Fatty Acid Biosynthesis in the Leaves of Barley, Wheat and Pea

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1. The incorporation of radioactivity from [1-¹⁴C]acetate into the leaf lipids of barley, pea and wheat has been studied in pulse-labelling experiments. 2. There was little increase in the total labelling of lipids after the leaves were transferred to non-radioactive medium. However, there was an increase in the relative labelling of unsaturated fatty acids. In addition, there was an increase in the relative labelling of diacylgalactosylglycerol. 3. The principal radioactively labelled acyl lipids were diacylgalactosylglycerol and phosphatidylcholine. Phosphatidylcholine showed a decreasing proportion of [¹⁴C]oleate and an increasing amount of [¹⁴C]linoleate with time. Diacylgalactosylglycerol also had decreasing amounts of [¹⁴C]oleate but, in addition, had an increasing proportion of [¹⁴C]linolenate with time. 4. The absence of significant amounts of [¹⁴C]linolenate in phosphatidylcholine appeared to exclude a role for this phospholipid in linoleate desaturation. 5. The specific radioactivities of oleate and linoleate in phosphatidylcholine, diacylgalactosylglycerol and diacylgalabiosylglycerol were very similar in any single experiment. It was concluded that these fatty acids can rapidly exchange between the three intact lipids.

The leaves of higher plants are a rich source of fatty acids, particularly linoleate and linolenate. Though these account for 70% or more of the total acyl moieties of leaf membrane lipids, the details of their biosynthesis are not known (cf. Givan & Harwood, 1976; Harwood, 1977).

Sequential desaturation of C₁₈ fatty acids was suggested from a study of the labelling kinetics from acetate (James, 1963) and was considerably reinforced by the finding that specifically labelled [1-¹⁴C]oleate was converted into [1-¹⁴C]linoleate and then [1-¹⁴C]linolenate (Harris & James, 1965). However, results in spinach and a number of other plants have suggested a second pathway for the synthesis of linolenate, that involves desaturation of C₁₂ fatty acids followed by elongation (Jacobson *et al.*, 1973; Stumpf, 1975).

Two types of substrates have been proposed for the desaturase enzymes. Evidence for thiol esters has been obtained from the desaturation of stearyl-(acyl-carried protein) in a number of tissues (cf. Stumpf, 1975; Harwood, 1977). The desaturation of oleoyl-CoA has been observed in two storage tissues (Abdelkader *et al.*, 1973; Vijay & Stumpf, 1971). The desaturation of linoleate has not, however, been demonstrated *in vivo*. The high rates of labelling of the fatty acid moieties of a number of complex lipids in photosynthetic tissues first led to the proposal that intact lipids (and, hence, oxygen esters) could act as substrates for desaturation. Thus diacylgalactosylglycerol has been implicated in desaturation in green and blue-green algae (Nichols & Moorhouse, 1969),

and phosphatidylcholine was suggested to be involved (possibly as the actual substrate) in linoleate synthesis in *Chlorella* (Gurr *et al.*, 1969). Direct evidence for phosphatidylcholine as a substrate for fatty acid desaturation has now been obtained by using various micro-organisms (Baker & Lynen, 1971; Talamo *et al.*, 1973; Pugh & Kates, 1975) and rat liver (Pugh & Kates, 1977). This has supported the hypothesis that this lipid is the substrate for oleate and linoleate desaturation in higher-plant leaves (Roughan, 1970, 1975; Slack & Roughan, 1975; Williams *et al.*, 1976). Phosphatidylcholine has also been suggested to act as a donor of linolenate for the galactolipids of chloroplasts (Roughan, 1975; Williams *et al.*, 1976).

Since no preparation has yet been found that will convert linoleate into linolenate *in vitro*, data for these proposals have had to be obtained from labelling studies with leaf preparations. Although these methods often produce equivocal results (cf. Tremolieres & Mazliak, 1974), they can also yield useful information. Thus there is general agreement that phosphatidylcholine is involved in some way during the desaturation of oleate (though not necessarily as the actual substrate). However, short incubation times revealed that linolenate could be synthesized without, apparently, the involvement of phosphatidylcholine (Heinz & Harwood, 1977; Siebertz & Heinz, 1977). These results were in apparent contradiction to results obtained with broad bean (Williams *et al.*, 1976) and with maize (Roughan, 1970). We have therefore investigated whether the lack of radio-

activity in the linolenate of the phosphatidylcholine of young leaves was due to the plant species used. The results indicate quite clearly that linolenate is synthesized without being esterified in phosphatidylcholine.

