bolic energy. This appears to be the first indication that, in some organisms, neutral fatty esters other than triglycerides may serve as the major form of storage of such reserves.

To test whether the mechanism of α-oxidation is involved in the metabolism of wax esters, imidazole, a potent inhibitor of fatty acyl peroxidase (*8*), was added to the culture medium (*4*). Entry of imidazole into the organism should block the α-oxidation of fatty acids, since it is a peroxidase-dependent process. In a culture of well-nourished euglenas growing in the dark in a complete medium, imidazole caused a lowering of the content of medium-chain fatty acids typically found in the wax esters, with a simultaneous accumulation of saturated 16-carbon (palmitic) acid and 18-carbon (stearic) acid, the chief saturated acids to be synthesized *de novo* (*9*) in *Euglena*. In starving dark-grown euglenas, imidazole clearly slowed the removal of their store of preformed wax esters (*4*). These observations are consistent with the interpretation that the wax esters of *Euglena* are produced and metabolized by a peroxidase-dependent process of fatty acid oxidation that can be blocked by exogenous imidazole.

The changing composition of lipids of starving dark-grown organisms provided a clue to an adaptive mechanism of general interest. As the euglenas starved and as their wax esters declined, arachidonic acid (*10*) and its homologs became the quantitatively predominant fatty acids (*4, 6*) in these organisms. The theoretical role of arachidonic acid as a basic component in expanded and readily hydrated membrane systems has recently been the subject of plausible speculation (*6, 11, 12*).

The four, all *cis*, double bonds in the hydrocarbon chain of arachidonic acid oblige the chain to assume a curled configuration when it is packed in a quasi-planar arrangement, or a helical configuration when packed in a linear arrangement (Fig. 3, Ia and Ib). With either configuration, a close packing with other, fully saturated, hydrocarbon chains is not possible, nor can there be strong London–Van der Waals bonding with other, fully saturated chains, since bonding necessitates the close approach of large enough numbers of methylene groups in adjacent chains that can, with such a close approach, form tight "hydrophobic" aggregates, as in myelin (*11, 12*). Rather than the formation of tightly aggregated, hydrophobic, limiting membrane systems, the formation of electron-rich, expanded, limiting membrane systems that are potentially receptive to the influx of metabolites from the surrounding aqueous medium would appear to be a necessity for the starving dark-grown euglenas. The introduction of a high percentage of arachidonic acid or similar long-chain polyunsaturated acids into the membranes of the starving organisms should help to build the needed systems. To examine this possibility, euglenas that had been maintained for a week in a medium that contained only potassium phosphate and magnesium chloride were fractioned and their pellicles isolated. Fully two-thirds of the fatty-acid components of the pellicles of starved etiolated euglenas turned out to be arachidonic acid and its homologs (*6*). Much of this was incorporated into complex lipids that were so firmly bound into the pellicle's structure that it was not easily extracted with a mixture of chloroform and methanol, the usual lipid extractant. Only after destruction of the pellicular architecture by prolonged heating in methanolic alkali, in the dark under nitrogen, was all of the arachidonic acid fraction released. In the pellicles of green photosynthesizing euglenas, there was only half the quantity of arachidonic acid and its homologs that was found in the pellicles of the starving dark-grown organisms. In the photosynthesizing organisms, arachidonic acid was also found in the chloroplasts, but nowhere else in the organisms. In the starving dark-grown euglenas, arachidonic acid and its homologs pervaded the organisms, with a great concentration in the pellicles (*6*). Thus, the long-chain polyenoic acids of *Euglena* were components of lipids that appeared to be located mostly in membrane systems close to the aqueous environment from which presumably hydrated metabolites had to be derived.

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10 July 1967

Galactosyl Diglycerides: Their Possible Function in Euglena Chloroplasts

Abstract. *Illumination of euglenas grown in the dark induces the formation of chloroplasts characterized by the simultaneous appearance of chlorophyll and galactosyl diglycerides in a relatively fixed ratio. The fatty acyl chains of the galactosyl diglycerides are constructed so that they can provide a stable lock-and-key fit with the phytol chains of chlorophyll in such a way as to localize the porphyrin structures of chlorophyll and space them for efficient photoreception. Light-starved photobiotic euglenas show chloroplast shrinkage with a concurrent partial loss of galactosyl diglycerides.*

The chloroplasts of green euglenas do not replicate in the dark. Once deprived of light, the progeny of these organisms will contain successively smaller stores of chlorophyll and, in the end, an inoculum of green euglenas grown in complete darkness will produce a dark-grown (etiolated) culture that is essentially achlorophyllous. Subsequent subcultures that can be produced repeatedly in the absence of light have the same characteristic. Exposure of etiolated euglenas to light induces them to redevelop chloroplasts. After this, the organisms are capable of an autotrophic existence once again.

