CHAPTER — III

LIPOLYTIC ACTIVITY IN THE FLIGHT MUSCLES AND IN THE FAT BODY OF THE BEETLE CYBISTER CONFUSUS AS WELL AS IN THE FLIGHT MUSCLES OF THE DRAGONFLY PANTALA FLAVESCENS AND ITS POSSIBLE SIGNIFICANCE IN SUSTAINED FLIGHT
The fact that in some insects during sustained flight their reserves of fat get reduced and the RQ values at this time are less than unity, indicates that fat might be serving as a metabolic fuel for muscular contractions (Williams, 1945; Beall, 1948; Krogh and Weis-Fogh, 1951; Weis-Fogh, 1952; Zebe, 1954; Cockbain, 1961 and Weis-Fogh, 1965). There is no evidence that neutral fat as such could be directly oxidized. Therefore, lipase action producing fatty acids as the first step leading to their oxidation should be considered as essential and a normal process in vivo. The level of lipase activity in a muscle is related to the extent of its fat utilization and its capacity for sustained action (George and Berger, 1966).

Quantitative assay of lipase by manometry with tributyrin as substrate in the skeletal muscles of birds and insects as well as in the fat body of some insects has been made by George and his associates (George et al., 1958; George and Eapen, 1959; George and Bhakthan, 1960, 1961 and 1963). George and Eapen (1959) have shown that the lipolytic activity in the fat body of Schistocerca gregaria was comparatively more than that in the pigeon adipose tissue as well as in its thoracic muscles. Lipase activity
of the wing-bearing segment muscles of the beetle *Heliocorpus bucephalus* was more than in the prothoracic muscles of the same insect (George and Bhakthan, 1960). High concentrations of lipase activity in flight muscles of some butterflies and moths which indulge in sustained activity was obtained by the same authors (1960a and b).

As early as 1948 Fodor demonstrated that the flight muscles preparations of *Schistocerca gregaria* were unable to hydrolyze olive oil, but did cleave monobutyrin and methyl butyrate. Similarly Baker and Paretsky (1958) have reported a lipase system or systems in the housefly *Musca domestica* which hydrolyzed triacetin, tributylin, trivalerin and tricaprylin. Gilbert et al., (1965) using radioassay were the first to demonstrate that the flight muscles as well as the fat body of the silkworm *Hyalophora cecropia* were capable of hydrolyzing diolein and triolien. They showed that the flight muscles were about five times as much active as the fat body in this respect.

However, Gilbert (1967) expressed the opinion that short chain fatty acids such as tributylin are hydrolyzed by an esterase and not by a lipase, since the latter has preference for long chain fatty acids in the acyl groups of glycerol esters. Okuda and Fujii (1968) on the other
hand have shown that, the purified rat liver lipase acts on short chain neutral fats more readily than on long chain neutral fats.

The dragonfly *Pantala flavescens* is capable of prolonged and sustained flight; so also the beetle *Cybister confusus* but occasionally only. That the flight muscles of these two insects utilize fat during their sustained flight was made quite evident in our previous (Chapter II) experiments. The significance of lipase activity in the flight muscles of these two insects could therefore be anticipated. The fat store in the *Cybister* beetle is distributed between the fat body, blood and flight muscles, unlike in the *Pantala* the fat store of which is confined to blood and flight muscles, as it has no fat body in the haemocoel. The present opportunity is availed of to test the following premises:

i. Lipolytic activity in the flight muscles of both *Pantala flavescens* and *Cybister confusus* as well as in the fat body of the latter insect.

ii. The nature of the flight muscle lipase action to find out if free fatty acids were released from the tributyrin - a short chain lipid more readily than from triolein - a long chain neutral fat.
iii. The nature of the lipolytic action to find out if mono-, di- and triglycerides were hydrolyzed equally well by the muscle lipase as well as fat body lipase.

iv. The stimulating effects of calcium and magnesium ions on the hydrolysis of glycerides.

