Light control of fatty acid synthesis and diurnal fluctuations of fatty acid composition in leaves

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1. Although isolated spinach chloroplasts were almost entirely (>99%) dependent on light for fatty acid synthesis, leaf discs were capable of fatty acid synthesis in the dark (up to 500nmol of $^3\text{H}/\text{h}$ per mg of chlorophyll equivalent to approx. 400nmol of carbon/h per mg of chlorophyll), which represented 12–20% of the corresponding 'light rates'. 2. Net fatty acid accumulation by greening maize leaves occurred largely or entirely during the light period. 3. There was a diurnal fluctuation in the proportions of C_{18} unsaturated fatty acids in the lipids of developing spinach leaves, where an increase in the concentration of oleate during the day and a subsequent decline at night was observed; a complementary change occurred in the concentration of α -linolenate. The rhythm is interpreted as reflecting the continuation of oleate and linoleate desaturation at high rates when oleate synthesis is markedly decreased at night. 4. Changes in the fatty acid composition of 3-*sn*-phosphatidylcholine accounted for at least 60% of the total decrease in oleate over the dark period. This result is consistent with suggestions that this lipid is the substrate for the leaf microsomal oleate desaturase and an intermediate in leaf glycerolipid biosynthesis.

Membrane biogenesis is of great importance during the growth and development of all plant tissues, yet in spite of the fact that fatty acids are major components of membrane glycerolipids, relatively little is known of the effects of light on the synthesis and metabolism of fatty acids at the level of the whole leaf. There is now considerable evidence that suggests that plastids are the sole site of long-chain fatty acid synthesis *de novo* in plants (Weaire & Kekwick, 1975; Vick & Beevers, 1978; Ohlrogge *et al.*, 1979). This implies that in chlorophyllous cells fatty acid, and therefore net glycerolipid and membrane, production is dependent on chloroplast fatty acid synthesis. However, isolated chloroplast preparations (as opposed to other plastids) always show a strong light dependence for fatty acid synthesis (Smirnov, 1960; Stumpf *et al.*, 1967; Nakamura & Yamada, 1975; Roughan *et al.*, 1979a, 1980). Roughan *et al.* (1979a, 1980) showed that the rate of [^{14}C]acetate incorporation in the dark into long-chain fatty acids by intact spinach chloroplasts was less than 0.5% of the light rate, and lysed chloroplasts supplied with NADPH, NADH and ATP achieved a dark rate that was only 2.5% of the corresponding light rate.

Under most conditions the major product of

chloroplast fatty acid synthesis *in vivo* and *in vitro* appears to be oleate (Slack & Roughan, 1975; Roughan *et al.*, 1976; Williams *et al.*, 1976; Slack *et al.*, 1977), but the sequential conversions of this compound to yield linoleate and α -linolenate do not appear to be directly controlled by light either in leaves *in vivo* (Harris *et al.*, 1967; Roughan, 1970; Hawke & Stumpf, 1980) or in microsomal (Slack *et al.*, 1976) or chloroplast (Roughan *et al.*, 1979b) preparations from green leaves.

In the present paper we investigate the consequences of the different effects of light on these component reactions of lipid biosynthesis in green leaves.

Experimental

Materials

Spinach (*Spinacea oleracea*, hybrid 102; Arthur Yates, Auckland, New Zealand) plants were grown under a controlled environment (constant 22°C temperature, 10h photoperiod, 600 $\mu\text{E}/\text{m}^2$ per s photosynthetically active radiation) as described by Slack & Roughan (1975). Maize plants (*Zea mays*; cultivar XL45) were grown singly in 5 litre pots under a controlled environment (constant 30°C

temperature, 12h photoperiod, $600\mu\text{E}/\text{m}^2$ per s photosynthetically active radiation) and were watered twice daily with nutrient solution (Hoagland & Arnon, 1938).

Sodium methoxide was prepared as described previously (Slack *et al.*, 1976) and diazomethane was generated by the method of Schlenk & Gellerman (1960). Sodium $[1\text{-}^{14}\text{C}]\text{acetate}$ and $^3\text{H}_2\text{O}$ were from The Radiochemical Centre, Amersham, Bucks., U.K. Triton X-100 from BDH Chemicals, Poole, Dorset, U.K., and other biochemicals were from Sigma, St. Louis, MO, U.S.A.

