Some Evidence for Light-induced Transfers of Fatty Acids in *Euglena gracilis*

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*Euglena gracilis*, acyl transfers, light-effect, lipids, fatty acids

*Euglena gracilis* was grown in the dark for 12 days. Subsequent incubation with sodium octanoate-l-14C in the dark for 6 hours resulted in a rather specific incorporation of radioactivity into the neutral lipids (primarily wax esters). Upon illumination of these cells in an identical medium without radiocarbon, the radioactivities in the neutral lipids decreased strongly, due to a decrease of the labeled wax acids (mainly 14:0) and wax alcohols (mainly C14). The radioactivities in phosphatidyl choline and phosphatidyl ethanolamine increased during the first 24 hours of illumination and thereafter decreased. This was caused by the initial increase and subsequent decrease of the labeled 14:0 and 16:0 acids in phosphatidyl choline and of the 14:0, 16:0, and 18:1 acids in phosphatidyl ethanolamine. The chloroplast lipids (sulfolipid, phosphatidyl glycerol, monogalactosyl diglyceride, and digalactosyl diglyceride) exhibited a steady increase in radiocarbon content. This was due to an increase of label in the 16:0 fatty acid of the sulfolipid, in the 16:0 and trans3-16:1 fatty acids of the phosphatidyl glycerol, and in the saturated and unsaturated C14 and C16 fatty acids of the monogalactosyl and digalactosyl diglycerides. The labeled fatty acids of the above phospho- and glycolipids had comparably high specific radioactivities. Incorporation of radiocarbon into the last two carbon atoms of their methyl ends, however, was low. The glycerol and sugar moieties of the individual lipids incorporated relatively little radiocarbon. It is concluded that in *Euglena gracilis* the biosynthesis of long chain fatty acids is associated with specific lipids. Upon illumination, oxidative breakdown of the neutral lipids as well as transfers of fatty acids from the neutral lipids via phosphatidyl choline and phosphatidyl ethanolamine to the chloroplast lipids seem to be induced. The lipids involved appear to function as parts of a “lipid-bridge” for the acyl transfers.

The findings of the preceding paper[^1] led us to the idea of fatty acid transfers between the individual lipids of *Euglena gracilis* grown initially in the dark and subsequently in light. Due to the conditions used in those experiments, we could not obtain clear evidence for such transfers. The main reason was that the cells had been grown with radioactive acetate from the very beginning of growth. Consequently, all parts of the cells had necessarily been labeled, among them the glycerol and sugar moieties and the acyl groups of the lipids. In order to obtain better evidence for transfers of fatty acids between the individual lipids, it was necessary to achieve a higher incorporation of radiocarbon into the fatty acids and a lower incorporation into the glycerol and sugar moieties. We therefore carried out similar experiments using radioactive precursors with carbon chains longer than acetate. Furthermore, the cells were at first grown on an unlabeled carbon source and then labeled by a “short-time” incubation with the radioactive precursor.

Experiments with labeled acids of different chain lengths (sodium butyrate-1-14C, sodium hexanoate-1-14C and sodium octanoate-1-14C) revealed that octanoate was incorporated most specifically into the total lipids during growth of the cells in the dark. The best results were obtained when the dark-grown cells were incubated with octanoate for only 6 hours (unpublished observations).

This paper reports the effects of illumination on the lipids and fatty acids of *Euglena gracilis* which were labeled by such a short-time incubation with radioactive octanoate.

**Material and Methods**

*Euglena gracilis* Klebs (strain *Z*) was obtained as an agar culture from the Pflanzenphysiologisches Institut, Universität Göttingen.

**Growth conditions**

The same medium (6.3 l) as described in the previous papers[^1][^2], containing 0.05% NH₄Cl and 0.1%

[^1]: Requests for reprints should be sent to Dr. P. Pohl, Institut für Pharmazeutische Arzneimittellehre der Universität, D-8000 München 2, Karl-Str. 29, Germany.

