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THE INTRACELLULAR DISTRIBUTION OF ENZYMES OF CARBOHYDRATE DEGRADATION IN THE FAT BODY OF THE ADULT MALE COCKROACH

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Abstract—The intracellular distribution and maximal activities of phosphorylase, trehalase, all the enzymes of glycolysis, NAD and FAD-linked glycerol 3-phosphate dehydrogenase, arginine kinase, and adenylate kinase have been determined in the fat body of the adult male cockroach *Periplaneta americana*. Phosphorylase activity is predominantly in the soluble form but some can be sedimented. Both forms of the enzyme exhibit large increases in activity due to the presence of AMP. Only low activities of trehalase are detected and the enzyme appears to exist in both active and inactive forms. FAD-linked glycerol 3-phosphate dehydrogenase is only found in the mitochondria whereas the NAD-linked enzyme and the glycolytic enzymes are located entirely in the cytosol. Of the glycolytic enzymes, phosphoglucomutase, phosphofructokinase and aldolase exhibit unusually low activities. The results suggest that conversion of hexose to triose phosphate may occur predominantly via the pentose phosphate pathway and not via glycolysis. Whereas considerable activities of both types of glycerol-3-phosphate dehydrogenase are present in the fat body only low activities of lactate dehydrogenase are detected in the tissue.

INTRODUCTION

WORK with whole insects and tissues from a variety of insects has established that the glycolytic pathway functions in insects (KILBY, 1963; CHERFURKA, 1965; SACKTOR, 1965, 1970). Studies, in recent years, on glycogen breakdown and glycolysis in flight muscle have led to a reasonable understanding of changes in such processes and their control during flight (CRABTREE and NEWSHOLME, 1975; SACKTOR, 1975). Unfortunately, no such comprehensive and detailed study of glycogenolysis and glycolysis has been carried out on the fat body of any one insect despite the central rôle played by this tissue in insect metabolism (BAILEY, 1975). As part of a wider study of fat body intermediary metabolism and its control, we now report an investigation of the intracellular distribution and maximal activities of enzymes of carbohydrate degradation in the adult male cockroach, *Periplaneta americana*.

MATERIALS AND METHODS

Insects

Newly emerged adult male *Periplaneta americana* were kept in groups of 6 to 12 at 27°C in a lighting schedule of 12 hr light and 12 hr dark. The insects were fed a rat cube diet (Oxoid diet 86, H.C. Styles (Bewdley) Ltd., Bewdley, Worcs) which contains lipid, carbohydrate and protein in the proportions 5:70:25, and were given water to drink *ad libitum*. The insects were killed at 10 days after adult emergence and fat body removed essentially as described by WALKER *et al.* (1970).

Chemicals

All substrates, cofactors and coupling enzymes were purchased from C. F. Boehringer Corp. (London) Ltd., London W5, U.K., except for fraction 5 (defatted) bovine serum albumin, acetyl-CoA, glycogen and dihydroxyacetone phosphate which were purchased from Sigma (London) Chemical Co., Ltd., London S.W.6., U.K. and arginine phosphate which was purchased from Calbiochem Ltd., London W1, U.K.

Preparation of tissue for enzyme assay and subcellular fractionation

To measure optimal total enzyme activity or to assay cytosolic enzyme activity, fat body was homogenized (1:5 or 1:10) in 100 mM Tris–HCl, pH 8.0, containing 15 mM mercaptoethanol (buffer 1). The homogenate was centrifuged at 105,000 *g* for 20 min and the supernatant solution used for enzyme assay. To separate fat body into subcellular fractions several different media were tested and contained (final concentrations):

Medium 1: 0.154M KCl, 0.05M Tris (pH 7.4), 0.005M $MgSO_4$, 0.001M EDTA.

Medium 2: 0.25M sucrose, 10 mM Tris (pH 7.4), 0.2 mM EGTA.

Medium 3: Medium 2 containing 2% defatted bovine serum albumin.

Medium 4: 0.06M sucrose, 240 mM mannitol, 0.2 mM EGTA, 10 mM Tris (pH 7.4).

Medium 5: Medium 4 containing 2% defatted bovine serum albumin.

Studies were conducted to determine the efficiency of the various media tested to stabilize enzyme activity in the cytosol. Statistically similar enzyme activities were found for the various soluble enzymes studied whether the fat body was homogenized in buffer 1 or in media 4 or 5.

