Labelling Studies *in vivo* on the Metabolism of the Acyl and Glycerol Moieties of the Glycerolipids in the Developing Maize Leaf

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1. When [2-³H]glycerol was supplied to developing maize-leaf laminae, label entered 3-*sn*-phosphatidylcholine at a linear rate essentially from zero time, whereas other lipids were labelled at accelerating rates. On transfer of laminae from [³H]glycerol to unlabelled glycerol, radioactivity was rapidly lost from 3-*sn*-phosphatidylcholine and accumulated in other lipids, principally monogalactosyl diacylglycerol. 2. Degradation of these lipids showed that ³H was present only in the glycerol moiety of the lipids. 3. In double-labelling pulse-chase experiments with [¹⁴C]acetate, which labelled essentially only fatty acids, and [³H]glycerol similar amounts of ¹⁴C and ³H radioactivity were lost from 3-*sn*-phosphatidylcholine and accumulated by monogalactosyl diacylglycerol. 4. The different molecular species of both lipids isolated from laminae during a double-labelled pulse-chase study were separated by argentation t.l.c., and the changes in the amount of radioactivity and the ¹⁴C/³H ratio in different species were compared. The greatest loss of radioactivity during the period in unlabelled substrates occurred from the 3-*sn*-phosphatidylcholine species containing oleate and from the dilinoleate species, and radioactivity accumulated by monogalactosyl diacylglycerol was mainly in the dilinolenate species. However, despite the considerable change in the radioactivity in these species during the chase, the ¹⁴C/³H ratio in each of them remained relatively unchanged. 5. It is proposed that 3-*sn*-phosphatidylcholine in the developing leaf may serve as a donor of linoleate-containing diacylglycerols which are incorporated into other lipids, principally monogalactosyl diacylglycerol.

Although there is considerable support for the belief that linolenate of the chloroplast galactolipids is derived from oleate by sequential desaturation via linoleate (Mazliak, 1973), the details of the pathway are unknown. Kinetic studies of the labelling of fatty acids in leaf tissue with [¹⁴C]acetate (Roughan, 1970; Slack & Roughan, 1975; Williams *et al.*, 1976) indicated that oleate of 3-*sn*-phosphatidylcholine, within the endoplasmic reticulum (Slack & Roughan, 1975), was a major precursor of the linolenate of chloroplast monogalactosyl diacylglycerol and that this phospholipid was probably involved in the initial desaturation of the oleate to linoleate. These studies supported, in part, the proposal of Trémolières & Mazliak (1974) that endoplasmic reticulum and chloroplasts function co-operatively in the desaturation of oleate to linolenate and indicated that a transfer of fatty acid, possibly linoleate, must occur between these organelles. It has been inferred that oleoyl-CoA is the substrate for oleate desaturation in higher plants (Vijay & Stumpf, 1971; Abdelkader *et al.*, 1973; Dubacq *et al.*, 1976). However, support for the belief that microsomal 3-*sn*-phosphatidylcholine is intimately involved in the desaturation of oleate in the leaf was obtained from an examination of the metabolism of oleoyl-CoA by pea-leaf microsomal preparations (Slack *et al.*, 1976). Oleate from the

thiol ester was found to be incorporated specifically into 3-*sn*-phosphatidylcholine before desaturation, and phosphatidylcholine linoleate was the product.

There is little information, however, about the means by which fatty acids could be transferred between different lipids located in different organelles. Diacylglycerols, containing polyunsaturated fatty acids, are the preferred substrates for a UDP-galactose-1,2-diacylglycerol galactosyltransferase (EC 2.4.1.46) (Mudd *et al.*, 1969; Joyard & Douce, 1976), localized in the chloroplast envelope (Douce, 1974). If this enzyme is responsible for the synthesis of monogalactosyl diacylglycerol *de novo* in leaves, then diacylglycerols liberated from 3-*sn*-phosphatidylcholine may provide the acceptors for galactosyl transfer as postulated by Trémolières & Mazliak (1970). Plant tissues also contain acylhydrolases that deacylate both phospho- and galacto-lipids (Galliard, 1971), and cell-free preparations of spinach leaf have been shown to acylate monogalactosyl monoacylglycerol (Safford *et al.*, 1971). It is conceivable, therefore, that the movement of fatty acid from 3-*sn*-phosphatidylcholine into monogalactosyl diacylglycerol observed *in vivo* could involve the deacylation of the phospholipid, then acyl transfer to a monoacyl galactolipid.

