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# STUDIES ON *TETRAHYMENA* MEMBRANES

# IN VIVO MANIPULATION OF MEMBRANE LIPIDS BY 1-*O*-HEXADECYL GLYCEROL-FEEDING IN *TETRAHYMENA PYRIFORMIS*

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## SUMMARY

1. *Tetrahymena pyriformis* NT-I cells were grown in the medium supplemented with 1-*O*-hexadecyl glycerol which is the precursor for alkyl ether-containing phospholipids; choline phosphoglyceride and 2-aminoethylphosphonolipid, and alterations in the plasma membrane and microsome lipid composition were examined. No incorporation of supplemented 1-*O*-hexadecyl glycerol was seen in ethanolamine phosphoglyceride.

2. The hexadecyl glycerol fed membranes contain more polyunsaturated fatty acids than do the native membranes. However, the level of oleic acid ($C_{18:1}$) drops strikingly in the phospholipids of plasma and microsome membranes.

3. The hexadecyl glycerol-feeding induced a remarkable alteration in the polar headgroup composition of plasma membrane, especially a large increase in 2-aminoethylphosphonolipid with a compensatory decrease in ethanolamine phosphoglyceride of plasma membranes.

4. The fatty acyl chain composition of phospholipids, especially ethanolamine phosphoglyceride, of the hexadecyl glycerol-fed plasma membranes and microsomes was found to be significantly different from that of the native membranes.

5. These results may indicate that marked alterations in polar headgroup as well as fatty acyl chain composition of membranes induced by glyceryl ether-feeding would be required for maintaining proper membrane fluidity.

## INTRODUCTION

Many experiments have recently been conducted to understand the relationship between the physical states of the biological membrane lipids and membrane functions, as reviewed by Singer [1].

In order to obtain direct evidence for proving such a relationship, we chose the ciliated protozoan, *Tetrahymena pyriformis* cell as useful model system, since this organism can undergo in vivo manipulation of membrane lipids in response to

growth conditions, such as temperature change [2–4], exogenous supplementation of fatty acids (ref. 5 and Martin, C. E., Skriver, L., Kitajima, Y., Nozawa, Y. and Thompson, G. A., in preparation), or sterol [6, 7], and starvation [8].

We have previously shown the marked alteration in the membrane lipid composition by changing the growth temperature [2, 3] and by replacing a triterpenoid tetrahymanol by ergosterol [7]. There was a striking change in the fatty acyl chain as well as polar headgroup composition of membrane phospholipids. Furthermore, it was demonstrated by electron spin resonance that such manipulated membranes are modified in the membrane fluidity [2].

As reported in the earlier papers, the *Tetrahymena* membranes, especially surface membranes, are rich in unusual lipids of 2-aminoethylphosphonolipid and glyceryl ether-containing phospholipid [9–11], yet the roles of these specific lipids in controling membrane fluidity are still unclear.

In the present study, we therefore examined the effects upon membrane lipid composition by feeding to *Tetrahymena* cells hexadecyl glycerol which is a precursor for glyceryl ether-containing phospholipids.

## MATERIALS AND METHODS

### *Growth of T. pyriformis NT-I cells*

A thermotolerant strain of *T. pyriformis* NT-I is grown at 39.5 °C in an enriched proteose-peptore medium as previously described [3, 9].

For the hexadecyl glycerol-feeding experiment, aqueous dispersion of 1-*O*-hexadecyl glycerol was prepared as follows; prior to adding sufficient water to give a concentration of 0.5 mg hexadecyl glycerol/ml, weighed amounts of hexadecyl glycerol were dissolved in a minimal amount of ethanol (approx. 1–2 drops per 5 mg of hexadecyl glycerol). Sonication for 3–5 min in an ice bath using a Branson sonifier produced a homogenous milky dispersion.

When the cell density reached $1 \cdot 10^5$ cells/ml, the hexadecyl glycerol solution was added to the growth medium (2 mg/200 ml of culture).

Subsequently, when the cell density became $2 \cdot 10^5$ and $4 \cdot 10^5$ cells/ml, 4 and 8 mg hexadecyl glycerol/200 ml culture were supplemented, respectively. Finally, 200 ml culture were harvested after 30–32 h inoculation, when cells were in late-logarithmic growth phase (approx. $6 \cdot 10^5$ cells/ml).

