The effect of temperature on glyceryl ethers in *Tetrahymena pyriformis* W[1]

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Abstract The effect of temperature on the ether content of the glycerophospholipids of *Tetrahymena pyriformis* W was examined. The only ether detected was 1-O-hexadecyl glycerol (α-chimyl alcohol). The data provide evidence that the class 1-O-alkyl-2-acyl-*sn*-glycero-3-(2-aminoethyl)-phosphate (1-alkyl PsE), in addition to the previously reported 1-O-alkyl-2-acyl-*sn*-glycero-3-(2-aminoethyl)-phosphonate (1-alkyl PnE) and 1-O-alkyl-2-acyl-*sn*-glycero-3-phosphorylcholine (1-alkyl PC), exists in this ciliate species. A comparison was made of the ether content of the glycerophospholipids from cells grown at 15° and 28.5°C. An elevation in the amount of ether was noted in all glycerophospholipids at the lower temperature with the largest proportional change in 1-alkyl PsE. *Tetrahymena* species have a high γ-linolenic acid content in the *sn*-1 position of the glycerophospholipids in addition to the usual saturated acids and ether. The replacement at low temperature of γ-linolenic acid by a saturated hydrocarbon at the *sn*-1 position of the glycerophospholipids of *Tetrahymena pyriformis* W should increase the microviscosity of the membranes; thus, it is difficult to envision this alteration in the glycerophospholipids as an adaptive change beneficial for growth. These findings are in direct contrast to the situation in *Tetrahymena thermophila* where the percentage of ether glycerophospholipids increases at the expense of γ-linolenate as the temperature rises.—**Lund-Katz, S., and R. L. Conner.** The effect of temperature on glyceryl ethers in *Tetrahymena pyriformis* W. *J. Lipid Res.* 1982. **23:** 1301–1307.

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Investigations with a variety of organisms have shown that the cellular lipid composition is responsive to changes in the environmental temperature (1). The alterations observed are believed to be directed toward maintenance of the physical state of the membranes that allows optimal membrane function (2). The most frequently encountered change involves the fatty acyl composition of the polar lipids, although reports of differences in polar head group content (3, 4) and quantity of sterol (5) have appeared.

Several species of the ciliated protozoon, *Tetrahymena*, have been examined with regard to temperature adaptation (4, 6–11). As in many organisms, a decrease in culture temperature of *Tetrahymena* leads to an increase in the proportion of unsaturated fatty acids in glycerophospholipids (6, 12); further, the derivatives of the palmitoleic acid pathway of unsaturated acid synthesis (13) increase at lower temperatures at the expense of stearic acid derivatives (11).

The polar lipids of the ciliate, *Tetrahymena*, are a complex grouping. The glycerophospholipids consist of a mixture of ethanolamine and choline derivatives.[3] Alkyl ether lipids, which are almost exclusively 1-O-hexadecyl-glycerol (α-chimyl alcohol), constitute approximately 25 mole % of the glycerophospholipids and account for 60 mole % of the PC and PnE fractions (14). Some investigators have reported that no ether is present in PsE (3, 15–17) while others have observed a small percentage of the alkyl derivative (18–20). A small decrease in glyceryl ethers has been observed in *T. pyriformis* NT-1 (3) and an increase of ~20 mole % has been noted in *T. thermophila* (4) when the temperature is raised from 15° to 39.5°C.

The saturated fatty acids in the glycerophospholipids of most organisms are usually esterified at the *sn*-1 position, while the highly unsaturated fatty acids are lo-

Abbreviations: TL, total lipid; NL, neutral lipid; PL, polar lipid; CA, α-chimyl alcohol, 1-O-hexadecyl-glycerol ether; batyl alcohol, 1-octadecyl-glycerol ether; ceramide-AEP, ceramide-2-aminoethylphosphonate; TLC, thin-layer chromatography; GLC, gas–liquid chromatography, GLC–MS, gas–liquid chromatography–mass spectroscopy; BHT, butylated hydroxytoluene; C–M, chloroform–methanol; HAc, glacial acetic acid; TMS, trimethylsilyl.

