DETERMINATION OF PALMITIC ACID UPTAKE BY THE FAT BODY OF THE MADERA ROACH

Franklin Chang, Nelson M. Esguerra and Eduardo A. Macion

Associate Professor, Department of Entomology, University of Hawaii at Manoa, Honolulu, Hawaii, USA; Associate Professor, Visayas State College of Agriculture, Baybay, Leyte, Philippines; and Entomologist, Dole Philippines, Polomolok, South Cotabato, Philippines.

ABSTRACT

Uptake of [1-^{14}C] palmitic acid by the fat body of female adult Leucophaea maderae **was measured** in vitro. **The fat body free fatty acid fraction showed the highest radioactivity, followed by triglyceride. The other lipid fractions showed low incorporation of label. The turnover of palmitate to triglyceride in the fat body of** L. maderae **appears to be slower than those reported for other insect species. A method of pre-labelling the fat body with a radioisotope was found satisfactory and reliable.**

Ann. Trop. Res. 32-36.

KEY WORDS: Madera roach. *Leucophaea madera*. Palmitic acid. Lipid. Fat body. Radioisotope. DPM

INTRODUCTION

Lipids are known to be very efficient energy sources for insects. Among the Lepidoptera, lipid appears to be the main source of respiratory fuel for flight, with carbohydrate being used during the initial stages of flight (Weis-Fogh, 1952; Beenakkers, 1965). Diglyceride complexed with hemolymph protein appears to be the major form in which lipid is transported in several insects studied (Chino and Gilbert, 1965), although free fatty acids and triglyceride have been implicated in other insects (Wlodawer, Lagwinska and Baranskay, 1966; Martin, 1969; Cook and Eddington, 1967; Chang, 1971).

Efforts during the past years have been made to determine the

hormonal factor(s) responsible for lipid mobilization from the fat body. In this regard, a hormonal factor has been isolated from locust corpora cardiaca and named adipokinetic hormone (Mayer and Candy, 1969). This hormone has been purified and found to be a blocked decapeptide (Cheeseman, Goldsworthy and Mordue, 1977). Its function is to mobilize specific diglycerides during the flight and stimulate their oxidation by the flight muscles (Robinson and Goldsworthy, 1974).

Many studies concerning the investigation of the endocrine control of lipid mobilization make widespread radioactively-labeled fat body (Gilbert, 1974). Information gained from insect fat body uptake experiments with radioactively-labeled simple precursors as acetate, glucose, or palmitate, has given investigators a clear picture of the rates of lipid biosynthesis.

This paper presented a method for pre-labeling insect fat body and a profile of the rate of incorporation of label into the various lipid classes of the fat body of adult *L. maderae.*

MATERIALS AND METHODS

Fat bodies from 10 adult female wood roaches, *L. maderae,* were carefully dissected and placed in cold saline (0.29% NaCl). Approximately 200 mg fat body tissues were placed in each of 7 glass scintillation vials. One ml of 0.2 M Na_2HPO_4 - NaH^2PO^4 buffer solution, pH 7.2, containing 2 x 10^5 DPM [1-^{14}C] palmitic acid, sodium salt (specific radioactivity, 14 mCu/mmole) was added. The fat bodies were incubated at 32°C in a shaker bath for 0, 5, 15, 30, 60, 120, and 180 min. Fat bodies were removed from the labeling medium after each incubation period, placed on Whatman No. 1 filter paper on a Buchler filter flask, and rinsed with cold saline to remove extraneous label. Approximately 150 mg of fat bodies were homogenized in an all-glass Potter-Elvehjem homogenizer with 5 ml chloroform-methanol solution. Additional 5 ml rinses of the homogenizer were pooled with the initial contents in the separatory funnel. One hundred twenty ml of 0.2% NaCl solution was added to the funnel, the contents shaken, and allowed to partition overnight. The bottom organic phase containing the lipids was transferred to a round bottom flask and the solvent removed *in vacuo.*

A minimum of diethyl ether was used as solvent to transfer the lipids to 20 ml glass scintillation vials, and then the ether removed under a stream of nitrogen. The lipid was then dissolved in 0.5 ml chloroform-methanol solution for spotting on thin layer plates.

