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QUANTITATIVE EFFECTS OF UNSATURATED FATTY ACIDS IN MICROBIAL MUTANTS

VII. INFLUENCE OF THE ACETYLENIC BOND LOCATION ON THE EFFECTIVENESS OF ACYL CHAINS

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Summary

The ability of a series of 18 carbon acetylenic fatty acids to fulfill the unsaturated fatty acid requirements of *Escherichia coli* and *Saccharomyces cerevisiae* was investigated. Despite their high melting points (>40°C), several isomers of the acetylenic fatty acids were as efficient or more efficient in supporting growth than the analogous fatty acid having a *cis*-double bond.

The efficiencies of the different positional isomers in supporting cell proliferation varied from essentially 0 cells per fmol for the 2—5 and 13—17 isomers to high values when the acetylenic bond was near the center of the chain: e.g. 45 *E. coli* and 5.5 *S. cerevisiae* cells/fmol for the 10 isomer. A striking ineffectiveness of the 9 isomer was observed with *E. coli*. The 7, 8 and 10 isomers were at least 10-fold more efficient than any of the other positional isomers in supporting the growth of *E. coli*. In contrast, the 9 isomer was among the most effective acetylenic fatty acids tested with the yeast mutant.

Chromatographic analysis of the extracted lipids indicated that each of the acetylenic isomers tested (except Δ2 and Δ3) could be esterified by the prokaryotic and eukaryotic microorganisms. The content of unsaturated plus cyclopropane acids observed when growth ceased in *E. coli* cultures supplemented with growth-limiting concentrations of the acetylenic fatty acids ranged from approx. 15 mol% for the 8 isomer to approx. 35 mol% for the 14 and 17 isomers. The 8—11 isomers were observed to be esterified predominantly at the two position in phosphatidylethanolamine of *E. coli* and in phosphatidylcholine of *S. cerevisiae*.

Introduction

Acetylenic fatty acids contain π-bonds in a configurational arrangement which is different from that in the more commonly occurring *cis*-ethylenic fatty acids and they have higher melting points [1]. Nevertheless, several reports have indicated that some acetylenic fatty acids are in some ways biochemically similar to their ethylenic counterparts. For example, Tamai et al. [2] showed that CoA thiolesters of the acetylenic fatty acids could serve as effective substrates in vivo for rat liver microsomal acyl-CoA:phospholipid acyltransferases. Furthermore, Meyer and Bloch [3] reported in 1963 that 9-octadecynoic acid could substitute for *cis*-9-octadecenoic acid (oleic acid) as a growth factor for anaerobically grown yeast. More recently, 9-octadecynoic acid has been reported [4—6] to support growth of microbial auxotrophs which require unsaturated fatty acids.

In this study we examine the ability of unsaturated fatty acid auxotrophs of *Escherichia coli* and *Saccharomyces cerevisiae* to grow with a series of positional isomers of octadecynoic acid. This approach was designed to test the limits of the sensitivity and selectivity of these microorganisms to small modifications in acyl structure in the hope that the results will yield further insight into the roles that fatty acids can play in maintaining cellular functions.

Materials and Methods

The synthesis and properties of the octadecynoic fatty acids used in this study have been described previously [1,7—9]. The octadecynoic acids were converted to the ammonium salts and dissolved in 80% ethanol. They were stored at −70°C at a concentration of 5—50 mM and were added to the microbial cultures in microliter amounts. The 2, 3 and 17 isomers were observed to decrease in concentration under these storage conditions.

E. coli 30E βox^- [10] was obtained from Drs. Carol Linden and C.F. Fox, and *S. cerevisiae* KD 46 [11] was obtained from Dr. Alex Keith. Both mutants are unable to synthesize or oxidize the nutrient unsaturated fatty acids, and they were grown and maintained as described elsewhere [12]. The relationship between cell density and absorbance of cultures grown with acetylenic fatty acids was not significantly different from that observed with cells grown with oleic acid. Procedures used for harvesting of cells and extraction and analysis of lipids have been described [12].

