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# ENERGY SUBSTRATES FOR FLIGHT IN THE BLISTER BEETLE *DECAPOTOMA LUNATA* (MELOIDAE)

LUTZ AUERSWALD AND GERD GÄDE*
*Zoology Department, University of Cape Town, Rondebosch 7700, South Africa*

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## Summary

**We investigated the substrates for flight in the blister beetle *Decapotoma lunata* by (a) establishing the patterns of maximum activities of enzymes of various metabolic pathways in the flight muscles, (b) measuring the respiratory rates of flight muscle mitochondria with various substrates and (c) determining metabolite concentrations in flight muscles and haemolymph during a flight period of up to 17 min and over a rest period of up to 2 h following 10 min of flight.**

**Activities of enzymes involved in proline metabolism (glutamate dehydrogenase, alanine aminotransferase, malic enzyme) were much higher in the blister beetle than in the migratory locust *Locusta migratoria*, whereas the activity of an enzyme responsible for fatty acid oxidation ($\beta$-hydroxyacyl-CoA dehydrogenase) was much lower.**

**Mitochondria from flight muscles of *D. lunata* have a much higher capacity to oxidise proline than those from *L. migratoria*. The glycerophosphate shuttle, however, was equally active in both insects.**

**Whereas lipid levels in the haemolymph did not change significantly during flight, there was a continuous decrease in proline levels from 34.8 to 6.6 $\mu$mol ml$^{-1}$ and a simultaneous increase in alanine concentration; carbohydrate levels dropped from 20.1 to 12.2 mg ml$^{-1}$.**

**In the thorax (flight muscles), glycogen levels were diminished between 2 and 17 min of flight from 25.9 to 6.7 $\mu$mol glucose equivalents g$^{-1}$ fresh mass. Proline concentration dropped continuously from an initial 49.5 to 10.1 $\mu$mol g$^{-1}$ fresh mass, whereas alanine levels rose concomitantly from 2.9 to 17.3 $\mu$mol g$^{-1}$ fresh mass.**

**After termination of a 10 min flight, pre-flight levels of proline in the haemolymph and flight muscles were only re-established after 2 h. In contrast, glycogen levels in the thorax were restored after 1 h.**

**Using the rates of utilisation of substrates during the first 10 min of flight to calculate rates of oxygen consumption during flight, it was shown that overall haemolymph substrates contribute 75 % and those of the flight muscles only 25 %. Although proline is an important substrate for flight in *D. lunata*, its role is secondary to that of carbohydrates. This type of substrate usage is different from that of the Colorado potato beetle *Leptinotarsa decemlineata* or the African fruit beetle *Pachnoda sinuata*, in which carbohydrates are of negligible or only slight importance, respectively.**

Key words: insect flight, energy metabolism, proline oxidation, enzyme activities, mitochondrial respiratory rates, metabolite changes, blister beetle, *Decapotoma lunata*.

## Introduction

Many insects have developed the capacity for fast and/or long-distance flight. This mode of locomotion requires large amounts of energy. Insect flight muscles perform their work completely aerobically and working flight muscles are known to be metabolically the most active tissue in nature with respect to oxygen uptake. During free hovering flight, rates of oxygen consumption of 40–150 ml $O_2$ g$^{-1}$ body mass h$^{-1}$ have been measured (Casey, 1989).

Various substrates can be oxidised and utilised as fuels for flight. Characteristically, Diptera and Hymenoptera power their flight muscles by the breakdown of carbohydrates. Lipids, however, are the predominant fuel for the contracting flight muscles in Lepidoptera and Orthoptera, especially in those moth species that do not feed during the adult stage and during sustained flight in migratory locusts (see reviews by Sacktor, 1975; Goldsworthy, 1983; Ziegler, 1984; Gäde, 1992*a*).

The amino acid proline can also be used as a substrate for flight. There are, however, major differences between insect species in the quantitative participation of proline in flight metabolism. Only a little proline is metabolised during the onset of flight in the blowfly *Phormia regina*, to provide tricarboxylic acid intermediates necessary for maximal

*Author for correspondence.

oxidation of pyruvate ('sparker function'; Sacktor and Wormser-Shavit, 1966; Sacktor and Childress, 1967). In the tsetse fly *Glossina morsitans*, however, proline is present in impressively high concentration in the flight muscles (160 $\mu$mol $g^{-1}$ fresh mass), and it is the major, or even exclusive, fuel during flight (Bursell, 1981). This amino acid is only partially oxidised and the alanine formed is transported to the fat body for resynthesis of proline (Bursell, 1981). A similar pattern of proline metabolism has been shown during flight in the Colorado potato beetle *Leptinotarsa decemlineata*. Although carbohydrates are concomitantly broken down with proline, the amounts of glycogen in the flight muscles and glucose/trehalose in the haemolymph are too small to be quantitatively important flight substrates (Weeda *et al.* 1979). Recently, we studied the flight metabolism of the African fruit beetle *Pachnoda sinuata* (Zebe and Gäde, 1993; Lopata and Gäde, 1994). We clearly established two phases of metabolite usage in the flight muscles of *P. sinuata*: proline is the most important substrate during the first phase of flight (2 min), whereas carbohydrates are mainly used thereafter (Zebe and Gäde, 1993). Hansford and Johnson (1975), working with mitochondria isolated from the Japanese beetle *Popillia japonica*, suggested that proline may be completely oxidised in this species. However, no direct evidence for the participation of proline during flight metabolism was given.

