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Effect of Exogenous Fatty Acids on Growth, Membrane Fluidity, and Phospholipid Fatty Acid Composition in Yeast

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The growth response of a double-mutant fatty acid auxotroph of yeast Saccharomyces cerevisiae to exogenous saturated fatty acids of a homologous series from 12:0 to 16:0, each supplied with oleate, linoleate, linolenate, or *cis*-Δ^{11}-eicosenoate, cannot be explained in terms of the efficiency of incorporation of the fatty acids into phospholipids or alteration of membrane fluidity. There is, however, a negative correlation between growth and levels of 12:0 plus 13:0 in phospholipids, as well as a positive correlation between growth and levels of 14:0, 15:0, and 16:0. We, therefore, conclude that the predominant factor in these phospholipid fatty acyl chain modifications is maintenance of an optimal concentration of C14:0 through C16:0 in phospholipids of this organism.

Key words: yeast growth, fatty acids, phospholipids, lipid fluidity

The importance of the structure of unsaturated fatty acids in membrane-associated properties has been well investigated [1–3]. By comparison, little is known of the importance of the structure of saturated fatty acids. In the present paper, we have modified the paraffinic portion of lipids in a mutant of yeast Saccharomyces cerevisiae that requires a saturated and an unsaturated fatty acid for growth and have studied the consequences of this modification on growth, membrane fluidity, and phospholipid acyl chain composition. We have found: 1) membrane fluidity remains unchanged despite extensive modification of the paraffinic portion of phospholipids; 2) there is no correlation between growth and membrane microviscosity; 3) there is no correlation between growth and level of unsaturated fatty acids in phospholipids. We found a negative correlation between growth and levels of C12:0 plus C13:0 in phospholipids and a positive correlation between growth and levels of C14:0, C15:0, and C16:0 in phospholipids. These observations indicate that there is a stringent requirement for an optimal concentration of saturated fatty acids with chain length of C14:0 to C16:0 in phospholipids for optimal growth of this organism.

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MATERIALS AND METHODS

Materials

Fatty acids were obtained from Applied Science Laboratories, Sigma Chemical Company and Supelco; 2-(10-carboxydecyl)-2-hexyl-4,4-dimethyl-3-oxazolidinyloxyl(12-doxyl stearate) methyl ester was purchased from Syva Research Chemicals. Tergitol NP-40 was purchased from Sigma Chemical Company. All other materials were of reagent grade and were obtained from various commercial sources.

Yeast Strain and Growth Condition

A double-mutant strain of yeast S cerevisiae defective in both fatty acid synthetase and desaturase [4] was used throughout these studies. Cells were grown aerobically at 30°C in the minimal salt medium of Wallace et al [5] supplemented with Bacto-Yeast extract (0.2%), glucose (5%), a saturated fatty acid (0.02%), an unsaturated fatty acid (0.02%), Tergitol NP-40 (3%), and inositol (5 μg/ml). For measuring the extent of growth, each medium (58-ml aliquots in 500-ml Nephalo culture flasks equipped with side arm) was inoculated with a washed suspension of cells (equivalent to 0.6 mg dry weight) from a culture growing exponentially on myristic and oleic acids. The course of growth was monitored turbidimetrically. As soon as maximal turbidity was noted, the yield in dry weight was measured by collecting the cells on preweighed Millipore filters (pore size 0.5 mμ) and drying to constant weight at 65°C.

Lipid Analysis

Cells were harvested during the exponential phase of growth, washed twice with water, and used for lipid extraction. The procedures for extraction of lipids, isolation of the phospholipid fraction, preparation of fatty acid methyl esters, and their gas-liquid chromatography have already been described [6, 7]; however, a Varian Series 1400 gas chromatograph was used in this study.

