Interrelationships between Fatty Acid Biosynthesis and Acyl-Lipid Synthesis in *Chlorella vulgaris*

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1. Fatty acid synthesis from [2-^{14}C]acetate by *Chlorella vulgaris* cells grown and incubated in the dark is limited almost entirely to the production of saturated and monoenoic acids. 2. In light-incubated cells, both saturated and polyunsaturated fatty acids are rapidly synthesized. 3. Two groups of lipids can be distinguished in both dark- and light-incubated cells. The first group, consisting of phosphatidylglycerol, monogalactosyl diglyceride, lecithin and neutral glyceride, has a very high turnover rate for certain fatty acids. The second group, consisting of digalactosyl diglyceride, sulpholipid, phosphatidylethanolamine and phosphatidylinositol, has a slow turnover of fatty acids. 4. The lipids with rapid fatty acid turnover may be involved in the sequences of saturated and unsaturated fatty acid synthesis. A classification of lipids is made on the basis of their suggested functions.

In an earlier report James & Nichols (1966) discussed the possible functions of the acyl-lipids of a variety of photosynthetic organisms as suggested largely by a comparison of their lipid and fatty acid compositions.

More definitive information can be obtained by following the rate of turnover of individual lipids or their structural components either during cellular development or under steady-state conditions. Studies under the latter condition have been carried out with green algae by using ^{32}P-labelled metabolites (Sastry & Kates, 1965) and $^{14}CO_2$ (Ferrari & Benson, 1961).

In the present paper we describe a study of the uptake of [2-^{14}C]acetate into the acyl-lipids of *Chlorella vulgaris* on changing from growth in the dark to photoautotrophic growth. We believe our results throw some light both on fatty acid synthesis and lipid function in a developing chloroplast system.

EXPERIMENTAL

Cell cultures. *Chlorella vulgaris* was cultured in the absence of light on the 'rich medium' described by Harris, Harris & James (1965).

Incubations in the light. Cells separated from the culture medium by centrifugation (15min. at 600*g*) were twice washed with 0·2M-phosphate buffer, pH 7·4, and then shaken with a small quantity of this buffer to give a thick suspension containing about 1·5g. wet wt. of cells in 10ml. Portions (15ml.) of this suspension were placed in 25ml. conical flasks to each of which 10μc of [2-^{14}C]acetate (2·2mg.) was added, and the mixture was then incubated by shaking in the light at 27° for the desired period. The light-source consisted of four 40w strip-lamps suspended about 25cm. above the incubation flasks.

Under the conditions employed, cell division of the alga occurred approximately once every 24hr. Appreciable net synthesis of lipid probably did not occur during the incubations except when the longer incubation periods were employed.

Incubations in the dark. Incubations in the dark were carried out under the same basic conditions as those described above with the exception that light was rigorously excluded from all operations whenever possible. A small quantity of light inevitably made contact with the cells during the harvesting operation, and to minimize the possible effects of this temporary illumination the labelled metabolites were sometimes added to the cell suspension 4hr. after harvesting.

No appreciable degree of cell division occurred during incubations under these conditions.

Isolation and separation of lipids. Incubations were stopped at the appropriate time by the addition of propan-2-ol (20vol.) to the reaction mixture, which was then shaken and filtered, and the residue was extracted with chloroform–methanol (2:1, v/v). The combined filtrates were concentrated *in vacuo*, washed with 0·9% NaCl to remove water-soluble impurities (Folch, Lees & Sloane-Stanley, 1957) and redissolved in chloroform.

The lipid extracts from each incubation were fractionated by a combination of column chromatography on DEAE-cellulose and by preparative thin-layer chromatography on silica gel (Nichols & James, 1964).

Radioactivity of individual fatty acids. The purified lipid fractions were refluxed with methanol–benzene–conc. H_2SO_4 (20:10:1, by vol.) for 90min. The methyl esters of

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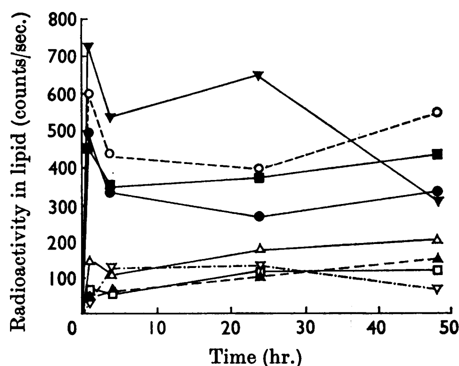


Fig. 1. Time-course of the incorporation of [2-^{14}C]acetate in the dark into the lipids of dark-grown cells of *Chlorella vulgaris*. ●, Monogalactosyl diglyceride; ▲, digalactosyl diglyceride; ○, phosphatidylglycerol; △, sulphoquinovosyl diglyceride; ■, phosphatidylcholine; ▽, phosphatidylethanolamine; □, phosphatidylinositol; ▼, neutral lipid.

the component fatty acids were extracted with light petroleum (b.p. 60–80°), dried and analysed on a radiochemical gas chromatograph with polyethylene glycol adipate as stationary phase. Comparative specific activities are expressed as ratios of radioactivity to mass peak area.

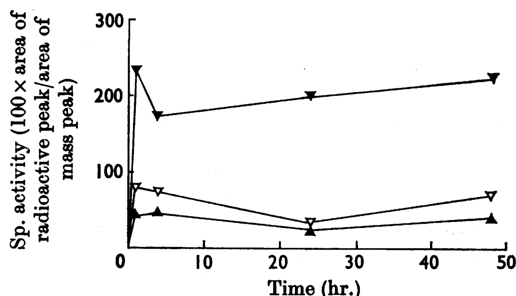


Fig. 2. Time-course of the incorporation of [2-^{14}C]acetate into the fatty acids of the phosphatidylglycerol of dark-grown *Chlorella vulgaris*. ▲, $C_{16:0}$ acid; ▼, $C_{18:0}$ acid; ▽, $C_{18:1}$ acid.