[^2]: Abbreviations: GLC, gas liquid chromatography; Radio-GLC, radio gas liquid chromatography; TLC, thin layer chromatography; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; SL, sulfolipid; MGDG, monogalactosyl diglyceride; DGDG, digalactosyl diglyceride; Card., cardiolipin.
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sodium acetate, was used. The cells were grown in the dark in a 101 flask at 25 °C under continuous aeration. After reaching the stationary phase of growth (12 days), the cells were centrifuged under sterile conditions for 10 min at 1,500 × g. The supernatant was decanted. The cells were resuspended in 1050 ml of new sterile medium containing 350 μCi of sodium octanoate-1-14C and kept in this medium for 6 hours. These operations were all carried out in the dark. After 6 hours of incubation with radioactive octanoate, the cells were harvested by centrifugation at 1,500 × g for 10 min and resuspended in 350 ml of new sterile medium without any radiocarbon. 900 ml of this cell suspension were immediately centrifuged at 10,000 × g for 5 min. The cell pellet was quickly ground in a mortar and extracted several times with chloroform—methanol 1:2, v/v), until no more radioactivity was detected in the solvent.

The remaining cell suspension (5.41) was immediately illuminated with white light (ca. 800 lux). A continuous stream of air was bubbled through the medium. 900 ml samples of the illuminated cell suspension were centrifuged at 10,000 × g after 1, 3, 8, 24, 68, and 120 hours of illumination, respectively.

**Lipid extraction**

The cell pellets were immediately ground in a mortar and extracted with chloroform—methanol (1:2, v/v) until no more radioactivity was detected in the solvent. The major portion of the solvent was evaporated under a stream of nitrogen at 40 °C. The total lipids were dried to constant weight in a vacuum desiccator.

Determination of chlorophylls, TLC, detection of the lipids, preparation of the fatty acid methyl esters, GLC and radio-GLC (both programmed from 120 °C to 190 °C), and determination of 14C were all carried out as described in the preceding paper 1.

**Radio-GLC of the wax alcohols**

10% UCCW 982 on chromosorb-W-AW-DMCS (80—100 mesh); column: 180 cm × 2 mm; temperature: programmed from 120 °C to 190 °C (2 °C/min).

Sodium octanoate-1-14C was purchased from New England Nuclear, Dreieichenhain, Germany.

**Oxidative degradation**

The component fatty acids derived from the individual lipids were hydrogenated in tetrahydrofurane with Adam's catalyst (PtO2). The hydrogenated fatty acids were oxidized according to the method of Kuhn-Roth as described by Lynen and co-workers 3: The fatty acids were kept in a sealed tube with 4 ml of oxidizing agent (10 ml H2CrO4, 10 ml H2O, 5 ml H2SO4 conc.) at 120 °C for 20 hours. After cooling, the mixture was extracted three times with 3 ml of hexane in order to remove unoxidized fatty acids (these extractions yielded about 1% of the radioactivities employed initially). The residue was reduced with a diluted hydrazine solution in an ice-bath in order to destroy excess chromate, and steam-distilled in a micro-Kjeldahl apparatus. The acetic acid distillate was trapped with an excess of N/1000 NaOH and the solution back-titrated against N/1000 HCl.

**Results**

Table I shows the formation of chlorophylls, total lipids, and lipid-free cell material by *Euglena gracilis* under the various conditions. During the “short-time” incubation (6 hours) with 14C-octanoate (350 μCi), a radioactivity of 70.51 × 10^6 dpm was taken up by the whole cells (Table II). 95.25% of this radiocarbon was incorporated into the total lipids, and the rest into the lipid-free cell material. 96.6% of the lipid radiocarbon was detected in the

<table>
<thead>
<tr>
<th></th>
<th>A (Dark)</th>
<th>B (Hours of illumination)</th>
<th>1</th>
<th>3</th>
<th>8</th>
<th>24</th>
<th>68</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>chlorophylls [% of the total lipids]</td>
<td>0.11</td>
<td>2.3</td>
<td>7.3</td>
<td>9.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total lipids [mg]</td>
<td>68.6</td>
<td>60.2</td>
<td>56.0</td>
<td>52.0</td>
<td>49.5</td>
<td>77.1</td>
<td>80.0</td>
<td></td>
</tr>
<tr>
<td>cell material (without lipids) [mg]</td>
<td>124</td>
<td>123</td>
<td>122</td>
<td>196</td>
<td>220</td>
<td>240</td>
<td>240</td>
<td></td>
</tr>
</tbody>
</table>

Table I. Formation of chlorophylls, total lipids, and cell material by *Euglena gracilis*. A. After growth in the dark (12 days) and incubation with sodium octanoate-1-14C in the dark (6 hours). B. During subsequent illumination.
Table II. Radioactivities in Euglena gracilis after growth in the dark (12 days) and incubation with sodium octanoate-1-14C (350 μCi) in the dark (6 hours).

