J. Lipid Research, October, 1962
Volume 3, Number 4

Fat transport in the locust*

ALISA TIETZ

Israel Institute for Biological Research, Ness-Ziona, Israel

[Received for publication May 25, 1962]

SUMMARY

When fat-body tissue from locusts was incubated with palmitate-1-C^{14} in phosphate-saline, the acid was readily taken up by the tissue; 80–90% of that taken up was esterified and recovered in the glyceride fraction. When the prelabeled tissue was incubated in hemolymph, glycerides were released from the tissue into the medium. The effect of hemolymph was specific; glycerides were not released into phosphate-saline, bovine serum, or buffered solutions of bovine serum albumin or egg albumin. The release of glycerides was inhibited by fluoride and by cyanide. The amount of glyceride released was proportional to the amount of hemolymph that was added. Effectiveness of the hemolymph in this regard was not affected by prolonged dialysis, but was destroyed by heating. The specific activity of the glycerides released was at least 10 times higher than the average specific activity of the glycerides inside the tissue. A considerable fraction of the released glycerides was incorporated into the lipoprotein fraction of the hemolymph. Uptake of glycerides by fat-body tissue was also demonstrated.

Much work has been done during the last few years on fat transport in man and other mammals (1). Although fat is an important source of energy in many other animals, especially in migrating birds and insects (2), relatively little is known about fat transport in these animals. Weis-Fogh (3) showed that fat was the principal source of energy during sustained flight in the desert locust, *Schistocerca gregaria;* the stores of carbohydrate were exhausted after 1 hr.

Since most of the fat in the locust is contained in the fat-body, fat must be mobilized during flight and transported to the flight muscles, presumably by way of the hemolymph. The present paper demonstrates that when fat-body tissue is incubated *in vitro* in hemolymph, glycerides are released from the tissue into the medium. A preliminary report of this work has appeared (4).

METHODS

Collection of Hemolymph and Preparation of Tissue. Locusts (*Locusta migratoria*) were bred in wooden cages (5) and fed grass and oatmeal. The cages were heated and illuminated by a 40- or 60-watt bulb for 16 hr each day. The temperature during the light period was 34–37°, and it dropped during the dark period to 25–30°. For most experiments, female locusts 8 to 15 days after the last molt were used.

To collect hemolymph, a small opening was cut between the eyes and the insect was placed in a precooled centrifuge tube with the head toward the pointed end. The insect was cooled for 5 min. and then centrifuged for 1 min at low speed. This was sufficient to collect most of the hemolymph (approximately 0.2 ml). To avoid contamination by digestive juice, a small piece of cotton was forced into the mouth of the insect. The hemolymph of several insects was pooled and centrifuged for 5 min at 400 × *g* to remove cells and fat. The clear hemolymph was then filtered through a small pad of cotton to remove small amounts of fat that accumulated on the top. To avoid blackening, 5 μmoles glutathione/ml of hemolymph were added.

After collecting the hemolymph, fat-body tissue was removed and placed in cold phosphate-saline (0.025M potassium buffer, pH 7.0 in a 0.9% solution of NaCl). The average weight of the fat-body of a well-fed locust was 150–250 mg, approximately 35–55% of which was fat. In most experiments, fat-body tissue from six or more insects was pooled.

Incubations. All incubations were carried out in small conical flasks for 1 hr at 30°, in air and with constant shaking. To measure palmitic acid uptake, 150–200 mg of fat-body tissue was incubated in 1 ml of phosphate-saline or hemolymph in the presence of 5 μmoles of glutathione and 0.3 μmole of potassium palmitate-1-C^{14} (60,000 cpm). At the end of the incubation, the tissues were removed from their respective flasks and rinsed twice with 10 ml fresh buffer. This tissue will be referred to in the following text as "pre-

* Supported by PHS Grant RG-8135 from the National Heart Institute, U. S. Public Health Service.

labeled tissue." When large amounts of prelabeled tissue were required, 500 mg tissue was incubated in 2 ml of buffer in the presence of 10 μmoles glutathione and 0.6 μmoles of palmitate-1-C^{14} (120,000 cpm). At the end of the incubation, tissues from several flasks were pooled and washed with a large excess of buffer. Lipids were extracted from a sample of these tissues to determine the amount of C^{14}-labeled palmitate incorporated into tissue glycerides.