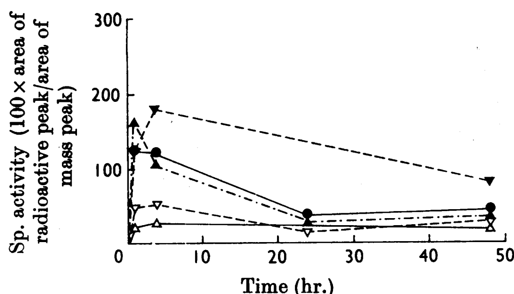


Fig. 3. Time-course of the incorporation of [2-^{14}C]acetate in the dark into the fatty acids of the monogalactosyl diglyceride of dark-grown *Chlorella vulgaris*. ●, $C_{14:0}$ acid; ▲, $C_{16:0}$ acid; △, $C_{16:1}$ acid; ▼, $C_{18:0}$ acid; ▽, $C_{18:1}$ acid.

RESULTS

Metabolism in the dark. After addition of labelled acetate to the dark-grown cells followed by incubation in the dark, all the lipids showed an initial pulse of labelling followed by a slight decline and then recovery during the following 48hr. (Fig. 1). Approx. 30% of the added acetate was converted into lipid. The maximal labelling (particularly between 1 and 4hr.) was found in the neutral lipid fraction (later found to consist primarily of a mixture of di- and tri-glycerides), phosphatidylglycerol, lecithin and monogalactosyl diglyceride. Sulpholipid, digalactosyl diglyceride, phosphatidylinositol and phosphatidylethanolamine were labelled comparatively slowly.

Only the neutral lipid fraction turned over sufficiently rapidly for the total activity to decline markedly between 24 and 48hr. In all lipids, the larger part of the radioactivity was incorporated into the fatty acid components, as might be expected from the growth conditions employed (Yung & Mudd, 1966). Of the fatty acids, stearic acid had the highest specific activity at all times (e.g. Figs. 2 and 3), but, as it is quantitatively only a minor component, the largest proportion of the radioactivity was found in oleic acid. The results are illustrated only for phosphatidylglycerol and monogalactosyl diglyceride (Figs. 2 and 3), since all the other lipids showed a very similar pattern. The peak of labelling in the first few hours was initially ascribed to allowing light to fall on the alga during addition of the labelled acetate. However, repetition of the experiment under entirely dark conditions gave essentially the same result except that the initial specific activities were lower, indicating that the pulse effect is produced by the addition of the acetate.

In the dark, little labelling of linoleic acid and linolenic acid was found in any of the lipids and synthesis stopped at oleic acid, although a small amount of labelled linoleic acid was found in the lecithin fraction. As observed by Nichols (1965*a*), no *trans*-hexadec-3-enoic acid was synthesized in the dark-grown cultures.

Effect of light. Incubation of the dark-grown cells in the phosphate buffer medium under high light-intensities gave a higher rate of incorporation (almost double that observed in the dark) into all the lipids (Fig. 4). Again two groups could be differentiated within the first few hours: (1) those with very high labelling, i.e. neutral lipid,

phatidylglycerol, lecithin and monogalactosyl diglyceride, and (2) those with much lower activities, i.e. phosphatidylinositol, phosphatidylethanolamine, sulpholipid and digalactosyl diglyceride. The maximum turnover was again achieved by the neutral glyceride fraction, though in this case the phosphatidylglycerol showed a similar pattern. However, by the end of 48hr., the activity of the phosphatidylethanolamine was equal to that of phosphatidylglycerol, and the activity of the digalactosyl diglyceride equal to that of the monogalactosyl diglyceride.

The change of specific activity with time of the fatty acids of the phosphatidylglycerol fraction is given in Fig. 5. There was an initial sharp rise in specific activity (in the first hour) of myristic acid, palmitic acid, oleic acid and stearic acid, this high specific activity decaying rapidly during the next 3hr. and then slowly up to 24hr. and stayed constant thereafter. The maximum specific activity of the only fatty acid specifically found in phosphatidylglycerol, namely *trans*-hexadec-3-enoic acid (Weenink & Shorland, 1964; Allen, Good, Davis & Fowler, 1964; Haverkate, 1965; Nichols, 1965*a*,*b*; Haverkate & van Deenen, 1965), was found at 4hr.

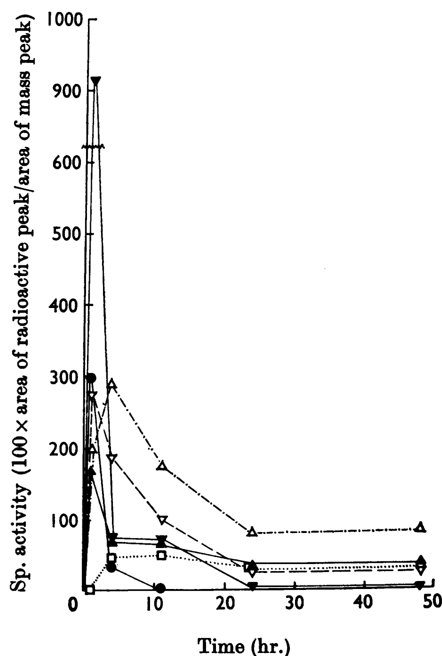


Fig. 5. Time-course of the incorporation of [2-^{14}C]acetate in the light into the fatty acids of phosphatidylglycerol, expressed as specific activity. ●, $C_{14:0}$ acid; ▲, $C_{16:0}$ acid; △, *trans*-Δ^3-$C_{16:1}$ acid; ▼, $C_{18:0}$ acid; ▽, $C_{18:1}$ acid; □, $C_{18:2}$ acid.

and decreased thereafter, its rate of decrease being similar to that of oleic acid. Stearic acid had a particularly rapid rate of fall of specific activity.

