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THE DISTRIBUTION AND BIOSYNTHESIS OF ARACHIDONIC ACID IN ALGAE

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Abstract—*Ochromonas danica* and *Porphyridium cruentum* differ from *Euglena gracilis* in synthesizing arachidonic acid by the pathway involving γ-linolenic acid. In *Monodus subterraneus* and *P. cruentum* the site of synthesis of arachidonic acid may be the chloroplast, but *O. danica* resembles *E. gracilis* in accumulating little of this acid in the photosynthetic apparatus.

TWO PATHWAYS leading to the synthesis of arachidonic acid (5,8,11,14-eicosatetraenoic acid) have been described. In a soil amoeba,[1] rat liver[2] and the alga *Euglena gracilis*,[3] linoleic acid is chain lengthened to the corresponding C_{20} acid which is then progressively desaturated to the tetraenoic acid:

$$18{:}2\ [9,12] \rightarrow 20{:}2\ [11,14] \rightarrow 20{:}3\ [8,11,14] \rightarrow 20{:}4\ [5,8,11,14].$$

In mammalian tissue, arachidonic acid is more commonly synthesized by a pathway involving γ-linolenic acid (6,9,12-octadecatrienoic acid),[4] according to scheme:

$$18{:}2\ [9,12] \rightarrow 18{:}3\ [6,9,12] \rightarrow 20{:}3\ [8,11,14] \rightarrow 20{:}4\ [5,8,11,14].$$

In addition to its presence in *E. gracilis*, arachidonic acid has been identified in several other classes of algae, notably in diatoms[5] and some members of the Chrysophyceae,[6, 7] Xanthophyceae[8] and Chlorophyceae.[9] However, the biosynthetic pathway leading to the formation of this acid in the last three classes of organism has not been established.

Moreover, the arachidonic acid in *E. gracilis* appears to be mainly a product of the metabolism associated with its heterotrophic growth, rather than the photosynthetic metabolism operative during photoautotrophic growth.[3] This would account for the absence of this acid from the galactosyl diglyceride fractions of *E. gracilis*[10] since these lipids predominate in the chloroplast lamellae, which would not be expected to accumulate fatty acids arising from heterotrophic metabolism. On the other hand, Nichols[11] has demonstrated that the arachidonic acid of mosses concentrates largely in the chloroplast lipids, and Wolf and co-workers

[1] E. D. KORN, *J. Biol. Chem.* **239**, 396 (1964).
[2] W. STOFFEL, *Z. Physiol. Chem.* **335**, 71 (1963).
[3] D. HULANICKA, J. ERWIN and K. BLOCH, *J. Biol. Chem.* **239**, 2778 (1964).
[4] J. F. MEAD and D. HOWTON, *J. Biol. Chem.* **229**, 575 (1957).
[5] M. KATES and B. E. VOLCANI, *Biochim. Biophys. Acta* **116**, 264 (1966).
[6] T. H. HAINES, S. AARONSON, J. L. GELLERMAN and H. SCHLENK, *Nature* **194**, 1282 (1962).
[7] J. ERWIN, D. HULANICKA and K. BLOCH, *Comp. Biochem. Physiol* **12**, 191 (1964).
[8] R. SHAW, *Advan. Lipid Res.* **4**, 107 (1966).
[9] E. KLENK, W. KNIPPRATH, D. EBERHAGEN and H. P. KOOF, *Z. Physiol. Chem.* **334**, 44 (1963).
[10] A. ROSENBERG and J. GOUAUX, *J. Lipid Res.* **8**, 80 (1967).
[11] B. W. NICHOLS, *Phytochem.* **4**, 769 (1965).

subsequently confirmed the presence of this acid in the chloroplasts of a variety of mosses.[12] Consequently, the sites of synthesis of arachidonic acid in *E. gracilis* and the mosses would seem to differ, and it is possible that the biosynthetic pathways involved are also dissimilar.

Even less information is available regarding the sites and biosynthetic pathways involved in the formation of this acid in marine algae and members of the Chrysophyceae.

