Insect Biochem., Vol. 11, No. 4, pp. 423–427, 1981.
Printed in Great Britain.

0020–1790/81/040423–05$02.00/0
© 1981 *Pergamon Press Ltd.*

ENZYME ACTIVITIES AND ISOZYME COMPOSITION OF TRIGLYCERIDE, DIGLYCERIDE AND MONOGLYCERIDE LIPASES IN *PERIPLANETA AMERICANA, LOCUSTA MIGRATORIA* AND *POLIA ADJUNCTA*

KEITH B. MALE and KENNETH B. STOREY*
Institute of Biochemistry, Carleton University, Ottawa, Ontario, Canada K1S 5B6

(*Received* 15 *September* 1980)

Abstract—1. Measurements of maximal enzyme activities were combined with an electrophoretic study of isozyme make-up in an examination of triglyceride, diglyceride and monoglyceride lipases from the flight muscle, fat body and gut of the cockroach, *Periplaneta americana* and the locust, *Locusta migratoria* and from the flight muscle and fat body of the moth, *Polia adjuncta.*

2. Tri-, di- and mono-glyceride lipases were present in all tissues of the insects with diglyceride lipase ≥ triglyceride lipase activity in all cases and monoglyceride lipase ≥ diglyceride lipase activity in locust and moth.

3. In the flight muscle, a strong correlation was found between the activities of lipases and the known use of lipid as a fuel for flight in these insects. Lipase activities were lowest in the cockroach (a carbohydrate-based flight metabolism), intermediate in the locust (both carbohydrate and lipid-fueled flight), and highest in the moth (a non-feeding, lipid-catabolizing adult) flight muscle.

4. Polyacrylamide gel electrophoresis, using substrate-impregnated gels and stained for fatty acids released by lipase action, demonstrated the presence of tissue specific isozymes of tri-, di- and mono-glyceride lipases in the three insects. In addition, some, but not all, tissues showed multiple molecular forms of one or more of the lipases.

5. Diglyceride and monoglyceride lipase activities in both flight muscle and fat body of the insects co-electrophoresed suggesting the possibility that these two lipase activities might be catalyzed by a single enzyme protein.

Key Word Index: Periplaneta americana, Locusta migratoria, Polia adjuncta, lipid metabolism, lipase isozymes, insect tissue lipases

INTRODUCTION

LIPIDS ARE important fuels for flight in a number of insect groups (GILBERT and CHINO, 1974; CRABTREE and NEWSHOLME, 1975). Many of the Lepidoptera, particularly those which are non-feeding as adults, rely entirely upon lipid oxidation to power flight (GILBERT, 1967) while amongst migratory species, lipid reserves are the fuel for long term flight. Lipid is stored in insects mainly as triglyceride deposits in the fat body. These are mobilized, most likely under the control of adipokinetic hormone (MAYER and CANDY, 1969) with diglyceride being released from the fat body and circulated to other tissues via the haemolymph. Haemolymph diglyceride appears to be the major lipid fuel oxidized by working flight muscle (GILBERT, 1967; MAYER and CANDY, 1969).

The initial steps in the oxidation of glycerides are catalyzed by the lipases, triglyceride, diglyceride and monoglyceride lipase which serially cleave the fatty acids from the glycerol backbone. Relatively few studies have been made of insect lipases (GILBERT *et al.*, 1965; CRABTREE and NEWSHOLME, 1972; STEVENSON, 1969, 1972; TIETZ and WEINTRAUB, 1978) despite their key role in lipid catabolism. In the present study measurements of the maximal activities of tissue lipases were combined with an electrophoretic analysis of the tissue specific compliment of lipases. The three insect species chosen differ widely in their use of lipid as a flight muscle fuel. The moth, *Polia adjuncta*, is a non-feeding adult and flight is dependent solely upon the oxidation of stored lipid reserves. The cockroach, *Periplaneta americana*, on the other hand, utilizes no lipid and flight is totally carbohydrate based (DOWNER and MATTHEWS, 1976). The locust, *Locusta migratoria*, has clearly defined stages of carbohydrate-powered and lipid-powered flight. Take off and the initial minutes of flight are fueled by carbohydrate (endogenous glycogen and haemolymph trehalose) but gradually, in flights exceeding 20–30 min, diglyceride takes over to become the muscle fuel for long term or migratory flight (WEIS-FOGH, 1952; WORM and BEENAKKERS, 1980). The inhibitory effects of lipid-related compounds upon the glycolytic enzyme, aldolase, in locust flight muscle have recently been suggested as one factor contributing to this transition from carbohydrate to lipid based flight (STOREY, 1980).

