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Lipogenesis in Rat and Guinea-Pig Isolated Epididymal Fat-Cells

By E. DAVID SAGGERSON
Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, U.K.

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Fat-cells were prepared from rat and guinea-pig epididymal adipose tissue and compared on the basis of the intracellular distributions and activities of enzymes and with respect to their utilization of various U-^{14}C-labelled substrates for lipogenesis. 1. Compared with the rat, guinea-pig extramitochondrial enzyme activities differed in that aconitate hydratase, alanine aminotransferase, ATP–citrate lyase, lactate dehydrogenase, NAD–malate dehydrogenase, NADP–malate dehydrogenase and phosphoenolpyruvate carboxykinase activities were appreciably lower, whereas aspartate aminotransferase, glucose 6-phosphate dehydrogenase, NADP–isocitrate dehydrogenase and 6-phosphogluconate dehydrogenase activities were appreciably higher. Mitochondrial activities of citrate synthase, NADP–isocitrate dehydrogenase and pyruvate carboxylase were appreciably lower, whereas mitochondrial activities of aspartate aminotransferase, glutamate dehydrogenase, NAD–malate dehydrogenase and phosphoenolpyruvate carboxykinase were higher in the guinea pig compared with the rat. 2. In general guinea-pig fat-cells incorporated acetate and lactate into fatty acids more readily than rat fat-cells, whereas rat fat-cells incorporated glucose and pyruvate more readily than guinea-pig fat-cells. 3. Acetate stimulated the incorporation of glucose into fatty acids in rat fat-cells, but had no appreciable effect upon this process in guinea-pig fat-cells. Acetate greatly decreased the incorporation of lactate into fatty acids in cells from both species. 4. Lactate/pyruvate ratios produced by incubation of guinea-pig cells with glucose+insulin were very low compared with those found with rat cells under the same conditions. 5. With glucose (+insulin) or with glucose+acetate (+insulin) as substrates guinea-pig cells produced enough NADPH by the hexose monophosphate pathway to satisfy the NADPH requirements of lipogenesis. In rat fat-cells under the same conditions, hexose monophosphate-pathway NADPH provision was not sufficient to meet the requirements of lipogenesis. 6. These results are discussed, particularly in relationship to the disposition of cytosolic reducing equivalents in the cells.

A considerable amount of information concerning the metabolic behaviour of rat epididymal adipose tissue *in vitro* has arisen from studies using both incubated fat-pad segments and isolated fat-cells to investigate the ability of the tissue to utilize various substrates (glucose, pyruvate, lactate, acetate) for lipogenesis, the nature of the pathways involved, and the effects of hormones and other physiological or non-physiological agents on the rate of operation of these pathways (Flatt & Ball, 1964, 1966; Katz *et al.*, 1966; Rognstad & Katz, 1966, 1969; Kneer & Ball, 1968; Del Boca & Flatt, 1969; Katz & Wals, 1970; Saggerson & Greenbaum, 1970*a*,*b*; Halperin, 1971; Saggerson, 1972*a*,*b*). These studies have given some insight into the metabolic potential of this particular tissue and the regulation of the lipogenic pathways. Studies with incubated rat adipose-tissue preparations have been complemented by studies of the properties and metabolism of isolated mitochondria (Martin & Denton, 1970, 1971) and of the intracellular activities and distributions of enzymes from rat adipose tissue (Martin & Denton, 1970).

A general picture of rat adipose tissue has emerged of a tissue able to readily synthesize glyceride fatty acids from glucose in a manner that is sensitive to insulin. Pyruvate may also be utilized as a lipogenic substrate, but lactate is poorly utilized. There is considerable evidence for the involvement of (*a*) a pathway of 'citrate cleavage' in the transport from mitochondria to cytosol of acetyl units generated from these substrates and (*b*) a 'malate–pyruvate' cycle in disposal of cytosolic, and regeneration of mitochondrial, oxaloacetate units. Provision of NADPH for fatty acid synthesis appears to be partly from the hexose monophosphate-pathway dehydrogenases and partly from other sources, presumably the NADP–malate dehydrogenase through its involvement in the 'malate–pyruvate' cycle. Two regulatory aspects are particularly pertinent to this study. There is evidence that the utilization for lipogenesis by rat adipose tissue of the physiological substrate glucose is restrained by limitation of the disposal of cytosolic NADH. Secondly, glucose utilization for lipogenesis may be 'self-limited' as, in isolation, it produces a

net ATP yield (Flatt, 1970). Simultaneous acetate utilization may relieve restraints imposed through the ATP balance.

These detailed investigations of rat adipose tissue have not been followed by investigations of similar depth into the metabolism of adipose tissues of other mammalian species. The relative utilization of glucose and acetate by ruminant adipose tissue has been investigated by Hanson & Ballard (1967) and by Ballard *et al.* (1972). In non-ruminants, besides the rat and the mouse, O'Hea & Leveille (1968, 1969) showed lipogenesis in porcine adipose tissue from various substrates, and human adipose tissue has been investigated by several workers (Galton & Wilson, 1970; Gries, 1970). Some information concerning pig, sheep and cow adipose-tissue enzyme activities has also been presented by these workers. Comparative studies with other lipogenic tissues, namely liver and lactating mammary gland, have been published for rat, guinea pig, rabbit, sow, goat, sheep and cow (Hardwick, 1966; Hanson & Ballard, 1968; Gul & Dils, 1969; Bauman *et al.*, 1970, 1972; Stanley, 1972; Strong & Dils, 1972; Gumaa *et al.*, 1973).

In the present study rat and guinea-pig fat-cells are compared with respect to their ability to utilize certain substrates in lipogenic and related pathways and with respect to differences in enzyme activity and distribution that may be correlated with the previous parameters. There is a general desirability to gain information concerning the regulation of triglyceride synthesis in species other than the rat. Also comparative regulatory studies of this kind, by presenting contrasts, may pick out and underline the more important regulatory features in each species under investigation. Two aspects are particularly considered. The guinea pig, though a non-ruminant, appears to more readily utilize a certain amount of short-chain fatty acids as lipogenic substrates than is the case found in the rat (Stanley, 1972). This property was therefore used to test the more general applicability of the hypothesis of Flatt (1970) considered above. Particular attention is also paid to the sources and disposal of reducing equivalents in fat cells from the two species and the relative effects of these on the regulation of lipogenesis.

Materials and Methods

Chemicals

Triethanolamine hydrochloride, Tris, sodium pyruvate, oxalacetic acid, GSH, all nucleotides and other phosphorylated compounds and all enzymes were obtained from the Boehringer Corp. (London) Ltd. (London W.5, U.K.). 5,5'-Dithiobis-(2-nitrobenzoic acid), amino-oxyacetic acid hemihydrochloride, L-aspartate (potassium salt), DL-3-hydroxybutyrate (sodium salt), L(+)-lactic acid, L-malic acid, 2-oxoglutaric acid, L-alanine, *threo*-D_s(+)-isocitrate (monopotassium salt), phenazine methosulphate and calf thymus DNA were obtained from the Sigma (London) Chemical Co. Ltd. (London S.W.6, U.K.). Bovine serum albumin (fraction V) from the Armour Pharmaceutical Co. Ltd. (Eastbourne, U.K.) was defatted as described by Saggerson (1972*a*). All radiochemicals were obtained from The Radiochemical Centre (Amersham, Bucks., U.K.), hyamine hydroxide from Nuclear Enterprises Ltd. (Edinburgh, U.K.), and 2,5-bis-(5-t-butylbenzoxazol-2-yl)thiophen from CIBA (A.R.L.) Ltd. (Duxford, Cambs., U.K.). (−)-Carnitine chloride was purchased from Koch–Light Laboratories (Colnbrook, U.K.), *NNN'N'*-tetramethyl-*p*-phenylenediamine from BDH Chemicals Ltd., Poole, Dorset, U.K., bovine insulin (6× recrystallized) from Boots Pure Drug Co. Ltd. (Nottingham, U.K.), and anti-insulin serum from the Wellcome Research Laboratories (Beckenham, Kent, U.K.). Insulin was freshly dissoved at a concentration of 1mg/ml in 3mM-HCl and diluted appropriately in incubation media. Acetyl-CoA was prepared by the method of Simon & Shemin (1953) and standardized by using phosphotransacetylase (Stadtman, 1957). All other chemicals were of A.R. grade and were used without further purification.

