CHAPTER 2

FIBRE DIAMETER AND CERTAIN ENZYME CONCENTRATIONS IN THE FLIGHT MUSCLES OF SOME MOTHS

By virtue of the structural peculiarities associated with the corresponding functional diversity, the striated muscles of insects could be considered a good material for studies directed towards a better understanding of the relationship between structure and function. Marked differences in the metabolism of the muscles of different insects have been found to exist and could be attributed to the differences in the structure and the mode of action of the muscles concerned. Differences in the tracheation, colour and variations in the ultra structure in the isolated fibrils in insects are also known (Edwards, Santos, Santos and Sawaya, 1954a, 1954b). However, little is known regarding the differences in the nature of the fuel store in different muscles and their bearing on the mode of flight, though it has long been an established fact that grasshoppers lay down large store of fat before making migratory flight and in the case of the beet-leafhopper it has been suggested (Fulton and Romney, 1940) that its fat content is an index of its flying capacity. Krogh and Weis-Fogh (1951) and Weis-Fogh,(1952) have shown that the R.Q. of the desert locust Schistocerca gregaria during sustained flight equals to 0.74 to 0.75 which is indicative of fat utilization. The lepidopteran insects seem to be even more specialized in this direction. They do not seem to utilize glucose, directly to a significant extent but convert it into fat before utilization

(Zebe, 1954, 1957). Obviously the demonstration of a fat-splitting enzyme (lipase) in considerably high concentrations in the flight muscles of some butterflies (Chapter 1) indulging in sustained flight is of special significance. The results of similar studies conducted on the flight muscles of various flying animals such as the pigeon, bat, locust and dragon fly have also shown high concentrations of fat and lipase in their muscles (George and Naik, 1958a, 1958b, 1959b; George and Scaria, 1956; George, Susheela and Scaria, 1958; George, Vallyathan and Scaria, 1958). It has been pointed out that in these muscles, there exist a correlation between the lipase content and fibre diameter greater the diameter lesser the lipase content and lesser the diameter greater the lipase content. It was also shown that the oxidative enzymes are concentrated more in narrow fibres than in the broad ones. It was therefore thought desirable to carry out similar investigations on the flight muscles of some moths and the results obtained are presented in this chapter.

Materials and Methods

The flight muscles of the following moths were examined.

Family	Name
Sphingidae	Cephonodes hylas hylas (L.)
"	Polyptychus dentatus (C.)
"	Hippotion rafflesi (L.)
"	Hippotion celerino (L.)
"	Deilaphila nerii (L.)
"	Herse convolvuli convolvuli (L.)

Family	Name
Sphingidae	Acherontia styx styx
Noctuidae	Ophlderes meterna (L.)
"	Nyctipao macrops (L.)

All the moths studied were caught at night during flight except Cephonodes hylas hylas which was caught during flight during the day and used after killing them by decapitation. Thorax was cut open on the ventral side and after pulling out the alimentary canal the muscles were carefully removed from their attachment by a pair of fine watchmaker's forceps and used for the different observations.

Measurement of fibre diameter:

Very thin hand sections of fresh forzen muscle, cut according to the method of George and Scaria (1958a) and mounted in glycerine jelly, were used for the meaaurement of fibre diameter. A microscope with occular eye piece and micrometer scale was used for the measurement.

Enzymes: Quantitative:

Lipase:

The concentration of lipase in the flight muscles of the above moths was studied quantitatively according to the method of Martin and Peers (1953) using the conventional Warburg apparatus, in a bicarbonate-carbon dioxide buffer system of pH 7.4 at 37°C. using tributyrin as substrate. The procedure is well described in Chapter 1. The reaction flask contained 1.5 ml of 0.025 M sodium bicarbonate solution and

1 ml of the enzyme solution in the main chamber and 0.5 ml of 4% (v/v) tributyrin in 0.0148 M sodium bicarbonate solution (emulsified by shaking with a drop of "Tween 80") in the side arm, thus making up the total valume of 3 ml in the reaction flask. The manometer and flask were gassed for 3 minutes with a mixture of 95% nitrogen and 5% carbon dioxide. After equilibration for 10 minutes in the constant temperature water bath of the apparatus, the substrate was tipped in and after 3 minutes the initial readings were noted. Readings were taken twice an hour.

