

## PROLINE SYNTHESIS IN FAT BODY OF *LEPTINOTARSA DECEMLINEATA*

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**Abstract**—Fat body isolated from the Colorado potato beetle is capable of synthesizing proline. Maximum rate of proline synthesis is achieved with alanine as substrate. A metabolic pathway in which stored lipid participates in proline synthesis is suggested. The close relationship between flight muscle and fat body metabolism is discussed.

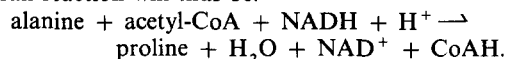
**Key Word Index:** *Leptinotarsa decemlineata*, fat body, proline synthesis, metabolic pathway

### INTRODUCTION

INSECTS are known to contain high levels of free amino acids in their haemolymph and tissues (FLORKIN and JEUNIAUX, 1974; SACKTOR, 1965). The physiological function of most of these amino acids is still not fully understood. Several studies indicate that certain amino acids play a role as energy source during flight. Proline is used as a main substrate for flight metabolism in the tsetse fly, *Glossina morsitans* (BURSELL, 1963, 1966, 1978) as well as in the Colorado beetle, *Leptinotarsa decemlineata* (MORDUE and DE KORT, 1978; BROUWERS and DE KORT, 1979; WEEDA *et al.*, 1979). Mitochondria isolated from the flight muscle of the latter insect display high rates of respiration with proline (DE KORT *et al.*, 1973 and WEEDA *et al.*, 1980), and alanine appeared to be the end product (WEEDA *et al.*, 1980). Also *in vivo* alanine is the end product of proline oxidation (BROUWERS and DE KORT, 1979; WEEDA *et al.*, 1979). Proline is conveyed to the flight muscles by the haemolymph. The proline concentration in haemolymph does not change dramatically during flight, which suggests that proline is replenished from other sources. Three possible processes can be suggested: firstly, resorption from the diet, secondly, the breakdown of fat body proteins and, finally, *de novo* synthesis in the fat body. Neither dietary amino acid intake nor protein degradation would lead to specific accumulation of large amounts of proline present in the Colorado beetle (DE KORT and KRAMER, 1976). Therefore, *de novo* synthesis by the fat body seems to be the most likely source for haemolymph proline.

Evidence obtained so far indicates that alanine, which is the product of proline oxidation in the flight muscles of the Colorado beetle participates in the synthesis of proline in the fat body (KHAN and DE KORT, 1978; WEEDA *et al.*, 1979). This has already been demonstrated in the tsetse fly where the pathways for

proline synthesis in the fat body has been elucidated (McCABE and BURSELL, 1975 and BURSELL, 1977). The overall reaction will thus be:



This paper describes an investigation on the substrates and enzymes necessary for *de novo* proline synthesis in the fat body of the Colorado potato beetle and on post-emergence development of proline synthetic capacity within the fat body.

### MATERIALS AND METHODS

#### *Insects*

Colorado potato beetles, *Leptinotarsa decemlineata* Say, 7–8 days old (unless otherwise stated) were reared under a long day photoregime as described by DE KORT (1969).

#### *Chemicals*

L-malate was obtained from Calbiochem Ltd. Amsterdam, The Netherlands. Aceto-acetyl CoA was prepared as described by DE KORT (1969). L-[U-<sup>14</sup>C] alanine, L-[U-<sup>14</sup>C] leucine, L-[U-<sup>14</sup>C] phenylalanine, [U-<sup>14</sup>C] glucose, and [U-<sup>14</sup>C] palmitate were obtained from the Radiochemical Centre, Amersham, UK (spec. activity 172, 335, 531, 310 and 403 mCi/mmol respectively). All other substrates, cofactors and coupling enzymes were purchased from Boehringer Ltd, Mannheim, W. Germany. All chemicals used were of analytical reagent grade.

#### *Dissection of the fat body tissue*

Before dissection, the beetles were chilled at 4°C for 30 min to prevent formation of excreta during dissection. Scissors and forceps were cleaned with 70% alcohol. The dorsal part of the abdominal cuticle with the attached fat tissue was dissected under ice cold *Leptinotarsa* saline (131 mM KCl, 2 mM NaCl, 1 mM CaCl<sub>2</sub> and 5 mM MgCl<sub>2</sub>). After dissection the fat bodies were washed three times in this saline solution.