In Fig. 6 the results are expressed as the percentage of total fatty acid radioactivity found in each fatty acid of the phosphatidylglycerol fraction, so taking account of the different masses of each acid. Figs. 5 and 6 show a steady synthesis of linoleic acid during the first 11hr. and a rapid decrease in the radioactivity of oleic acid in this lipid after 11hr. The larger part of the radioactivity at all times was found in palmitic acid, the fall of radioactivity in which in the first few hours was balanced by an increase in *trans*-hexadec-3-enoic acid. This is not surprising, since Nichols, Harris & James (1965) have already demonstrated that in *Chlorella vulgaris* palmitic acid is the direct precursor of this acid, and it is possible that the palmitic acid so desaturated is that already combined in the phosphatidylglycerol molecule.

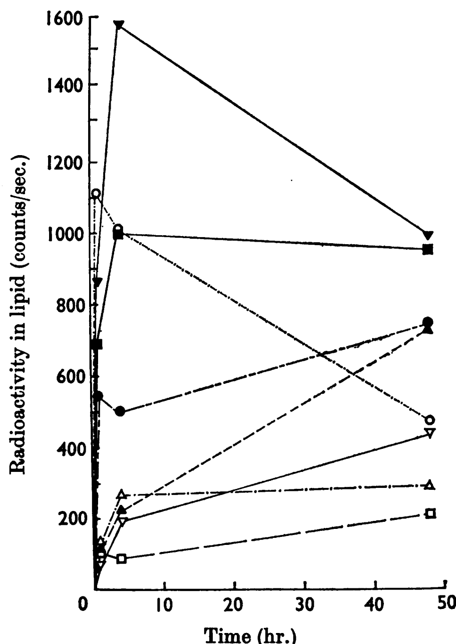


Fig. 4. Time-course of the incorporation of [2-^{14}C]acetate in the light into the lipids of dark-grown cells of *Chlorella vulgaris*. ●, Monogalactosyl diglyceride; ▲, digalactosyl diglyceride; ○, phosphatidylglycerol; △, sulphoquinovosyl diglyceride; ■, phosphatidylcholine; ▽, phosphatidylethanolamine; □, phosphatidylinositol; ▼, neutral lipid.

Similar results are given in Figs. 7 and 8 for the fatty acids of the monogalactosyl diglyceride. Again there was a very rapid flux of labelled stearic acid and of palmitic acid in the first 4hr. The radioactivity distribution data (Fig. 8) show that the loss

of radioactivity from oleic acid was, as expected, almost balanced by the gain in radioactivity of both linoleic acid and linolenic acid.

The changes in fatty acid composition of the monogalactosyl diglyceride during the incubation are shown in Fig. 9. As with the incorporation data, the major changes were completed within 24hr. Data on the fatty acid composition of all the *Chlorella* lipids at the beginning and after 24hr. are shown in Table 1. As described by Nichols (1965*a*),

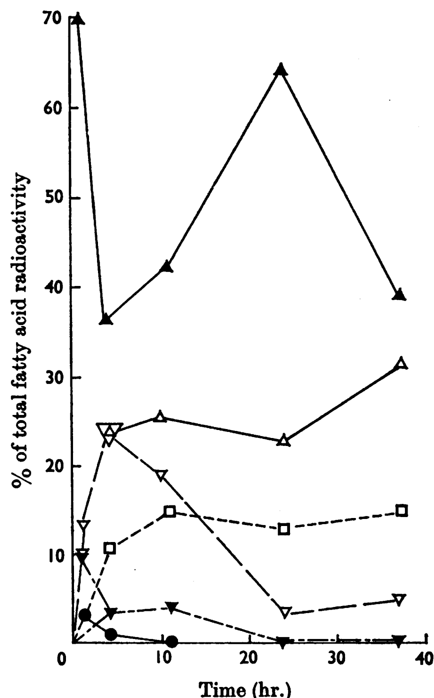


Fig. 6. Time-course of changes in the percentage distribution of total fatty acid radioactivity among the individual fatty acids of phosphatidylglycerol synthesized in the light. ●, $C_{14:0}$ acid; ▲, $C_{16:0}$ acid; △, *trans*-Δ^3-$C_{16:1}$ acid; ▼, $C_{18:0}$ acid; ▽, $C_{18:1}$ acid; □, $C_{18:2}$ acid.

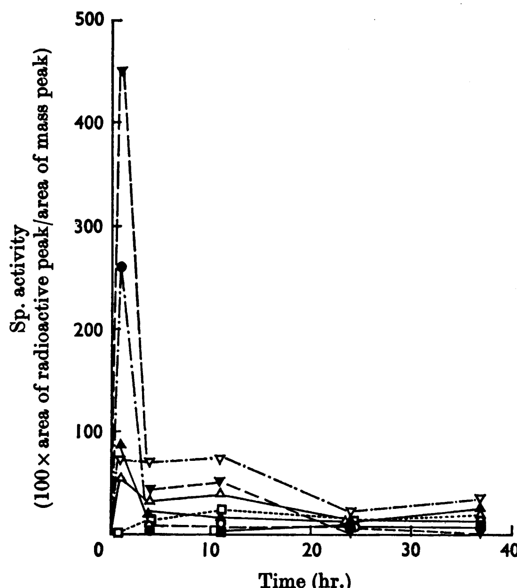


Fig. 7. Time-course of the incorporation of [2-^{14}C]acetate into the fatty acids of monogalactosyl diglyceride synthesized in the light, expressed as specific activity. ●, $C_{14:0}$ acid; ▲, $C_{16:0}$ acid; △, $C_{16:1}$ acid; ○, $C_{16:2}$ acid; ▼, $C_{18:0}$ acid; ▽, $C_{18:1}$ acid; □, $C_{18:2}$ acid; ■, $C_{18:3}$ acid.

