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Turnover of the Glycerolipids of Pumpkin Leaves

THE IMPORTANCE OF PHOSPHATIDYLCHOLINE

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Between 1 and 5% of the ^{14}C recovered from pumpkin leaves within 15–60min after pulse-labelling with $^{14}CO_2$ was in the lipids. The specific radioactivity of the phospholipids was higher than that of the glycolipids. Phosphatidylcholine had five times the specific radioactivity of monogalactosyl diglyceride, and the specific radioactivity of neither galactolipid changed significantly between 1 and 48h after labelling. It therefore seemed unlikely that the galactose moieties of the galactolipids were involved in the transport of assimilated compounds across the chloroplast membrane. Within 60min of the application of [1-^{14}C]acetate to the surfaces of mature, intact pumpkin leaves 70% of the recovered ^{14}C was in the lipid fraction. Of the separated glycerolipids, phosphatidylcholine had by far the highest specific radioactivity at the shorter time-intervals, and the glycolipids again had the lowest specific radioactivities. Phosphatidylcholine was the only lipid to show a significant turnover of radiocarbon as judged by the decrease in specific radioactivity with time. From a comparison of the changes with time of the labelling of fatty acid fractions from phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and monogalactosyl diglyceride, it is suggested that the primary site of linolenic acid biosynthesis in leaf cells is within the phosphatidylcholine molecule.

Although the glycerolipid compositions of leaves and algae are now fairly well established, the role of the individual lipid species in the cellular structure and function remains a mystery. It is generally accepted that glycerolipids make an essential contribution to the structure of biological membranes (Benson, 1966; Green & Perdue, 1966) and hence their fatty acids make up the bulk of the lipoid phase, but as yet no specific biochemical function can be confidently attributed to any constituent of the major glycerolipids found in higher plants.

It was once supposed that α-linolenic acid, the major fatty acid in leaves, was involved in photosynthetic electron transport (Erwin & Bloch, 1963), but more recent evidence (James & Nichols, 1966; Katayama & Benson, 1967) makes this seem unlikely. Even *trans*-hexadec-3-enoic acid, which is found only in photosynthetic tissue, is unlikely to be essential to photosynthesis, since it is absent from blue-green algae (Nichols & Wood, 1968).

Nichols, James & Breuer (1967) studied the uptake of [^{14}C]acetate into the separate glycerolipids of 'greening' *Chlorella vulgaris* and concluded that those glycerolipids showing a high turnover rate of certain fatty acids (monogalactosyl diglyceride, phosphatidylglycerol, phosphatidylcholine and triglyceride) were probably involved in fatty acid biosynthesis, whereas those with a low turnover [digalactosyl diglyceride, sulpholipid (sulphoquinovosyl diglyceride), phosphatidylethanolamine and phosphatidylinositol] were probably more important as structural units. Nichols (1968) later extended this study to include fully autotrophic *Chlorella* and suggested, from the reasonably rapid metabolism, that chain elongation and desaturation, at least, were taking place within the glycerolipids rather than as CoA or acyl-carrier-protein esters of the fatty acids (Nichols *et al.* 1967; Nichols, 1968).

Much less is known about the metabolism and function of the polar end-groups of the glycerolipids of photosynthetic tissue. Benson (1963), on the basis of the kinetics of $^{14}CO_2$ incorporation into the deacylated lipids of *Chlorella pyrenoidosa* (Ferrari & Benson, 1961), proposed that the galactose of the galactolipids may be involved in sugar transport across chloroplast membranes. It was also suggested that the glycerol of phosphatidylglycerol in *Chlorella* might provide a reservoir of glycerophosphate for hexose synthesis.

Since turnover studies provide a useful initial

approach for revealing possible functions of the glycerolipids of plants, and since the only studies from which information on such turnover can be obtained have been performed with growing cultures of *Chlorella* (Ferrari & Benson, 1961; Nichols *et al.* 1967; Nichols, 1968), it was considered that some very useful comparisons could be made if it were possible to measure turnover of the separate lipids of mature leaves under natural conditions.

EXPERIMENTAL

Pumpkin plants (*Cucurbita pepo*) were grown outside and the leaves used in late summer (February and through March, 1969). Experiments were begun when periods of warm settled weather were forecast and results reported here were obtained during sequences of warm sunny days (day length approx. 12h) for the duration of the experiment. The mean daily maximum air temperature during the experiments was 24°C, the mean nightly minimum was 14°C. Plants were copiously watered daily and the young fully expanded leaves used for labelling were 900–1000 cm^2 in surface area. Experiments were begun at noon on warm sunny days.