Mitochondria were isolated by the rapid method of KEELEY (1973) with the final centrifugation step being carried out at 39,000 *g* for 1 min. This method allowed for the isolation of intact mitochondria in less than 10 min and proved to be essential for accurate measurement of the activities of unstable enzymes such as NAD-dependent isocitrate dehydrogenase and glucose-6-phosphatase (STOREY and BAILEY, 1977). The nuclear fraction was prepared as KEELEY (1973) and the microsomes were isolated by taking the post-mitochondrial supernatant solution and centrifuging at 120,000 *g* for 30 min.

Of the media tried for mitochondrial isolation, media 4 and 5 proved the best for cockroach fat body. Medium 1 led to lower overall activities of fat body enzymes, both mitochondrial and soluble. Media 2 and 3 resulted in some (approximately 30%) mitochondrial rupture (as indicated by the distribution of glutamate dehydrogenase and citrate synthase among the subcellular fractions).

Medium 5 proved superior to medium 4 when long term (12 hr) stability of enzymes is taken into account. If osmotically shocked mitochondria were kept on ice for 12 hr significantly higher enzyme activities were obtained from mitochondria isolated in the presence of bovine plasma albumin. Soluble enzyme activities were also more stable in the presence of the added protein. In summary, mannitol seems essential in obtaining very good yields of intact cockroach fat body mitochondria and added bovine serum albumin is recommended when enzyme instability is a problem.

The activities of glutamate dehydrogenase, citrate synthase, FAD-dependent glycerol-3-phosphate dehydrogenase were used to assess the intactness of isolated mitochondria and to estimate the mitochondrial yield. These three enzymes are considered to be wholly mitochondrial, glycerol-3-phosphate dehydrogenase being attached to the inner mitochondrial membrane and the other two enzymes being soluble in the mitochondrial matrix (SACKTOR, 1975). Using either medium 4 or medium 5 it was found that less than 2% of citrate synthase activity and less than 0.5% of glutamate dehydrogenase activity were found in the non-mitochondrial fractions in routine preparations (STOREY and BAILEY, 1977). Glycerol-3-phosphate dehydrogenase was found to be essentially associated with the mitochondrial membrane, less than 1% of total activity appearing in the microsomal fraction and less than 0.1% in the nuclear fraction. From these biochemical criteria, the mitochondria isolated in this study are essentially intact and the method used results in essentially all the tissue mitochondria being isolated in the mitochondrial fraction. If mitochondria (intact or ruptured) was being isolated along with other subcellular fractions then the glycerol-3-phosphate dehydrogenase activity would be higher in the other fractions isolated. In this context it should be mentioned that glucose-6-phosphatase (considered to be microsomal) was located almost entirely in the microsomal fraction (STOREY and BAILEY, 1977) and less than 0.1% of the total enzyme activity was associated with the mitochondria.

Preparation of subcellular fractions for enzyme assay

Mitochondria were osmotically shocked by dilution in buffer 1 in order to rupture the membranes. Freeze-thawing of the mitochondrial pellet led to complete loss of some enzyme activities (acconitase, NAD-dependent isocitrate dehydrogenase) and low activities of many others. The osmotically shocked mitochondrial preparation was centrifuged at 100,000 *g* for 5 min and the supernatant solution used for enzyme assay. The pellet was found to contain no other activity than FAD-dependent glycerol-3-phosphate dehydrogenase. Microsomes were solubilized by suspension in buffer 1 followed by centrifugation at 100,000 *g* for 5 min and the supernatant solution used for enzyme assays. This treatment completely solubilizes glucose-6-phosphatase from the pellet. Initial homogenization of fat body in medium 4 and 5 led to glucose 6-phosphatase being confined to the microsomal membranes, whereas homogenization in buffer 1 led to complete solubilization of the enzyme activity. Nuclei were osmotically ruptured in buffer 1 followed by centrifugation at 100,000 *g* for 5 min and the supernatant used for assay of enzyme activities. None of the enzymes studied occurs in the nuclear fraction.

Assay of enzyme activities

All reactions were carried out at 25°C in 1 ml cuvettes in a recording spectrophotometer unless otherwise noted. All reactions were started by the addition of tissue preparation and unless otherwise noted were followed by measuring the change in E_{340} due to the oxidation or reduction of NAD(P)H or NAD(P).