In the present paper we address several questions arising from previous studies. (1) Is the use of proline as a fuel for flight restricted to some 'special' cases in the order Coleoptera, or is proline oxidation also important for the flight of the blister beetle *Decapotoma lunata*, a member of the family Meloidae? When feeding, for example on the yellow flowers of *Bolusafra bituminosa*, this beetle not only flies for short periods from flower to flower, but also for several minutes to flowers further away. (2) If there is a role for proline, is it used (a) as a 'sparker' or is it (b) partially oxidised as in tsetse flies (sole flight substrate), the Colorado potato beetle (mainly proline, some carbohydrates) or the African fruit beetle (first proline, later carbohydrates), or is it (c) completely oxidised as was proposed for the Japanese beetle?

## Materials and methods

### *Insects*

Blister beetles *Decapotoma lunata* (Pallas) of both sexes were caught on the slopes of Table Mountain, Cape Town, South Africa. Their average mass was 198±76 mg (mean ± S.D., *N*=17). Pilot experiments (see Results) showed that metabolite levels were affected by handling and the availability of food. Therefore, beetles were collected 1 day before experimentation and kept overnight with their food plant, flowers of *Bolusafra bituminosa* (L.) Kuntze (Fabaceae), in our insectary at 25 °C.

Male cockroaches *Periplaneta americana* (L.) and 2- to 3-week-old male *Locusta migratoria* L. were taken from our own colonies. They were reared as previously described (Gäde, 1991, 1992*b*). Male fruit beetles *Pachnoda sinuata flaviventris* (Gory & Percheron), were caught in the vicinity of Cape Town and were kept as outlined previously (Zebe and Gäde, 1993).

### *Flight experiments*

Experiments were performed at 28–32 °C in direct sunlight. Blister beetles were fixed on a thin thread between the head and prothorax, so that they could exhibit free flight. Although individuals occasionally flew for longer than 30 min (maximum 58 min), most were exhausted after 17 min. Exhaustion was recognised when the wings were extended but no longer moving. For recovery experiments, beetles were flown for 10 min and were subsequently rested in the dark without water and food for various periods.

Before and immediately after flight, and after various times of recovery following flight, 1 $\mu$l haemolymph samples were taken from the neck membrane for each of the metabolite determinations (see below). Beetles that showed reflex bleeding during handling (Nicolson, 1994) were discarded. Samples from locusts and cockroaches were taken from the membrane of one of the hind legs. Haemolymph of *P. sinuata flaviventris* was also taken from the neck membrane.

Immediately after flight, or after certain periods of recovery, beetles were dissected. The head, prothorax, legs, wings and abdomen were cut off and the gut was subsequently removed. The remaining whole thorax, which represents 24.0±2.1 % (mean±S.D., *N*=5) of the total body mass, was wrapped in aluminium foil and immediately frozen in liquid nitrogen. The whole procedure required approximately 90 s.

### *Preparation of samples and metabolite determination*

#### *Haemolymph*

1 $\mu$l of haemolymph was either blown immediately into 100 $\mu$l of concentrated $H_2SO_4$ for the determination of total lipids (Zöllner and Kirsch, 1962) or carbohydrates (Spik and Montreuil, 1964), or pipetted into 60 $\mu$l of 80 % acetonitrile for amino acid analysis (see below).

#### *Thorax*

Perchloric acid extracts from frozen thoraces were made according to Zebe and Gäde (1993).

*Glycogen determination*. Glycogen was extracted as previously described in Zebe and Gäde (1993) and analysed by the modified anthrone method (Spik and Montreuil, 1964) with glucose as a standard.

*Proline and alanine determination*. Derivatisation of extracts with dansyl chloride and determination of proline and alanine levels by HPLC were carried out as previously described (Zebe and Gäde, 1993).

### *Preparation of flight muscles for determination of enzyme activities*

Whole thoraces of the beetles (dissected as described above) were homogenised in an ice-cold medium containing triethanolamine/HCl buffer (100 mmol $l^{-1}$, pH 7.6) and 100 mmol $l^{-1}$ EDTA, using an Ultra Turrax twice for 10 s at maximum speed. Homogenates were subsequently sonicated

using a Branson sonifier B 30 for 10 s and centrifuged at 8000 ***g*** for 20 min. The supernatants were used within 1 h of preparation, to determine the activities of malic enzyme (ME), malate dehydrogenase (MDH), glutamate dehydrogenase (GluDH), β-hydroxyacyl-CoA dehydrogenase (HOAD), alanine aminotransferase (AlaT) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). For preparation of locust samples, the head and abdomen were cut off and the gut was removed. The thorax was cut open ventrally and the flight muscles were dissected out and treated as above.

