CHAPTER 9

The Function and Metabolism of Fatty Acids and Acyl Lipids in Chloroplasts

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I. Introduction

Although recent years have seen major advances in our knowledge of the lipid chemistry and biochemistry of photosynthetic tissues, much of the work has been performed on intact cell preparations rather than on isolated chloroplasts. To a large extent this is a reflection of the difficulty experienced in isolating plastids with their full metabolic capacities unimpaired but metabolic studies on whole cells can give data which, carefully interpreted, may yield much useful information regarding plastid metabolism.

II. Acyl Lipids of Chloroplasts

The photosynthetic tissues of plants contain a complex variety of neutral glycerides, phospholipids and glycolipids (Fig. 1). The higher algae possess similar lipids but lack both sterol glycosides and cerebrosides.

Several groups of workers, however, (Benson and Maruo, 1958; Nichols,

1963) have shown that leaf chloroplasts possess a much simpler acyl lipid composition than the whole cell and that they contain only four acyl lipids in major proportions. These comprise three glycolipids (mono- and di-galactosyl diglyceride and sulphoquinovosyl diglyceride) and one phospholipid (phosphatidyl glycerol), the structures of which are depicted in Fig. 2.

The presence of only these four acyl lipids in chloroplasts has now been established for a variety of plants and the generalization would seem to be valid. To our knowledge, the lipid compositions of isolated algal chloroplasts have not been studied and data for whole cells is usually assumed to

Phospholipids	
Phosphatidyl glycerol	Phosphatidyl inositol
Phosphatidyl choline (lecithin)	Cardiolipin
Phosphatidyl ethanolamine	Phosphatidic acid
Phosphatidyl serine	
Glycolipids	
Monogalactosyl diglyceride	Cerebroside
Digalactosyl diglyceride	Sterol glycoside ester
Sulphoquinovosyl diglyceride (Sulpholipid)	
Other lipids	
Diglyceride	Triglyceride
	Sterol ester

FIG. 1 The acyl lipids of plant leaves.

be sufficient. An indication that this assumption is acceptable comes from our own studies on the lipid composition of the blue-green algae where the major sub-cellular particles are chloroplast lamellae. The major acyl lipids of these blue-green algae have been shown to be the same four lipids found in leaf chloroplasts which are known to be primarily concentrated in the lamellae (Nichols *et al.*, 1965a; Allen *et al.*, 1966; Nichols and Wood, 1968).

The question of whether lecithin is present in chloroplasts is an interesting one and arises from the difficulty experienced in isolating uncontaminated chloroplasts from leaves using aqueous media. The proportion of lecithin in such preparations is relatively small compared with that of the major chloroplast lipids and the purer the preparation, the smaller does this proportion become. So far, there have been no reports of leaf chloroplast preparations which are free from lecithin and Allen and co-workers have even detected its presence in preparations of spinach chloroplast lamellae (Allen *et al.*, 1966). Indirect evidence for the presence of lecithin in chloroplasts is the reported relationship in photosynthetic tissue between lecithin and the synthesis of linoleic acid and the latter is known to be associated with the plastid fractions (Harris *et al.*, 1967).

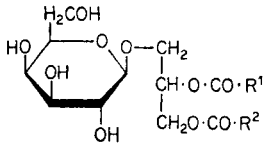
The major portion of the lecithin fraction from leaves, however, undoubtedly

originates from the mitochondrial-microsomal fractions and one might presume that its function in these particles would be different from that in the chloroplast. The lecithin fraction from these different classes of particle might, therefore, be expected to have differing fatty acid compositions and to be synthesized and metabolized at different rates. Such differences have yet to be established. We have studied the lecithin fractions from chloroplast and "mitochondrial" preparations from a variety of leaf tissues which had been incubated with ^{14}C-labelled acetate. At all times the fatty acid composition and the specific activities of these different fractions from the same plant were found to be identical. The lecithins are thus either freely exchangeable between cellular organelles or are synthesized in a single site and then transferred to other sites. Other evidence that lecithin is not always essential for normal photosynthetic function is provided by the blue-green algae which, although apparently photosynthesizing in a manner similar to that of green algae and higher plants, contain only the four "chloroplast lipids" and no lecithin (Nichols *et al.*, 1965a; Allen *et al.*, 1966; Nichols and Wood, 1968).

