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Lipid Composition and Metabolism of *Volvox carteri*[1]

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KARAN R. MOSELEY AND GUY A. THOMPSON JR.
Department of Botany, The University of Texas, Austin, Texas 78712

ABSTRACT

The membrane structural lipids of somatic cells and gonidia isolated from *Volvox carteri* f. *nagariensis* spheroids have been characterized. The principal polar lipid components of both cell types are sulfoquinovosyl diglyceride, mono- and digalactosyl diglyceride, phosphatidylglycerol, phosphatidylethanolamine, and 1(3), 2-diacylglyceryl-(3)-*O*-4'-(*N*,*N*,*N*,-trimethyl)homoserine. Light-synchronized cultures of spheroids were shown to incorporate [^{14}C]bicarbonate, [^{35}S]sulfate, [^{14}C]palmitic acid, and [^{14}C]lauric acid into complex lipids. [^{14}C]Palmitic acid was incorporated mainly into diacylglyceryltrimethylhomoserine and was not significantly modified by elongation or desaturation. In contrast, [^{14}C]lauric acid was incorporated into a wider variety of complex lipids and was also converted into longer chain saturated and unsaturated fatty acids. *Volvox* is a promising system for studying the role of membranes in algal cellular differentiation.

Studies of green algae have provided much of the information that we possess concerning the molecular aspects of photosynthesis and other biochemical processes of plants. In addition to the more familiar species of *Chlorella*, *Euglena*, and *Chlamydomonas* commonly used for metabolic investigations, the colonial alga *Volvox* has emerged as a valuable model system.

Each *Volvox* spheroid is composed of as many as 5,000 somatic cells, each similar in structure to the unicellular alga *Chlamydomonas* (16). Contained within the sphere of terminally differentiated somatic cells are approximately 16 much larger reproductive cells known as gonidia. These cells are of particular interest to biologists because they develop in the presence of an appropriate inducer into sexual spheroids containing either eggs or sperm, depending upon the strain of asexual spheroids (16).

Because cell division in some species of *Volvox* can be conveniently synchronized by varying the light regime, *Volvox* offers a particularly useful system for studying various aspects of development and differentiation. In this communication we describe initial experiments aimed at elucidating membrane lipid distribution and metabolism in cells of *Volvox carteri* f. *nagariensis*.

MATERIALS AND METHODS

Culture Conditions. *V. carteri* f. *nagariensis*, strain HK-10, was a gift from Dr. Richard C. Starr, Department of Botany, the University of Texas, Austin. Axenic cultures were grown with aeration in Starr's medium (21) on a schedule of 33 h light and 15 h darkness. Illumination with 3,000 to 5,000 lux in a Precision Scientific Incubator resulted in a culture temperature of 29 ± 0.5 C during the light period and 23 ± 0.5 C during the dark period. Growth was monitored by filtering 1-ml aliquots of cell suspension through a 4.5-cm-diameter Millipore gridded filter (No. HAWG04700) and counting the spheroids under a dissecting microscope.

Harvest and Disruption of Spheroids. Cultures were harvested by pouring the spheroid-containing medium through a No. HC3-90 nylon mesh screen (Tetko, Inc., Elmsford, N. Y.) having a pore size of 90 μm. The concentrated spheroids were washed and resuspended in deionized H_2O if intended for lipid extraction.

If gonidia and somatic cells were to be isolated, approximately 2×10^5 spheroids in stage 1 of development (see legend of Fig. 2), as determined by phase microscopy, were washed and resuspended in 100 ml cold 0.25 M sucrose in 50 mM Tris (pH 7.4). The suspension was then disrupted in the semimicro chamber of a Waring Blendor, model No. 1120, for 30 s at full power. This treatment released intact gonidia from 85–90% of the spheroids.

