Quantitative Measurement of the Effectiveness of Unsaturated Fatty Acids Required for the Growth of *Saccharomyces cerevisiae*

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The growth response of a mutant of *Saccharomyces cerevisiae* which is unable to synthesize unsaturated fatty acids has been measured in the presence of variable concentrations of exogenous unsaturated fatty acids. Final cell yields, doubling times, and lag times were all found to vary as a function of the initial concentration of the added unsaturated acid. The cell yield was found to be a convenient quantitative measurement to use in comparing the effectiveness of various unsaturated acids. Values for the acids ranged from 1.7 to 11 cells per femtomole with values for oleate and palmitoleate at 2.7 and 4.3 cells per femtomole, respectively. In general, the effectiveness of unsaturated acids was found to increase with an increasing number of double bonds. Saturated fatty acids of a chain length of 5 to 18 carbon atoms were completely ineffective. The varied efficiencies of different unsaturated fatty acids indicate that unsaturation per se was not the basis of the nutritional requirement and indicate certain acids that would be useful in further studies of the role of unsaturated acids in cell function.

Large differences in lipid composition exist among naturally occurring biomembranes. For example, the lipids of rat liver plasma membrane contain about 20 to 30% sterol by weight (30), whereas the membrane lipids of *Escherichia coli* and other bacteria lack sterol completely (14, 17). Similarly, phosphatidyl choline represents some 40% of the total phospholipid in rat liver membranes (30), but is absent from *E. coli* cells (12, 14), which contain about 85% phosphatidyl ethanolamine (12). In spite of the large compositional differences, some generalizations can be made concerning biological membranes; one is that all of these membranes contain phospholipids. In general, the phospholipids have been found to contain both saturated and unsaturated fatty acyl esters which are distributed nonrandomly between the available positions (Table 1, 8). Reports during the past 10 years have provided a more detailed base of information on how certain acids come to be located in certain positions of tissue phosphoglycerides. Not only have the specific enzymic steps for selectivity been suggested, but also some concept of the chemical features recognized by those enzymes is evolving. Furthermore, the selectivities for some acyltrans-ferases measured in vitro may occasionally allow a fairly accurate prediction of the compositions of saturated and unsaturated fatty acids in some membrane lipids (18, 19, 43). Most importantly, the enzymic selectivities for acids may not be simply classed as saturated or unsaturated, but may depend more upon ethylenic \( \pi \)-bonds at certain locations or upon configuration at certain locations depending upon the catalyst being considered (26, 40).

Although the compositional data always showed a consistent presence of some unsaturated or low melting acids in membrane phosphoglycerides, the recent isolation of mutants requiring exogenous unsaturated fatty acids of *E. coli* (5, 27, 35) and *Saccharomyces cerevisiae* (15, 32) has more clearly demonstrated the essentiality of the presence of unsaturated fatty acids in membrane lipids. Nevertheless, though several groups have shown that a variety of fatty acids can fulfill the nutritional requirement for an unsaturated fatty acid (4, 31, 36, 46, 47), no quantitative comparison of the effectiveness of various acids has been provided and the structural features that make an unsaturated acid beneficial to living cells have not been identified. A method
for quantitatively comparing the relative suitability of various unsaturated fatty acids would not only provide more precise information on the structure-function relationships of acids in a given organism, but would also provide a comparative measure of the acyl chain requirements for different organisms. We have, therefore, examined the ability of a variety of saturated and unsaturated fatty acids to promote the growth of *S. cerevisiae* KD46, a mutant requiring unsaturated fatty acid (15). *S. cerevisiae* was chosen because it is a eukaryote whose growth and genetic properties can be conveniently studied in liquid culture. The results provided reproducibly quantitative measures of the growth response as a function of the concentration of different added fatty acids.