$^{14}CO_2$ *labelling experiment.* The leaf was first shaded and then quickly enclosed in a plastic bag (36 cm × 45 cm) to which was connected inlet and outlet tubes of an air-circulating system. CO_2 containing 200 μCi of ^{14}C was generated by mixing 2ml of 1 M-H_2SO_4 with 1ml of a $NaH^{14}CO_3$ solution (26.7mCi/mmol) and circulated with air over the shaded leaf for 10min. The bag was then removed and the leaf exposed to full sunlight. Sampling was timed from the beginning of air circulation.

[1-^{14}C]*Acetate experiment.* A portion (2ml) of 1.33mM-sodium [1-^{14}C]acetate (27.4mCi/mmol), containing 100 μCi of ^{14}C, was applied evenly with a chromatogram sprayer over the surface of the leaf, which was in full sunlight. It was sometimes necessary to restrain the leaf in a horizontal position to ensure an even spread of the solution. Within 20–30min after application of the labelled solution the leaf surface appeared to be completely dry.

Sampling the leaves. At the times indicated three discs (diam. 1.4cm) were taken from each quadrant of the leaf, the selection of sites being random except that care was taken to avoid cutting a major vein. The 12 discs (fresh wt. approx. 0.5g) were ground with 6ml of chloroform–methanol (1:1, v/v) in a glass tissue grinder at room temperature and the slurry was filtered on a glass sinter under reduced pressure. Chloroform (3ml) was used to rinse out the grinder and to wash down the residue on the filter, and the filtrate (8.5ml) was washed with 0.2vol. of 0.73% NaCl (Folch, Lees & Sloane-Stanley, 1957). This gave three fractions from each sample: (1) a residue fraction, which could be recovered semi-quantitatively from the glass sinter and which consisted of cell-wall material along with most of the macromolecular constituents; (2) a water-soluble fraction, i.e. the aqueous-methanol phase of the washed chloroform–methanol extract, which represents the small-molecular-weight substances in the cells; (3) lipids, i.e. the chloroform phase of the washed chloroform–methanol extract.

Separation and analysis of labelled glycerolipids. The lipid extracts were dried in a rotary evaporator, made up to 1ml each in chloroform and samples (25–50 μl) taken for counting. Glycerolipids for analysis were separated from the remainder by a combination of DEAE-cellulose chromatography and t.l.c. (Roughan & Batt, 1968, 1969). Distributions of ^{14}C between the polar and fatty acid moieties of isolated lipids were determined after hydrolysis on the thin-layer adsorbent in 1 M-H_2SO_4 (Roughan & Batt, 1968). The ^{14}C in the hydrolysate and that remaining on the washed centrifuged silica gel were determined separately. Fatty acid methyl esters were prepared from glycerolipids eluted from thin-layer chromatograms by refluxing in methanol–benzene–conc. H_2SO_4 (30:15:2, by vol.) (Nichols, 1965) and were separated according to their number of double bonds by t.l.c. on $AgNO_3$-impregnated silica gel (Morris, 1966). Their positions on chromatograms were determined by radioautography so that appropriate zones could be recovered for scintillation counting. Qualitative analyses of the fatty acid constituents of the individual glycerolipids of pumpkin leaf were performed by separation of their methyl esters by g.l.c. (Nichols, 1965).

Measurement of radioactivity. Portions of lipid extracts were plated to infinite thinness on corrugated copper planchets and counted with 25% efficiency in a Nuclear–Chicago gas-flow counter. Residues were burned in an O_2 atmosphere to yield $^{14}CO_2$, which was trapped in methanolic ethanolamine for scintillation counting (Kalberer & Rutschmann, 1961). Glycerolipids, separated by t.l.c. and detected with iodine vapour, were transferred on the adsorbent to scintillation vials and counted in a toluene–Triton X-100–water (20:10:3, by vol.) scintillation emulsion (Patterson & Greene, 1965). Methyl esters on $AgNO_3$-impregnated silica gel, acid hydrolysates and fatty acids remaining on the silica gel after hydrolysis were all counted by the emulsion method. All scintillation counting was done at room temperature in a Packard liquid-scintillation spectrometer and counting efficiencies were continually checked by the channels-ratio method (Bush, 1963).

