Substrate preferences of mitochondria isolated from locust (*Locusta migratoria*) and blowfly (*Phormia regina*) fat bodies

J. S. BALLANTYNE AND K. B. STOREY[1]

Institute of Biochemistry, Carleton University, Ottawa, Ont., Canada K1S 5B6

Received April 18, 1983

BALLANTYNE, J. S., and K. B. STOREY. 1983. Substrate preferences of mitochondria isolated from locust (*Locusta migratoria*) and blowfly (*Phormia regina*) fat bodies. Can. J. Zool. **61**: 2351–2356.

The preferred substrate of the fat body mitochondria of both the locust (*Locusta migratoria*) and the blowfly (*Phormia regina*) based on state three rates of oxidation is palmitoyl-L-carnitine. The fat body mitochondria of the locust do not oxidize proline plus pyruvate, pyruvate plus malate, or α-glycerophosphate under the conditions used. The mitochondria of the blowfly fat body do oxidize these substrates at high rates. The mitochondria isolated from the locust fat body do not oxidize fumarate or oxaloacetate at measurable rates. The mitochondria isolated from the blowfly fat body do not oxidize citrate, isocitrate, or fumarate at measurable rates.

BALLANTYNE, J. S., et K. B. STOREY. 1983. Substrate preferences of mitochondria isolated from locust (*Locusta migratoria*) and blowfly (*Phormia regina*) fat bodies. Can. J. Zool. **61**: 2351–2356.

D'après les taux d'oxydation de niveau trois, le meilleur substrat pour les mitochondries du corps gras du criquet migrateur (*Locusta migratoria*) et de la mouche noire de la viande (*Phormia regina*) est la palmitoyl-L-carnitine. Les mitochondries du corps gras du criquet n'oxydent pas la proline additionnée de pyruvate, ni le pyruvate additionné de malate ou d'α-glycérophosphate dans les conditions de l'expérience. Les mitochondries du corps gras de la mouche oxydent ces substrats à des taux élevés. Les mitochondries isolées du corps gras du criquet n'oxydent ni le fumarate, ni l'oxaloacétate à des taux décelables. Les mitochondries isolées du corps gras de la mouche n'oxydent pas de quantités mesurables de citrate, d'isocitrate ou de fumarate.

[Traduit par le journal]

Introduction

The fat body fulfills a major role in the intermediary metabolism of insects, supplying fuel in various forms for use in other tissues especially flight muscle. Except for the work of Keeley (1971, 1972, 1973, 1981) on the hormonal and developmental influences on mitochondria from the cockroach fat body, little is known of the mitochondrial metabolism of the insect fat body.

In the locust, the fat body must supply lipid, proline, and carbohydrate in the form of trehalose to the flight muscle. Although the majority of lipid in the insect fat body is triglyceride (Bailey 1975) the fat body supplies lipid to the flight muscle in the form of diglyceride (Chino and Gilbert 1965) under the influence of adipokinetic hormone (Mayer and Candy 1969). Diglyceride levels may increase severalfold in locust haemolymph during flight (Mayer and Candy 1967). Carbohydrate in the form of trehalose is sent from the fat body to the flight muscle of the locust for use in the initial stages of flight. Smaller amounts of proline are used during flight and come from the fat body where they are synthesized from acetate units derived from endogenous lipid (Bursell 1981). For long term flight, however, fat in the form of diglyceride is the major fuel for flight muscle. Lipid oxidation is known to occur in the locust fat body from the 8th day of adult life (Walker and Bailey 1970; Walker et al. 1970).

Blowfly flight muscle does not utilize lipid (Sacktor 1970), relying instead on carbohydrate plus proline energy sources. Both of these, as in the case of locust, must ultimately come from the fat body since endogenous sources are inadequate to sustain prolonged flight.

The present study was undertaken as an investigation of the substrate preferences of the fat body mitochondria from these two insects whose flight muscles have different substrate requirements to determine if these differences would be reflected in the mitochondria of the fat bodies.

