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The biosynthesis of triacylglycerols in microsomal preparations of developing cotyledons of sunflower (*Helianthus annuus* L.)

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The synthesis of triacylglycerols was investigated in microsomes (microsomal fractions) prepared from the developing cotyledons of sunflower (*Helianthus annuus*). Particular emphasis was placed on the mechanisms involved in controlling the C_{18}-unsaturated-fatty-acid content of the oils. We have demonstrated that the microsomes were capable of: (1) the transfer of oleate from acyl-CoA to position 2 of *sn*-phosphatidylcholine for its subsequent desaturation and the return of the polyunsaturated products to the acyl-CoA pool by further acyl exchange; (2) the acylation of *sn*-glycerol 3-phosphate with acyl-CoA to yield phosphatidic acid, which was further utilized in diacyl- and tri-acylglycerol synthesis; and (3) the equilibrium of a diacylglycerol pool with phosphatidylcholine. The acyl exchange between acyl-CoA and position 2 of *sn*-phosphatidylcholine coupled to the equilibration of diacylglycerol and phosphatidylcholine brings about the continuous enrichment of the glycerol backbone with C_{18} polyunsaturated fatty acids for triacylglycerol production. Similar reactions were found to operate in another oilseed plant, safflower (*Carthamus tinctorius* L.). On the other hand, the microsomes of avocado (*Persea americana*) mesocarp, which synthesize triacylglycerol via the Kennedy [(1961) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **20**, 934–940] pathway, were deficient in acyl exchange and the diacylglycerol⇔phosphatidylcholine interconversion. The results provide a working model that helps to explain the relationship between C_{18}-unsaturated-fatty-acid synthesis and triacylglycerol production in oilseeds.

The triacylglycerols that accumulate in the developing seeds of certain species are important in providing vegetable oils for nutritional and industrial needs. Of particular importance are those oils rich in the C_{18} polyunsaturated fatty acid linoleic acid (*cis*, *cis*-$\Delta^{9,12}$-18:2). It is now well-established that linoleic acid is synthesized via the desaturation of oleic acid (*cis*-Δ^{9}-18:1) in the microsomal phosphatidylcholine in developing oilseeds (Stymne & Appelqvist, 1978; Slack *et al.*, 1979; Stymne, 1980) rather than the direct desaturation of an acyl-CoA substrate. We have previously provided evidence (Stymne & Glad, 1981) for a mechanism, in the microsomes (microsomal fractions) of developing oilseeds, which accounts for the transfer of oleate from acyl-CoA to position 2 of *sn*-phosphatidylcholine for the subsequent desaturation to linoleate. The mechanism also provides for the return of the polyunsaturated-fatty-acid products to the acyl-CoA pool. The linoleoyl-CoA so produced is then available for phosphatidic acid synthesis and triacylglycerol production by the reactions outlined by Kennedy (1961) for animal systems. We have shown that the quality of C_{18} fatty acids in the acyl-CoA pool is controlled by the acyl exchange between acyl-CoA and phosphatidylcholine and that this governs to a large extent the acyl components in the synthesized oils (Stymne *et al.*, 1983; Stobart *et al.*, 1983). These suggestions are slightly contradictory to the proposals for triacylglycerol synthesis put forward by Roughan & Slack (1982). The work presented here investigates triacylglycerol synthesis in the microsomes of developing cotyledons of sunflower (*Helianthus annuus* L.) and provides evidence which helps to integrate the schemes of Roughan & Slack (1982) and our own proposals.

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Materials and methods

Chemicals

1-^{14}C-labelled fatty acids, L-*sn*-[U-^{14}C]glycerol 3-phosphate and [γ-^{32}P]ATP were obtained from the Radiochemical Centre, Amersham, Bucks., U.K. Bovine serum albumin (fraction V, fatty-acid-free), CoASH, NADH, ATP, L-*sn*-glycerol 3-phosphate, glycerol kinase (EC 2.7.1.30), phospholipase A_2 [from Indian-cobra (*Naja naja*) venom] and various fatty acids were purchased from Sigma. SEP-PAK C_{18} cartridges were from Waters Associates, Framingham, MA, U.S.A.

