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CARBOHYDRATE USE IN THE FLIGHT MUSCLES OF *MANDUCA SEXTA* DURING PRE-FLIGHT WARM-UP

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SUMMARY

Although fat is the principal fuel for flight in moths and butterflies, some use of carbohydrate fuels during activity would be predicted on energetic and biochemical grounds, particularly in nectivores. The present study evaluates the use of carbohydrate fuels during pre-flight warm-up in the endothermic sphinx moth *Manduca sexta* (L.). Carbohydrate content of moths was measured at intermediate points during the pre-flight warm-up cycle and at take-off. Muscle glycogen content declined during the initial phases of warm-up, whereas glucose and trehalose concentrations were unchanged. Abdominal carbohydrates were not mobilized during warm-up. Energy budget analysis suggests that glycogen oxidation supplies about 39% of the energy needed for the initial phase of warm-up and about 6% of the total cost of warm-up. Glycogen use during warm-up may be correlated with the capacity for endothermic warm-up at low ambient temperatures. Carbohydrates appear to be more important as fuels for activity in some lepidopterans than has been previously reported for other members of this diverse Order.

INTRODUCTION

Fat is the principal fuel for prolonged flight activity in Lepidoptera (Kammer & Heinrich, 1978; Ziegler & Schulz, 1986*a*). These insects complete pupation and enter the adult stage with most of their fuel reserve in fat stores (Beall, 1948; Downer & Matthews, 1976*a*; Cookman, Angelo, Slansky & Nation, 1984) and available carbohydrate stores are limited (Domroese & Gilbert, 1964; Stevenson, 1968; Brown & Chippendale, 1974). However, fat cannot be the sole fuel for oxidative metabolism. The increase in metabolism accompanying the onset of flight depends upon priming of the citric acid cycle with catalytic amounts of 3- and 4-carbon compounds (Sacktor, 1975). These intermediates can only be obtained from the breakdown of glucose, amino acids or glycerol because insects, like vertebrates, lack a functional glyoxalate cycle and cannot convert long-chain fatty acids to glucose (Chino & Gilbert, 1965; Duve, 1977; Lehninger, 1975).

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Those moths and butterflies that feed upon floral nectars as adults consume a diet consisting primarily of water and sugars (Watt, Hoch & Mills, 1974), but these ingested sugars are rapidly converted to fat in many species (Zebe, 1954; Brown & Chippendale, 1974). Direct use of ingested or infused carbohydrates by moth flight muscles *in vivo* has been reported in a few instances (Van Handel & Nayar, 1972; Surholt & Newsholme, 1983; Ziegler & Schulz, 1986*b*) and the flight muscles of some other moth species have the capacity to oxidize carbohydrates *in vitro* (Stevenson, 1968; Beenakkers, 1969), but the functional significance of these observations has been unclear. Conversion of ingested sugars to fat rather than glycogen for storage reduces the mass per joule of fuel carried (Lehninger, 1975), an important consideration for flying animals (see Weis-Fogh, 1952), but the metabolic costs of fat synthesis will reduce the net energy gain by about 20 %. During pre-flight warm-up, metabolic rate and demand for fuel are increasing exponentially (Bartholomew, Vleck & Vleck, 1981). Concurrently, circulatory flow between the thorax and abdomen may be restricted (Heinrich & Bartholomew, 1971), increasing dependence on intrathoracic fuel stores. Thus, some direct use of carbohydrates might be expected, especially during warm-up at low temperatures.

The present study evaluates carbohydrate use during pre-flight warm-up at low temperatures in the endothermic sphinx moth, *Manduca sexta*. Carbohydrate contents of both the thoracic muscles and the abdomen were measured at intermediate points during the warm-up cycle. Energy budget analysis was used to estimate the quantitative significance of carbohydrate oxidation during warm-up and the relationship of carbohydrate fuels to activity patterns is also considered.

MATERIALS AND METHODS

Insect rearing

An initial stock of *M. sexta* eggs, obtained from Dr J. S. Buckner (Metabolism and Radiation Research Laboratory, Agricultural Research Service, USDA), was used to establish a breeding colony. Insects were reared in a temperature-controlled room according to a modification of the techniques of Bell & Joachim (1976) at 25 ± 2°C and a photoperiod of 17 h:7 h L:D, as described in Joos (1986).

