REGULATION OF SUBSTRATE UTILIZATION IN THE FLIGHT MUSCLE OF THE LOCUST, *LOCUSTA MIGRATORIA*, DURING FLIGHT

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Abstract—The concentrations of metabolites involved in carbohydrate metabolizing pathways, of amino acids and adenine nucleotides in the flight muscles of *Locusta migratoria* were determined during a flight of 2 hr. During the first 15 min of flight, glycogen and glucose concentrations decreased to reach approximately constant levels. The concentration of glucose-6-phosphate increased rapidly. After an initial rise the concentration of fructose-1, 6-diphosphate, dihydroxyacetone phosphate and pyruvate reached constant levels within 1–5 min of flight. No changes were found in concentrations of other intermediates of the glycolytic pathway. The concentration of malate increased sharply, then decreased slightly, and then remained constant throughout the entire flight. Glycerol concentration increased about threefold.

The concentrations of most amino acids were apparently not altered. Aspartate and alanine concentrations, however, showed a gradual increase during the first 10 min of flight, then decreased again to resting levels. The glutamate concentration decreased rapidly. The significance of this amino acid acting as a 'sparker' for citric acid cycle, is discussed along with the controlling sites of glycolytic flux.

Key Word Index: Locusta migratoria, metabolite, control enzyme, regulation, flight muscle

INTRODUCTION

IT IS WELL established that locusts *Locusta migratoria* and *Schistocerca gregaria* can use both carbohydrates and fatty acids for flight muscle metabolism (BEENAKKERS, 1969a; CANDY, 1970). Haemolymph trehalose concentration falls rapidly during the first 10 min of flight and is stabilized at around 50% of the initial concentration after 30 min (HOUBEN and BEENAKKERS, 1975; JUTSUM and GOLDSWORTHY, 1976). Concomitant with the decrease in trehalose level, the diglyceride content of the haemolymph is raised at the expense of fat body lipids under the action of the adipokinetic hormone (BEENAKKERS, 1969b; MAYER and CANDY, 1969). KROGH and WEIS-FOGH (1951) explained the changes in carbohydrate and lipid levels in the haemolymph as the result of their successive utilization for energy production. Trehalose is the main substrate during the first half hour of flight; however, during flights of longer duration, trehalose still appears to be consumed, although at a much lower rate than before (JUTSUM and GOLDSWORTHY, 1976; VAN DER HORST et al., 1978a), the main substrate then being fatty acids derived from haemolymph diglycerides. The initiator for the change in substrate consumption in the muscles is as yet unknown. Recently it has been argued that the adipokinetic hormone stimulates the oxidation of diglycerides, whereas there is a reduction of trehalose utilization (ROBINSON and GOLDSWORTHY, 1976). An enhanced rate of β-oxidation might inhibit glycolysis, this inhibition taking place under the influence of metabolic intermediates acting as modulators of glycolytic enzymes.

In mammalian heart muscle it is known that carbohydrate consumption is inhibited in the presence of long chain fatty acids (NEWSHOLME et al., 1962; BOWMAN, 1962). The first irreversible step of glycolysis is catabolized by phosphofructokinase, an enzyme under allosteric regulation, whose activity is inhibited by ATP and citrate (AHLFORS and MANSOUR, 1969). In the inhibition of glycolysis by β-oxidation high citrate and ATP levels, and low levels of AMP and P_i were concerned (NEELY et al., 1970).

Phosphofructokinases from flight muscles of insects, however, are not inhibited by citrate (WALKER and BAILEY, 1969). In the present paper the changes in concentration of twenty nine flight muscle metabolites of *L. migratoria* during flight are described. The possible participation of these metabolites in regulating substrate consumption is discussed.

MATERIALS AND METHODS

Adult male locusts, *Locusta migratoria migratorioides* (R&F), 12 days after ecdysis, were used for all experiments. The locusts were reared under crowded conditions at 30°C, 40% r.h. and a photoperiod of 12 hr. The insects were fed with float-grass, supplemented with rolled oats. Locusts were flown on motor-driven roundabouts, a gentle stream of air being passed over the locust. Immediately after flight the abdomen was cut off, the head removed together with the foregut, and the thorax was quickly plunged in liquid nitrogen.