* Correspondence and reprint requests should be addressed to: Dr. K. B. STOREY, Department of Biology, Carleton University, Ottawa, Ontario, Canada K1S 5B6.

MATERIALS AND METHODS

Chemicals and animals

Substrates and reagents were obtained from Sigma Chemical Co., St. Louis, Missouri. The mono olein used was 90% α-isomer while diolein was 85% 1,3-diolein, 15% 1,2-diolein. 1,2-Diglycerides are reported to be the naturally occurring substrate for insect diglyceride lipase (TIETZ and WEINTRAUB, 1980). However, our tests substituting purified 1,2-diolein for the 1,3-1,2 mixture produced no significant difference in the measured activities of diglyceride lipases. Adult male insects: cockroaches, *Periplaneta americana*, locusts, *Locusta migratoria*, and moths, *Polia adjuncta*, were used.

Assay of lipase activity

Tissues were dissected out, rinsed in homogenizing buffer, blotted, and homogenized in 3 vol 60 mM Tris–HCl buffer, pH 8.0. After centrifugation at 27,000 *g* (4°C) for 10 min, the supernatant (which contained > 95% of tri-, di- or mono-glyceride lipase activities present in the homogenate) was used as the source of enzyme.

The assay method used was that of MYRTLE and ZELL (1975) with modifications made in the type of substrates and emulsifying mixtures used. Triolein and monoolein substrate solutions were prepared by dissolving 330 mg triolein or 100 mg monoolein in 10 ml 60 mM Tris-HCl buffer, pH 8.0 containing 0.01 g deoxycholic acid, 0.75 g gum arabic and 0.01 g sodium benzoate. Diolein substrate was prepared by emulsifying 100 mg diolein in 10 ml 60 mM Tris–HCl buffer, pH 8.0 using a Tissuemizer homogenizer run at top speed for 3 min. Diolein substrate solution was used immediately after preparation.

Standard assays contained 0.15 ml triolein substrate, 0.5 ml diolein substrate or 0.2 ml monoolein substrate solution made up to 1 ml with 60 mM Tris–HCl buffer, pH 8.0. These were equivalent to substrate concentrations of 4.6 mM, 8.1 mM, and 5.6 mM for tri-, di- and mono-olein respectively. Assays were started by the addition of 50 μl of enzyme source and were run for 10 min at 23°C with continuous mixing using a Multi-purpose Rotator at maximal speed. Preliminary experiments were run to determine: (a) optimal substrate concentrations (ΔO.D./min against [S]) for V_{max} enzyme activities for all tissues and for all three substrates, (b) optimal assay time (ΔO.D. against *t*) within the linear range of the assay and (c) optimal amount of enzyme (ΔO.D./min against amount of extract) for the assay mixtures used.

Extraction and colorimetric determination of fatty acids produced was performed by the method of MYRTLE and ZELL (1975) using an oleic acid standard.

Gel electrophoresis of lipases

Tissues were homogenized in 3 vol 50 mM Tris–HCl buffer, pH 7.4 containing 0.5 mM EDTA, 15 mM β-mercaptoethanol and 0.25 M sucrose. After centrifugation at 2000 *g* (23°C) for 5 min, the supernatants (which contained > 95% of all lipase activities) were applied to the gels.

Substrate-impregnated gels were prepared in which 0.6 ml tributyrin, 0.3 ml triolein, 100 mg diolein or 100 mg monoolein were added per 28 ml acrylamide solution (one slab gel) just before polymerization was initiated. Slab polyacrylamide gels were run at 4°C and 200 V for 4 hr using Tris–glycine, pH 7.4 as the electrode buffer.