#### *Preparation of fat body tissue for enzyme assay*

Three dissected fat bodies were homogenized in 2 ml 100 mM Tris-HCl pH 7.8 and 15 mM mercaptoethanol in a Potter-Elvehjem tube and further disrupted with a Branson Sonifier for 30 sec. The homogenate was centrifuged at 100,000 g for 30 min at 0°C and the supernatant used for enzyme assays, except for glycogen phosphorylase. For the

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latter enzyme activity fat bodies were homogenized in 50 mM triethanolamine buffer pH 7.0, 5 mM EDTA (ethylenediaminetetraacetic acid), and 20 mM NaF. The supernatant obtained after high speed centrifugation as above was used for phosphorylase assay.

#### Assay of enzyme activities

All reactions were carried out at 25°C in 3 ml cuvettes in a recording spectrophotometer (Zeiss PM Q 3). They were started by addition of the tissue preparation. Optimal assay conditions for the different enzyme activities studied were as follows (final concentrations):

*Glycogen phosphorylase* (EC 2.4.1.1.): 42 mM Triethanolamine buffer pH 7.0, 5.5 mM imidazole, 1.3 mM EDTA, 1.4 mM dithiothreitol (DTT), 50 mM  $\text{KH}_2\text{PO}_4$ , 0.1 mM  $\text{MgCl}_2$ , 0.24 mM NADP, 0.2 mg/ml glycogen (omitted for control), excess of phosphoglucomutase and glucose-6-P-dehydrogenase. Total phosphorylase activity was measured in the presence of 1.6 mM AMP.

*Glyceraldehyde-3-phosphate dehydrogenase* (EC 1.2.1.9.): 100 mM Tris-HCl pH 7.3, 1 mM ATP, 0.9 mM EDTA, 2 mM  $\text{MgCl}_2$ , 20 mM glyceraldehyde-3-phosphate (omitted for control), 0.2 mM NADH and excess of 3-phosphoglycerate kinase.

*Pyruvate carboxylase* (EC 6.4.1.1.): 75 mM Tris-HCl, pH 7.0, 10 mM  $\text{MgCl}_2$ , 10 mM  $\text{K}_2\text{CO}_3$ , 2.5 mM ATP, 0.15 mM acetyl-CoA (omitted for control), 10 mM pyruvate, 0.1 mM NADH and excess of malate dehydrogenase.

*Oxaloacetate decarboxylase* (EC 4.1.1.3.): assayed by the method of WEEDA *et al.* (1980).

*Malate dehydrogenase* (EC 1.1.1.37.): 100 mM Tris-HCl, pH 7.5, 2 mM NAD, 10 mM L-malate (omitted for control).

*NAD-dependent malic enzyme* (EC 1.1.1.39.): 50 mM Tris-HCl, pH 7.8, 5 mM L-malate (omitted for control), 5 mM NAD and excess of malate dehydrogenase. After malate

dehydrogenase equilibrium, the reaction was started by addition of 5 mM  $\text{MgCl}_2$ .

*NADP-dependent malic enzyme* (EC 1.1.1.40.): 100 mM Tris-HCl pH 7.8, 2 mM L-malate (omitted for control), 0.05 mM NADP and 5 mM  $\text{MgCl}_2$ .

*Alanine dehydrogenase* (EC 1.4.1.1.): 50 mM Tris-HCl pH 10.0, 0.5 mM EDTA, 0.8 mM NAD and 10 mM L-alanine (omitted for control).

*Citrate synthase* (EC 4.1.3.7.): 200 mM Tris-HCl pH 8.0, 10 mM L-malate (omitted for control), 5 mM EDTA, 0.3 mM acetyl-CoA and excess of malate dehydrogenase.

*NAD-dependent isocitrate dehydrogenase* (EC 1.1.1.41.): 100 mM imidazole pH 7.0, 5 mM L-isocitrate (omitted for control), 4 mM  $\text{MgCl}_2$ , 1 mM ADP and 1 mM NAD.

*NADP-dependent isocitrate dehydrogenase* (EC 1.1.1.42.): as for the NAD-dependent enzyme except that the reaction was carried out at pH 7.5 and 0.8 mM NADP replaced the NAD in the reaction mixture.