Materials and methods

Animals

Adult male locusts aged 8 to 28 days postemergence were obtained from the breeding colony maintained at Queen's University, Kingston. Animals were fed a diet of bran and used at ages between 22 and 28 days postemergence.

Blowflies (*Phormia regina*) were obtained as pupae. The adults were used 2–14 days after emergence. During this period the adults were fed (sparingly) a diet of sucrose in water.

Tissue preparation

Fat bodies of 30 locusts were dissected free of the surrounding tissue before homogenization. Abdomens from 30 to 40 blowflies were separated from the rest of the body and tissues other than the fat body were removed under a dissecting microscope. The abdomen wall and adhering fat body was used in subsequent preparations.

[1]Author to whom reprint requests should be addressed.

TABLE 1. Oxidative phosphorylation in the mitochondria of the locust fat body

Substrate	State 3	State 4	RCR	ADP/O
Citrate, 5.71 m*M*	102.3±20.7	20.4±4.6	5.46±1.36	2.36±0.03
Isocitrate, 2.29 m*M*	131.4±88.7	47.7±25.6	2.65±0.69	2.16±0.13
α-Ketoglutarate, 5.71 m*M*	112.4±36.0	34.2±12.1	3.62±0.86	2.57±0.15
Succinate, 5.71 m*M*, plus rotenone 1 μ*M*	66.9±37.1	28.4±14.5	1.88±0.48	1.39 (2)
Fumarate, 5.71 m*M*	ND	ND	ND	ND
Malate, 5.71 m*M*	93±30.4	34.3±8.3	2.61±0.29	2.06±0.14
Malate, 5.71 m*M*, plus pyruvate, 0.11 m*M*	101.0±10.0	19.3±2.52	5.42±0.29	2.58±0.03
Oxaloacetate, 0.29 m*M*	ND	ND	ND	ND
α-Glycerophosphate, 5.71 m*M*, plus $CaCl_2$, 1.1 m*M*	ND	ND	ND	ND
Pyruvate, 5.71 m*M*	ND	ND	ND	ND
Pyruvate, 5.71 m*M*, plus malate 0.11 m*M*	ND	ND	ND	ND
Proline, 22.9 m*M*	ND	ND	ND	ND
Proline, 22.9 m*M*, plus pyruvate, 0.11 m*M*	ND	ND	ND	ND
Glutamate, 5.71 m*M*	57.5±30.5	19.6±9.3	2.73±0.23	2.34±0.27
Glutamate, 5.71 m*M*, plus malate 0.11 m*M*	130.6±35.2	49.6±10.1	2.81±0.17	2.16±0.04
Palmitoyl-L-carnitine, 10 μ*M*	ND	ND	ND	ND
Palmitoyl-L-carnitine, 10 μ*M*, plus malate, 0.11 m*M*	187.8±21.8 (4)	23.2±1.95 (4)	9.95±3.74	2.53±0.16
β-Hydroxybutyrate, 2.29 m*M*	ND	ND	ND	ND

NOTE: Values given are means ± SEM. Unless otherwise indicated by a number in parentheses the *n* value is 3. State 3 and state 4 rates are expressed as atoms O per minute per molecule cytochrome *a*. ND, not detected.

Isolation of mitochondria

Tissues were placed in 10 mL of ice-cold isolation medium (250 m*M* sucrose, 10 m*M* HEPES (pH 7.2 at 20°C), 2 m*M* EDTA, 1 m*M* EGTA, and 1% bovine serum albumin (essentially fatty acid free). Tissues were homogenized with three passes of a Potter-Elvehjem homogenizer with a loosely fitting Teflon pestle. The homogenate was centrifuged at 100 × *g* for 20 min (5 min in the case of the locust fat body preparation). The supernatant was decanted and centrifuged again at 9000 × *g* for 10 min. The resulting pellet was resuspended and centrifuged again for 10 min at 9000 × *g*. The final pellet was resuspended in a small volume of isolation medium.