Illuminated green euglenas live, but they cannot grow and divide, in a medium that contains no nitrogen, and,

in addition, in a medium that contains only minerals, they depend completely on light as a source of metabolic energy. When photobiotic euglenas in a mineral medium are deprived of light, their chloroplasts degenerate but do not disappear entirely.

There was a lag of several hours before the rapid accumulation of chlorophyll began in the continuously illuminated organisms. After the lag, monogalactosyl and digalactosyl diglycerides accumulated simultaneously with chlorophyll.

The galactosyl diglycerides (Fig. 1, B and C) appear to be integral components of the lipid matrix of the lamellae of the chloroplast structure (*1*, *2*). Their precise role in these lamellae (double membranes) has not been determined. The content of unsaturated fatty acids in the galactosyl diglycerides generally is so high that most galactosyl diglyceride molecules in chloroplasts of *Euglena* (*3*) and higher forms of plants (*4*) contain two unsaturated fatty acyl residues. They differ in this respect from the usual arrangement in the sulfolipids (*5*) and in most phosphatides (*6*), in which one saturated and one unsaturated fatty acyl residue per molecule are normally found.

In the galactosyl diglycerides of green *Euglena*, the fatty acids are mainly of the 16- or 18-carbon varieties with high proportions of methylene-interrupted dienes, trienes, and even tetraenes (*3*, *7*).

It has been proposed that the galactosyl diglycerides and smaller amounts of other surfactant lipids (*8*) aggregate to form a lipid or, in conjunction with protein, a lipoprotein matrix upon which chlorophyll molecules are dispersed to form photoreceptive surfaces in the chloroplast (*9*). Precisely how galactosyl diglycerides, as the major lipid components of the chloroplast lamellae, perform this function is still open to question.

The molecular structure (Fig. 1A) and physicochemical properties of chlorophyll provide clues to the probable function of the galactosyl diglycerides in the chloroplast lamellae. A complex, magnesium-containing, porphyrin structure (chlorophyllide) constitutes the portion of the chlorophyll molecule that absorbs visible light. Chlorophyllide is a hydrophilic structure. The lipophilic portion of the chlorophyll molecule is provided by phytol, a fatty alcohol whose hydrocarbon chain is linked through an ester bond to the propionic acid side chain of the chlorophyllide residue (Fig. 1A). It is reasonable to assume that the hydrocarbon tails of the phytol residues of chlorophyll molecules are buried in the lipid or lipoprotein layers of the chloroplast lamellae, with the chlorophyllide residues distributed at intervals on the surfaces of these layers. Here they may combine with fixed water molecules, proteins, and other factors to mediate photosynthesis.

The galactosyl diglycerides, then, appear to act as components of the lipid foundation that holds the phytol resi-