All enzyme activities reported are the maximal obtainable under the conditions employed. To arrive at optimal assay conditions, each variable was tested over a wide range of values. For any pH optimum reported for an enzyme, all the pH values ± 1 pH unit, have been similarly tested. For every enzyme reported at least 3 different buffer systems were tested (out of, Tris, Triethanolamine, Imidazole, Glycine, Phosphate) depending on the pH range involved. The concentration of each buffer was varied over at least a 10 fold range to obtain maximal enzyme activity. Finally, the concentrations of substrates, cosubstrates and all ions have been tested over at least a 10 fold range from their reported 'optimum' values to ensure that maximal activities are reported.

Optimal conditions of assay of the various enzymes studied were as follows (final concentrations):

Phosphorylase (E.C. 2.4.1.1.). 45 mM Phosphate buffer, pH 6.8, 0.1 mM EDTA, 2 mg/ml glycogen (omitted from control), 0.4 mM NADP, 4 μM glucose 1,6-diphosphate, 15 mM $MgCl_2$, excess phosphoglucomutase and glucose-6-phosphate dehydrogenase. To measure the total phosphorylase activity, 1.6 mM AMP was added to the assay. Activity was measured in a high speed supernatant solution (homogenization in buffer 1) and in the resultant pellet.

Trehalase (E.C. 3.2.1.28.). The enzyme was assayed by the method of CANDY (1974) which uses optimal conditions. The method involves using whole fat body homogenates as enzyme source and so subcellular distribution data for trehalase is not available.

Hexokinase (E.C. 2.7.1.1.). 50 mM Triethanolamine buffer pH 7.5, 1 mM glucose, 8 mM $MgCl_2$, 1 mM NADP, 1 mM ATP (omitted from controls) and excess glucose-6-phosphate dehydrogenase. This method determines the 'low K_m' hexokinase activity and for assay of the 'high K_m' enzyme 20 mM glucose was used in the assay mixture.

Phosphoglucomutase (E.C. 2.7.5.1.). 100 mM triethanolamine buffer pH 7.6, 3.5 mM glucose 1-phosphate (omitted from control), 0.02 mM glucose 1,6-diphosphate, 0.9 mM EDTA, 1.7 mM $MgCl_2$, 0.2 mM NADP and excess glucose 6-phosphate dehydrogenase.

Phosphoglucoisomerase (E.C. 5.3.1.9.). 100 mM Triethanolamine buffer, pH 7.6, 1.4 mM fructose 6-phosphate (omitted from control), 7 mM $MgCl_2$, 0.4 mM NADP and excess glucose glucose 6-phosphate dehydrogenase.

Phosphofructokinase (E.C. 2.7.1.11.). 50 mM Tris buffer, pH 8.0, 10 mM $MgCl_2$, 100 mM KCl, 0.15 mM NADH, 2 mM ATP, 5 mM fructose 6-phosphate (omitted from control) and excess glycerol-3-phosphate dehydrogenase, aldolase and triose phosphate isomerase.

Aldolase (E.C. 4.1.2.13.). 50 mM Triethanolamine buffer, pH 7.6, 0.15 mM NADH, 0.02 mM fructose 1, 6-diphosphate (omitted from control), and an excess of glycerol-3-phosphate dehydrogenase and triosephosphate isomerase. To test enzyme activity using fructose 1-phosphate as substrate, 10 mM fructose 1-phosphate was substituted for fructose 1,6-diphosphate in the assay.

Triosephosphate isomerase (E.C. 5.3.1.1.). 100 mM Triethanolamine buffer, pH 7.6, 0.2 mM NADH, 4 mM glyceraldehyde-3-phosphate (omitted from control) and excess glycerol-3-phosphate dehydrogenase.

Glyceraldehyde 3-phosphate dehydrogenase (E.C. 1.2.1.9.): 100 mM Tris buffer pH 7.8, 20 mM glycerate-3-phosphate (omitted from control), 1.1 mM ATP, 0.9 mM EDTA, 2 mM $MgSO_4$, 0.2 mM NADH and excess phosphoglycerate kinase.

