Lipid biosynthesis in chloroplast mutants of barley

LARS-ÅKE APPELQVIST,* JOHN E. BOYNTON,‡ KNUD W. HENNINGSEN, P. K. STUMPF and DITER VON WETTSTEIN

Department of Biochemistry and Biophysics, Department of Genetics, University of California, Davis, California 95616, and Institute of Genetics, University of Copenhagen, Copenhagen, Denmark

ABSTRACT The capacity of leaf slices from light-grown seedlings of wild type barley and 10 *xantha* mutants at six different gene loci to incorporate acetate-^{14}C into various lipids has been investigated. The fatty acid composition of the leaf lipids in these lethal mutants was similar to that of the wild type, but the fatty acid labeling pattern in the individual lipid classes can be drastically altered by these mutations, which affect chloroplast differentiation. A genetic block in chlorophyll synthesis, caused by mutations in the *xan-f* locus, leads to a repression of the formation of chloroplast membranes and of acetate incorporation into phospho-, sulfo-, and galacto-lipids (the acetate being preferentially channeled into a lipid fraction containing steroids and free fatty acids). Two leucine "auxotrophs" at different loci, which in the absence of leucine in the growth medium produce giant grana and accumulate some chlorophyll, differed considerably in the amount of labeling of their polar lipids during incubation.

Leaves of *xan-a*^{11}, containing plastids with little chlorophyll, highly disorganized membrane systems, and large bodies with osmiophilic deposits, were nonetheless equal to wild type in their capacity to incorporate acetate-^{14}C into phospho-, sulfo-, and galacto-lipids. The mutants at the *xan-m* locus have plastids with undispersed prolamellar bodies and osmiophilic packages of grana-like membranes associations. Leaf slices of these mutants synthesized considerably more linolenic acid-^{14}C, which was incorporated into monogalactosyl diglycerides, than did slices of the wild type. This led to a labeling pattern of the fatty acids in the monogalactolipids which was remarkably similar to their endogenous fatty acid composition.

KEY WORDS barley · *xantha* mutants · chloroplast structure · acetate incorporation · lipid biosynthesis · monogalactosyl diglyceride · digalactosyl diglyceride · steroids · radio–gas chromatography · linolenic acid

* Present address: Chemistry Department, Swedish Seed Association, Svalöf, Sweden.

‡ National Institute of Health Postdoctoral Fellow 1966–68.

Leaves from seedlings of lethal chloroplast mutants contain plastids with lamellar systems that are either partially developed or else organized as abnormal associations of membranes. Such gene mutants can be used in studies of the influence of the state of structural organization of the chloroplast on lipid metabolism of photosynthetic tissue. Conversely, the abnormal plastid development of some of these mutants may result from a defect in the capacity to synthesize chloroplast lipids.

We have explored the capacity of leaf slices from light-grown seedlings of the wild type and 10 *xantha* mutants of barley to incorporate acetate-^{14}C into the different leaf lipids. These mutants belong to six different gene loci: one locus (*f*) controls chlorophyll synthesis, mutants at two of the other loci (*b* and *c*) are leucine "auxotrophs," while the mutants at the other three loci have unidentified errors in metabolism. We have previously reported changes in the capacity of leaf slices of the wild type to synthesize various leaf lipids in the course of chloroplast development (1).

METHODS

Seedlings of the barley genotypes studied were grown in trays containing moist vermiculite at 20°C under cool white fluorescent lights (16 hr/day, ca. 500 ft-c). Primary leaves of 7–9-day old seedlings were sliced cross-wise into 1–2-mm long pieces, suspended in 5.2 ml of 0.2 M bicarbonate buffer containing 4 μc of sodium acetate-1-^{14}C (72 mμmoles), infiltrated in vacuo, saturated with O_2, and incubated for 3 hr in a Warburg incubator at 30°C with a light intensity of about 1000 ft-c (cf. 1). 2 g of tissue were used for the incubation, with the exception of *xan-f*^{10}, -*f*^{26}, and -*f*^{40} for which only 0.4, 1.0, and 0.2 g of tissue, respectively, were available.

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Leaf lipids were extracted according to Bligh and Dyer (2) in a Sorvall Omni-mixer with chloroform–methanol–water 2:2:0.8 as described previously (1). Radioactivity of the chloroform phase containing the total lipids was determined by counting aliquots in a liquid scintillation counter. The lipid classes were separated by ascending thin-layer chromatography on 20 × 20 cm glass plates coated with Merck's Silica Gel G in toluene–ethyl acetate–ethanol 2:1:1 (1). The six lipid fractions obtained conformed to those described for wild type barley namely A, phosphatidyl choline, phosphatidyl inositol, and other phospholipids; B, phosphatidyl glycerol and sulfoquinovosyl diglyceride; C, digalactosyl diglyceride; D, monogalactosyl diglyceride; E, free fatty acids and steroids (sterols and β-amyrin); and F, triglycerides and hydrocarbons. The radioactivity of the various lipid bands recovered from the thin-layer plates was determined by assaying aliquots of the eluates on planchettes with a thin window Geiger-Müller gas-flow counter (Nuclear-Chicago).

Fatty acid methyl esters were prepared from the lipid fractions with the aid of BF_3–methanol (cf. 1). Gas chromatographic analysis was performed on an Aerograph A 90 P 2 model equipped with a 5 ft × 1/4 inch stainless steel column containing 12% diethylene glycol succinate polyester on 60–70 mesh Anakrom ABS. The column was operated at 160–170°C with a helium flow rate of about 60 ml/min. Radioactivity of the effluent was monitored with a Nuclear-Chicago Biospan proportional counter, model 4998. Methyl palmitate-1-^{14}C (New England Nuclear Corp., Boston, Mass.) of known activity was used as standard for radioactivity determinations. For calibration of the chromatograph and identification of the fatty acid peaks, mixtures of pure fatty acid methyl esters (Applied Science Laboratories Inc., State College, Pa.) were injected. Amounts of fatty acids were determined by calculation of the peak areas as the product of peak height times width at half height.

The electron micrographs are from leaf material fixed in phosphate-buffered glutaraldehyde, subsequently fixed with buffered OsO_4, and embedded in an epoxy resin–acid anhydride mixture kindly supplied by Dr. A. R. Spurr, University of California at Davis. The thin sections have been contrasted with uranyl acetate and lead citrate.

