Inhibition of Growth of Microbial Mutants by trans-Octadecenoates*

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A series of positional isomers of trans octadecenoic acid were tested for their ability to support growth of microbial mutants that could not synthesize unsaturated fatty acids. The bacterial strain used (a variant of Escherichia coli 30E) grew with supplements of the trans isomers only at high temperatures (38°) and with acids containing the trans-ethylenic bond between carbon atoms 8 through 13. The yeast mutant (Saccharomyces cerevisiae KD46) grew only with the 8-trans-octadecenoate giving cell yields about one-fifth those obtained with oleate.

Although the trans isomers had little effect on the growth of the bacteria in the presence of oleate, they inhibited the growth of yeast with oleate. Inhibition was strongest for the 4, 6, 7, 11, and 12 isomers, almost negligible for the 8 isomer and of differing intermediate degrees for the others. The inhibitory effects had no correlation with the melting points of the acids and appeared to reflect selective action(s) on the metabolism of the cell.

When the net yield of the yeast cultures with oleate was lowered by the effect of added trans acids, there was a marked accumulation of triglycerides and nonesterified acids in the cells. The marked increase in triglyceride content while phospholipid per cell remained relatively constant suggest that trans acids, in addition to forming inadequate membrane lipids, may also interfere with a basic control point in lipid metabolism.

Although trans isomers of unsaturated fatty acids are relatively uncommon in natural lipids, they may be produced by partial hydrogenation of polyunsaturated acids by some rumen organisms (1) or by commercial processing of vegetable oils (2). Ingested trans acids were absorbed, oxidized, and transported across the placental membrane at rates comparable to other fatty acids (3, 4). Nevertheless, the finding of Sinclair (5) indicated that some selective exclusion of Δ9-trans-octadecenoate may occur in brain and testes. A recent detailed study of the selective esterification of trans-octadecenoyl-CoA isomers during lecithin biosynthesis by rat liver preparations showed that esterification proceeded at markedly different rates with the different positional isomers (6). Furthermore, those results suggested that the enzyme activity regarded generally to be selective for saturated fatty acids may be merely discriminating against cis-9 and cis-11 configurations rather than favoring saturated acyl chains. The chemical features that lead to the placement of saturated and unsaturated acids in their customary loci are still unknown. One perception developed from these in vitro studies is that no positional or geometric isomer of a fatty acid is certain to be a reliable model for the behavior of another isomer. Thus, results obtained with a certain saturated acid or a cis-unsaturated acid cannot be relied upon to predict the behavior of the various trans isomers. Furthermore, no single biochemical property is likely to characterize the action of different trans acids.

Recently, we have demonstrated that different fatty acids may elicit quantitatively different efficiencies in supporting cellular growth (7). The extent of growth in such experiments may reflect the effects of selective synthetic steps and of the resultant products upon cell function. Since in vitro acyltransferase results (6) have indicated large differences for different positional isomers, we examined the various trans-octadecenoate isomers in terms of the over-all cellular physiologic phenomenon of growth. The results with both the eukaryotic and prokaryotic mutant cells indicate that most trans isomers of octadecenoate are much less effective than the cis analogs in supporting growth, and that, particularly in the eukaryote Saccharomyces cerevisiae, certain positional isomers may have considerably toxic effects upon the cell.

MATERIALS AND METHODS

Reagents—Yeast extract, Bacto-agar, Casamino acids, and Bactopeptone were obtained from Difco Co. Mono- and dibasic potassium phosphate, sodium citrate, ammonium sulfate, and dextrose, all of reagent grade, were from J. T. Baker Chemical Co. Magnesium sulfate, Tween 40, and succinic acid (as disodium salt) were obtained from Allied Chemical Co. Tween 80 was obtained from Sargent Welch

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Scientific and thiamine hydrochloride from Merck. BFs in methanol was obtained from Applied Science Laboratories.

**Fatty Acids**—Commercial preparations of the following free fatty acids were obtained from either Hormel or NuCheck Laboratories and were the sodium salt obtained in aqueous 1 N sodium hydroxide for 1 hour at 65°C after which they were neutralized with acetic acid and extracted into petroleum ether. The other phase was washed twice with water, evaporated to dryness, a few drops of NH₄OH were added, and the excess ammonia was removed by evaporation to dryness. The ammonium soaps were then dissolved in 50% ethanol, and their concentrations were adjusted to about 0.5 mg.