Animals

Male Wistar rats and male guinea pigs were obtained from A. Tuck and Son Ltd. (Rayleigh, Essex, U.K.) or were bred in the animal colony at University College from animals originally obtained from Tuck's. The rats were maintained on Cube diet 41B (Bruce & Parkes, 1949) and the guinea pigs on diet SG1 (Oxoid Ltd., London S.E.1, U.K.) supplemented with green food (cabbage). All animals had water *ad libitum*. At death rats and guinea pigs weighed 150–200g and 600–800g respectively. All animals were killed by cervical dislocation.

Fat-cell techniques

Preparation. Fat-cells from rat and guinea-pig epididymal adipose tissue were prepared essentially as described by Rodbell (1964). The media used for the initial disaggregation of rat and guinea-pig tissues differed in that rat fat-pads were shaken for 30min in Krebs–Ringer bicarbonate (Krebs & Henseleit, 1932) containing collagenase (4mg/ml) and 2% (w/v) defatted albumin, whereas disaggregation of guinea-pig tissues was done by shaking for 15min in Ringer containing collagenase (2mg/ml) and 2% (w/v) defatted albumin. The washed cells were suspended in a final volume of Ringer containing 1.0% albumin and samples (1.0ml) were taken for determination of

DNA (Saggerson, 1972*a*) and dry weight. The concentration of washed cells in this final medium depended on the nature of the experiment as indicated below.

Incubation of cells and measurement of incorporation of ^{14}C-labelled substrates into metabolic products. Samples (0.5ml) of cells were dispensed into incubation flasks from suspensions of washed cells prepared as shown above. These 'stock' cell suspensions contained cells equivalent to one pair of rat or guinea-pig fat-pads per 3ml or per 9ml of Ringer respectively. All incubations were carried out at 37°C with shaking in 4ml volumes of Krebs–Ringer bicarbonate containing 1.0% albumin and the required ^{14}C-labelled substrate in 25ml Erlenmeyer flasks as described by Saggerson & Tomassi (1971). Incubation was for 60 or 90min as indicated in individual tables and figures. Procedures for determination of ^{14}C incorporation into glyceride fatty acids, glyceride glycerol and CO_2 and for measurement of lactate and pyruvate production were as described by Saggerson & Tomassi (1971) and by Saggerson (1972*a*,*b*).

Preparation of fat-cell extracts for determination of enzyme activities. Fat-cells from the pooled epididymal adipose tissues of six rats or two guinea pigs were suspended in a final volume of 12ml of Krebs–Ringer bicarbonate containing 1% albumin and samples (1.0ml) were taken for determination of DNA and dry weight. The remaining 10ml of cell suspension was centrifuged to remove as much Ringer as possible and the cells were disrupted in ice-cold sucrose medium (0.25M-sucrose containing 2mM-EGTA [ethanedioxybis(ethylamine)tetra-acetic acid], 20mM-Tris–HCl, 2% albumin) by agitation for 1min on a vortex mixer (Martin & Denton, 1970). The broken-cell suspension was centrifuged at 700*g* for 1min at 4°C to remove fat and a measured volume of the resulting infranatant centrifuged at 38000*g* for 30min. The two fractions produced were designated as 'supernatant' (S) and 'mitochondrial' (M) fractions. Washing of fraction M, used by Martin & Denton (1970), was not carried out. Fraction M was suspended in sucrose medium and subjected to ultrasonication at 0°C for four 30s periods over 4min. In most experiments the cell suspensions were disrupted in 15ml of sucrose medium and fraction M was sonicated in 3ml of the same medium. In experiments in which phosphoenolpyruvate carboxykinase activity was measured the cell disruption was done in 7ml and the sonication of fraction M in 1ml.

Measurement of enzyme activities

Spectrophotometric assays. These were carried out at 25°C in either 3.0ml or 1.2ml volume in cuvettes of 1cm light-path with a Unicam SP. 8000 recording spectrophotometer. Reactions were started by addition of a substrate. Simultaneous blanks were carried out by omission of this substrate. In all cases the rates of reaction were constant over the measured period and proportional to the volume of extract used. ATP–citrate lyase (EC 4.1.3.8), aconitate hydratase (EC 4.2.1.3), alanine aminotransferase (EC 2.6.1.2), aspartate aminotransferase (EC 2.6.1.1), carnitine acetyltransferase (EC 2.3.1.7), glucose 6-phosphate dehydrogenase (EC 1.1.1.49), glutamate dehydrogenase (EC 1.4.1.2), NAD–malate dehydrogenase (EC 1.1.1.37), NADP–isocitrate dehydrogenase (EC 1.1.1.42), NADP–malate dehydrogenase (EC 1.1.1.40) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) were assayed as described by Martin & Denton (1970). Citrate synthase (EC 4.1.3.7) was assayed by using the conditions described by Saggerson & Tomassi (1971) and lactate dehydrogenase (EC 1.1.1.27) as described by Saggerson & Greenbaum (1969). 3-Hydroxybutyrate dehydrogenase (EC 1.1.1.30) was assayed in the direction of 3-hydroxybutyrate oxidation by the method of Lehninger *et al.* (1960).

Radiochemical assays. Phosphoenolpyruvate carboxykinase (EC 4.1.1.32) was assayed essentially by using the method of Chang & Lane (1966). Samples (0.1ml) of tissue extracts were incubated for 10min at 30°C in stoppered tubes in a final volume of 1.05ml containing: 80μmol of imidazole chloride buffer, pH6.6; 50μmol of $KH^{14}CO_3$ (approx. 0.5μCi/mol); 1.25μmol of IDP; 1.8μmol of $MnCl_2$; 2.0μmol of GSH; 1.0μmol of NADH; 1.25μmol of phosphoenolpyruvate; 20 units of malate dehydrogenase; 0.25μg of rotenone. Phosphoenolpyruvate was omitted from the blanks which were assayed in parallel with every sample. Reactions were stopped by addition of 0.5ml of 2.5M-HCl. The tubes were placed in ice and 0.01ml of 1M-malic acid was added to each tube. Samples (0.2ml) of the tube contents were dried on squares of Whatman no. 1 filter paper in a cold-air blast, immersed in 15ml of scintillator [4g of 2,5-bis-(5-t-butylbenzoxazol-2-yl)thiophen/litre in toluene] and counted for radioactivity. The efficiency of counting was determined by counting standardized samples of [U-^{14}C]malic acid under identical conditions. Preliminary experiments established that these assay conditions gave linearity with respect to time of incubation and amount of tissue extract used.

Pyruvate carboxylase (EC 6.4.1.1) was assayed by using a modification of the method of Utter & Keech (1963). Acetyl-CoA in the assay was generated *in situ* (Wimhurst & Manchester, 1970). Samples (0.1ml) of tissue extracts were incubated for 10min at 30°C in stoppered tubes in a final volume of 1.25ml containing 120μmol of Tris–HCl buffer, pH7.4, 6μmol of $MgCl_2$, 30μmol of $NaH^{14}CO_3$ (approx. 0.2μCi/μmol), 3μmol of ATP, 0.5μmol of CoA, 1.2μmol of acetyl phosphate, 8μmol of sodium

pyruvate, 1.5 units of citrate synthase and 0.5 unit of phosphotransacetylase. Pyruvate was omitted from the blanks, which were assayed in parallel with every sample. Omission of ATP was found to be equally effective as a blank. Reactions were stopped by addition of 0.5ml of 2.5M-HCl, the tubes were placed in ice and 0.01ml of 1M-potassium citrate was added. Samples were dried on paper and counted for radioactivity as described for the phosphoenolpyruvate carboxykinase assay. Linearity of the assay with respect to time and quantity of tissue extract used was established in preliminary experiments.

A spectrophotometric assay (Martin & Denton, 1970) was used initially to assay pyruvate carboxylase and gave results for rat tissue extracts similar to those obtained previously (Saggerson & Tomassi, 1971). However, no pyruvate carboxylase activity was detectable in guinea-pig extracts by using this assay. Samples of rat extracts assayed in the presence and absence of equivalent amounts of guinea-pig extracts gave identical readings, suggesting that guinea-pig extracts did not contain inhibitory substances. The spectrophotometric assay was therefore discarded in favour of the CO_2-fixation assay.