To measure glyceride release, 150–200 mg of prelabeled tissue was incubated in 1 ml of phosphate-saline or hemolymph in the presence of 5 μmoles of glutathione. When less than 1 ml of hemolymph was used, the volume was brought up to 1 ml with phosphate-saline. Generally, 0.2 ml hemolymph was used. At the end of the incubation, the tissue was removed and rinsed as described above, and the remaining lipids were extracted.

To label the glycerides of the hemolymph, 1-C^{14}-labeled tripalmitin dissolved in 0.1 ml of ether was mixed with 2 ml hemolymph (6). The mixture was incubated for 30 min and the ether removed with a stream of nitrogen. Labeled hemolymph was also obtained by incubating samples of prelabeled tissue in 1 ml hemolymph. This hemolymph will be referred to in the following text as "prelabeled hemolymph."

To measure the oxidation of palmitate-1-C^{14}, incubations were carried out in Warburg flasks. Potassium hydroxide was placed in the center well and H_2SO_4 in the side arm. After a 1-hr incubation, the acid was tipped into the reaction mixture. Samples were converted to $BaCO_3$ for plating and counting.

Extraction and Separation of Lipids. Washed tissue was homogenized with ethanol–ether 3:1 (v/v), and the suspension was heated to the boiling point. The solvent was removed and the lipids were taken up with petroleum ether (b.p. 40–60°). Phospholipids were separated from the mixture by precipitation with acetone (7) after addition of carrier yolk phospholipids. The acetone supernatant was passed through a MgO-Celite column (7), and the glycerides were eluted with acetone. After it was shown that only negligible amounts of palmitate-1-C^{14} were incorporated into phospholipids, the lipid mixture was put directly onto a MgO-Celite column. The lipids of the incubation medium were extracted according to Dole with isopropanol-heptane (8). To separate free fatty acids (FFA) and glycerides, the heptane phase was washed with 0.1 N NaOH in 50% (v/v) ethanol (9). The FFA were extracted into petroleum ether, after acidification of the ethanol.

Paper Electrophoresis. Hemolymph (20 to 40 μl) was applied to 3-cm wide strips of Whatman 3 MM filter paper. Sodium diethyl barbiturate buffer pH 8.6, 0.1 ionic strength was used. A current of 1.0 ma/cm was applied for 18 hr. The strips were blotted and dried at 120°. Protein was detected by staining with Amido black 10B and lipoprotein with Sudan black according to Siakotos (10). Human serum was used as reference. The mobility of the protein fractions of the hemolymph were related to the mobility of the human serum albumin fraction.

C^{14} Assays. Plating and counting was done as previously described (11). To determine palmitate-1-C^{14} uptake and esterification by the tissue, the amount of C^{14} in the lipid extract was first measured. The C^{14} content of the triglyceride fraction (MgO-Celite column eluate) was then measured. Since the recovery of triglycerides from the columns was 95–100%, the amount of C^{14} in FFA could be calculated from the difference—total C^{14} in lipids minus C^{14} in triglycerides. In some experiments, the specific activity of the triglycerides in the medium and inside the tissue was compared. The amount of triglycerides was estimated by the hydroxamic acid method (12). Specific activity will be expressed as cpm/μEq of glyceride-fatty acids. FFA were estimated by titration (8).

To measure the incorporation of C^{14}-labeled lipids into the proteins of the hemolymph, the electrophoretic strips were cut into 1-cm pieces, and each piece was counted separately. C^{14}-containing material was then eluted from the pieces with isopropanol–heptane and extracted into heptane (8), and the C^{14} content of the extract was determined. Four successive pieces usually were pooled.

Materials. Palmitic acid-1-C^{14} and glyceryl tripalmitate-1-C^{14} were purchased from the Radiochemical Centre, Amersham, Bucks, England.