Heptadecanoyl-CoA (17:0-CoA); [1-^{14}C] oleoyl (Δ^9-18:1)-CoA (sp. radioactivity 2580d.p.m./nmol), [1-^{14}C]linoleoyl($\Delta^{9,12}$-18:2)-CoA (sp. radioactivity 2390d.p.m./nmol), [1-^{14}C]linolenoyl-($\Delta^{9,12,15}$-18:3)-CoA (sp. radioactivity 3410 d.p.m./nmol) and corresponding unlabelled acyl-CoA species were synthesized from their mixed anhydrides as described by Sanchez *et al.* (1973). *sn*-[^{32}P]Glycerol 3-phosphate was synthesized by incubation of 1.5μmol of [γ-^{32}P]ATP (100μCi) with 10μmol of glycerol and 10 units of glycerol kinase in 0.25ml of 0.1M-glycine/HCl buffer, pH9.8, at 37°C for 1h. The incubation mixture was then applied to a precoated cellulose t.l.c. plate (Merck; 0.1mm thickness) and glycerol 3-phosphate separated with ethanol/ammonium acetate (14:9, v/v), pH7.8. Reference glycerol 3-phosphate was detected with molybdate reagent and the corresponding sample area removed. The *sn*-[^{32}P]glycerol 3-phosphate was eluted from the gel with water. Before use, the *sn*-[^{32}P]glycerol 3-phosphate was mixed with unlabelled *sn*-glycerol 3-phosphate to give a sp. radioactivity of 15000d.p.m./nmol.

Plant material

Sunflower (*Helianthus annuus* L.) plants, varieties Bolero and Flambeau, were grown outdoors and the seeds were harvested 18–20 days after flowering. Safflower (*Carthamus tinctorius* L., var. Gila) plants were grown in a 16h photoperiod at 25°C and an 8h night at 15°C and the seeds were harvested 14–18 days after flowering. Avocados (*Persea americana*) were purchased locally.

Preparation of microsomes

The cotyledons from the seeds of sunflower and safflower were carefully removed and stored on ice. All further manipulations were carried out at 1–4°C. The cotyledons were ground in a mortar with two parts (w/v) of 0.1M-potassium phosphate, pH7.2, containing 0.1% bovine serum albumin and 0.33M-sucrose. The homogenate was filtered through a double layer of Miracloth, diluted five times with fresh grinding medium and centrifuged at 18000*g* for 20min. The supernatant was then filtered through Miracloth and centrifuged at 105000*g* for 90min. The resulting microsomal pellet was resuspended in 0.1M-phosphate buffer and stored at −70°C until used.

A microsomal fraction from avocado mesocarp was prepared by the same procedure as above, except that the mesocarp was homogenized in a Waring Blendor.

Enzyme assays

All assays were done at 30°C with constant shaking. Incubation mixtures contained 10mg of bovine serum albumin, substrates and cofactors at the concentrations stated in the Tables and Figures, and the microsomal preparation, adjusted to a final volume of 1ml with 0.1M-phosphate buffer. Acyl-CoA was added to the incubation mixture as an approx. 4mM aqueous solution.

Analytical procedures

Reactions were terminated by the addition of 3ml of 0.15M-acetic acid and the lipids extracted in a modification of the medium described by Bligh & Dyer (1959). The lower chloroform phase, which contains the complex lipids and free fatty acids, was removed and evaporated to dryness under N_2. The residue was redissolved in a small volume of chloroform and the polar and neutral lipids were purified by t.l.c. on precoated silica-gel plates (Merck; silica-gel 60) with chloroform/methanol/acetic acid/water (170:30:20:7, by vol.) or hexane/diethyl ether/acetic acid (70:30:1, by vol.) respectively. Lipid areas, located by lightly staining with I_2 vapour, were removed from the plates and either assayed for radioactivity or methylated *in situ* with methanolic 2.5% (v/v) HCl (Kates, 1964) for the analysis of fatty acids. The fatty acid methyl esters were analysed by g.l.c. and quantified by using methylheptadecanoic acid as an internal standard or analysed by radio-g.l.c. (Stymne & Appelqvist, 1978) for the determination of radioactivity in the fatty acids.

The acyl-CoA, which remains in the upper phase (methanol/water) after the Bligh & Dyer (1959) extraction, was purified by reversed-phase column chromatography on SEP-PAK C_{18}–silica-gel prepacked cartridges as previously described (Stymne & Glad, 1981; Stymne *et al.*, 1983). The acyl-CoA was transmethylated with sodium methoxide (Slack *et al.*, 1976) and identified and quantified, relative to heptadecanoyl-CoA (50nmol of which were added as an internal standard to the incubation mixtures just before extraction), by g.l.c. of the fatty acid methyl esters.

