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Lipid Biosynthesis in Green Leaves of Developing Maize[1]

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ABSTRACT

Successive leaf sections from the base to the tip of rapidly developing leaves of 7-day-old maize (*Zea mays* var. Kelvedon Glory), grown in the light, utilized acetate for fatty acid biosynthesis in a very divergent manner. Basal regions of the leaf containing proplastids synthesized insignificant proportions of unsaturated fatty acids and appreciable proportions of fatty acids with 20 or more carbon atoms. An increase in the light intensity during incubations with acetate-1-^{14}C resulted in very little enhancement of either total or polyunsaturated fatty acid biosynthesis in this tissue.

When the distal leaf sections, containing mesophyll chloroplasts with well developed grana and bundle sheath chloroplasts without grana, were incubated with acetate at 150 ft-c and 30 C, approximately 30% of the newly synthesized fatty acids were unsaturated (mainly 18:1 and 18:2). At 2800 ft-c and 20 C, 60% of the fatty acids were unsaturated and the total synthesis of fatty acids increased 4-fold. No detectable amount of fatty acids with 20 or more carbon atoms were synthesized in this morphologically mature tissue, and the proportions of newly synthesized fatty acids more closely resembled the endogenous fatty acids in the immature tissue.

Only 4% of the newly synthesized fatty acids were 18:3 but most of this was incorporated into monogalactolipid. In the distal sections, 20 to 25% of the newly synthesized fatty acids in monogalactolipid were 18:3 compared with the endogenous proportions of 85%. The differences in the composition of the newly synthesized fatty acids and the endogenous fatty acids appear to be related very largely to the low rate of 18:3 biosynthesis from 18:1 and 18:2. Phosphatidyl choline and phosphatidyl ethanolamine, with lower proportions of 18:3, contained radioactive fatty acids which resembled the endogenous composition more closely.

Phosphatidyl choline was quantitatively the most important acyl lipid synthesized under both light conditions. In addition, there was considerable stimulation of acetate incorporation into phosphatidyl glycerol and monogalactolipid, especially in the morphologically most mature regions of the tissue at the higher light intensity.

Lipid biosynthesis in tissue where plastids are developing is of special interest because of the quantitative importance of lipids in the lamellae of differentiated chloroplasts. One system extensively investigated is etiolated tissue greening in the light, in which etioplasts are differentiating rapidly into chloroplasts. Appelquist *et al.* (2), using such a system in the barley leaf, found that while polyunsaturated fatty acids in the constituent lipids, particularly those in monogalactosyl diglyceride, increased in the early stages of the formation of the chloroplast's lamellae system, biosynthesis of galactolipids and of the polyunsaturated fatty acids from acetate was well below expectations, based on the endogenous levels of these constituents. An alternative system in which to study lipid biosynthesis in plastids is the proplastid development in the normal green leaf, which differs from the etiolated system in that there is no extensive prolamellar body formation (7). Successive leaf sections from the base to the tip of a light-grown monocotyledonous leaf such as maize represent successive stages in cell and plastid differentiation (9). These sections offer an ideal material for the study of the relationship between morphological development and lipid biosynthesis. Increases in the amount of MGDG[3] and DGDG and their constituent polyunsaturated fatty acids were found to parallel closely plastid development in this system (10). Using rapidly developing leaves of the 7-day-old maize, we have now examined the ability of the tissue prepared from successive leaf sections to utilize acetate in the biosynthesis of the fatty acids of their constituent lipids.

MATERIALS AND METHODS

Plant Material. *Zea mays* (var. Kelvedon Glory) was grown for 7 days in constant environment cabinets at 28 C and 28,000 lux (0.8 ergs $\times$ 10^{-5}/sec·cm^2) as described elsewhere (10). After removal of the coleoptile and the first leaf, the remaining leaves (usually 10–14 cm long) were cut transversely into five sections—the four lower sections (A–D) were 2 cm each and the remaining section (E) was about 4 cm. The distal tissue was discarded.

Incubation with Acetate-1-^{14}C. Two g of fresh tissue taken from leaf sections A to E were cut into transverse slices about 0.5 mm in thickness, and were incubated with continuous shaking in 25-ml conical flasks with 5 μc (0.085 μmole) acetate-1-^{14}C (the Radiochemical Centre, Amersham, England) in 5 ml of 0.1 M phosphate buffer, pH 7.4, and 0.05 M sodium bicarbonate. Temperature was held at 15 C, 20 C, or 30 C and 150 ft-c (room light) or 2800 ft-c (tungsten lamps) illumination was used.

