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Adaptation of Membrane Lipids to Alcohols[1]

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The effects of alcohols of different chain lengths on the fatty acid composition of *Escherichia coli* K-12 have been examined. My results indicate that these cells radically change their fatty acid composition when grown in the presence of alcohols. These changes represent an adaptive membrane alteration compensating for the direct physicochemical interaction of alcohols with the membrane. Similar adaptive responses of membrane lipids are proposed as a possible biochemical basis for tolerance to alcohol and related drugs.

A fundamental property of all living organisms is their ability to adapt to their environment. In this respect, the organization of the cell membrane is of primary importance. Plant, animal, and bacterial cells have long been known to adjust their lipid compositions in response to environmental conditions, especially temperature (6, 20). At a molecular level within the cell membrane, a change in temperature is equivalent to a change in the degree of freedom or mobility of fatty acids and dissolved proteins (15). The primary enzymatic mechanism for the compensation of fatty acid composition in response to environmental temperature has been elucidated in *Escherichia coli* (26). Two enzymes are involved primarily, the sn-glycerophosphate transacylase and the monoacyl glycerophosphate transacylase. These two enzymes synthesize phosphatidic acid, the diglyceride precursor of phospholipids. Although the fatty acid composition of *E. coli* is modified by the abundance of respective fatty acid pools, this does not appear to be the major control point for the control of fatty acid composition (3).

Alcohols, local anesthetics, and other lipophylic agents interact directly with the lipid bilayer (24). Presumably, it is this interaction that is of primary importance in the anesthetic effect (21). Again at a molecular level, we are dealing with a change in the degree of freedom or fluidity of the membrane. This change is induced by the direct insertion of lipophylic agents into cellular membranes. Benzyl alcohol (10, 21), as well as ethanol and other alcohols (8, 11), has been shown to directly affect membrane fluidity. These changes in fluidity are analogous in many ways to those induced by changes in growth temperature. This study was undertaken to test the hypothesis that changes in the degree of membrane fluidity are the primary signal eliciting an adaptive response in fatty acid composition.

[1] Florida Agricultural Experiment Station Publication no. 7012.

MATERIALS AND METHODS

Strain and growth medium. *E. coli* K-12 strain CSH1, which requires thiamine and tyrosine, was obtained from the Cold Springs Harbor Laboratory (Cold Spring Harbor, N.Y.). Cultures were grown in Luria broth (17) containing glucose (2 g/liter), thiamine (10 mg/liter), and tyrosine (20 mg/liter).

Effects of alcohols on fatty acid composition. Overnight cultures grown at 37 C with forced aeration were diluted 1:100 into fresh medium containing appropriate concentrations of alcohols. These 200-ml batch cultures were incubated in 2-liter flasks at 30 C in a New Brunswick gyratory shaker for 3 h prior to harvesting. For time-course studies involving alcohol-induced changes in lipids, a 1:200 dilution of the overnight culture was used as inoculum, and the alcohol was added after 1.5 h of incubation. (T_0) For time-course studies involving the "removal" of alcohol, 1:100 dilution cultures were grown for 3 h in the presence of 4% (vol/vol) ethanol. At time zero, this culture was diluted 1:10 into fresh prewarmed medium lacking ethanol. The resulting cultures still contained 0.4% ethanol. For experiments involving fatty acid additions, these were added at 100 g/liter initially, prior to inoculation of the cultures. Growth was measured turbidometrically at 550 nm using a Spectronic 70 spectrophotometer (Bausch and Lomb, Inc., Rochester, N.Y.).

Lipid extraction. Cells were harvested by centrifugation, inactivated by the addition of 5% trichloroacetic acid, and extracted into chloroform-methanol, as described by Kanfer and Kennedy (14). The washed lipid extract was used for subsequent fatty acid analysis or for thin-layer chromatography.

Fatty acid analysis. The washed chloroform ex-

tract was evaporated to near dryness under dry N_2 and transesterified using 2% H_2SO_4 in methanol, as described by Silbert et al. (25). The methyl esters were extracted into pentane and concentrated under N_2 prior to analysis. One-microliter injections of the methyl esters were examined on a Packard series 7600 gas chromatograph (Packard Instrument Co., Downers Grove, Ill.) equipped with a flame ionization detector using a glass column (2 mm by 180 cm) packed with 10% diethyleneglycol succinate-coated Gas Chrom Q (100 to 120 mesh). The analyses were run isothermally at 175 C with a carrier gas (N_2) flow of 30 ml/min. Fatty acids were identified using authentic standards. Relative abundance of the various fatty acids is reported as a percentage of total peak areas. Peak areas were calculated by a Minigrator digital integrator (Spectra-Physics, Mountain View, Calif.).