To measure cytochrome oxidase (Cytox) activity, ten thoraces were cut into small pieces and homogenised in potassium phosphate buffer (50 mmol $l^{-1}$, pH 7.2) using a Potter–Elvehjem homogeniser. For locusts, the flight muscles of three insects were used (dissected as described above).

To ensure proportionality, all enzyme assays were carried out at least in duplicate with variable extract concentrations in a final volume of 1.0 ml at 25 °C. All enzymes activities (except Cytox) were measured by spectrophotometry at 340 nm using a Vitatron IFP instrument.

Assay conditions were as follows. MDH activity (EC 1.1.1.37) was determined according to Weeda *et al.* (1980), except that the pH was 9.4. GAPDH activity (EC 1.2.1.12) was measured according to Beenakkers (1969), except that glutathione was omitted. ME (EC 1.1.1.39), GluDH (EC 1.4.1.3), HOAD (EC 1.1.1.35) and AlaT (EC 2.6.1.2) activities were measured according to Zebe and Gäde (1993), except that the buffer used was triethanolamine/HCl for AlaT. To determine Cytox (EC 1.9.3.1) activity, the rate of oxygen consumption was measured polarographically according to Rafael (1985) using a YSI model 5300 instrument.

### *Preparation of flight muscle mitochondria and measurement of oxygen uptake*

Ten thoraces were cut into small pieces and homogenised in 10 ml of a phosphate buffer containing 250 mmol $l^{-1}$ sucrose, 150 mmol $l^{-1}$ potassium phosphate (pH 7.4) and 1 % bovine serum albumin (essentially fatty acid free) using a Potter–Elvehjem homogeniser. The homogenate was centrifuged for 5 min at 400 ***g*** and the pellet discarded. After further centrifugation for 10 min at 6000 ***g***, the supernatant was discarded and the sediment redissolved in 2 ml of medium. This solution was used for measuring respiratory rates by the YSI model 5300 oxygen monitoring system. The incubation medium contained 5 mmol $l^{-1}$ phosphate buffer (pH 7.4), 120 mmol $l^{-1}$ KCl, 40 mmol $l^{-1}$ sucrose, 3.5 mmol $l^{-1}$ $MgCl_2$, 0.01 % cytochrome *c* and 1.5 mmol $l^{-1}$ of the substrates tested (proline, pyruvate/malate, glycerol 3-phosphate). When glycerol 3-phosphate was used as a substrate, 1 mmol $l^{-1}$ $Ca^{2+}$ was added.

Protein determination was carried out using the method of Bradford (1976) with bovine serum albumin (fraction V) as a standard (Boehringer Mannheim).

### *Determination of haemolymph volume*

The procedure of Clegg and Evans (1961) was essentially used. In brief, a trace amount of [$^3$H-G]inulin ($1.315\times10^{10}$ Bq $g^{-1}$; DuPont, USA), dissolved in 5 μl of water (36992 cts $min^{-1}$), was injected ventrally into the abdomen between the last two segments using a 10 μl Hamilton syringe. After 20 min of incubation, a 1 μl sample of haemolymph was taken, pipetted into a scintillation vial containing 4 ml of scintillation fluid (Packard, Ultima Gold TR) and the radioactivity measured using a Tri Carb 460 instrument (Packard).

## Results

### *Concentration of metabolites in the haemolymph*

In the first series of experiments, we compared the levels of total carbohydrates and proline from beetles whose haemolymph was taken in the field immediately after capture, with those levels that had been measured after transferring the beetles from the wild to the laboratory and keeping them overnight without food but with access to water. Proline levels were about 30 % lower in the freshly caught individuals, probably because of flight activity just before capture, while carbohydrate levels decreased by about 35 % overnight, probably because of starvation (results not shown). Feeding the beetles held in the laboratory did help to keep the carbohydrate levels higher, but they were still 25 % lower than those of freshly caught beetles. For all further experiments, we kept the beetles as described in Materials and methods and all results shown are obtained from those beetles. As depicted in Table 1, haemolymph levels of carbohydrates and lipids in *D. lunata* are in approximately the same range as for *L. migratoria* and *P. americana*, which are known to use carbohydrates/lipids and carbohydrates, respectively, as energy substrates for flight. However, proline concentration in *D. lunata* is 3–4 times higher than in locusts and cockroaches. Compared with *P. sinuata*, which uses proline and carbohydrates during flight, the concentrations of carbohydrates and proline in *D. lunata* are twofold higher and 3.7-fold lower, respectively. As a first assessment from these blood values, it is suggested that proline in combination with carbohydrates may play a role during flight metabolism in *D. lunata*.

