Biochem. J. (1980) **190**, 183–189
Printed in Great Britain

Subcellular Distribution and Some Properties of *N*-Ethylmaleimide-Sensitive and -Insensitive Forms of Glycerol Phosphate Acyltransferase in Rat Adipocytes

E. David SAGGERSON, Carol A. CARPENTER, Christopher H. K. CHENG and Suren R. SOORANNA
Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, U.K.

(*Received 21 February 1980*)

1. Glycerol phosphate acyltransferase (GPAT) activities were measured in subcellular fractions obtained from rat epididymal adipocytes. These contained both *N*-ethylmaleimide-sensitive and *N*-ethylmaleimide-insensitive forms of the enzyme. 2. As shown by parallel measurements of marker enzymes, *N*-ethylmaleimide-insensitive GPAT is most probably a mitochondrial activity, whereas *N*-ethylmaleimide-sensitive GPAT is the microsomal enzyme. 3. Subcellular distributions are also reported for dihydroxyacetone phosphate acyltransferase (DHAPAT) (assayed with and without *N*-ethylmaleimide), monoacylglycerol phosphate acyltransferase (MGPAT) and Mg^{2+}-dependent and Mg^{2+}-independent forms of phosphatidate phosphohydrolase (PPH).

It is currently believed that the biosynthesis of glycerolipids can be initiated by the acylation of either glycerol phosphate or dihydroxyacetone phosphate. When glycerol phosphate is used as the initiating molecule, the initial acyl transfer is catalysed by GPAT. Studies, mainly with liver, have shown that this activity is located in both mitochondrial and microsomal fractions (Stoffel & Schiefer, 1968; Shephard & Hübscher, 1969; Daae, 1973; Van Tol, 1974; Bjerve *et al.*, 1976). The GPAT activities in these two fractions of liver differ in several important respects. The mitochondrial form has a lower K_m for glycerol phosphate (Nimmo, 1979), and a higher affinity for acyl-CoA substrates (Yamada & Okuyama, 1978; Bates & Saggerson, 1979). Also the mitochondrial activity has a higher preference for acylation at the 1-position of glycerol phosphate (Daae, 1972; Monroy *et al.*, 1972) and preferentially uses saturated fatty-acyl units (Monroy *et al.*, 1972, 1973). However, the most striking difference between the two GPAT activities is their differing response to thiol-group reagents. The microsomal GPAT from rat liver is almost completely inhibited by reagents such as iodoacetamide and *N*-ethylmaleimide (Lands & Hart, 1965; Monroy *et al.*, 1972; Haldar & Pullman, 1975; Yamada & Okuyama, 1978; Nimmo, 1979; Bates & Saggerson, 1979), whereas the mitochondrial activity is barely affected. It is therefore inferred that there are two distinct forms of GPAT with different intracellular locations (Nimmo, 1979). At present, the relative contributions of these two activities to the total glycerol phosphate acylation in any tissue is unknown. In rat liver the mitochondrial activity represents about half of the tissue total GPAT (Daae & Bremer, 1970; Sánchez *et al.*, 1973; Lloyd-Davies & Brindley, 1975), and it is this form that alters in diabetes, with changes in insulin status and after other changes in physiological state (Bates & Saggerson, 1977, 1979; Bates *et al.*, 1977).

In rat adipose tissue, where GPAT activity rapidly responds to hormonal treatment of the tissue (Sooranna & Saggerson, 1976, 1978), a far higher proportion of the total GPAT activity is reported to be in the microsomal fraction (Schlossman & Bell, 1976). Therefore characterization and accurate assessment of the amount of the mitochondrial form of the enzyme in adipose tissue is extremely difficult because of contamination of mitochondrial fractions with this very extensive microsomal activity. In the present work we have studied the subcellular distributions of *N*-ethylmaleimide-sensitive and -insensitive GPAT activities in rat adipocytes. It is concluded that the insensitive activity is a satis-

Abbreviations used: DGAT, diacylglycerol acyltransferase (EC 2.3.1.20); DHAPAT, dihydroxyacetone phosphate acyltransferase (EC 2.3.1.42); GPAT, glycerol phosphate acyltransferase (EC 2.3.1.15); MGPAT, monoacylglycerol phosphate acyltransferase (EC 2.3.1.51); PPH, phosphatidate phosphohydrolase (EC 3.1.3.4).