After electrophoresis, olein gels were incubated in 50 mM Tris–HCl buffer, pH 7.4, containing 10 mM $CaCl_2$, for 3 hr at 40°C to stimulate lipase activity. Free fatty acids released by lipase or esterase action were then stained with a solution of Nile Blue A (0.8 g dissolved in 0.2 M H_2SO_4) for 30 min and destained under running water. The electrophoresis and staining method is essentially that of LAKSHMI and SUBRAHMANYAM (1977).

Esterase and lipase activities were identified as blue bands on the destained gels. In the substrate-impregnated gels, non-specific esterases, as well as lipases, will cleave fatty acids from tri-, di- or mono-glycerides. Non-specific esterases were therefore identified using the control, tributyrin-impregnated gel and esterase bands were 'subtracted' from the tri-, di- or mono-olein gels to reveal the true lipase bands. Thus, esterase bands appeared on both tributyrin and glyceride gels while lipases appeared on only the glyceride gels. Typically between two to eight esterase bands were found per tissue, the highest numbers being found in gut and fat body. TURUNEN (1978) similarly reports multiple esterases in insect tissues.

RESULTS AND DISCUSSION

Distribution and activities of lipases

Triglyceride, diglyceride and monoglyceride lipases were present in all tissues tested, the flight muscle, fat body and gut of *P. americana* and *L. migratoria* and the flight muscle and fat body of *P. adjuncta*. V_{max} activities of the tissue lipases are presented in Table 1. In the tissues of all three insects, the activities of diglyceride lipase were greater than or equal to those of triglyceride lipase and in the moth and locust, monoglyceride lipase activity was similarly greater than or equal to diglyceride lipase levels. A similar relationship holds for flight muscle lipases from a variety of other insects (CRABTREE and NEWSHOLME, 1972). In addition, flight muscle lipase activities were in all cases higher than the corresponding lipase activities in fat body. In the cockroach and locust, from which gut tissue was available, gut lipase activities were in turn higher than the lipase activities in the other two tissues.

Table 1. Lipase activities in the tissues of the cockroach *P. americana*, the locust *L. migratoria*, and the moth *P. adjuncta*

		Enzyme activity (μmol fatty acid released/min/g wet wt.)		
Insect	Tissue	Triglyceride lipase	Diglyceride lipase	Monoglyceride lipase
Cockroach	Flight muscle	0.06 ± 0.01 (9)	0.14 ± 0.01 (3)	< 0.01
	Fat Body	0.05 ± 0.01 (8)	0.10 ± 0.02 (3)	< 0.01
	Gut	1.15 ± 0.12 (9)	1.17 ± 0.02 (4)	0.31 ± 0.02 (4)
Locust	Flight muscle	0.09 ± 0.01 (9)	0.11 ± 0.004 (4)	0.25 ± 0.03 (8)
	Fat body	0.03 ± 0.004 (6)	0.09 ± 0.03 (4)	0.08 ± 0.01 (10)
	Gut	0.14 ± 0.01 (9)	0.15 ± 0.03 (6)	0.84 ± 0.05 (8)
Moth	Flight muscle	0.27 ± 0.03 (7)	1.57 ± 0.03 (4)	3.20 ± 0.13 (8)
	Fat body	0.12 ± 0.02 (7)	0.22 ± 0.08 (4)	0.28 ± 0.02 (8)

Enzyme activity was measured as described in Materials and Methods.
Results are expressed as means ± S.E.M. with *n* in brackets.

The activities of lipases in the flight muscle appeared to be directly related to the known flight muscle metabolism of the three species. The cockroach, whose flight is powered by carbohydrate oxidation (DOWNER and MATTHEWS, 1976), had the lowest activities of flight muscle lipases of the three insects and in particular, monoglyceride lipase activity was extremely low. This is consistent with the absence of a need to oxidize lipid as a fuel for flight. But the presence of low levels of all three lipases demonstrated that cockroach flight muscle retains the ability to oxidize lipids, an ability perhaps important during periods of starvation when stored lipid reserves would be used to support basal metabolism (STOREY and BAILEY, 1978). Locust flight muscle catabolizes lipid during long term flight (WORM and BEENAKKERS, 1980) and correspondingly, our results show that the activities of lipases in locust flight muscle are overall higher than those in the cockroach flight muscle. Monoglyceride lipase in this muscle has activities more than two fold greater than those of either di- or triglyceride lipase. However, lipase activities were highest in the moth flight muscle. As this species has a non-feeding adult stage, the high lipase activities are consistent with the use of lipid as the sole fuel for both basal and working muscle metabolism Overall, of three lipase activities tested, flight muscle monoglyceride lipase shows the highest percentage change when one compares the three insect species (Table 1).