This paper presents the results of studies concerning the biosynthesis of C_{20} polyenoic acids in algae, which have comprised a comparison of the distribution of these and related acids in members of the Euglinaceae (*E. gracilis*), the Chrysophyceae (*Ochromonas danica*), the Rhodophyceae (*Porphyridium cruentum*) and the Xanthophyceae (*Monodus subterraneus*), and the metabolism of appropriate substrates by the three former organisms.

RESULTS

Acyl Lipids

Extracts from *Euglena gracilis*, *Monodus subterraneus*, *Porphyridium cruentum*, *Ochromonas danica* and *O. malhamensis* all contained triglyceride, mono- and di-galactosyl diglyceride, sulphoquinovosyl diglyceride, phosphatidyl glycerol, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol and cardiolipin. The relatively small proportions of the last three classes of phospholipid in extracts of *P. cruentum* did not permit an accurate determination of their fatty acid content.

The extracts from *O. danica* also contained three classes of lipid which we have not previously detected in photosynthetic organisms. One of these ("lipid A") was eluted from DEAE cellulose (acetate form) by chloroform at about the same rate as chlorophyll, that is to say after triglycerides and sterols, but before monogalactosyl diglyceride. During TLC on silica gel using chloroform–methanol–acetic acid–water (170:30:20:7 v/v), this lipid (R_f 0·85) migrated a little more slowly than monogalactosyl diglyceride (R_f 0·9). A second unidentified component ("lipid B") was eluted very slowly from DEAE cellulose acetate by 1 per cent methanol in chloroform, but rapidly with 5 per cent methanol in chloroform, i.e. it was eluted between monogalactosyl diglyceride and lecithin. Lipid B (R_f 0·75) migrated between monogalactosyl diglyceride and phosphatidyl ethanolamine (R_f 0·6) when examined by TLC as described above. Lipid C was eluted from DEAE cellulose acetate columns with the acidic lipid fraction using chloroform–methanol (2:1 v/v) saturated with ammonia, and had an R_f of 0·8 during TLC. A fourth unidentified component ("lipid D") was detected by two-dimensional TLC of the lipids extracted from *O. danica* but did not appear to be recovered from the DEAE cellulose.

Similar lipid classes were detected in *O. malhamensis*, but were not isolated.

Fatty Acids

Fatty acid analyses of the unfractionated lipid extracts from the algal preparations are included in Tables 1–4. Those for *E. gracilis* and *M. subterraneus* are in general agreement with data obtained by other workers[8, 14] for these algae. Only partial fatty acid analyses have previously been published for *O. danica*,[6] *O. malhamensis*[7] and *P. cruentum*[7] although these data are mainly in accordance with those we report here, except for the evidence relating to the C_{20} polyenoic acids of *P. cruentum*. Erwin and co-workers[7] did not detect arachidonic acid in this alga although 33 per cent of the fatty acids in their extracts were uncharacterized

[12] F. T. WOLF, J. G. CONIGLIO and R. B. BRIDGES, in *Biochemistry of Chloroplasts* (edited by T. W. GOODWIN), Vol. 1, p. 187, Academic Press, London (1966).

TABLE 1. PERCENTAGE FATTY ACID COMPOSITION OF THE LIPIDS OF *E. gracilis* GROWN HETEROTROPHICALLY IN THE LIGHT ON A SYNTHETIC MEDIUM

	Fatty acid																				
	14:0	?	?	16:0	9+7-16:1	3-16:1	16:2	17:0	16:3	16:4	18:0	18:1	18:2	9,12,15-18:3	20:2	20:2	20:3	20:4	20:5	22:5	22:6
Total unfractionated lipid	5	2	2	14	3	2	8	1	5	t	6	5	11	15	t	5	t	8	9	t	t
Monogalactosyl diglyceride	1		3	8	3	—	1	2	10	14	—	1	14	27	—	—	—	—	—	—	—
Digalactosyl diglyceride	—	—	—	11	4	—	20	1	15		4	4	23	20	—	—	—	—	—	—	—
Sulphoquinovosyl diglyceride	2	1	—	47	6	—	1	—	—	—	3	5	18	16	—	—	—	—	—	—	—
Phosphatidyl glycerol	2	1	—	—	16	26	1	—	—	—	3	5	31	11	5	—	—	—	—	—	—
Phosphatidyl choline	—	—	—	10	1	—	—	—	—	—	1	4	1	1	—	10	3	25	32	7	3
Phosphatidyl ethanolamine	—	—	—	13	9	—	4	4	3	—	4	28	9	6	—	4	—	11	6	—	—
Phosphatidyl inositol	—	—	—	41	4	—	2	—	—	—	7	11	4	3	—	15	—	14	—	—	—
Phosphatidyl serine	—	—	—	31	8	—	4	3	—	—	6	19	8	2	—	3	3	8	5	—	—
Cardiolipin	2	2	—	8	3	—	—	—	—	—	3	4	1	t	—	—	—	2	45	6	26
Triglyceride	—	3	10	22	3	—	5	2	3	—	5	6	4	8	—	3	—	10	9	—	—