The positional isomers of trans-octadecenoic acid (3-15) were prepared in the laboratory of F. D. Gunstone (8, 9). Since some of the preparations were methyl esters, all isomers were incubated in aqueous 1 N sodium hydroxide for 1 hour at 65°C after which they were neutralized with acetic acid and extracted into petroleum ether. The other phase was washed twice with water, evaporated to dryness, a few drops of NH₄OH were added, and the excess ammonia was removed by evaporation to dryness. The ammonium soaps were then dissolved in 50% ethanol, and their concentrations were adjusted to about 0.5 mg.

The concentration and degree of purity of each fatty acid solution was checked by gas chromatography after esterification in BF₃/methanol. The impurities were less than 1% in all fatty acid solutions except the 3- and 4-trans isomer preparations. A 5% impurity of low retention time was found in the 3-trans samples, and in the 4-trans sample, two peaks close together of about equal height were found. The stock solutions were designed for gas chromatography of the fatty acid preparations without the BF₃/methanol treatment. No esters were found in the stock solutions.

**Organisms and Media**—Two organisms requiring unsaturated fatty acids for growth were used. The first, *Saccharomyces cerevisiae* KD46 (ole 2), was deficient in the ability to desaturate saturated acids and was a generous gift from Dr. Alan Keith, Department of Biophysics, Pennsylvania State University. The basal medium was yeast extract, peptone/dextrose (YPED) described by Keith et al. (10). We found detergents to interfere with the quantitative determination of growth parameters and omitted them from all liquid culture media (7). The mutant was maintained on agar plates to which Tween 80 (2%, v/v) was added. Replicate plating on Tween 80 and Tween 40 plates maintained the strain free of revertants and other contaminants. A strain of *Escherichia coli* 30E (fab B), which could neither oxidize fatty acids nor synthesize unsaturated fatty acids (11), was a generous gift of Carol Linden and C. F. Fox (UCLA). The basal liquid medium for this strain contained Medium A (12) to which 1% Casamino acids were added. The inocula for bacterial growth tubes were prepared by transferring cells from an agar plate to liquid media containing oleic acid. The cells usually appeared in the liquid culture only after 15 hours, the growth tubes were of uniform character, KD46 cultures were prepared as follows. Cells were transferred from agar plates to liquid containing oleic acid and grown to mid-log phase. Then aliquots of the cells were added. In these cases, the growth rate was taken from the growth curve at mid-log phase expressed in doublings/hour.

**Growth Measurements**—To ensure that the inocula for the yeast growth tubes were of uniform character, KD46 cultures were prepared as follows. Cells were transferred from agar plates to liquid containing 50 μg/ml oleic acid and grown to mid-log phase. Then aliquots of the plus medium were transferred directly to several fresh tubes in such a way that the subsequent cultures would reach a desired cell concentration (in late log phase) at the end of a chosen time interval. The cells were then centrifuged and placed in new media which lacked oleic acid. This cell suspension was used to inoculate the experimental growth tubes. No esters were observed while the cells were in liquid, which was normally about 50 hours.

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The experimental growth cultures (8 ml) were shaken at 120 rpm on a rotary shaker in tubes 18 x 150 mm set at an angle of 50° at either 26°C (+0.3°) or 36°C (+0.5°) in a constant temperature room. At various times during the growth of the culture, the cell concentration was measured turbidimetrically by reading the absorbance at 660 nm in a Bausch and Lomb Spectronic-20, and cells/ml/A₆₆₀ were calculated as described elsewhere. Cell suspensions of KD46 grown on combinations of oleic acid and trans-fatty acids gave an average value of 62 ± 4 × 10⁹ cells/ml/A₆₆₀ as did suspensions grown on oleate alone. This average value did not differ for the growth observed at 26°C or 36°C.

| Isomer | Aldol ester fragment | Aldehyde fragment |
|--------|---------------------|------------------|
| Δ6     | 14.57 ± 0.02 (16)   | 11.42 ± 0.02 (10) |
| Δ7     | 15.43 ± 0.03 (15)   | 10.30 ± 0.04 (13) |
| Δ8     | 16.61 ± 0.06 (6)    | 9.48 ± 0.05 (19)  |
| Δ9     | 17.50 ± 0.02 (115)  | 8.40 ± 0.04 (37)  |
| Δ10    | 18.57 ± 0.04 (9)    |                  |
| Δ11    | 19.30 ± 0.02 (53)   |                  |
| Δ12    | 20.61 ± 0.05 (7)    |                  |
| Δ13    | 21.68 ± 0.06 (4)    |                  |
| Δ14    | 22.72 ± 0.02 (7)    |                  |

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second phase of growth between 5 and 20 hours after the initial phase was completed. Control cultures without any added fatty acid normally underwent about 1.5 to 2 doublings before stopping. This increase can be attributed partly to a small amount of oleate in the yeast extract present in the medium and to some extra nutrient introduced with the cells in the inoculum, but at least one-half of the increase appears to be an endogenous growth (see Fig. 1 and Table II) not influenced by fatty acids present in the growth medium.