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[3] In this paper both operational and chemical designations are employed to simplify the writing and to facilitate reading. PE refers to all ethanolamine derivatives in the glycerophospholipids; PsE refers to all glyceryl ethanolamine phosphates and PnE to all glyceryl ethanolamine phosphonates. The following symbols are employed to distinguish the glycerol esters from the ethers: 1-acyl PsE (1,2-diacyl-*sn*-glycero-3-(2-aminoethyl)-phosphate); 1-alkyl PsE (1-O-alkyl-2-acyl-*sn*-glycero-3-(2-aminoethyl)-phosphate); 1-acyl PnE (1,2-diacyl-*sn*-glycero-3-(2-aminoethyl)-phosphonate); 1-alkyl PnE (1-O-alkyl-2-acyl-3-(2-aminoethyl)-phosphonate). The glyceryl cholines are present only in the phosphoryl form; therefore, PC refers to all glyceryl cholines, 1-acyl PC refers to 1,2-diacyl-*sn*-glycero-3-choline phosphate and 1-alkyl PC refers to 1-O-alkyl-2-acyl-*sn*-glycero-3-choline phosphate.

calized primarily at the *sn*-2 position. The glycerophospholipids of *Tetrahymena* conform to the usual pattern in that the saturated fatty acids and ether are confined to the *sn*-1 position. Monoenoic acids are found at both carbons while the dienes are found at the *sn*-2 position. γ-Linolenic acid ($18:3^{\Delta 6,9,12}$), however, presents a striking exception to the usual positional distribution of fatty acids in that it is a major fatty acyl component of both the *sn*-1 and *sn*-2 positions of the major glycerophospholipids (20). The increase in glyceryl ether at higher temperatures in *T. thermophila* was found to be at the expense of the polyunsaturated acid, γ-linolenate (4). An increase in saturated hydrocarbon chains at the expense of unsaturated fatty acyl residues at *sn*-1 would be anticipated to have a significant effect on the physical characteristics of the glycerophospholipid and, thus, the cellular membranes involved. The large increases in the lipid glyceryl ether content at elevated temperatures found in *T. thermophila* may provide a means of thermal accommodation.

The striking difference between *T. pyriformis* NT-1 and *T. thermophila* in regard to glyceryl ether content as a function of temperature led us to examine a third commonly employed strain, *T. pyriformis* W. In the course of this study we were able to verify the presence of 1-alkyl PsE and found that the largest proportional change in ether occurs in this compound when the growth temperature is altered.

MATERIALS AND METHODS

General

All organic solvents and glacial acetic acid were distilled before use. Freshly distilled diethyl ether was stored in amber bottles over metallic sodium threads to insure an anhydrous solvent. Solvents employed in lipid extraction and column or thin-layer chromatography (TLC) contained an antioxidant, butylated hydroxytoluene (BHT) (Shell Fine Chemical Co., Houston, TX) at a final concentration of 8 μg/ml. Unisil, 100–200 mesh (Clarkson Chemical Co., Williamsport, PA) and Silic AR cc-4, 100–200 mesh (Mallinckrodt Chemical Works, St. Louis, MO) were washed with methanol at 65°C, air-dried for 24 hr, and reactivated overnight at 110°C. Removal of organic solvents was accomplished with a rotary evaporator operated under reduced pressure, or with a stream of nitrogen. Lipid samples were stored in the dark under nitrogen at −20°C.

Growth of *Tetrahymena pyriformis* W

Stock cultures of *Tetrahymena pyriformis* W were grown at 25 ± 1°C in an enriched proteose peptone culture fluid without added glucose (21).

Fernbach flasks (2800 ml), which contained 500 ml of culture medium, were equilibrated to the desired growth temperature, and each flask was inoculated with 4 ml of a 48-hr stock culture (early stationary phase) of *Tetrahymena* (2.6×10^6 cells). The cultures were grown at 15 ± 0.5°C for 62 hr and at 28.5 ± 0.5°C for 22 hr to obtain equivalent cell densities.