Phosphoglycerate kinase (E.C. 2.7.2.3.). 50 mM Tris buffer, pH 7.6, 0.9 mM EDTA, 0.2 mM NADH, 1.1 mM ATP, 10 mM glycerate 3-phosphate (omitted from control), 1.6 mM $MgSO_4$, and excess glyceraldehyde 3-phosphate dehydrogenase.

Phosphoglycerate mutase (E.C. 2.7.5.3.). 20 mM Tris buffer, pH 7.8, 1 mM $MgSO_4$, 0.12 mM glycerate 2,3-diphosphate, 0.2 mM NADH, 0.6 mM ADP, 6 mM glycerate-3-phosphate (omitted from control), and excess pyruvate kinase, lactate dehydrogenase, and enolase.

Enolase (E.C. 4.2.1.11.). 100 mM Triethanolamine buffer, pH 7.5, 3 mM $MgSO_4$, 0.2 mM NADH, 1.1 mM ADP, 1.1 mM 2-phosphoglycerate (omitted from control), and excess pyruvate kinase lactate dehydrogenase.

Pyruvate kinase (E.C. 2.7.1.40.). 50 mM triethanolomine buffer, pH 7.8, 100 mM KCl, 10 mM $MgCl_2$, 0.2 mM NADH, 5 mM ADP, 5 mM phosphoenolpyruvate (omitted from control), and excess lactate dehydrogenase. The addition of 0.2 mM fructose 1,6-diphosphate to the assay did not increase the enzyme activity.

Lactate dehydrogenase (E.C. 1.1.1.27.). 100 mM phosphate buffer, pH, 7.0, 10 mM pyruvate (omitted for control) and 0.2 mM NADH. The enzyme activity was unaltered by the use of 0.5 mM pyruvate in substrate.

Arginine kinase (E.C. 2.7.3.3.). The enzyme was assayed in both directions. Reverse direction: 100 mM Imidazole buffer, pH 6.7, 20 mM glucose, 10 mM $MgCl_2$, 1 mM ADP, 0.5 mM NADP, 10 mM glutathione (reduced), 20 mM arginine phosphate (omitted from control) and excess hexokinase and glucose-6-phosphate dehydrogenase. Forward direction: 20 mM glycine–NaOH buffer, pH 8.6, 20 mM $MgSO_4$, 200 mM KCl, 10 mM ATP, 2 mM phosphoenolpyruvate, 0.15 mM NADH, 20 mM arginine (omitted for control), and excess pyruvate kinase and lactate dehydrogenase.

Adenylate kinase (E.C. 2.7.4.3.). 50 mM Tris buffer, pH 7.8, 2 mM AMP (omitted from control) 2 mM ATP, 0.5 mM phosphoenol pyruvate, 4 mM $MgSO_4$, 150 mM KCl, 0.2 mM NADH, and excess pyruvate kinase and lactate dehydrogenase.

NAD-dependent glycerol-3-phosphate dehydrogenase (E.C. 1.1.1.8.). 100 mM thriethanolamine buffer, pH 7.8, 0.2 mM NADH, 0.4 mM dihydroxyacetone phosphate (omitted from controls).

FAD-dependent glycerol-3-phosphate dehydrogenase (E.C. 1.1.99.5.). The enzyme was assayed by the method of PENNINGTON (1961). Two preparations were used for enzyme assay, either whole, osmotically shocked mitochondria before centrifugation or the mitochondrial membrane fraction. Both fractions gave essentially the same results. Optimal conditions of assay were: 50 mM phosphate buffer, pH 7.5, 1 mM KCN, 1.7 mM acetaldehyde, 0.05% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyl-tetrazolium chloride), 20 mM α-glycerophosphate (omitted for controls) 10^{-5} M Ca^{2+} and excess alcohol dehydrogenase.

Protein determinations

Protein was determined by the method of WADDELL (1956).

RESULTS

The results for fat body phosphorylase and trehalase are shown in Table 1. The majority of the phosphorylase appears to be soluble and relatively little is sedimentable with the glycogen particles. Neither the soluble nor the precipitated form of the enzyme exhibits much activity in the absence of AMP, but the addition of this nucleotide causes considerable enhancement (8 fold) of activity in both cases. In contrast to the results for phosphorylase, only low activities of trehalase are detected in fat body. However, the low ('unactivated') activity can be increased somewhat by the addition of detergent to the tissue preparation ('activated'), suggesting possible release of bound 'inactive' enzymes from a membrane. Even in the presence of detergent the activity of trehalase is low compared to phosphorylase and other enzymes assayed.