The gonidia and somatic cells (many still embedded in fragments of broken spheroid matrix) were separated from any remaining whole spheroids by passage through the 90-μm pore size nylon screen and concentrated by centrifugation at 365*g* for 5 min. The pellets were resuspended in 35 ml of the disruption buffer, and 5 ml were loaded onto each of seven discontinuous gradients of sucrose in 50 mM Tris (pH 7.4). Each gradient was composed of 5 ml 0.34 M sucrose, 10 ml 1.0 M sucrose, 10 ml 1.46 M sucrose, and 10 ml 2.0 M sucrose. Centrifugation of the loaded gradients for 10 min at 365*g* produced three bands of green material. Layer 1, located on top of the 1.0 M sucrose, consisted of large sheets of somatic cells embedded in matrix material. Layer 2, located on top of the 1.46 M sucrose, was composed of single somatic cells and small sheets of matrix containing somatic cells. Layer 3, lying above the 2.0 M sucrose, consisted of gonidia, contaminated by a very occasional large sheet of somatic cell-containing matrix. The purity of the isolated fractions is illustrated in Figure 1.

Lipid Extraction and Analysis. Spheroids or isolated cell types were resuspended in a minimum volume of water or cell disruption medium, and lipids were extracted by the procedure of Bligh and Dyer (4). Water-soluble impurities were removed by washing the organic phase with a simulated Folch upper phase (10).

When a further resolution of the bulk lipid mixture was desired, the washed lipid extract was chromatographed on silicic acid (100 mesh, Mallinckrodt), eluting NL with $CHCl_3$, glycolipids with acetone, and PL with $CHCl_3$-methanol (1:1, v/v). TLC of polar lipids was performed, except where noted, on Silica Gel G plates, using the solvent system $CHCl_3$-acetic acid-methanol-H_2O (75:25:5:2.2, v/v/v/v). NL were chromatographed using the solvent system petroleum ether-ethyl ether-acetic acid (70:30:1, v/v/v). Lipid spots were detected on the developed plates by a H_2SO_4 spray followed by heating, by a ninhydrin spray (13), by the α-naphthol reagent (13), by the phosphate spray of Dittmer and Lester (7) or by exposing the plate to I_2 vapor. GLC of fatty acid methyl esters (18) was performed on a Varian model 3700 instrument equipped with a flame ionization detector, using a 1.83-m column of 10% diethyleneglycol succinate on Chromosorb W AW heated to 180 C. In some cases methyl ester samples were hydro-

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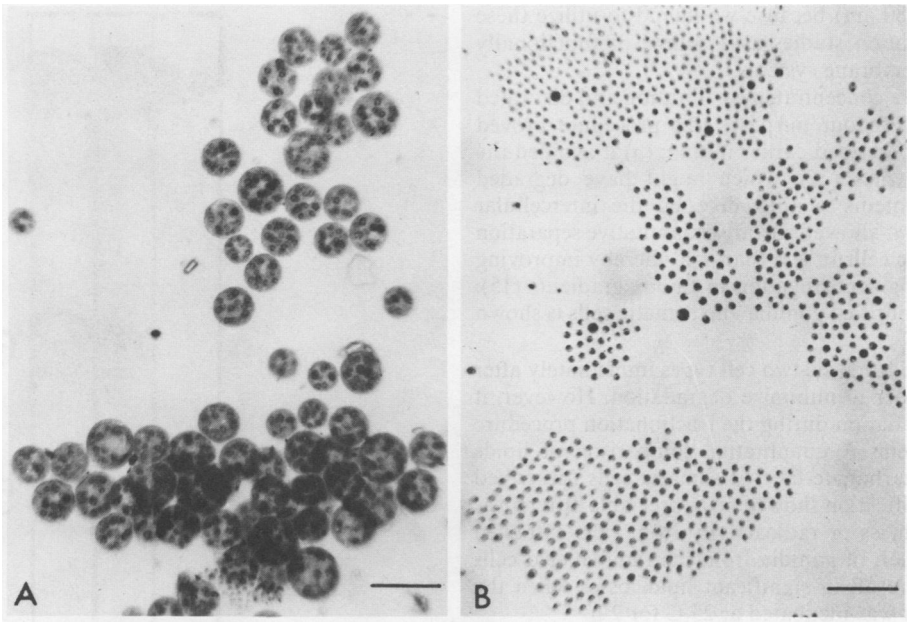


FIG. 1. A: photomicrograph of purified *Volvox* gonidia isolated as described under "Materials and Methods" (X 120). B: photomicrograph of purified somatic cells, still bound into small sheets of matrix material following their isolation (× 120). Bar = 100 μm.

genated with Pt (1). Radioactive peaks were trapped during gas chromatographic runs through the use of an effluent splitter.