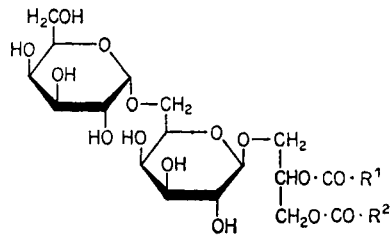
The proplastid bodies present in etiolated leaves are even more difficult to prepare than the chloroplasts. There is consequently little published information regarding their lipid composition although studies involving whole etiolated leaves indicate that they contain the same lipid classes but in different relative proportions (B. W. Nichols, unpublished results).

III. Fatty Acids of Chloroplasts

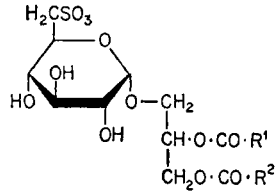
Leaf chloroplasts contain a high proportion of polyenoic acids, particularly those of the C_{18} series (James and Nichols, 1966) (Table I). There are greater variations in fatty acid composition of chloroplasts from different plant species than in the corresponding acyl lipid compositions. Thus, some chloroplasts such as those from spinach (Allen *et al.*, 1966) and tobacco (B. W. Nichols, unpublished results) contain significant quantities of the corresponding C_{16} polyenoic acids, while the chloroplasts of marine algae (Kates and Volcani, 1966; Klenk *et al.*, 1963) and the pteridophyta (Schlenk and Gellerman, 1965; Wolf *et al.*, 1966; Nichols, 1965a; Radunz, 1967) frequently contain polyenoic acids of the C_{20} series, including arachidonic acid. The major C_{16} monoenoic acids of leaves and the higher algae are of the Δ^9 and Δ^7 varieties (e.g. Klenk *et al.*, 1963; Schlenk and Gellerman, 1965) and these tissues also always contain small yet significant quantities of a unique C_{16} acid which contains a *trans* double bond in the 3-position (e.g. Nichols *et al.*, 1965b). The proplastid bodies of dark-grown leaves also contain relatively large proportions of polyenoic acids, although rather less than in chloroplasts, but the *trans*-3-hexadecenoic acid is absent from etiolated tissues (Nichols *et al.*, 1965c; Nichols, 1965b).



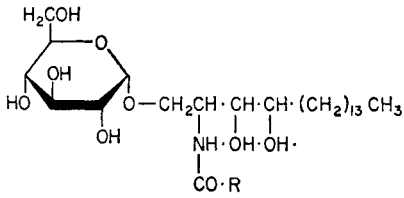
Monogalactosyl diglyceride [β-D-galactosyl-(1→1′)-2′, 3′-diacyl-D-glycerol]



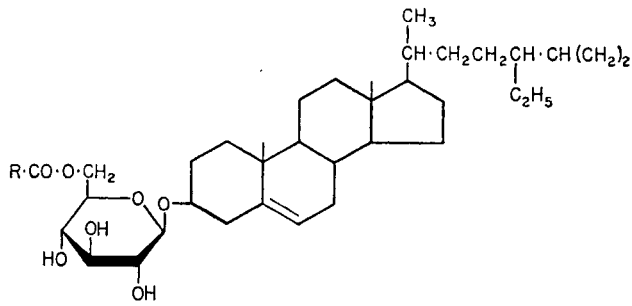
Digalactosyl diglyceride [α-D-galactosyl-(1-6)-β-D-galactosyl-(1-1′)-2′, 3′-diâcyl-D-glycerol]



Sulphoquinovosyl diglyceride [6-Sulpho-α-D-quinovosyl-(1-1′)-2′, 3′-diacyl-D-glycerol]

