A Comparison of Lipid Patterns in Photosynthesizing and Nonphotosynthesizing Cells of *Euglena Gracilis**

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The lipids found in green cells of *Euglena gracilis* grown in the light differ from the lipids found in etiolated cells grown in the dark. The fatty acids of the photosynthesizing green cells are mainly of the unsaturated variety. The major fractions are triply unsaturated 16-, 18-, and 20-carbon acids, doubly unsaturated 16- and 18-carbon acids, and an uncharacterized labile unsaturated fatty acid. In contrast, the etiolated cells have predominantly saturated fatty acids of chain lengths lower than 17 carbon atoms. The light-grown cells contain appreciably greater amounts of inosito-, uronido-, galacto-, and sulfolipids than the dark-grown cells. A wax constitutes approximately 25% by weight of the total lipid of the green cells and fully 50% of the lipid of the etiolated cells. Isolated in crystalline form, the wax has been found to consist of a mixture of the simple esters of saturated odd- and even-chain alcohols from 10 to 17 carbon atoms in length and saturated odd- and even-chain acids from 10 to 18 carbon atoms in length with myristyl myristate as the predominant species. A possible role for the waxy ester fraction in *Euglena gracilis* metabolism, the significance of an increase in unsaturated fatty acids in the green cells, and the retention of plant-typical lipids by the etiolated cells are discussed.

The feasibility of growing *Euglena gracilis* in large yield (Bach, 1960), the metabolic sufficiency of cells in the etiolated state, and the relative ease with which whole populations are subjected to a controlled environment, render the organism useful in the study of photosynthesis (Cramer and Myers, 1952; Lynch and Calvin, 1953).

Chloroplasts are rich in lipid (Chibnall, 1939) spatially arranged next to chlorophyll in the grana (Goedheer, 1961) where irradiation produces a rapid turnover of many lipid fractions (Sisikyan and Smirnov, 1958; Eberhardt and Kates, 1957). It is possible that fatty acids and their complex lipid derivatives participate in the conversion of radiant energy to chemical potential, or in attendant TPNH- and ATP-dependent reactions. The present report compares the lipid patterns of light-grown (green) and dark-grown (etiolated) cells of *Euglena gracilis*.

METHODS

Cultures of *Euglena gracilis*, strain Z, were a gift of Dr. George Brawerman who obtained them from Dr. S. H. Hutner (Haskins Laboratories, New York). The cells were grown at 26° (Brawerman and Chargaff, 1959; Wolken, 1961) and fractionated as described (Brawerman and Chargaff, 1959). Aside from growth of the cultures, all procedures were carried out at 4°. Harvested cells were washed in distilled water. Lipid extracts were made as follows:

The cells were minced at high speed in a Waring Blendor with 30 volumes of chloroform-methanol (2:1, v/v), the slurry was filtered with the aid of suction, and the filtrate was dialyzed through cellophane bags in the dark for 3 days against frequent changes of distilled water. The lower or chloroform phase of the resulting triphasic dialyzate in the cellophane bag was collected. The solvent was evaporated under nitrogen; the residue was dessicated for 18 hours in a vacuum over $CaCl_2$ and $CaSO_4$, weighed, and stored in the dark under nitrogen at −20°.

The total lipid was then analyzed as follows:

Phosphorus was estimated by the method of King (1932).

Preformed sulfate liberated by solvolysis (Burstein and Lieberman, 1958) and *total sulfur* (converted to sulfate by boiling to dryness suitable quantities of lipid in 1 ml concd nitric acid and reheating the deposit with 0.2 ml 30% hydrogen peroxide solution to a dry, white, inorganic residue) were estimated by a colorimetric method (Letonoff and Reinhold, 1936) adapted to a micro scale.

Hexose was isolated and the quantity present was determined as described previously (Rosenberg and Chargaff, 1958a, 1958b).

Inositol was split from the complex lipid by digestion with 6 N HCl at 100° in sealed tubes for 18 hours and was chromatographed (Böhm and Richarz, 1954). Quantitative analysis was made by planimetry. Fractions of hydrolyzate and increasing quantities of inositol (2–20 μg) were each applied to the paper in exactly 10 μl of solution. Spot areas on the developed chromatogram were plotted against logarithm of quantity and the resultant straight line was used as a calibration curve in estimating the quantity of inositol in the unknown spots.

Fatty acids were liberated by saponification in sealed tubes under nitrogen with 5% (w/v) KOH in methanol at 55° for 5 hours. Nonsaponifiable lipid and the fatty acids were extracted with hexane. Cooling is recommended during acidification prior to extraction of the fatty acid fraction. Fatty acids were converted to methyl esters and quantitatively estimated (Rosenberg and Chargaff, 1958a). Methyl esters and free fatty alcohols were chromatographed in a Barber-Coleman Model 10 instrument at 173° with ethylene glycol succinate as stationary phase and argon as mobile phase.

