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

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Euglena gracilis was grown in the dark for 12 days. Subsequent incubation with sodium octa-noate-1-^{14}C 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 C_{14}). The radioactivities in phosphatidyl choline and phosphatidyl ethanolamine increased during the first 24 hours of illumina-tion 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, mono-galactosyl 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 *trans*3-16:1 fatty acids of the phosphatidyl glycerol, and in the saturated and unsaturated C_{16} and C_{18} 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 con-cluded 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 chloro-plast 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 indi-vidual lipids of *Euglena gracilis* grown initially in the dark and subsequently in light. Due to the con-ditions used in those experiments, we could not obtain clear evidence for such transfers. The main reason was that the cells had been grown with radio-active 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" incuba-tion with the radioactive precursor.

Experiments with labeled acids of different chain lengths (sodium butyrate-1-^{14}C, sodium hexanoate-1-^{14}C and sodium octanoate-1-^{14}C) 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 pre-vious papers [1, 2], containing 0.05% NH_4Cl and 0.1%

sodium acetate, was used. The cells were grown in the dark in a 10 l 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 $1,500 \times g$. The supernatant was decanted. The cells were resuspended in 1050 ml of new sterile medium containing 350 μCi of sodium octanoate-1-^{14}C 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 $1,500 \times g$ for 10 min and resuspended in 6.3 l of new sterile medium without any radiocarbon. 900 ml of this cell suspension were immediately centrifuged at $10,000 \times g$ for 5 min. The cell pellet was quickly ground in a mortar in the dark and extracted several times with chloroform – methanol 1 : 2, v/v), until no more radioactivity was detected in the solvent.

The remaining cell suspension (5.4 l) 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 $10,000 \times g$ 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 weigth 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 ^{14}C 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: $180 \text{ cm} \times 2$ mm; temperature: programmed from 120 °C to 190 °C (2 °C/min).

Sodium octanoate-1-^{14}C 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 (PtO_2). 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 N H_2CrO_4, 10 ml H_2O, 5 ml H_2SO_4 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 radioactivies employed intially). 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 ^{14}C-octanoate (350 μci), a radioactivity of $70.51 \cdot 10^6$ 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 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-^{14}C in the dark (6 hours). B. During subsequent illumination.

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

Table II. Radioactivities in *Euglena gracilis* after growth in the dark (12 days) and incubation with sodium octanoate-1-^{14}C (350 μCi) in the dark (6 hours).

A. W h o l e C e l l s

Whole cells:	$70.51 \cdot 10^6$ dpm	= 100.0 %
a. Total lipids	$67.17 \cdot 10^6$ dpm	= 95.25%
b. Cell material (without lipids	$3.34 \cdot 10^6$ dpm	= 4.75%

B. T o t a l L i p i d s

Lipids	dpm	% of radio-activities in the total lipids	% of radio-activities in the whole cells
total lipids	$67.17 \cdot 10^6$	100.00	95.25
neutral lipids	$65.91 \cdot 10^6$	98.10	93.43
a. Wax esters	$64.90 \cdot 10^6$	96.60	92.00
b. Triglycerides	670,000	1.00	0.95
c. Carotenoids	336,000	0.50	0.48
PC	907,000	1.35	1.29
PE	275,000	0.41	0.39
PG	5,000	0.01	0.01
cardiolipin	6,500	0.01	0.01
SL	7,000	0.01	0.01
MGDG	62,000	0.09	0.09
DGDG	12,000	0.02	0.02

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 ^{14}C into the wax acids than into the alcohols.

As in the preceding paper, a drastic decrease of ^{14}C 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 ^{14}C 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 ^{14}C 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
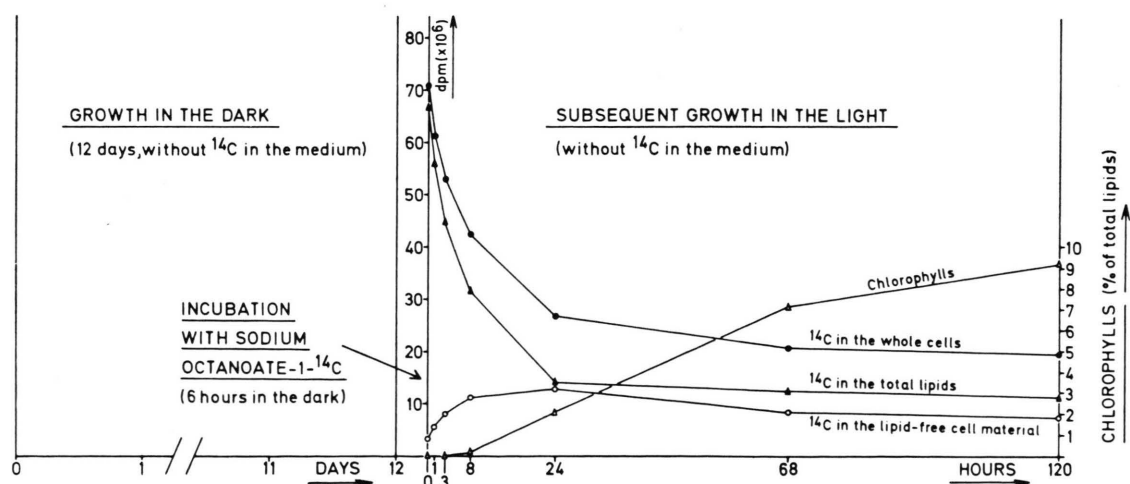

Fig. 1. Radioactivities in the whole cells, in the total lipids, and in the lipid-free cell material, and formation of chlorophylls in *Euglena gracilis* after growth in the dark (12 days) and incubation with sodium octanoate-1-^{14}C in the dark (6 hours).

