Journal of Supramolecular Structure 2:617–628 (1974)

THE EFFECT OF MEMBRANE-LIPID PHASE TRANSITIONS ON MEMBRANE STRUCTURE AND ON THE GROWTH OF ACHOLEPLASMA LAIDLAWII B

Ronald N. McElhaney

Department of Biochemistry, University of Alberta, Edmonton, Alberta (Canada) T6G 2E1

The physical state of the membrane lipids, as determined by fatty acid composition and environmental temperature, has a marked effect both on the temperature range within which A. laidlawii can grow and on the temperature coefficient of growth within the permissible temperature range. The minimum growth temperature under certain conditions is clearly defined by the lower boundary of the gel–to–liquid-crystalline phase transition of the membrane lipids. The physical state of the membrane lipids can also influence the optimum and maximum growth temperatures. An abrupt increase in the temperature coefficient of growth is noted at temperatures between the phase transition boundaries. Both the absolute rates and the temperature coefficients of cell growth are similar for cells whose membrane lipids exist entirely or predominantly in the liquid-crystalline state, but absolute growth rates decline rapidly and temperature coefficients increase when most of the membrane lipids become solidified. Some cell growth, however, can continue at temperatures at which less than 10% of the total lipid remains in the fluid state. Conversion of the membrane lipid from the liquid-crystalline to the gel state is accompanied by a progressive aggregation of intramembranous protein particles. An appreciable heterogeneity in the physical state of the membrane lipids can apparently be tolerated by this organism without a detectable loss of membrane function.

INTRODUCTION

If synthetic or natural phospholipids are dispersed in excess water at physiological temperatures, the phospholipid molecules normally become arranged in lamellar arrays (1). Studies utilizing a variety of physical techniques have confirmed that these lamallae consist of lipid bilayers separated by water-filled spaces (2). A unique property of these phospholipid bilayer structures is their ability to undergo a reversible, thermotropic gel–to–liquid-crystalline phase transition which arises from a cooperative melting of the

hydrocarbon chains in the interior of the bilayer. Phase transitions of this type have been studied by thermal analysis, infrared, laser-Raman, nuclear magnetic resonance and electron spin resonance spectroscopy, x-ray diffraction, and polarized light microscopy (for review see Ref. 3). Unlike phase transitions between the various liquid-crystalline phospholipid mesophases, the selective melting of the phospholipid hydrocarbon chains does not result in a gross molecular rearrangement; thus, the bilayer structure exists both above and below the transition temperature. The gel–to–liquid-crystalline transition temperature is of course markedly dependent on the structure and chain length of the fatty acid constituents of the phospholipid molecules.

We have shown previously that thermotropic gel–to–liquid-crystalline phase transitions can be detected by differential scanning calorimetry in the cell membrane of Acholeplasma laidlawii B (formerly Mycoplasma laidlawii B) grown in the absence of sterols (4). As expected, the transition temperature was dependent in a characteristic way on the fatty acid composition of the membrane lipid, was completely reversible, and occurred at nearly the same temperature when the membrane lipid was extracted and dispersed as a lamellar phase in excess water. A comparison of the enthalpies of the transition in the biomembrane and in the lipid bilayer-water dispersion revealed that approximately 90% of the membrane lipid participates in the phase transition. Since the melting of the hydrocarbon chains is a cooperative phenomenon which would be markedly affected by hydrophobic interactions with sterols or proteins, we concluded that the majority of the lipid hydrocarbon chains in this organism interact hydrophobically with the hydrocarbon chains of adjacent lipid molecules and not with membrane proteins. Support for these findings has recently come from studies utilizing x-ray diffraction (5, 6) and nuclear magnetic resonance (7) and electron spin resonance spectroscopy (7, 8).

Several investigators have suggested on theoretical grounds that the lipids in biological membranes must exist in the liquid-crystalline state in order for the cell membrane to function normally (1, 2), although until recently little direct experimental evidence was produced to support these speculations. Several years ago we presented evidence that the cell swelling and lysis which occurs when A. laidlawii is grown in the presence of long-chain saturated fatty acids may result from a crystallization of the hydrocarbon chains of the membrane lipids (4). However, these experiments were limited in scope and left a number of questions concerning the relationship between membrane lipid fluidity and membrane function unresolved. I have therefore undertaken a more detailed study of the relationship between the physical state of the membrane lipids, as influenced by environmental temperature and fatty acid composition, and the ability of A. laidlawii B to grow at various temperatures. By altering the fatty acid composition of this organism by manipulation of the fatty acid content of the growth medium (9), it has been possible to study cell growth not only at temperatures where either the gel or liquid-crystalline phase exists exclusively but also within the phase transition range where various proportions of both phases are present simultaneously.