Of the various trans isomers, only Δ8 consistently supported growth above that in the control tubes under the routine conditions used. Table II illustrates the strongly inhibitory action of three of the trans acids (Δ7, Δ7, and Δ12) on the total cell yields of KD46 cultures grown with 30 μM oleate. Other trans-18:1 isomers (Δ4, Δ9, Δ11, Δ13, Δ15) were also inhibitory to differing degrees. The total cell yield of 104 × 10⁶ cells/ml for cultures where 30 μM oleate alone was added decreased to a level of growth, which for cultures containing 15 or 30 μM Δ6 alone, represented about one-half of the control growth. The doublings/hour of this early basal growth, which is represented approximately by the growth rate at 3 to 4 hours, was also unaffected by fatty acids added to the culture medium. Beyond 4 hours, the growth rate was influenced by the acids added to the medium. Fig. 1 illustrates the inhibition noted in both the rate and extent of growth of KD46 after the first 4 hours when the trans-18:1 acids (Δ7 and Δ12 in this case) exerted their inhibitory effect on the culture.

A 28°, none of the trans acids, at any of the concentrations tested, supported growth of the Escherichia coli mutant above that of the controls (results not shown). At 36°, a small and inconsistent net increase in cells was observed with the Δ11 and Δ12 isomers. However, we confirmed that the mutant did grow on the Δ9-trans isomer at 38.8 ± 0.5° when we tested conditions similar to those of Wilson and Fox (13). We tested other trans acids at this temperature in the concentration range of 6 to 60 μM and found that isomers Δ8 through Δ12 also supported growth above the controls. The net cell yields were variable and small compared to those with oleate at the same concentration, but nonetheless indicated some consistent growth above the controls. The average efficiencies in cells/mmol were as follows: 12 ± 2 for Δ8, 10 ± 2 for Δ9, 8 ± 1 for Δ10, 9 ± 2 for Δ11, and 6 ± 1 for Δ12 (compared to 50 for Δ9-cis at 36°).

The cell yields for the E. coli mutant were a linear function of the concentration of added oleate up to about 30 μM, although the oleate apparently produced a somewhat less efficient (28 cells/mmol) yield phenotype at 26° than at 36° (50 cells/mmol). Cell yields for S. cerevisiae grown on added oleate also increased linearly up to about 30 μM nutrient acids. The linear responses at low concentrations were used to determine growth efficiencies for S. cerevisiae and E. coli grown at the two temperatures, 26° and 36°. These are summarized in Table III together with the correlation coefficients of the linear regressions. Although growth on oleate for E. coli was about one-half as efficient at the lower temperature, that for S. cerevisiae was relatively unaffected. Interestingly, the Δ8-trans isomer, which has a melting point of 52°, was somewhat more efficient for the yield of yeast cells at the lower temperature than at the higher temperature. The growth rate (0.07 ± 0.1 doublings/hour) observed for S. cerevisiae grown with Δ8-trans alone was about one-fourth that of cells grown on oleic acid (0.29 ± 0.01 doublings/hour) at all concentrations tested.

Inhibitory Effects of trans-Octadecenoates on Growth with Oleate—The cell yields of KD46 grown on 30 μM oleate at 36° in the presence of 30 μM amounts of the various isomeric trans-octadecenoic acids varied greatly (Fig. 2A). Isomers with the trans bond at positions 4, 6, 7, 11, and 12 completely inhibited the growth rate (Fig. 2B) and cell yield (Fig. 2A). The inhibition was very selective with regard to the position of the double bond; the Δ8-trans isomer gave little inhibition at 36° in comparison with the growth on oleic acid alone, and the other isomers showed different levels of inhibition of the growth on oleic acid. The pattern of inhibition of the rate of growth (Fig. 3B) was similar to that with the extent of growth. Additional studies (not shown) indicated stronger inhibitions at a lower temperature; the yield with oleic acid in the presence of the Δ6, Δ9, Δ10, Δ11, Δ12, Δ13, Δ14, and Δ15 isomers at 26° was 50% less than at 36°, although the yield with oleic acid alone was only about 15% lower. In further studies with a 15 μM level of trans acid, only the Δ4 isomer gave complete inhibition at both temperatures, and the other isomers gave either partial or no significant inhibition (results not shown).

