Hakomori, S. (1970), *Chem. Phys. Lipids 5,* 96.
Hakomori, S., Stellner, K., and Watanabe, K. (1972), *Biochem. Biophys. Res. Commun. 49,* 1061.
Hancock, R. A., Marshall, K., and Weigel, H. (1976), *Carbohydr. Res. 49,* 351.
Hites, R. A., and Biemann, K. (1970), *Anal. Chem. 42,* 855.
Hoffman, J., Lindberg, B., and Svensson, S. (1972), *Acta Chem. Scand. 26,* 661.
Holmgren, J., Lönnroth, I., Mansson, J. E., and Svennerholm, L. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 2520.
Kathan, R. H., and Winzler, R. J. (1963), *J. Biol. Chem. 238,* 21.
Kathan, R. H., Johnson, C. A., and Winzler, R. J. (1959), *Acta Soc. Med. Ups. 64,* 290.
Kaul, K., and Lester, R. L. (1975), *Plant Physiol. 55,* 120.
Kaul, K., and Lester, R. L. (1978), *Biochemistry 17* (preceding paper in this issue).
Klenk, E., and Uhlenbrook, G. (1958), *Z. Physiol. Chem. 312,* 144.
Laine, R. A., and Renkonen, O. (1975), *J. Lipid Res. 16,* 102.
Laine, R. A., Hodges, L. C., and Cary, A. M. (1977), *J. Supramol. Struct., Suppl. 15,* 31.
Lester, R. L., Smith, S. W., Wells, G. B., Rees, D. C., and Angus, W. W. (1974), *J. Biol. Chem. 249,* 3388.
McCrea, J. F. (1953), *Yale J. Biol. Med. 26,* 191.
McNeil, M., and Albersheim, P. (1977) *Carbohydr. Res.* **56,** 239.
Mullin, B. R., Pacuszka, T., Lee, G., Kohn, L. D., Brady, R. O., and Fishman, P. H. (1978), *Science 199,* 77.
Roseman, S., and Daffner, I. (1956), *Anal. Chem. 28,* 1743.
Seifter, S., Dayton, S., Novic, B., and Muntioyler, E. (1950), *Arch. Biochem. Biophys. 25,* 191.
Siddiqui, B., and Hakomori, S. (1971), *J. Biol. Chem. 246,* 5766.
Stellner, K., Saito, H., and Hakomori, S. (1973), *Arch. Biochem. Biophys. 155,* 464.
Taylor, R. L., Shively, J. E., Conrad, H. E., and Cifonelli, J. A. (1973), *Biochemistry 12,* 3633.
Van Heyningen, W. E. (1974), *Nature (London) 249,* 415.
Van Heyningen, W. E., and Miller, P. A. (1961), *J. Gen. Microbiol. 24,* 197.

Use of Fluorescence Polarization to Monitor Intracellular Membrane Changes during Temperature Acclimation. Correlation with Lipid Compositional and Ultrastructural Changes†

Charles E. Martin‡,* and Guy A. Thompson, Jr.

ABSTRACT: Fluorescence polarization of 1,6-diphenylhexatriene (DPH) was used to study the effects of temperature acclimation on *Tetrahymena* membranes. The physical properties of membrane lipids were found to be highly dependent on cellular growth temperature. DPH polarization in lipids from three different membrane fractions correlated well with earlier freeze-fracture and electron spin resonance observations showing that membrane fluidity progressively decreases in the order microsomes > pellicles > cilia throughout a wide range of growth temperatures. Changes in membrane lipid fluidity following a shift from high to low growth temperatures proceed rapidly in the microsomes, whereas there is a pronounced lag in the changes of peripheral cell membrane lipids. These data support previous observations that adaptive changes in membrane fluidity proceed via lipid modifications in the endoplasmic reticulum, followed by dissemination of lipid components to other cell membranes. The rapid changes in polarization observed in the microsomal lipids following a temperature shift correspond closely with the time-dependent alterations in both lipid fatty acid composition and freeze-fracture patterns of membrane particle distribution, suggesting that, in the endoplasmic reticulum, lipid phase separation is the primary cause of membrane particle rearrangements.