The basic toluene scintillation fluid, containing *p*-terphenyl (0.3%, w/v) and 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (0.01%) in toluene, gave a counting efficiency of 76–78% when tested with a [^{14}C]hexadecane standard (The Radiochemical Centre, Amersham, Bucks., U.K.) and a background of 16–17c.p.m. In agreement with Snyder (1964), a relatively high proportion of water was required in the scintillation-counting solutions in order to elute all classes of lipid from the thin-layer adsorbent. Mixtures of methanol or ethanol in toluene always produced lower count rates (d.p.m.) for the more polar lipids than the mixtures containing a high proportion of water and this was considered to be a result of absorption by the silica gel of β-particles emitted from adsorbed lipids. The emulsion technique was found to give higher efficiencies, was more stable, more economical for general use and gave the same results as those obtained with the dioxan–naphthalene–water mixture.

RESULTS

Glycerolipids of pumpkin leaf. Concentrations of the major glycerolipids of pumpkin leaf and their fatty acid compositions are shown in Table 1.

Table 1. *Glycerolipids of pumpkin leaf and their fatty acid compositions*

Glycerolipid	Amount (μmol/g fresh wt.)	% of total fatty acids							
		$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Monogalactosyl diglyceride	6.4	Trace	Trace	3	Trace	Trace	Trace	Trace	96
Digalactosyl diglyceride	4.2	Trace	Trace	9	Trace	Trace	Trace	Trace	89
Sulpholipid (sulphoquinovosyl diglyceride)	1.0	Trace	Trace	32	1	3	1	2	60
Phosphatidylcholine	1.4	Trace	Trace	23	1	6	2	8	60
Phosphatidylglycerol	1.1	Trace	Trace	27	38	5	5	5	20
Phosphatidylethanolamine	0.9	Trace	Trace	25	1	5	2	8	58
Phosphatidylinositol	0.5	7	3	37	1	6	1	3	41
Chlorophyll	2.6								

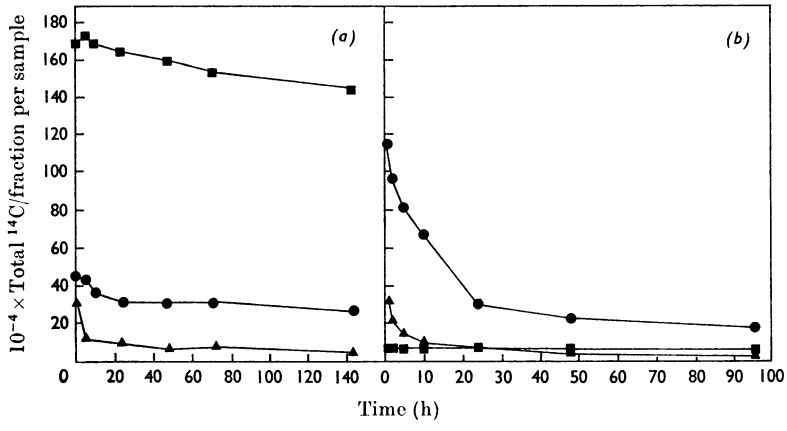

Fig. 1. Distribution of ^{14}C among the three fractions of pumpkin leaf after pulse-labelling with (*a*) [1-^{14}C] acetate and (*b*) $^{14}CO_2$. ■, Lipids; ●, residue; ▲, water-soluble fraction.

About 80–90% of the fatty acid residues of each molecular class, with the exception of phosphatidylglycerol, were made up of α-linolenic acid and palmitic acid. The large amount of a $C_{16:1}$ fatty acid in phosphatidylglycerol was presumed to be *trans*-hexadec-3-enoic acid (James & Nichols, 1966).

Distribution of ^{14}C *among leaf fractions.* At 60min after labelling with $^{14}CO_2$ the lipid fraction contained only 4.5% of the ^{14}C recovered from the leaf sample (Fig. 1*b*). In a similar experiment where the first sample was taken only 15min after the start of labelling the proportion of ^{14}C recovered in the lipids was less than 1%. The very high incorporation into the residue fraction presumably represented starch synthesis, and the subsequent loss of label from this fraction would then be attributable to starch turnover and translocation of hydrolysis products away from the leaf. A low turnover of lipids was suggested by the unchanging amount of ^{14}C in this fraction after the initial incorporation.