RESULTS

Palmitic Acid Uptake by Fat-Body Tissue. When locust fat-body tissue was incubated in phosphate-saline in the presence of palmitic acid-1-C^{14}, the acid was readily taken up by the tissue and incorporated into tissue glycerides. The rate of fatty acid uptake into glycerides was linear for approximately 30 min and then slowed down (Fig. 1). During the initial period of the incubation, more C^{14} was found in the FFA fractions than in the glycerides. After 1 hr of incubation, 80–90% of the label was found in the glyceride fraction of the cell. Only negligible amounts of C^{14} were found in the phospholipid fraction. When the tissue was incubated in hemolymph instead of phosphate-saline, much less C^{14} was recovered in the tissue glycerides. However, when the incubation media were analyzed,

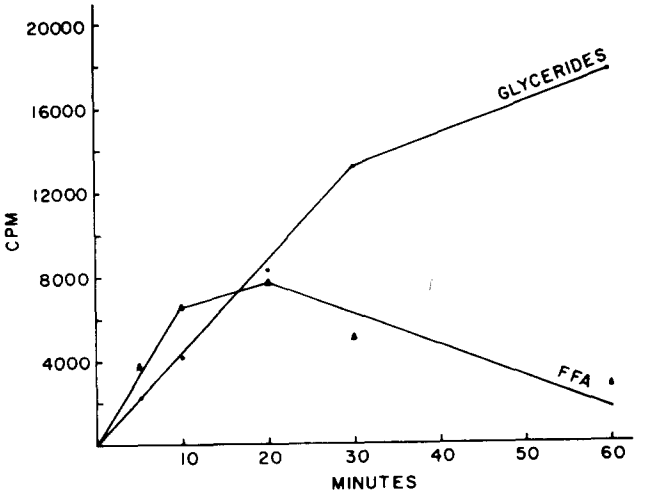

FIG. 1. Uptake and esterification of C^{14}-labeled palmitate. Samples (200 mg) of fat-body tissue were incubated in 1 ml of phosphate-saline medium containing 5 μmoles of glutathione and 0.3 μmoles of palmitate-1-C^{14} (60,000 cpm).

large amounts of C^{14}-labeled glycerides were found in the hemolymph, whereas only small amounts were found in the buffer (Table 1). These results suggested to us that when fat-body tissue was incubated in hemolymph, palmitic acid was taken up by the tissue, esterified, and then returned to the hemolymph as triglycerides.

Glyceride Release from Fat-Body Tissue. To study fat release, prelabeled tissue (see Methods) was incubated in fresh buffer or hemolymph and the distribution of label between tissue and medium was estimated. As can be seen from Table 2, only small amounts of C^{14}-labeled triglycerides and FFA were recovered in the medium when the tissue was incubated in phosphate-saline. Addition of crystalline bovine serum albumin or crystalline egg white to the incubation medium did not promote fat release. When the buffer was replaced by hemolymph, 48% of the C^{14}-labeled triglycerides inside the tissue at the start of the incubation were recovered in the hemolymph after 1 hr. Only small amounts of C^{14}-labeled FFA were detected. Release of C^{14}-labeled glycerides into the hemolymph continued throughout the incubation period (Fig. 2). When bovine serum was used instead of hemolymph, the amount of glycerides increased very slowly and only 2% was found in the medium after 1 hr of incubation. The amount of C^{14}-labeled FFA in both media remained constant or decreased slightly during the incubation.