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-^{14}C in the dark (6 hours). B. During subsequent illumination.

| Lipid	A (Dark)	B (Hours of illumination)					
		1	3	8	24	68	120
neutral lipids	7,350,000	7,000,000	6,020,000	4,220,000	475,000	145,000	130,000
PC	101,000	209,000	245,000	369,000	*788,000*	523,000	392,000
PE	31,100	50,000	86,200	116,300	*328,000*	150,000	137,000
PG	700	1,000	6,000	20,000	36,000	51,000	*78,500*
cardiolipin	800	2,000	2,300	5,000	18,000	32,000	28,300
SL	1,100	4,500	5,500	12,000	65,000	78,000	*84,000*
MGDG	6,500	15,000	28,400	43,500	215,000	293,000	*322,000*
DGDG	1,200	3,900	6,700	18,000	113,000	114,000	*116,500*
total	7,492,400	7,285,600	6,400,100	4,803,800	2,038,000	1,386,000	1,288,300
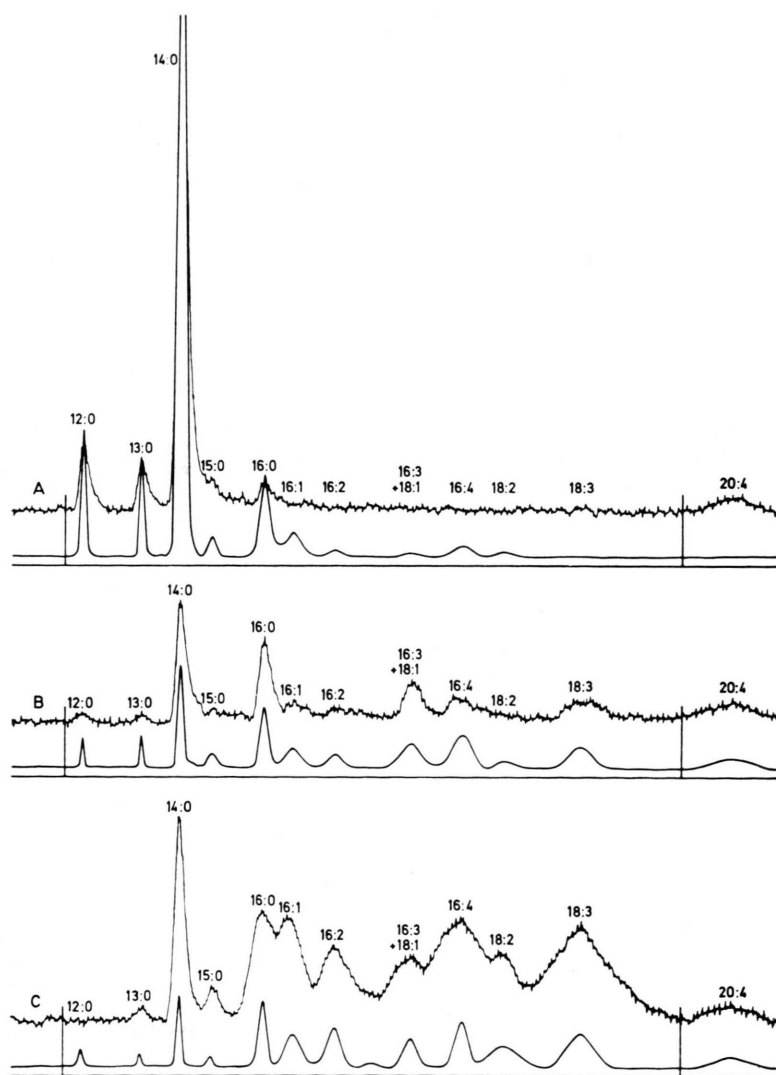
Fig. 2. *Euglena gracilis*; Radio-GLC of the total fatty acid methyl esters. A. After growth in the dark (12 days) and incubation with sodium octanoate-1-^{14}C in the dark (6 hours). B. After 24 hours of subsequent illumination. C. After 120 hours of subsequent illumination.

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-^{14}C in the dark (6 hours). B. After 24 hours of subsequent illumination. C. After 120 hours of subsequent illumination.