For measurements of the rates of incorporation of the acetylenic isomers with *E. coli*, aliquots of cell suspensions were filtered on Whatman GF/F filters and the cells on the glass fiber filters extracted as described previously for cell pellets [12].

Lipid analyses

Acetylenic fatty acids from lipid extracts were identified on the basis of their gas chromatographic retention times compared to the synthetically prepared compounds.

Phospholipase A_2 treatment. Phosphatidylethanolamine and phosphatidylcholine were isolated from *E. coli* and *S. cerevisiae* lipid extracts by thin-layer

chromatography on silica gel H developed in chloroform/methanol/water (65 : 25 : 4, v/v). The phosphatidylethanolamine and phosphatidylcholine which were eluted from the silica gel H were treated for 4 h with lyophilized *Crotalus admanteus* venom using a 100-fold reduction in the procedure of Brandt and Lands [13]. The reaction products were concentrated and applied to a second thin-layer chromatography plate which was developed in the same solvent system. Bands corresponding to free fatty acid, phospholipid and lysophospholipid were scraped into screw cap tubes and esterified with methanolic boron trifluoride (BF_3) at 80°C for 40 min or into scintillation vials and counted. The phospholipase reaction was 88—95% complete as judged from the fatty acids recovery in products and substrates.

Scintillation counting. The scintillation fluid contained 50 mg *p*-bis-[2-(5-phenyloxazolyl)]-benzene, 4 g 2,5-diphenyloxazole, 40 ml water, 200 ml Triton X-100, and 800 ml toluene. Scrapings from thin-layer chromatography plates were counted with 10 ml of the above scintillation fluid in a Packard Model 2002 TriCarb Scintillation Counter.

Results

Growth responses to nutrient acids

For *S. cerevisiae* cultures, only isomers 8—10 supported growth appreciably beyond control values. The cultures grew at increasing yields with increasing concentrations of the 9 isomer (Fig. 1A) in a manner observed previously for cultures supplemented with common unsaturated fatty acids [14]. All other acetylenic isomers gave limited cells yields above 5 μM nutrient and many gave yields less than the unsupplemented control. The rate of growth was not significantly different for all three isomers which supported high cell yields.

Cultures of *E. coli* supplemented with the 9-octadecynoate grew at slower rates than those with the 10 isomer (Fig. 1B). Increasing rates were observed with increasing concentrations of the 9 isomer, whereas the optimum growth rate observed for the 10 isomer did not vary with nutrient concentration. Opti-

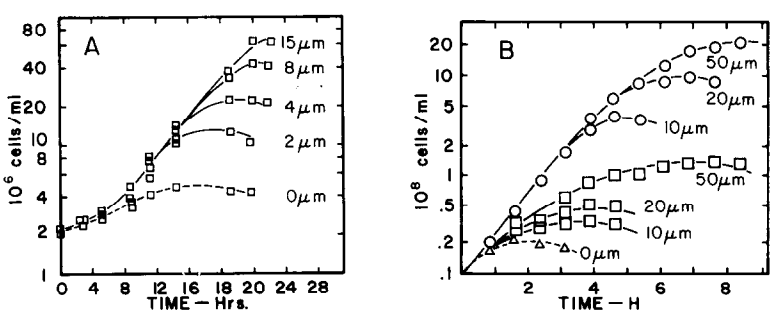

Fig. 1. Representative growth curves for (A) *S. cerevisiae* and (B) *E. coli* cultures supplemented with different concentrations of 9- (□———□) or 10-octadecynoic (○———○) acid. Inocula were grown overnight with 30—60 μM palmitoleate or oleate (*S. cerevisiae*) or 75—100 μM *cis*-9,10-methylene hexadecanoic acid (*E. coli*) as the unsaturated fatty acid supplement. Inocula were washed once with either unsupplement medium or 0.1 M NaCl before resuspending in the experimental growth media. The dashed line indicates results with no added acid supplement.