Acetolysis of lipid samples was achieved by refluxing the lipid in acetic acid-acetic anhydride (3:2, v/v) under a N_2 atmosphere for 10 h. After adding water, the lipid products were extracted with ethyl ether.

Lipid phosphorus was quantified by the procedure of Bartlett (3) as modified by Marinetti (17). Individual phospholipid species on TLC plates were analyzed according to Rouser *et al.* (19). Lipid nitrogen was estimated by the method of Sloane-Stanley (20). Lipid-bound carbohydrate in extracts and in TLC spots was measured by the technique of Dubois (8). Chl *a* and *b* were quantified by the spectrophotometric procedure of Arnon (2).

Radioisotope-labeling Procedures. 1-[^{14}C]Palmitic acid (55 Ci/mol) was obtained from New England Nuclear Corp., 1-[^{14}C]lauric acid (28.8 Ci/mol) and sodium [^{14}C]bicarbonate (40 Ci/mol) from Amersham/Searle, and $H_2[^{32}S]O_4$ (carrier-free) from ICN Pharmaceuticals. The water-soluble radioisotpes, [^{14}C]bicarbonate and $H_2[^{35}S]O_4$ (first neutralized with NaOH), were added directly to synchronous cultures of *Volvox* 2–3 h after the beginning of the light period, when the released juvenile spheroids still contained uncleaved gonidia. Radioactive fatty acids were added in 2–3 drops of ethanol to cultures of the same developmental stage, but following a 6-fold concentration of the spheroids by filtration. The cultures were diluted back to the normal density approximately 15 min after isotope addition.

Radioactivity was measured using a Packard Tri-Carb model 3310 scintillation spectrometer. Individual radioactive lipids on TLC plates were first visualized by exposing the plates to I_2 vapors and later quantified by counting the spots scraped from the plates.

RESULTS

Isolation of *Volvox* Lipids. When grown on a regime of 33 h light followed by 15 h of darkness, a complete cycle of spheroid development and release from the parent colony was accomplished with good synchrony each 48 h (Fig. 2). For most of the experiments described below, cultures were harvested when highly enriched in stage 1 spheroids, *i.e.* those spheroids containing gonidia which had not yet undergone cell division. These single-celled gonidia were analyzed when they neared their largest size (diam-

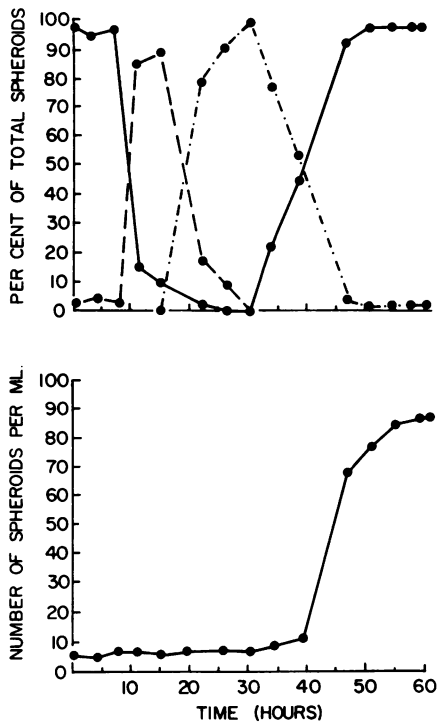


FIG. 2. Growth of asexual cultures of *Volvox* under conditions specified under "Materials and Methods." Upper graph shows proportions of stage 1 spheroids (●——●), stage 2 spheroids (●– –●), and stage 3 spheroids (●– · –●) found in aliquots removed at various times. Stage 1 was defined as the period of development beginning with the release of daughter spheroids from the parent spheroid and ending with the first division of the gonidia in the daughter spheroid. Stage 2 was designated as the period of development beginning with the first division of the gonidia and ending with the inversion of the young embryo. The period between inversion and release of daughter spheroids from the parent spheroid was defined as stage 3. Lower graph depicts number of spheroids/ml. Hours 1–33 are hours of the light period and 34–48 are hours of the dark period.

eter approximately 75–80 μm) because we intend to utilize these cells in future fractionation studies as a source of functionally distinct intracellular membrane systems.