Table 1. *Concentrations of proline, total carbohydrates and total lipids in the haemolymph of* Decapotoma lunata, Locusta migratoria, Periplaneta americana *and* Pachnoda sinuata

| Insect | Proline (μmol $ml^{-1}$) | Carbohydrates (mg $ml^{-1}$) | Lipids (mg $ml^{-1}$) |
|---|---|---|---|
| *D. lunata* | 34.8±12.6 (23) | 20.1±4.2 (19) | 10.5±3.2 (7) |
| *L. migratoria* | 9.2±1.5 (3) | 26.8±4.2 (4) | 9.9±1.4 (4) |
| *P. americana* | 13.6±5.8 (3) | 14.9±3.1 (4) | 15.7±0.9 (4) |
| *P. sinuata* | 128.9±24.8 (10) | 9.7±2.4 (8) | 8.7±3.7 (7) |

Values are means ± S.D. with *N* in parentheses.

Table 2. *Maximal enzyme activities in flight muscles of* Decapotoma lunata *in comparison with* Locusta migratoria *and* Pachnoda sinuata

| Enzyme | *N* | *D. lunata* | *N* | *L. migratoria* | *N* | *P. sinuata* |
|---|---|---|---|---|---|---|
| ME | 5 | 416±77<br>(17.9±3.3) | 6 | 88±22<br>(4.7±1.2) | 5 | (41.4±2.4) |
| MDG | 5 | 2974±436<br>(127.9±18.8) | 4 | 5860±900<br>(312±48.1) | | – |
| GluDH | 5 | 2732±225<br>(117.5±9.7) | 7 | 163±45<br>(8.7±2.4) | 5 | (80.7±4.9) |
| AlaT | 5 | 2141±273<br>(92.1±11.7) | 3 | 689±228<br>(36.8±12.2) | 5 | (323.6±45.7) |
| HOAD | 5 | 139±67<br>(6.0±2.9) | 6 | 833±115<br>(44.5±6.20) | 5 | (16.2±5.9) |
| GAPDH | 4 | 5053±285<br>(224.8±10.0) | 3 | 3529±545<br>(188.4±29.1) | | – |
| Cytox | 5 | 3981±357<br>(171.6±15.4) | 3 | 3649±503<br>(157.3±21.7) | 3 | (202.5±3.3) |
| Ratio | | | | | | |
| MDH/ME | | 7 | | 67 | | |
| GAPDH/HOAD | | 37 | | 4 | | |

Values are expressed as munits $mg^{-1}$ protein and (units $g^{-1}$ fresh mass), as mean values ± S.D., with the exception of Cytox, which is expressed as $\mu$mol $O_2$ $g^{-1}$ fresh mass $min^{-1}$ and ($\mu$mol $O_2$ $mg^{-1}$ protein $min^{-1}$).

Results for *P. sinuata* are from Zebe and Gäde (1993).

1 unit of enzyme activity = 1 $\mu$mole of substrate used per minute.

ME, malic enzyme; MDH, malate dehydrogenase; GluDH, glutamate dehydrogenase; AlaT, alanine aminotransferase; HOAD, $\beta$-hydroxyacyl-CoA dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Cytox, cytochrome oxidase.

### *Indirect measurements in flight muscles*

#### *Activities of certain enzymes*

Measurement of maximal activities of enzymes involved in a particular pathway can give an indication of the significance of certain metabolic pathways for flight metabolism (according to Pette, reviewed in Beenakkers *et al.* 1984). Such data are especially meaningful in direct comparison with another animal whose metabolism is well studied. Therefore, we included flight muscles from *L. migratoria* for a comparative investigation. Since we used a thorax preparation for *D. lunata*, values used for comparisons were calculated on the basis of the protein content of flight muscles in order to minimise errors resulting from chitin contamination. However, values calculated on the basis of fresh mass were used to compare the present results with those of our previous study on *P. sinuata*. Two enzymes, GAPDH and Cytox, have about the same high activity in blister beetle and locust flight muscles (Table 2), whereas the activities of MDH and HOAD are higher by twofold and sixfold, respectively, in the locust. Activities of the three enzymes AlaT, ME and GluDH, thought to be involved in proline metabolism, however, are about three-, five- and 17-fold higher, respectively, in the blister beetle. When the data for the two beetles are compared (Table 2), it is noteworthy that the levels of most of the enzymes responsible for proline oxidation are higher in *P. sinuata*, with the exception of GluDH, which has a similar activity in both beetles. HOAD, an enzyme responsible for the $\beta$-oxidation of fatty acids, also has a higher activity in *P. sinuata*.

#### *Respiratory rates of flight muscle mitochondria*

Another set of experiments was conducted to test for differences in the enzymatic design of the flight muscles of blister beetles and locusts. Here we measured the ability of isolated mitochondria to oxidise certain substrates; the rates were expressed as a percentage of Cytox activity, which is an excellent reference point with which to indicate the actual

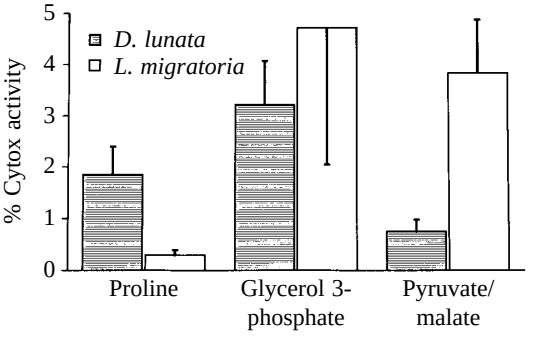


Fig. 1. Oxygen uptake of mitochondrial preparations of flight muscles from *Decapotoma lunata* (*N*=5) and *Locusta migratoria* (*N*=3). Data are given as a percentage of the cytochrome oxidase (Cytox) activity; mean values + S.D.