Isolation and Analysis of Lipids. Incubations were stopped by adding sufficient chloroform-methanol (4:3, v/v) to give one phase and then by heating on a water bath to boil the solvent. After standing overnight at room temperature, the

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[3] Abbreviations: MGDG: monogalactosyl diglyceride; DGDG: digalactosyl diglyceride; SL: sulpholipid; PC: phosphatidyl choline; PE: phosphatidyl ethanolamine; PG: phosphatidyl glycerol; PI: phosphatidyl inositol.

solvent was decanted and the tissue was extracted twice more with chloroform-methanol (2:1, v/v) warmed to 60 C for 30 min. The bulk extracts were washed in turn with 1% acetic acid, 0.1 M NaCl, and three times with distilled water. The final chloroform solution was dried under a stream of nitrogen at room temperature and the residue dissolved in a suitable volume of chloroform for subsequent analysis. Radioactivity was determined in toluene containing 0.6% PPO and 0.02% POPOP using a Nuclear-Chicago Unilux II liquid scintillation system.

Radioactive products were identified by scanning thin layer chromatograms of lipid extracts on a Nuclear-Chicago actigraph II Model 1006 and by autoradiography. Thin layer chromatograms of total lipids and lipid fractions on Silica Gel G were prepared using the following solvents: light petroleum-diethyl ether-acetic acid (70:30:1, v/v), toluene-ethyl acetate-95% ethanol (2:1:1, v/v), chloroform-methanol (9:1, v/v), and chloroform-methanol-acetic acid-water (85:15:10:4, v/v). TLC in the latter solvent was used to recover phospholipids and SL for subsequent analysis, and to isolate PC for analysis of ^{14}C labeled fatty acids by gas-liquid chromatography. PG and DGDG did not separate in this solvent. TLC in chloroform-methanol (9:1, v/v) was used in the determination and isolation of MGDG. PC and MGDG were extracted from Silica Gel G with chloroform-methanol-formic acid-water (97:97:4:2, v/v) as described by Abramson and Blecher (1).

Lipids on TLC plates were visualized by exposure to iodine or, when fatty acids were required for analysis, by spraying them with 0.2% 2′,7′-dichlorofluorescein in 50% aqueous ethanol, and viewing under UV light.

Glycolipids and phospholipids were assayed in the presence of absorbent by determination of galactose (14) and of total phosphorus (3), respectively. Bray's scintillation solvent (4) was used in the measurement of radioactivity in the presence of Silica Gel G.

Methyl esters of the fatty acids of total lipids were prepared after removal of the nonsaponifiable lipid (6), using BF_3-methanol (11), and were subsequently purified by TLC on silica gel (light petroleum-diethyl ether, 4:1, v/v). The methyl esters were then resolved by gas-liquid chromatography on a Hewlett-Packard 402 fitted with dual flame ionization detection and by using glass columns (2 m × 5 mm O.D.) packed either with 12% stabilized diethylene glycol succinate on Anachrom AS (50–60 mesh) or with 10% polyethylene glycol adipate on Chromosorb G (80–100 mesh). The column was held at 165 C with a flow of argon containing 5% CO_2 maintained at 45 ml/min. About 90% of the column effluent was passed into a Panax radiogas system (Panax Equipment Ltd., Redhill, Surrey, England) to determine the radioactivity of individual methyl esters. For quantitative measurement of radioactivity a Kienzle printer was used to record the scaler output.

In order to confirm the identity of fatty acids, methyl esters were sometimes fractionated prior to gas-liquid chromatography into saturated, monoenoic, dienoic, and trienoic compounds by TLC on Silica Gel G containing 5% silver nitrate, and were developed in light petroleum-diethyl ether (9:1 v/v).

RESULTS

Incorporation of Acetate into Total Lipids. The incorporation of acetate into lipids, by tissue prepared from the basal regions of the leaves (0–2 cm, A), was almost linear in incubations lasting up to 3 hr, when 124 nmoles of acetate had been incorporated per g dry weight (equivalent to 20% of the total acetate used). There was no further increase up to 5 hr (Table I). Labeling of acyl lipids reached a maximum of 15.4 nmoles acetate incorporated per g dry weight after 3 hr which is equivalent to 2.7% of the added substrate (Table I). Of the acyl lipids analyzed, PC and PE contained the most labeled carbon. The phospholipids also had higher specific activities (Table I) compared with MGDG or SL. In incubations longer than 3 hr there was a decline in the incorporation of acetate into acyl lipids, possibly due to degradative reactions such as lipolysis and oxidation. Consequently, 3-hr incubations were chosen to examine in detail acetate utilization for lipid biosynthesis by the five selected zones of maize leaves. At 30 C and 150 ft-c, approximately 20% (120–150 nmoles/g dry weight) of the label from the added acetate was incorporated into the total lipids of the three basal sections (A–C) and 11.5 and 7.7%, respectively, into the lipids of the distal D and E sections (Table II). Most of the radioactivity was associated with nonsaponifiable lipids, and the incorporation of acetate into the total fatty acids was in the range 9.6 to 17.2 nmoles acetate/g dry weight, with a tendency for greater incorporation in the basal leaf regions. Acetate incorporation into total lipids (including steroids and pigments) was much lower in sections A, B, and C when incubations were carried out at 20 C and 2800 ft-c but was considerably higher in section E (Table II). However, the incorporation of acetate into the fatty acid constituents of the lipids by tissue from sections A, B, and C was not greatly influenced by changed conditions of incubation. In contrast, in tissue from sections D and E, biosynthesis of fatty acids from acetate increased about 4-fold at the higher light

