The Relationship between Palmitoyl-Coenzyme A Synthetase Activity and Esterification of *sn*-Glycerol 3-Phosphate by the Microsomal Fraction of Guinea-Pig Intestinal Mucosa

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1. With microsomal fractions of guinea-pig intestinal mucosa the mean specific activity of palmitoyl-CoA synthetase was approx. 1.3-fold the esterification of *sn*-glycerol 3-phosphate with palmitoyl-CoA generated by the endogenous synthetase. The latter activity was approx. 2.5- and 5-fold that when palmitoyl-CoA was generated from palmitoylcarnitine or when it was added directly to the assay system. 2. There were significant correlations ($P < 0.001$) between the specific activities of palmitoyl-CoA synthetase and glycerolipid synthesis from either palmitate or palmitoylcarnitine. 3. The mean molar composition of glycerolipid synthesized from palmitate or palmitoylcarnitine was approx. 18% lysophosphatidate, 75% phosphatidate and 7% neutral lipid. 4. Glycerolipid synthesis from palmitate was inhibited by 80–90% after preincubation of microsomal fractions at 37°C for 40 min and was caused by inactivation of palmitoyl-CoA synthetase. 5. Addition of 100–400 mM-KCl inhibited palmitoyl-CoA synthetase activity and glycerolipid synthesis from palmitate but stimulated glycerol phosphate acyltransferase activity. 6. Diversion of palmitoyl-CoA synthesized by the endogenous synthetase to palmitoylcarnitine resulted in an almost stoichiometric decrease in glycerolipid synthesis. 7. Addition of *rac*-1-monopalmitin promoted utilization of palmitoyl-CoA by the monoglyceride pathway but did not inhibit phosphatidate biosynthesis. 8. With rate-limiting concentrations of CoA and Mg^{2+} the relative decreases in velocity for palmitoyl-CoA synthetase and glycerolipid synthesis from palmitate were almost identical. However, low concentrations of palmitate and ATP produced greater decreases in synthetase activity than in glycerolipid synthesis. 9. There appears to be a fine balance between the activities of palmitoyl-CoA synthetase and glycerol phosphate acyltransferase, with neither activity being in excess with respect to phosphatidate synthesis.

Earlier experiments indicated that palmitoyl-CoA synthetase (EC 6.2.1.3) might be rate-limiting in the synthesis of phosphatidate by the microsomal fraction of guinea-pig intestinal mucosa (Brindley & Ferrier, 1972). The relationship between palmitoyl-CoA synthetase activity and glycerolipid biosynthesis has now been investigated in more detail.

Palmitoyl-CoA synthetase and glycerol phosphate acyltransferase (EC 2.3.1.15) activities were assayed in the microsomal fractions prepared from the intestinal mucosa of a series of male guinea pigs. The relation between these activities and the ability to esterify *sn*-glycerol 3-phosphate with palmitoyl-CoA generated by the endogenous synthetase was then investigated. In addition, a series of kinetic experiments was performed to see which enzyme activity might be rate-limiting in phosphatidate biosynthesis. A further objective of this approach was to investigate whether metabolically distinct pools of palmitoyl-CoA exist in the endoplasmic reticulum.

The microsomal fraction has been used in this

study since the enzymes catalysing the synthesis of glycerides and phospholipids from *sn*-glycerol 3-phosphate are located predominantly if not exclusively in this fraction (Hübscher *et al.*, 1964; Gurr *et al.*, 1965; Brindley & Hübscher, 1965).

Experimental

Materials

Animals. Male guinea pigs (400–650 g) were supplied by the Joint Animal Breeding Unit, Sutton Bonington, Leics., U.K. Free access to food and water was allowed before the experiments.

Chemicals and enzymes. These were purchased or were prepared as described in the preceding paper (Sánchez *et al.*, 1973).

Methods

Subcellular fractionation. Microsomal fractions of intestinal mucosa were prepared by the method of

Hübscher *et al.* (1965) and assayed for enzyme activity on the day of preparation.

Enzyme assays. The method of incubation and the procedure for extracting and characterizing products are described in the preceding paper (Sánchez *et al.*, 1973) unless stated to the contrary.

