The Glycerol Phosphate, Dihydroxyacetone Phosphate and Monoacylglycerol Pathways of Glycerolipid Synthesis in Rat Adipose-Tissue Homogenates

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1. Fat-free homogenates from the epididymal fat-pads of rats were used to measure the rate of palmitate esterification with different substrates. The effectiveness of the acyl acceptors decreased in the order glycerol phosphate, dihydroxyacetone phosphate, 2-octadecenylglycerol and 2-hexadecylglycerol. 2. Glycerol phosphate and dihydroxyacetone phosphate inhibited their rates of esterification in a mutually competitive manner. 3. The esterification of glycerol phosphate was also inhibited in a partially competitive manner by 2-octadecenylglycerol and to a lesser extent by 2-hexadecylglycerol. However, glycerol phosphate did not inhibit the esterification of 2-octadecenylglycerol. 4. The esterification of dihydroxyacetone phosphate and 2-hexadecylglycerol was more sensitive to inhibition by clofenapate than was that of glycerol phosphate. Norfenfluramine was more effective in inhibiting the esterification of 2-hexadecylglycerol than that of glycerol phosphate or dihydroxyacetone phosphate. 5. It is concluded that rat adipose tissue can synthesize glycerolipids by three independent routes.

It has been suggested that triacylglycerol synthesis in adipose tissue can proceed via the esterification of three different precursors. These are respectively *sn*-glycerol 3-phosphate (Rose & Shapiro, 1960; Daniel & Rubenstein, 1968; Jamdar & Fallon, 1973), dihydroxyacetone phosphate (LaBelle & Hajra, 1972*a,b*) and monoacylglycerol (Schultz & Johnston, 1971). Little is known about the control of these pathways or their interrelationships. In fact, the relative activities of these three routes of metabolism measured simultaneously in the same tissue have not yet been reported. Neither is it known whether the incorporation of the three precursors into lipids is catalysed by the same or different enzymes.

There is considerable interest in the metabolism of adipose tissue both from the point of view of studying the normal processes of triacylglycerol storage and of investigating some of the events leading to obesity. The present study was designed to determine the relative activities of the three routes of glycerolipid synthesis, to characterize them, and to study their possible interrelationships. The experimental tissue chosen was the epididymal fat-pad of the rat.

Unit, Sutton Bonington, Leics., U.K. They were allowed free access to the 41B diet (Brindley *et al.*, 1976) and water before the experiments.

Materials

Unless stated to the contrary, materials were prepared or purchased as described previously (Sánchez *et al.*, 1973; Mangiapane *et al.*, 1973; Brindley & Bowley, 1975). Hepes [2-(*N*-2-hydroxyethylpiperazin-*N'*-yl)ethanesulphonic acid] was obtained from Hopkin and Williams Ltd., Chadwell Heath, Essex, U.K., and octadec-*cis*-9-enol and dihydroxyacetone phosphate (dimethylketal, cyclohexylamine salt) were from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. On the day before use, dihydroxyacetone phosphate was generated according to the manufacturer's instructions. The liberated methanol and the water from washings were removed by freeze-drying overnight. Immediately before use the solution was adjusted to pH 7-7.4 with solid KHCO_3 .

Preparation of alkylglycerols

2-*O*-Hexadecylglycerol was prepared by Mrs. V. J. Short (Short *et al.*, 1974). A sample (1 g) of this was labelled uniformly with ^3H gas by The Radiochemical Centre, Amersham, Bucks., U.K., by using the TR2

Materials and Methods

Animals

Male Wistar rats (250-320 g) were obtained from the University of Nottingham Joint Animal Breeding