MATERIALS AND METHODS

The males of the dragonfly Pantala flavescens used in the present investigation were selected from a number of them caught in the field during their flight. The males of Cybister confusus used were selected from a number of them kept in the laboratory aquarium. The fresh weight of the beetle and the dragonfly fell within a range of 3.53 to 3.58 gm. and 418 to 430 mg. respectively.

Enzyme Preparation:

The insects were killed by decapitation. The flight muscles were extirpated under cold insect ringer solution. The fat body of the Cybister beetle was collected mostly from the abdominal portion of the insect. The tissues thus collected from one to ten insects were then homogenized in cold saline water or tris buffer in Potter and Elevehjem glass homogenizer for 10 min. at 0°C to 4°C. These preparations were used almost immediately as lipase source.
Enzyme Assay:

Two methods have been followed to determine the lipase activity in the different tissue preparations, i) Titrimetric (ii) Radioassay.

Titrimetric Method:

The method of Fiore and Nord (1949) using glycerol tributyrate and purified triolein emulsified in polyvinyl alcohol was followed to estimate the rate of hydrolysis by the flight muscle preparations of the Pantala flavescens and of the Cybister confusus as well as of the fat body of the latter insect. A known quantity of flight muscles of each insect as well as fat body from Cybister beetle were homogenized in 70% glycerol in Potter and Elvehjem homogenizer at 0°C to 4°C. The incubation mixture consisted of 1 ml. emulsion; 1.0 ml. citrate buffer of pH 6.0 (0.1M) and 1 ml. enzyme source. The control preparations were run with each experiment by adding 2 ml. alcohol : acetone mixture (1:1) to the incubation mixture before the enzyme source was added to it. The mixtures were incubated at 37°C for 1 to 4 hr. The reaction in the experimental flasks was terminated by adding 2 ml. of alcohol-acetone mixture (1:1). The liberated acid moiety was titrated using microburett against aqueous 0.05 NaOH with phenolphthalein
as an indicator. The enzyme units were expressed as the amount of NaOH (0.05 N) required to neutralize the fatty acid produced in the reaction under the above-mentioned conditions.

Radioassay:

The method followed for radioassay of enzyme activity was essentially similar to that described by Gilbert et al., (1965) with little modification. In this procedure the radioactive substrates were prepared as follows:

Preparation of Radioactive Substrates:

\[ 1-C^{14} \text{ Triglyceride:} \]

For radioassay of enzyme activity, triglyceride uniformly labelled in the carboxyl position with C\textsuperscript{14} was obtained by germinating Soybeans in a medium containing 1-C\textsuperscript{14} sodium acetate (Subbaih, 1972). The procedure followed was that about 50 gm. of Soybeans were allowed to soak in running tap water for about two hours. The beans were then transferred to a beaker containing 10 milicuries of 1-C\textsuperscript{14} sodium acetate dissolved in 10 ml. of boiled water. They were left at room temperature for about 8 hr. with occasionally stirring. The acetate solution was
completely absorbed by the seeds during this time. They were then spread over a moistened thick blotting paper and allowed to germinate in the dark at 30°C for 48 hr. The seedlings were then homogenized in a Waring blender with 400 ml. of methanol and the lipids were extracted according to the method of Bligh and Dyer (1959). The lipid extract was concentrated and loaded on to an alumina column prepared in distilled chloroform. The neutral lipid fraction was eluted with one litre of chloroform. After concentrating the elutes the neutral lipid fraction which consists of triglyceride was further purified by subjecting it to an alumina column and eluted with 2% acetone in petrol. The purity of the triglyceride fraction was checked on silicagel thin layer chromatogram plate (tlc) with a standard triglyceride with a solvent system of n hexane : solvent ether : acetic acid (90:18:1.5 v/v/v).