### A. Whole Cells

**Whole cells:** 70.51 x 10⁶ dpm \(= 100.0\%\)

- a. Total lipids 67.17 x 10⁶ dpm \(= 95.25\%\)
- b. Cell material (without lipids) 3.34 x 10⁶ dpm \(= 4.75\%\)

### B. Total Lipids

<table>
<thead>
<tr>
<th>Lipids</th>
<th>dpm</th>
<th>% of radioactivities in the total lipids</th>
<th>% of radioactivities in the whole cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total lipids</strong></td>
<td>67.17 x 10⁶</td>
<td>100.00%</td>
<td>95.25%</td>
</tr>
<tr>
<td>Neutral lipids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Wax esters</td>
<td>64.90 x 10⁶</td>
<td>96.60%</td>
<td>92.00%</td>
</tr>
<tr>
<td>b. Triglycerides</td>
<td>275,000</td>
<td>0.41%</td>
<td>0.39%</td>
</tr>
<tr>
<td>c. Carotenoids</td>
<td>336,000</td>
<td>0.50%</td>
<td>0.48%</td>
</tr>
<tr>
<td>PC</td>
<td>907,000</td>
<td>1.35%</td>
<td>1.29%</td>
</tr>
<tr>
<td>PE</td>
<td>670,000</td>
<td>0.01%</td>
<td>0.01%</td>
</tr>
<tr>
<td>PG</td>
<td>6,500</td>
<td>0.01%</td>
<td>0.01%</td>
</tr>
<tr>
<td>SL</td>
<td>7,000</td>
<td>0.01%</td>
<td>0.01%</td>
</tr>
<tr>
<td>MGDG</td>
<td>62,000</td>
<td>0.09%</td>
<td>0.09%</td>
</tr>
<tr>
<td>DGDG</td>
<td>12,000</td>
<td>0.02%</td>
<td>0.02%</td>
</tr>
</tbody>
</table>

Wax esters (Table II). This means that 92% of the radiocarbon taken up by the whole cells was incorporated rather specifically into the wax esters. About 1% was found in the triglycerides, and 1.8% in PC and PE. Only 0.13% was detected in the TLC-spots of PG, SL, MGDG, and DGDG.

About 52% of the radiocarbon taken up by the wax esters was detected in the wax acids, and 48% in the wax alcohols, indicating a slightly higher incorporation of 14C into the wax acids than into the alcohols.

As in the preceding paper, a drastic decrease of 14C was observed in the whole cells and particularly in the total lipids upon illumination of the labeled cells (Fig. 1). The radioactivities in the lipid-free cell material increased during the first 24 hours of illumination and thereafter decreased. At all stages of the experiment, however, the content of 14C in the lipid-free cell material remained below 50% of the total radiocarbon in the whole cells.

The radioactivities in the carotenoids and triglycerides seemed to increase slightly during the first 8 – 24 hours of illumination and thereafter decreased (unpublished observations). These compounds are now being investigated more thoroughly. The chlorophylls did not incorporate significant amounts of radiocarbon.

After a varying number of hours of illumination (see Table III), the radioactivities were determined in each lipid. A drastic decrease of 14C was observed in the neutral lipids. The metabolic behaviour of the other lipids, however, differed markedly from that of the neutral lipids. The radioactivities in PC and PE increased during the first 24 hours of illumination and thereafter decreased. PG, SL, MGDG, and DGDG exhibited a significant increase of radiocarbon during the whole period of illumination.