MATERIALS AND METHODS

A. laidlawii B cells were grown in a lipid-poor growth medium as described previously (10) at 2° intervals from 4° to 48°C. Various single exogenous fatty acids (final

concentration 100 μM) were added to the growth medium as sterile ethanolic solutions before innoculation. All fatty acids were purchased from Analabs, Inc. (North Haven, Conn., U.S.A.). Cell growth was monitored by periodic determination of the absorbancy at 450 nm. The minimum and maximum growth temperatures were defined as the lowest and highest temperatures, respectively, at which an increase in the absorbancy of the culture with time was noted. The optimum growth temperature was taken as the temperature which produced the highest rate of absorbancy increase with time. During the logarithmic phase of growth of this organism absorbancy is a valid indicator of total cell protein and viable cell number, although this is not the case during the stationary and death phases (11, 12). As soon as cell growth ceased the cells were collected by centrifugation and the total lipids extracted according to the procedure of Bligh and Dyer (13), all as previously described (10). After removal of traces of nonlipid contaminants by silicic acid column chromatography, a portion of the purified membrane lipid was taken for fatty acid analysis and the remainder utilized for thermal analytical studies. Differential thermal analysis (DTA) of both isolated cell membranes and aqueous dispersions of the total membrane lipid was carried out on a DuPont 900 Thermal Analyzer exactly as recently described (14). The lower and upper boundaries of the membrane lipid phase transition were taken as the temperatures at which the DTA thermogram starts to depart from and returns to the baseline, respectively. The midpoint of the phase transition was defined as the temperature at which one half of the membrane lipid had been converted from the gel to the liquid-crystalline state, as determined from a planimetric measurement of the area of the thermogram peak. The freeze-fracture of intact cells of A. laidlawii was done essentially as described by James and Branton (15) using a Balzers Model BA 511 freeze etching device. Replicas were examined with a Philips Model 300 electron microscope.

RESULTS

Relationship Between Fatty Acid Composition and the Physical State of the Membrane Lipids

Temperature-based thermograms of isolated membranes derived from A. laidlawii cells grown in the presence of a variety of different fatty acids are shown in Fig. 1. In each case the exogenous fatty acid represents between 60 and 80 mole percent of the total esterified fatty acid in the membrane lipid (9). These thermograms depict the gel–to–liquid-crystalline membrane lipid phase transitions discussed previously. Because of the heterogeneous nature of the fatty acid esters present in each membrane system, these phase transitions occur over a 15–25°C range in temperature. Within the transition temperature range, both the gel and liquid-crystalline phases exist simultaneously, so that the membrane is heterogeneous with respect to the physical state of its lipids (3). As expected, the transition temperatures are profoundly influenced by the nature of the fatty acids esterified to the membrane lipids. Membranes enriched in linoleic acid, for example, undergo the phase transition at temperatures nearly 70°C below those observed for membranes enriched in stearic acid. The phase transitions were found to be completely reversible and could be abolished by the addition of cholesterol. Thermograms of disper-