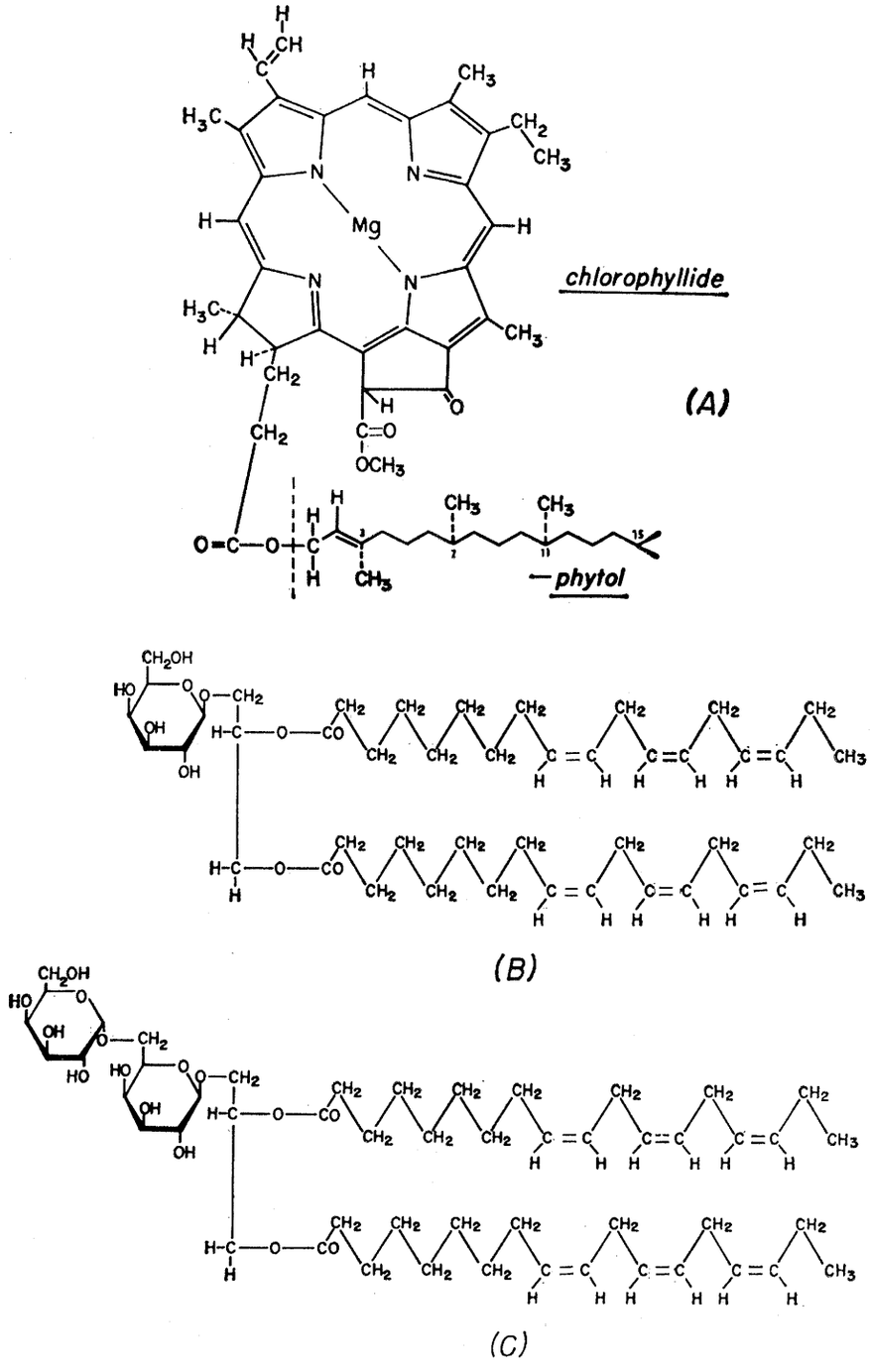

Fig. 1. Structural formulas of (A) chlorophyll *a* (the dotted line separates the photoreceptive porphyrin residue, chlorophyllide, from the lipophilic residue, phytol); (B) monogalactosyl diglyceride; and (C) digalactosyl diglyceride. The fatty acyl chains in (B) and (C) are shown as *cis*-9,12,15-hexadecatrienoyl (linolenoyl) chains.

dues of chlorophyll, and, as such, the galactosyl diglycerides provide a support for the photoreceptive residues of chlorophyll. But why so specialized a foundation? The chain lengths of the galactosyl diglycerides are generally, but not entirely, restricted to 16 or 18 carbon atoms. As stated previously, both of the fatty acyl residues in most of the molecules of galactosyl diglyceride in chloroplasts of *Euglena* are unsaturated, high proportions being dienes, trienes, and even tetraenes (*5*). The nature of the chlorophyll molecule provides the clue to the specific need for these unsaturated fatty acyl residues in the lamellae of the chloroplasts.