All enzyme assays were performed on the same day as the preparation of the tissue extracts.

Expression of results and calculation of ^{14}C fluxes

Rates of incorporation of ^{14}C-labelled substrates by cells are expressed throughout as μg-atoms of substrate carbon/h per 100μg of fat-cell DNA.

Fluxes of glucose carbon and, where appropriate, acetate carbon through various metabolic pathways were calculated from the yields of ^{14}C in fatty acids, CO_2 and glyceride glycerol and from the measured production of lactate and pyruvate with [1-^{14}C]-glucose, [6-^{14}C]glucose, [U-^{14}C]glucose and [U-^{14}C]-acetate as starting substrates (Flatt & Ball, 1964, 1966; Katz *et al.*, 1966).

Enzyme activities have been expressed throughout as munits/100μg of fat-cell DNA, where 1 munit of enzyme converts 1nmol of substrate/min at 25°C or 30°C in the spectrophotometric and radiochemical assays respectively.

Rat and guinea-pig fat-cells contained mean values of 196 and 93μg of DNA/g dry wt. respectively.

Results

Activities and intracellular distributions of enzymes

In all experiments rat and guinea-pig extracts were made and assayed for enzyme activities on the same day by using the same extraction and assay media. Glutamate dehydrogenase and lactate dehydrogenase activities were determined in S and M fractions in all experiments. It was assumed that glutamate dehydrogenase and lactate dehydrogenase are of exclusively mitochondrial and extramitochondrial origin respectively. These were used as marker enzymes to assess the cross-contamination of S and M fractions. In each determination S and M fraction enzyme activities were corrected for this cross-contamination to give corrected extramitochondrial and mitochondrial activities respectively. The mean contaminations of M fractions by lactate dehydrogenase and of S fractions by glutamate dehydrogenase were 4.3±0.3% and 7.6±1.0% respectively for rat experiments (16 determinations). The corresponding figures for guinea-pig experiments were 2.2±0.2% and 5.7±0.5% (18 determinations). Since these values were small, application of corrections for this cross-contamination of S and M fractions did not in general cause the calculated extramitochondrial and mitochondrial activities to be greatly different from the directly measured activities in S and M fractions.

The corrected mitochondrial and extramitochondrial activities of 16 enzymes in rat and guinea-pig fat cells are shown in Table 1. The values obtained in the rat tissue extracts may be compared with those obtained previously (Martin & Denton, 1970; Saggerson & Tomassi, 1971). Rat glutamate dehydrogenase, NADP–malate dehydrogenase, citrate synthase, pyruvate carboxylase and phosphoenolpyruvate carboxykinase activities were similar to those found by Saggerson & Tomassi (1971). In general total enzyme activities in rat cells in this study were lower than those found by Martin & Denton (1970). The activity of aconitate hydratase was particularly low in this study compared with the value of Martin & Denton (1970). The mitochondrial and extramitochondrial distributions of rat enzymes found in this study were not greatly different from those found by Martin & Denton (1970). NAD–malate dehydrogenase and NADP–isocitrate dehydrogenase distributions were similar to those found by Martin & Denton, (1970), whereas aconitate hydratase, alanine aminotransferase, aspartate aminotransferase, carnitine acetyltransferase and phosphoenolpyruvate carboxykinase in the present study had a greater mitochondrial percentage than found by Martin & Denton (1970) and Saggerson & Tomassi (1971).

Enzyme activities in guinea-pig fat-cells showed a different profile from those found in the rat. The following activities were appreciably lower in the guinea pig compared with the rat; lactate dehydrogenase, extramitochondrial aconitate hydratase, extramitochondrial alanine aminotransferase, citrate synthase, extramitochondrial NAD–malate dehydrogenase, NADP–malate dehydrogenase, ATP–citrate lyase and pyruvate carboxylase. However, the following enzyme activities were appreciably higher in the guinea pig: glutamate dehydrogenase, aspartate aminotransferase, extramitochondrial NADP–isocitrate dehydrogenase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and

Table 1. *Activities and distribution of enzymes in rat and guinea-pig fat-cells*

Extracts were made and enzyme activities determined in S and M fractions as described in the Materials and Methods section. Mitochondrial and extramitochondrial activities were calculated in each determination by correcting the activities in the M and S fractions respectively by using the determined percentage activities of lactate dehydrogenase in the M fraction and of glutamate dehydrogenase in the S fraction. — indicates that the activity in the original S or M fraction was too low to be satisfactorily detected or became zero after applying the above correction. The results are the means of the determinations ±S.E.M. Expts. 1 and 3 consisted of five determinations each for both species, Expt. 2 of six determinations for rat and eight for guinea pig.

		Rat				Guinea pig			
Expt. no.	Enzyme	Extramitochondrial activity (munits/100 μg of DNA)	Mitochondrial activity (munits/100 μg of DNA)	Total activity (munits/100 μg of DNA)	Percentage of activity that is mitochondrial	Extramitochondrial activity (munits/100 μg of DNA)	Mitochondrial activity (munits/100 μg of DNA)	Total activity (munits/100 μg of DNA)	Percentage of activity that is mitochondrial
1.	Glutamate dehydrogenase	—	131.5±8.6	131.5±8.6	100	—	264.0±18.0	264.0±18.0	100
	Lactate dehydrogenase	3010±225	—	3010±225	0	665.4±64.7	—	665.4±64.7	0
	Aconitate hydratase	12.51±0.51	5.20±0.31	17.72±0.79	29	4.66±0.83	4.39±0.77	9.05±1.46	49
	Alanine aminotransferase	349.8±10.1	74.2±8.8	424.0±18.7	17	189.1±12.0	89.7±3.1	278.8±15.0	32
	Aspartate aminotransferase	8.70±1.35	83.5±3.5	92.2±3.6	91	72.3±8.6	154.4±15.4	226.7±24.1	68
	Carnitine acetyltransferase	—	9.79±0.92	9.79±0.92	100	—	12.92±0.98	12.92±0.98	100
	Citrate synthase	—	390.7±20.9	390.7±20.9	100	—	206.1±20.3	205.1±20.3	100
	3-Hydroxybutyrate dehydrogenase	—	4.05±0.67	4.05±0.67	100	—	3.79±0.19	3.79±0.19	100
	NAD–malate dehydrogenase	12116±662	1178±11	13377±615	9	3204±110	1714±159	4918±247	35
	NADP–isocitrate dehydrogenase	86.8±3.2	31.1±0.8	117.9±3.7	26	181.9±11.5	18.0±1.3	199.9±12.1	9
	NADP–malate dehydrogenase	394.6±33.4	—	394.6±33.4	0	90.2±10.0	—	90.2±10.0	0
2.	Glutamate dehydrogenase	—	147.1±12.4	147.1±12.4	100	—	220.7±13.5	220.7±13.5	100
	Lactate dehydrogenase	2816±177	—	2816±177	0	657.7±48.9	—	657.7±48.9	0
	ATP–citrate lyase	141.0±7.9	—	141.0±7.9	0	45.0±3.5	—	45.0±3.5	0
	Glucose 6-phosphate dehydrogenase	169.2±11.5	—	169.2±11.5	0	553.2±23.3	—	553.2±23.3	0
	Phosphoenolpyruvate carboxykinase	8.70±1.03	1.77±0.16	10.48±1.19	17	0.30±0.09	7.39±0.57	7.69±0.57	96
	6-Phosphogluconate dehydrogenase	157.7±10.9	—	157.7±10.9	0	365.8±23.0	—	365.8±23.0	0
3.	Glutamate dehydrogenase	—	129.8±5.1	129.8±5.1	100	—	201.3±12.9	201.3±12.9	100
	Lactate dehydrogenase	4102±298	—	4102±298	0	527.5±49.6	—	527.5±49.6	0
	NADP–malate dehydrogenase	599.9±38.9	—	599.9±38.9	0	69.7±4.1	—	69.7±4.1	0
	Pyruvate carboxylase	—	146.5±15.0	146.5±15.0	100	—	19.25±1.15	19.25±1.15	100

mitochondrial phosphoenolpyruvate carboxykinase. These differences were reflected in the percentage distributions of these enzymes. In the guinea pig the mitochondrial components of aspartate aminotransferase and NADP–isocitrate dehydrogenase were found to be lower than in rat, whereas those of aconitate hydratase, alanine aminotransferase, NAD–malate dehydrogenase and phosphoenolpyruvate carboxykinase were higher.