Lipase activity was calculated on the basis of the protein concentration of the enzyme solution used and is expressed as the number of $\mu l CO_2$/mg. protein/hr. Protein was estimated according to the micro-Kjeldahl steam distillation method (Hawk, et al, 1954).

Histochemical:

The localization of the following enzymes were studied histochemically; lipase, acid and alkaline phosphatase, adenosine triphosphatase (ATPase) and succinic dehydrogenase.

Fresh frozen sections of the muscles prepared according to the method of George and Scaria (1958a), were used for the histochemical studies. Lipase was studied according to the method of Gomori using "Tween 80" as substrate at a pH of 8.4 . The revised method of Gomori using sodium glycerophosphate as substrate, was employed in the study of alkaline phosphatase at a pH of 9.2 (Pearse, 1954). Acid phosphatase was tested

according to Gomori's revised method (Glick, 1949) using sodium glycerophosphate as substrate at pH 5.1 . The procedure was the same as described in Chapter 1. In all the above experiments sections kept in boiling water for 10 minutes prior to incubation was used as control. Sections were incubated for 6 to 8 hours for lipase and 24 and 6 hours for alkaline and acid phosphatases respectively at 37°C. The presence of ATPase was tested by employing the method of Pearse and Reis (Pearse, 1954) using adenosine triphosphate (ATP) as substrate. Sections were incubated for 3 hours which was found to be the optimum period for these muscles. The following were the controls employed; sections for alkaline phosphatase incubated at pH 9.2 and 7.4 ,section kept in boiling water for 10 minutes before incubation.

Succinic dehydrogenase activity in the muscles was studied following the method of Straus et al (Pearse, 1954) using 2:3:5 triphenyl tetrazolim chloride (TTC) as the hydrogen acceptor. The procedure was exactly as described in the previous chapter.

Results

Fibre diameter:

The average diameter of the fibres corrected to the nearest whole number, in the different species is given in Table 1. It was observed that the fibre diameter of the muscle was more or less uniform for the species. The largest as well as the smallest fibres were found in the Sphingidae

ranging from 75 μ to 103 μ while in the other family studied (Noctuidae) the fibres were of more or less the same diameter (85-86 u). The figures given for the diameter represent the average of several hundred fibres measured.

Lipase activity of the muscle:

The results of the study made on the quantitative determination of lipase activity in the flight muscles of the different moths are presented in Table 1. It can be noticed that the greatest activity of 31.76 μl CO_2/mg. protein/hour is the Sphingid moth Cephonodes hylas hylas which possesses the narrowest fibres and the least (10.96 μl CO_2/mg. protein/hr.) also in a sphingid moth Acherontia styx styx having the largest fibres.

Table 1

Lipase activity in the flight muscles of some moths studied and their average fibre diameter. The value given for the lipase activity is the average of five experiments in each case.

Family	Name	Lipase activity μl CO_2/mg.protein/hr.	Average fibre diameter μ
Sphingidae	Cephonodes hylas hylas (L.)	31.76	75
"	Polyptychus dentatus (C.)	31.74	75
"	Hippotion rafflesi (B.)	26.81	80
"	Hippotion celerino (L.)	24.20	82
"	Deilephila nerii (L.)	20.23	86
"	Herse convolvuli convolvuli(L.)	15.37	95
"	Acherontia styx styx (W.)	10.96	103
Noctuidae	Nyctipao macrops (L.)	23.90	85
"	Ophideres meterna (L.)	22.32	86

Photomicrographs of transverse sections of the flight muscles of moths, treated histochemically to demonstrate the disposition of lipase.

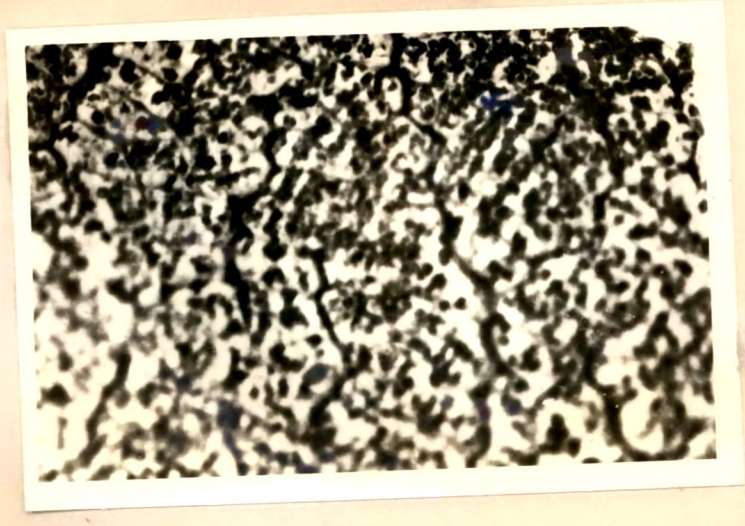


Fig. 1

Fig. 1 Cephonodes hylas hylas. Note the high concentration of lipase.