(Wilzbach) method (Radiochemicals catalogue of The Radiochemical Centre). Excess of ^3H and the catalyst were removed by The Radiochemical Centre and this product was then recrystallized twice from light petroleum (b.p. 40–60°C). The crystals were dissolved and stored in ethanol. Samples of this solution were purified by t.l.c. on plates (0.6 mm thick) of silica gel G by using two developments with chloroform/methanol (49:1, v/v). The product had a radiochemical purity of greater than 98% as determined by chromatography on t.l.c. plates of silica gel G in either chloroform/acetone (22:3, v/v) or chloroform/acetone/methanol (71:25:4, by vol.) as developing solvent. The $[\text{U-}^3\text{H}]\text{-2-hexadecylglycerol}$ ($42\ \mu\text{Ci}/\mu\text{mol}$) was diluted with non-radioactive 2-hexadecylglycerol to a specific radioactivity of $5\ \mu\text{Ci}/\mu\text{mol}$ as required. 2-*O*-Octadec-*cis*-9-enyl $[\text{1,3-}^3\text{H}]\text{glycerol}$ was prepared by the method of Bauman & Mangold (1964) by using $[\text{1,3-}^3\text{H}]\text{glycerol}$ ($1\ \mu\text{Ci}/\mu\text{mol}$) to synthesize the intermediate benzylidene- $[\text{1,3-}^3\text{H}]\text{glycerol}$. The 2-*O*-octadec-*cis*-9-enyl $[\text{1,3-}^3\text{H}]\text{glycerol}$ was finally purified by t.l.c. on plates (0.5 mm thick) of silica gel N (Macherey-Nagel, Düren, Germany) by using chloroform/methanol (49:1, v/v) for development (Thomas *et al.*, 1965). The proton and ^{13}C -n.m.r. spectra of the product were consistent with its being 2-*O*-octadecenyl $[\text{3H}]\text{glycerol}$ and its radiochemical purity was greater than 99.6%.

Preparation of fat-free homogenates

These were prepared essentially by the method of Jamdar & Fallon (1973), except that the epididymal fat-pads of single rats were homogenized in 0.25 M-sucrose containing 1 mM-Hepes buffer, adjusted to pH 7.4 with KOH.

Enzyme assays

The concentration of substrates and cofactors described below were those that gave maximum reaction rates except where specifically stated to the contrary. The rates of reaction were constant throughout the 30 min period of the incubations.

Determination of the rate of glycerolipid synthesis from *sn*- $[\text{1,3-}^3\text{H}]\text{glycerol 3-phosphate}$

Each assay contained in a volume of 0.25 or 0.50 ml: 25 mM-Hepes buffer, adjusted to pH 7.4 with KOH, 4 mM-dithiothreitol, 10 mM- MgCl_2 , 1.8 mM-ATP, 50 μM -CoA, 0.8 mM-potassium palmitate, 13.6 mM-*sn*- $[\text{1,3-}^3\text{H}]\text{glycerol 3-phosphate}$ ($1\ \mu\text{Ci}/\mu\text{mol}$) and 6 mg of fatty acid-poor bovine serum albumin/ml (approx. 90 μM). The reaction was started

with about 200 μg of homogenate protein/ml of assay medium. After incubation for 30 min at 37°C the reaction was stopped with 1.88 ml of chloroform/methanol (1:2, v/v). Lipids were extracted and analysed on t.l.c. plates as described by Brindley & Bowley (1975).

Measurement of dihydroxyacetone phosphate acyltransferase activity (EC 2.3.1.42)

The reaction conditions were identical with those used for measuring the esterification of glycerol phosphate, except that 0.8 mM-potassium $[\text{1-}^{14}\text{C}]\text{-palmitate}$ ($0.5\ \mu\text{Ci}/\mu\text{mol}$) was used and glycerol phosphate was replaced by 5 mM-dihydroxyacetone phosphate. The activity was defined as the dihydroxyacetone phosphate-dependent incorporation of $[\text{1-}^{14}\text{C}]\text{-palmitate}$ into acyldihydroxyacetone phosphate. After incubation at 37°C for 30 min, reactions were stopped with 1.88 ml of chloroform/methanol (1:2, v/v) and lipids extracted by the method of Hajra *et al.* (1968). Samples (100 μl) of the bottom phase were applied to t.l.c. plates of Kieselgel 60/Kieselguhr F254 (Merck, Darmstadt, Germany) and developed for 60% of the length with chloroform/methanol/acetic acid/10% (w/v) sodium metabisulphite (25:10:3:1, by vol.). This was followed by two developments with hexane/diethyl ether/acetic acid (60:40:1, by vol.) to remove $[\text{1-}^{14}\text{C}]\text{palmitate}$ from the phospholipid areas. Lipids were detected with I_2 vapour and spark-chamber photography. Acyldihydroxyacetone phosphate was taken as the band with R_f 0–0.1.