The activities of the glycolytic enzymes (which are only detected in the cytosol) are shown in Table 2. Although a comparison of these activities with those for insect flight muscle and mammalian tissues will be made in the discussion, several interesting points about the fat body results can be made at this point. Fat body contains both a low K_m and a high K_m hexokinase, i.e. equivalent to the hexokinase and glucokinase of mammalian liver. The conversion of glucose 6-phosphate, formed by hexokinases, to triose phosphate involves phosphoglucoisomerase, phosphofructokinase and aldolase and although the isomerase is quite active, only low activities of the other two enzymes are detected in fat body. Glucose 6-phosphate can also be metabolized via the pentose phosphate pathway (see discussion) or used in the synthesis of trehalose. The latter possibility involves conversion of glucose 6-phosphate to glucose 1-phosphate by the enzyme phosphoglucomutase which as

Table 1. The activities of phosphorylase and trehalase in fat body

Enzyme		Enzyme activity (μmole per min per g wet weight tissue)
Phosphorylase		
(a) Supernatant	−AMP	2.5 ± 0.11
	+AMP (1.6 mM)	17.5 ± 0.93
(b) Precipitate	−AMP	0.2 ± 0.02
	+AMP (1.6 mM)	2.0 ± 0.15
Trehalase		
(a) No additions		0.063 ± 0.008
(b) +Triton X 100		0.102 ± 0.01

The results are the means ± S.E.M. of at least 7 determinations and each determination involved pooled tissue from several insects. The preparation and assay of enzymes is as described in the Materials and Methods section.

Table 2. The activities of the enzymes of glycolysis in fat body

Enzyme		Enzyme activity (μmole substrate utilized per min per g wet weight tissue)	
Hexokinase (low K_m)		6.3 ± 0.17	
Hexokinase (high K_m)		6.5 ± 0.14	
Phosphoglucomutase		1.3 ± 0.06	
Phosphoglucoisomerase		27.3 ± 1.01	
Phosphofructokinase		1.0 ± 0.02	
Aldolase (substrate fructose 1,6-diphosphate)	(a)	1.2 ± 0.06	
Aldolase (substrate fructose 1, phosphate)	(b)	0.06 ± 0.01	ratio [(a)/(b)] = 20
Triosephosphate isomerase		133 ± 2.5	
Glyceraldehyde-3-phosphate dehydrogenase		21.7 ± 1.2	
Phosphoglycerate kinase		113 ± 3.3	
Phosphoglycerate mutase		22.5 ± 0.52	
Enolase		14.7 ± 0.31	
Pyruvate kinase		50.0 ± 0.71	

The results are the means ± S.E.M. of at least 7 determinations (each determination involving pooled tissue from several insects). Enzyme activities were only detected in the cytosol with none being found in the nuclear, mitochondrial or microsomal cell fractions.

can be seen from Table 2 has a very low activity. This enzyme is of course also involved in the breakdown of glycogen via the glycolytic or pentose phosphate pathways.

Phosphofructokinase is the lowest activity glycolytic enzyme in the fat body, being present at only 1 unit/g wet weight (Table 2). Experiments with crude preparations suggest that the phosphofructokinase of fat body is similar to that from other sources with respect to nucleotide control, e.g. at pH 7.0 fructose 6-phosphate saturation curves are sigmoidal with the affinity of the enzyme for substrate being increased by the presence of AMP. The fact that fat body aldolase is 20 fold more active towards fructose 1,6-diphosphate than fructose 6-phosphate suggests that it is of the type found in mammalian and insect muscle (ENDO *et al.*, 1970; BAUER *et al.*, 1974) rather than that of mammalian liver (ENDO *et al.*, 1970).

Conversion of triose phosphate to pyruvate involves the enzymes glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase and mutase, enolase, and pyruvate kinase. It is noteworthy that both the kinases involved are present in very high activity and that the activities of all the enzymes involved are in excess of those of the key enzymes involved in triose phosphate formation. Experiments with crude preparations of fat body suggest that pyruvate kinase is little affected by fructose 1,6-diphosphate, e.g. the K_m of the enzyme for phosphoenolpyruvate is 0.2 mM in the absence of and 0.15 mM in the presence of fructose 1,6-diphosphate. These results indicate that the enzyme is much more like that found in mammalian and locust muscle than that in mammalian liver and locust fat body (TAYLOR and BAILEY, 1967; BAILEY and WALKER, 1969).