C_{14}

C_{16}

A

C_{14}

C_{16}

B

C_{14} C_{16}

C

14:0

12:0

13:0

15:0   16:0 16:1   18:0   18:1   18:2   18:3

A

14:0

12:0

13:0

16:0

15:0   16:1   18:0   18:1   18:2   18:3

B

C

12:0 13:0   14:0   15:0   16:0 16:1   18:0   18:1   18:2   18:3

D

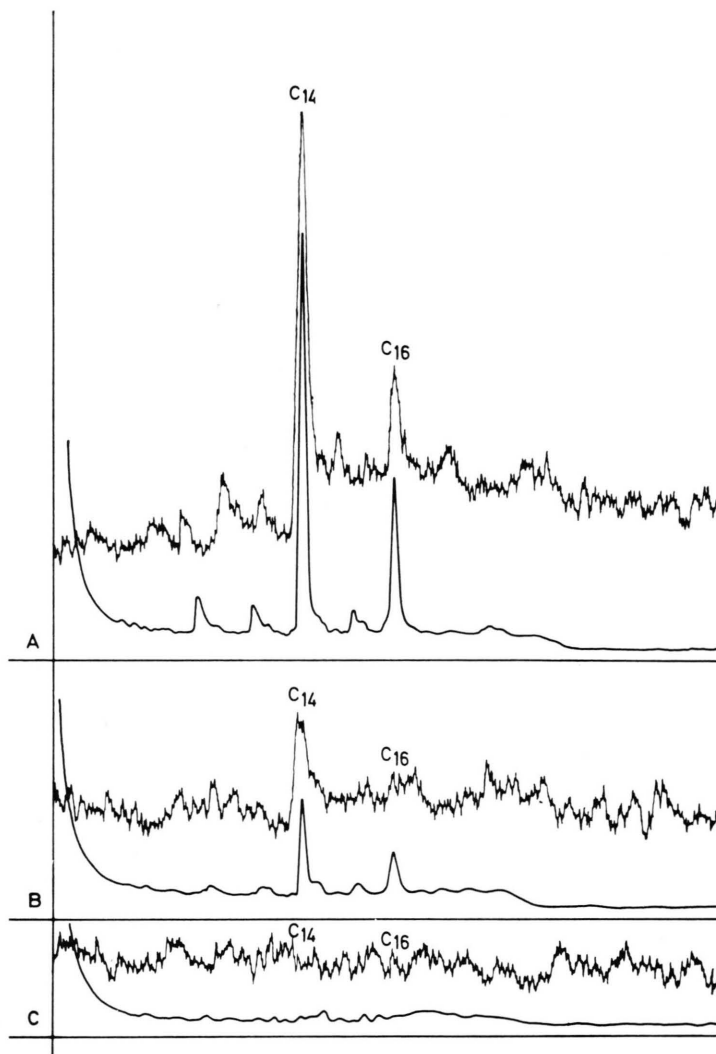12:0   13:0   14:0   15:0   16:0 16:1   18:0   18:1   18:2   18:3
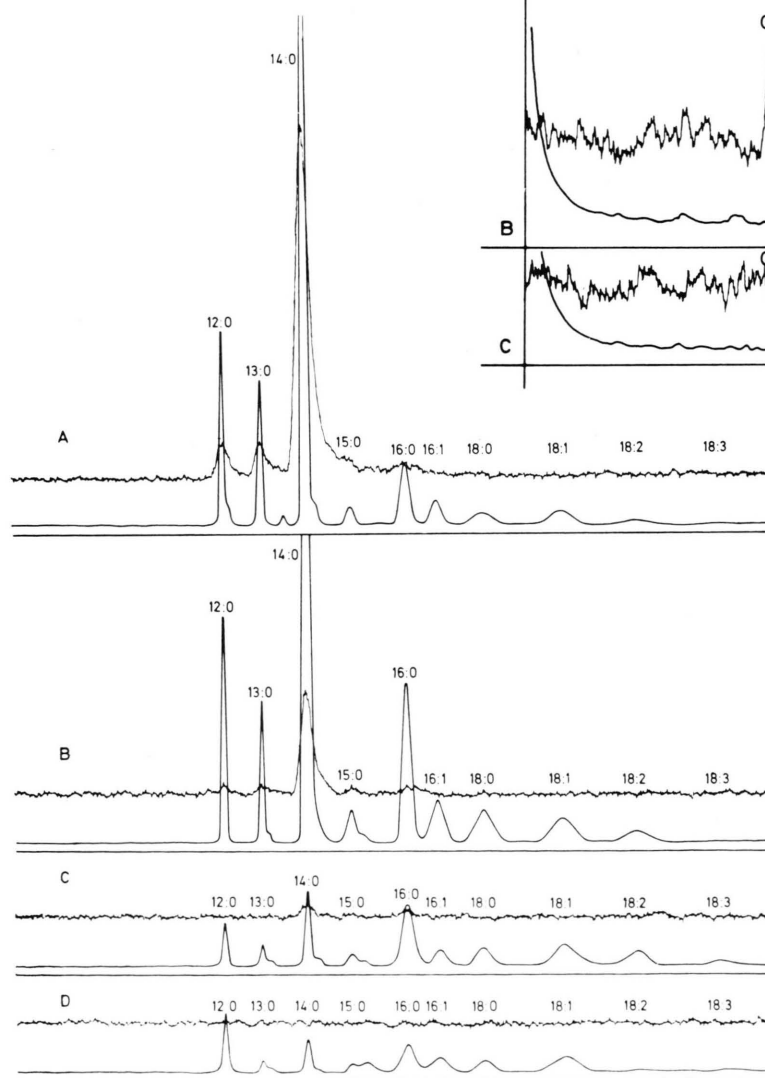
Fig. 3. *Euglena gracilis; wax esters:* Radio-GLC of aliquot amounts of the wax acids. A. After growth in the dark (12 days) and incubation with sodium octanoate-1-^{14}C in the dark (6 hours). B. After 3 hours of subsequent illumination. C. After 24 hours of subsequent illumination. D. After 120 hours of subsequent illumination.
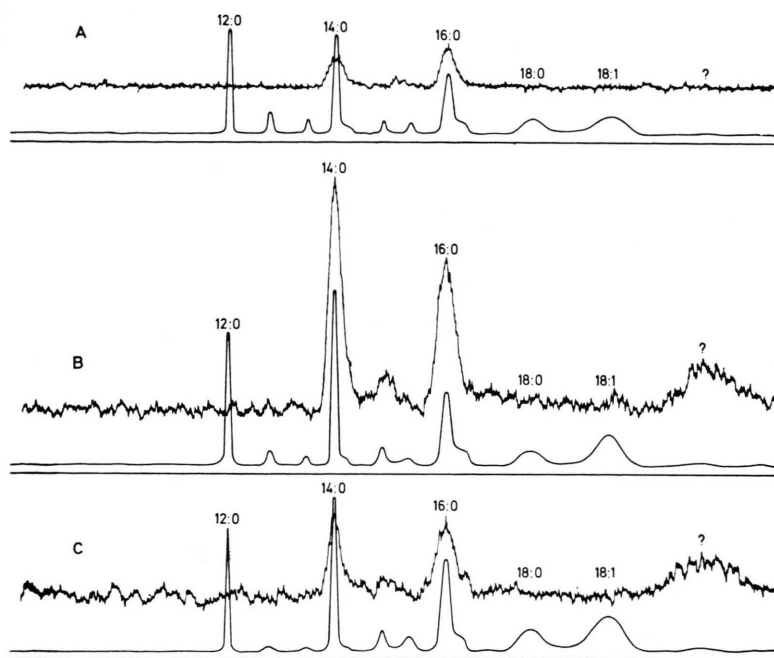
Fig. 5. *Euglena gracilis; phosphatidyl choline:* Radio-GLC of aliquot amounts of the fatty acid methyl esters. A. After growth in the dark (12 days) and incubation with sodium octanoate-1-^{14}C in the dark (6 hours). B. After 24 hours of subsequent illumination. C. After 120 hours of subsequent illumination.

