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Effects of Ethanol on the *Escherichia coli* Plasma Membrane†

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The effects of ethanol on the fluidity of *Escherichia coli* plasma membranes were examined by using a variety of fluorescent probes: 1,6-diphenyl-1,3,5-hexatriene, perylene, and a set of *n*-(9-anthroyloxy) fatty acids. The anthroyloxy fatty acid probes were used to examine the fluidity gradient across the width of the plasma membrane and artificial membranes prepared from lipid extracts of plasma membranes. Ethanol caused a small decrease in the polarization of probes primarily located near the membrane surface. In comparison, hexanol decreased the polarization of probes located more deeply in the membrane. Temperature had a large effect on probes located at all depths. The effects of ethanol on *E. coli* membranes from cells grown with or without ethanol were also examined. Plasma membranes isolated from cells grown in the presence of ethanol were more rigid than those from control cells. In contrast to plasma membranes, artificial membranes prepared from lipid extracts of ethanol-grown cells were more fluid than those from control cells. These differences are explained by analyses of membrane composition. Membranes from cells grown in the presence of ethanol are more rigid than those from control cells due to a decrease in the lipid-to-protein ratio. This change more than compensates for the fluidizing effect of ethanol and the ethanol-induced increase in membrane $C_{18:1}$ fatty acid which occurs during growth. Our results suggest that the regulation of the lipid-to-protein ratio of the plasma membrane may be an important adaptive response of *E. coli* to growth in the presence of ethanol.

Microorganisms are able to survive by adapting to adverse changes in their environment. The accumulation of ethanol as a product of fermentations represents an adverse environmental change to which organisms may have evolved an adaptive response (29). Ethanol changes the physical characteristics of the environment of a cell (21) and may alter the way in which a cell interacts with its environment. The primary site through which the cell maintains contact with its environment is the plasma membrane. Thus, the plasma membrane may be a primary site for the expression of an adaptive response to ethanol.

Escherichia coli undergoes dose-dependent and reversible changes in membrane lipid composition during growth in the presence of ethanol (7, 26, 27). The proportion of acidic phospholipids, such as phosphatidylglycerol and cardiolipin, increases, whereas the proprotion of phosphatidylethanolamine decreases (27). Also, the proportion of *cis*-vaccenic acid esterified in phospholipids increases, whereas the proportion of palmitic acid decreases (26). There is a decrease in the total amount of phospholipid in the cell (7). This reduction in the amount of total phospholipid and in the proportion of phosphatidylethanolamine results primarily from the preferential inhibition of phosphatidylethanolamine synthesis (27). The increase in *cis*-vaccenic acid and the decrease in palmitic acid seen in cells grown in the presence of ethanol results from the preferential inhibition of saturated fatty acid biosynthesis (8, 9, 28).

Ethanol has been shown to induce similar changes in the membranes of other microorganisms. *Bacillus cereus* changes both its fatty acid and phospholipid composition when grown in the presence of ethanol or propanol (37). The membranes of *Bacillus subtilis* exhibit an alcohol-induced decrease in total phospholipid and an increase in the mean fatty acyl chain length of the membrane lipids analogous to that in *E. coli* (50). *Mycobacterium smegmatis* undergoes an ethanol-induced decrease in total phospholipid and also exhibits an ethanol-induced decrease in phosphatidylethanolamine (56). In the protozoan *Tetrahymena pyriformis*, an ethanol-induced increase in mean fatty acyl chain length and in unsaturation has been observed (44). In the yeast *Saccharomyces cerevisiae*, an ethanol-induced change in membrane composition has been reported which is almost identical to that found in *E. coli* with an increase in $C_{18:1}$ at the expense of $C_{16:0}$ (6).

The ethanol-induced changes in the membrane composition of *E. coli* appear to be beneficial for growth and survival in the presence of ethanol (30). Alcohol-resistant mutants contain elevated levels of acidic phospholipids (15). The increase in *cis*-vaccenic acid also appears to be beneficial for cell survival. Mutants of *E. coli* that are unable to synthesize this fatty acid are hypersensitive to killing by ethanol (30). These same mutants can be rendered less sensitive to ethanol-induced killing by supplementation with *cis*-vaccenic acid.