In order to obtain more information about the changes of radioactivities in the lipids, the fatty acid methyl esters and alcohols derived from aliquot
Table III. Radioactivities [dpm] in the individual lipids of *Euglena gracilis*. A. After growth in the dark (12 days) and incubation with sodium octanoate-1-14C in the dark (6 hours). B. During subsequent illumination.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>A (Dark)</th>
<th>B (Hours of illumination)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>neutral lipids</td>
<td>7,350,000</td>
<td>7,000,000</td>
</tr>
<tr>
<td>PC</td>
<td>101,000</td>
<td>209,000</td>
</tr>
<tr>
<td>PE</td>
<td>31,100</td>
<td>50,000</td>
</tr>
<tr>
<td>PG</td>
<td>700</td>
<td>1,000</td>
</tr>
<tr>
<td>cardiolipin</td>
<td>800</td>
<td>2,000</td>
</tr>
<tr>
<td>SL</td>
<td>1,100</td>
<td>4,500</td>
</tr>
<tr>
<td>MGDG</td>
<td>6,500</td>
<td>15,000</td>
</tr>
<tr>
<td>DGDG</td>
<td>1,200</td>
<td>3,900</td>
</tr>
<tr>
<td>total</td>
<td>7,492,400</td>
<td>7,285,600</td>
</tr>
</tbody>
</table>

Fig. 2. *Euglena gracilis*; Radio-GLC of the total fatty acid methyl esters. A. After growth in the dark (12 days) and incubation with sodium octanoate-1-14C in the dark (6 hours). B. After 24 hours of subsequent illumination. C. After 120 hours of subsequent illumination.
Fig. 3. Euglena gracilis; wax esters: Radio-GLC of aliquot amounts of the wax acids. A. After growth in the dark (12 days) and incubation with sodium octanoate-1-14C in the dark (6 hours). B. After 3 hours of subsequent illumination. C. After 24 hours of subsequent illumination. D. After 120 hours of subsequent illumination.

Fig. 4. Euglena gracilis; wax esters: Radio-GLC of aliquot amounts of the wax alcohols. A. After growth in the dark (12 days) and incubation with sodium octanoate-1-14C in the dark (6 hours). B. After 24 hours of subsequent illumination. C. After 120 hours of subsequent illumination.
amounts of the total and individual lipids were analysed by radio-GLC. These investigations gave the following results (Figs. 2 – 10):

**Total fatty acids (Fig. 2)**

After growth in the dark and incubation of the cells with $^{14}$C-octanoate, myristic acid was the main fatty acid found to be labeled. Lesser amounts of $^{14}$C were detected in the 12 : 0 and 13 : 0 acids. The amounts and radioactivities of these compounds decreased upon illumination. After 120 hours of growth in the light, most of the radioactivities were located in saturated and unsaturated C16 and C18 fatty acids. Some radiocarbon was incorporated into the 20 : 4 acid in the dark. The radioactivities in this compound did not appear to change during illumination.

**Fatty acids of the wax esters (Fig. 3)**

The main fatty acid labeled was myristic acid, the concentration and radioactivities of which decreased strongly upon illumination.

**Alcohols of the wax esters (Fig. 4)**

After growth in the dark and incubation with $^{14}$C-octanoate, the C14 and to a lower extent the C16 alcohols were labeled. Both compounds decreased upon illumination.

**Fatty acids of PC (Fig. 5)**

Only the myristic and palmitic acids incorporated significant amounts of $^{14}$C in the dark. The other fatty acids present in this lipid in the dark did not appear to take up label. As was shown, PC had a maximum of radiocarbon incorporation after about 24 hours of illumination (see Table III). During this time the radioactivities increased in both myristic and palmitic acids and thereafter decreased again in both of these compounds.

**Fatty acids of PE (Fig. 6)**

Myristic and palmitic acids took up most of the label in the dark. PE, too, had a maximum of radiocarbon incorporation after 24 hours of illumination (see Table III). During this time there was a strong increase of $^{14}$C in palmitic acid, and to a smaller extent in the myristic and oleic acids. Thereafter the radioactivities decreased mainly in palmitic acid, and to a smaller extent in the myristic and oleic acids. It could not be determined clearly whether the 16 : 1 and 18 : 0 fatty acids were also labeled in this lipid.

**Fatty acids of the cardiolipin (without Figure)**

In this lipid either the 18 : 0 or the 18 : 1 fatty acid was labeled. Because of the low amounts
Fig. 6. *Euglena gracilis*: phosphatidyl ethanolamine: Radio-GLC of aliquot amounts of the fatty acid methyl esters. A. After growth in the dark (12 days) and incubation with sodium octanoate-1-14C in the dark (6 hours). B. After 24 hours of subsequent illumination. C. After 120 hours of subsequent illumination.