Ten microliters of lipid extract were spotted on each of 4 lanes marked on a 20 x 27cm plate coated with silica gel (E. Merck) at a thickness of 500 microns. Lipid standards consisting of a mixture of cholesteryl palmitate, monopalmitic, dipalmitin, tripalmitin, cholesterol, and palmitic acid in chloroform-methanol were spotted in the 5th outer lane. The plates were developed in the solvent system of

Feeman and West (1966): first solvent (polar) consisting of ether-benzene-ethanol-acetic acid (40:50:20:0.2, v/v), air-dried, then developed in the second solvent (non-polar) of diethyl ether-hexane (6:94, v/v). After the plates were developed and air-dried, the 4 lanes containing the radioactive lipid extracts were covered with aluminum foil. The exposed fifth lane was sprayed with 0.2% solution of 2', 7'- dichlorofluorescein in ethanol.

The lipid spots on silica gel plate corresponding with the lipid classes were circled after locating them with an ultraviolet lamp (254 nm). Areas on the other 4 lanes corresponding with each lipid standard were scraped off with a single-edge razor blade into 20 ml glass scintillation vials. Fifteen ml scintillation fluid containing 4 g PPO + 0.1 POPOP/ liter toluene were added to each vial. The samples were then counted in a Nuclear Chicago Unilux II-A liquid scintillation spectrometer with quench corrected with an external standard. All counts (CPM) were corrected to DPM (disintegrations per minute).

RESULTS AND DISCUSSION

Table 1 presents the amount of radioactivity (DPM's) found in each of the lipid classes after the fat body was inoculated for different time periods. Free fatty acids, monoglycerides, diglycerides, triglycerides, and phospholipids were present in fat bodies of the madera roach. Cholesterol, cholesterol esters and hydrocarbons were absent. With an increase in incubation time, lipid classes that were present also increased in rate of incorporation.

The highest rate of uptake was

Table 1. Time course of incorporation of [1 - ^{14}C] palmitic acid into lipids of the fat body of *Leucophaea madera*.

Incubation Time (min)	Disintegration Per Minute (DPM)							
	PL	MGL	FFA	CHOL	DGL	TGL	CE	HC
0	55	53	865	0	5	0	0	0
5	35	27	874	0	12	15	0	0
15	56	30	829	0	20	48	0	0
30	77	25	2289	0	34	25	0	0
60	106	7	1539	0	53	123	0	0
120	250	76	3940	0	129	887	0	0
180	342	103	3669	0	200	1407	0	0

PL - Phospholipid
MGL - Monoglyceride
FFA - Free Fatty Acid
CHOL - Cholesterol
DGL - Diglyceride
TGL - Triglyceride
CE - Cholesterol Ester
HC - Hydrocarbons

seen in the free fatty acid fraction, followed by the triglyceride fraction. However, free fatty acid incorporation began to decrease at 3-hr period, while triglyceride fraction remained high. The other lipid fractions showed a low rate of incorporation over the 3-hr period. The turnover rate of palmitate into triglyceride appeared to be slower than the rate of uptake of the fatty acid from the medium. In other studies on fat body uptake, rapid conversion to triglycerides from free fatty acid was seen with the free fatty acid radioactivity as well as the other lipid fractions remaining low throughout the period of incubation (Chang, 1971; Chino and Gilbert, 1965; Wlodawer, Lagwinska and Barinska, 1966). That the rate of biosynthesis to triglyceride is highest among the lipid classes in the wood roach fat body is understandable because triglyceride makes up the greatest fraction of the total fat body lipid content in the insects studied (Tietz, 1967; Chippendale, 1971; Chang, 1971; Chino and Gilbert, 1965).