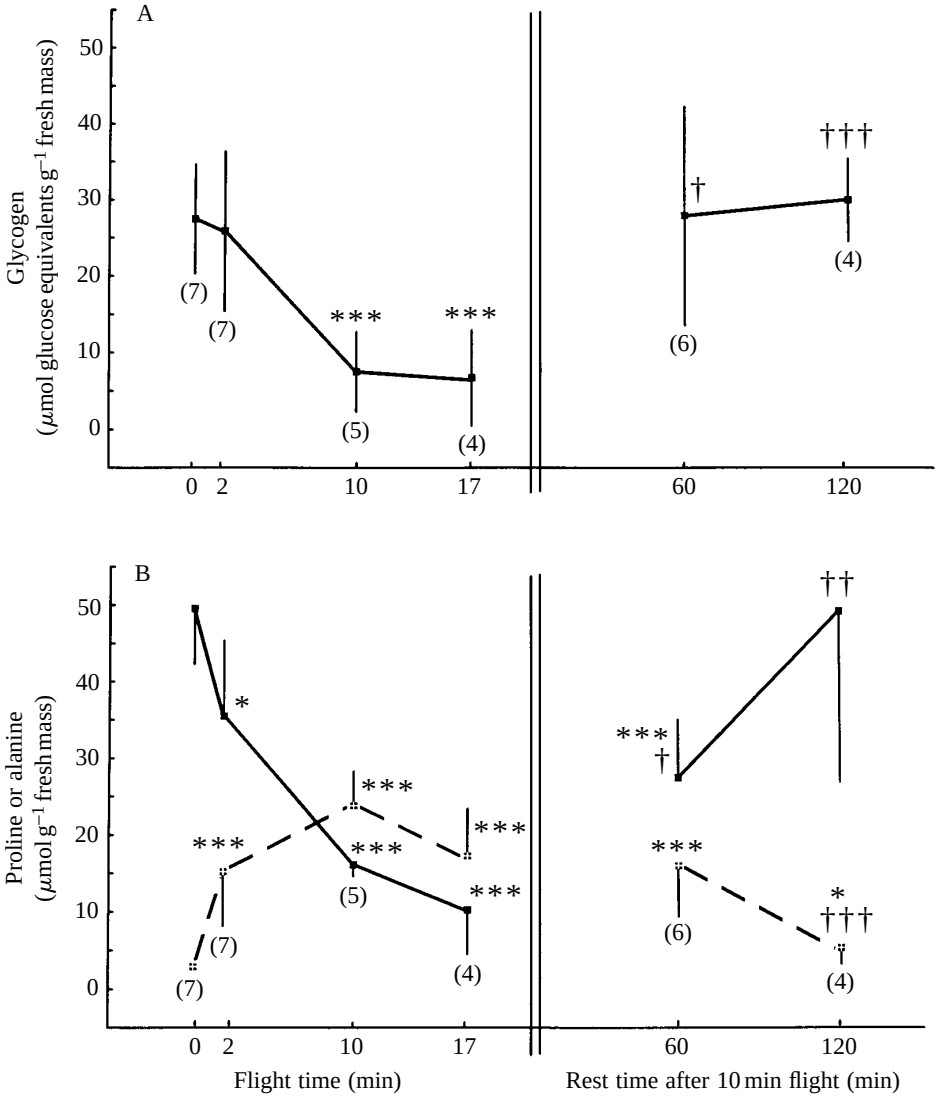


Fig. 2. Concentrations of glycogen (A) and proline (solid lines) and alanine (dashed lines) (B) in the flight muscles of *D. lunata* during various times of flight and during a rest period following 10 min of flight. Values are given as means ± S.D.; *N* is shown in parentheses for each value. Significance was calculated using Student's *t*-test. **P*≤0.05, ***P*≤0.01, ****P*≤0.005 compared with preflight levels; †*P*≤0.05, ††*P*≤0.01, †††*P*≤0.005 compared with the value after 10 min of flight.

maximal respiratory capacity of a particular mitochondrial preparation (van Hinsbergh *et al.* 1980). As illustrated in Fig. 1, mitochondria from blister beetles oxidise proline at a much higher rate than mitochondria from *L. migratoria* ($P \leq 0.05$). In contrast, pyruvate in combination with malate exhibited a high oxidation rate in locusts, but the rate was low in *D. lunata* ($P \leq 0.001$); respiration rate with pyruvate alone was negligible in both preparations. Glycerol 3-phosphate showed the highest rates in both species.

Both sets of indirect measurements are supportive of a major participation of both proline and carbohydrates during flight muscle contraction.