Fig. 7. *Euglena gracilis*: phosphatidyl glycerol: Radio-GLC of aliquot amounts of the fatty acid methyl esters. (Only trace amounts of PG could be detected by TLC after growth in the dark (12 days) and incubation with sodium octanoate-1-14C in the dark (6 hours). A. After 24 hours of subsequent illumination. B. After 120 hours of subsequent illumination.
available we could not determine unequivocally which of these two compounds was labeled.

**Fatty acids of PG (Fig. 7)**

After growth in the dark and incubation with 

\[ ^{14} \text{C}-\text{octanoate} \]

only trace amounts of this lipid could be detected by TLC. During illumination, an increase of radiocarbon was observed in palmitic acid and most probably in the \( \text{trans}3-16:1 \) acid. This latter compound, however, has so far been identified only by its relative retention time in GLC and radio-GLC.

**Fatty acids of SL (Fig. 8)**

Radioactivity was found almost exclusively in palmitic acid which increased during the whole period of illumination. The other fatty acids in the SL did not incorporate significant amounts of label.

**Fatty acids of MGDG (Fig. 9) and of DGDG (Fig. 10)**

In these two galactolipids an increase of radiocarbon was found in the \( 16:0; 7-16:1; 7, 10-16:2; 7, 10, 13-16:3; 4, 7, 10, 13-16:4; 9, 12-18:2, \) and \( 9, 12, 15-18:3 \) fatty acids. It could not be determined whether oleic acid \( (9-18:1) \) was also labeled in these two lipids.

In each lipid the percentage distribution of radioactivities was determined in the glycerol and sugar moieties, and in the acyl groups (Table IV). In PC, for example, the moieties contain about 19% of the total carbon atoms of the whole lipid, based on an
Fig. 9. *Euglena gracilis; monogalactosyl diglyceride*: Radio-GLC of aliquot amounts of the fatty acid methyl esters. A. After growth in the dark (12 days) and incubation with sodium octanoate-1-14C in the dark (6 hours). B. After 24 hours of subsequent illumination. C. After 120 hours of subsequent illumination.

Table IV. Incorporation of radiocarbon into the glycerol and sugar moieties and into the fatty acids of the individual lipids of *Euglena gracilis*. A. Percentage incorporation of 14C into the moieties and fatty acids after growth in the dark (12 days) and incubation with sodium octanoate-1-14C in the dark (6 hours). B. Percentage incorporation of 14C into the moieties and fatty acids during subsequent illumination.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>C-atoms in the moieties [%]</th>
<th>C-atoms in the fatty acids [%] (Theoretical percentage)*</th>
<th>14C in the moieties [%]</th>
<th>14C in the fatty acids [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A (Dark)</td>
<td>B (Hours of illumination)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>PC</td>
<td>ca. 19/81</td>
<td></td>
<td>26.2/73.8</td>
<td>15.4/84.6</td>
</tr>
<tr>
<td>PE</td>
<td>ca. 13/87</td>
<td></td>
<td>10.2/89.8</td>
<td>9.3/90.7</td>
</tr>
<tr>
<td>PG</td>
<td>ca. 15/85</td>
<td></td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>SL</td>
<td>ca. 21/79</td>
<td></td>
<td>19.1/80.9</td>
<td>14.2/85.8</td>
</tr>
<tr>
<td>MGDG</td>
<td>ca. 21/79</td>
<td></td>
<td>18.6/81.4</td>
<td>17.2/82.8</td>
</tr>
<tr>
<td>DGDG</td>
<td>ca. 30/70</td>
<td></td>
<td>32.2/67.8</td>
<td>28.3/71.7</td>
</tr>
</tbody>
</table>

* These data are based on an average occurrence of a C16 and a C18 fatty acid in each lipid.
The average occurrence of a C16 and C18 fatty acid in this lipid. At the time of maximum 14C incorporation (after 24 hours of illumination, see Table III) only 4.5% of the total radiocarbon of PC was found in the moieties (instead of theoretically 19%), and the rest in the fatty acids of PC. In PE the rate of radiocarbon uptake into the moieties was 5.9% at the time of maximum incorporation (24 hours) instead of the theoretical value of 13%. PG, SL, MGDG, and DGDG had the maximum of 14C incorporation after about 120 hours of illumination (see Table III). At this time the following rates of radiocarbon incorporation into the glycerol and sugar moieties were detected (Table IV): PG: 6.7% (theoretically 15%); SL: 7.5% (theoretically 21%); MGDG: 6.0% (theoretically 21%); DGDG: 9.2% (theoretically 30%).