Dorsolongitudinal flight muscles (muscles 81 and 112, ALBRECHT, 1953) were dissected in frozen state, freed from adhering fat body, and kept under liquid nitrogen. Muscles of ten locusts were pooled for each extract and powdered under liquid N_2 with pestle and mortar. The powdered tissue was immediately mixed with 5 ml of pre-cooled 6% perchloric acid solution in a pre-weighed centrifugal tube and re-

weighed; the difference in weight was taken as a measure of the muscle fresh weight. After standing for 10 min in an ice bath the tubes were centrifuged at 10,000 g for 5 min, 0°C. The residue was reextracted with 2 ml 3% perchloric acid, and after a second centrifugation; the washing was added to the supernatant fluid of the first centrifugation; the volume was adjusted to 8 ml with distilled water.

The solution was adjusted to the required pH with 5 M potassium carbonate (the pH depending on the metabolites to be measured). After 10 min all potassium perchlorate is precipitated and the supernatant fluid was pipetted off. Concentrations of the various intermediates were determined according to BERGMEYER (1974), using a Zeiss PM-6 spectrophotometer. All auxiliary enzymes were obtained from Boehringer, Mannheim.

Trehalose was assayed according to the anthrone method of MOKRASH (1954), after hydrolysis of glucose-1-phosphate with 0.05 M H_2SO_4 and destroying reducing sugars with 6 M NaOH according to the method of WYATT and KALF (1957). Analysis of free amino acids in the supernatant was performed with an automatic amino acid analyser (LKB-3201), using cation-exchange resin Durrum DC-6A, with lithium citrate buffers (column size 30 × 0.6 cm i.d.) according to BENSON et al. (1967). Ninhydrin reagent (MOORE, 1968) was added continuously to the eluate and extinction was measured at 570 and 440 nm. For all metabolites each value is the mean of two to six extracts. The significance of the apparent changes was computed by means of analysis of variance; the contrasts were judged according to the method of SCHEFFE (1953). P-Values, size of contrast and its interval at 0.95 probability level are given in parenthesis. Metabolite concentrations in the text are given in μmoles/g fresh weight ± standard deviation. Coefficients of variation are given in the legends of the figures.

RESULTS

The main reserve carbohydrates in the locust are glycogen and trehalose. In Fig. 1, sequential changes in the concentration of both substrates in the flight muscle extracts on initiation of flight and during continued flight are shown. Within 15 min of flight there is a fall in the concentrations of glycogen ($P < 0.01$; 11.1, 1.2–21.1) and glucose ($P < 0.001$; 2.4, 0.8–4.0) to approximately half the initial values. There was no significant change in trehalose as judged by the statistical method used. During prolonged flight, concentrations of glycogen and glucose do not show any further notable changes. Thus the major changes in carbohydrate reserves take place during the first 10 min of flight. Concomitant with these changes the concentration of glucose-6-phosphate (G-6-P) increases significantly ($P < 0.001$; 0.14, 0.05–0.23), until it reaches about twice the value found at rest, remaining at this level during continued flight. The concentration of glucose-1-phosphate (G-1-P) is low and does not appear to change during flight (Fig. 1).

The data of Fig. 2 show that the concentration of fructose-6-phosphate (F-6-P) is not significantly
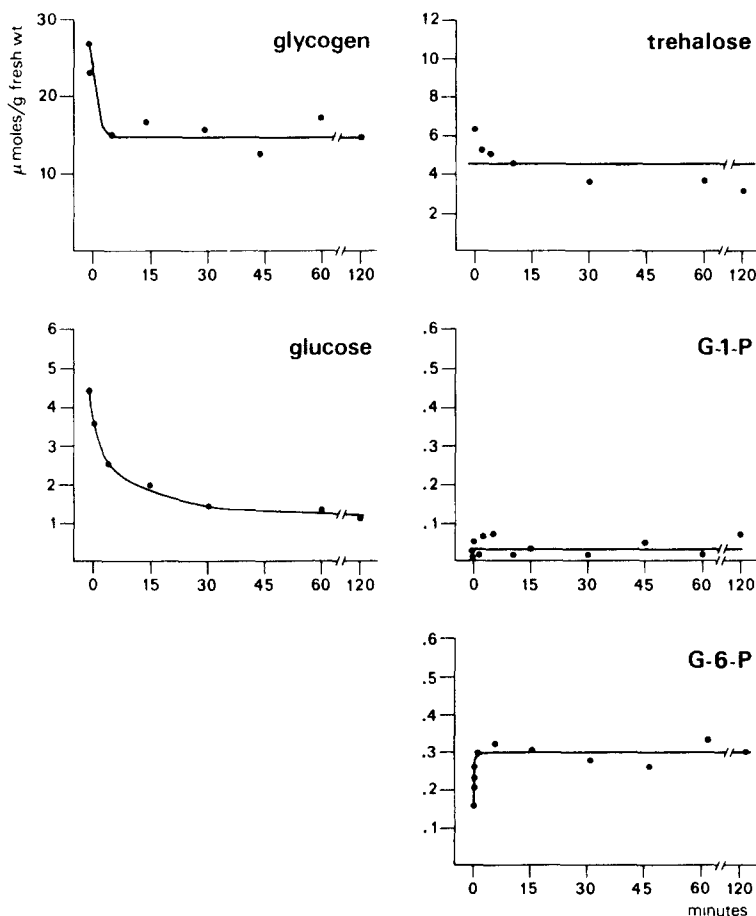