Almost 70% of the ^{14}C recovered from the [^{14}C]acetate-labelled leaf after 60min was located in the lipid fraction (Fig. 1*a*) and the bulk of the remainder was in the residue. This implied the ready penetration of the [^{14}C]acetate into the leaf cells under the conditions used and its very rapid incorporation into lipids. The radioactivity recovered in the separated glycerolipids was only 55% of the total for the whole lipid fraction and the pigments and non-polar lipid fractions did not contain significant activity. As no contamination of the lipid fraction by material from the 'water-soluble' fraction was observed, it seems possible that lipophilic protein material (Benson, 1966) was responsible for the radioactivity not accounted for in the lipid fraction.

Distribution of ^{14}C *among the glycerolipids of the* $^{14}CO_2$*-labelled leaf.* The phospholipids of pumpkin leaf incorporated label to a higher specific radioactivity than any of the glycolipids (Fig. 2). Phosphatidylcholine was more highly labelled than

all of the glycolipids combined and was the only glycerolipid to demonstrate a decrease in labelling during the experiment; the other non-chloroplast lipids, phosphatidylethanolamine and phosphatidylinositol, showed twofold increases in specific radioactivity. The main chloroplast lipids, monogalactosyl diglyceride, digalactosyl diglyceride, sulpholipid and phosphatidylglycerol, showed very little change in specific radioactivity between 1 and 48h. In terms of gross incorporation of ^{14}C from $^{14}CO_2$, phosphatidylcholine contained as much label after 60min as did monogalactosyl diglyceride and 1.5 times as much as did digalactosyl diglyceride (Table 2).

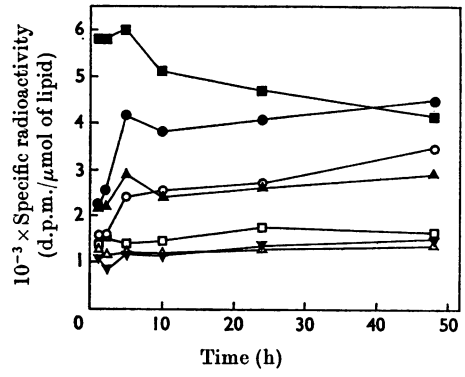

Fig. 2. Changes in labelling of the separated glycerolipids of pumpkin leaf after treatment of the leaf with $^{14}CO_2$. ●, Phosphatidylinositol; ■, phosphatidylcholine; ▲, phosphatidylglycerol; ○, phosphatidylethanolamine; □, digalactosyl diglyceride; ▼, sulpholipid; △, monogalactosyl diglyceride.

The distribution of ^{14}C between the polar and fatty acid regions of some of these lipids is shown in Table 3. The galactolipids were labelled almost exclusively in their polar moieties, and the fatty acids of the two major phospholipids contained a considerable proportion of their total ^{14}C. The apparent increase of the proportion of ^{14}C recovered in the polar region of phosphatidylcholine with time and the relatively high degree of fatty acid labelling within 60min is consistent with a high turnover of the fatty acids of this compound.

Distribution of ^{14}C among the various glycerolipids of [^{14}C]acetate-labelled pumpkin leaf. As with $^{14}CO_2$, the phospholipids of pumpkin leaf incorporated more ^{14}C from [^{14}C]acetate than the glycolipids (Fig. 3). Phosphatidylcholine was again the most highly labelled constituent 60min after application of the labelled precursor, the gross and specific radioactivities being three to four times that of any other glycerolipid (Fig. 3; Table 2). The ^{14}C content of phosphatidylcholine decreased with time exponentially, giving a half-life of approx. 20h. The other phospholipids showed much lower rates of decay of specific radioactivity and presumably had much lower turnover rates.

The specific radioactivity of monogalactosyl diglyceride, the major lipid of the leaf, increased

Table 2. *Percentage of ^{14}C recovered in individual glycerolipids of pumpkin leaves 60min after exposure to $^{14}CO_2$ or [^{14}C]acetate*

Results are expressed relative to the total ^{14}C recovered in the separated glycerolipids.