MATERIALS

The following lethal *xantha* mutants have been investigated: *xan-a*^{11} (alboxantha); *xan-b*^{12}; *xan-c*^{23}; *xan-d*^{31}; *xan-f*^{10}, *-f*^{26}, *-f*^{40}; and *xan-m*^{3}, *-m*^{48}, *-m*^{53}. *xan-m*^{3} was isolated from the variety Golden barley by Nilsson-Ehle in the 1920's. *xan-m*^{48}, *-m*^{53}, *xan-f*^{10}, *-f*^{40}, and *xan-d*^{31} were isolated from the variety Bonus; *xan-a*^{11} and *xan-b*^{12} from *ert-a*^{23} (an erectoides mutant of Bonus); *xan-f*^{26} from *ert-d*^{15} (an erectoides mutant of the variety Maja); and *xan-c*^{23} from *ert-c*^{14}*d*^{14} (an erectoides mutant of Maja). *xan-m*^{3} and *-m*^{48} appeared as spontaneous mutants. *xan-a*^{11}, *-b*^{12}, *-c*^{23}, *-d*^{31}, and *-f*^{26} have been induced with neutrons; *xan-f*^{10}, *-f*^{40} and *-m*^{53}, with sparsely ionizing radiations (X-, γ-rays).

The chloroplast structure, pigment content, and in some cases the metabolic blocks of the *xantha* mutants at these six loci are known. Figs. 2–10 are selected to demonstrate the type and amount of lamellar systems in the plastids of the mutant seedlings, which were grown in the dark for 7 days and subsequently exposed to 320 ft-c of white light for 24 hr at 22°C. Plastids in leaves of the wild type (Bonus) develop a complete lamellar system when greened under these conditions (Fig. 1). The capacity for structural development and pigment accumulation in these mutants is best evaluated in such synchronously developing plastids of dark-grown primary leaves. On the other hand the lipid-synthesizing capacity of the mutants has been investigated in light-grown seedlings (16 hr of 500 ft-c/day). Minor differences in plastid structure and pigment content between dark-grown mutant seedlings illuminated for 24 hr and light-grown seedlings can exist.

Locus *xan-f* controls chlorophyll synthesis. The alleles *xan-f*^{10} (3) and *-f*^{40} (4) are blocked at a step prior to protochlorophyllide and fail to aggregate the membrane discs of the plastids into grana structures. Although *xan-f*^{26} is a "leaky" mutant able to synthesize some chlorophyll, it accumulates the same porphyrins as the other alleles when fed δ-aminolevulinic acid (4). The widely spaced membrane discs seen in Fig. 2 are typical for the plastids of mutants at this locus. Compared to the wild type, the total amount of membrane material is strongly reduced. Spheroidal grana (Figs. 2 and 4) are found in plastids of leaves of *xan-f*^{26} that contain chlorophyll (in the present sample, about 10% of the wild type amount of chlorophyll).

Locus *xan-d:* The mutant *xan-d*^{31} can accumulate about 60% of the chlorophyll present in the wild type. Membrane discs in the plastids are cemented together into grana, some of which become exceedingly large in diameter (Fig. 3). The grana are not integrated into a regularly oriented lamellar system.

Locus *xan-b:* Mutants at this locus respond to leucine feeding by accumulating increased amounts of chlorophyll (5, 6). The discs in the plastids of untreated leaves are often aggregated into giant grana with an abnormal membrane spacing (7). A giant granum in a plastid of a *xan-b*^{12} leaf greened for 24 hr and containing about 60% of the amount of chlorophyll pigment of the wild type is shown in Fig. 6. Some spheroidal grana (Fig. 5) and numerous grana with diameters smaller than

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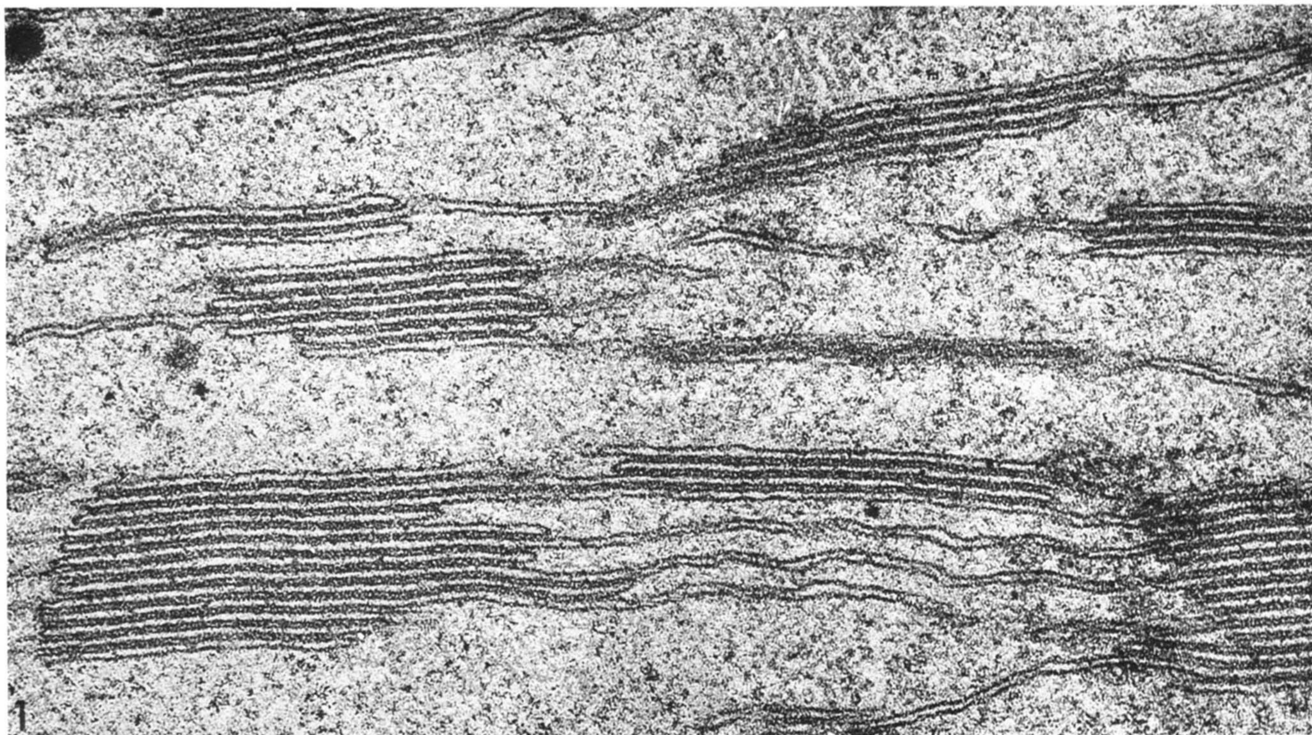