Fig. 1. The concentrations of glycogen, trehalose, glucose, glucose-1-P (G-1-P), and glucose-6-P (G-6-P) in flight muscles of locusts during flight. The average coefficients of variation are 0.17, 0.33, 0.06, 0.24 and 0.12, respectively.
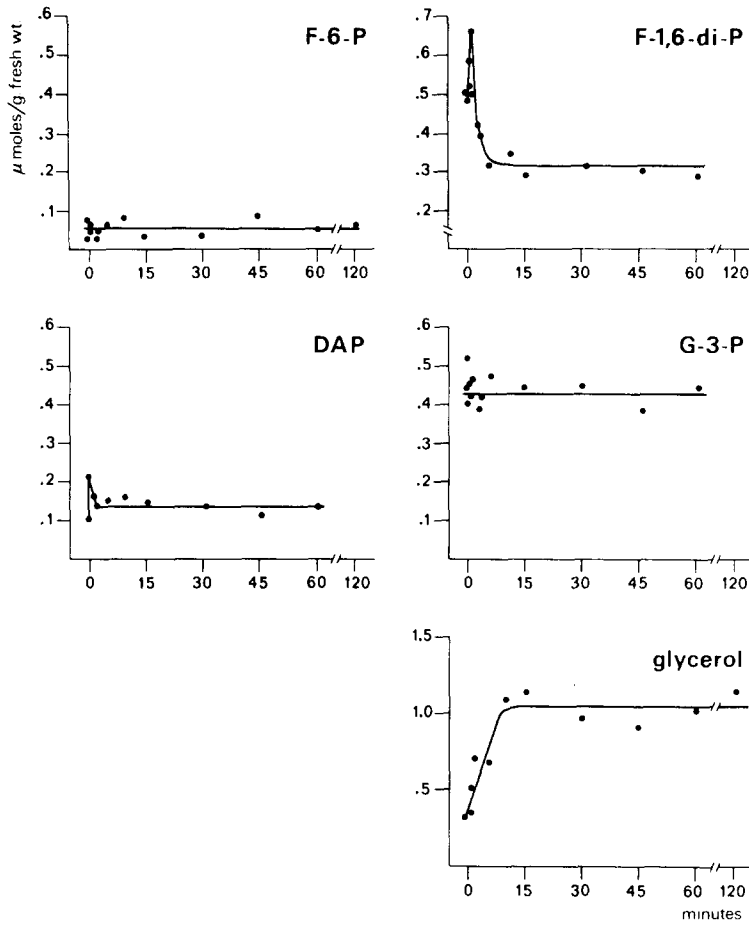
Fig. 2. The concentrations of fructose-6-P (F-6-P), fructose-1, 6-di-P (F-1, 6-di-P), dihydroxyacetone-P (DAP), glycerol-3-P (G-3-P), and glycerol in flight muscles of locusts during flight. The average coefficients of variation are 0.29, 0.10, 0.12, 0.30 and 0.27, respectively.
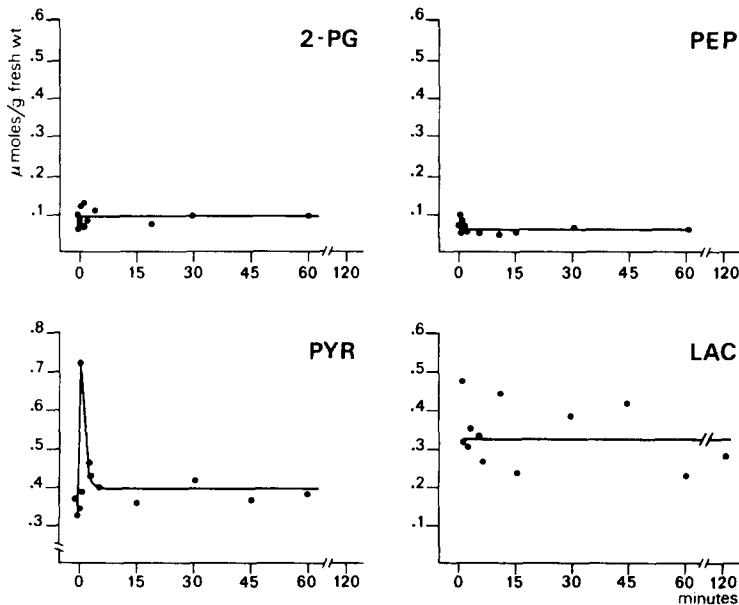

