

Light-induced Changes of Radioactivities in the ^{14}C -labeled Lipids and Fatty Acids of Dark Grown *Euglena gracilis*

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Euglena gracilis was grown in the dark for 12 days in a medium containing sodium acetate- $1\text{-}^{14}\text{C}$. The major amount of ^{14}C was incorporated into the neutral lipids. In these compounds myristic acid was the main fatty acid labeled. Subsequent growth in light (70 hours) in an identical medium (containing unlabeled acetate) led to a strong decrease of radiocarbon in the neutral lipids. The radioactivities in phosphatidyl choline and phosphatidyl ethanolamine increased during the first 28 hours of illumination and thereafter decreased. The radioactivities in phosphatidyl glycerol, sulfolipid, monogalactosyl diglyceride and digalactosyl diglyceride increased continuously. After 70 hours of illumination, most of the radiocarbon was detected in the saturated and unsaturated C_{16} and C_{18} fatty acids of these four lipids. The possibility of light-induced transfers of fatty acids from neutral lipids via phospholipids to the galactolipids is discussed.

In a recent paper¹ we presented some evidence for the existence of two pathways A and B of fatty acid biosynthesis in *Euglena gracilis*. Pathway A seems to be independent of light and is present in dark grown *Euglena cells*. The main fatty acids produced via this pathway are the saturated 14 : 0 and 16 : 0 and unsaturated C_{18} , C_{20} , and C_{22} fatty acids. This pathway seems to be associated with neutral lipids, PC and PE.

Upon illumination of the dark-grown cells, a second pathway B is developed by *Euglena gracilis*. It is light-dependent and requires a minimum amount of NH_4^+ ions in the nutrient medium. This pathway leads to the formation of unsaturated C_{16} and C_{18} fatty acids, and is associated with chloroplast lipids such as PG, SL, MGDG and DGDG.

The occurrence of these two pathways in *Euglena gracilis* is supported by the recent observations of Bloch and co-workers^{2,3}. They reported that dark grown cells of *Euglena gracilis* contain a single fatty acid synthetase similar to that found in yeast⁴. Upon illumination, a second fatty acid synthetase system is developed which is associated with the chloroplasts³.

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Abbreviations: GLC, gas liquid chromatography; TLC, thin layer chromatography; NL 1 and NL 2, neutral lipids; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; SL, sulfolipid; MGDG, monogalactosyl diglyceride; DGDG, digalactosyl diglyceride; Card., cardiolipin.

The data of the previous paper¹ prompted us to the idea of light-dependent transfers of fatty acids between the individual lipids. In order to provide some evidence for this hypothesis, *Euglena gracilis* was grown in the dark in a medium containing sodium acetate- ^{14}C . After reaching the stationary phase of growth, the cells were transferred to an identical medium containing unlabeled acetate and grown under white light. The effect of illumination on the fatty acids and lipids and on the radioactivities in these compounds is reported here.

Materials and Methods

Euglena gracilis Klebs was received as an agar culture from the Pflanzenphysiologisches Institut, Universität Göttingen, Nikolausberger Weg.

The same medium as reported in the previous paper¹ containing 0.05% NH_4Cl and 0.1% Na-acetate was used. *Euglena* was grown in the dark to the stationary phase of growth (12 days) in a 5 l flask containing 3 l of the above medium with 500 μCi of sodium acetate- $1\text{-}^{14}\text{C}$ added. Culturing was carried out at 25°C with a continuous stream of air bubbled through the medium. After reaching the stationary phase of growth, one sample of 400 ml was removed from the algal suspension. The cells were harvested by centrifugation at $10,000 \times g$ and freeze-dried. Four more portions of 400 ml were centrifuged in sterile and stoppered tubes at $1,500 \times g$ for 15 min. The supernatant was decanted, 100 ml of new sterile medium containing unlabeled sodium acetate (0.1%) were added to the centrifuge tubes and the cells resuspended by swirl-



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ing the tubes cautiously. The suspensions were then transferred under sterile conditions to flasks containing another 300 ml of new sterile medium with 0.1% of unlabeled acetate.