Methods

Light control of fatty acid synthesis by isolated chloroplasts. Spinach chloroplasts were isolated using low-ionic-strength buffers by the procedure of Nakatani & Barber (1977). The experiment was started by adding a portion of the chloroplast preparation to an assay mixture in a 25 ml conical flask to make a final volume of 1 ml containing 25 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/NaOH, pH 7.9, 0.33 M-sorbitol, 10 mM- NaHCO_3 , 2 mM-EDTA, 1 mM- MgCl_2 , 1 mM- MnCl_2 , 0.5 mM-dithiothreitol, 0.3 mM- K_2HPO_4 , 0.5 mM-CoA, 0.008% Triton X-100, 0.2 mM-sodium $[1\text{-}^{14}\text{C}]\text{acetate}$ (sp. radioactivity 8 Ci/mol) and chloroplasts equivalent to $188\mu\text{g}$ of chlorophyll. The incubation mixture was illuminated for 3 min in a Warburg apparatus (130 strokes/min, $200\mu\text{E}/\text{m}^2$ per s photosynthetically active radiation) at 25°C and then darkened for 3 min before being illuminated for a further 4 min. Portions ($50\mu\text{l}$) were transferred to tubes containing 1 ml of 10% (w/v) KOH in methanol plus 0.5 ml of water and the tubes were then purged with N_2 , capped and heated to 80°C for 90 min to saponify the lipids. After cooling 1 ml of 1.8 M- H_2SO_4 and 2.5 ml of light petroleum (b.p. $40\text{--}60^\circ\text{C}$) were added, the tubes shaken and the fatty acids recovered in the organic phase. These were concentrated under N_2 and purified by t.l.c. on silica gel G plates with light petroleum (b.p. $60\text{--}80^\circ\text{C}$)/diethyl ether/acetic acid (75:25:1, by vol.) as developing solvent before being scraped into scintillation vials and counted in 0.5% *p*-terphenyl in xylene/Triton X-100/water (6:3:1, by vol.).

Fatty acid synthesis by leaf discs. Spinach leaves harvested in the first half of the light period were floated on iced water and sets of 16 discs (total leaf area 1.50cm^2 , approx. 50 mg fresh weight) were transferred to tubes containing 0.45 ml of 50 mM-Mes (4-morpholine-ethanesulphonic acid)/NaOH buffer, pH 6.0, containing 0.37 M-sorbitol and 12.5 mM- KHCO_3 . Two additional sets of discs were used for chlorophyll analysis (Arnon, 1949). The tubes were stored on ice and when sufficient samples had been prepared, $50\mu\text{l}$ of labelled substrate was

added to each tube {100 nmol of aqueous sodium $[1\text{-}^{14}\text{C}]\text{acetate}$ (sp. radioactivity 58.7 Ci/mol) or 50 mCi of $^3\text{H}_2\text{O}$ } and the discs were vacuum infiltrated. Incubations were carried out at 25°C in the light and dark (tubes wrapped in aluminium foil) in the Warburg apparatus described above. Assays with $[^{14}\text{C}]\text{acetate}$ were stopped by addition of 1 ml of 10% (w/v) KOH in methanol and saponified immediately as described above, but this technique resulted in high radioactivity in control (zero-time) incubations when $^3\text{H}_2\text{O}$ was the substrate. The artefact was largely eliminated by removing the incubation medium and adding 0.5 ml of water before saponification.

Extraction, purification and radioactivity counting of fatty acids from $[^{14}\text{C}]\text{acetate}$ incubations were by the procedures described above. After $^3\text{H}_2\text{O}$ incubations the light-petroleum extract of fatty acids was mixed with $100\mu\text{l}$ of acetic acid before being evaporated to dryness under N_2 . The fatty acids were then taken up in 0.4 ml of ethanol/benzene/water/acetic acid (30:4:5:1, by vol.) and dried thoroughly again before being dissolved in light petroleum for application to the t.l.c. plate. After separation in light petroleum (b.p. $60\text{--}80^\circ\text{C}$)/diethyl ether/acetic acid (75:25:1, by vol.), the fatty acids were transferred to scintillation vials and counted in an emulsion containing 10% (v/v) water in the 'cocktail' described by Fricke (1975) since the *p*-terphenyl-based 'cocktail' is not suitable for ^3H counting.