The present paper describes some studies on the

kinetics of labelling the glycerolipids of developing maize leaf with [^3H]glycerol and [^{14}C]acetate, which suggest that the probable major form in which fatty acids are transferred between 3-*sn*-phosphatidylcholine and monogalactosyl diacylglycerol is as acylglycerol esters.

Materials

Seedlings of maize (*Zea mays* cultivar Wisconsin 436) were grown as previously described (Slack & Roughan, 1975), and segments of developing leaf lamina (12 cm long) were taken from the emerging sixth leaf of uniform seedlings as described in the above reference.

Unlabelled 3-*sn*-phosphatidylcholine and monogalactosyl diacylglycerol were purified from a lipid extract of developing maize-leaf lamina by a combination of silicic acid chromatography (Rouser *et al.*, 1967) and t.l.c. on silica gel G with chloroform/methanol/15M-NH₃ (65:25:2, by vol.). Thin-layer plates were stained with I₂ vapour, and bands containing the required lipids transferred to screw-cap tubes. The silica gel was suspended in 3.3 ml of methanol, and 2.0 ml of water was added to inactivate the gel, then 6.6 ml of chloroform was added and the lipid was extracted into the chloroform layer by shaking. When quantitative extraction of the lipid was required, the aqueous methanol was re-extracted with an additional 6.6 ml of chloroform. This procedure extracted more than 98% of labelled glycerolipids from silica gel and diacylglycerols from AgNO₃-impregnated silica gel (see below). The chloroform phase was dried by evaporation at 40°C, and the lipids were stored in chloroform at -20°C. The concentrations of the galacto- and phospho-lipids were determined as described by Roughan (1970) and Rouser *et al.* (1966) respectively.

Methanol and pyridine were dried by procedures outlined by Vogel (1957). Methanolic 3% HCl was made immediately before use by adding 5 ml of redistilled acetyl chloride to 100 ml of dry methanol. Sodium methoxide was prepared as previously described (Slack *et al.*, 1976). Hexamethyldisilazane and trimethylchlorosilane were from Pierce Chemical Co., Rockford, IL, U.S.A., and sodium [1- ^{14}C]acetate, [2- ^3H]glycerol and [U- ^{14}C]glycerol were from The Radiochemical Centre, Amersham, Bucks., U.K.

Methods

Labelling of maize-leaf lamina

Strips of developing maize lamina were supplied with labelled substrates under conditions previously described (Slack & Roughan, 1975). [^3H]Glycerol (140 mCi/mmol) was supplied at a concentration of

0.13 mM. In double-labelling experiments with [^3H]glycerol and sodium [^{14}C]acetate (59 mCi/mmol), the concentrations used were 0.26 and 0.15 mM respectively. With these concentrations, similar amounts of ^3H and ^{14}C radioactivity were incorporated into lipids from the two substrates.