Experimental

Materials

Barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) seeds were a generous gift from Dr. T. Galliard, A.R.C. Food Research Institute, Norwich, Norfolk, U.K. Pea (*Pisum sativum* cultivar Kelvedon Wonder) seeds were from Thompson and Morgan, Ipswich IP2 0BA, Suffolk, U.K. Seeds were grown in John Innes Seed Compost under normal daylight conditions (16h of daylight, 15–18°C). The monocotyledons were used after 6–7 days and the second true pair of developing pea leaves were used after approximately 10 days.

Sodium [^{14}C]acetate (sp. radioactivity 60mCi/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Fatty acid standards were obtained from Nu-Chek Prep., P.O. Box 172, Elysian, MN 56028, U.S.A., or Sigma (London) Chemical Co., Kingston upon Thames, Surrey KT2 7BH, U.K., and organic solvents were from Koch-Light Laboratories, Colnbrook, Bucks., U.K., or BDH Chemicals, Poole, Dorset, U.K. The solvents were redistilled (Bolton & Harwood, 1977). Complex-lipid markers were prepared from Swiss chard (Burns *et al.*, 1977).

Incubations

The leaves used in each experiment were from plants selected for uniformity on the basis of height and the extent of emergence. The leaves, or their stalks, were cut under water and were transferred to small tubes (12mm $\times$ 3mm) containing 1 or 2 μCi of sodium [^{14}C]acetate in 0.20ml of water. The leaves were incubated in a constant draught (approx. 5m/s) to aid transpiration and, hence, uptake of isotope. The temperature (25°C) was monitored throughout and illumination was approx. 105 (photosynthetically active wavelengths) $\mu\text{E}/\text{m}^2$ per s. After 30min the leaves were rinsed briefly with unlabelled solution and transferred to water for further incubation under the above conditions.

Extraction and analysis of lipid

Total lipids were extracted from leaves by the method of Garbus *et al.* (1963). The lower phase was washed with synthetic upper phase, and portions were removed for the determination of chlorophyll and radioactivity counting. The remaining lipid

extract was separated by t.l.c. on silica-gel G (E. Merck, Darmstadt, Germany) plates with acetone/benzene/water (91:30:8, by vol.) as solvent. Bands were revealed by spraying with aq. 0.001% (w/v) Rhodamine 6G. They were then scraped and methylated with 0.5M-sodium methoxide at 50°C for 10min.

Fatty acid methyl esters were separated on a glass column (2.5m $\times$ 0.7mm) packed with 15% (w/w) EGSS-X on Supelcoport (100–120 mesh; Supelco, Bellefonte, PA 16823, U.S.A.) at 185°C with a Pye 104 gas chromatograph fitted to a Panax radioactivity detector. Pentadecanoate was used as an internal standard.

Radioactivity measurement

Thin-layer chromatograms were examined with a spark-chamber radioautograph (Birchover Instruments, Hitchin, Herts., U.K.). Counting for radioactivity was carried out as described by Bolton & Harwood (1977).

Chlorophyll determination

Chlorophyll concentration was determined by the method of Bruinsma (1961).

Results

Incorporation of radioactivity into fatty acids of lipids

The distribution of the radioactivity incorporated from [^{14}C]acetate into the lipid fractions was examined by spark-chamber radioautography of the components that had been separated by t.l.c. In all three plant types, the principal labelled-lipid classes corresponded to fractions containing pigments plus neutral lipids, diacylgalactosylglycerol or phosphatidylcholine. Diacylgalabiosylglycerol and phosphatidylglycerol were also appreciably, though less, labelled. The proportion of the radioactivity present in the acyl moieties of diacylgalactosylglycerol, diacylgalabiosylglycerol and phosphatidylcholine is shown in Table 1. Whereas the two galactolipids contained significant radioactivity in the deacylated part of the molecule, phosphatidylcholine was greater than 94% labelled in its acyl moieties from [^{14}C]acetate. The distribution of radioactivity (Table 1) showed virtually no change with time in the case of barley leaves. However, in pea and wheat leaves there was a decrease in the labelling of the fatty acids of phosphatidylcholine and an increase in those of diacylgalactosylglycerol. In spite of their quantitative importance in leaves, the fatty acids of diacylgalabiosylglycerol were always considerably less labelled from [^{14}C]acetate than those of the two other lipids. By contrast the total lipid radioactivity/ μg of chloro-