TABLE 2. PERCENTAGE FATTY ACID COMPOSITION OF THE LIPIDS OF *O. danica*, GROWN HETEROTROPHICALLY IN THE LIGHT

	Fatty acid													
	14:0	16:0	9+7-16:1	18:0	18:1	18:2	6,9,12-18:3	9,12,15-18:3	18:4	20:0	20:3	20:4	22:4	22:5
Total lipid, unfractionated	13	12	1	2	14	20	14	4	4	t	5	6	1	4
Monogalactosyl diglyceride	5	2	3	t	7	31	25	9	14	—	1	1	—	—
Digalactosyl diglyceride	18	11	5	t	4	27	10	15	8	—	1	1	—	—
Sulphoquinovosyl diglyceride	4	12	2	2	16	30	15	7	6	—	1	1	—	—
Phosphatidyl glycerol	1	29	2	2	13	27	17	3	2	—	1	9	—	—
Phosphatidyl choline	8	9	t	2	15	25	16	2	4	—	5	13	t	4
Phosphatidyl ethanolamine	2	2	t	t	2	18	20	5	5	—	t	45	—	—
Phosphatidyl inositol	3	10	2	3	14	24	10	2	2	—	8	9	t	10
Cardiolipin	3	12	4	4	6	11	12	3	4	—	14	25	—	—
Lipid A	24	12	—	—	29	17	9	—	—	—	11	—	—	—
Lipid B	3	2	t	2	16	11	6	t	1	t	19	7	8	25
Lipid C	1	5	2	2	5	6	8	1	1	t	11	22	3	32
Triglyceride	21	26	1	4	15	16	15	t	2	1	t	t	t	t
Total lipid, *O. malhamensis*	13	12	1	3	9	26	4	5	1	t	2	7	t	16

TABLE 3. PERCENTAGE FATTY ACID COMPOSITION OF THE LIPIDS OF *P. cruentum*

	Fatty acid														
	14:0	?	16:0	9+7-16:1	3-16:1	16:2	18:0	18:1	18:2	9,12,15-18:3	6,9,12-18:3	20:2	20:3	20:4	20:5
Total lipid, unfractionated	1	—	23	2	t	—	2	3	16	—	t	t	2	36	17
Monogalactosyl diglyceride	1	1	24	1	—	—	1	2	4	—	—	—	—	26	40
Digalactosyl diglyceride	1	—	38	1	—	—	2	3	5	—	—	—	—	18	34
Sulphoquinovosyl diglyceride	—	—	49	1	1	—	2	6	3	—	—	—	—	16	23
Phosphatidyl glycerol	—	—	27	—	32	1	2	4	1	—	1	—	5	14	17
Phosphatidyl choline	1	t	22	1	—	—	1	3	4	—	1	—	6	59	10
Triglyceride	3	1	26	3	—	—	2	4	14	1	1	t	5	26	14