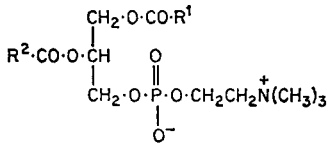


Glucocerebroside N-Acyl-α-D-glucosyl-(1-1′)-phytosphingosine

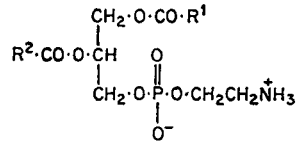


6-Acyl-β-D-glucopyranosyl (1-3′)-β-sitosterol

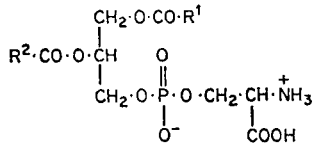
FIG. 2



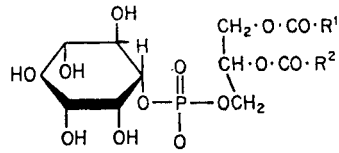
Phosphatidyl choline (Lecithin)



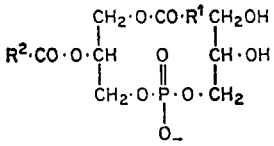
Phosphatidyl ethanolamine



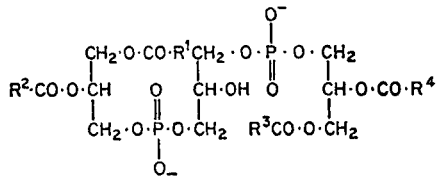
Phosphatidyl serine



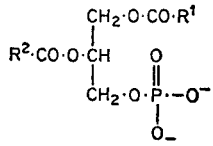
Phosphatidyl inositol



Phosphatidyl glycerol



Diphosphatidyl glycerol (Cardiolipin)



Phosphatidic acid

FIG. 2

TABLE I

Fatty acids of some leaf tissue.

Tissue	Fatty Acid, % of total								
	16:0[a]	Δ^{7+9} 16:1	Δ^{3} 16:1	16:3	18:0	18:1	18:2	18:3	22:0
Broad bean[1] (Etiolated leaf)	16·7	—	—	—	4·7	—	33·5	39·4	4·6
Broad bean[1] (Green leaf)	11·7	6·9		—	3·7	3·4	14·3	56·4	4·0
Broad bean[1] (Chloroplasts)	7·4	9·2		—	1·2	5·2	2·6	72·0	1·2
Spinach leaf[2]	12·9	—	2·6	4·6	t	6·6	16·3	56·2	
Holly leaf[3]	22·0	t	t	—	t	2·5	13·8	60·2	

[a] The figure before the colon denotes the number of carbon atoms; that after the colon, the number of double bonds.
[1] Crombie, 1958; [2] Debuch, 1961; [3] Nichols, 1965a; t = trace quantity.

The fatty acids of chloroplasts are not randomly distributed between the different acyl lipids but show a very high degree of specificity for certain lipids (Allen *et al.*, 1966; Nichols, 1965b). These specificities are common to all the photosynthetic tissues of higher plants and algae which have been examined in detail and are typified by the date presented in Table II. The

TABLE II

% Fatty acid composition of the major chloroplast lipids of *Chlorella vulgaris* (Nichols, 1965b).

	16:0	Δ^{9} 16:1	Δ^{3} 16:1	16:2	18:1	18:2	18:3
Partially etiolate							
Monogalactosyl diglyceride	3	11	—	28	17	33	5
Digalactosyl diglyceride	10	7	—	4	17	56	4
Sulphoquinovosyl diglyceride	33	11	—	4	16	28	3
Phosphatidyl glycerol	57	5	t	t	16	14	t
Photosynthetic							
Monogalactosyl diglyceride	5	2	—	19	3	17	45
Digalactosyl diglyceride	8	3	—	6	3	35	37
Sulphoquinovosyl diglyceride	32	5	—	3	10	25	15
Phosphatidyl glycerol	31	5	16	—	10	25	5

t = Trace quantity.

