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INTRACELLULAR DISTRIBUTION OF ENZYMES ASSOCIATED WITH LIPOGENESIS AND GLUCONEOGENESIS IN FAT BODY OF THE ADULT COCKROACH, *PERIPLANETA*

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Abstract—The intracellular distribution and maximal activities of some enzymes associated with lipogenesis, gluconeogenesis and fatty acid oxidation have been determined in the fat body of the adult male cockroach *Periplaneta americana*. Of the enzymes of lipogenesis, acetyl-CoA synthase, acetyl-CoA carboxylase, glucose 6-phosphate dehydrogenase, and ATP citrate lyase are located entirely in the cytosol. Of the other enzymes of citrate metabolism studied, citrate synthase and NAD-dependent isocitrate dehydrogenase are almost exclusively mitochondrial, whereas NADP-dependent isocitrate dehydrogenase and aconitase are predominantly cytosolic although significant mitochondrial activity is also present. The latter subcellular distribution is also observed for 'malic enzyme' and NAD and NADP dependent malate dehydrogenase. The enzyme of fatty acid oxidation studied, 3-hydroxyacyl-CoA dehydrogenase is entirely mitochondrial. Of the enzymes possibly involved in gluconeogenesis, glucose 6-phosphate is microsomal, fructose 1,6-diphosphatase cytosolic, pyruvate carboxylase mitochondrial and phosphoenolpyruvate carboxykinase predominantly cytosolic. Of the enzymes of amino acid metabolism studied, glutamate dehydrogenase is NAD-dependent and located in the mitochondria whereas glutamate/oxalacetate and glutamate/pyruvate transaminases are predominantly cytosolic but with significant activity in the mitochondria. Glycerol kinase and sorbitol dehydrogenase are cytosolic and the glyoxylate cycle enzymes malate synthetase and isocitrate lyase are not detected in the fat body. The distribution and relative activities of the enzymes are discussed in relation to fat body biosynthesis and in comparison to mammalian liver and adipose tissue.

INTRODUCTION

THE PATHWAY of lipogenesis in the insect fat body has previously been studied in the adult locust, *Schistocerca gregaria* (WALKER and BAILEY, 1969; 1970a, b) and is thought to be essentially similar to that occurring in mammalian tissues (KORNACKER and BALL, 1965). However, since the subcellular distributions of the enzymes of fat body lipogenesis have not previously been investigated we now report the results of such studies involving tissues from the adult male cockroach *Periplaneta americana*. The results complement those reported in a previous paper (STOREY and BAILEY, 1977) for the enzymes of carbohydrate degradation.

The fact that insects can convert certain amino acids into carbohydrate has been known for a long time, e.g. WIGGLESWORTH (1942) showed that glycogen stored in *Aedes aegypti*, depleted by starvation, could be restored by feeding casein, alanine, or glutamate. Further NAYAR and SAUERMAN (1971) have shown that glycerol can be converted to glycogen in mosquitoes. However, the pathway and the enzymes involved in gluconeogenesis in insect fat body appear not to have been investigated. Hence we now report on the maximal activities and the intracellular distribution of enzymes possibly involved in amino acid degradation and gluconeogenesis in the fat body of the adult cockroach.

MATERIALS AND METHODS

Insects and chemicals

The insects used were adult male cockroaches, *Periplaneta americana*, 10 days after adult emergence. Details of maintenance of the insects and purchase of chemicals are given in STOREY and BAILEY (1977).

Preparation of subcellular fractions

Fractionation of fat body was carried out as described by STOREY and BAILEY (1977). In the case of NAD-dependent isocitrate dehydrogenase, although the subcellular location was determined as described by STOREY and BAILEY (1977), because of the instability of the enzyme, maximal activities were determined in whole fat body homogenates prepared in the following medium:

5M glycerol, 30 mM mercaptoethanol, 20 mM Tris buffer, pH 8.0 This medium was determined to be optimal for enzyme stability. A number of different media were tested in an attempt to stabilize the enzyme. In all media tried optimal stability required 30 to 50 mM mercaptoethanol. The optimal pH for stability is 8.0 with Tris buffer being superior to phosphate, Imidazole or Triethanolamine buffers. Optimal stabilization ($t_{1/2}$ = 24 hr) was obtained with 5 M glycerol, other media tested (in order of stabilization) being: 3 M glycerol, 2 M sucrose, 0.1% bovine plasma albumin, 30% ammonium sulphate, polyethylene glycol, 100 mM KCl.

Assay of enzyme activity

All reactions were carried out in 1 ml cuvettes in a recording spectrophotometer at 25°C unless otherwise stated. All reactions were started by the addition of fat body preparation. Assays were followed by measuring the change in extinction at 340 nm due to the oxidation or reduction of NAD(P)H or NAD(P) unless otherwise noted. All enzyme activities reported are the maximal obtainable under the conditions employed. Optimal assay conditions were obtained as described by STOREY and BAILEY (1977).

