Molecular Control of Membrane Properties During Temperature Acclimation. Membrane Fluidity Regulation of Fatty Acid Desaturase Action?†

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ABSTRACT: Further studies on the molecular mechanisms of temperature acclimation have been carried out using the ciliate *Tetrahymena pyriformis*. The most prominent change in lipid metabolism during acclimation to high temperature—depression of fatty acid desaturase activity—could be simulated by supplementing the growth medium of isothermally-grown cells with polyunsaturated fatty acids. Such cells resisted the membrane-fluidizing effect of the incorporated exogenous acids by increased use of de novo synthesized saturated acids in their phospholipids. The data support the conclusions arising from earlier experiments with temperature-shifted cells (Martin, C. E., Hiramitsu, K., Kitajima, Y., Nozawa, Y., Skriver, L., and Thompson, G. A., Jr. (1976), *Biochemistry 15*), showing that, when membrane fluidity increased to a superoptimal level, the activity of membrane-associated fatty acid desaturases was decreased. Since the reaction is controlled by membrane fluidity, rather than temperature per se, we postulate that it is the general mechanism employed by cells adjusting to any fluidity-modifying factor, such as cations, drugs, etc.

Many organisms respond to variations in body temperature by altering the composition of their membrane lipids. A particularly dramatic example of lipid changes during temperature acclimation has been elucidated using the thermotolerant strain NT-1 of *Tetrahymena pyriformis* (Fukushima et al., 1976). As a first step towards identifying the molecular mechanism underlying the lipid changes, the effect was shown to be independent of temperature-related variations in the level of dissolved oxygen (Skriver and Thompson, 1976). More recent data, described in the preceding paper of this issue (Martin et al., 1976), are consistent with the hypothesis that the physical state of the membrane lipid bilayer is more directly responsible for changes in lipid metabolism than temperature itself. In this paper, we altered the cell's lipid composition isothermally by fatty acid supplementation. It was thereby possible to measure at a constant temperature distinctive changes in lipid metabolism coinciding with alterations in fluidity.

Materials and Methods

Most of the materials used and procedures followed for the culture, experimental manipulation, and lipid extraction of *Tetrahymena pyriformis*, strain NT-1, have been described in the preceding paper of this issue (Martin et al., 1976). Analysis of the lipids was also described in that communication. The only method that remains to be outlined is the supplementation of large amounts of linoleic acid (*cis,cis*,9,12-octadecadienoic acid), hereafter designated by the shorthand designation 18:2.

Cells were grown at 39.5 °C, as before, to a density of 75 000 cells/ml. An emulsion of 4.3 μmol of 18:2 plus 1 μCi of ^{14}C-18:2 in distilled water was added to each 200-ml medium, producing a concentration of 21.5 μM 18:2. Following a 100-min incubation at 39.5 °C, aliquots of the growth medium were removed for analysis and a second supplement of 18:2 was then added to the remainder in an amount equal, on a per ml of medium basis, to that fed in the first supplement.

Results

The preceding paper of this issue (Martin et al., 1976) shows that *Tetrahymena* cells shifted from a high to a low temperature quickly increased their rate of enzymatic fatty acid desaturation. The increase could be largely prevented by feeding the cells polyunsaturated fatty acids. In this paper, we describe similar feeding experiments designed to perturb the fatty acid composition of cells growing isothermally. The results shed more light on the mechanisms controlling fatty acid desaturation.