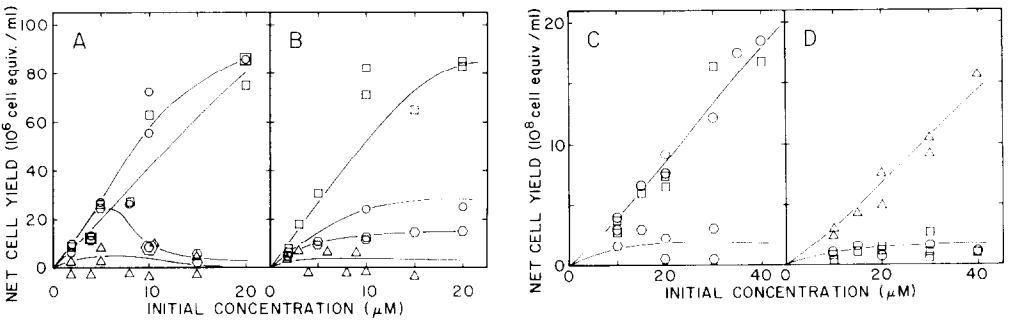

Fig. 2. Nutrient efficiency determinations from cell yields for *S. cerevisiae* and *E. coli*. Net cell yields were calculated from growth curves such as those shown in Fig. 1 and plotted against the initial concentration of added fatty acid. Values for *S. cerevisiae* are shown in panels A and B, and those for *E. coli*, in C and D. Isomers with even-numbered acetylenic bonds (A and C) are noted: 6, △——△; 8, □——□; 10, ○——○; 12, □——□ and the odd-numbered isomers (B and D) are: 7, △——△; 9, □——□; 11, ○——○; 13, □——□.

mum growth rates observed for the 7 and 8 isomer, were similarly unaffected by the concentration of nutrient, and were essentially the same as that with *cis*-9-octadecenoate (oleate). The lower growth rate with the 9 acetylenic isomer was accompanied with a plating efficiency that was about one half that for cells growing at a comparable stage of growth with the 10 isomer or with the 9-*cis*-ethylenic derivative.

The increase in net cell yield (cell yield minus the yield of an unsupplemented control) with increasing concentration of supplement was often a linear relationship (Fig. 2) with a slope characteristic of the fatty acid supplement [14]. Concentrations below 20 μM gave progressively increased cell yields with *S. cerevisiae*, whereas with *E. coli* some isomers gave increased cell yields up to 40 μM concentrations. In some cases (e.g. the 12 isomer) the net cell yield rose with increasing concentrations, but then fell to zero or negative values at higher concentrations. On the other hand, some isomers (e.g. 2, 7 or 15) often gave cell yields lower than the unsupplemented controls. These two aspects of diminished cell yields with *S. cerevisiae* were not observed with *E. coli*.

The greatest difference in the responses of the prokaryote *E. coli* and eukaryote *S. cerevisiae* occurred with the 7 and 9 isomers (Fig. 3). To determine whether the 9-isomer was inhibitory with *E. coli* or merely in effective, cultures were supplemented with a mixture of 12 μM *cis*-9-octadecenoate and either 10 or 20 μM of the 9-octadecynoate. The latter cultures had cell yields approx. 20% higher than cultures with the *cis*-9 isomer alone (results not shown), suggesting the absence of any inhibitory features of the acetylenic preparation. In addition, increasing amounts of the 9-acetylenic isomer seen in Figs. 1B and 2D suggest that the isomer may have been just ineffective rather than inhibitory.

Incorporation of the added acetylenic fatty acid

In nearly all cases examined, even those acids incapable of supporting growth of the cultures were esterified to some extent by the cells. Although the acetylenic acids tested were taken up by *S. cerevisiae*, only those that were appreciably esterified into cellular phospholipids supported growth. Ineffective isomers

