The Membrane Adaptivity of Cyanobacteria in the Presence of Normal Chain Alcohols

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OVERVIEW

The ability to adapt to environmental conditions is a fundamental property of living organisms. The adaptive ability of a cell membrane has been studied extensively in *E. coli* bacterial cells. This experiment focused on cyanobacterial cells and their ability to adapt to changes in temperature and solvent concentrations.

In the experiments described here, growth of cyanobacterial cells in different temperatures was shown to affect the observed lag time and the growth rate. Addition of normal chain alcohols was shown to affect both the growth rate and the lipid composition. The results indicated that during growth in the presence of methanol, the saturated fatty acids palmitic and capric acid both increased in the cell membrane. Similar fatty acid changes were observed in *E.coli* by Sullivan et al.(15); however, this was the first demonstration of this phenomenon in cyanobacterial cells.

The observed changes in the ratio of saturated to unsaturated fatty acid in the presence of alcohols were very similar to the ratio changes found previously with varying temperatures (9). This suggests that alcohols mimic the changes in membrane structure brought about by a change in temperature (15). The data from this experiment do not attempt to explain what mechanism caused the alcohol adaptation ; however, they did demonstrate that in the presence of methanol the saturated to unsaturated fatty acid ratio did increase.

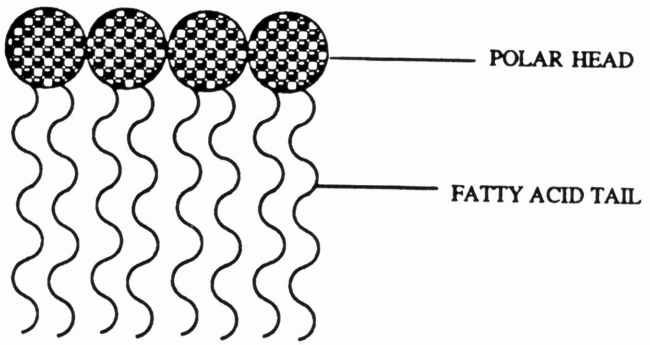
BACKGROUND INFORMATION

Lipids and proteins are the major components of the fluid mosaic structure of the cell membrane. The fluid mosaic model describes the membrane as a constantly moving and changing moiety. The membrane lipids contain two types of fatty acids,

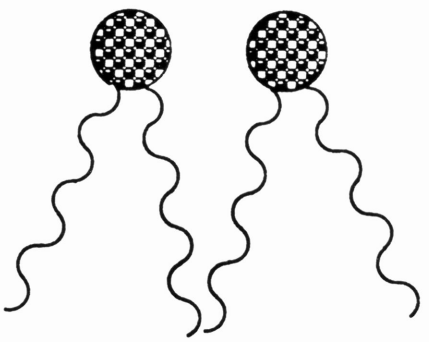
unsaturated and saturated. These two types of fatty acids differ in differing species of organisms.

Lipids are amphipathic; therefore, they have both hydrophobic and hydrophilic regions. Specifically these two regions are the polar phospholipid heads and the non-polar fatty acid tails (Figure 1).

FIGURE 1



SATURATED FATTY ACIDS



UNSATURATED FATTY ACIDS

Tails vary in length with unsaturated fatty acids containing at least one double bond. Usually one of the sides of the tail contains a double bond while the other side does not (1). Since the phospholipid heads are hydrophilic and the fatty-acid tails are

hydrophobic, the lipids spontaneously form a bilayer in an aqueous cell environment.

The membrane proteins extend through or are imbedded in the membrane. Proteins are also amphipathic; consequently, they can interact with the hydrophobic tails and the hydrophilic phospholipid heads. The hydrophobicity of some membrane proteins is increased by the covalent attachment of fatty acid chains that anchor the proteins in the bilayer (1). These proteins function as transport mechanisms and as enzymes for the cell. Previous research indicates that the addition of ethanol and tert-butanol has little effect on protein structure even though the alcohols substantially alter the lipid structure (4). The only effects alcohols seem to have on proteins are that they might alter the nature of a lipid-protein interaction.

Cyanobacteria are the most complex of the photosynthetic bacteria. They can be grown on simple mineral media. Some of their requirements include carbon dioxide, water, and light. Cyanobacteria use the electrons from water and the energy of light to convert carbon dioxide into organic compounds (1). Cyanobacteria have evolutionary significance since it is widely believed that chloroplasts are descendants of cyanobacteria .

Cyanobacterial membrane fatty acids are mainly unbranched chains with palmitic and palmitoleic acid being the most commonly found. In photosynthetic eukaryotes, polyunsaturated fatty acids are more prevalent than saturated fatty acids due to the large amount of polyunsaturated fatty acids found in the chloroplasts (7). Cyanobacteria contain mostly saturated and monounsaturated fatty acids, a characteristic they share with most of the non-photosynthetic bacteria (7). Many filamentous cyanobacteria have been shown to contain some polyunsaturated fatty acids; however, these compounds were not present in three particular unicellular

cyanobacteria (7). Among the three with low amounts of polyunsaturated fatty acids is Anacystis nidulans (R-2) which is used in this experiment.

Previous investigators had shown that the *E.coli* cell membrane has the ability to change its lipid composition to adapt to a wide range of environmental conditions. We hypothesized that cyanobacteria would respond in a similar manner. Cyanobacteria and *E. coli* are both gram negative organisms with respect to their cell walls. They both reproduce by binary fission and contain many of the same proteins, nucleic acids, and have no cytoskeleton. Despite these similarities, cyanobacteria have the ability to produce a plant type chlorophyll. They have additional cytoplasmic thylakoid membranes which are not found in *E. coli*.

Past research with *E. coli* and cyanobacteria exposed to different temperatures has shown that at a molecular level within the cell membrane a change in temperature causes a change in the degree of mobility of fatty acids and dissolved proteins (8). This change in the degree of mobility is followed by and counteracted by a change in the fatty acid composition. This fatty acid composition regulation occurs at a level of fatty acid incorporation into phospholipids and at fatty acid biosynthesis (2). It follows logically that at a particular species temperature optima, the growth rate would be the best. The end result in thermal regulation and adaption is that increasing the growth temperature increases the ratio of saturated to unsaturated fatty acids.

The obvious explanation of why this phenomenon has to happen involves saturated and unsaturated fatty acid structure. Since saturated fatty acids contain no double bonds they pack very tightly with their tails remaining fairly parallel to one another. Unsaturated fatty acids contain double and triple bonds, which cause them not to pack as tightly (Figure 2).

FIGURE 2

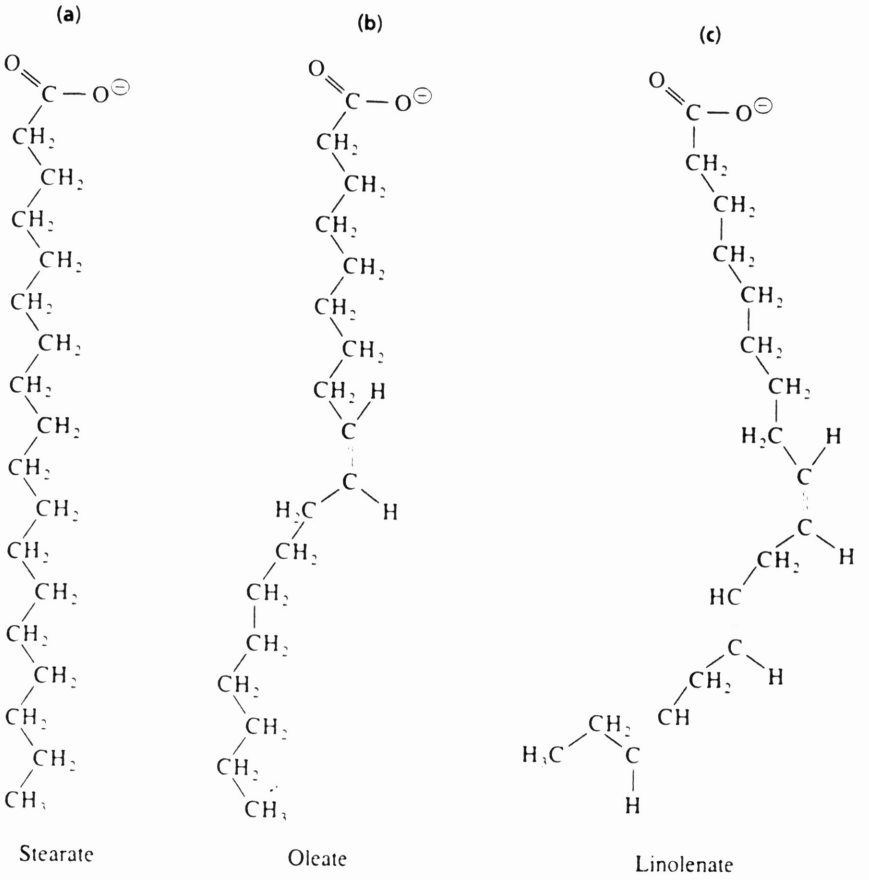


Figure 9·3
Structures of (**a**) stearate (octadecanoate), a saturated fatty acid; (**b**) oleate (*cis*-Δ^9-octadecenoate); and (**c**) linolenate (all *cis*-$\Delta^{9,12,15}$-octadecatrienoate). The *cis* double bonds put kinks in the tails of the unsaturated fatty acids. Thus, crystals of *cis* mono- and polyunsaturated fatty acids are not well ordered, and their melting points are much lower than those of saturated fatty acids containing the same number of carbon atoms.

Figure 2 copied from source 10 p. 211.

Fluidity depends in part on how tightly the fatty acids are packed together. Therefore, since saturated and unsaturated fatty acids pack differently, changing their ratios will affect fluidity. When the temperature or solvent concentration is increased, the fluidity of the membrane increases. This increased fluidity must be countered in some way to maintain normal cell functioning. The way a cell adapts to this new environmental condition somehow involves the incorporation of additional saturated fatty acids into the cell membrane. By increasing the saturated fatty acid composition, the increased membrane fluidity is decreased and the cell can maintain normal functioning.

Normal chain alcohols also affect the fatty acid composition; an increase in the concentration of alcohol in the growth media leads to an increase in the saturated to unsaturated fatty acid ratio. (5,6,15). The effects of temperature and solvents on the saturated to unsaturated fatty acid ratio are very similar. The increase in temperature or amount of added solvent is proportional to the increase of the saturated to unsaturated fatty acid ratio.

MATERIALS AND METHODS

Cell Cultures

Two species of cyanobacteria were used in the experiment, *Anacystis nidulans* (strain R-2) and *Nostoc macrozamiae* (strain Mac). The initial culture of R-2 was obtained from Dr. Susan Golden of the Texas A&M University Department of Biology. The initial culture of strain Mac was obtained from Dr. S.E. Stevens of the Memphis State University Department of Biology. Both cultures were derived from a single cell to ensure clonal populations.