The ciliates were harvested and washed by centrifugation at 200 *g* in a modified plankton centrifuge (22).

Fractionation of lipids

Procedures for estimation of cell numbers, harvesting, lyophilization of cells, and extraction of lipids have been described (22, 23). The lipids were extracted with chloroform–methanol (C–M) 2:1 (v/v) and purified through a Sephadex G-25 column (24).

Adsorption chromatography on activated silicic acid was utilized to separate the purified lipid into neutral lipid (NL) and polar lipid (PL) fractions (25). Further fractionation of the PL was achieved by adsorption chromatography on a Unisil column followed by either adsorption chromatography on a Silic AR cc-4 column (26) or by preparative TLC on a Silica Gel G plate (19). In a typical experiment, the PL was dissolved in chloroform (10 ml/100 mg PL) and chromatographed on a 4.5–6.0 g Unisil column. The following sequence of solvents was used: C–M 14:1 for the elution of PE; C–M 4:1 to elute ceramide-2-aminoethyl phosphonate (ceramide-AEP); and C–M 2:1 to remove the PC and lysoderivatives. The progress of the elution was followed by TLC analysis of the eluate fractions employing chloroform–methanol–water (C–M–W) 95:35:4 as the developing solvent mixture. The C–M 14:1 eluate was subjected to adsorption chromatography on Silic AR in order to separate PnE from PsE. Chromatographically pue PnE was eluted from the column with chloroform–acetic acid (C–HAc) 3:1. A mixture of PnE and PsE followed, and when no further PnE was detected, as demonstrated by TLC analysis, chromatographically pure PsE was eluted with C–M 2:1. Elution was terminated when no further primary amine emerged from the column as evidenced by ninhydrin staining. The progress of the elution was examined by TLC using C–HAc–M–W 75:25:5:1.5 (18). The C–M 14:1 fraction from Unisil column chromatography was also fractionated into PnE and PsE by preparative TLC using C–HAc–M–W 75:25:5:1.5 as developing solvent. The C–HAc 3:1 fractions from the Silic AR column which contained a mixture of PnE and PsE were resolved by TLC as well. All fractions were analyzed by TLC to determine the identity and purity of the various components. Standards corresponding to lipids found in *Tetrahymena pyriformis* were included (19). The PsE and PnE resolved

TABLE 1. Dry weight, lipid, and phosphorus content of *Tetrahymena pyriformis* grown at 15° and 28°C

	15°C	28.5°C
Dry weight (mg)[a] / 10^8 cells	300.1 ± 3.1	247.9 ± 7.9
Lipid content (mg) / 10^8 cells	37.1 ± 1.4	29.5 ± 1.1
μmol Lipid P[b] / 10^8 cells	35.6 ± 1.0	25.1 ± 0.8
Lipid content (mg)[c] / g dry weight cells	123.7 ± 4.8	118.8 ± 5.8
μmol Lipid P / g dry weight cells	98.6 ± 2.4	97.6 ± 1.7
% Lipid	12.4	11.9
Polar lipid (%)	89.1 ± 0.9	88.7 ± 0.7
Neutral lipid (%)	10.9 ± 0.4	11.3 ± 0.6
Polar lipid / Neutral lipid	8.2	7.8

[a] Mean ± S.D.; N = 4.
[b] Lipid phosphorus.
[c] Dry weight.

by TLC were rechromatographed on analytical Silica Gel G plates and found to be chromatographically pure.

The preparation of 1-alkyl-2-acyl- and 1,2-diacyl-3-acetyl-glycerol from PsE and PnE fractions was achieved by phospholipase C hydrolysis (Type III, *Bacillus cereus*, Sigma Chemical Company, St. Louis, MO) (27) followed by acetylation (28). The acetates were separated by TLC on Silica Gel G plates using a Skelly F–diethyl ether 75:25 mixture as the developing solvent.