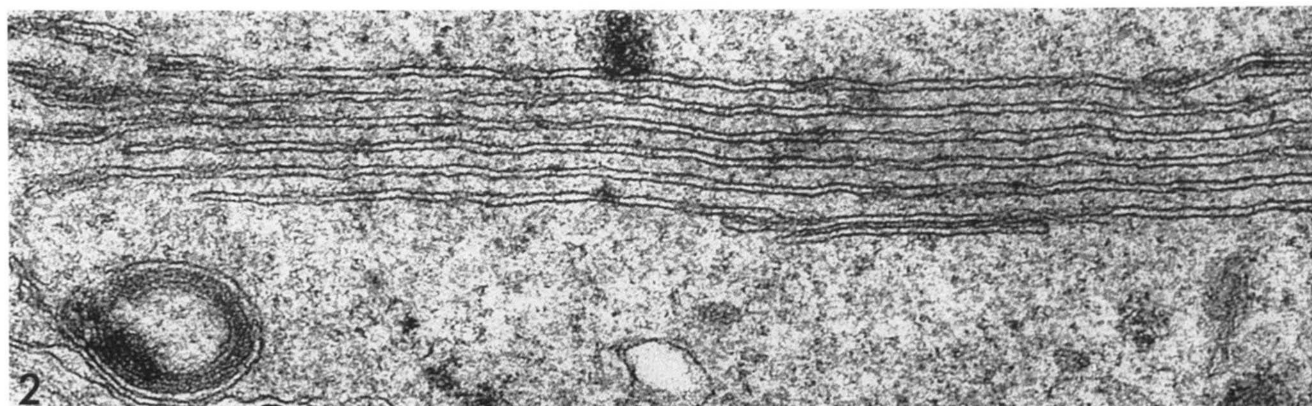

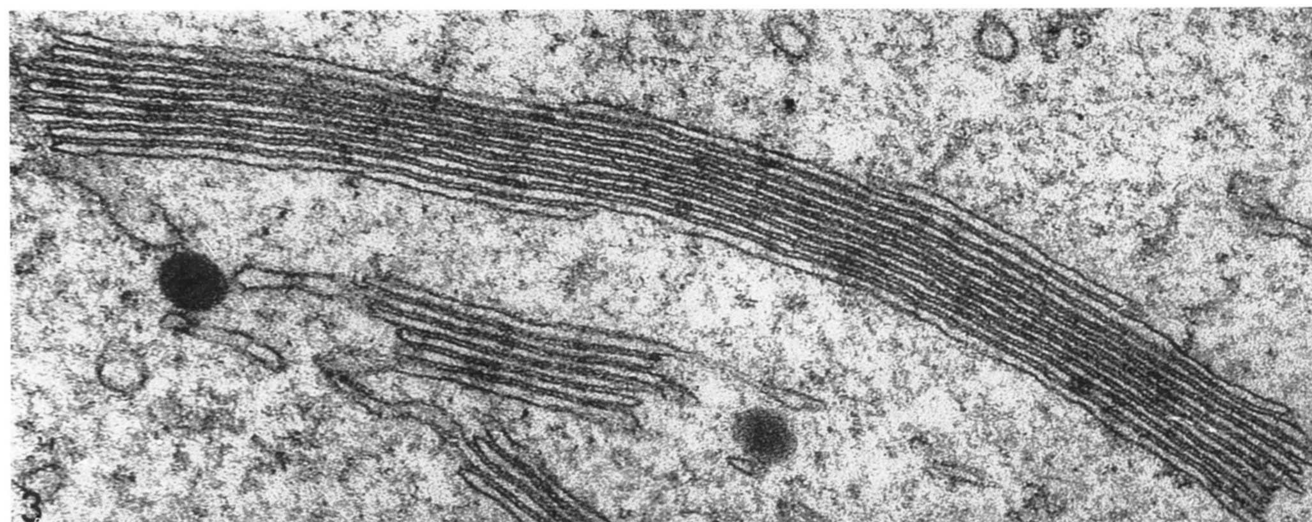

Figs. 1–10 are from plants grown for 7 days in the dark and then illuminated for 24 hr.

Fig. 1. Section through a chloroplast in a primary leaf of a wild type barley seedling. ×115,000.

Fig. 2. Section through plastid in a primary leaf of *xan-f*[26]. ×115,000.

Fig. 3. Section through plastid in a primary leaf of *xan-d*[31] with a giant granum. ×115,000.

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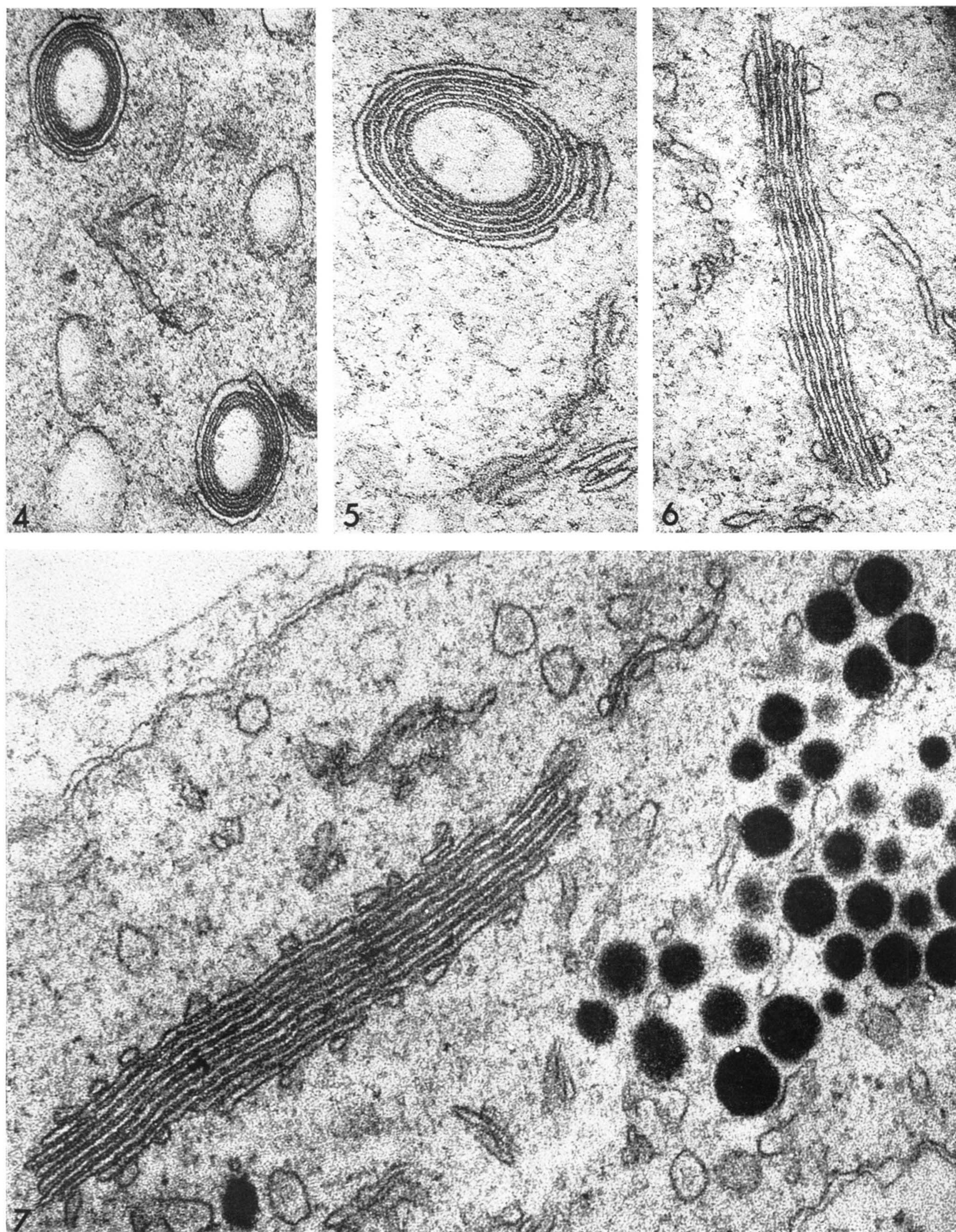

