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Changes in Lipid Composition of *Escherichia coli* Resulting from Growth with Organic Solvents and with Food Additives

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Cells of *Escherichia coli* contain an altered fatty acid and phospholipid composition when grown in the presence of sublethal concentrations of a variety of organic solvents and food additives. The diversity of compounds examined which caused these changes indicates that no single catabolic pathway is involved. Many of the observed changes are consistent with the hypothesis that cells adapt their membrane lipids to compensate for the presence of these compounds in the environment. Both sodium benzoate and calcium propionate caused the synthesis of unusual fatty acids.

Plant cells, animal cells, and bacterial cells all adapt their membrane lipids in response to growth temperature (11). This adaptation is accomplished in large part by changes in the relative abundance of saturated and unsaturated fatty acids in the polar lipids of the cell (8). In this way the lipids of the cell membrane are maintained in a fluid state, essential to biological function. An increase in growth temperature initially causes an increase in membrane fluidity (decrease in viscosity), which is antagonized by the synthesis of lipids containing an increased abundance of saturated fatty acids. In *Escherichia coli*, this adaptation is brought about primarily by the acylase enzymes, which synthesize phosphatidic acid from α-glycerol phosphate and fatty acyl-ACP (25), and to a lesser extent by the regulation of endogenous pools of fatty acids (6).

Previous studies in my laboratory have indicated that *E. coli* synthesizes lipids with an altered fatty acid composition when grown in the presence of alcohols (15). Alcohols are known to intercalate within biological membranes (23, 24) and alter their fluidity. The spontaneous insertion of long-chain alcohols (C5-C8) has been shown to increase membrane fluidity using both electron spin resonance (13, 22) and differential scanning calorimetry (14). *E. coli* adapts to the presence of these alcohols by an increased synthesis of phospholipids containing saturated fatty acids analogous to growth at an elevated temperature. Conflicting reports have been presented for the effects of short-chain alcohols (C1-C4) on membrane fluidity (13, 14, 22). Growth of *E. coli* in the presence of short-chain alcohols leads to the opposite change, an increase in the abundance of unsaturated fatty acids analogous to growth at a reduced temperature (19). This change in fatty acid composition would tend to increase membrane fluidity, suggesting that ethanol, like reduced temperature, acts to stabilize the cell membrane. Although this apparent stabilization by ethanol is contrary to the effects of most agents on membrane fluidity, recent physical studies by Papahadjopoulos et al. (21) have shown that the addition of dimethyl sulfoxide also decreases membrane fluidity. Thus, the cell responds to the presence of long-chain alcohols by decreasing the fluidity of its lipids and to short-chain alcohols by increasing the fluidity of its lipids.

The alteration of fatty acid content in response to alcohols suggests that the cell is able to perceive a change in membrane fluidity, per se. However, alternative possibilities related to the metabolism of these alcohols also exist. In this paper, we have surveyed the effects of a variety of common organic solvents of diverse structure as well as both organic and inorganic food additives on the fatty acid and phospholipid compositions of *E. coli*. The catabolism of these compounds involves a variety of different pathways. Indeed, some of these may not be metabolized at all by *E. coli* in rich media.

E. coli K-12 strain CSH2 was grown in Luria broth (18) at 30°C with forced aeration for five generations in the presence of various additives and harvested in log phase (2×10^8 cells/ml). The fatty acid composition of these cells was determined essentially as described previously (15). The mean standard deviation for all fatty acids from a variety of samples is 0.79%. Table 1 summarizes the effects of solvents and food additives on fatty acid composition. A series of concentrations of each agent was examined. The concentrations reported approach the max-

TABLE 1. *Effects of organic solvents and food additivies on the fatty acid and phospholipid composition of E. coli*