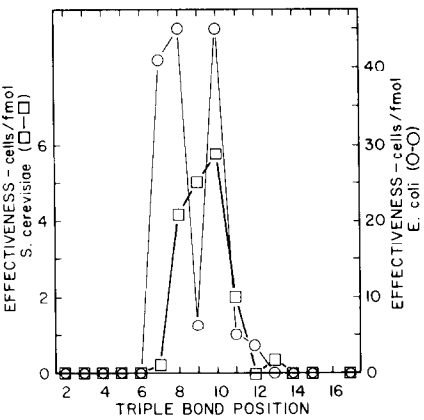

Fig. 3. Effectiveness of octadecynoic acids in supporting the growth of *S. cerevisiae* KD46 (□) and *E. coli* 30E βox^- (○). The ordinate values for the effectiveness of each isomer represent the slopes of the linear region of plots of net cell yield versus intial fatty acid concentration such as those shown in Fig. 2.

provided low cell yields with considerable nutrient acid remaining in the medium or with a majority of the fatty acid within the yeast cells in the non-esterified form (Table I). With *E. coli*, the added acetylenic acid was found either esterified to cellular phospholipid or as non-esterified acid remaining in the medium (Table I).

The fatty acid composition of cellular phospholipid esters in stationary phase cells grown with the 8, 9 and 10 isomers is shown in Table II. Inocula were grown with 17 : Cy (*E. coli*) or *cis*-16 : 1 (*S. cerevisiae*) as the "unsaturat-

TABLE I

INCORPORATION OF ACETYLENIC SUPPLEMENTS BY *S. CEREVISIAE* AND *E. COLI* CULTURES

Cultures supplemented with concentrations (20—30 μM) of the indicated isomers which limited the growth yield were harvested at stationary stage. The *S. cerevisiae* were extracted as described elsewhere [12] and the *E. coli* cultures were washed twice with 1% albumin and once with medium before extracting. The medium and washes were combined, acidified with formic acid and extracted three times with an equal volume of chloroform/methanol (1 : 1, v/v). Extracted lipids were separated by thin-layer chromatography and then methylated with BF_3/methanol prior to gas chromatography. The amount of acetylenic acid recovered in each major fraction is expressed as nmol/ml of total culture.

	S. cerevisiae					*E. coli*	
	6 (30 μM)	9 (30 μM)	10 (25 μM)	15 (30 μM)	17 (30 μM)	9 (30 μM)	10 (20 μM)
Phospholipid	2	12	15	1	1	8	12
Other lipid	1	2	3	1	1	0.4	0.4
Free fatty acid	11	1	1	12	6	0.1	0.1
Medium	3	1	1	5	10	19	2
Overall Recovery (%)	63	56	79	70	61	92	70

TABLE II

FATTY ACID COMPOSITION OF CULTURES SUPPLEMENTED WITH ISOMERIC OCTADECYNOIC ACIDS

Each isomer was added to the medium in concentrations which tended to limit the cell yield, and the cultures were harvested at stationary stage of growth. Values for *S. cerecisiae* represent the relative amounts of different fatty acids in the isolated phospholipid fraction whereas those for *E. coli* represent the amounts in the total esterified fatty acids of the cells expressed as mol %. Estimates of the functionality (ff) of the isomers were made using for *E. coli*, 8.0 for 16 : 1, 6.4 for 17 : Cy and 6 for 18 : 1. and for *S. cerevisiae*, 2,2 for 16 : 1, 2,3 for 18 : 1 and 3.2 for 18 : 2.

S. cerevisiae					*E. coli*			
Isomer (concentration)	8 (10 μM)	9 (10 μM)	10 (10 μM)	11 (10 μM)		8 (10 μM)	9 (30 μM)	10 (10 μM)
12 : 0	6.7	3.5	7	1.6	12 : 0	3.2	4.6	3
14 : 0	3.8	3	3	2.4	14 : 0	19	19	22
16 : 0	50	54	50	56	16 : 0	64	57	59
18 : 0	7.3	9	7.5	8	16 : 1	0.72	1	0.5
16 : 1	17	13	14	15.4	17 : Cy *	1.6	2.6	1.7
18 : 1	7.6	8.9	6.5	11	18 : 1	0.67	1.5	1.0
18 : 2	0.7	—	0.8	—	yne	10.5	12.7	12.1
yne **	4.6	6.2	6.5	5.1				
ff	2.5	3.4	3.0	1.8	ff	6.3	3.7	5.2

* 17:Cy; 9, 10-methylenehexadecanoic acid.