Fuel use during pre-flight warm-up

On the day of emergence, moths were placed in a 0·23 m^3 wire-mesh flight cage. Moths emerging on successive days were placed in separate cages. Each cage had a feeding station containing 20 % sucrose and moths could feed *ad libitum*. Individuals were held in these cages until the third day after emergence and were then used for warm-up experiments.

On the third day after emergence, moths were removed from the flight cage and placed in individual containers without food at approximately 09.30 h EST. The moths were held at 25°C until 13.00 h and then were held at 4–5°C under full light for 0·5–3 h prior to the start of an experiment. An environmental room at 15 ± 2°C with low (<100 lx) light levels was used for the warm-up experiments. A single moth

was placed in a plastic box filled with ice, the centre of the dorsal thorax was pierced with a 0·5 mm (26 gauge) hypodermic needle and a 0·2 mm (38 gauge) copper–constantan thermocouple was inserted to a depth of about 3 mm. The moth, with thermocouple attached, was moved to a shallow (approx. 5 cm deep) cardboard box and the thermocouple was connected to a Honeywell Elektronik 16 temperature recorder. The moths heated passively to room temperature and many began warm-up spontaneously after reaching room temperature. Others were stimulated by stroking the antennae or gently pinching the tip of the abdomen with forceps. Only those moths which warmed continuously were used for subsequent analysis. Duration of warm-up was timed to the nearest 0·1 s with a stopwatch and the average rate of thoracic temperature increase was estimated for each moth from the formula:

$$\text{Rate of warm-up} = (T_{th,final} - T_{th,initial})/\text{duration of warm-up}.$$

Upon reaching a specified thoracic temperature or at take-off, the moth was grasped by the thorax with forceps, cooled in an ethanol–dry ice bath, and then quickly plunged into the bath. Control moths were frozen immediately upon removal from the low-temperature chamber. The ethanol–dry ice technique allowed for rapid freezing of both the abdomen and the thorax; because glycolytic enzyme activities, particularly glycogen phosphorylase and trehalase, in *M. sexta* muscle are low (Joos, 1986), the technique is sufficiently fast to prevent changes in substrate concentrations. After the whole insect had been weighed, the head, wings, legs and abdomen were separated from the thorax, the thoracic scales were removed by rubbing with laboratory tissue, and the denuded thorax was split with a razor blade. Frozen muscle was separated from the exoskeleton with fine forceps, weighed to the nearest 0·0001 g, wrapped in aluminium foil, and refrozen between two blocks of dry ice. The whole abdomen was also weighed to the nearest 0·0001 g, wrapped and refrozen. Tissues were stored at −70°C until analysis.

Muscle samples and whole abdomens were analysed for glucose, glycogen and trehalose. Glycogen was assayed by the amyloglucosidase method (Keppler & Decker, 1974). Glucose in the glycogen digests was assayed by the glucose oxidase method (Sigma technical bulletin no. 510). Trehalose (1-*O*-alpha-D-glucopyranosyl-alpha-D-glucopyranoside) was determined on a sample of the neutralized tissue extract by the method of Wyatt & Kalf (1957) using the anthrone reagent (Mokrasch, 1954).

These procedures were slightly modified for the measurement of abdomen carbohydrates because of the high lipid concentrations. The supernatants from both the tissue extract and the glycogen digest formed two phases upon centrifugation; a lipid-rich upper phase and a lipid-poor lower phase. All samples for analysis were taken from the lower phase with minimal disturbance of the upper phase.

Statistics

Statistical analysis was carried out using the MIDAS statistical package (Statistical Research Laboratory, the University of Michigan) supported by the University of Michigan Computing Center. Since the carbohydrate concentration data were

heteroskedastic and standard transformation procedures did not eliminate heteroskedasticity, non-parametric statistical procedures were used. Mean values and the standard error of the mean are given for descriptive purposes only. The Mann–Whitney U-test and Kruskal–Wallis test were used to compare distributions and the non-parametric Student–Newman–Keuls test, as described by Zar (1974), was used for *a posteriori* multiple range testing. Rates of warm-up were analysed by simple linear correlation and regression procedures. Null hypotheses were rejected at the 95 % level of significance in all procedures.

RESULTS

Pre-flight warm-up behaviour

Moths began pre-flight warm-up after heating passively to a thoracic temperature slightly above chamber air temperature. Most moths climbed up onto the side of the holding box, positioned themselves at the top edge and warmed continuously to the final thoracic temperature without breaks or pauses.