Lipid and fatty acid analysis

Laminae were weighed and then steamed before extraction as previously described (Roughan & Boardman, 1972). The chloroform layer was dried at 40°C, redissolved in chloroform, dried under O₂-free N₂, dissolved in chloroform containing 0.005% butylated hydroxytoluene and stored at 4°C. To determine the radioactivity in individual lipids labelled with ^3H or with both ^3H and ^{14}C , samples of the chloroform extracts were chromatographed on silica-gel G plates in chloroform/methanol/acetic acid/water (85:15:10:3, by vol.) and stained with I₂ vapour. Each lipid-containing zone was transferred to a scintillation vial and scintillation fluid (Fricke, 1975) containing water (10% by vol.) was added. Counting efficiency was determined by the channels-ratio method. ^3H was counted at an efficiency of 12% and ^{14}C at an efficiency of 55%. When both ^{14}C and ^3H were present, they were counted at an efficiency of 25 and 7.5% respectively, with the windows of the scintillation counter adjusted to minimize spill-over of ^{14}C into the ^3H mode and vice versa. Alternatively, the amounts of ^3H and ^{14}C in double-labelled lipids were determined by transmethylation each lipid zone with 1.0 ml of 0.5M-sodium methoxide for 15 min at room temperature (20°C). Water (1.0 ml) and light petroleum (b.p. range 40–60°C) (2.0 ml) were then added and the fatty acid methyl esters extracted into the light petroleum. This phase was removed, and the aqueous methanol phase was neutralized with 0.5M-H₂SO₄. This procedure allowed the ^{14}C and ^3H radioactivity to be counted independently, since the fatty acid and non-acyl moieties of each lipid contained radioactivity derived only from the [^{14}C]acetate and [^3H]glycerol respectively (see below). Results obtained by the two methods were essentially the same.

To determine the ^{14}C radioactivity in individual fatty acids of 3-*sn*-phosphatidylcholine and monogalactosyl diacylglycerol, these lipids were separated by t.l.c. and transmethylated as above. Fatty acid methyl esters were separated by g.l.c. on columns fitted with effluent-stream splitters, and individual radioactive fatty acid methyl esters were collected as described previously (Slack & Roughan, 1975), except that columns contained Silar 10C on Gas-Chrom Q (100–120 mesh) (Applied Science Lab Inc., State College, PA, U.S.A.), and counted in 0.5% *p*-terphenyl in xylene (w/v) at an efficiency of 60%.

Degradation of 3-*sn*-phosphatidylcholine and monogalactosyl diacylglycerol

The lipids were separated by t.l.c., eluted from the silica gel with aqueous methanol and chloroform, and the radioactivity in the samples was determined as above. The remaining phosphatidylcholine was transferred to a screw-capped tube, and the chloroform evaporated under N_2 . Diethyl ether (2.0 ml) and 0.5 ml of 50 mM- H_3BO_4 /NaOH buffer, pH 6.5, containing 10 mM- $CaCl_2$ and 0.5 mg of phospholipase C from *Clostridium welchii* (type 1; Sigma Chemicals, St. Louis, MO, U.S.A.), were added to the tube and vigorously shaken by using a flask shaker [Baird and Tatlock (London) Ltd., Chadwell Heath, Essex, U.K.] for 2 h at 20°C. The ether was evaporated under N_2 , 2.5 ml of chloroform/methanol (2:1, v/v) was added, and diacylglycerols were extracted into the chloroform. Samples of this phase and of the aqueous methanol phase were counted to determine the radioactivity in diacylglycerols and choline phosphate respectively. Diacylglycerols were purified by t.l.c. on silica gel G with light petroleum (b.p. 60–80°C)/diethyl ether/acetic acid (75:25:1, by vol.), stained with I_2 vapour, then methylated, and the radioactivity in the chloroform and aqueous methanol phases was counted as above to determine radioactivity in fatty acids and glycerol.

Radioactive monogalactosyl diacylglycerol (approx. 0.2 μ mol) and unlabelled galactolipid 0.25 μ mol, prepared as above, were transferred in chloroform to screw-capped tubes, and the solvent was removed under N_2 . The methanolysis of this lipid, the subsequent separation of fatty acid methyl esters, formation of the trimethylsilyl derivatives of methyl galactosides and glycerol and their separation by temperature-programmed g.l.c. were performed as described by Williams *et al.* (1975), except that columns contained 5% OV17 on Chromasorb W (80–100 mesh) (Applied Sciences Lab Inc.). Trimethylsilyl derivatives were collected from the effluent and counted for radioactivity as above.