Additive	Concn (mM)	Fatty acid composition (% of total)						Phospholipid content		Phospholipid composition[a] (% of total)			
		12:0	14:0	16:0	16:1	Δ17	18:1	cpm/ml per A[b]	% of control	CL	PG	PE	LE
None	397.0	4.0	3.5	26.5	34.1	–	31.9	14,415	100	1.3	14.9	80.4	3.4
Acetone	48.0	3.5	2.3	23.8	32.7	1.7	36.0	12,542	87	8.4	11.5	73.2	6.9
Cyclohexanone	276.0	4.6	2.3	26.8	28.8	2.0	35.5	12,250	85	7.7	10.8	79.4	2.2
Dimethyl sulfoxide	450.0	3.5	3.6	23.8	34.8	–	34.3	14,549	101	1.1	15.3	80.1	3.5
Dioxane	852.0	2.6	1.6	24.4	26.9	2.6	41.9	13,989	97	1.9	17.8	79.3	1.0
Ethylene glycol		3.9	1.8	19.7	32.7	–	41.9	16,154	112	2.1	15.4	80.0	2.5
Ethylene glycol monomethyl ether	620.0	3.3	1.7	18.1	29.3	1.9	45.7	20,173	140	3.6	14.6	77.0	4.8
Methyl ethyl ketone	218.0	4.1	2.4	26.5	30.6	1.0	35.3	12,551	87	4.1	14.3	80.0	1.6
Sodium nitrite	40.0	4.7	1.4	26.0	23.9	2.6	41.4	14,234	91	2.4	19.3	76.4	1.9
Sodium sulfite[c]	113.0	4.1	2.1	22.1	22.4	10.4	37.8	14,214	91	6.0	16.8	75.0	2.2
Tetrahydrofuran	86.5	4.2	3.8	23.3	32.8	–	35.9	15,837	110	1.2	14.8	81.1	2.9
Amyl acetate	6.7	4.0	2.9	28.7	33.8	–	30.5	16,148	112	2.2	13.1	76.3	8.4
Aniline	32.9	4.3	7.8	54.6	21.7	4.8	6.7	17,881	124	5.5	9.8	83.3	1.5
Benzene	17.2	3.7	3.2	29.1	33.7	–	30.3	14,857	103	1.3	14.7	80.2	3.7
Carbon tetrachloride	10.3	3.5	3.4	28.7	33.4	–	31.0	14,125	98	1.8	16.0	78.7	3.5
Chloroform	18.7	3.9	3.6	28.3	34.2	–	29.9	14,836	103	2.5	14.7	76.1	6.7
Ethyl acetate	101.0	3.9	3.0	32.6	31.9	–	28.5	15,716	109	1.2	15.2	80.5	3.1
Methylene chloride	77.6	4.6	3.7	28.0	35.7	–	28.0	16,143	112	1.1	14.4	83.1	1.3
Methyl paraben	3.95	1.7	2.3	48.1	28.8	–	19.1	23,760	152	1.9	15.3	80.5	2.4
Pyridine	62.0	5.4	3.5	30.2	30.6	–	30.3	15,263	106	1.9	15.4	80.1	2.4
Sodium sorbate[d]	26.0	4.4	3.7	36.9	19.4	15.3	18.9	17,200	110	2.5	15.0	80.6	1.9
Toluene	4.67	4.2	3.7	27.5	34.4	–	30.3	14,653	102	1.9	15.6	79.2	3.3
Calcium propionate[e]	38.0	5.5	3.8	21.5	27.6	3.7	31.7	16,559	106	1.3	20.4	76.2	2.2
Sodium benzoate	20.0	52.1	1.0	18.8	5.9	4.8	17.4	12,025	77	2.9	19.1	75.3	2.6

[a] CL, Cardiolipin; PG, phosphatidyl glycerol; PE, phosphatidyl ethanolamine; LE, lysophosphatidyl ethanolamine.
[b] *A*, Absorbance unit at 550 nm.
[c] Δ19 = 1.0.
[d] Δ19 = 1.3%.
[e] Δ17 represents the sum of Δ17 and 17:1; 15:0 = 5.1%; 17.0 = 1.1%.

ima that allow growth. A concentration-dependent response was observed in all cases. Supplementation with acetone, cyclohexanone, dimethyl sulfoxide, dioxane, ethylene glycol, ethylene glycol monomethyl ether, methyl ethyl ketone, sodium nitrate, sodium sulfite, and tetrahydrofuran all cause an increased synthesis of lipids containing unsaturated fatty acids analogous to the changes observed with ethanol (15) or after a decrease in growth temperature (19). Amyl acetate, aniline, benzene, carbon tetrachloride, chloroform, ethyl acetate, methylene chloride, methyl paraben, and sodium sorbate all cause the opposite effect, an increased synthesis of lipids containing saturated fatty acids analogous to an increase in growth temperature (19). Little change in fatty acid composition was observed with toluene despite its potency as a growth inhibitor. Aniline, sodium nitrite, sodium sulfite, and sodium sorbate, in particular, cause a large increase in the proportion of cyclopropane fatty acid similar to that observed in late-log-phase and stationary-phase cells (5). Benzoate caused a dramatic increase in the proportion of lauric acid, usually a minor component in *E. coli*. Growth in the presence of propionate resulted in the production of fatty acids with odd chain lengths. Both 15-carbon and 17-carbon saturated fatty acids, as well as 17-carbon unsaturated fatty acid, were found. The identity of these has been confirmed by mass spectroscopy. The synthesis of these unusual fatty acids probably results from the utilization of propionyl-ACP as a primer for fatty acid synthesis instead of acetyl-ACP. Indeed, in vitro studies of a variety of organisms (1, 4, 28) indicate that propionyl-ACP or propionyl-coenzyme A can compete with their respective acetyl derivatives in this capacity.