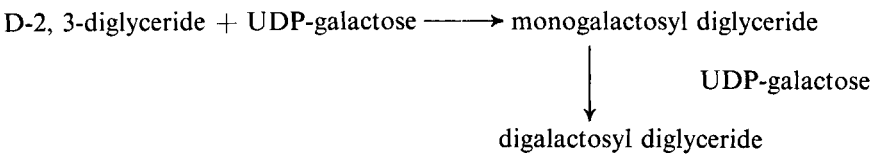
trienoic acids are commonly found in combination with the galactosyl diglycerides, especially in the monogalactosyl diglyceride. Palmitic acid is mainly found in both the phosphatidyl glycerol and sulpholipid fractions whereas *trans*-3-hexadecenoic acid is found only in the phosphatidyl glycerol. In those tissues where the C_{20} polyunsaturated acids occur, they are particularly associated with the plastidic galactosyl diglycerides (Nichols, 1965a).

While the tendencies we have just described appear to be fairly general for the chloroplasts of the higher plants and algae, they do not always hold for the blue-green algae which do not synthesize *trans*-3-hexadecenoic acid (Nichols *et al.*, 1965a; Nichols and Wood, 1968) nor in some cases polyenoic acids (Holton *et al.*, 1964; Parker *et al.*, 1967).

IV. Biosynthesis of the Acyl Lipids

A. GALACTOSYL DIGLYCERIDES

Ferrari and Benson (1961) observed a rapid incorporation of ^{14}C into monogalactosyl diglyceride and a slower entry into digalactosyl diglyceride during the growth of *Chlorella pyrenoidosa* in $^{14}CO_2$ and concluded that the digalactosyl diglyceride was synthesized by galactosylation of the monogalactosyl diglyceride. These authors proposed the following mechanism for biosynthesis of the galactosyl diglycerides:



Neufeld and Hall (1964) have demonstrated that spinach chloroplasts catalyse the transfer of galactose from UDP-galactose to an uncharacterized endogenous acceptor with the apparent formation of mono-, di-, tri- and possibly tetra-galactosyl diglyceride.

Although this and other kinetic data is consistent with the formation of digalactosyl diglyceride by galactosylation of monogalactosyl diglyceride, the fact that these two lipids almost invariably possess somewhat different fatty acid compositions when isolated from the same tissue or chloroplast preparation remains to be explained. If the pathway suggested by Ferrari and Benson (1961) is correct then there must be either a highly specific galactosylation mechanism for monogalactosyl diglyceride of a particular fatty acid composition or some degree of deacylation-reacylation *in vivo* of either, or both, of these lipids. The latter type of mechanism would require lipases capable of removing one or both acyl moieties from the galactosyl diglycerides and the

presence of such enzymes in the leaves of runner bean has been demonstrated by Sastry and Kates (1964).

B. SULPHOQUINOVOSYL DIGLYCERIDE

By analogy with the biosynthesis of monogalactosyl diglyceride from UDP-galactose, Benson (1963) has suggested that the sulpholipid might be synthesized by transfer of the sulphoquinovose group from a nucleoside diphosphosulphoquinovose (identified in extracts of *Chlorella*) to a diglyceride:

nucleoside diphosphosulphoquinovose + diglyceride ⟶ sulphoquinovosyl diglyceride + nucleoside diphosphate

C. PHOSPHATIDYL GLYCEROL

Haverkate and van Deenen (1964, 1965) demonstrated that the phosphatidyl glycerol fraction from spinach leaves has the same stereo-chemical configuration as the phosphatidyl glycerol from animals and bacteria and suggested that its synthesis might proceed by the same pathway, namely the reaction of CDP-diglyceride with glycerol-3-phosphate:

CDP-diglyceride + glycerol-3-phosphate ⟶ phosphatidyl glycerophosphate + CMP

phosphatidyl glycerophosphate ⟶ phosphatidyl glycerol + P_i

An alternative route for the synthesis of this lipid in chloroplasts has been suggested by two groups of workers (Benson *et al.*, 1967; Dawson, 1967) who found that plant tissues containing phospholipase D can catalyse the transfer of a phosphatidyl unit from lecithin to various alcohols such as glycerol, ethanolamine, methanol and ethylene glycol with the formation of the equivalent phospholipid. Thus phosphatidyl glycerol could be synthesized as follows:

phosphatidyl choline + glycerol ⟶ phosphatidyl glycerol + choline

As we have already indicated, all four chloroplast lipids show such different fatty acid compositions that it seems inconceivable that they could arise from a common diglyceride "pool", the two galactosyl diglycerides being a probable exception.

V. Biosynthesis of Fatty Acids

In early work we showed that acetate, octanoate, decanoate and tetradecanoate were utilized by chopped leaves to form longer chain saturated and unsaturated fatty acids and that the major site of such synthesis was the chloroplast (James, 1963; Stumpf and James, 1963). Although both acetyl-CoA and malonyl-CoA are effectively utilized, it is now known that acetyl-S-ACP and malonyl-S-ACP are the true substrates (Brooks and Stumpf, 1966). Isolated chloroplasts require ATP, Mg^{++}, CO_2, inorganic phosphate and CoA when synthesis is started from acetate.

The effects of light on fatty acid synthesis in chloroplasts are still unclear. Stumpf and James (1963) found that synthesis in isolated chloroplasts was greatly diminished in the dark and inhibited in the light by both NH_3 and PCMU. Such inhibitions could be explained by repression of the photosynthetic production of $NADPH_2$ and ATP. However Stumpf *et al.* (1963) also showed a coupling between non-photosynthetic production of $NADPH_2$, O_2 and ATP and lipid synthesis and was unable to replace light by addition of ATP, $NADPH_2$ and O_2 (Stumpf *et al.*, 1967). On the other hand, Mudd and McManus (1962) showed that two fractions could be obtained from disrupted spinach chloroplasts one of which was soluble and was able to incorporate acetyl-CoA into long chain fatty acids in the dark provided that

$$\text{Malonyl-S-CoA} + \text{ACP-SH} \underset{\text{transacylase}}{\overset{\text{malonyl}}{\rightleftharpoons}} \text{malonyl-S-ACP} + \text{CoA-SH}$$

$$\text{Acetyl-S-CoA} + \text{ACP-SH} \underset{\text{transacylase}}{\overset{\text{acetyl}}{\rightleftharpoons}} \text{acetyl-S-ACP} + \text{CoA-SH}$$

$$\text{Acetyl-S-ACP} + \text{malonyl-S-ACP} \underset{\text{synthetase}}{\overset{\beta\text{-ketoacyl-ACP}}{\rightleftharpoons}} \text{acetoacetyl-S-ACP} + CO_2 + \text{ACP-SH}$$

$$\text{Acetoacetyl-S-ACP} + \text{NADPH} + H^+ \underset{\text{reductase}}{\overset{\beta\text{-ketoacyl-ACP}}{\rightleftharpoons}} \text{D(-)-}\beta\text{-hydroxybutyryl-S-ACP} + NADP^+$$

$$\text{D(-)-}\beta\text{-hydroxybutyryl-S-ACP} \underset{\text{dehydrase}}{\overset{\beta\text{-hydroxyacyl-ACP}}{\rightleftharpoons}} \text{crotonyl-S-ACP} + H_2O$$

$$\text{Crotonyl-S-ACP} + \text{NADPH} \underset{\text{reductase}}{\overset{\text{enoyl-ACP}}{\rightleftharpoons}} \text{butyryl-S-ACP} + NADP^+ \quad \text{etc., etc.}$$

FIG. 3

$NADPH_2$ and ATP were present. The apparent contradictions in these results have yet to be explained.

Brooks and Stumpf (1966) have shown that synthesis of long chain fatty acids in chloroplasts involves malonyl-S-ACP rather than malonyl-S-CoA and it is probable that the fatty acid synthetase system of chloroplasts is essentially similar to that originally described by Vagelos and co-workers (e.g. Alberts *et al.*, 1963) for bacteria (Fig. 3).