Strain R-2 grows as single coccoid cells, which suspend uniformly in the media. It was chosen for its ease of growth. Mac is a filamentous strain that was chosen for comparison. It grows rapidly, but the filaments sometimes clump, making the culture less uniform. Both strains of cyanobacteria grew well at the same temperature and their reflectance could be measured at the same wavelength.

Growth media

The cyanobacterial growth media contained the following compounds:

TABLE 1. *Culture media for blue-green algae.*

Compound	Medium A	Medium B	Medium C[b]
Composition of culture media for blue-green algae (g/liter)			
Na_2EDTA	0.03	0.001	0.01
NaCl	18.0	—	—
$MgSO_4 \cdot 7H_2O$	5.0	0.075	0.25
KCl	0.6	—	—
$CaCl_2 \cdot 2H_2O$	0.37	0.037	—
$Ca(NO_3)_2 \cdot 4H_2O$	—	—	0.025
$NaNO_3$	1.0	1.5	—
KNO_3	—	—	1.0
KH_2PO_4	0.050	0.050	0.050
Na_2CO_3	—	0.020	—
Citric acid	—	0.006	—
Trizma base[a]	1.0	—	—
Glycylglycine	—	—	1.0
Trace element composition of culture media (mg/liter)			
Vitamin B_{12}	0.004	—	—
$FeCl_3 \cdot 6H_2O$	3.89	—	3.89
Ferric ammonium citrate	—	6.0	—
H_3BO_3	34.3	2.86	2.86
$MnCl_2 \cdot 4H_2O$	4.3	1.81	1.81
$ZnCl_2$	0.315	—	—
$ZnSO_4 \cdot 7H_2O$	—	0.222	0.222
MoO_3 (85%)	0.03	—	—
$Na_2MoO_4 \cdot 2H_2O$	—	0.39	0.39
$CuSO_4 \cdot 5H_2O$	0.003	0.79	0.79
$CoCl_2 \cdot 6H_2O$	0.0122	—	—
$Co(NO_3)_2 \cdot 6H_2O$	—	0.0494	0.0494

[a] Trizma base is adjusted to pH 8.2 with conc. HCl.
[b] pH is adjusted to pH 8.0 with NaOH.

Table was copied from source 13. The media used was in the last column (C) .

This growth media was developed by Stevens, Patterson, and Myers (13). Experience over the last twenty five years has shown that this media contains the necessary minerals and nutrients for proper growth. Twenty-five ml of growth media was carefully measured into pyrex growth tubes and autoclaved prior to cell addition.

Growth conditions

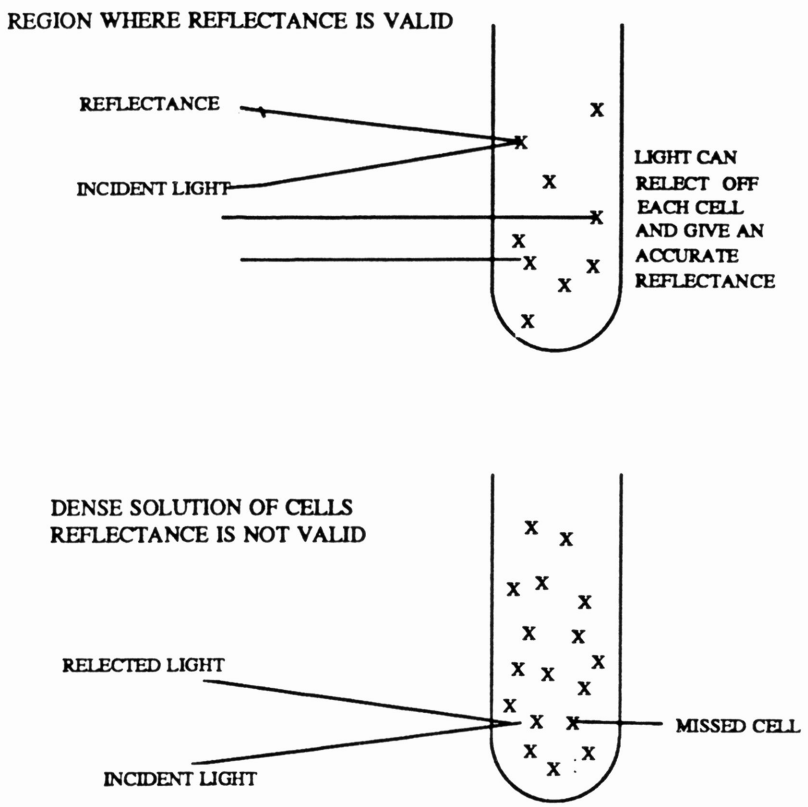
Cells (0.2ml) harvested during log phase were added to the growth media and their initial turbidity was recorded. This reading was considered the zero reading, and all other turbidity measurements were compared to this one.

The control group and the added alcohol groups were grown in the same position in the temperature bath. This insured that they were grown at the same temperature and with the same light intensity. The light used to illuminate the cells was a Philips F40CW 40 W bulb, placed a distance of 10 inches from the growth tubes. All pyrex 50 ml growth tubes were provided via bubblers with an excess of 1% carbon dioxide in air (v/v) to ensure proper growth and to keep the cells in suspension.

Measurement of growth rate

Cells were grown at constant temperature; growth rate was measured using spectrophotometry. The spectrophotomer used was a Bausch and Lomb Spectronic 20. Cells could either absorb or reflect the light. Since each cell was pigmented differently, absorbance was not an acceptable way to measure cell growth. The absorbance was minimized by selecting the wavelength of minimum absorbance to measure transmittance of the cells. The observed decreases in transmittance were due to increased cell concentration and thus increased light scattering. By measuring the transmittance of the cell suspension, the increased cell concentration was determined. By calculating turbidity from transmittance and plotting turbidity vs. time, the growth rate was determined. At high concentrations of cells, the transmittance measurement was less reliable. This can be explained using Figure 3.

FIGURE 3



At higher concentrations, cells got directly behind one another. This caused a lower transmittance reading than the actual transmittance due to increased cell concentration.

Solvent addition

Once the control group growth rate was determined, the addition of solvents to the media was employed. The solvents used were as follows: Fisher Methanol 99.9 Mol % pure, Fisher Butyl Alcohol certified, Photrex 2-Propanol (for UV spectrometry) 99% pure, and Analabs incorporated Isopropyl alcohol 99.9% pure.

The solvents were sterilized by filtration and added to the growth tubes using sterile techniques. The occurred after the media was autoclaved and prior to cell addition.

The alcohols were initially added to the growth tubes in concentrations of 1% (v/v). If growth occurred, then addition of 2% (v/v) alcohol was implemented. If the cyanobacteria did not grow in 1% (v/v) alcohol, the tube was discarded and another trial using lower alcohol concentrations was attempted. This continued until the maximum solvent concentration, which still allowed for cyanobacterial growth, was added. The strains were then extracted and later analyzed using gas chromatography.

Extraction procedure

The extraction method used in this experiment was developed by Sato and Murata (11).

1. Cells and growth media were centrifuged until only a pellet of cells remained in the bottom. The cells were centrifuged in a graduated conical centrifuge tube until 0.1 ml of cells had been obtained. The supernatent was discarded and the cells were quantified by wet weight using a twin beam balance. This was in addition to the quantification by volume.

2. 1.0 ml H_20 was added to the cells

3. 1.25 ml $CHCl_3$ and 2.50 ml CH_3OH were added and mixed by vortexing.

4. The solution was allowed to stand for 20 minutes at room temperature.

5. 1.25ml $CHCl_3$ and 1.25 ml H_2O were added and mixed by vortexing.

6. The solution was centrifuged for 15 minutes at 1000g.

7. The upper layer and intermediate fluff layer were withdrawn using a pipet and discarded.

8. 1.32 ml CH_3OH and 1.18 ml H_2O were added and mixed by vortexing.

9. The solution was centrifuged for 15 minutes at 1000g.

10. The lower layer was recovered and evaporated to near dryness using dry air.

11. To the extract was added 50 ml CH_3OH and 1 ml H_2SO_4 to form a 2% H_2SO_4 solution for methylation.

12. The solution was heated at 70 C for 1.5 hours

13. The fatty acid methyl esters were extracted into petroleum ether and concentrated to 10ml using dry air.

14. The concentrated methyl esters were refrigerated and used immediately for gas chromatography analysis.

Gas chromatography parameters

The gas chromatograph used for this experiment was a Hewlett Packard 5790A series equipped with a flame ionization detector and a Hewlett Packard 3390A integrator. The oven used the following temperature program:

Initial temperature 45C
Time at initial temperature 1 minute
Rate of temperature increase 20C/minute
Final temperature 199C
Time at final temperature 5 minutes

The detector temperature was 300C and the injector temperature was 200C. The needle used for injection was a Hamilton 7000 series syringe with a Chaney adapter in a volume of 3ul. The integrator parameters were the following:

Attenuation 2 times = 6
Chart speed = 1.0 cm/min
Peak width detected = 0.05 minutes
Signal to Noise ratio = 4 (corresponds to a minimum height area count for recogniton of 2000 counts)
Rejected Area Counts = 0

The main goal in selecting a gas chromatographic column was to choose one that would provide the best separation of mixtures of fatty acid methyl esters. The

column used for the gas chromatography analysis was a 5% DEGS-PS (diethylene glycol succinate packing) on 100/120 Supelcoport. The column tubing was glass and a 3 foot column length, 1/8 inch diameter OD, and 1.8mm ID was used. The reason for using glass tubing was because glass is the most inert; thus, the sample is less likely to interact with the walls of the tube. Glass also rarely causes tailing of the sample, decomposition of the sample, or problems due to injection directly onto the column. The 3 foot column was chosen over the 6 foot column primarily for financial reasons. The 6 foot column was recommended in the literature because it produced better peak resolution(16); however, for this experiment, the 3 foot column was used with some degree of success.

The stationary phase chosen was DEGS-PS which was a polyester type phase. The column support material chosen was important because it usually interacts with the sample and determines the efficiency of the column (16). Since the ideal gas chromatograph peak should be narrow and sharp, selection of the incorrect support could have caused peak tailing and wide peaks. The support material used in the column was Supelcoport, which consisted of acid washed and silanized diatomaceous earth. Acid washing was used to remove impurities from the support and it was recommended when a polar phase such as DEGS-PS was used in the column(16). Silanization was used to reduce peak tailing. The column was custom made by Supelco Inc. according to the specifications in Figure 4.