Positional analysis of the fatty acids in phosphatidylcholine was performed by treatment of the lipid with phospholipase A_2 from *Naja naja* as des-

cribed previously (Stymne *et al.*, 1983). After extracting the lipids in the medium described by Bligh & Dyer (1959) and separating out the lysophosphatidylcholine and the free fatty acids in the chloroform phase in the t.l.c. system for polar lipids (see above), the lysophosphatidylcholine and free fatty acids were removed and either assayed for radioactivity or methylated with methanolic HCl (Kates, 1964) for analysis by g.l.c.

Lipid samples were assayed for radioactivity in PCS (Amersham/Searle)/xylene (2:1, v/v) scintillant in a Beckman LS-230 liquid-scintillation counter with an efficiency of 98% for ^{14}C and 99% for ^{32}P. All radioactivity counts were corrected for background and quenching.

Results

Acyl exchange

Microsomes from the developing cotyledons of sunflower were tested for their ability to carry out acyl exchange between acyl-CoA and position 2 of *sn*-phosphatidylcholine. The microsomes (equivalent to 109nmol of phosphatidylcholine) were incubated with [^{14}C]oleoyl-CoA (200nmol) and at zero time and after 90min the [^{14}C]oleate incorporated into phosphatidylcholine and the mass of fatty acids in acyl-CoA and phosphatidylcholine were determined. The results (Table 1) show that some 60nmol of [^{14}C]oleate had entered phosphatidylcholine, of which 93.5% was in position 2. The mass of the oleate in phosphatidylcholine had increased by 33nmol, with a concomitant decrease of almost 30nmol in linoleate. The linoleate exchanged in the phosphatidylcholine was fully accounted for by the mass of linoleate in the acyl-CoA. Since 35% of the fatty acids in position 2 of *sn*-phosphatidylcholine consisted of oleate in these microsomes, a considerable exchange between oleoyl-CoA and oleoyl phosphatidylcholine must also have occurred. Microsomes from *H. annuus* (var. Bolero) were incubated with [^{14}C]oleoyl-CoA and NADH to test their ability to desaturate oleate to linoleate. After 60min., 66% of the oleate had been converted into linoleate in phosphatidylcholine (results not shown).

The oleate entering phosphatidylcholine from acyl-CoA by the mechanism of acyl exchange can fully account, therefore, for the observed incorporation of radioactivity and mass changes in the fatty acids in phosphatidylcholine and acyl-CoA. The oleate entering the phosphatidylcholine by acyl exchange was shown to be available for the synthesis of linoleate in the presence of NADH.

Utilization of linoleate and glycerol 3-phosphate

We have previously shown (Stymne *et al.*, 1983) that microsomal preparations of safflower will synthesize phosphatidate, diacylglycerol and triacylglycerol from [^{14}C]acyl-CoA and *sn*-glycerol 3-phosphate. Similar experiments were carried out with the sunflower preparations to assess their capacity for triacylglycerol synthesis. Microsomal preparations (equivalent to 153nmol of phosphatidylcholine) were incubated with [^{14}C]linoleoyl-CoA (200nmol) and *sn*-glycerol 3-phosphate (200nmol). At regular intervals the mass of [^{14}C]linoleate incorporated into the various lipids was determined. The results (Fig. 1) show that there was initially a rapid incorporation of linoleate into phosphatidylcholine, which reached a maximum after 40min incubation and then remained constant. Radioactivity also appeared in phosphatidic acid, and this continued to accumulate for 10min, then declined. The synthesis of triacylglycerol commenced slightly later than phosphatidic acid production and was almost linear for 40min incubation, after which the rate of [^{14}C]linoleate incorporation slightly decreased. The activity in diacylglycerol also increased linearly for 20min, then remained almost constant.

The results indicate that the microsomal preparations of sunflower were capable of an efficient synthesis of triacylglycerol by the initial acylation of *sn*-glycerol 3-phosphate to yield phosphatidic acid and the subsequent production of diacylgly-

Table 1. *'Acyl-exchange' between oleoyl-CoA and phosphatidylcholine in microsomes of developing sunflower (var. Flambeau) cotyledons*

Microsomes, eqivalent to 109nmol of phosphatidylcholine, were incubated with [^{14}C]oleoyl-CoA (200nmol) under non-desaturating conditions (i.e. minus NADH). At zero time and after 90min the mass of the fatty acids in phosphatidylcholine and acyl-CoA were determined. The data for the 90min incubation are given as mean values (±S.D.) for two incubations. For experimental details, see the Materials and methods section.