These four cultures were subsequently grown free of bacteria under white light (ca. 800 lux) at 25 °C with continuous shaking. After 4.5, 18, 28, and 70 hours one sample was centrifuged at $10,000 \times g$. The cells were freeze-dried.

Lipid extraction

The lipids were extracted with chloroform-methanol (1:2, v/v) according to the procedure described in the previous paper¹.

Thin layer chromatography of lipids

Solvent systems: Acetone – benzene – water (91 – 30 – 8)⁵ and chloroform – methanol – water (65 – 25 – 4). For good separations it was essential to use column-distilled solvents. The TLC-chambers contained paper saturated with solvents.

Thin layer plates

A slurry of 4 g silicagel HF 254 Merck in 9 ml water was used for preparing one plate (12 × 20 cm²). Thickness: 0.25 mm. The plates were activated for 2 hours at 120 °C and thereafter stored under vacuum in a desiccator over P₂O₅.

Detection of lipids

The total lipids were applied on the TLC-plates in a line by means of a soft brush. After TLC the lipids were sprayed with an alkaline solution of Rhodamin 6G (equal volumes of 0.006% Rhodamin 6G in water and of 8% NaOH⁶) and visualized under UV-light (366 nm).

Identification of the lipids

The lipids were identified with the aid of reference substances and by their content of galactose⁷, sulfoquinovose⁷, and of phosphorus⁸.

Preparation of the fatty acid methyl esters

The fatty acid methyl esters were obtained as described previously^{5,9} by transmethylation of the total lipids or of the TLC lipid zones with sodium methylate.

Gas chromatography

Model Packard, 20% Reoplex 400 on chromosorb WS (45 – 60 mesh), column 3 m × 4 mm; and 15% EGSS-X on gaschrom P (100 – 120 mesh), column 2 m × 5 mm; column temperature: 190 °C; flow rate: 60 ml argon/min; amounts injected: ca. 0.01 mg.

Radio gas liquid chromatography

Gas chromatograph: Model Packard, FID; Tri-carb combustion furnace model 325; Tricarb model 3004; column: 15% EGSS-X on gaschrom P (100 – 120 mesh); column temperature 190 °C; flow rate: 30 ml nitrogen/min; split ratio: ca. 1:100.

Sodium acetate-1-¹⁴C (500 µCi) was purchased from New England Nuclear, Dreieichenhain, Germany.

Determination of radioactivities

4 mg of total lipids were applied on one TLC-plate in a line. After TLC the plates were sprayed with an alkaline Rhodamine 6G solution and the lipid zones visualized under UV-light. Each lipid zone was removed from the plate together with the silica gel and measured in scintillation vials with cab-o-sil scintillation mixture. Aliquot amounts of the total lipids were measured in the same way. Counting was performed with a Tri-Carb liquid scintillation spectrometer. Channels: A: 70 – 1,000; B: 70 – 200; C: 200 – 1,000. Gain: 7%.

Chlorophyll determinations were carried out according to the method of Arnon¹⁰.

Results

During growth in the dark, the total radioactivities in the algal suspension (31) decreased from an initial value of 10.86×10^8 dpm to 5.08×10^8 dpm, indicating a high turnover rate of acetate-¹⁴C. When *Euglena* reached the stationary phase of growth, about one fourth of the total radiocarbon at that time was found in the cells (1.17×10^8 dpm). Microscopic examination revealed that the cells were colourless, spherical, and immobile. In the 400 ml sample analyzed after growth in the dark, the total lipids contained 4.84×10^6 dpm, or 256,000 dpm per 1 mg of lipids, respectively (Table I). TLC of the total lipids (Fig. 1*) showed that neutral lipids, PC, and PE predominated after growth in the dark. The upper spot of the neutral lipids in TLC (Fig. 1, NL 1) contained mainly wax esters^{11–14}. The composition of the second spot (NL 2) has so far not been determined. Only trace amounts of PG, SL, MGDG, and DGDG could be detected by TLC. More than 85% of the total lipid radiocarbon after growth in the dark was found in the neutral lipids, PC and PE (see Table II, first column).

* Fig. 1 s. Table on p. 226 a.