The activities of lipases in the fat body of the three species were lower than those in the corresponding flight muscles. Fat body lipase activities were highest in the moth, a consequence likely of a metabolism based largely upon the oxidation of stored lipid reserves. Cockroach fat body, like the flight muscle, again displayed very low activities of monoglyceride lipase. Overall there appears to be a correlation between the activities of fat body lipases and the activities of the corresponding flight muscle lipases and also therefore with the use of lipid as a fuel for flight in the three species. This could be expected because of the major role of the fat body in the provision of diglyceride fuel for the flight muscle from stored triglyceride reserves. Indeed TIETZ and WEINTRAUB (1978) have suggested that both triglyceride lipase and diglyceride lipase are necessary for this function. They have demonstrated that when fat body triglyceride stores are mobilized, triglyceride is first degraded to produce monoglycerides and then reacylated to form a 1,2-diacylglycerol which is released into the haemolymph. Hence fat body diglyceride and triglyceride lipase activities could, perhaps, reflect lipid use by the flight muscle. Other functions of fat body lipases would include (a) the breakdown and interconversion of dietary lipids and (b) the oxidation of lipids for endogenous energy supply.

All three lipases were present in gut tissue of cockroach and locust (gut in adult *P. adjuncta* is vestigial) and in both species gut displayed the highest tissue activities of lipases. Triglyceride and diglyceride lipase were present in approximately equal activities in gut of both species but monoglyceride lipase activity was five to six fold higher than either tri- or diglyceride lipases in the locust gut. However, in the cockroach gut, monoglyceride lipase, as in other cockroach tissues, displayed the lowest activity of the three lipases. Cockroach tri- and diglyceride lipase activities were about ten fold higher than those in locust gut and probably reflect the composition of the diet of these two species, that of the locust being much lower in fat. The role of lipases in the gut is thought to be largely in the breakdowm of dietary lipids which are then reconstituted as diglycerides and released into the haemolymph for transport to the fat body (WEINTRAUB and TIETZ, 1973; CHINO and DOWNER, 1979). Some lipid may also be converted into triglyceride and stored in the gut itself.

Electrophoresis and lipase isozymes

Slab polyacrylamide gel electrophoresis, using the substrate-impregnated gel technique of LAKSHMI and SUBRAHMANYAM (1977), was used to investigate the