*ATP citrate lyase* (EC 4.3.2.8.): assayed by the method of STOREY and BAILEY (1978b) except that the reaction was carried out with 50 mM triethanolamine buffer pH 7.5 and 10 mM citrate.

*Aspartate oxoglutarate aminotransferase* (EC 2.6.1.2.), *alanine oxoglutarate aminotransferase* (EC 2.6.1.1.), *glutamate dehydrogenase* (EC 1.4.1.2.) and *3-hydroxyacyl CoA dehydrogenase* (EC 1.1.1.35.) were carried out as described by KHAN and DE KORT (1978).

#### In vitro experiments

After dissection, groups of five fat bodies were incubated in 2 ml saline for 2 hr at 25°C under constant shaking. Proline synthesis was initiated by addition of various substrates either radiolabelled (2  $\mu\text{Ci}$ ) or unlabelled. At the end of each incubation period an aliquot of the medium was immediately transferred into ice-cold TCA (final concentration 2%), and

Table 1. The activities of enzymes involved in glycolysis, Krebs cycle, fatty acid oxidation, amino acid metabolism and lipogenesis in fat body of the Colorado beetle

| Enzyme                                     | Enzyme activity<br>( $\mu\text{mole/hr/mg protein}$ ) |
|--|---|
| <i>Glycolysis:</i>                         |   |
| Glycogen phosphorylase (– AMP)             | 1.5 $\pm$ 0.4   |
| Glycogen phosphorylase (+ AMP)             | 2.1 $\pm$ 0.3   |
| Glyceraldehyde-3-phosphate dehydrogenase   | 34.1 $\pm$ 6.9  |
| <i>Krebs cycle and other NAD- and NADP</i> |   |
| <i>Dependent enzymes:</i>                  |   |
| Citrate synthase                           | 4.9 $\pm$ 1.3   |
| NAD-dependent malic enzyme                 | < 0.3   |
| NADP-dependent malic enzyme                | 17.2 $\pm$ 2.5  |
| Oxaloacetate decarboxylase                 | < 0.3   |
| Pyruvate carboxylase                       | 3.3 $\pm$ 1.7   |
| Malate dehydrogenase                       | 52.9 $\pm$ 5.1  |
| Alanine dehydrogenase                      | 4.2 $\pm$ 1.2   |
| NAD-dependent isocitrate dehydrogenase     | 0.4 $\pm$ 0.1   |
| <i>Fatty acid oxidation:</i>               |   |
| 3-hydroxyacyl-CoA dehydrogenase            | 49.5 $\pm$ 3.7  |
| <i>Lipogenesis:</i>                        |   |
| ATP-citrate lyase                          | < 0.3   |
| NADP-dependent isocitrate dehydrogenase    | 8.3 $\pm$ 1.9   |
| <i>Amino acid metabolism:</i>              |   |
| Aspartate-oxoglutarate aminotransferase    | 18.1 $\pm$ 3.5  |
| Alanine-oxoglutarate aminotransferase      | 74.5 $\pm$ 8.5  |
| Glutamate dehydrogenase                    | 2.4 $\pm$ 0.6   |

Values are the mean  $\pm$  SD of at least four determinations, each determination involving pooled tissue from several insects.

Table 2. The effect of different additions on the rate of proline synthesis *in vitro* in the fat body of the Colorado beetle

| Additions    | Concentration (mM) | Proline synthesis ( $\mu\text{g/hr/mg protein}$ ) |
|--------------|--------------------|---|
| No additions | —                  | 13.5 $\pm$ 3.2 (10)                               |
| Alanine      | 4                  | 87.1 $\pm$ 11.7 (10)                              |
| Alanine      | 29                 | 78.5 $\pm$ 14.4 (10)                              |
| Leucine      | 5                  | 16.3 $\pm$ 2.3 (5)                                |
| Glutamate    | 5                  | 28.6 $\pm$ 8.9 (5)                                |
| Aspartate    | 5                  | 43.6 $\pm$ 7.4 (5)                                |
| Glucose      | 7                  | 13.9 $\pm$ 4.2 (5)                                |

Fat body tissue from five insects was incubated for 2 hr in *Leptinotarsa* saline with substrates indicated. The results are expressed as the mean  $\pm$  SD. In parentheses the number of determinations.

concentrations of alanine and proline were determined. Alanine was assayed as described by WILLIAMSON (1970) and proline according to BERGMAN and LOXLEY (1970) using toluene to extract the ninhydrin complex. Other amino acids in these samples were analysed on a Beckman type Multichrom amino acid analyser. Radioactivity present in proline and the other amino acids, was determined by the method of DE ZWAAN and VAN MARREWIJK (1973).

