroform-methanol (2 : 1, v/v) procedure of FOLCH *et al.* (1957). However, ether washing did not extract all the lipid from mature ovaries; total lipid content of mature ovaries was measured gravimetrically after extracting the lipid by the Folch procedure.

B. *Glycogen and protein content.* After ether extraction and determination of lipid-free dry weight the residues were homogenized in 1.0 ml of 1 N sodium hydroxide. The homogenate was divided into two equal parts for measurement of carbohydrate and protein content. Glycogen and other precipitable carbohydrates were measured with anthrone according to the method of SEIFTER *et al.* (1950). A glucose standard was included in every assay and measurements were made as glucose equivalents, using a Coleman spectrophotometer.

Protein was measured with Folin-Ciocalteu phenol reagent according to the method of LOWRY *et al.* (1951), using a Beckman DU spectrophotometer. A series of standard aqueous solutions of crystalline bovine serum albumin (Armour) was measured with every group of determinations.

Lipid analysis

A. *Amount of lipid in individual classes.* Lipid was extracted from the fat body and ovaries with chloroform-methanol (2 : 1, v/v) according to the method of FOLCH *et al.* (1957). After removal of the solvent under nitrogen at room temperature, the lipid was redissolved in 2 drops of chloroform-methanol (1 : 1, v/v). The lipid classes were separated by TLC using a 250 μ layer of silica gel-G (Warner-Chilcott Lab.) according to the procedure of FREEMAN and WEST (1966).

In routine TLC analysis a solvent mixture of anhydrous diethyl ether-benzene (thiophene free)-ethanol-acetic acid (40 : 50 : 2 : 0.2, v/v/v/v) was allowed to ascend 16 cm. At the concentrations used, TG, DG, MG, FFA, and sterols were separated without overlap. Phospholipid remained at the origin with other polar substances and was not measured. Steryl esters were separated from TG on a separate plate in anhydrous ethyl ether-hexane (6 : 94, v/v). All solvents used were reagent grade. Regions containing the separated lipids were located by

visualization of authentic samples in reference lanes, by spraying the latter with 2,7-dichlorofluorescein and examining under u.v. light. The desired regions were scraped on to aluminium foil and transferred to test-tubes. The amount of lipid was determined according to the procedure of AMENTA (1964), by measuring the amount of oxidized dichromate remaining, after the lipid reacted with potassium dichromate (2.5 g/l) in 36 N sulphuric acid. Measurement was made with a Beckman DU spectrophotometer at 350 m μ . A blank lane was included by applying an equivalent volume of chloroform-methanol (1 : 1, v/v) to the TLC plate and treating it identically to the experimental samples. This provided a silica gel-dichromate blank for each lipid class; distilled water was used to zero the spectrophotometer. Six standard samples containing 25 to 250 μ g of each authentic compound (see below) were separated by TLC and treated identically to the tissue lipids. The change in optical density was directly proportional to the concentration of each compound. The authentic compounds used were trilinolein (99+%) and cholesteryl palmitate (99+%) (from Applied Science Laboratories); palmitic acid (99+%), monopalmitin (99+%), and diolein (grade 2, 98%) (all from Sigma Chemical Company); and cholesterol (repurified, from Steraloids, Inc.).

B. Fatty acid composition of the lipids in whole insects and individual tissues.

Lipid extraction: Entire insects (about 100 g) were extracted in a Waring blender with two 500 ml portions of ether-methanol (3 : 1). The ether phase was washed once with a saturated sodium chloride solution, and then the wash solution was back washed once with ether. The combined extracts were dried with magnesium sulphate and were concentrated at reduced pressure on a rotary evaporator. A weight of lipid corresponding to 10.3 per cent of the wet weight of the insects was obtained.

Isolated fresh tissues from four to six females were pooled for each determination of fatty acid composition in the fat body and the ovaries. Lipids were extracted twice from these, and from the seeds, by homogenization in anhydrous ethyl ether. The extract was dried with anhydrous sodium sulphate and the ether removed over a steam-bath under nitrogen.

Separation of lipid classes: The lipid extracted from the sample of whole insects was separated into classes according to a modification of the method of CARROLL (1961). In a typical run 1.972 g of lipid, dissolved in a small volume of petroleum ether (b.p. 40-60°C, redistilled), was applied to a column of 100 g of Florisil (7.5% water). Hydrocarbons were eluted with petroleum ether, simple esters with ether-petroleum ether (5 : 95), TG with ether-petroleum ether (15 : 85), sterols with ether-petroleum ether (25 : 75), DG with ether-petroleum ether (50 : 50), MG and other polar lipids with methanol-ether (10 : 90), and FFA with acetic acid-ether (5 : 95). The FFA were further purified prior to GLC analysis by passage through Amberlite IRA-400(OH⁻) ion exchange resin.

Measurement of fatty acid composition: The separated lipid fractions and the oil extracted from the tissues and the seeds were converted to methyl esters for

identification and quantitative determination by GLC. Conversion was accomplished by mixing the lipid with 14 per cent (w/v) boron trifluoride-methanol (Applied Science Lab.) and heating under nitrogen for 30 min according to the method of MORRISON and SMITH (1964).