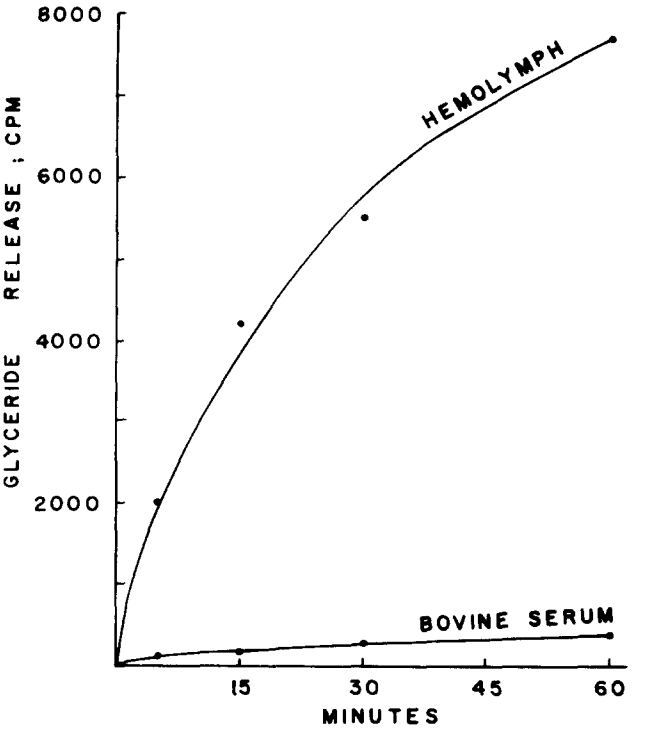

FIG. 2. Glyceride release from fat-body tissue into hemolymph and into bovine serum. Samples (170 mg) of prelabeled fat-body tissue containing 14,000 cpm in the glyceride fraction were incubated in 0.9 ml of hemolymph or bovine serum.

TABLE 1. METABOLISM OF PALMITIC ACID-1-C^{14} BY FAT-BODY TISSUE

Incubation Medium	Uptake by Tissue in 1 hr				Glyceride Release into Medium
	FFA	Glycerides	Phospholipids	CO_2	
	cpm	*cpm*	*cpm*	*cpm*	*cpm*
Phosphate-saline	4,600	19,800	500	1,950	980
Hemolymph	1,400	2,900	104	720	5,800

Conditions of incubation as described under Methods. Two hundred milligrams of tissue was added to each flask.

TABLE 2. FAT RELEASE FROM FAT-BODY TISSUE*

Incubation Medium	Glycerides in Medium		FFA in Medium (cpm)
	cpm	% of Total in Tissue at Zero Time	
Phosphate-saline	670	4.2	750
Bovine serum albumin	530	3.3	950
Egg albumin	150	1.0	50
Hemolymph	7,800	48.0	800

* Samples (180 mg) of prelabeled tissue containing 16,300 cpm in the glyceride fraction and 1,600 in FFA were transferred into 1 ml of phosphate-saline, 5% crystalline bovine serum albumin in phosphate-saline, 5% crystalline egg white in phosphate-saline or hemolymph.

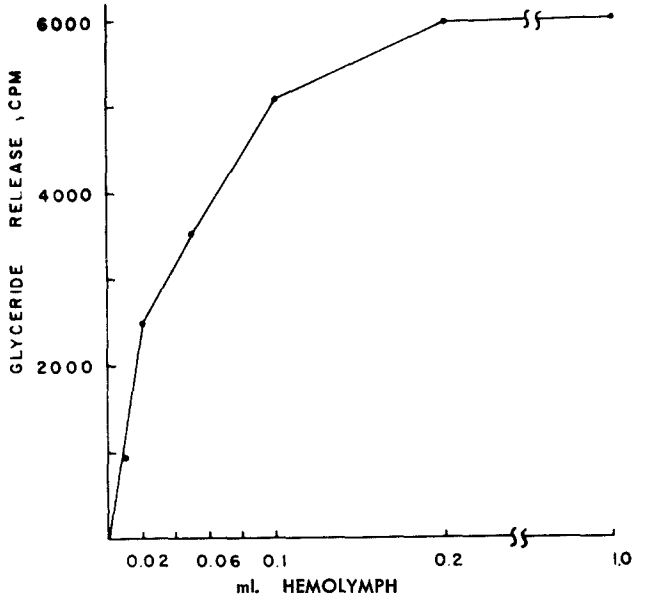

FIG. 3. The effect of increasing amounts of hemolymph in the medium on glyceride release. Samples (180 mg) of prelabeled fat-body tissue containing 18,000 cpm in the glyceride fraction were used.