The reaction mixtures (final concentrations) used were as follows:

Citrate synthase (E.C. 4.1.3.7.). 50 mM Tris buffer pH 8.1, 0.1 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), 0.3 mM acetyl-CoA (omitted for control) and 0.5 mM oxaloacetate. The reaction is followed at 412 nm, the DTNB and acetyl-CoA being added first to obtain a measure of acetyl-CoA deacylase activity (this is essentially zero in fat body) and the oxaloacetate added last to start the reaction. The molar extinction coefficient of DTNB is taken to be 13.6×10^3 l. mole^{-1} cm^{-1} (BIEBER *et al.*, 1972).

Aconitase (E.C. 4.2.1.3). 100 mM Tris buffer, pH 8.0, 1 mM NADP, 1 mM citrate (omitted for control), 2 mM $MgCl_2$ and excess isocitrate dehydrogenase.

ATP-citrate lyase (E.C. 4.2.3.8.). 20 mM Tris buffer, pH 7.5, 20 mM $MgCl_2$, 10 mM reduced glutathione, 0.2 mM NADPH, 20 mM citrate, 10 mM ATP (omitted for control), 0.4 mM CoASH (omitted for control) and excess malate dehydrogenase. The traditional method of extraction of ATP citrate lyase, involving homogenization in 20%. (v/v) ethanol (SRERE, 1959) failed to increase enzyme activity in fat body.

NAD-dependent isocitrate dehydrogenase (E.C. 1.1.1.41). 100 mM Imidazole buffer, pH 7.0, 4 mM $MgCl_2$, 6.0 mM isocitrate (omitted for control), 1.0 mM ADP and 1.0 mM NAD. ADP is essential for the activity of this enzyme.

NADP-dependent isocitrate dehydrogenase (E.C. 1.1.1.42). As for the NAD-dependent enzymes except that the reaction is carried out at pH 8.0 and 0.4 mM NADP replaced the NAD in the reaction mixture.

Glucose 6-phosphate dehydrogenase (E.C. 1.1.1.49). 50 mM Tris buffer, pH 7.6, 7 mM $MgCl_2$, 0.4 mM NADP, 1 mM glucose 6-phosphate (omitted for control).

'Malic enzyme' (E.C. 1.1.1.40). 50 mM Tris buffer, pH 8.2, 0.8 mM $MnCl_2$, 0.4 mM NADP, 1 mM malate (omitted for control).

Malate dehydrogenase (E.C. 1.1.1.37). 50 mM Phosphate buffer, pH 7.3, 0.2 mM NADH or 0.2 mM NADPH and 0.5 mM oxaloacetate (omitted for control).

Acetyl-CoA synthetase (E.C. 6.2.1.1.). 100 mM Tris buffer, pH 8.0, 2 mM ATP, 5 mM $MgCl_2$, 2 mM acetate (omitted for control), 2 mM CoA, 1 mM dithiothreitol, 0.2 mM NAD, 4 mM malate and excess citrate synthetase and malate dehydrogenase.

Acetyl-CoA carboxylase (E.C. 6.4.1.2). 100 mM Tris buffer, pH 7.5, 20 mM $MgCl_2$, 0.1 mM phosphoenolpyruvate, 0.05 mM NADH, 1 mM ATP, 10 mM citrate, 10 mM $KHCO_3$, 0.2 mM acetyl-CoA (omitted for control) and excess pyruvate kinase and lactate dehydrogenase. To obtain optimal activity for the enzyme the fat body must be homogenized in a medium containing bovine plasma albumin and it is also recommended that 10 mM citrate is added to ensure enzyme stability.

3-hydroxyacyl-CoA dehydrogenase (E.C. 1.1.1.35). 100 mM Phosphate buffer, pH 7.3, 0.2 mM NADH, 5 mM S-acetoacetyl-N-acetyl-cysteamine (omitted for control).

Glutamate/oxaloacetate transaminase (E.C. 2.6.1.1). 100 mM Phosphate buffer, pH 7.4, 40 mM aspartate, 7 mM α-oxoglutarate (omitted for control), 0.2 mM NADH and excess malate dehydrogenase.

Glutamate/pyruvate transaminase (E.C. 2.6.1.2). 50 mM Phosphate buffer, pH 7.5, 0.2 M alanine, 10 mM α-oxoglutarate (omitted for control), 0.2 mM NADH and excess lactate dehydrogenase.

Glutamate dehydrogenase (E.C. 1.4.1.2). 100 mM Imidazole buffer, pH 7.3, 250 mM ammonium acetate, 0.1 mM NADH or 0.26 mM NADPH, 0.1 mM EDTA, 1 mM ADP and 14 mM α-oxoglutarate (omitted for control).

Pyruvate carboxylase (E.C. 6.4.1.1.). 100 mM triethanolamine buffer, pH 8.0, 7 mM pyruvate, 2 mM ATP, 7 mM $MgSO_4$, 20 mM $KHCO_3$, 0.1 mM NADH, 0.1 mM acetyl-CoA (omitted for control) and excess malate dehydrogenase.

Phosphoenolpyruvate carboxykinase (E.C. 4.1.1.32). The enzyme was assayed essentially as described by SEUBERT and HUTH (1965). The optimal conditions of assay are: 50 mM Tris buffer, pH 7.5, 6 mM GTP, 5 mM oxaloacetate (omitted for control), 1 mM mercaptoethanol and 20 mM $MgCl_2$.