The five fat bodies were subsequently processed for determination of protein content by the method of BRADFORD (1976) using BSA (fraction V) as a standard.

## RESULTS

### Enzyme assays

The results of our enzyme studies on the fat body of the Colorado beetle are summarized in Table 1. When compared with the enzyme activities present in the fat body of the cockroach, *Periplaneta americana* and the locust *Locusta migratoria*, the activities of NADP-dependent malic enzyme, alanine-oxoglutarate aminotransferase, 3-hydroxyacyl-CoA dehydrogenase (HOAD), glyceraldehyde 3-P-dehydrogenase and malate dehydrogenase are relatively high. The activity of glycogen phosphorylase is relatively low. It is also noteworthy that 70% of the total phosphorylase is activated.

### Proline synthesis by fat bodies *in vitro*

In a preliminary experiment with alanine as a substrate it was shown that proline synthesis starts immediately after substrate administration and is linear up to at least 4 hr (results not shown). Routinely, proline synthesis was measured using 2 hr incubations.

As shown in Table 2, proline synthesis was stimulated between six- to seven-fold by addition of alanine. The other amino acids either do not stimulate

or are much less effective in stimulating proline synthesis. Glucose in the absence of alanine is completely ineffective. The results strongly indicate that alanine participates in proline synthesis.

### Metabolic pathway

Apart from participating in proline synthesis as indicated in the overall reaction given in the Introduction, alanine itself may also serve as a source of acetyl-CoA. In this view one molecule is carboxylated to give a four carbon moiety, while a second molecule will be decarboxylated to provide the two carbon moiety required for the condensation reaction. The overall reaction then would be:



To distinguish between the alternatives an *in vitro* experiment was performed in which proline formation was measured together with the disappearance of alanine. The results given in Table 3 show that the utilization of alanine and the accumulation of proline are nearly equimolar. This means that the two carbon moiety required for the condensation reaction with oxaloacetate must be derived from an other source, e.g. glycogen and/or fatty acids.

In order to examine the origin of this two carbon fragment, *in vitro* incubations were performed using [ $^{14}\text{C}$ ] labelled glucose or palmitate. In the absence of alanine in the assay system, glucose and palmitate are not capable of producing proline in the fat body. However, in the presence of 4 mM alanine radiocarbon from both substrates was incorporated into proline (Table 4). The incorporation from palmitate was much higher than from glucose. The results are not corrected for differences in the endogenous pool sizes of glucose and palmitate.

Table 4 shows proline synthesis in the presence of 4 mM alanine and [ $^{14}\text{C}$ ] leucine, [ $^{14}\text{C}$ ] alanine and [ $^{14}\text{C}$ ] phenylalanine. Radioactivity from phenylalanine is not incorporated into proline, while that from [ $^{14}\text{C}$ ] leucine is only weakly incorporated. The very high incorporation of radioactivity from [ $^{14}\text{C}$ ] alanine into proline is in agreement with Table 3 and indicates that the carbon moiety is incorporated into the proline molecule. Incorporation of the carbon moiety of these substrates into other amino acids could not be detected, except with [ $^{14}\text{C}$ ] glucose. In these incubations [ $^{14}\text{C}$ ] glucose radiocarbon becomes incorporated into alanine and aspartate as well (results not shown).

### Proline synthesis and enzyme activity in fat body during adult maturation

During growth and differentiation of the flight

Table 3. The relation between alanine utilization and proline synthesis in the fat body from the Colorado beetle *in vitro*

| Substrate (mM)      | Alanine utilization ( $\mu\text{mole/hr/mg protein}$ ) | Proline synthesis ( $\mu\text{mole/hr/mg protein}$ ) |
|---------------------|--|--|
| Alanine (4)         | 0.76 $\pm$ 0.1 (6)                                     | 0.75 $\pm$ 0.1 (6)                                   |
| Leptinotarsa saline | —  | 0.12 $\pm$ 0.1 (6)                                   |

The results are the mean  $\pm$  SD. In parentheses number of determinations. Each determination was performed on pooled tissue from five insects.