** yne; octadecyonic acid.

ed" fatty acid supplement. The 17 : Cy constituted varying mol % values in the final cultures depending on the number of generations of growth attained with the added acetylenic isomer. In general, the mol % of unsaturation observed at the stationary stage with *E. coli* varied inversely with the effectiveness of the nutrient isomer in supporting growth. For example, cultures grown with the efficient 8 isomer (approx. 45 cells/fmol) ceased growth with approx. 15 mol % unsaturated plus cyclopropane acids, whereas cultures grown with the inefficient 14 isomer (<1 cell/fmol) had approx. 35 mol % unsaturated plus cyclopropane acids when growth ceased. In *S. cerevisiae* the added acetylenic acid was present at greater levels in the extracted phospholipids (e.g. 6, 11 and 17 mol % of 9-yne) when greater nutrient levels (10, 20, 30 μM, respectively) were used. Although appreciable cell yields of *S. cerevisiae* were obtained with the 8—11 isomers, the increased cells represented no more than several generations. Thus, to secure adequate samples for analysis, heavy inocula were used to overcome the inability of the higher nutrient levels of acetylenic acids to provide greater cell growth.

Operational functionality of esterified acetylenic acid

If a nutrient unsaturated fatty acid is provided in growth-limiting amounts, its contribution to the "operational functionality" [15] of membrane lipids may be calculated from the mol % fatty acyl composition of the phospholipids at stationary stage. In Table III are presented functionality factors (calculated from the data such as shown in Table II) for the acetylenic nutrient fatty acids. For the *E. coli* cultures, the calculated values were consistent and did not appear to vary significantly with different concentrations (10—30 μM) of nutrient fatty acid. With *S. cerevisiae*, functionality factors for all except the 8 and

TABLE III

ESTIMATED FUNCTIONALITY FACTORS FOR POSITIONAL ISOMERS OF ACETYLENIC AND ETHYLENIC ACIDS

Functionality factors were calculated as described previously [15] from the acyl composition of stationary stage cultures supplemented with growth-limiting concentrations of the acetylenic isomers. The number of experiments averaged is shown in parentheses. Values for ethylenic acids are from ref. 12.

Isomer	*S. cerevisiae*		*E. coli*	
	Acetylenic	Ethylenic	Acetylenic	Ethylenic
3				—2.0
4	—	0.4	0.9(2)	0.2
5	—	2	1.9(3)	2.1
6	—	2.2	2.8(3)	2.3
7	—	3	4.4(5)	5.2
8	2.8(2)	2.2	5.5(4)	4.1
9	3.3(2)	1.7	3.4(4)	5.9
10	3.7(1)	2.2	5.1(4)	5.9
11	—	1.7	2.6(4)	4.5
12	—	0.6	2.2(5)	3.3
13	—	0.4	1.6(3)	2.5
14	—	—	1.2(3)	1.2
15	—	—	0.55(3)	0.1
17	—	—	—0.90(1)	—0.7

10 isomers were difficult to obtain since net cell yields were not proportional to added nutrient above 5 μM. In those cases there was no basis for assuming cell growth stopped because the membrane lipids had reached zero functionality.

Positional distributions of esterified nutrients

For both the prokaryotic and eukaryotic cells there was a clear predominance of acetylenic isomer esterified at the 2-position (Table IV), whereas exogenously added ^{14}C-16 : 0 was esterified primarily at the 1-position. ^{14}C-*trans*-9-18 : 1 was found in approximately equal amounts at the 1- and 2-positions of phosphatidylcholine of *S. cerevisiae* whereas in phosphatidylethanolamine of *E. coli* there was an approx. 3-fold preference for the 2-position (which was considerably less than the 8—10-fold observed with the acetylenic fatty acids).