Table I. Dry weights, total lipids, chlorophylls, and radioactivities in *Euglena gracilis*. A. After growth in the dark (12 days). B. During subsequent illumination.

	A (Dark)	B (Hours of illumination)			
		4.5	18	28	70
dry weight [mg]	79.0	78.4	79.5	87.3	89.2
total lipids [mg]	18.9	18.4	18.3	18.6	21.1
chlorophylls [% of total lipids]	—	0.01	0.64	3.60	8.35
radioactivity in the total lipids [dpm]	4.84×10^6	3.82×10^6	2.92×10^6	2.53×10^6	2.39×10^6
radioactivity in 1 mg of total lipids [dpm]	256,000	208,200	159,800	136,200	121,100

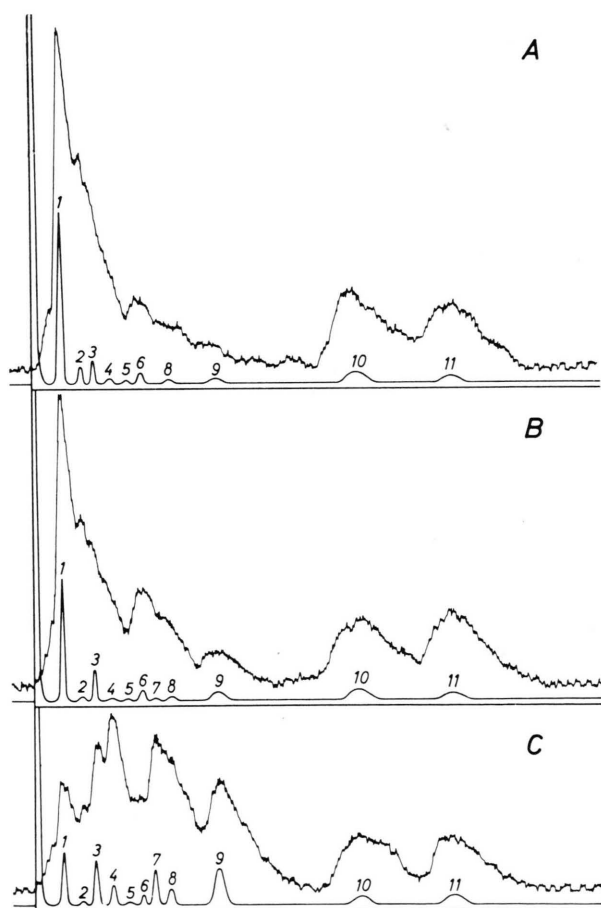


Fig. 2. Radio-GLC (EGSS-X) of aliquot amounts of the total fatty acid methyl esters of *Euglena gracilis*. A. After growth in the dark (12 days). B. After 18 hours of subsequent illumination. C. After 70 hours of subsequent illumination. 1 = 14 : 0; 2 = 16 : 0; 3 = 16 : 1; 4 = 16 : 2; 5 = 18 : 0; 6 = 16 : 3 + 18 : 1; 7 = 16 : 4; 8 = 18 : 2; 9 = 18 : 3; 10 = 20 : 4; 11 = 20 : 5.

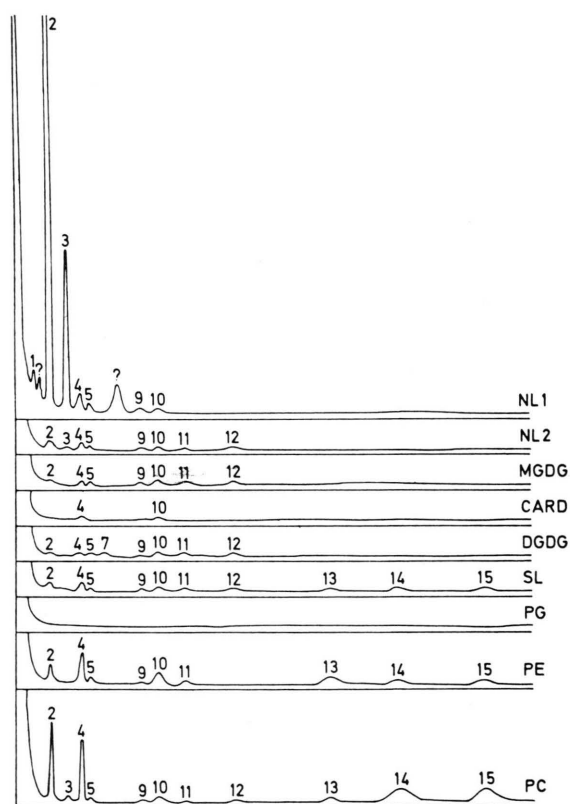


Fig. 3 A.

