*Insect Biochem.* Vol. 14, No. 3, pp. 261–266, 1984
Printed in Great Britain. All rights reserved

0020-1790/84 $3.00 + 0.00
Copyright © 1984 Pergamon Press Ltd

# LIPOLYTIC ACTIVITY IN THE FLIGHT MUSCLES OF *LOCUSTA MIGRATORIA* MEASURED WITH HAEMOLYMPH LIPOPROTEINS AS SUBSTRATES

C. H. Wheeler*, D. J. Van der Horst† and A. M. Th. Beenakkers†

*Department of Zoology, The University, Hull HU6 7RX, England and †Laboratory of Chemical Animal Physiology, State University of Utrecht, 8 Padualaan, 3508 TB Utrecht, The Netherlands

(*Received* 9 *June* 1983; *revised* 22 *August* 1983)

**Abstract**—A radiochemical assay is described in which neutral lipids presented as part of authentic haemolymph lipoproteins have been used as substrates to measure the lipolytic activity in the flight muscles of *Locusta migratoria*. The radiolabel in the substrate was located almost exclusively in the glycerol backbone of long chain acylglycerols (predominantly diacylglycerols) and the release of radiolabelled glycerol during incubation with muscle homogenates must therefore be due to "true" lipase activity rather than non-specific esterases. As the diacylglycerols were lipoprotein-bound, such enzymes may be regarded as lipoprotein lipases and this work therefore provides the first direct evidence for the existence of this group of enzymes in an insect tissue. Lipolytic activity in flight muscles has a single pH optimum with a peak at 7.5–8.0 and is only partially inhibited by 1 M NaCl which suggests dissimilarity with mammalian lipoprotein lipase. The activity and characteristics of the locust enzyme are discussed in relation to the energy requirements of flying locusts and in relation to what is known of the vertebrate enzyme.

*Key Word Index*: *Locusta migratoria*, lipase, lipoproteins, flight muscles, adipokinetic hormone

## INTRODUCTION

In locusts, long-term flight is maintained by the oxidation of fatty acids (Krogh and Weis-Fogh, 1951; Weis-Fogh, 1952; Beenakkers, 1965) which, under the action of adipokinetic hormone, are mobilized from triacylglycerols stored in the fat body (Beenakkers, 1969; Mayer and Candy, 1969). Lipids are released from the fat body in the form of specific (mainly 16:18 and 18:18) diacylglycerols (Jutsum and Goldsworthy, 1976; Spencer and Candy, 1976) and are incorporated into existing haemolymph lipoproteins (Mayer and Candy, 1967; Mwangi and Goldsworthy, 1977) which undergo a reorganization to form the higher molecular weight lipoprotein, $A^+$ (Mwangi and Goldsworthy, 1977, 1981; Van der Horst *et al.*, 1979). Lipoprotein $A^+$ is essential for the transfer of diacylglycerols to the flight muscles (Robinson and Goldsworthy, 1977; Mwangi and Goldsworthy, 1981) but the precise nature in which lipids enter the flight muscles is unknown. Locust flight muscles possess enzymes which can hydrolyse emulsions of neutral lipids (Crabtree and Newsholme, 1972; Male and Storey, 1981), thus uptake and intracellular hydrolysis of diacylglycerols could occur. In mammals, however, neutral lipids carried by lipoproteins are hydrolysed at the cell surface by lipoprotein lipases (Nilsson-Ehle *et al.*, 1980) and lipids enter the cells as fatty acids. The existence in *Locusta migratoria* of a sophisticated lipoprotein transport system which resembles superficially that in vertebrates (Wheeler and Goldsworthy, 1983a), together with the evidence that flight muscles can differentiate between specific lipoproteins (Robinson and Goldsworthy, 1976) suggest that a lipoprotein specific lipase may also be necessary to "unload" lipids from insect lipoproteins. The occurrence of an enzyme similar to mammalian lipoprotein lipase has been described in the moth *Deilephila nerii* (Chang, 1977) and Gilbert and Chino (1974) reported that lipase activity in moth flight muscles is higher when assayed using natural lipoprotein substrates than when using lipid emulsions, which may suggest the presence of lipoprotein specific lipases. As yet however, lipoprotein lipases in insect tissues have not been directly demonstrated. In this investigation, diacylglycerol carrying lipoproteins have been used as substrates to show the presence in locust flight muscles of enzymes capable of hydrolysing lipoprotein-bound neutral lipids.