Table 1. *Changes in the fatty acid composition of the individual lipids of* Chlorella vulgaris *when dark-grown cells are illuminated for 24hr.*

Lipid	Hours of light	Fatty acid composition (%, by wt.)									
		$C_{14:0}$ acid	$C_{16:0}$ acid	$C_{18:0}$ acid	Δ^9-$C_{16:1}$ acid	Δ^3-$C_{16:1}$ acid	$C_{18:1}$ acid	$C_{16:2}$ acid	$C_{18:2}$ acid	$C_{16:3}$ acid	$C_{18:3}$ acid
Monogalactosyl diglyceride	0	2·1	4·3	2·1	10·1	0	10·2	23·5	40·1	2·1	6·0
	24	2·1	3·2	2·1	5·2	0	3·8	20·9	32·6	4·1	26·0
Digalactosyl diglyceride	0	3·1	11·1	0	6·6	0	19·1	8·8	42·0	0	8·5
	24	1·9	6·2	0	4·9	0	6·2	8·0	48·5	2·1	22·0
Phosphatidylglycerol	0	1·3	54·3	1·3	0	2·0	15·8	2·5	21·0	0	2·3
	24	2·1	46·0	trace	0	14·0	7·8	2·1	24·3	0	5·8
Sulphoquinovosyl diglyceride	0	2·7	36·3	3·6	4·2	0	12·7	2·6	31·7	0	5·0
	24	2·7	36·9	1·5	5·3	0	8·6	2·7	33·2	0	8·7
Phosphatidylethanolamine	0	0	24·2	2·2	2·9	0	8·9	0	53·0	0	6·2
	24	0	29·6	1·4	2·2	0	7·1	0	54·1	0	4·6
Phosphatidylcholine	0	0	11·1	3·4	4·6	0	15·6	4·9	52·3	0	7·4
	24	0	14·3	2·4	6·0	0	11·3	3·4	53·5	0	10·2
Phosphatidylinositol	0	2·0	41·1	3·6	3·6	0	14·1	2·7	29·1	0	3·6
	24	1·3	52·0	2·0	4·0	0	9·6	2·5	24·0	0	3·3

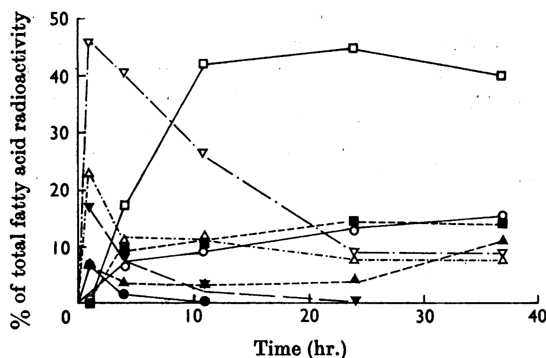


Fig. 8. Time-course of changes in the percentage distribution of total radioactivity among the individual fatty acids of monogalactosyl diglyceride synthesized in the light. ●, $C_{14:0}$ acid; ▲, $C_{16:0}$ acid; △, $C_{16:1}$ acid; ○, $C_{16:2}$ acid; ▼, $C_{18:0}$ acid; ▽, $C_{18:1}$ acid; □, $C_{18:2}$ acid; ■, $C_{18:3}$ acid.

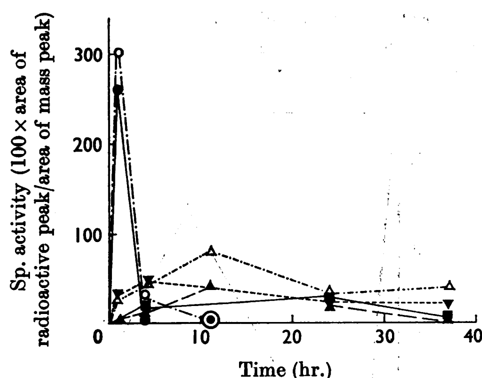


Fig. 10. Comparison of the changes in specific activity with time of myristic acid in each of the *Chlorella vulgaris* lipids during the incubation. ●, Monogalactosyl diglyceride; ▲, digalactosyl diglyceride; ○, phosphatidylglycerol; △, sulphoquinovosyl diglyceride; ■, phosphatidylcholine; ▼, neutral lipid.

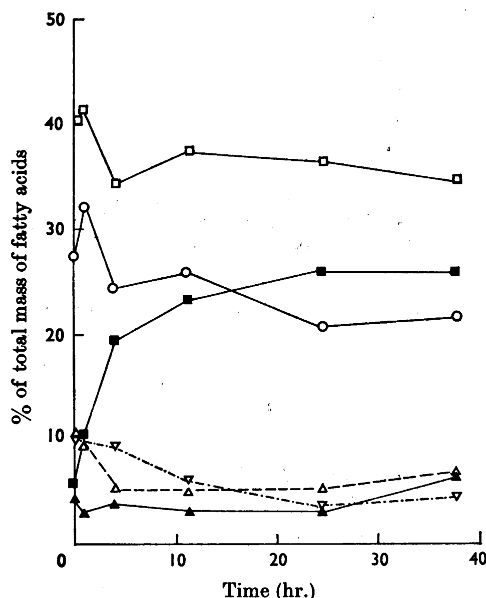


Fig. 9. Time-course of changes in percentage fatty acid composition of monogalactosyl diglyceride during the incubation. ▲, $C_{16:0}$ acid; △, $C_{16:1}$ acid; ○, $C_{16:2}$ acid; ▽, $C_{18:1}$ acid; □, $C_{18:2}$ acid; ■, $C_{18:3}$ acid.

