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MEMBRANE CHANGES DURING GROWTH OF *TETRAHYMENA* IN THE PRESENCE OF ETHANOL

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Summary

After a brief lag period for acclimation, *Tetrahymena pyriformis*, strain NT-1, is capable of growing in culture medium containing high levels of ethanol. When grown in a medium having 1.6% ethanol, the membrane phospholipid composition was significantly different from that of control cells. The principal changes included a reduction in hexadecenoic acids (16 : 1 and 16 : 2) from 23% to 5% and an increase in linoleic acid (18 : 2) from 14% to 25%.

Similar but less pronounced changes were observed in cells grown in lower ethanol concentrations. There was also a decrease in 2-aminoethylphosphonolipid in the ethanol-grown cells from 16% of the lipid phosphorus to 6% and a coincident rise in the phosphatidylethanolamine from 39% to 46%. The lipid pattern quickly reverted to normal when ethanol was removed.

In order to ascertain the effects of ethanol on membrane physical properties, freeze-fracture electron microscopy and fluorescence polarization studies were performed. Ethanol, as expected, had a clearly detectable fluidizing influence when present at 1.6%. However, changes induced in the membrane lipids by growth in the ethanol-containing medium led to a further fluidizing effect, whether they were tested in the presence or absence of ethanol. The alterations found here were qualitatively similar but quantitatively much more pronounced than those observed in mammals chronically exposed to ethanol.

Introduction

The remarkably efficient adaptation of living creatures to environmental extremes often involves modification of their membrane lipid composition

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[1—3]. The protozoan *Tetrahymena pyriformis* exhibits a typical acclimation response when exposed to decreased temperature [4—8]. In acclimating, the cell quickly provides additional unsaturated fatty acids for its membrane phospholipids, thereby increasing membrane fluidity to the same optimal range it occupied previously at the higher temperature [8].

We have established that the same basic molecular mechanism employed by *Tetrahymena* in adapting to changes in temperature is also used to adjust the physical properties of membranes perturbed by dietary fatty acids [4,10] and inhalation anesthetics [9].

In view of the known anesthetic effects of ethanol [11] and the possible long-term effects it might have on cellular membranes of chronic alcoholics, it seemed appropriate to examine the effect of ethanol on the extensively studied membranes of *Tetrahymena*. The findings described in this communication indicate that while ethanol induces large changes in the *Tetrahymena* membrane lipid composition, these changes do not appear to be compensatory in the sense discussed above.

Materials and Methods

Cell growth and culture conditions. Culture conditions for *Tetrahymena pyriformis*, strain NT-1, were as described elsewhere [5]. The organism was grown under sterile conditions at 28°C, with shaking, and the cell densities were monitored with a Coulter Counter Model B.

Cell growth in the presence of ethanol. Cells were grown in medium containing ethanol in concentrations varying from 0.4% to 1.6% (w/v). In the latter case, 10-ml suspensions of cells growing logarithmically in regular medium were innoculated into 200 ml of the same medium containing 4.2 ml absolute ethanol, for a final concentration of 1.6% (0.35 M). Cells were allowed to grow in the presence of ethanol for 3—4 days, after which 10-ml aliquots of this cell suspension were transferred to fresh flasks containing 200 ml growth medium plus 4 ml ethanol. Unless otherwise specified, 'ethanol adapted' cells refers to cells used for analysis after 8—10 days growth in ethanol medium.

Estimation of ethanol in the medium. The ethanol content in the medium was determined using a yeast alcohol dehydrogenase-NAD assay. Initial rates were determined by absorbance measurement at 340 nm in a Gilford spectrophotometer. 3.05 units of crystalline alcohol dehydrogenase (Sigma, St. Louis, MO) served as a source of the enzyme, and the NAD^+ (Sigma) concentration was 1.25 mM. Assays, performed at pH 8 and 21°C, were linear in the range of 1—20 mM ethanol.

Lipid isolation and analysis. Cells were harvested at specified cell densities by centrifugation, and cell fractionations were performed by the procedure of Nozawa and Thompson [12]. Lipids were extracted by the method of Bligh and Dyer [13]. Separation of phospholipids on silicic acid columns and preparation of fatty acid methyl esters are described elsewhere [5].