FIG. 4. Section through a spheroidal granum of *xan-f*[26]. ×105,000.
FIG. 5. Section through a spheroidal granum of *xan-b*[12]. ×110,000.
FIG. 6. Section through a giant granum of *xan-b*[12]. ×110,000.
FIG. 7. Section through a plastid of *xan-c*[23] with a giant granum. ×105,000.

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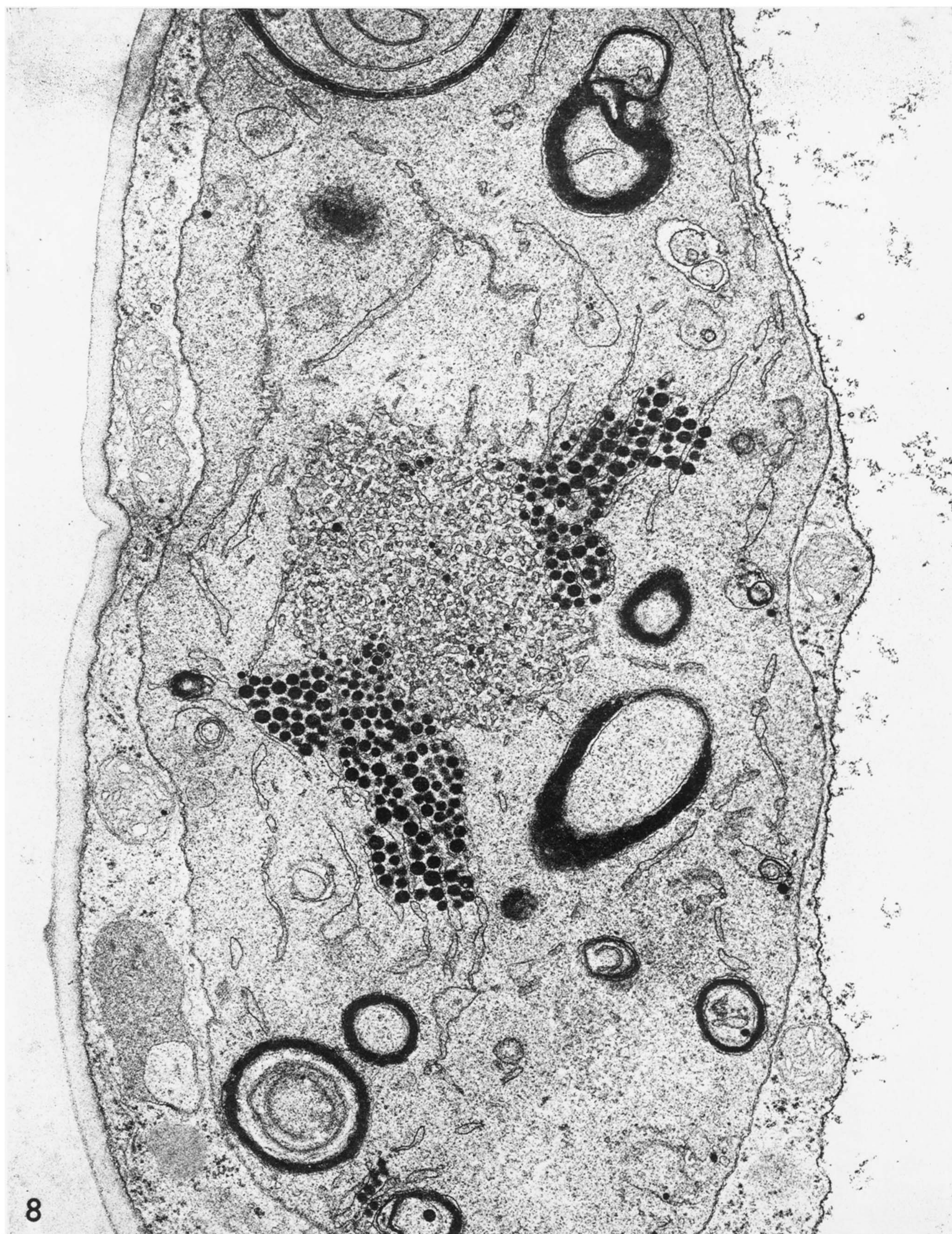

Fig. 8. Section through a plastid of *xan-a*[11] with an irregular membrane system and large bodies containing osmiophilic deposits. ×28,000.

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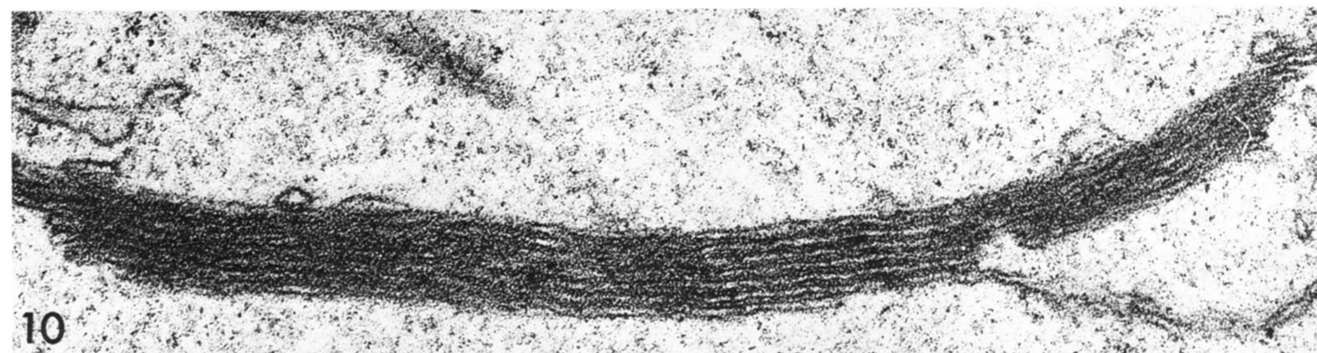

FIG. 9. Section through a plastid of *xan-m*^{53} with an undispersed prolamellar body. ×70,000.
FIG. 10. Section through a grana-like membrane association in a plastid of *xan-m*^{3}. ×95,000.

those in the wild type consisting of only 3 to 5 discs also occur. No regular orientation of the grana within a lamellar system is found.