The resulting methyl esters were dissolved in *n*-pentane and were separated on an F and M Model 810 dual-column gas chromatograph equipped with flame ionization detectors. The column of choice was a 6 ft \times $\frac{1}{8}$ in. 6% diethyleneglycol succinate (LAC-728) on acid-washed silanized Chromosorb. With the column at a constant temperature between 150 and 170°C, and a carrier gas flow rate of 33 ml/min (tissue and seed lipids) and 35 ml/min (entire insect lipids), resolution was satisfactory for quantitation. Peak areas were determined by triangulation, and identification was achieved through comparison of retention times of the methyl esters with those of authentic samples (Applied Science Lab.). The standards were run separately and in mixtures with the unknown samples.

Statistical analysis

The Mann-Whitney *U*-test (SIEGEL, 1956) was applied to comparable data on tissues of different ages, to ascertain the probability that groups of numerical data belong to the same numerical population. Two sets of numbers were considered significantly different if $P < 0.05$. Standard deviations were corrected for small sample size according to the table of BLISS and CALHOUN (1954).

RESULTS

Growth of adult fat body and ovaries

Dry weight provided a measure of changes in overall mass of fat body and ovaries and served as a reference for studies of changes in individual components such as lipid, glycogen, and protein. The changes in dry weight of the fat body are shown in Fig. 1. During the first 3 days after the larval-adult ecdysis the fat body more than doubled its mass, as measured by dry weight. In the subsequent 2 days before oviposition (days 3-5) the dry weight decreased, reaching a minimum value on the day of oviposition. In the somewhat shorter period which elapsed prior to the second oviposition (days 5-8) the dry weight underwent a similar increase and decrease, though of lesser magnitude than in the first cycle. Although measurements were not continued through the third reproductive cycle, an increase in dry weight was recorded on day 9, suggesting a repetition of the changes just described.

The fact that the fat body underwent an average reduction in dry weight of 36 and then 24 per cent, each in 2 days preceding oviposition, implies mobilization of materials for use by some other organ, or a decrease in feeding and assimilation. Since the only other organ which appears to change appropriately during this period is the ovary and since maturing oöcytes require a source of yolk components and energy for synthetic activity, attention was turned to the changes occurring in the ovaries at this time.

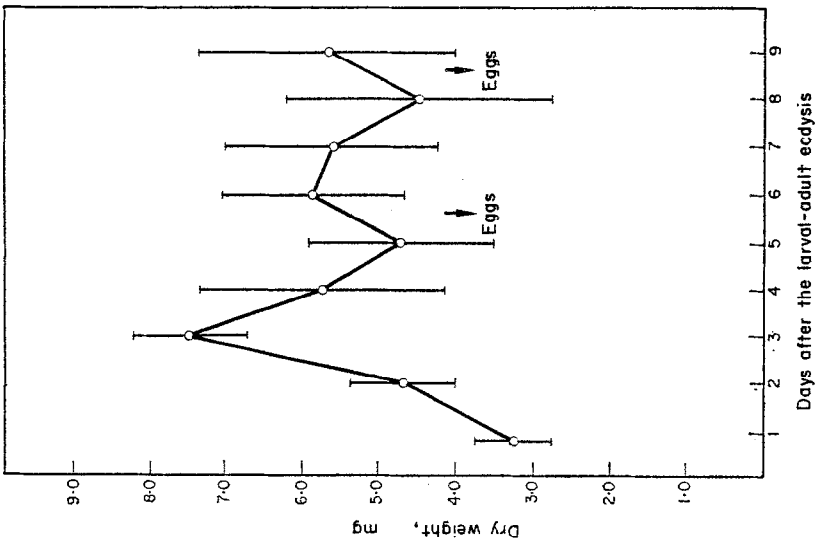


FIG. 1. Growth of the fat body in active females, represented by the means and standard deviations of four to six females at each age. The individual measurements used for this and all subsequent figures appear in MARRIN, 1968. Values of *P* for day 1 vs. day 3 = 0.008, for day 5 vs. day 6 = 0.062, and for day 6 vs. day 8 = 0.063.

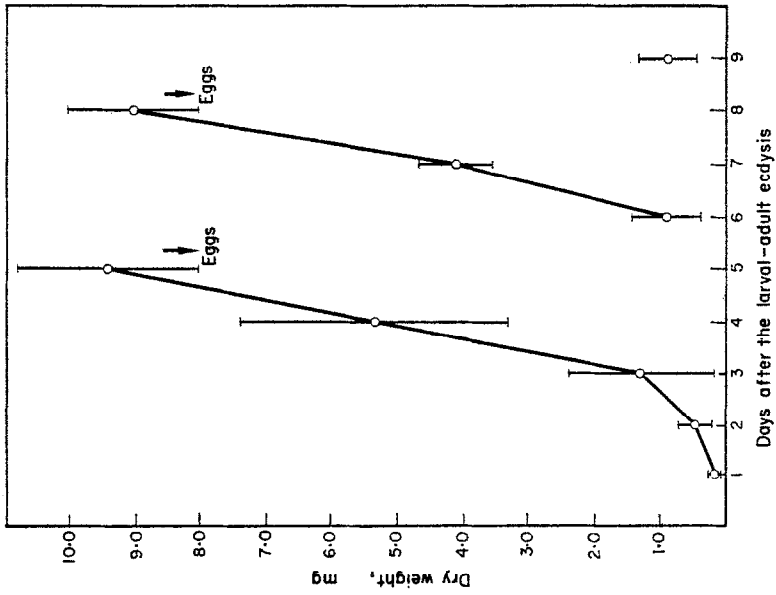


FIG. 2. Growth of the ovaries in active females, represented by the means of measurements of both ovaries and the standard deviations of five to ten females at each age.