**MATERIALS AND METHODS**

Yeast extract, agar, and peptone were all obtained from Difco. Fatty acids were purchased from the Hormel Institute or from Nu-Chek Preps and were of the highest purity available. Tergitol NP-40, a product of Union Carbide Company, was provided to us by Alec Keith. Tween 80 and Tween 40 were obtained from the Sargent Welch Scientific Company. BF₃ (14%) in methanol was obtained from Applied Science Laboratories, State College, Pennsylvania.

All other chemicals were obtained from commercial sources and were reagent grade or better.

**Organism and medium used.** *S. cerevisiae*, strain KD46 (ole 2), was a generous gift from Alec Keith. The basal medium used is the complex yeast extract-peptone-dextrose (YPEP) broth described by Keith et al. (15). The detergent Tergitol NP-40 was added at 1% (wt/vol) only in a few experiments where indicated. A solid medium for cell growth was prepared by adding 2% (wt/vol) agar to the basal system. For these agar plates, either Tween 80 or Tween 40 (2% wt/vol for both) was included as a source of unsaturated or saturated fatty acids, respectively (32). The KD46 mutant, because of its requirement for an unsaturated fatty acid, grows well on agar plates containing Tween 80 and very poorly on plates containing Tween 40. A suspected revertant, isolated by its ability to grow in liquid medium which contained no unsaturated fatty acid supplement, was shown to grow equally well on both Tween 80 and Tween 40 plates. In order to maintain the strain, fresh plates were prepared every 2 to 3 days by dispersing the cells from a single colony in sterile medium, diluting, and transferring about 100 cells in 0.1 ml to a fresh plate. The purity of the mutant colonies was checked by replicate plating onto both Tween 40 and Tween 80 plates. Revertants that no longer require unsaturated fatty acids as well as contaminants can be recognized and avoided by this procedure.

Growth experiments were performed as follows. Twenty hours before an experiment was begun, a tube containing 8 ml of medium (with 50 μM oleate as supplement) was inoculated to a density of approximately 10 × 10⁶ to 15 × 10⁶ cells per ml by using a sterile loop to transfer colonies grown on solid medium. After 8 h of growth at 30 C, samples were diluted to provide several tubes with cell densities between 3 × 10⁹ and 15 × 10⁹ cells per ml. After 12 additional h the tube that most nearly approximated ¹ log growth (approximately 10⁸ cells per ml) provided the inoculum for the growth experiment. Growth tubes were then incubated at 30 ± 2 C on a New Brunswick model G-25 gyrotary shaker at shaker speed "9". The above procedure was designed