Thin-layer chromatography. Washed chloroform extracts were separated into neutral lipid (includes free fatty acids), glycolipid, and polar lipid (phospholipids plus sulfolipids) fractions using solvent 1 described by Freeman and West (5). Samples were chromatographed on unactivated Silica Gel G-impregnated glass-fiber paper (Gelman ITLC-SG, Fisher Scientific Co., Pittsburgh, Pa.). With this system, pigments, neutral lipids, and free fatty acids run near the solvent front; polar lipids remain at the origin; and glycolipids run with an R_f of approximately 0.6. For phospholipid comparisons, lipid extracts were chromatographed on Silica Gel G plates (Merck & Co., Inc., Rahway, N.J.) containing $CaSO_4$ binder using the solvents described by Ames (1). Phospholipids were visualized using a molybdate spray reagent (4).

Chemicals. Tryptone and yeast extract were products of Difco Laboratories, Detroit, Mich. Tripalmitin, palmitic acid, phosphatidyl ethanolamine, phosphatidyl glycerol, cardiolipin, phosphatidyl serine, *n*-alcohols C5 through C10, and acetaldehyde were obtained from Sigma Chemical Co. (St. Louis, Mo.). Methanol, propanol, butanol, isopropanol, and isoamyl alcohol were products of the Fisher Chemical Co. Absolute ethanol was a product of the U.S. Industrial Chemical Co., New York, N.Y. Diethyleneglycol succinate, fatty acid methyl esters, and Gas Chrom Q were obtained from Applied Sciences (State College, Pa.).

RESULTS

Effects of alcohols on fatty acid composition. The presence of alcohols in rich growth media results in a considerable change in fatty acid composition (Table 1). The degree of this variation in fatty acid composition is strongly dependent upon alcohol concentration. For ethanol, this relationship is shown from 1 to 4%. The alcohol concentration required to cause a given change in fatty acid composition is inversely related to chain length, as is toxicity. The molar effectiveness of alcohols in eliciting an adaptive change in fatty acids is directly related to their lipid solubility, indicative of a direct membrane interaction (Fig. 1). The highest concentrations of alcohols reported in Table 1 represent concentrations of alcohols causing 30 to 50% growth inhibition after 3 h under the described conditions of incubation. In general, the maximum amount of fatty acid change that can be elicited declines with increasing chain length. Alcohols of chain lengths one thru four cause an increase in 18:1 fatty acid (vaccenic acid) with a decrease in 16:0 fatty acid (palmitic acid). Alcohols of chain lengths five thru ten cause just the opposite change, an increase in palmitic acid with a decrease in vaccenic acid. Similarly, isopropanol (C3) caused an increase in vaccenic acid (Table 2), and isopentanol (C5) caused an increase in palmitic acid (Table 2).

Some variations in the fatty acid composition of control cultures were observed from experiment to experiment. These are due primarily to the technical problems of keeping a hot-air incubator at 30 C. This growth temperature was initially chosen because of the approximately equal abundance to each of the three major *E. coli* fatty acids. Within a single experiment, the relative fatty acid concentrations of six control cultures agreed within 1% for each fatty acid.

The phospholipid compositions of 4% ethanol-grown cells and control cells were examined using thin-layer chromatography. Phospholipids were visualized using the phosphate spray reagent (4). No obvious differences in relative abundance of phosphatidyl ethanolamine, phosphatidyl glycerol, and cardiolipin were observed. No further attempt was made to quantitate these results.

Time course of induced fatty acid changes. The addition of ethanol (4%) to growing cultures of *E. coli* caused an immediate lag in growth for approximately 30 min, followed by a resumption of growth at a slightly reduced rate (Fig. 2A). Upon the addition of ethanol, changes in fatty acid composition also began immediately and continued through the growth lag into the growth period (Fig. 2B). Thus, the changes in fatty acid composition during the growth lag appears to represent membrane adaptations, which restore membrane function and allow growth.

Changes in the rate of growth upon 10-fold dilution of ethanol (4%)-grown cultures into fresh media lacking ethanol were also observed (Fig. 3A). Following dilution, cultures grew for approximately 30 min at an accelerated rate which slowed to that of normal cells. During this period, the relative abundance of the three major fatty acids rapidly readjusted to near normal levels (Fig. 3B). This growth inhibition

TABLE 1. *Effects of n-alcohols on the fatty acid composition of E. coli*[a]