Electron Spin Resonance Spectroscopy

Cells harvested from the exponential phase of growth were labeled with the methyl ester of 12-doxyl stearic acid as described previously [6, 7] except that 1-ml aliquots of the cell suspension (containing 50 mg dry weight of cells) in 0.02 M Tris HCl, pH 8.0, were incubated with 260 μg of the spin-labeled methyl ester. Under these conditions, approximately 3%–8% of the probe was taken up by the cells. Spectra were recorded at 30°C on a Varian E-12 EPR spectrometer operating at X-band as described previously [7].

RESULTS

Growth Response to Exogenous Fatty Acids

The effectiveness of each of a homologous series of saturated fatty acids plus oleic (18:1), linoleic (18:2), linolenic (18:3), or *cis*-Δ^{11}-ecosenoic acid (20:1) in meeting the auxotrophic requirement of the double-mutant strain of yeast S cerevisiae is shown in Tables I and II. The extent of growth depended on the structure of the saturated and unsaturated fatty acids, as well as on the combination in which the two were provided to the cells. In the series where oleic acid was used (Table I), growth yield in the medium containing myristate showed a mean value of 1.72 mg dry weight per ml. Relative to this value, considered a 100% yield, lauric (12:0), tridecanoic (13:0), pentadecanoic (15:0), and pal-

TABLE I. Cell Yields of the Yeast Double-Mutant Grown on an Unsaturated (ufa) and a Saturated (sfa) Fatty Acid

Fatty acids in growth media[a]		Cell yields[b] (mg/ml)	Fatty acids in growth media		Cell yields (mg/ml)
ufa	sfa		ufa	sfa	
18:1	12:0	0.70 ± 0.06	18:3	12:0	0.44 ± 0.08
18:1	13:0	1.58 ± 0.12	18:3	13:0	0.89 ± 0.12
18:1	14:0	1.72 ± 0.11	18:3	14:0	1.68 ± 0.12
18:1	15:0	1.98 ± 0.10	18:3	15:0	0.76 ± 0.10
18:1	16:0	1.40 ± 0.15	18:3	16:0	0.54 ± 0.05
18:2	12:0	1.05 ± 0.10	20:1	13:0	0.30 ± 0.05
18:2	13:0	2.00 ± 0.15	20:1	14:0	0.58 ± 0.08
18:2	14:0	2.28 ± 0.18	20:1	15:0	0.55 ± 0.06
18:2	15:0	2.04 ± 0.19	20:1	16:0	0.56 ± 0.07
18:2	16:0	1.36 ± 0.11			

[a]Figures before colon refer to the number of carbon atoms; that after colon refers to number of double bonds.

[b]Dry weight; each value is the average of values from two different cultures with deviations from means given after ± symbol. The average values were used to calculate relative growth (cf Table II).

TABLE II. Extent of Growth and Lipid Fluidity of Cells Grown on an Unsaturated (ufa) and a Saturated (sfa) Fatty Acid*

	Fatty acids in growth media		Parameters			Fatty acids in growth media		Parameters	
	ufa	sfa	Growth extent	T_c		ufa	sfa	Growth extent	T_c
A.	18:1	12:0	41	6.4	C.	18:3	12:0	26	9.1
	18:1	13:0	92	8.1		18:3	13:0	53	9.2
	18:1	14:0	100	6.6		18:3	14:0	100	7.8
	18:1	15:0	115	6.6		18:3	15:0	45	8.0
	18:1	16:0	81	6.3		18:3	16:0	32	6.7
B.	18:2	12:0	46	9.5	D.	20:1	13:0	52	6.7
	18:2	13:0	88	8.3		20:1	14:0	100	6.1
	18:2	14:0	100	6.5		20:1	15:0	95	6.1
	18:2	15:0	89	6.2		20:1	16:0	97	9.7
	18:2	16:0	60	8.7					

*Growth extent is given as relative to growth on (A) 18:1 and 14:0 (1.72 mg per ml of culture), (B) C18:2 and 14:0 (2.28 mg/ml), (C) 18:3 and 14:0 (1.68 mg/ml), and (D) 20:1 and 14:0 (0.58 mg/ml). Lipid fluidity is expressed as rotational correlation time (T_c) of the spin probe in nsec.