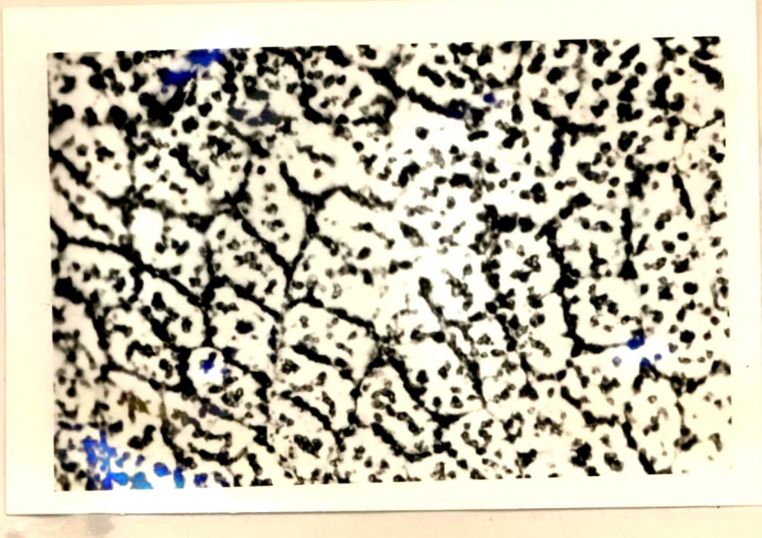


Fig. 2

Fig.2 Polyptycus dentatus. Note the equally high concentration of precipitate as in Fig. 1.

100 µ

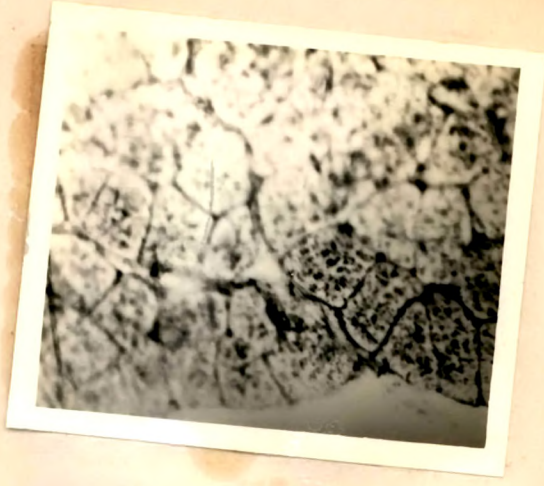
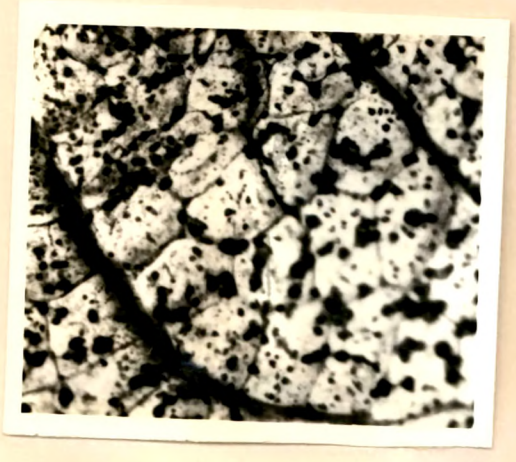


Fig.3 100µ Fig. 4

Fig. 3 Hippotion rafflesi. Fig. 4 Hippotion celerino.