The average rate of increase in thoracic temperature during warm-up (average rate of warm-up) did not differ significantly between moths allowed to take off and those groups in which warm-up was terminated prematurely, suggesting that thoracic temperature increases linearly with time. The mean value for all groups was $2{\cdot}30 \pm 0{\cdot}070\,^{\circ}\mathrm{C\,min^{-1}}$ (1 S.E.M., $N = 81$) and rate of warm-up was not correlated with body mass. Thoracic temperature at take-off was $37{\cdot}3 \pm 0{\cdot}49\,^{\circ}\mathrm{C}$ (1 S.E.M., $N = 13$). Take-off temperature was not correlated with body mass or with small variations in ambient temperature.

Fuel use during pre-flight warm-up

Muscle glycogen concentration declined from a mean of $23{\cdot}00\,\mu\mathrm{mol\,g^{-1}}$ tissue before warm-up to a mean of $17{\cdot}22\,\mu\mathrm{mol\,g^{-1}}$ at $T_{th} = 21\,^{\circ}\mathrm{C}$, but did not change significantly as warm-up proceeded further (Fig. 1; Table 1). Muscle glucose and trehalose concentrations during warm-up were not significantly different from the control value (Table 1).

Abdominal concentrations of glycogen and trehalose were much greater in females than in males, but neither glycogen nor trehalose concentrations changed significantly during warm-up. Glucose concentration was significantly lower at take-off than at earlier stages in the warm-up cycle or in controls (Table 2), but the change was small compared with the observed fluctuations in glycogen and trehalose concentrations. This decline may have resulted from local metabolism or may indicate incipient mobilization of fat body carbohydrate for use by the working muscle or other tissues.

DISCUSSION

Previous studies of the energy reserves of adult lepidopterans have found either no thoracic glycogen stores (*Hyalophora cecropia*, Domroese & Gilbert, 1964; *Danaus*

plexippus, Brown & Chippendale, 1974) or modest amounts which were considered quantitatively insignificant (Stevenson, 1968). Muscle glycogen levels of *M. sexta* (Table 1) and of a noctuid moth, *Spodoptera eridania* (Stevenson, 1968), are low

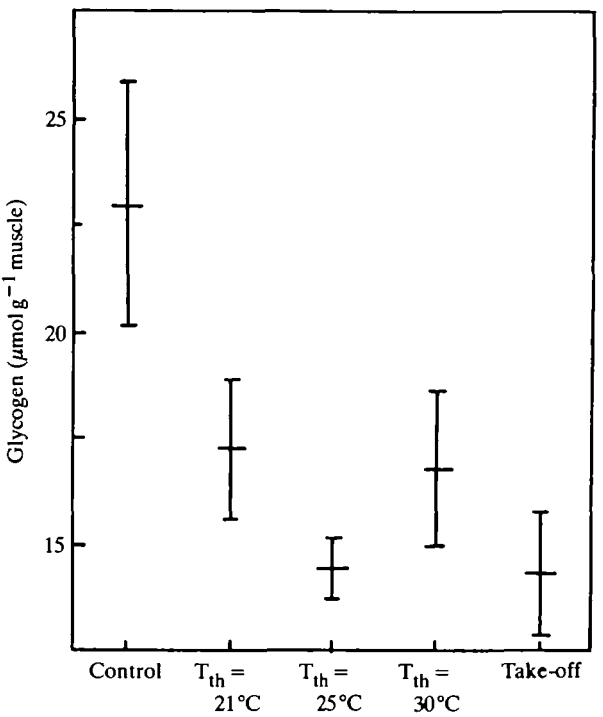

Fig. 1. Muscle glycogen concentration in *Manduca sexta* as a function of thoracic temperature (T_{th}) attained during pre-flight warm-up. Horizontal bars represent mean values and vertical bars represent one standard error of the mean. $N = 24$ for each group.

