LIPIDS OF LIGHT- AND DARK-TREATED PLASTIDS[1, 2]

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A number of publications have recently appeared in which are described the lipids of algal cells, leaves, and higher-plant plastids (Adhikari et al., 1961; Benson, 1961; Benson and Strickland, 1960; Debuch, 1961; Kates, 1959; Kates and Eberhardt, 1957; Newman, 1962; Wheeldon, 1960; Weenink, 1961; Wintermans, 1960; and Wolf et al., 1962). Most investigators have been concerned either with short-term photosynthetic events or with documenting the plastid lipids. The influence of various environmental factors on specific plastid lipids has received comparatively little attention. In addition, few reports have appeared concerning the specific lipids of yellow plastids. Wintermans (1960) found that leaves of *Phaseolus vulgaris* which were grown under longer daily light periods contained an increased amount of GPG-, G-gal-, and G-gal-gal-lipids.[2] Kates and Eberhardt (1957) reported from 75 to 116 mg of phosphatide P per 100 g dry weight of leaf material of *Phaseolus multiflorus* grown at various times of the year in a greenhouse in Canada. Bailey (1962) found that the season had a marked effect on the amount of lipid sugars of rye grass (*Lolium perenne*). Also, Newman (1962) found that the quality of fatty acids of bush bean (*Phaseolus vulgaris*) plastids was dependent upon the environment to which the plastids were subjected prior to isolation. It is suggested from these results and from those of other studies that the photoperiod and other light conditions may markedly influence the plastid lipid content. It, therefore, seemed of interest to examine the lipids of plastids subjected to different light conditions.

METHODS

Hubbard squash (*Cucurbita maxima* Duchesne, cultivar Burpee's true Hubbard) plants were grown in nutrient-irrigated vermiculite for 18 days under fluorescent-incandescent light of about 1350 ft-c intensity. The plants were exposed to light for 20 hr daily. Following this growing period one-half of the plants were subjected to four days of continuous darkness and then four days of a 20-hr photoperiod per 24 hr which was followed by another four days of darkness. The other one-half of the plants were subjected to a 20-hr photoperiod per 24 hr during the same period.

Plastids were isolated by sedimentation between 200 and 1,000×g in cold 0.35 M NaCl following release of the plastids with a Waring blendor. Aliquots of each

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[2]Abbreviations: GP, glycerophosphoric acid; GPC, glycerophosphoryl choline; GPE, glycerophosphoryl ethanolamine; GPG, glycerophosphoryl glycerol; GPI, glycerophosphoryl inositol; GPS, glycerophosphoryl serine; G-gal, monogalactosyl glycerol; G-gal-gal, digalactosyl glycerol.

isolate were taken for chlorophyll analyses (Koski et al., 1950). Total lipid extracts of the isolates which were boiled in ethanol were made by the method of Folch et al. (1957) using chloroform and methanol.

The dried isolates were chromatographed in a 15 g silicic acid column (Bio-Rad silicic acid minus 325 mesh). The lipids were eluted using a stepwise addition of 700 ml of chloroform, 350 ml of chloroform-methanol (5:1, v/v), 300 ml of chloroform-methanol (1:1, v/v), and finally 200 ml of methanol. Ten per cent of each 50 ml collected sample was used for ester determinations (Rapport and Alonzo, 1955) in order to identify the lipid-containing fractions. The results of phosphate analyses of a similar 10 per cent of each fraction were also used to identify lipid-containing fractions (Marinetti, 1962). Following the ester and phosphate analyses, samples containing lipids of similar mobilities were combined