## MATERIALS AND METHODS

*Locusta migratoria* were reared under conditions as described previously (Van der Horst *et al.*, 1978). Synthetic adipokinetic hormone (Peninsula Laboratories, San Carlos, California, U.S.A.) was prepared as a stock solution in absolute methanol (stored at −15°C) which was diluted with saline to the required concentration immediately before use. Solutions of NaCl were prepared in phosphate buffer (pH 7.65) on the day of use.

### *Substrate preparation*

Lipoprotein substrates were prepared from the haemolymph of mature (12–16-day-old) locusts of both sexes which had been injected 90 min previously with 4 pmol synthetic adipokinetic hormone. Animals were bled by the method of Jutsum and Goldsworthy (1976) and the haemolymph treated with ammonium sulphate following the method of Mwangi and Goldsworthy (1977). Radiolabelled lipoproteins were prepared in the same way from locusts which were injected with 0.5–1 μCi [U-$^{14}$C]glycerol (sp. act.

152 mCi/mmol; New England Nuclear) 5–10 min after hormone injection. Haemolymph (3–6 ml) from 100 to 150 animals (20 of which were injected also with [$^{14}$C]glycerol) were collected per assay. Before use, pooled haemolymph was desalted on Sephadex G-25 (Pharmacia; column dimensions 30 × 1.6 cm i.d.). Proteins were eluted in phosphate buffer (28 mM $Na_2HPO_4$, 5 mM $Na_2PO_4$, 81 mM NaCl 14 mM KCl, 4.3 Mm $MgCl_2$, 4.1 mM $NaHCO_3$; pH 7.65). Sodium azide (0.02%), which had no effect on lipase activity, was included in elution buffers to prevent microbial growth. After chromatography, proteins voided on G-25 were concentrated to approximately the original haemolymph volume by the method of Robinson and Goldsworthy (1977) and the resultant preparations were clear, coloured (usually yellow or green) solutions resembling fresh haemolymph. The pH of lipoprotein substrates was adjusted to 7.65 immediately before assay by the addition of 0.1 M $Na_2HPO_4$ solution. Specific activities (expressed as dis/min per mol dipalmitin) of the lipid moiety of lipoprotein substrates were determined from measurements of total radioactivity associated with washed TCA precipitable protein (after the method of Wheeler and Goldsworthy, 1983b) and total lipid (by the modified vanillin method of Holwerda *et al.*, 1977) in aliquots of substrates. Localization of radiolabel in substrate was confirmed by counting samples of individual lipid fractions scraped from plates after thin layer chromatography of extracted lipids (Bligh and Dyer, 1959) using the solvent system of Freeman and West (1966) and silica gel G plates. Visualization and elution of lipids from TLC plates was achieved by the methods of Van der Horst *et al.* (1983).

*Preparation of muscle homogenates*

Dorsal longitudinal flight muscles were dissected from mature male locusts and rinsed in phosphate buffer (see above) at 0°C. Adhering fat body and trachea were removed as far as was possible. Muscles were blotted dry with tissue paper, weighed and then homogenized in phosphate buffer (0°C, pH 7.65) using a glass/Teflon homogenizer (ten passes at 100 rpm, 0°C) and were kept at 0°C until required for assay (within 30 min of tissue removal). The inclusion of very small amounts of fat body tissue in flight muscle homogenates was unavoidable. Locust fat body homogenates can hydrolyse lipoprotein bound neutral lipids but the enzyme specific activity is only about one tenth of that in the flight muscles (C. H. Wheeler, unpublished observations). Since fat body constituted only a very small proportion of the total flight muscle homogenate, the lipolytic activity due to the fat body enzyme was negligible in these experiments. Preliminary experiments established that preparation of muscle tissues at pH 7.0, or homogenization in buffer containing 0.3 M sucrose, or sonication, or the addition of the cofactors such as CoA, cysteine, carnitine and ATP, all had no effect on the lipolytic activity of the preparations. Addition of albumin (fatty acid free) at concentrations between 1 and 5% did not increase lipolytic activity compared to controls during a 1 hr incubation.