Locus *xan-c:* The chlorophyll content and chloroplast structure of the mutant *xan-c*[23] are partially normalized in seedlings growing on a leucine-supplemented medium (5). A typical giant granum in a plastid from an untreated leaf of *xan-c*[23] greened for 24 hr and containing about 40% of the chlorophyll of the wild type is shown in Fig. 7. Many small grana and a discontinuous lamellar system similar to that found in plastids of mutants at the *xan-b* locus are also observed.

Locus *xan-a:* Mutants at this locus assemble highly irregular and variable membrane systems in their plastids (Fig. 8). The membrane material may be organized as branched reticulate structures, as individual groups of concentric primary layers, as undispersed prolamellar bodies, as swollen vesicles, or as large bodies containing osmiophilic deposits. Large aggregations of osmiophilic globuli can occur. The plastid of the mutant *xan-a*[11] shown in Fig. 8 is from a leaf containing about 1% of the chlorophyll of the wild type. If mutants of this locus are grown in dim light (ca. 2 ft-c) for prolonged periods they accumulate chlorophyll (up to 25% of wild type) and form normal grana within the irregular membrane systems.

Locus *xan-m:* The three mutants *xan-m*[3], *-m*[48], and *-m*[53] form packages of grana-like membrane associations and prolamellar bodies in plastids of dark-grown leaves. The prolamellar body is not dispersed by exposure to light for 24 hr (Fig. 9). During this period there appears to be no formation of grana or additional grana-like membrane associations, in spite of the fact that chlorophyll accumulation in the mutants reaches about 25% of that of the wild type. This suggests that the pigment synthesized during illumination is integrated into the grana-like membrane associations (Fig. 10) which have been formed in the dark. In certain developmental stages of plastids of the mutant *xan-m*[3], large numbers of globuli are present in addition to the grana-like packages (8).

RESULTS

The amounts of sodium acetate-^{14}C incorporated into lipids by slices of light-grown leaves of 10 *xantha* mutants and of the wild type during a 3 hr incubation period are presented in Table 1. Incorporation by the wild type varied between 10.2 and 15.1 mμmoles of acetate per g fresh weight in the four separate experiments. Some of the *xantha* mutants (e.g. *xan-a*[11] and *xan-f*[10]) incorporated as much of the labeled acetate into total lipids as the wild type, while others (e.g. *xan-c*[23] and *xan-d*[31]) gave slightly lower figures. The values for all mutants, however, fall within or very close to the range of 8.5–11.6 mμmoles/g

TABLE 1 Incorporation of Acetate-^{14}C into Lipids by Leaf Slices of Seedlings of Wild Type and Some *xantha* Mutants of Barley

Experiment	Genotype	Acetate Incorporation into: Total Lipids		Phospho-, Sulfo-, and Galactolipids
		mμmoles/g fresh weight		
I	Wild type (Bonus)	10.2	12.7 ± 1.0	6.2
II	" "	15.1		
III	" "	13.2		
IV	" "	12.1		
II	*xantha-b*[12]	10.1		1.3
I	*xantha-f*[10]	10.7		2.4
II	" *-f*[26]	9.0		2.6
II	" *-f*[40]	11.6		3.3
II	*xantha-d*[31]	7.6		3.3
III	*xantha-c*[23]	7.7		3.8
I	*xantha-a*[11]	12.7		6.3
IV	*xantha-m*[3]	9.0	8.8 ± 0.1	5.3
IV	" *-m*[3]	8.9		
IV	" *-m*[3]	8.4		
III	*xantha-m*[48]	8.5		3.9
III	" *-m*[53]	10.2		6.8

Incubation: 3 hr in light at 30°C. Means ± SEM.

fresh weight observed for leaf slices of the wild type at various stages of greening (1). On the other hand, very different amounts of label were channeled into the polar lipids of the various mutants (Table 1). The mutants at the *xan-b* and *xan-f* loci incorporated relatively little label into these lipids while mutants at the *xan-a* and *xan-m* loci behaved like wild type.

A comparison of the amount of labeled acetate incorporated into the individual classes of leaf lipids by the *xantha* mutants is presented in Fig. 11 as a percentage of that incorporated by the wild type; absolute incorporation values for the latter are given in Table 2. Low acetate incorporation into all polar lipids (Fractions A, B, C, and D) by mutants at the *xan-b* and *xan-f* loci is accompanied by four to five times higher incorporation into the steroid-containing "E" fraction (Fig. 11). Individual values for the three alleles at the *xan-f* locus are given in Table 2. This labeling pattern is similar to that of dark-grown tissues of the wild type barley after a 3 hr exposure to light (1): labeling of the polar lipid fractions A, B, C, and D was only 30, 24, 59, and 33%, respectively, of that of the light-grown wild type in the present experiment, whereas incorporation into the steroid-containing fraction was 380%.

Mutants *xan-c*[23] and *xan-d*[31] channeled relatively more labeled acetate into the phospho-, sulfo-, and galactolipids and less into the steroid-containing fraction than