FIGURE 4

ATTN:
Magie
Customer
Service

Column Configuration
for HP 5790A

Keri Keilberg
Texas A+M University

Return Authorization Number
491285

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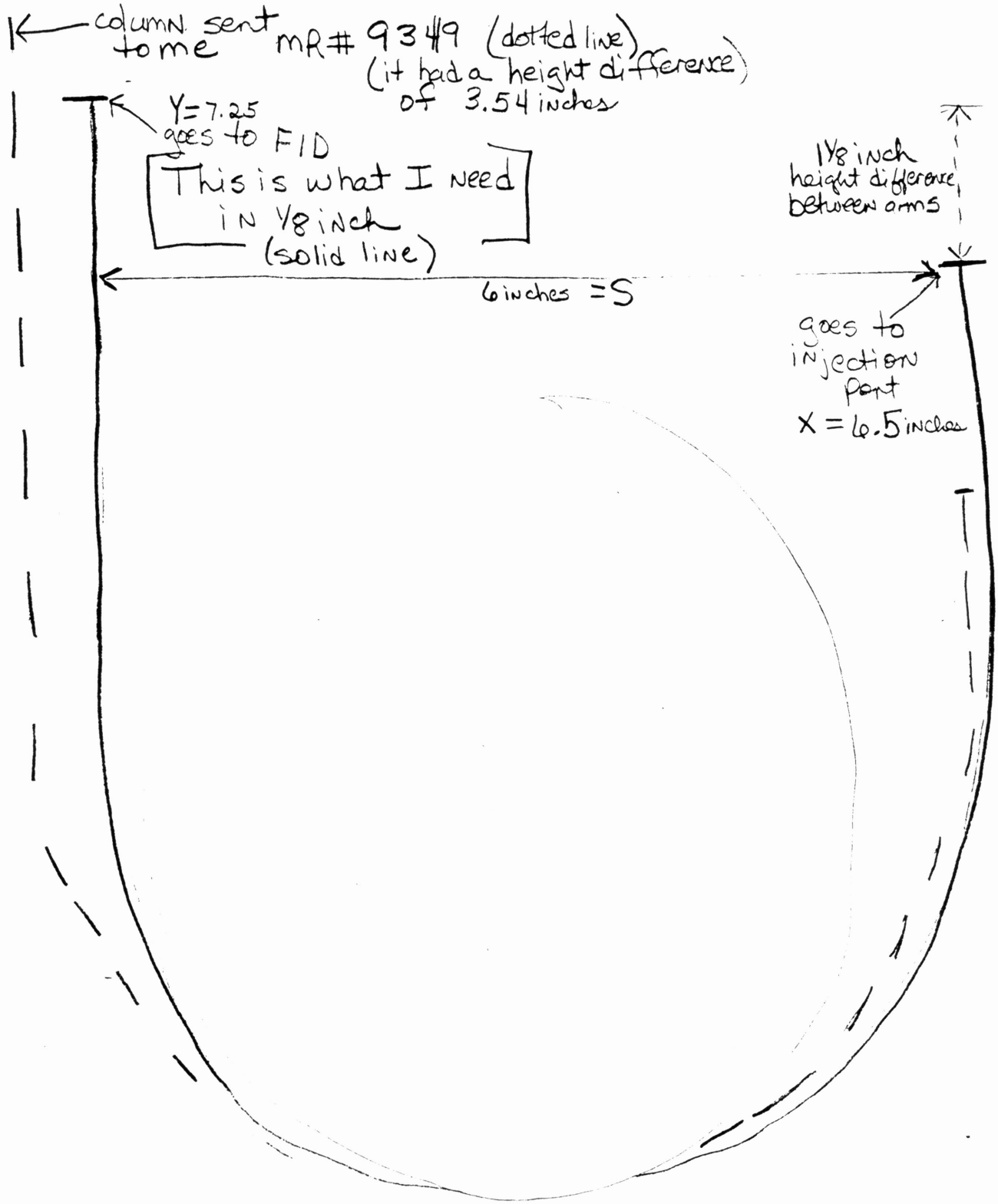


The mesh particle size related the size of the particle filter used and the amount of particles passing through the column. The one used for this experiment was 100/120 mesh, which corresponded to an actual particle size of 149-125 microns. This column was of higher efficiency than many others which could have been selected because the particle size was very small.

One of the most important parts of conducting the gas chromatography analysis was the column conditioning. Once the column was installed, the nitrogen carrier gas was calibrated to 20 ml/min. The calibration method used was volume of water displacement per unit time. The controls were adjusted until the nitrogen flowed at 20 ml/min. To properly condition the column, it was raised to the maximum operating temperature (199C) and left overnight. This step got rid of the solvents used in the column coating step and removed the very volatile portion of the stationary phase (16). If at any time during the experiment the gas flow had been removed from the column, the conditioning would have been repeated to keep stationary phase oxidation to a minimum.

Gases used in analysis

The gases used were of high quality and purity. The nitrogen carrier gas was of high purity and it was run through a drying tube packed with Supelco Activated Molecular Sieve 8/12 mesh. This packing actively absorbed water and other contaminants present in the purified nitrogen. The gases used for the flame ionization detector were compressed air and hydrogen. All gases were supplied by Bailey Oxygen Co. of Bryan, TX who acquired the gases from Big Three Industries Inc., of Houston, Texas. All the gases were routed through 1/8 inch OD copper tubing connected with copper ferrules.

Solvents used for analysis

The solvents used were as follows: Mallinckrodt Petroleum Ether analytical grade, Fisher Methanol 99.9 Mol % pure, Spectrum Chloroform reagent grade (contains 0.75% alcohol used as a stabilizer), and MCB Sulfuric Acid.

RESULTS

Control temperature growth

The first part of the fellows year was spent learning how to prepare the cyanobacterial growth media and obtaining a control group. The observed cyanobacterial growth rate in different temperature baths can be seen in Figures 5 and 6. These data indicated that as the temperature of the bath increased, so did the growth rate. The growth rate at 27 and 30 degrees was too slow for the purposes of this experiment. The growth rate in 36 degrees was so rapid that it would have been difficult to catch the cells in log phase. Therefore, the temperature chosen to conduct all analyses was 33-34 degrees. The R-2 and Mac cells both grew well in this temperature, yet they remained in log phase long enough to harvest, transfer, and analyze them.

The results from duplicate growth trials of the control group are shown in Figures 7 and 8. The curve for R-2 had a correlation of 0.99 while MAC had a correlation of 0.98. This was very logical when one considered that MAC was a filamentous strain of cyanobacteria; thus, the turbidity measurements should deviate more. This deviation was not as prevalent in the single cellular R-2 strain. This was due to the uniform nature of R-2 and the fact that the light reflected more evenly from uniform cells.

Alcohol addition results

The first alcohol explored was methanol since it was the shortest chain alcohol. The alcohol and cyanobacterial cells were added directly to the growth media. Both

FIGURE 5

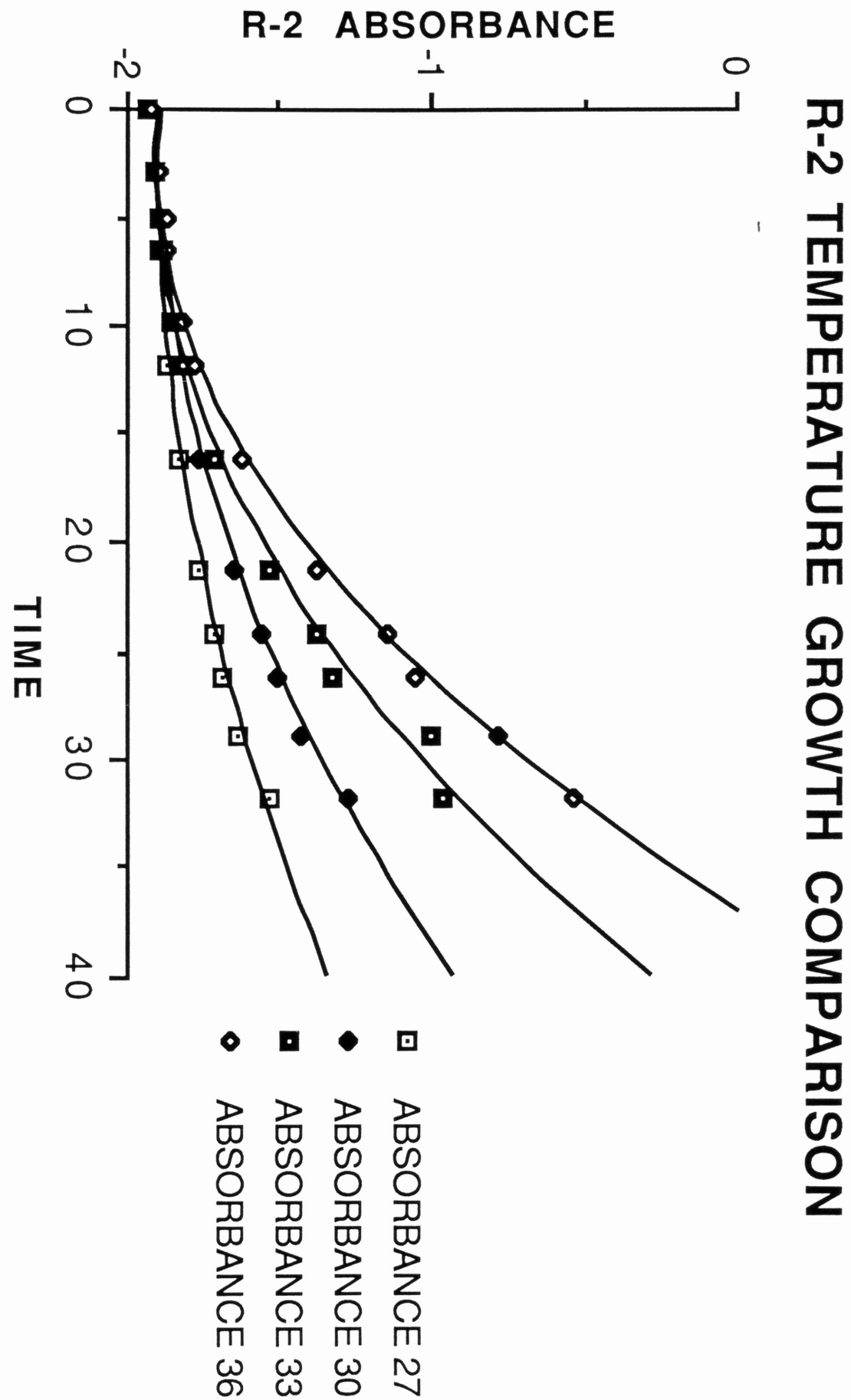

R-2 TEMPERATURE GROWTH COMPARISON
R-2 ABSORBANCE
-2
-1
0
0
10
20
30
40
TIME
ABSORBANCE 27
ABSORBANCE 30
ABSORBANCE 33
ABSORBANCE 36