*Assay for lipase activity*

Aliquots of muscle homogenates (20–30 $\mu$l containing normally less than 1 mg tissue) were pipetted into cold (0°C) reaction tubes, and assays were started by addition with mixing of an aliquot of substrate (60–100 $\mu$l). The tubes were kept agitated and the incubation was allowed to proceed at 35°C. Final lipid concentrations were greater than 12 $\mu$g/$\mu$l, unless otherwise stated. Due to the presence of endogenous lipolytic activity in all lipoprotein preparations, control tubes, identical to experimental groups except that the muscle preparation was heat "killed" (5 min at 90°C in sealed tubes) before addition of substrate, were run in parallel. Incubations lasting normally 16–60 min were terminated by addition with mixing of (600–1000 $\mu$l) 5% (w/v) trichloracetic acid (TCA). Precipitated proteins, lipids and lipoproteins were sedimented by centrifugation (12,000 *g* for 5 min at 20°C) and aliquots of the supernatants containing the liberated glycerol (confirmed by thin layer chromatography after the method of Van der Horst *et al.*, 1983) were added to scintillation vials containing 20 $\mu$l 5 M NaOH (to reduce quenching, following the method of Schotz and Garfinkel, 1972) and 4 ml Scintillater 299 (Packard) before liquid scintillation counting. Corrections for sample quenching were made by the channels ratio method. Specific activities of substrates were used to estimate the glycerol content of TCA supernatants on the basis that 1 $\mu$mol diacylglycerol yields 1 $\mu$mol glycerol upon complete hydrolysis. Lipase activities (at 35°C) were determined from the differences during incubation in release between control and experimental groups and are expressed as $\mu$mol glycerol released/min per g wet weight of tissue.

## RESULTS

*Substrate preparation*

Pre-injection of locusts with AKH causes preferential incorporation of injected [$^{14}$C]glycerol into haemolymph diacylglycerols (Candy *et al.*, 1976; Van der Horst *et al.*, 1983) rather than trehalose, so that 90 min after injection of hormone, 90% of haemolymph radioactivity was associated with TCA precipitable protein. After treatment of such haemolymph with ammonium sulphate followed by desalting, over 97% of the radioactivity voided on Sephadex G-25 was TCA precipitable though upon storage at room temperature (20°C) the amount of TCA soluble radioactivity increased slowly with time. Assays were, however, performed quickly after substrate preparation and controls were run in parallel with experimental groups so that errors which could have arisen from endogenous haemolymph lipase activity were avoided. The quality of haemolymph lipoprotein preparations was very important. In an experiment in which pooled haemolymph stabilized in ammonium sulphate was diluted with saline (1:1, v/v) and left overnight (16 hr at 20°C), the solution became cloudy. When the solution was desalted, reconcentrated and used as substrate in a lipase assay the maximum activity recorded was about 30% lower than when using haemolymph from the same pooled sample but which had not been diluted.

Thin layer chromatographic analysis of lipids from haemolymph substrates showed that about 95% of the radioactivity was recovered in acylglycerols with small amounts of activity being present in other lipid classes. Within the acylglycerol fraction, the majority of radioactivity was present in diacylglycerols though the amount of this radioactivity varied between preparations and was inversely proportional to the radioactivity present in the monoacylglycerol fraction.

*pH optimum of lipase activity*

A range of substrates at different pH values was prepared by mixing aliquots of concentrated desalted haemolymph with equal volumes of buffer prepared using different ratios of $Na_2HPO_4$ and $NaH_2PO_4$ solutions. The resultant pH values of the different substrate solutions were measured immediately before assay. Lipolytic activity in locust flight muscle was assayed between pH 4.5 and 8.5 and found to have a single pH optimum (Fig. 1) with the peak at 7.5–8.0. Endogenous haemolymph lipolytic activity

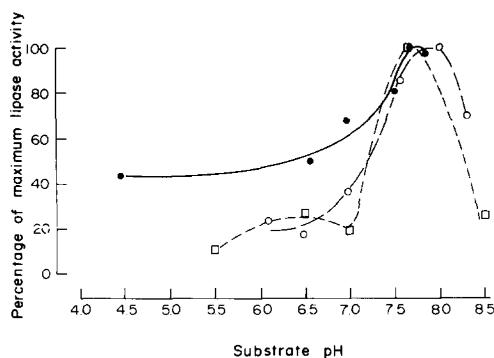


Fig. 1. Shows the pH dependency of flight muscle lipolytic activity when incubated with radiolabelled lipoprotein substrates. For easier comparison, results from three separate assays are plotted as a percentage of the maximum activity recorded in each experiment. Points represent the mean values for five replicate determinations.