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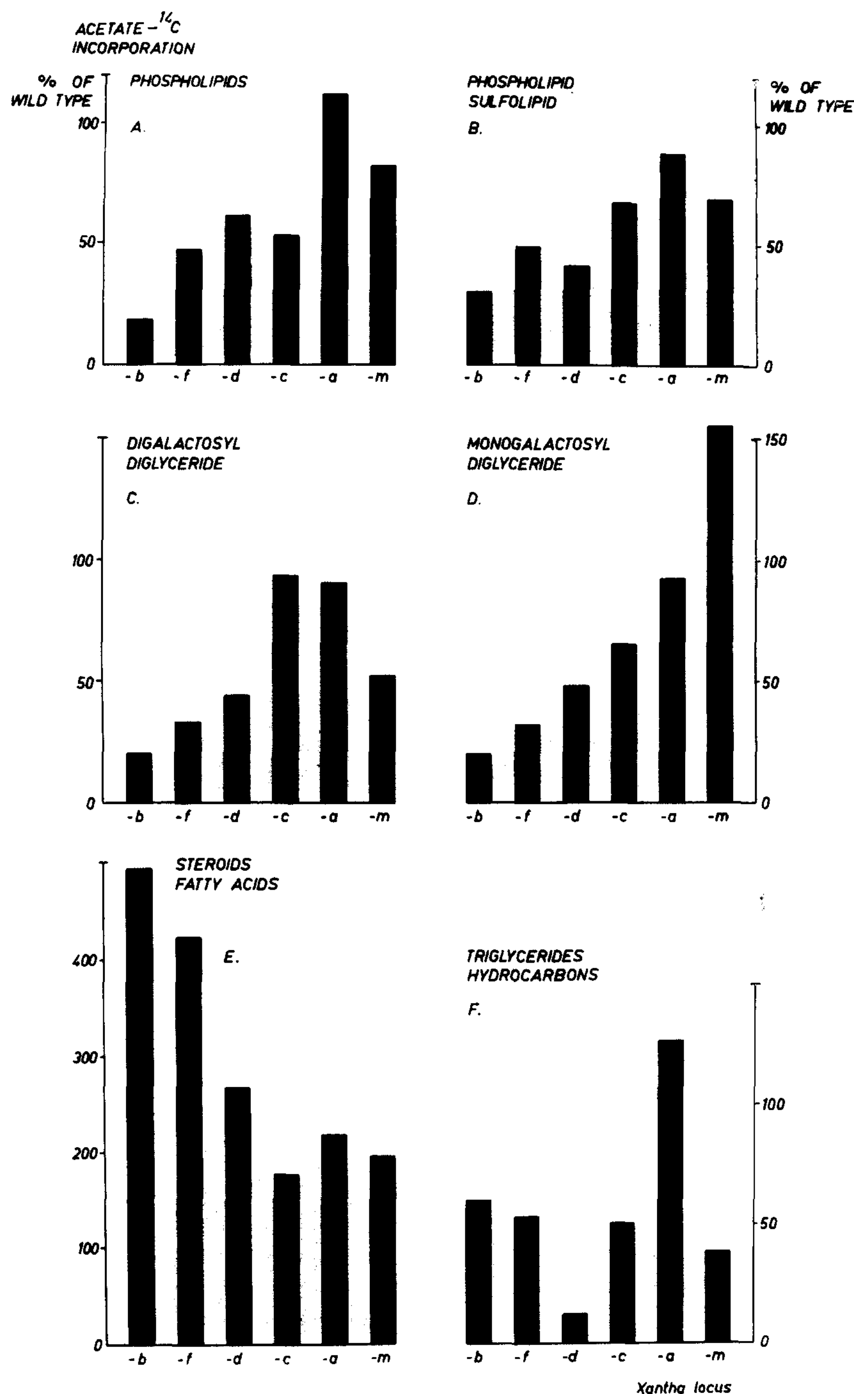

FIG. 11. Incorporation of acetate-^{14}C into the leaf lipids by the mutants of six *xantha* loci as a percentage of that incorporated by the wild type. Values given for the *xan-f* and *xan-m* loci are averages for three alleles.

did those at the *xan-b* and *xan-f* loci (Fig. 11). In comparison with the wild type, labeling of the different polar lipids by the mutants *xan-c*^{23} and *xan-d*^{31} was, with one exception, reduced to roughly 50%, while incorporation into steroids increased two to three fold. The mutant *xan-c*^{23} approached the capacity of the wild type for labeling digalactosyl diglycerides.

The mutants at the *xan-a* and *xan-m* loci also synthesized twice as much steroid plus free fatty acids from the labeled acetate as did the wild type. With respect to the phospho-, sulfo-, and galacto-lipids, the mutant *xan-a*^{11} showed a labeling pattern remarkably similar to that of the wild type. This is of special interest in view of the highly irregular membrane systems that are assembled in

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TABLE 2 Incorporation of Acetate-^{14}C into Various Lipid Classes by Leaf Slices of the Wild Type and Mutants at Two *xantha* Loci

Genotype	Acetate Incorporation into Fractions					
	A. Phospholipids	B. Phospho- and Sulfolipids	C. Digalactosyl Diglyceride	D. Monogalactosyl Diglyceride	E. Steroids, Fatty Acids	F. Triglycerides, Hydrocarbons
	mμmoles/g fresh weight					
Wild type	3.36	1.62	0.41	0.79	1.49	2.57
xantha-f^{10}	0.93	0.83	0.23	0.44	7.24	1.00
" *-f^{26}*	1.65	0.77	0.08	0.07	5.30	1.10
" *-f^{40}*	2.17	0.79	0.09	0.24	6.38	1.95
xantha-m^{3}	2.30	1.51	0.21	1.27	2.15	1.60
" *-m^{48}*	1.83	0.90	0.24	0.93	4.37	0.28
" *-m^{53}*	4.20	0.95	0.19	1.48	2.28	1.08

Incubation: 3 hr in light at 30°C.

the plastids of this mutant (Fig. 8). All mutants at the *xan-m* locus surpassed the wild type, on the average by 50%, in incorporation of acetate into monogalactosyl diglycerides. On the other hand, labeling of the digalactosyl diglycerides was only 50% of that of wild type. As seen in Table 2, all three alleles at this locus decreased the labeling of digalactosyl diglycerides and increased that of the monogalactosyl diglycerides. This may be significant in relation to the characteristic packages of grana-like membrane associations or occasional accumulations of globuli observed in plastids of these mutants (Figs. 9–10).

The fatty acid composition of the different lipids (fractions A, B, C, and D) of the primary leaves of all the *xantha* mutants was rather similar to that of the wild type (Table 3). No differences in the contents of the fatty acids could be established between the wild type, *xan-f^{26}*, *xan-c^{23}*, and *xan-a^{11}*. In *xan-b^{12}*, the phospholipid fraction (A) appears to contain relatively more linolenic acid than that of the wild type, as do the phospholipid fraction (A) and the galactolipid fractions (C and D) of *xan-d^{31}*. All three alleles at the *xan-m* locus contained a phospholipid fraction (A) with a higher linolenic acid content than in the wild type, whereas compositions of the galactolipid fractions (C and D) corresponded closely to those of wild type. (Values reported in Table 3 for the fraction C of *xan-m^{3}* are unreliable because of sample loss.) A fatty acid analysis of the mutants *xan-f^{10}* and *-f^{40}* was precluded by shortage of tissue.

The distribution of the label among the major fatty acids of the different lipids synthesized by the wild type and the various *xantha* mutants is compared in Table 4. Leaf slices of the mutant *xan-a^{11}* not only synthesized quantitatively as much phospho-, sulfo-, and galacto-lipids from acetate as did wild type slices, but also synthesized a spectrum of ^{14}C-labeled fatty acids very similar to that of wild type. Minor differences were found in the percentage distribution of label among the fatty acids of the phospho-, sulfo-, and galacto-lipids from the other mutants (Table 4). Some of these differences may represent analytical errors due to the small amount of label incorporated into these lipids by mutants such as *xan-b^{12}* and *xan-f^{26}*. It is, however, noteworthy that the monogalactosyl diglyceride fraction of all mutants, except *xan-a^{11}*, had a higher relative content of labeled linolenic acid than that of the wild type.