Oxidative phosphorylation

Oxygen uptake of mitochondrial suspensions was measured polarographically with a Clark-type oxygen electrode. The incubation medium consisted of 250 m*M* sucrose, 10 m*M* HEPES (pH 7.2 at 30°C), 10 m*M* KH_2PO_4, and 1% BSA for the locust fat body, and 150 m*M* KCl, 10 m*M* HEPES (pH 7.2 at 30°C), 20 m*M* KH_2PO_4, and 1% BSA for the blowfly fat body. All polarographic measurements were performed at 30°C. ADP/O and respiratory control ratios (RCR) were calculated as outlined by Estabrook (1967) with respiratory states of mitochondria as defined by Chance and Williams (1956). All substrates were adjusted to pH 7.2 before addition to the cuvette.

Cytochrome assays

Cytochrome *a* was assayed using the method of Williams (1964) with 2% Triton X-100 substituted for deoxycholate to solubilize the mitochondria. The difference spectra (reduced versus oxidized) were obtained with a Pye-Unicam SP8-100 recording spectrophotometer.

Chemicals

L-Ascorbate and potassium chloride were obtained from the J. T. Baker Chemical Co., Phillipsburg, NJ. All other chemicals were obtained from the Sigma Chemical Co., St. Louis, MO.

Results

Mitochondria with high respiratory control ratios (RCR) were isolated from the locust fat body. These mitochondria did not oxidize NADH at detectable levels. Rates of substrate oxidation by locust fat body are summarized in Table 1. Palmitoyl-L-carnitine was oxidized at the highest rate (188 atoms $O \cdot min^{-1} \cdot$ molecule cytochrome a^{-1}) but only when "sparked" with small amounts of malate. High mean RCR (9.95) and good ADP/O were obtained with this substrate. Citrate and isocitrate were also oxidized at high rates. However, the state 4 rate with isocitrate was more than two times that of citrate resulting in a lower RCR (2.65 versus

TABLE 2. Oxidative phosphorylation in the mitochondria of the blowfly fat body

Substrate	State 3	State 4	RCR	ADP/O
Citrate, 5.71 m*M*	ND	ND	ND	ND
Isocitrate, 2.29 m*M*	ND	ND	ND	ND
α-Ketoglutarate, 5.71 m*M*	227.0±96.6	119.9±54.7	2.16±1.25	2.56±0.11
Succinate, 5.71 m*M*, plus rotenone, 1 μ*M*	424.7±78.0	193.2±28.3	2.17±0.15	1.41±0.57
Fumarate, 5.71 m*M*	ND	ND	ND	ND
Malate, 5.71 m*M*	130.3±35.9	56.3±16.7	2.36±0.10	2.31±0.18
Malate, 5.71 m*M*, plus pyruvate, 0.11 m*M*	211.2±70.2	62.8±19.1	3.26±0.40	2.60±0.14
Oxaloacetate, 2.20 m*M*	283.9±170.9	82.0±30.0	3.13±1.00	2.67±0.12
α-Glycerophosphate, 5.71 m*M*, plus $CaCl_2$, 1.1 m*M*	170.5 (2)	—	—	—
Pyruvate, 5.71 m*M*	195.8±54.3	109.3±42.6	1.96±0.24	2.44±0.25
Pyruvate, 5.71 m*M*, plus malate 0.11 m*M*	283.3±82.6	102.9±67.9	3.33±1.05	2.07±0.43
Proline, 22.9 m*M*	101.3±52.9	60.63±30.4	1.87±0.27	2.51±0.05
Proline, 22.9 m*M*, plus pyruvate, 0.11 m*M*	165.0±41.5	79.2±5.48	2.36±0.43 (4)	2.60±0.12 (4)
Glutamate, 5.71 m*M*	190.4±104.9	62.6±33.6	2.34±0.68	2.30 (2)
Glutamate, 5.71 m*M*, plus malate 0.11 m*M*	133.8±71.5	75.8±40.7	1.51±0.26	2.14 (2)
Palmitoyl-L-carnitine, 10 μ*M*	441.0±18.2	118.9±22.3	3.68±0.57	2.60±0.10
Palmitoyl-L-carnitine, 10 μ*M*, plus malate, 0.11 m*M*	594.3±20.8	111.4±19.5	5.56±0.22	2.60±0.18

NOTE: Values given are means ± SEM. Unless otherwise indicated by a number in parentheses the *n* value is 3. State 3 and state 4 rates are expressed as atoms O per minute per molecule cytochrome *a*. ND, not detected.