1-C\textsuperscript{14} Diglyceride:

The 1-C\textsuperscript{14} diglyceride was prepared by hydrolysing C\textsuperscript{14} lecithin with phospholipase C. The incubation mixture consisted of C\textsuperscript{14} labelled lecithin (equivalent to 30,0000 cpm/min.) diluted with carrier lecithin, 2.0 ml. of tris maleate buffer; (pH 7.4) 2 mg. phospholipase C (Sigma) prepared in 5 ml. of 1% egg albumin and 1 ml. of 0.5 M CaCl\textsubscript{2}. 
The incubation was carried at 37°C for 60 min. with a constant shaking. The reaction was terminated by adding 25 ml. methanol and the lipid was extracted according to the method of Bligh and Dyer (1959). The extracted lipid was analysed on preparative silicagel tlc plate, along with the standard diglyceride with the solvent system of n-hexane : solvent ether : acetic acid (90:18:1.5 v/v/v). After exposing the plate in an iodine chamber, the diglyceride band formed was scraped out from the plate and transferred to a small chromatographic column and eluted with chloroform. The chloroform was evaporated off under reduced pressure; the diglyceride was then redissolved in a known vol. of chloroform and stored in a volumetric flask in a refrigerator until used.

1-\textsuperscript{14}C \textsuperscript{1-14} Monoglyceride:

1-\textsuperscript{14}C \textsuperscript{1-14} monoglyceride was obtained by hydrolyzing purified 1-\textsuperscript{14}C \textsuperscript{1-14} triglyceride with an acetone-dried powder of goat pancreas. The reaction mixture consisted of; 3.0 ml. 1M tris HCl buffer (pH 8); 0.2 ml. of (w/v) CaCl\textsubscript{2}; 0.5 ml. 0.1% (w/v) sodium taurocholate; 1-\textsuperscript{14}C \textsuperscript{1-14} triglyceride (20,0000 cpm/min.); and 25 mg. of pancrease powder. After 3 hr. of incubation at 40°C with a constant shaking, the reaction was terminated by adding 10 ml. methanol. The
lipid fraction was extracted according to the method of Bligh and Dyer (1959) and the extracted lipid was analysed on preparative silicagel tlc plate. The rest of the procedure was as described in the preceding section.

**Substrate Preparation:**

The substrates obtained as a result of the procedure mentioned above were emulsified in gum acacia sol. (1 gm./4 ml. distilled water). Purified tri-, di-, and monolein were used as carrier substrates. For the radioassay of enzyme activity, C\textsuperscript{14} tri-, di- and monoglycerides were emulsified with the carrier substrates by sonication for 2 min.

For the enzyme assay the standard reaction mixture contained in a final vol. of 2 ml., 0.5 milimoles of tris maleate buffer (pH 7.6 or 8.0), 0.5 ml. enzyme source and 1 ml. emulsified substrate containing 25 to 30 micromoles of tri-, di-, and monoglycerides equivalent to 30,000 counts/min. in each case. The incubation was carried out at 37°C for 1 to 9 hr. Control preparations were run with each experiment by adding appropriate vol. of methanol to the incubation mixture before the addition of homogenate and incubated along with the samples. The reaction in the experimental tubes was terminated by the addition of 2 ml.
of methanol, and the lipid portion was extracted following the method of Bligh and Dyer (1959). The reaction tubes were then centrifuged for 10 min. at 3,500 rpm. A known vol. of chloroform layer containing lipid was removed under reduced pressure from each reaction tube, and the lipid was redissolved in known vol. of chloroform. The free fatty acid (FFA) content of the lipid sample was separated on silicagel tlc plate using the solvent system of n-hexane : solvent ether : acetic acid (90:18:1.5 v/v/v). With this solvent system the monoglycerides (MG) and phospholipids remained at the origin, while 1, 2 and 1, 3 diglyceride (1, 2 and 1, 3 DG), FFA and triglycerides (TG) were resolved into distinct bands. The individual glycerides and FFA were identified with their respective standards (generously supplied by Dr.F.H.Mattson) after exposing the plate to iodine vapour. The FFA spot was scraped out from the plate and transferred to a counting vial containing liquid scintillation solution (12 mg. PPO/5 ml. toluene). The radioactivity in the FFA was measured on a Scintillation Counter (Beckman).