### *Direct measurements during flight and subsequent rest periods*

#### *Concentrations of metabolites in the thorax*

During the first 2 min of flight, glycogen levels remained unchanged, but they decreased significantly over the next 8 min from an initial 27 $\mu$mol glucose equivalents $g^{-1}$ fresh mass to 7.5 $\mu$mol $g^{-1}$ fresh mass, at which level they stayed until 17 min of flight (Fig. 2A). During a 1 h rest period after a 10 min flight, glycogen levels reached pre-flight values.

Proline concentrations decreased continuously during flight, apparently at a higher rate at the onset of flight (Fig. 2B). A significant drop from the initial 49.3 $\mu$mol $g^{-1}$ fresh mass to 35.5 $\mu$mol $g^{-1}$ fresh mass was clearly evident after 2 min of flight. Restoration of pre-flight levels was slow and took 2 h. The alanine concentration followed the proline pattern as a mirror image, resulting in a constant 40–50 $\mu$mol $g^{-1}$ fresh mass for the two amino acids together throughout the experiments, with the exception of the 17 min flight value (Fig. 2B), which was lower (30.5 $\mu$mol $g^{-1}$ fresh mass).

#### *Concentrations of metabolites in the haemolymph*

Total carbohydrate levels in the haemolymph showed a high degree of variability (Fig. 3A). A significant reduction was recorded after a flight of 10 min. The high scatter of values during the rest after flight experiments does not allow any conclusions to be drawn; it appears that recovery is a

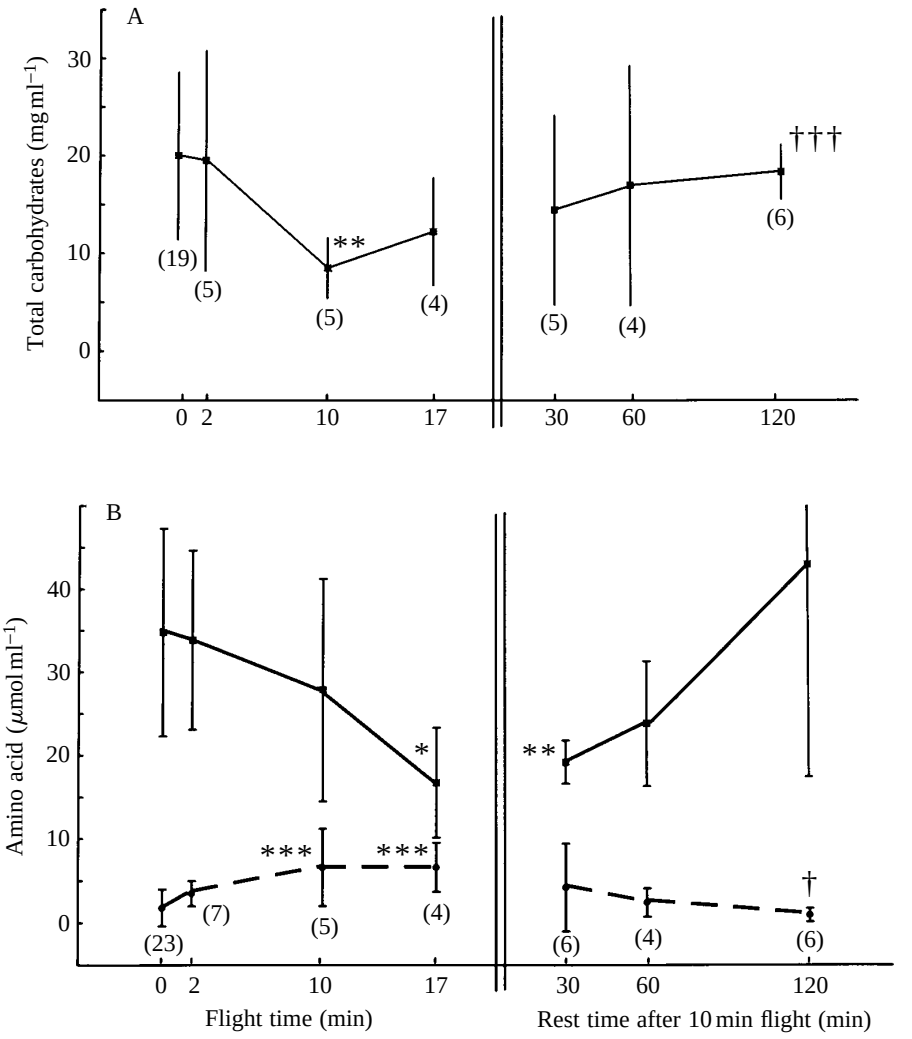


Fig. 3. Concentration of total carbohydrates (A) and proline (solid lines) and alanine (dashed lines) (B) in the haemolymph of *D. lunata* during various times of flight and a subsequent rest period after 10 min of flight. Values are given as means ± S.D.; *N* is shown in parentheses for each value. For levels of significance, see legend to Fig. 2.

protracted process, since only the value after 2 h is significantly different from the 10 min flight value. Haemolymph proline levels were also highly variable (Fig. 3B). There was a continuous decrease in concentration, and the concentration of 6.6 $\mu$mol ml$^{-1}$ after 17 min of flight was significantly lower than the initial 34.8 $\mu$mol ml$^{-1}$ level. It took 2 h of rest after 10 min of flight for the complete restoration of initial proline levels. The high variability of this value suggests that beetles do not recover simultaneously. The alanine levels were significantly increased from the initial 1.8 $\mu$mol ml$^{-1}$ to 6.6 $\mu$mol ml$^{-1}$ after 10 min of flight, when they reached a plateau (Fig. 3B). 2 h after a 10 min flight, alanine concentration had returned to the initial pre-flight levels.