0306-3283/80/070183-07$01.50/1

factory estimate of the mitochondrial form. By using *N*-ethylmaleimide to eliminate extensive cross-contamination of the mitochondrial enzyme by the microsomal activity, it is then possible to investigate some aspects of the acyl-CoA substrate concentration-dependence and specificity of this mitochondrial enzyme and to compare these with the *N*-ethylmaleimide-sensitive (microsomal) enzyme (Saggerson *et al.*, 1979). These studies are a necessary preliminary before it can be ascertained whether or not the mitochondrial and microsomal GPAT activities in adipose tissue respond similarly or differently to hormones and changes in physiological state.

Materials and Methods

Animals

Male Sprague–Dawley rats weighing 160–180g were used throughout. These were bred in the animal colony at University College London and had constant access to water and GR3-EK cube diet (E. Dixon and Sons, Ware, Herts., U.K.).

Chemicals

These were obtained and treated as described by Sooranna & Saggerson (1978), Cheng & Saggerson (1978) and Bates & Saggerson (1979). In addition, 1-palmitoyl-*sn*-glycerol 3-phosphate was obtained from Miles Laboratories (Slough, Bucks., U.K). Oleoyl-CoA, stearoyl-CoA and arachidoyl-CoA were obtained from International Enzymes (Windsor, Berks., U.K.). Bovine plasma albumin from Sigma (London) Chemical Co. (Kingston-upon-Thames, Surrey, U.K.) was subjected to a defatting procedure before use (Chen, 1967) with minor modifications (Saggerson, 1972).

Isolation of adipocytes

These were obtained by collagenase treatment of epididymal-fat-pad pieces (Rodbell, 1964) as described by Saggerson & Tomassi (1971) and washed twice with Krebs–Ringer bicarbonate medium (Krebs & Henseleit, 1932) containing fatty acid-poor albumin (10mg/ml).

Preparation of subcellular fractions

Adipocytes freshly isolated from six rats were centrifuged for 30s at $200\,g_{av.}$ and the underlying medium was removed by aspiration. The cells were transferred to a glass tube in ice containing 20ml of 0.25M-sucrose, 10mM-Tris/HCl buffer, pH7.4, 1mM-EDTA and 1mM-dithiothreitol. This is referred to as 'sucrose medium'. The tube contents were agitated for 1min on a vortex mixer (Martin & Denton, 1970) and then transferred to a plastic centrifuge tube in ice. Centrifugations were performed at 2°C in an SS-34 rotor ($r_{av.}$ = 10.8cm) of a Sorvall Superspeed RC2-B centrifuge. The broken-cell preparation was centrifuged by acceleration of the centrifuge to $3000\,g_{av.}$, maintaining that field for 1min and then decelerating with the brake on (integrated field-time = 4500*g*-min) to yield a top layer of fat, which was discarded, an infranatant and a pellet. The pellet was resuspended in 4.0ml of sucrose medium to give fraction N. The infranatant was re-centrifuged by accelerating to $20000\,g_{av.}$, maintaining that field for 1min and then decelerating with the brake on (integrated field-time = 30200*g*-min). The resulting pellet was resuspended in 10.0ml of sucrose medium and sedimented again at $20000\,g_{av.}$ for 1min as described above. The pellet was resuspended in 4.0ml of sucrose medium to give fraction M. The supernatant from this washing procedure was also kept (fraction W). The supernatant from the first $20000\,g_{av.}$ centrifugation was centrifuged for 1h at $105000\,g_{av.}$ in a Beckman model L ultracentrifuge fitted with a 50 Ti rotor. The resulting pellet was resuspended in 5.0ml of sucrose medium to give fraction E, and the supernatant (approx. 19.5ml) was also kept (fraction S).