**pH Optima:**

The effect of pH on the hydrolysis of different substrates was estimated by adjusting the reaction mixture to the various pH levels prior to the addition of enzyme source.
Time Course Hydrolysis:

The rate of hydrolysis of different substrates against incubation time was determined by incubating the standard reaction mixture at 37°C with a constant shaking. The tubes were removed at desired intervals of time for FFA analysis.

Effect of Ca and Mg on the Hydrolysis of di- and triglycerides:

This was tested by adding 5 to 10 μ.moles of Ca or Mg to the standard reaction mixture.

Determination of Dry Weight of Tissue:

The dry weight of the different tissues was determined by drying 1 ml. duplicate samples of the homogenate for 24 hrs. at 100°C and kept in vacuum desicator till constant weight was obtained.

Enzyme Units:

Except in radioactivity there is no differences between the unlabeled and the labeled substrates, since both were prepared in the same way. When this condition is met, and the amount of the substrate (μ.moles) as well as the radioactivity of the sample and FFA fraction are known the enzyme activity is calculated as usual following the formula given below.
RESULTS

The results of the titrimetric determination on the hydrolysis of tributyrin and triolein by the flight muscle preparations of *Pantala flavescens* and of *Cybister confusus* as well as by the fat body of the latter insect are summarized in the table V. Both the muscle preparations hydrolyzed tributyrin to a greater extent than triolein. The flight muscles of *Pantala* hydrolyzed tributyrin better (12.8 enzyme units) than the flight muscles of *Cybister* (9.0 enzyme units). The triolein hydrolysis on the other hand was almost similar in both the muscles preparations (3.90 and 3.95 enzyme units in *Pantala* and *Cybister* respectively) (table V). The fat body of the *Cybister* exhibited the greatest hydrolysis reaction with tributyrin (18.0 enzyme units) as well as with triolein (5.80 enzyme units) compared to the flight muscles of the same insect and of *Pantala*.

Table VI and VII indicate the results obtained by radioassay method on the hydrolysis of C\(^{14}\) TG, DG, and MG as substrates by the muscle preparations of the two insects studied as well as of the fat body of the *Cybister* beetle.
EFFECT OF PH ON THE HYDROLYSIS OF DI- AND TRIGLYCERIDES

A. BY FAT BODY LIPASE OF THE CYBISTI:

B. BY FLIGHT MUSCLE LIPASE OF THE CYBISTI.
Fig. IV.

HYDROLYSIS OF TRI-, DI- AND MONOGLYERIDE BY FLIGHT MUSCLE LIPASE OF THE DRAGONFLY PANTALA FLAVESCENS AT DIFFERENT pH
Fig. V HYDROLYSIS OF MONO-, DI- AND TRIGLYCERIDES BY THE FLIGHT MUSCLE LIPASE OF THE CYBISTER BEETLE IN RELATION TO INCUBATION TIME

\[ \text{Moles of fatty acid released per 100 mg dry tissue} \]

\[ \text{Time in hours} \]

- **MONOGLYCERIDE**
- **DIGLYCERIDE**
- **TRIGLYCERIDE**
HYDROLYSIS OF TRI-, DI- AND MONOGLYCERIDE
FLIGHT MUSCLE LIPASE OF THE DRAGONFLY
PANTALA FLAVESCENS

HOURS

μ MOLES OF FATTY ACIDS LIBERATED / 100 MG. DRY TISSUE

TIMING

TRIGLYCERIDE
MONOGLYCERIDES
DIGLYCERIDE
Fig. VII

Hydrolysis of MG-DG-and triglycerides by the fat body lipase in relation to incubation time.
Table VI. Hydrolysis of $^{14}$C Tri-, Di- and Monoglycerides by the flight muscle preparation of the dragonfly *Pantala flavescens*