Although *E. coli* does not alter its phospholipid composition extensively in response to growth temperature (9), other organisms such as *Clostridium butyricum* vary their phospholipid composition as part of their adaptation to growth temperature (17). Thus, the effects of the various additives on phospholipid content and composition were also examined (Table 1). Log-phase cells, uniformly labeled with ^{32}P (2 μCi/ml), were analyzed by the method of Ames

(2). Diverse changes were found. Acetone, aniline, cyclohexanone, methyl ethyl ketone, and sulfite all caused increases in the proportion of cardiolipin analogous to the changes observed in *E. coli* as cultures approached stationary phase (5). Similar increases in cardiolipin content in *E. coli* have previously been reported after the addition of phenethyl alcohol (3, 21). Acetone, aniline, and cyclohexanone caused a decrease in phosphatidyl glycerol, whereas dioxane, propionate, benzoate, and nitrite caused an increase in phosphatidyl glycerol. Acetone, amyl acetate, chloroform, and ethylene glycol monomethyl ether caused an increase in lysophosphatidyl ethanolamine. Phosphatidyl ethanolamine, however, remained the dominant phospholipid species in all cases.

The phospholipid content of cells grown with various additives was further examined by determining the amount of ^{32}P-labeled lipid (counts per minute) per milliliter per absorbance unit of cells at 550 nm (Table 1). Growth in the presence of methyl paraben and ethylene glycol monomethyl ether resulted in a considerable increase in total phospholipid. The phospholipid composition of these, however, is very close to that of the control. Growth in the presence of sodium benzoate results in cells depleted in phospholipid. Smaller differences were observed with a variety of other additives.

In *E. coli*, phosphatidyl glycerol contains much higher levels of vaccenic acid than found in phosphatidyl ethanolamine (16). Potentially, changes in the ratio of these two components could be responsible for some shifts in fatty acid composition such as those observed with amyl acetate and sodium sulfite. Clearly, this is not the basis for fatty acid changes with most additives. Indeed, in many cases such as acetone, cyclohexanone, and nitrite, the changes in phospholipids alone would lead to alterations in fatty acid composition opposite to those observed.

It is apparent from these studies that the lipid composition of cells is dramatically altered in response to the presence of lipophylic compounds in the environment as well as inorganic food additives and that these changes in lipid composition are not the result of any single catabolic pathway. Further, these changes are much too complex and diverse to be the result of any single mechanism. Compounds such as calcium propionate and sodium benzoate have dramatic effects probably at the level of fatty acid synthesis. Many of the other changes in fatty acid composition could be attributed to attempts by the cell to maintain a homeoviscous membrane (7, 26). The less spectacular changes in individual phospholipid components could be expected to alter the charge distribution of the membrane. This coupled with changes in fatty acid composition could affect protein-lipid interactions as well as organization. Although these lipid changes did not affect morphogenesis and cell division in *E. coli*, analogous alterations in lipid composition in more complex eukaryotic systems could potentially have many effects on complex morphogenetic processes. Indeed, a variety of lipophylic agents and organic acids are known to be teratogenic (10, 12, 27).