Gas chromatography was performed using a Varian-3700 chromatograph with a 6 ft long, 0.25 inch diameter stainless steel column containing 10% diethyleneglycol succinate on 100-mesh Chromosorb WAW support, at a column temperature of 176°C and carrier gas flow of 19 ml/min. Phospholipids

were estimated by the method of Rouser et al. [14], modified by the use of a different solvent system, chloroform/acetic acid/methanol/H_2O, 75 : 25 : 5 : 2.2 (by vol.), to separate individual phospholipid classes on silica gel-H thin-layer plates.

Freeze-fracture electron microscopy. 20-ml aliquots of cell suspensions treated as indicated below were chilled from 28°C to 9°C or 12°C and fixed with 1/3 volume of 4% glutaraldehyde in 0.2 M sodium phosphate buffer (pH 7.2) [8].

The fixed cells were examined by freeze-fracture electron microscopy [8]. Prior to chilling and fixation, different aliquots of cells were pretreated as follows: (a) The cells in a 200 ml culture growing logarithmically in ethanol-free medium were centrifuged, resuspended in 20 ml fresh medium and incubated with shaking for 30 min. (b) Cells grown identically to experiment (a) (above) were centrifuged, resuspended in 20 ml fresh medium containing 0.35 M ethanol and incubated with shaking for 30 min. (c) Cells in a 200 ml culture grown in 0.35 M ethanol for ten days were centrifuged, resuspended in 20 ml medium containing 0.35 M ethanol and incubated for 30 min. (d) Cells grown in ethanol-containing medium as in experiment (c) (above) were centrifuged, resuspended in 20 ml ethanol-free medium and incubated for 30 min.

Platinum replicas made from the fractured preparations were examined for particle-free areas in endoplasmic reticulum (microsomes). The extent of intramembraneous particle aggregation in the outer alveolar membrane was calculated as the particle density index [8]. Higher particle density index values indicate lower fluidity.

Fluorescence depolarization. Dipalmitoylphosphatidylcholine (Serdary, London, Ontario) revealed only a single spot at the expected position by thin-layer chromatography. The fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (Aldrich Chemicals, Milwaukee, WI) was utilized without further purification.

The cells were grown at 25.5°C in the presence or absence of ethanol and the lipids were extracted as described above.

Multilamellar liposomes were prepared by evaporating a mixture of lipid and the fluorescent probe, in $CHCl_3$ under N_2. A vacuum was then applied to the dried sample for 30 min during which the system was intermittently purged with N_2. 2 ml of 15% sucrose/50 mM KCl solution containing in some cases 0.35 M ethanol was added to the tube along with a glass bead and the sample was then sonicated for 1 min under a stream of N_2 in a sonicator bath maintained at 25°C.

The fluorescence intensities of 1,6-diphenylhexatriene-labeled liposomes were measured in a device described previously [18] after passing part of the emitted light through one polarizer oriented parallel ($I_\parallel$) and part through another oriented perpendicular ($I_\perp$) to a plane polarized excitation beam. The lipid phosphorus concentration in the sample was 0.5 mM, and the molar ratio of lipid to probe was 500 : 1. Sample temperature was controlled to within ±0.1°C. Suitable corrections in polarization values to compensate for light scattering by the sample were made as described elsewhere [18]. Polarization values, P, were calculated according to $P = I_\parallel - I_\perp / I_\parallel + I_\perp$ which is related to the rotational relaxation of the molecule [19]. It has previously been shown that relaxation of 1,6-diphenylhexatriene in *Tetrahymena* lipid liposomes is com-

plex, and the polarization appears to be a sensitive function of the degree of order and the packing structure of the bilayer [20].

Results

Effect of ethanol on cell behavior

Tetrahymena cells can grow and divide in the presence of high concentrations of ethanol. The cells appear morphologically normal in media containing ethanol concentrations of 0.4%, 0.8% or 1.6%. All of these concentrations are well below the level of approximately 4.6% beyond which further increases produce unexpected and non-physiological responses in a number of standard tests, such as protection against hemolysis of erythrocytes, membrane disordering, and levels of membrane-bound calcium [11,21]. At ethanol levels of 2% or higher, *Tetrahymena* cells appear shrunken and move slowly with an abnormal swimming pattern.