Incorporation of ^{14}C-labelled substrates

In the experiments summarized in Fig. 1 and in Table 2 rat and guinea-pig fat-cells were incubated with various glucose concentrations in the presence and absence of insulin to establish conditions under which glucose incorporation into fatty acids is maximal. Production of lactate + pyruvate by the cells was also measured. The essential features emerging from these experiments were: glucose incorporation into fatty acids and production of lactate+pyruvate by guinea-pig cells was maximal below 5mM-glucose, as is the case found in rat cells (Saggerson, 1972*a*). Insulin had little effect upon glucose incorporation into fatty acids by guinea-pig cells at any concentration of glucose tested. This lack of insulin sensitivity was surprising, but was reproducible and did not appear to be an artifact of collagenase treatment in

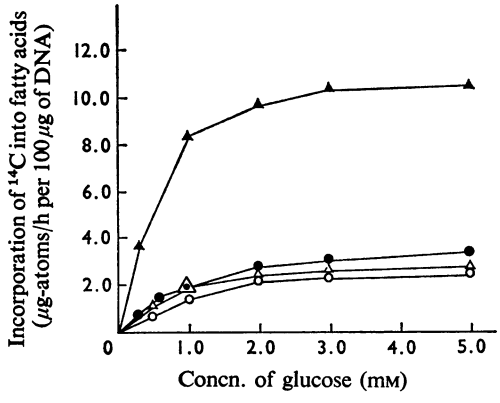

Fig. 1. *Effect of glucose concentration upon [U-^{14}C]-glucose incorporation into glyceride fatty acids by fat-cells from rats and guinea pigs in the presence and absence of insulin*

Fat-cells were incubated for 1h in Krebs–Ringer bicarbonate containing 1.0% (w/v) albumin together with the required concentration of [U-^{14}C]glucose and insulin (20munits/ml) or anti-insulin serum (10munits/ml). The results are the means of four determinations. The results presented in Table 2 were obtained in the same experiment. ●, Rat and anti-insulin serum; ▲, rat and insulin; ○, guinea pig and anti-insulin serum; △, guinea pig and insulin.

Table 2. *Effect of glucose concentration on lactate and pyruvate production by fat-cells from rats and guinea pigs in the presence and absence of insulin*

Fat-cells were incubated for 1h in Krebs–Ringer bicarbonate containing 1.0% (w/v) albumin together with the required concentration of glucose and insulin (20munits/ml) or anti-insulin serum (10munits/ml). The results are the means±S.E.M. of four determinations. The results presented in Fig. 1 were obtained in the same experiment.

	Rat				Guinea pig			
	Production of lactate and pyruvate (μg-atoms/h per 100μg of DNA)		Lactate/pyruvate ratio		Production of lactate and pyruvate (μg-atoms/h per 100μg of DNA)		Lactate/pyruvate ratio	
Concn of glucose (mM)	With anti-insulin serum	With insulin	With anti-insulin serum	With insulin	With anti-insulin serum	With insulin	With anti-insulin serum	With insulin
0.3	5.71±0.46	9.13±0.65	16.41	8.98	—	—	—	—
0.5	—	—	—	—	4.96±0.27	3.54±0.47	2.55	0.99
0.6	6.91±0.82	11.96±0.94	18.51	8.13	—	—	—	—
1.0	7.14±0.91	13.67±1.18	13.41	7.48	6.68±0.53	5.13±0.60	1.72	0.88
2.0	8.19±0.93	14.66±1.48	11.04	7.75	7.56±0.45	6.31±0.25	1.60	1.08
3.0	8.93±0.98	15.11±1.60	11.95	8.32	8.20±0.64	7.09±0.14	1.43	0.86
5.0	9.15±0.61	14.81±1.47	10.15	8.01	7.92±0.71	6.85±0.50	1.15	0.68

preparation of the cells since insulin also failed to appreciably stimulate glucose incorporation into fatty acids in guinea-pig epididymal adipose-tissue pieces incubated under similar conditions; i.e. in an experiment in which fat pieces and fat-cells were obtained from the same guinea-pig tissue samples and incubated under identical conditions (5mM-glucose), insulin stimulated glucose incorporation into fatty acids by 31% in fat pieces and by 35% in fat-cells (three determinations in each case). Rates of total lipid formation from glucose were similar in fat pieces and fat-cells in this experiment (6.10±0.32 and 8.77μg-atoms/h per g wet wt. of tissue for fat pieces incubated with anti-insulin serum and insulin respectively, and 6.15±0.43 and 8.42±0.12μg-atoms/h per g dry wt. of cells for similarly treated fat-cells). In other preliminary experiments using only guinea-pig fat-cells it was established that increasing the insulin concentration beyond that used in this study (20munits/ml) did not further increase the incorporation of glucose into fatty acids in these cells. In the presence of glucose, rat cells produced lactate considerably in excess of pyruvate, whereas guinea-pig cells produced roughly equal proportions of the two products. In cells from both species the lactate/pyruvate ratio was decreased by insulin at all glucose concentrations.

The experiment summarized in Fig. 2 contrasts the effects of acetate addition on glucose incorporation into fatty acids by cells from the two species. Acetate, which was itself more readily incorporated into fatty acids by guinea-pig cells, had no appreciable effect upon glucose incorporation into fatty acids at any concentration tested in guinea-pig cells. In other experiments (Tables 3 and 4) slight stimulations of glucose incorporation on acetate addition were found in guinea-pig cells, but these were never of the magnitude found in rat cells. It may therefore be concluded that acetate utilization by guinea-pig fat-cells, though extensive, does not permit the removal of restraints on glucose utilization as is found in rat cells (Flatt, 1970). The effects of acetate on glucose utilization were further studied (Table 3) by examination of the effect of one concentration of acetate (2.5mM) on glucose carbon flux through various pathways. The incorporation of acetate carbon was measured simultaneously.

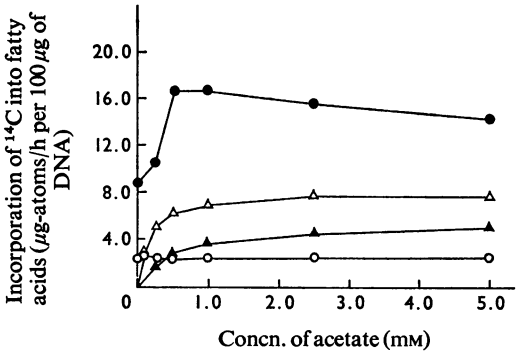

Fig. 2. *Effect of sodium acetate concentration upon [U-^{14}C]-glucose and [U-^{14}C]acetate incorporation into glyceride fatty acids by fat-cells from rats and guinea pigs*

Fat-cells were incubated for 1h in Krebs–Ringer bicarbonate containing 1.0% (w/v) albumin and insulin (20munits/ml) together with either 5mM-[U-^{14}C]glucose and the required concentration of sodium [^{12}C]acetate or 5mM-[^{12}C]glucose and the required concentration of sodium [U-^{14}C]acetate. The results are the means of four determinations. ●, Rat, glucose incorporation; ▲, rat, acetate incorporation; ○, guinea pig, glucose incorporation; △, guinea pig, acetate incorporation.

The effects of acetate on glucose incorporation in rat cells were in accord with those found previously with isolated cells and with incubated fat-pad segments (Flatt & Ball, 1966; Saggerson & Greenbaum, 1970*a*,*b*; Saggerson, 1972*b*). Preliminary experiments established that with both rat and guinea-pig cells summation of the measured metabolic products (^{14}C-labelled total lipid+$^{14}CO_2$+lactate+pyruvate) gave an adequate estimation of the total utilization of glucose in the incubations as determined by enzymic assays of glucose before and after the period of incubation. This method of quantification of glucose utilization was previously found to be satisfactory for rat fat cells by Kather *et al.* (1972), and was used in the present study in the calculation of hexose monophosphate-pathway fluxes (methods II and III). In both rat and guinea-pig cells the increased total fatty acid synthesis found after addition of acetate was accompanied by an increase in the hexose monophosphate-pathway flux. The three methods used for calculation of this parameter were in quite good agreement. In rat cells glucose/acetate ratios for incorporation into fatty acids and citric acid-cycle CO_2 were 1:0.41 and 1:1.26 respectively. In guinea-pig cells glucose/acetate ratios were considerably different and were 1:1.61 for fatty acids and 1:0.63 for citric acid-cycle CO_2.