Fig. 3. The concentrations of 2-phosphoglycerate (2-PG), phosphoenolpyruvate (PEP), pyruvate (PYR), and lactate (LAC) in flight muscles of locusts during flight. The average coefficients of variation are 0.13, 0.17, 0.18 and 0.17, respectively.
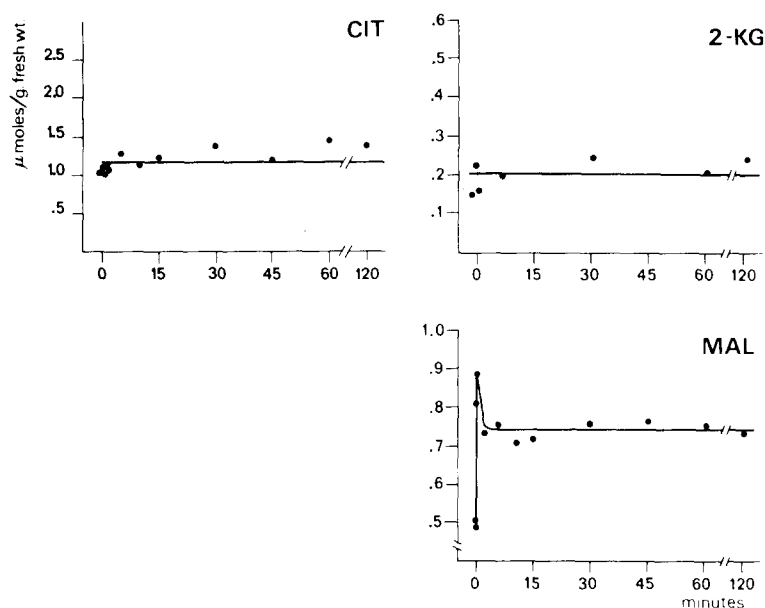
Fig. 4. The concentrations of citrate (CIT), 2-ketoglutarate (2-KG) and malate (MAL) in flight muscles of locusts during flight. The average coefficients of variation are 0.14, 0.22, and 0.10, respectively.

altered throughout flight; the concentration of fructose-1, 6-diphosphate (F-1, 6-di-P) increased during the first 0.5 min of flight ($P < 0.001$; 0.18, 0.04–0.33); then, after a sharp decrease, it is stabilized at about two thirds of the resting value during the remainder of the flight with a mean value of 0.315 ± 0.038 ($P < 0.001$; 0.17, 0.05–0.29). Dihydroxyacetone-phosphate (DAP) concentration increases in the first 0.5 min of flight ($P < 0.001$; 0.09, 0.02–0.16), but falls rapidly to a value of 0.130 ± 0.011, not significantly higher than the resting value.

The relatively high concentration of glycerol-3-phosphate (G-3-P), about four times those of other phosphorylated glycolytic intermediates, does not appear to change during flight (Fig. 2). Accumulation of glycerol (Fig. 2) was demonstrated during the first 15 min of flight ($P < 0.001$; 0.8, 0.2–1.3). This intermediate may be derived from diacylglycerol and most likely represents a value for the extracellular space (cf. Discussion). The concentration of 2-phosphoglycerate (2-PG) and phosphoenolpyruvate (PEP) are low and do not change significantly (Fig. 3). Since only a catalytic quantity of DAP is needed in the G-3-P-cycle, the excess triosephosphate will be metabolized to pyruvate. The concentration of the latter intermediate increases strikingly on the initiation of flight ($P < 0.001$; 0.4, 0.2–0.6), but already after 5 min a steady state level with a mean value of 0.403 ± 0.025, not significantly higher than the resting level, is achieved (Fig. 3). The concentration of lactate (0.345 ± 0.060) (Fig. 3) appears to remain unchanged during exercise. This may indicate no conversion of pyruvate to lactate which is in accordance with the very low activity of LDH in locust flight muscle (BEENAKKERS, 1969a; CRABTREE and NEWSHOLME, 1972).