To investigate whether differences in the effectiveness of the 9, 10 and 17 isomers observed with *E. coli* (Fig. 3) were correlated with differences in the rates of incorporation of these fatty acids, the amount of each isomer esterified versus time was studied. A culture was washed once with 1% albumin and then with medium to remove fatty acid from the cells. This washed culture was used to inoculate media containing 30 μM of either 9-, 10- or 17-octadecynoic acid. Cells were harvested by filtration on Whatman GF/F filters at various times after inoculation and the amount of octadecynoic acid esterified was deter-

TABLE IV

POSITIONAL DISTRIBUTION OF SUPPLEMENTAL ACIDS IN PHOSPHATIDYLCHOLINE OF *S. CEREVISIAE* AND PHOSPHATIDYLETHANOLAMINE OF *E. COLI*

Cultures grown with the supplements indicated below were harvested during logarithmic growth, the lipids extracted and the phospholipids separated by thin-layer chromatography on silica gel H (chloroform/methanol/water (65 : 24 : 4, v/v)). The phosphatidylcholine and phosphatidylethanolamine fractions were eluted and treated with *C. adamantus* venom. The reaction products were separated by thin-layer chromatography and either counted in a scintillation counter or methylated with boron trifluoride/methanol and the methyl esters quantitated by gas-liquid chromatography. Results are expressed as % nmol recovered of the acetylenic acid or % cpm recovered of the radioactive control acids.

Supplement isomer	*S. cerevisiae*		*E. coli*	
	Position		Position	
	2	1	2	1
	% nmol			
8-yne	70	30	93	7
9-yne	93	7	89	11
10-yne	93	7	99	1
11-yne	70	30	90	10
	% cpm			
^{14}C-12 : 0	71	29	95	5
^{14}C-16 : 0	13	87	33	67
^{14}C-18 : 1t *	45	55	77	23
^{14}C-18 : 1c **	93	7	90	10

* 18:1t; *trans*-9-octadecenoate.

** 18:1c; *cis*-9-octadecenoate.

mined by gas chromatography after sodium methoxide transmethylation of the extracted lipids. The 10 isomer which was effective for growth was esterified at approximately twice the rate of the ineffective 9 isomer, but at a rate indistinguishable from 9-*cis*-octadecenoate. On the other hand, the ineffective 17 isomer was esterified at approximately two times the rate of the 10-yne and 9-*cis* isomers.

Discussion

In considering what physicochemical attributes of a fatty acid allow it to support membrane function, melting point has often been suggested as an indication of such ability. The low effectiveness of *trans*-fatty acids in supporting membrane function [16] is consistent with their higher melting point (for example, *trans*-9-18 : 1, m.p. = 45°C vs. *cis*-9-18 : 1, m.p. = 11°C). The melting points of the acetylenic fatty acids are in general slightly higher than those of the corresponding *trans*-octadecenoic isomers [1]. On the basis of this criterion, the acetylenic structure might also be expected to have a poor effectiveness in meeting the growth requirements of *S. cerevisiae* and *E. coli* for unsaturated fatty acids. However, the effective isomers, 8 and 10, have melting points of 47 and 46°C, respectively, whereas ineffective isomers (6 and 11) have similar melting points (51 and 47°C, respectively). Furthermore, the 8 and 10 isomers had nutrient efficiency values (Fig. 3) comparable to those observed (42 *E. coli* cells/fmol and 6 *S. cerevisiae* cells/fmol) for *cis*-9-18 : 1 which has a melting point of 11°C. Thus, the data demonstrate that the bulk phase melting point of the free acid is a poor indicator of a fatty acid's ability to fulfill the growth requirements for unsaturated acids in *E. coli* and *S. cerevisiae*.

Results from two earlier studies suggested that membranes containing acetylenic fatty acids have higher phase transition temperatures than those containing a corresponding *cis*-unsaturated fatty acid. From Arrhenius plots, Eletr and Keith [6] reported a transition at 27°C for a nitroxide spin label in yeast membranes containing 9-octadecynoate as compared to 16°C for membranes containing *cis*-9-octadecenoate (oleate). Schairer and Overath [4] measured the temperature characteristics for thiomethylgalactoside accumulation in *E. coli* cultures grown with *cis*-9-octadecenoate, 9-octadecynoate and *trans*-9-octadecenoate and over the temperature range of 15—25°C obtained values of 9, 16 and 40 kcals, respectively, from the slopes of the Arrhenius plots.