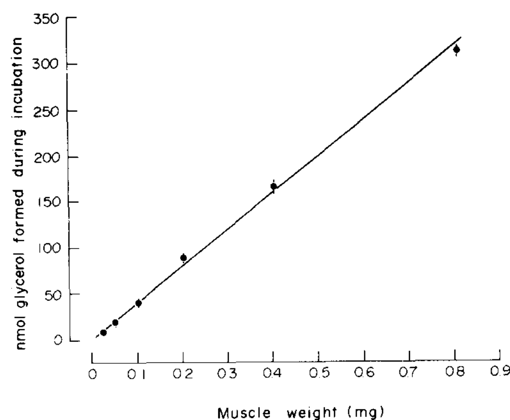


Fig. 3. The relationship between muscle weight and glycerol release from radiolabelled lipoprotein substrates. Points represent the means ± SE for five replicate determinations.

also appeared to be pH dependent with one pH optimum at pH 7.5, and a second between pH 5.5 and 6.5, but this was not investigated further.

*Time course for glycerol production*

The release of radiolabelled glycerol increased linearly with incubation time up to at least 30 min after the start of the assay (Fig. 2), although in some cases the reaction was linear up to 1 hr under the conditions employed. Consequently, most assays were performed using a 30 min incubation period.

*Effect of muscle weight on glycerol production*

Muscle homogenates were diluted serially with homogenization buffer and the resultant range of enzyme concentrations assayed for lipase activity. A linear relationship was found between muscle weight and radiolabelled glycerol production (Fig. 3).

*Effect of substrate concentration on enzyme activity*

Identical aliquots of muscle homogenate were assayed with a range of substrate concentrations prepared by serial dilution with phosphate buffer (pH 7.65) of desalted elevated haemolymph. Maximum lipase activities were recorded at lipid concentrations in excess of about 10 $\mu$g/$\mu$l (Fig. 4).

*Enzyme stability at 35°C*

Aliquots of homogenized muscle (pH 7.65) were pre-incubated in a shaking waterbath (35°C) for 30, 60, 90 and 120 min before addition of substrate and assay for lipolytic activity using a short (15 min) incubation period. Lipolytic activity of muscle samples assayed after pre-incubation did not differ significantly from those which were assayed without prior incubation (Table 1).

*The effect of sodium chloride concentration on lipase activity*

Muscle homogenates were assayed using haemolymph substrates to which increasing concentrations (0–0.8 M) of NaCl had been added. Enzyme activity was reduced in relation to the concentration of NaCl present (Table 2). The degree of inhibition however, varied between experiments; 1 M NaCl causing between 19 and 45% inhibition of activity

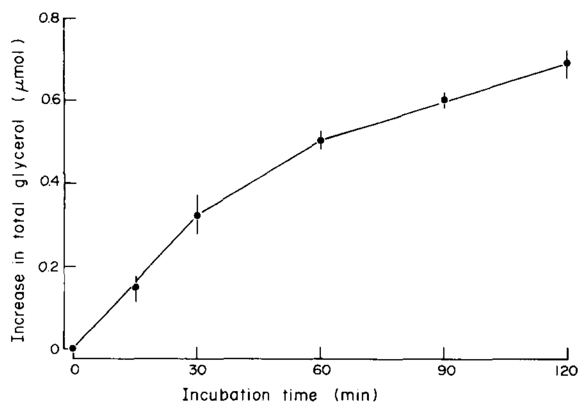


Fig. 2. The time course for glycerol formation during incubation of locust flight muscle homogenates with lipoprotein substrates. Points represent the means ± SE for five replicate determinations.

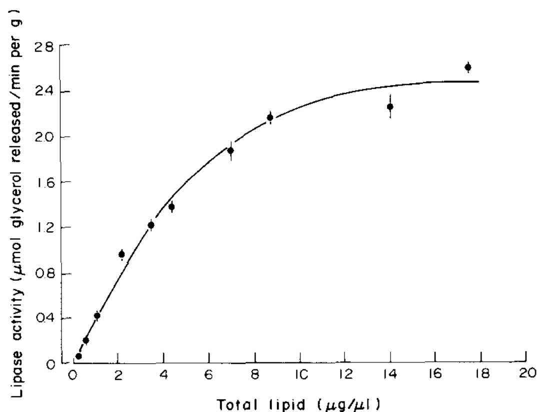


Fig. 4. The relationship between substrate total lipid concentration and total lipolytic activity. Points represent means ± SE for five replicate determinations.