Uronic acid was split from suitable quantities of lipid by hydrolysis in 1 N HCl in sealed tubes at 100°. Hydrolysis for 3 hours provided optimal release. Interfering substances were extracted with chloroform

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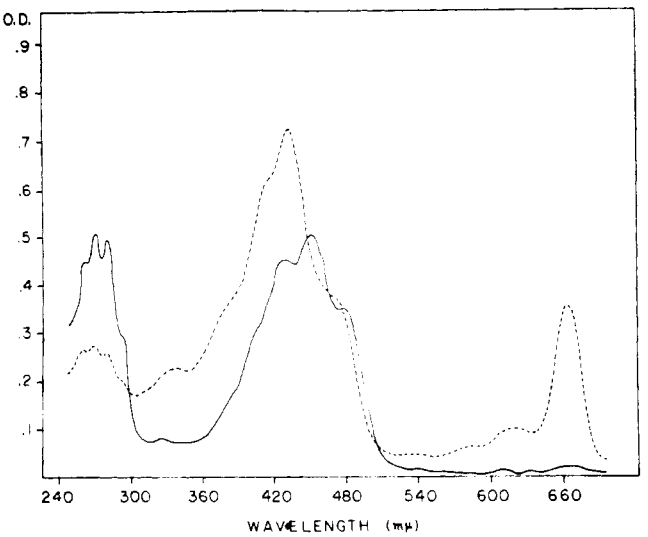

FIG. 1.—Absorption spectra of the total lipid extracts of green and etiolated cells of *Euglena gracilis*, in hexane: ------, green cells; ———, etiolated cells.

and quantitative estimation (Ritter and Ewins, 1961) was made.

Waxy esters were separated from the total lipid by a thin-layer chromatographic arrangement with hexane-ether-acetic acid (Malins and Mangold, 1960). Synthetic myristyl myristate or a portion of the waxy ester fraction obtained by low-temperature crystallization from chloroform solutions of the total lipid can serve as reference guides. Spots were made visible by spraying with an indicator dye (Malins and Mangold, 1960) and viewing under ultraviolet light. Quantitative recovery was made by scraping the spots from the glass plate, transferring the scrapings to a glass-stoppered tube, adding 5 ml 0.05 N HCl, and extracting the esters with hexane. The total pure waxy ester fraction is obtained on evaporation of the hexane extract with nitrogen. Total recovery of the waxy esters could also be made, with less ease, by application of suitable quantities of lipid to columns of dried, lipid-free cellulose powder, followed by irrigation of the columns with anhydrous heptane. After elution of carotene with the solvent front, the waxy ester fraction is obtained by collection of a volume of effluent equal to 2 retention volumes of the column.

RESULTS

Absorption spectra showed the virtual lack of chlorophyll and the persistence of a carotene fraction in the etiolated cells (Figure 1). The gross lipid contents of green and etiolated cells grown on the Pringsheim medium (Brawerman and Chargaff, 1959) are given in Table I. Comparable amounts of lipid were found in both cell types.

The percentage by weight for each fatty acid species identified by gas-liquid chromatography on a single stationary phase is given in Table II. The values show the state of the fatty acids in mature 10- to 14-day-old cultures in the resting phase of cell multiplication. Large differences were noted in the total fatty acid complements of green and etiolated cells. As an aid to identification, saturated and unsaturated methyl ester fractions were separated through the acetoxymercurimethoxy adducts (Jantzen and Andreas, 1959) and rechromatographed separately. Eighty per cent of the fatty acids of the green cells are of the unsaturated variety, a major proportion having multiple double bonds. Thirty-two per cent of the fatty acids of the etiolated cells are unsaturated with roughly a 1:2 distribution between fatty acids containing single and multiple double bonds.

The initial total fatty acid content of the Pringsheim culture medium (Brawerman and Chargaff, 1959) was less than 4% of the amount found in the harvested cells and was distributed among a number of molecular species with 16, 16:1, 16:2, 18, and 18:2 as the major fractions. In a synthetic growth medium devoid of fatty acid (Wolken, 1961) the qualitative differences in the fatty acids of green and etiolated cells were the same as those of cells grown on the Pringsheim medium (84% unsaturated fatty acids in green, 33% in etiolated cells) with some alteration in the quantitative

TABLE I

LIPID CONTENT OF CELLS FROM ETIOLATED AND GREEN CULTURES OF *Euglena gracilis*

	Etiolated	Green
Cell harvest (total number of cells/liter)	4.40×10^{7}	6.64×10^{7}
Lipid content (g/cell)	1.01×10^{-10}	1.08×10^{-10}
Lipid-free dry residue (g/cell)	2.75×10^{-10}	3.17×10^{-10}