The ethanol-induced increase in vaccenic acid in the membranes of *E. coli* is paradoxical. More unsaturated lipids are produced at the expense of saturated lipids during growth in the presence of ethanol. Ethanol is known to fluidize cell membranes (62), and such a change would be expected to further fluidize the cell membrane (16). Based on the hypothesis of "homeoviscous adaptation" (1, 53), the production of membrane lipids enriched in saturated fatty acids would be expected as compensation for the fluidizing action of ethanol. The present study was undertaken to resolve this apparent paradox and to investigate the differential effects of ethanol across the width of the plasma membrane.

MATERIALS AND METHODS

Organism and growth conditions. *E. coli* K-12 strain TB4 was used in this study (8). This strain is wild type for fatty

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acid synthesis with a defect in fatty acid degradation (*fadE*). Strain TB4 was grown at 37°C in Luria broth (41) containing 0.3 M NaCl without added carbohydrate (29). Ethanol-adapted cells were grown in the same medium containing 4% (vol/vol) ethanol. Growth was monitored by measuring optical density at 550 nm.

Lipid-starved cells were prepared by growth in the presence of cerulenin, a potent inhibitor of fatty acid biosynthesis (61). Cultures were grown to an optical density of 0.4. At this time cerulenin was added (35 mg/liter), and cells were harvested after one additional mass doubling.

Isolation of plasma membranes. Cells were harvested in log phase (3×10^8 cells per ml) by centrifugation and washed in 0.03 M Tris buffer (pH 7.5) containing 0.58 M sucrose. This and all subsequent steps were carried out at 0 to 4°C. Plasma membranes were isolated using a modification of the procedure described previously by Ito et al. (31). Whole cells were resuspended in the 0.58 M sucrose solution and lysed by ultrasonic disruption (Sonicator Cell Disruptor model W-220F, Plainview, N.Y.) in the presence of lysozyme (Sigma Chemical Co., St. Louis, Mo.). The crude membrane fraction was layered onto a sucrose step gradient and spun at $100,000 \times g$ for 2 h. The uppermost light band was harvested as plasma membrane. This plasma membrane fraction was 8- to 22-fold enriched in NADH-oxidase activity as compared with the outer membrane fraction (lower band). NADH oxidase assays were performed as described previously by Osborn et al. (46). The plasma membrane fraction was washed with 10 mM Tris (pH 7.5) and stored frozen at −20°C.

Preparation of liposomes. Frozen cell membranes (about 3 mg of protein) were lyophilized for 2 h and then extracted overnight in a mixture of chloroform and methanol as described previously by Kanfer and Kennedy (36). Particulate matter remaining in the lipid extract was filtered, using a small glass wool column with a 0.5-ml dead volume. The chloroform-methanol extract was dried down in a glass tube under a stream of nitrogen and placed under high vacuum for 2 h to remove the final traces of solvent. The lipids were suspended in 0.2 ml of 10 mM Tris (pH 7.5) and agitated for 5 min in a low-power sonic cleaning bath at 40°C to form liposomes. Tris (10 mM, pH 7.5) was added to the solution to bring the final volume up to 5 ml. The liposome solution was allowed to equilibrate at room temperature overnight before use. These preparations will be referred to as membrane-derived liposomes.

Fluorescent probes. A set of *n*-(9-anthroyloxy) fatty acid probes ($n = 2, 3, 6, 9, 12$, or 16) consisting of a 9-anthroyloxy fluorophore in an ester linkage with different carbons along the fatty acyl chain of either stearic (*n*AS) or palmitic (*n*AP) acid was used in this study. Nuclear magnetic resonance (NMR) (48) and fluorescence quenching (57) investigations have shown that the anthroyloxy portion of these probes is localized at different depths within the membrane corresponding to the position of attachment of the fluorophore to the fatty acid chain. Other probes used were 1,6-diphenyl-1,3,5-hexatriene (DPH) and perylene. DPH and perylene were obtained from the Aldrich Chemical Company (Milwaukee, Wis.). Of the anthroyloxy probes, 3AS, 9AS, and 16AP were obtained from Molecular Probes, Inc. (Pano, Tex.). The 2AP, 6AS, and 12AS probes were synthesized as described previously by Thulborn and Sawyer (57).