### *Isolation of surface membranes from T. pyriformis NT-I cells*

The harvested cells were resuspended in 0.2 M phosphate buffer containing 0.1 M NaCl/3 mM EDTA, and plasma membranes and microsome were isolated according to Nozawa and Thompson's procedure [9, 12]. The purity of isolated membranes was examined by electron microscopy.

Lipids were extracted from individual membrane fractions by the method of Bligh and Dyer [13] and the resultant lipid solutions were stored in chloroform/methanol (6 : 1 v/v) at −20 °C.

Lipid phosphorus was determined by the procedure of Bartlett [14] as modified by digestion with perchloric acid according to Marinetti [15]. Thin-layer chromatography on Silica Gel H was done as described earlier [9]. The estimation of

phospholipid distribution was carried out by a minor modification of Rouser's method [16]. Individual phospholipids were extracted three times with chloroform/methanol/water (1 : 2 : 0.8). The recovery of this extraction was more than 95 % [17]. The quantitative analysis of glyceryl ether-containing phospholipids was carried out as previously described [3]. Tetrahymanol was analysed quantitatively according to the method described previously [11]. For fatty acid analysis, methyl esters were prepared by Morrison's method [18]. These samples were examined with a JEOL Model JGC-1100 gas chromatograph. Each sample was injected onto a glass column (200 cm) packed with 15 % diethyleneglycol succinate supported on chromosorb W (80-100 mesh, Gaschro Kogyo Co., Tokyo). The column temperature was 185 °C and the pressure of the carrier gas, $N_2$, was 0.5 kg/cm$^2$. The unsaturation index of fatty acid composition is defined as $\Sigma$(number of double bonds in each fatty acid) $\times$ (mol % of each fatty acid) [19].

## RESULTS

*Effect of hexadecyl glycerol-feeding upon glyceryl ether content in Tetrahymena membranes and phospholipids*

No abnormality of cell growth and motility was observed after feeding hexadecyl glycerol.

The glyceryl ether content in the membrane phospholipids from the cells grown in the presence and absence of hexadecyl glycerol is presented in Table I. It is interesting that the glyceryl ether content of plasma membrane phospholipid was greatly increased by hexadecyl glycerol-feeding; 32.7 % for the native and 40.7 % for the fed-membranes. A smaller increase of glyceryl ether phospholipid was observed in microsomes.

Table II gives the glyceryl ether content in the major phospholipids from cells grown in the presence and absence of hexadecyl glycerol. Unlike the case of *T. pyriformis* W cells as reported by Berger et al. [20], choline phosphoglyceride has a higher glyceryl ether content than does 2-aminoethylphosphonolipid in the thermo-

TABLE I

GLYCERYL ETHER CONTENT IN NATIVE AND HEXADECYL GLYCEROL FED-*T. PYRIFORMIS* NT-I MEMBRANE PHOSPHOLIPIDS

Lipids were extracted from isolated membranes according to the method of Blig and Dyer [13]. The extracted lipids were hydrolyzed with 1M KOH in 90 % ethanol, extracted with diethyl ether and total phosphorus in the aqueous and ether phases was determined to estimate the glyceryl ether content in phospholipids [3]. The numbers in parentheses represent the number of different experiments. Values are means $\pm$S.D.

| Fraction | Glyceryl ethers (mol/100 mol of lipid phosphorus) | |
|---|---|---|
| | Native | Hexadecyl glycerol-fed |
| Whole cells | 28.8$\pm$7.1(10) | 33.0$\pm$6.3(8) |
| Plasma membranes | 32.7$\pm$4.0(6) | 40.7$\pm$3.5(5) |
| Microsomes | 33.1$\pm$2.5(5) | 39.3$\pm$3.4(4) |

TABLE II

GLYCERYL ETHER CONTENT IN THE MAJOR PHOSPHOLIPIDS FROM NATIVE AND HEXADECYL GLYCEROL FED-*T. PYRIFORMIS* NT-I CELLS

Total lipids were extracted by the method of Bligh and Dyer [13]. Individual phospholipids were separated on Silica Gel H thin-layer chromatographic plates and eluted three times with chloroform methanol water (1:2:0.8). The content of glyceryl ether phospholipids was estimated by the same method as in Table I. The numbers in parentheses represent the number of different experiments. Values are means ±S.D.