### Table 1. Effectiveness of some common fatty acids in supporting the growth of *S. cerevisiae* KD46

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Double bond position (Δ)</th>
<th>ϵ (cells per fmol)</th>
<th>ϵ⁻¹ (amol per cell)</th>
<th>Tₘᵣₐₓ(h)</th>
<th>Kₐ (μM)</th>
<th>Kₐ' (μM)</th>
<th>Maximum yield (cells/ml × 10⁻⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:1</td>
<td>9</td>
<td>4.26 ± 0.36 (11)</td>
<td>234</td>
<td>1.8</td>
<td>6.3</td>
<td>47</td>
<td>110</td>
</tr>
<tr>
<td>18:1</td>
<td>9</td>
<td>2.73 ± 0.16 (22)</td>
<td>366</td>
<td>2.4</td>
<td>11.3</td>
<td>900</td>
<td>125</td>
</tr>
<tr>
<td>18:2</td>
<td>9, 12</td>
<td>5.32 ± 0.43 (11)</td>
<td>199</td>
<td>2.0</td>
<td>12</td>
<td>22</td>
<td>120</td>
</tr>
<tr>
<td>18:3</td>
<td>9, 12, 15</td>
<td>4.64 ± 0.24 (8)</td>
<td>216</td>
<td>1.6</td>
<td>5.4</td>
<td>40</td>
<td>125</td>
</tr>
<tr>
<td>20:2</td>
<td>11, 14</td>
<td>4.74 ± 0.39 (7)</td>
<td>211</td>
<td>6.1</td>
<td>1.0</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>20:3</td>
<td>11, 14, 17</td>
<td>4.36 ± 0.49 (10)</td>
<td>230</td>
<td>3.6</td>
<td>5.0</td>
<td>17</td>
<td>40</td>
</tr>
<tr>
<td>20:4</td>
<td>5, 8, 11, 14</td>
<td>8.80 ± 0.63 (20)</td>
<td>114</td>
<td>2.2</td>
<td>4.5</td>
<td>170</td>
<td>150</td>
</tr>
<tr>
<td>20:5</td>
<td>22:1</td>
<td>9.20 ± 0.75 (7)</td>
<td>109</td>
<td>2.4</td>
<td>4.2</td>
<td>100</td>
<td>130</td>
</tr>
<tr>
<td>22:6</td>
<td>22:2</td>
<td>10.99 ± 0.65 (10)</td>
<td>600</td>
<td>6.0</td>
<td>1.2</td>
<td>(4)</td>
<td>30</td>
</tr>
<tr>
<td>5:0</td>
<td></td>
<td>0.007</td>
<td>&gt;140,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6:0</td>
<td></td>
<td>0.012</td>
<td>&gt;83,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8:0</td>
<td></td>
<td>0.062</td>
<td>&gt;16,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:0</td>
<td></td>
<td>0.063</td>
<td>&gt;16,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td></td>
<td>0.122 ± 0.029 (10)</td>
<td>91</td>
<td>2.2</td>
<td>5.0</td>
<td>75</td>
<td>110</td>
</tr>
<tr>
<td>18:0</td>
<td></td>
<td>0.044 ± 0.082 (11)</td>
<td>&gt;23,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values are given as mean ± standard error (number of determinations). Tₘᵣₐₓ, Kₐ, and Kₐ' values are determined from intercepts of plots such as those shown in Fig. 4 and 5. Values for ϵ and maximum yield for each acid were determined from yield plots such as those shown in Fig. 3.*
to insure uniformity among the inocula from experiment to experiment.

**Measurements of cell growth.** Cell growth in liquid culture was monitored turbidimetrically by reading the absorbance at 660 nm in a Bausch & Lomb Spectronic-20 spectrophotometer. Tubes, 18 mm in diameter, containing 8 ml of medium were used throughout. Cell cultures having absorbance at 660 nm less than 1.0 were read directly, whereas those having absorbance greater than 1.0 were evaluated by diluting cultures 1 to 9 in 0.1 M NaCl and by determining absorbance at 660 nm of this diluted solution. Cell numbers (cells per milliliter) were also determined by direct microscope observation and counting in a Levy-Hausser hemacytometer (42). In addition, viable cell counts were obtained by plating a known dilution of liquid culture on Tween 80 plates and counting the number of colonies developing after 48 to 72 h.

**Fatty acid supplements.** The concentration and purity of all fatty acid stock solutions was checked by using standard qualitative gas chromatographic techniques with methyl esters prepared by modification of the method originally described by Metcalfe and Schmitz (24) with a known amount of pentadecanoic acid included as an internal standard in each run.

The purity of the acids used in these studies is given below: palmitoleate (16:1), olate (18:1), and eicosadienoic acid (20:2) were all >99% pure with no impurities detectable on the chromatograms. Linoleate (18:2) contained about 1% 16:1 and the linolenate (18:3) preparation was contaminated with 1% 18:2 and 0.6% 18:1. Arachidonate (20:4) was also found to contain small amounts of linolenate (0.8%) and olate (1.5%). Eicosatrienoic (20:3) and eicosapentaenoic (20:5) acids each contained trace (<1%) impurities whose retention times did not correspond with any common unsaturated fatty acid. Docosahexaenoic acid (22:6) contained a 3.2% impurity whose retention time was longer than that of 22:6 itself. Fatty acid stock solutions were prepared as soaps in ethanol and stored at -14 C.