Table 1. *Carbohydrate concentration in the flight muscle of* Manduca sexta *at different points during the pre-flight warm-up cycle*

Group	Glucose	Glycogen	Trehalose
Control	0·70 ± 0·25	23·00 ± 2·87	17·95 ± 3·49
$T_{th} = 21°C$	0·39 ± 0·16	17·22 ± 1·67†	10·45 ± 1·78
$T_{th} = 25°C$	0·83 ± 0·21	14·40 ± 0·70†	10·96 ± 1·92
$T_{th} = 30°C$	0·65 ± 0·23	16·77 ± 1·79†	11·21 ± 1·79
Take-off	0·49 ± 0·13	14·35 ± 1·47†	11·59 ± 1·58
All groups*	0·61 ± 0·09	—	12·43 ± 1·01

Carbohydrate concentrations are expressed as μmol glucose equivalents g^{-1} muscle. $N = 24$ (12 males, 12 females) in each group. The mean and one standard error of the mean are shown.

* Combined mean and standard error of the mean for all groups. This mean was computed when there was no significant difference between groups.

† Significantly lower than control, $P < 0·001$.

T_{th}, thoracic temperature.

Table 2. *Carbohydrate concentration of the abdomen of* Manduca sexta *at different points during the pre-flight warm-up cycle*

Group	Glucose	Glycogen (males)	Trehalose (males)	Glycogen (females)	Trehalose (females)
Control	14·27 ± 8·90†	4·51 ± 0·69	14·15 ± 2·29	110·75 ± 17·95	151·12 ± 16·01
T_{th} = 21 °C	2·91 ± 1·40†	3·93 ± 0·30	10·17 ± 2·73	78·71 ± 18·08	151·05 ± 28·15
T_{th} = 25 °C	1·56 ± 1·11†	4·24 ± 0·59	12·21 ± 5·74	100·90 ± 15·44	170·24 ± 24·77
T_{th} = 30 °C	3·71 ± 3·21†	12·79 ± 8·08	20·10 ± 7·31	77·02 ± 24·78	136·26 ± 29·44
Take-off	0·52 ± 0·19	4·74 ± 0·53	17·00 ± 3·93	74·80 ± 18·22	94·29 ± 14·14
All groups*	—	6·04 ± 1·63	14·73 ± 2·12	88·44 ± 8·48‡	140·59 ± 10·62‡

Carbohydrate concentrations are expressed as μmol glucose equivalents g^{-1} tissue. $N = 24$ (12 males, 12 females) in each group. Mean value and one standard error of the mean are given.

* Combined mean and one standard error of the mean for all groups. This mean was computed when there were no significant differences between groups.

† Significantly higher than at take-off, $P < 0·01$.

‡ Significantly greater in females than in males, $P < 0·0001$.

T_{th}, thoracic temperature.

Table 3. *Comparison of the energy supplied by glycogenolysis with total heat production during pre-flight warm-up in* Manduca sexta

	Complete warm-up cycle	$T_{th} = 21°C$
Glycogen depletion*	2·09 μmol	2·09 μmol
Equivalent heat production from glycogen†	6·02 J	6·02 J
Total heat production	96·02 J‡	15·30 J§

Estimates are for a 2·2-g moth, thoracic mass of 0·57 g. 50 % of the thoracic mass is assumed to be muscle.

* In μmol glucose equivalents. Taken from Table 1.

† Assuming complete oxidation and a caloric equivalent of 5·09 kcal l^{-1} O_2.

‡ From Hegel & Casey (1982).

§ Estimated using data from Hegel & Casey (1982), assuming no heat transfer to the abdomen.

T_{th}, thoracic temperature.

compared with those of insects such as blowflies which depend heavily upon carbohydrates for flight fuels (Norden & Patterson, 1969). However, the glycogen levels of these moth species approximate to those in the pectoralis muscles of several species of passerine birds (see Marsh, 1979). As in passerine birds, such relatively modest carbohydrate stores are probably not a major energy store for flight, but may have important qualitative roles. In addition to serving as a source of 3- and 4-carbon compounds for the priming of the citric acid cycle (Sacktor, 1975), essentially instantaneous access to intramuscular carbohydrate fuels may be important in the early phases of muscle activity before extrathoracic fuels, principally lipids, can be mobilized (Ziegler & Schulz, 1986*a*).