FIGURE 6

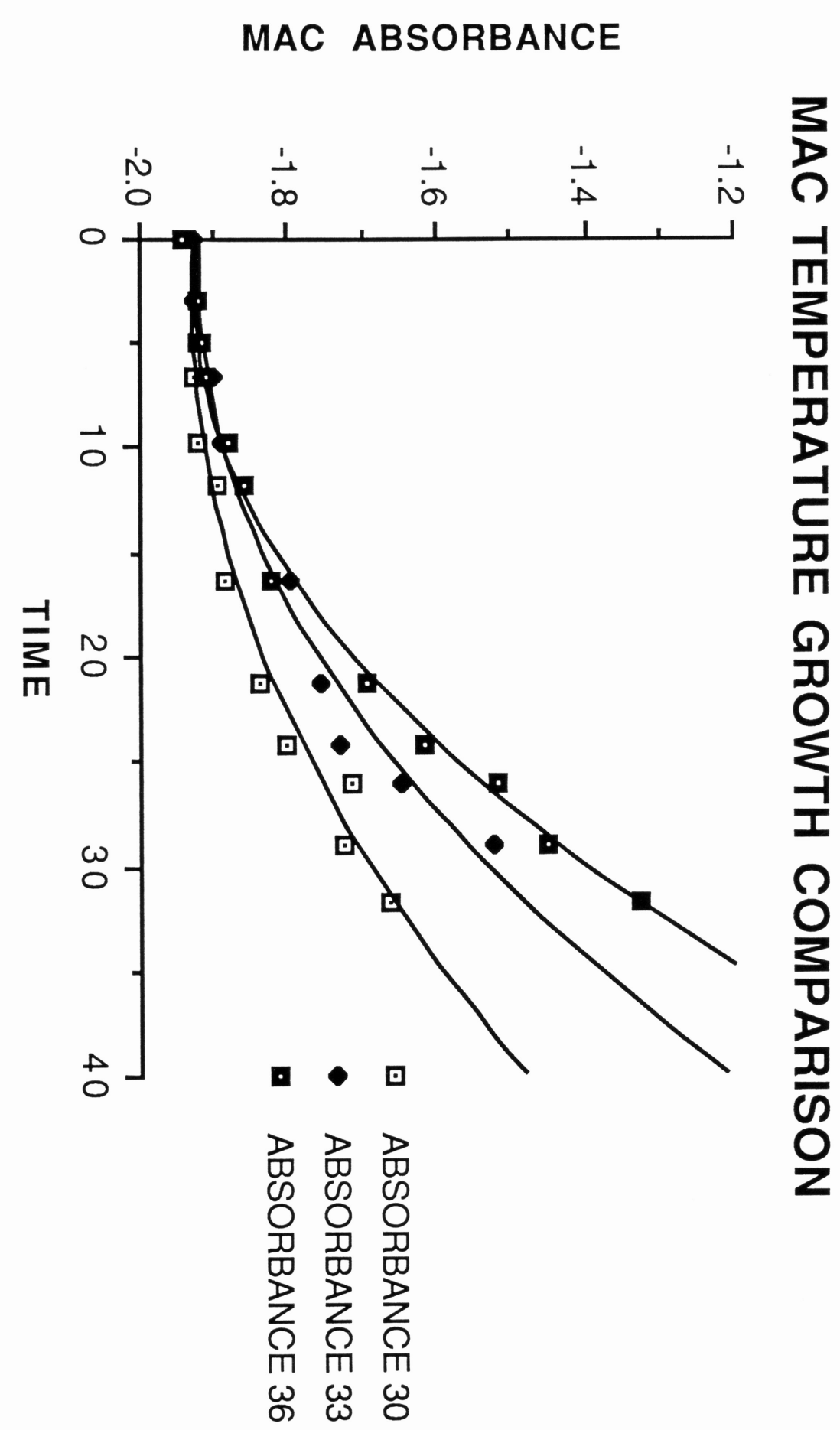

MAC TEMPERATURE GROWTH COMPARISON
MAC ABSORBANCE
-1.2
-1.4
-1.6
-1.8
-2.0
0
10
20
30
40
TIME
ABSORBANCE 30
ABSORBANCE 33
ABSORBANCE 36

FIGURE 7

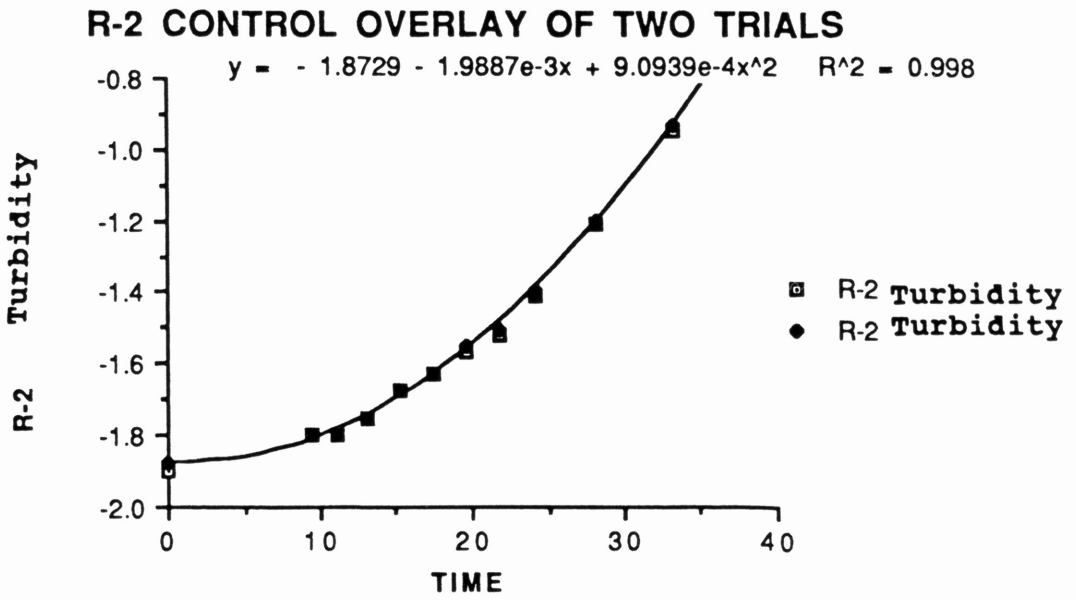
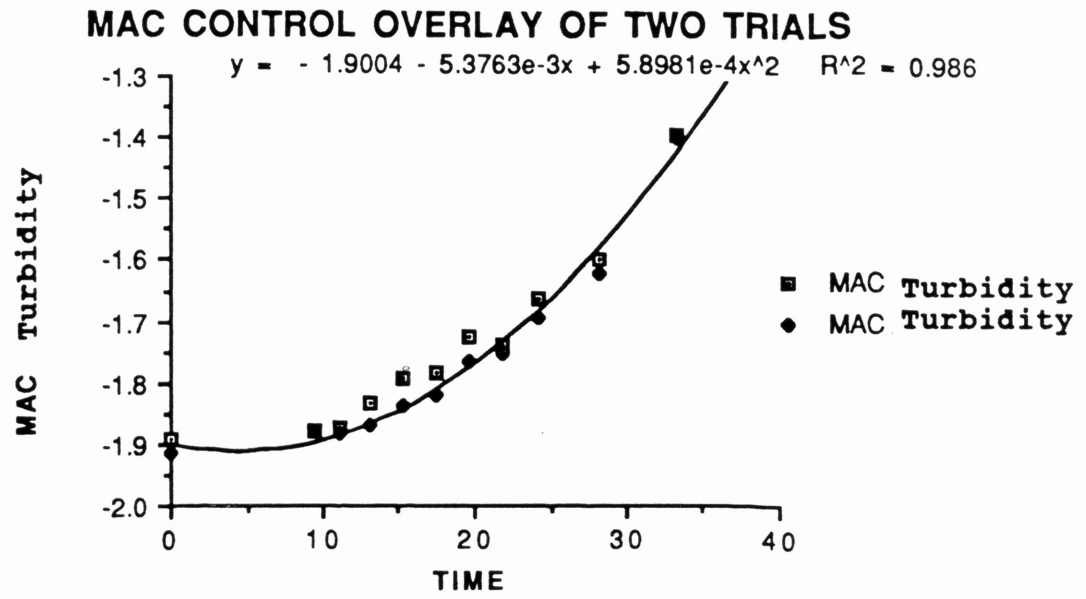


FIGURE 8



R-2 and MAC grew well in methanol up to a growth concentration of 2% methanol. Figure 9 compares the growth rates of R-2 in standard culture media and no added methanol, and with methanol added in a 2% concentration. Figure 10 compares the growth rates of Mac in standard culture media and no added methanol, with methanol added to a 2% concentration, and with methanol added to a 4% final concentration. Similar growth rate measurements made with various concentrations of other alcohols, can be seen in Figures 11-16.

It was difficult to obtain growth in butanol. When concentrations of 1 and 2% butanol were added to the media, cyanobacterial cell bleaching and death occurred. To counter the harsh effects that the butanol had on the cells, a "training" technique was developed and employed. First the cells were exposed to much lower concentrations of butanol (0.2%). Once the cells were growing in the low concentrations, they were transferred into a tube of fresh media with a slightly higher concentration of butanol. This procedure was successful until 0.8% butanol was reached, at which point bleaching occurred. We suspected that the cell bleaching was caused by a combination of the addition of butanol to the growth media, and the cell exposure to intense light. In subsequent experiments, cells were grown in the dark for two days to lessen combination shock to the cells. This technique proved successful up to 0.8 % butanol; however, in this experiment no higher percentages were reached successfully.

All of these trials indicated that as the concentration of alcohol increased, there was an increase in the lag time and a decrease in the growth rate. This could be due to a situation in which the growth was slowed while the cells were adapting to the alcohol. The gas chromatography determined that the adaptation period (decrease in growth rate) was accompanied by a lipid compositional change.