TABLE 4. PERCENTAGE FATTY ACID COMPOSITION OF THE LIPIDS OF *M. subterraneous*

	Fatty acid												
	14:0	16:0	9+7-16:1	3-16:1	18:0	18:1	18:2	6,9,12-18:3	9,12,15-18:3	20:0	20:3	20:4	20:5
Total lipid, unfractionated	2	24	24	t	1	9	4	t	t	t	1	5	29
Monogalactosyl diglyceride	2	12	16	—	t	2	2	t	t	t	t	6	62
Digalactosyl diglyceride	1	25	39	—	t	7	3	—	t	—	t	2	24
Sulphoquinovosyl diglyceride	3	49	42	—	t	6	t	—	—	—	—	—	—
Phosphatidyl glycerol	1	40	8	8	t	4	—	—	—	—	—	t	39
Phosphatidyl choline	2	28	15	—	3	18	16	2	—	—	—	5	11
Phosphatidyl ethanolamine	1	21	10	—	2	8	2	—	—	—	t	16	40
Phosphatidyl inositol	3	44	30	—	2	19	3	—	—	—	t	t	t
Triglyceride	3	29	28	—	3	21	t	—	—	—	—	3	12

C_{20} unsaturated fatty acids. Extracts from our cultures of *P. cruentum* contained 36 per cent of an acid, the methyl ester of which co-chromatographed with authentic arachidonic during GLC when either diethylene glycol adipate or silicone SE-30 were used as the stationary phase. Oxidation of the acid from the red alga yielded a mixture of monobasic and dibasic acids which was qualitatively similar to that obtained with authentic arachidonic acid under comparable conditions.

The fatty acid composition of *P. cruentum* as detailed in Table 3 is similar to data obtained by Klenk and co-workers for other red algae.[9]

TABLE 5. ^{14}C ACTIVITY IN THE COMPONENT FATTY ACIDS OF *E. gracilis* AFTER INCUBATION OF THE ALGA WITH ^{14}C-LABELLED SUBSTRATES. THE FIGURES INDICATE THE ACTIVITY IN INDIVIDUAL ACIDS EXPRESSED AS A PERCENTAGE OF THE TOTAL ADDED ACTIVITY

	^{14}C-labelled substrate							
	Oleate		Linoleate		γ-Linolenate		11,14-Eicosadienoate	
Acid	9 hr	23 hr	9 hr	23 hr	9 hr	23 hr	9 hr	23 hr
Total C_{14} acids	—	—	—	—	—	—	—	—
Total C_{16} acids	—	—	—	—	—	—	—	—
18:0	—	—	—	—	—	—	—	—
18:1	68·8	48·1	—	—	—	—	—	—
18:2	6·1	8·1	42·1	17·8	—	—	—	—
γ-18:3	—	—	—	—	100·0	100·0	—	—
α-18:3	—	3·7	2·6	6·7	—	—	—	—
20:2	11·1	13·3	28·7	14·4	—	—	—	73·0
20:3	5·5	4·6	11·4	11·2	—	—	—	15·0
20:4	8·5	22·3	15·2	49·8	—	—	—	12·0
Other C_{20} and C_{22} acids	—	—	—	—	—	—	—	—

TABLE 6. ^{14}C ACTIVITY IN THE COMPONENT FATTY ACIDS OF *O. danica* AFTER INCUBATION OF THE ALGA WITH ^{14}C-LABELLED SUBSTRATES. THE FIGURES INDICATE THE ACTIVITY IN INDIVIDUAL ACIDS EXPRESSED AS A PERCENTAGE OF THE TOTAL ADDED ACTIVITY

	^{14}C-labelled substrate							
	Oleate		Linoleate		γ-Linolenate		11,14-Eicosadienoate	
Acid	9 hr	24 hr	9 hr	24 hr	9 hr	24 hr	9 hr	24 hr
Total C_{14} acids	—	—	—	—	—	—		
Total C_{16} acids	—	—	—	—	—	—		
18:0	—	—	—	—	—	—		
18:1	16·2	10·6	—	—	—	—		
18:2	18·7	12·1	47·7	35·0	—	—	11·5	13·5
γ-18:3	21·2	13·2	19·0	15·4	70·7	52·6		
α-18:3	6·0	9·3	9·9	17·0	—	—		
18:4	7·1	10·5	4·5	9·5	11·8	17·0		
20:2	—	—	—	—	—	—	88·5	86·5
20:3	15·6	13·3	9·7	10·6	9·1	15·6	—	—
20:4	15·2	16·2	9·2	12·6	8·4	14·8	—	—
Other $C_{20}+C_{22}$ polyenoic acids	—	14·7	—	—	—	—	—	—