After the larval-adult ecdysis, the oöcytes grew progressively, the dry weight of both ovaries increasing from 0.1 mg to 1.3 mg (Fig. 2) in the first 3 days. During the following 2 days the oöcytes grew more rapidly to maturity, at which time the ovaries weighed 9.4 mg. This rapid increase in mass was repeated during the 2 days before laying the second batch of eggs. Analysis of the ovarian tissue revealed that lipid and protein were present in the ovaries in approximately equal amounts by weight and that together they accounted for 66 per cent of the tissue dry weight on day 5 (Fig. 3). Glycogen made up a much smaller proportion of the tissue

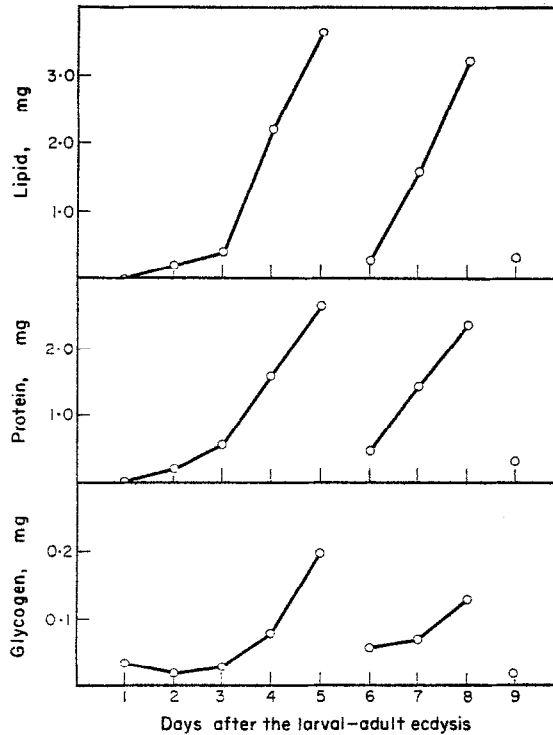


FIG. 3. Composition of the ovaries during growth.

(2 per cent on day 5) but the factor of increase was comparable to that of the whole tissue and the lipid. Since ovarian growth occurred at the time when the fat body was decreasing in size, the composition of the fat body and its growth under various conditions were examined further to investigate the possibility that the fat body was contributing to the enormous increase in ovarian weight.

Fat body growth when ovaries were inactive or absent

The initial growth of the fat body was unchanged in ovariectomized females, increasing 3.7 mg by day 3 (Fig. 4). However, after day 3 the pattern of growth was markedly different from normal females. No decrease in mass occurred after

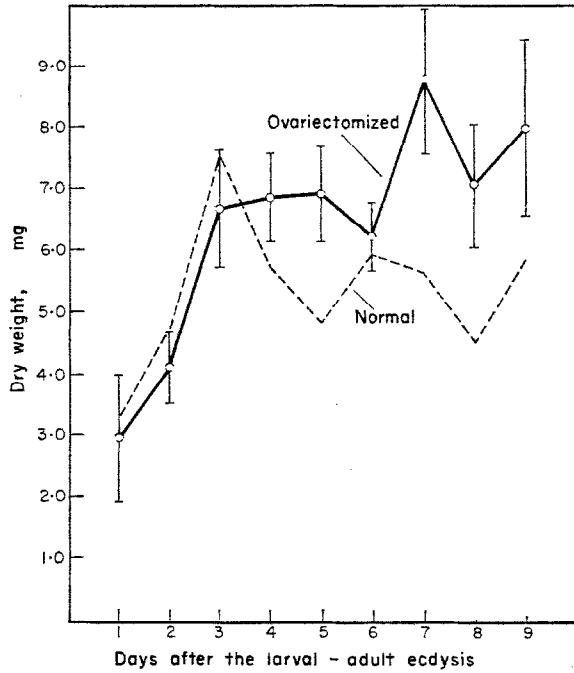


FIG. 4. Growth of the fat body in ovariectomized females, represented by the means and standard deviations of four to five females at each age.