Biological membranes are formed through complex interactions between lipids and proteins. Such associations allow for the existence of stable, highly ordered structures while permitting remarkable mobility of their individual molecular components (Singer & Nicolson, 1972; Edidin, 1974). Because the unique physical characteristics of membrane lipids account in large part for the observed fluid properties of cellular membranes, a great deal of effort has recently been applied to the study of lipid contributions to cell structure and function. A persuasive body of evidence now indicates that the function of many membrane-bound enzymes is dependent upon specific associations with lipids (Finean, 1973) and is strongly dependent upon the physical state of the surrounding lipid environment (Singer, 1974; Vik & Capaldi, 1977).

Where highly purified membranes have been studied, the lipids have been found to consist of heterogeneous mixtures of many molecular species. In fact, each functionally distinct membrane in eukaryotic cells appears to be maintained with its own specific lipid composition (for reviews, see Ansell et al., 1973). The physical properties of artificial membranes composed of single phospholipid molecular species have been determined by a number of corroborating physical techniques

† From the Department of Botany, The University of Texas, Austin, Texas 78712. *Received February 27, 1978.* Supported by grants from the National Institute of General Medical Sciences (GM20148), the National Cancer Institute (1 T32 CA09182), the Robert A. Welch Foundation (F-350), and the National Science Foundation (01P74-22029).
‡ National Cancer Institute Postdoctoral Trainee (I T32 CA09182).

0006-2960/78/0417-3581$01.00/0

(Lentz et al., 1976; Shimshick & McConnell, 1973; Mabry & Sturtevant, 1976). The properties of the complex mixtures found in natural membranes are less clearly understood, however, although it is clear that differences in lipid compositions produce widely divergent membrane physical properties.

In order to gain insight into the functional role of membrane fluidity, we have been engaged in a continuing study of the regulation of this property in response to factors which perturb the physical state of membranes in *Tetrahymena*. Since the physical state of membrane lipids is strongly temperature dependent, alterations in environmental temperature provide a uniform and convenient perturbation method. In fact, it has been known for a number of years that poikilothermic organisms alter their membrane lipids in response to temperature change in a manner which suggests an attempt is being made to maintain the fluidity of the membrane within an optimal, if not constant, range (Sinensky, 1974; Esser & Souza, 1976).

We have previously shown that a number of different membrane fractions of *Tetrahymena thermophila,* strain NT-1, possess distinct phospholipid, sterol, and fatty acid compositions which are markedly dependent on the growth temperature. Shifting cells from one growth temperature to another resulted in rapid, dramatic changes in the membrane lipid compositions. In conjunction with this study, we were able to infer from freeze fracture electron microscopy observations that these changes radically altered the thermotropic properties of each membrane (Martin et al., 1976; Kasai et al., 1976).

We have now proceeded to examine this regulatory response in a more direct manner in order to determine how changes in membrane lipid fluidity occur during temperature acclimation. The fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) incorporated into artificial bilayers of lipids isolated from purified membrane fractions was used as a probe of the contribution of the lipids to the fluidity of that particular membrane. The degree of DPH polarization found within a particular lipid environment is a function of the rotational relaxation times and the fluorescent lifetime of the probe molecules (Andrich & Vanderkooi, 1976; Vanderkooi et al., 1977). Since these factors are determined by the degree of order and the packing structure of lipids within the bilayer, this method gives a sensitive indication of the physical environment surrounding the probe molecules.

Materials and Methods

Cell Growth, Membrane Fractionation, and Lipid Analysis. Culture conditions for strain NT-1 of *Tetrahymena thermophila* (formerly designated as *T. pyriformis*) have been reported by Fukushima et al. (1976). Cell fractionations were performed by the methods of Nozawa & Thompson (1971), and lipids were extracted by a modification of the Bligh & Dyer (1959) procedure. The lipid preparations were routinely examined by thin-layer chromatography to confirm the absence of free fatty acids or triglycerides in levels high enough to compromise polarization measurements. Phospholipid fractions were purified by silicic acid chromatography prior to methylation for fatty acid analyses. Thin-layer chromatography of lipids and gas–liquid chromatography of fatty acid methyl esters have also been described previously by Fukushima et al. (1976). Total lipid phosphorus was determined by the procedure of Bartlett (1959) as modified by Marinetti (1962). The procedure used for temperature shift experiments was that of Martin et al. (1976).