mitic acids (16:0) were 41%, 92%, 115%, and 81% as effective, respectively (Table II). When supplied with 18:2, 14:0 yielded 2.28 mg dry weight of cells per ml. C12:0 yielded 46% of that; while 13:0 and 15:0 each were 90%, and 16:0 was 60% as effective as 14:0 (Tables I, II). In the series where 18:3 was used to provide the unsaturated fatty acid requirement, again the maximal cell yield (1.68 mg dry weight per ml) was obtained in the culture supplemented with 14:0, with relative values of 26%, 53%, 45%, and 32% for 12:0, 13:0, 15:0, and 16:0, respectively (Table II). When *cis*-Δ^{11}-eicosanoic acid was used, growth,

in general, was very poor as has already been reported by Walenga and Lands [8] for an unsaturated fatty acid auxotroph of S cerevisiae. A maximal yield of 0.58 mg dry weight per ml was recorded for 14:0, which was nearly the same as that for 15:0 or 16:0, and twice that of 13:0 (Tables I and II). Cells supplied with this unsaturated fatty acid and 12:0 failed to grow. For any given saturated fatty acid, the effectiveness of the unsaturated fatty acid in supporting growth decreased from 18:2 to 18:1, to 18:3, to 20:1. In addition, the relative effectiveness of each of the saturated fatty acids depended on the type of unsaturated fatty acid supplement, namely, 41:92:100:115:81 when 18:46:88:100:89:60 when 18:2, 26:53:100:45:32 when 18:3, and 0:50:100:95:97 when 20:1 was added to growth medium.

Rotational correlation time (T_c) of methyl 12-doxyl stearate incorporated into the cells were also calculated [9–12]. A representative spectrum is shown in Figure 1, and correlation times are given in Table II. Altogether, T_c values ranged from 6.1 to 9.7 with a mean value of 7.5 nsec.

Effect of Exogenous Fatty Acids on Fatty Acid Composition of Phospholipids

Tables III through VI show the fatty acid composition of the phospholipid fraction. The proportions of the exogenous unsaturated fatty acid ranged from 5%–69%, and that of unmodified exogenous saturated fatty acid ranged from 8%–40%. The sum of exogenous saturated fatty acids plus their elongation product(s) also varied substantially and ranged from 29%–68% of the total.

Dependence of Growth on the Physicochemical Characteristics of Lipids

Since there were five combinations (n = 5) in series supplied with 18:1, 18:2, or 18:3 and four in the group containing 20:1, the maximal number of degrees of freedom for any correlation or regression analysis would be n = 2 or too few to permit any meaningful analysis for each group separately. They may be all treated as one group of nineteen samples if growth within each group is expressed as the percentage of that on the same saturated fatty acid, for example, 14:0. By so doing, variation due to exogenous unsaturated fatty acids is eliminated, and then the effect due to saturated fatty acid supplementation can be evaluated. In other words, growth extent is converted to relative growth (Table II). By treating the data in this manner, we have tried to find a correlation between growth response and physicochemical properties of lipids. The results are shown in Figures 2 and 3 and Table VII.

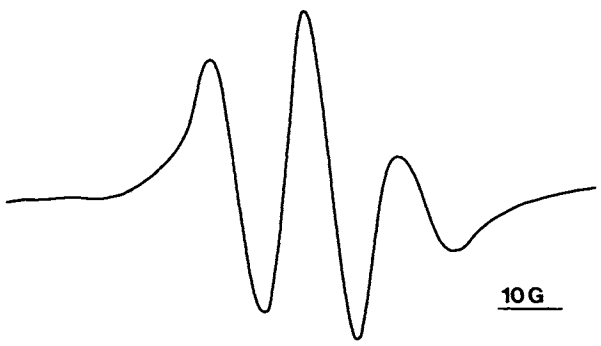

Fig. 1. EPR spectrum of cells labeled with methyl ester of 12-doxyl stearate.