Determination of palmitoyl-CoA synthetase activity. The assay was described in the preceding paper (Sánchez *et al.*, 1973). Each incubation contained, in a volume of 0.25 ml, 25 mM-Tris buffer adjusted to pH 7.4 with HCl, 5 mM-dithiothreitol, 25 μ M-CoA, 7.5 mM-ATP, 10 mM-MgCl₂, 1.2 mM-potassium palmitate, 1.5 mg of fatty acid-poor bovine serum albumin, 6.0 mM-($\pm$)-[³H]carnitine and excess of carnitine acyltransferase (EC 2.3.1.23) (420 μ g of protein). Reactions were at 37°C; they were started with 25–50 μ g of microsomal protein and were stopped after 7 min.

Determination of sn-glycerol 3-phosphate esterification with palmitoyl-CoA generated from palmitate. Assays were performed and lipid classes were analysed as described in the preceding paper (Sánchez *et al.*, 1973) except that lipids were extracted by the method of Hajra *et al.* (1968), which gave recoveries about 1.6-fold those from the extraction with butan-1-ol (Daee & Bremer, 1970; Sánchez *et al.*, 1973). This was probably because butan-1-ol is a less-efficient solvent for phosphatidate, which was the major product of the microsomal system (Table 1). Each assay contained, in a volume of 0.25 ml, 25 mM-Tris buffer adjusted to pH 7.4 with HCl, 5 mM-dithiothreitol, 25 μ M-CoA, 7.5 mM-ATP, 10 mM-MgCl₂, 1.2 mM-potassium palmitate, 1.5 mg of fatty acid-poor bovine serum albumin and 15.5 mM-sn-[³H]glycerol 3-phosphate (1 μ Ci/ μ mol). Reactions were started by the addition of 25–50 μ g of microsomal protein and after 7 min at 37°C were stopped with 1.88 ml of chloroform-methanol (1:2, v/v). The mixture was then acidified with 0.25 ml of 2 M-HCl before extraction of lipids (Hajra *et al.*, 1968).

Determination of sn-glycerol 3-phosphate esterification with palmitoyl-CoA generated from palmitoyl-($\pm$)-carnitine. Assays were performed and lipid classes were extracted as described above for sn-glycerol 3-phosphate esterification with palmitate. Each incubation mixture contained, in a final volume of 0.25 ml, 25 mM-Tris buffer adjusted to pH 7.4 with HCl, 5 mM-dithiothreitol, 50 μ M-CoA, 824 μ M-palmitoyl-($\pm$)-carnitine, 1.5 mg of fatty acid-poor bovine serum albumin, 15.5 mM-sn-[³H]glycerol 3-phosphate (1 μ Ci/ μ mol) and excess of carnitine palmitoyl-transferase (approx. 130 μ g of protein). Reactions were started with 25–50 μ g of microsomal protein, incubated at 37°C for 7 min and stopped with chloroform-methanol.

Determination of sn-glycerol 3-phosphate esterification with exogenous palmitoyl-CoA. Assays were performed and lipids extracted as described above

for sn-glycerol 3-phosphate esterification with palmitate. Each incubation mixture contained, in a final volume of 0.25 ml, 25 mM-Tris buffer adjusted to pH 7.4 with HCl, 5 mM-dithiothreitol, 26 μ M-palmitoyl-CoA and 2 mM-sn-[³H]glycerol 3-phosphate (4 μ Ci/ μ mol). Reactions were started with 50 μ g of microsomal protein and were for 4 min at 37°C.

Determination of protein. Protein was determined by a biuret method (Brindley & Hübscher, 1965).

Results

Comparison of the activities of palmitoyl-CoA synthetase and of esterification of sn-glycerol 3-phosphate with palmitate

To investigate the relationship between palmitate activation and its subsequent esterification to sn-glycerol 3-phosphate these activities were measured and compared in the intestinal mucosa of a series of male guinea pigs.

The specific activities of palmitoyl-CoA synthetase and for sn-glycerol 3-phosphate esterification are shown in Table 1. Analysis of the results with a paired *t* test showed that the activity of the synthetase was significantly greater ($P < 0.01$) than the rate of sn-glycerol 3-phosphate esterification with palmitoyl-CoA generated by the endogenous synthetase. However, the mean rate of palmitate activation was only approx. 1.3-fold the rate of esterification of sn-glycerol 3-phosphate from free palmitate. This latter activity was approx. 2.5-fold that when palmitoylcarnitine was used as acyl donor and 5.0-fold that when exogenous palmitoyl-CoA was used. The activities of glycerol phosphate acyltransferase determined by these last two methods underestimate the maximum activity that can be obtained when palmitate is activated by the endogenous synthetase. This demonstrates the problems encountered when attempting to compare specific activities that have inevitably to be assayed under slightly different conditions. Comparison of specific activities is an especially difficult task when the substrates of the reactions are lipids that also exhibit detergent properties. Because of this much of the comparison of different enzyme activities will be discussed below in terms of relative activities.