		Mass of fatty acids (nmol) in:					
		Phosphatidylcholine		Acyl-CoA			
Incubation time (min)	[^{14}C]Oleate in phosphatidylcholine (nmol)	Oleate	Linoleate	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
0*	1.2	50.4	109.8	2.8	0.9	211	6.4
90	64.6±1.6	83.5±3.5	79.6±3.0	2.5±0.2	1.6±0.3	130±1	42.2±0.2

* About 5s.

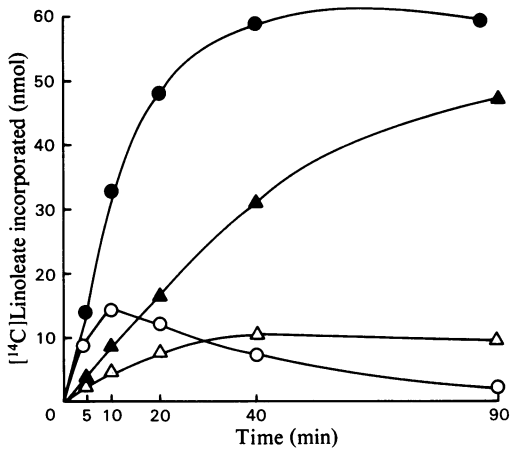


Fig. 1. *Incorporation of* [^{14}C]*linoleate from* [^{14}C]*linoleoyl-CoA into various lipids in the presence of sn-glycerol 3-phosphate in microsomes of developing sunflower (var. Flambeau) cotyledons*

Microsomes, equivalent to 153nmol of phosphatidylcholine, were incubated with [^{14}C]linoleoyl-CoA (200nmol) in the presence of glycerol 3-phosphate (200nmol). The radioactivity incorporated into various lipids was determined at regular intervals. For experimental details, see the Materials and methods section. ●, Phosphatidylcholine; ○, phosphatidic acid; △, diacylglycerols; ▲, triacylglycerols.

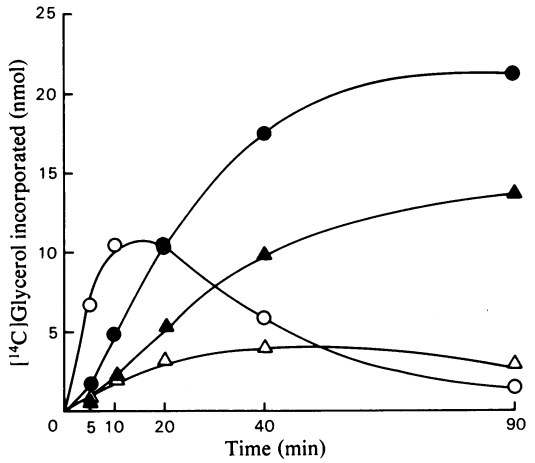


Fig. 2. *Incorporation of* [^{14}C]*glycerol from* [^{14}C]*glycerol 3-phosphate into various lipids in the presence of linoleoyl-CoA in microsomes of developing sunflower (var. Flambeau) cotyledons*

Microsomes, equivalent to 153nmol of phosphatidylcholine, were incubated with [^{14}C]glycerol 3-phosphate (200nmol) in the presence of linoleoyl-CoA (200nmol). The radioactivity incorporated into the various lipids was determined at regular intervals. For experimental details, see the Materials and methods section. ●, Phosphatidylcholine; ○, phosphatidic acid; △, diacylglycerols; ▲, triacylglycerols.

cerol. The rapid incorporation of linoleate in phosphatidylcholine could be accounted for by the acyl exchange that occurs between the acyl-CoA and phosphatidylcholine and that will utilize linoleic and linolenic acids.

To confirm these interpretations further, experiments were carried out with *sn*-[^{14}C]glycerol 3-phosphate and non-radioactive acyl-CoA at the same concentrations and conditions as in the previous experiment. The results (Fig. 2) show that radioactivity first appeared in phosphatidic acid and that this reached a maximum in 10–20min and then decreased. After a slight delay, however, glycerol was incorporated in phosphatidylcholine, and this continued to accumulate radioactivity throughout the incubation period. After a further delay, radioactivity in triacylglycerol appeared and was then incorporated at an almost linear rate for 40min.

It is noteworthy that the incorporation of [^{14}C]glycerol into phosphatidylcholine appeared after that in phosphatidic acid, the reverse of the situation when [^{14}C]linoleate was used as the radioactive substrate.