Stage 1 spheroids were concentrated by filtration and disrupted in a Waring Blendor. For our purposes this procedure proved superior to techniques described earlier in that: (*a*) it avoided the use of proteolytic enzymes (25), which might have degraded intrinsic membrane proteins as they digested the intercellular matrix material; and (*b*) it allowed a nearly quantitative separation of gonidia from somatic cells in less than 1 h, thereby improving on a technique requiring centrifugation in Ludox gradients (15). The appearance of the purified gonidia and somatic cells is shown in Figure 1.

Lipids were extracted from the two cell types immediately after their purification in order to minimize degradation. However, it appears that lipid degradation during the fractionation procedure is not a serious problem. A quantitative comparison of lipids extracted from [^{14}C]bicarbonate-labeled somatic cells (described later) 7 h after their purification showed no appreciable differences in the distribution of mass or radioactivity from cells extracted after only 1 h. Disruption of gonidia from the radiolabeled cells by sonication did not result in significant lipid losses when the broken cell preparation was incubated at 25 C for 2 h.

Characterization of Lipids. The identity of the *Volvox* lipids was determined using extracts of whole spheroids. The TLC behavior of the lipids is illustrated in Figure 3. As shown, the lipid pattern is similar to that found in the related alga *Chlamydomonas reinhardi.*

Two-dimensional TLC using chloroform-methanol-water (95:35:5, v/v/v) in the first dimension and chloroform-acetic acid-methanol-water (75:25:5:2.2, v/v/v/v) in the second dimension showed no additional spots. Therefore, the one-dimensional plates were used for characterizing the various components. Spot 1, a relatively minor component, was negative to the phosphate spray reagent and to ninhydrin but positive to the α-naphthol spray for sugars. Because its mobility suggested that it was the plant SL[2] (13), spheroids were analyzed after incubation for 3 h in the light with [^{35}S]sulfate. Sixty-four per cent of the ^{35}S recovered in lipids was found in spot 1, confirming its identity as SL.

Spots 2 and 6 were phosphate- and ninhydrin-negative and α-naphthol-positive. Acid hydrolysis (in sealed tube with aqueous 2 N HCl for 48 h) of the two combined lipids after column chromatography (see below) produced fatty acids, glycerol, and galactose, as determined by descending paper chromatography, using ethyl acetate-pyridine-H_2O (2.5:1:2.5, upper phase), and TLC on plates prepared with Silica Gel H suspended in 0.3 M KH_2PO_4, using 1-butanol-acetone-H_2O (4:5:1, v/v/v). The relative TLC mobility of the intact lipids indicated that spot 2 was DGDG while spot 6 was MGDG.

Spots 3 and 4 were both positive for phosphorus, and spot 4 was also positive for amino groups reactive with ninhydrin. Lipids 3 and 4 were identified as PG and PE, respectively, by co-chromatography with authentic samples of these two compounds.

Because spot 5 was negative to all of the spray reagents mentioned above, its characterization required that it be purified and studied in further detail. Column chromatography on silicic acid allowed a separation of the total spheroid lipids into the following fractions: NL, eluted with $CHCl_3$; glycolipids (spots 2 and 6), acetone; unknown lipid (spot 5), $CHCl_3$-methanol (9:1, v/v); PL (spots 3 and 4) and SL (spot 1), $CHCl_3$-methanol (1:1, v/v). The $CHCl_3$-methanol (9:1) fraction was used for further examination of the unidentified compound.

In addition, the unknown lipid could be purified by an alternative procedure. After elution of glycolipids from the silicic acid column, all remaining polar lipids were quickly removed batchwise with $CHCl_3$-methanol (1:1, v/v). The resulting lipid mixture was then concentrated to dryness and subjected to acetolysis. This converted all polar lipids except the unknown compound into diglyceride acetates, which could be removed easily from the unknown compound by rechromatography on silicic acid.