**RESULTS**

The relationship between cell abundance and absorbance at 660 nm derived from the entire range of values encountered in these studies gave a value of (38.4 ± 2.0) × 10⁴ cells per ml for a culture of A = 1.0. A series of viable cell-count data obtained by plating diluted cultures onto plates containing Tween 80 gave a value of (35.0 ± 2.6) × 10⁴ cells per ml per A₆₆₀.

**Effect of fatty acid concentration on the growth of KD46.** Growth curves obtained in the presence of various concentrations of palmitoleic, arachidonic, oleic, and linoleic acids are presented in Fig. 1. Results obtained when no exogenous fatty acid was added or when palmitate was added are also included in Fig. 1 for comparison. All of the curves can be characterized by the lag, log, and stationary growth phases outlined by Monod (25). The control cultures which were unsupplemented, or supplemented with palmitate, exhibited some growth but generally failed to exceed one doubling the initial cell number. Jollow et al. (11) and Proudlock et al. (31) report similar growth of *S. cerevisiae* on unsupplemented complex growth medium and were able to trace the source of this growth to small amounts of unsaturated fatty acids present in yeast extract. The cells in our control experiments may well have been utilizing such contaminants.

It can be seen in Fig. 1 that the final cell number attained by a given culture depended upon the initial concentration of the unsaturated fatty acid supplement. It can also be seen that the duration of the initial lag phase was extended at increasing fatty acid concentrations. This phenomenon seemed particularly striking for palmitoleate (Fig. 1a).

The rates of cell growth also varied with the initial concentration of fatty acid supplement. For example, in Fig. 1b the doubling times of the cultures growing on arachidonate are 4.5 h at 2.6 μM, 3.0 h at 5.2 μM, and 2.0 h at 13.1 or 26.1 μM. Similar dependencies were observed for the other acids and, in addition, some acids were found to slow the rate of growth at high concentrations. Such a situation can be seen in Fig. 1d where the doubling times of cultures growing on olate are 3.0 h at 5.9 μM 18:2, 2.8 h at 11.7 μM, 2.5 h at 17.5 μM, and then 3.3 h at 58.5 μM. The dependencies of the cell yields and growth rates on fatty acid concentration will be presented in some detail to show the reproducible and quantitative nature of the phenomenon for added fatty acids.

Figure 2a shows the final cell yield plotted as a function of fatty acid concentration for olate and arachidonate and for olate in the same medium containing added 1% Tergitol. The cell yields were calculated as the difference in final and initial cell numbers from growth curves such as those shown in Fig. 1. The same procedure was applied to the control growth curves obtained in the absence of any fatty acid supplementation. This latter value was subtracted from those observed with added fatty acids. The data in Fig. 2, then, represent changes in cell density over and above that seen in the control experiments, and negative values (such as those for palmitate) are a result of cultures which show less increase than the controls. This procedure corrects for growth arising from traces of olate carried over from the inoculum or from materials other than the added fatty acid that is under study. The data in Fig. 2a are composites of six experiments for olate and five for arachidonate. Thus, the variability in the data may reflect small varia-
Fig. 1. Growth curves of KD46 on four different fatty acid supplements. The log of the cell number (determined turbidimetrically) is plotted against the time after inoculation. All cultures were grown in YEPD medium at 30 ± 2 C. The number to the right of each curve is the micromolar concentration of the fatty acid supplement. (a), Palmitoleate, cis Δ9 hexadecenoate; (b), Arachidonate, cis Δ5, 8, 11, 14 eicosatetraenoate; (c), Oleate, cis Δ9 octadecenoate; (d), Linoleate, cis Δ9, 12 octadecadienoate. Control cultures containing palmitate (a) or no added fatty acid (b, c) are also shown.
tions in growth medium from day to day.