TABLE III. Fatty Acid Composition of Phospholipids of Cells Grown on Oleic Acid and a Saturated Fatty Acid*

Phospholipid fatty acid composition	Saturated fatty acid in growth medium				
	12:0 [3]	13:0 [2]	14:0 [2]	15:0 [2]	16:0 [2]
12:0	18 (5)				
13:0		23 (6)			
14:0	10 (0)		17 (3)	1 (0)	
15:0		34 (2)		30 (3)	
16:0	16 (1)	4 (2)	28 (6)	4 (1)	30 (6)
16:1	13 (2)		16 (6)		22 (5)
17:0		6 (0)		10 (2)	
17:1		2 (2)		13 (3)	
18:0	3 (0)		6 (0)		6 (1)
18:1	41 (4)	30 (6)	34 (4)	41 (2)	42 (0)

*As percentage of total fatty acids in phospholipids. The figure before the colon refers to the number of carbon atoms; that after colon refers to the number of double bonds; the number in brackets refers to the number of cultures analyzed. Each value is the average with mean deviations given in parentheses.

TABLE IV. Fatty Acid Composition of Phospholipids of Cells Grown on Linoleic Acid and a Saturated Fatty Acid*

Phospholipid fatty acid composition	Saturated fatty acid in growth medium				
	12:0 [3]	13:0 [2]	14:0 [2]	15:0 [2]	16:0 [3]
12:0	13 (1)				
13:0		14 (2)			
14:0	5 (0)		8 (2)	1 (1)	1 (0)
15:0		35 (1)		30 (4)	1 (1)
16:0	12 (2)		38 (0)	8 (2)	31 (6)
17:0		8 (0)		15 (3)	
18:0	3 (0)		10 (2)	2 (2)	10 (4)
18:2	66 (4)	40 (0)	46 (0)	44 (0)	56 (10)

*See footnote to Table III.

TABLE V. Fatty Acid Composition of Phospholipids of Cells Grown on Linolenic Acid and a Saturated Fatty Acid*

Phospholipid fatty acid composition	Saturated fatty acid in growth medium				
	12:0 [2]	13:0 [2]	14:0 [5]	15:0 [3]	16:0 [2]
12:0	14 (2)				
13:0		34 (11)			
14:0	3 (0)		10 (1)	2 (1)	1 (0)
15:0		18 (4)	1 (1)	34 (3)	1 (1)
16:0	12 (0)	2 (1)	41 (10)	4 (3)	27 (5)
17:0		7 (2)		23 (5)	
18:0	14 (1)		6 (3)		2 (2)
18:3	57 (4)	40 (8)	41 (11)	37 (12)	69 (3)

*See footnote to Table III.