Separation and analysis of molecular species of 3-*sn*-phosphatidylcholine and monogalactosyl diacylglycerol

Radioactive 3-*sn*-phosphatidylcholine (approx. 0.1 μ mol) and an unlabelled sample of this phospholipid (0.6 μ mol) were incubated with 1 mg of phospholipase C for 2 h, and the diacylglycerols were extracted and purified by t.l.c. as above, then eluted from silica gel with ether. About 95% of the radioactivity was associated with the diacylglycerols and the remainder with monoacylglycerols and unesterified fatty acids. The diacylglycerol preparation and radioactive monogalactosyl diacylglycerol (approx. 0.2 μ mol) plus 0.5 μ mol of unlabelled galactolipid were separated into different molecular species by argen-

tation t.l.c. by the methods of Gurr & Brawn (1970) and Nichols & Moorhouse (1969) respectively. Plates were sprayed with 0.02% dichlorofluorescein in 80% (w/v) ethanol and viewed under u.v. light. Individual bands of lipid-containing silica gel were eluted with aqueous methanol plus chloroform as above, and the lipid was stored in dry chloroform at –20°C. The radioactivity and mass of individual fatty acids in each of these bands were determined as above.

Results

Kinetics of labelling of leaf lipids with [3H]glycerol

When [3H]glycerol was supplied to developing maize-leaf laminae, the label entered 3-*sn*-phosphatidylcholine at a linear rate from zero time, whereas other lipids were labelled at accelerating rates with time (Fig. 1). Radioactivity was lost from 3-*sn*-phosphatidylcholine and accumulated by other glycerolipids, principally monogalactosyl diacylglycerol, when laminae were transferred from [3H]glycerol to unlabelled glycerol (Fig. 2). Between 96 and 98% of the radioactivity present in the total lipid extracts of the experiment described in Fig. 2 was recovered in the aqueous methanol phase when samples were transmethylated with sodium methoxide and the fatty acid methyl esters extracted into light petroleum. This observation indicated that essentially all the glycerol incorporated into lipids was located in non-acyl moieties. Further, more than 95% of the label

Fig. 1. Time-course of incorporation of [2-^3H]glycerol into some lipids of immature maize lamina

Strips from immature maize leaf were supplied with [2-^3H]glycerol, and sampled and analysed as described in the Methods section. ○, 3-*sn*-Phosphatidylcholine; ●, 3-*sn*-phosphatidylglycerol + digalactosyl diacylglycerol; □, monogalactosyl diacylglycerol.

Fig. 2. Changes in the distribution of radioactivity among the glycerolipids of immature maize lamina after transfer from $[2\text{-}^3\text{H}]\text{glycerol}$ to unlabelled glycerol

Lamina strips were supplied with $[2\text{-}^3\text{H}]\text{glycerol}$ for 35 min, then transferred to unlabelled glycerol, and one strip was removed at each of the times indicated and analysed as described in the Methods section. ○, 3-*sn*-Phosphatidylcholine; ●, 3-*sn*-phosphatidylglycerol+digalactosyl diacylglycerol; □, monogalactosyl diacylglycerol; ■, 3-*sn*-phosphatidylethanolamine. Laminae were in darkness from 5.5 to 18 h after transfer to unlabelled glycerol.

present in 3-*sn*-phosphatidylcholine was retained in the diacylglycerol that was liberated by phospholipase C, indicating that the glycerol rather than the choline phosphate moiety was labelled. A similar proportion of the label present in monogalactosyl diacylglycerol was recovered in glycerol after methanolysis of the lipid. These results suggested that glycerol was initially incorporated into the glycerol moiety of 3-*sn*-phosphatidylcholine and subsequently transferred from this lipid to become the glycerol moiety of monogalactosyl diacylglycerol and probably other lipids. A similar conclusion was reached from labelling experiments with $[\text{U}\text{-}^{14}\text{C}]\text{glycerol}$. However, with this substrate, in contrast with $[2\text{-}^3\text{H}]\text{glycerol}$, a significant amount of label entered the acyl moiety of the lipids.