Feeding linoleic acid (18:2) and γ-linolenic acid (18:3) in the amounts used previously (Martin et al., 1976) yielded unexpected results. Only minor changes were detected in the cells growing isothermally at 39.5 °C, partly because the rapid growth at that temperature led to a dilution of the supplemented acids by other fatty acid species synthesized de novo. In an effort to maintain a high level of 18:2 and 18:3 and thereby force a change in the fatty acid composition, we tried increasing the amount administered but, at higher doses, 18:3 proved toxic, as shown earlier by Lees and Korn (1966). Ultimately, a protocol was developed which did cause significant perturbations in the normal fatty acid pattern. This required feeding twice the amount of fatty acids used earlier for the temperature-shifted cells, but with the supplement, in this case, consisting entirely of 18:2. Additional 18:2 was provided 100 min after the first feeding. As it so happened, extensive

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TABLE I: Proportions of Major Fatty Acids[a] Found in Phospholipids of Strain NT-1 Grown Isothermally at 39.5 °C with and without Linoleic Acid.[b]

Fatty Acid	100 min No 18:2	100 min 18:2	345 min No 18:2	345 min 18:2
14:0	11.8 ± 0.0	9.6 ± 0.7	11.3 ± 0.7	10.5 ± 1.7
16:0	14.6 ± 1.9	13.5 ± 1.4	13.5 ± 1.5	15.5 ± 1.1
16:1	12.9 ± 2.3	6.8 ± 0.6	13.1 ± 2.6	4.8 ± 1.3
16:2+ 17:0	8.3 ± 1.8	5.2 ± 1.6	7.2 ± 0.9	2.4 ± 0.6
18:1	5.8 ± 0.7	3.8 ± 0.7	5.4 ± 1.5	3.5 ± 0.8
18:2	12.9 ± 1.4	28.5 ± 2.6	12.7 ± 1.1	30.5 ± 2.4
18:3	23.0 ± 1.7	24.3 ± 1.3	26.4 ± 1.1	25.0 ± 3.0

[a] Fatty acid species less than 5% of the total weight of fatty acids are not shown; complete fatty acid compositions for this strain have been published previously (Fukushima et al., 1976). Each column represents the averages and standard deviations for at least four experiments. [b] Cells were grown at 39.5 °C to a density of approximately 75 000 ml^{-1}. Linoleic acid was added as a sonicated dispersion in 1–3 ml of inorganic medium (Hamburger and Zeuthen, 1957) or sterile distilled water in the ratio of 4.1–4.6 μmol of 18:2/200 ml of culture medium. After 100-min incubation, aliquots were removed and a second supplement of 18:2 was added in similar proportions to the remaining medium.

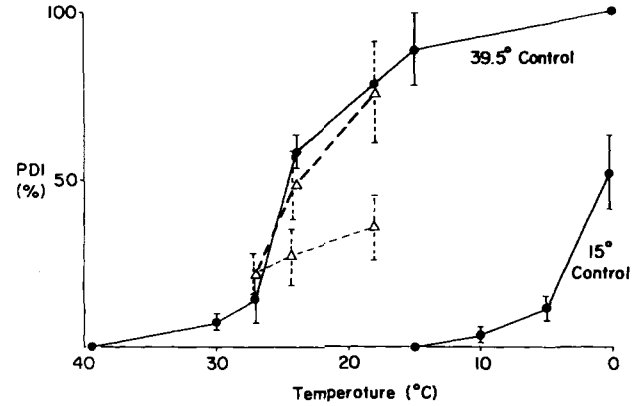

FIGURE 1: The effects of 18:2 supplementation on the particle density index (PDI) of outer alveolar membranes of *Tetrahymena* growing at 39.5 °C. The PDI, calculated as described in the preceding paper of this issue (Martin et al., 1976), is plotted vs. the temperature at which cell aliquots were fixed with glutaraldehyde after chilling from their growth temperature over a 4-min period. The filled circles (●) and solid lines are the PDI curves for unsupplemented 39.5 °C grown and 15 °C grown cells (Martin et al., 1976). The open triangles (△) and thick broken lines represent the PDI curve for cells 100 min following 18:2 supplementation, while the open triangles and thin broken lines represent the PDI curve for cells 345 min following the initial 18:2 supplementation.

desaturation of 18:2 to 18:3, as measured by feeding ^{14}C-18:2, compensated to a large extent for the absence of 18:3 in the supplement.