The 14 : 0, and to a lower extent, the 15 : 0 and 16 : 0, were the main fatty acids labeled in the dark (Fig. 2). They were found predominantly in the neutral lipids, PC and PE (Figs. 3/A and 3/B).

When the four samples of 400 ml were subsequently grown under light in the medium with unlabeled acetate, the cells became green, ellipsoid

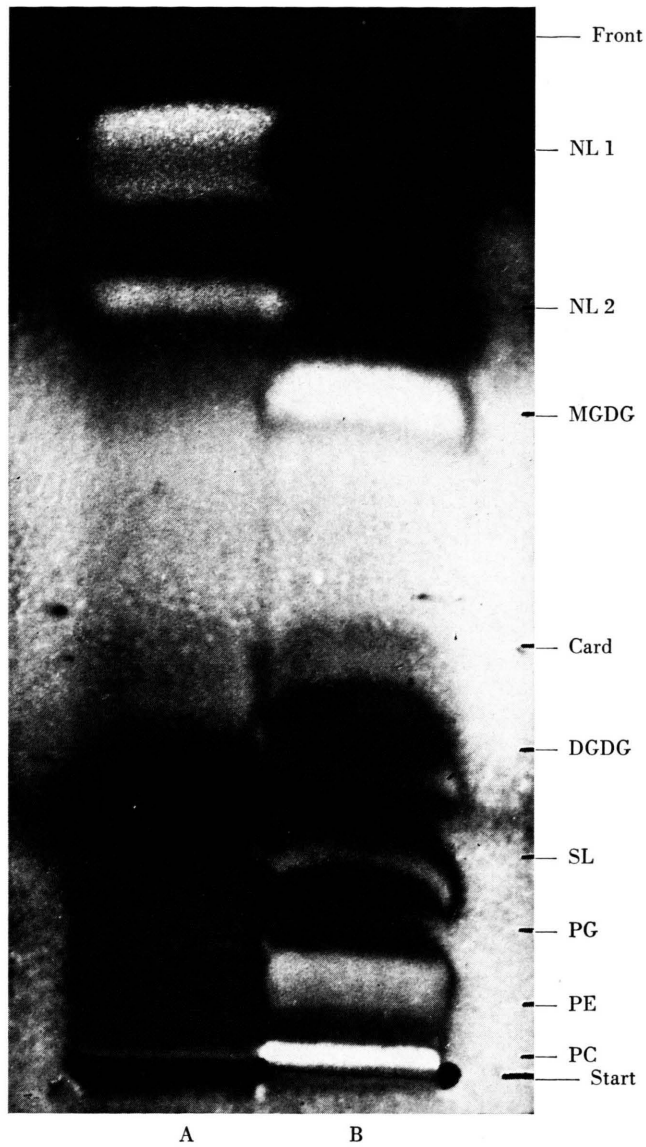


Fig. 1. TLC of the total lipids (0.5 mg each) of *Euglena gracilis*. Solvent system: Acetone—benzene—water (91—30—8). A. After growth in the dark (12 days). B. After 70 hours of subsequent illumination.