To gain a better understanding of the inhibitory effectiveness of the trans isomers on the eukaryotic yeast cell, we examined a range of concentrations as shown in Fig. 3. The inhibition was readily observed with most acids at levels of inhibitor above 10 μM, except for the Δ6 isomer, which was strongly inhibitory above 2 to 5 μM. The unusually marked inhibition by the Δ6-trans isomer did not appear due to some nonacid contaminant, since chromatographic purification of the Δ6 isomer subsequently gave similar reproducible results.

Growth of the E. coli mutant on oleic acid at 30 μM in the presence of 30 μM amounts of the various trans isomers is described in Fig. 4. There were no strong inhibitors of growth of this procaryotic organism among any of the trans isomers tested, and the added trans isomer seemed to have little effect on the utilization of the oleate which was present. The Δ13-trans isomer consistently gave a moderate inhibition of cell yield at 26° but not at 36°. The rates of growth of the bacterial cultures were not significantly different with the various fatty acid mixtures.

Incorporation of trans-Octadecenoates into Cellular Lipids—We studied the incorporation of oleic acid and the various trans-18:1 acids into cellular lipids of KD46 under
Log phase KD46 cells (previously grown with oleic acid) were inoculated into yeast extract/peptone/dextrose culture media to which were added. The total cell yield was followed until a maximum was achieved. The growth rates (doublings/hour) at 4 and 10 to 20 hours were graphically estimated from linear sections of the growth curve plotted on semilog paper. The average values of these parameters and the associated standard errors are given, together with the number of experiments in parentheses.

### Table II

*Inhibition of total maximum cell yield of KD46 trans-18:1 acids Δ6, Δ7, and Δ12*

| Fatty acids added to culture medium | Maximum total cell yield | Growth rate at 0 to 4 hours | Growth rate at 10 to 20 hours |
|------------------------------------|--------------------------|-----------------------------|-----------------------------|
| Control (no added acid)            | (10^6 cells/ml)          | 0.27 ± 0.04 (17)            | 0 ± 0 (17)                  |
| 15 μM Δ6                          | 18 ± 1 (17)              | 0.27 ± 0.02 (3)             | 0 ± 0 (3)                   |
| 30 μM Δ6                          | 8 ± 1 (3)                | 0.19 ± 0.001 (3)            | 0 ± 0 (3)                   |
| 30 μM oleate                      | 104 ± 5 (13)             | 0.30 ± 0.01 (13)            | 0.29 ± 0.01 (13)            |
| + 15 μM Δ0                        | 11 ± 2 (7)               | 0.27 ± 0.01 (7)             | 0 ± 0 (7)                   |
| + 30 μM Δ6                        | 11 ± 2 (4)               | 0.28 ± 0.001 (4)            | 0 ± 0 (4)                   |
| + 12 μM Δ7                        | 47 ± 1 (4)               | 0.28 ± 0.02 (4)             | 0.1 ± 0.1 (2)               |
| + 20 μM Δ7                        | 18 ± 3 (3)               | 0.24 ± 0.04 (3)             | 0 ± 0 (3)                   |
| + 30 μM Δ7                        | 14 ± 2 (3)               | 0.25 ± 0.007 (3)            | 0 ± 0 (3)                   |
| + 15 μM Δ12                       | 68 ± 9 (1)               | 0.27 ± 0.02 (4)             | 0.13 ± 0.05 (4)             |
| + 20 μM Δ12                       | 14 ± 2 (3)               | 0.23 ± 0.02 (4)             | 0 ± 0 (3)                   |
| + 30 μM Δ12                       | 17 ± 3 (3)               | 0.23 ± 0.02 (4)             | 0 ± 0 (3)                   |

### Table III

*Growth efficiencies and maximum net cell yields of KD46 and 30E box− on individual fatty acids at 26° and 36°*

The growth efficiencies for KD46 and 30E box− are the slopes of the net cell yield versus fatty acid concentration for concentrations up to 30 μM Δ9 cis and up to 10 μM Δ8 trans. The maximum net cell yields represent the values at high fatty acid concentrations where a horizontal plot is obtained. Correlation coefficients are given in brackets and the number of observations in parentheses.