Diurnal increase in the fatty acid content of maize leaves. A region 35 mm long was selected at the proximal end of the partially emerged tenth leaf on each of six maize plants. This region on each side of the mid-vein of each leaf was sampled (5 discs, 0.47cm^2 leaf area) at the start and end of each light period over 48 h and the samples were saponified as described above. The light-petroleum extracts of free acids recovered from the saponification mixtures were dried under N_2 and methylated directly by treatment with 1 ml of diazomethane solution for 40 min. Unreacted diazomethane and solvent were removed under N_2 and the fatty acid methyl esters taken up in $100\mu\text{l}$ of light petroleum (b.p. $40\text{--}60^\circ\text{C}$) and their masses were estimated after g.l.c. at 180°C on a column ($2\text{m} \times 2\text{mm}$) of 15% ethylene glycol succinate on Chromosorb W by comparison with a standard fatty acid methyl ester mixture.

Diurnal variation in the fatty acid composition of spinach leaf lipids. Five leaves were sampled over a 3 day period. Each sample contained 10 discs (one from each side of the mid-rib of each leaf; total area 0.94cm^2 , approx. 40 mg fresh weight) and four samples were taken at each time. The samples were steamed for 10 min (Roughan & Boardman, 1972) and extracted by the method of Bligh & Dyer

(1959). The chloroform phase was recovered, evaporated to dryness under N_2 and the lipids were taken up in 0.5 ml of chloroform. A 100 μ l portion from each sample was transmethylated by incubation with 1 ml of sodium methoxide for 20 min after which time the fatty acid methyl esters were extracted into light petroleum (b.p. 40–60°C) and analysed by g.l.c. as described above. The replicates at each sampling time were then pooled and the lipids separated either by two-dimensional t.l.c. as described by Roughan *et al.* (1978b) or by one-dimensional t.l.c. on silica gel G in chloroform/methanol/15 M- NH_3 (65:25:2, by vol.). Lipids were detected by staining lightly with I_2 vapour, transferred into tubes and transmethylated on the silica gel with sodium methoxide (Slack *et al.*, 1976). The fatty acid methyl esters were analysed by g.l.c. as described above.

Results

Light control of fatty acid synthesis by isolated chloroplasts

The strong light dependency reported previously (Roughan *et al.*, 1979a) from long-term incubations of isolated spinach chloroplasts was confirmed (Fig. 1). In addition, the rapid nature of the dark/light and light/dark transitions is demonstrated. In both instances this transition occurs in less than 10 s and the rate of fatty acid synthesis, determined by linear regression, is approximately the same before and after the dark period (2086 and 1723 nmol of acetate/h per mg of chlorophyll respectively).

Fatty acid synthesis by leaf discs

To find out whether the light requirement of long-chain fatty acid synthesis exhibited by chloroplasts *in vitro* is also a feature of leaf systems *in vivo*, the ability of infiltrated leaf discs to incorporate exogenous [^{14}C]acetate into long-chain fatty acids was investigated. Despite the fact that addition of label to, and vacuum infiltration of, the samples took 10 min, linear rates of incorporation in both the light and dark were not achieved until 10–15 min after transferring the samples from the ice bucket to the Warburg bath (Fig. 2). Fatty acid synthesis was linear throughout the remaining 50 min of the experiment and the rate of synthesis from exogenous [^{14}C]acetate in the dark was 12% of that in the light (Table 1). However, the rate observed in the light was only 4–6% of that obtained by isolated spinach chloroplasts (Roughan *et al.*, 1979a, and Fig. 1). It is likely that this was the result of the extensive use of unlabelled endogenous carbon reserves by the leaf discs and it follows that it is not possible to determine the absolute rates of fatty acid synthesis by leaf tissue using this method.

Fig. 1. Fatty acid synthesis by isolated intact spinach chloroplasts in the light and the dark

Chloroplasts were prepared by the method of Nakatani & Barber (1977) and incubated with [$1-^{14}C$]acetate as described under 'Methods'. The assay was started by addition of the chloroplasts to the illuminated reaction mixture, which was subsequently darkened at 3 min and then re-illuminated at 6 min reaction time. At the times indicated 50 μ l portions of the reaction mixture were transferred to tubes containing 1 ml of 10% (w/v) KOH in methanol plus 0.5 ml of water and saponified under N_2 . Fatty acids were purified and their radioactivities were measured as described under 'Methods'.