In order to provide an efficient photoreceptive surface, chlorophyll molecules must be spread into a rather thin film (*10*). However, the stability of films composed of pure chlorophyll is low; the molecules readily undergo reaggregation into a compact crystalloidal arrangement not very suitable for the interception of visible light. Solution in a lipid matrix separates the chlorophyll molecules, permitting them to form a surface that can properly absorb visible light (*11*). This appears to occur in the lamellae of the native chloroplast. The requirements are exacting with regard to the nature of the hydrocarbon chains of lipids that have the ability to dissolve chlorophyll, since, to do so, the hydrocarbon chains of such lipids must either be capable of close association with phytol, or, if not, they must at least be packed loosely enough to permit the intrusion of phytol chains. Along its 16-carbon chain, phytol bears four separate protruding methyl groups at positions 3, 7, 11, and 15. These methyl groups should effectively prevent its close association with saturated hydrocarbon chains (*12*). Without such close association, bonding between the hydrocarbon chains of phytol and the hydrocarbon chains of the lipid matrix through London–Van der Waals dispersion-attraction forces (*13*) would be so weak as to be negligible in the face of the thermal agitation that acts to dissociate the chains at physiological temperatures. If fully saturated chains in the lipid matrix are present in high concentration, they should form stable intermolecular associations that exclude chlorophyll, and the chlorphyll should then aggregate.

Occurrence of *cis* double bonds in hydrocarbon chains of the lipid matrix causes the formation of pockets or twists in the chains. Such twisted chains should not be capable of the close intermolecular association typical of linearly extended saturated chains. Therefore, they should permit good phase mixing with the branched chains of the phytol residues of chlorophyll. The pockets can provide a fit for the methyl groups of phytol. The close association of methyl group and the double bond may also be stabilized through induced polar interaction of double bonds and the methyl groups. A calculation of the order of magnitude of this effect is given below (see *14*). The fit of methyl group into *cis* double bond can permit close association of the remaining (methylene) groups in the backbones of both phytol and fatty acyl chains, further promoting the stability of the aggregate through the action of London–Van der Waals dispersion-attraction forces. An examination of molecular models indicates that the bulky form of the methyl groups at positions 7, 11, and 15 in phytol disturbs the normal relaxed configuration of the hydrocarbon backbone of the molecule so that, where the methyl groups occur, an extended relaxed planar arrangement of the linked carbon atoms of the backbone is not possible; the methyl groups introduce pockets or twists in the phytol chain in a manner similar to that in which pockets or twists are introduced by the methylene-interrupted *cis* double bonds in the fatty acyl chains of the galactosyl diglycerides. The potential complementarity of configuration of the methyl-terminal sections of the hydrocarbon chains of phytol and the *cis*-unsaturated fatty acids (Fig. 2) of the galactosyl diglycerides should make it possible for many types of close approach to occur between the lipophilic portions of chlorophyll molecules and galactosyl diglyceride molecules in the lamellae of the chloroplast. Presumably, the final, most stable packing of the molecules would involve a space-saving arrangement in which there is close fit of methyl group and *cis* double bond as developed above.

The problem may be examined in more detail. The first (*cis*) double bond in the unsaturated fatty acyl chains of the galactosyl diglycerides almost always occurs at position 9 in the chain; that is, the first seven carbon atoms after the ester bond (to glycerol, Fig. 1, B and C) constitute a fully saturated unbranched section of the chain. This section is capable of an extended planar configuration. Similarly, in the phytol chain in chlorophyll, the first five carbon atoms in the chain after the ester bond to the propionic acid side chain of the chlorophyllide residue are capable of an extended planar configuration. This configuration is possible because the first methyl group of phytol, at carbon atom No. 3 (Fig. 1A), lies adjacent to the *trans* double bond between carbon atoms No. 2 and No. 3. This is the only double bond in phytol and, being a *trans* double bond, it does not interfere with the capability of the chain in this region to assume an unstrained, extended planar configuration. Furthermore, the double bond provides space to accommodate the bulk of the methyl group lying adjacent to it on carbon atom No. 3. Arranging the front ends of phytol and fatty acyl groups alongside each other in an unstrained, extended planar configuration brings respective methyl groups and *cis* double bonds in the methyl-terminal sections of the chains into almost exact juxtaposition, and permits their lock-and-key fit in a space-saving arrangement (Fig. 2).