Temperature programing

FIGURE 9

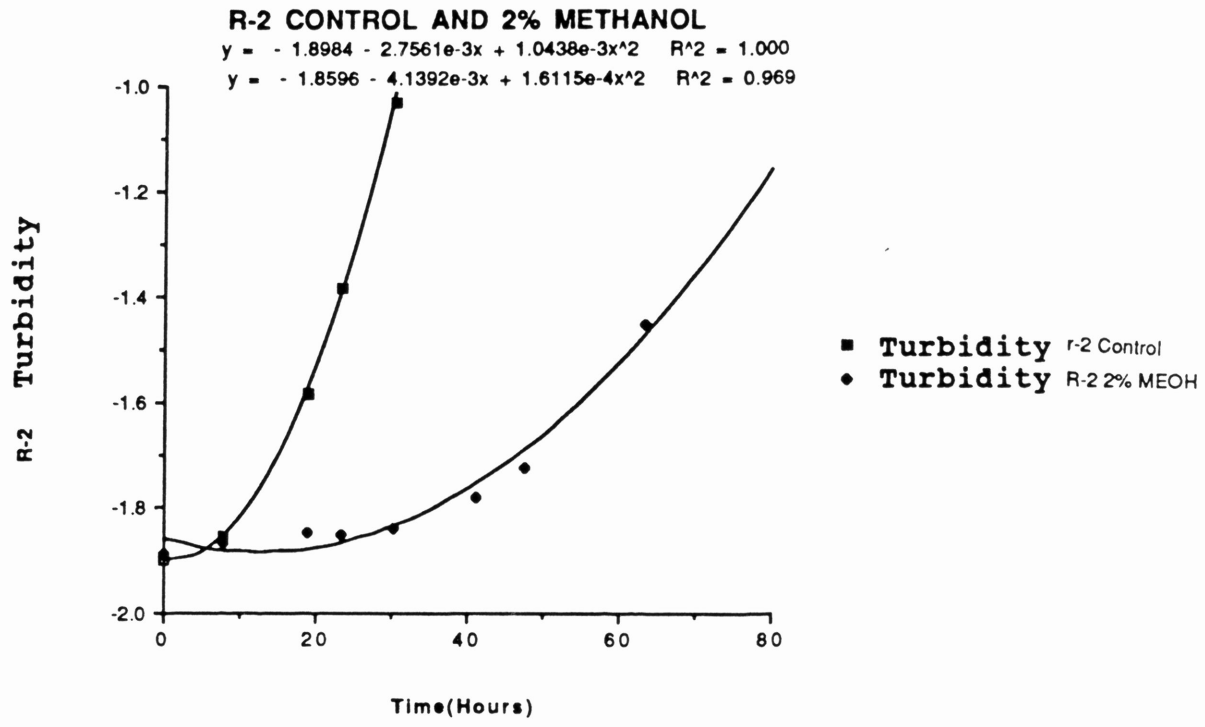


FIGURE 10

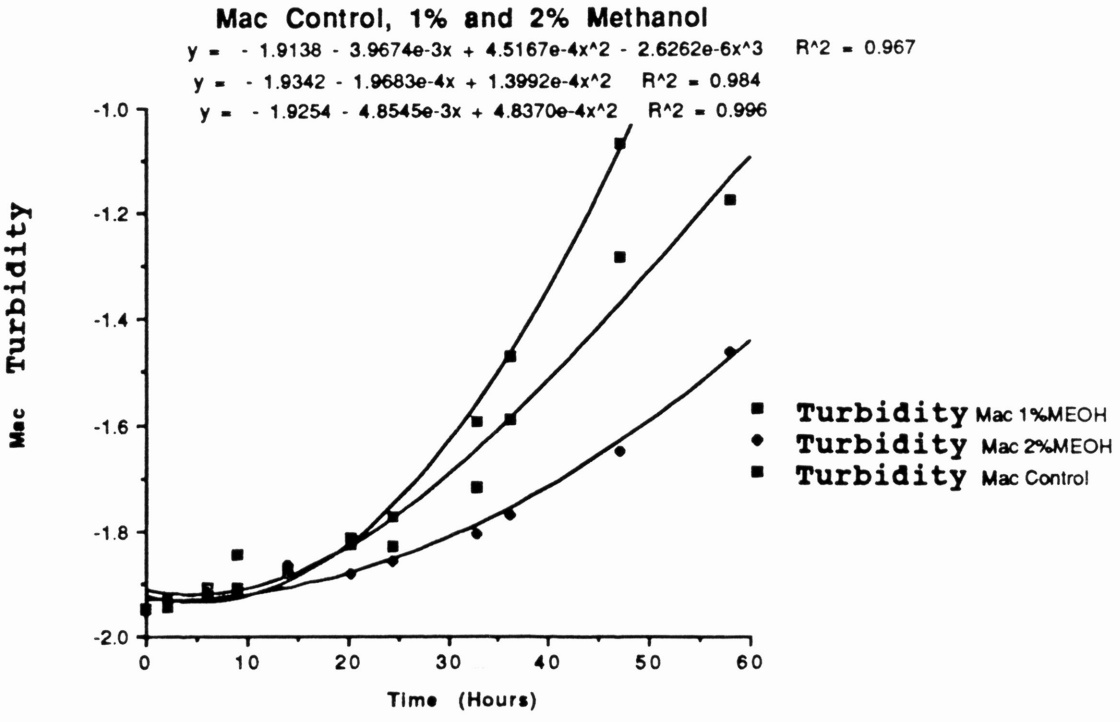


FIGURE 11

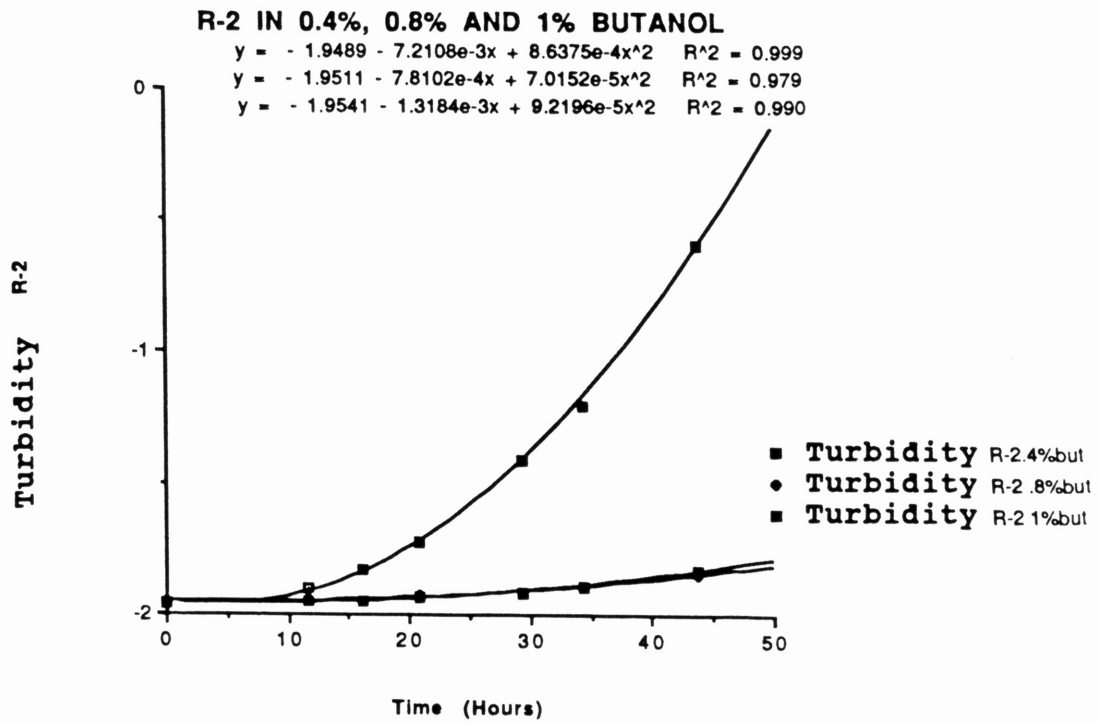


FIGURE 12

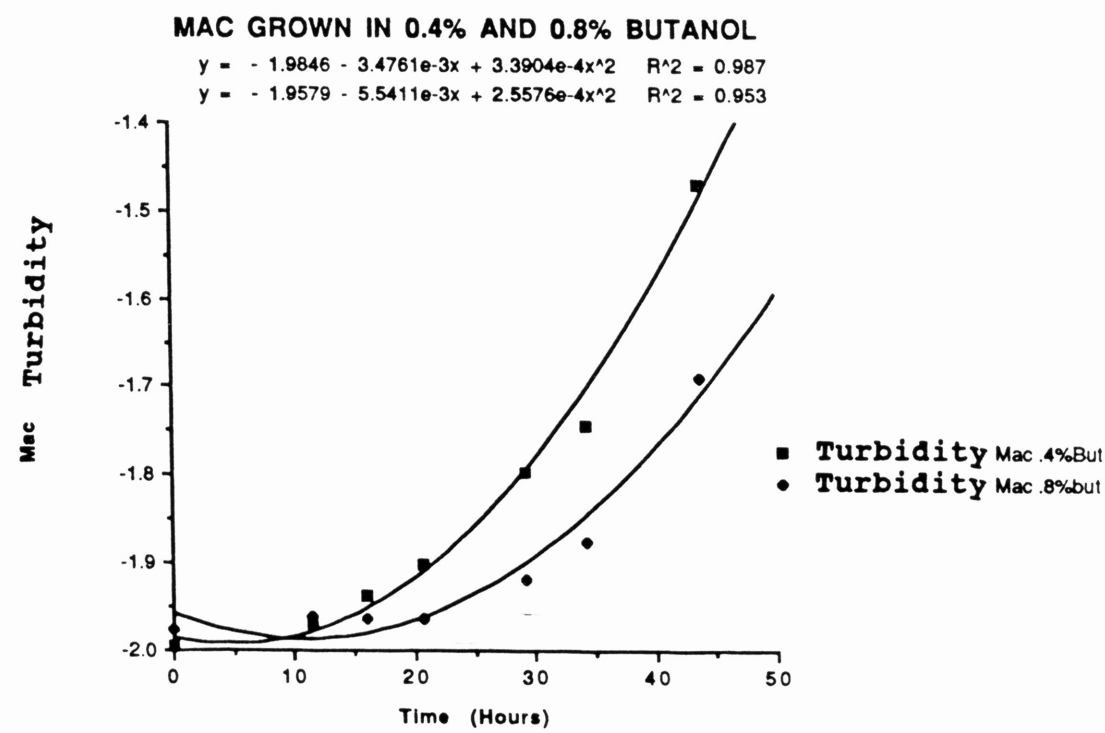


FIGURE 13

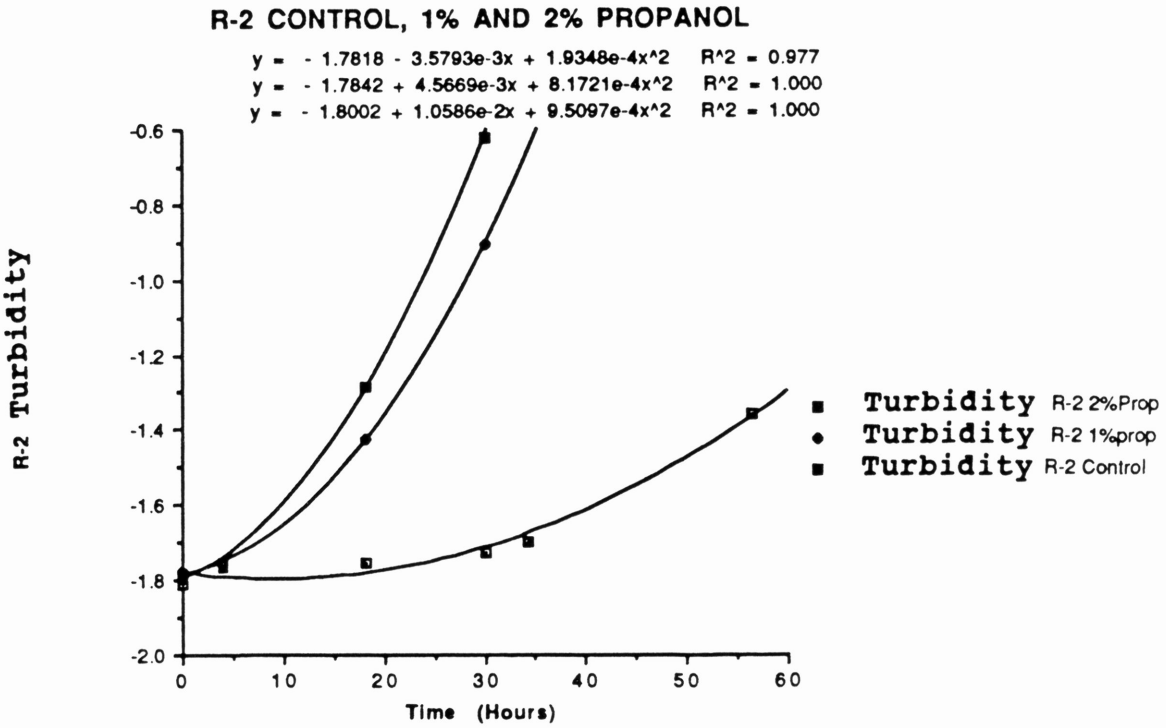


FIGURE 14

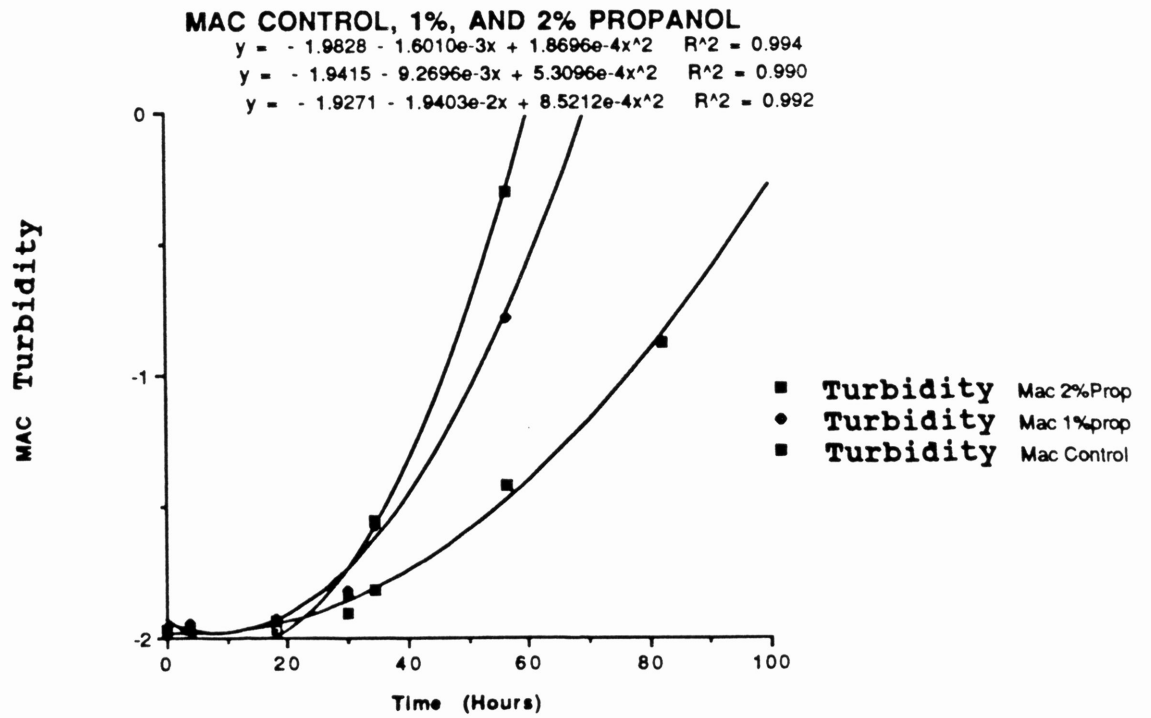


FIGURE 15

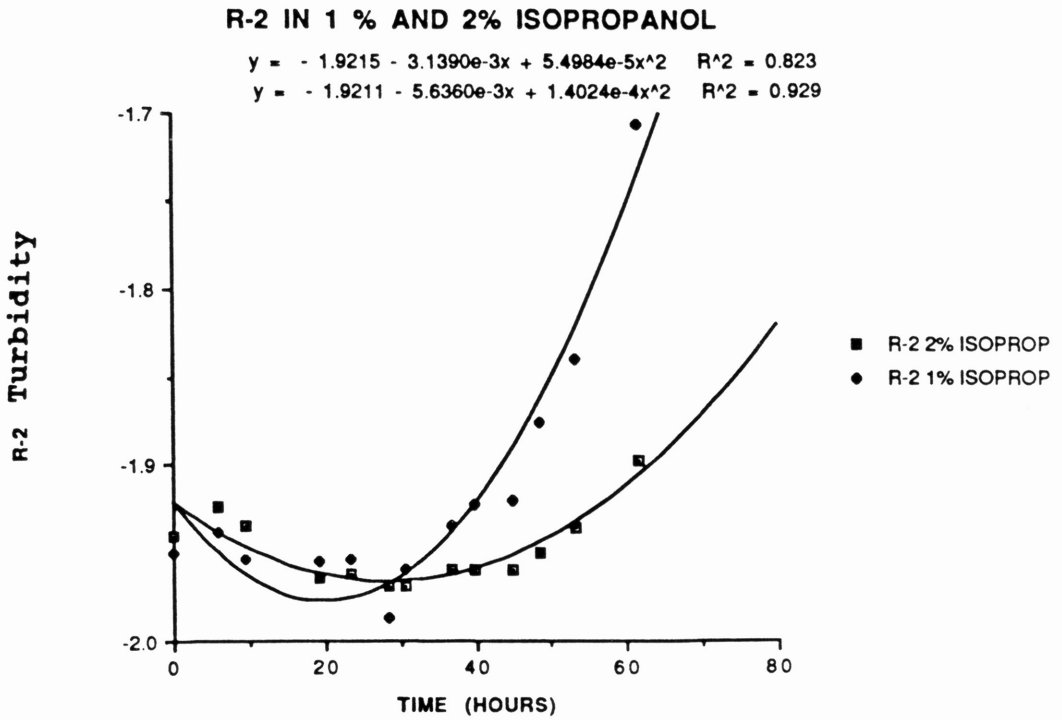
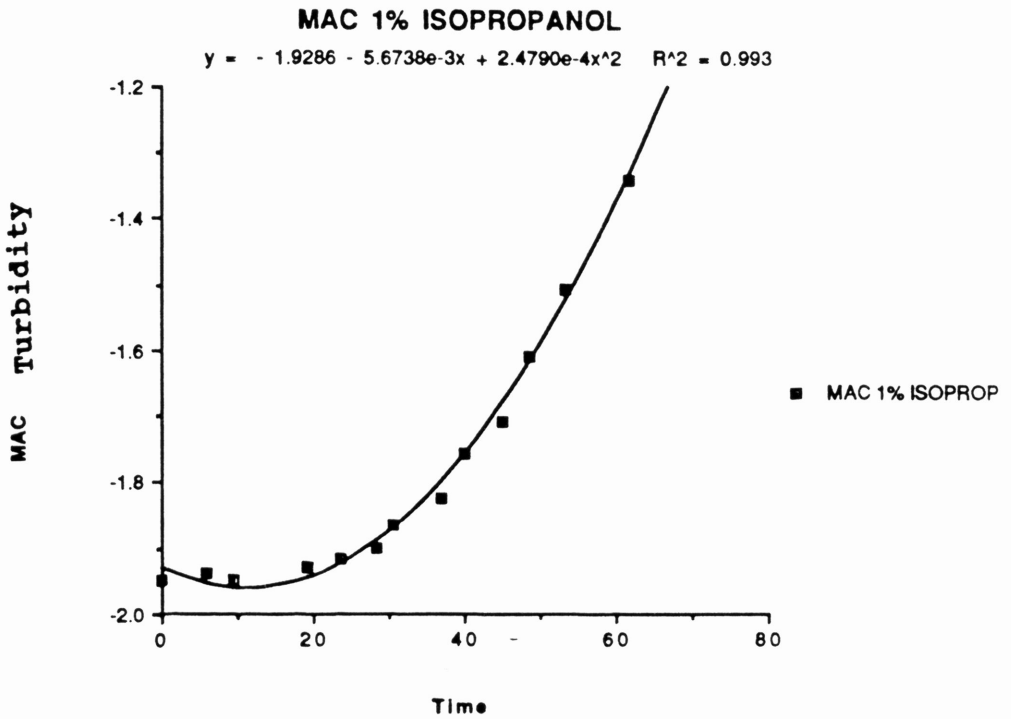


FIGURE 16



Many different trials were performed before the parameters on the gas chromatograph were satisfactory. Previous researchers had analyzed lipid fractions by chromatography under isothermal conditions at 175C (6). The methylated fatty acids in this experiment could not be separated using isothermal runs; consequently, a temperature program was developed. The temperature program began at 45 degrees and went to 199 degrees with a 20degrees/min rate as described in Materials and Methods. This situation allowed the best separation of the different components.

Standards results