Table I presents data on the effect of the larger amounts of 18:2 upon the phospholipid fatty acid distribution. In these experiments, an average of 2.5 μmol of 18:2 was incorporated into phospholipids of fed cells by 100 min (this accounted for 33.6% of the fatty acid complement at that time) and 8.3 μmol by 345 min (accounting for 30% of the fatty acid complement at that time). The differences between fed and control cell fatty acid patterns are, in general, comparable to those found in temperature-shifted cells (Martin et al., 1976) with major polyunsaturated acids (18:2 plus 18:3) being increased from approximately 35–40% of the total to well over 50%. In the isothermal 18:2 feeding experiments (unlike the previous temperature shift ones), the increase was largely due to a rise in 18:2, despite the fact that as much as 30% of the administered 18:2 recovered in phospholipids had been desaturated to 18:3 by 100 min.

A further similarity between the present isothermal experiments and the ones involving a temperature shift is the maintenance of the major saturated fatty acids (14:0 and 16:0) at their normal level, while the monounsaturates (16:1 and 18:1) fell significantly.

The level of polyunsaturated fatty acids in phospholipids of the fed, isothermally grown cells was even higher than the level found in the cells acclimated to 15 °C. One might therefore suspect that the fluidity of membranes in these cells would be in the same range as that of 15 °C cells. To gain information on this parameter, we employed freeze-fracture electron microscopy, which has been found to be a reliable indicator of phase separations of membrane structural lipids (Wunderlich et al., 1975, Kleeman and McConnell, 1976). While one can only infer a degree of fluidity based upon the alterations in membrane particle distribution visualized by this technique, the method may be applied in much the same way that differential scanning calorimetry is used to estimate relative changes in the physical state of membrane lipids. In Figure 1, the particle density index values for the outer alveolar membranes of the isothermally fed cells are compared with equivalent values for control 39.5 and 15 °C cells. The replicas prepared from cells taken 100 min after feeding 18:2 showed very little difference in particle aggregation from control cells. By 345 min after feeding, there was a significant change in the degree of aggregation, despite the fact that the fatty acid distribution in phospholipids had undergone little further change from that experienced by 100 min (Table I). This apparent inconsistency between the two types of data was resolved when it was determined that the fed fatty acids were initially incorporated very selectively into certain subcellular organelles.

The Selective Effect of Exogenous Fatty Acids Upon Functionally Different Membranes. From our previous analyses of cells utilizing ingested fatty acids (Martin et al., 1976), we realized that the most pronounced initial effect would be in the endoplasmic reticulum, where phospholipid synthesis takes place. Although the particle density index method of estimating the extent of lipid bilayer phase separation cannot, at present, be rigorously applied to membranes other than the outer alveolar membrane, some idea of the relative fluidity of other membranes was gained by determining the temperature at which particle-free zones first appear. In unsupplemented 39.5 °C cells, the endoplasmic reticulum first exhibited particle-free domains when cells were chilled to 24 °C. In cells supplemented with 18:2 for 100 min, equivalent signs of phase separation were not seen until the temperature dropped to 18 °C. The feeding of 18:2 had a similar fluidizing effect on nuclear membranes but, surprisingly, mitochondrial outer membranes of fed cells were very little different from those of controls.

As time passed, the highly unsaturated phospholipids synthesized from assimilated 18:2 and its desaturation product 18:3 moved out into other membranes of the cell. Freeze-fracture data of the type described above indicated that, by 345 min after feeding, the fluidity of the endoplasmic reticulum had decreased (first phase separation seen at 21 °C), while that of the outer alveolar membrane had increased in comparison with equivalent membranes 100 min after feeding (Figure 1).

TABLE II: Major Fatty Acids[a] of Phospholipids from Fractions of 18:2-supplemented, 39.5 °C grown cells.