As indicated in Table 3, fat body contains considerable activity of adenylate kinase but none of arginine kinase in the cytosolic cell fraction. Low activities of lactate dehydrogenase are also found in the cytosol and the enzyme activity is unaffected by high concentrations of pyruvate suggesting that the enzyme is of the type found in mammalian muscle and liver (EUERGE and KAPLAN, 1972). Although fat body only has low lactate dehydrogenase activity it does contain considerable activities of cytosolic NAD-dependent glycerol-3-phosphate dehydrogenase and mitochon-

Table 3. The activities of lactate and glycerol-3-phosphate dehydrogenases and adenylate and arginine kinases in fat body

Enzyme	Enzyme activity (μmole substrate utilized per min per g wet weight tissue)	
	Cytosol	Mitochondria
Lactate dehydrogenase		
pyruvate—0.5 mM	0.4 ± 0.01	0
pyruvate—20 mM	0.4 ± 0.01	0
NAD-linked glycerol 3-phosphate dehydrogenase	14.2 ± 0.80	0
FAD-linked glycerol-3-phosphate dehydrogenase	0	4.45 ± 0.41
Arginine kinase	0	0
Adenylate kinase	16.7 ± 1.1	0

The results are the means ± S.E.M. of at least 7 determinations (each determination involving pooled tissue from several insects). Enzyme activities were detected in the cell fractions indicated but none in the nuclear or microsomal fractions.

drial FAD-dependent glycerol-3-phosphate dehydrogenase, the essential enzymic components of the glycerophosphate shuttle for the oxididation of cytosolic NADH (SACKTOR, 1965).

DISCUSSION

In contrast to the fat body of *Locusta migratoria*, in which the majority of the phosphorylase activity is sedimentable (APPLEBAUM and SCHLESINGER, 1973), the enzyme activity in the cockroach fat body is predominantly in the soluble form. However, in common with the locust enzyme and that from the fat bodies of *Samia cynthia* and *Hyalophora cecropia* (STEVENSON and WYATT, 1964), AMP has a marked stimulatory effect on the activity of the enzyme. The rôle of this nucleotide in the control of glycogenolysis differs in various organisms, playing a central rôle in mammalian systems via activation of phosphorylase b, but having no effect on protozoan or yeast phosphorylase (BLUM, 1970; SAGARDIA *et al.*, 1971). As with the phosphorylase of mammalian tissues that in the flight muscles of *Phormia regina* exists in two forms (a and b). However, in the case of the blowfly flight muscle it is the a form of the enzyme rather than the b form which is readily activated by AMP (CHILDRESS and SACKTOR, 1970). Evidence for the existence of a and b forms of phosphorylase in the fat body of *Hyalophora cecropia* has also been obtained (WIENS and GILBERT, 1967). Clearly our studies with crude preparations only deal with the maximal activity of the cockroach fat body phosphorylase and give no indication of the number of enzymic forms present in the tissue.

Although the cockroach fat body only contains low activities of trehalase it is of interest that the enzyme can be activated by detergent as has been reported for the flight muscle enzyme from *Hyalophora cecropia* (GUSSIN and WYATT, 1965) and *Schistocerca gregaria* (CANDY, 1974). It is paradoxical that the fat body which is the site of formation of trehalose (WYATT, 1967) should also possess the capacity to degrade the carbohydrate. Presumably, either, control mechanisms exist to prevent a futile cycle of trehalose synthesis and degradation or, the two enzymic systems are spatially separated within the fat body. Since the fat body contains several different types of cells it is conceivable that some cells produce trehalose and others utilize it. Further, if the degrading cells were only a small proportion of the total then this would explain the relatively low activity of trehalose in whole tissue homogenates. At the moment however such comments can only be speculative since there have as yet been no reports of studies on enzyme distribution within fat body cell types.