Determination of protein content of fractions

The method of Lowry *et al.* (1951) was used, with fatty acid-poor albumin as a standard. The mean (±S.E.M.) protein contents of each fraction were: fraction N, 4.08±0.28mg (8 experiments); fraction M, 3.72±0.16mg (19 experiments); fraction W, 3.0±0.20mg (8 experiments); fraction E, 5.05±0.30mg (15 experiments); fraction S, 33.3±1.6mg (8 experiments). The mean (±S.E.M.) percentage distributions of protein between these fractions were: N, 8.2±0.6%; M, 7.4±0.2%; W, 6.1±0.5%; E, 9.3±0.3%; S, 69.0±1.2%.

Enzyme assays

Glutamate dehydrogenase (EC 1.4.1.2) and lactate dehydrogenase (EC 1.1.1.27) were assayed spectrophotometrically at 25°C and 340nm as described by Martin & Denton (1970) and by Saggerson (1974) respectively, except that Triton X-100 (1mg/ml) was included in the assay of glutamate dehydrogenase. $NADP^+$–cytochrome *c* reductase (EC 1.6.2.4) and succinate–cytochrome *c* reductase (EC 1.3.99.1) were assayed spectrophotometrically at 25°C and 550nm by the methods of Phillips & Langdon (1962) and Sottacasa *et al.* (1967) respectively. MGPAT (EC 2.3.1.51) was assayed at 25°C by observing the transfer of CoA from oleoyl-CoA to 5,5′-dithiobis-(2-nitrobenzoic acid) at 420nm (Okuyama *et al.*, 1971). The assay contained, in a volume of 1.0ml, 50mM-Tris/HCl buffer, pH7.4, 120mM-KCl, fatty acid-poor albumin (2mg/ml), 100μM-5,5′-dithiobis-(2-nitrobenzoic acid) and 120μM-oleoyl-CoA. The reaction was

started by addition of 65nmol of 1-palmitoyl-*sn*-glycerol 3-phosphate dispersed in 0.04ml of 33mM-Tris/HCl buffer, pH8.0. PPH (EC 3.1.3.4) was assayed for 10min at 37°C in the presence and absence of 2.5mM-$MgCl_2$ as described by Cheng & Saggerson (1978). Monoamine oxidase (EC 1.4.3.4) was assayed radiochemically at 30°C by the method of Aas (1971) with [*side-chain*-2-^{14}C]tyramine hydrochloride as substrate. CTP–phosphatidate cytidylyltransferase (EC 2.7.7.41) was assayed radiochemically at 37°C by the method of Carter & Kennedy (1966) followed by extraction of the product by the method of Hajra (1974). DHAPAT (EC 2.3.1.42) was assayed radiochemically at 30°C by using dihydroxy[^{14}C]acetone phosphate generated *in situ* from [U-^{14}C]fructose 1,6-bisphosphate as described by Bates & Saggerson (1979). DGAT (EC 2.3.1.20) was assayed radiochemically at 30°C by the method of Coleman & Bell (1976) by measurement of the dioleoylglycerol-dependent incorporation of [^{14}C]palmitoyl-CoA into hexane-soluble products. GPAT (EC 2.3.1.15) was assayed radiochemically at 30°C essentially as described by Sooranna & Saggerson (1976), except that the reaction was terminated by addition of 2ml of butan-1-ol saturated with 0.1M-HCl. After mixing and centrifugation (5min at 2000$g_{av.}$), the butanol layer was washed twice with butanol-saturated 0.1M-HCl containing 10mM-*rac*-glycerol phosphate, and a 1.0ml portion of the butanol layer taken for liquid-scintillation counting in 10ml of toluene containing 2,5-bis-(5-t-butylbenzoxazol-2-yl)thiophen (4g/litre). Unless stated otherwise, GPAT assays contained, in a 1ml volume, 50mM-Tris/HCl buffer, pH7.4, 120mM-KCl, fatty acid-poor albumin (1.75mg/ml), 1mM-[U-^{14}C]glycerol phosphate (0.5–2.0μCi/μmol) and 65μM-palmitoyl-CoA.