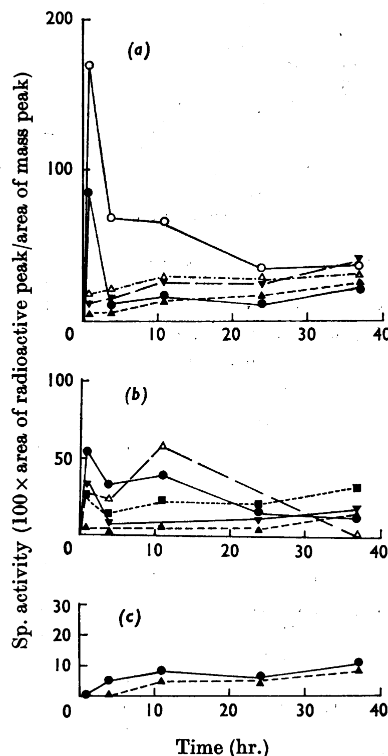


Fig. 11. Comparison of the changes in specific activity with time of (*a*) palmitic acid, (*b*) the C_{16} monoenoic acids and (*c*) hexadeca-7,10-dienoic acid synthesized from [2-^{14}C]acetate in each of the *Chlorella* lipids during the incubation. ●, Monogalactosyl diglyceride; ▲, digalactosyl diglyceride; ○, phosphatidylglycerol; △, sulphoquinovosyl diglyceride; ■, phosphatidylcholine; ▼, neutral lipid.

the major changes were found in the monogalactosyl and digalactosyl diglycerides and in phosphatidylglycerol (particularly so far as *trans*-hexadec-3-enoic acid is concerned). Sulpholipid and phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol showed comparatively minor changes in composition.

Comparison of the fatty acid labelling in the

different lipids is given in the next series of Figures, where the specific activities of a selected acid at different times are plotted for each of the *Chlorella* lipids. Fig. 10 shows that myristic acid had a high turnover rate only in phosphatidylglycerol and monogalactosyl diglyceride.

Figs. 11(*a*), 11(*b*) and 11(*c*) show the results obtained for palmitic acid, palmitoleic acid and the hexdecadienoic acid. Both phosphatidylglycerol and monogalactosyl diglyceride showed a considerable turnover of palmitic acid, less so for palmitoleic acid, and the other lipid fractions showed in both cases an increase in specific activity that levelled off after 11hr. The hexadecadienoic acid was appreciably labelled only in the galactosyl glycerides.

In Fig. 12, the results for stearic acid show it to have a very fast turnover in phosphatidylglycerol, phosphatidylcholine, monogalactosyl diglyceride and neutral glyceride, with its maximum occurring after only 1hr. The peak of specific activity of the stearic acid in the digalactosyl lipid occurred much later. All other lipids showed distinct but slower turnover of stearic acid, whose specific activity dropped almost to zero at 24hr.

Fig. 13 shows the data for oleic acid; again the maximum turnover was found in phosphatidylglycerol, a lower rate occurring in lecithin and monogalactosyl diglyceride. In this case, the maximum labelling in the lecithin was found at 11hr., whereas in the other two lipids it was found at 1hr. together with a faster rate of labelling. A similar but lower maximum was found in digalactosyl diglyceride and phosphatidylethanolamine fractions.

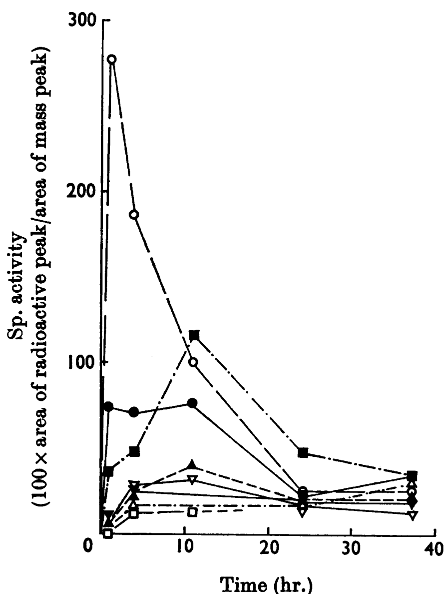


Fig. 13. Comparison of the changes in specific activity with time of the oleic acid synthesized from [2-^{14}C]acetate in each of the *Chlorella* lipids. ●, Monogalactosyl diglyceride; ▲, digalactosyl diglyceride; ○, phosphatidylglycerol; △, sulphoquinovosyl diglyceride; ■, phosphatidylcholine; ▽, phosphatidylethanolamine; □, phosphatidylinositol; ▼, neutral lipid.

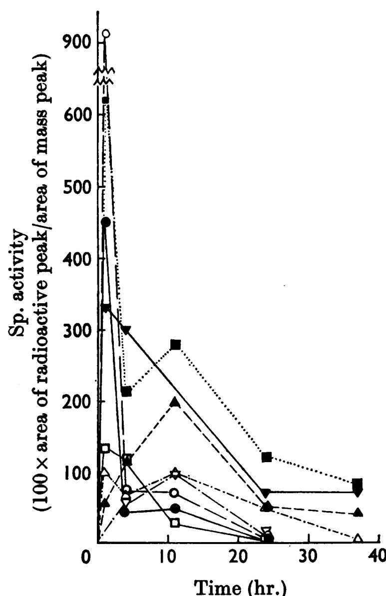


Fig. 12. Comparison of the changes in specific activity with time of the stearic acid synthesized from [2-^{14}C]acetate in each of the *Chlorella* lipids. ●, Monogalactosyl diglyceride; ▲, digalactosyl diglyceride; ○, phosphatidylglycerol; △, sulphoquinovosyl diglyceride; ■, phosphatidylcholine; ▽, phosphatidylethanolamine; □, phosphatidylinositol; ▼, neutral lipid.

The results for linoleic acid given in Fig. 14 show a similar pattern to those for oleic acid except that the digalactosyl diglyceride, neutral glyceride, sulpholipid, phosphatidylethanolamine, phosphatidylinositol and phosphatidylcholine showed no peak of labelling but instead a slow climb. Only phosphatidylglycerol and monogalactosyl diglyceride showed a peak of specific activity, both at about 21hr.