TABLE 3. NET GLYCERIDE RELEASE FROM FAT-BODY TISSUE

Expt. No.	Time (min)	Hemolymph Glycerides: cpm	Hemolymph Glycerides: μEq Glyceride-Fatty Acids	Hemolymph Glycerides: Average Specific Activity of Glycerides Released*	Tissue Glycerides: Specific Activity
1	0	...	5.8		200
	5	1,650	9.2	485	197
	30	7,025	10.0	1,675	129
	60	10,320	12.5	1,540	125
2	0		4.6	...	208
	5	1,860	7.8	581	...
	30	7,380	9.2	1,600	...
	60	10,450	9.2	2,270	...
3	0		2.8		125
	5	1,375	5.4	530	...
	30	4,930	5.4	1,890	...
	60	6,930	5.4	2,660	...
4	80		5.7		105
	5	1,900	7.5	1,055	101
	30	9,100	11.5	1,570	79
	60	12,600	14.4	1,450	69

* Specific activity: cpm divided by the μEq of glycerides released. Glyceride release was calculated by substracting the μEq of glyceride-fatty acids present in the hemolymph before the incubation from the amounts found after 5, 30, and 60 min.

The amount of labeled glyceride released was proportional to the amount of hemolymph added to the incubation medium (Fig. 3). As little as 0.01 ml of hemolymph stimulated release. Maximum stimulation was obtained with 0.2 ml. The hemolymph could be dialyzed overnight against phosphate-saline without loss of activity. Most of the activity, however, was lost when the hemolymph was kept for 1 min at 100°.

Since 25–40% of the labeled glycerides inside the tissue at the start of the incubation were released into the hemolymph during 1 hr of incubation, attempts were made to detect net release of glyceride-fatty acids. In all experiments, an initial increase of glyceride-fatty acids of relatively low specific activity was observed in the medium (see Table 3). During subsequent periods a further net increase occurred in some experiments (see experiments 1 and 4); in others (see experiments 2 and 3) little or no increase was found although C^{14}-labeled glycerides were continuously released. In all experiments, the specific activity of the glyceride-fatty acids released during 1 hr was at least 10 times higher than the average specific activity of the glycerides inside the tissue.

Glyceride Uptake by Fat-Body Tissue. To determine whether an exchange of glycerides occurred between the hemolymph and fat-body, the tissue was incubated in hemolymph containing dispersed C^{14}-labeled tripalmitin. It can be seen from Table 4 that labeled tripalmitin was readily taken up by the tissue, although net release of glyceride-fatty acid had occurred. Similar results were obtained when the tissue was incubated in prelabeled hemolymph. Approximately 10% of the labeled glycerides was taken up by the tissue.

The Effect of Tissue Poisons. The addition of fluoride and cyanide markedly inhibited glyceride release from fat-body tissue incubated in hemolymph (Table 5). This inhibitory effect was not found, however, when the tissue was incubated in a buffered solution of Tween-20 (cf. Table 5). Under these conditions, glyceride-fatty acids of low specific activity were found in the medium in contrast to the highly labeled glycerides found in the hemolymph. Since the average specific activity of tissue glyceride-fatty acids is low, damage to the tissue would result in a leakage of glycerides of low specific activity into the medium. These results, therefore, seem to indicate that glyceride release into hemolymph is an active process that is inhibited by fluoride and cyanide, in contrast to the leakage of glycerides into Tween-20 that was increased by cyanide.

Interaction of Lipids with Proteins of the Hemolymph. The hemolymph of females 8 to 15 days after the last molt contained an average of 60 mg protein/ml (range 55–65 mg) and 28.8 μEq of esterified fatty acids (range

TABLE 4. UPTAKE OF C^{14}-LABELED TRIPALMITIN AND OF LABELED HEMOLYMPH TRIGLYCERIDES

Expt. No.*	Time (min)	Hemolymph Glycerides cpm	Hemolymph Glycerides μEq Glyceride-Fatty Acids	Glyceride Uptake cpm	Glyceride Uptake μEq Glyceride-Fatty Acids†
1	0	30,000	6.3		
(tripalmitin-C^{14})	5	23,000	7.4	3,600	...
	60	14,600	11.1	14,200	...
2	0	5,250	10.1		
(prelabeled	5	5,750	12.6	323	0.7
hemolymph)	60	5,040	13.2	725	1.9
3	0	12,650	14.4		
(prelabeled	5	13,400	14.8	550	0.6
hemolymph)	60	12,700	15.6	990	1.2

* In experiment 1, 1 ml hemolymph was diluted with 1 ml phosphate-saline and mixed with 0.08 μmoles of C^{14}-labeled tripalmitin (150,000 cpm); 0.8 ml of labeled hemolymph was used per flask. In experiments 2 and 3, prelabeled hemolymph was used. Each flask was prepared separately.