An alternative technique for quantifying fatty acid synthesis is to measure the incorporation of 3H from 3H_2O into lipids (Jungas, 1968; Yamada & Nakamura, 1975; Roughan *et al.*, 1978a). Results obtained using this method with isolated chloroplasts (Roughan *et al.*, 1978a) gave rates in close agreement with those obtained with [$1-^{14}C$]acetate, although Jungas (1968) noted a small isotopic discrimination against 3H during fatty acid synthesis by rat adipose tissue.

The high activities of control (zero-time) incubations noted in previous investigations (Yamada & Nakamura, 1975; Roughan *et al.*, 1978a) were largely eliminated by techniques (detailed under 'Methods') designed to allow carrier removal of non-covalently bound 3H atoms. Radioactivity associated with the non-esterified fatty acid region of t.l.c. separations from saponified zero-time incubations

Fig. 2. Fatty acid synthesis from $^3\text{H}_2\text{O}$ by spinach leaf discs in the light or the dark

Leaf discs were infiltrated in 0.5 ml of 50 mM-Mes/NaOH buffer, pH 6.0, containing 0.33 M-sorbitol, 10 mM- NaHCO_3 and 50 mCi of $^3\text{H}_2\text{O}$, and incubated at 25°C in the light (□) or the dark (■). At the times shown samples were harvested, the medium replaced with 0.5 ml of unlabelled water and 1 ml of 10% (w/v) KOH in methanol was added to the discs, which were immediately saponified under N_2 . Non-covalently bonded ^3H atoms were removed and the fatty acids purified and their radioactivity measured as described under 'Methods'.

was decreased from 50 000 to <10 000 d.p.m. per assay by these procedures.

^3H incorporation showed a 10–15 min lag, similar to that observed when ^{14}C acetate was the substrate, and was then linear up to at least 60 min in the light and 180 min in the dark. The rate of labelling was not dependent on added acetate (results not shown), but was strongly affected by the developmental stage of the leaves selected (Table 1).

Table 1. Light and dark rates of fatty acid synthesis by spinach leaf discs from $[1-^{14}\text{C}]$ acetate or $^3\text{H}_2\text{O}$

Experiments with $^3\text{H}_2\text{O}$ were carried out as described in the legend to Fig. 2. For incubations with $[1-^{14}\text{C}]$ acetate the medium contained 200 μM -sodium $[1-^{14}\text{C}]$ acetate in place of $^3\text{H}_2\text{O}$ and procedures for carrier removal of ^3H were not required. The rate is measured as nmol of acetate/h per mg of chlorophyll (Expt. 1) or nmol of ^3H /h per mg of chlorophyll (Expt. 2 and 3).

Experiment	Rate of linear isotope incorporation into fatty acids		
	Light	Dark	Dark/light ratio
(1) $[1-^{14}\text{C}]$ Acetate	70.2	8.3	0.12
(2) Older leaves + $^3\text{H}_2\text{O}$	852	173	0.20
(3) Young leaves + $^3\text{H}_2\text{O}$	3200	500	0.16

Older leaves achieved average rates of 852 and 173 nmol of ^3H /h per mg of chlorophyll in the light and dark respectively, whereas young leaves similar to those used in the preparation of chloroplasts showed corresponding rates of 3200 and 500 nmol of ^3H /h per mg of chlorophyll. The chlorophyll per unit leaf area for the two classes of leaves were within 15% of one another. The rate of $^3\text{H}_2\text{O}$ incorporation into fatty acids by discs from young leaves in the light is similar to that achieved by isolated chloroplasts (Roughan *et al.*, 1978a).

Neglecting any isotopic effect, the rate of ^3H incorporation by the discs would be equivalent to approx. 2560 nmol of carbon/h per mg of chlorophyll (assuming 22.5 ^3H molecules are incorporated in each C_{18} chain of oleate). This compares with a rate of 1704 nmol of carbon/h per mg of chlorophyll obtained from $^{14}\text{CO}_2$ labelling of intact spinach leaves (Murphy & Leech, 1978).