TABLE I. *Incorporation of Acetate-1-^{14}C into Lipids*

Incorporation of acetate-1-^{14}C was measured in 1- to 5-hr incubations at 30 C and 150 ft-c by tissue slices prepared from 0- to 2-cm section (A) of 7-day-old maize leaves.

Lipids	Incubation Time					Specific Activities After 3 Hr
	1 hr	2 hr	3 hr	4 hr	5 hr	
	nmoles/g dry wt					*dpm/μmole*
Total lipid	55.5	88.0	124.0	122.0	116.0	
Acyl lipid	6.1	10.2	15.4	11.7	8.6	
MGDG	0.9	1.4	1.5	2.7	1.1	19,800
DGDG + PG	0.8	1.3	1.8	1.1	1.1	
SL	0.1	0.3	0.2	0.1	0.2	3,900
PC	1.8	2.7	5.8	3.4	2.2	24,800
PE	1.6	2.8	3.8	3.1	2.8	46,500
PI	0.9	1.7	2.3	1.3	1.2	66,700

Table II. *Incorporation of Acetate-1-^{14}C into Total Lipid and Fatty Acids by Tissue Slices*

Slices were prepared from successive 2-cm leaf sections of 7-day-old green maize seedlings during 3 hr incubations.

Leaf Section	Chlorophyll		Acetate-1-^{14}C Incorporation			
			150 ft-c at 30 C		2800 ft-c at 20 C	
			Total lipids	Fatty acids	Total lipids	Fatty acids
cm from base	*μg/2 cm section*	*μg/μg dry wt*	*nmoles/g dry wt*		*nmoles/g dry wt*	
0–2 (A)	7.5	2.4	119	16.5	35.2	14.2
2–4 (B)	8.9	3.2	150	17.2	34.5	16.5
4–6 (C)	18.4	5.2	141	16.7	46.0	22.4
6–8 (D)	29.3	6.8	65	9.6	59.0	31.0
8–12 (E)	43.1	2.1	34	10.2	64.2	42.0

intensity, and there was a large decrease in the amounts of nonsaponifiable lipid synthesized from acetate. The scans showed that the distribution of label between the newly synthesized lipids was unaltered when tissues were incubated with acetate in the range of 15 to 30 C, so that the differences in incorporation and labeling of lipid may be attributed to the effect of light. The only effect of temperature was to decrease slightly the proportions of 18:1 and 18:2 synthesized in the total lipid at higher temperatures of incubation (8).

Incorporation of Acetate into Phosphatidyl Choline and Monogalactosyl Diglyceride. At 150 ft-c there was little variation in the pattern of labeling of the major lipid constituents by tissue from cells of different ages. PG and DGDG were not analyzed separately but together contained about 10% of the radioactivity in acyl lipids. The specific activity of PC (Table III) was found to be fairly constant in the different sections, although a small increase in sections A to C and a slight decrease in sections D and E was apparent. The specific activity of MGDG decreased markedly from the basal to the distal sections of the leaves because of the larger endogenous levels of this lipid in the mature leaf tissue (9). The total incorporation into MGDG is also shown in the table.

When tissue from each of the sections A to E was incubated with acetate 1-^{14}C at 2800 ft-c and 20 C, there was a similar distribution of label in the acyl lipid constituents in sections A, B, and C which qualitatively resembled those obtained at the lower light intensity in all sections; PC, PI, and PE contained most label. However, in sections D and E there was a relatively greater incorporation of label into PG, MGDG, and SL as well as into PC at the higher light intensity. These between-section differences in distribution at the higher light intensity are shown in the autoradiographs of sections C and D (Figure 1). Analysis of the combined PG and DGDG zone showed that most of the radioactivity was in PG. There was approximately a 4- and a 6-fold stimulation in the utilization of acetate by section E for the biosynthesis of PC and MGDG, respectively, at the higher light intensity (see also Table II for total incorporation into fatty acids).