Fluorescent-labeled liposomes were prepared by combining 2 μmol phospholipid and 2 nmol of DPH dissolved in $CHCl_3$:MeOH (6:1) in a HNO_3-washed 12 × 100 mm test tube. Solvents were removed by prolonged evaporation under N_2. Sufficient time was taken during this process to ensure complete removal of the organic solvents. In some experiments a vacuum was applied to the sample to remove possible traces of solvent; however, no differences were observed in the behavior of the lipids prepared by the two procedures. Lipids were vortexed with a glass bead in 2 mL of 15% sucrose–50 mM KCl for 30 s followed by immersion of the tube for 1 min in a low power sonicator cleaning bath in which the water was maintained at 39.5 °C. This procedure resulted in uniform preparations which yielded constant polarization values. The concentration of lipid phosphorus in these samples was 1 mM. Lipid to probe ratio was 1000:1.

Steady-State Polarization Measurements. Steady-state polarization measurements were performed with a device constructed so that vertical and horizontally polarized emission components could be measured simultaneously. Excitation light was selected by an Oriel 7240 monochrometer at 360 nm with a 12-nm bandwidth. The excitation beam was polarized through a Glan-Thompson prism. Emission polarization was observed through sheet polarizers oriented both parallel and perpendicular to the polarized excitation beam. $NaNO_2$ (4 M) was used to cut off emission wavelengths below 390 nm. Precise temperature control of the sample was provided by circulating water through the jacketted cuvette, and the sample temperature was monitored by a thermistor placed in the sample just above the excitation beam.

Fluorescence emission was measured by using two matched photomultiplier tubes, one for each polarized component. Each tube was powered by a separate high voltage power supply and the PM responses were measured independently by digital voltmeters. Both voltmeter readings could be locked to record the simultaneous output from each photomultiplier tube, eliminating fluctuations in lamp intensity from consideration. The voltage output from each PM tube was linear with respect to emission intensity well beyond the regions used for measurements.

In order to compensate for differences in filter transmissions, the gains of the photomultiplier tubes were adjusted so that they were identical when the emission polarizers were oriented parallel to the excitation beam. Parallel and perpendicular emission intensities were then recorded at least 10 times for each data point and mean polarization values were calculated according to the formula:

$$p = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

where $I_{\parallel}$ is the parallel intensity and $I_{\perp}$ is the perpendicular intensity. Standard deviations for these measurements were on the order of 0.1–0.4% of the mean value. Scattering was measured by using identical lipid samples without DPH. This was found to contribute less than 2% of the total emitted light and therefore no corrections were employed.

Results

DPH polarization measured in lipids isolated from the microsomes, pellicles, and ciliary membranes of cells shows the marked differences in physical behavior of the lipids from these membrane fractions. Figure 1 plots the fluorescence polarization vs. temperature for these lipids from cells fully acclimated to growth at 39.5 °C. Higher polarization values indicate that the movement of probe molecules is more restricted; hence, a more highly ordered bilayer structure can be assumed. Several conclusions can be drawn from these data. Probe mobility, for example, is more restricted in the pellicle and

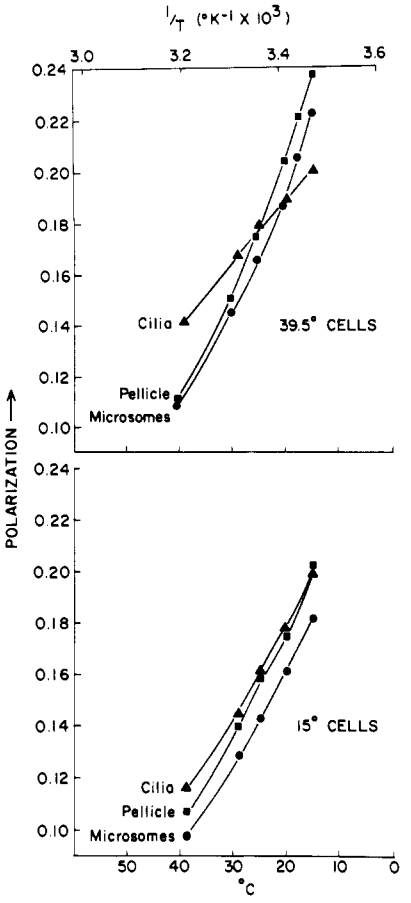

FIGURE 1: Effect of temperature on DPH polarization in lipids isolated from membranes of 39.5 °C cells (top) and 15 °C cells (bottom).