The depletion of muscle glycogen observed in *M. sexta* during pre-flight warm-up (Table 1; Fig. 1) indicates that glycogen was mobilized as a muscle fuel during this period. The accompanying stability of glucose levels and statistically insignificant decline in trehalose levels (Table 1) further indicate that glycogen was catabolized rather than converted to oligosaccharides. During warm-up, little carbohydrate appears to be mobilized from stores in the abdomen (Table 2). Qualitatively similar patterns of differential glycogen depletion occur at the onset of flight activity in two species which do not warm up, a cockroach, *Periplaneta americana* (Downer & Matthews, 1976*b*; Elliot, Hill & Bailey, 1984), and a locust, *Schistocerca gregaria* (Rowan & Newsholme, 1979). In *M. sexta*, however, the actual change in glycogen concentration is about 30 % of the resting value (Table 1), a much smaller proportional change than in either *P. americana* or *S. gregaria*.

The quantitative significance of the observed glycogen depletion can be evaluated by comparing a heat budget for pre-flight warm-up in *M. sexta* (Hegel & Casey, 1982) with the energy derived from catabolism of muscle glycogen. For a 2·2-g moth with 0·285 g of thoracic muscle, glycogen depletion supplies about 6 % of the total cost of endothermic warm-up (Table 3). However, glycogen depletion did not occur uniformly throughout warm-up but, rather, was confined to the initial phases of warming (Fig. 1). When the cost of this first portion of warm-up is compared with

the glycogen depletion during that period (Table 3), energy derived from muscle glycogen can account for about 39% of the total heat production. On this basis, muscle glycogen can be considered a quantitatively, as well as a qualitatively, significant fuel during the initial phases of endothermic warm-up at low ambient temperatures.

The present study is the first report of muscle glycogen use in a lepidopteran and one of very few studies on fuel use during normal behaviour. Beenakkers (1969) suggested, on the basis of glycolytic enzyme activities, that carbohydrate fuels might be important during the pre-flight 'wing-whirring' of some noctuid moths, but although fuel use in tethered flight has been examined in *M. sexta* (Ziegler & Schulz, 1986*a*,*b*) and in another sphingid, *Acherontia atropos* (Surholt & Newsholme, 1983), warm-up has not been investigated. During tethered flight body temperature (T_b) rises but there is no obligatory pre-flight warm-up, other thermoregulatory responses are reduced or absent, and metabolic rates are lower than in free flight or at comparable T_b during pre-flight warm-up (see Heinrich, 1971; Bartholomew *et al.* 1981). It may be that muscle glycogen is, in part, a reserve fuel, which is only mobilized under the more demanding conditions imposed by the combination of an energetically demanding activity, pre-flight warm-up, with low ambient temperatures and attendant limitations on mobilization of extrathoracic fuels (see Heinrich & Bartholomew, 1971).

The large range of observed carbohydrate concentrations in *M. sexta* tissue is problematic. These data were collected over a period of several months and, although rearing procedures were standardized and all insects were descended from the same starting stock, the magnitude of the variance about the mean fluctuated considerably over the duration of the study. This fluctuation could result from genetic differences in successive generations, subtle differences in rearing conditions, or differences in behaviour. Although all moths had the same opportunities to feed and the duration of the period from the last feeding opportunity was similar in all cases, the nutritional status of any individual was unknown. *M. sexta* vary in their ability to learn to feed at artificial feeders (R. D. Stevenson, personal communication), and their level of feeding could also be influenced by the presence of other moths using the same feeding site. Individuals with the highest levels of muscle carbohydrates also had the highest levels of abdomen carbohydrates, consistent with differences in nutritional status among individuals. Also, lepidopteran eggs have a high carbohydrate content (Stevenson, 1968), and the wide range of abdomen glycogen and trehalose concentrations in female *M. sexta* may result from the presence of varying numbers of eggs at various stages of maturation. Although variation in the data may reduce the impact of the data set, the goal of the present study was to evaluate fuel use during normal, voluntary behaviour. This goal may have to be achieved at the cost of greater variation than might result from experimental protocols relying upon inherently less variable behaviour such as involuntary, tethered flight.

The extent to which the results obtained in the present study can be extended to other moths and butterflies is uncertain. Those moths which do not feed as adults,

such as the saturniids, have little carbohydrate available (Domroese & Gilbert, 1964) and would be less likely to rely on trehalose or glycogen as fuels. Among nectivorous moths and butterflies, the absence of thoracic glycogen stores in monarch butterflies (Brown & Chippendale, 1974) and the low respiratory exchange ratios (R) found in most Lepidoptera (Zebe, 1954) are more difficult to reconcile. Differences in methodology may account for some of the differences between the present study and Zebe's (1954) results. Zebe reported R for flights of several minutes to 2 h and any elevation of R resulting from initial use of glycogen could have been obscured in cumulative measurements over the entire activity period. Also, Zebe's study was carried out at higher temperatures (21–24°C), where carbohydrate fuels may be quantitatively less important.