For spinach leaves of both ages the ratio of fatty acid synthesis in the dark to that in the light was approximately the same as that observed when ^{14}C acetate was the label used (Table 1) and in both cases the ^3H incorporation (equivalent to approx. 138 and 400 nmol of carbon/h per mg of chlorophyll) is considerably higher than could be achieved by chloroplast preparations supplied with cofactors in the dark (50 nmol of carbon/h per mg of chlorophyll; Roughan *et al.*, 1980). Significant rates of fatty acid synthesis in the dark by sliced spinach leaves have also been reported by Yamada & Nakamura (1975).

It seems unlikely that the ^3H incorporation by leaf discs in the dark reported here is the result of non-chloroplastic fatty acid synthesis *de novo* or of light-insensitive chain-elongation reactions. Ohlrogge *et al.* (1979) have provided convincing evidence

that chloroplasts are the only sites of fatty acid synthesis in green spinach leaves and essentially all of the ^3H radioactivity in fatty acids from both light and dark incubations of leaf discs in $^3\text{H}_2\text{O}$ was accounted for by C_{16} and C_{18} species after gas-chromatographic separation (results not shown).

Diurnal increase in the fatty acid content of maize leaves

The large differences between the light and dark rates of fatty acid synthesis by green leaves would be expected to result in a measurable day/night difference in the rate of fatty acid accumulation during the period of rapid glycerolipid synthesis that accompanies greening of recently emerged maize leaves. The maize leaf emerges gradually from the surrounding leaves and leaf sheaths due to the activity of an intercalary meristem and is fully expanded shortly after emergence and well before greening is completed. This contrasts with the situation in spinach leaves where cell division, cell expansion and chloroplast production occur throughout the leaf lamina. Measurements of the fatty acid content of maize leaves at 'lights-on' and 'lights-off' were made over 2 days during which time the mean fatty acid content increased from 320 to 460 nmol of fatty acid/cm² leaf area. The results (Table 2) indicate that net fatty acid accumulation was largely or entirely confined to the light period. The synthesis of chlorophyll from protochlorophyllide is also light-dependent (Goodwin, 1971) so that the production of these two important chloroplast components appears to be synchronized by a common light requirement for their syntheses.

Table 2. *Diurnal increase in the fatty acid content of greening maize leaves*

Maize plants were grown in a controlled environment at constant 30°C and 12h days (08:30h to 20:30h). Samples of five discs (0.47 cm² total leaf area) were taken over consecutive days from 35 mm long sections of the emerging tenth leaf of six plants starting when the sections had emerged 50 mm and the tips of the leaves were 500 mm from the point of emergence. Each sample was saponified as described under 'Methods', the fatty acids methylated with diazomethane and their masses determined by gas chromatography. Results are means $\pm$ S.E.M. ($n = 12$).

Time period	Mean increase in fatty acid content (nmol/cm ²)
Day 1	80.1 $\pm$ 19.5
Night 1	1.5 $\pm$ 11.5
Day 2	74.8 $\pm$ 20.3
Night 2	-16.7 $\pm$ 11.3

Diurnal variation in the fatty acid composition of spinach leaf lipids

Available evidence suggests that oleate and linoleate desaturation are not directly controlled by light (Harris *et al.*, 1967; Roughan, 1970; Slack *et al.*, 1976; Roughan *et al.*, 1979b; Hawke & Stumpf, 1980). If no other rapidly acting mechanism restricts desaturation at night then the concentrations of unsaturated C_{18} fatty acids will vary diurnally as a result of the differences in the light and dark rates of fatty acid synthesis and desaturation.

Fig. 3. *Diurnal variations in the fatty acid composition of total lipids (a) and 3-sn-phosphatidylcholine (b) of spinach leaves*

Four samples of discs were taken at each time from spinach leaves of plants growing in a controlled environment of 25°C for a 10h photoperiod. Samples were steamed before extraction of the lipids. A portion of each total lipid extract was transmethylated with sodium methoxide and the fatty acid composition was determined by g.l.c. The four extracts from each time were pooled, the 3-sn-phosphatidylcholine purified by t.l.c. as described under 'Methods' and the fatty acid composition of this lipid determined by g.l.c. after transmethylation using sodium methoxide. Results are shown for oleate (●), linoleate (▲) and α -linolenate (■) expressed as a percentage of the total mass in these three fatty acids.