TABLE II

FATTY ACIDS IN ETIOLATED AND GREEN CELLS OF *Euglena gracilis*

Number of Carbons in Fatty Acid[a]	Weight Per Cent of Total[b] Etiolated	Green
6:1	—[e]	0.3
8	—	0.1
8:1	—	1.0
10	—	0.1
11	—	0.1
12	9.0	1.1
12:3	—	0.1
13	7.9	2.6
14	31.1	4.5
14:1	2.2	—
14:2	13.2	—
15	1.4	2.0
15:1	—	0.1
16	12.3	6.7
16:1	5.1	6.1
16:2	2.2	9.2
16:3	—	4.5
17	—	0.1
17:1	—	0.1
18	4.5	1.4
18:1	5.6	1.5
18:2	2.8	12.0
18:3	—	15.0
18:4	—	2.0
19.1	—	8.4[c]
20	1.1	—
20:2	1.4	0.8
20:3[d]	—	8.1
22:1	—	1.4
22:2, 23:1 ?	—	5.8
22:3	—	1.4
24:1	—	2.0
25	—	1.1
26	—	0.4

[a] Number after colon shows number of double bonds in molecule. [b] Calculated from integrated elution curve areas. [c] Unidentified unsaturated acid with retention time corresponding to 19.1 carbon atoms. [d] Arachidonate has the same retention time. [e] Dashes indicate less than 0.05%.

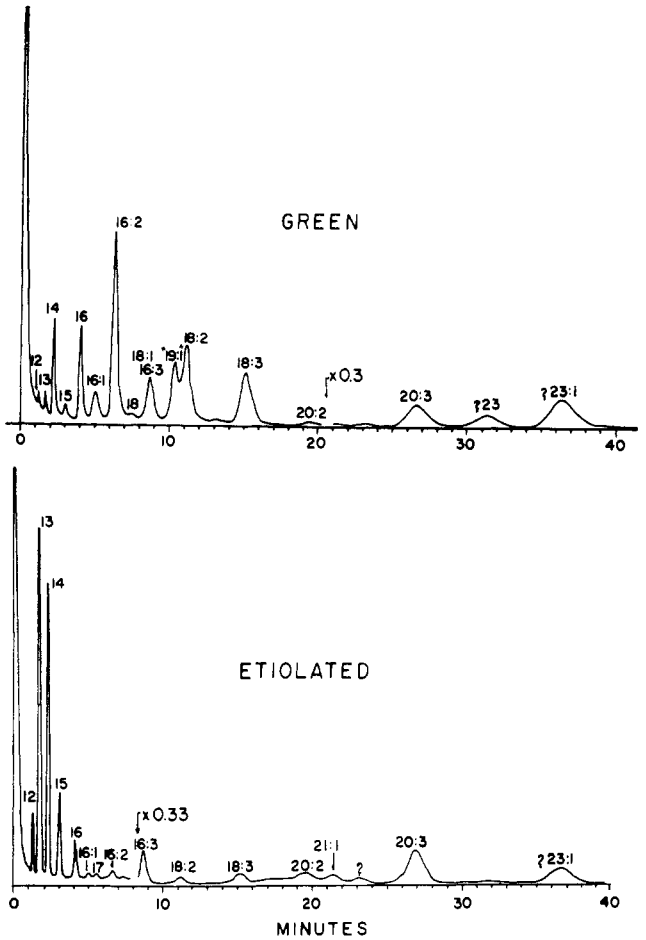

FIG. 2.—Methyl esters of the total fatty acid complements of resting stage cells of green and etiolated *Euglena gracilis*. Gas-liquid chromatograph on ethylene glycol succinate at 173°. The cells were grown on a fully synthetic medium.

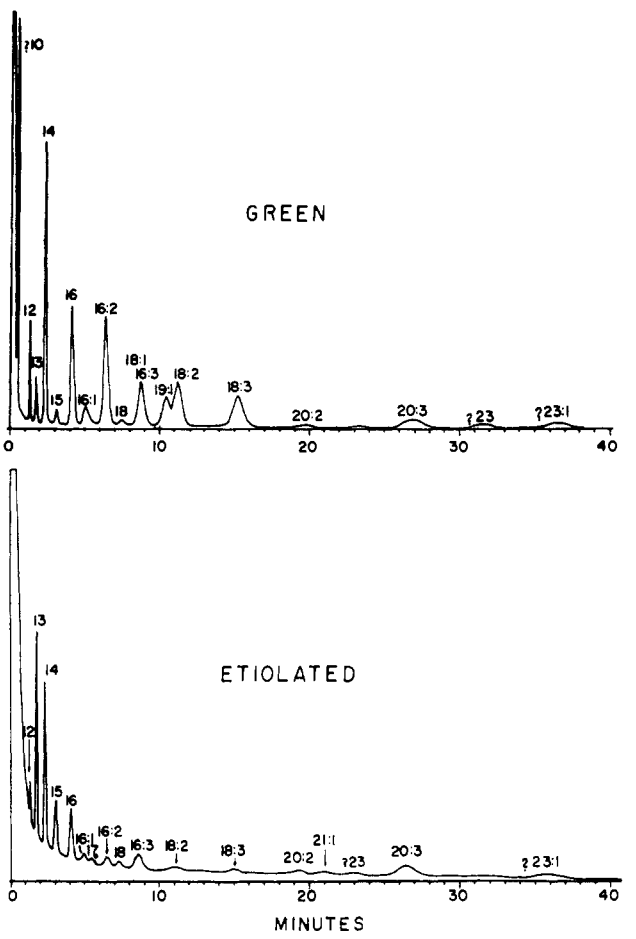

FIG. 3a.—Methyl esters of the total fatty acid complements of logarithmic stage cells of green and etiolated *Euglena gracilis*. Gas-liquid chromatograph on ethylene glycol succinate at 173°. The cells were grown on a fully synthetic medium.