Biosynthesis of Fatty Acids from Acetate by Leaf Sections. Increased synthesis of unsaturated fatty acids paralleled the increasing abundance of polyunsaturated fatty acids towards the distal region of the leaf (10). This trend was particularly evident at the higher light intensity (Table IV). However, none of the leaf sections utilized acetate to synthesize fatty acids in the same proportions as observed in the endogenous lipids. The difference in the distribution of ^{14}C in the fatty acids synthesized by sections B and E is shown in the radiogas-liquid chromatograms (Fig. 2). There was a very marked increase in the biosynthesis of unsaturated fatty acids and decrease in the biosynthesis of the long chain saturated fatty acids by the distal sections.

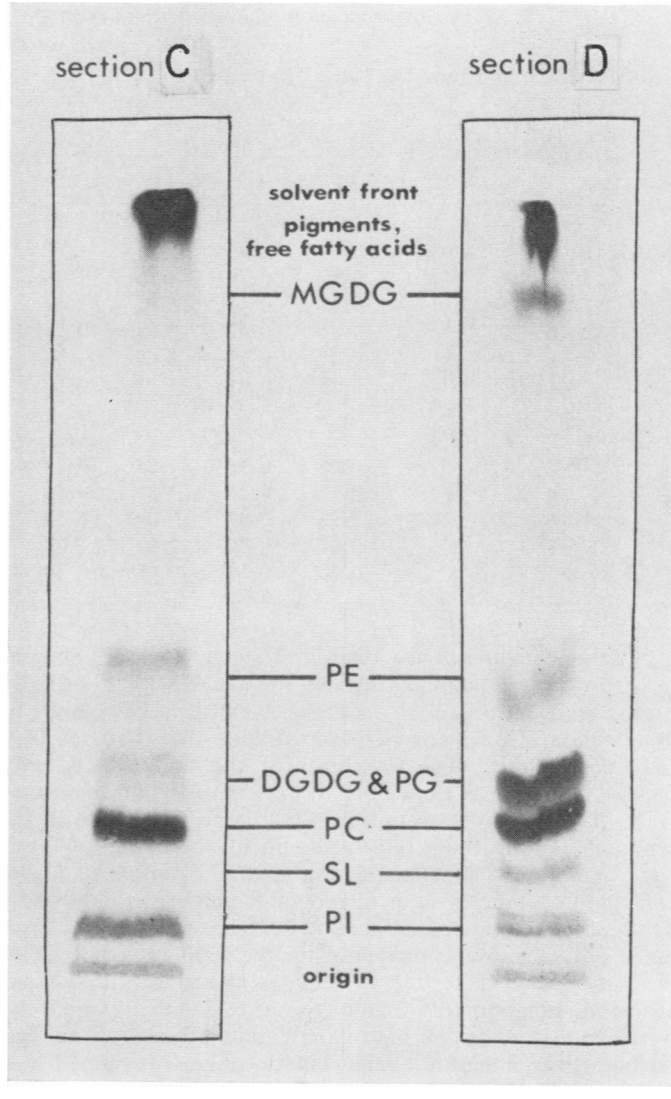

FIG. 1. Autoradiograph of TLC separations of the lipids synthesized from acetate-1-^{14}C by sections C and D of maize leaves after incubation for 3 hr at 2800 ft-c (approximately 20,000 dpm were applied to each plate).

Table III. *Specific Activities of Phosphatidyl Choline and Monogalactosyl Diglyceride Isolated from Tissue Slices*

Slices were from 7-day-old maize seedlings incubated with acetate-1-^{14}C for 3 hr at 150 ft-c and 30 C.

Leaf Section	PC			MGDG		
	Acetate incorporation		Specific activity	Acetate incorporation		Specific activity
cm from base	%[1]	*nmoles/g dry tissue*	*dpm/μmole*	%[1]	*nmoles/g dry tissue*	*dpm/μmole*
0–2 (A)	20.2	3.4	38,040	7.2	1.2	19,100
2–4 (B)	30.5	5.3	42,500	10.5	1.8	11,350
4–6 (C)	37.2	6.3	65,900	11.8	2.1	14,040
6–8 (D)	47.4	4.6	44,200	16.2	1.5	3,560
8–12 (E)	22.0	2.1	51,170	7.0	0.7	1,840

[1] Percentage of the radioactivity incorporated into acyl lipid.

At low light illumination (150 ft-c), no polyunsaturated fatty acids were synthesized from acetate by section A. Ninety-five per cent of the label entered saturated fatty acids (Table IV), the remainder being recovered in 18:1. Section B also synthesized very little labeled unsaturated fatty acids. However, there was a small increase in the incorporation of label into unsaturated fatty acids proceeding from section C to section E. In both sections D and E about 25% of the radioactivity was in 18:1 and 18:2. Only 2 to 3% of the activity incorporated into fatty acids was detected in 18:3.