The two experiments with [^{14}C]linoleoyl-CoA and *sn*-[^{14}C]glycerol 3-phosphate respectively can be compared directly, since the same microsomal preparation and substrates were used. Table 2 gives the ratio of [^{14}C]linoleate to [^{14}C]glycerol in the various lipids throughout the incubation period. The ratio remained constant at all times in phosphatidic acid and diacylglycerol, being about 1.3 and 2.5 respectively. The ratio in triacylglycerol, on the other hand, was high in the short-term incubations and then rapidly decreased to about 3.4 after 10min incubation. The ratio in phosphatidylcholine, however, exhibited a most pronounced change, being initially high after 5min incubation and then showing a progressive decline throughout the remainder of the experiment.

The relatively high linoleate/glycerol ratio in phophatidylcholine in the early periods of incubation suggests that the predominant label entering phosphatidylcholine is from linoleate via acyl exchange. This is further verified by the rapid incorporation of linoleate into phosphatidylcholine (before phosphatidic acid; see Fig. 1) in the early periods of incubation.

However, with increasing incubation times the contribution to phosphatidylcholine via [^{14}C]glycerol markedly increases, and the linoleate: glycerol ratio appreciably declines. The high ratio

Table 2. *Ratios of [^{14}C]linoleate to [^{14}C]glycerol in various lipids after incubating microsomes from developing sunflower (var. Flambeau) cotyledons with [^{14}C]linoleoyl-CoA and [^{14}C]glycerol 3-phosphate*

The ratios in the various lipids are calculated from the amounts of [^{14}C]linoleate and [^{14}C]glycerol obtained in the experiments presented in Figs. 1 and 2 respectively. For experimental details, see the legends to Figs. 1 and 2 and the Materials and methods section.

Incubation time (min)	[^{14}C]Linoleate/[^{14}C]glycerol ratio in:			
	Phosphatidylcholine	Phosphatidic acid	Diacylglycerols	Triacylglycerols
5	8.7	1.3	2.6	14.0
10	6.8	1.3	2.3	4.0
20	4.6	1.2	2.4	3.2
40	3.3	1.2	2.8	3.4
90	2.8	1.5	2.8	3.4

Table 3. *Incorporation of ^{32}P into lipids in microsomes from developing sunflower (var. Flambeau) cotyledons incubated with sn-glycerol 3-[^{32}P]phosphate and linoleoyl-CoA*

Microsomes, equivalent to 153nmol of phosphatidylcholine, were incubated with glycerol 3-[^{32}P]phosphate (200nmol $\equiv 3 \times 10^6$ d.p.m.) and 200nmol of unlabelled linoleoyl-CoA. At regular intervals the radioactivity in phosphatidylcholine, phosphatidic acid and other chloroform-soluble compounds was determined. For experimental details, see the Materials and methods section.

Incubation time (min)	Glycerol 3-[^{32}P]phosphate (nmol) in:		
	Phosphatidylcholine	Phosphatidic acid	Other chloroform-soluble compounds
5	0.1	8.4	0.9
10	0.0	11.7	0.8
20	0.0	10.8	1.0
40	0.0	6.6	0.6
90	0.0	1.7	0.1

observed in triacylglycerol at the early incubation time reflects the initial acylation of an active, pre-existing, pool of diacylglycerol.

Similar experiments were carried out with microsomal preparations from developing safflower seeds (var. Gila, a high-linoleate variety) and avocado mesocarp (rich in oleate). The results for safflower (results not shown) were almost identical with those obtained for the sunflower microsomal preparations. On the other hand, the avocado microsomal preparations, although capable of high rates of triacylglycerol synthesis, incorporated very little, if any, glycerol into phosphatidylcholine in the presence of linoleoyl-CoA (results not shown). It is also noteworthy that we have been unable to demonstrate acyl exchange between acyl-CoA and position 2 of *sn*-phosphatidylcholine in avocado preparations, unlike the situation in the microsomes from the developing oilseeds.