The specific radioactivities were determined in the fatty acids of the individual lipids at the time of maximum 14C-incorporation, i.e. in the wax esters after 6 hours of incubation with 14C-octanoate, in PC and PE after 24 hours of illumination, and in PG, SL, MGDG, and DGDG after 120 hours of illumination (Table V). The specific radioactivities in the labeled fatty acids of PC were slightly lower than in the wax acids. The labeled fatty acids of PE had about half the specific radioactivities of the wax acids. After 120 hours of illumination, the labeled fatty acids in PG, SL, MGDG, and DGDG had an average specific radioactivity of about 8–10% of the specific radioactivity of the wax acids.

Oxidative degradation of the hydrogenated fatty acids was expected to yield one µmole of acetic acid from the methyl ends of one µmole of fatty acids employed. Table VI shows that the specific radioactivities of the acetic acid distillates were considerably lower than the average specific radioactivities of the hydrogenated fatty acids.

**Discussion**

When *Euglena gracilis* is grown heterotrophically in the dark with organic substances such as acetate as carbon source, the main lipids produced are wax esters. They are composed of saturated fatty acids and alcohols with 12–16 carbon atoms and probably serve as storage products in the dark. Upon illumination of the cells, the metabolism becomes adapted to photoautotrophic growth. This
process involves the metabolic degradation of the wax esters, the formation of chloroplasts from the proplastids present in the dark, and the production of chloroplast lipids (PG, SL, MGDG, DGDG), and of unsaturated C\textsubscript{16} and C\textsubscript{18} fatty acids in these lipids. The results obtained here are in full agreement with those of the preceding paper and more significant. After growth in the dark and incubation with radioactive octanoate, the wax esters were the main pool of radiocarbon in the cells because they contained 92\% of the radioactivities incorporated into the whole cells (Table II). Upon illumination, the wax esters were rapidly metabolized, as indicated by the decrease of both the labeled wax acids and alcohols. Simultaneously an increase of radiocarbon was observed in all of the other lipids. This radiocarbon could be derived either from the labeled wax esters or from the labeled cell material. The strong decrease of \(^{14}\)C in the neutral lipids and the increase in the labeled cell material (Fig. 1) indicate that the newly incorporated radiocarbon in the other lipids was mainly derived from the neutral lipids.

There are two possible explanations for this light-induced flow of \(^{14}\)C from the wax esters to the other lipids: A. Oxidative breakdown of the wax acids and alcohols (leading to the formation of a pool of radioactive acetyl-CoA), followed by de novo biosyntheses of the other lipids. B. Direct transfer of fatty acids from the wax esters to the other lipids. The wax alcohols might also be involved in such transfers after oxidation to the corresponding fatty acids.

If in our experiments there had been only breakdown of the wax esters followed by de novo biosyntheses of the other lipids, all of the fatty acids present in the other lipids should have been labeled according to their molecular portion in each individual lipid. This was not the case. In each lipid only certain fatty acids were labeled although all of the individual lipids contained a variety of other fatty acids (Figs. 3 – 10).

Furthermore, after oxidative breakdown followed
by de novo biosynthesis, the glycerol and sugar moieties should also have been labeled according to their molecular portion in each lipid. This, too, could not be confirmed. At the time of maximum $^{14}$C incorporation, all of the individual lipids contained considerably less radioactivities in their moieties than one should have expected theoretically (Table IV).

Thirdly there was a striking structural interrelationship of the involved labeled fatty acids with respect to their carbon chains and to their degree of unsaturation: The decrease of the 14:0 fatty acid in the neutral lipids was followed by the increase of the 14:0 and 16:0 fatty acids in PC, of 14:0, 16:0, and 18:1 in PE, of 16:0 in SL, of 16:0 and trans3-16:1 in PG, and of saturated and unsaturated C$_{16}$ and C$_{18}$ fatty acids in MGDG and DGDG.