Johnston *et al.* (1967a) reported that palmitoyl-CoA could not act as a substrate for the glycerol phosphate acyltransferase of hamster intestinal mucosa. In the present experiments and those reported previously (Brindley & Ferrier, 1972) the specific activities obtained with exogenous palmitoyl-CoA and microsomal fractions of guinea-pig intestinal mucosa were low but could be used as a valid measure of acyltransferase activity. However, higher activities and more precise kinetics were obtained

Table 1. *Specific activities of the enzymes involved in palmitate activation and sn-glycerol 3-phosphate esterification by microsomal fractions of guinea-pig small-intestinal mucosa and composition of the products of the reactions*

The mean $\pm$ S.E.M. of enzyme activities are given as nmol of palmitate activated or esterified to *sn*-glycerol 3-phosphate/min per mg of microsomal protein. The means $\pm$ S.E.M. of the product composition are expressed in mol/100 mol. Numbers in parentheses indicate the number of guinea pigs used. The product composition of each experimental system was used to convert the results from *sn*-glycerol 3-phosphate into palmitate incorporations.

Enzyme system	Sp. activity	Product composition (mol/100 mol)		
		Lysophosphatidate	Phosphatidate	Neutral lipid
(1) Palmitoyl-CoA synthetase	65.6 $\pm$ 5.8 (18)	—	—	—
(2) Esterification of glycerol phosphate with				
(a) palmitoyl-CoA generated from palmitate	51.6 $\pm$ 7.1 (18)	18 $\pm$ 2 (18)	75 $\pm$ 2 (18)	7 $\pm$ 1 (18)
(b) palmitoyl-CoA generated from palmitoylcarnitine	21.0 $\pm$ 3.9 (15)	20 $\pm$ 2 (15)	71 $\pm$ 2 (15)	9 $\pm$ 2 (15)
(c) exogenous palmitoyl-CoA	10.2 $\pm$ 1.2 (4)	5 $\pm$ 2 (4)	85 $\pm$ 2 (4)	10 $\pm$ 5 (4)

when palmitoyl-CoA was generated from palmitoylcarnitine, and for these reasons this method has been preferred.

When the specific activities of *sn*-glycerol 3-phosphate esterification from free palmitate and from palmitoyl-CoA generated from palmitoylcarnitine were subjected to linear regression analysis (Fig. 1a) a positive correlation ($r = 0.83$, $P < 0.001$) was found. The intercept was not significantly different from zero. A similar comparison of palmitoyl-CoA synthetase with glycerolipid synthesis from either palmitate or palmitoylcarnitine (Figs. 1b and 1c) also showed significant correlations ($r = 0.87$, $P < 0.001$ and $r = 0.80$, $P < 0.001$ respectively). However, the intercepts on the palmitoyl-CoA synthetase axes were respectively 29.4 ± 6.1 (S.E. with 18 animals) and 37.3 ± 7.5 (S.E. with 15 animals). If it is valid to extrapolate to zero activity of the *sn*-glycerol 3-phosphate pathway then there is a significant ($P < 0.001$ when the hypothesis of a zero intercept is tested) activity of palmitoyl-CoA synthetase, which does not appear to be related to phosphatidate biosynthesis.

Table 1 shows that the complex lipids synthesized from *sn*-glycerol 3-phosphate in all three systems consisted mainly of phosphatidate plus lysophosphatidate. Only small quantities of neutral lipid were synthesized by isolated microsomal fractions, because the phosphatidate phosphohydrolase that is active in glycerolipid synthesis is recovered in the particle-free supernatant (Johnston *et al.*, 1967a; Smith *et al.*, 1967). The neutral lipid that was isolated in the present experiments consisted almost equally of di- and tri-glycerides.

The results shown in Fig. 1 demonstrate that the

activities of both palmitoyl-CoA synthetase and glycerol phosphate acyltransferase are correlated with the ability to synthesize phosphatidate from *sn*-glycerol 3-phosphate and from palmitate.