Table 1. Enzyme activities in a homogenate of locust flight muscles after preincubation at 35°C

| Time of pre-incubation (min) | Mean values for total glycerol (nmol) after incubation | Muscle lipase activity (μmol/min per g) |
|---|---|---|
| 0 | 17.10 ± 0.6 | 1.81 ± 0.03 |
| 30 | 17.76 ± 0.9 | 1.94 ± 0.1 |
| 60 | 18.77 ± 0.8 | 2.05 ± 0.08 |
| 90 | 18.39 ± 0.8 | 2.01 ± 0.08 |
| 120 | 17.62 ± 0.7 | 1.92 ± 0.07 |

Muscle homogenates were pre-incubated at 35°C before addition of substrate followed by assay in the normal manner using a 15 min incubation. Each value represents the mean ± standard error for five replicate determinations of enzyme activity. No significant differences in enzyme activity were recorded.

compared with buffer treated controls. In experiments where muscle homogenates were pretreated with 1 M NaCl, followed by addition of haemolymph substrate so that the final NaCl concentration during incubation was only 0.16 M, total activity of NaCl treated groups was still significantly ($P < 0.1$) less than in buffer treated groups (Table 3). Preincubation of muscle in either 1 M NaCl or buffer for an hour at 0°C before assay did not significantly alter the activity of the preparation compared to replicate groups assayed without prior pre-incubation (Table 3).

## DISCUSSION

During long term flight in locusts, energy for the flight muscles is provided largely in the form of diacylglycerols transported as part of lipoprotein $A^+$ (Mwangi and Goldsworthy, 1977, 1978, 1981; Van der Horst *et al.*, 1979). For this reason, haemolymph from locusts injected with adipokinetic hormone which contains large amounts of this lipoprotein, was used as substrate throughout this investigation. Haemolymph was treated with ammonium sulphate before desalting, and substrates therefore consisted of lipoprotein $A^+$ (which carried the majority of lipid), but small quantities of $A_{yellow}$ and other minor lipoproteins (Wheeler and Goldsworthy, 1983a) would also have been present together with any other haemolymph proteins soluble in 50% ammonium sulphate (see Mwangi and Goldsworthy, 1977).

The majority of substrate radioactivity was present in the acylglycerol fraction, and from previous work it is known that over 99% of the activity is present in the glycerol backbone of acylglycerols (Candy *et al.*, 1983; Van der Horst *et al.*, 1983). It was demonstrated that increases in TCA soluble radioactivity during incubations was due mainly to the release of glycerol after the complete hydrolysis of acylglycerols. As the neutral lipids in *L. migratoria* haemolymph consist mainly of long chain (16:18 and 18:18) diacylglycerols (Beenakkers, 1965; Jutsum and Goldsworthy, 1976) then lipolytic activity was due to "true" lipases (see Downer and Steele, 1973; Brockerhoff and Jensen, 1974). Complete hydrolysis of lipoprotein diacylglycerol would require either the presence of both monoacyl- and diacylglycerol lipases, or of a single non-specific enzyme. Since both radiolabelled monoacyl- and diacylglycerols were present in substrate preparations (although diacylglycerols predominate) it is impossible to say anything of the specificity of the enzymes operating in this system, though previous work (Crabtree and Newsholme, 1972; Male and Storey, 1981) has shown the presence in locust flight muscles of enzymes capable of hydrolysing all classes of neutral lipids.

Preliminary experiments showed that the lipoprotein substrate was completely precipitable by 5% TCA, but that any free glycerol produced by complete acylglycerol hydrolysis was TCA-soluble, and hence lipoproteins and free glycerol were easily separable. Due to the use of specific radioactive substrates the assay was extremely sensitive and as such may be applicable to the measurement of (lipoprotein?) lipase activities in a variety of insects or tissues. The free glycerol present in substrate solutions immediately after desalting was negligible, but this increased steadily with time. This is thought to be due either to spontaneous diacylglycerol degradation, or hydrolysis by haemolymph lipases or esterases which have been reported to occur in a variety of insects (Downer and Steele, 1973; Chang, 1977). The observation that the release of glycerol in control incubations was pH-dependent (possibly with two pH optima) would suggest that it is, in part, due to endogenous enzyme activity. Although the production of free glycerol in control incubations was substantial, the total endogenous lipase activity in haemolymph was low in comparison with that in the flight musculature; very small amounts of muscle were used in the enzyme assays relative to the large volumes of haemolymph employed as substrate.