| Major phospholipids | Glyceryl ethers (mol/100 mol of lipid phosphorus) | |
|---|---|---|
| | Native | Hexadecyl glycerol-fed |
| Choline phosphoglycerides | 66.0±2.5(4) | 71.2±7.6(3) |
| 2-Aminoethylphosphonolipids | 20.2±3.1(4) | 27.4±4.8(3) |
| Ethanolamine phosphoglycerides | 0 | 0 |

tolerant NT-I cells used in this study. This discrepancy may be due to the difference of the strain employed between two groups. By hexadecyl glycerol-feeding both choline phosphoglyceride and 2-aminoethylphosphonolipid became richer in glyceryl ether level, indicating that most of choline phosphoglycerides are of glyceryl ether-type lipid. Nevertheless, no incorporation of glyceryl ether into ethanolamine phosphoglycerides was found.

*Alteration of polar headgroup composition in the hexadecyl glycerol-fed plasma membrane and microsome*

The total amount of phospholipids in the hexadecyl glycerol-fed cells is slightly higher than the normal cells; 3.885 $\mu g/10^6$ cells and 4.988 $\mu g/10^6$ cells for the native and the fed cells, respectively. However, the molar ratio of tetrahymanol to phospholipid was not found changed. It was found that the glyceryl ether-feeding induced alterations in membrane phospholipid class composition. The phospholipid composition of plasma membranes and microsomes isolated from cells grown in the presence and absence of hexadecyl glycerol is presented in Table III. Although the change in percent of choline phosphoglyceride was not noted, there was a large increase in 2-aminoethylphosphonolipid with a corresponding decrease in ethanolamine phosphoglyceride of fed-plasma membrane. In contrast, as for microsomes, small changes were found in phospholipid composition and there was a slight drop in the percent of ethanolamine phosphoglyceride accompanied by a small increase of choline phosphoglyceride plus 2-aminoethyl-phosphonolipid.

*Alteration by hexadecyl glycerol-feeding in fatty acyl chain composition in the plasma membrane and microsome lipids*

Table IV shows the fatty acid composition of total lipids from native and hexadecyl glycerol-fed plasma membranes and microsomes. A remarkable increase of palmitic acid ($C_{16:0}$) together with a slight elevation of the content of polyunsaturated fatty acids, linoleic ($C_{18:2}$) and $\gamma$-linolenic ($C_{18:3}$) acids were noted in both membranes, thus not causing a noticeable change in the ratio of total unsaturated to

TABLE III

ALTERATIONS INDUCED BY HEXADECYL GLYCEROL-FEEDING IN PHOSPHOLIPID COMPOSITION IN PLASMA MEMBRANES AND MICROSOMES OF *T. PYRIFORMIS* NT-I

Lipids were extracted from the isolated membranes according to the method of Bligh and Dyer [13]. Individual phospholipids were separated on Silca Gel H thin-layer chromatographic plates and quantitatively analyzed by the Rouser's method [16]. The numbers in parentheses represent the number of different experiments. Values are means ±S.D.

| Membranes | Mol % of total phospholipids | | | | | |
|---|---|---|---|---|---|---|
| | 2-Aminoethyl phosphonolipids | Lyso-ethanolamine phosphoglycerides plus lyso-2-amino-ethyl phosphono-lipids | Ethanolamine phosphoglycerides | Lyso-choline phosphoglycerides | Choline phosphoglycerides | Cardiolipin |
| Plasma membranes | | | | | | |
| Native (6) | 21.9±4.5 | 0.9±1.6 | 46.8±3.1 | 2.6±0.6 | 25.0±1.7 | 1.0±0.6 |
| Hexadecyl glycerol-fed(6) | 30.5±1.8 | 1.1±0.5 | 37.1±1.6 | 2.7±0.8 | 25.5±2.0 | 1.3±0.9 |
| Microsomes | | | | | | |
| Native (3) | 20.9±0.7 | 0.6±0.3 | 40.9±0.7 | 1.5±1.3 | 33.2±1.0 | 1.9±1.0 |
| Hexadecyl glycerol-fed(3) | 22.6±1.1 | 1.0±0.7 | 36.1±0.9 | 1.9±0.9 | 35.5±0.5 | 2.1±0.1 |