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 C_{16} and C_{18} 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 C_{14} and to a lower extent the C_{16} 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
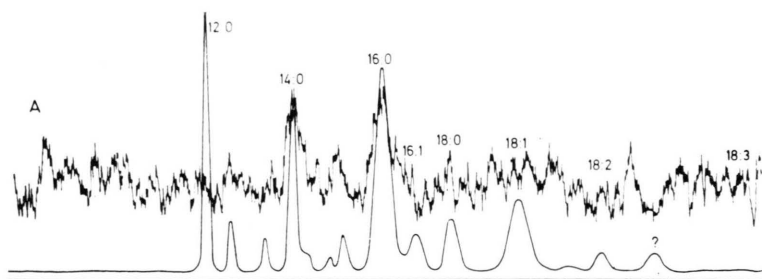
Fig. 6. *Euglena gracilis; phosphatidyl ethanolamine:* Radio-GLC of aliquot amounts of the fatty acid methyl esters. A. After growth in the dark (12 days) and incubation with sodium octanoate-1-^{14}C in the dark (6 hours). B. After 24 hours of subsequent illumination. C. After 120 hours of subsequent illumination.
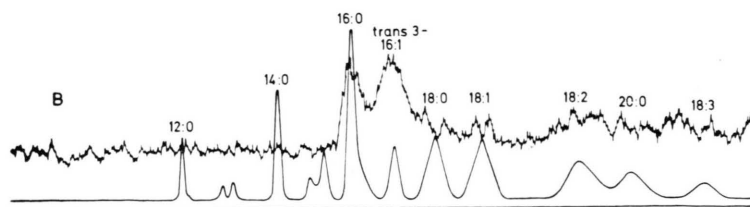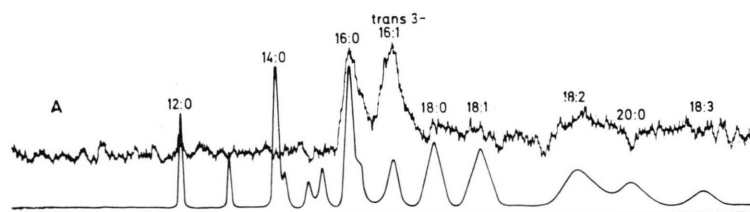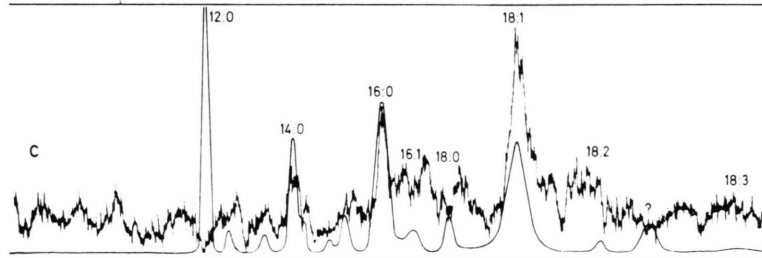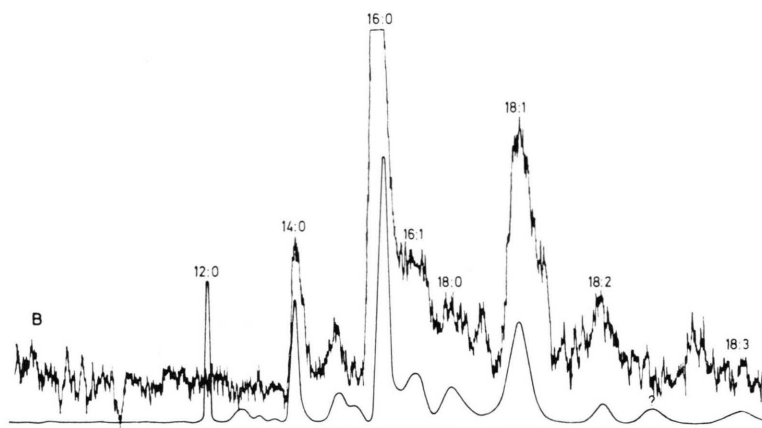