The most significant effect of increasing the light intensity was to stimulate greatly the synthesis of 16:0, 18:1, and 18:2 in sections C, D, and E, the greatest effect occurring with the distal tissue (Table IV). At the same time, the proportions of 18:0 decreased to the very low levels which occur endogenously (10). However, there was only slight stimulation of 18:3 biosynthesis by increased illumination even in the distal sections of tissue.

Table IV. *Incorporation of Radioactivity from Acetate-1-^{14}C into Fatty Acids by Tissue from Maize Leaves at Two Light Intensities*

Incubation conditions: A, 3 hr at 20 C; B, 3 hr at 30 C.

Leaf Section	Radioactivity											
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0	24:0	26:0
cm from base	*nmoles/g dry wt*											
A. 150 ft-c (room light)												
0–2 (A)	0.10	0.78	2.16		1.71	0.79			2.64	5.21	3.10	
2–4 (B)	0.17	0.17	1.98		3.66	1.00	0.28	0.28	2.72	2.40	3.30	1.30
4–6 (C)		0.42	2.67	0.18	3.29	1.17	0.89	0.60	1.40	2.10	3.97	
6–8 (D)	0.07	0.19	3.09		1.20	1.40	1.31	0.33	0.47	0.50	1.05	
8–12 (E)	0.25	0.37	4.28	0.10	1.27	1.26	1.27	0.30	0.15	0.39	0.54	
B. 2800 ft-c												
0–2 (A)	0.06	0.11	1.23	0.23	0.50	1.85	0.68		2.74	5.44	1.36	
2–4 (B)	0.17	0.26	1.81	0.18	0.79	2.97	1.45		2.95	3.53	2.36	
4–6 (C)	0.38	0.47	6.09	0.34	0.67	6.04	3.29	0.36	1.01	1.77	1.97	
6–8 (D)	0.47	0.71	11.07	0.37	0.65	7.35	6.70	1.08	0.56	0.78	1.27	
8–12 (E)	0.50	0.67	12.98	0.50	0.50	12.50	11.90	1.76	0.34	0.38		

A notable feature of acetate utilization was the large amount of label incorporated into saturated fatty acids with 20 or more carbon atoms, especially by sections A and B at both levels of illumination. Under low light conditions the 20:0 to 24:0 fatty acids contributed two-thirds of the radioactivity with section A (Table IV) but only a minor proportion in section E (Fig. 2). Section C occupied an intermediate position in the synthesis of long chain fatty acids, in that levels of synthesis were high after incubations at low light (7.5 nmoles) and decreased to 4.8 nmoles on incubation at the higher light intensity. Synthesis of saturated fatty acids with 20 or more carbon atoms made only a minor contribution to the total radioactivity of the fatty acids synthesized by sections D and E at 2800 ft-c: at low illumination only 2 nmoles and 0.9 nmole, respectively, were synthesized and in high illumination 2.5 and 0.8 nmoles. At both light intensities, radioactivity in 16:0 remained at a high level and tended to increase in quantitative importance progressively from section A to section E (Table IV).

Distribution of Labeled Fatty Acids in Acyl Lipids. The radioactive polyunsaturated fatty acids synthesized from acetate in increasing amounts by tissue towards the distal ends of maize leaves, especially at the higher light intensity (Table IV), are associated mainly with MGDG and PC and, to a lesser extent, with PE. In particular, most of the linolenate synthesized by tissue from sections C, D, and E was channelled into MGDG with only small proportions (less than 5%) of the total radioactivity in the phospholipids present as 18:3 (Table V). Oleate and linoleate together contributed 60% of the radioactivity in MGDG isolated in section E, and thus 80% of the radioactive fatty acids were unsaturated with 18 carbon atoms (Table V). The proportions of radioactive 18:3 in MGDG were very similar in these three sections (20–26% of the total radioactivity) but endogenous fatty acids consisted of 72 to 85% 18:3 (10). In contrast, MGDG isolated from sections A and B contained no detectable radioactive 18:3 and very little radioactive 18:2, although labeled 20:0 and 22:0 (absent from sections C, D, and E) were detected. At the lower level of illumination, the fatty acids synthesized from acetate by sections C, D, and E had a lower over-all level of unsaturation than at 2800 ft-c.

The distribution of radioactive fatty acids in PC underwent a marked transition from sections A to E and, as with MGDG, the greatest change occurred between section B and section C. PC in section A and, to a lesser extent in section B, contained considerable proportions of radioactive fatty acids with 20 or more carbon atoms; these fatty acids occurred in minor proportions in section C and were virtually absent from PC in sections D and E. Only trace amounts of these acids were present endogenously and were not included in the analyses given in an earlier publication (10). The proportion of 18:2 synthesized from acetate increased from 10.5% in section B to 30.3% in section C, and was greater than 40% in sections D and E. Inasmuch as there was a parallel increase in the total synthesis of fatty acids towards the distal end of the leaf at 2800 ft-c (Table II), there was a substantially greater synthesis of the polyunsaturated fatty acids associated with PC in the distal sections.