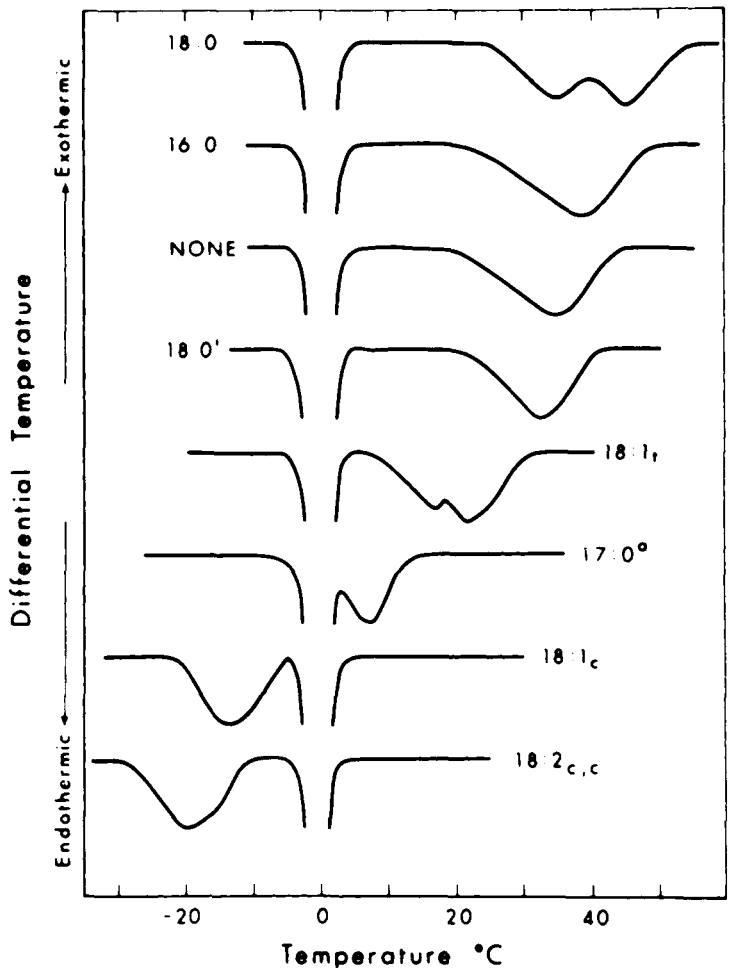

Fig. 1. Temperature-base thermograms of isolated A. laidlawii plasma membranes grown without fatty acid supplementation or in the presence of various exogenous fatty acids. The temperature differentials between the samples and inert reference material are plotted as a function of the temperature of the reference, using a heating rate of 5°C per minute. Cooling the samples at a rate of 5°C results in essentially identical curves except that the entire thermogram is shifted by 2–3°C to lower temperatures. The large endotherm centered around 0°C results from the melting of the ice from excess water associated with the membrane preparations. Fatty acids are designated by the number of carbon atoms followed by the number of double bonds, if any, present in the molecule; the subscripts *c* and *t* denote the *cis* or *trans* configurations, respectively, of these double bonds. The superscipts i and a indicate a methyl group attached to the penultimate carbon atom (an isobranched fatty acid) and the antepenultimate carbom atom (an anteisobranched fatty acid), respectively.

TABLE I. The Minimum, Optimum, and Maximum Growth Temperatures and the Membrane-Lipid Phase Transition Parameters of Acholeplasma laidlawii B Cells Grown in Various Fatty Acids.

Fatty acid	Growth temperatures (°C)			Transition midpoint (°C)	Transition range (°C)
	Minimum	Optimum	Maximum		
18:0*	28	38	44	41	25–55
16:0	22	36	44	38	20–50
None	20	36	44	34	18–45
$18:0^{i}$	18	36	44	32	18–42
$18:1_{t}$	10	36	44	21	5–32
$17:0^{a}$	8	36	44	7	0*–15
$18:1_{c}$	8	34	40	−13	−22 to −4*
$18:2_{c,c}$	8	32	38	−19	−30 to −10

*These temperatures are estimates because a portion of the lipid phase transition endotherms was obscured by the melting of the ice from the excess water associated with the membrane preparations.

sions of the total membrane lipid in excess water were nearly identical to those obtained from isolated membranes of similar fatty acid composition.

Relationship Between the Minimum, Optimum, and Maximum Growth Temperatures and the Physical State of the Membrane Lipids

The minimum, optimum, and maximum growth temperatures of A. laidlawii cells grown in the presence of a variety of exogenous fatty acids are presented in Table I, along with the phase transition temperature range and the transition midpoint temperature of the isolated membranes of corresponding fatty acid composition. It will be noted that the minimum growth temperature *can* be most strongly and directly dependent on the physical state of the membrane lipids. The minimum growth temperature of cells grown in the lower-melting fatty acids does not appear to be determined by the lipid components of the membrane since cells supplemented with linoleic, oleic, or anteisoheptanoic acid could not grow below 8°C. However, the minimum growth temperatures of cells supplemented with all other fatty acids or grown without fatty acid always falls above 8°C and between the lower end of the phase transition range and the transition midpoint, always being closer to the former. Thus, cells whose lipids exist wholly or even in small part in the liquid-crystalline state at any temperature above 8°C are capable of growth at that temperature. An inspection of the DTA thermograms indicates, in fact, that at the minimum growth temperatures only 5–10% of the total membrane lipid still exists in the liquid-crystalline state. As the environmental temperature is lowered to the point where the hydrocarbon chains of the membrane lipids approach the fully crystalline state, cell growth ceases.