The concentration of total lipids in the haemolymph did not change significantly during flight; 12.7±2.2 mg ml$^{-1}$ (mean ± S.D.; *N*=7) was measured in resting beetles, 13.7±4.6 mg ml$^{-1}$ (*N*=15) after 10 min of flight and 14.4±4.0 mg ml$^{-1}$ (*N*=8) 30 min after a flight of 10 min.

When the haemolymph volume was determined by the inulin dilution method, a significant linear relationship between the beetles' total mass (*M*) and haemolymph volume (*V*) was established, following the equation: $V$ ($\mu$l) = 0.34$M$ (mg) + 7.15; $r$=0.934; $P \leqslant 0.001$ (Fig. 4). Thus, a mean haemolymph volume of 63.2±15.3 $\mu$l (± S.D., *N*=7) was established for beetles with an average mass of 166.9±42.6 mg.

## Discussion

This study provides evidence that the blister beetle *D. lunata* uses proline as a substrate during flight. The special features of this type of energy metabolism were indicated by assaying the activities of enzymes important for the degradation of proline. When compared with the flight muscles of *L. migratoria*, which are a typical example of a muscle specialised to oxidise fatty acids, these differences in maximal activities are highlighted. This can be clearly seen from comparison of the activities of the enzymes of glycolysis/fatty acid oxidation (the ratio GAPDH/HOAD) in Table 2. In the flight muscles of locusts this ratio is low (4; or 3.1 measured by Beenakkers, 1969), whereas the flight muscles of *D. lunata* have a ninefold higher ratio.

Since MDH and ME compete for malate, the ratio MDH/ME can be used as an indicator as to whether pyruvate can be

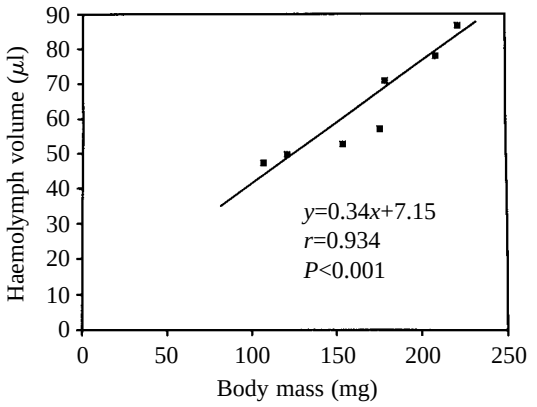


Fig. 4. Relationship between body mass and haemolymph volume (assayed by the inulin dilution method) in *D. lunata*.

obtained from the Krebs cycle in quantitative amounts to serve as acceptor for the ammonia released during deamination of glutamate (Bursell, 1981). The lowest ratio (1) was found in tsetse flies (Hoek *et al.* 1976), which are known to utilise proline exclusively. Weeda *et al.* (1980) found a ratio of 8 in *L. decemlineata*. This is similar to the ratio (7) we measured in *D. lunata*. Our ratio for *L. migratoria* (67) is in the range reported for *Schistocerca gregaria* (29) and *Periplaneta americana* (101) (Hoek *et al.* 1976). In these insects, proline is not an important flight substrate.

Measurements of the respiratory rates of isolated mitochondria from flight muscles supported the interpretation that proline might be an energy substrate for flight. Proline oxidation was much more pronounced in the blister beetle, whereas locusts showed high rates of pyruvate/malate oxidation. Glycerol 3-phosphate oxidation, an indicator of the functioning of the glycerophosphate cycle, which reoxidises NADH formed glycolytically from the cytosol, and thus carbohydrate breakdown, was similar in both insects.

The direct measurement of changes in metabolite levels in haemolymph and flight muscles during flight demonstrated unequivocally that proline was indeed an important substrate, and thus did not only serve a 'sparker' function (see Introduction). It also became clear that proline was not completely oxidised, but was only partially oxidised to alanine. Furthermore, carbohydrates in the haemolymph and glycogen in the flight muscles contributed to flight metabolism. Thus, as a first conclusion, we can state that flight metabolism of *D. lunata* is different (a) from that of the blowfly, where proline performs a 'sparker' function (Sacktor and Wormser-Shavit, 1966; Sacktor and Childress, 1967), (b) from the proposed complete oxidation of proline in the Japanese beetle (Hansford and Johnson, 1975) and; (c) from that of the tsetse fly, which uses proline exclusively (Bursell and Slack, 1976). It has a closer resemblance to the situation in the Colorado potato beetle and the fruit beetle (Weeda *et al.*1979; Zebe and Gäde, 1993). But what exactly are the contributions of the two substrates, proline and carbohydrates?