distribution between the various molecular species. The gas-liquid chromatographic patterns of methyl esters of the fatty acids from resting phase cultures of green and etiolated *Euglena gracilis* grown on the synthetic medium are shown in Figure 2. Figure 3a, shows the fatty acid patterns of cells grown on the same medium at the stage of logarithmic cell multiplication. Results at this stage of growth are comparable with those obtained with cells grown on the rich medium.

Figure 3b shows the fatty acid patterns obtained with synthetic medium cultures at the stage of logarithmic cell multiplication after treatment for a period of 3 hours with 0.05 μmole of imidazole per ml of culture medium. While little change was noted in the photosynthesizing cell, events in the etiolated cell were marked by a great accumulation of palmitate and stearate as well as a secondary accumulation of unsaturated homologs. On prolonged incubation with no further addition of imidazole, palmitate reverted to a low level with an accompanying rise in tridecanoate and myristate. Brief treatment of the cells (approximately 45 minutes) with 0.01 μmole of hydrogen peroxide per ml of culture medium resulted in an approximate doubling of the 12- to 15-carbon fatty acid content of both green and etiolated cells, with the relative ratios of the quantities of these acids remaining essentially unchanged. The significance of these findings will be discussed.

Solutions of the total lipid of both green and etiolated cells in chloroform-methanol (2:1, v/v) deposited a crystalline waxy material on standing at −10°. Infrared spectra (Figure 4) of the crystals from both cell types were identical. Absorption maxima at 13.92 μ and 13.73 μ showed the crystal packing typical of aliphatic hydrocarbon compounds (Chapman, 1960). Seven major plus an eighth minor progressive evenly spaced absorption maxima in the 8.5–7.4 μ region indicated a simple saturated aliphatic ester with a 14-carbon moiety as a major component and a 16-carbon moiety as a minor component. The ester function was also indicated by the carbonyl absorption maximum at exactly 5.77 μ.

The waxy ester fraction migrated with myristyl myristate on thin-layer chromatography. It was separated from the other lipid fractions and isolated with complete recovery by the methods outlined in the preceding section. By weight, the waxy ester fraction constituted 50% of the lipid of well-nourished (Brawerman and Chargaff, 1959) etiolated cells and from 20 to 25% of the lipid of actively photosynthesizing cells. The ratio of the acid to the nonsaponifiable moeity was 1:1 both by hydroxamic acid analysis and by weight. The major species of fatty acid and alcohol were isolated in roughly 30% yield by low temperature fractional crystallizations from heptane and methanol, respectively. The alcohol melted at 37°; myristyl alcohol, 37.6°. The acid melted at 58°; myristic acid, 58°. The methyl ester froze at 18°; methyl myristate, 18.4°. Gas-liquid chromatograms of the alcohol fraction and of the methyl esters of the acid fraction

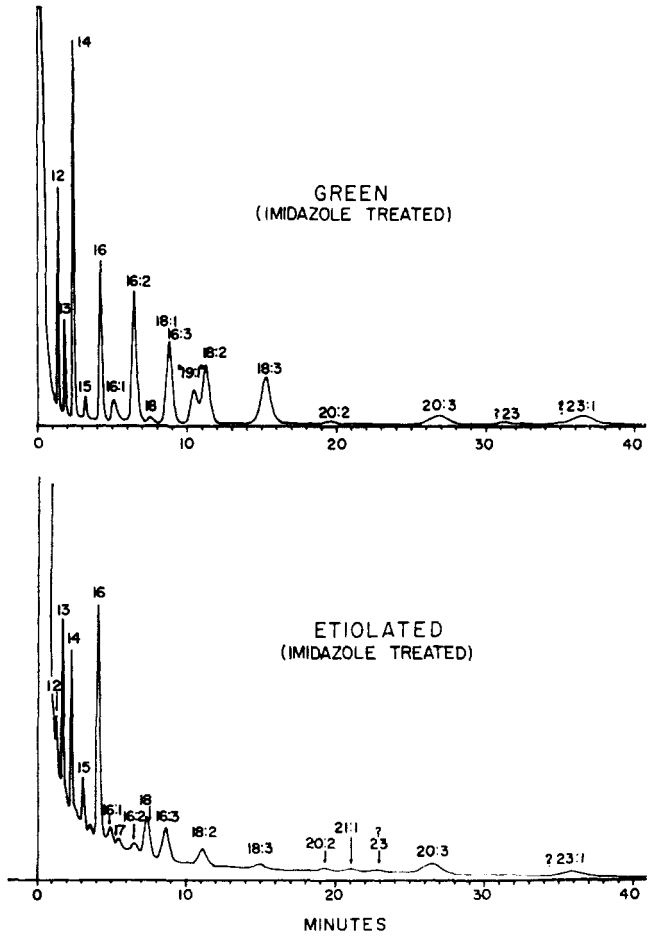

FIG. 3b.—The same as Figure 3a after treatment of the cells with 0.05 μmoles of imidazole per ml of culture medium for a period of 3 hours.