A. MONOENOIC ACIDS

Despite earlier work which in some cases gave apparently contradictory results, it now seems reasonably certain that oleic acid is synthesized in both leaf and algal tissues by direct desaturation of stearic acid, probably in the form of its ACP thiol ester (Harris *et al.*, 1965; Nagai and Bloch, 1965). A similar mechanism has been established for the synthesis of 9-hexadecenoic acid in algae by desaturation of palmitic acid (Harris *et al.*, 1965) but this route has yet to be established in leaves. The *trans*-3-hexadecenoic acid has been shown to be derived by direct dehydrogenation of palmitic acid (Nichols *et al.*, 1965b).

There is as yet no clear evidence that the 7-hexadecenoic and 11-octadecenoic acid of leaves and algae are synthesized by the direct desaturation of palmitic and stearic acids, respectively, and Bloch and his associates (Nagai and Bloch, 1965; Bloch *et al.*, 1967) regard their presence as being indicative of a route involving chain elongation of β, γ-unsaturated C_{10} or C_{12} acids produced by an oxygen-requiring desaturation:

$$C_{10} \xrightarrow{O_2} \Delta^3\text{–}C_{10} \longrightarrow \Delta^5\text{–}C_{12} \longrightarrow \Delta^7\text{–}C_{14} \longrightarrow \Delta^9\text{–}C_{16} \longrightarrow \Delta^{11}\text{–}C_{18}$$

$$C_{12} \xrightarrow{O_2} \Delta^3\text{–}C_{12} \longrightarrow \Delta^5\text{–}C_{14} \longrightarrow \Delta^7\text{–}C_{16} \longrightarrow \Delta^9\text{–}C_{18}$$

Such steps have yet to be verified experimentally.

B. POLYUNSATURATED ACIDS

The polyunsaturated fatty acids such as linoleic acid and linolenic acid are produced by the stepwise oxygen-requiring dehydrogenation of the corresponding monoenoic acids (James, 1962; Harris and James, 1965), e.g.:

$$\text{Oleic acid} \xrightarrow{O_2} \text{linoleic acid} \xrightarrow{O_2} \text{linolenic acid}$$

This desaturation system is very sensitive to disruption of the tissue and functional cell-free systems have been produced only from *Chlorella vulgaris*

and safflower seeds. The system is particle bound, presumably to the plastid.

Pathways leading to the formation of the C_{16}, C_{18}, C_{20} and C_{22} tetraenoic acids observed in some chloroplast preparations have not been investigated.

VI. Function of Lipids in Chloroplasts

Although the structure and relative stoichimetry of the lipids present in chloroplasts are now fairly well understood, their functions have yet to be clearly defined. That these compounds contribute some essential function in photochemical processes has been recently demonstrated by Shibuya and Maruo (1966) who succeeded in restoring much of the electron transport activity of delipidized chloroplast lipoprotein by recombining aqueous suspensions of the lipid and the protein. These lipids could function as either chemical or structural components of the photochemical apparatus and might serve a dual purpose.

The difficulties experienced in the isolation and study of the different units of the photosynthetic apparatus has meant that any data relevant to lipid function in these systems has usually been of an indirect nature.

A. STRUCTURAL FUNCTION

We shall consider first the possible structural rôle of the chloroplast lamella lipids. These lipids could have two types of structural rôle in the protein-pigment-lipid complex of the chloroplast.

The first possibility is that they might represent specific structural components which could maintain the pigments in correct steric orientation with one another and their associated enzymes. In such a case there would be fairly specific requirements for lipid structure and ionic charge and one might consequently expect similar lipids or groups of lipids to occur in all photosynthetic systems of a given type.

Alternatively, they could provide an organized micellar medium of low dielectric constant in which the pigment-protein complexes could be embedded and in which the electron transport sequences could operate. Such a medium could be provided by a variety of ampiphatic substances and highly specific structures and charge distributions would not be involved. Thus, similar photosynthetic processes could be operated by complexes in which the nature of the lipid components *could* be fairly variable.