Fluorescence depolarization. Fluorescence depolarization measurements were made essentially as described previously by Abruzzini et al. (1) using a modified procedure of Esko et al. (20). Membranes were resuspended in 10 mM Tris (pH 7.5) at a protein concentration of 0.1 mg/ml. The equilibrated liposome suspension was diluted in the same manner, using lipid extracted from an equivalent amount of membranes. Equal volumes of membranes or liposomes and a 2 μM microcrystalline dispersion of the appropriate fluorescent probe in 10 mM Tris (pH 7.5) were mixed. This mixture was equilibrated at the chosen assay temperature for 45 min to allow probe insertion. Fluorescence was measured with an SLM series 4000 polarization spectrofluorometer in the ratio mode with the excitation wavelength set by a monochromator (1). The emission wavelengths were set with Schott cutoff filters. The anthroyloxy probes were excited at 363 nm and the fluorescence emission above 418 nm was measured. DPH was excited at 360 nm and the emission was measured above 418 nm. Perylene was excited at 410 nm with the emission measured above 470 nm. Polarization was calculated as described previously by Chen and Bowman (12). Sample temperature was regulated with a Neslab circulating water bath. Ethanol was pipetted into the sample cuvette, mixed, and allowed to equilibrate for 5 min before measurement. No further changes were observed with longer incubation times. Because of the low solubility of hexanol in aqueous solution, the microcrystalline probe dispersion was made in buffer containing 0.2% hexanol for the hexanol experiments. Fluorescence polarization was used as a relative measure of lipid fluidity. Under the conditions of these experiments, fluorescence polarization is inversely related to fluidity (57), although other factors such as polarity and lifetime also affect the behavior of probe fluorescence (57, 58).

To examine the effects of a specific treatment (the addition of ethanol, hexanol, etc.) on probe fluorescence polarization, the fluidity gradient of an untreated bilayer was measured and then subtracted from that of the same bilayer exposed to the specific treatment. These differences were plotted on an expanded scale.

In dose-response experiments, ethanol concentrations of 0, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, and 4.0% were used. In experiments examining the effect of alcohols on lipid bilayer fluidity profiles, an ethanol concentration of 4% (vol/vol) and a hexanol concentration of 0.1% (vol/vol) were used. Growth in the presence of these alcohol concentrations resulted in significant changes in the lipid composition of strain TB4 (7, 26).

To examine the physical effects of membrane adaptation to growth in ethanol, we studied the polarizations of a variety of fluorescent probes in both plasma membranes and liposomes prepared from ethanol-adapted and normal *E. coli*. An array of probes was used to show that the effects observed were not due to a specific property of any individual probe molecule.

Analysis of membrane composition. Protein content of the plasma membrane fraction was determined by the method of Lowry et al. (40). Phospholipid samples were digested with perchloric acid as described previously by Dawson (17). Inorganic phosphate was then determined by the method of Bartlett (4).

RESULTS

Effects of protein, ethanol, hexanol, and a change in temperature on membrane fluidity. Figure 1a shows the fluidity gradient of *E. coli* plasma membranes and their derived liposomes. The anthroyloxy probes with their fluorophore localized closer to the surface of the bilayer exhibited a higher polarization and were immobilized to a greater extent than the anthroyloxy probes with their fluorophore localized