3.46). α-Ketoglutarate was oxidized at a rate similar to that of citrate. Succinate was oxidized at a lower rate (67 atoms $O \cdot min^{-1} \cdot$molecule cytochrome a^{-1}) than any other citric acid cycle intermediate except for fumarate and oxaloacetate which were not oxidized under the conditions used. Malate was oxidized at a high rate (93 atoms $O \cdot min^{-1} \cdot$molecule cytochrome a^{-1}) with high state 4 rates also. The addition of small amounts of pyruvate (0.11 m*M*) increased the state 3 rate of malate oxidation and lowered the state 4 rate resulting in a substantially higher RCR. Pyruvate alone at high concentration (5 m*M*) was not oxidized by locust fat body mitochondria nor was α-glycerophosphate. Proline was not oxidized in the presence or absence of pyruvate. Glutamate alone was oxidized at low rates (57 atoms $O \cdot min^{-1} \cdot$molecule cytochrome a^{-1}) and at high rates (130 atoms $O \cdot min^{-1} \cdot$molecule cytochrome a^{-1}) in the presence of small amounts of malate (0.11 m*M*). β-Hydroxybutyrate was not utilized by the mitochondria. ADP/O ratios above 2.00 were obtained for all substrates oxidized except for succinate which gave a value of 1.39.

The mitochondria isolated from blowfly fat body were more permeable to NADH than those of the locusts. The rate of NADH oxidation was as high as 30% of the state 3 rate in preparations with very low RCR. The rates of oxidation of substrates by blowfly fat body mitochondria are presented in Table 2. Overall the rates were about twice as high as those found in the locust fat body. The highest rates (594 atoms $O \cdot min^{-1} \cdot$molecule cytochrome a^{-1}) were for the oxidation of palmitoyl-L-carnitine plus malate. Palmitoyl-L-carnitine alone was oxidized at high rates in contrast to the situation in the locust fat body mitochondria. RCR's for the oxidation of palmitoyl-L-carnitine were the highest of all the substrates tested. Succinate was oxidized at very high rates in state 3 but also had high rates in state 4 resulting in a low RCR. α-Ketoglutarate was oxidized at a rate about one half that of succinate. Malate alone was oxidized at low rates but when small amounts of pyruvate were added the rate almost doubled. Oxaloacetate was oxidized at a higher rate than malate plus pyruvate. Citrate, isocitrate, and fumarate were not oxidized at a measurable level by blowfly fat body mitochondria. α-Glycerophosphate was utilized at a higher rate than malate alone when stimulated by $CaCl_2$ (1 m*M*). Pyruvate alone at high concentrations (5.7 m*M*) was oxidized at about the same rate as α-glycerophosphate. The rate increased substantially when a small amount of malate (0.11 m*M*) was added. Unlike the locust fat body mitochondria, proline was oxidized at a low rate which increased when pyruvate (0.1 m*M*) was added. Glutamate was utilized but glutamate oxidation seemed to be inhibited by the

addition of the small amounts of malate (0.11 m*M*). Overall the RCR's for the substrates oxidized by the blowfly fat body mitochondria were lower than those obtained for the locust fat body mitochondria for the same substrates.

Discussion

One of the important functions of the fat body in insects is in the synthesis and storage of lipid, carbohydrate, and amino acid and protein reserves (Bailey 1975). During flight the reserves of the fat body are mobilized to supply the energy needs of the flight muscle.