Table 1. *Distribution of radioactivity in the fatty acids of complex lipids of leaf tissue labelled from [1-¹⁴C]acetate*
Leaves were incubated and complex lipids extracted and separated as detailed in the Experimental section. Incorporation rates were in the range 824–928 d.p.m./μg of chlorophyll, 688–2641 d.p.m./μg of chlorophyll and 586–1468 d.p.m./μg of chlorophyll for the total lipids of wheat, pea and barley respectively. Means ± s.d. are shown with the numbers of independent experiments in parentheses. The values for 1–5 h were compared with the values for ½ h and the significances of their difference analysed by Student's *t* test: **P* < 0.025; ***P* < 0.01.

		¹⁴ C-labelled fatty acids (% of total lipid radioactivity)				
Plant	Incubation time (h) ... Lipid	½	1	2	3	5
Pea	Phosphatidylcholine	11.1 ± 1.8 (6)	10.0 ± 1.1 (6)	5.8 ± 0.4 (6)**	5.9 ± 0.3 (6)**	5.7 ± 2.0 (6)**
	Diacylgalactosylglycerol	9.0 ± 1.6 (6)	11.2 ± 3.1 (6)	12.8 ± 2.8 (6)	14.7 ± 2.4 (6)*	14.0 ± 0.8 (6)**
	Diacylgalabiosylglycerol	1.8 ± 0.3 (6)	1.7 ± 0.2 (6)	1.4 ± 0.1 (6)	1.4 ± 0.3 (6)	1.3 ± 0.3 (6)
Barley	Phosphatidylcholine	2.0 ± 0.1 (6)	2.4 ± 0.3 (5)	1.8 ± 0.3 (5)	2.4 ± 0.4 (6)	2.0 ± 0.2 (6)
	Diacylgalactosylglycerol	16.8 ± 1.7 (6)	21.7 ± 2.8 (6)	20.2 ± 2.1 (6)	19.8 ± 2.0 (6)	17.4 ± 1.0 (6)
	Diacylgalabiosylglycerol	2.5 ± 0.1 (4)	2.2 ± 0.5 (6)	1.8 ± 0.4 (4)	2.0 ± 0.2 (6)	1.8 ± 0.5 (4)
Wheat	Phosphatidylcholine	13.8 ± 4.9 (4)	12.2 ± 3.8 (4)	13.4 ± 4.8 (4)	10.8 ± 1.0 (4)	10.0 ± 3.2 (4)
	Diacylgalactosylglycerol	8.5 ± 1.5 (4)	15.4 ± 2.2 (4)**	16.9 ± 2.1 (4)**	12.5 ± 2.0 (4)*	19.1 ± 1.1 (4)**
	Diacylgalabiosylglycerol	1.8 ± 0.5 (3)	2.2 ± 0.4 (4)	2.3 ± 1.9 (3)	2.1 ± 0.3 (4)	4.6 ± 1.5 (3)

Fig. 1. *Changes in the distribution of radioactivity in the fatty acids of glycerolipids in barley leaves labelled by pulse-chase from [1-¹⁴C]acetate*
Leaves were incubated under conditions described in the Experimental section. After ½ h exposure to [1-¹⁴C]acetate, they were transferred to non-radioactive medium. Fatty acids from diacylgalactosylglycerol (a) and phosphatidylcholine (b) were analysed. Symbols: ●, palmitate; ○, oleate; △, linoleate; □, linolenate. Results show means ± s.e.m. (*n* = 6 for diacylgalactosylglycerol and *n* = 5 for phosphatidylcholine in independent experiments).

Fig. 2. *Changes in the distribution of radioactivity in the fatty acids of glycerolipids in wheat leaves labelled by pulse-chase from [1-¹⁴C]acetate*
For a definition of the symbols see the legend to Fig. 1. Results show means ± s.e.m. (*n* = 3 for phosphatidylcholine and *n* = 5 for diacylgalactosylglycerol in independent experiments). Fatty acids from diacylgalactosylglycerol (a) and phosphatidylcholine (b) were analysed.

phyll showed no significant increase during the period of non-radioactive pulse-chase (J. Wharfe & J. L. Harwood, unpublished work).