When crude flight muscle homogenates were incubated with lipoprotein substrates, glycerol was always released at faster rates than in controls with heat-"killed" muscle. Glycerol release was proportional to the weight of muscle used and was linear for up to 30 min incubation and hence the enzymes would appear to have been fully saturated under the conditions employed. The non-linear product formation after 30 min incubation did not appear to be due to inhibition of the enzyme by fatty acids as addition of albumin, which normally relieves such inhibition, did not increase glycerol production during a 1 hr incu-

Table 2. Effect of NaCl addition on flight muscle lipase activity

| Added NaCl concentration | Lipase activity* (μmol/min per g) | Percentage activity compared to controls |
|---|---|---|
| 0.0 | 2.08 ± 0.09 | 100.00 |
| 0.1M | 2.02 ± 0.02 | 97.47 |
| 0.2M | 1.89 ± 0.05 | 91.12 |
| 0.4M | 1.88 ± 0.02 | 90.45 |
| 0.8M | 1.72 ± 0.03 | 82.60 |

*Values represent the means ± standard error for 5 replicate determinations of enzyme activity.

Table 3. Effect of pre-incubation of flight muscle homogenates with NaCl on lipase activity

| Muscle treatment | Lipase activity measured before pre-incubation | Lipase activity measured after pre-incubation 1 hr at 0°C |
|---|---|---|
| Saline | 2.90 ± 0.03 | 2.96 ± 0.06 |
| 1 M NaCl | 2.39 ± 0.09 | 2.39 ± 0.05 |

Muscle samples were treated with saline or 1 M NaCl and assayed immediately or after 60 min pre-incubation at 0°C. Assay conditions were such that the final NaCl concentration during incubation was 0.16 M. Values represent the mean ± standard errors for five replicate determinations of enzyme activity and are expressed as μmol glycerol released/min per g tissue.

bation. It is probable that due to the quantities of haemolymph proteins present there was sufficient endogenous fatty acid binding capacity in the medium to overcome such inhibition. During pre-incubation at 0 or 35°C, enzyme activity was very stable and thus enzyme degradation during assay would not appear to be the cause of non-linearity, but it is possible that incubation of the enzyme with the substrate causes its degradation/inactivation, but this was not investigated.

The pH optimum for flight muscle lipolytic activity was between pH 7.5 and 8.0. The physiological significance of this is difficult to assess since the pH in the flight muscles during rest and flight is unknown but optimum flight muscle enzyme activities in this range agree with earlier work on lipases (Crabtree and Newsholme, 1972; Male and Storey, 1981), hexokinase (Storey, 1980a) and aldolase (Storey, 1980b).

In this work, homogenates of locust flight muscles are capable of hydrolysing lipoprotein-bound acylglycerols and the enzymes responsible may therefore be termed lipoprotein lipases. Vertebrate lipoprotein lipase is completely deactivated by 1 M NaCl (Korn, 1955; Fielding, 1968) and this is used as one diagnostic feature of the enzyme (Nilsson-Ehle *et al.*, 1980). Inhibition of lipolytic activity by NaCl has also been used to infer the presence of lipoprotein lipases (Crabtree and Newsholme, 1972; Chang, 1977) in insect tissues, but in the present study total flight muscle lipolytic activity was not fully destroyed by 1 M NaCl and some of the decreased in activity was apparently reversible (C. H. Wheeler, unpublished observations). Though the activities studied in this report may be due to more than one enzyme (one or more of which could be more sensitive to inhibition by NaCl), there is clearly some enzyme activity capable of lipoprotein lipid hydrolysis in the presence of 1 M NaCl and therefore the use of criteria such as inhibition by NaCl for the identification of lipoprotein lipases in insects may be premature until further characterization of these enzymes is achieved. Some preliminary observations suggest a further dissimilarity between the vertebrate lipoprotein lipase and the lipase enzyme described here, since heparin injections into locusts did not cause the release of tissue enzyme activity into the haemolymph (D. J. Van der Horst and J. M. Van Doorn, personal communication).

Lipase activities varied considerably between experiments. This variation may be due to the problems associated with using natural lipoprotein substrates. Though haemolymph donor animals of the same age were injected with a standard dose of synthetic adipokinetic hormone and bled at the peak of lipid mobilization it is likely that there were some variations in the quality of the haemolymph obtained. Lipid loading of specific lipoprotein particles may be important in their binding of apoproteins (Wheeler and Goldsworthy, 1983a) and this may depend on the physiological state of the animals involved.