All reaction rates were proportional to the amount of tissue extract added and were linear with time.

Results and Discussion

Distribution of N-ethylmaleimide-sensitive and -insensitive GPAT activities between centrifugation fractions

In preliminary experiments (Saggerson *et al.*, 1979) it was found that the uncorrected specific activity of GPAT in fraction E was approx. 2½ times that of fraction M: 10mM-*N*-ethylmaleimide abolished 96% of the activity in fraction E, whereas 1–10mM-*N*-ethylmaleimide only abolished 50% of the activity in fraction M. Inhibition of adipocyte microsomal GPAT by *N*-ethylmaleimide has been shown previously by Schlossman & Bell. (1976). For further experiments 10mM-*N*-ethylmaleimide was chosen as a suitable concentration, and *N*-ethylmaleimide-sensitivity or -insensitivity refers to the response of GPAT activities to this concentration of thiol-group reagent. A similar concentration of *N*-ethylmaleimide is sufficient to abolish 91–92% of the GPAT activity in liver microsomal fraction under similar assay conditions (Bates & Saggerson, 1979).

Table 1 shows that the percentage distribution of *N*-ethylmaleimide-sensitive GPAT between the various fractions was similar to that of the microsomal marker enzyme NADP$^+$–cytochrome *c* reductase. *N*-Ethylmaleimide-insensitive GPAT, however, showed a distribution pattern quite similar to that of any of the three mitochondrial marker enzymes, namely glutamate dehydrogenase (matrix), succinate–cytochrome *c* reductase (inner membrane) and monoamine oxidase (outer membrane). The 50% of fraction-M GPAT that was inhibited by *N*-ethylmaleimide (see above) almost certainly represents microsomal GPAT contaminating the mitochondrial fraction. Even if this contamination is a small percentage of the microsomal activity, it is relatively large in absolute terms, since the specific activity of the microsomal GPAT is very high (15–20 times that found in liver microsomal fraction under similar assay conditions; Bates & Saggerson, 1979). The 'true' mitochondrial GPAT activity may be obtained by subtraction of the calculated microsomal contamination as follows:

$$\text{'True' mitochondrial GPAT} = \text{total GPAT in fraction M} - \frac{\text{GPAT in fraction E} \times \text{NADP}^+\text{–cytochrome } c \text{ reductase in fraction M}}{\text{NADP}^+\text{–cytochrome } c \text{ reductase in fraction E}}$$

This calculation is feasible, since the mitochondrial contamination of fraction E is only approx. 20% (Table 1) and the GPAT activity in fraction M is small compared with that in fraction E. This calculated 'true' mitochondrial GPAT activity was in good agreement with the *N*-ethylmaleimide-insensitive activity that was directly measured (Saggerson *et al.*, 1979). It is concluded therefore that, as in the liver, *N*-ethylmaleimide-insensitive GPAT represents the mitochondrial form of the enzyme and that assay with and without the thiol-group reagent is a suitable way of investigating differences in the properties of the mitochondrial and microsomal forms of the enzyme. Throughout the present study the specific activity per mg of protein of *N*-ethylmaleimide-insensitive GPAT in fraction M had

Table 1. *Subcellular distribution of enzymes of glycerolipid synthesis and marker enzymes in adipocytes*

Centrifugation and assay procedures are described in the Materials and Methods section. The values are means ± S.E.M. of the numbers of observations shown in parentheses. NEM-insensitive GPAT and DHAPAT refers to the activities observed in the presence of 10mM-*N*-ethylmaleimide (NEM).