Effect of preincubation on the activation of palmitate and its subsequent esterification to sn-glycerol 3-phosphate

Earlier experiments (Brindley & Ferrier, 1972) showed that when microsomal fractions from small-intestinal mucosa were preincubated at 37°C for 50 min, both palmitoyl-CoA synthetase and the esterification of palmitate with *sn*-glycerol 3-phosphate were inhibited by 80–90%. In contrast the esterification of *sn*-glycerol 3-phosphate with exogenous palmitoyl-CoA (Brindley & Ferrier, 1972) and palmitoyl-CoA generated from palmitoylcarnitine (present study) was inhibited by less than 10%. The results indicate that the inability to synthesize phosphatidate after preincubation was caused by an inhibition of palmitoyl-CoA synthetase.

This conclusion is confirmed by the following observation. Additions of partially purified acyl-CoA synthetase (3–5 μ g of protein), which did not exhibit glycerol phosphate acyltransferase activity, to preincubated microsomal fractions almost completely restored glycerolipid synthesis to its original value.

In contrast, similar additions of synthetase to untreated microsomal fractions did not enhance the esterification of *sn*-glycerol 3-phosphate with palmitoyl-CoA generated from palmitate. This indicates that the untreated microsomal fraction does not contain an excess of glycerol phosphate acyltransferase activity. The combined results of these studies

Fig. 1. Relationship between the specific activities of palmitoyl-CoA synthetase and glycerolipid synthesis in the microsomal fraction of guinea-pig intestinal mucosa

The specific activities shown in Table 1 were plotted relative to each other and subjected to linear regression analysis (Sánchez *et al.*, 1973). The relationships between (a) glycerolipid synthesis from palmitate and palmitoylcarnitine, (b) palmitoyl-CoA synthetase and glycerolipid synthesis from palmitate and (c) palmitoyl-CoA synthetase and glycerolipid synthesis from palmitoylcarnitine are shown. Specific activities are expressed as nmol of palmitate activated or incorporated into glycerolipids/min per mg of microsomal protein.

show that the activities of palmitoyl-CoA synthetase and glycerol phosphate acyltransferase are finely balanced, and that either activity could easily become rate-limiting in phosphatidate biosynthesis.

Effect of KCl on palmitoyl-CoA synthetase and on the esterification of sn-glycerol 3-phosphate

Aas (1970) demonstrated that addition of KCl to the assay systems would stimulate the palmitoyl-CoA synthetase of rat liver. This phenomenon was confirmed in the present investigation although the optimum stimulation was only in the order of 25% with microsomal and mitochondrial fractions of rat liver. However, when microsomal fractions of guinea-pig intestinal mucosa were used, the activity of palmitoyl-CoA synthetase was inhibited (Fig. 2). This inhibition was accompanied by a similar inhibition of *sn*-glycerol 3-phosphate esterification with palmitoyl-CoA generated from palmitate. If the palmitoyl-CoA synthetase was bypassed by generating palmitoyl-CoA from palmitoylcarnitine or by adding exogenous palmitoyl-CoA, then KCl increased glycerolipid biosynthesis (Fig. 2) by stimulating glycerol phosphate acyltransferase.

The results shown in Fig. 2 indicate that palmitoyl-CoA synthetase can easily become the rate-limiting reaction of phosphatidate biosynthesis with the microsomal fraction of guinea-pig intestinal mucosa.

Effect of carnitine palmitoyltransferase and (–)-carnitine or palmitoyl(–)-carnitine on the incorporation of palmitate into glycerolipids

The palmitoyl-CoA pool produced by the endogenous synthetase can be decreased or increased by

Fig. 2. Effect of KCl on palmitate activation and *sn*-glycerol 3-phosphate esterification by the microsomal fraction of guinea-pig intestinal mucosa

Potassium chloride was added as indicated to the assays used to determine the activities of palmitoyl-CoA synthetase (●, guinea-pig no. 1; ○, guinea-pig no. 2) and glycerolipid synthesis from *sn*-glycerol 3-phosphate with palmitoyl-CoA generated from palmitate (▲, guinea-pig no. 1; △, guinea-pig no. 2) or from palmitoylcarnitine (■, guinea-pig no. 1) or with exogenous palmitoyl-CoA (□, guinea-pig no. 1; □, guinea-pig no. 2). Each enzyme activity is expressed as a percentage of its own activity measured in the absence of KCl.

adding carnitine palmitoyltransferase (130 μ g of protein) and either (–)-carnitine or palmitoyl(–)-carnitine respectively.