Though there were between-assay differences, in most experiments the lipase activity was between 2 and 4 $\mu$mol/min per g (mean $\pm$ SE for 16 assays, $2.5 \pm 0.3$ $\mu$mol/min per g). The absolute values must, however, be treated with some caution as they probably depend on the quality of the substrate preparations; for example the presence of lipids as part of minor lipoproteins (e.g. residual $A_{yellow}$) which may not act as true substrates, would make the values high. A more accurate determination of activity will be achieved by use of purified lipoprotein preparations. Interestingly, our values are generally higher than those previously recorded for locust flight muscle (Crabtree and Newsholme, 1972; Male and Storey, 1981) using lipid emulsions. On the basis that a locust containing an average 180 mg of flight muscle uses 4.1 mg of fatty acids per hour during long term flight (Beenakkers, 1965), it can be estimated that a minimum hydrolysis of 0.69 $\mu$mol diacylglycerol/min per g (i.e. a total diacylglycerol lipase activity of 0.69 $\mu$mol glycerol released/min per g) would be required to provide sufficient energy to the flight muscles. The lipase activities determined in the present work are well in excess of this figure, and appear to confirm the observation by Gilbert and Chino (1974) that higher lipase activities may be recorded when using natural substrates rather than lipid emusions. It is possible that the activities recorded may be due to a number of different enzymes, but the presence of a highly active enzyme, which could rapidly unload lipoprotein diacylglycerol, may be an adaptation to overcome problems associated with energy supply in an open circulation (Crabtree and Newsholme, 1975).

Flight muscle lipolytic activity was fully activated at total lipid concentrations in excess of approx. 10 $\mu$g/$\mu$l which in mature locusts would be achieved within 15–30 min of the onset of flight (Jutsum and Goldsworthy, 1976). Hence during the flight period (after 30 min) when lipids are the major fuel used, lipase activity would be maximal.

The finding that muscle preparations can hydrolyse lipids associated with lipoproteins at higher rates than in studies using lipid emulsions, suggests that components of the lipoprotein complex may aid lipolysis, either directly by increasing the binding of lipase to the substrate, or by an allosteric action on the enzyme. Such a situation would be analagous to that of the action of lipoprotein lipase in vertebrates (Bengtsson and Olivecrona, 1982). The possible role of locust lipoprotein apoproteins in directing such events is at present under investigation.

*Acknowledgements*—The authors wish to thank J. M. Van Doorn for his technical assistance and Dr G. J. Goldsworthy for his critical reading of the manuscript. C.H.W. acknowledges the support of SERC/NATO for the provision of an Overseas Fellowship, and of the Faculty of Biology, University of Utrecht for research facilities.

## REFERENCES

Beenakkers A. M. Th. (1965) Transport of fatty acids in *Locusta migratoria* during sustained flight. A comparative study. *J. Insect Physiol.* **11,** 879.

Beenakkers A. M. Th. (1969) The influence of corpus allatum and corpus cardiacum on lipid transport in *Locusta migratoria. Gen. comp. Endocr.* **13,** Abstract 12.

Bengtsson G. and Olivecrona T. (1982) Activation of lipoprotein lipase by apoliprotein CII. Demonstration of an effect of the activator on the binding of tht enzyme to milk fat globules. *FEBS Lett.* **147.** 183–187.

Bligh E. G. and Dyer W. J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37,** 911–917.

Brockerhoff H. and Jensen R. G. (1974) *Lipolytic Enzymes*, Academic Press, New York.

Candy D. J., Hall L. J. and Spencer I. M. (1976) The metabolism of glycerol in the locust *Schistocerca gregaria* during flight. *J. Insect Physiol.* **22,** 583–587.

Chang F. (1977) The presence of lipoprotein lipase activity and its relationship to lipid transport in the oleander hawkmoth, *Deilephila nerii. Comp. Biochem. Physiol.* **57B,** 209–214.

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* (Edited by Usherwood P. N. R.), pp. 405–500. Academic Press, New York.

Downer R. G. H. and Steele J. E. (1973) Haemolymph lipase activity in the American cockroach, *Periplaneta americana. J. Insect Physiol.* **19,** 523–532.

Fielding C. J. (1986) Inactivation of lipoprotein lipase in buffered saline solutions. *Biochim. biophys. Acta* **159,** 94–102.

Freeman C. P. and West D. (1966) Complete separation of lipid classes on a single thin-layer plate. *J. Lipid Res.* **7,** 324–327.

Gilbert H. I. and Chino H. (1974) Transport of lipids in insects. *J. Lipid Res.* **15,** 439–456.

Holwerda D. A., Van Doorn J. and Beenakkers A. M. Th. (1977) Characterization of the adipokinetic hormone and hyperglycaemic substances from the locust corpus cardiacum. *Insect Biochem.* **7,** 151–157.