The relationship between the optimum and maximum growth temperatures and the physical state of the membrane lipids appears to be an indirect one, since no definite

and constant relationship is discernible between these growth parameters and the membrane lipid phase transitions. The maximum growth temperature is likely determined by the thermal stability of one or more cellular proteins, since cells grown in a variety of the higher-melting fatty acids all show the same maximum growth temperature of 44°C. However, the membrane lipids have some effect on these parameters as shown by the fact that cells supplemented with fatty acids which show the lowest phase transition temperatures also exhibit definitely lower optimum and maximum growth temperatures. Apparently an upper as well as a lower limit exists on the membrane lipid fluidity which is compatible with the growth of this organism.

It is of interest to note that the optimum growth temperature of cells grown without fatty acid supplementation and of cells grown in the presence of many of the higher-melting exogenous fatty acids falls within the phase transition range of the membrane lipids, indicating that in this organism heterogeneous lipid domains exist even in cells growing near the upper limit of their growth temperature range.

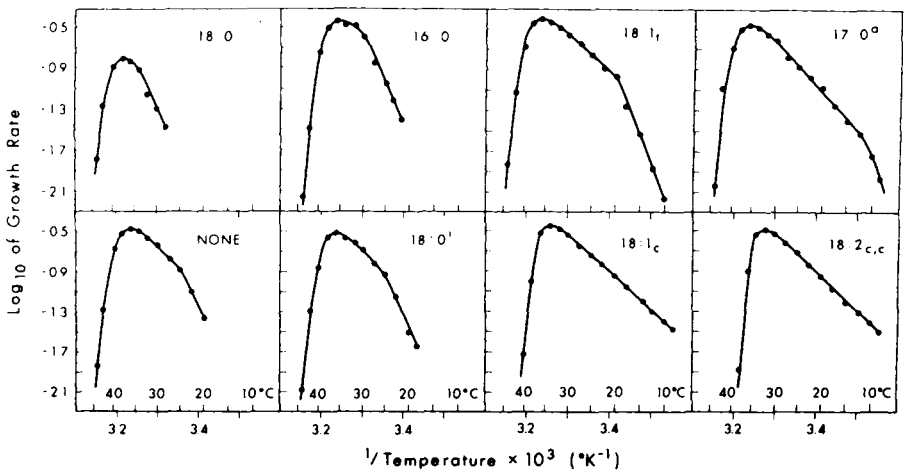

Fig. 2. Arrhenius plots of the relative growth rate of A. laidlawii cells expressed in generations per hr vs the temperature of growth.

Relationship Between Growth Rates and the Physical State of the Membrane Lipids

Arrhenius plots of the growth rate, expressed in generations per hour as a function of temperature are presented in Fig. 2 for cells grown with a variety of exogenous fatty acids. In all cases the rate of growth declines with decreasing temperature, but not in the same manner for cells enriched with different fatty acids. Arrhenius plots of three distinct shapes can be noted. Cells grown in oleic or linoleic acids and whose lipid phase transition midpoint temperatures are well below the minimum growth temperature of these cells exhibit a simple linear dependence of the logarithm of the growth rate on reciprocal temperature over the entire temperature range between the minimum and optimum growth temperatures. The apparent temperature characteristic of growth calculated from the slopes of these plots is 16–17 kcal/mole. Cells grown in palmitic or stearic acid

and whose phase transition midpoint temperatures fall above the optimum growth temperatures for these cells also show a linear relationship between the logarithm of the growth rate and reciprocal temperature in the temperature range between the minimum and optimum growth temperatures. With palmitate- and stearate-enriched cells, however, the apparent temperature characteristic of growth is much higher, being approximately 45 kcal/mole. In contrast, cells grown in anteisoheptadecanoic, elaidic, or isosteric acid or with no fatty acid additions and whose lipid phase transition midpoint temperatures fall between the minimum and optimum growth temperatures of these cells show "breaks" or abrupt changes in the slopes of their Arrhenius plots at temperatures of about 12°, 20°, 24°, and 25°C, respectively. At temperatures above those at which the break in slope occurs, the temperature coefficient of growth is approximately 16–18 kcal/mole, while at lower temperatures this temperature coefficient rises to values of 40–50 kcal/mole. The temperature at which the break in the Arrhenius plots is noted always falls within the phase transition boundaries, usually being closer to the phase transition midpoint than to either the upper or lower temperature limits of the phase transition. However, these breaks in the Arrhenius plots do not appear to correlate with a particular and discrete region of the lipid phase transition to the extent observed for the minimum growth temperatures. Thus the rate of cell growth appears to be unaltered, as compared to cells having all the membrane lipids in the liquid-crystalline state, when as much as half of the membrane lipid exists in the gel state, but the rate of growth declines rapidly with decreasing temperatures when most of the membrane lipid becomes crystalline.