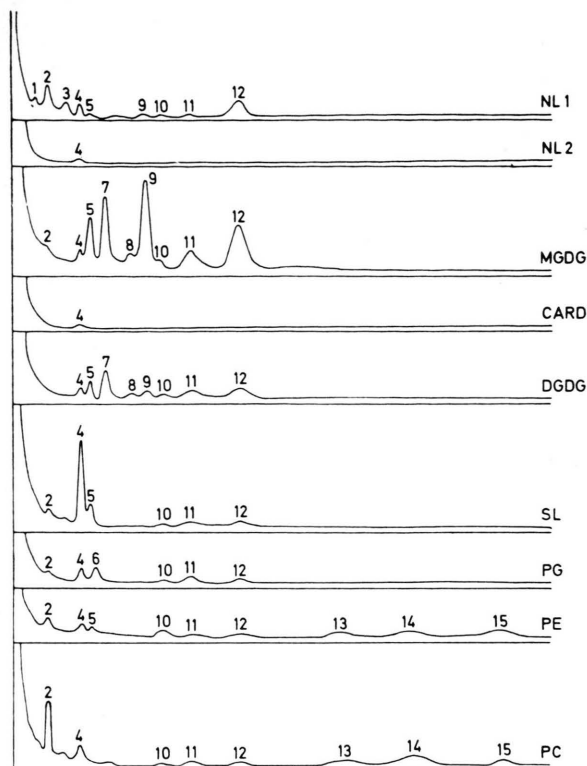


Fig. 3 B.

Fig. 3 A and 3 B. GLC (Reoplex) of aliquot amounts of the fatty acid methyl esters derived from the individual lipids of *Euglena gracilis*. 3 A. After growth in the dark (12 days). 3 B. After 70 hours of subsequent illumination. 1 = 12 : 0; 2 = 14 : 0; 3 = 15 : 0; 4 = 16 : 0; 5 = 16 : 1; 6 = *trans*-3-16 : 1; 7 = 16 : 2; 8 = 16 : 3; 9 = 16 : 4 + 18 : 0; 10 = 18 : 1; 11 = 18 : 2; 12 = 18 : 3; 13 = 18 : 4; 14 = 20 : 4; 15 = 20 : 5.

and mobile. The chlorophyll content increased to 8.35% of the total lipids (Table I). The radioactivities of the total lipids decreased from 4.84×10^6 to 2.39×10^6 dpm within 70 hours (Table I). Per 1 mg of total lipid, the radioactivities decreased from 256,000 to 121,100 dpm.

Table II shows the radioactivities in the individual lipids after a varying number of hours of illumination. The strong overall decrease was mainly due to the loss of radioactivities in the neutral lipids (NL 1 and NL 2). Within 70 hours of illumination, the dpm values of these compounds decreased from about 580,000 to about 100,000.

In contrast to this significant decrease, the situation with the other lipids was quite different. The radioactivities in PC and PE increased and thereafter decreased, having a maximum of radio-

carbon incorporation after about 28 hours of illumination. The radioactivities in PG, SL, MGDG, and DGDG increased during the whole time of illumination. No significant change could be observed in the cardiolipin.

The major part of the lipid radiocarbon was located in the acyl groups, as is shown by the percent radioactivities in the fatty acid moieties of the individual lipids (Table II).

The above findings are in agreement with those from TLC of the total lipids (Fig. 1). In the dark, neutral lipids, PC, and PE predominated. Upon illumination, the neutral lipids almost disappeared whereas PG, SL, MGDG, and DGDG were synthesized in higher amounts.

According to the radio gas chromatograms of the total fatty acids (Fig. 2), the 14 : 0, 16 : 0, 16 : 1, 20 : 4, and 20 : 5 were the main fatty acids labeled in the dark. Upon illumination particularly the saturated compounds with carbon chains of medium length (14 : 0, 16 : 0) decreased, whereas those with longer carbon chains (20 : 4 and 20 : 5) maintained their radioactivities. After 70 hours of illumination, the total fatty acids consisted primarily of saturated and unsaturated C_{16} and C_{18} fatty acids.

Figs. 3/A and 3/B show the gas chromatograms of the fatty acid methyl esters in the individual lipids of the first sample (grown in the dark) and of the last sample (after 70 hours of illumination). These chromatograms were obtained from aliquot amounts of lipids. The fatty acids can therefore be compared quantitatively by the size of their peak areas in GLC. These chromatograms show that during growth in the dark the neutral lipids contained high amounts of saturated fatty acids, mainly the 14 : 0 (Fig. 3/A). After 70 hours of illumination there were only very low amounts of fatty acids derived from the neutral lipids (Fig. 3/B).