The fatty acid at the end of the synthetic sequence, α-linolenic acid, gave a very different pattern (Fig. 15). In this case, phosphatidylglycerol was virtually unlabelled and the major linolenic acid-containing lipid, digalactosyl diglyceride, had a slow steady increase in labelling throughout the whole period of incubation. Linolenic acid was labelled most rapidly in the mono-

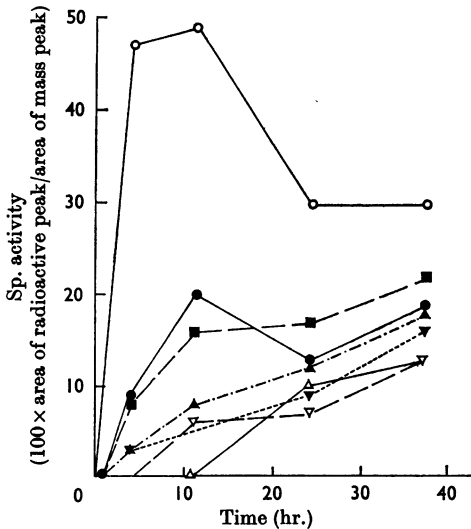


Fig. 14. Comparison of the changes in specific activity with time of the linoleic acid synthesized from [2-^{14}C]acetate in each of the *Chlorella* lipids. ●, Monogalactosyl diglyceride; ▲, digalactosyl diglyceride; ○, phosphatidylglycerol; △, sulphoquinovosyl diglyceride; ■, phosphatidylcholine; ▽, phosphatidylethanolamine; ▼, neutral lipid.

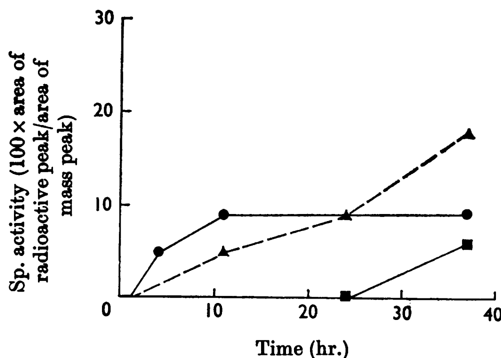


Fig. 15. Comparison of the changes in specific activity with time of the linolenic acid synthesized from [2-^{14}C]acetate in *Chlorella* lipids. ●, Monogalactosyl diglyceride; ▲, digalactosyl diglyceride; ■, phosphatidylcholine.

galactosyl diglyceride and neutral lipid fractions, reaching its maximum at 11hr. for the monogalactosyl diglyceride and at 24hr. for the neutral lipid, thereafter both remaining steady and identical. Lecithin, on the other hand, showed labelling only after 37hr.

Repetition of the above experiments but with sample withdrawal times of 10, 20, 40 and 80min. gave results in agreement with those above.

DISCUSSION

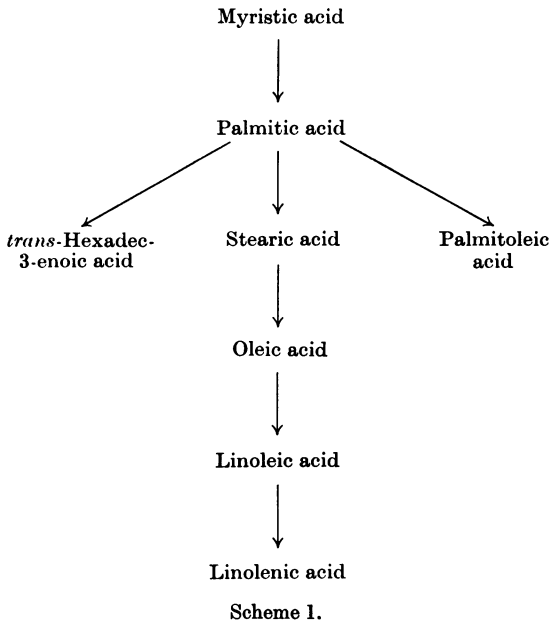
Lipid turnover in the dark. The division of the lipids into two groups based on the rate of labelling of their fatty acids (Fig. 1) suggests that they have a difference in function. A plausible explanation would be that the phosphatidylethanolamine, phosphatidylinositol, digalactosyl diglyceride and sulpholipid act under these growth conditions as structural components of cellular organelles, whereas the phosphatidylglycerol, neutral glyceride, monogalactosyl diglyceride and phosphatidylcholine may have some metabolic as well as structural function. The fall in radioactivity of most of the lipids between 1 and 4hr. could be explained by an initial stimulation of lipid synthesis by the added labelled acetate causing withdrawal of carbon from other metabolic sources to give unlabelled acetate. This would cause a dilution of the acetate pool followed by an apparently slower lipid synthesis from the acetate of lower specific activity.

The consistently high specific activity of stearic acid in phosphatidylglycerol and monogalactosyl diglyceride shows that in the dark synthesis of oleic acid was partially inhibited and that of linoleic acid and linolenic acid was almost completely inhibited. There was distinct labelling of linoleic acid in the phosphatidylcholine fraction at 24hr., but elsewhere only trace labelling could be detected (cf. Harris, James & Harris, 1967).