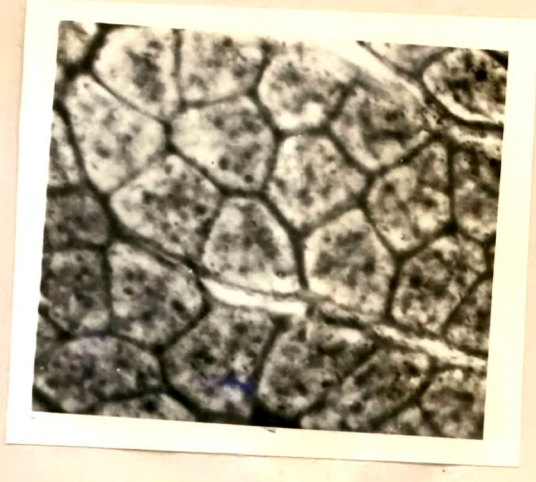


Fig. 5 100µ Fig. 6

Fig. 5 Deilephila nerii. Fig. 6 Nyctipao macrops.

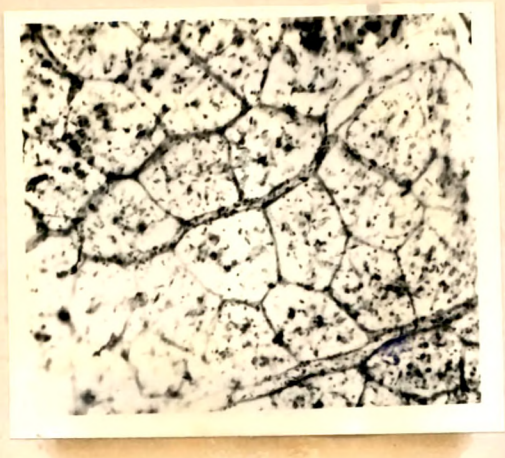


Fig. 7

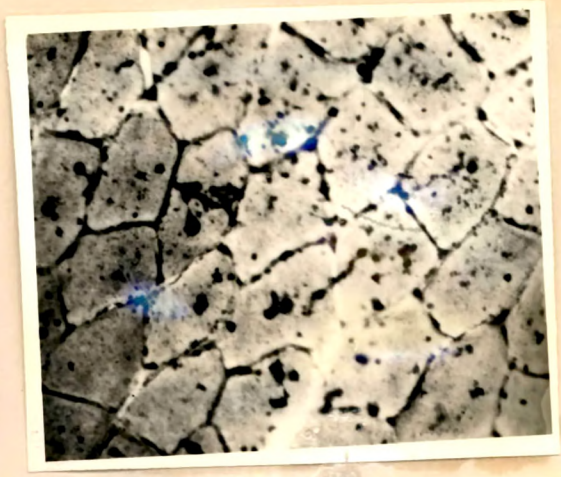


Fig. 8

Fig. 7 Ophideres meterna.

Fig. 8 Herse convolvuli convolvuli.

100µ

Fig. 9

Fig. 9 Acherontia styx styx. Note the low concentration of the precipitate and the large size of the fibres.

Histochemical observations:

Lipase:

The flight muscles of all the moths studied gave a positive staining reaction for lipase. However, clear histochemical distinction between those showing greater or lesser activity was possible only in those that formed the highest and the lowest amounts of precipitate obtained in the two species with the smallest and largest fibres respectively. The quantitative study revealed the exact difference in the level of lipase activity in them (Figs: 1 to 9).

Phosphatases:

All the phosphatases tested for, namely acid and alkaline phosphatases and adenosine triphosphatase could be demonstrated histochemically in the flight muscles of all the moths studied. The localization of the enzyme was uniform in the fibres. For ATPase a very dark colour was obtained in all the cases denoting a very strong positive reaction for the enzyme.

Succinic dehydrogenase:

The colour developed due to formazan was uniform in all the fibres of the flight muscles of all the moths observed. A quantitative estimation of this enzyme would be more confirmatory in assessing the relative differences (Chapter 10).

Discussion

The utilization of fat as a source of energy in insect flight is probably better understood then is generally realized, for the majority of studies hitherto made, happened to have been concerned with insects which use carbohydrate as the chief fuel

(Chadwick, 1953a). But in several insects it is known or suspected that fat is largely utilized; e.g. the desert locust (Weis-Fogh, 1952) beet-leafhopper (Fulton and Romney, 1940) Lepidoptera (Williams, 1945; Beall, 1948; Zebe, 1954, 1957) dragon fly and bumble bee (George, Vallyathan and Scaria, 1958).