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Experimental</th>
<th>Control</th>
<th>Net release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoglyceride (5)</td>
<td>6.25 ± 3.25</td>
<td>2.19 ± 1.23</td>
<td>4.86</td>
</tr>
<tr>
<td>Diglyceride (5)</td>
<td>10.50 ± 1.1</td>
<td>1.50 ± 1.11</td>
<td>9.00</td>
</tr>
<tr>
<td>Triglyceride (5)</td>
<td>4.95 ± 1.4</td>
<td>2.95 ± 1.36</td>
<td>2.00</td>
</tr>
</tbody>
</table>

The number of insects used in each determination is indicated in the parentheses. The results are the mean ± standard error.

* The results are the mean ± standard error.
Table VII. Hydrolysis of $^{14}$C Tri-, Di- and Monoglycerides by the flight muscle as well as by fat body of the beetle Cybister confusus

<table>
<thead>
<tr>
<th>Substrates</th>
<th>u.moles of fatty acids liberated/100 mg. dry tissue/hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FLIGHT MUSCLE</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
</tr>
<tr>
<td>Monoglyceride (3)</td>
<td>5.88 $\pm$ 3.22</td>
</tr>
<tr>
<td>Diglyceride (3)</td>
<td>5.46 $\pm$ 3.5</td>
</tr>
<tr>
<td>Triglyceride (3)</td>
<td>8.86 $\pm$ 2.1</td>
</tr>
</tbody>
</table>

The number of insects used in each case is presented in the parenthesis.

* The results are the mean $\pm$ standard error.
The lipase activity with DG as substrate by the flight muscles of Pantala was about four and half times (9.0 µ.moles) that with TG (2.0 µ.moles). In the Cybister beetle on the other hand the differences between DG (4.84 µ.moles) and TG (5.94 µ.moles) hydrolysis by flight muscle lipase was insignificant. In the fat body of the latter insect the hydrolysis of the TG was almost double (22.57 µ.moles) that of the DG (9.93 µ.moles) by its lipase (table VII). The fat body however, exhibited the greatest lipolytic activity with all the substrates tested so far.

Fig. (III) demonstrates the effect of different pH on the hydrolysis of TG and DG by the fat body and flight muscle preparations of Cybister beetle. The pH optima for hydrolysis of TG and DG of the fat body was 8.0, while the flight muscle preparation exhibited pH optima of 8.0 and 7.5 for TG and DG hydrolysis respectively. Fig. (IV) demonstrates the pH optima obtained in respect of TG, DG and MG hydrolysis by the flight muscles of the dragonfly Pantala. The MG and DG showed the pH optima of 7.5, for hydrolysis, while TG hydrolysis was most active at pH 8.0.

Time Course of Hydrolysis:

In the case of Cybister beetle the hydrolysis of TG, DG and MG was almost zero order for the first one hour, (Fig.V).
Table VIII. Effect of Ca and Mg on the hydrolysis of Di- and Triglycerides by the flight muscles of *Pantala flavescens* and *Cybister confusus* as well as fat body of the latter insect.

<table>
<thead>
<tr>
<th>Substrates</th>
<th>μ.moles of fatty acids liberated/100 mg. dry tissue/hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flight muscle (beetle)</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
</tr>
<tr>
<td>Diglyceride (3)</td>
<td>8.7 ± 0.9</td>
</tr>
<tr>
<td>Triglyceride (3)</td>
<td>4.95 ± 1.4</td>
</tr>
</tbody>
</table>

The number of insects used in each case is presented in the parenthesis.