No significant change in citrate or 2-ketoglutarate concentration was observed (Fig. 4). Malate concentration is quickly raised at the onset of flight ($P < 0.015$; 0.37, 0.02–0.73), but then falls to a level

significantly different from the resting level ($P < 0.015$; 0.22, 0.05–0.49) (Fig. 4). This increase in TCA-cycle intermediates might be at the expense of free amino acids. The concentration of proline decreases during the first 20 min of flight ($P < 0.045$; 0.16, 1.5–22.6). Also there is a rapid fall in glutamate concentration at the onset of flight ($P < 0.001$; 2.1, 0.8–3.4) (Fig. 5). Synthesis of 2-ketoglutarate from these amino acids has to be accompanied by removal of amino-N. Indeed concomitant with the decrease in proline (and glutamic acid) an increase in alanine concentration is measured although no stoichiometric relationship exists. After about 3 min of flight, alanine reaches a maximal value ($P < 0.001$; 2.6, 1.2–3.9), followed by a gradual decrease during the remainder of the flight. The concentration of aspartate is temporarily raised ($P < 0.001$; 1.4, 0.8–2.0) and then falls to a value not significantly higher than the resting value (Fig. 5). The concentrations of threonine (0.8 μmoles), serine (2.5 μmoles), asparagine and glutamine (1 μmole each), and glycine (15 μmoles) (values per g fresh weight) do not change significantly during a 2-hr flight period.

Figure 6 shows the path of the concentrations of the adenine nucleotides. ATP, ADP and AMP levels do not change significantly throughout flight with mean values of 5.05 ± 0.44, 1.67 ± 0.24 and 0.42 ± 0.12, respectively.

DISCUSSION

Studies on the dynamics of locust haemolymph trehalose and diglycerides during flight revealed that at the onset of flight the proportional contributions of carbohydrate to flight muscle metabolism is high, whereas after 15–30 min of flight, diglycerides become the predominant fuel (VAN DER HORST et al., 1978a, b). Our findings, which show that in the flight muscles neither glycogen nor trehalose are depleted
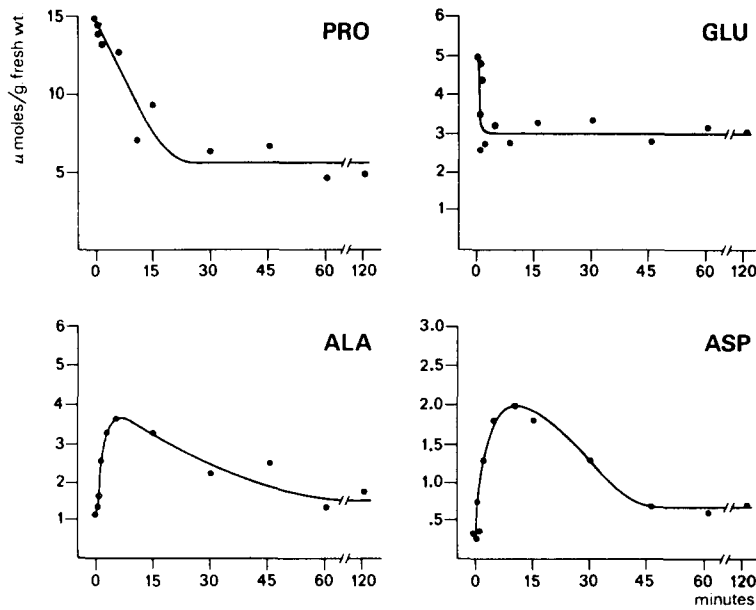
Fig. 5. The concentrations of proline (PRO), glutamate (GLU), alanine (ALA), and aspartate (ASP) in flight muscles of locusts during flight. The average coefficients of variation are 0.21, 0.10, 0.12, and 0.11, respectively.