Measurement of monoacylglycerol acyltransferase activity (EC 2.3.1.22)

The activity was determined by using 2-alkylglycerols as model substrates for the reaction (Short *et al.*, 1974). These were prepared by sonication (Short *et al.*, 1974) in 0.6 mg of fatty acid-poor bovine serum albumin/ml. The assays contained in a final volume of 0.25 or 0.5 ml: 25 mM-Hepes buffer, adjusted to pH 7.4 with KOH, 4 mM-dithiothreitol, 10 mM- MgCl_2 , 1.8 mM-ATP, 50 μM -CoA, 1 mM-potassium $[\text{1-}^{14}\text{C}]\text{-palmitate}$ ($0.5\ \mu\text{Ci}/\mu\text{mol}$), 6 mg of fatty acid-poor bovine serum albumin/ml and either 2 mM-2-hexadecylglycerol or 2 mM-2-octadecenyl $[\text{3H}]\text{glycerol}$ ($1\ \mu\text{Ci}/\mu\text{mol}$). Reactions were stopped after 30 min at 37°C with 1.88 ml of chloroform/methanol (1:2, v/v) and the water content was adjusted to 0.5 ml. Lipids were extracted by the method of Bligh & Dyer, (1959). Neutral lipids were isolated by column chromatography with 8 g of aluminium oxide by elution with 12 ml of chloroform. The products were also analysed on t.l.c. plates of silica gel G by using hexane/diethyl ether/acetic acid (60:40:1, by vol.) as a developing solvent.

Measurement of the competition for palmitate esterification by glycerol phosphate, dihydroxyacetone phosphate and 2-alkylglycerols

In these experiments the conditions were essentially the same as in the assays described above, except that two acyl acceptors were added at the concentrations indicated in Figs. 2-4. Antimycin A (4 µg/ml) was included in the assays that contained both glycerol phosphate and dihydroxyacetone phosphate in order to inhibit any glycerol phosphate dehydrogenase (EC 1.1.99.5) activity. Incubations involving alkylglycerols and glycerol phosphate were extracted by the method of Bligh & Dyer (1959) and those involving alkylglycerols and dihydroxyacetone phosphate by the method of Hajra *et al.* (1968). Samples of the bottom phase (100 µl) in the latter system were analysed as above by t.l.c. to detect acyldihydroxyacetone phosphate. The bottom phase was then washed twice with 2 ml of synthetic top phase made by mixing 1 litre of chloroform, 1 litre of methanol, 400 ml of water and 500 ml of 100 mM-Tris buffer, adjusted to pH 8 with HCl. Portions of the bottom phase were then applied to an alumina column as above to determine the radioactivity in neutral lipids.

Radioactivity

Radioactivity was determined by liquid-scintillation counting (Brindley *et al.*, 1976).

Protein

Protein was determined by a biuret method (Hübscher *et al.*, 1965).

Enzyme kinetics

Lineweaver & Burk (1934) analysis was performed by using a weighted regression procedure (Wilkinson, 1961).

Experimental and Results

Characterization of the glycerol phosphate, dihydroxyacetone phosphate and monoacylglycerol acyltransferase activities of rat adipose tissue

The mean specific activity of glycerol phosphate acyltransferase activity was 9.1 ± 5.5 (s.d. from ten independent experiments) nmol/min per mg of homogenate protein. The molar composition of the products was approx. 15% lysophosphatidate, 61% phosphatidate, 6% diacylglycerol and 18% triacylglycerol. This product composition indicates that phosphatidate phosphohydrolase activity is rate-limiting in the synthesis of triacylglycerol under these conditions. The assay system had been specifically optimized for the acylation of glycerol phosphate rather than for the synthesis of neutral lipids.