Analysis of the purified unknown on a Varian model EM-360 NMR spectrometer gave a spectrum characterized by major peaks at 1.26, 2.1, 2.33, 2.83, 3.32, and 5.4 ppm. The first four peaks indicated the presence of long chain unsaturated fatty acids, while the peak at 3.32 and 5.4 ppm suggested trimethylammonium protons and protons associated with a glycerol moiety, respectively. These properties, coupled with the TLC behavior of the

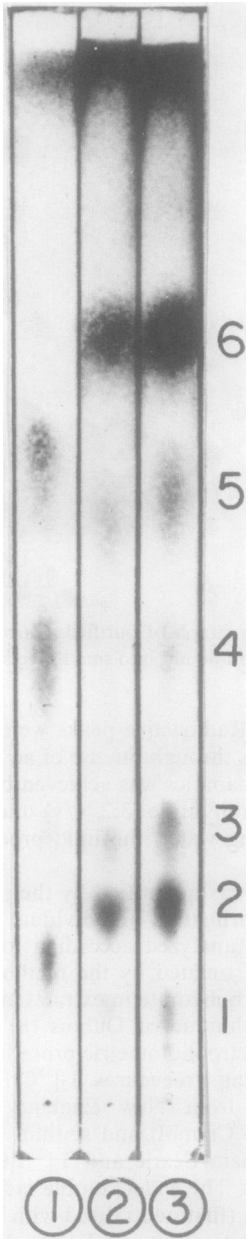
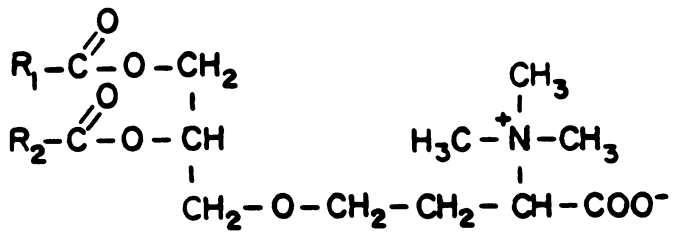


FIG. 3. Thin layer chromatogram of *Volvox* total lipids developed in the system chloroform-acetic acid-methanol-H_2O (75:25:5:2.2, v/v/v/v). Lane 1 represents *Tetrahymena pyriformis* phospholipids, lane 2 *Volvox* total lipids, and lane 3 *C. reinhardi* total lipids. Identification of the components are: 1, SL; 2, DGDG; 3, PG; 4 (not clearly visible in *Volvox* lipids), PE; 5, DGTH; 6, MGDG. Upper band represents NL at solvent front.

[2] Abbreviations: NL: neutral lipids; PL: phospholipid; SL: sulfolipid; DGDG: digalactosyl diglyceride; MGDG: monogalactosyl diglyceride; PG: phosphatidylglycerol; PE: phosphatidylethanolamine; DGTH: 1(3),2-diacylglyceryl-(3)-*O*-4′-(*N*,*N*,*N*-trimethyl)homoserine; GL: lipid galactose.



SCHEME I.

compound, indicated its strong resemblance to DGTH (Scheme I), as characterized by Brown and Elovson (5) in *Ochromonas danica* and more recently by Eichenberger and Boschetti (9) in *C. reinhardi*. An analysis of methyl esters prepared from the compound eluted from a silicic acid column with $CHCl_3$-methanol (9:1) yielded the following results: 16:0, 34%; 16:1, 2%; 18:0, 5%; 18:1, 9%; 18:2, 8%; unknown I, 16%, α18:3, 16%; and unknown II, 8%. Hydrogenation studies indicated that unknown I is a C_{18} unsaturated fatty acid.

Hydrolysis of 22 mg of the lipid in 2 N methanolic HCl under reflux for 1 h yielded an ether soluble residue of fatty acids accounting for 68% of the original weight and a water-soluble residue equivalent to 36% of the starting weight (theoretical for DGTH: 73 and 32%, respectively). The water-soluble residue released no glycerol either during mild base hydrolysis (6) or strong acid hydrolysis (3 N HCl in sealed tube at 125 C for 48 h), as reported for DGTH by Brown and Elovson (5).

Direct comparison of the unknown *Volvox* lipid with *Chlamydomonas* DGTH (Fig. 3) confirmed its identity. Examination of charred TLC plates indicated that this lipid was present in whole spheroids at levels comparable to those of the PL and SL but less than those of the galactosyldiglycerides. A quantitative nitrogen analysis of two samples of the *Volvox* DGTH purified from total spheroid lipids by the acetolysis procedure indicated a molar ratio of DGTH nitrogen to PL phosphorus of 1.13. Although quantitative analyses of DGTH in isolated gonidia and somatic cells were not performed, visual inspection of TLC plates indicated the presence of the lipid in both cell types.