The above mentioned unsaturated C_{16} and C_{18} fatty acids prevailing after illumination were located in MGDG and DGDG. In addition, there was a remarkable increase of the 16 : 0 fatty acid in the SL and — to a smaller extent — of 16 : 0 and *trans*-3-16 : 1 in PG (Figs. 3/A and 3/B).

Discussion

In the experiments described here, *Euglena* was grown in the dark in a medium containing Na-acetate- ^{14}C . Thereafter the cells were transferred to

Table II. A. Radioactivities [dpm] in the individual lipids of *Euglena gracilis*. B. Radioactivities [% of A] in the fatty acid moieties. a. After growth in the dark (12 days). b. During subsequent illumination. (Separation of 4 mg of total lipids on one TLC-Plate.)

Lipid	a (Dark)	b (Hours of illumination)			
		4.5	18	28	70
NL 1	A. 558,100	453,100	259,100	115,000	89,500
	B. 85%	83%	74%	79%	77%
NL 2	A. 21,600	22,500	13,200	14,500	13,000
	B. 90%	83%	85%	84%	79%
PC	A. 81,000	114,100	132,800	156,700	106,800
	B. 95%	94%	94%	96%	96%
PE	A. 52,300	56,300	57,500	59,000	38,400
	B. 79%	89%	88%	81%	68%
Card	A. 19,200	18,200	15,100	20,300	17,700
	B. 95%	94%	90%	82%	95%
PG	A. — *	— *	8,400	12,300	17,900
	B. —	—	62%	65%	68%
SL	A. 29,600	11,000	12,000	15,400	24,500
	B. 87%	84%	86%	84%	89%
MGDG	A. 28,100	29,700	40,800	71,300	104,000
	B. 74%	70%	65%	65%	72%
DGDG	A. 12,000	18,000	23,000	28,800	47,500
	B. 83%	81%	68%	65%	67%
total	801,9900	722,900	561,900	493,300	459,300

* PG not detectable by TLC.

an identical medium containing unlabeled acetate. All of the radioactivities detected in the lipids and fatty acids during subsequent illumination could therefore only be derived from radiocarbon sources built up in the dark within the cells. Upon illumination two different processes seemed to be induced:

1. Degradation of the neutral lipids, indicated by the strong decrease of radioactivities in these lipids.
2. A flow of radiocarbon into the other lipids, indicated by the increase of radioactivities in these compounds.

There are two possible explanations for this light-induced flow of ^{14}C into the other lipids:

- A. Oxidative breakdown of lipids and of other cell material (leading to a pool of radioactive acetyl-CoA) followed by *de novo* biosyntheses of the other lipids.
- B. Direct transfer of labeled fatty acids from the neutral lipids to the other lipids.

As for the fatty acids involved in the above light-induced processes, the decrease of labeled fatty acids with a "medium" chain length (14 : 0, 15 : 0, 16 : 0) in the neutral lipids seemed to end with the appearance of saturated and unsaturated fatty acids with elongated carbon chains (C_{16} , C_{18}) in the

galactolipids. This structural interrelationship of the involved fatty acids seems to favour the idea of fatty acid transfers.

An important criterion for the existence of transfers of labeled fatty acids between lipids is the distribution of label between the fatty acids and the glycerol and sugar moieties of each individual lipid. Theoretically, these moieties should incorporate relatively low amounts of ^{14}C , and the fatty acids relatively high amounts, with regard to the number of carbon atoms in both parts of the lipids.

In the experiments described here, ^{14}C -acetate was used as a precursor. Consequently, all parts of the cells became labeled during growth in the dark and could function as a source of radiocarbon during the illumination period. Although, upon illumination, the major part of the lipid radiocarbon was detected in the acyl groups of the individual lipids, MGDG and DGDG seemed to incorporate relatively high amounts of ^{14}C into their glycerol and sugar moieties (Table II). Hence, from the data of this paper, no clear conclusion can be drawn as to whether fatty acid transfers do exist in *Euglena gracilis*. For this reason, further experiments were carried out using radioactive precursors with carbon chains longer than acetate in order to achieve a more specific incorporation into the neutral lipids

and fatty acids. These experiments are described in the following paper.

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