With four microsomal preparations out of seven, the addition of 150 μ M-palmitoyl(–)-carnitine produced a marginal stimulation (about 10%) in the rates of *sn*-glycerol 3-phosphate esterification. However, with three other microsomal preparations examined the addition of 50–200 μ M-palmitoyl(–)-carnitine did not significantly alter the rates of *sn*-glycerol 3-phosphate acylation. Concentrations of palmitoyl(–)-carnitine greater than 200 μ M inhibited glycerolipid biosynthesis in all preparations examined. This inhibition was probably caused by the detergent action of palmitoyl(–)-carnitine and also the excess of palmitoyl-CoA that was produced.

Since phosphatidate synthesis from palmitate was not stimulated by more than 10% when additional palmitoyl-CoA was generated from palmitoyl(–)-carnitine or with added exogenous acyl-CoA synthetase, the activity of the endogenous synthetase should be sufficient or almost sufficient to satisfy the maximum reaction velocities or *sn*-glycerol 3-phosphate and monoacyl-*sn*-glycerol 3-phosphate acyltransferases.

When [14 C]carnitine was added to the assays instead of palmitoyl(–)-carnitine some of the palmitoyl-CoA synthesized by the endogenous synthetase was converted into palmitoyl(–)-[14 C]-carnitine, which cannot be directly acylated with *sn*-glycerol 3-phosphate. The palmitoyl(–)-[14 C]-carnitine was measured by extraction into butanol (Sánchez *et al.*, 1973). However, the recovery of 3 H-labelled lipid in butanol was incomplete (see the Experimental section) and the acylation of *sn*-glycerol 3-phosphate was measured in parallel experiments in which non-radioactive carnitine was used and lipid was extracted into chloroform–methanol (Hajra *et al.*, 1968).

The quantity of palmitoyl(–)-carnitine produced was of the same order as the decrease in palmitate

esterified to *sn*-glycerol 3-phosphate (Table 2). These results confirm that a decreased net synthesis of palmitoyl-CoA is accompanied by a decreased esterification of palmitate to *sn*-glycerol 3-phosphate.

Effect of rate-limiting concentrations of ATP, palmitate, CoA and MgCl₂ on palmitoyl-CoA synthetase activity and glycerolipid biosynthesis

In these experiments, the concentrations of cofactors and substrates for palmitoyl-CoA synthetase were decreased so as to decrease the net synthesis of palmitoyl-CoA.

The curves obtained for synthetase activity and glycerolipid biosynthesis with rate-limiting concentrations of CoA and MgCl₂ are of a similar shape and very close together (Fig. 3). This would indicate that the synthetase is in fact the rate-determining step in phosphatidate biosynthesis. However, with the same microsomal preparations, limiting concentrations of ATP and palmitate (except with guinea-pig no. 5) produced significantly greater decreases in palmitoyl-CoA synthesis than in *sn*-glycerol 3-phosphate esterification.

The results of glycerolipid biosynthesis were measured in terms of the incorporation of *sn*-[3 H]-glycerol 3-phosphate into lipids. However, as discussed in the preceding paper (Sánchez *et al.*, 1973), differences in the proportion of mono-, di- and tri-acyl-lipids could not explain the differences in the relative positions of the curves for palmitate activation and esterification to *sn*-glycerol 3-phosphate with different limiting cofactors and substrates.

The differences in the relative positions of these two curves might be explained for limiting concentrations of palmitate (guinea-pigs nos. 3 and 4) if endogenous fatty acids were both activated and incorporated into glycerolipids during the incubations. It is possible that the specificity of the acyltransferase reactions might have favoured the esterification of

Table 2. *Effect of adding carnitine palmitoyltransferase and (–)-carnitine on the synthesis of glycerolipids from sn-glycerol 3-phosphate and palmitate*

Partially purified carnitine palmitoyltransferase (130 μ g of protein) and various concentrations of (–)-[14 C]-carnitine (0.12 μ Ci/ μ mol) were added to the assay system used for measuring the esterification of *sn*-glycerol 3-phosphate from free palmitate (see the Experimental section). Specific activities are expressed as nmol of palmitate incorporated/min per mg of microsomal protein.