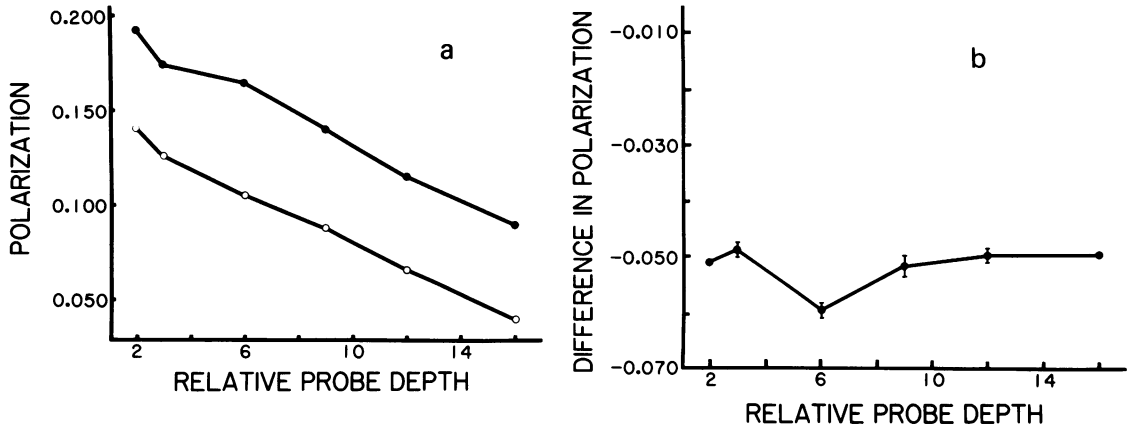

FIG. 1. Effect of depth on probe polarization in isolated membranes and in membrane-derived liposomes. (a) Polarization gradients for the *n*-(9-anthroyloxy) fatty acid probes assayed at 37°C. Relative probe depth refers to the carbon on the fatty acid to which the anthroyloxy fluorophore is attached. (b) Difference in polarization of the fatty acid probes in liposomes and plasma membranes assayed at 37°C. The error bars refer to the error of the polarization measurements carried through a propagation of errors treatment for a difference of two means. Symbols: ●, *E. coli* plasma membranes; ○, liposomes derived from *E. coli* plasma membranes.

more deeply within the lipid bilayer. By subtracting the fluidity gradient of plasma membranes from that of their derived liposomes, the effect of bulk protein on lipid fatty acyl chain mobility can be deduced. The removal of protein caused a large decrease in polarization (increase in fluidity) (Fig. 1b). This change occurred almost equally at all depths within the *E. coli* lipid bilayer.

Figure 2a shows the effects of a 7°C increase in temperature on fluorescence polarization as a function of probe position for both membranes and liposomes. This change in temperature affected all probes but caused the largest decrease in the polarization of the 6AS, 9AS, and 12AS probes. Although this effect was similar for both the plasma membranes and their derived liposomes, the polarization of plasma membrane-bound probes appeared to be more sensitive to the increase in temperature. The change observed in the polarization of the 6AS probe in liposomes was only 0.4 that of the probe in plasma membranes.

The ethanol-induced changes in polarization for both plasma membranes and their derived liposomes are shown in Fig. 2b. Ethanol decreased the fluorescence polarization of all probes. As compared with the effect of temperature, however, ethanol preferentially reduced the polarization of probes localized near the membrane surface. Similar effects were observed with whole membranes and liposomes. The magnitude of the ethanol effect on the fluorescence polarization of probes in plasma membranes was similar to that of probes in liposomes and was ca. 0.2 that of the temperature effect (7°C increase).

The effects of ethanol on the fluorescence polarization of the fatty acid probes in plasma membranes were further examined by determining dose-response curves for the 6AS and 16AP probes in *E. coli* plasma membranes. Increasing concentrations of ethanol progressively fluidized the membrane (Fig. 3). The slopes of these curves are a measure of the sensitivity of the fluorescence polarization of each probe to ethanol. The slope of the dose-response curve for 6AS was more negative than that of 16AP, indicating that the position of 6AS in the membrane is more sensitive to the presence of ethanol than that of 16AP.

The effects of hexanol on probe fluorescence polarization in plasma membrane-derived liposomes are shown in Fig. 2c. Hexanol decreased the fluorescence polarization of probes located more deeply within the bilayer than those affected by ethanol. The magnitude of the hexanol effect is about three times that of the ethanol effect and about twice that of the temperature effect (7°C increase) as seen in plasma membrane-derived liposomes for the 6AS probe.

Effects of ethanol on membranes from cells grown in the presence of ethanol. Figure 4 compares membranes and liposomes from cells grown in the presence and absence of ethanol. The plasma membranes from alcohol-grown cells appeared more rigid (higher polarization) than the plasma membranes from control cells with all probes tested. In contrast, liposomes prepared from the plasma membranes of alcohol-grown cells were more fluid than those from control cells, as measured by two of the anthroyloxy probes (6AS and 12AS).

To further investigate this phenomenon, we used the 2AP, 3AS, 6AS, 9AS, 12AS, and 16AP probes to examine the effect of growth in the presence of ethanol on the fluidity gradient of the plasma membranes and their derived liposomes. The polarizations of all of the probes in membrane-derived liposomes except 16AS (localized in the center of the lipid bilayer) were lower in preparations from alcohol-grown cells than in liposomes from control cells (Fig. 5). Figure 5 confirms the results of Fig. 4 by showing that ethanol-adapted plasma membranes were more rigid than control membranes (positive changes in probe polarization) due to growth in the presence of alcohol. The profiles of the curves for plasma membranes and membrane-derived liposomes from Fig. 5 are similar. Probes located at the surface of membranes or liposomes have more negative changes in polarization due to growth in the presence of ethanol than probes located closer to the center. The change in polarization due to growth in the presence of ethanol, however, increased more rapidly for liposomes than for plasma membranes.