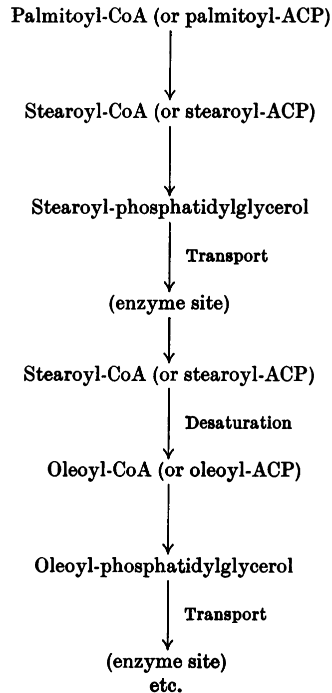
Effect of light. The suggestion of the two functions of the two lipid groups in the dark incubation is supported by the similar picture of lipid synthesis found in the light-incubated system (Fig. 4). However, the turnover rate in each lipid varies with the fatty acid. The decreasing order of turnover rate is: stearic acid, myristic acid, oleic acid, palmitic acid, palmitoleic acid, linoleic acid, linolenic acid, hexadecadienoic acid (Figs. 11, 12, 13, 14 and 15). In Table 2 are shown those lipids having a high rate of turnover of the intermediary acids of the synthetic sequence shown in Scheme 1. This sequence has been described in a number of papers (Harris & James, 1965; Harris *et al.* 1965). Thus all the lipids in Table 2 can be considered as 'carriers' of stearic acid and palmitoleic acid, whereas only phosphatidylglycerol and monogalactosyl diglyceride appear to function for myristic acid and palmitic acid. Oleic acid and linoleic acid turn over rapidly in phosphatidylglycerol, monogalactosyl diglyceride and lecithin. The other lipids, namely phosphatidylethanolamine, phosphatidylinositol, sulpholipid and digalactosyl diglyceride, have either lower turnover rates or a steady increase in specific activity, suggesting slow synthesis. The maximum turnover rates are so high as to suggest that the acyl-lipid is behaving in a manner similar to that expected

Table 2. *Lipids showing high turnover rate of specified fatty acids*

Lipid	Myristic acid	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid
Phosphatidylglycerol	+	+	+	+	+	+
Monogalactosyl diglyceride	+	+	+	+	+	+
Phosphatidylcholine			+	+	+	+
Neutral glyceride			+	+		

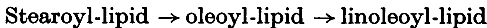


Scheme 1.



Scheme 2. ACP, Acyl-carrier protein.

for a true intermediate in the fatty acid synthetic sequence. This could be achieved in two distinct ways. First, the acyl-lipid could act as a transport agent moving the acid between enzyme locations, giving a sequence as shown in Scheme 2. Secondly, but less likely, the acyl-lipid could act as the true substrate for the desaturase, in which case the sequence would be:


Stearoyl-lipid → oleoyl-lipid → linoleoyl-lipid

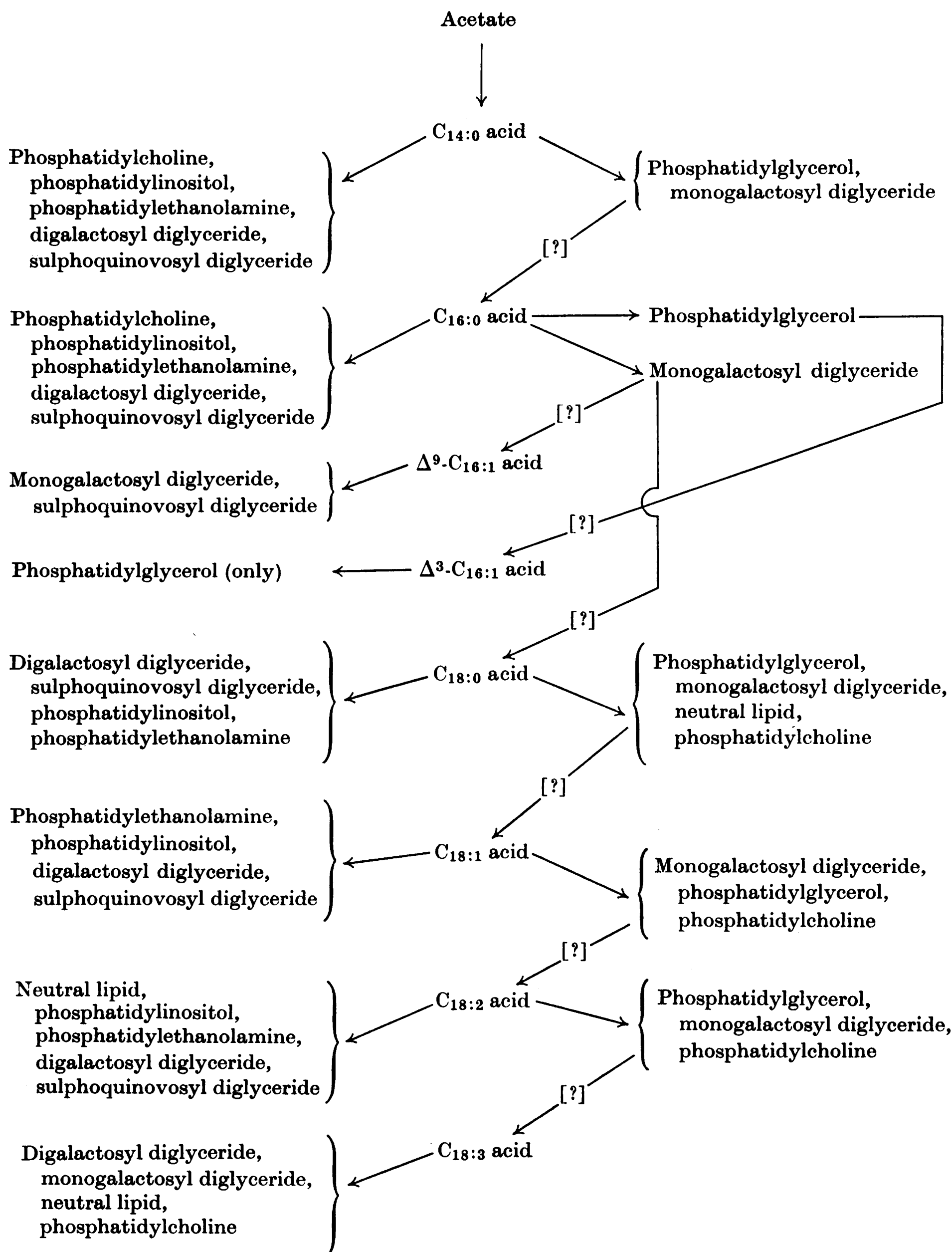
Scheme 3 shows sufficient detail to account for the experimental results.