Alcohol	Concn (% vol/vol)	Fatty acid						
		12:0	14:0	16:0	16:1	Δ17	18:0	18:1
Methanol	0	0.8	1.0	26.0	37.2	0.7		34.3
Methanol	1	0.6	1.0	24.2	37.4	0.9		35.9
Methanol	2	0.9	1.3	22.6	37.3	0.6		37.3
Methanol	4	0.9	1.0	17.2	34.8	1.3		44.8
Methanol	6	0.9	0.9	11.2	31.1	2.3		53.6
Ethanol	0	0.9	1.9	24.0	36.6	0.6		36.0
Ethanol	1	0.6	1.3	20.4	36.7	0.6		40.4
Ethanol	2	0.8	1.2	17.7	36.4	1.0		42.9
Ethanol	3	0.9	0.9	15.1	34.5	1.4		47.2
Ethanol	4	0.7	0.9	11.5	33.9	2.1		50.9
Propanol	0	1.3	1.7	27.3	34.0	1.0		34.7
Propanol	0.2	0.6	1.4	26.0	34.5	0.5		37.0
Propanol	0.4	1.0	0.4	25.5	34.0	0.8		38.3
Propanol	0.6	1.2	tr	23.1	33.7	1.3		40.7
Propanol	1.0	1.0	tr	21.5	33.0	1.5		43.0
Propanol	1.5	1.5	0.3	19.0	32.5	1.7		45.0
Butanol	0	1.4	2.6	25.3	35.8	1.1		33.9
Butanol	0.2	2.2	2.2	25.0	35.1	1.0		34.5
Butanol	0.4	1.1	1.6	24.0	34.2	1.5		37.6
Butanol	0.6	1.6	1.4	22.7	32.7	1.8		39.8
Butanol	0.8	1.6	1.9	22.0	31.6	2.0		40.9
Butanol	1.0	1.0	1.7	21.6	29.2	2.3		44.2
Pentanol	0	1.2	1.0	26.7	34.3	1.1		35.7
Pentanol	0.025	0.8	1.3	27.0	34.6	1.4		34.9
Pentanol	0.050	1.1	1.6	28.2	35.1	1.0		33.0
Pentanol	0.100	1.8	2.6	30.6	31.8	2.0	1.5	29.7
Pentanol	0.150	1.4	2.0	32.2	30.8	3.0	2.1	28.5
Pentanol	0.200	2.1	3.2	33.0	25.4	3.8	7.4	25.1
Hexanol	0	2.0	2.4	27.5	34.8			33.3
Hexanol	0.013	2.5	3.3	28.8	34.0			31.4
Hexanol	0.025	2.1	3.4	31.9	33.5			29.1
Hexanol	0.038	2.0	3.9	33.0	33.0			28.1
Hexanol	0.050	2.1	3.0	35.0	32.5			27.4
Hexanol	0.100	3.1	3.0	36.7	30.4			26.8
Heptanol	0	1.5	1.9	26.1	35.8	0.6		34.1
Heptanol	0.013	1.3	1.6	30.3	33.4	0.6		32.8
Heptanol	0.025	1.6	2.2	32.3	33.0	0.6	1.1	29.2
Heptanol	0.038	1.7	2.1	33.3	30.8	1.0	1.2	29.9
Octanol	0	3.5	3.1	24.3	34.0	0.5		34.6
Octanol	0.0025	3.0	3.0	24.5	35.4			34.1
Octanol	0.0050	3.0	3.1	25.0	35.8			33.1
Octanol	0.0100	3.0	3.1	26.7	34.7			32.5
Octanol	0.0250	3.0	3.1	30.3	33.5			30.1
Nonanol	0	2.1		26.6	35.0	1.0		35.3
Nonanol	0.0025	2.7		28.0	37.1	0.5		31.7
Nonanol	0.0050	2.5		28.4	37.3	1.3		30.5
Nonanol	0.0100	2.9		33.6	35.4	0.8		27.3
Nonanol	0.0150	2.9		35.7	34.5	1.5	0.9	24.5
Decanol	0	2.0	2.4	26.6	35.2	0.6		33.2
Decanol	0.0050	—[b]	4.0	25.6	34.7	1.3		34.4
Decanol	0.0100	—	2.8	25.5	34.5	1.0		36.2
Decanol	0.0150	—	2.7	24.0	34.4	1.1		37.8

[a] Expressed as percentage of total fatty acids.
[b] Decanol interferes with 12:0 determination.

following ethanol addition and growth stimulation upon its removal suggests that the basis of the fatty acid changes is not related to ethanol as a major source of carbon and energy but rather to a membrane adaptation.