						GPAT	
	Protein (8)	Glutamate dehydrogenase (8)	Monoamine oxidase (8)	Succinate–cytochrome *c* reductase (8)	$NADP^+$–cytochrome *c* reductase (8)	NEM-insensitive (8)	NEM-sensitive (8)
Total activity (nmol/min per mg of protein)	Total protein = 49.6 ± 1.2 mg	42.3 ± 2.0	0.50 ± 0.02	22.6 ± 1.5	18.5 ± 1.1	1.06 ± 0.23	7.16 ± 0.60
Percentage distribution in fraction:							
N	8.2 ± 0.6	24 ± 2	19 ± 1	21 ± 1	8 ± 1	21 ± 2	8 ± 1
M	7.4 ± 0.2	51 ± 2	39 ± 2	60 ± 1	7 ± 0	48 ± 2	8 ± 1
W	6.0 ± 0.5	6 ± 0	10 ± 0	5 ± 1	6 ± 0	7 ± 1	7 ± 1
E	9.3 ± 0.3	14 ± 1	28 ± 1	14 ± 2	68 ± 1	20 ± 1	72 ± 1
S	69.1 ± 1.1	5 ± 0	4 ± 1	0	11 ± 1	4 ± 1	5 ± 0

		DHAPAT				CTP–phosphatidate cytidylyltransferase	PPH		
	Protein (3)	NEM-insensitive (3)	Total (3)	MGPAT (3)	DGAT (3)		Mg^{2+}-independent (3)	Mg^{2+}-dependent (3)	Lactate dehydrogenase (3)
Total activity (nmol/min per mg of protein	Total protein = 48.2 ± 1.4 mg	0.22 ± 0.03	1.31 ± 0.37	55.0 ± 6.1	3.76 ± 1.41	0.083 ± 0.004	2.81 ± 0.18	12.9 ± 5.2	501 ± 70
Percentage distribution in fraction:									
N	9.7 ± 0.2	13 ± 2	13 ± 1	9 ± 0	15 ± 1	24 ± 3	20 ± 2	7 ± 2	4 ± 1
M	7.4 ± 0.4	17 ± 1	10 ± 2	7 ± 0	9 ± 0	13 ± 1	25 ± 3	2 ± 0	0 ± 0
W	7.2 ± 0.8	11 ± 4	6 ± 0	7 ± 0	9 ± 2	8 ± 1	14 ± 4	1 ± 1	1 ± 0
E	9.5 ± 0.4	56 ± 4	69 ± 3	66 ± 0	66 ± 4	52 ± 5	26 ± 3	21 ± 6	1 ± 0
S	66.2 ± 1.3	3 ± 0	2 ± 0	11 ± 0	1 ± 1	3 ± 1	15 ± 6	69 ± 8	94 ± 1

values 2–3 times that of liver mitochondrial GPAT assayed under similar conditions (Bates & Saggerson, 1979).

The distributions referred to in the present study are expressed as percentages of the sum of activities in the five fractions rather than as percentages of the activity in the starting homogenate, which contained many pieces of fat and was therefore heterogeneous. A quite significant proportion of the aqueous part of the homogenate is entrained in this fatty mass. The first centrifugation is sufficient to compact the fat, so that at least 95% of the aqueous portion is recovered, but at the same time sediments fraction N. Two subsidiary experiments were performed which gave reassurance that the sum of activities in fractions N + M + W + E + S related to the activity in the starting homogenate. Homogenates were filtered through glass wool to yield a reasonably homogenous milky suspension. A sample of this was saved and the rest was subjected to the usual centrifugation procedures. On summing the activities in the fractions, the mean recoveries of protein and of the three marker enzymes, lactate dehydrogenase, glutamate dehydrogenase and $NADP^+$–cytochrome *c* reductase, were 109%, 95%, 85% and 115% respectively. It was concluded that the means of expression employed here was sound, was more convenient and avoided the problems inherent in attempting to assay enzymes in a fatty homogenate.