LITERATURE CITED

1. **Alberts, A. W., P. W. Marjerus, and P. Vagelos.** 1969. β-Ketoacyl acyl acyl carrier protein synthase. Methods Enzymol. 14:57-60.
2. **Ames, G.** 1968. Lipids of *Salmonella typhimurium* and *Escherichia coli*: structure and metabolism. J. Bacteriol. 95:833-843.
3. **Barbu, E., J. Poulonovski, C. Rampini, and M. Lux.** 1970. Modifications of phospholipid composition of cells grown in the presence of phynethyl alcohol. C.R. Acad. Sci. Ser. D 270:2596-2599.
4. **Bloch, K.** 1975. Fatty acid syntheses from *Mycobacterium phlei*. Methods Enzymol. 35:84-90.
5. **Cronan, J. E., Jr.** 1968. Phospholipid alterations during growth of *Escherichia coli*. J. Bacteriol. 95:2054-2061.
6. **Cronan, J. E., Jr.** 1975. Thermal regulation of the membrane lipid composition of *Escherichia coli*. J. Biol. Chem. 250:7074-7077.
7. **Cronan, J. E., Jr., and E. P. Gelman.** 1975. Physical properties of membrane lipids: biological relevance and regulation. Bacteriol. Rev. 39:232-256.
8. **Cronan, J. E., Jr., and P. R. Vagelos.** 1972. Metabolism and function of membrane phospholipids of *Escherichia coli*. Biochim. Biophys. Acta 265:25-60.
9. **Desiervo, A. J.** 1969. Alterations in the phospholipid composition of *Escherichia coli* B during growth at different temperatures. J. Bacteriol. 100:1342-1349.
10. **Freeze, E., C. W. Sheu, and E. Galliers.** 1973. Function of lipophylic acids as antimicrobial food additives. Nature (London) 241:321-325.
11. **Fulco, A. J.** 1974. Metabolic alterations of fatty acids, p. 215-241. *In* E. S. Snell (ed.), Annual review of biochemistry, vol. 43. Annual Reviews Inc., Palo Alto, Calif.
12. **Ginsburg, E., D. Salomon, T. Sreevalsan, and E. Freeze.** 1973. Growth inhibition and morphological changes by lipophylic acids in mammalian cells. Proc. Natl. Acad. Sci. U.S.A. 70:2457-2461.
13. **Grisham, C. M., and R. E. Barnett.** 1973. The effects of long-chain alcohols on membrane lipids and the (Na^+ and K^+)-ATPase. Biochim. Biophys. Acta 311:417-422.
14. **Hui, F. K., and P. G. Barton.** 1973. Mesomorphic behaviour of some phospholipids with aliphatic alcohols and other nonionic substances. Biochim. Biophys. Acta 296:510-517.
15. **Ingram, L. O.** 1976. Adaptation of membrane lipids to alcohols. J. Bacteriol. 125:670-678.
16. **Kito, M., S. Aibara, M. Kato, and T. Hata.** 1972. Differences in fatty acid composition among phosphatidylethanolamine, phosphatidylglycerol and cardiolipin of *Escherichia coli*. Biochim. Biophys. Acta 260:475-478.
17. **Khuller, G. K., and H. Goldfine.** 1974. Phospholipids of *Clostridium butyricum*. V. Effects of growth temperature of fatty acids, alk-1-enyl ether group and phospholipid composition. J. Lipid Res. 15:500-507.
18. **Luria, S. E., and M. Delbruck.** 1943. Mutations of

bacteria from virus sensitivity to virus resistance. Genetics 28:491-511.
19. **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.
20. **Nunn, W. D., and B. E. Tropp.** 1972. Effects of phenethyl alcohol on phospholipid metabolism of *Escherichia coli*. J. Bacteriol. 109:162-168.
21. **Papahadjopoulos, D., S. Hui, W. J. Vail, and G. Poste.** 1976. Studies on membrane fusion. I. Interactions of pure phospholipid membranes and the effect of myristic acid, lysolecithin, proteins and dimethylsulfoxide. Biochim. Biophys. Acta 448:245-264.
22. **Paterson, S. J., K. W. Butler, P. Huang, J. Labelle, I. C. P. Smith, and H. Schneider.** 1972. The effect of alcohols on lipid bilayers: spin label study. Biochim. Biophys. Acta 266:597-602.
23. **Roth, S., and P. Seeman.** 1972. The membrane concentrations of neutral and positive anesthetics (alcohols, chlorpromazine, morphine) fit the Meyer-Overton rule of anesthesia; negative narcotics do not. Biochim. Biophys. Acta 255:207-219.
24. **Seeman, P.** 1972. The membrane actions of anesthetics and tranquilizers. Pharmacol. Rev. 24:583-655.
25. **Sinensky, M.** 1971. Temperature control of phospholipid biosynthesis in *Escherichia coli*. J. Bacteriol. 106:449-455.
26. **Sinensky, M.** 1974. Homeoviscous adaptation. A homeoviscous process that regulated the viscosity of the membrane lipids in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 71:522-525.
27. **Shepard, T. H.** 1973. Catalog of teratogenic agents. The Johns Hopkins University Press, Baltimore.
28. **Smith, S., and S. Abraham.** 1975. Fatty acid synthase from lactating rat mammary gland. Methods Enzymol. 35:65-74.