Table 4 summarizes the salient points arising from an experiment designed to compare the relative abilities of rat and guinea-pig cells to incorporate glucose (+insulin), pyruvate and lactate into fatty acids and to test the relative abilities of these substrates to support acetate incorporation. At 5mM concentrations rat cells incorporated pyruvate into fatty acids more readily than guinea-pig cells, whereas guinea-pig cells incorporated lactate more readily than rat cells. Rat cells incorporated substrates (in the absence of acetate) to the following extent: glucose (+insulin) $\simeq$ pyruvate $\gg$ lactate. However, the order of incorporation of these substrates was reversed in guinea-pig cells and was lactate > pyruvate > glucose. In all experiments lactate was readily incorporated by

Table 3. *Measurement of fluxes of glucose and acetate carbon through metabolic pathways in fat-cells from rats and guinea pigs*

Fat-cells were incubated for 90min in Krebs–Ringer bicarbonate containing 1.0% (w/v) albumin, 5mM-glucose and 20munits/ml of insulin. Sodium acetate (2.5mM) was added where indicated. All flasks with glucose alone contained [1-^{14}C]glucose, [6-^{14}C]glucose or [U-^{14}C]glucose. Flasks containing glucose and acetate contained either [1-^{14}C]glucose, [6-^{14}C]glucose, [U-^{14}C]glucose or sodium [U-^{14}C]acetate. The results are expressed in every case as μg-atoms of glucose or acetate carbon incorporated/h per 100μg of DNA and are the means ± S.E.M. of five determinations. Hexose-monophosphate-pathway CO_2 production was calculated by three methods. Method I: from [6-^{14}C]glucose and [U-^{14}C]glucose yields in fatty acids and CO_2 (Flatt & Ball, 1964). Method II: from [1-^{14}C]glucose and [6-^{14}C]glucose yields in fatty acids and CO_2 (method C of Katz *et al.*, 1966). Method III: from [1-^{14}C]glucose, [6-^{14}C]glucose and [U-^{14}C]glucose yields in fatty acids and glyceride glycerol (method D of Katz *et al.*, 1966).

Animal	Additions to incubation medium	Total glucose utilization	Fatty acid synthesis from glucose	Fatty acid synthesis from acetate	Glyceride glycerol synthesis from glucose	Lactate and pyruvate production	Lactate/pyruvate ratio	Glucose-derived citric-acid-cycle CO_2	Acetate-derived CO_2	Hexose monophosphate-pathway CO_2		
										Method I	Method II	Method III
Rat	—	36.21 ± 1.47	8.89 ± 0.94	—	2.22 ± 0.15	15.51 ± 1.45	8.41	0.22	—	2.73	2.59	3.00
										Mean = 2.77		
	2.5mM-Sodium acetate	45.62 ± 1.57	11.56 ± 1.07	4.73 ± 0.21	2.61 ± 0.12	18.11 ± 1.30	6.14	0.39	0.49 ± 0.14	4.92	5.06	5.43
										Mean = 5.14		
Guinea pig	—	17.00 ± 1.46	3.68 ± 0.40	—	1.42 ± 0.06	6.76 ± 0.40	0.98	0.24	—	2.42	2.08	2.46
										Mean = 2.32		
	2.5mM-Sodium acetate	23.20 ± 2.80	4.12 ± 0.52	6.62 ± 0.98	1.62 ± 0.10	9.00 ± 1.10	0.90	0.49	0.31 ± 0.04	5.16	4.28	5.26
										Mean = 4.90		

Table 4. *Effect of acetate on glucose, pyruvate and lactate incorporation into glyceride fatty acids and the effect of glucose, pyruvate and lactate upon acetate incorporation in fat-cells from rats and guinea pigs*

Fat-cells were incubated for 1 h in Krebs–Ringer bicarbonate containing 1.0% (w/v) albumin and various substrate additions as indicated in the table. The results are expressed as μg-atoms of substrate carbon incorporated/h per 100 μg of DNA and are the means ± S.E.M. of five determinations.

Animal	Addition to incubation medium ...	5mM-Sodium acetate	5mM-Glucose+20 units of insulin/ml	5mM-Glucose+20 units of insulin/ml+5mM-sodium acetate		5mM-Sodium pyruvate		5mM-Sodium pyruvate+5mM-sodium acetate		5mM-Sodium lactate		5mM-Sodium lactate+5mM-sodium acetate	
	Substrate incorporated into fatty acids ...	[U-^{14}C]-Acetate	[U-^{14}C]-Glucose	[U-^{14}C]-Glucose	[U-^{14}C]-Acetate	[U-^{14}C]-Pyruvate		[U-^{14}C]-Acetate		[U-^{14}C]-Lactate		[U-^{14}C]-Lactate	[U-^{14}C]-Acetate
Rat		0.76 ± 0.11	7.86 ± 0.62	13.59 ± 1.14	7.19 ± 0.64	7.09 ± 0.49		1.19 ± 0.16		1.45 ± 0.19		0.47 ± 0.10	0.99 ± 0.10
Guinea pig		0.83 ± 0.12	2.72 ± 0.05	3.45 ± 0.08	8.01 ± 1.14	3.53 ± 0.14		2.47 ± 0.14		1.48 ± 0.10		5.80 ± 0.32	1.06 ± 0.08	2.96 ± 0.32

guinea-pig cells and was extremely poorly incorporated by rat cells. In the absence of other substrates, acetate was poorly incorporated into fatty acids by both rat and guinea-pig cells. Glucose (+insulin) greatly stimulated acetate incorporation into rat and guinea-pig cells, whereas acetate incorporation was poorly stimulated in the presence of pyruvate in rat and guinea-pig cells, and moderately stimulated by lactate in guinea-pig cells only. At 5mM concentrations, the ratio of acetate carbon incorporated to that of glucose, pyruvate or lactate was always greater in the guinea pig than in the rat. Insulin had virtually no effect upon pyruvate or lactate incorporation by cells of either species and did not influence the ability of these substrates to support acetate incorporation. Acetate had little effect on pyruvate incorporation, but greatly decreased lactate incorporation into both rat and guinea-pig cells. This effect of acetate upon lactate incorporation was considerable at acetate concentrations as low as 0.5mM (results not shown).

Discussion

General considerations

The results indicate that guinea-pig fat-cells are able to utilize glucose as a lipogenic substrate, though to a considerably lesser extent than rat cells. The guinea-pig fat cells used in this study were far less sensitive to insulin than rat cells in terms of the ability of this hormone to stimulate glucose incorporation into fatty acids (Fig. 1). This insulin insensitivity did not appear to arise in the course of the preparation of the cells and was just as apparent in incubated adipose-tissue pieces and fat-cells from considerably younger guinea pigs than those used in this study (E. D. Saggerson, unpublished work). The rats and guinea pigs used in the present study appeared to be at approximately the same stage of development.

Since the guinea pig is a herbivorous animal, it was not surprising that guinea-pig fat-cells showed a greater ability to incorporate acetate than rat cells. Presumably this metabolite is produced to some extent through the action of symbiotic bacteria in the enlarged caecum. Acetate incorporation into fatty acids in rat and guinea-pig cells was very low in the absence of other substrates and required the support of glucose in the case of the rat, and glucose or lactate in the case of the guinea pig, for an appreciable incorporation of acetate (Table 4). Appreciable incorporation of acetate alone occurs in pig, sheep and cow adipose tissue, though this incorporation is increased in the presence of glucose (Hanson & Ballard, 1967; O'Hea & Leveille, 1968; Ballard *et al.*, 1972). It is interesting that in both rat and guinea-pig cells pyruvate had little ability to support acetate incorporation and acetate had little effect on pyruvate

incorporation. The effect of acetate in greatly decreasing lactate incorporation into both rat and guinea-pig cells (Table 4) was surprising and not readily explained. In particular, in the rat, in which lipogenesis is nowhere near maximal with lactate as sole substrate, it might have been expected that lactate incorporation would have been increased in the presence of acetate through the utilization of reducing equivalents for acetate incorporation.