As Table 5 shows, the leaf slices of the mutants *xan-c^{23}*, *xan-d^{31}*, and *xan-m^{3}*, *-m^{48}*, *-m^{53}* actually synthesized more labeled linolenic acid, which was incorporated into monogalactosyl diglyceride, than did the slices of the wild type. This phenomenon is most pronounced for the three alleles at the *xan-m* locus, which stimulated the acetate incorporation into monogalactosyl diglycerides (cf. Fig. 11). Leaf slices of these mutants showed a three to four fold increase in the amount of labeled acetate incorporated into the linolenic acid of this monogalactolipid. Such a preferential labeling of linolenic acid of the monogalactolipid was not characteristic for slices of greening wild type leaves (1). Slices greened for 3 hr incorporated as little as 0.08 mμmole of acetate into linolenic acid of the monogalactosyl diglycerides, which is half the amount incorporated by the light-grown wild type of the present experiment.

The labeling patterns of the major fatty acids in the different lipid classes, averaged for the three *xan-m* mutants, are contrasted with those of the wild type in Fig. 12. More labeled stearic acid was found in all lipids of these mutants than in those of the wild type. Conspicuous differences in the amount of labeled oleic, linoleic, and linolenic acids in the monogalactosyl and digalactosyl diglycerides from the mutants and from the wild type are evident. The spectrum of labeled fatty acids of the monogalactosyl diglycerides in the leaf slices of the *xan-m* mutants is more similar to that of the endogenous fatty acids of this lipid fraction than is the labeling pattern of the wild type. The *xan-m* mutants demonstrate that leaves

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TABLE 3 Content of Palmitic, Linoleic, and Linolenic Acids in Lipid Classes A to D from Leaves of Wild Type Barley and Some *xantha* Mutants

Genotype	A. Phospholipids			B. Phospho- and Sulfolipids			C. Digalactosyl Diglyceride			D. Monogalactosyl Diglyceride		
	16:0	18:2	18:3	16:0	18:2	18:3	16:0	18:2	18:3	16:0	18:2	18:3
							% of total fatty acids					
Wild type	25	25	20	28	11	37	18	5	52	10	4	71
xantha-b^{12}	23	22	43	24	18	42	22	5	59	10	3	70
xantha-d^{31}	24	22	37	21	10	43	14	5	69	5	3	82
xantha-m^{3}	21	11	58	25	7	51	[13	4	80]	15	2	68
" -m^{48}	23	12	51	32	8	51	25	5	59	13	2	67
" -m^{53}	21	15	51	27	10	37	24	6	42	14	3	53

Fatty acids are designated by chain length: number of double bonds.

TABLE 4 Distribution of ^{14}C-Label among Fatty Acids of Lipids Synthesized from Acetate by Slices of Leaves of Wild Type Barley and Some *xantha* Mutants

Genotype	A. Phospholipids					B. Phospho- and Sulfolipids					C. Digalactosyl Diglyceride					D. Monogalactosyl Diglyceride				
	16:0	18:0	18:1	18:2	18:3	16:0	18:0	18:1	18:2	18:3	16:0	18:0	18:1	18:2	18:3	16:0	18:0	18:1	18:2	18:3
											% total label in fatty acids									
Wild type	19	3	36	39	2	30	tr.	29	34	8	34	—	28	26	12	14	—	24	40	20
xantha-b^{12}	22	18	14	40	6	27	11	8	17	9	22	—	—	78	—	21	—	18	13	48
xantha-f^{26}	13	9	13	49	13	23	6	8	32	15	37	—	—	63	—	21	—	9	22	48
xantha-d^{31}	18	—	24	58	—	29	2	13	31	19	21	6	18	34	21	6	—	7	25	62
xantha-c^{23}	16	7	23	48	6	27	3	11	41	18	17	3	14	53	10	10	4	8	29	49
xantha-a^{11}	17	11	27	42	3	25	3	29	39	4	25	1	38	28	5	17	1	22	37	22
xantha-m^{3}	17	19	19	36	7	24	12	15	33	14	16	20	22	22	20	18	6	8	26	42
" -m^{48}	22	8	18	44	8	38	4	10	34	13	18	11	17	41	14	17	3	9	20	51
" -m^{53}	22	7	21	43	7	28	4	15	35	18	22	6	19	40	13	21	—	11	22	47

Incubation: 3 hr in light at 30°C.

TABLE 5 Incorporation of ^{14}C-Label into Fatty Acids of Monogalactosyl and Digalactosyl Diglycerides by Leaf Slices of Wild Type Barley and Some *xantha* Mutants

Genotype	Digalactosyl Diglyceride				Monogalactosyl Diglyceride			
	16:0	18:1	18:2	18:3	16:0	18:1	18:2	18:3
					$m\mu$moles label/g fresh weight			
Wild type	0.14	0.12	0.11	0.05	0.11	0.19	0.31	0.16
xantha-b^{12}	0.02	—	0.06	—	0.03	0.03	0.02	0.08
xantha-f^{26}	0.03	—	0.05	—	0.01	0.01	0.02	0.03
xantha-d^{31}	0.05	0.03	0.06	0.04	0.02	0.03	0.09	0.24
xantha-c^{23}	0.06	0.05	0.20	0.04	0.05	0.04	0.15	0.25
xantha-a^{11}	0.09	0.14	0.10	0.02	0.12	0.16	0.27	0.16
xantha-m^{3}	0.03	0.05	0.05	0.04	0.22	0.10	0.33	0.53
" -m^{48}	0.04	0.04	0.10	0.03	0.16	0.09	0.18	0.47
" -m^{53}	0.04	0.03	0.07	0.02	0.30	0.16	0.32	0.70

Incubation: 3 hr in light at 30°C.

of higher plants contain enzyme systems that allow the rapid synthesis of linolenic acid and its specific incorporation into monogalactosyl diglycerides.

DISCUSSION

Surprisingly, most of the light-grown yellow mutants studied here did not differ appreciably from the wild type in the amount of linolenic acid in their primary seedling leaves. A deficiency of this fatty acid is characteristic for the monogalactosyl diglycerides from dark-grown barley seedlings (1), for the lipids of dark-grown seedlings of other plants (9–11), and for dark or heterotrophically grown algae such as *Euglena* (12). Lipids deficient in