After a brief lag period, cells transferred from normal medium to medium

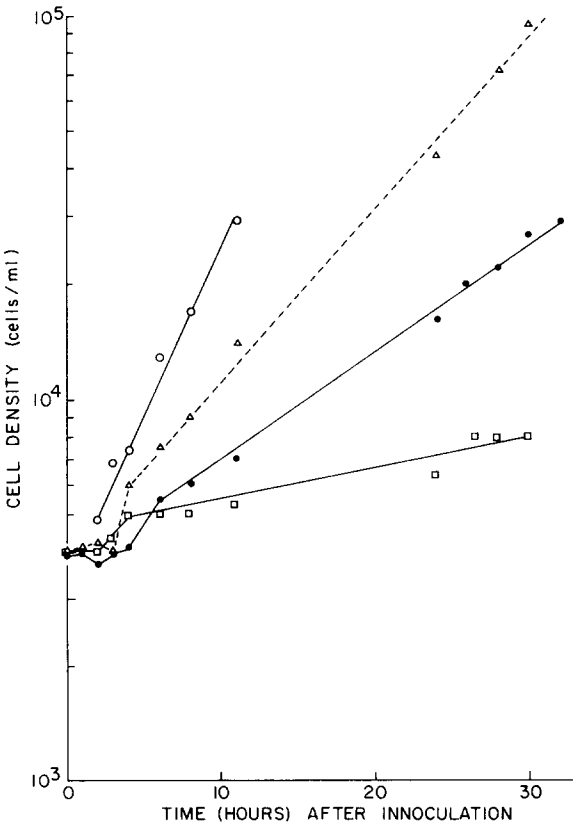

Fig. 1. Growth curve of *Tetrahymena* at 28°C under varying concentrations of ethanol. 10-ml suspensions of logarithmic phase cells were transferred to flasks of medium containing the final concentrations of ethanol indicated below. The flasks were then incubated at 28°C with shaking, and 2-ml aliquots of cells were removed at regular intervals for microscopic observation and counting. ○, control; △, 0.25 M ethanol; ●, 0.35 M ethanol; □, 0.45 M ethanol.

TABLE I

CONCENTRATION OF ETHANOL IN GROWTH MEDIUM AS DETERMINED BY THE ALCOHOL DEHYDROGENASE ASSAY

A, 200 ml enriched medium containing 0.35 M ethanol and no cells; B, 200 ml enriched medium innoculated with cell suspension and 0.35 M ethanol; C, 200 ml enriched medium innoculated with cell suspension but no ethanol. Aliquots of samples were centrifuged from each of three flasks at the beginning of incubation and after four days of incubation with shaking at 28°C,

Sample	Time (days)	ΔAbsorbance/ min	μmol alcohol/ 100 ml sample	% loss of alcohol in four days
A	0	0.82	11.1	
	4	0.79	9.76	10
B	0	0.75	8.83	
	4	0.71	7.14	15
C	0	0	0	
	4	0	0	—

containing ethanol resume growth at slower rates (Fig. 1). The average generation time increases with ethanol concentration (control, 3.5 h; 1.1% ethanol, 5.5 h; 1.6% ethanol, 8 h; 2.06% ethanol, 36 h).

A concentration of 1.6% (0.35 M) ethanol was chosen for most subsequent experiments. Cells could be maintained with a constant generation time and without any apparent abnormal behavior for at least 20 days in this concentration of ethanol. To prevent depletion of the ethanol, transfers to fresh ethanol medium were made every 3—4 days (before the cell density exceeded 100 000 cells/ml). No large losses of ethanol, either through evaporation or utilization by the cells, were evident during a four-day cell growth period as indicated by estimating the amount of ethanol in the medium initially and after four days (Table I).

Ethanol-induced lipid changes

Membrane phospholipid fatty acid composition was determined using cells grown in several ethanol concentrations and at various times after cell growth began in the presence of 0.35 M ethanol. A gradual change in composition was evident as the level of ethanol and the time in its presence increased (Table III). Significant decreases in 16 : 1 and 16 : 2 and a marked increase in 18 : 2 developed over the first eight days in 0.35 M ethanol, but no further fatty acid changes accrued during an additional 12 days.

The fatty acid composition reverted to control values within three generations of growth (12 h) when the long term ethanol-adapted cells were transferred back to the normal medium (Table III). This observation rules out the possibility that the ethanol-induced changes were due merely to the selective proliferation of a minority of cells naturally high in 18 : 2 and low in 16 : 1.