The standards were obtained from Sigma Chemical Company, and they contained the following components:

Standard mix 189-2 palmitic acid methyl ester, stearic acid methyl ester, oleic acid methyl ester (unsaturated), linolenic acid methyl ester, and Arachidic acid methyl ester.

Standard mix 189-3 caprylic acid methyl ester, capric acid methyl ester, lauric acid methyl ester, myristic acid methyl ester, and palmitic acid methyl ester.

Standard mix 189-4 plalmitoleic acid methyl ester (unsaturated), oleic acid methyl ester (unsaturated), 11-eicosenoic acid methyl ester (unsaturated), and erucic acid methy ester (unsaturated).

All the above fatty acids were saturated fatty acids unless otherwise noted. For a description of the fatty acid methyl esters see Table 2.

TABLE 2

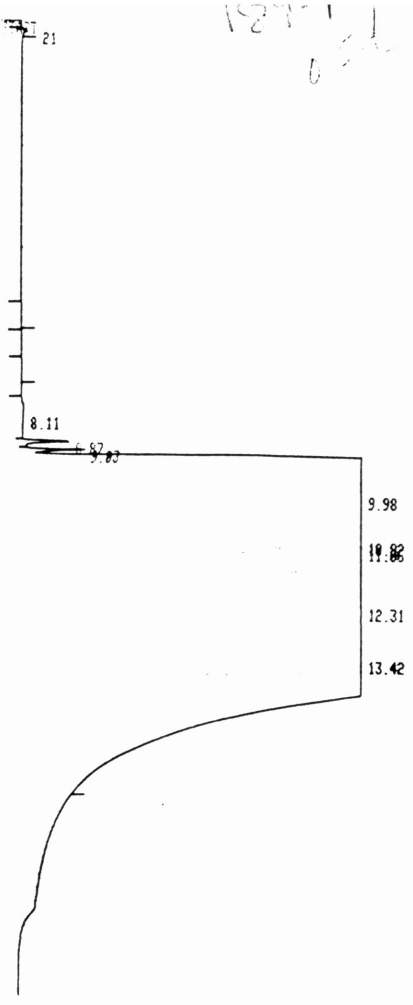
Fatty Acid Name	Carbon chain length	Degree of Unsat.
palmitic	16	0
stearic	18	0
oleic	18	1
linolenic	18	3
arachadonic	20	0
caprylic	8	0
capric	10	0
lauric	12	0
myristic	14	0
palmitoleic	16	1
11-eicosenoic	20	1
erucic	22	1

Sample chromatograms of the standards can be seen in Figures 17-19.