Effects of growth in the presence of ethanol on plasma membrane composition. We have examined the phospholipid content of plasma membranes from cells grown in the presence or absence of ethanol (Table 1). The phospholipid/protein ratio of membranes from control cells was over twice that of membranes from cells grown in the presence of 4% ethanol, consistent with our earlier observations (27). Assuming an average phospholipid molecular weight of 750, membranes from control cells and ethanol-grown cells were ca. 28 and 14% phospholipid, respectively.

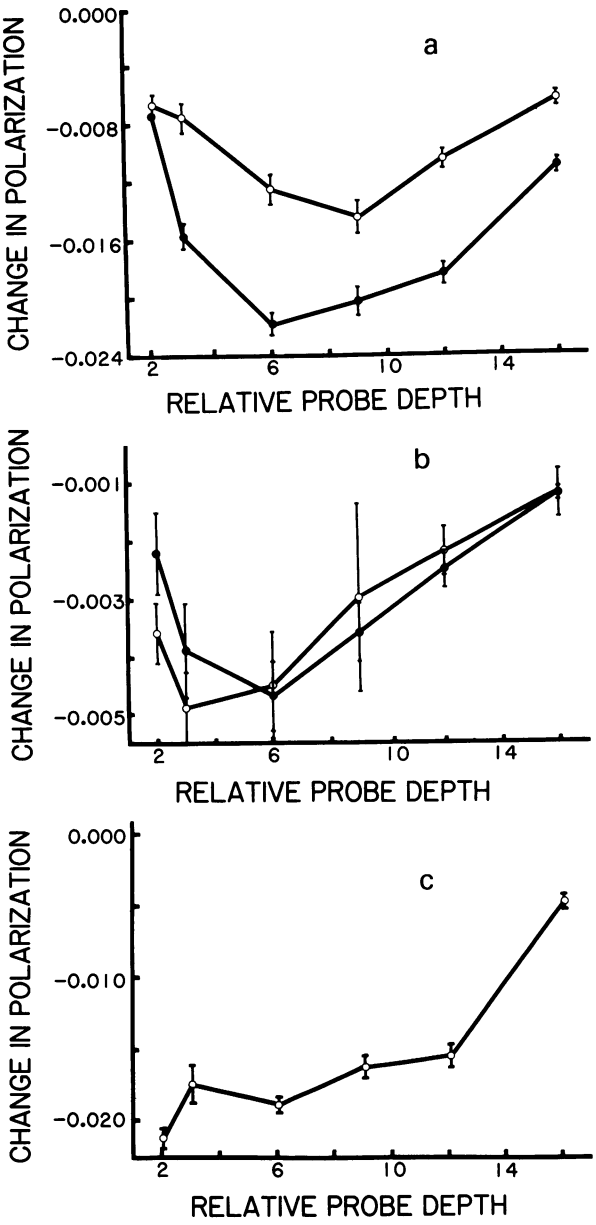

FIG. 2. Effects of alcohols and an increase in temperature on probe polarization. These changes in polarization of the anthroyloxy fatty acid probes in *E. coli* plasma membranes and plasma membrane-derived liposomes are due to a 7°C increase in temperature (a), the addition of 4% ethanol (b) or the addition of 0.1% hexanol (c). Ethanol and hexanol treatments were assayed at 37°C. The significance of difference of all points of (a), (b), and (c) from baseline polarization values is at least $P < 0.1$. Degree of significance was calculated by a one-tailed *t*-test. Symbols: ●, *E. coli* plasma membranes; ○, plasma membrane-derived liposomes.

Effect of lipid starvation on membrane fluidity. The relative fluidity of membranes from ethanol-grown cells was considerably lower than that of control membranes (Fig. 4). However, the opposite trend was observed with liposomes prepared from lipid extracts of these same membranes, as would be expected based upon fatty acid composition. These results suggested that bulk membrane protein is of major importance in determining the physical properties of the