completely within the first flight period, support the idea that a change from carbohydrate to lipid utilization is not due to absence of carbohydrate but the result of regulatory processes. It must be taken into consideration that the trehalose concentration measured in the present experiments most likely includes the trehalose present in the extracellular space, FORD and CANDY (1972) showed that in the locust, *Schistocerca gregaria*, 50% of the flight muscle liquid volume is occupied by cytosol, whereas 30% is accounted for by mitochondria and 20% by extracellular space. Attributing the changes in metabolite levels to cytosol only, is probably incorrect

for metabolites that are known to be at high concentration in haemolymph such as trehalose and glycerol, but it is of value to make comparisons of such changes even though the calculated values are not absolutely confined to muscle cytosol. Haemolymph concentrations of glycogen and other carbohydrates are known to be very low (WYATT and KALF,, 1957).

In heart muscle, NEWSHOLME and RANDLE (1964) found an increase in the concentrations of glucose-6-phosphate and citrate under conditions of decreased glycolytic flux caused by oxidation of fatty acids and ketone bodies. They supposed a near equilibrium for phosphoglucose isomerase (PGI) in this muscle, thus
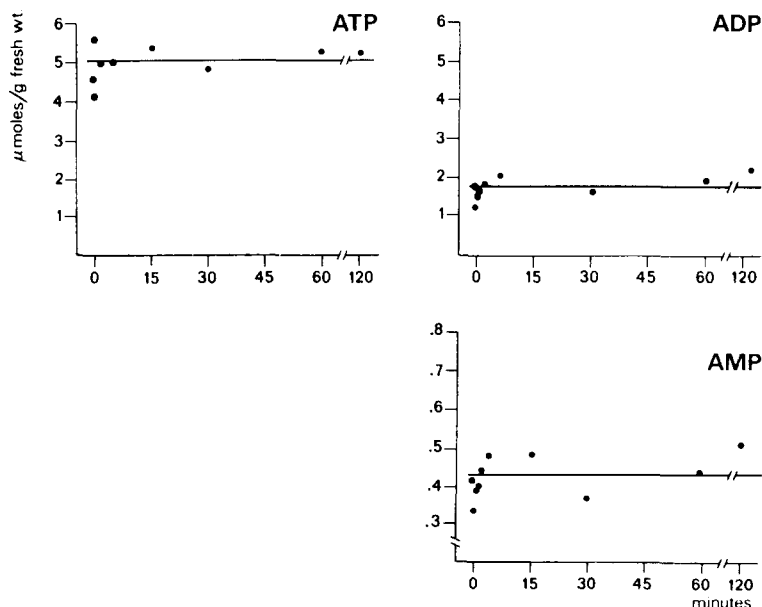

Fig. 6. The concentrations of ATP, ADP, and AMP in flight muscles of locusts during flight. The average coefficients of variation are 0.13, 0.28, and 0.29, respectively.

changes in the level of glucose-6-phosphate reflect changes in the level of fructose-6-phosphate. The increased level of glucose-6-phosphate then will be the result of a lowered activity of phosphofructokinase (PFK). Since citrate is an inhibitor of phosphofructokinase in heart muscle they supposed that the elevated level of citrate is responsible for the inhibition of phosphofructokinase and hence of glycolysis.

In *Locusta migratoria*, we found an increase in glucose-6-phosphate concentration. The change in citrate concentration was not significant. Moreover, WALKER and BAILEY (1969) found no inhibition of phosphofructokinase by citrate in flight muscle of locusts.

Glucose-6-phosphate can be metabolized either by phosphoglucose isomerase in the Embden–Meyerhof Parnas pathway or by glucose-6-phosphate dehydrogenase in the pentose phosphate cycle. The latter pathway, however, is of minor importance in muscle tissue of locusts; VOGELL et al. (1959) reported that the activity of glucose-6-phosphate dehydrogenase in flight muscle of *L. migratoria* was only 0.1% of the activity of the glycolytic chain.

A pathway of considerable importance is the glycerol-3-phosphate cycle, functioning in reoxidizing the NADH formed. Whereas under experimentally induced anaerobic conditions glycerol-3-phosphate accumulates in insect flight muscles, during flight the relatively high concentration of this intermediate remains stable, indicating complete aerobic metabolism during exercise. In the flight muscle of *L. migratoria* the mitochondrial glycerol-3-phosphate dehydrogenase seems not to be rate limiting (33 μmole/min/g fresh weight) nor is the cytoplasmic enzyme (124 μmole/min/g fresh weight) (CRABTREE and NEWSHOLME, 1972). The present study shows that dihydroxyacetone phosphate is raised for only a very short period but then decreases again and stabilizes at a value not significantly higher than the resting value. It was shown previously that only a catalytic quantity of the regenerated DAP was needed in the glycerol-3-phosphate cycle (SACKTOR and DICK, 1962), the excess triosephosphate being metabolized to pyruvate.