Phosphorus determinations were achieved by a modification of the method of Aalbers and Bieber (29) which incorporated the ashing method of Ames (30) with the sulfuric acid digestion procedure described by Bartlett (31). A total and an ester phosphorus value could be obtained for any given preparation. 2-Aminoethylphosphonate and NaH_2PO_4 were employed as standards. The proportion of phosphonate was calculated by subtracting the micromoles of phosphate obtained by the H_2SO_4 digestion (ester phosphorus) from the value obtained from the ashing procedure (total phosphorus), and dividing by the total phosphorus value (19, 20).

The ether content of the various fractions was determined by a modification of the method of Wood and Snyder (32). 1-Octadecyl glycerol ether (batyl alcohol) (Applied Science Laboratories, State College, PA) was added as an internal standard. The methyl boronate derivatives were prepared by the addition of 0.2 ml of methyl boronic acid (Applied Science Laboratories) (4 mg/ml acetone). The derivatives were examined after a reaction period of 20 min at 25°C by gas–liquid chromatography (GLC) on a 6-foot glass column packed with either 0.75% SE-52 silicon gum rubber coated on 80–100 mesh Gas Chrom S (Applied Science Laboratories) (190°C) or 10% EGSS-X coated on 100–120 mesh Gas Chrom P (Supelco, Inc.) (170°C). The amount of α-chimyl alcohol was calculated on the basis of the internal standard, batyl alcohol. Both internal and external standards carried through the entire procedure consistently gave recoveries of 95% or greater.

The TMS derivative of the reduced phospholipid samples was examined by gas–liquid chromatography–mass spectroscopy (GLC–MS) with a Perkin-Elmer Model 990 gas chromatograph combined with a Perkin-Elmer Hitachi RMS-4 mass spectrometer; the gas–liquid chromatograph was equipped with a 6-foot glass column packed with a 3% OV-1 (dimethyl silicone) on 100–120 mesh Gas Chrom Q and operated isothermally at 150°C.

RESULTS

Effect of temperature on cells and lipids

The ciliates were grown at 15° and 28.5°C. Cells were larger at the lower temperature, as indicated by an increase in dry weight per cell (**Table 1**). A direct correlation was observed between dry weight and lipid content per cell. The polar to neutral lipid ratio was unaltered. No change based on phosphorus values was seen in the C–M 14:1 (PE) or the C–M 2:1 (PC) fractions. PE constituted 53.9 ± 1.2 and 52.1 ± 1.6% of the polar lipids at 15° and 28.5°C, respectively, and PC was 18.2 ± 0.7 and 18.1 ± 0.8%, respectively, at the two temperatures.

The ether content was measured by direct polar lipid reduction with $LiAlH_4$ to generate chimyl alcohol, which was quantitated by GLC. The results of the analysis of various lipid fractions from 15° and 28.5°C cells are shown on **Table 2**. Only α-chimyl alcohol was present; β-chimyl, batyl, and selachyl alcohol were not detected. The polar lipids from cells grown at 15°C were richer in glyceryl ether content than the material isolated from cells grown at 28.5°C. At the lower temperature, an increase in ether content occurred in both the PE and PC fractions without a concomitant increment in lipid phosphorus.

Berger, Jones, and Hanahan (15), Stoffel, Bauer, and Stahl (16), and Fukushima et al. (3) have reported that chimyl alcohol is found only in PnE, whereas Ferguson et al. (19) and Pieringer and Conner (20) have suggested that an additional small amount of the ether is present in PsE. Consequently, it was of importance to determine

TABLE 2. Glyceryl ether and phosphorus content of various lipid fractions of *Tetrahymena pyriformis* grown at 15° and 28.5°C