Fructose 1,6-diphosphatase (E.C. 3.1.3.11). 50 mM Tris buffer, pH 7.3, 6 mM $MgCl_2$, 0.02 mM fructose 1,6-diphosphate (omitted for control) 0.2 mM NADP and excess phosphoglucoseisomerase and glucose 6-phosphate dehydrogenase.

Glucose 6-phosphatase (E.C. 3.1.3.9). 100 mM Triethanolamine buffer, pH 7.8, 1.5 mM glucose 6-phosphate (omitted for control), 1.5 mM ATP, 10 mM phosphate, 10 mM $MgCl_2$, 0.15 mM NADH and excess lactate dehydrogenase, pyruvate kinase, and hexokinase.

Glycerol kinase (E.C. 2.7.1.30). 200 mM glycine–hydrazine buffer, pH 9.8, 2 mM $MgCl_2$, 0.4 mM NAD, 3 mM glycerol (omitted for control), 2 mM ATP and excess α-glycerophosphate dehydrogenase.

Sorbitol dehydrogenase (E.C. 1.1.1.14). 100 mM Triethanolamine buffer, pH 7.8, 500 mM fructose (omitted for control) and 0.2 mM NADH.

Malate synthase (E.C. 4.1.3.2.). This reaction is followed by measuring the change in extinction at 232 nm. The cuvette contains, 10 mM $MgCl_2$, 0.2 mM acetyl-CoA and 100 mM Tris buffer, pH 8.0. ΔE 232 is followed for acetyl CoA deacylase activity (essentially zero in fat body) then 0.2 mM glyoxylate is added and ΔE 232 followed again.

Isocitrate lyase (E.C. 4.1.3.1.). This assay is followed by measuring the change in extinction at 324 nm. To a cuvette, 50 mM imidazole buffer, pH 7.0, 20 mM $MgCl_2$, 0.01 mM EDTA and 0.01 mM phenylhydrazine HCl are added and the ΔE 324 followed, then 10 mM isocitrate is added and again ΔE 324 followed.

RESULTS

The activity and the intracellular distribution of the enzymes associated with lipogenesis are shown in Table 1. Of the enzymes of citrate metabolism, reasonably similar total activities of citrate synthase, aconitase, NAD-dependent isocitrate dehydrogenase and ATP citrate lyase are observed. Somewhat higher activities of the NADP-dependent isocitrate dehydrogenase are obtained. Whereas citrate synthase and NAD-dependent isocitrate dehydrogenase are predominantly or exclusively mitochondrial the other enzymes of citrate metabolism are predominantly or exclusively cytosolic in location.

Since ATP citrate lyase activity probably supplies the acetyl-CoA for cytosolic fatty acid biosynthesis it is of interest that the activity of the enzyme is the same as that of acetyl-CoA carboxylase, the first enzyme in the fatty acid biosynthesis sequence. A similar activity is also detected of the enzyme acetyl-CoA synthetase which would bring about the conversion to acetyl-CoA of any acetate arising in the fat body. Both acetyl-CoA carboxylase and acetyl-CoA synthetase are detected only in the cytosol.

As noted earlier considerable activities of NADP dependent isocitrate dehydrogenase are detected in the cytosol. Other enzymes possibly involved in the provision of NADPH for fatty acid biosynthesis are glucose 6-phosphate dehydrogenase and 'malic enzyme'. As can be seen from Table 1 considerable activities of both those enzymes are also detected in the cytosol. Glucose 6-phosphate dehydrogenase activity is only detected in the cytosol, whereas small but significant 'malic enzyme' activity is also present in the mitochondria. NAD-dependent malate dehydrogenase is detected in considerable activity in the mitochondria

Table 1. The activities of enzymes of lipogenesis and fatty acid oxidation in the fat body

Enzyme	Enzyme activity (μmole/min/g wet weight tissue) Mitochondria (M)	Cytosol (C)	M/C ratio
Enzymes of citrate metabolism:			
Citrate synthase	0.47 ± 0.02	0.01	47
Aconitase	0.06 ± 0.003	0.95 ± 0.04	0.06
ATP citrate lyase	0	0.50 ± 0.01	0
Isocitrate dehydrogenase (NADP)	0.09 ± 0.003	2.83 ± 0.10	0.03
Isocitrate dehydrogenase (NAD)	0.50 ± 0.04	0	∝
Other NAD and NADP dependent enzymes:			
Glucose 6-phosphate dehydrogenase	0	3.84 ± 0.09	0
'Malic enzyme'	0.13 ± 0.01	5.33 ± 0.20	0.02
Malate dehydrogenase (NAD)	5.26 ± 0.23	126.7 ± 3.8	0.04
Malate dehydrogenase (NADP)	0.14 ± 0.005	0.82 ± 0.04	0.17
Other enzymes of lipogenesis:			
Acetyl-CoA carboxylase	0	0.50 ± 0.02	0
Acetyl-CoA synthase	0	0.50 ± 0.02	0
Fatty acid oxidation:			
3-Hydroxyacyl-CoA dehydrogenase	0.13	0	∝

The results are the means ± S.E.M. of at least 7 determinations, each determination involving pooled tissue from several insects. No activity of any of the enzymes studied was detected in the nuclear or microsomal cell fraction.

and extremely high activity in the cytosol. In contrast, only low activities of NADP-dependent malate dehydrogenase are detected, and a considerably higher proportion of the total cellular activity is located in the mitochondria than for the NAD-dependent enzyme.