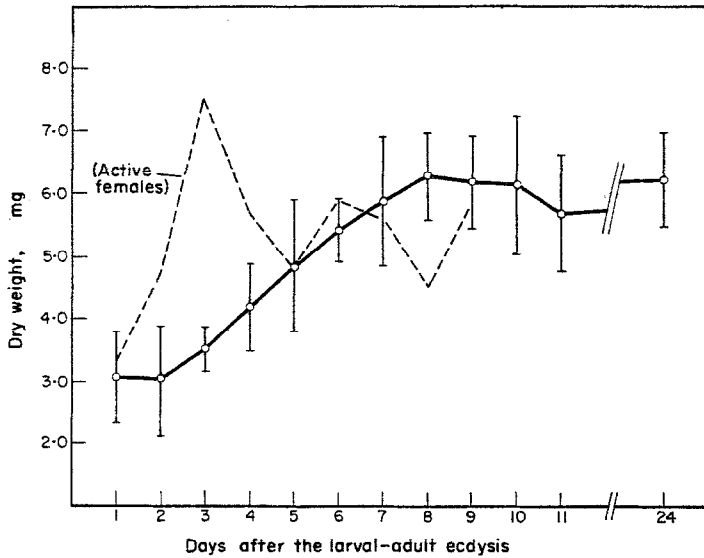


FIG. 5. Growth of the fat body under diapause conditions represented by the means and standard deviations of five females at each age.

day 3 as it had when the ovaries began their rapid growth in normal females. Instead, the dry weight fluctuated at or above the usual maximum.

The rate of growth of the fat body was considerably reduced in females entering diapause (Fig. 5). Female *Pyrrhocoris apterus* reach a full state of diapause, as indicated by a characteristically reduced respiration rate, by day 11 (SLÁMA, 1964). Maximum dry weight of the fat body was not attained until day 8 and no cyclic changes in weight occurred.

The fat bodies of most of the allatectomized females were larger than the normal fat body at its maximum growth (Table 2) and failed to show the cyclic changes in mass which occur in normal fat bodies. Although no normal fat bodies were measured on days 13 to 18, SLÁMA (1964) found that sham-operated females are very similar to the normals in body weight and that the weight on days 13 to 16 is very similar to that on days 4 to 9.

When the females were starved after ecdysing into adults the fat body underwent no increase in mass (Fig. 6). In fact, it slowly decreased below the usual minimum of 3.0 mg. Since the ovaries in these females showed no sign of growth whatsoever, the fat body's reserves were apparently slowly mobilized in response to demands of some other tissue.

Composition of the fat body

Two trends are evident in the measurements of fat body content of protein and glycogen (Table 3). The proportion of protein remained essentially constant with an average of approximately 7 per cent despite the considerable changes occurring in the mass of the fat body during the reproductive cycle in normal females. This was true of diapausing and ovariectomized females as well. Glycogen, on the other hand, showed much greater variation within each group of females but no apparent trend of either increase or decrease. In diapausing and in allatectomized females (Table 2) the glycogen content of the fat body was significantly elevated ($P < 0.002$) compared both with normal females and with ovariectomized females.

JANDA and SLÁMA (1965), in studies on the entire insect, found that the amount of glycogen in the diapausing adult *Pyrrhocoris* female was approximately doubled as compared with the active female. The same effect was found when both the corpora cardiaca and the corpus allatum had been removed; when only the corpus allatum was extirpated glycogen was increased to an even greater degree. In the few fat bodies examined after allatectomy in the present study (Table 2), the glycogen content was uniformly very high, although not different from those in diapause. This suggests either a direct or indirect hormonal control of glycogen storage or utilization. Apparently it is the absence of the corpus allatum, and not the corpora cardiaca, which increases glycogen storage or decreases glycogen utilization.

The lipid content was considerably reduced by more than 10 days of starvation, falling to 59 per cent on day 20 (Table 3). The lipid content of the normal female fat body was never less than 68 per cent in any insect examined. In females starved more than 8 days the lipid content of the fat body was significantly less than in fed females ($P = 0.002$).

TABLE 2—DRY WEIGHT AND COMPOSITION OF THE FAT BODY IN ALLATECTOMIZED FEMALES

Age	Dry wt. (mg)	Lipid		Glycogen		Protein	
		mg	%	mg	%	mg	%
Day 13	8.4						
	8.6	5.9	69	1.56	18.1	0.77	8.9
Day 14	9.9	6.1	62				
	8.6	6.7	78	1.56	18.2	0.51	5.9
	7.4	5.3	72	1.57	21.2	0.73	9.9
Day 15	7.3	5.1	70	1.80	24.8	0.58	8.0
Day 16	10.8	7.1	66				
	8.9	6.3	71	2.50	28.1	0.89	10.0
Day 17	6.8	4.3	63				
	13.8	9.1	66				
Day 18	8.5	6.1	72				
	12.4	9.6	77				
Mean \pm S.D.	9.3 \pm 2.1		70 \pm 5		22.1		8.5