Concn. of (–)-carnitine (mM)	Incorporation of palmitate	
	Into palmitoylcarnitine	Into glycerolipid
0	0	80.3
0.8	16.3	61.1
1.6	16.8	59.8

Fig. 3. Effect of rate-limiting concentrations of ATP, palmitate, CoA and $MgCl_2$ on palmitoyl-CoA synthetase and on the synthesis of glycerolipids from *sn*-glycerol 3-phosphate with palmitoyl-CoA generated from palmitate

The concentrations of (a) ATP, (b) palmitate, (c) CoA and (d) $MgCl_2$ were varied while the concentrations of other cofactors and substrates were kept at optimum concentrations. Palmitoyl-CoA synthetase (●, guinea-pig no. 3; ○, guinea-pig no. 4; ○, guinea-pig no. 5) and glycerolipid synthesis from palmitate (▲, guinea-pig no. 3; ▲, guinea-pig no. 4; ▲, guinea-pig no. 5) were measured and the results expressed relative to the maximum velocity for the appropriate activity. Maximum velocities were 62.3, 54.8 and 63.0 nmol of palmitate activated/min per mg of microsomal protein and 26.5, 32.2 and 36.2 nmol of palmitate incorporated into glycerolipids/min per mg of microsomal protein for guinea-pigs nos. 3, 4 and 5 respectively.

these acids to *sn*-glycerol 3-phosphate relative to the ability of the palmitoyl-CoA synthetase to activate them. Despite these difficulties of interpretation the results do indicate that decreased synthesis of pal-

mitoyl-CoA readily leads to a lowered esterification of *sn*-glycerol 3-phosphate. This was most pronounced when limiting concentrations of CoA and $MgCl_2$ were employed.

Table 3. *Effect of rac-1-monopalmitin on the esterification of palmitate with sn-glycerol 3-phosphate*

The assay system was that used to measure *sn*-glycerol 3-phosphate esterification with palmitate (see the Experimental section) except that [$1\text{-}^{14}\text{C}$]palmitate ($0.5\text{ }\mu\text{Ci}/\mu\text{mol}$) was added to measure palmitate esterification with either *sn*-glycerol 3-phosphate or *rac*-1-monopalmitin. Phospholipids and glycerides were separated with silicic acid and alumina column chromatography (Mitchell *et al.*, 1971). Specific activities are expressed as nmol of substrate incorporated into complex lipids/min per mg of microsomal protein.

Concn. of <i>rac</i> -1-monopalmitin (mM)	Incorporation into phospholipids		Incorporation into glycerides	
	Glycerol phosphate	Palmitate	Glycerol phosphate	Palmitate
0	15.9	31.9	1.6	5.8
0.8	16.7	30.2	1.3	9.8
2.4	16.4	30.0	0.7	11.7

Effect of rac-1-monopalmitin on the rate of glycerolipid synthesis from sn-glycerol 3-phosphate

rac-1-Monopalmitin was added in the form of an emulsion (Brindley & Ferrier, 1972) to the assay system used for measuring *sn*-glycerol 3-phosphate esterification from palmitate, to divert some of the palmitoyl-CoA away from phosphatidate biosynthesis by providing substrate for the monoglyceride pathway.

The results are shown in Table 3. Addition of 0.8 mM- and 2.4 mM-*rac*-1-monopalmitin resulted in an increased incorporation into glycerides of 4.0 and 5.9 nmol of palmitate/min per mg of microsomal protein respectively. The increased incorporation of palmitate into glycerides was caused by the enzymes of the monoglyceride pathway, since the incorporation of *sn*-glycerol 3-phosphate into glycerides showed a slight decrease. The increased utilization of palmitoyl-CoA by the monoglyceride pathway did not lead to a significant decrease in incorporation of palmitate or *sn*-glycerol 3-phosphate into phospholipids by the glycerol phosphate pathway. This was true even when the emulsion concentration of *rac*-1-monopalmitin was raised to 5.2 mM. The total incorporation of palmitate into glycerolipids increased from 37.7 to 41.7 nmol/min per mg of microsomal protein after the addition of 2.4 mM-*rac*-1-monopalmitin, whereas the maximum rate of palmitoyl-CoA synthesis was 45.1 nmol/min per mg of microsomal protein.