Few data are available in the literature concerning the intracellular concentrations of ethanol in cells exposed to it. In the present study, the effect of ethanol on lipids of intracellular membranes were perhaps even more pronounced than on membranes directly facing the medium. Table IV illustrates the alteration in fatty acid composition in ciliary membranes, pellicles (the complex of surface membranes), and microsomes. Thus we suspect that the

TABLE II

FATTY ACID COMPOSITION OF PHOSPHOLIPIDS FROM CONTROL CELLS AND CELLS GROWN IN THE PRESENCE OF ETHANOL

Fatty acid	Ethanol concentration (%)					Control ***
	0.4 *	0.8 *	1.6 **			
			for two days	for four days	for eight days ***	
14 : 0	9.7 ± 1.1	10.7 ± 0.8	9.2	8.7	10.4 ± 1.3	13.9 ± 3.3
16 : 0	10.7 ± 0.6	12.4 ± 0.5	13.0	14.5	16.4 ± 3.1	11.5 ± 1.5
16 : 1	15.8 ± 1.1	15.0 ± 0.9	11.3	6.4	7.1 ± 1.9	17.1 ± 2.4
16 : 2	4.1 ± 0.7	5.1 ± 0.2	3.8	2.5	1.5 ± 1.0	5.9 ± 1.1
18 : 0	1.4 ± 0.7	0.9 ± 0.2	2.3	2.5	2.6 ± 2.1	2.5 ± 1.3
18 : 1	7.7 ± 0.6	6.8 ± 1.0	4.5	3.5	2.7 ± 1.3	4.7 ± 2.2
18 : 2	22.1 ± 1.6	20.7 ± 1.2	19.1	22.2	21.8 ± 2.5	12.5 ± 1.4
18 : 3	24.6 ± 0.4	23.1 ± 0.9	20.9	24.9	24.3 ± 1.1	21.9 ± 3.6

* Cells were grown for approximately the same number of generations as those analyzed after eight days in 1.6% ethanol (see following footnote), with transfer to fresh medium whenever cells reached a density of $1 \cdot 10^5$—$1.5 \cdot 10^5$ cells/ml. Values are average mass % ± S.D. of four determinations from two separate experiments.

** A 10 ml cell suspension of logarithmically growing cells was transferred to each of four 500 ml flasks containing 200 ml enriched medium with 4.2 ml ethanol and incubated with shaking at 28°C for two days. 10-ml aliquots of this suspension were then transferred to a new set of four flasks containing 0.35 M ethanol while the remaining cells were used for fatty acid analysis. The second set of flasks was incubated for two days (total four days in ethanol) and 10-ml suspensions transferred to a new set of flasks from which lipids were extracted at the end of four days (total eight days).

*** Values are average mass % ± S.D. of four separate experiments.

cytoplasmic ethanol concentration is fairly high.

In addition to the changes in fatty acid composition brought on by growth in ethanol, there was a dramatic change in the relative proportions of the major phospholipid classes. Growth in ethanol induced a large reduction in 2-amino-

TABLE III

CHANGES IN FATTY ACID COMPOSITION OF PHOSPHOLIPIDS FROM *TETRAHYMENA* CELLS GROWN IN ETHANOL MEDIUM AND TRANSFERRED BACK TO NORMAL MEDIUM

Cells for sample (A) were grown in 0.35 M ethanol for 14 days. 10-ml aliquots ($1 \cdot 10^5$ cells/ml) of this culture were transferred either to 200 ml medium containing 0.35 M ethanol for an additional three generations of growth (B) or two 200 ml medium containing no ethanol for three generations of growth (C).

Fatty acid	Ethanol adapted (A)	Ethanol → ethanol (B)	Ethanol → normal (C)
14 : 0	11.4	12.5	14.2
15 : 0 ai	5.2	4.5	1.6
15 : 0	0.8	2.1	1.5
16 : 0	15.9	17.6	11.5
16 : 1	7.6	7.5	15.5
16 : 2	0.6	2.6	4.8
18 : 0	0.5	0.3	1.8
18 : 1	1.3	1.8	5.0
18 : 2	20.2	18.2	11.5
18 : 3	23.6	24.5	21.2

TABLE IV

MAJOR FATTY ACIDS IN DIFFERENT MEMBRANE FRACTIONS ISOLATED FROM *TETRAHYMENA* GROWN IN THE PRESENCE OR ABSENCE OF ETHANOL

A 10-ml cell suspension from logarithmically growing cells was transferred to 200 ml of medium containing 4.2 ml ethanol. The culture was allowed to grow for four days, at which time a 10 ml suspension of these cells was transferred to each of six flasks of medium containing 4 ml ethanol. Incubation continued with shaking for an additional four days before the cells were fractionated.