It is intriguing that those moth species in which carbohydrate metabolism has been demonstrated belong to the Sphingidae and certain of the Noctuidae. These groups have relatively smaller wings which operate at higher wingbeat frequencies than do representatives of other moth families of similar body mass (Bartholomew & Casey, 1978; Casey & Joos, 1983): consequently, members of these groups have higher power requirements when airborne. Many are also endothermic in flight and require a period of pre-flight warm-up (Beenakkers, 1969; Bartholomew & Casey, 1978). In contrast, monarch butterflies (*Danaus plexippus*), which rely more on behavioural thermoregulation (Kammer & Bracchi, 1973) and have comparatively low power requirements for flight (Kammer, 1970), have no thoracic glycogen (Brown & Chippendale, 1974). This possible relationship between use of carbohydrate fuels and physiological thermoregulation in the Lepidoptera should be explored further.

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REFERENCES

BARTHOLOMEW, G. A. & CASEY, T. M. (1978). Oxygen consumption of moths during rest, pre-flight warm-up, and flight in relation to body size and wing morphology. *J. exp. Biol.* **76**, 11–25.

BARTHOLOMEW, G. A., VLECK, D. & VLECK, C. M. (1981). Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. *J. exp. Biol.* **90**, 17–32.

BEALL, G. (1948). The fat content of a butterfly, *Danaus plexippus* (Linn.) as affected by migration. *Ecology* **29**, 80–94.

BEENAKKERS, A. M. T. (1969). Carbohydrate and fat as a fuel for insect flight. A comparative study. *J. Insect Physiol.* **15**, 353–361.

BELL, R. A. & JOACHIM, F. G. (1976). Techniques for rearing laboratory colonies of tobacco hornworms and pink bollworms. *Ann. ent. Soc. Am.* **69**, 365–373.

Brown, J. J. & Chippendale, G. M. (1974). Migration of the monarch butterfly *Danaus plexippus*: energy sources. *J. Insect Physiol.* **20**, 1117–1130.

Casey, T. M. & Joos, B. (1983). Morphometrics, conductance, thoracic temperature, and flight energetics of noctuid and geometrid moths. *Physiol. Zool.* **56**, 160–173.

Chino, H. & Gilbert, L. I. (1965). Studies on the interconversion of carbohydrate and fatty acid in *Hyalophora cecropia*. *J. Insect Physiol.* **11**, 287–295.

Chippendale, G. M. (1973). Metabolic reserves of larvae and pupae of the Angoumois grain moth, *Sitotroga cerealella*. *Insect Biochem.* **3**, 1–10.

Cookman, J. E., Angelo, M. J., Slansky, F. & Nation, J. L. (1984). Lipid content and fatty acid composition of larvae and adults of the velvetbean caterpillar, *Anticarsia gemmatalis*, as affected by larval diet. *J. Insect Physiol.* **30**, 523–527.

Domroese, K. A. & Gilbert, L. I. (1964). The role of lipid in adult development and flight-muscle metabolism in *Hyalophora cecropia*. *J. exp. Biol.* **41**, 573–590.

Downer, R. G. H. & Matthews, J. R. (1976*a*). Patterns of lipid distribution and utilization in insects. *Am. Zool.* **16**, 733–745.

Downer, R. G. H. & Matthews, J. R. (1976*b*). Glycogen depletion of thoracic musculature during flight in the cockroach, *Periplaneta americana* L. *Comp. Biochem. Physiol.* **55**B, 501–502.

Duve, H. (1977). Test for the operation of the glyoxalate cycle in the blowfly *Calliphora erythrocephala*. *Insect Biochem.* **7**, 381–385.

Elliot, J., Hill, L. & Bailey, E. (1984). Changes in tissue carbohydrate content during flight of the fed and starved cockroach, *Periplaneta americana* L. *Comp. Biochem. Physiol.* **78**A, 163–165.

Hegel, J. R. & Casey, T. M. (1982). Thermoregulation and control of head temperature in the sphinx moth, *Manduca sexta*. *J. exp. Biol.* **101**, 1–15.