† To calculate the μEq of glyceride-fatty acids taken up, it was assumed that complete mixing of the glycerides in the hemolymph had occurred. The cpm taken up were divided by the mean specific radioactivity of the fatty acids of the medium glycerides.

TABLE 5. THE EFFECT OF TISSUE POISONS ON GLYCERIDE RELEASE

Expt. No.	Incubation Medium	Inhibitor (0.01M)	Glyceride Release cpm	Glyceride Release μEq Glyceride Fatty Acids	Glyceride Release Specific Activity	Tissue Glycerides Specific Activity
1	Phosphate-saline	...	510			85
	Hemolymph	...	9,850	4.6	2,140	...
	Hemolymph	KF	1,950	3.1	630	...
	Hemolymph	KCN	2,800	4.2	667	...
	Tween-20*	...	3,080	21.8	147	...
	Tween-20	KF	3,380	34.0	98	...
	Tween-20	KCN	7,650	65.0	118	...
2	Hemolymph	...	12,650	8.7	1,455	69
	Hemolymph	KF	1,720	4.2	410	122
	Hemolymph	KCN	2,320	7.4	314	118
	Tween-20	...	1,860	12.5	149	129
	Tween-20	KF	1,470	10.6	138	114
	Tween-20	KCN	8,450	63.4	133	132

* Incubations with Tween-20 (2 mg/ml) were carried out by using the phosphate-saline medium.

21.7–34.6). By paper electrophoresis, two protein fractions with a relative mobility of 0.75 and 0.60 were resolved. In some samples, a third fraction with a relative mobility of 0.47 was found. Staining with Sudan black revealed that the slow fractions contained lipids.

To test whether a specific interaction occurred *in vitro* between lipids and the proteins of the hemolymph, 0.1 ml hemolymph was incubated with 0.01 μmoles 1-C^{14}-labeled potassium palmitate (30,000 cpm) and a sample separated by electrophoresis. The labeled material remained near the origin. Similar results were obtained when hemolymph was incubated with C^{14}-labeled tripalmitin (see Methods), and a sample separated by electrophoresis. When samples of hemolymph that had been incubated with prelabeled tissue were likewise separated, C^{14}-containing lipids were found spread along the electrophoretic strip. In some experiments, however, large amounts of C^{14}-labeled glycerides were concentrated in the lipoprotein fraction (cf. Fig. 4). Thus, lipids are introduced into the lipoproteins only in the presence of fat-body tissue.

DISCUSSION

The fat-body of the locust extends throughout the abdominal and thoracic cavities. It can be divided into a peripheral portion that is firmly attached to the overlying epidermis and a more central mass that exists as a loose meshwork of connected lobes in the space between the gut and the abdominal wall (13). The latter part was used in the experiments. The tissue is mostly composed of fat cells; some large cells with a single nucleus-oenocytes are also found (13). In well fed insects, the fat-body contains large reserves of glycogen and fat. Since the fat-body lies in the hemocoele, it is