Fatty acid	Microsomes		Pellicle		Cilia	
	Control	Ethanol	Control	Ethanol	Control	Ethanol
14 : 0	9.8	6.4	12.2	8.7	7.1	6.4
16 : 0	8.5	13.5	13.1	20.3	16.4	32.6
16 : 1	16.4	4.0	14.6	4.9	12.9	5.9
16 : 2	5.8	1.3	4.9	1.3	2.5	1.4
18 : 0	4.4	1.5	4.2	2.2	7.1	10.8
18 : 1	5.9	5.7	7.2	5.8	9.2	11.6
18 : 2	13.5	32.5	9.2	27.6	13.2	11.4
18 : 3	24.1	31.2	19.6	22.8	23.3	15.7

ethylphosphonolipid and a coincident rise in phosphatidylethanolamine of whole cell phospholipids (Table V). Although no quantitative phosphorus analyses were carried out on cell fractions, a decrease in phosphonolipid was also evident there by visually examining charred thin-layer chromatograms of lipids from isolated cilia, pellicles, and microsomes. The differences were more striking in the microsomes and pellicles as compared to the cilia. The possibility that this decrease in phosphonolipid, with its own characteristic fatty acid composition, might be responsible for the observed change in total phospholipid fatty acid distribution was discarded because 18 : 2, the fatty acid species showing the largest increase, has been shown to be even more prevalent in the phosphonolipid than in other components [17].

Ethanol effects on membrane physical properties

It has been shown previously that alterations in *Tetrahymena* membrane fluidity caused by fatty acid changes can be detected with great sensitivity through the use of freeze-fracture electron microscopy of cells suddenly chilled to lower temperatures [15,16]. Quantitative estimates are readily made by calculating the degree to which sudden chilling induces intramembraneous pro-

TABLE V

THE DISTRIBUTION OF MAJOR *TETRAHYMENA* PHOSPHOLIPIDS IN CONTROL AND ETHANOL-ADAPTED CELLS

Values represent averages ± S.D. of three separate experiments and are expressed as percent of total lipid phosphorus.

Phospholipid class	Control cells	Ethanol-adapted cells
Phosphatidylcholine	28.6 ± 3.9	29.1 ± 5.3
Phosphatidylethanolamine	37.6 ± 2.6	46.4 ± 4.3
2-Aminoethylphosphonolipid	15.8 ± 2.4	6.3 ± 1.6
Cardiolipin	4.8 ± 1.8	4.3 ± 1.2

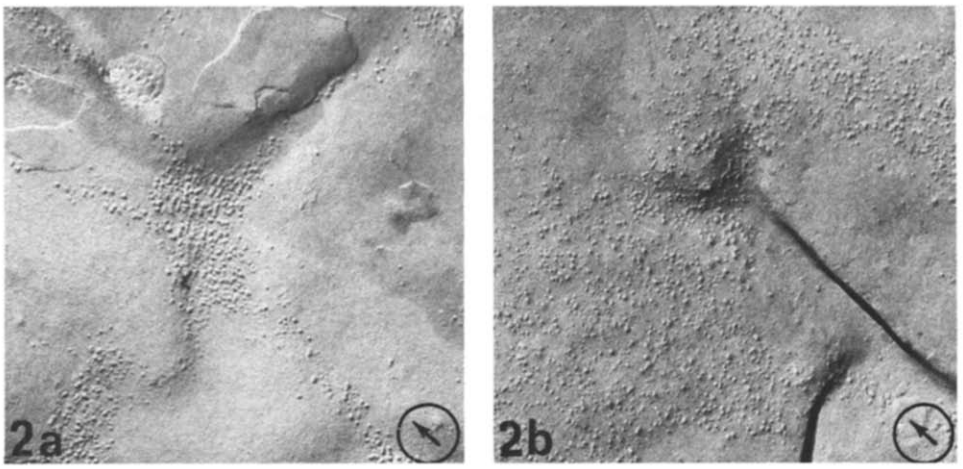

Fig. 2. Freeze-fracture photomicrographs of the outer alveolar membrane (OAM) protoplasmic face of cells grown at 28°C, chilled to 9°C over a 4 min period and fixed. Replicas were examined with a Hitachi HS-8 microscope at 50 kV. (a) X63 000. Typical pattern of control cell OAM showing tightly aggregated intramembranous particles. (b) X63 000. The OAM pattern found in cells grown for at least ten days in 0.35 M ethanol. The particle aggregation is much less pronounced than in controls (see Table VI).

tein particles of the outer alveolar membrane to aggregate by moving laterally in the plane of the membrane. Fluorescence depolarization measurements [18] have confirmed that increasing particle aggregation in that membrane means lower fluidity of the lipid bilayer.