| Organism | Fatty Acid | Temperature | Growth Efficiency | Maximum Cell Yield |
|----------|------------|-------------|-------------------|--------------------|
| KD46     | Δ8-trans   | 36°         | 1.31 [0.78 (9)]   | 13 ± 2 (6)         |
| KD46     | Δ8-trans   | 26°         | 1.96 [0.90 (9)]   | 18 ± 1 (7)         |
| KD46     | Δ9 cis     | 36°         | 2.60 [0.88 (34)]  | 92 ± 2 (20)        |
| KD46     | Δ9 cis     | 26°         | 2.21 [0.90 (14)]  | 81 ± 5 (10)        |
| 30E box− | Δ9 cis     | 36°         | 50 [0.93 (26)]    | 1700 ± 130 (16)    |
| 30E box− | Δ9 cis     | 26°         | 28 [0.95 (11)]    | 1700 ± 210 (7)     |

![Fig. 2. A and B, inhibition of the growth of KD46 cultures containing 30 μM oleate by 30 μM trans-18:1 acids at 36° and 26°](http://www.jbc.org/)
FIG. 3. A to J, effect of the concentration of trans-18:1 acids on the net cell yield of KD46 grown with 30 Μ oleate. The preparation of KD46 cell inoculum is described in Fig. 2, and the measurement of net cell yields in this figure. The mean net cell yield (O) of cultures with only 30 Μ oleate is given on the left ordinate of each figure. All other points represent single observations for cultures with the level of trans-18:1 acid noted plus 30 Μ oleate.

Accumulation of the trans isomers in phospholipids, in general, did not follow their pattern of inhibition of cell growth. For instance, 30 Μ Δ7 inhibited growth completely, whereas the Δ8 isomer inhibited the growth very little if at all, even though both acids were incorporated at comparable mole fractions.

We found a striking correlation between the inhibition by the trans acids and the total amount of the combined 18:1 that was recovered in the neutral lipid fraction which was somewhat less than their content of 15 to 50% in the phospholipid fraction. A large increase was observed for 18:1 accumulated in the free fatty acid fraction: from 9 to 27 amol/cell in the noninhibited cultures to more than 200 amol/cell in the strongly inhibited cultures. An additional characteristic of the free fatty acid fractions was the relatively high proportion of the trans isomer in the 18:1 compared to its proportion in the esterified forms of 18:1. In most cases, the trans isomer was found to be a higher relative component in the 18:1 of the nonesterified acids than it was in the initial 18:1 nutrient mixture.

A similar increased accumulation of total 18:1 in neutral lipids and free fatty acids was observed when the concentration of one trans acid, Δ11, was increased from noninhibitory to severely inhibitory levels (Table V). The pattern of fatty acids in the phospholipids, as well as the 18:1 distribution among the lipid classes of relatively uninhibited cultures, resembled that seen for cultures with oleate only. However, starting at levels of 20 to 30 Μ trans Δ11, the amount of total 18:1 in the neutral lipid and free fatty acid fractions increased as the amount of added Δ11 was increased up to the highest concentration tested, thus, the changes in lipid content noted in Table V among cultures that were inhibited to different degrees by different abundances of the same acid paralleled the changes seen in Table IV where the different degree of inhibition of growth was caused by trans positional isomers.

Fig. 4. Inhibition of the cell yield of E. coli 30E box- grown on 30 Μ oleate by 30 Μ trans-18:1 acids at 26° and 36°. E. coli 30E box- inoculum was prepared as in Fig. 3 and used to inoculate medium tubes containing 30 Μ oleate and 30 Μ trans-18:1 acids. The growth tubes were then incubated at 26° (△) and 36° (Δ). Average values with 30 Μ oleate alone (C) are shown on right.

Growth-supporting Properties of trans-18:1 Acids—Both trans- and cis-unsaturated fatty acids can support the growth of microbial cultures that are auxotrophic for unsaturated fatty acids, although fewer trans isomers than their cis-counterparts are recognized to support growth. Cis-octadecenoate isomers from Δ6 to Δ13 supported substantial growth of the S. cerevisiae mutant KD46, and the Δ7 through Δ13 isomers did likewise for a strain of the E. coli mutants 30E. In the present study using the trans-18:1 isomers with the same strains, we have observed that only the Δ8 isomers supported growth of S. cerevisiae, and the Δ8 to Δ12 supported growth of E. coli 30E box- (at 38°) with a much lower cell yield than did their cis-counterparts. Some differences in the growth requirements of yeast and bacterial mutants can be recognized in comparing reports of other workers (14–16) although different temperatures have been used.