TABLE I: DPH Polarization in Membrane Lipids of Cells Acclimated to 39.5 and 15 °C.

membrane fraction	polarization measured at 15 °C	polarization measured at 39 °C
39 °C microsomes	0.231	0.118
15 °C microsomes	0.176	0.094
39 °C pellicles	0.248	0.121
15 °C pellicles	0.203	0.101
39 °C cilia	0.211	0.142
15 °C cilia	0.200	0.116

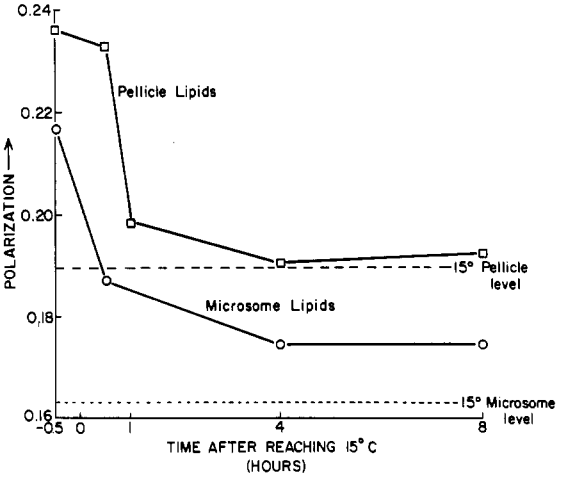

FIGURE 2: Time course of changes in DPH polarization in membrane lipids of 39.5 °C acclimated cells following a shift to 15 °C. Cells were grown at 39.5 °C, shifted to 15 °C over a 30-min period, and then harvested and fractionated at the indicated times for membrane lipid isolation and DPH polarization measurements at 15 °C. Dashed lines show the polarization values in cells fully acclimated to 15 °C.

ciliary membrane fractions than in the microsomes, indicating that lipid order and its contribution to membrane fluidity are greater in the latter fraction. Furthermore, although the thermal properties of pellicle and microsomal lipids parallel each other over a wide range of temperatures, the ciliary lipids deviate markedly from this behavior. This is probably due to the large amounts of tetrahymanol, a sterol analogue, which is found in these membranes (Fukushima et al., 1976). Nozawa et al. (1974) have previously demonstrated that tetrahymanol behaves similarly to cholesterol in phospholipid bilayers by exerting a condensing effect at high temperatures and a fluidizing effect at low temperatures.

Figure 1 shows the corresponding polarization values found in these membranes of cells which were acclimated to 15 °C. In this case, each membrane lipid fraction appears to exhibit similar thermal characteristics, with the hierarchy of structural order progressing from the cilia (most ordered) to the microsomes (least ordered) and with the pellicles assuming an intermediate value throughout the range of the temperature scan. In contrast to the findings with 39.5 °C cells, there is little difference in the slope of ciliary lipid polarization compared with the other 15 °C cell membrane lipids. This agrees with our previously published observations of a lower tetrahymanol content of the cilia in cells of this strain grown at 15 °C (Fukushima et al., 1976).

Although the pellicular and microsomal lipids from cells grown at both temperatures exhibit similar thermal characteristics, DPH polarization is markedly lower in the lipids from 15 °C acclimated cells. A clearer appreciation of the differences produced by high and low growth temperatures can be gained by comparing polarization values measured at the same temperature. Table I summarizes the data compiled in this manner. These data agree closely with the electron spin resonance observations of Nozawa et al. (1974) of the thermotropic properties of similar membrane fractions from a different strain of *Tetrahymena*. The lower polarization values of each 15 °C membrane fraction emphasize the highly fluid nature of these lipids which appears to be a necessary characteristic of cells grown at low temperatures.

Kinetics of Changes in Lipid Properties during Temperature Acclimation. In order to correlate the temperature-induced changes in the physical properties of membrane lipids with the other cellular events which we have observed, such as the redistribution of intramembranous protein particles and the changes in phospholipid fatty acid saturation, both of which occur following a shift in growth temperature, cells were grown at 39.5 °C and then shifted to 15 °C over a 30-min period as in previous experiments (Martin et al., 1976). This procedure produces a 10–12-h growth lag period during which the membrane lipids change toward the 15 °C cell pattern and the initial pronounced particle aggregation seen in freeze-fracture replicas of a number of the cell membranes reverts to a random particle distribution. Pellicle and microsomal membrane lipids were isolated from these cells at intervals following the temperature shift and transformed into multilamellar vesicle preparations. DPH polarizations measured in these lipids at the new environmental temperature (15 °C) showed marked differences in the responses of the two membrane fractions. The time course of these changes is shown in Figure 2.