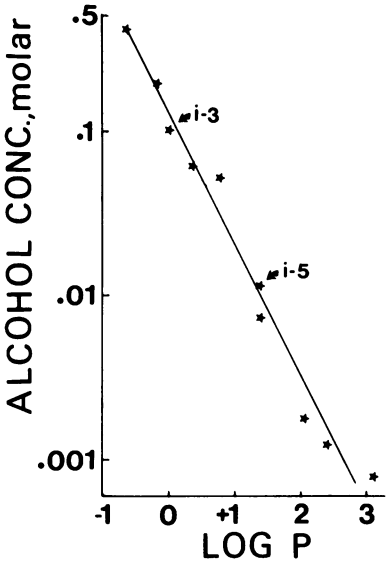

FIG. 1. *Correlation of octanol/water partition coefficients with the concentration of alcohol required to elicit a 10% change in the average abundance of 16:0 fatty acid and 18:1 fatty acid, as compared to untreated controls. Points include aliphatic alcohols of chain lengths one thru eight, isopropyl alcohol (i-3), and isopentyl alcohol (i-5).*

Alcohol synergism and antagonism. The two short-chain alcohols, ethanol and methanol, were found to have roughly additive effects on membrane-fatty acid composition (Table 3). The combination of ethanol and methanol produces a change roughly equivalent to the sum of their independent effects. Long-chain alcohols (chain lengths five to ten) induce changes in fatty acid composition the opposite of those induced by ethanol. Thus, it was of little surprise to find that long-chain alcohols, such as heptanol, antagonized the effect of ethanol (Table 4). A similar antagonism of ethanol by long-chain alcohols was reported previously (12). Ethanol-stimulated cell division in a blue-green bacterial mutant was partially blocked by

TABLE 2. *Effects of isopropyl and isopentyl alcohols on the fatty acid composition of E. coli*[a]

Alcohol	Concn (% vol/vol)	Fatty acid					
		12:0	14:0	16:0	16:1	Δ17	18:1
Isopropanol	0	3.1	3.8	25.5	32.1	0.8	34.7
Isopropanol	0.25	3.4	3.9	25.0	31.8	1.2	34.7
Isopropanol	0.50	3.5	4.0	23.2	32.0	1.0	36.3
Isopropanol	1.0	2.4	2.8	21.5	31.6	3.1	38.6
Isopropanol	1.5	2.5	2.8	19.4	28.7	1.1	45.5
Isopentanol	0	2.2	2.2	27.6	35.1	1.0	31.9
Isopentanol	0.05	2.7	2.5	29.0	35.2	1.2	29.4
Isopentanol	0.10	2.7	2.8	30.1	34.9	0.5	29.0
Isopentanol	0.15	2.8	2.8	34.5	31.2		28.6

[a] Expressed as percentage of total fatty acids.

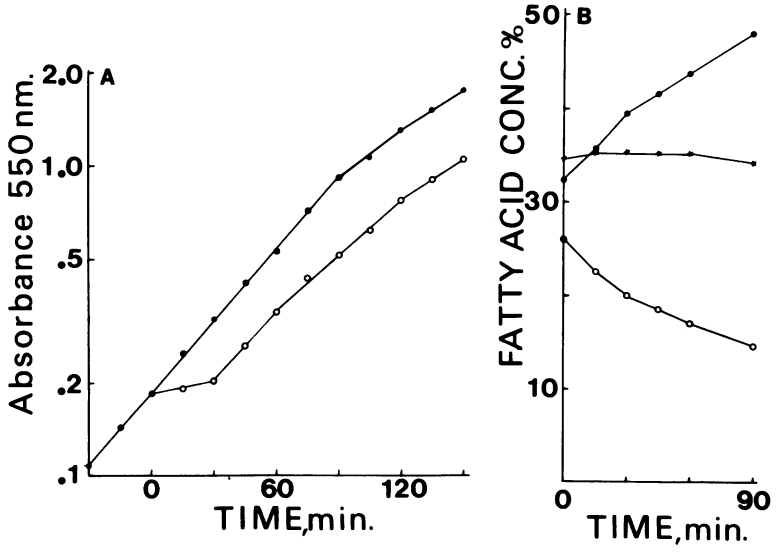

FIG. 2. *Effects of the addition of ethanol (4% vol/vol) on the growth and fatty acid composition of E. coli. (A) Ethanol was added after 1.5-h preincubation of cultures under growth conditions (30 C). This point is time zero. (A) Growth: ●, control; ○, ethanol. (B) Fatty acid composition: ●, 18:1; ○, 16:0; *, 16:1.*