TABLE 7. ^{14}C ACTIVITY IN THE COMPONENT FATTY ACIDS OF *P. cruentum* AFTER INCUBATION OF THE ALGA WITH ^{14}C-LABELLED SUBSTRATES. THE FIGURES INDICATE THE ACTIVITY IN INDIVIDUAL ACIDS EXPRESSED AS A PERCENTAGE OF THE TOTAL ADDED ACTIVITY

	^{14}C-labelled substrate							
	Oleate		Linoleate		γ-Linolenate		11,14-Eicosadienoate	
Acid	9 hr	23 hr	9 hr	23 hr	9 hr	23 hr	9 hr	23 hr
Total C_{14} acids	—	—	9·3	14·7	—	—	—	
Total C_{16} acids	—	—			—	—	—	
18:0	—	—	—	—	—	—	—	
18:1	16·2	17·9	—	—	—	—	—	6·5
18:2	49·1	43·9	61·1	60·6	—	—	—	
γ-18:3	17·4	16·2	15·5	9·3	100	42·2	—	
α-18:3	—	—	—	—	—	—	—	
20:3	—	—	—	—	—	—	100	93·5
20:3	—	—	—	—	—	7·1	—	—
20:4	17·3	22·0	14·2	15·1	—	50·7	—	—
20:5	—	—	—	—	—	—	—	—

The distribution of the individual fatty acids among the component lipids of the four algae are presented in Tables 1–4. The results from the two cultures of *E. gracilis* were sufficiently similar that for the sake of brevity only one series of results is presented here. Data derived from the incubation of the algae with various ^{14}C-labelled substrates are presented in Tables 5–7.

DISCUSSION

Whether synthesis of the C_{20} and C_{22} tetra- and penta-enoic acids occurs in the chloroplasts or elsewhere within the algal cells was impossible to establish directly because of difficulties experienced in isolating undamaged chloroplasts free from other cell debris.

In the circumstances, we preferred to isolate those lipids which are known to be mainly concentrated in the plastids, namely the galactosyl diglycerides, phosphatidyl glycerol and sulphoquinovosyl diglyceride.[13] The absence of a particular acid from all four lipids might then be regarded as indicative that the fatty acid is not present in the chloroplast in significant proportions.

The data for *Euglena gracilis* presented here, and the partial data reported by Rosenberg and co-workers, are illustrative of this principle. Arachidonic acid is quantitatively a major fatty acid when this organism is grown heterotrophically, but is less abundant when it is cultured photo-autotrophically.[3, 14] Consequently, the acid is regarded as being a product of the animal-like metabolism which predominates in dark-grown *E. gracilis*[3] and therefore should predominate in the non-chloroplast lipids. The results presented in Table 1 show this to be the case. In agreement with the partial data of three other groups of workers,[10, 15, 16] we found no C_{20} or C_{22} polyenoic acids in either of the galactosyl diglycerides. They were also

[13] B. W. NICHOLS, *Biochim. Biophys. Acta* **70**, 417 (1963).
[14] A. ROSENBERG, M. PECKER and E. MOSCHIDES, *Biochemistry* **4**, 680 (1965).
[15] G. CONSTANTOPOULOS and K. BLOCH, *J. Biol. Chem.* **242**, 3538 (1967).
[16] L. L. M. VAN DEENEN and F. HAVERKATE, in *Biochemistry of Chloroplasts* (edited by T. W. GOODWIN), Vol. 1, p. 117, Academic Press, London (1966).

absent from the two other chloroplast lipids. On the other hand, these acids were major components of several of the other cellular lipids. The absence of γ-linolenic acid from all our lipid fractions from *E. gracilis* is also in agreement with previously published observations,[3, 17] although Hulanicka and co-workers were unable to establish whether this acid can be an intermediate in the biosynthesis of arachidonic acid in this organism.[3]

The other heterotroph studied, *Ochromonas danica*, showed distinct differences from *E. gracilis* in both acyl lipid and fatty acid composition. The former organism contained major proportions of two novel lipids (lipid A and lipid B) which were absent from our cultures of *E. gracilis*, nor were they present in any other class of algae examined. The structures of these two lipids are currently under investigation, and the results will be published separately.