In the initial stages of flight in the locust, carbohydrate serves as the main energy source (Jutsum and Goldsworthy 1976). Both endogenous glycogen and glucose and haemolymph trehalose are used during this phase (Worm and Beenakkers 1980). The flight muscle of *Locusta* contains large amounts of glucose and trehalose but only small amounts of glycogen (Worm and Beenakkers 1980; Bucher and Klingenberg 1958). After 5 min of flight, muscle glycogen may drop by 50% (Worm and Beenakkers 1980). Proline concentration in the muscle also drops sharply in this period followed by a more gradual decline (Mayer and Candy 1969). This initial sharp decrease occurs as proline augments the concentrations of Krebs cycle intermediates (Sacktor and Childress 1967). Proline is not the major energy source in the locust muscle as it is in the tsetse fly (Bursell 1981). In the locust flight muscle during prolonged flight (5 h) lipid is the major energy source. During prolonged flight, the fat body must supply 85–90% of the lipid used (Weis-Fogh 1952). Beenakkers (1965) suggests that the fat body has sufficient lipid stored for 5 h of sustained flight. During this period the fat body must supply carbohydrate for the initial stages of flight, lipid for prolonged flight, and proline for anaplerotic requirements.

These functions of the fat body are reflected in the substrate preferences of the mitochondria. Lipid in the form of palmitoyl-L-carnitine is the preferred substrate. This agrees with the findings of Walker et al. (1970). The oxidation of lipid by fat body mitochondria may be very important in providing the basal energy needs for the synthetic processes which are the main functions of the fat body. Some lipids must be partially oxidized to acetate for the synthesis of proline via a mechanism suggested by Bursell (1981) in which, in the muscle, alanine is stoichiometrically produced as proline is oxidized. Alanine is then sent to the fat body through the haemolymph where it is used in the resynthesis of proline using acetate from endogenous lipid. The absence of significant oxidation of pyruvate, α-glycerophosphate, or proline in locust fat body suggests that both carbohydrate and proline are exclusively exported to the flight muscle. The extreme importance of fat body carbohydrate during flight is indicated by the fact that total carbohydrate reserves may be completely depleted in locusts flown overnight (Tietz 1965).

In the blowfly flight muscle, carbohydrate serves as the major energy source even during prolonged flight. The flight muscle uses both the high endogenous glycogen and haemolymph trehalose (Sacktor and Wormser-Shavit 1966). Clegg and Evans (1966) have calculated that the blowfly uses carbohydrate at such a rate that the total pool of trehalose in the haemolymph is only sufficient to supply the energy requirements of the muscle for 15 min. Since the blowfly can sustain flight for 2 to 3 h, the fat body must supply trehalose at a similar rate. As in the case of the locust, proline levels fall markedly in the initial stages of flight and then decline more slowly (Sacktor and Wormser-Shavit 1966). This proline is also thought to fulfill an anaplerotic role (Johnson and Hansford 1975). The fat body of the blowfly must therefore provide both trehalose as a major energy source to the flight muscle and proline for Krebs cycle augmentation.

The main features of the blowfly fat body mitochondria which distinguish them from those of the locust fat body are the high rate of utilization of proline and carbohydrate substrates (pyruvate and α-glycerophosphate). In this respect, the blowfly fat body more closely resembles that of the cockroach which contains significant amounts of glycerol-3-phosphate dehydrogenase (Storey and Bailey 1978). In the fat body mitochondria of both insects, the preferred fuel is lipid based on state 3 rates of respiration. Since the fat body of *Phormia* does not provide lipid for oxidation in the flight muscle (Sacktor 1970) virtually all endogenous lipid is available to provide acetate for proline synthesis. This may explain why proline is oxidized in the blowfly fat body mitochondria as well as being sent to the flight muscle.

The maximal rates of oxidation of pyruvate and proline by blowfly fat body mitochondria were also substantially less than those for lipid oxidation. The α-glycerophosphate cycle provides the only means for the transfer of reducing equivalents from cytosol to mitochondria in flight muscle but evidence from the present study indicates that the blowfly fat body mitochondrion could make use of both the α-glycerophosphate cycle and the malate-aspartate shuttle. Carbohydrate is oxidized in the blowfly fat body mitochondria suggesting that the blowfly fat body more closely resembles mammalian liver mitochondria where all three major energy sources are used (Hansford 1975).