Fig. 7. *Euglena gracilis; phosphatidyl glycerol:* Radio-GLC of aliquot amounts of the fatty acid methyl esters. (Only trace amounts of PG could be detected by TLC after growth in the dark (12 days) and incubation with sodium octanoate-1-^{14}C in the dark (6 hours). A. After 24 hours of subsequent illumination. B. After 120 hours of subsequent illumination.

Fig. 8. *Euglena gracilis; sulfolipid:* 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-^{14}C in the dark (6 hours). B. After 24 hours of subsequent illumination. C. After 120 hours of subsequent illumination.
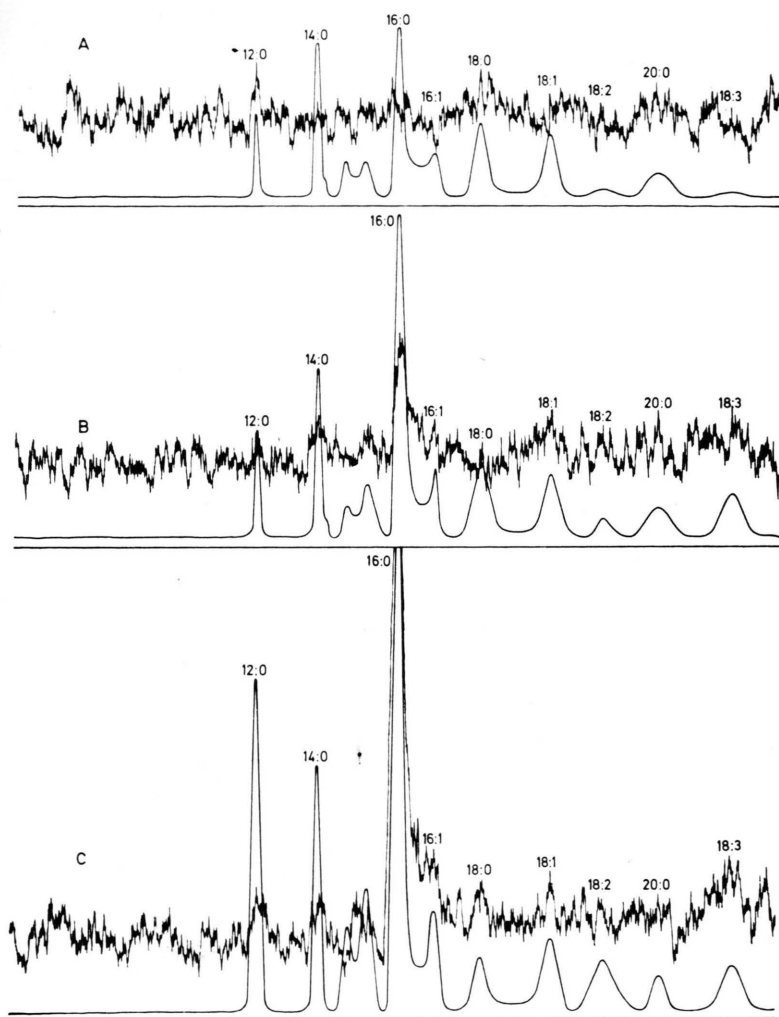
available we could not determine unequivocally which of these two compounds was labeled.