Qualitatively, the major differences between the fatty acids of *O. danica* and *E. gracilis* are the presence of major quantities of 6,9,12,15-octadecatetraenoic acid and γ-linolenic acid in *O. danica*, and the absence from this organism of hexadecatetraenoic acid (Table 2).

A study of the fatty acid composition of the individual lipid classes from *O. danica* showed only very small relative quantities of C_{20} tetra- and penta-enoic acids in the four chloroplast lipids, suggesting that, as in *E. gracilis*, synthesis of these acids occurs mainly outside the chloroplast. The small proportion of these acids present in the chloroplast lipids may result either from a very limited synthesis of C_{20} polyenoic acids by the photosynthetic apparatus, or alternatively may be attributable to the small proportions of these lipids which may be present in organelles other than plastids, such as mitochondria. The fatty acid compositions of the chloroplast lipids from *O. danica* are very different from those of the equivalent lipid fractions from *Euglena* grown under comparable conditions, particularly with regard to the presence in the former of γ-linolenic and octadecatetraenoic acids, and the absence of hexadecatetraenoic acid. In containing a high proportion of γ-linolenic acid but little C_{20} pentaenoic acid, the chloroplast lipids of *O. danica* resemble those of the blue-green alga *Spirulina platensis*[18] and it is possible that the fatty acid metabolism in the photosynthetic apparatus of the two organisms is similar, although *O. danica* contains more α-linolenic acid than does *Sp. platensis*. Another outstanding feature of the fatty acid distribution in *O. danica* is the extremely high level of C_{20} and C_{22} polyenoic acids in two of the uncharacterized lipids.

In the other classes of algae studied, both the red alga (*Porphyridium cruentum*) and the yellow alga (*Monodus subterraneus*) contained large quantities of C_{20} tetra-and penta-enoic acids, and these comprised a major proportion of the component acids of the chloroplast lipids (Tables 2 and 4). Radunz has also observed a high concentration of C_{20} polyenoic acids in the galactosyl diglyceride fractions of the red alga *Batrachospermum moniliforme*.[19] It therefore appears that in *P. cruentum* and *M. subterraneus*, synthesis of arachidonic acid occurs in the photosynthetic apparatus, as is probably the case in mosses.

Our results concerning the utilization of γ-linolenic acid and 11,14-eicosadienoic acid by the algae under consideration, show distinct differences between *E. gracilis* on the one hand and *O. danica* and *P. cruentum* on the other. With *E. gracilis*, our findings were similar to those of Erwin and co-workers,[7] namely that this alga readily desaturates the eicosadienoic acid to eicosatrienoic acid and arachidonic acid, but does not desaturate γ-linolenic acid. Also, no mass or radioactive fractions corresponding to γ-linolenic acid were detectable in extracts from this alga following its incubation with ^{14}C-linoleic acid or ^{14}C-oleate (Table 5).

[17] E. D. KORN, *J. Lipid Res.* **5**, 352 (1964).
[18] B. W. NICHOLS and B. J. B. WOOD, *Lipids* **3**, 46 (1968).
[19] A. RADUNZ, *Z. Physiol. Chem.* **349**, 1091 (1968).

Reference to Tables 6 and 7 shows that in comparable experiments employing *O. danica* and *P. cruentum* the eicosadienoic acid was not desaturated, but in both cases the added γ-linolenic acid was sequentially desaturated to eicosatrienoic acid and arachidonic acid. That the γ-linolenate pathway to arachidonic acid operates almost exclusively in these algae was also suggested less directly by the appearance of radioactive fractions corresponding to γ-linolenate, eicosotrienoate and arachidonate, but not eicosadienoate, when these algae were incubated with ^{14}C-oleate and ^{14}C-linoleate. The absence of significant quantities of radioactivity in acids of chain length shorter than C_{18} (e.g. of the C_{14} and C_{16} series) during these incubations is a strong indication that the γ-linolenate was directly converted to eicosatrienoic acid and arachidonic acid by these algae, rather than first being broken down into smaller units followed by synthesis from these fragments.