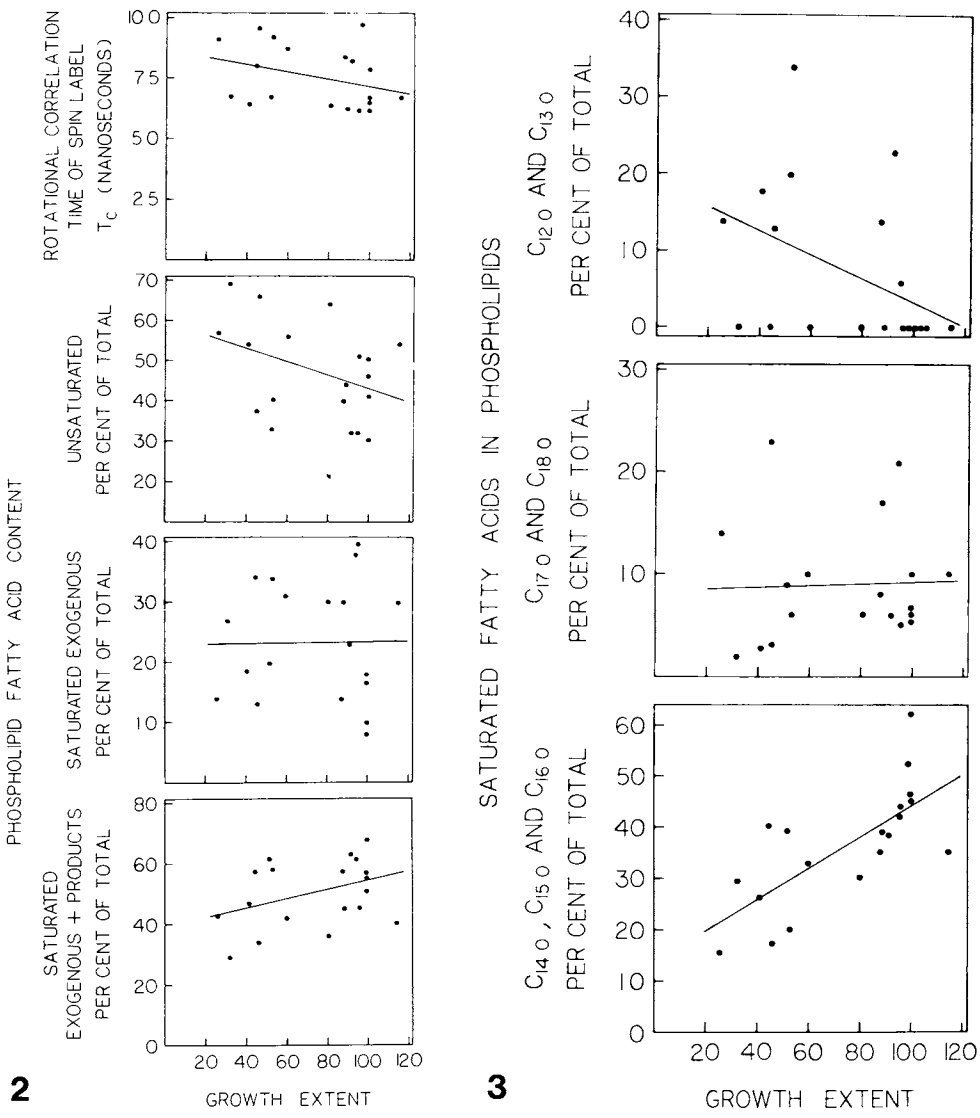

Fig. 2. Correlation between growth extent and some physiochemical properties of membrane lipids. Growth extent refers to relative growth as given in Table II. T_c is rotational correlational time of the spin-label probe also given in Table II. Phospholipid fatty acid content refers to the average values shown in Tables III–VI. Lines of regression were drawn according to Alder and Roessler [26].

Fig. 3. Correlation between growth extent and levels of saturated fatty acids in phospholipids. Growth extent refers to relative growth given in Table II. The data were taken from Tables II–VI and regression or correlation lines were drawn according to Alder and Roessler [26].

As shown in Table VII, the correlation coefficient between growth and T_c values or growth and the level of unsaturated fatty acids in phospholipids was statistically insignificant. Likewise, no correlations were found between growth and the levels of exogenous saturated fatty acids or growth and the levels of exogenous saturated fatty acids plus their elongation product(s).

TABLE VI. Fatty Acid Composition of Phospholipids of Cells Grown on *cis*-Δ^{11}-Eicosenoic Acid and a Saturated Fatty Acid*

Phospholipid fatty acid composition	Saturated fatty acid in growth medium			
	C13:0 [2]	C14:0 [2]	C15:0 [2]	C16:0 [2]
C13:0	20 (2)		6 (2)	
C14:0	1 (0)	18 (2)	1 (1)	1 (0)
C15:0	34 (3)		38 (4)	1 (0)
C15:1	14 (6)			
C16:0	4 (2)	44 (4)	5 (3)	40 (2)
C16:1	2 (0)	18 (2)	2 (0)	30 (1)
C17:0	8 (2)		17 (2)	
C17:1	8 (0)		17 (3)	
C18:0	1 (1)	6 (1)	4 (2)	5 (0)
C18:1	4 (0)	8 (0)	6 (0)	10 (0)
C20:1	5 (0)	4 (4)	7 (5)	11 (0)

*See footnote to Table III.