Fatty acids of PG (Fig. 7)

After growth in the dark and incubation with ^{14}C-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 *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
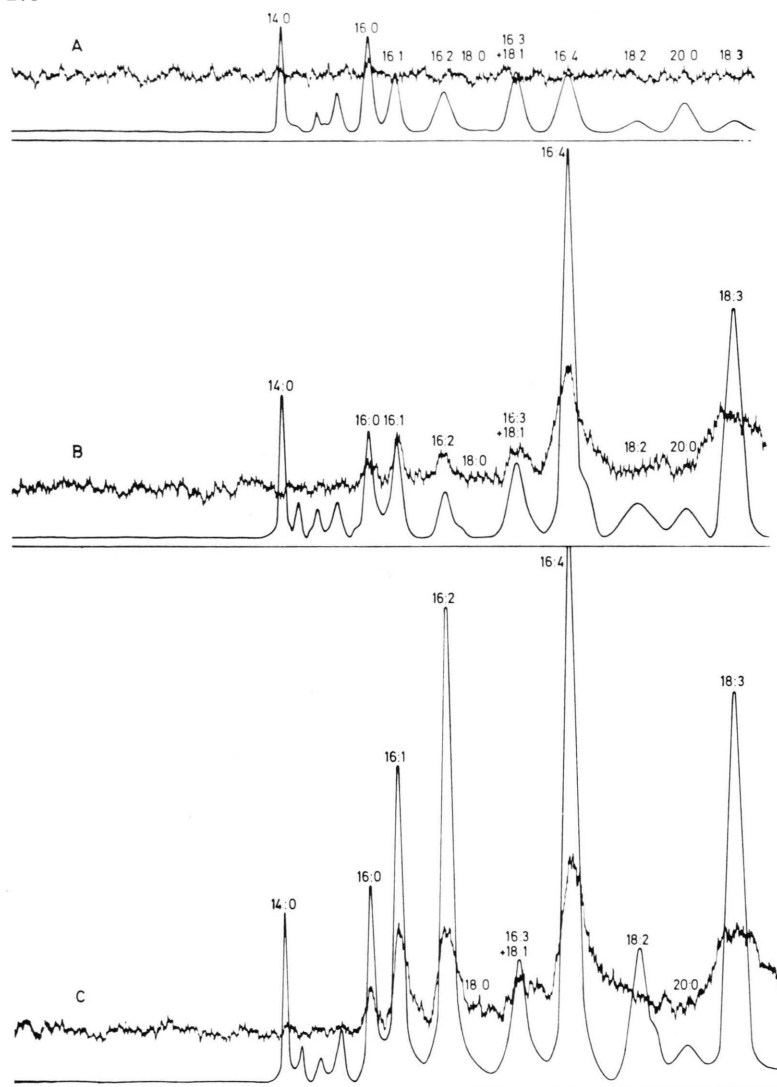
Fig. 9. *Euglena gracilis; monogalactosyl diglyceride:* Radio-GLC of aliquot amounts of the fatty acid methyl esters. A. After growth in the dark (12 days) and incubation with sodium octanoate-1-^{14}C in the dark (6 hours). B. After 24 hours of subsequent illumination. C. After 120 hours of subsequent 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 ^{14}C into the moieties and fatty acids after growth in the dark (12 days) and incubation with sodium octanoate-1-^{14}C in the dark (6 hours). B. Percentage incorporation of ^{14}C into the moieties and fatty acids during subsequent illumination.

Lipid	C-atoms in the moieties [%]	^{14}C in the moieties [%]/^{14}C in the fatty acids [%]						
	C-atoms in the fatty acids [%] (Theoretical percentage) *	A (Dark)	B (Hours of illumination)					
			1	3	8	24	68	120
PC	ca. 19/81	26.2/73.8	15.4/84.6	13.2/86.8	9.6/90.4	*4.5/95.5*	8.7/91.3	9.6/90.4
PE	ca. 13/87	10.2/89.8	9.3/90.7	8.5/91.5	7.8/92.2	*5.9/94.1*	10.9/89.1	12.1/87.9
PG	ca. 15/85	?	?	?	?	18.9/81.1	11.9/88.1	*6.7/93.3*
SL	ca. 21/79	19.1/80.9	14.2/85.8	10.0/90.0	9.1/90.9	7.0/93.0	7.2/92.8	*7.5/92.5*
MGDG	ca. 21/79	18.6/81.4	17.2/82.8	14.1/85.9	9.5/90.5	5.9/94.1	5.8/94.2	*6.0/94.0*
DGDG	ca.30/70	32.2/67.8	28.3/71.7	28.0/72.0	14.7/85.3	7.2/92.8	7.7/92.3	*9.2/90.8*

* These data are based on an average occurrence of a C_{16} and a C_{18} fatty acid in each lipid.

average occurrence of a C_{16} and C_{18} fatty acid in this lipid. At the time of maximum ^{14}C 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

Table V. Specific radioactivities in the fatty acids of the individual lipids at the time of maximum ^{14}C-incorporation. *Wax esters:* After growth in the dark (12 days) and incubation with sodium octanoate-1-^{14}C in the dark (6 hours). *PC and PE:* After 24 hours of subsequent illumination. *PG, SL, MGDG, and DGDG:* After 120 hours of subsequent illumination.