Fig. 1. Effect of NADPH on the esterification of dihydroxyacetone phosphate with palmitate

The rate of esterification of dihydroxyacetone phosphate was measured as described in the Materials and Methods section, except that NADPH was added to some incubations. The rates of synthesis of acyldihydroxyacetone phosphate (▲), phosphatidate (■) and total glycerolipid (●) were calculated by assuming that 1 mol of palmitate incorporated is equivalent to 1 mol of acyldihydroxyacetone phosphate and 0.5 mol of phosphatidate. Negligible amounts of neutral lipid were synthesized. The rate of synthesis of acyldihydroxyacetone phosphate was 2.3 ± 0.4 (s.d. from three independent experiments) nmol/min per mg of homogenate protein, and this is shown as 100 on the graph. The other rates of incorporations are expressed relative to this and s.d. values are indicated by the error bars.

The rate of esterification of glycerol phosphate was significantly greater than that for dihydroxyacetone phosphate ($P < 0.001$). The latter activity was 2.2 ± 1.6 (s.d. from 14 independent experiments) nmol of dihydroxyacetone phosphate esterified/min per mg of homogenate protein. The product was almost entirely palmitoyl-dihydroxyacetone phosphate. As reported for liver (LaBelle & Hajra, 1974), the addition of NADPH resulted in the formation of phosphatidate (Fig. 1). Optimum concentrations (0.2–0.4 mM) of NADPH did not alter the rate of esterification of dihydroxyacetone phosphate (Fig. 1), but, as expected, resulted in a doubling of the rate of palmitate incorporation. The rate of esterification of dihydroxyacetone phosphate was greater than that of 2-hexadecylglycerol ($P < 0.01$), but not significantly different from the rate of 2-octadecenylglycerol esterification.

The rate of glycerol phosphate esterification was also greater ($P < 0.005$) than the rates of esterification of 2-hexadecylglycerol and 2-octadecenylglycerol. These were respectively 0.8 ± 0.3 (s.d. from 11 independent experiments) and 1.1 ± 0.5 (s.d. from seven independent experiments) nmol of alkylglycerol esterified/min per mg of homogenate protein. For both precursors the products were about 98% acyl-alkylglycerol, with the remainder being diacylalkylglycerol. Similar product compositions were obtained

Fig. 2. Interaction of glycerol phosphate and dihydroxyacetone phosphate on the rate of palmitate esterification

(a) Effect of dihydroxyacetone phosphate concentration on the rate of esterification of glycerol phosphate as measured by the incorporation of [¹⁴C]palmitate into phosphatide. The concentrations of dihydroxyacetone phosphate used were 0 (Δ), 1 (○), 2.5 (■), 4 (▲) and 5 (●) mM. (b) Effect of glycerol phosphate concentration on the rate of synthesis of [¹⁴C]palmitoyldihydroxyacetone phosphate. The concentrations of *sn*-glycerol 3-phosphate used were 0 (○), 1.3 (■), 2.75 (▲) and 4.75 (●) mM. The effects of 6.75, 10 and 15 mM *sn*-glycerol 3-phosphate are not shown in the Figure, since the rates of palmitoyldihydroxyacetone phosphate synthesis were not significantly different from those that were obtained at 2.75 and 4.75 mM *sn*-glycerol 3-phosphate. The error bars indicate the relative errors based on the S.D. obtained from three independent experiments. To compare the results from different experiments, the results in (a) and (b) respectively are expressed relative to the maximum rates of esterification of *sn*-glycerol 3-phosphate (at 15 mM) and of dihydroxyacetone phosphate (at 5 mM). These were given a value of 1 and both were obtained in the absence of the other acyl acceptor. The absolute activities were 10 ± 3 (S.D. from three experiments) and 1.8 ± 0.17 (S.D. from three experiments) nmol of palmitate incorporated/min per mg of homogenate protein. $V_{\max}$ values were not calculated by extrapolating to infinite substrate concentrations, since dihydroxyacetone phosphate caused substrate inhibition at concentrations in excess of about 10 mM (not shown).

with preparations from guinea-pig intestinal mucosa (Short *et al.* 1974). However, Schultz & Johnston (1971) obtained relatively more diacylalkylglycerol (50%) by using enzymes derived from hamster adipose tissue. In the present work the rate of diacylalkylglycerol synthesis might have been greater if the ionic strength of assay system had been increased and if an unsaturated fatty acid had been used as acyl donor (Coleman, 1976).