TABLE VII. Correlation Between Cell Growth and Physicochemical Properties of Membrane Lipids

Variables	Correlation coefficient[a,b]
Membrane fluidity[c]	−0.33
Phospholipid fatty acid content[d]:	
Unsaturated	−0.38
Saturated	
Exogenous	+0.02
Exogenous plus products	+0.35
C12:0 plus C13:0	−0.40[e]
C14:0 through C16:0	+0.71[f]
C17:0 plus C18:0	+0.03

[a]No correlation for coefficients from −0.38 to +0.38.
[b]Calculated from data of Tables II–VI using formula of Alder and Roessler [26]. Please also see Figures 2 and 3.
[c]As monitored by rotational correlation time (T_C) of methyl 12-doxyl stearate.
[d]As percentage of total fatty acids in phospholipids.
[e]A regression, with a confidence level between 90% and 95%.
[f]A correlation, with a confidence level greater than 99.9%.

We then examined the correlation between growth and the levels of three categories of saturated fatty acids, ie, 12:0 plus 13:0, 14:0 through 16:0, and 17:0 plus 18:0, in phospholipids (Table VII, Fig. 3). We found that with a confidence level of 90%–95%, there is a negative correlation between growth and the level of lauric plus tridecanoic acids; with a confidence level of greater than 99.9%, there is a positive correlation between growth and the level of saturated fatty acids 14:0, 15:0, and 16:0 and no correlation between the level of 17:0 plus 18:0 in phospholipids and growth. The lack of correlation with 17:0 and 18:0 may be explained in terms of their levels being too low to exercise a positive or negative effect.

It should be noted that we also used exponential rate of growth, instead of growth extent, as an index of optimal growth and reached the same conclusions. In other words, whether we used growth extent or growth rate, the correlation patterns remained the same.

Further, when the cells were harvested prior to the onset of exponential growth, the level of saturated fatty acids with chain length of 14 to 16 was found to be very low. Thus, cells first grown on 18:1 plus 14:0, then transferred to media supplemented with 18:1 plus 12:0, or 18:2 plus 12:0 and harvested prematurely contained no more than 14%–17% 14:0 plus C16:0 in their phospholipids. These observations further indicate that a minimal amount of such fatty acids must be present in the phospholipids to obtain optimal growth.

It should also be noted that plasma membranes were isolated, as described previously [7], from cells growth on 12:0 plus 18:1, 12:0 plus 18:2, and C16:0 plus 18:1, and their phospholipid fatty acid composition analyzed. In all cases, the compositions were very similar to those obtained for the overall acyl chain composition of the cells. Plasma membrane, therefore, should be useful for studies of the relationship between acyl chain length and membrane properties.

In addition to phospholipids, two other acyl-containing compounds, namely, steryl esters and triglycerides, have been found in wild-type strains of S cerevisiae [13–17]. Concentration of both appears to be dependent on growth conditions. The content of steryl esters in cells growing exponentially on glucose, conditions similar to those we employed, is insignificant and ranges from 0%–10% of total sterol content [13]. The level of triglycerides, while quite high in anaerobically grown cells [15], amounted to less than 10% of that of phospholipids in cells grown at 30°C in chemostat cultures [16], and it has been suggested that triglycerides accumulate only in the stationary phase of growth [17]. We determined the contents of steryl esters and triglycerides in the strain used in the present studies. Total lipids isolated from cells grown on various fatty acids were fractioned by thin-layer chromatography [18], and the concentrations of different classes of lipids were determined according to Amenta [19]. In all cases, combined levels of steryl esters and triglycerides were less than 1% of the weight of total lipids. By comparison, phospholipids accounted for 80 ± 5% of the total. This negligible amount of triglycerides and steryl esters under our conditions may be due to growth conditions we employed and/or the yeast strain used.