In the present report, the two types of cells were compared at the same temperature with different isomeric trans-
TABLE IV
Incorporation of different trans-octadecenoates into cellular lipids

| Supplement | Net Cell Yield | mole % | % trans in 18:1 | 18:1 amoles/cell |
|------------|---------------|--------|----------------|----------------|
| 18:1c (30µM) | 49            | 12:0 4  | 37 11 14 35 | 133 24 9 |
| 18:1c (30µM) | 41            | 14:0 4  | 37 11 21 38 | 296 47 9 |
| 18:1c + 5 µM Δ6t | 37            | 16:0 4  | 5 35 11 31 8 | 14 8 22 14 95 167 10 |
| 18:1c + 15 µM Δ6t | -14.3         | 18:0 -2 | - - - - - 133 24 9 |
| 18:1c + 10 µM Δ7t | 40            | 18:1c 39 | 8 29 2 4 10 28 25 | 114 95 12 |
| 18:1c + 13 µM Δ7t | 7             | 18:1t 11 | 6 41 12 6 12 4 - 30 347 594 27 |
| 18:1c + 30 µM Δ7t | -9.3          | Plip 4  | 2 4 12 10 22 45 50 293 1700 531 |
| 18:1c + 12 µM Δ8t | 61            | Neutr 4  | 6 3 27 5 17 11 42 29 | 77 29 13 |
| 18:1c + 50 µM Δ8t | 58            | FFA 4  | 6 3 14 10 30 44 73 50 | 397 56 24 |
| 18:1c + 12 µM Δ10t | 35            | Initial 4  | 3 3 38 8 39 18 | 176 76 26 |
| 18:1c + 30 µM Δ10t | 20            | Plip 4  | 1 2 23 6 45 10 | 22 21 60 50 | 430 124 21 |
| 18:1c + 13 µM Δ11t | 44            | Neutr 4  | 3 2 10 10 41 8 | 18 25 38 30 268 45 12 |
| 18:1c + 30 µM Δ11t | 4             | FFA 4  | 2 2 10 5 46 12 | 30 25 74 50 | 594 407 240 |
| 18:1c + 12 µM Δ12t | 25            | Initial 4  | 3 4 34 7 42 11 | 20 16 47 29 201 145 26 |
| 18:1c + 50 µM Δ12t | 0             | Plip 4  | 1 1 10 5 50 27 | 30 44 73 50 | 600 770 166 |
| 18:1c + 12 µM Δ13t | 55            | Neutr 4  | 8 4 45 7 27 2 | 7 22 17 29 67 33 12 |
| 18:1c + 50 µM Δ13t | 45            | FFA 4  | 6 5 45 7 31 2 | 7 13 15 29 92 52 21 |

* Percentage of recovered octadecenoate as the trans isomer in the phospholipid (Plip); di- and triacylglycerols (Neutr); and nonesterified acids (FFA). ** Values not determined.

TABLE V
Effect of exogenous supply of trans-11-octadecenoate on its incorporation into cellular lipids

Contents are expressed as in Table IV.

| Supplement | Net Cell Yield | mole % | % trans in 18:1 | 18:1 amoles/cell |
|------------|---------------|--------|----------------|----------------|
| 18:1c (30µM) | 41            | 12:0 4  | 4 39 11 38 0 0 | 296 47 9 |
| 18:1c + 7 µM | 44            | 14:0 4  | 2 37 11 38 0 0 | 267 33 11 |
| 18:1c + 13 µM | 44            | 16:0 4  | 2 31 10 40 8 18 | 268 45 12 |
| 18:1c + 20 µM | 33            | 18:0 4  | 3 26 8 42 12 23 23 29 36 40 | 290 57 14 |
| 18:1c + 20 µM | 16            | 18:1c 4  | 3 23 6 31 26 24 22 62 40 | 438 259 56 |
| 18:1c + 30 µM | 4             | 18:1t 4  | 2 18 5 46 9 30 21 74 50 | 504 407 248 |
| 18:1c + 40 µM | 0             | Plip 4  | 2 2 15 4 47 22 32 51 - 56 | 542 1150 - |
| 18:1c + 55 µM | 0             | Neutr 4  | 2 2 14 4 47 23 33 29 80 65 | 547 857 2250 |

octadecenoates. The inhibitions noted for certain isomers in our study do not clearly reflect the melting points of those acids and suggest that selective metabolic effects occurred that were not necessarily dependent upon a solid-liquid transition. Apparently, the cell yield of the E. coli mutant was limited by an event very sensitive to temperature, whereas the extent of growth of the S. cerevisiae mutant was not. The trans isomers (8 to 12) that could support growth of the bacteria were equally effective, although they had different melting points and showed dramatically different effects upon the yields of the yeast. Thus, the growth-limiting step(s) for yeast exhibited a much more selective interaction with the positional isomers.