In support of the foregoing theoretical formulation, one finds that chlorophyll has been shown to be essentially immiscible with the 18-carbon, fully saturated, straight-chain alcohol, stearyl alcohol, but chlorophyll is quite miscible with the 18-carbon, straight-chain alcohol, oleyl alcohol, which bears a *cis* double bond at position 9 in the chain (*10*). Not unexpectedly, a film of chlorophyll is not stabilized by superposition on a film of stearyl alcohol, but it is greatly stabilized by superposition on a film of oleyl alcohol. An ideal solution (with no excess energy of mixing) is formed, and the ability of the spaced chlorophyll–oleyl alcohol film to react with visible light is vastly enhanced, compared with a compact film composed of chlorophyll alone (*10*). Theoretically, the same structural arrangement as the one proposed here for chlorophyll in the chloroplast lamellae may also pertain to the plastoquinones and perhaps to the carotenoids, whose lipid chains are composed of units of isoprene that can present an array of methyl groups similar to that presented by the isopentane units in the lipid

residue of chlorophyll. Since plastoquinones and similar molecules (*15*) are thought to function as electron sinks, or receptors, for electrons produced by illuminated chlorophyll, their positioning close to chlorophyll in the photoreceptive surface of the galactosyl diglyceride matrix may be a necessary feature of the chloroplast. It should be emphasized that galactosyl diglycerides in *Euglena* contain 16- and 18-carbon fatty acyl components, plus smaller amounts of the 20-carbon component, all with varying degrees of *cis*-unsaturation (*3*). Solution of the phytol residues of chlorophyll in such a mélange of *cis*-polyenoic structures should not per se result in a completely uniform orientation of the chlorophyllide residues of chlorophyll on the surface of the lipid matrix. From light polarization measurements (*16*), this appears to be the case.

The theoretical packing arrangement proposed here for lipophilic residues in the lamellae of the chloroplast should result in a surface in which there is functional contiguity of the photoreactive porphyrin residues of chlorophyll, the electron-receptive quinone residues of plastoquinone, and the water-fixing hexose residues of galactosyl diglyceride. Therefore, arrangement of the basic photoreceptive aggregate should not initially require the participation of molecules of protein. However, for stabilization of the aggregates and for building the complex ultrastructure of the chloroplast (*17*), interaction of the lipid aggregates with chloroplast lamellar protein (*18*), shown to be rich in lipophilic side chains, undoubtedly is quite necessary, as is a superposition of the enzyme systems and the numerous other factors needed on the photoreceptive surfaces in order that the primary reactions of the photosynthetic process (*19*) may take place.

A consideration of the general modes of interaction of the various chloroplast components recently has been set forth by Benson in a detailed and informative treatment of the molecular nature of chloroplast membranes (*9*), and a dimensional analysis has been developed by Wolken (*20*).

If the model given here for the spaced photoreceptive aggregate in the chloroplast is correct, one might expect that chlorophyll and galactosyl diglycerides would accumulate simultaneously during chloroplast assembly and that during this process a relatively constant ratio of chlorophyll to galactosyl diglycerides would be maintained. From spatial considerations, the minimal theoretical ratio of galactosyl diglyceride to chlorophyll necessary to stabilize all of the chlorophyll molecules in a film-like arrangement would be two molecules of galactosyl diglyceride for each molecule of chlorophyll; that is, one phytol chain of chlorophyll for four *cis*-unsaturated fatty acyl chains of galactosyl diglyceride. Such a ratio would space the chlorophyll molecules sufficiently to permit good photoreception. These concepts were tested in the living intact organisms.

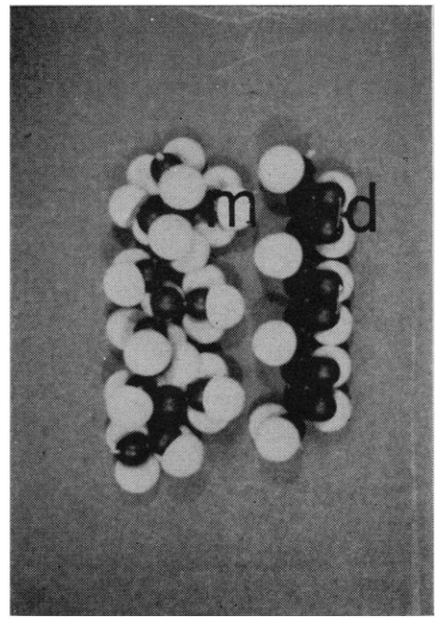

Fig. 2. (Left) Triisopentyl chain as it occurs in the phytyl residue of chlorophyll; *m* indicates the first of the three consecutive methyl groups. (Right) Methylene-interrupted *cis*-trienyl chain as it occurs in a fatty acyl residue of galactosyl diglyceride; *d* indicates the first of the three consecutive methylene-interrupted double bonds. The models have been arranged to show a positional correspondence between the respective methyl groups and double bonds in adjacent chains.