Interaction between the three pathways of glycerolipid synthesis

These experiments examine whether glycerol phosphate, dihydroxyacetone phosphate and 2-hexadecylglycerol are mutually competitive for the esterification of palmitate.

Fig. 2 shows that glycerol phosphate and dihydroxyacetone phosphate inhibit the esterification of each other in a competitive manner. The apparent K_m value for glycerol phosphate based on the incorporation of *sn*-[³H]glycerol 3-phosphate into lipid was 0.6 ± 0.2 mM (S.D. of five independent experiments), whereas that based on the incorporation of [¹⁴C]-palmitate into phosphatide was 0.09 ± 0.02 mM

(S.D. of three independent experiments). These two methods for determining the requirement for glycerol phosphate measure slightly different parameters. The incorporation of palmitate is affected by a contribution from the endogenous fatty acids of the preparation and by the fatty acid specificities of the three enzymes responsible for phosphatide synthesis. The apparent K_m for dihydroxyacetone phosphate was 2.9 ± 0.9 mM (S.D. from four independent experiments) and the respective K_i values for glycerol phosphate and dihydroxyacetone phosphate were 0.16 and 0.24 mM. There was a residual activity of dihydroxyacetone phosphate acyltransferase activity which could not be further inhibited by concentrations of glycerol phosphate between 2.75 and 15 mM (Fig. 2b). Since homogenates were used in these studies the K_m and K_i values should be treated with caution. However, the results do show the competitive interactions between glycerol phosphate and dihydroxyacetone phosphate.

Schultz *et al.* (1971) and Polheim *et al.* (1973) have shown that octadecenylglycerol and octadecenylglycerol inhibit the esterification of glycerol phosphate in preparations of hamster adipose tissue and small-intestinal mucosa. In our hands the addition of

Fig. 3. Effect of 2-hexadecylglycerol on the rate of glycerol phosphate esterification

The Figure shows the effect of varying the concentration of 2-hexadecylglycerol in incubations containing either 1 (Δ) or 13.5 ($\bullet$) mm-*sn*-glycerol 3-phosphate. The results are expressed relative to the maximum rate of glycerol phosphate esterification which is taken as 100 and which was 8.2 ± 2.1 (s.d. from four independent experiments) nmol/min per mg of homogenate protein. The error bars at 13.5 mm-glycerol phosphate represent the s.d. from the four experiments, whereas those at 1 mm-glycerol phosphate indicate the ranges from two experiments.

Fig. 4. Effect of 2-octadecylglycerol on the rate of glycerol phosphate esterification

The Figure shows the effect of varying the concentrations of 2-octadecylglycerol in incubations containing 1 ($\circ$), 3 ($\blacksquare$), 5 ($\triangle$) or 13.5 ($\bullet$) mm-*sn*-glycerol 3-phosphate. The results are expressed relative to the rates of glycerol phosphate incorporation at the various glycerol phosphate concentrations in the absence of 2-octadecylglycerol. The error bars indicate the means and range of activities from two independent experiments.

increasing concentrations of 2-hexadecylglycerol to the incubations, which were optimum for glycerol phosphate esterification, produced up to a twofold stimulation of lipid synthesis from glycerol phosphate (Fig. 3). It was expected that an inhibition of glycerol phosphate esterification might be observed if the concentration of enzyme protein was increased to make it rate-limiting in the assay (W. C. Hülsmann, personal communication). Such an inhibition was not observed in the present work, but an inhibition of up to 35% was obtained when the concentration of glycerol 3-phosphate was made suboptimum in the assay system (Fig. 3).

This result indicates that the inhibition of glycerol phosphate esterification by alkylglycerols might be of a competitive nature. This possibility was tested extensively by using 2-octadecyl[^3H]glycerol, which was expected to be a more potent inhibitor. When this unsaturated analogue was used, consistent inhibition of glycerol phosphate esterification was observed (Fig. 4). The kinetics were not of the normal hyperbolic type, which makes conventional analysis impossible. However, Fig. 4 demonstrates the partially competitive nature of the inhibition, which was more pronounced at low concentrations of

glycerol phosphate. The esterification of 2-octadecyl[^3H]glycerol, which was measured simultaneously, was not significantly affected by varying the concentration of glycerol phosphate.