The response of cell yield to fatty acid concentration was found to be reasonably linear at low fatty acid concentrations, whereas the response at higher concentrations of fatty acid was somewhat variable. In some cases, the final cell yield became independent of fatty acid concentration (e.g., olate, arachidonate); in other cases a decrease in the yield was observed as fatty acid concentration was raised (e.g., palmitoleate, docosahexaenoate). In the former cases, we presume that some other component of the growth medium such as glucose or amino acids become growth limiting at a cell density of about 150 million cells per ml. In fact, we have calculated by using the constant presented by Beauchop and Elsdon (1) that 2.3 mg (dry weight) per ml is the expected cell yield for fermentative growth on 110 mM glucose (2% wt/vol). This value is similar to the yield obtained by Clark-Walker and Linnane (2).

Measurements taken from electron micrographs (2) reveal that petite mutant cells are about one-third the volume of the wild-type cells which have a dry weight of \(37 \times 10^{-12}\) g per cell (13). Thus, the yields of petite cell could be expected to be limited to 186 million cells per ml (2.3 mg/ml + 12.3 \(\times 10^{-9}\) mg/cell) under our growth conditions. Table 1 shows that the maximum cell yields obtained with high levels of nutrient acid were in fair agreement with this calculation. However, for three of the acids we tested, namely 20:2, 20:3, and 22:1, the cell yield became independent of the fatty acid concentration at 30 to 40 million cells per ml. The explanation of this phenomenon is not readily apparent since we know that the medium was usually capable of supporting more extensive growth.

The data in Fig. 2 show that for the low ranges of fatty acid concentration there is a linear dependence of cell yield on fatty acid concentration. Such linear dependencies have been observed for a variety of carbon sources (31) and growth factors (10) and represent the basis for one type of bioassay (37, 38). The dimensions of the slope of such a line are cells (produced) per mass of nutrient added and this slope, which we have called epsilon (\(\epsilon\)), therefore, can be thought of as an efficiency factor. The values of \(\epsilon\) for various unsaturated fatty acids are given in Table 1.

The growth rate can be characterized by a doubling time or generation time (T) which is related to the specific growth rate (ks) by the equation: \(T = \ln 2/k_s = 0.69/k_s\) (29).

The relationship between the doubling time (T) and the reciprocal of the initial substrate concentration is shown in Fig. 3 for palmitoleate, olate, arachidonate, and docosahexaenoic acids. The linearity of these plots suggest that the rate data are related to fatty acid concentration by a hyperbolic equation. From these plots, the concentration of fatty acid giving one half of the maximum growth rate (ks) can be obtained for each fatty acid and the maximum growth rate can be estimated from the ordinate intercept. The kinetic constants \(K_s\) and \(T_{\text{min}}\) are tabulated in Table 1 for each acid.

Also notable in Fig. 1, 3c, and 3d is a distinct
increase in doubling time (inhibition of the growth rate) by high concentrations of fatty acid. When the data are replotted as $T$ versus $(S)$, by analogy with the procedure used for excess substrate inhibition of enzymatic velocities (Ref. 3, p. 78), a substrate inhibition constant ($K_s'$) can be determined for each acid. Examples of such plots are shown in Fig. 4 and the resulting $K_s'$ values determined in this way for all acids tested are tabulated in Table 1.

**Fig. 3.** The doubling times ($T$) in hours (per generation) of various cultures are shown plotted against the reciprocal of the concentration of the fatty acid.

**Fig. 4.** Determination of the excess (nutrient) substrate inhibition constants ($K_s'$) on the growth rate of KD46. The doubling times are plotted against the concentrations of oleate (a) and arachidonate (b). $K_s'$ can be determined from the abscissa intercepts.

supplement for arachidonate (a), oleate (b), docosahexaenoate (c), and palmitoleate (d). Solid circles represent data obtained in YEPD medium, whereas the data denoted by $\times$ were obtained in YEPD medium containing 1% (wt/vol) Tergitol NP-40. The substrate activation constant, $K_a$, can be determined from each abscissa intercept and the minimum doubling time ($T_{\text{min}}$) can be determined from the ordinate intercepts.
DISCUSSION

Included in Fig. 2 and 3 are some data obtained in the presence of 1% Tergitol NP-40, a non-ionic detergent. This detergent was included in some early experiments as a solubilizing agent for added fatty acids. In each case the growth response of the mutant is different in the presence of Tergitol than that observed in its absence. In general, we feel that these differences in response can be explained by the formation of hydrophobic complexes (mixed micelles) which render a variable portion of the added fatty acid unavailable to the organism. In support of this hypothesis is the observation that although higher levels of added fatty acid were needed to attain a given growth rate in the presence of detergent, the \( T_{\text{min}} \) values obtained were independent of the presence of detergent (Fig. 3a, b).