TABLE IV

FATTY ACID COMPOSITION OF TOTAL LIPID FROM NATIVE AND HEXADECYL GLYCEROL-FED *T. PYRIFORMIS* MEMBRANES

Lipids were extracted according to the method of Bligh and Dyer [13] from the membranes from cells grown in the presence and absence of hexadecyl glycerol. For fatty acid analysis the lipids were methylated and analysed by gas-liquid chromatography. Minor components not shown in this Table contain $C_{15:0}$, iso $C_{15:0}$, $C_{17:0}$, iso $C_{17:0}$. The numbers in parentheses represent the number of different experiments.

| Fatty acids | Percentage fatty acid composition of total lipids from: | | | |
|---|---|---|---|---|
| | Plasma membranes | | Microsomes | |
| | Native(6) | Hexadecyl glycerol-fed(6) | Native(3) | Hexadecyl glycerol-fed(3) |
| $C_{12:0}$ | 2.7 | 2.6 | 0.8 | 0.5 |
| $C_{14:0}$ | 10.4 | 6.9 | 7.8 | 5.5 |
| $C_{16:0}$ | 16.9 | 21.0 | 12.4 | 19.9 |
| $C_{16:1(\Delta^9)}$* | 9.6 | 10.7 | 11.1 | 13.5 |
| $C_{18:0}$ | 2.7 | 2.8 | 1.6 | 1.8 |
| $C_{18:1(\Delta^9)}$ | 11.9 | 9.4 | 14.4 | 4.5 |
| $C_{18:2(\Delta^{6,11})}$** | 3.0 | 3.3 | 5.6 | 3.1 |
| $C_{18:2(\Delta^{9,12})}$ | 11.9 | 14.2 | 14.5 | 16.7 |
| $C_{18:3(\Delta^{6,9,12})}$ | 19.4 | 21.5 | 21.4 | 23.6 |
| Unsaturation index*** | 109 | 119 | 129 | 128 |
| Total unsaturated | 55.8 | 59.1 | 67.0 | 61.4 |
| Total saturated | 32.7 | 33.3 | 22.6 | 27.7 |
| $\Sigma U/\Sigma S$ | 1.70 | 1.78 | 2.96 | 2.21 |

* The peak contains small amount of iso $C_{17:0}$

** The peak had been previously designated as $C_{18:2(\Delta^{6,9})}$, but was recently identified to be $C_{18:2(\Delta^{6,11})}$ by Ferguson et al. [6]

*** The unsaturation index is defined as $\Sigma$(number of double bonds in each fatty acid) × (mol % of each fatty acid).

saturated fatty acid content. The level of oleic acid ($C_{18:1}$) content was found to be much lower in hexadecyl glycerol-fed microsomes than in native membranes.

*Alteration of fatty acyl chain composition in individual phospholipids of hexadecyl glycerol-fed membranes*

The fatty acyl chain composition of major phospholipids from native and hexadecyl glycerol-fed plasma membrane is given in Table V. Each phospholipid has a characteristic fatty acid composition. For example, 2-aminoethylphosphonolipid of native plasma membrane has a higher content of $\gamma$-linolenic acid (38.5 %), thereby being the most unsaturated phospholipid, whereas ethanolamine phosphoglyceride contains small amounts of $C_{18:3}$ (7.9 %). The hexadecyl glycerol-feeding produced a general trend towards an increase of polyunsaturated fatty acids, linoleic ($C_{18:2}$) and $\gamma$-linolenic ($C_{18:3}$). A marked decline in the level of oleic acid ($C_{18:1}$) was observed in ethanolamine and choline phosphoglycerides from the hexadecyl glycerol-fed plasma membranes. The acyl chain composition of these major phospholipids

TABLE V

FATTY ACYL CHAIN COMPOSITION OF PHOSPHOLIPIDS FROM NATIVE AND HEXADECYL GLYCEROL-FED PLASMA MEMBRANES OF *T. PYRIFORMIS* NT-I