Experiments were also performed to test the interactions between the dihydroxyacetone phosphate and monoacylglycerol pathways. Varying the concentration of dihydroxyacetone phosphate (0–5 mm) and 2-hexadecylglycerol (0–4 mm) had no significant effect on the rate of the esterification of the other acyl acceptor.

Effects of clofenapate and norfenfluramine on glycerolipid synthesis

Clofenapate [4-(4'-chlorophenyl)phenoxyisobutyrate] and norfenfluramine [1-(*m*-trifluoromethylphenyl)-2-aminopropane] are two drugs that interfere with lipid metabolism. They were added to assay systems containing the three different precursors for glycerolipid synthesis to see whether they could be used to detect differences in properties between the respective acyltransferase activities (Table 1). Clofenapate, an amphiphilic anion, inhibited all three acyltransferase activities. By analogy with *p*-chloro-

Table 1. *Effects of clofenopate and norfenfluramine on the activities of the acyltransferases which act on glycerol phosphate, dihydroxyacetone phosphate and 2-hexadecylglycerol*

The mean concentrations of clofenopate and norfenfluramine that were required to produce a 50% inhibition of the enzyme activities are listed. The values were obtained from three independent preparations with at least five different concentrations of the drugs for each enzyme. The combined results were plotted as $\ln$ concentration (abscissa) against the percentage inhibition (ordinate). An inhibition of 50% was arbitrarily given a value of 0 and the results were subjected to linear regression analysis. The mean concentration of drug giving a 50% inhibition was defined as the intercept on the abscissa. The values in parentheses show the range of ± 1 s.d. from these means. Some compounds produced no significant inhibition up to the concentration shown and this is indicated by *.

	Concentration (mM) of drug required to produce a 50% inhibition of enzyme activity	
	Clofenopate	Norfenfluramine
Glycerol phosphate acyltransferase	1.5 (1.4-1.6)	20*
Dihydroxyacetone phosphate acyltransferase	0.91 (0.84-1.00)	16*
Monoacylglycerol acyltransferase	0.87 (0.78-0.98)	15 (7-30)

phenoxyisobutyrate, this effect is probably non-competitive with respect to the acyl acceptor (Lamb & Fallon, 1972). The activity of the glycerol phosphate acyltransferase appeared to be less sensitive to inhibition ($P < 0.001$) than the activity of dihydroxyacetone phosphate acyltransferase, which resembles the situation in rat liver (Bowley *et al.*, 1973; Bowley & Brindley, 1976). Monoacylglycerol acyltransferase activity was also more sensitive to inhibition ($P < 0.001$) than was glycerol phosphate acyltransferase. The clofenopate inhibition of monoacylglycerol acyltransferase has not been reported before, but clofenopate does appear to be a general inhibitor of acyltransferase activities (Brindley & Bowley, 1975).

In contrast with clofenopate, norfenfluramine is an amphiphilic cation. It was a poor inhibitor of acyltransferase activities (Table 1). Dannenburg *et al.* (1973) reported that norfenfluramine inhibited the activity of monoacylglycerol acyltransferase in the small intestine by 50% at about 1.7 mM. The equivalent inhibition was not obtained until about 15 mM in the present work (Table 1). Wilson & Galton (1971) reported an inhibition of neutral-lipid synthesis by human adipose tissue by 2-3 mM-fenfluramine, which they attributed to an inhibition of glycerol phosphate acyltransferase. In the present work concentrations of norfenfluramine in this range stimulated glycerol phosphate acyltransferase activity by up to 2.5-fold. However, the proportion of neutral lipid synthesized relative to phosphatidate was decreased. This indicates that an inhibition of phosphatidate phosphohydrolase is the likely explanation for the decreased rate of synthesis of neutral lipids. This conclusion is supported by work with rat liver (Brindley & Bowley, 1975).