Acyl migration, transferring fatty acids between lipids, could occur at any stage. However, the time-sequence of labelling in the two groups of lipids is so different that acyl migration of the rapidly turning-over fatty acids between the groups seems unlikely, though within a group this is possible.

The results given here do not permit decision to be made between these alternatives, but would best fit the second proposal. Since we do not know the precise nature of the enzyme substrates, which could be acyl-CoA, acyl-carrier protein (Vagelos, Alberts & Majerus, 1965; Nagai & Bloch, 1965, 1966) or acyl-lipid, they are labelled in the schematic diagram comprising Scheme 3 as '[?]'.

Ferrari & Benson (1961) described the uptake of $^{14}CO_2$ into *Chlorella pyrenoidosa* during steady-state synthesis. The maximum labelling in periods up to 6hr. was found in monogalactosyl diglyceride, a mixture of phosphatidylglycerol and phosphatidylcholine, digalactosyl diglyceride, and a mixture of sulpholipid and phosphatidylinositol. The time curves for incorporation of label into mono- and digalactosyl diglyceride suggested the following relationship:

UDP-galactose + monogalactosyl diglyceride → digalactosyl diglyceride



Scheme 3.

Our results would agree with this sequence, since the specific activities of all the major acids in the two lipids coincide at the end of the incubation period, but the process of equilibration is very slow. The fact that the two galactosyl diglycerides invariably have significant differences in fatty acid composition could be accounted for either by a certain amount of deacylation and reacylation of

these lipids or by a specific metabolic conversion of distinct molecular species of monogalactosyl diglyceride (Nichols, 1965*b*).

The results reported by Ferrari & Benson (1961) for the phospholipids are also in general agreement with ours in that the decreasing order of labelling was: phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine. Our results do not agree with the suggestion that phosphatidylglycerol provides an acylated phosphatidyl group for phosphatidylinositol. Our fatty acid data show that phosphatidylethanolamine and phosphatidylinositol have fatty acids of similar specific activities and turnover rates, but that phosphatidylglycerol and phosphatidylcholine turn over more rapidly. This is in general agreement with the ^{32}P-uptake studies carried out with *Chlorella vulgaris* by Sastry & Kates (1965). They found phosphatidylglycerol to have a very fast turnover, with phosphatidylcholine the next most active phospholipid. However, it should be remembered that these authors were studying steady-state photosynthesis in light-grown cultures, whereas we were studying a transition from dark-grown cultures to autotrophic growth in the light.

By combining all these results, the lipids of a green alga might be classified in their possible functions as shown in Table 3.

The great variation of fatty acid turnover rate among these lipids does not support the concept of a common diglyceride pool except for phosphatidylethanolamine and phosphatidylinositol, but even these have radically different fatty acid compositions (Table 1).

At all times during the studies reported here the composition and specific activities of the acids from the corresponding diglyceride and triglyceride fractions showed little similarity, although both fractions were fairly rapidly labelled (faster than phosphatidylethanolamine, phosphatidylinositol and digalactosyl diglyceride).

No direct evidence has been obtained with regard to the function of the diglyceride, but in all probability this fraction is a mixture of that diglyceride which acts as a precursor of many of the complex lipids, and that derived from these lipids by the action of lipases. It is surprising that the triglyceride fraction is so rapidly labelled, since it would be normally considered to have the rather static role of energy storage.

Our results, in conjunction with those of others, point to the necessity for future work designed for

Table 3. *Classification of suggested functions of lipids*

Lipid	Metabolic function	Structural function
Monogalactosyl diglyceride	(*a*) Involved in biosynthesis of the C_{14}, C_{16} and C_{18} saturated acids, and the C_{16} and C_{18} unsaturated acids (*b*) Involved in galactose metabolism (Ferrari & Benson, 1961)	Major component of chloroplast lamellae (Allen, Hirayama & Good, 1966)
Digalactosyl diglyceride	Involved in galactose metabolism (Ferrari & Benson, 1961)	Major component of chloroplast lamellae (Allen *et al.* 1966)
Sulpholipid	Suggested function as a sulphur and carbon reserve material (Miyachi & Miyachi, 1966)	Major component of chloroplast lamellae (Allen *et al.* 1966)
Phosphatidylglycerol	(*a*) Involved in biosynthesis of the C_{14}, C_{16} and C_{18} saturated acids, *trans*-hexadec-3-enoic acid, and C_{18} mono- and di-enoic acids (*b*) Involved in phosphate metabolism (Sastry & Kates, 1965)	Major component of chloroplast lamellae (Allen *et al.* 1966)
Phosphatidylcholine	(*a*) Involved in the biosynthesis of the C_{18} unsaturated fatty acids (*b*) Involved in phosphate metabolism (Sastry & Kates, 1965)	Possibly minor component of chloroplast (Allen *et al.* 1966)
Phosphatidylethanolamine	Probably no function in chloroplasts; may be involved in phosphate metabolism in other organelles	Absent from chloroplasts (Allen *et al.* 1966)
Phosphatidylinositol	As for phosphatidylethanolamine; possibly involved in inositol metabolism (Ferrari & Benson, 1961)	May be very minor component of chloroplast lamellae (Allen *et al.* 1966)
Neutral glyceride	Diglyceride involved in C_{18} acid metabolism and probably involved in phospholipid synthesis; triglyceride is involved in C_{18} acid metabolism	Minor component of lamellae

relating function to individual molecular species within a lipid class. The simplest structural functions may reside mainly in those molecular species in a lipid class having a low turnover rate. However, because chloroplast lamellae are presumably involved not only in photosynthetic processes but also in transport processes for the products, certain of the lipids having a high turnover rate might also have an important structural function.

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