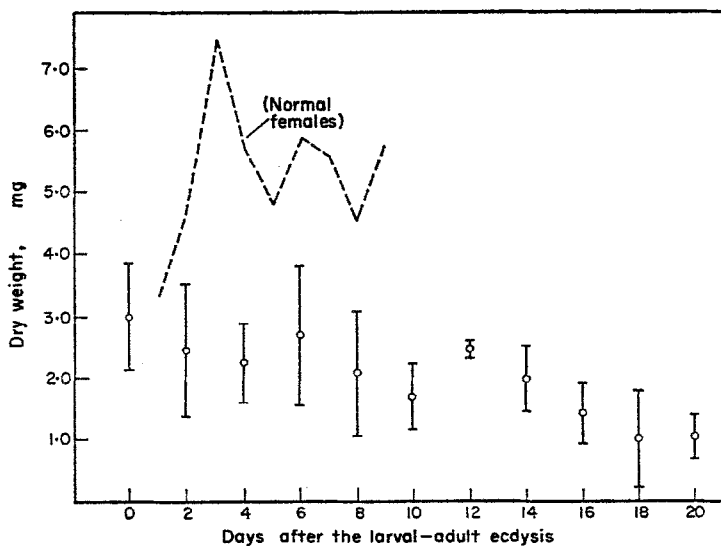


FIG. 6. Growth of the fat body in females starved since the adult moult represented by the means and standard deviations of three to five females at each age.

TABLE 3.—PROPORTION OF LIPID, PROTEIN, AND GLYCOGEN IN ADULT FEMALES UNDER VARIOUS PHYSIOLOGICAL CONDITIONS

Age (days)	A. Normal			B. Ovariectomized			C. Diapause			D. Starved	
	Lipid %	Protein %	Glycogen %	Lipid %	Protein %	Glycogen %	Lipid %	Protein %	Glycogen %	Age (days)	Lipid %
1	75	4.9 3.8		75	8.6 8.1	8.4 6.4	84 (3)			0	83 (4)
2	78	8.4 8.9	12 8.4	76	6.2 6.1	3.3 3.2	72 (2)			2	76 (4)
3	81 (4)	7.7 8.7	4.7 4.1	78	8.4 7.8	8.1 3.4	76	8.2 6.7	20.3 12.5	4	81 (3)
4	81	8.1 7.2	15 5.9	76	5.2 3.9	7.0 3.0	74			6	78 (4)
5	81	5.9 5.8	1.7 1.6	71	10 4.4	3.2 3.1	72	8.1 6.6	18.7 14.5	8	80 (4)
6	77	6.3 5.5	6.2 3.2	82	6.5	4.2	74 (4)			10	66 (4)
7	78	6.9 5.2	2.7 2.2	71	7.7 5.3	14.9	71	7.4 7.1	24.6 19.3	12	73 (3)
8	84		2.6 2.6	78	6.3 5.8	3.1 2.5	71	9.3 7.5	20.3 18.6	14	72 (2)
9	81		6.2 5.9	81	7.1 6.1	11.9 8.6	72	7.8 5.0	19.1 18.5	16	63 (4)
Average	80	7.1	5.2	76	6.7	5.9	74	7.4	18.6	18	65 (5)
										20	59 (4)

Values shown are percentage of dry weight. Values for lipid are the average of measurements on five fat bodies except where noted in parentheses following the mean. Values for protein and glycogen are individual measurements on single animals.

Although the ovarian growth cycle, in terms of dry weight, encompassed a much larger increase than the concomitant decrease in weight of the fat body, the increase in ovarian lipid was similar to the amount of lipid lost by the fat body during the first reproductive cycle and these two processes occurred at the same time (Fig. 7). With this in mind the composition of fat body lipid was examined further and the relationship of lipid in the fat body and lipid in the ovaries was considered.

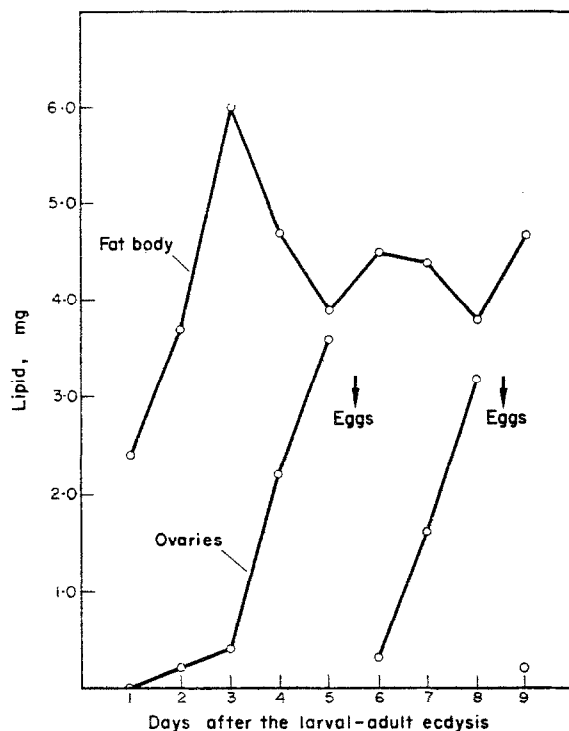


FIG. 7. Lipid content of the fat body and ovaries in active females. These data are from the females represented in Figs. 1 and 2.

Major classes of lipid in the fat body

Lipids were extracted from the fat bodies of four active females and the major distribution of lipid classes was examined. In three of the samples (containing 85, 263, and 278 μg lipid) only TG was detected. The fourth sample, containing 319 μg lipid, included relatively low but detectable amounts of DG and FFA (8 μg each). MG, sterol ester, and sterol were not detected. The range of accuracy of the method is 15 to 300 μg of each lipid class (AMENTA, 1964). Thus at least 95 per cent of the lipid (exclusive of phospholipid) in the fat body was TG while DG and FFA were minor components accounting for no more than 5 per cent of the total lipid.