Fatty Acid	Unsupplemented Cells[b]			100 min following Supplementation (Average of Two Experiments)			345 min following Supplementation (Average ± S.D. of Four Experiments)		
	Cilia	Pellicles	Microsomes	Cilia	Pellicles	Microsomes	Cilia	Pellicles	Microsomes
14:0	6.7	8.9	7.3	5.2	12.1	7.8	7.4 ± 2.5	12.5 ± 2.3	9.8 ± 0.8
16:0	16.8	16.4	13.2	20.9	17.8	14.4	19.5 ± 0.8	18.8 ± 1.7	15.8 ± 2.6
16:1	8.3	7.1	8.7	8.1	8.4	9.5	7.6 ± 1.9	5.8 ± 1.9	6.4 ± 2.2
17:0 + 16:2	2.3	4.0	4.0	3.0	2.9	2.7	1.4 ± 0.7	2.4 ± 0.9	2.2 ± 0.7
18:0	6.1	3.0	2.2	5.2	2.0	1.6	5.6 ± 1.9	2.8 ± 0.6	2.3 ± 0.7
18:1	11.7	12.6	13.9	21.8	6.4	6.0	13.5 ± 1.2	4.0 ± 1.8	3.7 ± 1.5
18:2	8.1	10.5	13.0	14.8	23.3	29.2	17.5 ± 4.6	22.1 ± 3.0	26.5 ± 3.4
18:3	20.7	18.5	21.4	12.9	18.5	21.2	18.5 ± 1.6	22.0 ± 3.1	26.1 ± 3.7

[a] Only those fatty acids averaging more than 5% are shown here. [b] Data from Fukushima et al. (1976).

TABLE III: Phospholipid Composition of Cells and Cell Fractions from 39.5 °C Cultures Fed 18:2 for a Total of 345 min.[a]

Phospholipid	Whole Cells (n = 5)	Pellicles (n = 8)	Microsomes (n = 8)	Unfed[b] 39.5 °C Whole Cells	Unfed[b] 15 °C Whole Cells
Cardiolipin	5.9 ± 1.1	3.9 ± 1.9	3.5 ± 1.9	7.8 ± 1.9	5.2 ± 0.6
2-Aminoethyl phosphonolipid	15.6 ± 1.6	23.9 ± 5.5	17.2 ± 4.6	15.6 ± 1.7	29.0 ± 0.6
Ethanolamine phosphatides	40.7 ± 1.8	38.9 ± 6.6	39.1 ± 5.0	42.5 ± 1.3	25.6 ± 4.4
Choline phosphatides	30.3 ± 1.1	26.2 ± 2.3	34.0 ± 2.4	26.8 ± 3.4	27.2 ± 1.3

[a] See Materials and Methods for experimental details. Values are mole % of total phospholipid ± standard deviation. Minor components not shown. [b] From Fukushima et al. (1976).

This pattern of change was verified by analysis of fatty acids from the phospholipids of three isolated membrane systems; pellicles, endoplasmic reticulum (microsomes), and cilia. Table II presents the data showing the gradual flow outward of the newly synthesized lipids. Not only was there a tendency for the fed 18:2 to decrease in relative percent in the endoplasmic reticulum containing microsomes and increase in the externally situated ciliary membranes, but there was a clear-cut desaturation of some 18:2 to 18:3 in all fractions. However, despite the large influx of 18:2 and its product 18:3, the relative amounts of 14:0 and 16:0 increased as compared with nonfed control cells. Decreases were sustained primarily by the monounsaturates.

Effects of Fed Fatty Acids Upon the Distribution of Phospholipid Head Groups. One of the most striking differences between membrane lipids of this *Tetrahymena* strain acclimated to high and to low temperatures is the marked change in the relative proportions of the major phospholipid types of whole cells and of several isolated organelles (Fukushima et al., 1976). It was not possible, then, to decide whether the variation in phospholipid polar head group proportions was caused by temperature itself or by the change in phospholipid fatty acid composition. Analysis of the phospholipids from whole *Tetrahymena* cells and from isolated pellicles and microsomes (Table III), 345 min following fatty acid supplementation, revealed little difference in head group distribution from that found in unsupplemented 39.5 °C cells (Fukushima et al., 1976). A much wider range of values in replicate analyses (see large standard errors) than was found in controls suggested the possibility of some unrecognized variable, but it seems clear that fluidity changes in isothermally-grown cells do not effect an immediate alteration of the phospholipid patterns.