Under these conditions it therefore seems that the rate of palmitoyl-CoA synthesis was adequate to satisfy the maximum rates of palmitate esterification with *sn*-glycerol 3-phosphate even when optimum concentrations of *rac*-1-monopalmitin were used to provide a substrate for the enzymes of the monoglyceride pathway.

Discussion

Studies were undertaken in which the specific activities of palmitoyl-CoA synthetase and *sn*-

glycerol 3-phosphate esterification were measured in the microsomal fraction of guinea-pig intestinal mucosa. On average 78% of the palmitoyl-CoA produced by the synthetase could be esterified to *sn*-glycerol 3-phosphate to form mainly phosphatidate (Table 1). In contrast, rates of palmitoyl-CoA production in rat liver mitochondria (Sánchez *et al.*, 1973) and microsomal fractions (Lloyd-Davies & Brindley, 1973) were respectively five- and four-fold the rates of esterification. With the mucosal preparations there were significant correlations ($P < 0.001$) between the specific activities of palmitoyl-CoA synthetase, glycerol phosphate acyltransferase and the esterification of *sn*-glycerol 3-phosphate with palmitoyl-CoA generated by the endogenous synthetase (Fig. 1). However, if the line for the esterification of *sn*-glycerol 3-phosphate from either palmitate or palmitoylcarnitine was extrapolated to zero, then there was a significant ($P < 0.001$) synthetase activity remaining, which did not appear to be related to the activity of the glycerol phosphate pathway. If this extrapolation is valid then the remaining synthetase activity might be that concerned with the synthesis of activated fatty acids necessary for the activities of the monoglyceride pathway, or for fatty acid oxidation, desaturation and elongation.

The specific activities shown in Fig. 1 vary considerably from one animal to another. A small variation might have been caused if the purification of the microsomal fractions varied in different preparations (Hübscher *et al.*, 1965), but the observed ranges of specific activity are greater than could be accounted for in this way. The differences in specific activity probably reflect the different physiological states of the guinea pigs used in these experiments. It is already known that starvation (McManus & Isselbacher, 1970), a high-fat diet (Hoving & Valkema, 1969) and adrenalectomy (Isselbacher, 1965) alter the activities of the enzymes concerned in intestinal glycerolipid biosynthesis. In addition removal of the jejunum increased the activity of the enzymes of fatty acid esterification in the ileum (Rodgers & Bochenek,

1970). The results shown in Fig. 1 demonstrate that the activities of palmitoyl-CoA synthetase and glycerol phosphate acyltransferase in our experiments alter in parallel.

The results shown in Table 1 and Fig. 1 suggest that palmitoyl-CoA synthetase activity might be rate-limiting in phosphatidate synthesis, since the rates of activation were only marginally greater than the rates of esterification. Further evidence to support this hypothesis was provided by the kinetic experiments where the production of palmitoyl-CoA was impaired by preincubation, addition of KCl, diversion of palmitoyl-CoA to palmitoylcarnitine or by employing rate-limiting concentrations of Mg^{2+} or CoA. The decreased palmitoyl-CoA synthesis was paralleled by a decrease in phosphatidate synthesis with palmitoyl-CoA generated by the endogenous synthetase as suggested by Brindley & Ferrier (1972).

There were, however, situations in which the palmitoyl-CoA pool produced by the synthetase was decreased without similar decreases in phosphatidate synthesis being observed. When *rac*-1-monopalmitin was added to divert palmitoyl-CoA to the monoglyceride pathway, no inhibition of *sn*-glycerol 3-phosphate esterification was observed (Table 3). It is possible that the palmitoyl-CoA utilized by the monoglyceride and glycerol phosphate pathways is produced by different acyl-CoA synthetases. For instance, when the enzyme complex that catalyses the esterification of monoglycerides in hamster intestinal mucosa is purified 70-fold, it retains acyl-CoA synthetase activity (Rao & Johnston, 1966). Also, although the enzymes of the monoglyceride and glycerol phosphate pathways are isolated in the same microsomal fractions of cat intestinal mucosa (Brindley & Hübscher, 1965), histochemical evidence suggests that the subcellular sites of the two pathways are different (Higgins & Barnett, 1971). The enzymes of the monoglyceride pathway were found mainly in the smooth endoplasmic reticulum whereas those of the glycerol phosphate pathway were located in the rough endoplasmic reticulum. In addition, the reaction sequence of both pathways involves conversion of diglyceride into triglyceride. However, the two pools of diglyceride are not interchangeable (Johnston *et al.*, 1967*b*, 1970) and there thus appear to be two different diglyceride acyltransferases (EC 2.3.1.20).