Euglenas grown in the dark on a fully defined medium (*7*) were collected at the end of the logarithmic phase of growth. The organisms were washed free of medium and suspended in a 0.01*M* solution of magnesium chloride and potassium phosphate (*7*). They were now photobiotic, and aside from endogenous stores, they depended upon light for their metabolic energy. They were exposed to continuous illumination at 990 lu/m^2.

Samples of culture were taken at regular intervals. The organisms were harvested by centrifugation, and the lipids were extracted from the organisms by blending them with 20 volumes of a mixture of chloroform and methanol (2 : 1), followed by cooling to −20°C overnight. This procedure extracts all of chlorophyll and galactosyl diglycerides. Chlorophyll in the extract was determined spectrophotometrically; monogalactosyl and digalactosyl diglycerides were isolated by a combination of column and thin-layer chromatography, and they were analyzed as described (*3*).

After the lag, chlorophyll and galactosyl diglycerides were found to accumulate together in the illuminated organisms (*7*). Throughout the process of active greening, a relatively constant molecular ratio of one molecule of chlorophyll to two to three molecules of galactosyl diglyceride was maintained. Thus, there always appeared to be sufficient galactosyl diglyceride needed theoretically to stabilize, in a film, all of the chlorophyll molecules present, and the photoreceptive porphyrin residues of the chlorophyll molecules in the chloroplasts should have been in a spaced arrangement on the surfaces of the lipid matrix.

Excess galactosyl diglycerides in the chloroplasts of *Euglena* conceivably could serve as a source of metabolic energy in time of need. The fate of the galactosyl diglycerides of green euglenas, deprived of light in a mineral medium, was tested; in this medium, they had no exogenous source of metabolic energy. Since the organisms were in total darkness, the photoreceptive structures in their chloroplasts were nonfunctional. The euglenas were unable to grow, divide, and thereby lose their chloroplasts (by virtue of nonreplication of chloroplasts in the dark) as they do in a normal, etiolated, dark-grown culture, obtained from an inoculum of green organisms in a complete medium.

The light-starved cultures were observed over a period of several days. The chlorophyll of the euglenas diminished very slowly; after 7 days in the dark, they still retained over 85 percent of their original content. However, their content of excess galactosyl diglycerides diminished precipitously as light-starvation proceeded. A clearly visible progression of chloroplast

shrinkage, with concurrent increase in density of pigmentation, was observed with the light microscope (Fig. 3). The great stability of chlorophyll has also been noted in mature higher plants (*21*); neither resynthesis nor degradation was observed to occur.

Disappearance of galactosyl diglycerides in the light-starved euglenas proceeded for 100 hours until the ratio approached that of one molecule of chlorophyll for two molecules of galactosyl diglyceride. Beyond this point, chlorophyll and galactosyl diglycerides disappeared together, although slowly, and the organisms began to become dormant and to disintegrate.

In a precise analysis of the quantosomes (*22*) (main subunits of the chloroplast) from spinach leaves, the values reported for chlorophyll and galactosyl diglycerides indicate a precise ratio of one molecule of chlorophyll for every two molecules of galactosyl diglyceride. Is this the normal ratio in higher plants? Or were the leaves obtained from the usual commercial sources, and hence kept in poor illumination until the maximum permissible ratio of chlorophyll to galactosyl diglyceride was reached, as in the light-starved euglenas?

The recent observations of Nichols, Harris, and James (*23*), with regard to the galactosyl diglycerides of blue-green algae, are of great interest. In these prokaryotic algae, the structure of the chloroplasts is not compact and lamellar, as it is in eukaryotes like *Euglena* and higher plants but is, instead, diffuse and distributed around the periphery of the organisms (*24*). The galactosyl diglycerides of these organisms are very rich in saturated and monoenoic fatty acyl residues. One of the blue-green algae (*Anacystis nidulans*) studied had only saturated and monoenoic fatty acyl residues in its galactosyl diglycerides and, therefore, no fatty acyl residues with more than one double bond. It would appear that the fatty acyl groups of such galactosyl diglycerides are not constructed so as to form compact aggregates with phytol chains of chlorophyll, for the reasons proposed. The diffuse structure of the chloroplast material in the blue-green algae indicate that this may be so.