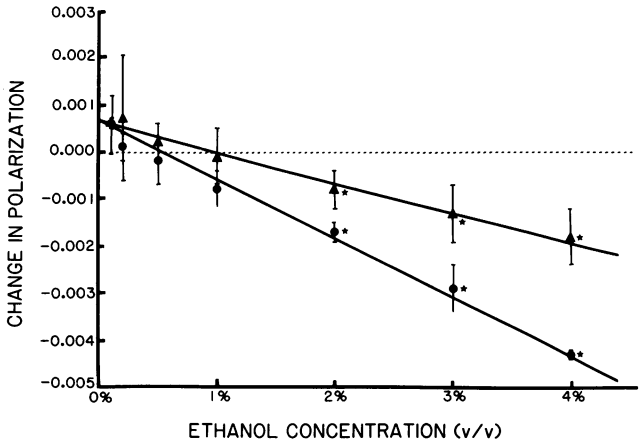

FIG. 3. Ethanol dose-response curves for 6AS and 16AP in *E. coli* plasma membranes assayed at 37°C. Points above the broken line at the 0.000 change in polarization indicate decreased fluidity, whereas those below the line indicate increased fluidity. The error bars indicate standard errors of the mean. A degree of significance of $P < 0.05$ between points at identical ethanol concentrations is indicated by ⋆. Degree of significance was calculated by a one-tailed *t*-test. Symbols: ●, 6AS; ▲, 16AP.

plasma membrane. Membranes from ethanol-grown cells contained roughly half the phospholipid of control membranes, suggesting that a general decrease in phospholipid/protein ratio may be responsible. To test this hypothesis, cells grown in the presence of cerulenin, an inhibitor of fatty acid synthesis (61), were used as a source of membranes with a decreased proportion of phospholipid (Table 1). Membranes from cerulenin-treated cells were indeed deficient in phospholipid and contained less than half the phospholipid of control cells. Like ethanol-grown cells, membranes from cerulenin-grown cells were considerably more rigid than those of control cells, consistent with the importance of bulk membrane protein in determining membrane fluidity.

DISCUSSION

Our results with *E. coli* and the anthroyloxy fatty acid series of fluorescent probes provide further evidence for the existence of a fluidity gradient across the membrane, confirming the results of studies by Tilley et al. (59) and Thulborn et al. (58). This fluidity gradient has also been measured by electron paramagnetic resonance (EPR) probes, ^{13}C NMR, and ^{2}H NMR (22, 39, 54). Although the fluidity gradients measured by all of these techniques are qualitatively similar, the gradients measured by fluorescent and EPR probes are more fluid closer to the bilayer surface than those measured by NMR methods. The intrinsic perturbing nature of the fluorescent and EPR probes (3, 51), as well as a difference in the frequencies of the types of motions being measured (42), are two possible explanations for this discrepancy.

The effect of a change in temperature on the fluidity at different depths within both *E. coli* plasma membranes and their derived liposomes was examined as a control to which the specific effects of ethanol could be compared. Both increasing temperature (2, 45, 54) and ethanol (34, 35, 62) are known to fluidize the fatty acyl chains of lipid bilayers. As anticipated, these treatments caused a decrease in probe fluorescence polarization. However, the decrease caused by ethanol (4%, vol/vol) was much smaller and was shifted

toward the surface of the lipid bilayer in comparison to the effects of an increase in temperature (7°C). Also, isolated membranes were more sensitive to temperature-induced changes than liposomes, whereas both membranes and liposomes were equally affected by ethanol. These results suggest that lipid-protein interactions may be more sensitive to temperature than lipid-lipid interactions. In contrast, lipid-protein interactions do not appear to be more sensitive to ethanol than lipid-lipid interactions.

The anthroyloxy probes most disturbed by ethanol (near the bilayer surface) may represent the site of the localization of ethanol. Alternatively, ethanol could be localized in the hydrophobic membrane interior, thus creating a gap where the lipid fatty acyl carbons have less-restricted motion. To resolve this problem of localization, we examined the effects of hexanol on the fluorescence polarization of the fatty acid probes. Hexanol, being a longer-chain alcohol than ethanol, should occupy more space within the lipid bilayer than ethanol. If the positions of the probes which are the most disturbed by ethanol represent a gap created by the absence of alcohol, then bilayers treated with hexanol should have a smaller gap than bilayers treated with ethanol. This was not observed. Alternatively, if the positions of the probes most affected by ethanol are the region of high ethanol concentration, then hexanol should disturb probes located more deeply within the bilayer. Figure 2c provides evidence for this latter hypothesis, suggesting that the position of the probes most affected by the alcohols represents the position of the greatest intramembrane concentration. These results are consistent with the findings of other studies on the localization of alcohols in lipid bilayers. Using proton NMR, Metcalfe (43) has shown that benzyl alcohol is localized near the surface of the lecithin bilayer, possibly by the hydrophilic alcohol hydroxyl group. Similarly, Presti et al. (49) has postulated that the hydroxyl function of cholesterol hydrogen bonds to the glycerol ester oxygen, localizing the sterol rings near the surface of the lecithin bilayer.