Effects of Fed Fatty Acids Upon de Novo Fatty Acid Synthesis. The experiments described thus far suggest that the dramatic change in lipid composition and membrane properties induced in the endoplasmic reticulum of *Tetrahymena* by feeding 18:2 was gradually counteracted by dilution with endogenously synthesized lipids. In order to return the average membrane lipid composition and fluidity to the optimal values for 39.5 °C, the pattern of newly made fatty acids appeared to be enriched in saturated species and decreased in unsaturated ones (Tables I and II). Direct evidence for this has been obtained by following [^{14}C]acetate incorporation into fatty acids by the fed cells. Figure 2 shows the effect of feeding 18:2 upon the incorporation of [^{14}C]acetate administered at the time of the first feeding (Figure 2A) and at the time of the second feeding (Figure 2B). The results, in both cases, were similar—the proportion of radioactivity going into saturated fatty acids was increased while that going into unsaturated acids decreased. One exception to the rule was oleic acid (18:1), which showed little change in ^{14}C incorporation with feeding.

The total incorporation of [^{14}C]acetate into lipids by fed cells was 60–70% of that utilized by control cells. A determination of lipid phosphorus in eight cell samples each of fed and corresponding control cells taken at various times revealed an average 18% increase in phospholipid/cell in supplemented cultures. Thus, it seems that fatty acid supplementation stimulated a degree of membrane proliferation. Further work will be needed to confirm this apparent increase in membrane content and ascertain whether it is associated with a general increase in cell mass and volume, rather than a selective growth of a particular organelle.

Changes in Nonmembrane Lipids of the Cell. Unsupplemented *Tetrahymena* cells growng logarithmically contain