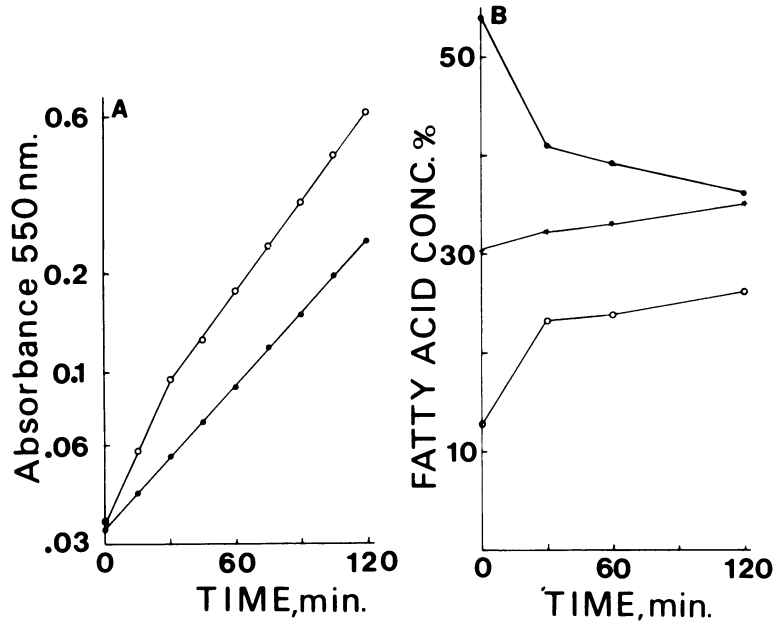

FIG. 3. *Effects of ethanol (4% vol/vol) removal on growth and fatty acid composition. (A) Cultures were grown for 3 h in the presence of 4% (vol/vol) ethanol. These were diluted 1:10 into medium lacking ethanol (O) and into medium containing ethanol (●) and incubated under growth conditions (30 C). (B) Cultures grown for 3 h in the presence of ethanol were diluted 1:10 with fresh medium and incubated under growth conditions. Samples were removed at intervals for fatty acid analyses. Cultures were diluted at zero times. Symbols: ●, 18:1; O,16:0; *, 16:1.*

TABLE 3. *Combined effects of ethanol and methanol*[a]

Fatty acid	No addition	Ethanol (2%)	Methanol (3%)	Ethanol (2%) + methanol (3%)
12:0	2.4	2.3	2.4	2.1
14:0	2.9	2.1	2.3	2.1
16:0	24.9	20.1	21.6	16.4
16:1	36.3	37.2	37.1	36.5
Δ17	0.8	0.7	0.7	0.8
18:1	32.7	37.6	35.9	42.1

[a] Expressed as percentage of total fatty acids.

TABLE 4. *Antagonism of ethanol effects on fatty acid composition by heptanol*[a]

Fatty acid	No addition	Ethanol (2%)	Heptanol (0.025%)	Ethanol (1%) + heptanol (0.025%)	Ethanol (2%) + heptanol (0.025%)
12:0	2.5	3.6	3.0	3.5	3.2
14:0	3.2	3.0	3.5	2.9	2.8
16:0	24.5	15.8	28.3	24.0	21.4
16:1	34.8	33.1	31.0	33.1	33.2
Δ17	1.0	0.7	1.3	1.3	0.9
18:1	34.0	43.8	32.8	35.5	38.6

[a] Expressed as percentage of total fatty acids.

low concentrations of octanol. The results with *E. coli* suggest that the basis of this antagonism is a direct membrane interaction, as suggested earlier (12).

Effects of acetaldehyde and acetic acid on fatty acid composition. To further explore the possibility that the ethanol effect on membrane composition may be related to an increased availability of a readily oxidizable substrate or of two-carbon compounds for fatty acid elongation (increased 18-carbon fatty acid abundance), we examined the effects of two likely products of ethanol (Table 5). Both acetaldehyde and acetate caused changes very different from those induced by ethanol. Acetaldehyde caused changes different from those induced by any alcohol. Increasing concentrations of acetate caused a shift from 16:1 to 18:1. Such a shift could be a result of increased availability of two-carbon fragments for fatty acid elongation.

Effects of ethanol and palmitic acid upon phospholipid fatty acids. Lipids were extracted from cells grown in the presence and absence of ethanol, with and without palmitic acid, and chromatographed on ITLC. The phos-

TABLE 5. *Effects of acetaldehyde and acetic acid on the fatty acid composition of E. coli*[a]

Additive	Concn (% vol/vol)	Fatty acid							
		12:0	14:0	16:0	16:1	Δ17	18:0	18:1	Δ19
Acetaldehyde	0	4.7	3.1	24.7	33.7	1.3		32.5	
Acetaldehyde	0.025	4.1	4.0	29.1	30.7	1.4		30.7	
Acetaldehyde	0.050	7.0	6.0	28.6	23.7	3.6	1.6	24.9	4,6
Acetaldehyde	0.100	8.3	6.6	29.0	18.2	5.9	2.9	20.9	8.2
Acetate	0	2.8	3.3	26.0	37.4			30.5	
Acetate	0.8	3.8	2.8	26.2	31.0	0.4		35.8	
Acetate	1.6	4.4	2.6	26.4	28.6	1.2		36.8	
Acetate	2.4	6.5	3.7	25.3	27.2	2.6	1.2	32.4	1.1

[a] Expressed as percentage of total fatty acids.