Our major conclusions therefore are that *O. danica* and *P. cruentum* differ from *E. gracilis* in synthesizing arachidonic acid by the pathway involving γ-linolenic acid. *P. cruentum* and *M. subterraneus* differ from *O. danica* and *E. gracilis* in synthesizing major quantities of C_{20} polyenoic acids, including arachidonic acid, within their chloroplasts.

EXPERIMENTAL

Algae

Stock cultures were obtained from the Cambridge Collection of Algae and Protozoa. *Euglena gracilis* strain No. 1225/53 was separately cultured under two differing sets of conditions. One culture was grown in the dark on the complex medium of Haverkate[20] while the second was grown in the light on a synthetic medium.[21] *Ochromonas danica* (strain No. 933/2) and *O. malhamensis* (strain No. 933/1a) were grown in the light on a purely synthetic medium[22] while *Porphyridium cruentum* (strain No. 1380/1) and *Monodus subterraneus* (strain No. 848/1) were grown photoautotrophically by established methods.

Extraction of Lipids

Algal cultures were centrifuged and the culture media decanted from the packed cells, which were then shaken with about 100 vol. of $CHCl_3$–methanol (2:1 v/v). After standing at room temperature for about 1 hr, the extraction mixtures were filtered and the residues re-extracted with $CHCl_3$–methanol. The extracts were combined, concentrated *in vacuo*, and the residues redissolved in a small volume of $CHCl_3$–methanol (2:1 v/v) which was then washed with 1/5 volume of 0·7% saline to remove water-soluble material.

When this extraction procedure was applied to harvested cells of *M. subterraneus* little chlorophyll and only about 75 per cent of the total lipids were extracted, even after extraction at room temperature for 24 hr. (The extraction efficiency was determined by comparing the total free and combined fatty acids in a $CHCl_3$–methanol extract, which the amount of fatty acid extracted by this solvent only after hydrolysis of the solvent-extracted residues with 6 N HCl.) However, gas-chromatographic analysis (GLC) of the extractable lipid fatty acids and those removable only after acid hydrolysis showed them to have almost identical compositions. Consequently, the lipids obtained by $CHCl_3$–methanol extraction of cells of *M. subterraneus* were regarded as being representative of those present in the whole cell.

Fractionation of Lipid Extracts

Lipid extracts were fractionated into their component lipid classes by a combination of column chromatography on DEAE cellulose (acetate form) and TLC on silica gel free from gypsum binder.[23] Lipid fractions were stored either in $CHCl_3$ or $CHCl_3$–methanol, both solvents containing 0·005% BHT antioxidant.

Fatty Acid Analyses

The fatty acid composition of lipid fractions, and the location of double bonds in certain acids, were established by standard techniques which are described elsewhere.[18]

[20] F. HAVERKATE, Thesis: Phosphatidyl Glycerol from Photosynthetic Tissues, p. 35, Univ. of Utrecht (1965).
[21] J. J. WOLKEN, *Euglena*, p. 13, Rutgers University Press (1961).
[22] S. AARONSON and S. SCHER, *J. Perotozol.* **7**, 156 (1960).
[23] B. W. NICHOLS and A. T. JAMES, *Fette Seifen Anstrichmittel* **66**, 1003 (1964).

^{14}C-labelled Substrates

1-^{14}C oleic acid (57·8 mc/mM) and 1-^{14}C linoleic acid (529 mc/mM) were purchased from the Radiochemical Centre, Amersham. 1-^{14}C γ-linolenic acid (approx 10 mc/mM) was prepared biochemically by incubating cells of *Tetrahymena pyriformis* with 1-^{14}C oleic acid. 11,14-[1-^{14}C]eicosadienoic acid (approx. 1 mc/mM) was the generous gift of Dr. L. J. Morris.

Incubation of Algae with Labelled Substrates

Algal cells were harvested by centrifugation and resuspended in their culture media in the approximate ratio of 1 ml pcv of cells to 10 ml of media. To this suspension was added approximately 2 μc of the substrate in the form of an aqueous sonicated suspension of the sodium salt, and the mixtures were incubated at 30° in the light on a reciprocating water bath for the required period. The lipids were then extracted from the algae in the usual way and the component fatty acid methyl esters analysed by radio-GLC.