Time course of sn-glycerol 3-[^{32}P]phosphate incorporation into lipids

The experiments on the incorporation of *sn*-[^{14}C]glycerol 3-phosphate into phosphatidylcholine by the microsomal preparations leave it unclear as to the direct lipid precursor of the phosphatidylcholine. Numerous time-course experiments have been performed, and it proved impossible to discern whether the radioactivity in phosphatidylcholine arose from phosphatidic acid or diacylglycerol. To clarify the situation, microsomal preparations were incubated with ^{32}P-labelled *sn*-glycerol 3-phosphate. The *sn*-glycerol 3-[^{32}P]phosphate was synthesized and purified as given in the Materials and methods section. Microsomal preparations (equivalent to 153nmol of phosphatidylcholine) were incubated with 200nmol of linoleoyl-CoA and 200nmol of *sn*-[^{32}P]glycerol 3-phosphate of known specific radioactivity. At regular time intervals the radioactivity in phosphatidylcholine and phosphatidic acid was determined. The results (Table 3) show that the *sn*-glycerol 3-[^{32}P]phosphate skeleton was incorporated intact in phosphatidic acid and this reached a maximum within 10–20min incubation and thereafter substantially declined. The incorporation of ^{32}P into phosphatidylcholine, on the other hand, was negligible throughout the experiment. A small incorporation of radioactivity was recovered in the remaining lipids and this remained at a similar level during the incubation.

The results indicate that phosphatidic acid is

synthesized directly by the acylation of *sn*-glycerol 3-phosphate, from which diacylglycerol is produced by the action of a phosphatidate phosphatase. The incorporation of glycerol into phosphatidylcholine, therefore, appears to arise directly from diacylglycerol.

Effect of sn-glycerol 3-phosphate on acyl exchange

In previous experiments with safflower microsomes (Stymne *et al.*, 1983) we observed that the incorporation of ^{14}C-labelled fatty acids from acyl-CoA into phosphatidylcholine appeared to be stimulated by *sn*-glycerol 3-phosphate. In the light of our findings on the back-transfer of diacylglycerol to phosphatidylcholine, this was re-examined with the sunflower microsomal preparations. Microsomes were incubated for short periods with [^{14}C]oleoyl-CoA with or without *sn*-glycerol 3-phosphate, and after 5 and 10min the incorporation into phosphatidylcholine was determined. The results (Table 4) show that a small quantity of glycerol was incorporated into phosphatidylcholine after 5min, and this increased 4-fold after a further 5min. The incorporation of [^{14}C]oleate, from [^{14}C]oleoyl-CoA, into phosphatidylcholine occurred in the absence of *sn*-glycerol 3-phosphate, and this increased by 40 and 100% in the presence of *sn*-glycerol 3-phosphate after 5 and 10min respectively. Similar results were obtained in experiments with other microsomal preparations of developing sunflower seeds. The total incorporation of oleate into phosphatidylcholine was, therefore, almost entirely accounted for by the direct incorporation of oleate from oleoyl-CoA by acyl exchange, together with the synthesis of phosphatidylcholine via the acylation of *sn*-glycerol 3-phosphate and the subsequent back-transfer from diacylglycerol.

Positional incorporation of [^{14}C]acyl-CoA into phosphatidylcholine

Acyl exchange between acyl-CoA and position 2 of *sn*-phosphatidylcholine in conjunction with the acylation of *sn*-glycerol 3-phosphate to yield phosphatidic acid and diacylglycerol and the further back-transfer from diacylglycerol to phosphatidylcholine should result in the increase of radioactive fatty acid in position 1 of *sn*-phosphatidylcholine. To test this, microsomal preparations were incubated with [^{14}C]linoleoyl-CoA and *sn*-glycerol 3-phosphate and the percentage incorporations of radioactivity in positions 1 and 2 of *sn*-phosphatidylcholine determined. The results (Table 5) show a relative increase in radioactivity in position 1 from 11% after 5min incubation to 26% after 90min. The percentage of radioactivity in position

Table 4. *Effect of sn-glycerol 3-phosphate on the incorporation of [^{14}C]oleate and [^{14}C]glycerol into phosphatidylcholine in microsomes from developing sunflower (var. Flambeau) cotyledons*

Microsomes, equivalent to 76nmol of phosphatidylcholine, were incubated with 200nmol of ^{14}C- or un-labelled oleoyl-CoA in the presence or absence of 200nmol of ^{14}C- or un-labelled glycerol 3-phosphate for 5 and 10min. At these times the amounts of [^{14}C]oleate and [^{14}C]glycerol in phosphatidylcholine was determined. For experimental details, see the Materials and methods section.