showed that the wax was composed of saturated fatty acids with both odd and even numbers of carbon atoms and fatty alcohols of a similar chain length distribution. In both moieties, a chain length of 14 carbon atoms predominated. The chromatographic tracings are reproduced in Figure 5. Careful oxidation of the alcohol fraction with chromic acid produced a series of fatty acids whose methyl esters migrated with the predicted mobilities in the gas-liquid chromatograph. All the alcohols in the nonsaponifiable fractions of the total lipid of green and etiolated cells could be found in the waxy ester fractions except for an unidentified alcohol with an elution time corresponding approximately to that of a 20-carbon alcohol. It was found in the green cell lipid and not in the etiolated cell lipid, and is presumed to be phytol.

Galactose was found to be the sole lipid hexose on paper chromatograms of lipid hydrolyzed in 3 N HCl at 100° for 1 hour. Although glucose was also found consistently in fractions eluted from lipid-free cellulose, it proved to be an exchange artifact. Caution is indicated in the interpretation of a finding of glucose in glycolipid fractions subjected to a prolonged contact with cellulose in a nonaqueous medium. Also present in the lipid hydrolyzates were a pentose migrating with xylose, a highly polar compound giving the reaction of pentose on paper (Rosenberg and Chargaff, 1958a), and a trace quantity of a reducing compound migrating more rapidly than galactose. Relative to galactose, the migrations of the pentose and polar pentose-reacting substance were, respectively, 1.60 and 0.17 in ethyl acetate–pyridine–water, 10:7:3, v/v/v; 1.46 and 0.20 in isopropanol–formic acid–water, 5:3:2, v/v/v; and 1.80 and 0.13 in *n*-butanol-ethanol-water, 4:1:1, v/v/v. Taken together, these observations were suggestive of the presence of a uronic acid which would migrate slowly but react as a pentose on paper, and in hot 3 N HCl would undergo decarboxylation to a pen-