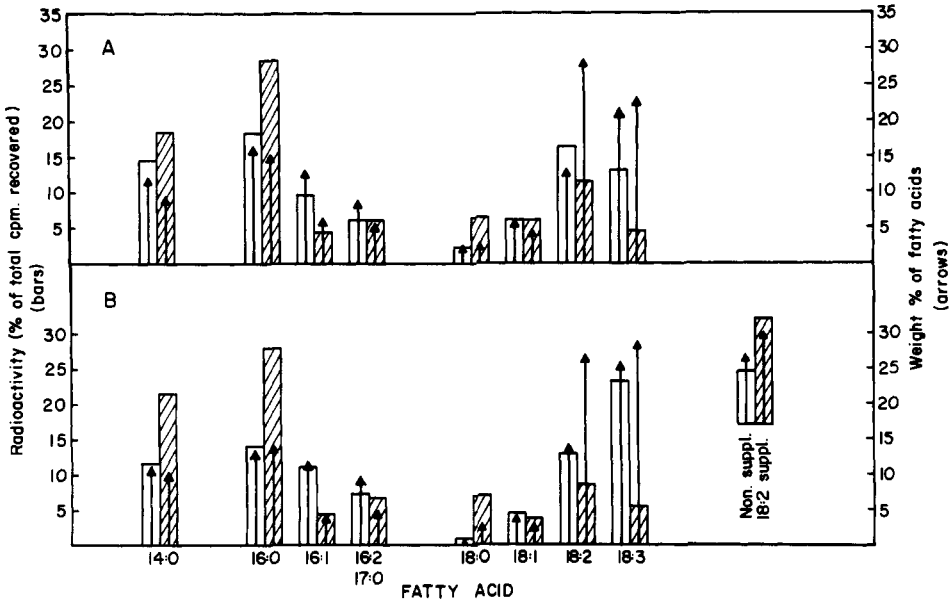

FIGURE 2: The effects of 18:2 supplementation on [^{14}C]acetate incorporation into fatty acids of phospholipids in 39.5 °C grown cells. Panel A depicts the radioactivity (bars) and the composition by weight (arrows) of fatty acids from cells supplemented with 4.3 μmol of 18:2 and a trace of [^{14}C]acetate for 100 min. Panel B shows the equivalent data for cells supplemented with 18:2 as in A but supplied with an additional 4.3 μmol of 18:2 and, for the first time, [^{14}C]acetate at the 100-min point. Lipids were extracted from these cells 345 min after the initial supplementation. In each case, unsupplemented control cells were analyzed for comparison. The data are averages of two experiments.

negligible amounts of triglycerides. On the other hand, thin-layer chromatography of total lipid extracts from cells made at various times following massive feedings of ^{14}C-18:2 revealed an initial pattern of fatty acid uptake into both phospholipids and triglycerides. By 100 min after the first feeding, over 40% of the intracellular radioactivity was present as triglycerides vs. 40% present as phospholipids. After a total of 345 min (including a second feeding), the triglycerides had declined to 13% of the lipid radioactivity, while phospholipids rose to 70%. Of that radioactivity in triglycerides of the fed cells, 7% or less was in the form of 18:3, although the desaturation of ^{14}C-18:2 to ^{14}C-18:3 had proceeded more rapidly (approximately 25% of the total cpm) in the phospholipids of the same cells.

Complementary experiments involving nonradioactive 18:2 feeding coupled with measurements of [^{14}C]acetate incorporation into fatty acids confirmed the relative isolation of the triglyceride pool. By 100 min, or longer following 18:2 feeding, less than 10% of the cellular fatty acids formed de novo from [^{14}C]acetate were recovered from triglycerides. Except for a slightly decreased level of radioactivity in 16:0 after 100 min, the triglyceride distribution of fatty acid radioactivity (but not mass) was quite similar to that in phospholipids. Such findings give assurance that the altered fatty acid distribution in membrane phospholipids was not achieved simply by a large-scale selective migration of certain fatty acid species from phospholipids into the triglyceride droplets.

Discussion

Previous reports from this laboratory (Fukushima et al., 1976) and many others (see Fulco, 1974; Erwin, 1973), including some working with cells from homeothermic animals (Ferguson et al., 1975), have established that variations in the degree of membrane-bound fatty acid desaturation are invariably a part of the temperature acclimation phenomenon. The preceding paper in this issue (Martin et al., 1976) presented data consistent with the hypothesis that temperature acts upon fatty acid desaturation largely in an indirect way. It seemed likely that membrane fluidity, rather than temperature proper, modulates the rate of desaturation. In the present paper, we have held temperature constant while altering membrane fluidity through fatty acid supplementation. The results provide further support for the concept of membrane fluidity-controlled fatty acid desaturation. The most pronounced difference between the findings using cells grown isothermally at 39.5 °C and those obtained using 39.5 °C cells shifted to 15 °C (Martin et al., 1976) lay in the ability of isothermal cells to resist major alteration of membrane physical properties. Polyunsaturates supplied in a concentration sufficient to produce drastic changes in fatty acid composition and freeze-fracture membrane particle distribution in temperature-shifted cells had little effect on cells maintained at 39.5 °C. Even when much higher amounts of fatty acids were fed, the isothermal cells counteracted the forced increase in polyunsaturated components by reducing the level of monounsaturates and raising the content of saturated acids (Tables I and II). The same general reaction of isothermal cells to polyunsaturates occurred in the study of Lees and Korn (1966). In the present case, the incorporation pattern of [^{14}C]acetate into fatty acids (Figure 2) confirms the metabolic basis for the response.