As discussed in the previous section, Fig. 2 also illustrates that the minimum, optimum, and maximum growth temperatures are altered in a characteristic way by variations in the fatty acid composition of the membrane lipids. However, it is interesting to note that at the optimum growth temperature the absolute rates of growth are very similar for cells grown in the various exogenous fatty acids or with no fatty acid additions to the growth medium. In all cases the generation time was approximately 3 hr. The only exception was noted for cells grown in stearic acid, where the maximum growth rate was only half that obtained with all other fatty acid compositions tested.

Relationship of Maximum Cell Density and the Physical State of the Membrane Lipids

The maximum cell densities obtained by cell cultures grown in the presence of a variety of exogenous fatty acids or without fatty acid supplementation at their respective optimum growth temperatures are very similar, thus indicating that maximum cell density does not depend on the physical state of the membrane lipids. Cultures grown in the presence of stearic acid again proved to be exceptional in that the maximum cell density obtained by these cells is normally 40–50% of that obtained with other fatty acid supplementations (data not presented).

Relationship of Membrane Structure to the Physical State of the Membrane Lipids

Freeze-fracture electron micrographs of A. laidlawii plasma membranes enriched in either elaidic or oleic acid and maintained at various temperatures from 0° to 55°C are presented in Fig. 3. The plasma membrane has been fractured in such a way that the