On the basis of these results, we propose a model for the interaction of ethanol and hexanol with lipid bilayers. In this model, the alcohol hydroxyl groups are proposed as being

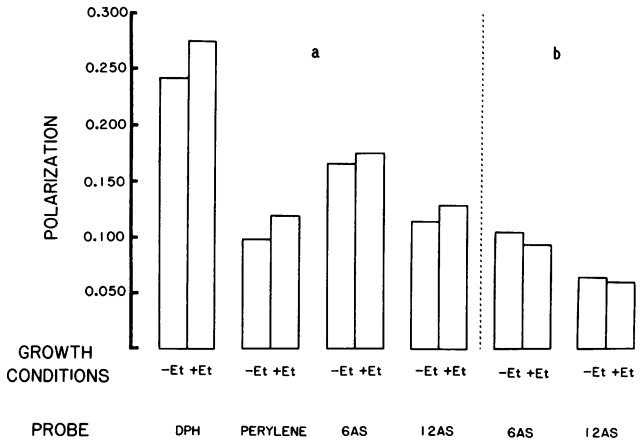

FIG. 4. Comparison of the effects of growth in the presence or absence of ethanol. The bars show the polarization of fluorescent probes in *E. coli* plasma membranes (a) and membrane-derived liposomes (b) from cells grown in the absence (−Et) or presence (+Et) of ethanol. Assay temperature was 37°C. All bars represent the mean of three sets of 10 determinations. In all cases, the polarization for −Et bars differs significantly by at least $P < 0.1$ from +Et bars. Degrees of significance were calculated by a one-tailed *t*-test.

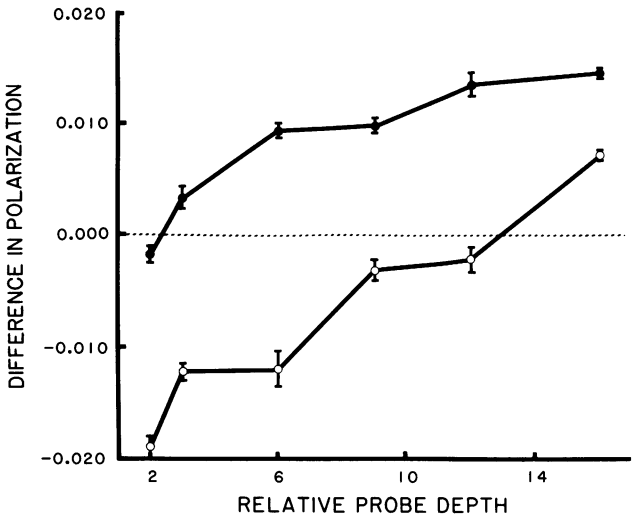

FIG. 5. Difference in polarization of the anthroyloxy fatty acid probes in *E. coli* plasma membranes and membrane-derived liposomes as a result of growth in the presence of ethanol. Plotted values represent the polarization of probe fluorescence in membranes or membrane-derived liposomes from alcohol-grown cells minus that of control cells. Assay temperature was 37°C. The corresponding points of each line differ significantly ($P < 0.005$) from each other. Symbols: ●, *E. coli* plasma membranes; ○, membrane-derived liposomes.

localized near the surface of the bilayer due to hydrogen bonding with polar surface groups such as the ester oxygens of the lipids, water, or proteins. The hydrocarbon chains would be expected to penetrate toward the center of bilayer. This model is further supported by the partition coefficient data of Seeman (52) for short-chain alcohols from buffer to erythrocyte ghosts. The partition coefficient predicts that seven times as much ethanol should be located in the buffer as in the hydrophobic region of the membrane. Thus, a gradient exists within the membrane with the highest concentration of ethanol near the surface. The hydrocarbon chain of hexanol would be expected to penetrate to a greater

TABLE 1. Comparison of membrane depolarization and lipid/protein ratio of isolated membranes[a]

Growth condition	Lipid/protein ratio (μmol of lipid phosphorus per mg of protein)	Fluorescence depolarization
Control	0.506 ± 0.010	0.257 ± 0.003
+Ethanol (4%, vol/vol)	0.221 ± 0.004	0.290 ± 0.001
+Cerulenin	0.148	0.289 ± 0.002