Incubation time (min)	*sn*-Glycerol 3-phosphate	[^{14}C]Oleate in phosphatidylcholine (nmol)	[^{14}C]Glycerol in phosphatidylcholine (nmol)
5	–	3.4	–
5	+	4.9	0.6
10	–	5.3	–
10	+	11.0	2.0

Table 5. *Effects of sn-glycerol 3-phosphate on the positional distribution of [^{14}C]linoleate in phosphatidylcholine in microsomes of developing sunflower (var. Flambeau) cotyledons*

Microsomes, equivalent to 153nmol of phosphatidylcholine, were incubated with [^{14}C]linoleoyl-CoA (200nmol) and *sn*-glycerol 3-phosphate (200nmol) for the times stated. The phosphatidylcholine was purified, treated with phospholipase A_2, and the radioactivity in the non-esterified fatty acids and lysophosphatidylcholine determined. For experimental details, see the Materials and methods section.

Incubation time (min)	*sn*-Glycerol 3-phosphate	Positional distribution of [^{14}C]linoleate in *sn*-phosphatidylcholine (%)	
		Position 1	Position 2
5	+	10.9	89.1
40	+	14.2	85.8
90	+	26.0	74.0
90	–	8.7	91.3

1 of *sn*-phosphatidylcholine in the absence of *sn*-glycerol 3-phosphate was about 9% after 90min incubation. The results confirm that, in the absence of *sn*-glycerol 3-phosphate, the incorporation of fatty acid into phosphatidylcholine from acyl-CoA is via acyl exchange; however, in the presence of *sn*-glycerol 3-phosphate, an ever-increasing contribution is made by the back-transfer from diacylglycerol.

Discussion

Microsomal preparations from the developing cotyledons of sunflower were active in catalysing the acyl exchange between acyl-CoA and position 2 of *sn*-phosphatidylcholine. The oleate entering phosphatidylcholine by acyl exchange was available for desaturation to linoleate. These results are in accord with our previous observations on microsomes of developing seeds of safflower (Stymne *et al.*, 1983; Stobart *et al.*, 1983).

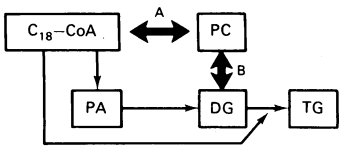
The experiments with radioactive *sn*-glycerol 3-phosphate and acyl-CoA show that the microsomes were also capable of phosphatidic acid, diacylglycerol and triacylglycerol synthesis essentially by the reactions outlined by Kennedy (1961) for the synthesis in rat liver microsomes. Our results also show that phosphatidylcholine could be synthesized directly from diacylglycerol, and this was further confirmed in the analysis of fatty acids in positions 1 and 2 of *sn*-phosphatidylcholine in the presence of *sn*-glycerol 3-phosphate and acyl-CoA substrates. The microsomal preparations also contained a small active diacylglycerol pool that was, initially, rapidly acylated to yield triacylglycerol.

The diacylglycerol → phosphatidylcholine transfer appears to be reversible and in a tightly controlled equilibrium. In many experiments (results not shown) we have been unable to demonstrate a significant change in the total mass of phosphatidylcholine in the microsomes, even after substantial incorporation of [^{14}C]glycerol, and again this would imply a tightly coupled exchange of glycerol backbone and acyl components between the pools of phosphatidylcholine and diacylglycerol.

Acyl exchange between acyl-CoA and position 2 of *sn*-phosphatidylcholine, the acylation of *sn*-glycerol 3-phosphate, and the equilibrium between diacylglycerol and phosphatidylcholine, all appear to be essential reactions in the microsomes of developing oilseeds, particularly those rich in linoleate. It is interesting that microsomes from avocado mesocarp, which produce triacylglycerol rich in oleate, were found to possess only a functional Kennedy (1961) pathway, with little or no acyl exchange and diacylglycerol⇌phosphatidylcholine interconversion. Further work is needed to determine the extent of acyl exchange and to investigate the diacylglycerol → phosphatidylcholine transfer in '*in-vitro*' systems from oilseeds rich in oleate.

Previously, (Stymne *et al.*, 1983) we proposed that the C_{18}-polyunsaturated-fatty-acid content in triacylglycerol of oilseeds could be explained on the basis of a differential selectivity for acyl-CoA species between the *sn*-glycerol 3-phosphate-acylating enzymes and the acyl-CoA⇌phosphatidylcholine acyl-exchange system. The acyl exchange was found to exhibit a bias for the entry of oleate into phosphatidylcholine for desaturation and offered a mechanism for the return of the linoleate product to the acyl-CoA pool. The linoleate was then preferred in the acylation of *sn*-glycerol 3-phosphate, from which triacylglycerol was eventually synthesized. These proposals are in contrast with those of Roughan & Slack (1982), who envisaged that the acylation of *sn*-glycerol 3-phosphate would predominantly be by oleate, as opposed to our observations that acyl exchange produces a phosphatidic acid pool relatively more rich in linoleate. It should be emphasized, however, that the phosphatidic acid so synthesized can still contain significant quantities of oleate.