At 150 ft-c, stearate contained 31 to 45% of the radioactivity in PC isolated from sections A, B, and C. Increasing the intensity of the illumination to 2800 ft-c reduced 18:0 to minor proportions, and the corresponding increases in 18:1 and 18:2 suggest that desaturation of 18:0 was stimulated under these conditions.

The distribution of radioactivity in fatty acids of PE showed somewhat similar trends to that of PC throughout the leaf but with a slightly lower over-all level of unsaturation. However, the proportions of unsaturated fatty acids incorporated into PG were relatively constant in all sections and were much lower than in the other acyl lipids. Palmitate contributed up to 78% of the total radioactivity and about 27% of the endogenous fatty acids (10). The small amount of 16:1 synthesized was probably Δ^{3t} 16:1 which is a characteristic constituent of PG.

The specific effects of the increased light intensity on the utilization of acetate by maize leaf tissue can be summarized as follows. (*a*) There was a 4-fold increase in acetate incorporation into fatty acids which was particularly marked with sections taken from the distal areas of the leaf. (*b*) The fatty acids synthesized became more unsaturated giving higher proportions of 18:1 and 18:2 and lower proportions of the fatty acids with 20 or more carbon atoms. In the distal sections the total synthesis of unsaturated fatty acids was increased about 8-fold by increasing the light intensity. (*c*) At 2800 ft-c the newly synthesized fatty acids were most extensively incorporated into PC and PG and, to a lesser extent, into MGDG