Pattern of incorporation into fatty acids

The ^{14}C -labelled fatty acids present in diacylgalactosylglycerol, diacylgalabiosylglycerol and phosphatidylcholine during the pulse-chase experiments were then analysed. The results obtained for barley, wheat and pea are presented in Figs. 1, 2 and 3 respectively. It is interesting to compare the change with time in labelling pattern for the two major labelled acyl lipids in the three plants. Certain

Fig. 3. Changes in the distribution of radioactivity in the fatty acids of glycerolipids of pea leaves labelled by pulse-chase from $[1-^{14}\text{C}]$ acetate

Fatty acids from diacylgalactosylglycerol (a), diacylgalabiosylglycerol (b) and phosphatidylcholine (c) were analysed. For a definition of the symbols see the legend to Fig. 1. Results show means $\pm$ S.E.M. ($n = 4$ independent experiments).

generalizations can be made. For diacylgalactosylglycerol, palmitate represented less than 20% of the total radioactivity and gradually decreased in relative amount during the experiments. Likewise, oleate contained the highest percentage of radioactivity in the shortest time intervals. On the other hand, the linoleate in diacylgalactosylglycerol contained an increasing proportion of the radioactivity, which reached a maximum about 2h after exposure to $[^{14}\text{C}]$ acetate, when its label declined somewhat. $[^{14}\text{C}]$ Linolenate was detectable in the shortest time intervals examined and was increasingly labelled throughout the experiment. Oleate and linoleate were the principal radioactive fatty acids in phosphatidylcholine. The proportion of radioactivity in oleate decreased during the experiment and that in $[^{14}\text{C}]$ linolenate increased. $[^{14}\text{C}]$ Linolenate was only occasionally detectable in phosphatidylcholine even at the longer time intervals. The amounts of this ^{14}C -labelled fatty acid in phosphatidylcholine were also very small when compared with diacylgalactosylglycerol.

Of the two monocotyledons, wheat contained a higher percentage of radioactivity, which was initially in the palmitate moiety of diacylgalactosylglycerol. This plant also exhibited very rapid labelling of linolenate. The distribution of radioactivity within the fatty acids of wheat diacylgalactosylglycerol remained constant after a 3h pulse-chase. On the other hand, when comparing the three plant types, pea contained the higher proportion of radioactivity in linolenate of diacylgalactosylglycerol.

Specific radioactivities of fatty acids

To gain more information about the possible source of fatty acids, as well as the interrelationship between lipids during pulse-chase experiments, we also calculated the specific radioactivities of individual fatty acids. Results for individual experiments are shown for both the monocotyledons (Table 2) and pea (Table 3). In all cases there is a very close agreement in the specific radioactivities of oleate and linoleate in different lipids isolated during the same experiment. The specific radioactivity of palmitate in phosphatidylcholine and in galactolipids was also similar for a given time interval, though agreement was not as good as for oleate and linoleate. Since linolenate was rarely labelled in phosphatidylcholine during the time course of these experiments, its specific radioactivity is only of interest with regard to the galactolipids.

Discussion

It is of interest to compare the present work with previously published data on the labelling of plant leaf lipids *in vivo*. The rapid labelling of phosphatidylcholine and diacylgalactosylglycerol has been ob-

Table 2. *Specific radioactivities of the fatty acids of phosphatidylcholine and diacylgalactosylglycerol from barley and wheat leaves labelled with [1-¹⁴C]acetate*

Specific radioactivities are calculated from radio-g.l.c. separations of the fatty acid methyl esters of purified lipids from individual experiments. Abbreviation: n.d., not detected. Values are the means for duplicate estimations that had a maximum deviation of 9% from the mean.