A sharp transition in the Arrhenius plot of the relative growth rate was observed at 37° for an E. coli mutant grown on trans 18:1 (17) and similar transitions in the temperature range of 36-41° were also reported for respiration, efflux of [14C]methyl β thiogalactoside, and in force area isotherms of extracted phosphatidylethanolamine on monolayers (17). It was suggested that the transition observed was due to a change in membrane lipids (from a condensed to an expanded state) as the temperature increased. trans-Unsaturated fatty acids are regarded as much less disruptive of hydrocarbon chain packing than cis acids (18), and would appear to cause membranes to undergo such transitions at a higher temperature than cis acids. When the E. coli mutant (civ-2 f40-6) was grown on elaidate at 37° and the temperature then lower to 27°, the culture lost viability rapidly, but when oleate was added, the viability loss was apparently reversed (19). Even at 37°, membranes of the E. coli containing elaidate were considered to be less stable than those containing oleate; the phospholipid turnover in the membranes was higher and their sensitivity to lysozyme lysis was greater (20, 21). In our studies, the trans
isomers Δ8 to Δ12, that supported growth of the *E. coli* caused no diminution in cell yield with 30 μM oleate at 37° and only slight decreases at 27°. Furthermore, although the growth rate was different at different temperatures, we did not discern differential effects of the various *trans* isomers upon the growth rates of the *E. coli* strain examined. Thus, different growth-limiting events may not consistently reflect the melting properties of the *trans* acids.

The growth-supporting properties of *trans*-unsaturated acids appear to reflect physical properties midway between those of the cis-unsaturated and the saturated fatty acids for *Mycoplasma* (22, 23) and *E. coli* (24). Apparently in *S. cerevisiae*, the structural requirement for fatty acids is more stringent since *trans*-18:1 alone did not support growth of a saturated fatty acid mutant (25).

**Effects of trans Fatty Acids on Lipid Metabolism—trans-**

Unsaturated fatty acids might influence cellular metabolism in two general ways. First, as free acids, or simple derivatives, they could interfere with transport, activation, or acylation of other fatty acids. Second, their esterified products could influence membrane-modulated processes and enzymic reactions. Examples for each of these possible modes of interaction with cellular metabolism have been described and will be summarized here.

*trans* acids in their free acid form, might influence cells by competitively inhibiting enzymic processes such as fatty acid activation and acylation. However, recent reports in the literature have not indicated this type of inhibition. Wissieski and Kiyomoto (15) described no inhibitory effect on the growth of wild type yeast with any of several *trans* acids. Palmitoyl-CoA:glycerol-3-phosphate acyltransferase was reported to be not inhibited by either the Δ9-*trans* or Δ11-*trans*-18:1 (26), and saturated and *trans*-unsaturated fatty acids actually gave a lower level of inhibition of fatty acid activation than did cis acids. Although Sinclair (5, 27) first reported a limited incorporation of dietary elaidic acid into the phospholipids of brain and testes, the overall metabolism of *trans*-fatty acids in animal tissue has been described to be generally similar to that of cis-fatty acids when either or both acids are present in the animal diet. Dietary elaidic acid was incorporated into all lipoprotein classes studied (28) and can become up to 17% (29) to 24% (30) of the acyl groups in adipose triacylglycerols. No significant differences in cis and *trans* acids were found with respect to intestinal absorption, transport from plasma, oxidation to CO₂, and placental transfer in the rat (4) or incorporation into the free fatty acid, triglyceride, and phospholipid classes of perfused chicken liver (31). However, a recent, more detailed study indicated that the 9-*trans* isomer may be incorporated into phospholipids in preference to the 9-cis isomer, and the reactions involved were more complex than those for incorporation into triglycerides (32).

Activation of the *trans*-fatty acids to their CoA derivatives by rat liver microsomes showed a minimum rate for the Δ9 and Δ10 isomers (33). This pattern did not resemble the one for acylation obtained by Okuyama et al. (6) and has no recognized physiological significance.

The acyltransferase systems that form lecithin in rat liver microsomes handle the naturally occurring Δ9-cis-18:1 differently from the corresponding *trans* isomer. Esterification of *trans*-Δ9 was comparable to that for saturated acids at position 1 and to cis-Δ9 at position 2 (34). A somewhat similar selectivity appears to place Δ9-*trans*-18:1 at both positions 1 and 2 of phosphatidylethanolamine of *E. coli* (35, 36).

**Effects of trans-Fatty Acids on Membrane-bound Enzymes—trans-**

Unsaturated fatty acids esterified into membrane lipids may influence cell metabolism by modulating the activity of membrane-associated enzymes. Two types of modulation have been observed: a change in a particular enzyme activity over a temperature range without any visible transitions on an Arrhenius plot, and a distinct transition or transitions on the Arrhenius plot, most often ascribed to phase changes in the bulk lipid mass of the membrane.