The results for 3-hydroxyacyl-CoA dehydrogenase are also shown in Table 1. This representative enzyme of the β-oxidation sequence of fatty acid oxidation is present in quite low activity in the fat body and as would be expected is only present in the mitochondrial cell fraction.

The activities and the intracellular distribution of enzymes possibly associated with fat body gluconeogenesis is given in Table 2. Considerable activities of glutamate/oxaloacetate and glutamate/pyruvate transaminases are detected in the fat body. Although for both enzymes more activity is detected in the cytosol than the mitochondria, the mitochondria/cytosol activity ratio is much lower for the glutamate/oxaloacetate than the glutamate/pyruvate enzyme. Glutamate dehydrogenase is present in much less activity than are the transaminases, and the enzyme is only located in the mitochondria. Further, the enzyme is NAD-dependent and has no activity towards NADP.

Reasonable activities of the enzymes necessary for the reversal of glycolysis are present in the fat body. Lowest activities are noted for pyruvate carboxylase (which is located in the mitochondria) and glucose 6-phosphatase (only located in the microsomal fraction). Higher activities of phosphoenolpyruvate carboxykinase and fructose 1,6-diphosphatase are found in the fat body, the former enzyme being detected primarily in the cytosol but with significant activity being found in the mitochondria whereas the latter enzyme is only detected in the cytosol. The cytosol also contains moderate activity of glycerol kinase and quite high activity of sorbitol dehydrogenase. As can also be seen from Table 2 no activity of the key enzymes of the glyoxylate cycle, malate synthase and isocitrate lyase could be detected in the fat body, supporting the previous suggestion (BADE, 1962) that the glyoxylate cycle is not operative in insects.

DISCUSSION

Figure 1 outlines the pathways possibly involved in fat body lipogenesis and gluconeogenesis. With regard to carbohydrate conversion to lipid, the activities and the intracellular distribution of the enzymes

Table 2. The activities of enzymes associated with gluconeogenesis in the fat body.

Enzymes	Enzyme activity (μmole/min/g wet weight tissue) Mitochondria (M)	Cytosol (C)	M/C ratio	Microsomes
Enzymes of amino acid catabolism:				
Glutamate/oxaloacetate transaminase	0.57 ± 0.02	8.5 ± 0.40	0.07	0
Glutamate/pyruvate transaminase	2.9 ± 0.18	9.6 ± 0.55	0.30	0
Glutamate dehydrogenase (NAD)	0.53 ± 0.02	0	∝	0
Glutamate dehydrogenase (NADP)	0	0	—	0
Enzymes of glycolysis reversal:				
Pyruvate carboxylase	0.25 ± 0.01	0	∝	0
Phosphoenolpyruvate carboxykinase	0.30 ± 0.02	1.78 ± 0.11	0.17	0
Fructose 1,6-diphosphatase	0	2.83 ± 0.10	0	0
Glucose 6-phosphatase	0	0	—	0.25 ± 0.02
Other enzymes:				
Glycerol kinase	0	0.20 ± 0.01	0	0
Sorbitol dehydrogenase	0	15.0 ± 0.96	0	0
Glyoxylate cycle enzymes:	Whole homogenate			Mitochondria
Malate synthase	0			0
Isocitrate lyase	0			0

The results are the means ± S.E.M. of at least 7 determinations, each determination involving pooled tissue from several insects. No activity of any of the enzymes studied was detected in the nuclear cell fraction.

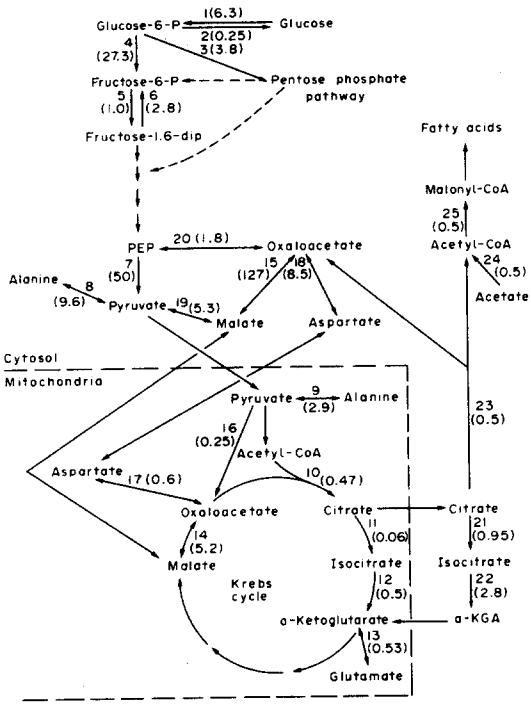

Fig. 1. Pathways possibly involved in fat body lipogenesis and gluconeogenesis. The enzymes involved are numbered as indicated below and the activities (μmole substrate transformed/min/g tissue at 25°C) are indicated in brackets. 1 Hexokinase 2 Glucose 6-phosphatase 3 Glucose 6-phosphate dehydrogenase 4 Phosphoglucoisomerase 5 Phosphofructokinase 6 Fructose 1,6-diphosphatase 7 Pyruvate kinase. 8 and 9 Glutamate/pyruvate transaminase 10 Citrate synthase 11 and 21 Aconitase 12 NAD-dependent isocitrate dehydrogenase 13 NAD-dependent glutamate dehydrogenase 14 and 15 NAD-dependent malate dehydrogenase 16 Pyruvate carboxylase 17 and 18 Glutamate/oxaloacetate transaminase 19 'Malic enzyme' 20 Phosphoenolpyruvate carboxykinase 22 NADP-dependent isocitrate dehydrogenase 23 ATP citrate lyase 24 Acetyl-CoA synthetase 25 Acetyl-CoA carboxylase. The data for the glycolytic enzymes is taken from STOREY and BAILEY (1977).