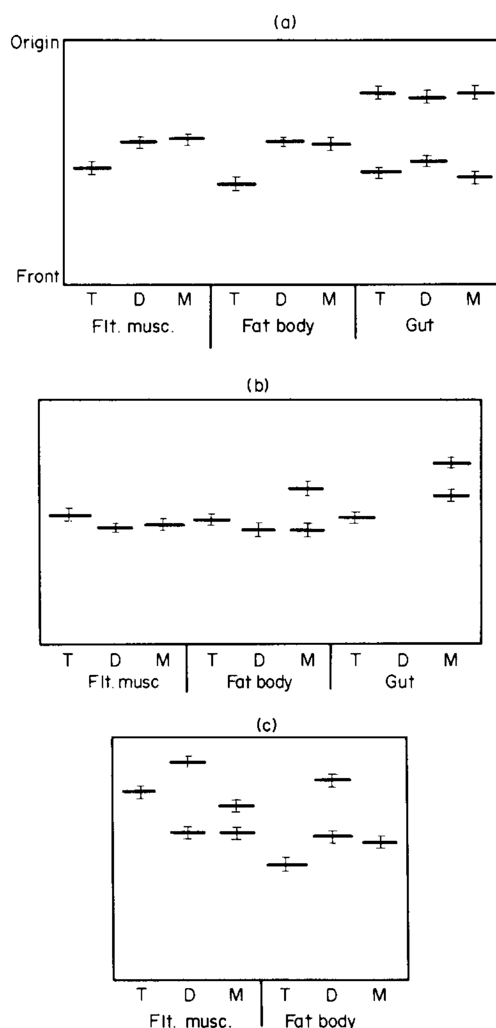

Fig. 1. Polyacrylamide gel electrophoresis of insect tissue lipases from (A) cockroach, *P. americana* (B) locust, *L. migratoria* and (C) moth, *P. adjuncta*. Substrate-impregnated gels were run, incubated, and stained as described in Materials and Methods. Figures are composite drawings showing the mean R_f (of eight to ten gels) for each lipase band with the range indicated by the error bars. Lipase enzymes migrated towards the anode. T, D and M denote triglyceride, diglyceride and monoglyceride lipase, respectively.

Table 2. R_f values for tissue lipases of the cockroach, *P. americana*, the locust *L. migratoria*, and the moth, *P. adjuncta*

Insect	Tissue		R_f Triglyceride lipase		R_f Diglyceride lipase		R_f Monoglyceride lipase
Cockroach	Flight muscle		0.52 (0.50–0.54)		0.41 (0.39–0.43)		0.40 (0.38–0.42)
	Fat body		0.58 (0.56–0.60)		0.41 (0.39–0.43)		0.42 (0.40–0.44)
	Gut	(a)	0.21 (0.19–0.23)		0.23 (0.21–0.25)		0.21 (0.19–0.23)
		(b)	0.53 (0.51–0.55)		0.49 (0.47–0.51)		0.55 (0.53–0.57)
Locust	Flight muscle		0.47 (0.45–0.49)		0.52 (0.50–0.54)		0.51 (0.49–0.53)
	Fat body		0.49 (0.47–0.51)		0.53 (0.51–0.55)	(a)	0.36 (0.34–0.38)
						(b)	0.53 (0.51–0.55)
	Gut		0.48 (0.46–0.50)		n.d.	(a)	0.26 (0.24–0.28)
						(b)	0.39 (0.37–0.41)
Moth	Flight muscle		0.22 (0.20–0.24)	(a)	0.10 (0.08–0.12)		0.28 (0.26–0.30)
				(b)	0.39 (0.37–0.41)		0.39 (0.37–0.41)
	Fat body		0.52 (0.50–0.54)	(a)	0.17 (0.15–0.19)		0.43 (0.41–0.45)
				(b)	0.40 (0.38–0.42)		

Slab gel electrophoresis was performed as outlined in Materials and Methods.
R_f values were determined relative to mobility of the tracker dye bromothymol blue. Results are the mean R_f for eight to ten gels of each substrate with the range given in brackets.
n.d. = not detected.

molecular form(s) of lipases in the tissues of the three insects. The results are shown in Fig. 1 with R_f values for tissue lipases given in Table 2. Electrophoresis indicated the presence of both tissue specific isozymes of tri-, di- and mono-glyceride lipase and multiple molecular forms of lipases within a single tissue. By contrast, LAKSHMI and SUBRAHMANYAM (1977), who first applied this technique to insect lipases, found only a single molecular form of tri-, di- or mono-glyceride lipase in whole animal extracts of the mosquito, *Culex*.

Tissue specific isozymes of triglyceride lipase were found in each of the three cockroach tissues examined (Fig. 1a). Two forms of triglyceride lipase were found in the gut. One form appeared to be the flight muscle isozyme (R_f values of the two enzymes were the same) while the other form was a gut specific enzyme. Cockroach flight muscle and fat body each had a single form of diglyceride lipase which would appear to be the same isozymic form (identical R_f values) in both tissues. Two electrophoretically separable isozymes of diglyceride lipase were found in gut; both of these were different from the flight muscle/fat body isozyme. The results for monoglyceride lipase in the cockroach paralleled those for diglyceride lipase. Flight muscle and fat body each had a single form of monoglyceride lipase, apparently the same isozyme in both tissues. Gut displayed two isozymes of monoglyceride lipase, both of which had different R_f values from that of the flight muscle/fat body isozyme. In this study, whole gut (foregut + midgut + hindgut) was used as source of lipase enzymes. Each of the three different areas of gut have separate cell types and separate functions. The finding of multiple molecular forms of tri-, di- or mono-glyceride lipase in gut could reflect this mixing of cell types with each of the two forms of these lipases originating from a different cell type.