In order to test the hypothesis that ethanol affects membrane fluidity, freeze-fracture electron microscopy was performed using ethanol-grown and control cells. By comparing the degree of particle aggregation in control cells with that in ethanol-acclimated cells fixed while growing in ethanol, significantly greater fluidity was indicated in the ethanol-grown cells (Fig. 2). The fluidizing effect of ethanol was also apparent in other membranes of the cells. Whereas particle-free areas, representing phase separation of lipids in these membranes, were detected in the microsomal membranes of control cells fixed after chilling to 12°C, none were evident in the ethanol-grown cells, even at 9°C (Table VI).

TABLE VI

THE PHYSICAL STATE OF *TETRAHYMENA* MEMBRANES IN THE PRESENCE OR ABSENCE OF ETHANOL AS OBSERVED BY FREEZE-FRACTURE ELECTRON MICROSCOPY

Treatment	Presence of particle-free regions in endoplasmic reticulum at:		Particle density index in outer alveolar membranes, chilled to 9°C
	9°C	12°C	
(a) Control	+	+	63.4
(b) Incubated with ethanol 30 min	+	+	58.4
(c) Grown in ethanol for ten days	—	—	30.0
(d) Grown in ethanol and transferred to normal medium for 30 min	—	—	45.8

Efforts were made to differentiate between the fluidizing action of the ethanol molecules themselves and any effect due to the altered lipid composition. Cells grown in the absence of ethanol were fixed for freeze-fracture analysis only 30 min after being placed in 0.35 M ethanol medium (see Materials and Methods). Conversely, ethanol-grown cells were shifted into ethanol-free medium and fixed after 30 min. The object was to determine, in the first case, the effect of ethanol on membranes that had not yet altered their lipid composition in response to ethanol and, in the second case, the effect on membrane fluidity of ethanol-induced lipid alterations tested in the absence of ethanol.

Examination of freeze-fracture replicas from the above experiments revealed that membranes of the cells briefly exposed to ethanol were, on the average, somewhat more fluid than controls but not so fluid as membranes of ethanol-adapted cells. The cells fixed shortly after transfer out of ethanol medium exhibited aggregation patterns which were also intermediate between those of control cells and ethanol-acclimated cells fixed while still in the presence of ethanol (Table VI).

The results of the freeze-fracture experiments clearly suggested the incorporation of a more fluid assortment of membrane lipids in the presence of ethanol. But the quantitative extent of the change was equivocal in certain of the experiments because it was impossible to accurately determine whether cells shifted to (or from) ethanol medium for 30 min had fully gained (or lost) ethanol to equilibrium levels. In an effort to complement the findings, we carried out fluorescence polarization studies on lipids extracted from control and ethanol-grown cells.

The technique employed the preparation of liposomes from lipids of interest plus 1 mol % of the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene. Fig. 3