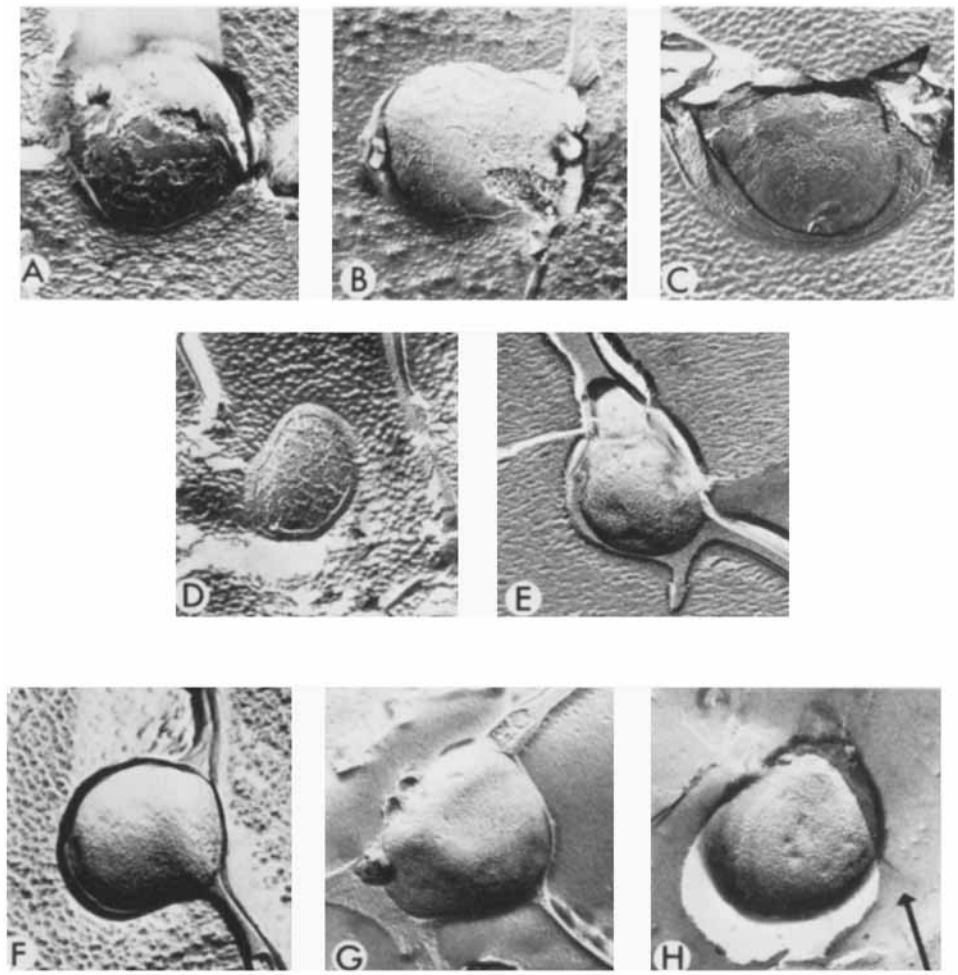

Fig. 3. Freeze-fracture electron micrographs of intact cells of A. laidlawii showing the hydrophobic face of the inner half of the plasma membrane. Micrographs A–E are cells grown in elaidic acid and subsequently maintained at temperatures of 0°C (A), 10°C (B), 20°C (C), 30°C (D), and 40°C (E). Micrographs F–H are cells grown in oleic acid and maintained at 5°C (F), 40°C (G), and 55°C (H). Magnification is 40,000 ×. Shadows appear white in these micrographs, and the direction of shadowing is indicated by the arrow in H.

hydrophobic face of the inner half of the membrane is exposed in each case. The presence of intramembranous particles of 100 Å diameter can be noted, as originally reported by Tourtellotte et al. (8). Tourtellotte and Zupnik (16) have recently presented evidence that these particles are protein in nature and may represent transport proteins which span the A. laidlawii membrane. Cells grown in the presence of elaidic acid exhibited a temperature-dependent aggregation of these intramembranous particles which could be correlated with the membrane-lipid phase transition boundaries. Cells maintained at 40°C (slightly above the upper boundary of the phase transition) show a near-random distribution of particles,

but progressive aggregation is noted as the temperature at which the cells are maintained is reduced to 10°C (just above the lower boundary of the phase transition). Elaidate-enriched cells maintained at a temperature near 0°C exhibit nearly complete segregation of the intramembranous particles in tightly aggregated arrays separated by particle-free areas. Cells enriched in oleic acid, however, which do not undergo a membrane-lipid phase transition at temperatures above 0°C, exhibit a random distribution of particles at all temperatures between 5° and 40°C. I believe that the most likely explanation of these observations is that the aggregation of intramembranous particles results from the squeezing-out of membrane proteins from areas of the lipid bilayer which have been converted to the crystalline state. Thus, the particle-free areas in these electron micrographs are thought to represent areas of lipid existing in the gel state, from which intramembranous particles have been excluded. Qualitatively similar observations and interpretations have been made by Verkleij et al. (17) and James and Branton (15), although neither of these groups related their morphological observations to the boundaries of the membrane-lipid phase transition.

It is of interest to note that cells maintained for 30 min at 55°C, a treatment resulting in irreversible cellular damage and extensive denaturation of membrane protein (4), nevertheless showed the same total number and distribution of intramembranous particles as cells maintained in the normal growth temperature range.

DISCUSSION

A definite relationship exists between the physical state of the membrane lipids and the temperature range over which A. laidlawii cells of various fatty acid compositions can grow. The absolute minimum growth temperature of 8°C is not defined by the physical state of the membrane lipid when cells are enriched in fatty acids giving rise to membrane lipid phase transitions occurring entirely or in part below this temperature. At temperatures below 8°C some other factor, such as the existence of a cold-labile enzyme or regulatory protein, becomes growth limiting. However, the growth of A. laidlawii in fatty acids giving rise to lipid phase transitions occurring above the absolute minimum growth temperature results in a new, elevated minimum growth temperature which is clearly determined by the fatty acid composition of the cell membrane. That this organism should not be able to grow at temperatures at which its membrane lipids exist entirely in the gel state is not surprising and confirms our earlier observation that some proportion of the lipid hydrocarbon chains must be in a liquid-crystalline state to support proper membrane function (4). The optimum and maximum growth temperatures also proved to be influenced to a significant degree by the physical state of the membrane lipids, shifting to lower temperatures when cells are grown in the presence of the lower-melting unsaturated fatty acids. If thermal denaturation of some essential cell protein (or proteins) defines the optimum and maximum growth temperature, this observation suggests that this key protein is an intrinsic membrane protein since its conformation would appear to be intimately related to the physical state of the membrane lipids. Alternatively, the decrease in the optimum and maximum growth temperatures noted with the lower-melting fatty acids could be due to a breakdown in the structure of these more fluid lipid bilayers at higher tempera-

tures. In earlier studies of the effect of variations in fatty acid composition on the cellular permeability to nonelectrolytes, however, the permeability barrier of A. laidlawii cells grown in oleic or linoleic acids appeared to remain intact at temperatures well above the maximum growth temperatures noted in this study (14). I therefore favor the idea that the effect of the fatty acid composition on the optimum and maximum growth temperatures is the result of their effects on the conformation of one or more membrane proteins.