Locust tissues had a somewhat less complicated isozymic make-up (Fig. 1b). There appeared to be only a single form of triglyceride lipase (as judged by identical R_f values) and this form was found in all three tissues. The same result was found for diglyceride lipase. However, at least three isozymic forms of monoglyceride lipase were identified in locust tissues. Flight muscle had a single, tissue specific isozyme of the enzyme while both fat body and gut displayed dual isozymic forms. One of the fat body isozymes appeared to be the same one found in flight muscle (R_f values were identical within experimental error) while the other isozyme was identical with one of the isozymes occurring in gut. The second isozyme found in gut was tissue specific.

Moth tissues displayed tissue specific isozymic forms of all three lipases (Fig. 1c). Flight muscle and fat body each had a single tissue specific form of triglyceride lipase. However, diglyceride lipase occurred in two electrophoretic forms in both tissues. One diglyceride lipase isozyme was common to both tissues while the other was a tissue specific form. Dual forms of monoglyceride lipase were found in flight muscle. One of these forms co-electrophoresed with the monoglyceride lipase of the fat body while the second was a tissue specific form. Of note is the fact that the moth was the only one of the three insects to show multiple molecular forms of lipases in the flight muscle. Whether this occurrence is related to the use of lipid as the sole fuel for flight in the moth is at present unknown.

Molecular nature of lipases

Little is known about the molecular nature of lipases within the cell and virtually nothing is known about insect lipases in this regard. Are triglyceride, diglyceride and monoglyceride lipases each separate enzymes? Are the enzymes aggregated to form a lipase complex *in vivo*? Does a single enzyme protein exhibit one or more lipase activities? A recent study by BERGLUND *et al.* (1980) showed that hormone-sensitive lipase from chicken adipose tissue occurs as a large lipid–protein complex containing triglyceride, diglyceride, monoglyceride and cholesterol ester hydrolases. This complex was resolved into several proteins, one of which displayed both triglyceride and diglyceride lipase activities, indicating that in this

instance both of these activities is performed by a single enzyme protein. A separate protein displayed monoglyceride lipase activity. It is clear, therefore, that a single enzyme protein may show more than one lipase activity. In all three insects studied, the diglyceride and monoglyceride lipase activities of both flight muscle and fat body co-electrophoresed (identical R_f values) suggesting that these enzyme activities may be associated with a single protein. Triglyceride lipase of flight muscle and fat body, however, always had an R_f value clearly different from those of the other lipases, indicating that a separate protein catalyzes the triglyceride lipase reaction. In cockroach gut, all three lipase activities could be found associated with a band running with an R_f of 0.21–0.23 suggesting that in this case a single protein may possess tri-, di- and mono-glyceride lipase activities. While these results can in no way be conclusive in determining that more than one lipase activity can be associated with a single protein, the results form a good basis from which to further investigate this question. Indeed, the data of LAKSHMI and SUBRAHMANYAM (1977) provide a similar suggestion of a single protein catalyzing more than one lipase activity. However, unlike our results, their work on *Culex* would suggest that triglyceride and diglyceride lipase activities are associated with a single protein species with monoglyceride lipase occurring as a separate enzyme.

In summary this study has demonstrated the presence of triglyceride, diglyceride and monoglyceride lipases in the tissues of three species of insect. A close correlation has been demonstrated between the measured activities of lipases in flight muscle and the utilization of lipid as a fuel for insect flight. The study is unique in its electrophoretic examination of insect tissue lipases and in its demonstration of the presence of tissue specific lipases isozymes and of multiple isozymic forms of lipases within single tissues. The data support the idea that di- and mono-glyceride lipase activities in insects may be catalyzed by a single enzyme protein. Further work in this area will include studies to purify and characterize the various tissue specific isozymes of the lipases and to further investigate the possibility that single enzyme proteins can have multiple lipase activities.