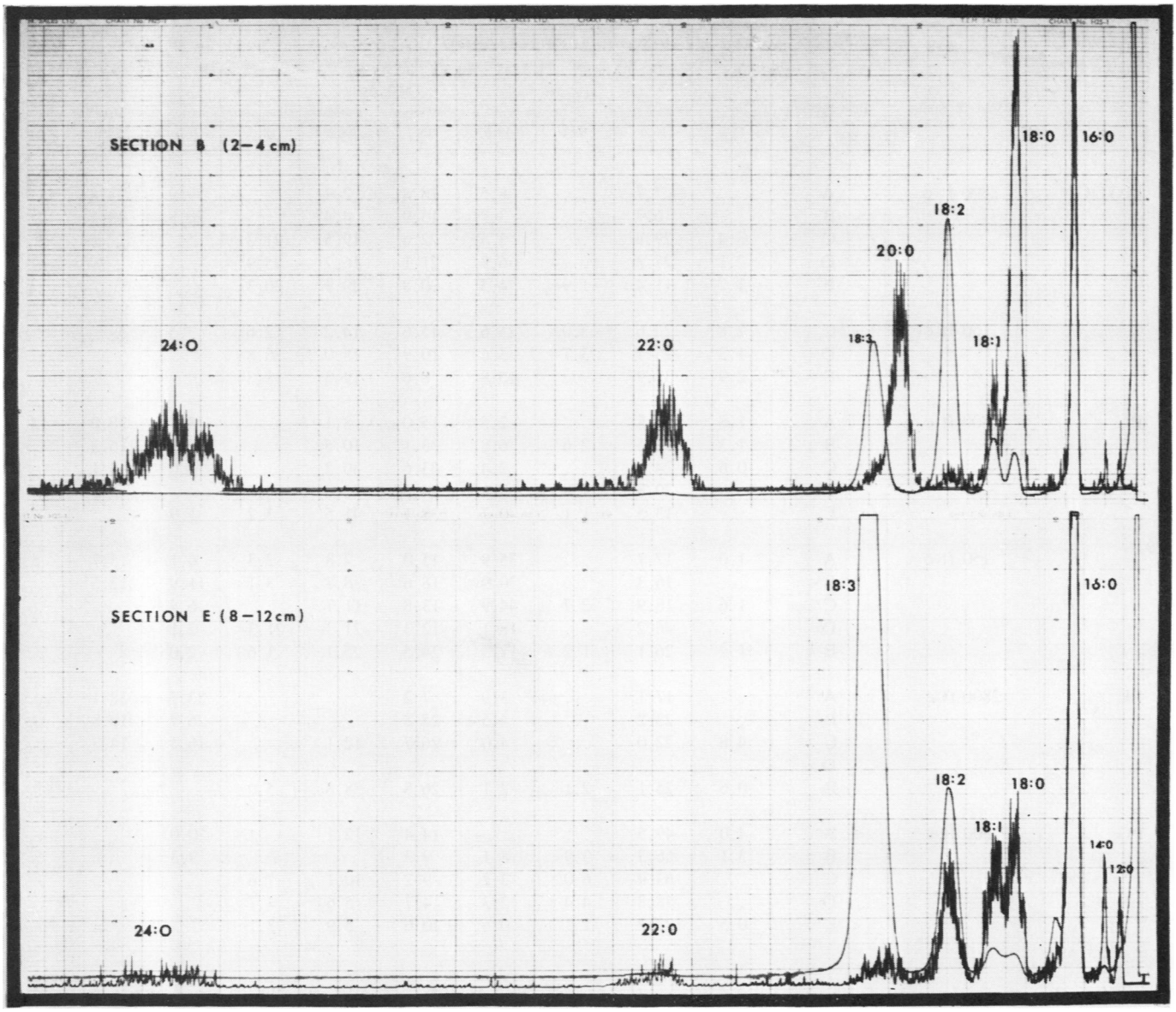

FIG. 2. Radiogas-liquid chromatography traces showing mass (smooth trace) and radioactivity (spiked trace) distributed in fatty acids of total lipid extracts from section B and section E of 7-day-old maize leaves, following incubation of tissue with acetate-1-^{14}C for 3 hr at 150 ft-c.

by distal sections, whereas at 150 ft-c the distribution of the label between the constituent lipids was fairly similar in all sections. (*d*) The response to light increased from the base to the leaf tip, being minimal in sections A and B but of major significance in sections D and E.

DISCUSSION

The description of the ultrastructural differences in plastid structure of the developing maize leaf (9, 10) makes it possible to relate the capabilities of this tissue for lipid biosynthesis with a particular phase of chloroplast differentiation. Section C marks the transition between the proplastid-containing tissue (sections A and B) and the tissue in sections D and E, containing mesophyll chloroplasts (grana of 12–15 compartments) and agranal bundle sheath chloroplasts. Parallel with these morphological changes, clear differences in the utilization of acetate by different sections at both light intensities have now been found. The response of the two basal sections (A and B) to acetate results in an appreciable synthesis of saturated fatty acids with 20 or more carbon atoms, and is very similar to the response previously observed with young etiolated tissue prepared from a number of Gramineae species including maize (6). In addition, insignificant amounts of unsaturated fatty acids were synthesized, their formation being only slightly enhanced by increased light intensity during incubation. In earlier experiments, Hawke and Stumpf (6) found no light response in etiolated tissue. The distal sections of the green maize leaf (beyond 4 cm from the base) utilized acetate to give greater proportions of 16:0, 18:1, and 18:2. The most mature tissue (section E) gave the greatest response to light, leading to the synthesis of greatly enhanced proportions of 18:1 and 18:2, a minor increase in 18:3, and negligible amounts of 18:0.

The intersection diversity of fatty acid composition (10) and the type of fatty acids synthesized from acetate are much greater than the changes observed when dark-grown barley is greened for varying lengths of time (2). In light-grown maize, linoleate comprised 55% and 16% and linolenate 15% and 65% of the fatty acids in the basal and distal sections, re-

Table V. *Distribution of Radioactivity in the Fatty Acids of Acyl Lipids after Incubation of Leaf Tissue from Maize Seedlings with Acetate-1-^{14}C*

Lipid Class	Light Intensity	Leaf Section	Fatty Acids									
			14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0	24:0
			%									
MGDG	2800 ft-c	A	...	7.7	...	8.5	18.8	2.7	...	29.9	27.4	...
		B	...	6.7	...	3.7	15.9	4.4	...	20.8	48.5	...
		C	3.4	29.8	...	3.4	22.6	19.5	21.3	...	...	...
		D	2.1	27.3	...	6.9	21.1	16.7	25.9	...	...	...
		E	1.2	11.4	1.9	4.5	30.8	29.9	20.3	...	...	...
	150 ft-c	C	2.8	22.6	3.0	25.6	15.6	14.2	12.6	3.5	...	...
		D	1.3	25.7	3.7	3.6	10.9	18.0	36.8	...	...	...
		E	2.9	31.9	...	15.8	8.6	19.3	18.3	...	...	...
PC	2800 ft-c	A	1.8	7.8	...	2.5	15.0	8.1	...	13.7	38.0	12.1
		B	1.3	16.5	2.6	6.8	33.1	10.5	...	12.9	16.3	...
		C	0.6	24.0	...	2.4	33.6	30.3	...	5.0	4.1	...
		D	...	20.8	1.8	3.1	24.3	45.8	4.2	...	...	...
		E	...	13.5	1.1	0.7	38.1	41.5	3.2	1.9	...	...
	150 ft-c	A	1.0	17.7	...	35.9	15.0	2.8	2.3	6.1	13.7	5.5
		B	...	16.3	...	30.9	18.6	8.7	3.1	11.9	12.5	...
		C	1.6	18.9	2.7	44.9	13.8	11.7	...	6.4	...	...
		D	...	46.2	...	18.1	12.3	21.3	21.3	2.1	...	...
		E	1.2	26.1	1.2	17.5	24.5	25.1	1.6	2.0	...	...
PE	2800 ft-c	A	...	17.1	...	3.9	7.2	...	...	33.7	38.1	...
		B	...	23.2	...	4.5	25.8	...	...	26.7	19.8	...
		C	4.8	22.0	...	4.0	26.7	12.1	...	16.1	14.3	...
		D	...	...	...	...	...	...	...	...	...	...
		E	0.6	25.1	2.1	2.1	26.8	38.3	5.0	...	...	...
PG	2800 ft-c	A	4.0	49.5	...	...	14.4	12.1	...	20.0	...	...
		B	3.1	66.3	3.9	8.1	9.3	...	...	9.3	...	...
		C	...	63.9	6.0	3.2	9.2	10.1	7.6	...	...	...
		D	...	78.3	4.4	2.3	4.7	5.6	4.7	...	...	...
		E	0.5	77.2	2.1	0.6	10.6	5.9	3.1	...	...	...