Compared with the normal pattern of phospholipid fatty acids made by 39.5 °C cells (see control cells, Figure 3), 18:2-supplemented cells underwent a sharp decrease in desaturation, particularly that leading to the formation of 16:1 and 18:3. Thus, the cells were able to offset the influx of 18:2 and minimize changes in membrane fluidity, as estimated by freeze-fracture electron microscopy.

By subtracting the quantity of each fatty acid in the cells initially and the amount of exogenous 18:2 incorporated (using ^{14}C-18:2 uptake data) from the fatty acid complement after 100 min, we obtained rough figures for the net synthesis of each acid during the period following supplementation. The results, expressed as percentages of the total production, are: 14:0, 19%; 16:0, 33%; 16:1, 0%; 16:2 plus 17:0, 5%; 18:1, 4%; 18:2, 14%; and 18:3, 16%. These figures, while merely approxima-

TABLE IV: Summarized Effects of Membrane Fluidity on Fatty Acid Desaturation.

Type of Experiment	Fluidity of Endoplasmic Reticulum	Effect on Enzymatic Desaturation
(A) Shifting 39.5 to 15 °C	Suboptimal	Higher than 15 °C controls
(B) Feeding 18:2 + 18:3 before shifting from 39.5 to 15 °C	Optimal → Superoptimal (?)	Lower than 15 °C controls
(C) Feeding large amounts of 18:2 to 39.5 °C cells	Superoptimal	Lower than 39.5 °C controls
(D) Shifting 15 °C cells to 39.5 °C	Superoptimal	Lower than 39.5 °C controls

tions, are in general agreement with independent measurements of net synthesis derived from the [^{14}C]acetate incorporation experiments.

Virtually identical figures are obtained when the same calculations are made (with fewer assumptions needed) from the experiment in the preceding paper of this issue (Martin et al., 1976) in which 15 °C cells were quickly shifted up to 39.5 °C and analyzed for the net increase of each fatty acid after 2 h. In neither instance do our data agree with the report of Conner and Stewart (1976), postulating that in *T. pyriformis*, strain WH-14, a lowering of environmental temperature inhibits the entire pathway 16:0 → 18:0 → 18:1 Δ9 → 18:2 Δ9, 12 → 18:3 Δ6, 9, 12 and stimulates the complementary scheme 16:0 → 16:1 Δ9 → 18:1 Δ11 → 18:2 Δ6, 11.

While there appears to be a more pronounced inhibition of some desaturation steps than others in our studies, uncertainties arising from varying pool sizes and other unknown factors have, thus far, not allowed us to establish this point with certainty. It is not clear, for example, whether calculations of the pool sizes of fatty acid substrate available for desaturation should be based on the distribution of fatty acids in the whole cell or in the microsomal fraction, where the reaction primarily occurs. There is considerable evidence that a family of desaturases exists, each enzyme having its own substrate specificity (Fulco, 1974). It is our ultimate aim to show whether certain desaturases are more sensitive to membrane fluidity changes than others.

In testing the influence of the membrane's physical state on desaturation, we have conducted various experiments in an effort to separate the effects of fluidity from those of temperature per se. The experiments, described above and in the preceding paper of this issue (Martin et al., 1976), are summarized in Table IV. Whereas it is not yet possible to quantify the rapidly changing fluidity in these cases, it can be clearly determined (with the possible exception of experiment B) whether the fluidity is above or below the optimal level for cells acclimated to the appropriate temperature. The data show variations in the relative desaturation rates often greater than the differences found between 39.5 °C grown cells and 15 °C grown cells. This is not unexpected, since, in the latter two cases, the cells are operating at a steady state and an optimal fluidity for their respective temperatures.

Virtually identical results for [^{14}C]acetate incorporation by cells in experiments C and D argue in favor of the common property, fluidity, rather than temperature, as the regulatory factor. In both cases, desaturation is much less than found in control 39.5 °C grown cells. Making allowances for the slower metabolism at 15 °C, the experiments of type B gave results remarkably similar to those in cases C and D. Here again, decreased desaturation coincides with superoptimal fluidity.