It is pertinent at this point to discuss the possible role of the equilibrium observed between phosphatidylcholine and diacylglycerol and its relationship in the control of the acyl quality in triacylglycerol. We consider that such a mechanism offers further opportunity for the enrichment of the C_{18} polyunsaturated fatty acids in the oils and perhaps other phospholipid membrane components. This, of course, would confer distinct physiological advantage on those species capable of such reactions. Since the selectivity for polyunsaturated fatty acids of the *sn*-glycerol 3-phosphate-acylating enzymes is not complete (Stymne *et al.*, 1983) the diacylglycerol⇌phosphatidylcholine equilibrium provides a mechanism by which lipid species containing oleate can be returned to the phosphatidylcholine pool for further opportunities to participate in desaturation. It is possible that diacylglycerol species containing oleate are preferred in the transfer to phosphatidylcholine and this requires investigation. Slack *et al.* (1979) have shown that oleate can be desaturated in the 1-position of *sn*-phosphatidylcholine as well as on the 2-position. We have also observed this in sunflower microsomes, although more work is required to substantiate it. However, assuming that desaturation does occur in both positions in phosphatidylcholine, the diacylglycerol⇌phosphatidylcholine equilibrium must be considered as an important mechanism for returning oleate to phosphatidylcholine for desatu-



Scheme 1. *Movement of C_{18}-unsaturated-fatty-acyl groups in the synthesis of triacylglycerols in developing oil seeds*

The acyl exchange between acyl-CoA and phosphatidylcholine (reaction A) provides oleate in position 2 of *sn*-phosphatidylcholine for desaturation to linoleate as well as linoleoyl-CoA for the acylation of *sn*-glycerol 3-phosphate. The equilibrium between diacylglycerols and phosphatidylcholine (reaction B) provides the phosphatidylcholine with oleate in both positions for desaturation, as well as providing diacylglycerols with linoleate in both positions for further acylation to triacylglycerols. The equilibrium reactions A and B co-operate to give triacylglycerols with an enriched polyunsaturated fatty acid content. Abbreviations used: PC, phosphatidylcholine; PA, phosphatidic acid; DG, diacylglycerol; TG, triacylglycerol.

ration. This, of course, would further lead to the availability of linoleate in the 2-position of *sn*-phosphatidylcholine for acyl exchange. Thus the diacylglycerol ⇌ phosphatidylcholine equilibrium coupled to the acyl exchange between acyl-CoA and phosphatidylcholine provides the continuous enrichment of the glycerol backbone with polyunsaturated fatty acids for triacylglycerol synthesis. These proposals are summarized in Scheme 1.

The enzyme(s) involved in the diacylglycerol ⇌ phosphatidylcholine exchange remain conjecture for the moment. However, work with animal systems (Kanoh & Ohno, 1975) has demonstrated a CDP-choline:1,2-diacylglycerol choline phosphotransferase (choline phosphotransferase, EC 2.7.8.2) enzyme that will catalyse the reaction of diacylglycerol with CDP-choline to produce CMP and phosphatidylcholine. These reactions appear to be freely reversible (Goracci *et al.*, 1981). Roughan & Slack (1982) have implicated a similar reaction in a scheme for triacylglycerol synthesis in plants in which they depict the reversible movement of substrate between diacylglycerol and phosphatidylcholine. It is possible that it is this enzyme which is responsible for the observations presented here. It should be emphasized, however, that the cofactors and substrates (e.g. CDP-choline, CMP) do not appear to be rate-limiting in our microsomal preparations and so are presumably bound to the enzyme and only required in catalytic amounts. This, of course, would offer further energy conservation in a system geared to the production of storage oils. It is also noteworthy that, in our experiments, the diacylglycerol ⇌ phosphatidylcholine exchange could only be demonstrated when glycerol backbone was flowing towards triacylglycerol through the Kennedy (1961) pathway.

Since this paper was originally submitted, Slack *et al.* (1983) have produced evidence, in experiments *in vivo* with linseed (*Linum usitatissimum*) cotyledons, that indicates an exchange of glycerol backbone between diacylglycerol and phosphatidylcholine.

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