DISCUSSION

From the results we have presented here, it is evident that growth response of the mutant to exogenous fatty acids cannot be explained in terms of alteration of membrane fluidity. This statement was based on the following observations: the narrow range in variation of rotational correlation times, the lack of correlation between growth and level of unsaturated fatty acids in phospholipids, and the lack of correlation between growth and mobility of the spin probe. Thus, despite the substantial modification of acyl chains of phospholipids, bulk membrane lipid viscosity at growth temperature was fairly constant.

Another parameter that potentially could explain, for any given unsaturated fatty acid, the relative effectiveness of the saturated fatty acids in supporting the growth of the mutant is the ease or difficulty with which phospholipids are synthesized from these acids. From studies with yeast mutants defective in fatty acid synthetase [20, 21], it has been concluded that the capacity of such cells to incorporate and elongate medium chain fatty acids does not adequately explain the very selective growth response of these mutants to chain length. Furthermore, Orme et al [21] compared the chain length specificity of fatty acyl-CoA synthetase with growth response of one such mutant to saturated fatty acids and concluded that fatty acid activation is not a rate-limiting step in fatty acid metabolism. Also from the extensive incorporation of radioactivity from ^{14}C-laurate into lipids, it was

suggested that failure of the mutant to effectively utilize short or medium chain fatty acids may not be due to impaired assimilation, but most likely to the secondary consequences of defective membrane biosynthesis [21]. Based on these reports and our findings that there was no correlation between growth response and the level of exogenous saturated fatty acids or these plus their elongation product(s), and that the relative effectiveness of saturated fatty acid depended on the nature of unsaturated fatty acid in growth medium, we conclude that the ease or difficulty of incorporation of the saturated fatty acids into phospholipids cannot satisfactorily provide an explanation for their growth-promoting capacity either.

Finally, we examined the dependence of growth on the chain length of saturated fatty acids and found a negative correlation between growth and the levels of 12:0 and 13:0, a positive correlation with those of 14:0 through 16:0, and no correlation between growth and levels of 17:0 and 18:0 (probably because of their low levels in phospholipids). We interpret these findings as a stringent requirement of the organism for an optimal concentration of saturated fatty acids with optimal chain length in phospholipids for optimal growth. Inversely, the linear regression between growth and the level of 12:0 and 13:0 indicates that if chain elongation could be prevented, these fatty acids would not support growth and that phospholipids containing these fatty acids lack properties necessary for maintenance of optimal membrane structure and function.

Based on studies of model membranes [22–25] and our observation that there is a linear correlation between growth and the levels of 14:0 through 16:0 in phospholipids, we expect that some properties of these membranes, such as permeability, stability, and lipid-protein interaction, to be influenced by the length of saturated fatty acids. It has been shown that lecithin liposomes become increasingly permeable to glycerol and glucose as chain length decreases [22]. On the other hand, there is a lower limit to this reduction in chain length since stability of vesicles of didecanoyl, or dilauryl, lecithin is substantially less than that of vesicles of lecithins with long-chain fatty acids [23]. It has been calculated that, in the liquid-crystalline state, the interaction energy of dimyristoyl lecithin and higher homologs is sufficient to permit a stable association of molecules in the bilayer [24]. Finally, the significance of the length of paraffinic chains in lipid-protein interactions is well illustrated by studies of reactivation of β-hydroxybutyrate apodehydrogenase by lecithins of saturated fatty acids [25], where it was shown that the apoenzyme binds to vesicles of lecithins containing short-chain fatty acids with low affinity and poor reactivation efficiency. These were attributed more to impaired hydrophobic interactions and molecular fit than to the phase characteristics or phospholipids.

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