	% of total glycerolipid ^{14}C						
	Phosphatidylcholine	Phosphatidylglycerol	Phosphatidylethanolamine	Phosphatidylinositol	Monogalactosyl diglyceride	Digalactosyl diglyceride	Sulpholipid
$^{14}CO_2$	30	9	5	4	28	19	4
[^{14}C]Acetate	61	14	10	3	9	2	<1

Table 3. *Percentage of glycerolipid ^{14}C recovered in the polar moieties of glycerolipids from $^{14}CO_2$-labelled pumpkin leaf*

Time after labelling (h)	% of glycerolipid ^{14}C recovered			
	Monogalactosyl diglyceride	Digalactosyl diglyceride	Phosphatidylglycerol	Phosphatidylcholine
1	95	98	65	40
2	95	95	60	45
24	93	90	55	45
48	—	93	—	47

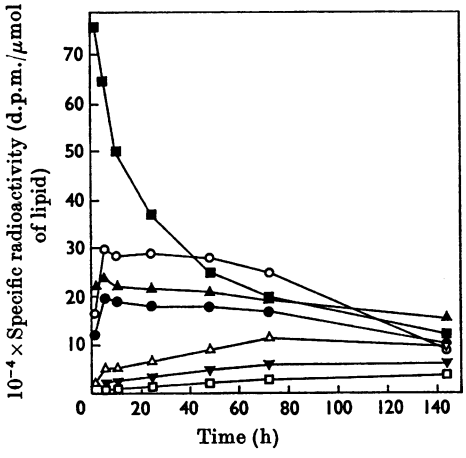

Fig. 3. Changes in labelling of the separated glycerolipids of pumpkin leaf after treatment of the leaf with [1-^{14}C]-acetate. Symbols are as in Fig. 2.

Table 4. *Percentage of* ^{14}C *recovered in fatty acid fractions of phosphatidylcholine and monogalactosyl diglyceride from* [^{14}C]*acetate-labelled pumpkin leaf*

Time after labelling (h)	% of ^{14}C recovered: Phosphatidylcholine	% of ^{14}C recovered: Monogalactosyl diglyceride
1	85	90
10	80	80
24	75	78
48	68	77
72	67	70

at a low linear rate from 10 to 72h after application of the labelled precursor and then remained unchanged during the following 72h, whereas the specific radioactivity of digalactosyl diglyceride and sulpholipid increased slowly over the whole 144h of the experiment. In fact, after 25 days the specific radioactivity of digalactosyl diglyceride was twice what it was at 144h whereas that of monogalactosyl diglyceride was 80% of the 144h value, thus emphasizing the very slow turnover of ^{14}C in these lipids.

At the shorter time-periods the greater bulk of the ^{14}C incorporated into phosphatidylcholine and monogalactosyl diglyceride was in the fatty acid moieties of the molecules (Table 4), but with increasing time the polar moieties of both glycerolipids became relatively more heavily labelled.

Labelling of specific fatty acid fractions of the [^{14}C]*acetate-labelled glycerolipids.* Radioactivities recovered in the fatty acid fractions of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and monogalactosyl diglyceride, expressed in terms of the concentration of the glycerolipid, are shown in Fig. 4. The very high incorporation of ^{14}C into linoleic acid and the monoenoic acid fraction ($C_{16:1}$+$C_{18:1}$) of phosphatidylcholine and its subsequent rapid dilution suggest a very high turnover rate for these acids in this particular glycerolipid. No other lipid demonstrated such a high rate of fatty acid turnover. Even the closely related phosphatidylethanolamine had a different pattern of incorporation and an apparently much decreased fatty acid metabolism. The rapid decrease in specific radioactivity of linoleic acid and monoenoic acids and the initial increase in linolenic acid is consistent with the biosynthetic sequence leading to linolenic acid in higher plants, namely $C_{18:1} \rightarrow C_{18:2} \rightarrow C_{18:3}$ (Harris, James & Harris, 1967), but the overall loss of ^{14}C from phosphatidylcholine would appear to require a transfer of newly synthesized linolenic acid to other glycerolipids. In support of this idea it could be shown (Table 5) that the total ^{14}C being lost from phosphatidylcholine closely matched the total ^{14}C appearing in monogalactosyl diglyceride