Lipid	Fatty acid	Hours of illumination	μMoles of fatty acids	Specific radio-activities [dpm/μmole]
wax esters	12 : 0	0	0.20	$1.1 \cdot 10^6$
	13 : 0		0.22	$1.1 \cdot 10^6$
	14 : 0		2.98	$1.1 \cdot 10^6$
PC	14 : 0	24	0.32	$1.04 \cdot 10^6$
	16 : 0		0.39	$1.03 \cdot 10^6$
PE	14 : 0	24	0.096	319.000
	16 : 0		0.31	493.000
	18 : 1		0.23	496.000
PG	16 : 0	120	0.59	62.000
	*trans*3-16 : 1		0.22	192.000
SL	16 : 0	120	0.79	93.000
MGDG	16 : 0	120	0.20	65.000
	16 : 1		0.36	114.000
	16 : 2		0.71	74.000
	16 : 3		0.23	97.000
	16 : 4		1.06	88.000
	18 : 2		0.31	61.000
	18 : 3		0.88	93.000
DGDG	16 : 0	120	0.21	105.000
	16 : 1		0.21	123.000
	16 : 2		0.29	90.000
	16 : 3		0.09	96.000
	16 : 4		0.09	60.000
	18 : 2		0.14	8.000
	18 : 3		0.14	127.000

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 hat the maximum of ^{14}C 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 ^{14}C-incorporation, *i. e.* in the wax esters after 6 hours of incubation with ^{14}C-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.

Table VI. Oxidative degradation of the hydrogenated fatty acids of the individual lipids of *Euglena gracilis*.

Lipid	Hours of illumination	Hydrogenated fatty acids average specific radioactivity [dpm/		Acetic acid (formed by oxidative degradation) specific radioactivity [dpm/	
		[μmoles]	μmole]	[μmoles]	μmole]
wax esters	0	ca. 2.9	940,000	3.3	13,000
PC	24	ca. 1.8	344,000	2.2	5,600
PE	24	ca. 1.4	183,000	1.7	2,850
PG	120	ca. 4.0	25,600	4.6	211
SL	120	ca. 3.9	31,200	4.6	304
MGDG	120	ca. 7.0	83,500	8.0	820
DGDG	120	ca. 3.4	70,500	3.9	650

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 [4-8] They are composed of saturated fatty acids and alcohols with $12 - 16$ carbon atoms and probably serve as storage products in the dark [7]. Upon illumination of the cells, the metabolism becomes adapted to photoauxotrophic growth. This
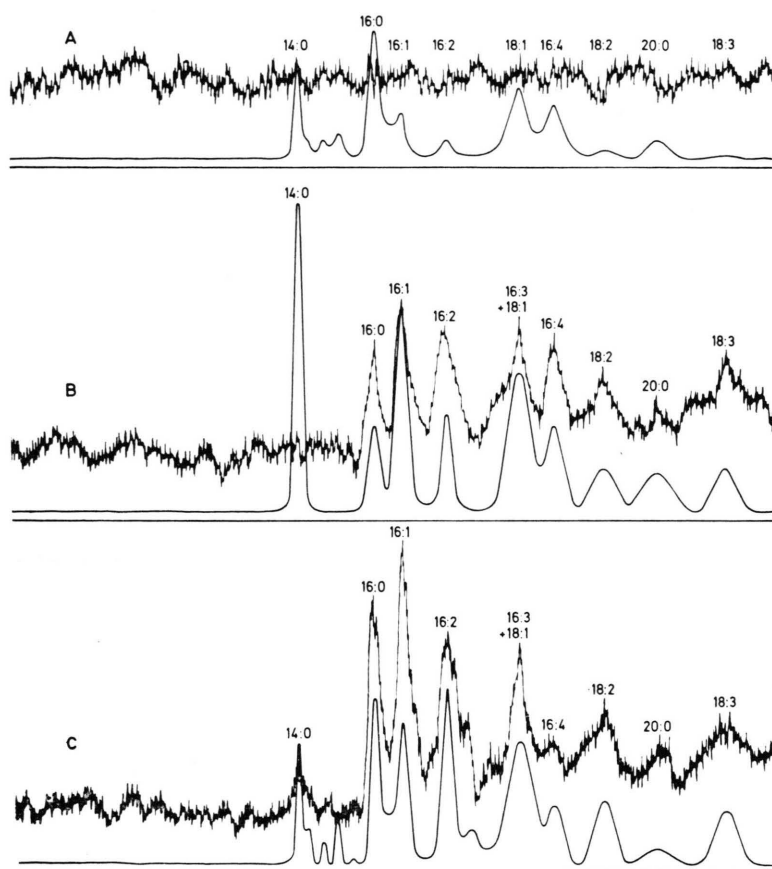
Fig. 10. *Euglena gracilis; digalacto-syl diglyceride:* Radio-GLC of aliquot amounts of the fatty acid methyl esters. A. After growth in the dark (12 days) and incubation with sodium octanoate-1-^{14}C in the dark (6 hours). B. After 24 hours of subsequent illumination. C. After 120 hours of subsequent illumination.

process involves the metabolic degradation of the wax esters, the formation of chloroplasts from the proplastids present in the dark [9], and the production of chloroplast lipids (PG, SL, MGDG, DGDG) [2, 9], and of unsaturated C_{16} and C_{18} fatty acids in these lipids [2]. The results obtained here are in full agreement with those of the preceding paper [1] 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 radioactivies in their moieties than one should have expected theoretically (Table IV).