This possibility was investigated by taking samples over a period of 3 days from a single set of spinach leaves growing at constant temperature. When the masses of oleate, linoleate and α -linolenate in the whole leaf lipids are expressed as percentages of their sum (Fig. 3a) it is clear that oleate increases in proportion during the day and declines at night. α -Linolenate shows complementary changes in proportion, whereas linoleate (which is the intermediate in the synthesis of α -linolenate from oleate) undergoes smaller changes. Superimposed on these periodic changes is a net increase in the concentrations of α -linolenate at the expense of oleate and linoleate (results for 08:30h or 18:30h in Fig. 3a). Similar overall changes in lipid fatty acid composition have been recorded in maturing maize leaves (Leech *et al.*, 1973).

The fatty acid composition of 3-*sn*-phosphatidylcholine displayed similar fluctuations to those observed in the whole lipids, but the magnitude of the change shown by each fatty acid was larger (Fig. 3b). The magnitude and speed of these changes are consistent with the suggestions that 3-*sn*-phosphatidylcholine is the substrate for leaf microsomal oleate desaturase and that this lipid is an important intermediate in leaf lipid metabolism (Nichols *et al.*, 1967; Roughan, 1970; Slack & Roughan, 1975; Slack *et al.*, 1976, 1977; Williams *et al.*, 1976).

Complete analyses of the fatty acid content of

each lipid from the samples taken at 18:30h and 08:30h each day were made and the mean values are shown in Table 3. In contrast with the proportions (Fig. 3) the masses indicate that there is a substantial (16.5%) drop in the amount of lipid per unit leaf area in the spinach leaves during the night. This is interpreted as lipid dilution due to continued cell expansion in the dark, whereas fatty acid synthesis is decreased by 80–90% (Tables 1 and 2). The total decrease in the mass of oleate during the night was 14.8 nmol/cm² leaf area and of this 60% is accounted for by the change in the mass of oleate on 3-*sn*-phosphatidylcholine. If allowance is made for the decrease in total lipid mass as a result of leaf expansion, 3-*sn*-phosphatidylcholine accounts for 73% of the total change in oleate.

The only known substrate for a linoleate desaturase in leaves is diacylgalactosylglycerol (Roughan *et al.*, 1979b; Hawke & Stumpf, 1980). This lipid shows only small absolute changes in its oleate and linoleate contents (Table 3), but it is noteworthy that the content of both oleate and linoleate in this lipid was decreased by 75% during the dark period.

Discussion

In isolated intact spinach chloroplasts the light control of fatty acid synthesis is rapid and absolute

Table 3. *Diurnal variation in the fatty acid content of spinach leaf lipids*

The pooled extracts of leaf disc samples taken at 18:30h and 08:30h during the experiments described in the legend to Fig. 3 were separated by two-dimensional t.l.c. as described under 'Methods', the individual lipids trans-methylated with sodium methoxide and the mass of the fatty acid methyl esters determined by g.l.c. Each value is the mean for the three different morning or evening samplings.

Lipid	Time (h)	Fatty acid content (nmol of fatty acid/cm ² leaf area)						
		Total	C _{16:0}	C _{16:1}	C _{16:3+18:0}	C _{18:1}	C _{18:2}	C _{18:3}
1,2-Diacyl-3-galactosylglycerol	18:30	146.6	1.9	—	24.8	2.4	5.1	112.4
	08:30	110.9	0.6	—	16.5	0.7	1.8	91.3
1,2-Diacyl-3-digalactosylglycerol	18:30	91.6	10.6	1.2	3.3	2.8	2.9	70.8
	08:30	82.9	8.4	1.0	2.9	2.4	2.8	65.3
Phosphatidylcholine	18:30	62.9	10.5	—	1.5	14.8	20.9	15.3
	08:30	47.6	9.0	—	0.7	5.8	17.6	14.4
Phosphatidylglycerol	18:30	25.0	3.4	6.5	0.4	1.4	3.1	10.2
	08:30	24.5	2.9	7.5	0.3	0.6	2.8	10.4
Sulphoquinovosyldiacylglycerol	18:30	24.9	9.6	—	0.6	1.0	2.2	11.6
	08:30	20.5	8.2	—	0.5	0.5	1.7	9.5
Phosphatidylethanolamine	18:30	21.8	5.2	—	0.5	2.4	9.4	4.3
	08:30	19.8	5.6	—	0.3	1.5	8.7	3.6
Phosphatidylinositol	18:30	14.1	5.0	—	0.5	1.7	3.4	3.3
	08:30	11.4	4.3	—	0.3	1.0	3.1	2.7
Non-polar lipids	18:30	67.2	11.0	—	3.9	8.7	10.4	29.9
	08:30	61.5	9.0	—	3.6	7.7	12.0	27.6
Total	18:30	453.8				35.0		
	08:30	379.0				20.2		