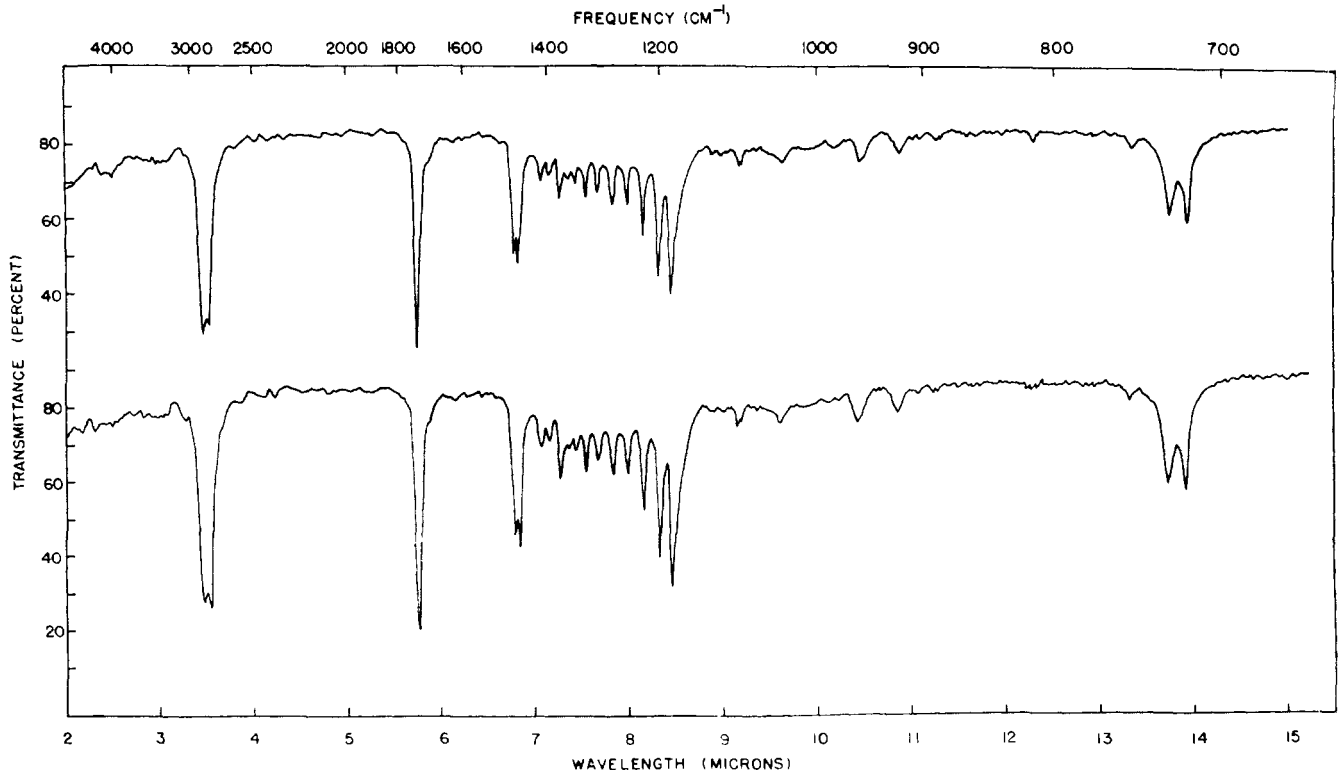

FIG. 4.—Infrared spectra of the crystalline waxy ester fraction from green and etiolated cells of *Euglena gracilis*. KB plate. Upper curve: green cells; lower curve: etiolated cells.

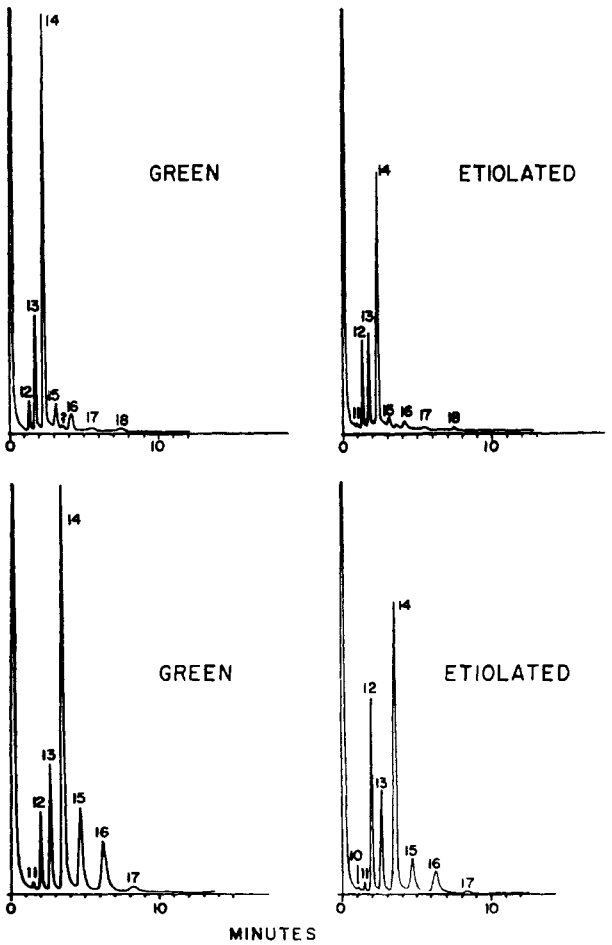

FIG. 5.—Gas-liquid chromatographs of the fatty acid moiety (as the methyl esters) and of the fatty alcohol moiety of the wax fraction of green and etiolated *Euglena gracilis* cells. Upper curves: methyl esters of the fatty acids: lower curves: the fatty alcohols. Ethylene glycol succinate column at 173°.

tose as well as some cyclization to a rapidly migrating lactone. Analysis of lipid hydrolyzates showed that a uronic acid was a component of the lipids of green and etiolated cells. Lengthy hydrolysis of the total lipid in 6 N HCl revealed inositol as a lipid component.

Total lipid phosphorus varied considerably in the two cell types, ranging from 0.5 to slightly over 1.0%, w/w. A small but apparently fixed proportion of the total lipid sulfur existed as preformed sulfate. Table III lists the analytical values obtained for some of the polar and ionic components of the lipids of green and etiolated cells of *Euglena gracilis*. The occurrence of choline and of numerous amino acids found in acid hydrolyzates of purified lipid fractions will be the subject of a separate report.

TABLE III

ANALYSES OF SOME LIPID FRACTIONS OF GREEN AND ETIOLATED *Euglena gracilis* CELLS

Fraction Estimated	Per Cent of Total Lipid (g/100 g) Etiolated	Green
Galactose	1.44	8.20
Total sulfur[a]	2.86	4.16
Preformed sulfate	0.37	0.53
Inositol	0.18	0.29
Uronic acid	0.10	0.13

[a] As sulfate.

TABLE IV

RELATIVE WEIGHT PERCENTAGE OF SOME FATTY ACID SPECIES[a] IN CHLOROPLASTS AND LEUCOPLASTS OF *Euglena gracilis*

Carbon Atoms[b] in Fatty Acid	Relative Per Cent by Weight Chloroplast	Leucoplast
12	1	5
13	1	6
14	4	33
15	1	8
16	9	16
16:1	9	4
18	1	14
18:1	12	9
18:2	10	Trace
18:3	26	Trace
"19.1"[c]	15	5
20:3[d]	9	Trace

[a] Higher chain length homologs and a "21.1"-carbon acid (see footnote *c*) can also be demonstrated. [b] Number after colon gives number of double bonds in molecule. [c] Unidentified unsaturated fatty acid with retention time of a hypothetical acid with 19.1 carbon atoms. [d] Not fully identified; arachidonate has the same retention time.