As we have already indicated, the evidence available shows that all photosynthetic apparatus which perform the Hill reaction have the same acyl lipid composition, even although the relative stoicheiometry and individual fatty acid composition may show slight variations. Thus, this similarity is either of evolutionary significance or else these lipids are acting as specific structural components. Recently Weier and Benson (1966) and Mühlethaler (1966)

have suggested how these compounds and other components of the photochemical apparatus could be arranged in the chloroplast lamellae. It is proposed that those lipids which are devoid of charged groups, i.e. the galactosyl diglycerides may participate in hydrophobic interactions with structural protein of the chloroplast while the negatively charged lipids (phosphatidyl glycerol and sulpholipid) may play a prominent part in attaining charge-charge interactions between lipid micelles and proteins (van Deenen and Haverkate, 1966).

Whether or not the lipids may be partially responsible for the ultrastructural geometry of an organelle is not clear. Changes in ultrastructure are usually accompanied by changes only in the relative concentrations of the chloroplast lipids. Thus the conversion of proplastids to chloroplasts is accompanied by the rapid synthesis of phosphatidyl glycerol (Miller, 1963), monogalactosyl diglyceride (Bloch *et al.*, 1967) and sulpholipid (Rosenberg and Pecker, 1964) in addition, of course, to that of chlorophyll. Synthesis of digalactosyl diglyceride is not appreciably accelerated during this process (Bloch *et al.*, 1967). We also know that the major subcellular particles of some yellow petals are derived from chloroplasts, such as the polymembranous particle of narcissus trumpet (Nichols *et al.*, 1967). During this transition the relative proportions of the mono- and di-galactosyl diglycerides change significantly. On the other hand, maturation of the buttercup petal involves the breakdown of the chloroplast lamellae into large globuli (Frey-Wyssling and Kreutzer, 1958) quite unlike the particles of daffodil trumpet and in buttercup tissue the relative proportions of the two galactosyl diglycerides are entirely reversed. It is thus unlikely that the lipids control the ultrastructure of any of these organelles.

B. METABOLIC FUNCTION

We might now consider the possibility that lipids could be chemically involved in the various metabolic processes carried out within the chloroplast.

One suggestion made in the past is that part of the acyl lipids might be involved in the electron transport chain of photosynthesis. This would require a readily oxidizable component such as a highly unsaturated fatty acid and since high levels of polyenoic acids are characteristic of the chloroplasts of higher plants and algae it was frequently speculated that these acids might have such a function (Erwin *et al.*, 1964). However, the observation by Holton and co-workers (1964), and subsequently by others (Nichols and Wood, 1968; Parker *et al.*, 1967), that some blue-green algae contain no polyenoic acids and yet seem to function photosynthetically in a perfectly normal manner would seem to invalidate this proposal.

As an alternative explanation for the wide distribution of polyunsaturated fatty acids it might be pointed out that leaves and, to a lesser extent, algae,

must sometimes be able to function at low ambient temperatures and a high proportion of unsaturated fatty acids might ensure that their lipoprotein structures were fully mobile over a wide temperature range.

The observation that in leaves and green algae *trans*-3-hexadecenoic acid is specifically located on phosphatidyl glycerol (Allen *et al.*, 1964; Weenink and Shorland, 1964; Haverkate *et al.*, 1964), metabolically the most active chloroplast lipid, and that it is absent from the corresponding etiolated tissue (Nichols *et al.*, 1965c; Nichols, 1965b), led us to suggest that this acid might have some specific active rôle in photosynthesis. However, we found subsequently that this acid does not occur in the blue-green algae (Nichols *et al.*, 1965a; Nichols and Wood, 1968) so that unless there is some discrete difference between the mechanisms of photosynthesis in green algae and leaves and that in blue-green algae, involvement of this acid cannot be obligatory for photosynthesis.