Component identification

The fatty acids obtained from membranes in strain R-2 were identified by comparison with standards. Things that were considered when determining similar peaks and retention times were: in different concentrations of samples, the same component will have varied retention times, it was assumed that the shorter chained fatty acids in the standards would elute before the longer chained fatty acids, and sometimes peaks would split (give two retention time readings very close together) when actually they were the same component.

It proved difficult to obtain consistent results from strain Mac. It appeared that fatty acid methyl esters from Mac were changing upon exposure to air following extraction. Filamentous bacteria are known to contain highly unsaturated fatty acids. Excessive exposure to air causes oxidation of fatty acids; thus, lipid compositional changes occur (11). For long term storage of lipids containing unsaturated fatty acids an antioxidant such as butylated hydroxytoluene(BHT) should be used (11). To remove the added BHT would have required Thin Layer Chromatography and the development of a new methylation procedure;



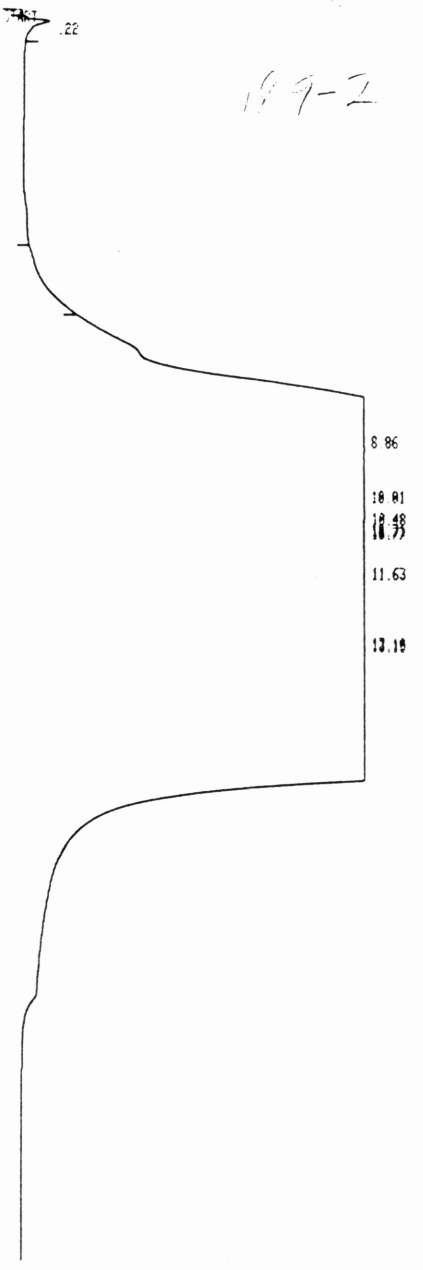
STOP

RUN # 196

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.21	26814	PB	0.061	0.008
8.11	52709	PP	0.268	0.016
8.87	208998	PV	0.054	0.065
9.03	282350	VV	6.053	0.088
9.98	8.5079E+07	VV	1.097	26.464
10.92	3.3850E+07	VV	0.416	10.280
11.86	6.0167E+07	VV	0.753	18.715
12.31	7.7635E+07	VV	1.200	24.148
13.42	6.4994E+07	VB	1.372	20.216

FIGURE 17



STOP

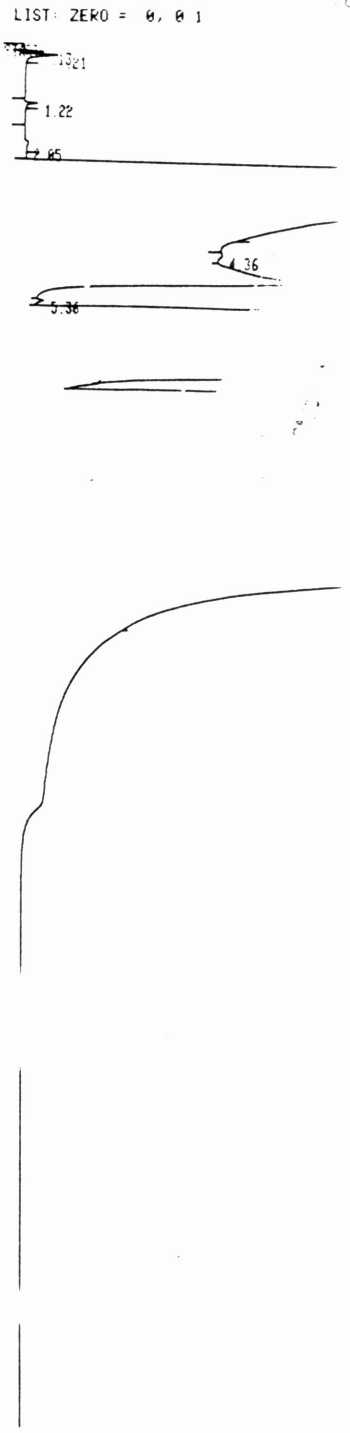
RUN # 203

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.22	405110	BB	0.170	0.062
8.86	0	PP	6.000	0.000
10.01	5.9433E+07	PV	0.417	9.148
10.48	1.1496E+08	VV	0.401	17.694
10.71	1.5519E+07	VV	0.131	2.389
10.77	1.1595E+07	VV	6.098	1.785
11.63	1.7284E+08	VV	0.720	26.603
13.10	1.5300E+08	VV	1.043	23.558
13.15	1.2195E+08	VB	0.837	18.770

TOTAL AREA= 6.4969E+08
MUL FACTOR= 1.0000E+00

FIGURE 18



STOP

RUN # 188

AREA%

RT	AREA	TYPE	AR/HT
0.13	50874	BV	0.029
0.21	188100	VB	0.062
1.22	44297	PB	0.039
2.05	15060	PB	0.062
3.12	7.6862E+07	PB	0.884
4.36	397890	BV	0.219
4.95	5559700	VP	0.382
5.36	41061	PP	0.059
5.67	1.2346E+08	SPH	0.323
7.80	2.2327E+08	SHB	1.902
9.40	8.6321E+07	TRV	1.010
10.71	1.0070E+08	TxB	0.601

TOTAL AREA= 6.1691E+08
MUL FACTOR= 1.0000E+00

FIGURE 19

consequently, it was decided that in this experiment R-2 would be the only strain analyzed for lipid changes.

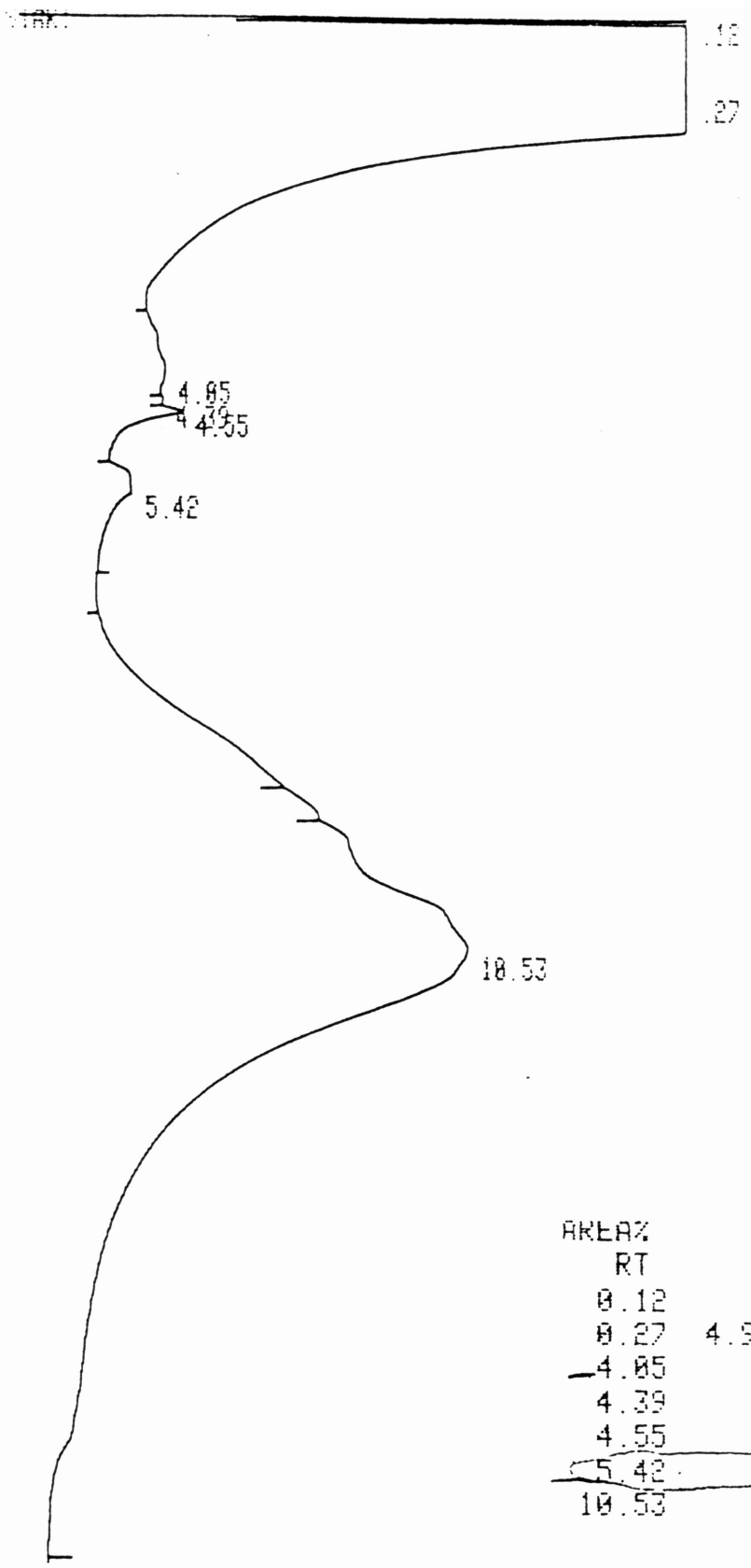
Seen in Figure 20 is the resulting chromatogram of cells grown in alcohol-free media. Particular peaks of interest include the ones at 5.42 and 10.57. In comparison to the standards, these peaks appear to be capric and palmitic acid respectively. The other peaks could not be positively identified; however, we suspect they all were caprylic acid that was split into several components by the integrator.

Analysis of fatty acids derived from cells grown in 2% methanol can be seen in Figure 21. Of particular interest were the peaks at 5.24 and 10.26. When comparing the area counts under the curve for the capric peak, one finds an increase of 77,430 area counts in the 2% methanol added chromatogram. The data from the palmitic acid peak was not printed due to a malfunction of the integrator; however, when placing one graph on top of the other, one can definitely see an increase in the 2% methanol added chromatogram. Both capric and palmitic acid are saturated fatty acids, and both increased upon the addition of 2% methanol.