The substrate preferences of fat body mitochondria reflect the function of the tissue. Mitochondria from both locust and blowfly fat body oxidize a variety of Krebs cycle intermediates indicating the presence of transporters for these compounds, transporters which could alternatively be used for the biosynthetic function of the Krebs cycle in providing di- and tri-carboxylic

acids for biosynthesis. The oxidation of citrate and isocitrate by locust fat body mitochondria indicates the presence of a citrate transporter which would also have a major role in the transport of citrate out of the mitochondria for the biosynthesis of fatty acids in the cytosol. The oxidation of malate, glutamate, and α-ketoglutarate by mitochondria from both species may suggest the presence of the malate–aspartate shuttle for the transportation of reducing equivalents across the mitochondrial membrane. Blowfly fat body mitochondria also may possess a functional α-glycerophosphate cycle as evidenced by the oxidation of α-glycerophosphate. However, the rate of α-glycerophosphate oxidation was amongst the lowest of the substrates utilized.

The blowfly fat body mitochondria differ from the mitochondria of the flight muscle in several ways. The most obvious is the oxidation of lipid in the fat body mitochondria. Additionally, mitochondria from the flight muscle are impermeable to Krebs cycle intermediates (Sacktor 1970). In the fat body, on the other hand, the mitochondria can oxidize α-ketoglutarate, succinate, malate, and oxaloacetate at high rates. It is unlikely that the enhanced permeability of the blowfly fat body mitochondria is due to damage during preparation, since several substrates of similar size (citrate, isocitrate, and fumarate) to those readily oxidized were not oxidized at detectable levels. Both the blowfly flight muscle and fat body mitochondria oxidize proline to a limited extent. The flight muscle mitochondria oxidize α-glycerophosphate and pyruvate at the highest rates while in the fat body the highest rates were found with palmitoyl-L-carnitine. This demonstrates that while the major energy source in the flight muscle is carbohydrate in the fat body it is lipid.

The higher rates of oxidation of substrates found in the fat body mitochondria of the blowfly than in those of the fat body of the locust may reflect the higher metabolic rates of the smaller insect.

Acknowledgements

This research was funded by an NSERC operating grant to K.B.S. and an NSERC postdoctoral fellowship to J.S.B. We gratefully acknowledge the kind donation of adult male locusts from Professor G. R. Wyatt, Department of Biology, Queen's University, Kingston, and the gift of blowfly pupae from Ms. C. I. McGregor Smith, Research Centre, Agriculture Canada, London, Ontario. K.B.S. wishes to thank Dr. R. Hansford (NIA, Baltimore) for many helpful discussions on mitochondrial function and his critical comments on the manuscript.

BAILEY, E. 1975. Biochemistry of insect flight. 2. Fuel supply. *In* Insect biochemistry and function. *Edited by* D. J. Candy and A. Kilby. Chapman and Hall, London. pp. 89–176.

BEENAKKERS, A. M. T. 1965. Transport of fatty acids in *Locusta migratoria* during sustained flight. J. Insect Physiol. **11**: 879–888.

BUCHER, T., and M. KLINGENBERG. 1958. Wege des Wasserstoffs in der lebendigen Organization. Angew. Chem. **70**: 552–570.

BURSELL, E. 1981. The role of proline in energy metabolism. *In* Energy metabolism of insects. *Edited by* G. H. Downer. Plenum Publishing Corp., London. pp. 135–154.

CHANCE, B., and R. G. WILLIAMS. 1956. The respiratory chain and oxidative phosphorylation. Adv. Enzymol. **17**: 65–134.

CHINO, H., and L. J. GILBERT. 1965. Lipid release and transport in insects. Biochim. Biophys. Acta, **98**: 94–110.

CLEGG, J. S., and D. R. EVANS. 1961. The physiology of blood trehalose and its function during flight in the blowfly. J. Exp. Biol. **38**: 771–792.

ESTABROOK, R. W. 1967. Mitochondrial respiratory control and the polarographic measurement of ADP/O ratios. Methods Enzymol. **10**: 41–47.