It might be inferred from the two above-mentioned studies that membranes containing acetylenic fatty acids have less fluid properties than those containing the corresponding *cis*-ethylenic fatty acids, and that this phenomenon is due to inclusion of the high melting acetylenic acid in cellular lipids. However, the high effectiveness of several other acetylenic isomers reported in the present study (Fig. 3) emphasizes that caution is warranted before extrapolating data from Arrhenius plots with one positional isomer toward a prediction of the physiologic suitability of any certain type of fatty acid at an isothermally maintained higher growth temperature. We cannot envision great differences in the chain-chain interactions for closely related positional isomers such as the 8-, 9- and 10-octadecynoates, yet the acids produce greatly different cellular phenomena. Furthermore, the estimated functionality (ff) of the 9-yne acid

when esterified in membrane phospholipids of either *S. cerevisiae* or *E. coli* (3.5 and 3.9, respectively) was comparable to that of other effective nutrients reported earlier [12,15].

Many isomers which were ineffective in supporting growth of *S. cerevisiae* were recovered in the cells to a considerable degree in the form of non-esterified fatty acids (Table I). Therefore it appears likely that the rates of uptake of these acetylenic isomers into the cells provided an adequate supply of membrane precursors, but subsequent activation or acyl transfer may have been rate limiting thereby leading to an increased free fatty acid pool. The increase in intracellular non-esterified fatty acid may possibly have led to a disruption of cellular functions that led to or contributed to the ineffectiveness of these acetylenic isomers in supporting growth of the eukaryotic yeast cells.

On the other hand, we did not detect an increase in free fatty acids in *E. coli* grown with ineffective isomers (Table I). This result may be consistent with the process of "vectorial acylation" proposed by Klein et al. [17] for the uptake of acid by *E. coli.* It might also reflect that free fatty acids in *E. coli* were removed by washing with albumin or that activation and esterification were generally faster than uptake by these cells. In this regard the ineffective 17 isomer was taken up and esterified by *E. coli* at a rate comparable to the effective 10 isomer. Thus the inability of the 17 isomer to support growth may best be attributed to properties of its derivatives after it is esterified rather than to an inability to enter into phospholipid biosynthesis.

One clue to the basis of the high effectiveness of the acetylenic isomers might be revealed by a consideration of their molecular architecture. Although the *trans* conformation of methylene units is generally the most stable in bulk phases, NMR data obtained from lipid bilayers (18) has indicated that a *gauche* conformation will be favored if it helps eliminate a kink in the acyl chain [19] and thereby allows more interchain interactions. Possibly conformational arrangements for both the ene and yne acyl chains in a bilayer could have similar kinks, and therefore might have similar tendencies to break up interchain interactions. Further effects including chain substrate interactions were described by Welles et al. [20] who observed that monolayers of acetylenic fatty acids were more expanded than those of either the corresponding *cis* or *trans* acid.

Tamai et al. [2] showed a very selective pattern of esterification of acetylene fatty acids. The selectivities supported the concept that acyltransferases acting at the 2-hydroxyl may respond to the presence of π-bonds. Transfer to the 1-hydroxyl, however, was sensitive to configuration of the acyl chains, and a *cis*-rotamer may be the form of acyl chain that is primarily involved in enzymatic transfer to the 1-position. For example, the *cis*-rotamer of 10-octadecynoic acid is superimposable on the structure of *cis*-9-octadecenoic acid but not on *cis*-10-octadecenoic acid. Such a rotamer could help interpret the alternating selectivity between the 8 and 13 acetylenic isomers that was opposite to the pattern observed for *cis*-octadecenoyl transfers. The selective growth response of *E. coli* thus resembled the pattern of selectivity of the rat liver system for acetylenic acid transfer to the 1-position with a shift down of one carbon atom. (i.e. 7, 8 and 10 $\gg$ 9 rather than 8, 9 and 11 $\gg$ 10). Ironically, the 1-position generally contains saturated rather than unsaturated acids. The acetylenic struc-

ture may be "perceived" as if similar to a *cis*-ethylenic acid since the 8, 9, 10 and 11 acetylenic isomers were esterified predominantly at the 2-hydroxyl of the phospholipids.