Quantitative Analysis of Different Cell Types. Analysis of the Stage 1 spheroids gave an average of 2.4 ± 1.5 μmol lipid P/10^6 spheroids. The value was dependent upon the size of the rapidly growing gonidia. In spheroids containing mature but still unicellular gonidia, approximately 70% of the PL were present in these reproductive cells. In whole spheroids and in purified gonidia and somatic cells, the major phospholipid was PG (40-60% of the total), with PE being present in smaller amounts.

Molar ratios of the major lipid classes relative to phospholipids are given in Table I. We found considerable variation in these ratios, and it is not clear at this time whether there are lipid compositional changes during the course of developmental stage 1. Table I also shows results from replicate analyses of a single preparation each of purified gonidia and somatic cells. Except in the MGDG/DGDG ratios, the values for the isolated cell types are not significantly different from those of whole spheroids.

Aliquots of the three lipid fractions from gonidia and somatic cells were converted to fatty acid methyl esters and analyzed by GLC (Table II). All fractions contained a high proportion of palmitic acid, and the somatic cell fatty acids were surprisingly low in polyunsaturates.

Biosynthesis of *Volvox* Lipids. The first radioactive precursor of membrane lipids tested with *Volvox* was sodium [^{14}C]bicarbonate. A 100-μCi aliquot of the radiotracer was added to the flask of illuminated stage 1 spheroids after concentrating them to six times their normal density. Half of the spheroids were harvested after 2 h and the other half after 4 h. The lipids of the two samples contained 3 and 4% of the added radioactivity, respectively. Most of the ^{14}C was found by TLC to be associated with the NL and the galactosyldiglycerides (Table III).

[^{14}C]Palmitate was also examined as a precursor of *Volvox* membrane lipids. Fifteen min after adding 2.5 μCi 1-[^{14}C]palmitate to a 12-fold concentrated suspension of stage 1 spheroids, the culture was diluted 5-fold and returned to the illuminated incubator. Aliquots of 100 ml were harvested and extracted at the times indicated in Figure 4. The [^{14}C]palmitate was rapidly incorporated into complex lipids by the spheroids (4). Most of the radioactivity was associated with DGTH, whose characterization

Table I. *Molar Ratios of Some Principal Volvox Lipids*

Lipid Ratios	Spheroids[a]	Gonidia	Somatic Cells
PL/Chl/GL/SL	1/2.4 ± 1.0/10.1 ± 4.1/0.5 ± 0.2	1/1/8/0.4	1/1/6/0.3
Chl *a*/Chl *b*	1.8 ± 0.4	2.21	2.09
MGDG/DGDG	9.0 ± 3.2	3.29	3.55

[a] Average ± SD of four experiments.

Table II. *Fatty Acid Distribution in Volvox Lipid Classes*

Fatty Acid	Retention Time	Whole Spheroid Total Lipid	Gonidia			Somatic Cells		
			NL	GL	PL + DGTH	NL	GL	PL + DGTH
	min							
12:0	2.0	0.7	2.8		0.2			
14:0	3.3		2.2	0.6	0.6	0.6	1.6	
16:0	5.9	20.9	20.3	31.8	32.4	35.6	66.4	43.4
16:1	7.1		4.0	4.5	10.3	0.6		8.1
16:2?[a]	9.0	1.3		0.6				
16:4?	14.1	12.9		9.3				
18:0	10.5	2.5	8.8	2.1	6.9	17.0	6.6	11.6
18:1	12.2	13.3	50.7	8.8	11.2	34.1	14.5	12.3
18:2	15.8	10.7	3.5	6.0	10.1		3.7	9.7
X[b]	17.9	4.3			10.5			9.3
18:3	21.4	32.6	4.5	35.8	14.0		7.1	5.4
18:4?	24.4				3.8			
Per cent of:								
Saturated fatty acids		24.1	34.1	34.5	39.1	53.2	74.6	55.0
Unsaturated fatty acids		70.8	62.7	65.0	49.4	34.7	25.3	35.5

[a] Question marks indicate the fatty acids tentatively identified by extrapolation of semilogarithmic plots of retention time *versus* number of carbon atoms in the fatty acids standards.