Table 4. *In vitro* incorporation of radioactivity from [ $^{14}\text{C}$ ]-labelled substrates into proline with alanine as an accessory substrate in the fat body of the Colorado beetle

| $^{14}\text{C}$ -compound added | Incorporation of [ $^{14}\text{C}$ ] carbon moiety into proline (dpm/ $\mu\text{g}$ proline/hr/mg protein) |     |     |        |
|---------------------------------|--|-----|-----|--------|
|                                 | a  | b   | c   | $\chi$ |
| Alanine*                        | 256  | 347 | 454 | 352    |
| Phenylalanine                   | nd   | nd  | nd  | nd     |
| Leucine                         | 37   | 22  | 21  | 27     |
| Glucose                         | 37   | 52  | 39  | 43     |
| Palmitate                       | 110  | 98  | 108 | 105    |

a, b and c refer to separate determinations. Each experiment was performed on pooled tissue from five insects. Reaction medium contained 4 mM unlabelled alanine and 2  $\mu\text{Ci}$  of the appropriate substrate. Abbreviations: nd; not detectable,  $\chi$ , the mean of three determinations.

\* From the incorporation of radioactivity the specific activity of proline could be calculated if the proline synthesis and the amount of protein (440–500  $\mu\text{g}$  and 5 mg) is taken into account.

muscle, which in this insect occurs mainly after adult emergence (DE KORT, 1969), the specific activity of proline oxidation increased quite strongly (DE KORT *et al.*, 1973). This suggests that the capacity of proline synthesis in the fat body may change during adult maturation. In order to investigate this the activities have been measured of a number of key enzymes for proline synthesis at different times after adult emergence. In addition, the capacity of the fat body to synthesize proline at various days after adult emergence has been determined. The results (Table 5) show a correlation between the capacity of proline synthesis and the activities of these enzymes. It is quite obvious that proline synthesis increases after adult emergence in particular between days 4 and 8.

## DISCUSSION

The present work demonstrates the ability of the fat body of the Colorado potato beetle to synthesize proline and identifies possible pathways (Fig. 1).

Table 2 shows that *in vitro* fat body synthesizes proline only in the presence of alanine or, to a lesser extent, aspartate. The rate of proline synthesis *in vitro* is in fair agreement with the data of BURSELL (1977) for the tsetse fly. The proline synthetic capacity of 400–500  $\mu\text{g}$  proline per hour per beetle is more than sufficient to account for the observed half-life of [ $^{14}\text{C}$ ]-proline in the resting beetle (DE KORT and KRAMER, 1976), if it is assumed that the fat body represents 5 mg of protein per beetle. However the

synthetic capacity is low compared with the rate of proline oxidation by isolated mitochondria from flight muscle. From the rate of oxygen consumption with proline as substrate this can be calculated to be 1650  $\mu\text{g}$  proline per hour per beetle, as the thorax contains about 1 mg of mitochondrial protein. This could mean that flight duration in Colorado potato beetle is limited by availability of this energy substrate, as it has been shown to be in the tsetse fly (HARGROVE, 1976).

Table 3 shows that during incubation of fat body with alanine, the disappearance of alanine is accompanied by the formation of equimolar amounts of proline. Moreover the results of the incorporation experiment in Table 4 with [ $^{14}\text{C}$ ]-alanine fit in with the role of this amino acid as the main precursor for proline synthesis as shown in the overall equation in the Introduction. These results rule out the function of fat body alanine dehydrogenase in proline synthesis; therefore alanine itself cannot serve as a source of acetyl-CoA for proline synthesis. The origin of this two carbon moiety must be derived from other sources within the fat body. To determine the origin of these acetyl-CoA units, [ $^{14}\text{C}$ ]-labelled glucose, palmitate, phenylalanine and leucine were used. Radioactive carbon from all compounds, except [ $^{14}\text{C}$ ]-phenylalanine, was incorporated into proline (Table 4). The rate of incorporation into proline was highest in the presence of [ $^{14}\text{C}$ ]-palmitate. So these experiments, together with the results in Table 2, support our conclusion that fatty acids must be considered as the main source of acetyl-CoA for proline synthesis.