DISCUSSION

When cultures were grown on the enriched medium (Brawerman and Chargaff, 1959) developed by Pringsheim for etiolated cells (Neff, 1960), comparable amounts of lipid per cell were found in both green and etiolated cells in the resting phase as shown in Table I. It is not known whether such a relationship holds for cells grown under other conditions. The quantity of preformed fatty acid in the enriched medium is insufficient to directly influence to a marked degree the cellular fatty acid patterns observed, although selective absorption and retention of fatty acid from the medium may possibly occur.

Photosynthetic organisms are our major biological source of unsaturated fatty acids. The mechanisms by which photosynthesizing cells produce these fatty acids are still obscure. There are differing reports (Mead, 1960) indicating that synthesis of unsaturated fatty acids may either precede, follow, or stem from a metabolic precursor in common with the saturated analogs.

It seems that in *Euglena gracilis* synthesis of the greater part of the cellular unsaturated fatty acids accompanies photosynthesis. Although it is not known that unsaturated fatty acids participate directly in the photosynthetic mechanism, their synthesis is an important factor in the economy of the photosynthesizing cell. Experimental verification should be sought to determine whether this involves direct participation in light-induced chemical reactions, utilization of early products of photosynthesis such as O_2, TPNH, and ATP (Arnon, 1961), or intermediates further along ensuing metabolic chains. The formation of Δ-9 unsaturated fatty acids has been demonstrated to occur in yeast cells (Bloomfield and Bloch, 1960). The mechanism requires TPNH and molecular oxygen. A similar mechanism may be operative in the chloroplast.

A comparison of the relative proportions of fatty acid species in a chloroplast fraction of green *Euglena gracilis* cells and a corresponding leucoplast fraction of etiolated cells shows (Table IV) a greatly increased ratio of unsaturated to saturated homologs in the chloroplast. An unidentified major fatty acid fraction found principally in the chloroplasts is an unsaturated

fatty acid with an elution time corresponding to a fatty acid with "19.1" carbon atoms. This labile fraction diminishes slowly on storage under nitrogen and more rapidly on exposure to air. Not listed in Table IV, but present only in the chloroplast, is a fatty acid with an elution time corresponding to that of a fully saturated 20-carbon fatty acid. This substance is demonstrable in the gas-liquid chromatograph only at very high flow rates because of rapid destruction at the elevated column temperature. It sometimes occurs in quantity great enough to mask the major 18:3 peak lying adjacent and is very rapidly lost on storage, saponification in air, or even brief heating above room temperature.

As shown by Tables II and IV, photosynthesizing cells of *Euglena gracilis* and their chloroplasts have an increased content of a number of species of unsaturated fatty acids as compared with etiolated cells. A recent communication has stressed the importance of 18:3 as a product of active photosynthesis in *Euglena* (Erwin and Bloch, 1962). The results reported here show that 18:3, although sometimes predominant, is not the only unsaturated fatty acid to undergo a major increase during photosynthesis. However, variations due to strain differences have yet to be explored. The increase in unsaturated fatty acids during photosynthesis seems to be independent of the nature of the culture medium, as long as the latter provides sufficient nourishment, and relates in some way to the influx of radiant energy to the cell.

It has been noted that although both the green and etiolated cells contained comparable amounts of lipid, waxy esters account for 50% of the total weight of cellular lipid in the etiolated form and only 25% in the actively photosynthesizing form. A possible mechanism leading to the formation of the waxy ester fraction may be found in the peroxide-dependent α-oxidative decarboxylation of fatty acids to aldehydes which has been shown to occur in plant cells (Martin and Stumpf, 1959). The waxy ester fraction of *Euglena gracilis* cells contains equivalent quantities of fatty acids and fatty alcohols of comparable chain length distribution and rich in odd-chain homologs. These acids and alcohols may stem from aldehydic precursors formed by successive α-oxidative decarboxylations of fatty acids. When the energy requirements of the cell no longer require their oxidation, the aldehydes may undergo dismutation to acids and alcohols of equivalent chain length which may be combined as waxy esters for storage purposes. In the etiolated cell, twice as much waxy ester is found as in the green cell. It is conceivable that in the absence of photosynthesis the etiolated cell maintains its supply of reduced pyridine nucleotide by an emphasis on the α-oxidation of fatty acids, resulting in an increased accumulation of waxy ester in the cell. When etiolated cells in the resting stage are maintained on the fully synthetic medium rather than on the enriched medium an increased proportion of tridecanoate is observed in the fatty acid complement of the waxy ester fraction. An equivalent increase in tridecanol is noted. Tridecanyl tridecanoate now constitutes the bulk of the waxy esters. Acids containing up to 19 carbon atoms are also found. This suggests a high degree of activation of the α-oxidative mechanism. In irradiated cultures undergoing cell multiplication at the logarithmic rate, newly divided cells predominate. Such cells should have a submaximal number of functional chloroplasts. In Figure 3a it may be seen that the fatty acid pattern of the logarithmic stage cells partially reverts to that of the etiolated cells with an increase in the proportion of waxy ester–typical fatty acids.