The threefold increase of glycerol in the flight muscle extract is most likely present in the extracellular space and probably is the result of the hydrolysis of diacylglycerol (CANDY et al., 1976). In both locusts, *L. migratoria* and *S. gregaria*, the activity of glycerol kinase, the first enzyme in the pathway of glycerol metabolism is low (NEWSHOLME and TAYLOR, 1969). During flight, haemolymph glycerol concentration is raised; probably glycerol is transported back to the fat body for re-esterification with fatty acids.

The decrease in the concentration of proline could be explained in terms of its utilization by the flight muscles. SACKTOR and CHILDRESS (1967) demonstrated a stoichiometric relationship between the utilization of proline and the formation of alanine in the blowfly, *Phormia regina*, indicating oxidation of proline to glutamate and a subsequent transamination by means of alanine–ketoglutarate aminotransferase. Our studies do not give support to the existence of such a stoichiometry. There is, however, an approximate stoichiometry between the decrease in glutamate and

the concomitant rise in alanine. Mitochondria of *L. migratoria* are permeable to glutamate which can be metabolized by the action of either alanine–ketoglutarate aminotransferase or glutamate dehydrogenase (VAN DER BERGH, 1967). The relatively high activity of the aminotransferase suggests that indeed glutamate decrease is related to the increase in alanine. The intramitochondrial 2-ketoglutarate is then further metabolized via the TCA-cycle to form oxaloacetate. The fact that the 2-ketoglutarate level is not significantly raised indicates that 2-ketoglutarate dehydrogenase activity is adequate to cope with a faster supply of 2-ketoglutarate. Our studies make it plausible that on the initiation of flight TCA-cycle intermediates are supplied by glutamate. The increase in malate should also be viewed in that light.

The observed increase in aspartate is difficult to reconcile with this view because it could be the result of a transamination of oxaloacetate with glutamate by means of glutamate–oxaloacetate aminotransferase; this would, however, counteract the 'sparker' function of glutamate.

DE KORT et al. (1973) found a very low proline oxidation in isolated mitochondria of *L. migratoria*, and CRABTREE and NEWSHOLME (1970) measured a proline dehydrogenase activity in the flight muscle of this locust of only 1.7 μmole/min/g fresh weight. The latter figure, however, does not conflict with the rate of proline decrease we measured within the first 20 min of flight (0.5 μmole/min/g muscle). It must be emphasized that this contribution of proline to energy production would be small, compared with the contribution of trehalose. In *L. migratoria* haemolymph proline level is low (4.46 $\pm$ 0.89 μmole/ml; HOUBEN, personal communication) and does not change significantly during flight. From the data given by VAN DER HORST et al. (1978a) it can be calculated that in the first flight period, trehalose consumption amounts to 2 μmoles/min/g muscle; thus in every minute of flight up to 7 μmoles ATP are derived from proline against 144 μmoles/g muscle from trehalose.

Phosphofructokinase activity is susceptible to adenine nucleotides and fructose-1, 6-diphosphate concentrations. No significant changes in the levels of the adenine nucleotides were found in our study, this does not clearly point to an inhibitory effect on the phosphofructokinase catabolized step. FORD and CANDY (1972) showed that the flight muscle of *S. gregaria* possesses several control enzymes such as phosphorylase, trehalase, hexokinase, phosphofructokinase, aldolase and pyruvate kinase. The relative importance of some of the control sites may be different and not all the sites will be operative under various conditions. Although a number of metabolites show initial or enduring changes, our findings do not give an answer with respect to the supposed mutual interrelationship between the reduction of trehalose utilization and the increasing consumption of diglycerides.

Note: During the last phase of the preparation of this paper, a paper was published (ROWAN and NEWSHOLME, 1979) dealing with changes in metabolite concentrations within 3 min of flight of the locust, *S. gregaria*. Although a number of the metabolites show similar changes to those found in the present study, others do not and generally

metabolite concentrations reported here are somewhat higher than those of ROWAN and NEWSHOLME (1979).

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