Pronounced alterations in lipid properties were readily ap-

TABLE II: Fatty Acid Composition of Microsome Phospholipids during Temperature Acclimation from 39 to 15 °C (Weight % Total Fatty Acid Species from 14:0 to 18:3).

fatty acid species	control cells at 39 °C	growth conditions for 39–15 °C shifted cells time (min) after reaching 15 °C 30	240	480	control cells at 15 °C
14:0[a]	16.1	12.0	15.8	10.2	11.0
15:0 (a.i.)	4.7	6.1	2.9	2.3	0.8
15:0	1.4	1.9	0.8	0.9	0.3
16:0	18.4	12.7	9.6	11.3	13.1
16:1	10.8	12.1	13.5	13.0	9.2
16:2	5.6	8.9	8.4	8.7	2.9
?	1.1	1.3	3.8	3.6	3.2
18:0	3.8	0.9	3.5	4.2	3.7
18:1	7.5	4.2	6.8	10.1	7.8
18:2 (6,11)					4.2
18:2 (9,12)	11.4	12.0	6.7	9.5	12.8
18:3	18.9	23.3	26.3	23.8	29.5
double bonds/100 molecules	109	128	129	131	145

[a] The figure preceding the colon represents the length of the carbon chain, while that following the colon indicates the number of double bonds present.

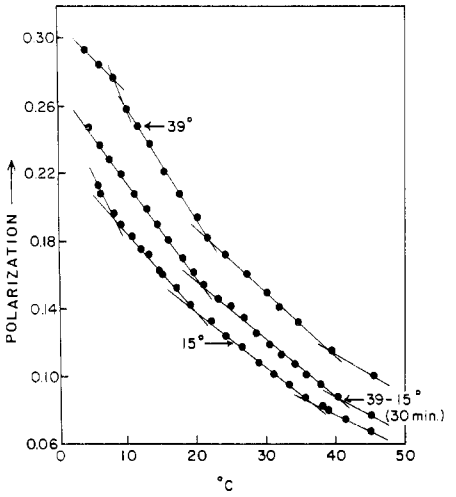

FIGURE 3: Detailed scan of dph polarization vs. temperature in microsomal membrane lipids from cells acclimated to 39.5 and 15 °C (top and bottom curves, respectively). Middle curve is DPH polarization in microsomal lipids of 39.5 °C cells acclimating to 15 °C 30 min after reaching the lower temperature. (See Martin et al. (1976) for experimental details for temperature shift.)

parent from the first measurement of the microsomal fraction, while large changes in DPH polarization of pellicle lipids were seen only after a period of about 1 h. The changes in microsomal polarization initially proceeded much more rapidly than that of the pellicular membranes, but, rather unexpectedly, they did not reach the state found in the 15 °C cells during the 8-h period examined. However, the pellicular membranes, following an initial lag period, proceeded directly to the level of 15 °C acclimated cell pellicles.

In the microsomal lipids, the DPH polarization changes correlate strikingly well with changes in fatty acid unsaturation (Table II). The degree of polarization in the microsomal lipids corresponds particularly closely with the number of double bonds found in the phospholipid fatty acids. Previous analyses of the fatty acid compositional changes in whole cell phospholipids (Martin et al., 1976) did not allow this precipitous rise in microsomal fatty acid unsaturation to be detected because it is masked by a more gradual rise in the other fractions.

Thermotropic Properties of Lipids during Acclimation. Studies of the temperature effect on numerous membrane phenomena have revealed abrupt breaks in the slopes of Arrhenius plots of both membrane-bound enzymatic activities and thermotropic properties of membrane lipids (recent papers include Thilo & Overath, 1976; Linden et al., 1973; Esfahani et al., 1971; Morrisett et al., 1975; Tecoma et al., 1977; Schroeder et al., 1976). It has been widely held that these breaks represent the temperatures at which some of the lipids in the bilayer undergo a gel to liquid-crystalline transition with their segregation into physically separate domains within the bilayer. A detailed examination of DPH polarization in *Tetrahymena* microsomal lipids also shows such abrupt changes with respect to temperature.