Plant	Lipid	Fatty acid ... Incubation time (h)	10 ⁻³ × Fatty acid radioactivity (d.p.m./μg)			
			C _{16:0}	C _{18:1} (9)	C _{18:2} (9, 12)	C _{18:3} (9, 12, 15)
Barley	Diacylgalactosylglycerol	1	3.1	3.0	0.7	0.1
	Phosphatidylcholine	1	3.8	2.4	0.7	n.d.
	Diacylgalactosylglycerol	1½	4.7	5.3	13.4	0.7
	Phosphatidylcholine	1½	4.6	5.6	13.7	n.d.
	Diacylgalactosylglycerol	2½	9.3	19.3	64.1	2.2
	Phosphatidylcholine	2½	12.8	21.8	68.0	0.5
	Diacylgalactosylglycerol	3½	6.5	24.0	65.3	2.3
	Phosphatidylcholine	3½	7.4	25.5	64.8	n.d.
	Diacylgalactosylglycerol	5½	5.6	15.4	4.3	0.9
Wheat	Phosphatidylcholine	5½	4.9	11.7	6.1	n.d.
	Diacylgalactosylglycerol	1	5.8	7.2	5.7	0.2
	Phosphatidylcholine	1	2.3	6.7	10.6	n.d.
	Diacylgalactosylglycerol	1½	2.6	4.3	3.0	0.1
	Phosphatidylcholine	1½	1.4	4.3	3.2	n.d.
	Diacylgalactosylglycerol	2½	1.7	7.6	10.4	0.5
	Phosphatidylcholine	2½	12.7	5.0	10.4	n.d.
	Diacylgalactosylglycerol	3½	8.2	34.8	45.0	3.9
	Phosphatidylcholine	3½	6.8	21.0	51.1	n.d.
	Diacylgalactosylglycerol	5½	2.6	8.8	18.9	2.2
	Phosphatidylcholine	5½	2.5	8.7	10.9	2.7

Table 3. *Specific radioactivities of the fatty acids of phosphatidylcholine, diacylgalactosylglycerol and diacylgalabiosylglycerol from pea leaves labelled with [1-¹⁴C]acetate*

For details see Table 2. Abbreviation: n.d., not detected.

Lipid	Incubation time (h)	Fatty acid ...	10 ⁻³ × Fatty acid (d.p.m./μg)			
			C _{16:0}	C _{18:1}	C _{18:2}	C _{18:3}
Diacylgalactosylglycerol	1½		2.7	14.3	3.0	0.3
Diacylgalabiosylglycerol	1½		1.9	15.1	2.7	n.d.
Phosphatidylcholine	1½		3.9	15.3	3.2	n.d.
Diacylgalactosylglycerol	2½		2.6	58.7	11.2	0.8
Diacylgalabiosylglycerol	2½		2.3	53.7	11.5	0.4
Phosphatidylcholine	2½		6.3	55.4	14.2	n.d.
Diacylgalactosylglycerol	3½		2.9	24.2	5.7	0.8
Diacylgalabiosylglycerol	3½		1.2	22.9	6.1	0.9
Phosphatidylcholine	3½		3.5	19.4	6.9	n.d.
Diacylgalactosylglycerol	5½		5.4	45.4	10.7	1.7
Diacylgalabiosylglycerol	5½		2.3	43.0	11.4	1.4
Phosphatidylcholine	5½		6.7	46.3	13.2	n.d.

served in most species studied, whether they be algae (Ferrari & Benson, 1961; Nichols *et al.*, 1967) or higher plants (Hawke *et al.*, 1974; Heinz & Harwood, 1977; Roughan, 1970; Slack & Roughan, 1975; Williams *et al.*, 1976). The relative amounts of radioactivity incorporated from [¹⁴C]acetate varied from plant to plant, and the two monocotyledons used in

the present study contained less heavily labelled phosphatidylcholine than some other species. These variations were not, apparently, due to differences in the concentrations of [¹⁴C]acetate that were used (J. Wharfe & J. L. Harwood, unpublished work).

During the experiments no significant increase was observed in the total radioactivity incorporated into

lipids on a chlorophyll basis during the pulse-chase period. This contrasted with the data of Slack & Roughan (1975), who found an increase in labelling for some time after [^{14}C]acetate had been replaced by unlabelled precursor. This difference may relate to differences in experimental methods or to the plant species used. However, our results agree in showing a small decrease in the amount of radioactivity in phosphatidylcholine and an increase in that of diacylgalactosylglycerol in pea and wheat (Table 1) that were similar in this respect to maize (Slack & Roughan, 1975).