Acknowledgements—The authors wish to thank Dr. G. WYATT, Queen's University for his kind gift of the locusts, Dr. A. HUDSON, Agriculture Canada for her gift of the moths and Dr. D. GARDNER, Carleton University for the cockroaches. Dr. R. G. H. DOWNER, University of Waterloo, is thanked for helpful discussions. The work was supported by an N.S.E.R.C. operating grant to KBS.

REFERENCES

BERGLUND L., KHOO J. C., JENSEN D. and STEINBERG D. (1980) Resolution of hormone-sensitive triglyceride/diglyceride lipase from monoglyceride lipase of chicken adipose tissue. *J. biol. Chem.* **255**, 5420–5428.

CHINO H. and DOWNER R. G. H. (1979) The role of diaclyglycerol in absorption of dietary glyceride in the american cockroach, *Periplaneta americana* L. *Insect Biochem*, **9**, 379–382.

CRABTREE B. and NEWSHOLME E. A. (1972) The activities of lipases and carnitine palmitoyltransferase in muscles from vertebrates and invertebrates. *Biochem. J.* **130**, 697–705.

CRABTREE B. and NEWSHOLME E. A. (1975) Comparative aspects of fuel utilization and metabolism by muscle. In *Insect Muscle* (Ed. by USHERWOOD P. N. R.) pp. 405–500. Academic Press, New York.

DOWNER R. G. H. and MATTHEWS J. R. (1976) Glycogen depletion of thoracic musculature during flight in the american cockroach, *Periplaneta americana* L. *Comp. Biochem. Physiol.* **55B**, 501–502.

GILBERT L. I. (1967) Lipid metabolism and function in insects. *Adv. Insect Physiol.* **4**, 69–211.

GILBERT L. I., CHINO H. and DOMROESE K. A. (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.

LAKSHMI M. B. and SUBRAHMANYAM D. (1977) Separation and detection of lipases using polyacrylamide gel electrophoresis. *J. Chromatog.* **130**, 441–443.

MAYER R. J. and CANDY D. J. (1969) Control of haemolymph lipid concentration during locust flight: an adipokinetic hormone from the corpora cardiaca. *J. Insect Physiol.* **15**, 611–620.

MYRTLE J. F. and ZELL W. J. (1975) Simplified photometric copper–soap method for rapid assay of serum lipase activity. *Clin. Chem.* **21**, 1469–1473.

STEVENSON E. (1969) Monoglyceride lipase in moth flight muscle. *J. Insect Physiol.* **15**, 1537–1550.

STEVENSON E. (1972) Haemolymph lipids and fat body lipases of the southern armyworm moth. *J. Insect Physiol.* **18**, 1751–1756.

STOREY K. B. (1980) Kinetic properties of purified aldolase from locust flight muscle. Role of the enzyme in the transition from carbohydrate to lipid-fueled flight. *Insect Biochem.* **10**, 647–655.

STOREY K. B. and BAILEY (1978) Intracellular distribution of enzymes associated with lipogenesis and gluconeogenesis in fat body of the adult cockroach, *Periplaneta. Insect Biochem.* **8**, 125–131.

TIETZ A. and WEINTRAUB H. (1978) Hydrolysis of glycerides by lipases of the fat body of the locust, *L. migratoria. Insect Biochem.* **8**, 11–16.

TIETZ A. and WEINTRAUB H. (1980) The stereospecific structure of haemolymph and fat body 1,2-diacylglycerol from *Locusta migratoria. Insect Biochem.* **10**, 61–63.

TURUNEN S. (1978) Multiplicity of tissue esterases in *P. brassicae. Ann. Zool., Fennici* **15**, 89–93.

WEINTRAUB H. and TIETZ A. (1973) Triglyceride digestion and absorption in the locust, *L. migratoria. Biochim. biophys. Acta* **306**, 31–41.

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

WORM R. A. A. and BEENAKKERS A. M. Th. (1980) Regulation of substrate utilization in the flight muscle of the locust, *Locusta migratoria*, during flight. *Insect Biochem.* **10**, 53–59.