(Fig. 1; Roughan *et al.*, 1979a, 1980). This control is not significantly relieved by supplying cofactors either directly to lysed chloroplasts (which are capable of rapid fatty acid synthesis in the light) or via a dihydroxyacetone phosphate shuttle (Krause & Heber, 1976; Roughan *et al.*, 1980; J. Browse, unpublished work). However, spinach leaf discs are capable of fatty acid synthesis in the dark at rates up to the equivalent of 400 nmol of carbon/h per mg of chlorophyll (Table 1). If chloroplasts are the only sites of fatty acid synthesis in green leaves as suggested by Ohlrogge *et al.* (1979) then whatever the light block is, it is partially overcome in the leaf discs used in the present study. This points to a possible role in the whole leaf of cytoplasmic factors interacting with the light control of this chloroplast activity.

The dark rates of fatty acid synthesis by the discs were nevertheless only about 15% of the light rates (Table 1; Yamada & Nakamura, 1975). This fact, together with the continuation of oleate and linoleate desaturation in the dark (Harris *et al.*, 1967; Roughan, 1970; Slack *et al.*, 1976; Roughan *et al.*, 1979b; Hawke & Stumpf, 1980), is sufficient to explain the diurnal variation in the fatty acid composition of lipids observed in developing spinach leaves (Fig. 3, Table 3). The rates of fatty acid synthesis from $^3\text{H}_2\text{O}$ given in Table 1 are equivalent to 6.12 and 1.39 nmol of fatty acid/h per cm^2 leaf area in the light and 0.96 and 0.28 nmol of fatty acid/h per cm^2 leaf area in the dark for young and older leaves respectively. Assuming that the activity of the oleate desaturase is constant and sufficient to metabolize all the oleate formed in a 24 h period (see Fig. 3a) and that oleate and the fatty acids derived from it account for 78% of the total fatty acids, then these rates would result in a diurnal fluctuation of 5.0 and 23.4 nmol of oleate/ cm^2 or 1.6 and 7.7 mol% of the mean $\text{C}_{18:1} + \text{C}_{18:2} + \text{C}_{18:3}$ fatty acid content of the spinach leaves sampled (Table 3). The average fluctuation shown in Fig. 3(a) is 3.1%. 3-*sn*-Phosphatidylcholine, which is a major structural lipid of the endoplasmic reticulum and other cell membrane systems, exhibited particularly large changes in its fatty acid composition through the 24 h cycle (Fig. 3b). Results of radio-tracer studies *in vivo* indicate that this compound is a rapidly turned over intermediate of glycerolipid biosynthesis in leaves, that it is the substrate of oleate desaturation and that the metabolically active pool is a large proportion of the total leaf complement of this lipid (Nichols *et al.*, 1967; Roughan, 1970; Slack & Roughan, 1975; Slack *et al.*, 1976, 1977; Williams *et al.*, 1976). Experiments *in vitro* with a pea leaf microsomal preparation (Slack *et al.*, 1976) suggested that the entire microsomal pool of 3-*sn*-phosphatidylcholine is available as a substrate for the oleoyl phosphatidyl-

choline desaturase, which is associated with this cell fraction. The large changes shown in Fig. 3(b) are consistent with 3-*sn*-phosphatidylcholine being the substrate for oleate desaturation, but the size of the 3-*sn*-phosphatidylcholine pool in the leaf changed only slightly (from 13.9 to 12.6% of the total lipids, equivalent to approx. 5 nmol of fatty acid/ cm^2) during the night period, suggesting that if metabolism of linoleoyl phosphatidylcholine to other lipids does occur in the dark it is balanced by resynthesis, perhaps of linolenyl-containing species. The fact that the oleate content of 3-*sn*-phosphatidylcholine (Fig. 3b) and of the total leaf lipids (Fig. 3a) changes more rapidly immediately after a light/dark or dark/light transition than in the later part of the light or dark period suggests that the ratio of fatty acid synthesis to desaturation may not vary as a simple step function during the day/night cycle, but that the activities may be regulated so that the oleate content alters only within certain limits.

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