Table 5. Specific activities of some enzymes and capacity of proline synthesis of fat body at different stages of adult maturation of the male Colorado beetle

| Age (day) | Malic enzyme   | 3-hydroxyacyl-CoA dehydrogenase | Alanine-oxoglutarate aminotransferase | Aspartate-oxoglutarate aminotransferase | Glyceraldehyde-3-P dehydrogenase | Proline synthesis |
|-----------|----------------|---------------------------------|---------------------------------------|---|----------------------------------|-------------------|
| 0         | 1.6 $\pm$ 0.3  | 11.8 $\pm$ 1.8                  | 16.7 $\pm$ 3.2                        | 14.2 $\pm$ 2.0                          | 12.5 $\pm$ 3.0                   | 11.7 $\pm$ 5.3    |
| 2         | 7.1 $\pm$ 1.1  | 20.5 $\pm$ 4.1                  | 20.5 $\pm$ 3.8                        | 27.8 $\pm$ 0.8                          | 28.0 $\pm$ 2.5                   | 18.3 $\pm$ 6.2    |
| 4         | 7.3 $\pm$ 0.7  | 20.6 $\pm$ 2.1                  | 25.1 $\pm$ 3.5                        | 13.1 $\pm$ 2.1                          | 16.5 $\pm$ 1.5                   | 26.1 $\pm$ 3.5    |
| 6         | 10.4 $\pm$ 1.2 | 31.4 $\pm$ 3.5                  | 36.0 $\pm$ 3.2                        | 17.4 $\pm$ 3.0                          | 21.4 $\pm$ 2.0                   | 42.0 $\pm$ 5.2    |
| 8         | 15.2 $\pm$ 1.5 | 42.5 $\pm$ 5.4                  | 60.0 $\pm$ 9.5                        | 14.0 $\pm$ 0.5                          | 28.2 $\pm$ 3.0                   | 60.3 $\pm$ 4.5    |

Results are expressed as mean  $\pm$  SD of four determinations. Enzyme activities are expressed as  $\mu\text{mole}$  of substrate utilized/hr/ mg protein. Proline synthesis is expressed as  $\mu\text{g}$  proline synthesized/hr/mg protein.

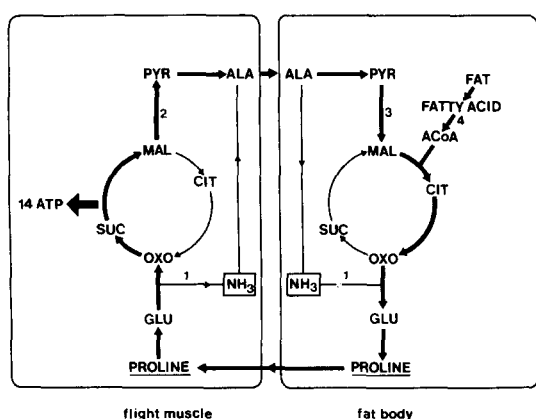


Fig. 1. The proposed relationship between flight muscle and fat body metabolism in the Colorado potato beetle. 1 = alanine-oxoglutarate aminotransferase, 2 = NAD-dependent malic enzyme, 3 = NADP-dependent malic enzyme, 4 = 3-hydroxyacyl-CoA dehydrogenase. For further explanation see Discussion.

The enzymic studies also support the view that the pathway of proline synthesis in the Colorado potato beetle operates as outlined in Fig. 1. The participating enzymes in proline synthesis studied are: alanine-oxoglutarate aminotransferase (1); NADP-dependent malic enzyme (3) and 3-hydroxy-acyl-CoA dehydrogenase (4). These enzymes show higher activities in the fat body of the Colorado potato beetle than in the fat body of the American cockroach or the migratory locust, insects which use carbohydrates, and carbohydrates or lipid, for flight metabolism respectively (Table 6). In addition, the activities of these enzymes increase with adult age (Table 5). Age-related changes in the activities of malic enzyme, 3-hydroxyacyl-CoA dehydrogenase and alanine-oxoglutarate amino-transferase correlate nicely with the capacity of the fat body to synthesize proline (Table 5). The proline-synthetic capacity of the fat body also correlates with age changes in the capacity of mitochondria from flight muscles to