TABLE 4—FATTY ACID COMPOSITION OF *Pyrrhocoris apterus* AND OF *Tilia cordata* SEEDS

	< C ₁₄					18:0	18:1	18:2	18:3	> C ₁₈
	a	b	c	(?)	(17:1?)					
Individual tissues (females)										
Fat body on:										
Day 1	7.4	1.0			1.6	3.1	21.7	62.7	2.7	
Day 2	10.3	0.8			1.0	8.1	20.3	56.4	3.3	
Day 3	10.0	0.8			1.3	8.8	21.1	54.9	3.0	
Day 4	11.7	0.8			1.2	7.5	20.3	56.5	2.1	
Day 5	9.0	0.7			1.2	8.5	20.5	58.2	2.9	
Ovaries on:										
Day 3	11.6	0.7			2.2	11.3	20.6	48.1	Trace	
Day 4	9.8	0.7	1.1		1.0	7.8	20.5	54.7	2.2	2.6
Day 5	9.6	0.6			1.5	10.7	20.5	53.6	3.6	
Linden seeds										
Entire insect* (males and females)										
Lipid classes:										
simple esters										
TG	Trace	0.7	12.5	0.9	0.7	5.5	19.3	59.2	1.0	
DG		0.6	12.9	0.3	0.8	5.1	22.3	56.4	1.1	
MG		1.1	15.8	1.0	0.5	4.8	18.9	57.0	1.5	
FFA		1.5	10.5	0.5	Trace	5.4	23.6	58.4	Trace	
		3.8	3.6	0.7	0.7	9.8	19.2	47.8		

Quantities are percentage of total. Fatty acids designated by a question mark are placed or given tentative identification appropriate to their retention time; standards for such fatty acids were not chromatographed.

* Data of Mr. DAVID E. TSONG, who was a National Science Foundation Undergraduate Research Participant in the Department of Chemistry, University of Michigan, during the summer 1967.

Fatty acid composition of lipids in the fat body, ovaries, whole insect, and diet

As seen in Table 4, the fatty acid profiles were very similar in the fat body, ovaries, the whole insect, and the diet, with linoleate (18:2) accounting for more than half of the total fatty acid. Oleate (18:1) accounted for 20 per cent and palmitate (16:0) accounted for 10 per cent, while stearate (18:0), linolenate (18:3), palmitoleate (16:1), and an unidentified acid (17:1 ?) accounted for a total of about 12 per cent. The only substantial difference between the insect and its diet with respect to fatty acid composition is in stearate, which measured 8 to 11 per cent in the isolated tissues and closer to 5 per cent in the diet and the whole insect.

In the fat body and ovaries, the alteration in fatty acid composition during the first reproductive cycle was small and without apparent pattern. In the whole insect, the FFA fraction had a higher proportion of stearate and a lower proportion of linoleate than was found in the other lipid classes and, in addition, two unidentified fatty acids were present in measurable amounts only in the FFA. The significance, if any, of this finding is not understood. In all other respects the fatty acid composition of the separate lipid classes was uniform.

The predominant lipid class in the extract of entire insects was TG, amounting to nearly 60 per cent of the extracted lipid (Table 4). Triglyceride is known to be the major class of insect lipids (summarized in the review by GILBERT, 1967b). The lipid classes included in the measurement of fatty acid composition (TG, DG, MG, FFA, and esters) accounted for 90.4 per cent of the total lipid extracted. The hydrocarbon fraction was 1.2 per cent and the sterol fraction (contaminated with TG) was 5.3 per cent of the lipid extract. The unrecovered portion (3.1 per cent) would include phospholipid and other highly polar compounds.

Amount of lipid consumed during vitellogenesis

Daily food consumption was measured in two normal adult females. The lipid content of the seeds, without the seed coat, was determined by Folch extraction to be 33 per cent of fresh weight. The lipid consumed by each of two adult females during vitellogenesis (days 3 to 5) was 1.5 and 1.9 mg respectively, and during days 1 to 3 was 2.9 and 2.2 mg, respectively.

Fat body growth when females were not fed during the period of ovarian growth

In order to investigate whether the ovary assimilated lipid from the digestive tract for normal growth during vitellogenesis and whether the fat body released an increased amount of lipid when the amount of food was reduced, five normal females from a group which were laying eggs on day 6 were deprived of food during the last 2 (three females) or 3 (two females) days prior to their first oviposition. All developed mature oöcytes in the normal length of time, showing that feeding need not be concurrent with oöcyte maturation. In the animals thus deprived of food, the dry weight of the fat body on the day of oviposition was $3.5 \text{ mg} \pm 0.7$ (range was 2.7–4.3 mg), significantly smaller than normal ($P = 0.028$ when compared with normal day 5 fat body weighing 4.8 mg), strongly suggesting increased mobilization of reserves.