Results with auxotrophic mutants allow recognition of differences among acyl chains in maintaining normal cell physiology. We have attempted to quantitate the degree to which the effects could be attributed to additive, non-specific interactions of the sort attributed to membrane fluidity. In the case of *cis*-octadecenoate isomers [12], we found that the effectiveness as a nutrient, as well as the estimated functionality of the esterified nutrient in membranes, was generally related to the pattern of excess molar volume of the phospholipid esters. Only in the case of the *cis*-5 and *cis*-6 isomers was there good indication of additional selective effects of the acyl chains. In the present study of acetylenic acids, the more effective isomers with either prokaryote or eukaryote cells tended to be those with the functional group between carbon atoms 7 and 11. The acetylenic isomers, however, exhibited signs of selective nutritional effects on cell growth that could not be attributed to general, additive actions reflected by the functionality factors. Thus, the quantitative studies with *cis* [12], *trans* [16] and acetylenic acids have now provided ample indication that selective actions of fatty acyl chains upon cell metabolism will be found to accompany the more widely recognized phenomenon of non-selective acyl chain interactions.

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References

1 Barve, J.A. and Gunstone, F.D. (1971) Chem. Phys. Lipids 7, 311–323
2 Tamai, Y., Lands, W.E.M., Barve, J.A. and Gunstone, F.D. (1973) Biochim. Biophys. Acta 296, 563–571
3 Meyer, F. and Bloch, K. (1973) J. Biol. Chem. 283, 2654–2659
4 Schairer, H.O. and Overath, P. (1969) J. Mol. Biol. 44, 209–214
5 Wisnieski, B.J. and Kiyomoto, R.K. (1972) J. Bacteriol. 109, 186–195
6 Eletr, S. and Keith, A.D. (1972) Proc. Natl. Acad. Sci. U.S. 69, 1353–1357
7 Gunstone, F.D. and Ismail, I.A. (1967) Chem. Phys. Lipids 1, 337–340
8 Barve, J.A., Davies, J.E.D., Gunstone, F.D., Hodge, P. and Ismail, I.A. (1972) J. Chem. Soc. 2, 1557
9 Barve, J.A., Gunstone, F.D., Jacovsberg, F.R. and Winlow, P. (1972) Chem. Phys. Lipids 8, 117–126
10 Linden, C.D., Wright, K.L., McConnell, H.J. and Fox, C.F. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2271–2275
11 Keith, A.D., Resnick, M. and Haley, A.B. (1969) J. Bacteriol. 98, 415–420
12 Ohlrogge, J.B., Barber, E.D., Lands, W.E.M., Gunstone, F.D. and Ismail, I.A. (1976) Can. J. Biochem. 54, 736–745
13 Brandt, A. and Lands, W.E.M. (1967) Biochim. Biophys. Acta 144, 605–612
14 Barber, E.D. and Lands, W.E.M. (1973) J. Bacteriol. 115, 543–551
15 Holub, B. and Lands, W.E.M. (1975) Can. J. Biochem. 53, 1262–1277
16 Vandenhoff, G., Gunstone, F.D., Barve, J.A. and Lands, W.E.M. (1975) J. Biol. Chem. 250, 8720–8727
17 Klein, K., Steinberg, R., Fiethen, B. and Overath, P. (1971) Eur. J. Biochem. 19, 442–450
18 Seelig, A. and Seelig, J. (1974) Biochemistry 13, 4839–4845
19 Trauble, H. (1971) J. Membrane Biol. 4, 193–208
20 Welles, M.L., Zografi, G., Scrimglour, C.C. and Gunstone, F.D. (1975) Adv. Chem. Ser. (ACS) No. 144, 135–152.