Comparison of the labelling of lipids with $[^3\text{H}]\text{glycerol}$ and $[^{14}\text{C}]\text{acetate}$

The kinetics of glycerol labelling of the glycerolipids in developing maize lamina (Figs. 1 and 2) resembled those previously observed with similar tissue when the acyl moieties of these lipids were labelled from $[^{14}\text{C}]\text{acetate}$ (Slack & Roughan, 1975). To examine the degree of similarity between the labelling of glycerol-

Fig. 3. Changes in the distribution of ^3H and ^{14}C among the glycerolipids of immature maize lamina after transfer from a solution of $[2\text{-}^3\text{H}]\text{glycerol}$ and $[1\text{-}^{14}\text{C}]\text{acetate}$ to unlabelled substrates

Lamina strips were supplied with $[2\text{-}^3\text{H}]\text{glycerol}$ and $[1\text{-}^{14}\text{C}]\text{acetate}$ for 40 min, then transferred to unlabelled substrates, and strips removed for analysis, as described in the Methods section, at the times shown after transfer. Open symbols, ^3H radioactivity; closed circles, ^{14}C radioactivity. ○, 3-*sn*-Phosphatidylcholine; □, ■, 3-*sn*-phosphatidylglycerol+digalactosyl diacylglycerol; △, ▲, monogalactosyl diacylglycerol. Laminae were in darkness from 6 to 20 h after transfer to unlabelled substrates.

lipids with $[^{14}\text{C}]\text{acetate}$ and $[^3\text{H}]\text{glycerol}$, we carried out double-labelling experiments. In the pulse-chase studies described (Fig. 3 and Table 1) it was apparent that a similar proportion of the ^{14}C and of the ^3H that had originally been incorporated into the acyl and glycerol moieties of 3-*sn*-phosphatidylcholine respectively was lost from the phospholipid after laminae were transferred to unlabelled substrates and that the two isotopes entered individual lipids in similar proportions. Although such observations would be expected if a double-labelled acylglycerol ester was transferred from the phospholipid, it is not inconceivable that similar observations could be obtained if the fatty acid and glycerol moieties were transferred independently.

To examine these possibilities we determined the change in $[^3\text{H}]\text{glycerol}$ label and $[^{14}\text{C}]\text{fatty acid}$ label of the different species of 3-*sn*-phosphatidylcholine and monogalactosyl diacylglycerol (Tables 2a and 2b) from the experiment described in Table 1. The redistribution of ^{14}C label among the fatty acids of 3-*sn*-phosphatidylcholine and monogalactosyl diacylglycerol during the 27 h period after transfer from unlabelled substrates (Table 1) showed similar, but

Table 1. *Changes in the proportion of the total ^3H and ^{14}C radioactivity in glycerolipids present in 3-sn-phosphatidylcholine and monogalactosyl diacylglycerol and the distribution of ^{14}C radioactivity among the fatty acids of these lipids of immature maize lamina after the transfer from labelled to unlabelled substrates*

Six lamina strips from immature maize leaf were supplied with $[1-^{14}\text{C}]$ acetate and $[2-^3\text{H}]$ glycerol for 60 min, then transferred to unlabelled substrates. Two lamina strips were removed at the time of transfer and others at the times shown, and bulked and the lipids analysed as described in the Methods section. Laminae were illuminated from 0 to 6 h and from 20 to 27 h during the period in unlabelled substrates.

Lipid	Time after transfer from radioactive substrates (h)	Radioactivity in individual lipid (% of total incorporated)		Distribution of ^{14}C radioactivity amongst fatty acids of individual lipids (%)			
		^{14}C	^3H	$\text{C}_{16:0}$	$\text{C}_{18:1}$	$\text{C}_{18:2}$	$\text{C}_{18:3}$
3-sn-Phosphatidylcholine	0	54.0	52.3	6.1	43.0	50.4	0.6
	20	23.5	24.1	8.9	21.2	65.1	4.8
	27	15.8	16.2	13.3	9.4	63.7	13.6
Monogalactosyl diacylglycerol	0	17.0	16.0	7.7	19.3	36.3	36.7
	20	37.6	32.4	1.9	2.8	10.5	84.8
	27	40.5	37.6	1.5	1.2	10.9	86.3

more pronounced, changes than those observed previously (Slack & Roughan, 1975) during a shorter chase. In particular, not only did the amount of radioactivity in the oleate of the phospholipid fall by about 15-fold, but there was also about a 2.5-fold decrease in the label in the linoleate of this lipid. The increase in the ^{14}C radioactivity in the galactolipid was attributable to a sixfold increase in the label in the linolenate moiety.