In addition to defining the temperature range over which growth can occur, the physical state of the membrane lipids can also markedly affect the temperature coefficient of growth within this temperature range. At temperatures below the optimum growth temperature, cells which contain up to about half of their membrane lipids in the gel state are able to function quite as well as cells whose membrane lipid exists entirely in the liquid-crystalline state within the same temperature range, as judged both by their similar absolute growth rates and by their similar temperature coefficients of growth of 16–18 kcal/mole. Thus a heterogeneous state of lipids in itself seems not to be detrimental to membrane function in this organism, provided that the proportion of solidified lipid does not become too high. However, if the environmental temperature is lowered so that more than about half of the total membrane lipid exists in the gel state, the temperature coefficient of growth rather suddenly increases to values of 40–45 kcal/mole. If the proportion of crystallized lipid rises still further to values of approximately 90–95%, cell growth ceases entirely. My interpretation of these observations is as follows. At temperatures within the phase transition range but above the midpoint of the lipid phase transition, a sufficiently high proportion of the membrane lipids exists in a functional (i.e., a fluid) state so that the normal growth rate characteristic of cells whose membrane lipids are fully liquid-crystalline may be maintained. The temperature coefficient of growth is determined solely by the temperature-dependence of one or more processes occurring in those parts of the membrane which remain fluid. As the environmental temperature falls below the midpoint of the lipid phase transition, physiologically significant proportions of plasma membrane now become nonfunctional (solidified) and do not permit the potential growth rate characteristic of predominantly liquid-crystalline cells to be obtained. The temperature coefficient now increases because both the total area of membrane remaining functional and the temperature-dependent physiological processes occurring with the remaining functional areas of the membrane become growth-limiting. Eventually, as the lower end of the phase transition is approached, there are almost no functional fluid membrane regions and cell growth ceases. The rapid decline in the growth rate below the phase transition midpoint may also result, at least in part, from structural perturbations caused by the accommodation of rigid, planar arrays of crystallized lipid into small spherical cells. Indeed, evidence for a transient breakdown in the permeability barrier apparently correlated with the lower end of the membrane-lipid phase transition has recently been reported for A. laidlawii (14) and for a fatty acid auxotroph of Escherichia coli (18). The aggregation of intramembranous protein particles and the resulting alteration in the state of the boundary lipid associated with these particles may also contribute to the loss of membrane functionality (19).

It is of interest to note that while the physical state of the membrane has a definite effect on the absolute growth rate at any given temperature, the maximum growth rates and the growth yields obtained at the optimum growth temperature did not vary significantly with the fatty acid composition of the membrane lipids. Cells grown on

stearic acid were the only exception to these rules. With regard to the postulate discussed above, that cells growing at temperatures above their phase transition midpoint temperature have an "excess" of functional liquid-crystalline bilayer, it should be noted that stearate-enriched cells are the only cells in this study whose optimum growth temperature falls below their phase transition midpoint. Thus cells grown in the presence of stearic acid, even at their relatively high optimum growth temperature of 38°C, still have the bulk of the membrane lipid existing in the gel state. The suboptimal areas of functional fluid membrane which may exist only in the case of cells supplemented with stearic acid could account for the reduced growth rate and cell yield obtained at the optimum growth temperature of these cells. Although at temperatures above 38°C the fraction of membrane lipid in the liquid-crystalline state increases, the thermal inactivation of some cellular component rapidly becomes growth-limiting, so that the potential growth rate that is obtained with other cells containing an "excess" of fluid membrane is never obtained in stearate-enriched cells.