The stimulatory effect of acetate on glucose incorporation seen in rat cells (Tables 3 and 4; also Flatt & Ball, 1966; Saggerson, 1972*b*) was not observed in guinea-pig cells. In rat adipose tissue this effect has been explained through the action of acetate incorporation into fatty acids utilizing ATP and thus removing a restraint on glucose utilization (Flatt & Ball, 1966; Flatt, 1970). It is noteworthy that in the guinea pig, an animal in which acetate is probably a physiological lipogenic substrate, acetate has no appreciable effect upon glucose incorporation into fatty acids, especially since the greater provision of NADPH by the hexose monophosphate pathway in this animal (see below) renders lipogenesis less 'expensive' in terms of ATP utilization (Flatt, 1970). The hypothesis of Flatt (1970) may therefore not have general applicability to all fat-cell types. A complete ATP balance of guinea-pig fat-cell pathways of glucose utilization would prove interesting in this respect.

Pathways related to lipogenesis from glucose in rat and guinea-pig fat-cells

General. In rat adipose tissue it is generally accepted that the synthesis of fatty acids from the physiological substrate glucose involves the following procedures: glycolytic formation of pyruvate in the cytosol, mitochondrial oxidation of pyruvate to acetyl-CoA, mitochondrial condensation of this acetyl-CoA with oxaloacetate to form citrate, transport of citrate from mitochondria to cytosol, and cleavage of citrate to yield cytosolic oxaloacetate and acetyl-CoA, the acetyl unit of which is incorporated into long-chain fatty acids. The provision of NADPH for the reductive synthesis of these fatty acids appears to be shared between the dehydrogenases of the hexose monophosphate pathway and NADP–malate dehydrogenase in a manner dependent on the rate of lipogenesis. This leaves one molecule of cytosolic oxaloacetate for each glucose-derived acetyl unit incorporated into fatty acids. The fate of this oxaloacetate is uncertain (see following discussion).

Generation and utilization of cytosolic reducing equivalents. In rat fat-cells, the hexose monophosphate pathway supplied 71% of the NADPH required for fatty acid synthesis from glucose in the presence of insulin (Table 5). This may be compared with other published values of 63% and 60% for

Table 5. *Balance of extramitochondrial production and utilization of reducing equivalents in rat and guinea-pig fat-cells*

The values are expressed as μmol/h per 100 μg of DNA and are derived from the data of Table 3. The balance is based on the following considerations. Utilization of reducing equivalents: for the formation of 1 μmol of glyceride glycerol or lactate 1 μmol of NADH is required. For formation of 1 μg-atom of carbon of fatty acid 0.875 μmol of NADPH is required (Kather *et al.*, 1972). Production of reducing equivalents: 1 μmol of NADH is formed for every μmol of glyceraldehyde 3-phosphate oxidized to pyruvate as calculated by Flatt & Ball (1964). NADPH (2 μmol) is formed for every μmol of CO_2 produced in the hexose monophosphate pathway. Percentage hexose monophosphate pathway contributions to glucose catabolism were calculated as described by Katz *et al.* (1966).

Animal	Additions to incubation medium	NADPH production (hexose monophosphate pathway)	Contribution of hexose monophosphate pathway to glucose catabolism (%)	NADH production (glyceraldehyde 3-phosphate dehydrogenase)	NADPH utilization (fatty acid synthesis)	NADH utilization: Lactate formation	NADH utilization: Glyceride glycerol formation	Total reducing equivalents produced	Total reducing equivalents utilized	Utilization (%)	NADPH required for fatty acid synthesis produced by hexose monophosphate pathway (%)
Rat	—	5.54	15.3	9.72	7.78	4.62	0.74	15.26	13.14	86	71
	2.5 mM-Sodium acetate	10.30	22.6	12.01	14.22	5.19	0.87	22.31	20.28	91	73
Guinea pig	—	4.64	27.3	4.21	3.22	1.12	0.47	8.85	4.81	54	145
	2.5 mM-Sodium acetate	9.80	42.2	5.30	9.40	1.42	0.54	15.10	11.36	75	104

fat-pads (Flatt & Ball, 1966) and fat-cells (Kather *et al.*, 1972) respectively. In the presence of glucose and insulin the hexose monophosphate pathway contributed approx. 15% to the catabolism of glucose in the rat cells, a value similar to that found by Kather *et al.* (1972) but lower than that found for fat-pads by Katz *et al.* (1966). The amount and proportion of glucose catabolized by the hexose monophosphate pathway is increased when acetate is provided in addition to glucose in rat cells (Tables 3 and 5). As noted by Flatt & Ball (1966), the increase in the hexose monophosphate pathway is sufficient to supply the extra NADPH needed for the incorporation of acetate into fatty acids, leaving enough NADPH to supply 61% of that needed for the synthesis of glucose-derived fatty acids. However, in guinea-pig cells incubated with glucose+insulin the calculated provision of NADPH by the hexose monophosphate pathway was more than sufficient to satisfy the observed rate of fatty acid synthesis. When acetate was present together with glucose+insulin the NADPH requirement was just satisfied by the hexose monophosphate pathway (Table 5). The contribution of the hexose monophosphate pathway to glucose catabolism is greater in guinea pig than in rat and is particularly large (42%) in the presence of glucose+acetate.

These differences in metabolic pattern between the two types of cells may be correlated with differences in enzyme profile (Table 1). The activities of the hexose monophosphate-pathway dehydrogenases in guinea-pig cells are considerably higher than those found in rat cells. In the rat NADP–malate dehydrogenase was the highest cytosolic NADP-linked dehydrogenase activity measured. In the guinea pig NADP–malate dehydrogenase was the lowest activity and glucose 6-phosphate dehydrogenase the highest. It was unexpected to find appreciable NADP–malate dehydrogenase in the guinea-pig cells. This enzyme is not detectable in guinea-pig liver or mammary gland (Stanley, 1972) or in rabbit mammary gland (Gul & Dils, 1969). The activity also appears to be very low or undetectable in ruminant mammary gland, liver and adipose tissue (Hardwick, 1966; Hanson & Ballard, 1967; Bauman *et al.*, 1970) under normal feeding conditions (Ballard *et al.*, 1972). It is noteworthy, however, that in the sow, similarly to the guinea pig, the activity of this enzyme is negligible in the liver and the mammary gland, but is appreciable in adipose tissue (O'Hea & Leveille, 1968, 1969; Bauman *et al.*, 1970; Anderson & Kauffman, 1973). Table 1 shows that the activities of the other three cytosolic NADP-linked dehydrogenases are lower relative to glucose 6-phosphate dehydrogenase in the guinea pig compared with the rat. Although it is now generally accepted that measured maximal activities of enzymes cannot be used to predict their rate of operation in intracellular metabolic pathways, differences in the relative amounts or activities of these dehydrogenases in the two species should indicate the relative abilities of the various NADPH-supplying systems to compete for a common substrate (Sols & Marco, 1970), namely NADP. The provision of NADP as a result of a reductive synthesis of fatty acids in lipogenic tissues is believed to govern the rate of NADPH production and not the converse (Greenbaum *et al.*, 1971; Kather *et al.*, 1972). The differences in the profiles of cytosolic NADP-linked dehydrogenases between rat and guinea pig are consistent with the greater relative importance of the hexose monophosphate pathway in guinea-pig adipose tissue. NADP-linked isocitrate dehydrogenase was the lowest of the four cytosolic NADP-linked dehydrogenase activities measured in the rat. This enzyme is not generally included in discussions concerning the disposition of lipogenic reducing equivalents in the rat. The activity of this enzyme was higher in the guinea-pig cells than in the rat, although the activity relative to glucose 6-phosphate dehydrogenase was less. Whether this enzyme plays a role in the lipogenic process in guinea-pig adipose tissue is unclear. It has been suggested that NADP–isocitrate dehydrogenase is involved in provision of NADPH in rabbit, sheep and cow mammary gland (Gul & Dils, 1969; Bauman *et al.*, 1970, 1972; Gumaa *et al.*, 1973). The observation that acetate alone is poorly incorporated into fatty acids in rat and guinea-pig fat-cells (Table 4) suggests, however, that lipogenic schemes, based partly on NADP–isocitrate dehydrogenase as a supplier of NADPH, similar to that proposed for sheep mammary gland by Gumaa *et al.* (1973), would not be operative in fat-cells in these species. Stanley (1972) proposed an opposite role for this enzyme in guinea-pig mammary gland, namely that it may be involved in the reoxidation rather than production of cytosolic NADPH (see below).