A comparison of 39.5 and 15 °C microsomal lipids with those from 39.5–15 °C shifted cells (30 min) is shown in Figure 3. A particularly striking aspect of these data is the parallel nature of all three temperature scans throughout the entire temperature range examined. The lipids from temperature-shifted cells showed intermediate polarization within this range in the same manner shown by relative comparisons measured at 15 °C (Figure 2). Each profile exhibits a characteristic break in the slopes of the plots in the region between 22 and 18 °C. There appears to be a downward shift in the inflection temperature as the cells acclimate to the lower growth temperature. However, the exact inflection temperature shown in Figure 3 cannot be considered as being biologically significant at the present time. Changes of several degrees were observed in some preparations, depending upon the heating or cooling rates, the equilibration time, and the previous history of the lipid dispersion. Nevertheless the existence of an inflection point in the 18–22 °C range is well established. We have observed similar breaks in this temperature region using *trans*-parinaric acid as a fluorescent probe (Martin & Thompson, unpublished data). Experiments are now in progress using time resolved methods to study the molecular events which cause such abrupt changes in the thermal behavior of these lipids.

The breaks in the temperature profiles correspond well with those reported by Wunderlich et al. (1975) for microsomal membrane vesicles from another strain of *Tetrahymena* grown at 28 °C. These workers found consistent breaks in Arrhenius plots of glucose-6-phosphatase activity, the fluorescence intensity of 8-anilino-1-naphthalenesulfonate, and the electron

spin resonance spectrum of 5-doxylsteric acid, all of which centered in the region between 17 and 20 °C. These breaks corresponded with the emergence of particle-free areas on the fracture face of endoplasmic reticulum membranes and were postulated to result from clustering of membrane lipids. No correlation seems to exist between the breaks in DPH polarization scans and membrane particle rearrangements observed by us, however, since particle redistribution occurs in 15 °C acclimated cells, approximately 16 °C lower than the corresponding polarization break point (Martin et al., 1976).

Discussion

From a physiological point of view, perhaps the most important finding of this study is the unexpectedly rapid and pronounced response of *Tetrahymena* microsomes to a downward shift in culture temperature. It is clear from both the compositional changes of the membrane lipids (Table II) and the alteration of lipid physical properties (Figure 2) that microsomal membranes respond to the lowered temperature much more quickly than either the whole cell in general (Martin et al., 1976) or one of the other principal organelles, the pellicle (Table II, Figure 2). The rapidity of the microsomal reaction to the temperature drop is also closely reflected in observations made by freeze-fracture electron microscopy. Plotting the data obtained by these three independent methods on a single graph illustrates the marked similarity in the rate of change of each parameter (Figure 4).

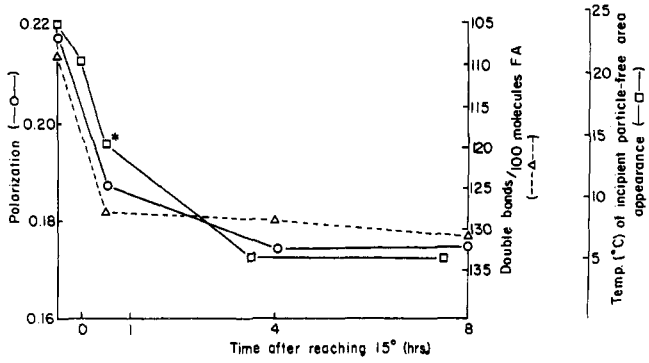

FIGURE 4: Comparison of the changes of three independently measured properties of microsomal membranes during temperature acclimation from 39.5 to 15 °C. Data from freeze-fracture observations of membrane particle redistribution (□), fluorescence polarization of DPH in membrane lipids (○), and the number of double bonds in phospholipid fatty acids (△) have been plotted to show the correspondence in the rates of change of the three parameters. Freeze-fracture data were recalculated from previous experiments of Kitajima & Thompson (1977); the point marked with an asterisk represents a new observation of freeze-fracture replicas of cells fixed at 15 °C only. Since no particle-free areas were present this can be taken as an upper limit. The true temperature of incipient particle-free area appearance is probably 1–2 °C lower.

Because the fatty acid desaturase enzyme systems responsible for temperature acclimation are associated with the endoplasmic reticulum, it is logical to expect that any temperature-induced alteration in membrane lipid composition would be initiated there. Our data confirm that changes resulting from the temperature shift are manifested in the endoplasmic reticulum itself considerably earlier than they are in other parts of the cell. The relatively slow dissemination of newly modified lipids throughout the remainder of the cell means that the microsomal fraction may be considered as a nearly closed system during the first few minutes of the temperature response. As such, it constitutes a valuable system for studying the detailed molecular mechanisms of temperature acclimation of eukaryotic cells.