Mavis and Vagelos (37) have reported unique changes in several membrane-associated enzyme activities caused by esterified *trans*-Δ9 and Δ11 acids. Apparently, the presence of a *trans* isomer in the membrane phospholipids changed the acyl-CoA:glycerol phosphate acyltransferase specificity by some selective interaction with the unsaturated fatty acid alky chain, but not by any sharply defined phase change of the bulk lipids. The membrane-bound uridine diphosphate galactose lipopolysaccharide galactosyltransferase of *E. coli* showed discontinuity in Arrhenius plots associated with cis, but not with *trans* acids (38).

Distinct transition points or discontinuities in Arrhenius plots are often ascribed to a phase change from a solid hexagonal to a liquid crystalline state of the bulk lamellar-structured lipids or at least of defined lipid domains in the membrane. Wilson and Fox (10) reported that each unsaturated fatty acid tested gave a characteristic transition point on Arrhenius plots for induced β-glucoside transport systems, and a modification of the above experiment produced two transition points when cell growth on oleate was followed by induction of the transport system with elaidate (39). Two transition temperatures observed with a single acid supplement were recently ascribed to the onset and completion of a lateral phase transition within the membrane lipid (11). Emphasis on a range in which transitions may occur brings attention to the great variety of lipid molecular species that exist in membranes. The concept that these can constitute a particular single solid phase is difficult to define with precision. Reports by Esfahani et al. (40) and Oldfield and Chapman (41) urged caution regarding the idea that simple phase changes of the bulk membrane lipids determine the transition temperature of enzyme activities on Arrhenius plots. In our studies with *S. cerevisiae*, the marked differences in inhibition between positional isomers that can be expected to have similar melting points do not seem easily reconciled with a simple phase transition phenomenon.

**Physiology and Metabolism of trans Acids in Animals—**

Although natural milk (42) and beef fat (43) may contain up to 9.7% *trans* acids from the action of rumen bacteria, partially hydrogenated vegetable oils represent a major source of *trans* fatty acids in the North American diet. The *trans*-octadecenoate isomers commonly present in commercially available margarines and shortenings (18 to 55% *trans* acid content (44, 45)) are principally the 10 and 11 isomers with 10 > 11 > 9 > 8 > 12 > 13 (2).

The clearly toxic effect of certain positional *trans* isomers upon *S. cerevisiae* make it of some interest to consider the possible consequences of the various dietary *trans* acids upon higher eukaryotic systems. While no immediate toxic effect has been imputed to dietary intake of *trans* acids, certain physiological and long term pathological consequences have been reported (46-48). Mattson (49), however, reported that when all cis and either of two *trans*-18:2 isomers were included in equal amounts in the diet of rats deficient in essential fatty acids,
the trans isomers did not inhibit the weight gain facilitated by the all cis isomers. Apparently, the ratio of cis to trans acids in the nutrient can influence the effectiveness of the trans isomer inhibitors since an 8-fold excess of the all trans-18:2 inhibited anabolic reactions of the all cis compound (50).

In our studies with S. cerevisiae, many trans-octadecenoate isomers gave significant toxic effects when present at one-third the level of the cis-nutrient and markedly toxic effects when added in equimolar amounts. Some evidence links intake of trans-fatty acids and saturated, higher melting acids with development of atherosclerosis and increased serum triglycerides and cholesterol levels (51). In this regard then, it may be significant that trans acids caused a major increase in triglyceride in the eukaryotic yeast cell. Such an increased glyceride formation by mammalian liver cells might lead to an increased secretion of low density lipoproteins, a process apparently not available to S. cerevisiae. A hypertriglyceridemic response reported for liver reflected the amount of exogenous fatty acids, as palmitate or linoleate (52, 53). If saturated or cis- and trans-fatty acids and saturated, higher melting acids with available to S. cerevisiae. A hypertriglyceridemic response might lead to an increased secretion of low density lipoproteins, a process apparently not available to S. cerevisiae. A hypertriglyceridemic response reported for liver reflected the amount of exogenous fatty acids, as palmitate or linoleate (52, 53). If saturated or cis- and trans-unsaturated acids can, in fact, exert different effects on cellular lipid metabolism, perhaps S. cerevisiae mutants will prove to be a useful, sensitive model for examining acyl chain modulated control of hypertriglyceridemia. The results obtained in this survey of effects of trans isomers indicate that very selective, detrimental effects on cellular lipid metabolism and cellular function may be further examined in these eukaryotic cells.

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