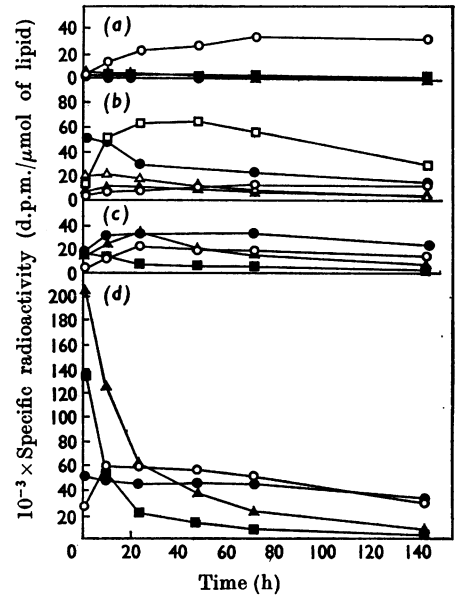

Fig. 4. Changes in labelling of fatty acid fractions from glycerolipids of [1-^{14}C]acetate-labelled pumpkin leaf. (*a*) Monogalactosyl diglyceride; (*b*) phosphatidylglycerol; (*c*) phosphatidylethanolamine; (*d*) phosphatidylcholine. Fatty acids: ●, saturated; ■, monoenoic (*cis* + *trans*); □, monoenoic (*trans*); △, monoenoic (*cis*); ▲, dienoic; ○, trienoic.

Table 5. *Amount of* ^{14}C *recovered in phosphatidylcholine, monogalactosyl diglyceride and digalactosyl diglyceride of* [^{14}C]*acetate-labelled pumpkin leaf*

Time after labelling (h)	10^{-4} × Radioactivity (d.p.m./lipid per leaf sample)			
	Phosphatidyl-glycerol	Monogalactosyl diglyceride	Digalactosyl diglyceride	Total
1	57	9	0.5	66.5
10	40	19	1.5	60.5
24	30	26	3	59
48	21	36	5	62
72	18	43	7	68

and digalactosyl diglyceride, as might be expected if the linolenic acid in those lipids came largely by acyl transfer from phosphatidylcholine.

Of the fatty acids of phosphatidylglycerol only *trans*-hexadec-3-enoic acid showed a significant turnover. The specific radioactivity of linolenic acid in monogalactosyl diglyceride increased steadily during the experiment. Saturated fatty acids of all the glycerolipids examined had lower apparent turnover rates than the unsaturated acids, and this was particularly noticeable in phosphatidylcholine. It therefore seemed likely that the bulk of the [^{14}C]acetate was incorporated, at least into phosphatidylcholine, by the chain-elongation process rather than by synthesis of long-chain fatty acids *de novo* (Stumpf & Barber, 1957).

Extended radioautography of the non-polar lipids of [^{14}C]acetate-labelled pumpkin leaf separated by t.l.c. showed that there was no significant radioactivity in those regions of the chromatograms where free fatty acids, mono-, di- and tri-glycerides would be expected.

DISCUSSION

It appears from the very slow turnover of ^{14}C in the galactolipids of pumpkin leaf that the role of these compounds in carbon dioxide assimilation processes of photosynthetic tissues does not follow that proposed by Benson (1963). Had the $^{14}CO_2$ fixed in photosynthesis been passing through the galactose moiety of these compounds then they should have had specific radioactivities at least as high as, and probably very much higher than, any of the other glycerolipids. However, the polar moiety of digalactosyl diglyceride had the lowest specific radioactivity per carbon atom of all the glycerolipids, and that of monogalactosyl diglyceride was only slightly higher. Nor did phosphatidylglycerol show a turnover rate that might suggest for it a dynamic role in the carbohydrate economy of the cells (Benson, 1963), since it had probably the slowest turnover of the phospholipids.

The discrepancies between my observations and those of Ferrari & Benson (1961) can probably best be explained in terms of the different biological systems under study and the expression of results in terms of specific radioactivity (the present study) rather than gross ^{14}C content (Ferrari & Benson, 1961). These authors were studying the uptake of $^{14}CO_2$ by growing *Chlorella* cells, and it follows that relatively large amounts of radioactivity would have been incorporated into the lipid fraction as a result of net synthesis of the individual components. Such incorporation does not necessarily denote turnover. The leaves used in the present study were fully expanded, so that uptake of label and decay of specific radioactivities were not expected to be seriously complicated by net synthesis or breakdown of the components under study.

Secondly, Ferrari & Benson (1961) based their interpretation of high turnover rates for the galactolipids and phosphatidylglycerol on the basis of gross ^{14}C content of these compounds. However, turnover can be expressed only in terms of specific radioactivity, and when relative specific radioactivities were calculated from their published data for glycerolipids separated from cells grown for 6h in $^{14}CO_2$ the order of decreasing specific activity was phosphatidylethanolamine > sulpholipid and phosphatidylinositol > digalactosyl diglyceride > phosphatidylglycerol and phosphatidylcholine > monogalactosyl diglyceride. Thus the galactolipids of *Chlorella* do not appear to occupy any relatively more important position than the same glycerolipids in pumpkin leaf. I conclude that their role in chloroplasts is largely, if not wholly, structural and that their galactose moieties are probably not in equilibrium with photosynthetic assimilation products.