Jutsum A. R. and Goldsworthy G. J. (1976) Fuels for flight in *Locusta. J. Insect Physiol.* **22,** 243–249.

Korn E. D. (1955) Clearing factor, a heparin-activated lipoprotein lipase. *J. biol. Chem.* **215,** 1–14.

Krogh A. and Weis-Fogh T. (1951) The respiratory exchange of the desert locust, *Schistocerca gregaria*, before, during and after flight. *J. exp. Biol.* **28,** 344–357.

Male K. B. and Storey K. B. (1981) Enzyme activities and isozyme composition of triglyceride, diglyceride and monoglyceride lipases in *Periplaneta americana*, *Locusta migratoria* and *Polia adjuncta. Insect Biochem.* **11,** 423–427.

Mayer R. J. and Candy D. J. (1967) Changes in haemolymph lipoproteins during locust flight. *Nature, Lond.* **215,** 987.

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.

Mwangi R. W. and Goldsworthy G. J. (1977) Diglyceride-transporting lipoproteins in *Locusta. J. comp. Physiol.* **14,** 177–190.

Mwangi R. W. and Goldsworthy G. J. (1978) Studies on diglyceride-transporting lipoproteins in *Locusta*. In *Comparative Endocrinology* (Edited by Gaillard P. J. and Boer H. H.), pp. 459–463. Elsevier, Amsterdam.

Mwangi R. W. and Goldsworthy G. J. (1981) Diacylglycerol-transporting lipoproteins and flight in *Locusta. J. Insect Physiol.* **27,** 47–50.

Nilsson-Ehle P., Garfinkel A. S. and Schotz M. C. (1980) Lipolytic enzymes and plasma lipoprotein metabolism. *A. Rev. Biochem.* **49,** 667–693.

Robinson N. L. and Goldsworthy G. J. (1976) Adipokinetic hormones and flight metabolism in the locust. *J. Insect. Physiol.* **22,** 1559–1564.

Robinson N. L. and Goldsworthy G. J. (1977) Adipokinetic hormone and the regulation of carbohydrate and lipid metabolism in a working flight muscle preparation. *J. Insect Physiol.* **23,** 9–16.

Schotz M. C. and Garfinkel A. S. (1972) A simple lipase assay using trichloroacetic acid. *J. Lipid Res.* **13,** 824–826.

Spencer I. M. and Candy D. J. (1974) The effect of flight on the concentrations and composition of haemolymph diacyl glycerols in the desert locust. *Biochem. Soc. Trans.* **2,** 1093–1096.

Storey K. B. (1980a) Regulatory properties of hexokinase from flight muscle of *Schistocerca americana gregaria*. Role of the enzyme in control of glycolysis during the rest-to-flight transition. *Insect Biochem.* **10,** 637–645.

Storey K. B. (1980b) Kinetic properties of purified aldolase from flight muscle of *Schistocerca americana gregaria*. Role of the enzyme in the transition from carbohydrate to lipid-fuelled flight. *Insect Biochem.* **10,** 647–655.

Van der Horst D. J., Baljet A. M. C., Beenakkers A. M. Th. and Van Handel E. (1978) Turnover of locust haemolymph diglycerides during flight and rest. *Insect Biochem.* **8,** 369–373.

Van der Horst D. J., Van Doorn J. M. and Beenakkers A. M. Th. (1979) Effects of the adipokinetic hormone on the release and turnover of haemolymph diglycerides and on the formation of the diglyceride-transporting lipoprotein system during locust flight. *Insect Biochem.* **9,** 627–635.

Van der Horst D. J., Abbink J. H. M., Van Doorn J. M., Van Marrewijk W. J. A. and Beenakkers A. M. Th. (1983) Glycerol dynamics and metabolism during flight of the locust, *Locusta migratoria. Insect Biochem.* **13,** 45–55.

Weis-Fogh T. (1952) Fat combustion and metabolic rate of flying locusts. *Phil. Trans. R. Soc. Lond. Ser. B* 237.

Wheeler C. H. and Goldsworthy G. J. (1983a) Protein-lipoprotein interactions in the haemolymph of *Locusta* during the action of adipokinetic hormone: The role of $C_L$-proteins. *J. Insect Physiol.* **29,** 349–354.

Wheeler C. H. and Goldsworthy G. J. (1983b) Qualitative and quantitative changes in *Locusta* haemolymph proteins and lipoproteins during ageing and adipokinetic hormone action. *J. Insect Physiol.* **29,** 339–347.