Heinrich, B. (1971). Temperature regulation of the sphinx moth, *Manduca sexta*. I. Flight energetics and body temperature during free and tethered flight. *J. exp. Biol.* **54**, 141–152.

Heinrich, B. & Bartholomew, G. A. (1971). An analysis of pre-flight warm-up in the sphinx moth *Manduca sexta*. *J. exp. Biol.* **55**, 223–239.

Joos, B. (1986). Biochemical correlates of pre-flight warm-up in the sphinx moth, *Manduca sexta*. Ph.D. dissertation, The University of Michigan.

Kammer, A. E. (1970). Thoracic temperature, shivering, and flight in the monarch butterfly, *Danaus plexippus* (L.). *Z. vergl. Physiol.* **68**, 334–344.

Kammer, A. E. & Bracchi, J. (1973). Role of the wings in the absorption of radiant energy by a butterfly. *Comp. Biochem. Physiol.* **45**A, 1057–1063.

Kammer, A. F. & Heinrich, B. (1978). Insect flight metabolism. *Adv. Insect Physiol.* **13**, 133–228.

Keppler, D. & Decker, K. (1974). Glycogen. In *Methods of Enzymatic Analysis*, vol. 3 (ed. H. U. Bergmeyer), pp. 1127–1131. New York: Academic Press.

Lehninger, A. L. (1975). *Biochemistry*, pp. 560–578. New York: Worth.

Marsh, R. L. (1979). Seasonal adjustments in size and biochemistry of the flight muscles in a long distance migrant, the grey catbird (*Dumetella carolinensis*). Ph.D. dissertation, The University of Michigan.

Mokrasch, L. C. (1954). Analysis of hexose phosphates and sugar mixtures with the anthrone reagent. *J. biol. Chem.* **208**, 55–59.

Norden, D. A. & Patterson, D. J. (1969). Carbohydrate metabolism in flight muscle of tsetse fly (*Glossina*) and blowfly (*Sarcophaga*). *Comp. Biochem. Physiol.* **31**, 819–827.

Rowan, A. N. & Newsholme, E. A. (1979). Changes in the contents of adenine nucleotides and intermediates of glycolysis and the citric acid cycle in flight muscle of the locust upon flight and their relationship to control of the cycle. *Biochem. J.* **178**, 209–216.

Sacktor, B. (1975). Biochemistry of insect flight. In *Insect Biochemistry and Function* (ed. D. J. Candy & B. A. Kilby), pp. 1–88. New York: Wiley.

Stevenson, E. (1968). Carbohydrate metabolism in the flight muscle of the southern armyworm moth *Prodenia eridania* (Cramer). *J. Insect Physiol.* **14**, 179–188.

Surholt, B. & Newsholme, E. A. (1983). The rate of substrate cycling between glucose and glucose-6-phosphate in muscle and fat body of the hawk moth (*Acherontia atropos*) at rest and during flight. *Biochem. J.* **210**, 49–54.

VAN HANDEL, E. & NAYAR, J. K. (1972). Direct use of carbohydrates during sustained flight in the moth, *Spodoptera frugiperda*. *Insect Biochem.* **2**, 203–208.

WATT, W. B., HOCH, P. C. & MILLS, S. G. (1974). Nectar resource use by *Colias* butterflies. Chemical and visual aspects. *Oecologia* **14**, 353–374.

WEIS-FOGH, T. (1952). Fat combustion and metabolic rate of flying locusts. *Phil. Trans. R. Soc. Ser.* B **237**, 459–510.

WYATT, G. R. & KALF, G. F. (1957). The chemistry of insect hemolymph. II. Trehalose and other carbohydrates. *J. gen. Physiol.* **40**, 833–847.

ZAR, J. H. (1974). *Biostatistical Analysis*, pp. 151–162. Englewood Cliffs: Prentice-Hall Inc.

ZEBE, E. (1954). Über den Stoffwechsel der Lepidopteren. *Z. vergl. Physiol.* **36**, 290–317.

ZIEGLER, R. & SCHULZ, M. (1986*a*). Regulation of lipid metabolism during flight in *Manduca sexta*. *J. Insect Physiol.* **32**, 903–908.

ZIEGLER, R. & SCHULZ, M. (1986*b*). Regulation of carbohydrate metabolism during flight in *Manduca sexta*. *J. Insect Physiol.* **32**, 997–1001.