In rat liver, mitochondrial GPAT has been assigned to the outer membrane (Stoffel & Schiefer, 1968; Zborowski & Wojtczak, 1969; Daae & Bremer, 1970; Monroy *et al.*, 1972). It is therefore noteworthy that, in the experiments summarized in Table 1, the percentage distributions of *N*-ethylmaleimide-insensitive GPAT and monoamine oxidase were at variance with each other. Fraction M contained 48.4 ± 1.7% of the total *N*-ethylmaleimide-insensitive GPAT, but only 38.9 ± 1.8% of the monoamine oxidase (means ± S.E.M.). These were significantly different ($P < 0.01$). The reason for this difference is unknown at present.

In preliminary experiments it was established that the rate of [^{14}C]glycerol phosphate incorporation was constant between 1 and 10 min when mitochondrial fractions were assayed for GPAT. For microsomal fractions, the chosen assay conditions did not sustain a linear rate of incorporation after 7 min. For this reason, assays were limited to 7 min. In these assays zero-time values were always determined and subtracted from all other values. Despite this, none of the time courses could be extrapolated back to pass through the origin, but gave a small intercept on the ordinate (activity axis). The reason for this is unknown, but the implication is that there is an initial burst of glycerol phosphate acylation which diminishes to a steady rate within 1 min. These experiments also showed that the inhibitory effect of *N*-ethylmaleimide on microsomal GPAT contaminating the mitochondrial fraction is fully established within 1 min.

Distribution of enzymes of glycerolipid synthesis between centrifugation fractions.

In three cases the subcellular fractions generated in the course of the above experiments were also assayed for other enzymes associated with glycerolipid synthesis. We are unaware of any previous measurements of MGPAT and CTP–phosphatidate cytidylyltransferase activities in adipose tissue. The subcellular distributions of Mg^{2+}-independent and Mg^{2+}-dependent PPH activities have been examined in whole adipose tissue by Jamdar & Fallon (1973), but we are unaware of any such measurements in adipocytes. Since PPH has a fairly wide distribution in various tissues and cell types, and adipocytes may represent as little as 50% of adipose-tissue cells (Rodbell, 1964), we decided to make these measurements in adipocytes. The results of these experiments are also presented in Table 1.

DHAPAT. The subcellular distribution of this activity has been studied in adipocytes by Schlossman & Bell (1976), who used different techniques for fractionation and assay. That previous study found a greater proportion of activity in the microsomal fraction (92%). The most noteworthy finding here is that the distribution of the residual activity in the presence of 10 mM-*N*-ethylmaleimide is not greatly different from that of the total activity. It is unlikely therefore that there is an appreciable *N*-ethylmaleimide-insensitive adipocyte DHAPAT activity in the mitochondria, or perhaps in the peroxisomes as in the liver (Jones & Hajra, 1977; Bates & Saggerson, 1979).

MGPAT. The percentage distribution of MGPAT (assayed with oleoyl-CoA as acyl substrate) clearly paralleled that of $NADP^+$–cytochrome *c* reductase. Its distribution also was not dissimilar from that of DGAT (which has been suggested as a suitable microsomal marker by Sarzala *et al.*, 1970), except that MGPAT activity had an appreciably higher percentage of its activity in fraction S. In these experiments the specific activities of MGPAT and *N*-ethylmaleimide-sensitive GPAT in fraction E were 384 and 54.6 nmol/min per mg of protein respectively. In this respect it is noteworthy that MGPAT specific activity in rat liver microsomal fraction has been reported as being 12 (Yamashita *et al.*, 1973) or 6 (Lamb & Fallon, 1974) times that of GPAT. The MGPAT specific activity found here in adipocyte microsomal fraction is 10–15 times the activity reported for liver microsomal fraction (Yamashita *et al.*, 1973; Lamb & Fallon, 1974). It is not possible to establish from the data of Table 1 whether adipocyte mitochondria contain any in-

trinsic MGPAT activity. However, t.l.c. of the products of a GPAT assay on fraction M performed with 10mM-*N*-ethylmaleimide present showed these to be 64% monoacylglycerol phosphate and 36% phosphatidate + neutral lipids [the chromatography solvent system was chloroform/methanol/acetic acid/water (65:25:8:4, by vol.)]. An almost identical distribution of products was found in a parallel assay of GPAT in liver mitochondria. It is therefore likely that adipocyte mitochondria do have some intrinsic MGPAT activity.