possibly concerned supports the view of WALKER and BAILEY (1969, 1970a, b) that in the fat body the pathway utilized is essentially the same as that suggested for mammalian lipogenic tissues (KORNACKER and BALL, 1965). Thus the appropriate enzymes exist in the correct cellular compartments to allow cytosolic conversion of glucose to pyruvate via the glycolytic and pentose phosphate pathways. Within the mitochondria acetyl-CoA is formed from pyruvate and transported into the cytosol in the form of citrate (formed by the action of citrate synthase). Within the cytosol acetyl-CoA for fatty acid synthesis is regenerated by the action of ATP citrate synthase. However, since the fat body cytosol also contains acetyl-CoA synthetase activity it is possible that acetyl-CoA for lipogenesis arises in the cytosol by the action of acetyl-CoA synthase on acetate transported from the mitochondria (in which it could arise by deacylase activity on acetyl-CoA) as has been suggested for mammalian liver (MURTHY and STEINER, 1971). Although this is a possible rôle for acetyl-CoA synthetase it seems likely that the enzyme is predominantly concerned with the metabolism of acetate arising in the diet or by the action of the gut flora.

Fatty acid biosynthesis requires NADPH and WALKER and BAILEY (1969, 1970b) have suggested that in the locust fat body this is provided by the action of the pentose phosphate pathway and NADP-dependent isocitrate dehydrogenase. This is supported by the results presented here indicating considerable cytosolic activity of glucose 6-phosphate dehydrogenase and NADP-dependent isocitrate dehydrogenase. Indeed the relatively high activity of glucose 6-phosphate dehydrogenase compared to phosphofructokinase and aldolase (STOREY and BAILEY, 1977) suggests that in the fat body conversion of hexose to triose occurs primarily by the pentose phosphate and not the glycolytic pathway. With regard to NADP-dependent isocitrate dehydrogenase, the high activities of this enzyme and cytosolic aconitase compared to the relatively low activities of mitochondrial aconitase and NAD-dependent isocitrate dehydrogenase may indicate that an appreciable portion of flow in the span citrate to α-oxoglutarate in the tricarboxylic acid cycle occurs outside the mitochondria, forming NADPH for biosynthesis, α-Oxoglutarate may then re-enter the mitochondria as indicated in Fig. 1.

In mammalian tissues it has been suggested (KORNACKER and BALL, 1965) that some of the NADPH for lipogenesis is derived from the conversion of oxaloacetate (formed by ATP citrate lyase) to malate by cytosolic NAD-dependent malate dehydrogenase and conversion of the malate formed to pyruvate (which can enter the mitochondria) with the concomitant formation of NADPH by 'malic enzyme'. The formation of acetyl-CoA and NADPH for lipogenesis via the ATP citrate lyase pathway and 'malic enzyme' leads to a depletion of mitochondrial oxaloacetate which is thought to be replaced in mammalian tissues by the action of pyruvate carboxylase (KORNACKER and BALL, 1965). WALKER and BAILEY (1969, 1970b) have presented evidence to suggest that in the locust fat body 'malic enzyme' functions in the direction of malate formation (i.e. NADPH utilization) in order to give rise to mitochondrial oxaloacetate since in the locust fat body pyruvate carboxylase is of very low activity when lipogenesis is high (WALKER and BAILEY, 1970c). In the cockroach fat body also the pyruvate carboxylase activity is considerably less than are those of ATP citrate lyase and acetyl-CoA carboxylase. However the activity of pyruvate carboxylase is not so low that one has to postulate that 'malic enzyme' operates in the direction of malate formation, especially as some of the oxaloacetate formed by ATP citrate lyase may be returned to the mitochondria as malate or aspartate since considerable cytosolic activities of NAD-dependent malate dehydrogenase and glutamate/oxaloacetate transaminase are observed.

The discussion above concerning lipogenesis and that below concerned with gluconeogenesis assumes that fat body mitochondria are permeable to and probably have transport systems for several di- and tricarboxylic acids and amino acids as indicated in Fig. 1. Although such transport systems have been shown to occur in the mitochondria of mammalian liver (CHAPPELL, 1968) and adipose tissue (MARTIN

and DENTON, 1970) such systems have not yet been studied in insect fat body mitochondria. However a consideration of the enzyme distribution observed makes the assumption of mitochondrial permeability to certain metabolites a reasonable one.