The study outlined here clearly shows the involvement of lipid structures in an adaptive morphogenetic process. The field is new; results are of a preliminary nature; interpretations necessarily are tenuous. By their structure, lipids are endowed with the property of aggregation in an aqueous environment to form lamellar or micellar matrices that can range, depending on the structure of their fatty acyl components, from solid to semisolid to fluid at physiological temperatures.

They are found to be among the basic structuro-functional components of all physiological membrane systems; in energy-producing organelles, they seem to be necessary to provide the solid state organization upon which the polar transfer of electronic energy or charge in these organelles no doubt depends (*20*). To remove the lipids from such organelles, be they retinal cones or mitochondria or chloroplasts, is to remove their energy-producing capability; to restore their lipid components under proper conditions is to restore, at least in part, those capabilities.

There is an ever-growing realization of the importance of lipids in providing an organization to the myriad enzyme-catalyzed reactions that go to make the living cell. This realization has excited a burgeoning interest in the chemical nature of the complex lipids.

As I have attempted to show here in small part, even a fragmentary knowledge of the chemical composition of the lipid components of cellular

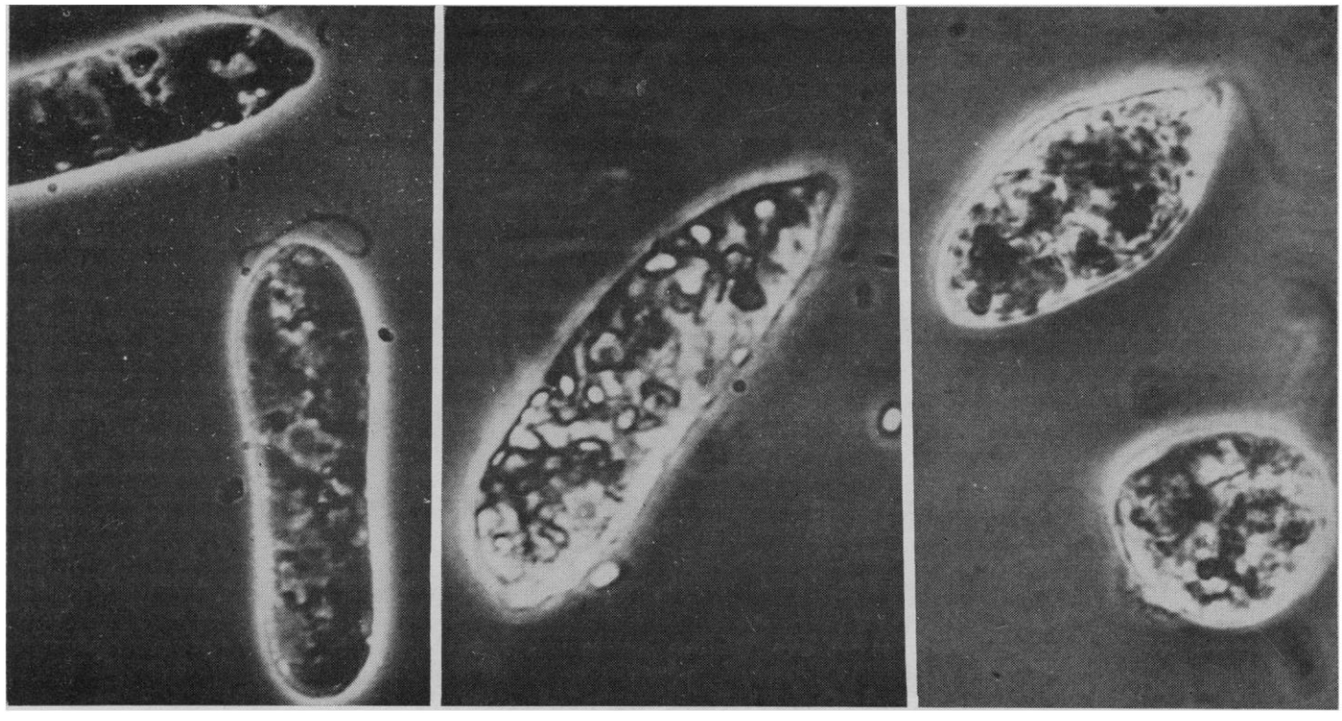

Fig. 3. Degenerative morphological changes in the chloroplast structures of green photobiotic *Euglena gracilis* kept in the dark. Left, 0 days in the dark; center, 3; and right, 7 days.

structures, coupled with a quite facile consideration of the physicochemical attributes of these components, can help to bring a new dimension to our understanding of organismic behavior.