* The results are the mean ± standard error.
while the *Pantala* the hydrolysis occurred almost after 40 min. (Fig. V). A fairly linear rate of hydrolysis for all the three substrates tested for, was observed in the flight muscle preparations of both the insects (Fig. V and VI). In the fat body of *Cybister* beetle on the other hand the rate of hydrolysis of MG and DG was linear even after 8 hr. of incubation, whereas TG hydrolysis was linear for about 6 hr. and after which the hydrolysis rate gradually decreased. This decrease in the TG hydrolysis may perhaps be due to the saturation of the medium by the FFA released by the fat body.

**Effect of Ca++ and Mg++ ions on the Lipase Action:**

As can be seen from the table (VIII) that calcium as well as magnesium ions have no stimulatory effect on the muscle as well as fat body lipases.

**DISCUSSION**

Quantitative assay of lipase by manometry with tributyrin as substrate in some insects has been made by George and his associates (George *et al.*, 1958; George and Eapen, 1959; George and Bhakthan, 1960; 1961; and 1963). George and Bhakthan (1960a, b, 1961 and 1963) have obtained a higher lipolytic activity in those insects indulging in sustained
flight. Considerably greater activity of lipase was obtained in the flight muscles of the dragonfly Pantala than in the muscles of the locust Locusta migratoria (George et al., 1958). It has been demonstrated in the previous study (Chapter II) that the flight muscles of the Cybister beetle as well as of the Pantala use their lipid reserves during their sustained flight. It could be expected then that the flight muscles of these two insects should possess lipase. It can be seen from the table (V) that the hydrolysis of the tributyrih by the flight muscles of Pantala was comparatively more than what takes place in the flight muscles of Cybister.

Gilbert (1967) is of the opinion that the reports of the earlier workers on lipase activity using tributyrin as substrate may not be one to a true lipase but an esterase, since the former enzyme has a preference for long chain in the acyl groups of glycerol esters (viz, triolein) and latter one attacks short chain groups namely tributyrin. By using radioassay method Gilbert et al., (1965) were the first to demonstrate a true lipase acting on long chain triolein in the flight muscles and fat body of Cecropia and Periplaneta americana. In the present investigation it has been possible to show by titrimetric method that the flight muscle preparations of Pantala as well as of Cybister
could hydrolyze triolein although the rate of hydrolysis was very much slow. The radioassay method was also revealed that the flight muscle preparations of both insects could hydrolyze C\textsuperscript{14} TG-, DG- and MG. In Pantala the hydrolysis of all the three substrates occurred almost after 40 min. of incubation, whereas it was almost zero order for first one hr. in the case of Cybister, (Fig. IV and VII). It is also evident from the table (VI) that the flight muscle lipase of Pantala was more active towards MG (4.86 μ.moles) and DG (9.0 μ.moles) than towards TG (2.0 μ.moles). It is known that the dragonfly Pantala is a sustained flier and depends more on fat for flight energy. The aquatic Cybister beetle takes to flight occasionally. The significance of greater lipolytic activity in the flight muscles of the dragonfly is understandable.

The fat body of the Cybister confusus exhibited a greatest hydrolytic activity with tributyrin as well as with C\textsuperscript{14} TG (22.57 μ.moles), DG (9.93 μ.moles) and MG (16.7 μ.moles) compared to flight muscles of the same insect and of Pantala (table VI and VII). In this context the observations of George and Eapen (1959) that in Schistocerca gregaria the hydrolysis of tributyrin by the fat body was twice than that of pigeon adipose tissue and many times more than the flight muscles of the locust is of some significance during
the migratory flight of the locust. Stevenson (1972) has demonstrated that the flight muscles of *Prodenia* contains a very active lipase which hydrolyzed MG at a faster rate, while its ability to hydrolyze DG and TG is very low. The fat body on the other hand contains a lipase able to hydrolyze TG, DG and MG. The author has suggested that TG of the fat body is completely hydrolyzed by its active lipase, and resulting FFA is carried to the flight muscles to provide energy for flight. Similarly Beenakkers (1965) obtained a considerably increase in the fatty acid content in the haemolymph during flight of the locust indicating efficient transport of the acids from the fat body to the muscles. A similar phenomenon could be expected in the *Cybister* beetle since the lipase activity in the fat body of this beetle is about four times more with TG compared to that found in the flight muscles of the same insect. In *Pantala* on the other hand there is no distinct fat body in the haemocoel. It is therefore assumed that its fat reserves are distributed between the muscles and the blood.