Measurements of microbial growth have been used for many years in the assay of vitamins and other nutrients (37, 38) and a wide variety of essential metabolites have been recognized and purified using such assays. For example, McIlwain (23) used streptococcal growth to study the metabolic interrelationship between pantoyltaurine and pantothenate. More recently Johnston et al. (10) used the growth of \( S. carlsbergensis \) to demonstrate the biological activity of several oxidation products of myo-inositol. Proudblock et al. (31) and Kormanickova et al. (16) and many others have used total growth to measure the efficiency of utilization of various energy sources by yeast cells.

In our studies, we have used measurements of the total growth of KD46 to calculate a relative effectiveness for each fatty acid tested. Appreciable amounts of carbohydrate and amino acid are present in the growth medium and any needed saturated fatty acids are presumably formed from these sources. Since KD46 does not have the mitochondrial oxidative pathway to oxidize the acids, the limiting amounts of the nutritionally required unsaturated acids that were added seem most likely to be incorporated into elements needed in cell function rather than representing in themselves a limit to the available energy. If so, the reciprocal of the efficiency term \( (1/e) \) represents, in part, a minimal requirement for forming cellular components. This concept was also presented by Monod (25) as the "amount of limiting nutrient used up in the formation of a standard cell." Values for each fatty acid (Table 1) range between 100 amol (atomoles) per cell for 22:6 and 600 amol per cell for 22:1, the most and least effective fatty acids tested, respectively.

It is of interest to compare the amounts of acids necessary for growth with the reported content of lipids in \( S. cerevisiae \). Using the report that this organism contains about 3% phospholipid on a dry weight basis (11) and that the dry weight per cell may be \( 37 \times 10^{-12} \) g (13), and assuming a molecular weight of 750 for an average phospholipid molecule, it can be calculated that each cell may contain about \( 1.4 \times 10^{-17} \) mol of phospholipid. In other words, about 2,800 amol of total fatty acids might be required to form the phospholipids present in one wild-type cell.

Experimental results with wild-type cells grown in rich media show that palmitoleate and oleate normally represent 50 and 25%, respectively, of the yeast fatty acids (9, 20, 39). However, the values for 16:1 and 18:1 obtained in this study indicate that the minimal amounts of these unsaturated acids needed is much less than that accumulated in cells with an abundant supply. In this way, studies with restricted nutrient supplies provide a clearer index of the degree of essentiality and efficiency of certain acids for cell growth and function. In this regard, Proudblock et al. (31) reported that mitochondrial function was not appreciably altered by a shift of unsaturated fatty acid content from 70 to 20%.

It should be noted that the substrate saturation constants \( (K_s) \) in Table 1 for the various fatty acids are relatively similar. With the exceptions of 18:1, 20:2, and 22:1, the value is about 5 \( \mu \)M for all the acids. Thus, the rate-limiting effects were less selective than the extent-limiting properties. The values for inhibitory effects \( (K_s') \) were more variable than the \( K_s \) constants and, again, the value for oleate was very atypical.

Stimulation of the rate of growth at low essential nutrient concentrations can, as a first approximation, be fitted by the equation presented by Monod (25, 42): \( v = V_m/(1 + K_s/S) \). Recently, Shehata and Marr (34) have shown that the response of the growth rate of \( E. coli \) to glucose, inorganic phosphate, and amino acids shows a biphasic activation which cannot be fitted by the simple Monod equation, but requires a more complex description involving a summation of individual terms. Our rate data could be explained by such a summation in which one or more of the substrate terms is of opposite sign. Although the inhibitory effect could be due to a detergent action of the added substrate, we find it hard to explain the great variances in inhibition by such structurally similar acids.