As with carbohydrate conversion to lipid in fat body, the data presented in this paper supports the view that gluconeogenesis in fat body proceeds via the pathway operative in mammalian tissues (SCRUTTON and UTTER, 1968). Thus fat body possesses the enzymes which circumvent the essentially irreversible steps of glycolysis i.e. pyruvate carboxylase and phosphoenolpyruvate carboxykinase to convert pyruvate to phosphoenolpyruvate, fructose 1,6-diphosphatase and glucose 6-phosphatase to convert glucose 6-phosphate to glucose (although it seems likely that in a gluconeogenic situation in fat body much of the glucose 6-phosphate would be converted to trehalose). With regard to phosphoenolpyruvate formation it is of interest that this occurs predominantly in the cytosol since phosphoenolpyruvate carboxykinase is located mainly in the cytosol. This distribution of the enzyme is similar to that found in the liver of rats and mice but in contrast to that for the chicken and rabbit in which the enzyme is mitochondrial (SCRUTTON and UTTER, 1968). Thus in the fat body oxaloacetate which is formed from pyruvate in the mitochondria by pyruvate carboxylase must pass into the cytosol. Mammalian liver mitochondria are impermeable to oxaloacetate and movement occurs in the form of malate or aspartate (SCRUTTON and UTTER, 1968). Although the permeability properties of fat body mitochondria have not yet been studied the movement of oxaloacetate in the form of malate or aspartate is quite likely since considerable activities of NAD-dependent malate dehydrogenase and glutamate/oxaloacetate transaminase occur in both the mitochondria and cytosol. Movement of malate from the mitochondria to the cytosol has the advantage that reoxidation of malate by malate dehydrogenase in the cytosol leads to the generation of NADH required for the reversal of glycolysis that occurs in gluconeogenesis.

Potential substrates for gluconeogenesis in the fat body are glycerol (derived from lipids) and amino acids which can give rise to pyruvate or a tricarboxylic acid cycle intermediate and hence oxaloacetate. Glycerol enters glycolysis at the triose level via the action of glycerokinase and NAD-dependent α-glycerphosphate dehydrogenase both of which are present in the fat body (Table 2 and STOREY and BAILEY, 1977). Degradation of amino acids involves transaminases and glutamate dehydrogenases (BAILEY, 1975). It is of interest that the fat body glutamate dehydrogenase is NAD-dependent as has previously been shown for the enzyme of cockroach (MILLS and COCHRAN, 1963) tsetse fly and blowfly (BURSELL, 1975) muscle mitochondria. The occurrence of transaminases in the mitochondria and cytosol of fat body was first demonstrated in locusts by KILBY and NEVILLE (1957). However, previous assays of these enzymes in fat body have been performed on whole fat body preparations from cockroaches (MCALLAN and CHERFURKA, 1961) and locusts (MORDUE and GOLDSWORTHY, 1973). The values obtained in these earlier studies were considerably lower than the total activities reported here, possibly due to sub-optimal enzyme assay conditions having been used since the authors did not report any attempt to optimize assay conditions. Since intracellular distribution studies have not previously been undertaken in insects the data presented here can only be compared with that for the well investigated mammalian systems and it is of interest that in a study of the distribution of glutamate/pyruvate transaminase isoenzymes in various mammalian tissues (DE ROSA and SWICK, 1975) it was shown that in gluconeogenic tissues the occurrence of the enzyme in the cytosol could be correlated with the presence of phosphoenolpyruvate carboxykinase in the same compartment as is clearly also the case for the cockroach fat body.

Since insect fat body has been compared to a combined mammalian liver and adipose tissue (KILBY, 1963) the activities of the enzymes of lipogenesis and gluconeogenesis have been compared in fat body and rat liver and adipose tissues. Table 3 presents the

Table 3. A comparison of the activities of enzymes associated with lipogenesis in fat body and rat liver and adipose tissue

Enzyme	Fat body		Liver		Adipose tissue	
	Total activity	M/C ratio	Total activity	M/C ratio	Total activity	M/C ratio
Enzymes of citrate metabolism:						
Citrate synthase	0.48	47	3	x	0.51	x
Aconitase	1.01	0.06	1.5	0.14	0.11	0.17
ATP citrate lyase	0.5	0	0.9	0	0.51	0
Isocitrate dehydrogenase (NADP)	2.92	0.03	15	0.1	0.37	0.3
Isocitrate dehydrogenase (NAD)	0.5	x	1	x	0.04	x
Other NAD and NADP-dependent enzymes:						
Glucose 6-phosphate dehydrogenase	3.84	0	3.5	0	0.58	0
'Malic enzyme'	5.46	0.02	1.1	0.06	0.2	0.11
Malate dehydrogenase (NAD)	131.9	0.04	387	0.5	32.9	0.1
Other enzymes:						
Acetyl-CoA carboxylase	0.5	0	0.2	0	0.01	0
Acetyl-CoA synthase	0.5	0	2.4	0	0.01	0.25

M/C = mitochondria/cytosol.

The results for rat liver and adipose tissue have been taken from the work of ERNSTER and NAVAZIO (1956), SHONK and BOXER (1964), SCRUTTON and UTTER (1968), MARTIN and DENTON (1970), BRDICZKA and PETTE (1971), ROBINSON (1973), and KNOWLES *et al.* (1974). The enzyme activities have been adjusted to the same units as those for the fat body enzymes i.e. μmole/min/g wet weight tissue at 25°C.