Diacylglycerols, liberated from the 3-sn-phosphatidylcholine by treatment with phospholipase C, were separated into six bands and the monogalactosyl diacylglycerol into five bands by argentation chromatography. Analysis of the fatty acid composition of these bands (Table 2b) indicated that complete separation of the lipids into individual molecular species had not been achieved. However, the fatty acid composition of the individual bands from the 0 h and 27 h harvests were very similar, and, since our aim was to compare the changes in radioactivity between harvests, no attempt was made to purify individual molecular species by repeated chromatography. From the fatty acid composition of the diacylglycerol bands we have assumed that the major molecular species in bands 1–6 (numbered from the top of plate) were saturated fatty acid/oleate plus saturated fatty acid/linoleate, saturated fatty acid/linoleate, oleate/linoleate, dilinoleate, saturated fatty acid/linolenate and linoleate/linolenate respectively. Maize-leaf monogalactosyl diacylglycerol contains mainly linolenate and predictably the major band obtained by argentation t.l.c. of this lipid was a dilinolenate species (band 5). Two of the four minor bands obtained contained dilinolenate-containing galactolipid as well as galactolipid containing linoleate (bands 3 and 4) and the others a mixture of species containing saturated, mono-, di- and tri-enoic acids.

As would be expected from the redistribution of ^{14}C radioactivity among the fatty acids of 3-sn-phosphatidylcholine and monogalactosyl diacylglycerol during the chase in unlabelled substrates (Table 1), the largest loss of ^{14}C from the different species of the phospholipid occurred from those that contained oleate (bands 1 and 3), and the accumulation of ^{14}C label by the galactolipid was into the dilinolenate species (band 5) (Table 2a). The kinetics of labelling of 3-sn-phosphatidylcholine linoleate *in vivo* and studies of oleate desaturation by leaf microsomal preparations (discussed above) indicate that oleate esterified to 3-sn-phosphatidylcholine is desaturated to linoleate in leaves, as in fungi (Baker & Lynen, 1971; Talamo *et al.*, 1973) and *Chlorella vulgaris* (Gurr *et al.*, 1969). The present finding that the oleate in different molecular species was desaturated with apparent equal facility is in agreement with the observation for *Chlorella* (Gurr & Brawn, 1970). We suggest that the saturated fatty acid/oleate and oleate/linoleate species (bands 1 and 3) are the probable precursors of the saturated fatty acid/linoleate and dilinoleate species (bands 2 and 4) respectively. Our failure to identify a diacylglycerol derived from phosphatidylcholine dioleate suggests that only a small proportion of the oleate of maize-leaf 3-sn-phosphatidylcholine may exist in this species under steady-state conditions. A dioleoylglycerol would have a mobility on AgNO_3 -impregnated silica gel very similar to that of a saturated fatty acid/linoleate species (Gurr & Brawn, 1970).

The ratio of ^{14}C label in fatty acid to ^3H label in the glycerol moieties varied between species and changed to different degrees during the chase. Predictably, the 3-sn-phosphatidylcholine species with the highest $^{14}\text{C}/^3\text{H}$ ratios at the start of the chase were those containing oleate and the dilinoleate species,

Table 2. Distribution of (a) ^{14}C and ^3H radioactivity and (b) fatty acids among different molecular species of 3-sn-phosphatidylcholine and monogalactosyl diacylglycerol during a double-labelled pulse-chase with [^{14}C]acetate and [^3H]glycerol

3-sn-Phosphatidylcholine and monogalactosyl diacylglycerol were isolated from lipid extracts of immature laminae harvested at 0, 20 and 27 h after pulse-labelling with [^{14}C]acetate and [^3H]glycerol in the experiment described in Table 1. (a) The galactolipid and diacylglycerols, prepared from the phospholipid by treatment with phospholipase C, were separated by argentation t.l.c. into different bands and the radioactivity in each was determined. The ratios of $^{14}\text{C}/^3\text{H}$ radioactivity are given in parentheses. The fatty acid composition of the different bands from the two lipids are shown in (b). Fatty acid methyl esters were prepared by trans-methylation from each of the bands separated by argentation t.l.c. from the 0 and 27 h harvests and analysed by g.l.c. as described in the Methods section. Values given are the means of the two measurements.