CTP–phosphatidate cytidylyl transferase. This activity was essentially confined to particulate fractions, the majority being microsomal. Fractions N and M contained a higher percentage of this activity than of $NADP^+$–cytochrome *c* reductase or DGAT. It is likely therefore that this enzyme is also found in the nuclei and/or the mitochondria. In rat liver the subcellular-distribution pattern of this is not totally agreed on, but the consensus would appear to be that it has a bimodal distribution between the mitochondrial inner membrane and the microsomal fraction (Van den Bosch, 1974).

PPH. The Mg^{2+}-independent activity showed a general distribution between all fractions. The majority of the Mg^{2+}-dependent activity was in the soluble fraction, although an appreciable microsomal activity was apparent. The distribution of lactate dehydrogenase precluded this microsomal activity being due to contamination of the microsomal fraction by cytosol. The mitochondrial fraction contained essentially no Mg^{2+}-dependent PPH. These findings are in general agreement with those obtained with whole adipose tissue by Jamdar & Fallon (1973).

Concluding remarks

This study demonstrates the presence of *N*-ethylmaleimide-sensitive and *N*-ethylmaleimide-insensitive forms of GPAT in the rat adipocyte. It is likely that the *N*-ethylmaleimide-insensitive activity shows a strong preference for palmitoyl-CoA as acyl substrate and has little activity, compared with the *N*-ethylmaleimide-sensitive microsomal enzyme for unsaturated substrates (Saggerson *et al.*, 1979). Furthermore, as in the liver (Yamada & Okuyama, 1978; Bates & Saggerson, 1979) the mitochondrial GPAT has a higher affinity for palmitoyl-CoA than does the microsomal enzyme (Saggerson *et al.*, 1979). Although it is quite obvious that the intact adipocyte esterifies a spectrum of fatty acids rather than just palmitate, and also that the selectivity of the mitochondrial GPAT for palmitoyl-CoA is greater than the microsomal enzyme, these findings are of interest. A lowering of fatty-acyl-CoA concentration in the cell may favour the use of the mitochondrial GPAT to initiate glycerol phosphate acylation. Addition to incubated rat epididymal fat-pads of insulin to produce a lipogenic metabolic profile is accompanied by decreases in the total tissue content of long-chain fatty-acyl-CoA (Denton & Halperin, 1968; Saggerson & Greenbaum, 1970). At present, however, the relative contributions of the two forms of GPAT to the initiation of adipocyte glycerolipid synthesis and their relative roles in the control of this process remain to be established.

The British Diabetic Association and the Shell Company of Hong Kong are thanked for their financial support.