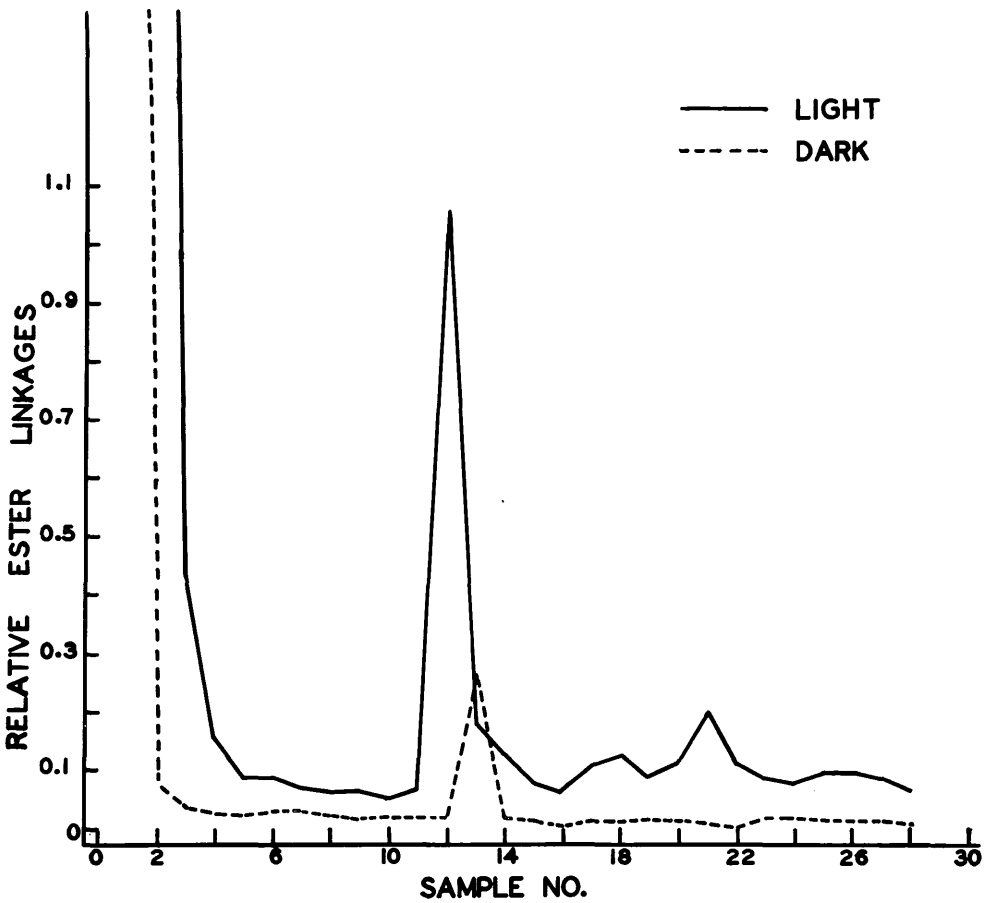

FIGURE 1. Ester concentration of eluant fractions.

and were labeled I, II, etc. One-half of each combined fraction was used for fatty acid analyses; one-half was used for chromatography on silicic acid-impregnated paper and in thin layers of silica gel G (Merck).

For the fatty acid analyses lipids were saponified in aqueous ethanolic KOH and then were esterified with methanolic BF_3 (Metcalfe and Schmitz, 1961).

Fatty acid methyl esters were analyzed using gas-liquid chromatography. The conditions were as follows:

Chromatograph	F & M Model 500.
Column dimensions	305×0.6 cm stainless steel coiled tube.
Solid support	Firebrick (60/80 mesh).
Stationary phase	20% diethylene glycol succinate.
Temperatures	Column: 200 C; Detector: 268 C; Injector: 270 C.
Carrier gas	Helium at 80 ml/min measured at outlet. Inlet pressure: 30 psig; outlet pressure: atmospheric.
Detector	Hot wire.
Recorder	Honeywell 1 mv; 1 sec; ¼ inch/min.
Sample size	150 to 250 μl.
Analysis time	About 50 min to linolenic.