Table 4. A comparison of the activities of enzymes associated with gluconeogenesis in fat body and rat liver and adipose tissue

Enzyme	Fat body		Liver		Adipose tissue	
	Total activity	M/C ratio	Total activity	M/C ratio	Total activity	M/C ratio
Glutamate/oxaloacetate transaminase	9.07	0.07	64	3	0.17	3.1
Glutamate/pyruvate transaminase	12.5	0.30	34	0.2	0.61	0.09
Glutamate dehydrogenase	0.53	α	150	α	0.18	α
Pyruvate carboxylase	0.25	α	6	12	0.30	α
Phosphoenolpyruvate carboxykinase	2.08	0.17	3	0.12	0.02	0
Fructose 1,6-diphosphatase	2.83	0	8	0	0	—
Glucose 6-phosphatase	0.25	—	8	—	0	—
Glycerol kinase	0.20	0	2	0	0.1	0

M/C = mitochondria/cytosol

The results for rat liver and adipose tissue are taken from the work of BOYD (1961), ROBINSON and NEWSHOLME (1967, 1969), SCRUTTON and UTTER (1968), BALLARD and HANSON (1967) and MARTIN and DENTON (1970). The enzyme activities have been adjusted to the same units as those used for the fat body enzymes i.e. μmole/min/g wet weight tissue at 25°C.

values for the enzymes associated with lipogenesis. Of the enzymes of citrate metabolism, similar activities of citrate synthase and ATP citrate lyase are found in the fat body and adipose tissue with the liver having higher activities. For aconitase and NAD- and NADP-dependent isocitrate dehydrogenase however the fat body much more resembles the liver having much higher activities than are found in adipose tissue. The intracellular distribution of citrate synthase, NAD-dependent isocitrate dehydrogenase and ATP citrate lyase is similar in all three tissues. However fat body differs slightly from liver and adipose tissue with respect to the distribution of aconitase and NADP-dependent isocitrate dehydrogenase with a higher proportion of both enzymes being found in the cytosol than is the case for the mammalian tissues. Despite this difference all these tissues have a considerable amount of their aconitase activity in the cytosol and this appears to be characteristic of lipogenic tissues (ROBINSON, 1973). With regard to the activities of glucose 6-phosphate dehydrogenase 'malic enzyme', NAD-linked malate dehydrogenase and acetyl-CoA synthase, much higher values are found for fat body and liver than for adipose tissue and in the case of 'malic enzyme' fat body has a particularly high activity. With respect to the intracellular distribution of these enzymes fat body is more like liver than adipose tissue with regard to acetyl-CoA synthase and 'malic enzyme' but the reverse is true for NAD-dependent malate dehydrogenase.

From Table 4 it can be seen that the activities of glutamate/oxaloacetate and glutamate/pyruvate transaminases in fat body are intermediate between those for liver and adipose tissue. Further the insect glutamate/oxaloacetate transaminase is less mitochondrial and the glutamate/pyruvate transaminase more mitochondrial in distribution than the mammalian enzymes. Glutamate dehydrogenase is solely mitochondrial in each tissue and the enzyme is much less active in fat body and adipose tissue than in liver. Pyruvate carboxylase, similarly, is less active in fat body and adipose tissue than in liver and the intracellular distribution also differs being solely mitochondrial in the former two tissues and being mainly, but not solely, mitochondrial in the latter tissue. Phosphoenolpyruvate carboxykinase is of similar activity and intracellular distribution in liver and fat body, with the activity being much greater than the low activity found in adipose tissue. Fructose 1,6-diphosphatase and glucose 6-phosphatase are not detected in adipose tissue but have the same intracellular distribution in liver and fat body although the mammalian tissue has considerably higher activity. Finally glycerol kinase the same intracellular distribution in all three tissues considered but is much higher in activity in liver than in fat body or adipose tissue. The relatively low enzyme activity in fat body is also observed from cockroach flight and femoral muscles (NEWSHOLME and TAYLOR, 1969).

The data presented in this paper and by STOREY and BAILEY (1977) indicates that the fat body exhibits many similarities to mammalian liver and adipose tissue with regard to enzymic composition and intracellular distribution. Clearly this is to be expected considering the many similar functions carried out by the tissues. However many of the enzymic features studied appear to be unique to fat body and presumably reflect the rôle played by the tissue in the metabolism of the whole insect.

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REFERENCES

BADE M. L. (1962) Metabolic conversions during preparation of the Cecropia silkworm. 2. Tests for the operation of the glyoxylate cycle. *Biochem. J.* **83**, 478–482.

BAILEY E. (1975) Biochemistry of insect flight. Part 2: fuel supply. In *Insect Biochemistry and Function* (Ed. by CANDY D. and KILBY B. A.) pp. 91–176. Chapman & Hall, London.

BALLARD F. J. and HANSON R. W. (1967) Phosphoenolpyruvate carboxykinase and pyruvate carboxylase in developing rat liver. *Biochem. J.* **104**, 866–871.

BIEBER L. L., ABRAHAM T., and HELMRATH T. (1972) A rapid spectrophotomertic assay for carnitine palmytoyltransferase. *Analyt. Biochem.* **50**, 509–518.