All the commonly used physical techniques for measuring the fluidity of membranes suffer from uncertainties of interpretation. It was therefore encouraging to derive reinforcement of our previous electron microscopy observation from such a dissimilar approach as fluorescence polarization. Agreement of results from these two techniques and the time course of changing fatty acid unsaturation tend to validate the freeze-fracture technique as a bona fide approach to the quantitative estimation of membrane fluidity characteristics. Comparison of the results also suggests that the rates of protein particle redistribution seen in the freeze-fracture replicas are triggered primarily by physical changes in the associated lipids rather than such factors as the contraction of underlying protein structures. Similar results have recently been observed in mitochondrial membranes by Hackenbrock et al. (1976).

A point of considerable interest to us is the observation that, during the 8-h test period, microsomal lipid mobility in acclimating cells never reached the value typifying microsomes of fully acclimated 15 °C cells (Figure 2). It is tempting to speculate that failure of the microsomal membranes to return fully to the 15 °C state is a necessary part of the programmed sequence of events leading to complete cellular acclimation. Our previous studies (Martin et al., 1976; Kasai et al., 1976) indicated that the fatty acid desaturase in *Tetrahymena* microsomes are regulated through changes in the fluidity of their membrane environment rather than by changes in the temperature per se. If this is indeed the case, it would seem mechanistically desirable to maintain the microsomal membranes in a somewhat more rigid state than normal, thereby assuring a continued high level of fatty acid desaturase activity until all other cellular membranes could be adequately supplied with unsaturated lipid species.

The fine structure seen in the detailed polarization vs. temperature plots (Figure 3) offers an opportunity for probing the actual mechanics of acclimation. Despite the rapid changes taking place in membrane fatty acids, the shapes of the polarization curves for at least the microsomal lipids remain relatively uniform, suggesting a change in the proportions but not the types of molecular species present. Whereas some physical analyses of two component phospholipid mixtures have been made (Shimshick & McConnell, 1973; Mabry & Sturtevant, 1976; Lentz et al., 1976), it would be premature at this time to attempt a detailed molecular interpretation of the slopes and inflection points of such a complex mixture as found in actual membranes. However, it is worth noting that the most prominent inflection points found in the microsomes in this study have also been found by other workers using quite different analytical techniques (Wunderlich et al., 1975). The fact that these inflections are detected by methods which probe different regions of the bilayer indicates that major structural rearrangements occur in the membranes at this particular temperature.

Although we have emphasized the agreement between our diverse experimental approaches, the data to this point do not offer a precise description of membrane fluid properties under actual growth conditions. Our earlier findings (Kitajima & Thompson, 1977) indicated that lipid phase separation in microsomal membranes began at 24 °C in 39.5 °C grown cells, at 12 °C in 27 °C grown cells, and at 0.5 °C in 15 °C grown cells. The unfailing appearance of particle-free domains at approximately 15 °C below the growth temperature led us to speculate that, if the physical state of the differently acclimated cells' microsomal membranes could be directly measured at their respective growth temperatures, identical fluidity values would be found. This interpretation would be consistent with the concept of homeoviscous adaptation proposed by Sinensky (1974). However, our present evidence using polarization

techniques would not seem to be in full agreement with this conclusion. Examination of Figure 1 shows that the polarization of each membrane of 39.5 °C grown cells measured at the growth temperature (39.5 °C) is not identical with the polarization value for the corresponding cell fraction of 15 °C grown cells measured at their growth temperature of 15 °C. At least part of this discrepancy is due to the fluorescent properties of the probe molecule, which vary not only with the fluidity of the membrane but also with the absolute temperature, making it impossible to directly equate the polarization values measured by the steady-state techniques used here. This prevents us from addressing the interesting question of whether acclimation restores fluidity to some optimal value.

In order to resolve this uncertainty, we have recently completed the first of a series of experiments using time-resolved fluorescence polarization methods to examine and compare in more nearly absolute terms the environments of membrane lipids at their respective growth temperatures (Martin & Foyt, accompanying paper). This study of 39.5 and 15 °C microsomal lipids indicates that, although the lipid environments exhibit very similar properties at their respective growth temperatures, there appear to be some differences in the packing structure, or "bulk viscosity" between similar membranes of cells grown at the two temperatures. Such differences may not be entirely unexpected, since our freeze-fracture techniques measure the properties of intact membranes while our fluorescence polarization measurements have been confined until now to membrane lipids. We now intend to use time-resolved polarization measurements of intact membrane preparations with recently developed picosecond laser methods to examine the interaction of lipids and proteins in determining the physical state of the bilayer.