Since we did not measure the turnover rates of the substrates, we based our calculations for $O_2$ consumption on the utilisation rates measured during 10 min of flight (data shown in Figs 2 and 3). We assumed that partial oxidation of proline in *D. lunata* follows the equation given for tsetse flies and Colorado beetles (McCabe and Bursell, 1975; Weeda *et al.* 1980):

$$\text{proline} + 2.5O_2 + 14\text{ADP} + 14\text{Pi} \rightarrow \text{alanine} + H_2O + 2CO_2 + 14\text{ATP}.$$

The results of our calculations are shown in Table 3 for an individual 'model' beetle of 166.9 mg fresh mass, containing 40.1 mg of thorax (24 % of total body mass) and 63.2 μl of haemolymph (see Results). Overall, it is clear that carbohydrates play a more important role in flight than proline (3.8 *versus* 0.6 ml $O_2$ beetle$^{-1}$ h$^{-1}$, respectively; combined values for haemolymph plus thorax; see Table 3). Although the differences between the contribution of proline and glycogen are only minor in the flight muscles (41 % *versus* 59 %), carbohydrates are the major source (96 %) in the haemolymph. Interestingly, 75 % of the calculated rate of oxygen consumption stems from the haemolymph substrates and only 25 % from the flight muscles. This strikingly illustrates the importance of the haemolymph as a reservoir for energy

Table 3. *Oxygen consumption calculated from substrate utilisation during 10 min of flight*

| Compartment | Utilisation rate of substrates | Molar ratio | $O_2$ consumption (μmol $O_2$ g$^{-1}$ fresh mass min$^{-1}$) | $O_2$ consumption (ml $O_2$ beetle$^{-1}$ h$^{-1}$) | % of compartment $O_2$ consumption | % of total $O_2$ consumption |
|---|---|---|---|---|---|---|
| Haemolymph | | | | | | |
| Proline | 0.69[a] | 2.5 | 1.73 | 0.14 | 4.5 | 75 |
| Carbohydrates | 6.44[a] | 6.0 | 38.6 | 3.14 | 95.5 | |
| Thorax | | | | | | |
| Proline | 2.35[b] | 2.5 | 8.4 | 0.45 | 40.9 | 25 |
| Glycogen | 2.0[c] | 6.0 | 12.0 | 0.64 | 59.1 | |
| Total | | | | 4.4 | | 100 |

[a]Expressed as μmol ml$^{-1}$ min.
[b]Expressed as μmol g$^{-1}$ fresh mass min$^{-1}$.
[c]Expressed as μmol glucose g$^{-1}$ fresh mass min$^{-1}$.

substrates. If we calculate, from our data, the mass-specific oxygen consumption, the respiratory rate of 26.2 ml $O_2$ $g^{-1}$ $h^{-1}$ is in the low range of rates measured for various scarabaeid beetles: 38–40 ml $O_2$ $g^{-1}$ $h^{-1}$ in *Melolontha melolontha* and *Amphimallus solstitialis* (Kittel, 1941; Ludwig, 1960), 45–90 ml $O_2$ $g^{-1}$ $h^{-1}$ in *Cotinus texana* (Chappell, 1984) and 52 ml $O_2$ $g^{-1}$ $h^{-1}$ in *Popillia japonica* (Oertli and Oertli, 1990). Of course, our calculations are only an estimate of respiratory rates and they have to be considered as minimum values. First, we have not taken any contribution of abdominal tissue into account, i.e. resynthesis of proline during flight as reported for the tsetse fly and Colorado beetle (Bursell, 1977; Weeda *et al.* 1980). However, we measured the restoration of reserves in flight muscles and haemolymph during rest after flight (Figs 2, 3). Resynthesis of proline appears to be more protracted than in the fruit beetle (Zebe and Gäde, 1993), whereas glycogen is replenished more rapidly. Nevertheless, overall resynthesis taking place during the flight period will only contribute negligibly to our balance. Second, some other tissue such as the gut wall may contribute. In the locust, a flight of 15 min diminished the glycogen and glucose concentrations in this tissue by 50 % (Mayer and Candy, 1969). Thus, it seems likely that we have underestimated oxygen consumption. The contribution of carbohydrates, in this case, would be even larger. There were no indications that lipids participated as a fuel for flight in a significant manner.

During future studies we will investigate whether the flight metabolism of this insect is hormonally regulated. We will inject extracts of the beetles' corpora cardiaca and determine their influence on levels of carbohydrates and proline in the haemolymph. Additionally we will determine the activation state of glycogen phosphorylase, which is an indicator for hormonal regulation of carbohydrate metabolism.

In conclusion, we have shown that the blister beetle *D. lunata* powers the contraction of the flight muscles *via* oxidation of proline, but the contribution of proline to overall flight metabolism is small compared with that of carbohydrates. The latter are provided mainly from the haemolymph. Therefore, the blister beetle's flight metabolism, although generally similar to that of the Colorado beetle and fruit beetle, is another variation on the same theme.

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