BOYD J. W. (1961) The intracellular distribution, latency and electrophoretic mobility of L-glutamate-oxaloacetate transaminase from rat liver. *Biochem. J.* **81**, 474–481.

BRDICZKA D. and PETTE D. (1971) Intra- and extramitochondrial isoenzymes of (NADP) malate dehydrogenase. *Eur. J. Biochem.* **19,** 546–551.

BURSELL E. (1975) Glutamic dehydrogenase from sarcosomes of the tsetse fly (*Glossina morsitans*) and the blowfly (*Sarcophaga nodosa*) *Insect Biochem.* **5,** 289–297.

CHAPPELL J. B. (1968) Systems used for the transport of substrates into mitochondria. *Br. med. Bull.* **24,** 150–157.

DE ROSA G. and SWICK R. W. (1975) Metabolic implications of the distribution of the alanine aminotransferase isoenzymes. *J. biol. Chem.* **250,** 7961–7967.

ERNSTER L. and NAVAZIO F. (1956) The cytoplasmic distribution of isocitric dehydrogenase. *Exp. Cell Res.* **11,** 483–486.

KILBY B. A. (1963) The biochemistry of the insect fat body. *Adv. Insect Physiol.* **1,** 111–174.

KILBY B. A. and NEVILLE E. (1957) Amino acid metabolism in locust tissues. *J. exp. Biol.* **34,** 276–289.

KNOWLES S. E., JARRETT I. V., FILSELL D. H. and BALLARD F. J. (1974) Production and utilization of acetate in mammals. *Biochem. J.* **142,** 401–411.

KORNACKER M. S. and BALL E. G. (1965) Citrate clearage in adipose tissue. *Proc. natn. Acad. Sci. U.S.A.* **54,** 899–904.

MARTIN B. R. and DENTON R. M. (1970) The intracellular localization of enzymes in white adipose-tissue fat cells and permeability properties of fat cell mitochondria. *Biochem. J.* **117,** 861–877.

MCALLEN J. W. and CHERFURKA W. (1961) Some physiological aspects of glutamate-aspartate transamination in insects. *Comp. Biochem. Physiol.* **2,** 290–299.

MILLS R. R. and COCHRAN D. G. (1963) Purification and properties of certain glutamic acid metabolizing enzymes from cockroach muscle mitochondria. *Biochim. biophys Acta* **73,** 213–221.

MORDUE W. and GOLDSWORTHY, G. J. (1973) Transaminase levels and uric acid production in adult locusts. *Insect Biochem.* **3,** 419–427.

MURTHY U.K. and STEINER C. (1971) Acetic thiokinase, a possible regulatory enzyme in liver. *Fed. Proc.* **30,** 207.

NAYAR J. K. and SAUERMAN D. M. (1971) Physiological effects of carbohydrates on survival, metabolism and flight potential of female *Aedes Taeniorhynchus. J. Insect Physiol.* **17,** 2221–2233.

NEWHOLME E. A. and TAYLOR K. (1969) Glycerol kinase activities in muscles from vertebrates and invertebrates. *Biochem. J.* **112,** 465–474.

ROBINSON B. H. (1973) The role of mitochondrial tricarboxylate anion transport in metabolism. *Proc. Soc. exp. Biol.* **27,** 195–214.

ROBINSON J. and NEWSHOLME E. A. (1967) Glycerol kinase activities in rat heart and adipose tissue. *Biochem. J.* **104,** 2C–4C.

ROBINSON J. and NEWHOLME E. A. (1969) Some properties of hepatic glycerol kinase and their relation to the control of glycerol utilization. *Biochem. J.* **112,** 455–464.

SCRUTTON M. C. and UTTER M. F. (1963) The regulation of glycolysis and gluconeogenesis in animal tissues. *A. Rev. Biochem.* **37,** 249–302.

SEUBERT W. and HUTH W. (1965) On the mechanism of gluconeogenesis and its regulation II. The mechanism of gluconeogenesis from pyruvate and fumarate. *Biochem. z.* **343,** 176–191.

SHONK C. E. and BOXER G. E. (1964) Enzyme pattern in human tissues. 1. Methods for the determination of glycolytic enzymes. *Cancer Res.* **24,** 709–721.

SRERE P. A. (1959) The citrate clearage enzyme. 1. Distribution and purification. *J. biol. Chem.* **234,** 2544–2547.

STOREY K. B. and BAILEY E. (1977) The intracellular distribution of enzymes of carbohydrate degradation in the fat body of the adult male cockroach. *Insect Biochem.* **8,** 75–81.

WALKER P. R. and BAILEY E. (1969) Role of 'malic enzyme' in lipogenesis. *Biochim. biophys. Acta* **187,** 591–593.

WALKER P. R. and BAILEY E. (1970a) 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. and BAILEY E. (1970b) Changes in enzymes associated with lipogenesis during development of the adult male desert locust. *J. Insect Physiol.* **16,** 679–690.

WALKER P. R. and BAILEY E. (1970c) Fat body pyruvate carboxylases of the adult male desert locust. *Comp. Biochem. Physiol.* **36,** 623–626.

WIGGLESWORTH V. B. (1942) The storage of protein, fat, glycogen and uric acid in the fat body and other tissues of mosquito larvae. *J. exp. Biol.* **19,** 56–77.