For quantitative analysis of individual phospholipids, total lipids extracted according to Bligh and Dyer's method [13] from plasma membranes from cells grown in the presence and absence of hexadecyl glycerol were applied and separated on Silica Gel H thin-layer plates. For fatty acid analysis, each phospholipid fraction eluted from the plate was methylated and examined by gas-liquid chromatography. The numbers in parentheses represent the number of different experiments

| Fatty acyl chains | Percentage fatty acid composition of phospholipids | | | | | |
|---|---|---|---|---|---|---|
| | 2-Aminoethyl phosphonolipids | | Ethanolamine phosphoglycerides | | Choline phosphoglycerides | |
| | Native (3) | Hexadecyl glycerol-fed (3) | Native (3) | Hexadecyl glycerol-fed (3) | Native (3) | Hexadecyl glycerol-fed (3) |
| $C_{12:0}$ | 0.5 | 0.4 | 3.8 | 3.5 | 0.4 | 0.2 |
| $C_{14:0}$ | 4.5 | 2.6 | 14.1 | 11.5 | 5.9 | 3.4 |
| $C_{16:0}$ | 8.2 | 7.9 | 19.1 | 25.9 | 12.6 | 14.0 |
| $C_{16:1(\Delta^9)}$* | 6.7 | 7.2 | 11.2 | 12.7 | 8.2 | 8.4 |
| $C_{18:0}$ | 2.1 | 1.6 | 2.6 | 2.3 | 3.0 | 2.4 |
| $C_{18:1(\Delta^9)}$ | 11.6 | 11.3 | 13.3 | 8.0 | 15.7 | 9.9 |
| $C_{18:2(\Delta^{6,11})}$** | 6.1 | 6.4 | 0.5 | 0.6 | 4.5 | 3.7 |
| $C_{18:2(\Delta^{9,12})}$ | 14.4 | 19.6 | 11.1 | 14.4 | 11.7 | 16.0 |
| $C_{18:3(\Delta^{6,9,12})}$ | 38.5 | 37.7 | 7.9 | 10.2 | 28.5 | 33.2 |
| Unsaturation index*** | 174 | 183 | 71 | 81 | 141 | 157 |
| Total unsaturated | 77.3 | 82.2 | 44.0 | 45.9 | 68.6 | 71.2 |
| Total saturated | 15.3 | 12.5 | 39.6 | 43.2 | 21.9 | 20.0 |
| $\Sigma U/\Sigma S$ | 5.05 | 6.57 | 1.10 | 1.06 | 3.13 | 3.56 |

* The peak contains small amount of $isoC_{17:0}$

** The peak had been previously designated as $_{(18:2(\Delta^{6,9}))}$, but was recently identified to be $C_{18:2(\Delta^{6,11})}$ by Ferguson et al. [6]

*** The unsaturation index is defined as $\Sigma$(number of double bonds in each fatty acid) × (mol % of each fatty acid).

TABLE VI

FATTY ACYL CHAIN COMPOSITION OF PHOSPHOLIPIDS FROM NATIVE AND HEXADECYL GLYCEROL-FED MICROSOMES OF *T. PYRIFORMIS* NT-I

Lipids were extracted from microsomes by the method of Bligh and Dyer [13]. Fatty acid analysis of individual phospholipids was performed by the same procedure as described in Table V. The numbers in parentheses represent the number of different experiments.

| Fatty acyl chains | Percentage fatty acid composition of phospholipids | | | | | |
|---|---|---|---|---|---|---|
| | 2-Aminoethyl phosphonolipids | | Ethanolamine phosphoglycerides | | Choline phosphoglycerides | |
| | Native (2) | Hexadecyl glycerol-fed (2) | Native (2) | Hexadecyl glycerol-fed (2) | Native (2) | Hexadecyl glycerol-fed (2) |
| $C_{12:0}$ | trace | trace | 1.0 | 0.9 | 0.3 | 0.3 |
| $C_{14:0}$ | 3.2 | 2.5 | 7.7 | 6.7 | 5.3 | 3.0 |
| $C_{16:0}$ | 7.1 | 8.0 | 13.8 | 22.9 | 10.9 | 16.1 |
| $C_{16:1(\Delta^9)}$* | 8.1 | 9.6 | 14.5 | 18.7 | 11.0 | 12.8 |
| $C_{18:0}$ | 1.2 | 1.6 | 1.8 | 1.5 | 2.1 | 1.5 |
| $C_{18:1(\Delta^9)}$ | 12.1 | 5.9 | 18.0 | 4.8 | 18.1 | 5.5 |
| $C_{18:2(\Delta^{6,11})}$** | 8.2 | 6.2 | 1.7 | 1.2 | 4.7 | 3.1 |
| $C_{18:2(\Delta^{9,12})}$ | 15.1 | 18.9 | 14.9 | 18.4 | 13.5 | 17.7 |
| $C_{18:3(\Delta^{6,9,12})}$ | 37.0 | 38.0 | 13.7 | 13.2 | 22.5 | 27.5 |
| Unsaturation index*** | 177 | 179 | 106 | 102 | 133 | 142 |
| Total unsaturated | 80.5 | 78.6 | 62.8 | 56.3 | 69.8 | 66.6 |
| Total saturated | 11.5 | 12.1 | 24.3 | 32.0 | 18.6 | 20.9 |
| $\Sigma U/\Sigma S$ | 7.00 | 6.49 | 2.58 | 1.75 | 3.75 | 3.18 |