[b] X represents an unidentified fatty acid. Data are typical of several analyses.

Table III. *Incorporation of [^{14}C]Bicarbonate into Lipids of Volvox Spheroids*

Lipid	Total Radioactivity[a] in Lipid after:	
	2 h	4 h
	%	
SL	2.2	1.3
DGDG	8.3	13.1
PG	1.9	0.7
PE	1.8	2.1
DGTH	7.1	7.4
MGDG	10.8	17.1
NL[b]	64.5	55.3

[a] The data are from thin layer chromatographic plates containing at least 26,000 cpm total radioactivity.

[b] Radioactivity was mostly in area of plates containing sterols.

is described in a previous section. The [^{14}C]palmitate-labeling experiment was repeated, with samples of spheroids being extracted not only at short time intervals but also at 12 and 24 h following isotope addition. The labeling pattern attained by 6 h (Fig. 4) showed little change over the longer period.

A similar incorporation pattern was observed in spheroids which were disrupted prior to [^{14}C]palmitate addition in order to assure that the gonidia as well as the somatic cells were exposed to the radiotracer. The radioactivity in membrane lipids of both cell types 90 min following the [^{14}C]palmitate was again primarily in DGTH.

Following its incorporation into complex lipids, the [^{14}C]palmitate acid underwent little further metabolic alteration. Fatty acids recovered from the polar lipid fraction of spheroids incubated for 24 h with [^{14}C]palmitate still contained 86% of their radioactivity in the combined 16:0-16:1 peaks eluted from the gas chromatograph. Most of the remainder (10% of the total ^{14}C recovered) was found in the 18:0 peak.

Thus, [^{14}C]palmitate was selectively incorporated into a lipid fraction in which it was not readily available for elongation, desaturation, or exchange into different complex lipids. A similar phenomenon has been reported in other plants (11, 12), and has been postulated to result from the inability of the cells to convert exogenously supplied long chain fatty acids to the metabolically active acyl-ACP derivatives (11). Since shorter chain fatty acids, such as lauric acid (12:0), have been found to enter the metabolically accessible pool of *Chlorella* (12), we examined the incorporation of [^{14}C]lauric acid into *Volvox* lipids.

Spheroid lipids extracted 0.5 and 24 h following the addition of 1 μCi 1-[^{14}C]lauric acid contained a large proportion of their radioactivity in DGTH (Table IV). However, unlike the finding with [^{14}C]palmitate, there was a significant and increasing content of radioactivity in other membrane lipids. Furthermore, there was considerably more elongation and desaturation of the incorporated [^{14}C]lauric (Table V) than was observed with [^{14}C]palmitate. It seems that lauric acid entered a lipid compartment much more active metabolically than that occupied by exogenous longer chain fatty acids.

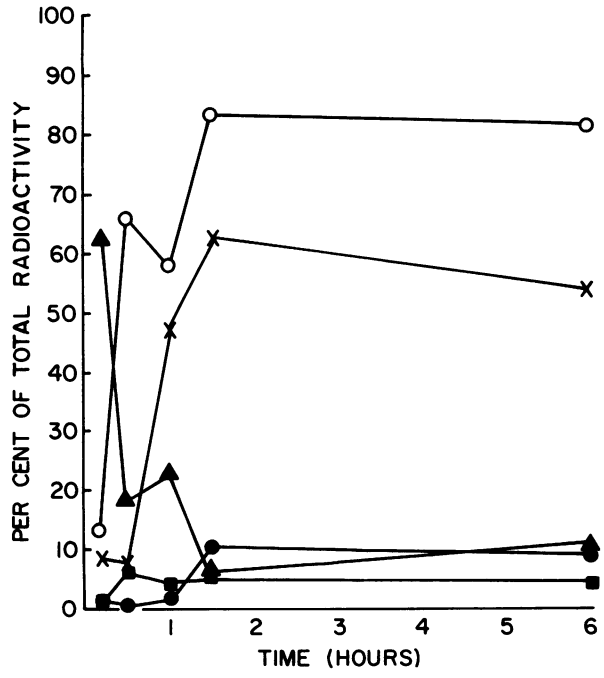


FIG. 4. Incorporation of 1-[^{14}C]palmitate into lipids of *Volvox*. (▲): Free fatty acids; (○): polar lipids; (×): DGTH; (■): glycolipids; (●): phospholipids.