The results of the [1-^{14}C]acetate study provide strong evidence that phosphatidylcholine is directly involved in the biosynthesis of α-linolenic acid, the major fatty acid of all leaves. The very high incorporation of [^{14}C]acetate (equivalent to 0.1 μmol of acetate/μmol of phosphatidylcholine) within 60min of application of the label to the leaf surface

Table 6. *Changes in specific radioactivity of α-linolenic acid associated with different pumpkin-leaf glycerolipids*

Time after application of [^{14}C]acetate (h)	10^{-3} × Radioactivity (d.p.m./μmol of $C_{18:3}$ acid)				
	Phosphatidylcholine	Phosphatidylethanolamine	Monogalactosyl diglyceride	Phosphatidylglycerol	Total
1	23	2.4	1.6	5.2	32
10	54	10	6.6	10	81
24	53	20	11.5	11.3	96
48	51	18	13.7	16.3	99
72	46	17	18	20.7	102
144	26	13	18	20.7	78

and the subsequent rapid decrease in the specific radioactivity of the lipid suggests a rapid turnover of constituent fatty acids. The changes in the labelling of the fatty acids within phosphatidylcholine showed that the reaction sequence $C_{18:1} \rightarrow C_{18:2} \rightarrow C_{18:3}$ was probably occurring, as the specific radioactivities of the phosphatidylcholine fatty acids remained much higher than those of fatty acids in the other glycerolipids and showed little tendency to equilibrate with them (Table 6). If α-linolenic acid were being synthesized by an enzyme system using CoA or acyl-carrier-protein derivatives then such large differences (14-fold between phosphatidylcholine and monogalactosyl diglyceride at 1h) in the specific radioactivities of the same material at different sites would not be expected.

If α-linolenic acid is synthesized primarily within the phosphatidylcholine molecule then it follows that this fatty acid would be incorporated into other glycerolipids by acyl transfer (Table 5). Since chloroplasts probably do not contain phosphatidylcholine (Nichols, 1963), such a mechanism may help to explain the inability to demonstrate α-linolenic acid biosynthesis in isolated chloroplasts (Stumpf & James, 1963; Stumpf, Brooks, Galliard, Hawke & Simoni, 1967), even though these organelles are particularly rich in this fatty acid.

Acyl-lipids have been suggested as possible intermediates in the biosynthesis of fatty acids in *Chlorella vulgaris* (Nichols *et al.* 1967; Nichols, 1968; Nichols & Moorehouse, 1970), but no specific glycerolipid was implicated. More recently, Gurr, Robinson & James (1969) have shown that [1-^{14}C]-oleic acid is incorporated almost exclusively into phosphatidylcholine in *Chlorella* and that desaturation to linoleic acid occurs only after a large proportion of the label has been incorporated into this lipid. My results are in agreement with this finding. Blue-green algae, which do not contain phosphatidylcholine, have no α-linoleic acid (*Anacystis nidulans*) or contain the fatty acid in much smaller amounts compared with leaves (Nichols & Wood, 1968).

This technique of labelling and then repeatedly sampling very large leaves has been successful, judged from the relative smoothness of the curves described by the experimental points: had there been any heterogeneity in the sampling then this would probably have shown up as irregularities in these curves. The application of [^{14}C]acetate to leaf surfaces was considered necessary when preliminary experiments indicated that leaves would have to be kept alive for several days after labelling, thus precluding petiole uptake as a means of introducing the precursor into the leaf (Kates, 1960; James, 1963). The main objection to this method of application would be the possibility of a net (forced) synthesis of lipids by a localized increase in the acetate concentrations in palisade and upper mesophyll cells. For this reason very low concentrations of acetate were used, and if one can assume a rapid equilibration of this material through the free space of the leaf then this objection should be largely overcome. The method does provide a means of obtaining highly labelled phosphatidylcholine which, when recovered soon after application of the precursor to the leaf surface, should provide an excellent substrate for a study of the enzymology of fatty acid desaturation in leaf preparations.

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