Fraction	μmol Lipid P[a] / g Dry Weight		μmol Chimyl Alcohol[b] / g Dry Weight		μmol Chimyl Alcohol / μmol Lipid P × 100[c]	
	15°C	28.5°C	15°C	28.5°C	15°C	28.5°C
TLS[d]	98.6 ± 2.4	97.6 ± 1.7	28.9 ± 0.9	18.9 ± 0.7	29.3	19.4
NL			1.0 ± 0.3	0.8 ± 0.2		
PL	95.1 ± 2.9	93.5 ± 3.0	28.0 ± 0.7	18.0 ± 0.4	29.4	19.3
C–M 14:1[e]	62.7 ± 3.0	63.2 ± 1.9	15.0 ± 0.6	9.2 ± 0.6	24.0	14.6
C–M 4:1[e]	4.6	2.1				
C–M 2:1[e]	22.8 ± 1.2	23.5 ± 0.9	13.3 ± 0.8	10.0 ± 0.7	58.3	42.6

[a] Mean ± S.D.; N = 12.
[b] Mean ± S.D., N = 16, chimyl alcohol (CA).
[c] This number is equivalent to the percentage of glyceryl ether phospholipid present in the fraction.
[d] Total lipids after Sephadex.
[e] The C–M 14:1 eluate contained PE, the C–M 4:1 eluate contained ceramide-AEP, and the C–M 2:1 eluate contained PC and lyso-derivatives.

in which PE component(s) the increase in ether content occurred and, in fact, to ascertain if 1-alkyl PsE existed as a lipid class in *Tetrahymena pyriformis*.

The PE fraction was resolved by Silic AR cc-4 column chromatography by the procedure of Berger and Hanahan (26) into pure PnE and PsE and an overlap region. The overlap portion, which consisted of a mixture of the two, was separated into chromatographically pure PnE and PsE by preparative TLC (20). Differential phosphorus analysis showed the PnE fractions to be >99% phosphonolipid, while the PsE fractions consisted of >99% phosphate ester. An enrichment in ether content of the PnE eluted from the Silic AR cc-4 column early compared to PnE in the overlap fraction was noted (**Table 3**). This finding is in agreement with the data of Berger and Hanahan (26). Examination of the PsE fraction, which eluted last from the Silic AR cc-4 column, revealed 5% of 1-alkyl PsE (Table 3). PsE from the column overlap fraction had a higher ether content. This would be anticipated if the less polar ether phospholipid migrated ahead of the diacyl phosphatides in a fashion analogous to the phosphonate derivatives. Studies on the lipids from cells grown at 15°C gave a PsE fraction that contained less than 1% phosphonate lipid and that had a 10% chimyl alcohol content (Table 3). These data clearly indicate that 1-alkyl PsE is present as a lipid class in *Tetrahymena pyriformis*.

Structural analysis of glyceryl ether

The chromatographically pure PsE (>99% ester phosphorus) and PnE (>99% phosphonate) were reduced with $LiAlH_4$, and the α-chimyl alcohol was con-

TABLE 3. Glyceryl ether and phosphorus content in PE, PnE, PsE, and the overlap fraction of *Tetrahymena pyriformis* grown at 15° and 28.5°C

Fraction	μmol Lipid P[a] / g Dry Weight		μmol Chimyl Alcohol[a] / g Dry Weight		μmol Chimyl Alcohol / μmol Lipid P × 100	
	15°C	28.5°C	15°C	28.5°C	15°C	28.5°C
PE	60.5 ± 1.9	59.3 ± 1.4	15.1 ± 0.8	9.1 ± 0.6	25.0	15.3
PnE[b]	10.5 ± 0.8	8.4 ± 0.4	8.3 ± 0.2	5.3 ± 0.4	79.0	63.1
PsE[b]	37.5 ± 1.2	30.2 ± 1.5	3.7 ± 0.3	1.5 ± 0.2	9.9	5.0
PnE + PsE (Overlap fraction)	14.0 ± 0.6	22.0 ± 0.9	2.6 ± 0.4	2.4 ± 0.3	18.6	10.9
PnE[c]	3.6 ± 0.5	7.1 ± 0.4	0.8 ± 0.2	1.1 ± 0.2	22.2	15.5
PsE[c]	10.9 ± 0.7	16.0 ± 0.6	1.5 ± 0.3	1.2 ± 0.2	13.8	7.5
Total PnE	14.1 ± 0.8	15.5 ± 0.4	9.1 ± 0.2	6.4 ± 0.4		
Total PsE	48.4 ± 1.2	46.2 ± 1.5	5.2 ± 0.3	2.6 ± 0.2		

[a] Mean ± S.D.; N = 6.
[b] Obtained by subjecting the PE fraction to absorption chromatography on Silic AR. The chloroform–acetic acid (C–HAc) 3:1 eluate contained chromatographically pure PnE and the C–M 2:1 eluate contained chromatographically pure PsE.
[c] Chromatographically pure PnE and PsE were secured by subjecting the overlap fraction to preparative TLC.