Table IV. *Incorporation of [^{14}C]Lauric Acid into Lipids of Volvox Spheroids*

Lipid	Total Radioactivity[a] in Lipid after:	
	0.5 h	24 h
	%	
SL	1.6	4.0
DGDG	6.3	15.1
PG	2.0	
PE	1.6	0.8
DGTH	30.8	20.6
MGDG	11.2	21.1
NL[b]	30.5	27.0

[a] The data are from thin layer chromatographic plates containing at least 2,400 cpm total radioactivity.

[b] Radioactivity was mostly in triglycerides and other lipids less polar than free fatty acids.

Table V. *Incorporation of [^{14}C]Lauric Acid into Fatty Acids of Volvox Lipids*

The data represent analyses of the combined 0.5-h and 24-h samples.

Fatty Acid	Total Radioactivity in Fatty Acid			Relative Specific Radioactivity[a]		
	NL	GL	PL + DGTH	NL	GL	PL + DGTH
	%					
14:0	6.3	4.7	25.2	1.8	5.9	28.0
16:0	10.6	23.5	23.1	0.9	0.7	0.7
16:1	1.9	1.7	2.6	4.4	1.5	1.4
18:0	29.5[b]	13.2[b]	7.4	1.0[b]	0.7[b]	0.08
18:1			8.1			1.
18:2	12.3	17.8	10.4	4.5	1.5	1.1
18:3	23.1	18.8	5.0	1.6	0.7	0.5
Others[c]	16.3	20.3	18.2			

[a] Computed by dividing the per cent of total radioactivity in each fatty acid by the weight per cent of that fatty acid present in the mixture.

[b] Peaks not resolved.

[c] Includes 12:0.

DISCUSSION

Experimental studies carried out on *Volvox* during the past few years have established it as a useful system for studies of cellular differentiation. As shown by earlier workers (25) and confirmed in the present work (Fig. 2), cultures of *Volvox* grow relatively fast and are easily synchronized so that all spheroids advance from one developmental phase to the next in unison. Development of new spheroids from the unicellular gonidia involves a number of morphologically interesting but physiologically uncharacterized processes, such as inversion, a complex series of cell movements by which the embryonic spheroid turns inside out (16, 23). Additional interest has been focused on the *Volvox* system since the discovery of a hormonally controlled transformation of certain strains from an asexual to a sexual form of reproduction (22).

We have begun an evaluation of *Volvox* as a model system for studying the role of membranes in algal differentiation. For this reason, we have undertaken the characterization of *Volvox* membrane lipids.

The lipids are generally similar in structure to those found in the noncolonial alga *C. reinhardi* (9). One of the principal components has been identified as DGTH, a lipid hitherto reported only in *Chlamydomonas* (9), *Ochromonas danica* (5), and *Epidermophyton floccosum* (24).

The quantitative relationships among some of the key lipid species in whole spheroids are mirrored by similar ratios deter-

mined in a single analysis of somatic cells and gonidia (Table I). The one apparent difference, a much lower MGDG/DGDG ratio in gonidia and somatic cells, will have to be confirmed by more extensive analyses of the purified cell types. The possible presence of galactolipids in the extracellular matrix will also be sought. The degree of unsaturation in the membrane lipids of gonidia is considerably higher than in somatic cells (Table II). This may result in more fluid membranes in the gonidia.

The radioisotope-labeling experiments described above establish the feasibility of examining *Volvox* lipid metabolic pathways by these means. It will be particularly interesting to study the biosynthesis of DGTH, a compound whose metabolic origin is completely unknown.

The long range goal of our work is to determine what changes occur in the lipid composition and metabolism of *Volvox* membranes and how any resulting alterations in membrane fluidity (14) affect differentiation in this organisms. Work is now underway to fractionate gonidia and embryonic spheroids into preparations of structurally and functionally homogeneous membranes for further study.

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