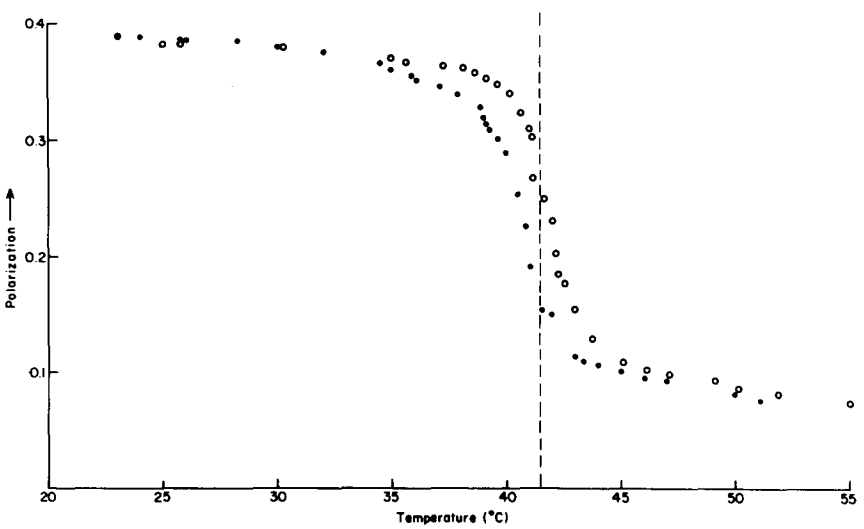

Fig. 3. 1,6-Diphenylhexatriene depolarization in multilamellar liposomes of dipalmitoylphosphatidylcholine prepared in the presence (•) or absence (○) of 0.35 M ethanol. The generally accepted mid-point (41.5°C) of the transition in the absence of ethanol is shown as a dashed line.

illustrates the response of an extensively studied synthetic phospholipid, dipalmitoylphosphatidylcholine, to the presence of ethanol. The large differences in polarization values between dipalmitoylphosphatidylcholine measured in the presence and in the absence of 0.35 M ethanol reveal a significant fluidizing effect of ethanol on the multibilayer membranes at temperatures near the phase transition.

Fig. 4 compares the polarization scans of microsomal phospholipid liposomes from control cells and ethanol-adapted cells measured in the absence (solid lines) and in the presence (dashed lines) of 0.35 M ethanol. The effect of ethanol was, as expected, to decrease the polarization (implying an increase in fluidity) of both control and ethanol-grown preparations. The effect of chronic exposure of cells to the ethanol-containing medium upon the inherent fluidity of the microsomal lipids confirmed the freeze-fracture observations. The lipid environment surrounding the probe was slightly but significantly more fluid in liposomes from ethanol-grown cells than in those from controls. Essentially identical results were obtained in three independent experiments.

In addition to their phospholipids, the native *Tetrahymena* membranes contain the pentacyclic triterpenoid, tetrahymanol, which exerts the same kind of stabilizing effect on membrane physical properties that cholesterol does in

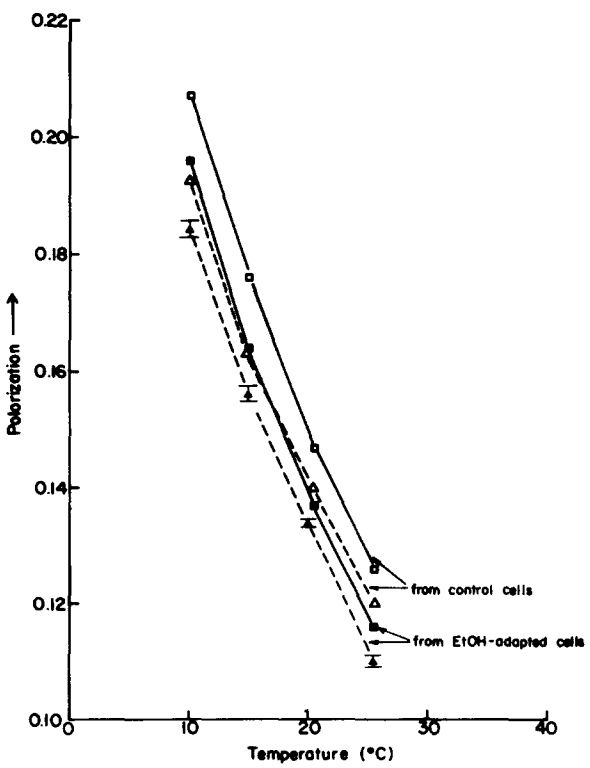

Fig. 4. 1,6-Diphenylhexatriene polarization in microsomal membrane phospholipids of control and ethanol-adapted cells grown at 25.5°C. ———, phospholipids without ethanol; - - - - -, phospholipids measured in the presence of 0.35 M ethanol. Error bars (shown on lower curve only) show the standard deviations observed generally for all points on the figure. Data for each point were taken from four separate experiments utilizing three different lipid preparations of adapted and non-adapted cells.