Thirdly there was a striking structural inter-relationship 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 radio-activity as the wax acids. In PE, the specific radio-activities 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 radio-activities: 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 each sample (900 ml). At that time, each sample contained about 68.6 mg of total lipids (see Table I) of which an estimated 66 mg (ca. 155 μmoles) were wax esters, according to the distribution of label among the total lipids (see Table II).

During the first 24 hours of illumination, the radioactivities in the wax esters decreased about 94% (Table III). In case of oxidative breakdown, this would have led to a total formation of ap-proximately 2,000 μmoles of ^{14}C-acetyl-CoA in each sample (900 ml), or 1.4 μmoles per min, respec-tively, assuming that the newly formed acetyl-CoA was again used up in the cell metabolism within one minute. Considering the 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 H_2CrO_4/H_2SO_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 spe-cific 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. Accord-ing 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. Phospho-lipids* (PC and PE), *3. Chloroplast lipids* (PG, SL, MGDG, DGDG). One part of the lipid-bridge (neu-tral 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 follow-ing light-induced steps of fatty acid biosynthesis in *Euglena gracilis*: Upon illumination, the wax esters are hydrolized, yielding mainly myristate and C_{14} alcohol. Part of the myristate seems to be trans-
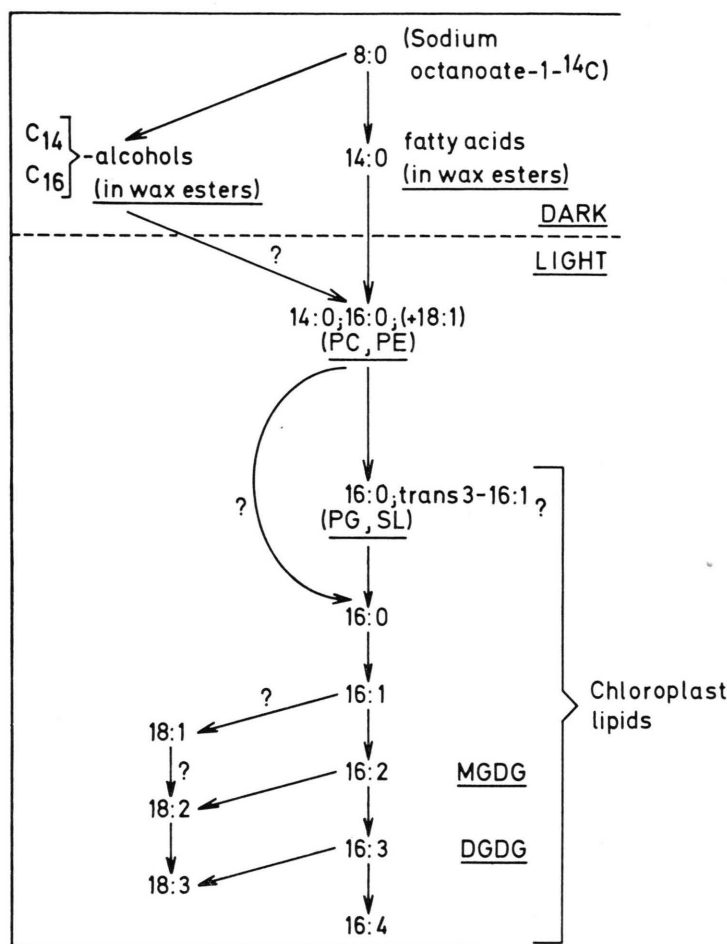
Scheme 1. Model of light-induced transfers of fatty acids via a "lipid-bridge" in *Euglena gracilis*.

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 *trans*3-16 : 1 fatty acid in PG. In *Chlorella vulgaris*, the *trans*3-16 : 1 acid is synthesized from palmitic acid in the presence of light[10], but is also hydrogenated to give again palmitic acid[11]. Both the 16 : 0 and *trans*3-16:1 are fatty acids of an intermediary type. In contrast to SL and PG, the galactolipids are charac-

terized by the accumulation of labeled saturated and unsaturated C_{16} and C_{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_{16} and C_{18} fatty acids in these lipids. NICHOLS and co-workers [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[14, 15] have recently shown that dark-grown *Euglena* cells contain a single fatty acid synthetase similar to that found in yeast[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 *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[17−20]. Chloroplasts, however, contain very little PC and PE[21]. These two phospholipids are typical lipids of plant[22−25] and animal[26−40] microsomes and mitochondria, containing mainly saturated fatty acids. In a light-grown *Euglena*, the chloroplasts are surrounded by mitochondria[41]. Hence, the pathways of fatty acid biosynthesis proposed in this and in the previous papers[1, 2] possibly proceed in different organells of the cell, *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[42] observed the *in vitro* exchange of fatty acids between mitochondria and microsomes of potato tuber and cauliflower tissues. Shohet[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[44] and animal[40, 45−48] mitochondria and microsomes.

So far our experiments present evidence that light-induced transfers of fatty acids take place in *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 *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 transdesaturation (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_{16} and C_{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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