A number of recent studies support the idea that the liquid-crystalline state is necessary to support growth (20), membrane transport (20, 21), the assembly of transport proteins (22), and the activity of membrane-associated enzymes (23). Particularly pertinent to the present study is the report of De Kruyff et al. (24) that the temperature coefficient of the ATPase of the A. laidlawii B plasma membrane shows a sharp increase at temperatures which depend on the fatty acid composition of the membrane lipids and correlates well with the lower portion of the gel–to–liquid-crystalline membrane-lipid phase transition. The very rapid decline in ATPase activity with temperature as the conversion to the gel state nears completion may well be related to the similar decline in the growth rate of this organism described in the present study. It would be of interest also to study the effect of variations in the fatty acid composition of the membrane lipids on the temperatures at which optimal enzymic activity and thermal denaturation of this key membrane-bound enzyme occur, to determine if these parameters might also correlate with the optimum and maximum growth temperatures of cells grown in the same fatty acids.

In summary, previous suggestions that the liquid-crystalline state of the membrane lipids is essential to the proper function of the plasma membrane are confirmed, with the important qualifications that, in A. laidlawii at least, up to about half of the total membrane lipid may exist in the gel state without apparent adverse effects on the parameters of cell growth and that the existence of less than a tenth of the membrane lipid in a fluid state is sufficient to support some cell growth and replication, albeit at greatly reduced rates.

ACKNOWLEDGMENTS

I thank Bonnie Gordashko for her excellent technical assistance and Dr. Douglas Scraba and Mr. Roger Bradley for producing the freeze-fracture electron micrographs. This work was supported by a grant (MA-4261) from the Medical Research Council of Canada.

REFERENCES

1. Luzzati, V., and Husson, F., J. Cell. Biol. 12:207–219 (1962).
2. Reiss-Husson, F., and Luzzati, V., Adv. Biol. Med. Phys. 11:87–107 (1967).
3. Oldfield, E., and Chapman, D., FEBS Lett. 23:285–297 (1972).
4. Steim, J. M., Tourtellotte, M. E., Reinert, J. C., McElhaney, R. N., and Rader, R. L., Proc. Nat. Acad. Sci. Wash. 63:104–109 (1969).
5. Engelman, D. M., J. Mol. Biol. 47:115–117 (1970).
6. Engelman, D. M., J. Mol. Biol. 58:153–165 (1971).
7. Metcalf, J. C., Birdsall, N. J. M., and Lee, A. G., FEBS Lett. 21:335–340 (1972).
8. Tourtellotte, M. E., Branton, D., and Keith, A. D., Proc. Nat. Acad. Sci. Wash. 66:909–916 (1970).
9. McElhaney, R. N., and Tourtellotte, M. E., Science 164:433–434 (1969).
10. McElhaney, R. N., and Tourtellotte, M. E., Biochim. Biophys. Acta 202:120–128 (1970).
11. Maniloff, J., Microbios 2:125–135 (1969).
12. McElhaney, R. N., and Tourtellotte, M. E., J. Bacteriol. 101:72–76 (1970).
13. Bligh, E. G., and Dyer, W. J., Can. J. Biochem. Physiol. 37:911–917 (1959).
14. McElhaney, R. N., De Gier, J., and Van Der Neut-Kok, Biochim. Biophys. Acta 298:500–512 (1973).
15. James, R., and Branton, D., Biochim. Biophys. Acta 323:378–390 (1973).
16. Tourtellotte, M. E., and Zupnick, J. S. Science 179:84–86 (1973).
17. Verkleij, A. J., Ververgaert, P. H. J., Van Deenen, L. L. M., and Elbers, P. F., Biochim. Biophys. Acta 288:326–332 (1972).
18. Haest, C. W. M., De Gier, J., Van Es, G. A., Verkleij, A. J., and Van Deenen, L. L. M., Biochim. Biophys. Acta 288:43–53 (1972).
19. Jost, P. C., Capaldi, R. A., Vanderkooi, G., and Griffith, O. H., J. Supramol. Struc. 1:269–280 (1973).
20. Overath, P., Schairer, H. U., and Stoffel, W., Proc. Nat. Acad. Sci. Wash. 67:606–612 (1970).
21. Machtiger, N. A., and Fox, C. F., Ann. Rev. Biochem. 42:575–600 (1973).
22. Tsukagoshi, N., and Fox, C. F., Biochemistry 12:2816–2822 (1973).
23. Kimelberg, H. K., and Papahadjopoulos, D., Biochim. Biophys. Acta 282:277–292 (1972).
24. De Kruyff, B., De Greef, W. J., Van Eyk, R. V. W., Demel, R. A., and Van Deenen, L. L. M., Biochim. Biophys. Acta 330:269–282 (1973).