(a) Time after transfer from labelled substrates (h)	$10^{-4} \times ^{14}\text{C}$ and ^3H radioactivity in different bands (d.p.m./g fresh wt. of lamina)										
	3-sn-Phosphatidylcholine						Monogalactosyl diacylglycerol				
	1	2	3	4	5	6	1	2	3	4	5
0 ^{14}C	20.9	18.0	24.5	25.1	4.1	6.1	4.8	6.2	4.9	9.3	7.8
	(0.49)	(0.31)	(0.58)	(0.45)	(0.16)	(0.25)	10.4	(0.46)	(0.5)	(0.6)	(0.49)
^3H	42.7	58.1	42.2	55.8	25.6	24.4	10.4	12.4	8.2	19.0	21.1
20 ^{14}C	4.7	11.9	7.0	14.0	2.6	3.6	2.3	3.4	3.1	7.5	53.9
	(0.42)	(0.32)	(0.50)	(0.43)	(0.18)	(0.25)	6.1	(0.38)	(0.43)	(0.39)	(0.42)
^3H	11.2	37.2	14.0	32.6	14.4	14.4	6.1	7.9	7.9	17.8	114.6
27 ^{14}C	2.6	9.2	3.8	9.0	3.2	2.5	0.6	2.3	2.2	12.5	59.4
	(0.39)	(0.31)	(0.45)	(0.52)	(0.30)	(0.25)	0.6	(0.22)	(0.22)	(0.21)	(0.44)
^3H	6.7	29.7	8.4	17.3	10.7	10.0	2.7	10.4	10.4	28.4	152.3
(b)											
Fatty acids	Amount of individual fatty acids in the different bands (μg)						Monogalactosyl diacylglycerol				
	3-sn-Phosphatidylcholine						Monogalactosyl diacylglycerol				
	1	2	3	4	5	6	1	2	3	4	5
$\text{C}_{16:0}$	12.4	43.6	1.8	3.8	11.6	1.4	2.4	2.6	—	—	—
$\text{C}_{18:0}$	2.0	4.6	0.6	1.2	1.2	0.6	0.8	0.8	—	—	—
$\text{C}_{18:1}$	7.8	1.4	4.0	—	—	—	0.8	0.4	0.4	—	—
$\text{C}_{18:2}$	7.4	49.2	6.4	53.8	2.2	7.6	3.2	2.6	1.6	6.8	1.0
$\text{C}_{18:3}$	—	—	—	—	12.2	7.2	1.0	4.0	13.6	29.0	226.0

fatty acids that contained the bulk of the ^{14}C radioactivity present in this lipid (Table 1). The low ratio in the linolenate-containing species of this phospholipid (bands 5 and 6) may reflect the synthesis of these species from a linolenate pool having a low ^{14}C specific radioactivity. The small change in the $^{14}\text{C}/^3\text{H}$ ratio of bands 1–4 from 3-*sn*-phosphatidylcholine and band 5 of the monogalactosyl diacylglycerol during the chase, concomitant with the relatively large loss of radioactivity from these phospholipid bands and gain in radioactivity by the galactolipid, are not consistent with acyl exchange being the major means of fatty acid transfer between these lipids. However, the $^{14}\text{C}/^3\text{H}$ ratio of most bands was not constant and in general tended to decrease, particularly in bands 1–3 of the galactolipid. Such a change indicates some exchange of labelled fatty acid from the lipids with unlabelled fatty acid.