The distinction drawn by Gilbert *et al.*, (1965) and others (Okuda and Fugii, 1968) between lipase which hydrolyzes glycerides of long chain lipids and esterase that hydrolyzes short chain lipids is certainly not clear. According to Gilbert *et al.*, (1965) "the use of substrates such as
tributyrin, triacetin has little physiological significance since, these substances are not usually present in the animal tissue, although the rate of hydrolysis of these esters may be an indicator of general metabolic activity." In this context the findings of Van Aspren (1959) who demonstrated the presence of potent aliesterase activity in various body regions of Musca domestica and found 22% of esterase activity responsible for the hydrolysis of tributyrin to be localized in the head region of the insect was of considerable interest. On the other hand Okuda and Fugii (1967) have demonstrated that the rat liver lipase could easily be converted into an esterase by the acetone treatment or by pancreatic lipase. The same authors (1968) have further shown that the short chain lipids were more readily hydrolyzed than those of long chain fatty acids by the purified rat liver lipase. The authors have suggested a single enzyme that catalyzes the hydrolysis of both short and long chain lipids. In mammalian adipose tissue Schnatz and Williams (1962) have reported different enzymes that are responsible for the hydrolysis of long and short chain fatty acid glycerides, one of which has been named as tributyrinase and has a specificity for short chain glycerol ester such as mono-, di- and tributyrin. It appears therefore that in the flight muscles and in the fat body of the two insects mentioned hitherto, there appear to
be different enzymes responsible to catalyze the hydrolysis of the short chain fatty acid-tributyrin and the long chain fatty acid triolein. Tributyrinase and lipase respectively are the enzymes mediating the hydrolysis of these two substrates. However, further research is required to understand whether tributyrin is naturally occurring substrate in the muscles as well as in the fat body of the insects studied under the present investigation.

The importance of lipase in the fat utilization by the flight muscles of these two insects is evident. The dragonfly *Pantala flavescens* which remains air-borne for many hours must depend exclusively on the lipid metabolism. The high concentration of tributyrinase and lipase in the flight muscles of this insect is expected. The *Cybister confusus* on the other hand takes to flight occasionally only, yet the flight muscles as well as its fat body contain considerable amount of tributyrinase and lipase. This may perhaps be due to the fact that the beetle is a heavier insect and requires to spend more amount of energy during its short flight.

**SUMMARY**

1. Lipolytic activity in the flight muscles of *Pantala flavescens* and of the *Cybister confusus* as well as
the fat body of the latter insect was tested both titrimetrically and by radioassay. All the three tissues showed positive lipolytic activity.

ii. Titrimetric assay revealed that free fatty acids are more readily released from tributyrin than from triolein by hydrolysis with all these tissue preparations.

iii. Radioassay technique has revealed that the flight muscle lipase of Cybister confusus is capable of hydrolyzing mono-, di- and triglycerides equally well. On the other hand the flight muscle lipase of Pantala flavescens showed most lipolytic activity on di-, little less on mono- and least on triglycerides. The enzyme activity in the fat body of the former insect was most on tri-, less on mono- and least on diglycerides.

iv. The effect of calcium and magnesium ions on the lipolytic activity showed that both these ions have no stimulatory effect on the hydrolysis of di- and triglycerides.

v. The somewhat higher amount of tributyrinase and lipase in the dragonfly flight muscles compared to that in the flight muscles of the beetle appears to account for, the fact that the former insect is a more sustained flier and its major lipid store is, in the flight muscles.