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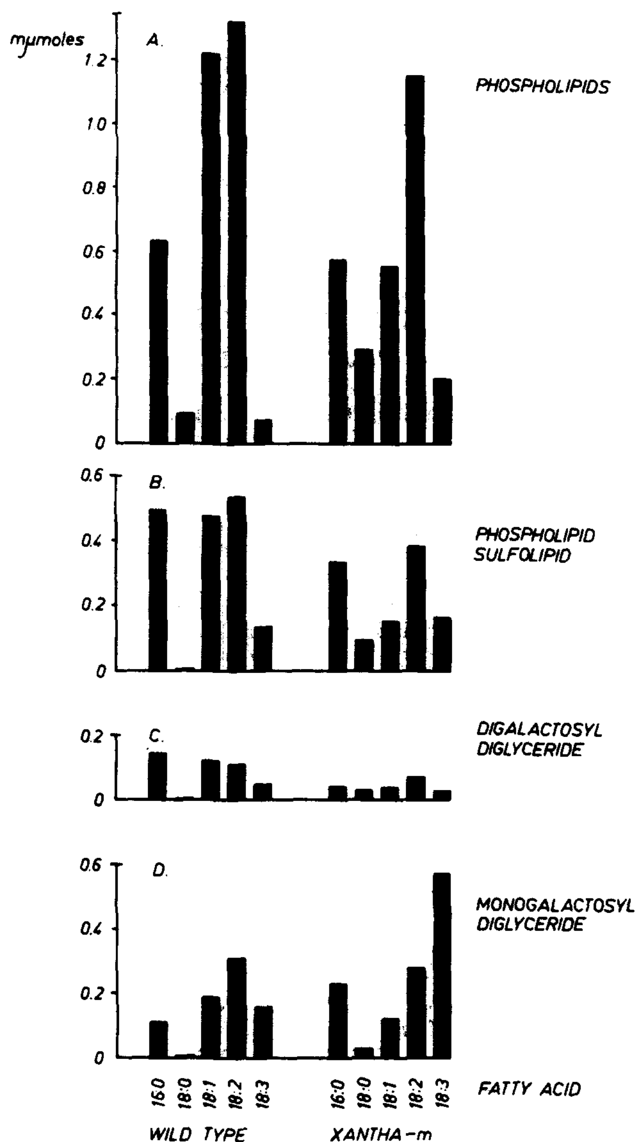

FIG. 12. Labeling patterns of the major fatty acids in the different lipid classes of the wild type compared to the average of the three mutants at the *xan-m* locus.

linolenic acid are also reported for white parts of variegated plants from *Acer negundo*, *Zea mais*, and *Ilex aquifolium*, for yellow leaves of *Ligustrum ovatifolium* (9), and possibly for a slow-greening plastome mutant of *Antirrhinum majus* (13). Naturally occurring, or experimentally produced, pigment-deficient strains of *Euglena* and *Chlamydomonas* synthesize lipids practically devoid of α-linolenic acid in the light (12). Furthermore, a strongly reduced α-linolenic acid content has been detected (12) in a *Scenedesmus* mutant that is blocked in the Hill reaction (14).

Erwin and Bloch (12) have demonstrated that the metabolic state of an algal cell can determine whether or not it synthesizes α-linolenic acid. The low level of α-linolenic acid in the pigment-deficient algal strains, in mature white leaves from variegated plants, and in dark-grown seedlings could thus be a consequence of the fact that these cells and tissues depend on respiration, and thereby on oxidative phosphorylation, for their major energy source. However, the "normal" linolenic acid content of the monogalactosyl diglycerides in the *xantha* mutants, e.g. $xan\text{-}a^{11}$ and $xan\text{-}f^{26}$, has been achieved, in the light, by tissues that must depend to a dominating extent on respiration for energy. This suggests that either the aforementioned control mechanism for linolenic acid synthesis is upset in all these mutants or, more likely, that factors other than the activity of photosynthetic versus respiratory metabolism can govern the synthesis of α-linolenic acid and its specific incorporation into galactolipids.

The reduction in linolenic acid found for the *Scendesmus* mutant blocked in the Hill reaction did not lead to a detectable change in its chloroplast lamellar structure (14). Similarly, removal of large amounts (about 50%) of linolenic acid from isolated lamellar systems of barley chloroplasts did not change the membrane structure visible in electron micrographs (15). Thus integrity of chloroplast disc membranes is not necessarily dependent on a high content of linolenic acid.

A series of the *xantha* mutants mimicked wild type seedlings at different greening stages (1) with regard to the different patterns of acetate labeling of the phospho-, sulfo-, and galacto-lipids on the one hand and of the steroid-containing fraction on the other. However, quite different labeling patterns were shown by some mutant leaves, such as the disproportionately high linolenic acid labeling of the monogalactolipids by the three *xan-m* mutants.

The effect of mutations in the individual *xantha* loci on lipid metabolism and chloroplast structures can be summarized as follows.

(*a*) A genetic block in chlorophyll synthesis as found in the *xan-f* mutants led, in the light-grown seedling leaves, to a repression of the formation of chloroplast membranes and of the acetate-^{14}C incorporation into phospho-, sulfo-, and galacto-lipids.

(*b*) The temperature-sensitive leucine "auxotroph" $xan\text{-}b^{12}$ showed a strong repression in labeling of all the polar lipids despite the presence of giant grana and significant amounts of chlorophyll.

(*c*) $xan\text{-}c^{23}$, another temperature-sensitive leucine "auxotroph" with giant grana and some chlorophyll, allowed considerable labeling of the polar lipids during the incubation period.

(*d*) The mutant $xan\text{-}d^{31}$, which also produces giant

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grana, showed a labeling pattern in most cases intermediate between the mutants at the *xan-b* and *xan-c* loci.

(*e*) The leaves of *xan-a*[11], containing plastids with little chlorophyll and highly disorganized membrane structures, were nonetheless equal to wild type in their capacity to incorporate acetate into phospho-, sulfo-, and galacto-lipids. It will be of great interest to determine whether the chloroplast-specific lipids synthesized by the leaves of this mutant are deposited in the conspicuous osmiophilic bodies in the plastids, when these lipids cannot be incorporated into membranous discs.

(*f*) The packages of grana-like membrane associations and the occasional accumulations of globuli in the plastids of the *xan-m* mutants occur in leaves that showed an exceptional capacity for rapid linolenic acid synthesis and for preferential insertion of this fatty acid into monogalactosyl diglycerides. It is an attractive hypothesis that these mutants, in an unbalanced fashion, accumulate monogalactolipids which coat the lamellar discs and glue these into the abnormal osmiophilic membrane packages or, alternatively, become deposited as osmiophilic globuli. Such globuli, which contain 90% lipid by dry weight, are known to be rich in galactolipids (16, 17). If indeed a causal relationship exists between the osmiophilic membrane associations and the preferential monogalactolipid synthesis, we expect the *xan-m* mutants to be able to synthesize monogalactosyl diglycerides with a high linolenic acid content in the dark, since they are able to form the abnormal membrane associations in the dark, i.e. in the absence of chlorophyll. Whatever the cause for this seemingly "uncontrolled" synthesis of monogalactosyl diglycerides turns out to be, the mutants at the *xan-m* locus attract attention since they provide the first example of a higher plant leaf in which the fatty acids of a given lipid could be labeled from acetate-^{14}C, during a brief incubation, in proportions corresponding roughly to the endogenous pattern.

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