DISCUSSION

Although the yolk of insect eggs includes both protein yolk bodies and lipid yolk bodies (DAVEY, 1965), nearly all the recent literature on the source of yolk components is concerned with protein yolk. The literature dealing with lipid yolk formation relies entirely on cytological and histochemical studies of the ovary, in which the concern has been to identify the structures where lipid is first observed and to determine whether lipid may be transferred from the trophocytes or follicle cells to the oöcyte (review by BONHAG, 1958). The possibility that lipid originates outside the ovary has apparently not been considered in the course of the histological studies mentioned. However, the concomitant decrease in fat body lipid with the maturation of lipid-rich eggs in various insects has led several other authors to suggest a transfer of lipid from the fat body to the ovaries (HARLOW, 1956; ORR, 1964; GILBERT, 1967a). This is certainly implicated in *P. apterus*.

The cyclic pattern of growth in the adult, female *Pyrrhocoris* fat body strongly suggests a temporary storage of lipid in the fat body and subsequent transfer of lipid to the maturing oöcytes. During the first reproductive cycle in normal females the fat body more than doubles its lipid content prior to the major growth of the oöcytes and then decreases its lipid content by one-third, an amount of lipid nearly equal to the amount accumulated by the ovary during the same period (days 3 to 5, Fig. 7).

When the ovaries are inactive or absent a profound and characteristic change occurs in the growth cycle of the fat body. While the initial growth of the fat body (days 1 to 3) is not altered by ovariectomy, the usual decrease in fat body weight after day 3 does not occur in ovariectomized females (Fig. 4). Thus, when the ovaries were absent, fat body reserves were not depleted and the dry weight, although it did not remain constant, did not drop below the usual maximum level of 6.0 mg. The fluctuations in weight which occurred after day 5 may reflect hormonal activity but were not pursued further.

The initial growth rate of the fat body and the subsequent retention of reserves in ovariectomized females show that in this species the accumulation of fat body reserves is independent of the ovaries while the normal mobilization of these reserves is functionally related to the growth of the ovary. SLÁMA (1964) found that ovariectomy affected the growth of the whole adult, female *Pyrrhocoris* in the same way; the initial growth pattern was unaltered but the normal periodic fluctuation in weight was lost.

As in the whole insect (SLÁMA, 1964), the fat body grew more slowly in females entering diapause than in active females. This probably results from a reduced rate of feeding or digestion as suggested by SLÁMA (1964), although there is no reason to either reject or favour some hormonal control directly on the fat body. In diapausing females the ovaries are inactive and the fat body does not undergo the decrease in mass which is characteristic of reproductively active females. Thus, when the ovaries are absent or inactive the fat body does not undergo a net mobilization of reserves as it does during the period of yolk formation.

Composition of the fat body

The major non-aqueous component of the fat body in adult *Pyrrhocoris* females is lipid which is present in greater concentrations than has been found in other reproducing, female insects. In active female *Pyrrhocoris* lipid accounts for 75 to 84 per cent of the fat body dry weight and the content is similar during diapause and after ovariectomy. ORR (1964) found that during the reproductive cycle in the female blowfly (*Phormia regina*) lipid accounted for 52 to 69 per cent of the dry weight of the fat body, HILL *et al.* (1968) found that the fat body of the desert locust was 54 to 71 per cent lipid during the first reproductive cycle, and GILBERT (1967a) found that during oögenesis in the female cockroach *Leucophaea maderae* the lipid content of the fat body varied from 29 to 46 per cent of the dry weight. As has been found in other insects (CHINO and GILBERT, 1965; GILBY, 1965; WLODAWER and ŁAGWIŃSKA, 1967) the primary class of lipid in the fat body of *Pyrrhocoris* is TG.

P. apterus follows the general trend of insects in its high content of 18-carbon unsaturated fatty acids, with palmitate and stearate being the only detectable saturated fatty acids. However, linoleate is unusually abundant in *P. apterus*, accounting for more than 50 per cent of the fatty acid content. The only other insects in which such a large proportion of this acid has been found are the carabid beetle *Harpalus caliginosus*; an unidentified cicadid and *Stictocephala deciros* (BARLOW, 1964a); and the bug *Oncopeltus fasciatus* (KINSELLA, 1966b).

Linoleate is a dietary requirement for several unrelated species of insects and can be synthesized by only one of the several species examined (review by GILBERT, 1967b). Deficiency of linoleate and linolenate results in profound developmental and reproductive defects, such as resorption of egg cases, failure of adult emergence or unfolding wings, and loss of wing scales (review by FAST, 1964). This requirement is not universal, however; BARLOW (1964b, 1966a, b) reports that three species of flies do not require polyunsaturated acids. The only species reportedly able to synthesize linoleate is the cockroach, *Periplaneta americana* (LOULOUDES *et al.*, 1961); however, NELSON and SUKKESTAD (1968) consider that the data of Louloudes *et al.* leave several other conclusions possible.