pholipid portion of the chromatogram was excised and used for fatty acid analysis. The changes in the fatty acid composition of phospholipids from ethanol-grown cells (Table 6) are very similar to those found in the total lipids, demonstrating that the observed membrane adaptation was not due to unusual effects on neutral lipids or free fatty acids. Lauric acid was consistently absent from all phospholipid samples, indicating that it probably occurs only as a free fatty acid and/or neutral lipid. In the presence of ethanol, myristic acid also was absent. Growth in the presence of palmitic acid, both in the presence and absence of ethanol, resulted in an increase in esterified 16:0. Cells grown in the presence of both 16:0 and ethanol have a near normal fatty acid composition. This can be interpreted in a variety of ways. The addition of palmitic acid could replace a deficit in 16:0 biosynthesis caused by ethanol. Alternatively, the fatty acid composition of cells grown in the presence of both palmitic acid and ethanol could reflect an adaptive response to the combined presence of two mutually antagonistic membrane-active compounds. In the latter case, a further assumption, that the presence of ethanol facilitates an enhanced response to exogenously supplied 16:0, is necessary. This would not seem unreasonable, however. The intercalation of ethanol into a phospholipid bilayer may well facilitate an increased insertion of exogenously added 16:0, which could then become available for transesterification in addition to eliciting an increased adaptive response.

TABLE 6. *Effect of exogenous palmitic acid on ethanol-induced changes in fatty acid composition of E. coli phospholipids*[a]

Fatty acid	No addition	Palmitic acid (100 mg/liter)	Ethanol (3%)	Palmitic acid (100 mg/liter) + ethanol (3%)
12:0	0	0	0	0
14:0	2.8	2.6	0	0
16:0	27.0	29.0	20.0	29.1
16:1	36.6	35.3	34.9	34.6
Δ17	0.9	0.6	0.5	0.3
18:1	32.7	32.5	44.6	36.0

[a] Expressed as percentage of total fatty acids.

DISCUSSION

Our results indicate that *E. coli* K-12 alters its fatty acid composition when grown in the presence of lipophylic agents, such as alcohols in the environment. This change in fatty acid composition may be an adaptive response to the physicochemical interactions of these alcohols with membrane lipids. Upon addition of ethanol to growing cultures, growth is inhibited and does not resume until the cells have begun to change their fatty acid composition. This strongly suggests that the fatty acid alterations are essential for the function of many membrane-associated enzymes. Indeed, the addition of ethanol has long been known to inhibit membrane enzymes such as adenosine triphosphatase (2, 7–9). Other evidence for a direct physicochemical effect of alcohols as a basis for adaptation includes the direct relationship of alcohol potency to their lipid solubility (octanol/water partition coefficients), the strict concentration dependence of the adaptive response in many cases at levels that would be expected to saturate metabolism, the additive nature of the response among short-chain alcohols, and the direct antagonism by long-chain alcohols of the adaptive responses to short-chain alcohols.

The rationale of the adaptive response in

long-chain alcohols (chain lengths five to ten) can be readily explained based upon current concepts of membrane structure and function (24). These alcohols are still six or more carbons shorter than the dominant fatty acid in the membrane. Insertion of these into a lipid bilayer with any orientation would cause an increase in the fluidity of the membrane, as demonstrated by both electron-spin resonance (8, 21) and differential scanning calorimetry (11). The observed increase in saturated fatty acids within the membrane causes the opposite effect, a decrease in membrane fluidity. Thus, the increase in saturated fatty acids will act to compensate for the presence of the long-chain alcohols. The most likely candidates for the mediation of this response are the membrane-bound transacylase enzymes (26), although fatty acid synthesis may also be effected. Independent of transcriptional or translational controls, the transacylase enzymes are able to synthesize phosphatidic acid with the fatty acid composition appropriate to their incubation temperature. Thus, these membrane-bound enzymes are able to shift their substrate specificities in response to their lipid environment. Undoubtedly, this must involve conformational changes determined by some aspect of membrane fluidity within these proteins. The insertion of long-chain alcohols causes an increase in fluidity of the membrane. Disregarding specific protein interactions, the lipid environment of the transacylase enzyme is modified in a fashion very analogous to that caused by an increase in temperature. Indeed, *E. coli* compensates for an increase in growth temperature by increasing the relative abundance of saturated fatty acids, primarily palmitic acid (18).