Acknowledgments

We would like to thank Dr. Roger Walser of the Electrical Engineering Department of The University of Texas for his technical assistance and consultations concerning fluorescence polarization measurements. Most of this work was performed at the Center for Fast Kinetics Research at The University of Texas at Austin. The CFKR is supported by National Institutes of Health Grant RR-00886 from the Biotechnology Branch of the Division of Research Resources and by The University of Texas at Austin. We would also like to acknowledge the assistance of Ms. Sharon Mattox in making the analysis of freeze-fracture electron micrographs.

References

Andrich, M. P., & Vanderkooi, J. M. (1976) *Biochemistry 15,* 1257.

Ansell, G. B., Dawson, R. M. C., & Hawthorne, J. N., Eds. (1973) *Form and Function of Phospholipids,* Amsterdam, Elsevier.

Bartlett, G.R. (1959) *J. Biol. Chem. 234,* 466.

Bligh, E. C., & Dyer, W. J. (1959) *Can. J. Biochem. Physiol. 37,* 911.

Edidin, M. (1974) *Annu. Rev. Biophys. Bioeng. 3,* 179.

Esfahani, M., Limbrick, A. R., Knutton, S., Oka, T., & Wakil, S. J. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68,* 3180.

Esser, A. F., & Souza, K. A. (1976) in *Extreme Environments: Mechanisms of Microbiological Adaptation* (Heinrich, M. R., Ed.) p 283, Academic Press, New York, N.Y.

Finean, J. B. (1973) in *Form and Function of Phospholipids* (Ansell, G. B., Hawthorne, J. N., & Dawson, R. M. C., Eds.) p 171, Elsevier, Amsterdam.

Fukushima, H., Martin, C., Iida, H., Kitajima, Y., Thompson, G. A., Jr., & Nozawa, Y. (1976) *Biochim. Biophys. Acta 431,* 165.

Hackenbrock, C. R., Höchli, M., & Chau, R. M. (1976) *Biochim. Biophys. Acta 455,* 466.

Kasai, R., Kitajima, Y., Martin, C. E., Nozawa, Y., Skriver, L., & Thompson, G. A., Jr. (1976) *Biochemistry 15,* 5228.

Kitajima, Y., & Thompson, G. A., Jr. (1977) *J. Cell Biol. 72,* 744.

Lentz, B. R., Barenholtz, Y., & Thompson, T. E. (1976) *Biochemistry 15,* 4529.

Linden, C. D., Wright, K. L., McConnell, H. M., & Fox, C. F. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70,* 2271.

Mabry, S., & Sturtevant, J. M. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73,* 3862.

Marinetti, G. V. (1962) *J. Lipid Res. 3,* 1.

Martin, C. E., Hiramitsu, K., Kitajima, Y., Nozawa, Y., Skriver, L., & Thompson, G. A., Jr. (1976) *Biochemistry 15,* 5218.

Morrisett, J. D., Pownall, H. J., Plumlee, R. T., Smith, L. C., Zehner, Z. E., Esfahani, M., & Wakil, S. J. (1975) *J. Biol. Chem. 250,* 6969.

Nozawa, Y., & Thompson, G. A., Jr. (1971) *J. Cell Biol. 49,* 712.

Nozawa, Y., Iida, H., Fukushima, H., Ohki, K., & Ohnishi, S. (1974) *Biochim. Biophys. Acta 298,* 39.

Schroeder, F., Holland, J. F., & Vagelos, P. R. (1976) *J. Biol. Chem. 251,* 6747.

Shimshick, E. J., & McConnell, H. M. (1973) *Biochemistry 12,* 2351.

Sinensky, M. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71,* 522.

Singer, S. J. (1974) *Annu. Rev. Biochem. 43,* 805.

Singer, S. J., & Nicolson, G. L. (1972) *Science 175,* 720.

Tecoma, E., Sklar, L. A., Simoni, R. D., & Hudson, B. (1977) *Biochemistry 16,* 829.

Thilo, L., & Overath, P. (1976) *Biochemistry 15,* 328.

Vanderkooi, J. M., Landesberg, R., Selick, H., II, & McDonald, G. G. (1977) *Biochim. Biophys. Acta 464,* 1.

Vik, S. B., & Capaldi, R. A. (1977) *Biochemistry 16,* 5755.

Wunderlich, F., Ronai, A., Speth, V., Seelig, J., & Blume, A. (1975) *Biochemistry 14,* 3730.