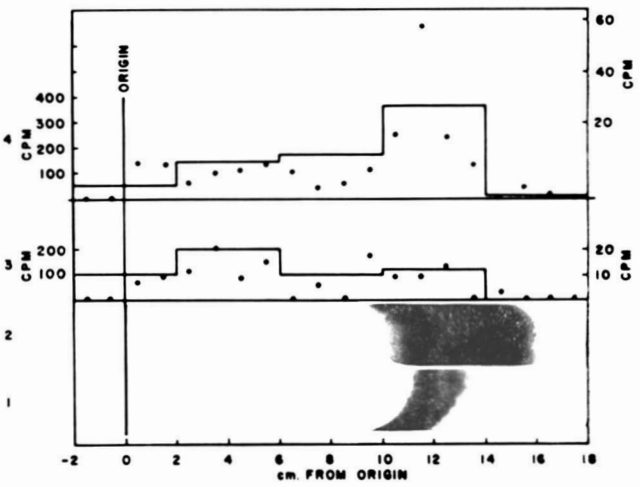

FIG. 4. Separation of hemolymph by paper electrophoresis. 1. Strip stained with Sudan black. 2. Strip stained with Amido black. 3. and 4. Distribution of C^{14}-labeled glycerides. In the first experiment (line 3), 0.05 ml of hemolymph containing 530 cpm was applied onto the electrophoretic strip at the starting line. In the second experiment (line 4), 0.05 ml of hemolymph containing 890 cpm was separated similarly. Right ordinate: radioactivity measured on 1-cm pieces of the electrophoretic strip. Left ordinate (continuous line): radioactivity of the isopropanol-heptane eluates.

immersed in hemolymph, which also circulates through the interspaces of the tissue. The close contact between the cells of the fat-body and the hemolymph allows free exchange of metabolite. It was shown by Treherne (14) that the fat-body *in vivo* removed glucose, mannose, and fructose from the hemolymph and converted these sugars into trehalose, which was subsequently released into the hemolymph. When palmitate-1-C^{14} was injected into the hemocoele of a resting locust (15), the acid was rapidly taken up by various tissues, incorporated into tissue glycerides, and returned into the hemolymph as C^{14}-labeled glycerides. Over 50% of the labeled glycerides in the hemolymph was concentrated in the lipoprotein fraction. *In vitro*, a considerable fraction of the glycerides released from fat-body tissue were incorporated into the lipoprotein fraction of the hemolymph. It seems likely, therefore, that *in vivo* glycerides are mobilized from the fat-body and incorporated by the tissue into hemolymph-lipoproteins.

Very little is known about the nature of the proteins of insect hemolymph (16). On the basis of specific staining methods, Siakotos (10) detected neutral lipids, phospholipids, and sterols associated with protein fractions of the hemolymph of the American cockroach, *Periplaneta americana L.* In the present studies, lipoproteins have been demonstrated in the hemolymph of the locust, *Locusta migratoria*. Varying amounts of esterified fatty acids, 21.7–34.6 μEq/ml hemolymph, were also found. The differences in the amounts of esterified fatty acids were reflected by differences in the staining intensity of the lipoproteins. Only very small amounts of FFA were detected in the hemolymph of resting locust (approximately 0.01 μEq/0.1 ml). Insignificant amounts of FFA were released into the hemolymph during incubation *in vitro*. In contrast to mammalian serum, the proteins of the hemolymph cannot bind free fatty acids.

It is difficult to calculate exactly the amounts of glycerides released or taken up by the tissue, since the values obtained are the result of two opposite processes occurring simultaneously. An approximate calculation can be made from the results of experiments 1 and 4 (Table 3). Assuming that the glycerides found in the hemolymph after 5 min of incubation were released from cells damaged by handling the tissue, and disregarding uptake, 3.3 and 6.9 μEq of glyceride fatty acids (or 1 and 2.2 mg fat) were released during 55 min of incubation. Glyceride uptake can be calculated from the experiments described in Table 4. If complete mixing of the glycerides in the hemolymph is assumed in experiments 2 and 3, 1.9 and 1.2 μEq of glyceride fatty acids were taken up by the tissue during 1 hr of incubation. Thus, considerable amounts of glycerides were removed from the hemolymph by the tissue. This observation can explain the differences obtained when net glyceride release was estimated.

Since it was shown by Meyer *et al.* (17) that a particulate fraction from the flight muscle of the desert locust could completely oxidize fatty acid *in vitro*, it can be assumed that fat in the locust is utilized without prior conversion to carbohydrates (2). Our results seem to indicate that glycerides can be released from the fat-body into the surrounding hemolymph. It is suggested that glyceride release is of great physiological importance, especially during flight. Since Weis-Fogh (3) showed that an average locust oxidized approximately 17 mg fat/hr during flight, a higher rate of fat release than that obtained so far in resting locusts would be necessary to meet the energy requirements of flying locusts. Fat mobilization in flying locusts is at present under investigation.

We are indebted to Professor G. Frankel, Department of Entomology, University of Illinois, for suggesting to us the method for collecting hemolymph.

The technical assistance of Mrs. Elana Bogoslavsky is gratefully acknowledged.

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