The lack of inhibition of phosphatidate synthesis by monoglycerides might have been expected, since during fat absorption the intestinal cells would need to synthesize efficiently and simultaneously glycerolipids through the monoglyceride and glycerol phosphate pathways. In contrast to this, 2-monoethers, which have been used as analogues of monoglycerides, inhibited the synthesis of phosphatidate from either palmitate or palmitoyl-CoA in adipose tissue (Schultz *et al.*, 1971). It was postulated that the monoglyceride

produced by the hormone-sensitive lipase could inhibit *sn*-glycerol 3-phosphate esterification and thus ensure the release of fatty acids from adipose tissue. The synthesis of glycerides by the glycerol phosphate pathway in this tissue would therefore not occur simultaneously with the accumulation of monoglycerides (Schultz *et al.*, 1971).

The other situation in which a decreased production of palmitoyl-CoA did not lead to an immediate decrease in phosphatidate synthesis was obtained when rate-limiting concentrations of ATP and palmitate were employed (Fig. 3). If there was one pool of palmitoyl-CoA in the microsomal system then one would expect an equal decrease in phosphatidate biosynthesis when the palmitoyl-CoA production was decreased by limiting ATP, CoA, Mg^{2+} or palmitate. This was not observed with the microsomal fraction of guinea-pig intestinal mucosa (Fig. 3), or the mitochondrial (Sánchez *et al.*, 1973) and microsomal fractions (Lloyd-Davies & Brindley, 1973) of rat liver. One explanation of these discrepancies might be that there are different palmitoyl-CoA synthetases with different K_m values for cofactors and substrates. The multiple forms of the synthetase might have different abilities to donate activated fatty acids to the enzymes of the glycerol phosphate pathway. More direct evidence for the existence of multiple forms of palmitoyl-CoA synthetase is provided by the work of Bar-Tana *et al.* (1972).

If the different enzymes that catalyse palmitoyl-CoA synthesis were to possess different metabolic functions, there is no evidence to suggest that acyl-CoA has to be generated in the immediate vicinity of the glycerol phosphate acyltransferase. Palmitoyl-CoA generated by partially purified acyl-CoA synthetase or by carnitine palmitoyltransferase in addition to exogenous palmitoyl-CoA could be used as substrates for phosphatidate biosynthesis. Conversely it is assumed that carnitine palmitoyltransferase can efficiently accept the palmitoyl-CoA produced by the microsomal synthetase.

The kinetic results discussed so far indicate that palmitoyl-CoA production can easily become rate-limiting in phosphatidate synthesis by the microsomal fraction of intestinal mucosa. The question arose as to whether *sn*-glycerol 3-phosphate acyltransferase activity was greatly in excess of the rate of palmitoyl-CoA synthesis. When the latter rate was increased by adding partially purified acyl-CoA synthetase or by generating additional palmitoyl-CoA from palmitoylcarnitine, there was no significant increase in phosphatidate biosynthesis with microsomal fractions that had not been preincubated. This indicated that the utilization of palmitoyl-CoA for phosphatidate biosynthesis approximately balances the ability of the palmitoyl-CoA synthetase to donate activated fatty acid.

The relationship between palmitoyl-CoA produc-

tion and the subsequent esterification of *sn*-glycerol 3-phosphate in the microsomal fraction of guinea-pig intestinal mucosa differs from that in the mitochondria of rat liver (Sánchez *et al.*, 1973). In the latter organelle the rate of palmitoyl-CoA synthesis was significantly in excess of the rate of palmitate esterification to *sn*-glycerol 3-phosphate. A similarly large excess in the total production of palmitoyl-CoA over esterification was observed with the microsomal fraction of rat liver, but there were indications that under certain conditions palmitoyl-CoA synthesis could become rate-limiting in phosphatidate synthesis (Lloyd-Davies & Brindley, 1973). Taken together these observations show that the relationship between palmitoyl-CoA synthetase and *sn*-glycerol 3-phosphate esterification might be different in different organelles from the same tissue, in different organs, and possibly in the same organs of different animals.

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