TABLE VII

DIPHENYLHEXATRIENE POLARIZATION OF MICROSOMAL TOTAL LIPIDS

	Sample temperature (°C)		
	10	20	25
Control cells	0.234	0.182	0.157
Ethanol-adapted cells	0.223	0.170	0.144
Difference in polarization values	0.011	0.012	0.013

mammalian cells. When liposome preparations made from total lipids (containing phospholipids, tetrahymanol and traces of triglycerides) were analyzed, the polarization differences (Table VII) between ethanol-grown lipids and control lipids were identical to those measured for purified phospholipids (Fig. 4).

Discussion

Since the popularization of the fluid mosaic model for membrane structure, a number of workers have proposed that membranes may be the focal point of cellular adaptation to ethanol. If ethanol produces a membrane perturbation, the membrane's lipid composition may well be altered because of it, as happens in the case of certain other stresses. Considerable effort has been spent testing this hypothesis. Short-chain alcohols have indeed been shown to exert a fluidizing effect on lipid bilayers [22—24]. Some evidence for minor physical changes in native membranes has also been reported [25,26]. Furthermore, small changes in lipid composition have been found in tissues of mammals chronically exposed to intoxicating levels of ethanol [27—29]. But the lipid changes and changes in membrane physical properties detected thus far have been of such low magnitude that their physiological consequences remain uncertain.

In the experiments reported here, we have probed the molecular effects of chronic ethanol exposure on membranes using *Tetrahymena* as a model system. In this organism, the fluidizing effect of ethanol, as inferred by freeze-fracture electron microscopy, is quite pronounced. Fluorescence polarization measurements confirmed the perturbing effect of ethanol, both on naive cells and on cells acclimated to growth in the presence of ethanol.

The principal ethanol-induced changes noted in *Tetrahymena* fatty acids, namely an increase in 18 : 2 and a decrease in 16 : 2 and 16 : 1, are in some respects similar to, but much greater than, the changes observed in mammalian tissues, which sustained a slight increase in 18 : 2 at the expense of 20 : 4 (Ref. 28, and Thompson, G.A., Jr. and Shorey, R.L., unpublished observations).

A perplexing observation in all the eukaryotic systems known to respond to ethanol is the increase in 18 : 2, which is generally considered to have a fluidizing effect. Cells adjusted to the fluidizing influence of high temperature [6—8], unsaturated fatty acids [4—10], or general anesthetics [9], experience a seemingly more logical increase in saturated fatty acids coupled with a decrease in polyunsaturates. A probable explanation is apparent from the interesting work of Ingram [30]. By growing *Escherichia coli* in various concentrations of

a homologous series of normal alcohols, he was able to discern a clear pattern of metabolic responses. Growth in alcohols having five to ten carbon atoms led to a large increase in saturated fatty acids, evidently aimed at compensating for the fluidizing effects of alcohols. However, growth in the presence of the lower alcohols (C_1—C_4) induced a different response altogether. Vaccinic acid (18 : 1), the major unsaturate (*E. coli* contains no polyunsaturates), underwent a major increase at the expense of saturated acids. It might therefore appear likely that ethanol and other short chain alcohols interact with the membrane in a fashion differing from that of their longer chain homologs. This concept is supported by extensive calorimetric measurements of Jain and Wu [22], which indicated that the effects of C_1—C_4 alcohols on the melting profiles of dipalmitoylphosphatidylcholine differed from those of long chain alcohols. It has been suggested on the basis of this and other physical chemical studies [31—33] that ethanol acts on the outer edge of the membrane hydrophobic core region. This is not unreasonable because ethanol is a moderately polar and hydrophilic molecule.

Even assuming that the fluidizing action of ethanol is brought to bear on the outer fringes of the membrane core, an explanation for the observed pattern of fatty acid unsaturation that results is not immediately apparent. We have no evidence to indicate that the fatty acid changes compensate for the ethanol-induced perturbation in a way that would restore membrane physical properties towards the normal state. But since the increase of 18 : 2 would fluidize the membranes in a region different from that affected by ethanol, it may serve to equalize the altered fluidity throughout the bilayer. There is presently no indication that such a balance is needed for membrane function.

Comparison of the compositional changes found here with the responses of higher animal lipids to ethanol reveals patterns that are qualitatively similar but quantitatively much greater in *Tetrahymena*. Perhaps due to the quantitatively less pronounced ethanol-induced changes in fatty acid composition observed in mammals, the few studies of membrane physical alterations caused by chronic ethanol exposure have been rather inconclusive [26]. Hopefully, further analysis using more sensitive techniques will permit a comparison with the *Tetrahymena* response.

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