Discussion

The preferential incorporation of [^3H]glycerol into the glycerol moiety of 3-*sn*-phosphatidylcholine and of ^{14}C -labelled fatty acids into this lipid in developing maize-leaf lamina, together with the transfer of label from both moieties to monogalactosyl diacylglycerol and other lipids indicates a rapid turnover of this phospholipid. In this leaf tissue there is considerable lipid synthesis associated with the accumulation of chloroplast lipids (Leech *et al.*, 1973; Slack *et al.*, 1974), and it is noteworthy that a high rate of turnover of acyl and glycerol moieties of phospholipids has also been observed in developing soya-bean cotyledons, concomitant with the accumulation of triacylglycerol (Wilson & Rinne, 1976). Whether or not a rapid turnover of 3-*sn*-phosphatidylcholine is peculiar to plant tissues in which lipid synthesis is occurring at a high rate has not to our knowledge been determined.

Our present studies do not conclusively show whether the accumulation of [^3H]glycerol and [^{14}C]linolenate in monogalactosyl diacylglycerol from radioactivity originally present in the glycerol and fatty acids of 3-*sn*-phosphatidylcholine occurred by the complete degradation of the phospholipid and the resynthesis of diacylglycerol precursors of the galactolipid or via diacylglycerol liberated from the phospholipid. However, in view of the similarity between the $^{14}\text{C}/^3\text{H}$ ratio in the oleate- and linolenate-containing species of the phospholipid and of dilinolenyl monogalactosyl diacylglycerol during the transfer of the labelled moieties, we suggest that the latter possibility is more likely. For the $^{14}\text{C}/^3\text{H}$ ratio to be maintained during a complete degradation of 3-*sn*-phosphatidylcholine and resynthesis of monogalactosyl diacylglycerol, one would have to postulate that the pools of fatty acid and glycerol used for the synthesis of the galactolipid were of similar size, and

further, that similar proportions of fatty acid and glycerol liberated from the phospholipid entered these pools.

It is implicit in our above suggestion, that oleate esterified to 3-*sn*-phosphatidylcholine is the substrate for oleate desaturation in leaves, that linoleate should be the major fatty acid exported from this phospholipid to monogalactosyl diacylglycerol. However, since palmitate and stearate comprise only a few per cent of the fatty acids in the maize-leaf galactolipid, it seems unlikely that species of 3-*sn*-phosphatidylcholine containing linoleate and these fatty acids could provide diacylglycerols for incorporation directly into the galactolipid. A comparison of the ^{14}C radioactivity lost from the predominantly C_{18} species of 3-*sn*-phosphatidylcholine in the experiment described in Table 2a (bands 3, 4 and 6) with that gained by the dilinolenate species of monogalactosyl diacylglycerol indicates that only 74% of the radioactivity gained by the latter could have been derived from the former species. However, if the ^{14}C -labelled fatty acids lost during the chase from other species of monogalactosyl diacylglycerol were desaturated and incorporated into the dilinolenate species of this lipid, as has been suggested from studies with *Chlorella vulgaris* (Nichols *et al.*, 1967; Nichols & Moorehouse, 1969), then the radioactivity from bands 3, 4 and 6 from 3-*sn*-phosphatidylcholine could account for more than 90% of the remaining ^{14}C radioactivity gained by the dilinolenyl monogalactosyl diacylglycerol.

A better understanding of the possible role of 3-*sn*-phosphatidylcholine as a donor of polyunsaturated diacylglycerols must await a greater understanding of the processes of synthesis and breakdown of this lipid. Evidence for the presence of the enzymes glycerol kinase and glycerol phosphate acyltransferase (Barron & Stumpf, 1962; Chenaie, 1965; Sastry & Kates, 1966), phosphatidate phosphatase (Konigs & Heinz, 1974) and choline phosphotransferase (Devor & Mudd, 1971; Lord, 1976; Moore, 1976) in plants has been obtained; these enzymes have been implicated in the synthesis of the phospholipid. However, the mechanism by which diacylglycerols could be liberated from 3-*sn*-phosphatidylcholine is not known, but could conceivably involve the concerted action of phospholipase D, an enzyme widespread in plant tissues (Quarles & Dawson, 1969; Roughan & Slack, 1976), and phosphatidate phosphatase.

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