[a] The extent of plasma membrane enrichment was estimated by measuring the specific activity of NADH oxidase. The specific activity of the total membrane fraction from control cells, ethanol-grown cells, and cerulenin-grown cells was 0.81, 0.90, and 2.20 μmol/(mg protein-min), respectively. In comparison to total membranes, enrichment factors of 2.4-, 2.4-, and 1.7-fold, respectively, were obtained. The ratios of specific activity in the upper band (plasma membrane fraction) to the lower band (outer membrane fraction) were 22, 22, and 8, respectively. These results indicate that equivalent purification was obtained with control and ethanol-treated cells, whereas the separation of plasma membranes from cerulenin-treated cells was less effective. Unlike the other cells, cerulenin-treated cells and crude membranes tended to aggregate. This aggregation may have adversely affected purification.

depth than ethanol because it can participate more extensively in van der Waals interactions with lipid fatty acyl chains. Ethanol has about one-third the hydrophobic character of hexanol (two methylene groups as compared with the six methylene groups of about similar hydrophobic character for hexanol) and, thus, may be less efficient at participating in van der Waals interactions.

In contrast to our results, EPR studies by Chin and Goldstein (14, 23) have suggested that ethanol disorders the membrane interior to a greater extent than the membrane surface. This apparent discrepancy can be ascribed to the use of different types of membranes (containing and lacking cholesterol) or to the use of different fluidity probes. The doxyl group used in the EPR study is more polar than the anthroyloxy group used in our fluorescence study (10, 51). Because it is more polar, the doxyl group may, itself, cause a localized increase in polarity at different depths within the membrane and participate in hydrogen bonding with alcohols. Alternatively, the order parameter calculated from EPR measurements may reflect a different aspect of lipid organization than the polarization values calculated from fluorescence intensity ratios. Fluorescence depolarization primarily reflects the rate of probe motion (5, 32), whereas the EPR order parameter primarily reflects the homogeneity of probe orientation (51).

Growth in the presence of ethanol resulted in the biosynthesis of *E. coli* plasma membranes which appeared more rigid than control membranes with all of the fluorescent probes tested. However, liposomes from alcohol-grown cells appeared more fluid than liposomes from control cells with all but the 16AP probe. The increased fluidity of the lipid is consistent with the increase in $C_{18:1}$ fatty acid at the expense of $C_{16:0}$ that has been shown to occur in *E. coli* during growth in the presence of ethanol (26). The decrease in the mobility of the 16AP probe in liposomes from alcohol-grown cells may also be caused by this change in fatty acid composition. The replacement of $C_{16:0}$ by $C_{18:1}$ in ethanol-grown cells may make the center of the bilayer more rigid (38). The ethanol-induced change in plasma membrane fluidity is qualitatively consistent with homeoviscous adaptation to the fluidizing effects of ethanol on membrane lipids. Growth in ethanol, however, causes a much larger increase in membrane rigidity than was needed to compensate for the small ethanol-induced increase in fluidity. Membranes from alcohol-grown cells contained a higher proportion of protein than membranes from control cells. This finding is consistent with our previous studies which have demonstrated an alcohol-induced decrease in cellular phospholipid (27). ^{13}C NMR, FTIR, and other fluorescence studies have concluded that membrane-bound proteins have a marked rigidifying effect on lipid fatty acyl chains (11, 24, 25, 33, 55, 60). Thus, the decrease in the lipid/protein ratio in membranes from alcohol-grown cells appears to compensate for the fluidizing action of increased lipid unsaturation and is responsible for the observed decrease in membrane fluidity.

Decreasing the proportion of membrane lipid may be of selective advantage to *E. coli* during growth in the presence of ethanol. Alcohols shorter than octanol decreased membrane resistance (13) and increased ion leakage in liposomes (47). A study by Enequist et al. (19) has provided evidence that ethanol acts to uncouple various cellular processes from the proton-motive force generated across the plasma membrane of *E. coli* by increasing ion leakage. Other studies in our laboratory have implicated leakage as the mechanism of ethanol killing in *E. coli* (18). Bulk protein may serve as a barrier to ion movement. By decreasing the amount of lipid in the membrane, less area would be available for the passive diffusion of ions during growth of *E. coli* in the presence of ethanol.

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