* The peak contains small amount of $isoC_{17:0}$

** The peak had been previously designated as $C_{18:2(\Delta^{6,9})}$, but was recently identified to be $C_{18:2(\Delta^{6,11})}$ by Ferguson et al. [6]

*** The unsaturation index is defined as $\Sigma$(number of double bonds in each fatty acid) $\times$ (mol % of each fatty acid).

from the fed-membranes is more unsaturated as shown by a higher unsaturation index.

Table VI shows the fatty acid composition of phospholipid from native and hexadecyl glycerol-fed microsomes. The increase in palmitic ($C_{16:0}$) and decrease in oleic ($C_{18:1}$) acids of choline and ethanolamine phosphoglycerides from fed-microsomes were more noticeable than those of the fed-plasma membranes. Unlike the case of plasma membranes the relative percentage of total unsaturated fatty acid declined in the hexadecyl glycerol-fed microsomes, thereby the ratio of total unsaturated to saturated fatty acids being higher as compared with that of native microsomes.

## DISCUSSION

It is now widely appreciated that the dynamic structure (fluidity) of the membrane lipid bilayer may be closely associated with membrane functions [1]. Much interest has recently been focussed upon obtaining direct evidences for such close interrelationship between fluidity and function in biological membranes. A possible approach is to see whether membrane functions are influenced by alteration of membrane lipid composition. For this purpose *Tetrahymena* cell is a potentially useful model system, since, besides several advantages such as bacteria-like rapid growth, easy isolation of membrane components [9, 12], presence of unusual lipids [9–11], easy labeling of membranes with isotope [21], spin-probe [2], and fluorescence, one big advantage is the easiness to manipulate in vivo the lipid composition of different membrane components. In fact, we have undertaken a series of in vivo lipid manipulations using *T. pyriformis*, and presented several lines of evidence that phospholipid as well as its acyl chain composition can be altered by growing cells at hot (39.5 °C) and cold (15 °C) temperatures [3], by temperature-shift [2], and also by ergosterol-replacement [7]. More recently it was found that exogenous supplementation of fatty acids, e.g., linoleic, γ-linolenic acid, altered membrane lipid composition, resulting in the change in membrane fluidity (Martin, C. E., Skriver, L., Kitajima, Y., Nozawa, Y. and Thompson, G. A., in preparation). In our earlier work we have shown that membranes from cells grown at 15 °C are more fluid than those grown at 34 °C [2], and ergosterol-replaced surface membranes are less fluid than the native membranes by fluorescence and electron spin resonance.

The present study was undertaken in order to modify lipid composition by hexadecyl glycerol in a thermotolerant *T. pyriformis* NT-1 which has a higher level of glyceryl ether phospholipid. The results presented here show that hexadecyl glycerol-feeding produced a larger proportion of glyceryl ether phospholipid in membranes, probably due to stimulated biosynthesis of ether lipids; 2-aminoethylphosphonolipid and choline phosphoglyceride. However, no incorporation of hexadecyl glycerol into ethanolamine phosphoglyceride was observed. The mechanism is unresolved by which such a strict selective incorporation of glyceryl ether can occur. A marked increase of glyceryl ether-containing 2-aminoethylphosphonolipid was found in plasma membrane, but with a concurrent decrease of ethanolamine phosphoglycerides, while a small increase of 2-aminoethylphosphonolipid and choline phosphoglyceride content is compensated by the ethanolamine phosphoglyceride decrease in microsomes. Furthermore, in addition to such a striking alteration of polar head-group composition, the fatty acyl chain composition of individual phospholipids did