Our present observations of the flight muscles of some moths and the data obtained on the concentration of lipase in them, suggest that moths too mobilize fat in their flight muscles for energy and that lipase value could be regarded as an index of the measure of fat utilization. If this is accepted it is seen that moths are comparatively more active than butterflies. Among the butterflies studied (Chapter 1) the highest lipase value of 22.92 µl CO_2/ mg. protein/hr. was obtained for the migrant danaid butterfly, Danais chrysippus possessing the narrowest muscle fibres with an average diameter of 80 µ and in the two sphingid moths Cephonodes hylax hylas and Polyptychus dentatus were found to have the highest lipase value of 31.76 and 31.74 µl CO_2/mg. protein/hr. respectively and having the narrowest fibres with a diameter of 75 µ. Cephonodes hylas hylas which is popularly known as the bumble bee hawk moth is a very swift and darting flier maintaining a steady degree of activity throughout the day while Polyptychus dentatus is also an active moth but nocturnal and readily attracted by light. The moths in general show a higher lipase value for their flight muscles than the butterflies. With the exception of two species, all the moths

studied were hawk moths belonging to the family Sphingidae. The hawk moths in general with a few exceptions can be easily distinguished from other lepidopterans by their swiftness and body form in having long narrow pointed fore wings, stout triangular hind wings, powerful thorax and sharply pointed abdomen. Several species of hawk moths are reported to be migrants (Williams, 1958) viz. Hippotion celerino, Deilephila nerii, Herse convolvuli convolvuli and Acherontia styx and related species. The minimum lipase value of 10.96 µl CO_2/mg. protein/hr. was obtained for Acherontia styx styx which is often included among the migrating hawk moths and was found to possess the largest muscle fibres with a diameter of 103 µ. In all the other hawk moths studied it was observed that with a decrease in lipase value of the muscle, there was a corresponding increase in the diameter of the respective muscle fibres (Table 1). The two Noctuidae moths, Nyctipao macrops and ophideres materna have an average diameter of 85 u and 86 µ and lipase values of 23.9 µl CO_2/mg. protein/hr. and 22.3 µl CO_2/mg. protein/hr. respectively which as regards lipase values amount to more or less the highest figures obtained for the butterflies.

In classifying insect muscles on histological basis Tiegs (1955) and Pringle (1957) included the lepidopteran flight muscles among the thinly fibrillated or closely packed type which stands far below in evolutionary status than the coarsely fibrillated fibres of the higher insect orders, such as Coleoptera, Hymenoptera and Diptera. Tiegs (1955) also

observed that though the thrips and aphids are equipped with coarse sarcostyles in their flight muscle fibres, they are some of the feeblest fliers, while of the butterflies with their thinly fibrillated fibres some were well know for their migratory flights. Stroboscopical examinations of the wing movements and the contractions of the flight muscles of some butterflies and moths (Tiegs, 1955) revealed that the moths have a greater wing beat frequency than the butterflies, showing thereby that the moths possess a more active flight musculature than the butterflies. These observations also lend support to the conclusion that the changes in the biochemical and physiological properties of a tissue, are often accompanied by histological changes as well as its component cells and muscular tissue is not an exception tothis.

The amount of lipase present in a muscle may be regarded as an indirect indication of the extent to which fat could be mobilized and utilized in that muscle. The data presented in this chapter is further evidence in favour of such an inference. The comparatively higher concentration of lipase detected in the flight muscles of the moths studied, suggest the utilization of fat for energy during flight. Williams (1945) examined the fat content of two migrant moths and suggested that fat was the chief fuel for their migratory flight. Other experimental data (Williams, 1958) also show that a large number of the hawk moths indulge in migratory flights. An extensive study by Beall (1948) on the fat content of a butterfly (Danais plexippus) also is suggestive of the

cumbustion of fat for energy. A study on the metabolism of some Lepidoptera (Zebe, 1954) showed that especially during flight, fat was used in the flight muscles. In the light of these observations it can be suggested that the amount of lipase activity in the flight muscles of the moths examined, could be considered as an index of the extent of fat utilization in the muscle for energy.

The presence of the acid and alkaline phosphatases, ATPase and succinic dehydrogenase in considerably large concentrations, demonstrated by histochemical techniques, shows that these muscles are metabolically very active. Although the precise physiological role of the alkaline and acid phosphatases is not so well understood as that of ATPase, the former two have been shown to be important in the metabolism of lipids and carbohydrates and in the active transport of materials across a membrane barrier.