In rat cells summation and comparison of the total cytosolic reducing equivalents produced and utilized (Table 5) indicates a near balance of production and utilization, consistent with the findings of Katz *et al.* (1966), Rognstad & Katz (1966) and Kather *et al.* (1972), suggesting that transfer of reducing equivalents from cytosol to mitochondria is either unnecessary or restricted. On the other hand, in guinea-pig cells incubated with glucose+insulin there was no balance of production and utilization of cytosolic reducing equivalents, nearly 50% needing to be disposed of by means other than fatty acid synthesis and lactate and glyceride glycerol formation (1.34 μmol of NADPH/h per 100 μg of DNA and 2.61 μmol of NADH/h per 100 μg of DNA). Martin & Denton (1971) suggested that restricted utilization of malate by rat fat-cell mitochondria would prevent loss of cytosolic reducing power (NADH). A higher activity (Table 1) of mitochondrial NAD–malate dehydro-

genase may account for the apparent lessening of this restriction in guinea-pig cells. This difference between rat and guinea-pig cells is also shown by the ready utilization of lactate as a lipogenic substrate by guinea-pig cells (Table 4), and because the artificial electron acceptors *NNN'N'*-tetramethyl-*p*-phenylenediamine and phenazine methosulphate have no effect on and inhibit respectively lactate and glucose incorporation by guinea-pig cells (E. D. Saggerson, unpublished work). In rat fat-cells lactate is poorly utilized (Table 4), and the electron acceptors considerably stimulate glucose and lactate incorporation into fatty acids (Saggerson, 1972*b*). These results are in accord with an inability to dispose of cytosolic NADH in rat cells that is not shared by the guinea-pig cells and could also explain the considerable differences in lactate/pyruvate ratios produced by the two cell types incubated with glucose (Table 1). It is noteworthy though that there is at least an order of magnitude difference between the lactate/pyruvate ratios found with guinea-pig fat-cells and those found in guinea-pig liver preparations (Garber & Hanson, 1971; Arinze *et al.*, 1973); however, lactate/pyruvate ratios in rat fat-cells and fat-pad segments incubated with glucose+insulin (Denton *et al.*, 1966; Saggerson & Greenbaum 1970*a*; Saggerson 1972*b*) are similar to those reported for directly freeze-clamped rat liver (Williamson *et al.*, 1967) and isolated perfused rat liver (Williamson *et al.*, 1969).

With respect to the need to dispose of excess of cytosolic NADPH in guinea-pig fat-cells, a similar situation is encountered in guinea-pig mammary-gland slices incubated with glucose, where it is suggested by Stanley (1972) that excess of NADPH is reoxidized by a transhydrogenase system involving cytosolic NADP–isocitrate dehydrogenase (NADPH →NADP) and mitochondrial NAD–isocitrate dehydrogenase (NAD→NADH), thus permitting mitochondrial reoxidation of cytosolic NADPH reducing equivalents.

Cleavage of citrate. In adult ruminants glucose does not appear to be able to be readily utilized as a lipogenic substrate, whereas acetate may be utilized. This may be correlated with low activity of ATP–citrate lyase in these animals (Hardwick, 1966; Hanson & Ballard, 1967). The observations that guinea-pig fat-cells have considerably lower ATP–citrate lyase activity than rat fat-cells (Table 1), and that the guinea-pig cells utilize acetate more readily and glucose less readily *in vitro* than rat cells (Fig. 2; Tables 3 and 4) are consistent with these earlier findings and suggest that *in vivo* the citrate-cleavage pathway plays a lesser role in providing acetyl units for adipose-tissue lipogenesis in the guinea pig than in the rat. Attempts to measure the activity and distribution of acetyl-CoA synthetase in the rat and guinea-pig cell extracts prepared in the present study by using either the spectrophotometric assay of Martin & Denton (1970) or the colorimetric assay of Kornacker & Lowenstein (1965) were unsuccessful. Citrate-cleavage-independent lipogenesis from acetate requires the participation of extramitochondrial acetyl-CoA synthetase.

'Malate–pyruvate' cycle. Guinea-pig fat-cells compared with rat cells contained considerably lower activities of the necessary enzymes (Table 1) for the conversion of cytosolic oxaloacetate into pyruvate followed by the mitochondrial carboxylation of pyruvate to regenerate oxaloacetate. The operation in rat cells of such a 'malate–pyruvate' cycle involving cytosolic NAD- and NADP-malate dehydrogenases and mitochondrial pyruvate carboxylase permits the removal of excess of cytosolic NADH through the transhydrogenase action of the NAD- and NADP-malate dehydrogenases, supplies NADPH for fatty acid synthesis, and disposes of cytosolic oxaloacetate produced by cleavage of citrate. In guinea-pig fat-cells incubated with glucose there is less requirement for these operations, since disposal of cytosolic NADH, presumably by other means, appears not to be restricted, NADPH requirements for fatty acid synthesis can all be met by the hexose monophosphate pathway and less cytosolic oxaloacetate is likely to be generated as a result of citrate cleavage since glucose is not so readily utilized for lipogenesis by guinea-pig cells. However, since guinea-pig fat-cells can utilize pyruvate as a lipogenic substrate (Table 4) it would seem likely that the NADP–malate dehydrogenase is capable of providing NADPH for fatty acid synthesis under certain circumstances, although there appears to be no need to invoke its involvement when glucose or glucose+acetate are the lipogenic substrates.

Utilization of cytosolic oxaloacetate. Fatty acid synthesis from glucose generates cytosolic oxaloacetate as a result of citrate cleavage. Cytosolic oxaloacetate will also be produced in the course of lipogenesis from pyruvate as sole substrate *in vitro* (Kneer & Ball, 1968). To maintain the flow of pathway metabolites this must be removed or utilized in some way. Since it is generally held that direct transfer of oxaloacetate between cytosol and mitochondria is unlikely (Lardy *et al.*, 1965), other means of disposal of this metabolite in rat fat-cells have been considered (Martin & Denton, 1970), namely: $oxaloacetate_{cyt.} \rightarrow phosphoenolpyruvate_{cyt.} \rightarrow pyruvate_{cyt.}$ utilizing cytosolic phosphoenolpyruvate carboxykinase; $oxaloacetate_{cyt.} \rightarrow malate_{cyt.} \rightarrow pyruvate_{cyt.}$ (i.e. the malate–pyruvate cycle discussed above); $oxaloacetate_{cyt.} \rightarrow aspartate_{cyt.} \rightarrow aspartate_{mit.} \rightarrow oxaloacetate_{mit.}$; or $oxaloacetate_{cyt.} \rightarrow malate_{cyt.} \rightarrow malate_{mit.} \rightarrow oxaloacetate_{mit.}$ by using cytosolic and mitochondrial NAD–malate dehydrogenases, thus removing oxaloacetate and a reducing equivalent from the cytosol. In guinea-pig fat-cells conversion of cytosolic oxaloacetate into pyruvate

would appear unlikely either via malate (discussed above) or via phosphoenolpyruvate because of the virtual absence of cytosolic phosphoenolpyruvate carboxykinase (Table 1). Guinea-pig fat-cells had an even lower proportion of the activity of this enzyme in the cytosol than has been found for guinea-pig liver (Lardy, 1965; Garber & Hanson, 1971). Removal of cytosolic oxaloacetate as aspartate requires the participation of cytosolic and mitochondrial aspartate aminotransferases together with the simultaneous transport of 2-oxoglutarate and glutamate and is therefore a rather cumbersome process. This process would appear to be of little importance in rat fat-cells owing to the very low cytosolic activity of aspartate aminotransferase. In guinea-pig cells the activity of aspartate aminotransferase was higher in cytosol and mitochondria by factors of 8 and 2 respectively compared with the rat (Table 1). Amino-oxyacetate, an inhibitor of aspartate aminotransferase (Braunstein, 1964), however, had no appreciable effect on fatty acid synthesis from glucose (+insulin) or pyruvate in guinea-pig cells and rat fat-cells when tested over a 0.5–3.0mM range (E. D. Saggerson, unpublished work), suggesting that removal of cytosolic oxaloacetate as aspartate is not an important route in either species. The possibility that amino-oxyacetate was without effect through lack of penetration into the cells was precluded by control experiments demonstrating that 1mM-[U-^{14}C]aspartate incorporation into fatty acids in the presence of glucose+insulin by guinea-pig fat-cells was 70% inhibited by 2mM-amino-oxyacetate. Denton & Martin (1970) have presented similar evidence that inhibition of aspartate aminotransferase by amino-oxyacetate does not prevent glucose incorporation into fatty acids by incubated rat fat-pads.