oxidize proline (DE KORT *et al.*, 1973). As development and differentiation of flight muscles in Colorado potato beetle occurs mainly after adult emergence (DE KORT, 1969), the time relations between development of flight muscle and the capacity to mobilize energy substrates in fat body suggests a close correlation between these two events. This emphasizes the relation between metabolism in flight muscles and fat body during flight. During rest, the cycle of proline oxidation and proline synthesis will be in steady state because the capacity of fat body to synthesize proline is more than enough to meet the energy demands. The system will shift far from steady state after the onset of flight. The capacity to synthesize proline then might change drastically and this process might be under neuroendocrine control. Hormonal influences on proline synthesis in fat body will be the subject of another paper.

Taken together, the results presented in this paper show convincingly that alanine is the main amino acid substrate for proline synthesis in fat body of the Colorado potato beetle. Alanine, produced during proline oxidation in flight muscles (WEEDA *et al.*, 1980) is transported via the haemolymph to the fat body, where proline is resynthesized from alanine and acetyl-CoA derived from fatty acid oxidation. The proline passes back to the haemolymph for transport to the flight muscles, so completing the cycle. Thus this system can be regarded as a special mechanism for the utilization of fatty acids from the fat body. The present results are, in general, similar to those obtained with the tsetse fly. MCCABE and BURSELL (1975) and BURSELL (1977) suggested in this fly a similar cycle of proline synthesis and utilization with alanine acting as the recycling nitrogen intermediate. However there is one difference between Colorado potato beetle and tsetse fly. BURSELL and SLACK (1976) suggested that about 20% of proline oxidation by mitochondria of the tsetse fly flight muscle is complete, which means that ammonia is formed due to the participation of glutamate dehydrogenase. The studies with mitochondria from flight muscles of the Colorado potato beetle clearly established alanine as the only

Table 6. A comparison of activities of the enzymes involved in glycolysis, fatty acid oxidation, Krebs cycle and amino acid metabolism in fat body of some insects

| Enzyme                                     | <i>Leptinotarsa decemlineata</i><br>fat body | <i>Periplaneta americana</i><br>fat body | <i>Locusta migratoria</i><br>fat body |
|--|--|--|---------------------------------------|
| Glycogen phosphorylase (– AMP)             | 1.5  | 1.6 <sup>a</sup>                         | 2.0 <sup>c</sup>                      |
| Glycogen phosphorylase (+ AMP)             | 2.1  | 9.8 <sup>a</sup>                         | 18.0 <sup>c</sup>                     |
| 3-hydroxyacyl-CoA<br>dehydrogenase         | 49.5   | <0.1 <sup>b</sup>                        | 4 <sup>d</sup>                        |
| Citrate synthase                           | 4.9  | 0.3 <sup>b</sup>                         | 1 <sup>d</sup>                        |
| Alanine-oxoglutarate<br>aminotransferase   | 74.5   | 8.5 <sup>b</sup>                         | 9 <sup>d</sup>                        |
| Aspartate-oxoglutarate<br>aminotransferase | 18.1   | 5.8 <sup>b</sup>                         | 23 <sup>d</sup>                       |
| Glutamate dehydrogenase                    | 2.4  | 0.3 <sup>b</sup>                         | 1 <sup>d</sup>                        |
| NADP-dependent malic enzyme                | 17.2   | 3.6 <sup>b</sup>                         | nd                                    |

<sup>a</sup> Taken from STOREY and BAILEY (1978a).

<sup>b</sup> Taken from STOREY and BAILEY (1978b).

<sup>c</sup> Taken from VAN MARREWIK and BEENAKKERS (unpublished observations).

<sup>d</sup> Taken from KHAN and DE KORT (1978).

The enzyme activities have been adjusted to the same units as those for the fat body enzymes in the Colorado potato beetle, i.e.  $\mu\text{mole/hr/mg protein}$ . All activities were measured at 25°C. Abbreviation: nd; not determined.

nitrogenous end-product of proline oxidation (WEEDA *et al.*, 1980).

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