The minimum \( T \) value (maximum growth rate) was reasonably constant throughout the series except for 20:2, 20:3, and 22:1 which
gave higher T values. This observation can be interpreted to mean that some reaction step involved in the conversion of these acids from free exogenous acids to lipid products still limits the rate of growth of the organism even when fatty acid is fully saturating.

It is not clear whether the magnitude of the efficiency term, $\epsilon$, is due to the suitability of the acid as a precursor of cellular materials or to the suitability of the resultant products containing that acid for growth and membrane function. Since this measure of effectiveness ($\epsilon$) was determined by total growth and was independent of the rate of growth, the suitability of an acid as a substrate could be important only if some reaction step showed a very high discrimination against that acid. Conversely, if all acids tested have a similar rate of incorporation (exogenous acid $\rightarrow$ lipid products), then the structural or functional nature of the lipid products must be the principal determinant of the value of $\epsilon$. In the case of short- and medium-chain saturated acids, the esterified products might be expected to resemble those of typical monounsaturated acids (41). The fact that no saturated acid tested could fulfill the requirement for growth suggests that exogenous supplies of those acids are not suitable substrates. We expect that the different efficiencies observed will in some cases reflect both kinetic features of utilization and the subsequent features of the products formed.

Several groups have shown that the properties of the lipid products correlate with observed cellular properties. Fox and co-workers (6, 44, 45), using mutants of E. coli K-12 that require unsaturated fatty acids, have shown that the thermal transition temperature for glycoside transport is dependent upon the fatty acid incorporated into the lipid during induction. Using a different mutant of E. coli, Overath and co-workers (27, 28, 33) have correlated transition temperatures of lipid monolayers with a number of physiological transitions including those for growth and respiration. Silbert and Vagelos (35) and Mavis and Vagelos (22) have described fatty acid incorporation and the thermal behavior of three membrane-bound enzymes, also in E. coli. Proudlock et al. (31) have shown that the content of unsaturated fatty acids affects the efficiency of oxidative phosphorylation in yeast mitochondria. Thus, there is a growing body of evidence which implicates the properties of the acids of membrane lipids as playing an important role as determinants of membrane function. Such functions may determine the viability of KD46 with various fatty acids and thereby cause the quantitatively different efficiency noted for each different acid.

The effectiveness data in Table 1 show that KD46 will grow on a variety of unsaturated fatty acids. No particular chain length requirement is evident nor can any general statement be made as to a positional requirement for the double bond(s). Acids containing 16 to 22 carbon atoms and acids containing double bonds located in various positions from the $\Delta 4$ position through $\Delta 19$ all support growth. Thus, no single location of the unsaturated group is apparent as a required feature of the nutrient acid in these studies, although it has been suggested elsewhere (46), and further modified (47). The effectiveness of these acids, however, does vary and inefficient acids can be distinguished from those of moderate or high efficiency. In general, there was a direct correlation between the number of double bonds present and the effectiveness of the acid. Esfahani et al. (4), Silbert et al. (36), and Overath et al. (27), showed that the mole percent of unsaturated fatty acids accumulated in lipids of E. coli mutants was inversely proportional to the number of double bonds in the acid. Our data suggest that a similar phenomenon may be operating in yeast and that it can be regulating the yield of cells in a culture.

Interestingly, the unsaturated fatty acids normally found in yeast, palmitoleate and oleate (20, 39), were among the least effective of the fatty acids tested. On the other hand, the polyenoic fatty acids (20:4, 20:5, and 22:6), which had the highest observed efficiencies, are not normal constituents of yeast cell lipids (20). In fact wild-type yeasts should be expected to have little exposure to such acids, given their normal ecological niche (21). It is thus of some interest that yeasts can so effectively utilize the polyunsaturated acids that are found more abundantly in cells of higher eukaryotic organisms.

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LITERATURE CITED