James and his co-workers (Harris & James, 1965; Harris *et al.*, 1967) established the sequential desaturation of plant fatty acids at the C_{18} level. The individual steps of this process have not, however, been completely detailed as yet. In particular, the possible involvement of phosphatidylcholine in the desaturation of oleate and linoleate is an area of controversy. Results from algae first implicated phosphatidylcholine in oleate desaturation (Harris *et al.*, 1967; Gurr *et al.*, 1969), and this phospholipid has been shown unequivocally to be a substrate for desaturation in micro-organisms (Baker & Lynen, 1971; Pugh & Kates, 1975; Talamo *et al.*, 1973) and mammals (Pugh & Kates, 1977). Roughan (1970) suggested that the phosphatidylcholine in maize leaves could also be involved in linoleate desaturation. In previous data obtained from broad bean (Heinz & Harwood, 1977) and rye grass (Bolton & Harwood, 1978) we have argued that the lack of appreciable [^{14}C]linolenate in phosphatidylcholine during short time intervals is evidence against such an involvement. The results in Figs. 1–3 agree with this conclusion since linolenate was very poorly, if at all, labelled in phosphatidylcholine in spite of being highly radioactive in diacylgalactosylglycerol. Other data from different systems confirm this observation (Appelqvist *et al.*, 1968; Hawke *et al.*, 1974; Siebertz & Heinz, 1977; Tremolieres, 1972). The intermediate amounts of [^{14}C]linolenate in diacylgalactosylglycerol also agree with results from broad bean (Heinz & Harwood, 1977; Williams *et al.*, 1976) and may be partly due to the slow galactosylation of diacylgalactosylglycerol (Williams *et al.*, 1975).

The pulse-chase experiments by Slack & Roughan (1975) in maize gave similar overall data to the present results for fatty acid changes (Figs. 1–3) in that phosphatidylcholine was highly labelled in linoleate and showed a declining percentage of [^{14}C]oleate with time. They also observed an increase in the percentage of [^{14}C]linolenate in diacylgalactosylglycerol with time. Although this fatty acid was also poorly labelled in maize phosphatidylcholine (Hawke *et al.*, 1974; Slack & Roughan, 1975), the relatively high incorporation of radioactivity into the phospholipid in maize meant that it contained a significant proportion of the total [^{14}C]linolenate. We conclude

that it is highly unlikely, in the plant species we have studied (barley, broad bean, pea, rye grass and wheat), that phosphatidylcholine is involved in linolenate formation. Indeed, if a complex lipid is involved, then diacylgalactosylglycerol is the best candidate (Figs. 1–3 and Heinz & Harwood, 1977; Nichols, 1968; Safford & Nichols, 1970). However, in the absence of direct evidence this must remain merely as a hypothesis (cf. Harwood, 1977).

The rapid incorporation of [^{14}C]oleate and [^{14}C]linoleate into phosphatidylcholine was expected since this phospholipid characteristically contains large amounts of these radioactive acids (e.g. Gurr, 1971; Slack & Roughan, 1975). However, since oleate and linoleate thioesters will rapidly donate their fatty acids to phosphatidylcholine (Vijay & Stumpf, 1971; Abdelkader *et al.*, 1973; Tremolieres & Mazliak, 1974) this labelling may not necessarily indicate that oleate desaturation takes place on the complex lipid. The rather constant specific radioactivity of oleate and linoleate in galactolipids as well as phosphatidylcholine (Tables 2 and 3) would tend to argue against the idea that the latter is a specific substrate for oleate desaturation. Rather, they indicate that the diacylglycerol for condensation into the three lipids is from the same pool or, alternatively, that acyl exchange ensures a rapid equilibration between different lipids. In *Chlorella*, Gurr *et al.* (1969) found that the oleate and linoleate of phosphatidylcholine did not exchange, and, if a similar situation exists in the higher plants, then a common pool of diacylglycerol may be indicated. Since Joyard & Douce (1976) have observed a large quantity of diacylglycerol present in the chloroplast envelope, one possibility is that this lipid has been formed from extrachloroplastic lipids (of which phosphatidylcholine is the principal radioactive compound) by phospholipase C and other hydrolytic enzymes. Diacylgalactosylglycerol would be formed from this diacylglycerol, and such a mechanism could account for the similar specific radioactivity of the oleate and linoleate of diacylgalactosylglycerol and phosphatidylcholine. It cannot, however, explain the specific radioactivity of these acids in pea leaf diacylgalactosylglycerol (Table 3) because the latter is formed by galactosylation of diacylgalactosylglycerol. These considerations suggest that rapid fatty acyl exchange must be taking place in the systems we have studied.

Whether or not complex lipids (Slack & Roughan, 1975) or thioesters (Stumpf, 1975; Shine *et al.*, 1976) are used as substrates for desaturation in leaf tissue must await some studies with purified desaturases. The data presented in this paper seem to exclude such a role for phosphatidylcholine in linoleate desaturation.

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