The changes in membrane fatty acids in response to ethanol and alcohols of chain length of less than four carbons are not as intuitively obvious. The cellular response is the incorporation of more unsaturated fatty acids into phospholipids, which would tend to increase membrane fluidity. These same short-chain alcohols have been shown to increase the mobility of electron-spin probes (8, 10), suggesting that they too cause an increase in membrane fluidity. However, another measure of membrane fluidity (which does not involve the addition of lipophylic probe molecule), differential scanning calorimetry, did not reveal significant changes in membrane melting temperature over a wide range of ethanol concentrations (11). These apparent discrepancies in physical effects of ethanol and other short-chain alcohols can be rationalized as having a number of trivial explanations, such as differences in the systems under investigation. Alternatively, the apparent ethanol-induced increase in fluidity (electron-spin resonance) could merely reflect changes caused by the spin-label probe with increased solubilization of this probe in membranes containing short-chain alcohols. In the cells, however, the net effect of ethanol and other short-chain alcohols is to cause changes in the membrane in some ways equivalent to a decrease in membrane fluidity. That is, the growth data indicate that the increased abundance of 18:1 may be required to restore overall membrane function. Cellular adaptive changes probably represent the best indication of the true effect of ethanol on the cell membrane. A number of possible interactions that might cause changes equivalent to a decrease in fluidity can readily be visualized for these short-chain alcohols. These short-chain alcohols could act to replace water molecules associated with polar head groups of lipids, thus decreasing their repulsion, or to replace water molecules associated with membrane enzymes, leading to conformational changes as the dominant effects. Alternatively, these short-chain alcohols could be inserted not only into the exterior of the bilayer but also into the hydrophobic interior. Here, within the hydrophobic interior, these small alcohols could fill many of the gaps caused by unsaturated fatty acids and fatty acid-protein interactions and produce an apparent decrease in fluidity (Fig. 4). Insertion of small alcohols into gaps within the hydrophobic interior would restrict the movement of fatty acid chains. This latter hypothesis is supported in part by the effects of a more highly charged product of ethanol. Neither acetaldehyde nor acetic acid induces an increase in unsaturated fatty acid composition as do short-chain alcohols. Being more highly charged, acetaldehyde and acetic acid would be more restricted to the exterior portion of the bilayer. Alcohols of five carbons and larger are visualized as being too large to fill gaps in the hydrophobic membrane interior without themselves creating additional spaces thereby increasing membrane fluidity. Thus, only small uncharged molecules would be capable of decreasing fluidity in this manner.

Cellular adaptation of membrane lipids to the presence of alcohols could provide a biochemical basis for the acquired tolerance of man and experimental animals to alcohol. The molar effectiveness of alcohol-induced adaptation is directly related to their octanol/water partition coefficient. A nearly identical relationship has been demonstrated both for the ability of these

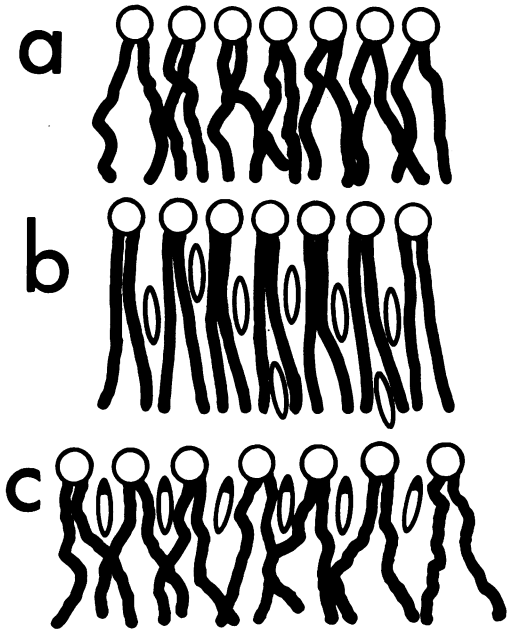

FIG. 4. *Model for the insertion of short-chain alcohols (C1 to C4) and acetaldehyde into a lipid bilayer based upon their effects on cellular fatty acid composition. Ethanol and other short-chain alcohols are proposed as inserting into the hydrophobic interior, thus restricting the movement of fatty acyl chains. Longer chain alcohols (not shown) are envisioned as inserting into the hydrophobic interior also but, due to their size, introducing additional gaps in phospholipid packing and increasing the freedom of fatty acyl chains. More polar compounds, such as acetaldehyde, are proposed as being restricted to the more polar region of the membrane, increasing the spacing between phospholipids, and thus increasing the freedom of fatty acyl chains. (a) Bilayer with no addition; (b) bilayer with ethanol inserted; (c) bilayer with acetaldehyde inserted.*

alcohols to disorder bilayers (8) and for their anesthetic potency (21). It is generally concluded that alcohol tolerance reflects some as yet unknown adaptation of the central nervous system (13, 16, 23, 27). Membrane fluidity is particularly important to nerve cell function, as indicated by their high concentrations of unsaturated fatty acid (22). Here, too, ethanol may cause adaptive changes in fatty acids or complex lipids. Such changes would be reversible upon withdrawal of ethanol. Other drugs such as barbituates and Δ^9-tetrahydrocanabinol (2, 19) are cross-tolerant with ethanol and could potentially function in the same manner.