Some of the fatty acids were collected and brominated in order to identify

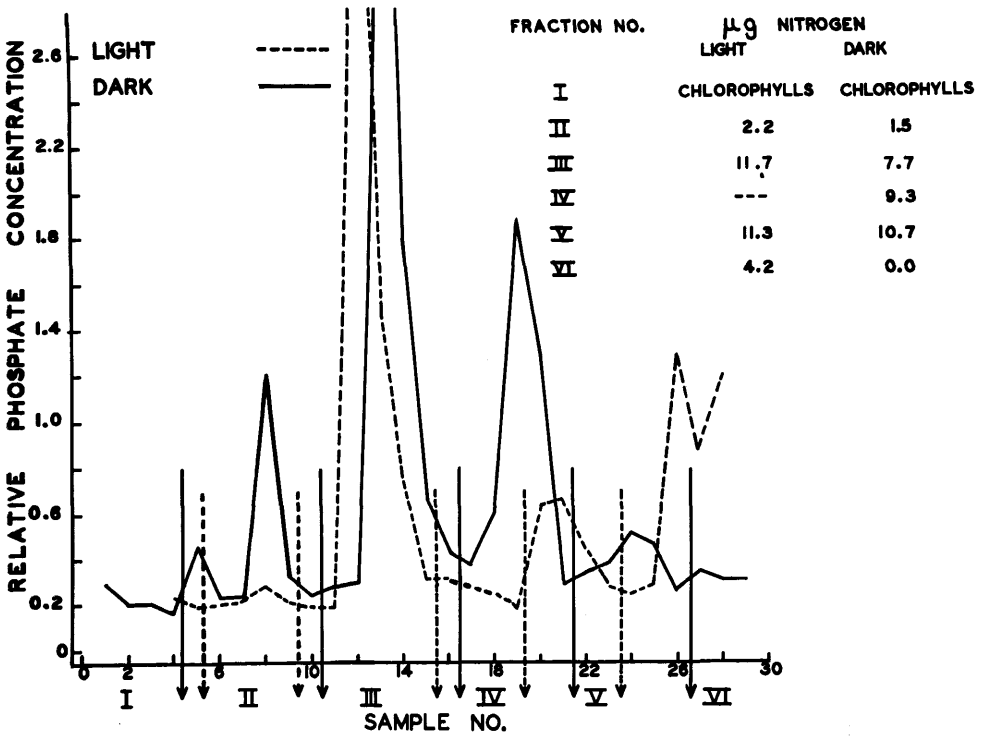

FIGURE 2. Phosphate concentration of eluant fractions. Nitrogen concentrations of combined fractions-μg per 18 per cent of the fraction.

unsaturated materials. The detector was calibrated using several mixtures of highly purified fatty acid methyl esters (a gift from NIH).

Of the other one-half of each combined fraction 10 per cent was used for nitrogen analysis (Marinetti, 1962). The remaining amount was divided into four equal portions, three portions of which were chromatographed on silicic acid-impregnated paper and the other portion of which was chromatographed in a thin layer of silica gel G. The solvent system for the silicic acid-impregnated paper chromatography was diisobutyl ketone-acetic acid-water (40:25:5, v/v/v) at about 25 C. One chromatographed fraction was stained with Rhodamine 6G and viewed under ultraviolet light (360 mμ) in order to identify the position of

the spots. Similar spots were eluted with 25 per cent chloroform in methanol and used for phosphate and sugar determinations (Marinetti, 1962; Bailey, 1962).

It was found that ultraviolet absorption spectra of sulfuric acid-treated glycolipids were not exactly similar to sulfuric acid-treated, pure galactose. The portion used for thin-layer (0.3-mm thickness) chromatography was chromatographed in one dimension, ascending, using chloroform-methanol-water (70:30:3, v/v/v). The chromatograms were visualized after spraying with 50 per cent (v/v) aqueous sulfuric acid and heating at 180 C.

RESULTS

The results from the ester and phosphate analyses are given in figures 1 and 2. Five, or possibly six, major groups of phosphorus-containing compounds were found in the column eluant. It has been suggested that a stepwise elution of lipids from a silicic acid column is somewhat less reproducible than gradient elution but satisfactory gradient elution schemes have been more difficult to achieve (Bader and Morgan, 1962). Two solvent fronts containing the same materials may appear with the use of a stepwise elution scheme.

Results of the fatty acid analyses are given in tables 1 and 2. The isolate of

TABLE 1

Fatty acid composition of light- and dark-treated plastids

Treatment	Fraction	C_{12}	C_{14}	C_{16}	*$C_{16^{1=}}$	C_{18}	$C_{18^{1=}}$	$C_{18^{2=}}$	$C_{18^{3=}}$	C_{20}	% of total	Amount mg×10^2	Postulated moieties to which fatty acids were esterified	No. spots from thin-layer chromatography
		Percentages of each fraction												
Light	I	15.5	13.2	19.9	5.5	4.3	7.2	4.7	29.8	—	5.5	89.5	———	2
	II	—	—	—	—	—	—	—	—	—	0.0	0.0	———	3
	III	—	—	9.3	3.0	1.1	2.3	2.2	80.8	1.3	89.4	1448.6	GPI, G-gal, G-gal-gal GPG, GPC**, GPE**	8
	IV	—	2.6	27.9	6.0	5.6	6.3	10.8	40.9	—	1.7	26.9	G-gal-gal, GPC	1
	V	—	—	28.7	4.9	4.6	4.9	8.1	48.9	—	1.9	30.7	GPC, G-gal-gal	1
	VI	21.0	—	22.5	—	8.7	12.7	11.5	23.7	—	1.6	25.3	Glycoside	3
% of total		1.2	0.8	10.7	3.2	1.6	2.9	2.7	75.8	1.2				
amt. mg×10^2 for 40% of isolate		19.2	12.5	173.8	51.7	25.5	46.5	43.8	1228.7	19.3		1621.0		
Dark	I	3.2	—	65.4	—	—	6.5	5.0	19.9	—	4.1	53.8	———	1
	II	—	—	3.4	—	4.4	9.8	7.4	75.0	—	1.5	20.4	suL/olipid	1
	III	—	—	10.8	3.3	1.3	1.7	2.1	80.9	—	85.4	1127.5	G-gal-gal, G-gal GPG, GPC**	6
	IV	5.1	0.9	24.8	2.1	3.1	2.7	4.0	57.3	—	8.0	105.2	G-gal-gal, GPC	1
	V	—	—	34.0	—	41.2	24.7	—	—	—	0.7	9.7	G-gal-gal	1
	VI	45.5	—	18.2	—	36.4	—	—	—	—	0.3	4.4	Glycoside	0
% of total		0.7	0.1	14.2	2.9	1.9	2.2	2.4	75.6	—				
amt. mg×10^2 for 40% of isolate		9.1	0.9	187.5	38.8	24.6	29.6	32.2	998.3	—		1321.0		