The determinations of the specific radioactivities (Table V) showed that after 24 hours of illumination, the labeled fatty acids in PC (14:0 and 16:0) had approximately the same specific radioactivity as the wax acids. In PE, the specific radioactivities were about 55% lower than in the wax acids. In the fatty acids of the chloroplast lipids, the average specific radioactivity was about 90,000 dpm/µmole. As compared with the specific radioactivities of the wax esters, this was a decrease by a factor 1:12.

There is, however, one fact which has to be taken into consideration with regard to the specific radioactivities: After growth in the dark and incubation with $^{14}$C-octanoate, the cells had been transferred to a new medium containing 0.1% of unlabeled acetate, giving a practically constant concentration of 11,000 µmoles of unlabeled acetate in the nutrient solvent, this acetyl-CoA would have been diluted by an average factor of about 1:8000 during the first 24 hours of illumination. Hence, in comparison to the wax acids ($1.1 \cdot 10^6$ dpm/µmole), the specific radioactivities of the fatty acids in PC, PE, and in the chloroplast lipids should have been diminished by approximately the same factor to about 150 dpm/µmole. Our experiments showed that this was not the case.

Oxidative degradation of the hydrogenated fatty acids with $\text{H}_2\text{CrO}_4/\text{H}_2\text{SO}_4$ yielded about one µmole of acetic acid per µmole of fatty acid employed for oxidation. The low specific radioactivities of the resulting acetic acid distillates (Table VI) indicate that very little radiocarbon was incorporated into the last two C-atoms of the methyl ends of the fatty acids. Hence, the predominant pathway for the biosynthesis of these compounds did not seem to involve $^{14}$C-acetyl-CoA.

These observations (specific labeling of only certain fatty acids in each individual lipid, relatively high incorporation of $^{14}$C into the acyl groups, structural interrelationship and relatively high specific radioactivities of the involved fatty acids, distribution of label within the fatty acid molecules) suggest that part of the radioactivities of the wax esters was brought to the other lipids by means of a direct transfer of fatty acids. As indicated by the overall decrease of radiocarbon in the cells, both processes (oxidative breakdown as well as acyl transfers) seem to be induced by light in Euglena gracilis. In Scheme 1 a tentative model is proposed for the acyl transfers.

The lipids involved seem to function as parts of a "lipid-bridge" for the fatty acid transfers. According to the chronological order of radioactivity maxima in the individual lipids, this bridge seems to be established by the following sequence of lipids: 1. Neutral lipids (mainly wax esters), 2. Phospholipids (PC and PE), 3. Chloroplast lipids (PG, SL, MGDG, DGDG). One part of the lipid-bridge (neutral lipids, PC, PE) seemed to be already present in the dark. The second part (PG, SL, MGDG, DGDG) was built up upon illumination.

The data of this paper seem to indicate the following light-induced steps of fatty acid biosynthesis in Euglena gracilis: Upon illumination, the wax esters are hydrolyzed, yielding mainly myristate and C$_{14}$ alcohol. Part of the myristate seems to be trans-
ferred directly to PC and PE. Another part seems to be elongated to palmitate and then transferred to one of the other lipids. The rapid increase of the labeled 14:0 and 16:0 fatty acids in PC and PE (Figs. 5 and 6) within the first 24 hours of illumination suggests that PC and PE are the fatty acid acceptors next to the wax esters. This transfer step would require the presence of lyso-PC and lyso-PE to accept the fatty acids from the wax esters. The next step seems to be a transfer of palmitic acid to either SL or PG, or to both of them, involving trans-desaturation of this compound to the trans3-16:1 fatty acid in PG. In Chlorella vulgaris, the trans3-16:1 acid is synthesized from palmitic acid in the presence of light\textsuperscript{10}, but is also hydrogenated to give again palmitic acid\textsuperscript{11}. Both the 16:0 and trans3-16:1 are fatty acids of an intermediary type. In contrast to SL and PG, the galactolipids are characterized by the accumulation of labeled saturated and unsaturated C\textsubscript{16} and C\textsubscript{18} fatty acids. Some of these compounds seem to be end-products (16:4, 18:3). This suggests that MGDG and DGDG are the final lipids of the "lipid-bridge", and that PG and SL have intermediary functions. Presumably there are several "species" of MGDG and DGDG rendering possible the formation of the various unsaturated C\textsubscript{16} and C\textsubscript{18} fatty acids in these lipids. NICHOLS and co-workers\textsuperscript{12,13} separated MGDG from Chlorella vulgaris into 5 species which all differed in their fatty acid compositions. Similar investigations should be done with DGDG. From our experiments it could not be determined whether PC was labeled earlier than PE, PG earlier than SL, and MGDG earlier than DGDG. Furthermore, no indications were obtained as to whether or not the wax alcohols are involved in the transfers, too.
Bloch and co-workers\textsuperscript{14,15} have recently shown that dark-grown \textit{Euglena} cells contain a single fatty acid synthetase similar to that found in yeast\textsuperscript{16}. This synthetase produces mainly palmitate and shorter-chain acids. Upon illumination of the cells, a second fatty acid synthetase system is developed which is associated to the chloroplasts and which yields stearate and longer-chain fatty acids. These reports seem to indicate that \textit{Euglena} has two centres of fatty acid biosynthesis. In the dark, fatty acids are produced by the mitochondrial and microsomal system. In the light, a second centre is developed in the chloroplasts. It is unknown whether there is an exchange of fatty acids between these two centres.