References

Aas, M. (1971) *Biochim. Biophys. Acta* **231**, 22–47
Bates, E. J. & Saggerson, E. D. (1977) *FEBS Lett.* **84**, 229–232
Bates, E. J. & Saggerson, E. D. (1979) *Biochem. J.* **182**, 751–762
Bates, E. J., Topping, D. L., Sooranna, S. R., Saggerson, E. D. & Mayes, P. A. (1977) *FEBS Lett.* **84**, 225–228
Bjerve, K. S., Daae, L. N. W. & Bremer, J. (1976) *Biochem. J.* **158**, 249–254
Carter, J. R. & Kennedy, E. D. (1966) *J. Lipid Res.* **7**, 678–683
Chen, R. F. (1967) *J. Biol. Chem.* **242**, 173–179
Cheng, C. H. K. & Saggerson, E. D. (1978) *FEBS Lett.* **87**, 65–68
Coleman, R. & Bell, R. M. (1976) *J. Biol. Chem.* **251**, 4537–4543
Daae, L. N. W. (1972) *Biochim. Biophys. Acta* **270**, 23–31
Daae, L. N. W. (1973) *Biochim. Biophys. Acta* **306**, 186–193
Daae, L. N. W. & Bremer, J. (1970) *Biochim. Biophys. Acta* **210**, 92–104
Denton, R. M. & Halperin, M. L. (1968) *Biochem. J.* **110**, 27–38
Hajra, A. K. (1974) *Lipids* **9**, 502–505
Haldar, D. & Pullman, M. E. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 632
Jamdar, S. C. & Fallon, H. J. (1973) *J. Lipid Res.* **14**, 517–524
Jones, C. L. & Hajra, A. K. (1977) *Biochem. Biophys. Res. Commun.* **76**, 1138–1143
Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
Lamb, R. G. & Fallon, H. J. (1974) *Biochim. Biophys. Acta* **348**, 166–178
Lands, W. E. M. & Hart, P. (1965) *J. Biol. Chem.* **240**, 1905–1911
Lloyd-Davies, K.A. & Brindley, D. N. (1975) *Biochem. J.* **152**, 39–49
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Martin, B. R. & Denton, R. M. (1970) *Biochem. J.* **117**, 861–877
Monroy, G., Rola, F. H. & Pullman, M. E. (1972) *J. Biol. Chem.* **247**, 6884–6894

Monroy, G., Keller, H. C. & Pullman, M. E. (1973) *J. Biol. Chem.* **248**, 2845–2852
Nimmo, H. G. (1979) *Biochem. J.* **177**, 283–288
Okuyama, H., Eibi, H. & Lands, W. E. M. (1971) *Biochim. Biophys. Acta* **248**, 263–273
Phillips, A. H. & Langdon, R. G. (1962) *J. Biol. Chem.* **237**, 2652–2660
Rodbell, M. (1964) *J. Biol. Chem.* **239**, 275–380
Saggerson, E. D. (1972) *Biochem. J.* **128**, 1057–1067
Saggerson, E. D. (1974) *Biochem. J.* **140**, 211–224
Saggerson, E. D. & Greenbaum, A. L. (1970) *Biochem. J.* **119**, 193–219
Saggerson, E. D. & Tomassi, G. (1971) *Eur. J. Biochem.* **23**, 109–117
Saggerson, E. D., Sooranna, S. R. & Cheng, C. H. K. (1979) *INSERM Colloq.* **87**, 223–238
Sánchez M., Nicholls, D. G. & Brindley, D. N. (1973) *Biochem. J.* **132**, 697–706
Sarzala, M. G., van Golde, L. M. G., De Kruyff, B. & van Deenen, L. L. M. (1970) *Biochim. Biophys. Acta* **202**, 106–119
Schlossman, D. M. & Bell, R. M. (1976) *J. Biol. Chem.* **251**, 5738–5744
Shephard, E. H. & Hubscher, G. (1969) *Biochem. J.* **113**, 429–440
Sooranna, S. R. & Saggerson, E. D. (1976) *FEBS Lett.* **64**, 36–39
Sooranna, S. R. & Saggerson, E. D. (1978) *FEBS Lett.* **90**, 141–144
Sottocasa, G. L., Kuylenstierna, B., Ernster, L. & Bergstrand, A. (1967) *J. Cell Biol.* **32**, 415–438
Stoffel, W. & Schiefer, H. G. (1968) *Hoppe-Seyler's Z. Physiol. Chem.* **349**, 1017–1026
Van den Bosch, H. (1974) *Annu. Rev. Biochem.* **43**, 243–277
Van Tol, A. (1974) *Biochim. Biophys. Acta* **357**, 14–23
Yamada, K. & Okuyama, H. (1978) *Arch. Biochem. Biophys.* **190**, 409–420
Yamashita, S., Hosaka, K., Taketo, M. & Numa, S. (1973) *FEBS Lett.* **29**, 235–238
Zborowski, J. & Wojtczak, L. (1969) *Biochim. Biophys. Acta* **187**, 73–84