TABLE 4. Glyceryl ether and phosphorus content in PE, PnE, and Pse of *Tetrahymena pyriformis* grown at 15° and 28.5°C

Lipid Content	μmol Lipid P[a] / g Dry Weight		μmol Chimyl Alcohol[a] / g Dry Weight		μmol Chimyl Alcohol / μmol Lipid P × 100	
	15°C	28.5°C	15°C	28.5°C	15°C	28.5°C
PE	60.1 ± 0.7	62.9 ± 0.9	15.5 ± 0.4	10.4 ± 0.6	25.8	16.5
PnE[b]	14.0 ± 0.9	16.8 ± 0.5	9.5 ± 0.5	7.9 ± 0.7	67.9	47.0
PsE[b]	46.6 ± 1.0	45.1 ± 0.8	5.1 ± 0.6	2.4 ± 0.3	10.9	5.3

[a] Mean ± S.D.; N = 6.
[b] Chromatographically pure fractions obtained by TLC of the PE fraction as described in Materials and Methods.

verted to the trimethylsilyl (TMS) derivative and analyzed by GLC–MS.

The fragmentation patterns of both the PsE and PnE derivatives were superimposable on that of an authentic α-chimyl alcohol standard. The spectra could be divided into hydrocarbon and silicon-containing peaks. Molecular ions, in accordance with previous reports, were not observed (33–35). The highest mass present was the $[M-15]^+$ peak. This weak ion peak corresponds to the loss of a methyl radical from the TMS group of the parent ion and is characteristic of the TMS derivatives of 1-alkyl-2-acyl-phosphoglycerides. This component is 14 mass units less than that of the corresponding 1,2-diacyl derivative (36). A strong peak at m/e 235, which was due to cleavage of the C-O bond, corresponds to $[R]^+$. The peak at m/e 219 was present in all spectra and was due to the ion $[M-RO]^+$ for the hexadecyl derivatives. The complementary alkoxy ion, [RO], which would occur at m/e 241, was not present.

One additional verification of the presence of 1-alkyl PsE was established by preparation of a second known derivative. The purified PsE was treated with phospholipase C to generate diglycerides (27). After acetylation, the 1-alkyl-2-acyl-3-acetylglycerol was separated from the 1,2-diacyl-3-acetylglycerol by analytical TLC (28). Two components were detected when the thin-layer plates were stained with iodine vapor. The 1-alkyl-2-acyl-3-acetylglycerol migrated ahead of the 1,2-diacyl analogue, as demonstrated by comparison to authentic standards. The lipids were extracted from the plates and saponified to obtain chimyl alcohol, which was analyzed by GLC as the methyl boronic derivative. Ether analogues accounted for 9.8% of the PsE in cells grown at 15°C at 4.6% in 28.5°C cells. These values are in good agreement with those reported above (10.9 vs. 5.3%, **Table 4**).

These investigations have demonstrated that in PsE, as well as in PnE, two forms exist, namely, the 1,2-diacyl and the 1-O-alkyl-2-acyl components.