*$C_{16^{1=}}$ palmitoleic acid—16 carbon atoms in the molecule containing 1 unsaturated position.

**Note—the identification of these compounds is less positive due to the presence of other components with similar mobilities and compositions. Other minor components were also observed in these fractions.

light-treated plastids contained approximately two times as much chlorophyll and about 23 per cent more fatty acid material than the isolate of dark-treated plastids. The lipids eluted with a 5:1 (v/v) chloroform-methanol mixture (fractions III) contained most (85.4 and 89.4 per cent) of the fatty acid materials. Linolenic acid (75.6 and 75.8 per cent) was the predominant fatty acid. Palmitic acid (10.7 and 14.2 per cent) was also found in significant amounts. Sastry and Kates (1963) found that linolenic acid was the most abundant fatty acid esterified in galactolipids of runner-bean leaves (96 and 93 per cent). The acetone-soluble lipids of red clover (*Trifolium pratense*) leaves consist largely of galactosyl-l-glycerol and digalactosyl-l-glycerol linolenate (Weenink, 1961). Weenink (1962) concluded that the glycolipids could be distinguished from the phospholipids by the simpler fatty acid composition of the glycolipids.

Both fractions I probably contained some free fatty acids. Fraction II of the lipids from light-treated plastids contained only traces of a few fatty acids but small amounts of linolenic, oleic, and other fatty acids were present in this fraction from the lipids of dark-treated plastids. Fractions III contained large amounts of palmitic, palmitoleic, linolenic acids, and small amounts of other fatty acids. From the phosphate and sugar analyses and silicic acid-impregnated paper chromatography it appeared that fractions III contained GPG-, GPC-, GPE-, G-gal-, and G-gal-gal-lipds. Fractions IV probably contained G-gal-gal-lipid, GPC-lipid, and probably another glycolipid. Fractions V probably contained GPC-lipid materials from the light-treated plastids and G-gal-gal-lipid from the dark-treated plastids. Fractions VI may have contained a glycolipid.

TABLE 2

General relationships of fatty acids of plastids isolated from light- and dark-treated plants

Treatment	Saturates/ unsaturates	C_{16}/ C_{18}	Stearic/ C_{18} unsaturates
Light	0.18	0.17	0.19
Dark	0.20	0.21	0.23

The light-treated plastids had a lower ratio of saturates to unsaturates, of C_{16} to C_{18} fatty acids, and of stearic to C_{18} unsaturates (table 2). These findings are consistent with those of Newman (1962). The actual ratios of saturates to unsaturates and of C_{16}/C_{18} fatty acids reported here are considerably less than those previously reported since different plants were used and other fatty acids were considered in the previous report.

It is apparent from the above data that consideration should be given to the influence which the environment may have on the plastid lipid content. However, the differences in the fatty acids of light- and dark-treated plastids reported here are not nearly as marked as the differences reported previously. Subjecting plastids to appropriate environmental conditions may result in biodegradation of the lipids. More is known concerning the biosynthesis of plastid lipids than about the pathways of degradation (Stumpf et al., 1963). However, more extended periods of darkness and investigations of the other plastid lipids will have to be made before the pathways of breakdown or conversion in the dark may be suggested. It is interesting, however, that under the conditions of the treatments few marked relative differences in the plastid fatty acids existed.

Longer dark periods may result in decreased unsaturates (Bloch et al., 1961) with a concomitant change in the ratio of saturates/unsaturates without any net

synthesis of fatty acids in the dark. The literature suggests that at least some oleic acid arises from acetate and not from long chain saturates—palmitic or stearic acids (Stumpf and James, 1962).

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