The chloroplast lipids might also function as required substrates or co-factors for the enzymes synthesizing fatty acids and they could also be involved in the mobilization of fatty acids in an analogous manner to CoA and ACP derivatives. The former class of function seems particularly plausible in the formation of *trans*-3-hexadecenoic acid from palmitic acid. Haverkate and van Deenen (1965) have shown that in spinach leaves this acid is specifically bound to the β-hydroxyl group of the glyceride moiety of phosphatidyl glycerol, which is otherwise most usually occupied by palmitic acid. Thus it is possible that the desaturation occurs either on the molecule or in its immediate environment. Support for this hypothesis has recently been found in our laboratory (Bartels *et al.*, 1967) where it was shown that added free *trans*-3-hexadecenoic acid was very rapidly reduced to palmitic acid by algal and leaf tissue but that before this reduction was complete, some of the *trans*-acid was incorporated into all the other lipid classes. Thus the specific association of the *trans*-3-hexadecenoic acid with the phosphatidyl glycerol fraction of photosynthetic tissue is most convincingly explained by invoking the palmityl phospholipid as the required substrate for the dehydrogenation. Any tendency for the acid to be split from the phosphatidyl glycerol molecule by lipase action would presumably result in a rapid hydrogenation of the *trans*-acid before it could be incorporated into the other lipid classes.

We have also obtained evidence which suggests that, in particular, phosphatidyl glycerol and monogalactosyl diglyceride might be similarly involved in the synthesis and metabolism of other fatty acids in the chloroplast. In studies involving the incorporation of ^{14}C-labelled acetate into the lipids of *Chlorella vulgaris*, we noted that the uptake and turnover of certain fatty acids in these lipids was faster than one would normally expect from that due to *de novo* synthesis of these lipids during cell growth and division (Nichols and James, 1967). Thus it appears that certain fatty acids are continually

TABLE III

Classification of possible lipid function based on metabolic studies.

Lipid	Metabolic function	Structural function
Monogalactosyl diglyceride	(a) Involved in fatty acid 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.	Major component of chloroplast lamellae.
Digalactosyl diglyceride	Involved in galactose metabolism.	Major component of chloroplast lamellae.
Sulpholipid	Involved in hexose metabolism. Suggested function as a sulphur and carbon reserve material.	Major component of chloroplast lamellae.
Phosphatidyl glycerol	(a) Involved in fatty acid biosynthesis of the C_{14}, C_{16} and C_{18} saturated acids, and the *trans*-3-hexadecenoic acid, as well as the C_{18} mono- and dienoic acids. (b) Involved in phosphate metabolism.	Major component of chloroplast lamellae.
Phosphatidyl choline	(a) Involved in the biosynthesis of the C_{18} unsaturated fatty acids. (b) Involved in phosphate metabolism.	Possibly a minor component of chloroplasts.

fluxing through these lipids suggesting that they might be required "carriers" or substrates in certain fatty acid conversions. On this basis, the lipids would not be merely acceptors of the end-products from a fatty acid synthetase but an integral part of the system.

Ferrari and Benson (1961) have also noted that the fatty acids of monogalactosyl diglyceride and phosphatidyl glycerol were rapidly labelled when *Chlorella pyrenoidosa* was incubated with $^{14}CO_2$. These authors also observed a rapid turnover of label in the sugar moieties of the three plastid glycolipids which, in the case of the digalactosyl diglyceride and the sulpholipid, was considerably faster than that in the fatty acid portion of the molecule. They therefore concluded that these lipids, particularly the galactosyl diglycerides, might be intimately involved in sugar metabolism and transport.

Miyachi and Miyachi (1966) have observed that starving cells of *Chlorella* utilize the carbon and sulphur of the sulpholipid which therefore serves as an emergency reserve for these elements but it is debatable whether this observation is indicative of the main function of the lipid in the healthy cell.

Thus the available evidence is that the acyl lipids of chloroplasts have both a metabolic and structural rôle and these possible functions are summarized in Table III.

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