As was the case in cells shifted to 15 °C (Martin et al., 1976), the first measurable impact of the exogenous fatty acids on membrane physical properties of 39.5 °C cells was in the endoplasmic reticulum. Electron microscopic evidence (Kitajima and Thompson, 1977) suggests that the fluidity of these membranes increased more rapidly than did that of the others as the 18:2 level rose. In those cases where its location has been established (Fulco, 1974) fatty acid desaturation takes place predominantly in the endoplasmic reticulum. Phospholipids synthesized from the desaturated fatty acids ultimately move out into all organelles of the cell (Nozawa and Thompson, 1971, 1972). We propose that the easily altered fluidity of these particular membranes regulates the pattern of enzymatic desaturation occurring there.

Unfortunately, there is little information available concerning the effect on fatty acid desaturase activity of the molecular environment surrounding the enzymes. Fulco (1974) has summarized much of what is known about the regulation of lipid unsaturation. Our work eliminates some of the proposed mechanisms, as far as *Tetrahymena* is concerned. Thus, the apparent control of desaturase action by molecular oxygen, as reported to occur in yeasts (Brown and Rose, 1969) and higher plants (Harris and James, 1969), has already been ruled out in *Tetrahymena* (Skriver and Thompson, 1976). Increases in the destruction of certain fatty acids by β oxidation is most unlikely in our system. The low recovery of radioactivity from fed longer chain acids (such as ^{14}C-18:2 or ^{14}C-16:0) in shorter chain acids through recycling of [^{14}C]acetate confirms the observations of Borowitz and Blum (1976) that β-oxidation activity is minimal. Likewise, the regulation of phospholipid fatty acid composition by diverting undesirable acids into triglycerides is improbable because (1) the triglyceride content in some instances is too low to account for the observed effects, and (2) the change in triglyceride fatty acid composition is usually not compatible with such an explanation. Finally, the absence of radioactive saturated acids following ^{14}C-18:2 administration virtually eliminates biohydrogenation as an important determinant.

The effect of temperature upon desaturase activity is clearly nonlinear over the range examined. Little change in lipid composition occurred between 39.5 and 26 °C (Fukushima et al., 1976). It is the further drop to 15 °C that caused the striking increase in unsaturation. Based upon our present findings, it is reasonable to postulate that desaturase activity is less inhibited by a lipid phase separation than is net fatty acid synthesis. Wunderlich et al. (1975) have shown and we have confirmed a disappearance, rather than an aggregation, of membranous particles in chilled *Tetrahymena* endoplasmic reticulum. This was thought to indicate a perpendicular movement of proteins outward toward the side of the membranes facing the cytoplasm (Wunderlich et al., 1975). Perhaps, such a movement could increase the exposure of the fatty acid desaturases, thereby lessening the inactivating effect of the increased lipid rigidity. Physical-chemical analyses by electron spin resonance (Nozawa, Y., and Iida, H., in preparation) and fluorescence polarization spectroscopy (Martin, C. E., and Thompson, G. A., Jr., in preparation) are providing more direct evidence correlating the increasing degree of enzymatic desaturation with declining fluidity.

We propose that the fluidity of a membrane, such as the endoplasmic reticulum, which contains the cell's fatty acid desaturase molecules, is self-regulating. The normal inter-

membrane lipid exchange, known to be very rapid in *Tetrahymena* (Nozawa and Thompson, 1972) would presumably be capable of equilibrating any altered pattern of lipids with other organelles. The novelty of the fluidity-controlled desaturase hypothesis is that it can be used to explain the fatty acid changes induced by other environmental factors, such as salinity, pressure, and drugs. Preliminary experiments indicate that the altered fatty acid composition of *Tetrahymena* acclimated to grow in 0.3 M NaCl may be provoked by salt-induced fluidity changes (Mattox and Thompson, unpublished observations). This versatility of *Tetrahymena* assures that the applicability of the proposed control system to diverse variables can be tested.

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