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10 July 1967

Induction of Mutants with Altered DNA Composition: Effect of Ultraviolet on Bacterium paracoli 5099

Abstract. *The culture of* Bacterium paracoli *5099 represents a favorable system for induction of mutants with altered DNA base composition. The frequency of induction of these mutants by ultraviolet radiation is strongly dose-dependent, and has a peak at the ultraviolet exposure equal to 860 ergs. On both sides of this maximum the rate of appearance of mutants decreases, and with the exposures less than 350 and more than 1900 ergs per square millimeter, mutants with altered DNA base composition do not appear at all.*

Investigation of mutants with altered DNA base composition in bacteria has been hindered by the rarity of these events, and a summary of previous studies has been published (*1*). A system has become accessible in which such mutants are induced with greater frequency, and therefore can be reproduced routinely (*2*). Work with this system has made it possible to conclude that the frequency of induction of mutants with altered DNA base composition strongly depends on the dosage of ultraviolet radiation, as outlined in this report.

For the investigation we used *Bacterium paracoli* 5099 from the Type Culture Collection of U.S.S.R. (State Control Institute of Medical Biological Preparations of the Ministry of Health, Moscow). As a source of ultraviolet radiation (2540 Å) we used an ultraviolet lamp giving a dose rate of 11.53 erg sec^{-1} mm^{-2}; distance from the target was 33 cm. Bacteria were grown on nutrient agar at 37°C for 24 hours and suspended in water to the density 10^9 cells per milliliter; the suspensions were placed in dishes in layers 1-mm thick, and irradiated for various intervals between 30 and 165 seconds. Samples of irradiated suspensions were plated on nutrient agar that contained 1 percent glucose and were incubated at 37°C for 7 days.

We had observed earlier (*2*) that mutants appearing as small yellowish colonies on agar plates were approximately 200 times more sensitive to the action of trypaflavine than the cells of the parent culture, and that there is complete correlation between the drastic increase of sensitivity to trypaflavine and distortion of DNA base composition in the cells of mutants. We therefore used "gradient" agar plates containing trypaflavine (10 μg/ml in the upper layer) in order to evaluate the

Table 1. Guanine-cytosine (percent GC) content in DNA of the parent culture *B. paracoli* 5099 and of small yellowish-colony mutants susceptible to trypaflavine as determined from the melting temperature (T_m). Fifty mutants (1416 to 2570) were induced by ultraviolet. Two mutants (266 Fu and 426 Fu) were induced by 5-fluorouracil. Mutants 73 and 161 are representatives of a group of 13 mutants induced by ultraviolet in the parent culture resistant to kanamycin.

Strain	T_m (°C)	GC (%)	Strain	T_m (°C)	GC (%)
5099 (parent)	89.0	48.0	2450	97.5	68.8
1416	97.4	68.5	2451	98.2	70.5
1417	97.4	68.5	2452	97.5	68.8
1707	97.5	68.8	2453	97.5	68.8
1799	97.6	69.0	2454	97.9	69.8
1926	97.5	68.8	2455	97.8	69.5
1927	97.3	68.3	2456	97.8	69.5
1975	98.2	70.5	2457	97.4	68.5
2074	96.7	66.8	2458	98.5	71.2
2244	97.5	68.8	2459	97.6	69.0
2263	97.7	69.3	2462	98.2	70.5
2264	98.2	70.5	2464	97.7	69.3
2312	97.5	68.8	2467	98.0	70.0
2329	97.7	69.3	2468	97.5	68.8
2341	98.5	71.2	2469	98.1	70.2
2346	98.1	70.2	2472	97.8	69.5
2393	97.6	69.0	2484	97.8	69.5
2420	98.0	70.0	2488	97.9	69.8
2421	98.6	71.5	2497	97.8	69.5
2423	97.5	68.8	2503	98.1	70.2
2430	97.8	69.5	2519	97.6	69.0
2434	97.7	69.3	2528	97.3	68.3
2439	98.2	70.5	2570	98.5	71.2
2442	97.4	68.5	266 Fu	97.8	69.5
2444	98.0	70.0	426 Fu	97.2	68.0
2446	97.9	69.8	73	97.9	69.8
2448	97.5	68.8	161	98.0	70.0
2449	97.5	68.8			