spectively, whereas in the etiolated system the fatty acids of the lipids underwent only slight changes between 3- and 36-hr greening; 18:2 decreased from 29% to 19% while 18:3 increased from 41% to 55%. In contrast, when etiolated red clover was exposed to light during acetate feeding (15), the nature of the fatty acids remained unaltered (mainly unsaturated and monoenoic), although there was a greatly stimulated utilization of substrate for fatty acid and lipid synthesis.

Differences in the biosynthetic characteristics of the etiolated system and of the light-grown system studied here apply to both the endogenous and newly synthesized fatty acids of individual lipids. Appelquist *et al.* (2) found that the distribution of label in newly synthesized phospholipids and galactolipids was fairly constant, irrespective of the greening time. This contrasts with the wide differences in the nature of the fatty acids of the acyl lipids synthesized from acetate by the morphologically diverse tissue along the length of green maize leaves, particularly when the light intensity was increased. In the present study, PC and PG were the two lipids most strongly labeled in response to light. In the etiolated system on greening, Tremolieres and Mazliak (15) demonstrated increases in the labeling of PC and PE, while Appelquist and co-workers (2) found increased labeling in the total phospholipids.

Although the response to acetate of the maize tissue, differentiated under normal physiological conditions, resembles more closely the barley (2) than the red clover system (15), it is clear from the present work that the behavior of leaf cells during artificial greening is very much simpler and might not necessarily provide relevant information on the response during differentiation.

The inability to obtain newly synthesized fatty acids in the same proportions as exist endogenously appears to be related to a low rate of 18:3 biosynthesis from 18:1 and 18:2. This is best illustrated in MGDG isolated from section E in which 18:1, 18:2, and 18:3 comprise 30.8, 29.9, and 20.3% of the radioactive fatty acids (total 81.0%) compared with endogenous proportions of 1.5, 3.7, and 91.2%, respectively (total 96.4%). As a consequence of low 18:3 biosynthesis, the composition of the newly synthesized fatty acids in MGDG was markedly different from the endogenous fatty acids.

The similarity in the fatty acid composition of the newly synthesized PC and PE to that of the endogenous PC and PE is probably due to 18:3 not being such a dominant fatty acid constituent as in MGDG. In PC in section E 18:1, 18:2, and 18:3 together comprise 80% of the label whereas these fatty acids total about 71% endogenously; the main difference in this case being an altered 18:1/18:2 ratio. These findings related to both PC and MGDG, appear to be consistent with a

slow or impaired desaturation of 18 carbon atom fatty acids in the transformation 18:1→18:2→18:3.

The greater incorporation of fatty acids into galactolipids and PG in the distal than in the basal sections, especially noticeable in the PG of sections D and E, is consistent with the increasing quantitative importance of these lipids as the chloroplasts develop. However, PC was the most highly labeled of the acyl lipid components, despite a cellular distribution which favors the nonchloroplastic fractions (12). PC also had the highest specific activity of the glycerolipids of quantitative importance, although PI was higher in section A. Furthermore, there was an appreciable light stimulation of PC synthesis in section E despite the greatly decreased quantitative importance of PC in the more mature tissue (9). Arising from turnover studies with older photosynthetic tissues, it has been suggested that PC may be of special significance in fatty acid biosynthesis (5, 13). If PC is involved as an acyl carrier in the desaturation of 18:1 to 18:2 (5), increased labeling of PC could be expected to result from the greater demands for polyunsaturated fatty acids for the galactolipids and for PG which are required for granal formation. Therefore, the increased labeling of PC in sections D and E is not necessarily inconsistent with its greatly decreased quantitative importance in these sections. PG is highly labeled but the incorporation of label into MGDG remained rather low, and perhaps this is associated with the relatively low synthesis of 18:3, the major fatty acid of the MGDG.

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