PG, SL, MGDG, and DGDG are chloroplast lipids, containing mainly unsaturated fatty acids\textsuperscript{17–20}. Chloroplasts, however, contain very little PC and PE\textsuperscript{21}. These two phospholipids are typical lipids of plant\textsuperscript{22–25} and animal\textsuperscript{26–40} microsomes and mitochondria, containing mainly saturated fatty acids. In a light-grown \textit{Euglena}, the chloroplasts are surrounded by mitochondria\textsuperscript{41}. Hence, the pathways of fatty acid biosynthesis proposed in this and in the previous papers\textsuperscript{1,2} possibly proceed in different organelles of the cell, \textit{i.e.}, in the mitochondria, microsomes, and chloroplasts, involving acyl transfers between these organelles. There is now growing evidence that transfers of fatty acids and lipids occur between plant and animal mitochondria and microsomes. Mazliak and Abdelkader\textsuperscript{42} observed the \textit{in vitro} exchange of fatty acids between mitochondria and microsomes of potato tuber and cauliflower tissues. Shohet\textsuperscript{43} reported the transfer of fatty acids from PC to PE in human erythrocytes. Several authors have shown that even entire phospholipids are exchanged between plant\textsuperscript{44} and animal\textsuperscript{45–48} mitochondria and microsomes.

So far our experiments present evidence that light-induced transfers of fatty acids take place in \textit{Euglena gracilis} only as long as the wax esters built up in the dark are being metabolized and are still available in the cells during growth in light. At present, it is not known whether such transfers occur in \textit{Euglena} cells which have only been grown in light and which contain no or very few wax esters. Experiments in our laboratory are aimed at incorporating specifically labeled lipids (for example PC labeled in the myristic and palmitic acids) in order to determine the individual steps in the fatty acid transfers described here, and to find evidence for transfers of fatty acids from microsomes and mitochondria to the chloroplasts.

A striking result of our experiments is the finding that under the conditions employed here, the individual lipids have a high affinity for certain fatty acids. Furthermore, they appear to be specifically associated with biochemical conversions of these compounds, such as carbon chain elongation and desaturation. The wax esters, PC, and PE seem to be involved in transfers and in chain elongation of mostly saturated fatty acids with 14 to 16 carbon atoms, and PG and SL in the transfer and trans-desaturation (only PG) of palmitic acid. Possibly PE is also involved in the formation of monoenoic fatty acids as indicated by the labeling of oleic acid in this lipid. From the radio gas liquid chromatograms (Fig. 6) it could not be concluded unequivocally whether the 16 : 1 and 18 : 0 fatty acids in this lipid were labeled, too. MGDG and DGDG seem to be associated with the transferring, desaturating, and elongating enzyme systems producing the polyunsaturated C\textsubscript{16} and C\textsubscript{18} fatty acids of the chloroplasts.

These considerations bring up the question as to how the fatty acid transfers described here might really function. One can visualize such transfers only if one assumes the participation of enzymes transferring the acyl groups from one lipid to the other. As a consequence, lipids and proteins should be closely associated with one another in these systems. Such arrangements of lipids and proteins are found throughout the membranes of all living cells. Hence, the above observations might reveal a new function of such membranes as bases for acyl transfers.

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