It is reasonable to presume that *Pyrrhocoris* does not synthesize linoleate, and that the linoleate in the fat body and ovaries is obtained entirely from the diet. If the bug were synthesizing other fatty acids to any significant extent, the proportion of linoleate in the tissues would be consequently reduced while those fatty acids synthesized would be present in larger proportions than found in the diet. Since the proportions of all the fatty acids except stearate are essentially identical in the fat body, ovaries, and in the seeds, it would appear that under the conditions in the present study, the fat body and ovaries are not synthesizing fatty acids to any appreciable extent (except perhaps, stearate) unless those fatty acids synthesized are selectively utilized or released. Moreover, it appears that lipids are being utilized in the proportions in which they occur, with no preferential breakdown of particular fatty acids. The same situation apparently exists in the ichneumonid parasite *Exeristes comstockii* (Hymenoptera). BRACKEN and BARLOW (1967) found that the

fatty acid composition of the larvae of this parasite paralleled that of three unrelated insect hosts whose fatty acid patterns were profoundly different from one another.

The extent of similarity in fatty acid profile between insect lipids and their diet varies considerably among different species with an apparent consistency along taxonomic lines. Several species of Orthoptera and Diptera and three species of Lepidoptera maintain a fatty acid composition which is different from their diet (BARLOW, 1964b, 1966a, b; MCGUIRE and GUSSIN, 1965; KEITH, 1966; KINSELLA, 1966a; SVOBODA *et al.*, 1966; NAKASONE and ITO, 1967; HUTCHINS and MARTIN, 1968; NELSON and SUKKESTAD, 1968; SCHAEFER, 1968). Those few insect species which have been found to have the same fatty acid composition as their diet include two Hemiptera, one Hymenoptera, and one Coleoptera (LAMBREMONT *et al.*, 1964; BRACKEN and BARLOW, 1967; KINSELLA, 1966b; this paper). The species which do not alter the fatty acid pattern of their food are feeding on a diet which satisfies their fatty acid requirements and may save them the metabolic cost of synthesizing particular fatty acids. This observation, however, is in no way intended to suggest that such insects are incapable of fatty acid synthesis or alteration.

Thus, it appears that the ovaries and fat body of *Pyrrhocoris* are not synthesizing fatty acid to any extent under the present conditions. The fat body apparently accumulates lipid consumed during the first 3 days after ecdysis and then releases lipid without any selectivity or appreciable synthesis of fatty acids. The results of the present study do not demonstrate whether the ovary takes up digested lipid directly or whether it only receives lipid which has been in the fat body. However, the amounts of lipid which both sources made available implicate both the diet and the fat body. On days 3 to 5 of adult life the two females measured ate a total of only 1.5 and 1.9 mg lipid. The ovaries increased their lipid at this time by 3.2 mg. Since the fat body underwent a net loss of 2.1 mg during the same time, it seems that the ovaries must have obtained lipid both from the fat body and from the diet, neither one of which alone provided a sufficient amount. The increased mobilization of fat body reserves when the females were not allowed to feed during vitellogenesis substantiates this theory. Five females which were deprived of food during the 2 or 3 days before oviposition developed mature oöcytes, apparently at the expense of the fat body. The average net reduction in fat body dry weight during vitellogenesis was increased by 1.3 mg or approximately 1.1 mg of lipid. This is exactly the amount by which the ovarian increase in lipid exceeds the lipid loss by the fat body in normally feeding females.

The behaviour of the fat body in the adult female when the ovaries are not growing also indicates that fat body lipid is utilized by the growing oöcytes. In both diapausing and ovariectomized females no oöcytes developed and the fat body did not undergo a net loss of lipid during the first 5 days of growth.

It appears, then, that the growing oöcytes incorporate lipid from the fat body and that the fat body mobilizes its reserves in conjunction with the developing oöcytes. The extent of mobilization seems to depend on the demand. When the dietary source of lipid is limited (as discussed above) mobilization from the fat body is increased.

The most attractive explanation for lipid accumulation in the fat body prior to incorporation by the ovary is that the rate of food ingestion during the actual period of ovarian growth (days 3 to 5) is insufficient to provide the ovaries with sufficient lipid; thus the fat body provides an added store of lipid, accumulated prior to vitellogenesis, for incorporation by the growing ovary. More food was consumed by the insects prior to vitellogenesis (days 1 to 3, a total of 6.7 and 8.6 mg by each insect) than during vitellogenesis (days 3 to 5, a total of 5.7 and 4.6 mg, respectively). The same result was found by HRUBEŠOVÁ and SLÁMA (1967) who measured the amount of food in the intestine of adult, female *Pyrrhocoris* during the first reproductive cycle. They found that the intestine contained twice as much food during days 1 to 3 as during days 3 to 5 and concluded that the ovaries occupy so much space in the abdominal cavity during vitellogenesis that there simply is not room for the gut to distend sufficiently to contain much food. In addition, lipid storage in the fat body would be advantageous if food were not constantly available to the insect, enabling her to reproduce after feeding only intermittently. Furthermore, uptake and release of lipid by the fat body may provide a greater degree of regulation of lipid transport and utilization. It is realized that this explanation is somewhat speculative and other possibilities exist. Although no alteration in fatty acid content of the lipid occurs in the fat body, there may be some other sort of transformation occurring, such as formation of lipoprotein. Another possibility is that the fat body releases a different form of glyceride than the form released from the digestive tract. This possibility will be examined in a subsequent communication.

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