Discussion

Palmitate esterification by homogenates of rat adipose tissue was measured by using four precursors for lipid glycerol. The rates of esterification obtained with these precursors decreased in the order glycerol phosphate, dihydroxyacetone phosphate, 2-octadecenylglycerol and 2-hexadecylglycerol. These rates were dependent on the presence of the other precursors in the assay system. For instance, glycerol phosphate and dihydroxyacetone phosphate were mutually competitive (Fig. 2). A similar effect has been demonstrated with the microsomal fraction of guinea-pig liver (Hajra, 1968). However, these precursors were not mutually competitive with respect to their esterification by the mitochondrial fractions of guinea-pig (Hajra, 1968) and of rat liver (Bowley *et al.*, 1973). Hajra (1968) also showed that the fatty acid specificity of the mitochondrial dihydroxyacetone phosphate acyltransferase differed from that of the microsomal enzyme. Schlossmann & Bell (1976) reported that more than 85% of the activity of dihydroxyacetone phosphate acyltransferase in rat adipose cells was microsomal. LaBelle & Hajra (1972b) have shown that the specific activity of dihydroxyacetone phosphate acyltransferase in the microsomal fraction of rat adipose tissue exceeds that in the mitochondrial fraction by a factor of 2.5. The failure of glycerol phosphate (2.75-15 mM) to inhibit dihydroxyacetone phosphate acyltransferase completely is compatible with there being a dual localization of this enzyme in adipose tissue, the glycerol phosphate-insensitive activity being located in the mitochondria. Schlossmann & Bell (1976) concluded from studies with *N*-ethylmaleimide, trypsin, Triton X-100 and deoxycholate that the

esterification of glycerol phosphate and dihydroxyacetone phosphate is catalysed in the microsomal fraction of rat adipocytes by a single enzyme. Although a mutual competition for palmitate esterification was also demonstrated between glycerol phosphate and dihydroxyacetone phosphate (Fig. 2; Schlossmann & Bell, 1976), the observation that the acyltransferase activities towards these substrates varied independently of each other (Dodds *et al.*, 1976) indicates that there are two different enzymes. This is also confirmed by the greater sensitivity of the dihydroxyacetone phosphate acyltransferase activity to inhibition by clofenapate.

2-Octadecenylglycerol also inhibited the activity of glycerol phosphate acyltransferase. The inhibition appeared to be of a competitive type (Fig. 4), but the effect of this lipid substrate cannot be described in terms of conventional kinetics (Brindley & White, 1974). 2-Hexadecylglycerol only produced relatively small inhibitions of glycerol phosphate acyltransferase when the concentration of glycerol phosphate in the assay system was suboptimum (Fig. 3). At optimum concentrations of glycerol phosphate a stimulation was observed (Fig. 3) and this has not been reported before. In previous work with the microsomal fraction of guinea-pig intestinal mucosa, 1-monopalmitoylglycerol did not significantly affect the rate of glycerol phosphate esterification (Brindley, 1973). Schultz *et al.* (1971) proposed that the inhibition of glycerol phosphate acyltransferase by monoacylglycerol, as demonstrated with their ether analogues, might constitute a control mechanism in the cell. This hypothesis is very attractive, but the inhibition might be caused by a non-specific detergent effect.

The following paper (Dodds *et al.*, 1976) shows a close correlation between the activities of glycerol phosphate acyltransferase and monoacylglycerol acyltransferase. This may mean that the activities are catalysed by one enzyme. However, the inability of glycerol phosphate to inhibit the esterification of octadecenylglycerol and the greater sensitivity of the monoacylglycerol acyltransferase to inhibition by norfenfluramine indicate that the two reactions are catalysed by separate enzymes as they are in the enterocyte.

The present paper demonstrates that adipose tissue, like the small intestine (Brindley, 1974), can synthesize glycerolipids from glycerol phosphate, dihydroxyacetone phosphate and monoacylglycerols. The relative specific activities of the three acyltransferases have been measured and their interactions described. Evidence has been presented to show that these acyltransferases are separate enzymes. The following paper (Dodds *et al.*, 1976) shows how these activities in adipose tissue vary when rats are fed on different diets and discusses the possible physiological significance of these different routes of glycerolipid synthesis.

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