Remaining is the possible removal of cytosolic oxaloacetate by the route oxaloacetate$_{cyt.}$ → malate$_{cyt.}$ → malate$_{mit.}$ → oxaloacetate$_{mit.}$. This would appear feasible for guinea-pig cells, but not for rat cells (Martin & Denton, 1971). It would also create a route for removal of cytosolic NADH reducing equivalents and presumably would be facilitated by the greater proportion of NAD–malate dehydrogenase that is mitochondrial in the guinea pig. Also, since Martin & Denton (1971) have demonstrated that rat fat-cell mitochondria will only take up malate and convert it into oxaloacetate if pyruvate carboxylase activity is restricted, it is to be expected that the considerably lower activity of pyruvate carboxylase in guinea-pig fat-cells would facilitate the process. Katz & Wals (1972) have suggested the existence of a similar metabolic route of mitochondrial utilization of cytosolic malate in rat mammary gland, which has a profile of reducing-equivalent disposition that is dissimilar to that found in rat adipose tissue, but similar to that found in guinea-pig adipose tissue in the present study.

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References

Anderson, D. B. & Kauffman, R. G. (1973) *J. Lipid Res.* **14**, 160–168

Arinze, I. J., Garber, A. J. & Hanson, R. W. (1973) *J. Biol. Chem.* **248**, 2266–2274

Ballard, F. J., Filsell, O. H. & Jarrett, I. G. (1972) *Biochem. J.* **126**, 193–200

Bauman, D. E., Brown, R. E. & Davis, C. L. (1970) *Arch. Biochem. Biophys.* **140**, 237–244

Bauman, D. E., De Kay, D. E., Ingle, D. L. & Brown, R. E. (1972) *Comp. Biochem. Physiol. B* **43**, 479–486

Braunstein, A. E. (1964) *Vitam. Horm. (New York)* **22**, 451–484

Bruce, H. M. & Parkes, A. S. (1949) *J. Hyg.* **47**, 202–206

Chang, H.-C. & Lane, M. D. (1966) *J. Biol. Chem.* **241**, 2413–2420

Del Boca, J. & Flatt, J. P. (1969) *Eur. J. Biochem.* **11**, 127–134

Denton, R. M. & Martin, B. R. (1970) in *Adipose Tissue: Regulation and Metabolic Functions* (Jeanrenaud, B. & Hepp, D., eds.), pp. 143–151, Academic Press, London

Denton, R. M., Yorke, R. E. & Randle, P. J. (1966) *Biochem. J.* **100**, 407–419

Flatt, J. P. (1970) *J. Lipid. Res.* **11**, 131–143

Flatt, J. P. & Ball, E. G. (1964) *J. Biol. Chem.* **239**, 675–685

Flatt, J. P. & Ball, E. G. (1966) *J. Biol. Chem.* **241**, 2862–2869

Galton, D. J. & Wilson, J. P. D. (1970) in *Adipose Tissue: Regulation and Metabolic Functions* (Jeanrenaud, B. & Hepp, D., eds.), pp. 172–174, Academic Press, London

Garber, A. J. & Hanson, R. W. (1971) *J. Biol. Chem.* **246**, 589–598

Greenbaum, A. L., Gumaa, K. A. & McLean, P. (1971) *Arch. Biochem. Biophys.* **143**, 617–663

Gries, F. A. (1970) in *Adipose Tissue: Regulation and Metabolic Functions* (Jeanrenaud, B. & Hepp, D., eds.), pp. 167–171, Academic Press, London

Gul, B. & Dils, R. (1969) *Biochem. J.* **112**, 293–301

Gumaa, K. A., Greenbaum, A. L. & McLean, P. (1973) *Eur. J. Biochem.* **34**, 188–198

Halperin, M. L. (1971) *Biochem. J.* **124**, 615–621

Hanson, R. W. & Ballard, F. J. (1967) *Biochem. J.* **105**, 529–536

Hanson, R. W. & Ballard, F. J. (1968) *Biochem. J.* **108**, 705–713

Hardwick, D. C. (1966) *Biochem. J.* **99**, 228–231

Kather, H., Rivera, M. & Brand, K. (1972) *Biochem. J.* **128**, 1089–1096

Katz, J. & Wals, P. A. (1970) *J. Biol. Chem.* **245**, 2546–2548

Katz, J. & Wals, P. A. (1972) *Biochem. J.* **128**, 879–899

Katz, J., Landau, B. R. & Bartsch, G. E. (1966) *J. Biol. Chem.* **241**, 727–740

Kneer, P. & Ball, E. G. (1968) *J. Biol. Chem.* **243**, 2863–2870

Kornacker, M. S. & Lowenstein, J. M. (1965) *Biochem. J.* **94**, 209–215

Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66

Lardy, H. A. (1965) *Harvey Lect.* **60**, 261–278

Lardy, H. A., Paetkau, V. & Walter, P. (1965) *Proc. Nat. Acad. Sci. U.S.* **53**, 1410–1415
Lehninger, A. L., Sudduth, H. C. & Wise, J. B. (1960) *J. Biol. Chem.* **235**, 2450–2455
Martin, B. R. & Denton, R. M. (1970) *Biochem. J.* **117**, 861–877
Martin, B. R. & Denton, R. M. (1971) *Biochem. J.* **125**, 105–113
O'Hea, E. K. & Leveille, G. A. (1968) *Comp. Biochem. Physiol. B* **26**, 1081–1089
O'Hea, E. K. & Leveille, G. A. (1969) *J. Nutr.* **99**, 338–344
Rodbell, M. (1964) *J. Biol. Chem.* **239**, 375–380
Rognstad, R. & Katz, J. (1966) *Proc. Nat. Acad. Sci. U.S.* **55**, 1148–1156
Rognstad, R. & Katz, J. (1969) *Biochem. J.* **111**, 431–444
Saggerson, E. D. (1972*a*) *Biochem. J.* **128**, 1057–1067
Saggerson, E. D. (1972*b*) *Biochem. J.* **128**, 1069–1078
Saggerson, E. D. & Greenbaum, A. L. (1969) *Biochem. J.* **115**, 405–417
Saggerson, E. D. & Greenbaum, A. L. (1970*a*) *Biochem. J.* **119**, 193–219
Saggerson, E. D. & Greenbaum, A. L. (1970*b*) *Biochem. J.* **119**, 221–242
Saggerson, E. D. & Tomassi, G. (1971) *Eur. J. Biochem.* **23**, 109–117
Simon, E. J. & Shemin, D. (1953) *J. Amer. Chem. Soc.* **75**, 2520–2522
Sols, A. & Marco, R. (1970) *Curr. Top. Cell. Regul.* **2**, 227–273
Stadtman, E. R. (1957) *Methods Enzymol.* **3**, 935–938
Stanley, P. I. (1972) Ph.D. Thesis, University of London
Strong, C. R. & Dils, R. (1972) *Comp. Biochem. Physiol. B* **43**, 643–652
Utter, M. J. & Keech, D. B. (1963) *J. Biol. Chem.* **238**, 2603–2608
Williamson, D. H., Lund, P. & Krebs, H. A. (1967) *Biochem. J.* **103**, 514–527
Williamson, J. R., Browning, E. T. & Scholz, R. (1969) *J. Biol. Chem.* **244**, 4607–4616
Wimhurst, J. M. & Manchester, K. L. (1970) *Biochem. J.* **120**, 79–93