remarkedly change. A general trend towards increase in polyunsaturated fatty acids ($C_{18:2}$, $\gamma$-$C_{18:3}$) and palmitic acid ($C_{16:0}$) and decrease in oleic acid ($C_{18:1}$) was observed. This was especially obvious for ethanolamine phosphoglycerides. It is easy to understand that since C-1 positions of 2-aminoethylphosphonolipid and choline phosphoglycerides are occupied by incorporated glyceryl ethers, acyl chains at C-2 positions must be changed to maintain the equivalent roles to diacyl-type phospholipids in lipid bilayers of the non-fed membranes. However, it should be noted that the most striking alteration in acyl chain composition occurred to ethanolamine phosphoglycerides containing no glyceryl ether. This may indicate that the altered polar headgroup composition by glyceryl ether-feeding induced the rearrangement of acyl chain distribution in phospholipid molecules. We should consider involvement of glyceryl ether at C-1 position in a phospholipid in controlling the membrane fluidity. There has been only one report demonstrating that diether-type phospholipid displayed consistently higher transition temperatures than their corresponding diacyl-type phospholipids [22]. Thus, one would expect that the marked alteration induced by glyceryl ether-feeding in polar headgroup as well as acyl chain composition would be required for keeping the membrane fluidity proper to conduct membrane functions properly. The finding of such lipid alteration prompted us to examine its influence upon membrane fluidity and some preliminary experiments are now in progress.

It would therefore, be hoped that some useful information could be obtained about the role of glyceryl ether lipid in membrane fluidity. Furthermore, since *Tetrahymena* membrane lipid can be easily in vivo manipulated by feeding exogenous sterol, fatty acid or glyceryl ether, and growth temperature change, this cell is a potentially useful model system for studies to elucidate the relationship between the physical state of lipids and their functions in biological membranes.

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REFERENCES

1 Singer, S. J. (1974) Ann. Rev. Biochem. 43, 805–833
2 Nozawa, Y., Iida, H., Fukushima, H., Ohki, K. and Ohnishi, S. (1974) Biochim. Biophys. Acta 367, 134–147
3 Fukushima, H., Martin, C. E., Iida, H., Kitajima, Y., Thompson, G. A. and Nozawa, Y. (1976) Biochim. Biophys. Acta in the press.
4 Wunderlich, F. and Ronai, A. (1975) FEBS Lett. 55, 237–241
5 Lees, A. M. and Korn, E. D. (1966) Biochemistry 5, 1475–1481
6 Ferguson, K. A., Davis, F. M., Conner, R. L., Landrey, J. R. and Mallory, F. B. (1975) J. Biol. Chem. 250, 6998–7005
7 Nozawa, Y., Fukushima, H. and Iida, H. (1975) Biochim. Biophys. Acta 406, 248–263
8 Thompson, G. A., Bambery, R. A. and Nozawa, Y. (1972) Biochim. Biophys. Acta 206, 630–638
9 Nozawa, Y. and Thompson, G. A. (1971) J. Cell Biol. 49, 712–721
10 Nozawa, Y. and Thompson, G. A. (1971) J. Cell Biol. 49, 722–730

11 Thompson, G. A., Bambery, R. J. and Nozawa, Y. (1971) Biochemistry 10, 4441–4447
12 Nozawa, Y. (1975) Method in Cell Biology (Prescott, D. M., ed.), Vol. X, pp. 105–133, Academic Press, New York
13 Bligh, E. G. and Dyer, W. J. (1959) Can. J. Biol. Physiol. 31, 911–917
14 Bartlett, G. R. (1959) J. Biol. Chem. 234, 466–468
15 Marinetti, G. V. (1962) J. Lipid Res. 3, 1–11
16 Rouser, G., Siakotos, A. N. and Feischer, S. (1966) Lipids 1, 85–86
17 Kates, M. (1972) Laboratory Techniques in Biochemistry and Molecular Biology (Work, T. S. and Work, E., eds.), Vol. III, pp. 444–446, American Elsevier Publishing Co., Inc., New York
15 Morrison, W. R. and Smith, L. M. (1964) J. Lipid Res. 5, 600–608
19 Williams, M. A., Stancliff, R. C., Packer, L. and Keith, A. D. (1972) Biochim. Biophys. Acta 267, 444–456
20 Berger, H., Jones, P. and Hanahan, D. J. (1972) Biochim. Biophys. Acta 260, 617–629
21 Thompson, G. A. (1972) J. Protozool. 19, 231–236
22 Vaughan, D. J. and Keough, K. M. (1974) FEBS Lett. 47, 158–161