The synthesis of triglycerides by the insect fat body apparently follows the glycerophosphate pathways as uncovered in either animals (Tietz, 1967). It appears that diglycerides are released from the fat body and are eventually transported to the flight muscles where the fatty acids may be released by lipases located in the flight muscle or hemolymph (Chang, 1977; Stevenson, 1969; Gilbert, Chino and Domroese, 1965). The fatty acids are then broken down in the fat body cells *via* B-oxidation for flight energy.

This study indicates that absorption of radioactivity-labeled palmitic acid occurred in fat bodies of the madera roach, and that its absorption by the fat bodies is influenced greatly by incubation time. Among the lipid classes, faster rate of incorporation occurred in free fatty acid and triglyceride fractions. This method, therefore, for pre-labeling the insect fat body is both reliable and satisfactory.

LITERATURE CITED

BEENAKKERS, A.M.T. 1965. Transport of fatty acids in *Locusta migratoria* during sustained flight. *J. Insect Physiol.* 11: 879-888.

CHANG, F. 1971. A developmental analysis of the uptake and release of lipids by the fat-body of the tobacco hornworm, *Manduca sexta*. *Insect Biochem.* 1: 63-80.

CHANG, F. 1977. The presence of lipoprotein lipase activity and its relationship to lipid transport in the oleander hawkmoth, *Deilephila nerii*. *Comp. Biochem. Physio.* 57B: 209-214.

CHEESEMAN, P., GOLDSWORTHY, G.J. and MORDUE, W. 1977. Studies on the purification of locust adipokinetic hormone. *Life Sciences* 21: 231-236.

CHINO, H. and GILBERT, L.I. 1965. Lipid release and transport in insects. *Biochem. Biophys. Acta*. 98: 94-110.

CHIPPENDALE, G.M. 1971. Fat body and hemolymph lipids of the southwestern corn borer, *Diatraea grandiosella*, during metamorphosis. *Insect Biochem*. 1: 39-46.

COOK. B.J. and EDDINGTON, L.C. 1967. The release of triglycerides and free fatty acids from the fat body of the cockroach, *Periplaneta americana*. *J. Insect Physiol*. 13: 1361-1372.

FREEMAN, C.P. and WEST, D. 1966. Complete separation of lipid classes on a simple thin layer plate. *J. Lipid Res*. 1: 324-328.

GILBERT, L.I., CHINO, H. and DOMROESE, K.J. 1965. Lipolytic activity of insect tissues and its significance in lipid transport. *J. Insect Physiol*. 11: 1057-1070.

GILBERT, L.I. and CHINO, H. 1974. Transport of lipids in insects. *J. Lipid Res*. 15: 439-456.

MAYER, R.J. and CANDY, D.J. 1969. Control of hemolymph lipid concentration during locust flight: Adipokinetic hormone from the *corpora cardieca*. *J. Insect Physiol*. 15: 611-620.

MARTIN, J.S. 1969. Studies on assimilation, mobilization, and transport of lipids by the fat body and hemolymph of *Pyrrhocoris apterus*. *J. Insect Physiol*. 15: 2319-2344.

ROBINSON, N.L. and GOLDSWORTHY, G.J. 1974. The effects of locust adipokinetic hormone on flight muscle metabolism *in vivo* and *in vitro*. *J. Comp. Physiol*. 89: 369-377.

STEVENSON, E. 1969. Monoglyceride lipase in moth flight muscle. *J. Insect Physiol*. 15: 1537-1550.

TIETZ, A. 1967. Fat transport in the locust: The role of diglycerides. *Eur. J. Biochem*. 2: 236-242.

WEIS-FOGH, T. 1952. Fat combustion and metabolic rate of flying locusts *(Shistocerca gregaria* Forskal). *Phil. Trans. R. Soc. Ser. B*. 237: 1-36.

WLODAWER, D., LAGWINSKA, E. and BARANSKA, J. 1966. Esterification of fatty acids in the wax moth hemolymph and its possible role in lipid transport. *J. Insect Physiol*. 12: 547-560.