It is of great interest that cockroach fat body contains not only a low K_m hexokinase but also a high K_m (glucokinase) enzyme which is characteristic of mammalian liver (SCRUTTON and UTTER, 1968). Clearly in the fat body as with the liver the possession of the two glucose phosphorylating enzymes is of great advantage when dealing with the large variations in the amounts of glucose arriving at the tissues from the gut due to changes in the dietary intake of glucose and glucose containing carbohydrates. In the fat body, as with the liver, glucose-6-phosphate formed by the action of hexokinase can be converted to glycogen and lipid and in the fat body the phosphorylated glucose can also be converted to trehalose to maintain the level of this disaccharide in the haemolymph (BAILEY, 1975).

Conversion of dietary carbohydrate to lipid in the fat body involves glyeolytic breakdown (BAILEY, 1975) and it is of interest that the activities of three of the four enzymes involved in glycolytic conversion of glucose-6-phosphate to triose phosphate, i.e. phosphoglucomutase, phosphofructokinase and aldolase, are very low whereas that of the other enzyme phosphoglucoisomerase, is not. The enzymes involved in triose phosphate conversion to pyruvate are all present in considerable activity. Further, the rate limiting enzyme of the pentose phosphate pathway, glucose-6-phosphate dehydrogenase, is also quite active in fat body (STOREY and BAILEY, 1977) and it seems likely that in this lipogenic tissue conversion of glucose-6-phosphate to triose phosphate occurs predominantly via the pentose phosphate pathway, thus generating NADPH for lipogenesis. This would explain the considerable activities of phosphoglucoseisomerase detected in fat body since this enzyme is essential for the recycling of the fructose-6-phosphate formed in the pentose phosphate pathway. The results of CHERFURKA *et al.* (1970) on the assessment of pentose phosphate pathway activity is whole insects is also consistent with this explanation if one assumes that is the whole insect, fat body makes a major contribution to total pentose phosphate pathway activity.

In common with the more thoroughly studied insect flight muscle systems (SACKTOR, 1965, 1975; CHERFURKA, 1965) the cockroach fat body contains only a low activity of lactate dehydrogenase but in contrast high activities of the glycerophosphate shuttle enzymes, NAD and FAD dependent glycerol-3-phosphate dehydrogenase. Thus oxidation of glycolytically generated NADH may be achieved via this shuttle system. However, fat body is a lipogenic tissue and both NAD-dependent glycerol-3-phosphate dehydrogenase and cytosolic NADH generated during glycolytic breakdown of carbohydrate, will be involved in lipogenesis (STOREY and BAILEY, 1977). Although the fat body lactate dehydrogenase activity is quite low it may still be sufficient to cope with lactate produced by lactate dehydrogenase activity in the leg muscle and mid gut (CHERFURKA, 1965) in much the same way that the mammalian liver deals with lactate produced by skeletal muscle.

It has been shown that in a wide range of tissues from different forms of life the relative activities of many of the enzymes of glycolysis are the same with triosephosphate isomerase always having high activity, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase and phosphoglycerate mutase having similar medial activities and enolase having relatively low activity (PETTE *et al.*, 1962). However, consideration of the activities of the fat body glycolytic enzymes reported in this paper indicated that these enzymes do not contain any such constant proportion group. Thus phosphoglycerate kinase was well as triose phosphate isomerase has relatively high activity whereas all the other enzymes quoted in the work of PETTE *et al.* (1962) have medial activity.

Table 4. A comparison of the activities of the enzymes of glycolysis in insect fat body and flight muscle and rat liver and adipose tissue

Enzyme	Enzyme activity						
	Cockroach fat body	Locust flight muscle		Rat liver		Rat adipose tissue	
		(a)	(b)	(a)	(b)	(a)	(b)
Phosphorylase (total)	22.2	8	0.4	18	12	0.2	1.7
Hexokinase (low K_m)	6.3	12	0.7	0.4	0.2	0.17	1.4
Hexokinase (high K_m)	6.5	0	0	2	1.3	0	0
Phosphoglucomutase	1.3	—	—	21	14	0.85	7.1
Phosphoglucoisomerase	27.3	20	1.2	112	74	3.2	27
Phosphofructokinase	1.0	17	1	1.5	1	0.12	1
Aldolase	1.2	13	0.8	5	3.3	0.35	2.9
Triosephosphate isomerase	133	2,200	130	476	315	—	—
Glyceraldehyde-3-phosphate dehydrogenase	21.7	330	19	85	57	0.15	1.3
Phosphoglycerate kinase	113	340	20	75	50	1.5	13
Phosphoglyceromutase	22.5	425	25	36	24	2.2	18
Enolase	14.7	42	3	8	5.3	0.3	2.5
Pyruvate kinase	50	90	5	25	17	2.5	21
Lactate dehydrogenase	0.4	1.5	0.1	115	76	3.5	29
Glycerol-3-phosphate dehydrogenase (NAD)	14.2	140	8	65	44	3.1	26
Glycerol-3-phosphate dehydrogenase (FAD)	4.4	34	2				