A separate set of experiments was undertaken at 15° and 28.5°C in order to determine the quantity of ether in PnE and PsE. The PE fraction was obtained by Unisil column chromatography and had a phosphonate content of 23.3 ± 0.4% and 26.7 ± 0.5% at 15° and 28.5°C, respectively (Table 4). The PE fractions were resolved directly by preparative TLC into PnE and PsE fractions which contained greater than 99% phosphonate and ester phosphorus, respectively. Each fraction showed only a single component when examined by analytical TLC. Glyceryl ether analysis revealed a marked increase in ether content of both the PnE and PsE fractions at 15°C (Table 4). Further, at 28.5°C, 23.6%, and at 15°C, 31.8% of the ether derivative in the PE fraction was associated with PsE. 1-Alkyl PsE, therefore, was elevated in amount more than 1-alkyl PnE at the lower temperature.

DISCUSSION

It has been recognized that not all membranes of a particular cell have the same lipid composition; further, it is now widely appreciated that the physical state of the membrane lipid bilayer may be closely associated with membrane function (37). Temperature accommodation in many diverse organisms appears to lead to the adjustment of the membrane lipids to maintain a "fluidity" (microviscosity) compatible with satisfactory membrane function.

The polar lipids of the ciliated protozoon genus, *Tetrahymena*, are unusual in that phosphonate derivatives, as well as the usual phosphoryl esters, are present (14, 38). An increase in PnE at the expense of PsE occurred over a temperature range of 15° to 39.5°C in *T. thermophila*, while the opposite is true in *T. pyriformis* NT-1. *Tetrahymena pyriformis* W cells appear to differ from the other two species (3, 4) in that little or no change in the glycerophospholipid polar head groups was observed with a change in temperature. It should be noted that the temperature span employed for *T. pyriformis* W was smaller (15° to 28.5°C) since 39.5°C is lethal to these cells.

The response of *T. pyriformis* W cells to a temperature shift is opposite to that of cultures of *T. thermophila* in regard to glyceryl ether synthesis. The latter species shows a progressive increase as the temperature is raised (4), while *T. pyriformis* W, in common with *T. pyriformis* NT-1 (3), shows an elevation at low temperatures. Some investigators have reported that the ether is associated only with PnE and with PC, while others have indicated the presence of 1-alkyl PsE. Isotopic labeling studies also have suggested the presence of this derivative (18). The current investigation has examined the lipids that contain ether groups in addition to carboxylic acid esters. The data have provided evidence that the class 1-alkyl PsE, in addition to the previously reported 1-alkyl PnE and 1-alkyl PC, exists in the ciliate species, *Tetrahymena pyriformis* W. A lowering of temperature leads to the greatest proportional increase in ethers in 1-alkyl PsE. Further, 1-alkyl PsE has been found in the thermotolerant strain, *Tetrahymena thermophila*.[4] The presence of 1-alkyl PsE in two diverse species of *Tetrahymena*, one amicronucleate (*T. pyriformis*) and the other micronucleate (*T. thermophila*), suggests the widespread occurrence of this compound in the genus. This leads to the conclusion that all of the major glycerophospholipids occur as both the diacyl and monoalkyl-monoacyl derivatives. Electron spin resonance studies of *T. thermophila* membrane systems indicate a lower degree of fluidity when the cells are grown at elevated temperatures (9). These organisms appear to have a novel mechanism for temperature accommodation, namely, replacement of the fatty acyl residues at the *sn*-1 position by an ether-bonded saturated hydrocarbon chain. The replacement of the polyunsaturate, γ-linolenic acid, in the *sn*-1 position of the glycerophospholipids by the saturated ether hydrocarbon can be seen to be a useful device to regulate membrane fluidity (4). The increment observed in saturated hydrocarbons would be anticipated to enhance van der Waal's interactions, which would increase membrane microviscosity and, thus, counteract the influence of higher temperature on membrane fluidity. The elevated ether content seen at low temperatures in *T. pyriformis* W and NT-1 cells would not appear to be a beneficial adaptive change. From the argument stated above, one would anticipate an increase in microviscosity with the rise in ether content. This would seem to be to the detriment of cells that are attempting to overcome the impact of lower temperatures and reduced membrane fluidity. We are continuing our investigations into the nature of the changes in lipid composition seen in *T. pyriformis* W with altered environmental temperatures to deduce what accommodation, if any, occurs in the lipids of these cells.

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