HANSFORD, R. G. 1975. Properties of mitochondria from animal and plant tissues. Part 1. Respiratory carrier content. *In* Biological handbooks. Cell biology. *Edited by* P. L. Altman and D. S. Dittmer. Federation of American Societies for Experimental Biology, Bethesda, MD. pp. 144–152.

JOHNSON, R. N., and R. G. HANSFORD. 1975. The control of tricarboxylate cycle oxidations in blowfly flight muscle. The steady-state concentrations of citrate, isocitrate, 2-oxoglutarate and malate in flight muscle and isolated mitochondria. Biochem. J. **146**: 527–535.

JUTSUM, A. K., and G. J. GOLDSWORTHY. 1976. Fuels for flight in *Locusta*. J. Insect Physiol. **22**: 243–249.

KEELEY, L. L. 1971. Endocrine effects on the biochemical properties of fat body mitochondria from the cockroach, *Blaberus discoidalis*. J. Insect Physiol. **17**: 1501–1515.

——— 1972. Biogenesis of mitochondria: neuroendocrine effects on the development of respiratory functions in fat body mitochondria of the cockroach *Blaberus discoidalis*. Arch. Biochem. Biophys. **153**: 8–15.

——— 1973. Characterization of insect fat body mitochondria isolated by a rapid procedure. Comp. Biochem. Physiol. B, **46**: 147–151.

——— 1981. Neuroendocrine regulation of mitochondrial development in the insect fat body. *In* Energy metabolism in insects. *Edited by* R. G. H. Downer. Plenum Publishing Corp., London. pp. 207–237.

MAYER, R. J., and D. J. CANDY. 1967. Changes in haemolymph lipoproteins during locust flight. Nature (London), **215**: 987.

——— 1969. Control of haemolymph lipid concentration during locust flight: an adipokinetic hormone from the corpora cardiaca. J. Insect Physiol. **15**: 611–620.

SACKTOR, B. 1970. Regulation of intermediary metabolism with special reference to the control mechanisms in insect flight muscle. Adv. Insect Physiol. **7**: 267–348.

SACKTOR, B., and C. C. CHILDRESS. 1967. Metabolism of proline in insect flight muscle and its significance in stimulation of the oxidation of pyruvate. Arch. Biochem. Biophys. **120**: 583–588.

SACKTOR, B., and E. WORMSER-SHAVIT. 1966. Regulation of metabolism in working muscle *in vivo*. 1. Concentration of

some glycolytic, tricarboxylic acid and amino acid intermediates in insect flight muscle during flight. J. Biol. Chem. **241**: 624–631.

STOREY, K. B., and E. BAILEY. 1978. The intracellular distribution of enzymes of carbohydrate degradation in the fat body of the adult male cockroach. Insect Biochem. **8**: 73–79.

TIETZ, A. 1965. Metabolic pathways in the insect fat body. Handb. Physiol. Sect 5: Adipose Tissue 1965. pp. 45–54.

WEIS-FOGH, T. 1952. Fat combustion and metabolic rate of flying locusts *Schistocerca gregaria* Forskal. Philos. Trans. R. Soc. London, **237**: 1–36.

WALKER, P. R., and E. BAILEY. 1970. Metabolism of glucose, trehalose, citrate, acetate, and palmitate by the male desert locust during adult development. J. Insect. Physiol. **16**: 499–509.

WALKER, P. R., L. HILL, and E. BAILEY. 1970. Feeding activity, respiration, and lipid and carbohydrate content of the male desert locust during adult development. J. Insect. Physiol. **16**: 1001–1015.

WILLIAMS, J. 1964. A method for the simultaneous quantitative estimation of cytochromes *a*, *b*, c_1 and *c* in mitochondria. Arch. Biochem. Biophys. **107**: 537–543.

WORM, R. A. A., and A. M. T. BEENAKKERS. 1980. Regulation of substrate utilization in the flight muscle of the locust *Locusta migratoria* during flight. Insect Biochem. **10**: 53–59.