Several precautions taken to prevent questions about other factors causing an effect like this should be reiterated. First both of these samples were grown at the same temperature and light intensity. Both samples began the extraction process with exactly equal amounts of cells (they were weighed). No cells were lost in the extraction process and both samples were treated the same in the extraction and methylation processes. The samples were each concentrated to exactly 10 ml using a volumetric flask. The same amount of sample was injected into the chromatograph (3 ul). Therefore, the changes observed had to be the result of a physiochemical change.

Even using a temperature program and immediately analyzing the lipids on the gas chromatograph, the final data were not conclusive. The gas chromatograph was

FIGURE 20

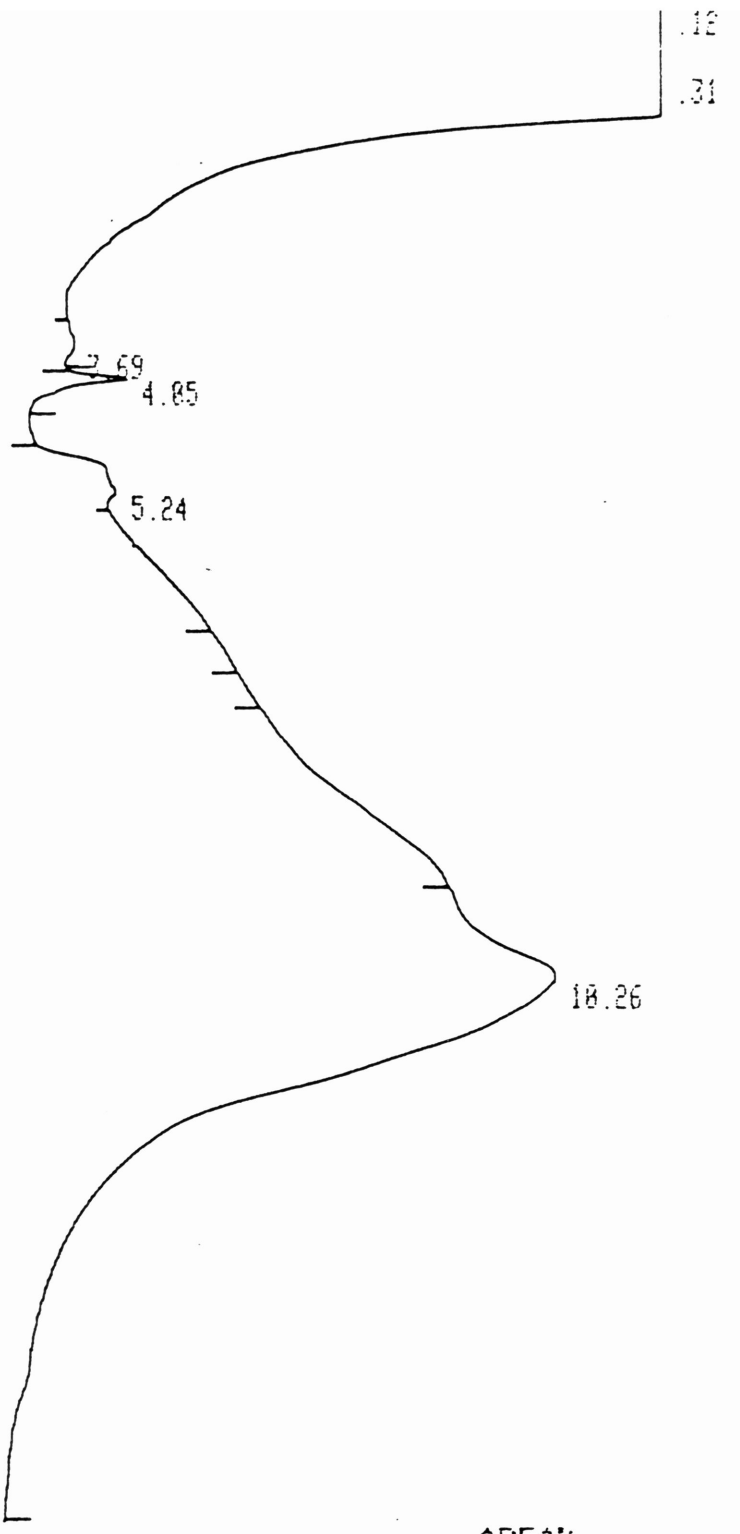


AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.12	1046500	SBH	0.027	0.208
0.27	4.9277E+08	↑SHH	0.947	98.134
4.05	1088500	TBV	0.625	0.217
4.39	275000	TVV	0.142	0.055
4.55	559080	TVP	0.187	0.111
5.42	563620	TPP	0.449	0.112
10.53	5838000	BB	0.616	1.163

TOTAL AREA= 5.0214E+08
MUL FACTOR= 1.0000E+00

FIGURE 21



AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.12	2.4295E+07	↑SBH	0.047	5.504
0.31	4.1602E+08	↑SHB	0.800	94.247
3.69	135270	TBB	0.321	0.031
4.05	323920	BB	0.105	0.073
5.24	641850	PB	0.621	0.145
10.26	0	BB	0.000	0.000

TOTAL AREA= 4.4142E+08
MUL FACTOR= 1.0000E+00

quite old and the air circulation door on the column oven was not working properly; therefore, the door had to be closed manually. This introduced some error, particularly into the retention times reported by the integrator. As seen in Figures 22 and 23 , the retention times differed by 0.3 minutes, and it was only through comparison that conclusions concerning component identification were reached.

Another concern seen in the data was that there was extreme baseline drift, particularly in the palmitic acid peak (10.26/10.53). There was a possibility that there were additional components which were never resolved around the palmitic acid peak. This could have been caused by several factors. If there were unresolved peaks, increasing the column length could increase the peak resolution. The column used was only a 3 foot column and the recommended length for an experiment like this one was 6 feet. Co-chromatography was an additional way that resolution could be improved. Since several extraction methods were explored, this was probably not a factor in the peak resolution problem. Thin Layer Chromatography could have been employed, particularly since in most previous experiments this was a step in their component separation analysis.

Despite the above concerns, one can see from the data that the increase in saturated to unsaturated fatty acid ratios occurred to some extent. More experimentation using additional equipment will greatly enhance the credibility of these results. The most obvious change seen in these data was the definite compositional changes observed from the control chromatogram and the 2% methanol chromatogram. Possibly the baseline shift seen prior to the capric acid peak was due to the addition of alcohol.

DISCUSSION

The results obtained from this experiment verified the hypothesis that as the amount of alcohol in the growth media increased so did the ratio of saturated to unsaturated fatty acids. These observations agreed with those of Sullivan et al when working with *E. coli* (14), but they differed from those reported by Ingram (6). Ingram found that when cells were grown in the presence of alcohols of chain lengths one to four, there was a decrease in palmitic acid and an increase in vaccenic acid (unsaturated fatty acid). However, when he exposed the *E.coli* to alcohols of chain length five through ten, the opposite occurred. According to Sullivan et al, Ingram used an unextracted media where fatty acid assimilation from the media was a possibility. Sullivan et al found consistently that the addition of alcohols at any chain length caused an increase in the saturated to unsaturated fatty acid ratio. The results found in this experiment using cyanobacteria appear to be similar to those found by Sullivan in *E.coli* (15).

Several models are currently being tested to determine the mechanism producing membrane compositional changes in response to environmental conditions. One model developed by Sinensky to describe how temperature affects the fatty acid composition deals with membrane bound acyltransferases (12). He demonstrated in vitro that these transferases have different temperature optima and at different temperatures, different ratios of fatty acids are produced. This model says that the enzymes do not detect and respond to the increased fluidity at increased temperature; rather that different acyltransferases have temperature dependent specificity which cause the incorporation of different fatty acids at different temperatures.

These enzymes might be good sites for normal alcohols to exert their affects. According to Sinensky, the alcohols might alter the activity of the transferases by

indirectly perturbing the packing of the lipids surrounding the enzymes (12). This perturbation activity would change the lipid bilayer so much that an increase in the saturated fatty acid composition would be necessary to compensate for this alteration (12). Currently there is no proof that enzymes can detect fluidity or that fluidity can directly influence enzyme activity. Previous researchers have failed to propose a mechanism which actually accounts for the cellular response to increased membrane fluidity in the presence of normal chain alcohols.

One model suggested by C.O. Patterson (personal conversation) to account for cellular responses to increased membrane fluidity in the presence of normal chain alcohols involves the allosteric action of the membrane bound acyltransferases. If there is a allosteric site on the acyltransferase for unsaturated fatty acids, as long as an unsaturated fatty acid is bound to the site, the acyltransferase continues incorporating the unsaturated fatty acid into the hydrocarbon chain. However, when the fluidity of the membrane is increased, the site is altered and the unsaturated fatty acid can no longer be incorporated into the hydrocarbon chain by the acyltransferase. This model suggests that there is a conformational change in the acyltransferase due to the increased fluidity of the membrane. A repressor would work in the opposite way. When a repressor site has an unsaturated fatty acid bound to it, the acyltransferase stops incorporating unsaturated fatty acids. This allows another acyltransferase to incorporate saturated fatty acids, thus compensating for the increased membrane fluidity.

Another possible model which would be extremely difficult to demonstrate in vitro, yet is a very good possibility, deals with the position of the active site of an acyltransferase which incorporates saturated fatty acids. (C.O. Patterson personal conversation). This mobility model could behave in two ways. First consider an active site for the incorporation of saturated fatty acids. Suppose that the active site is embedded in the membrane. When the membrane is exposed to an

environmental condition that increases membrane fluidity (such as increased temperature or increased solvent) the protein can rotate; consequently, the active site is exposed. When the site becomes exposed, saturated fatty acids are incorporated into the phospholipids. This increased incorporation of saturated fatty acids causes a decrease in membrane fluidity and thus a more rigid membrane. Once the active site of the enzyme is no longer exposed, the incorporation of saturated fatty acids ceases or slows and the cell functions normally.

The second way the active site mobility model could behave deals with an imbedded active site where membrane fluidity regulates access to the acitve site. When the fluidity of the membrane increases, there is increased saturated fatty acid penetration to the active site. This causes the acyltransferase to incorporate the saturated fatty acids into the hydrocarbon chain.

The proposed active site mobility model and conformational change model would account for the increase in membrane saturated fatty acids observed in the presence of normal chained alcohols. However, it should be noted that these models are only possible explanations since no data were collected to test either model.

The purpose of this experiment was to extend knowledge on membrane fatty acid alterations in the presence of normal alcohols to an organism not previously studied. This was of particular interest since no photosynthetic prokaryote had previously been examined. This organism should be ideal to study membrane adaptations since it has not only a plasma membrane, but also thylakoid membranes. In theory, the more membrane material available for study, the more the environmental changes can be observed.

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