The data for locust flight muscle, except for aldolase (K. B. STOREY, unpublished observations), was obtained from SACKTOR (1965) and CRABTREE and NEWSHOLME (1975) and the data for rat tissues from SHONK and BOXER (1964) and SCRUTTON and UTTER (1968). In (a) the results are expressed as μmoles per min per g weight of tissue at 25°C and in (b) the results are expressed relative to a value of 1 for phosphofructokinase.

Since insect fat body has been compared to a combined mammalian liver and adipose tissue (KILBY, 1963) the activities of the glycolytic and glycerophosphate shuttle enzymes in the three tissues have been compared in Table 4. Also included in the table is data for locust flight muscle which is the only other insect tissue for which the activities of most of the enzymes of glycolysis and glycerophosphate shuttle are available. The absolute activities of most of the enzymes are higher in flight muscle than in fat body except for pyruvate kinase, phosphoglucoisomerase and total hexokinase which are similar in both tissues and phosphorylase which is higher in fat body than flight muscle. When the activities of the enzymes are considered relative to the activity of the key regulatory enzyme phosphofructokinase then similar values are found for many enzymes in both tissues but with enolase and phosphoglycerate kinase in addition to those enzymes mentioned in the previous sentence being relatively low in flight muscle.

Both the absolute activities and the activities relative to phosphofructokinase of many of the enzymes of glycolysis are similar in liver and fat body. However, the activities of essentially all the enzymes of the initial portion of glycolysis (phosphoglucomutase to glyceraldehyde 3-phosphate dehydrogenase) are higher in liver than fat body. This may be indicative of the fact that liver utilizes this sequence of reaction both in glycogen breakdown as well as gluconeogenesis, whereas fat body may use alternative pathways of carbohydrate degradation (such as the pentose phosphate pathway) preferentially. Lactate dehydrogenase levels are also much higher in the liver than fat body, indicating that lactate is a much more prevalent metabolite in vertebrate metabolism. Insects, such as the cockroach, are essentially obligate aerobes and consequently lactate production will be minimal.

Difficulties arise when attempting comparisons of fat body enzyme levels with those from vertebrate adipose tissue since there are few reports in the literature concerning the appropriate adipose tissue enzymes. However, from the available data, absolute activities of the glycolytic enzymes other than lactate dehydrogenase appear to be much lower in adipose tissue than in fat body. Even when expressed relative to the activity of phosphofructokinase, apart from lactate dehydrogenase, only phosphoglucomutase, phosphoglucoisomerase, aldolase and phosphoglyceromutase have values similar to or greater than those in fat body.

In conclusion it may be said that fat body resembles each of liver, adipose tissue and flight muscle in certain respects. Like liver it contains high levels of phosphorylase and has a 'high K_m' hexokinase (or glucokinase activity). Like adipose tissue it has a higher hexokinase activity than phosphofructokinase activity. However, it also resembles flight muscle in several respects: it has similar phosphofructokinase to triose phosphate isomerase and phosphofructokinase to glyceraldehyde 3-phosphate dehydrogenase ratios (ratios very different to the one found in liver and adipose tissue). Further aldolase and phosphofructokinase occur in a one to one activity ratio in muscle and fat body whereas aldolase has much higher activity in the other two tissues. The fat body also resembles the flight muscle in having very low lactate dehydrogenase activity and a very active α-glycerophosphaste cycle potential, indicating the essential aerobic metabolism of these two tissues. Unlike any of the other three tissues of comparison, however, fat body lacks phosphagen kinase. Vertebrate muscles as well as insect flight muscle contain phosphagen kinases and recently creatine kinase has been detected in liver and adipose tissue (BERLET *et al.*, 1976).

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