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LITERATURE CITED

1. Ames, G. 1968. Lipids of *Salmonella typhimurium* and *Escherichia coli:* structure and metabolism. J. Bacteriol. **95**:833-843.
2. Caldwell, J., and P. S. Sever. 1974. The biochemical pharmacology of abused drugs. II. Alcohol and barbiturates. Clin. Pharmacol. Ther. **16**:737-749.
3. Cronan, J. E., Jr. 1974. Regulation of fatty acid composition of the membrane phospholipids of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **71**:3758-3762.
4. Dittmer, J. C., and R. L. Lester. 1964. A simple, specific spray reagent for the detection of phospholipids on thin-layer chromatograms. J. Lipid Res. **5**:126-127.
5. Freeman, C. P., and D. West. 1966. Complete separation of lipid classes on a single thin-layer plate. J. Lipid Res. **7**:324-327.
6. Fulco, A. J. 1974. Metabolic alterations of fatty acids. Annu. Rev. Biochem. **43**:215-241.
7. Grisham, C. M., and R. E. Barnett. 1972. The interrelationship of membrane and protein structure in the functioning of the ($Na^+ + K^+$)-activated ATPase. Biochim. Biophys. Acta **266**:613-624.
8. Grisham, C. M., and R. E. Barnett. 1973. The effects of long-chain alcohols on membrane lipids and the ($Na^+ + K^+$)-ATPase. Biochim. Biophys. Acta **311**:417-422.
9. Grisham, C. M., and R. E. Barnett. 1973. The role of lipid-phase transitions in the regulation of the (sodium + potassium) adenosine triphosphatase. Biochemistry **12**:2635-2637.
10. Hubbel, W. L., J. C. Metcalfe, S. M. Metcalfe, and H. M. McConnell. 1970. The interaction of small molecules with spin-labeled erythrocyte membranes. Biochim. Biophys. Acta **219**:415-427.
11. Hui, F. K., and P. G. Barton. 1973. Mesomorphic behavior of some phospholipids with aliphatic alcohols and other nonionic substances. Biochim. Biophys. Acta **296**:510-517.
12. Ingram, L. O., and W. D. Fisher. 1973. Stimulation of cell division by membrane-active agents. Biochem. Biophys. Res. Commun. **50**:200-210.
13. Kalant, H. 1971. Adsorption, diffusion, distribution and elimination of ethanol: effects on biological membranes, p. 1-62. *In* B. Kissin and H. Begleiter (ed.), The biology of alcoholism, vol. 1. Plenum Press, New York.
14. Kanfer, J., and E. P. Kennedy. 1963. Metabolism and function of bacterial lipids. J. Biol. Chem. **238**:2919-2922.
15. Ladbrooke, B. D., and D. Chapman. 1969. Thermal analysis of lipids, proteins and biological membranes. A review and summary of some recent studies. Chem. Phys. Lipids **3**:304-367.
16. Lieber, C. S. 1973. Hepatic and metabolic effects of alcohol (1966 to 1973). Gastroenterology **65**:821-846.
17. Luria, S. E., and M. Delbruck. 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28**:491-511.
18. Marr, A. G., and J. L. Ingraham. 1962. Effect of temperature on the composition of fatty acids in *Escherichia coli*. J. Bacteriol. **84**:1260-1267.
19. Newman, L. M., M. P. Lutz, and E. F. Domino. 1974. Δ^9-Tetrahydrocanabinol and some CNS depressants: evidence for cross-tolerance in the rat. Arch. Int. Pharmacodyn. Ther. **207**:254-259.
20. Olson, G. J., and L. O. Ingram. 1975. Effects of temperature and nutritional changes on the fatty acids of *Agmenellum quadruplicatum*. J. Bacteriol. **124**:373-379.
21. Paterson, S. J., K. W. Butler, P. Huang, J. Labelle, I. C. P. Smith, and H. Schneider. 1972. The effects of alcohols on lipid bilayers: a spin label study. Biochim.

Biophys. Acta **206**:597-602.
22. Rao, P. S., and K. S. Rao. 1973. Fatty acid composition of phospholipids in different regions of developing human fetal brain. Lipids **8**:374-377.
23. Rubin, E., and C. S. Lieber. 1971. Alcoholism, alcohol, and drugs. Science **172**:1097-1102.
24. Sheetz, M. P., and S. J. Singer. 1974. Biological membranes as bilayer couples. A molecular mechanism of drug-erythrocyte interactions. Proc. Natl. Acad. Sci. U.S.A. **71**:4457-4461.
25. Silbert, D. F., R. C. Ladenson, and J. L. Honegger. 1973. The unsaturated fatty acid requirement in *Escherichia coli*. Biochim. Biophys. Acta **311**:349-361.
26. Sinensky, M. 1971. Temperature control of phospholipid biosynthesis in *Escherichia coli*. J. Bacteriol. **106**:449-455.
27. Woods, J. H., and G. D. Winger. 1974. Alcoholism and animals. Prev. Med. **3**:49-60.