It has been demonstrated (Martin and Stumpf, 1959) that α-oxidation, dependent on peroxide, is greatly inhibited by the presence of imidazole. The addition of imidazole to cultures vigorously growing on the synthetic medium resulted in a great accumulation of palmitate and stearate in the etiolated cell. See Figure 3b. The proportions of lower-chain homologs remained relatively static. This is interpreted to indicate the uninhibited synthesis of palmitate by the malonyl CoA pathway (Wakil, 1958; Wakil and Ganguly, 1959) with subsequent degradation by the α-oxidative mechanism blocked in the presence of imidazole. Increased amounts of palmitate would then become available for desaturation and elongation as Figure 3b appears to show. Palmitate may occur at a metabolic fork leading either to α-oxidative utilization for the production of reduced pyridine nucleotide or to chain elongation. On recovery from the imidazole treatment, the cells display their original fatty acid pattern. In irradiated cells treated with imidazole, only minor changes in dodecanoate and tridecanoate are observed. When both irradiated and etiolated cells are treated briefly with exogenous peroxide, their waxy ester–typical fatty acids rapidly increase in quantity. This may indicate that the availability of endogenous peroxide mediates the rate of α-oxidation of fatty acids in the cell.

In explaining the accumulation of odd and even intermediate chain length fatty acids in etiolated cells, it may be proposed alternatively that such acids are produced by the malonyl CoA pathway (Wakil, 1958; Wakil and Ganguly, 1959), but that an elongation mechanism either functions less adequately or is dependent on a prior desaturation of substrate. The assumption of this possibility renders difficult an explanation of the imidazole and peroxide effect and of the accumulation of both fatty acid and fatty alcohol of comparable odd and even chain length distribution. Also, it would not be easy to understand the source of an abundance of propionyl CoA to act as a receptor for malonyl CoA in the synthesis of odd-chain fatty acids, especially since *Euglena gracilis* is not by preference a carbohydrate utilizing organism (Wolken, 1961). Yet, the possibility of odd-chain synthesis by the malonyl CoA pathway or some other mechanism is not excluded in these studies.

The fatty acids of both the green and etiolated cells are present in the waxy ester fraction and in some twenty discrete phospholipid, sulfolipid, and galactolipid fractions which can be separated by thin-layer chromatography. The distribution of the various molecular species of fatty acids among these lipid fractions is now under investigation. In the green cells, practically all the saturated fatty acids in the 12- to 15-carbon range are found in the waxy ester fraction. Alterations in phospholipid composition and a net increase in sulfo- and galactolipids (see Table III) appear to account for a major part of the increase in unsaturated fatty acids in the green cell. In the etiolated cell, the fatty acids of the waxy ester fraction account for 25% of the total cellular fatty acid content. Yet, this fraction does not contain all the 12- to 15-carbon fatty acids in the cell (see Table II). These are now found in numerous complex lipid fractions along with unsaturated fatty acids which are present in diminished quantity in the etiolated cell. It appears that in the etiolated cell the lower chain homologs are utilized to build complex lipids, and excess production causes the storage of these homologs as waxy esters which eventually become the quantitatively predominant lipid fraction.

There is not yet sufficient evidence upon which pre-

cise functions can be ascribed to the lipids whose involvement in photosynthesis has been demonstrated (Benson, 1961). At least for *Euglena gracilis*, unsaturated fatty acids should perhaps be added to the list of active participants.

The values in Table III compare the quantities of some ionic and polar lipid components of green and etiolated cells. The presence of galactose in the lipid of green cells has been shown previously (Carter *et al.*, 1961). The report of its absence in etiolated cells (Carter *et al.*, 1961) was possibly due to the solubility of the small quantity of galactolipid in acetone in the presence of a very large amount of acetone-soluble waxy esters. It is of interest that the ratio of quantity of preformed lipid sulfate to total lipid sulfur remains constant for both cell types although absolute quantities are greater in the green cell. Taking note of the similar ionic and structural properties of glucuronic and 6-deoxygalactose-6-sulfonic acids, it is tempting to speculate concerning an associated role for sulfo- and uronidolipids in the photosynthesizing cell. While galactose and the other components listed in Table III are reduced in quantity, they do not disappear from etiolated cells or from a thermally induced apochlorotic mutant (Rosenberg, unpublished results) no longer capable of photosynthetic activity. This, coupled with evidence for an emphasis on an oxidative mechanism typical of plant cells, casts some doubt on the often supposed likelihood of the conversion of dark-grown cells of *Euglena gracilis* to an "animal" metabolism.

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