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NOTES

Failure of *Escherichia coli* to Alter Its Fatty Acid Composition in Response to Cholesterol-Induced Changes in Membrane Fluidity†

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Substantial amounts of exogenously supplied cholesterol were incorporated into the membranes of *Escherichia coli* during growth and caused a large decrease in membrane fluidity. Although no compensatory changes in fatty acid composition were observed, the incorporation of cholesterol did not affect the rate of growth of *E. coli* or interfere with the changes in fatty acid composition which normally occur during growth at different temperatures.

Temperature-induced changes in fatty acid composition occur in the membranes of both eucaryotic and procaryotic organisms (9). In general, the degree of unsaturation of membrane fatty acids decreases with increasing growth temperature and is often accompanied by a small decrease in mean fatty acid chain length. These changes have been termed "homeoviscous adaptation" (25) and have been well documented in the bacterium *Escherichia coli* (15, 23, 24).

Sterols are essential lipid components of eucaryotic cells, where they function as major determinants of membrane fluidity (20). Studies with mammalian cells (3, 4, 22, 26) have shown that sterols are required for membrane function. Changes in the sterol levels of mammalian cells in culture result in compensatory changes in fatty acid composition (4, 20).

Although sterols are not synthesized by most procaryotes, *Mycoplasma* species are known to require sterols for growth (2, 6, 19) and to adjust their fatty acid composition in response to sterol availability (21). *Acholeplasma laidlawii*, a related organism, has also been shown to incorporate sterols but does not require sterols for growth (7). Recent studies by Razin (18) have shown that sterol incorporation is not limited to these mycoplasma-like organisms and that some eubacteria also appear to incorporate exogenously supplied sterols into their membranes. In this study, we have investigated the effects of cholesterol on the lipid composition of *E. coli*.

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After the growth of *E. coli* in rich media with exogenous cholesterol, substantial amounts of cholesterol were recovered in purified membranes (Table 1). Washing did not remove this cholesterol, which appeared to be localized in the cytoplasmic membrane after sucrose gradient centrifugation, as previously reported for *Proteus mirabilis* (18). The amount of cholesterol recovered with isolated membranes was greatly influenced by growth temperature. Membranes from cells grown at 25°C contained only 1/3 of that contained in membranes from cells grown at 37°C. A similar temperature dependence of cholesterol incorporation has been reported for *Mycoplasma mycoides* (21). Regardless of the extent of cholesterol incorporation, no changes in cell morphology or cell motility were observed. Further, the rate of growth in the presence of cholesterol was identical to that in its absence.

The relative fluidity of membranes from both control and cholesterol-grown cells was determined, using the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (Table 2). Cells grown at 37°C without cholesterol contained more rigid membranes than cells grown at 25°C (0.03-U increase in polarization), reflecting the greater abundance of saturated fatty acids incorporated into the phospholipid molecular species at higher growth temperatures (5, 16). Membranes from cells grown with cholesterol were more rigid than those from control cells grown at the same temperature in its absence. The cholesterol-induced increase in rigidity was much larger in cells

TABLE 1. *Incorporation of cholesterol into membranes of E. coli TB4*[a]

Membrane sample	Growth temp (°C)	Membranes		Ratio (cholesterol/protein)
		Cholesterol (mg)	Protein (mg)	
Total[b]	25	6.5	130	0.050
Total[b]	37	5.0	34	0.147
Cytoplasmic[c]	37	0.651	1.2	0.543
Outer[c]	37	0.267	23.7	0.011

[a] Cells were grown in Luria broth (14) without added carbohydrate in a reciprocating shaker and were harvested during exponential growth (approximately 2×10^8 cells per ml). Where indicated, cultures were supplemented with cholesterol (20 mg/liter, final concentration) by adding 1/1,000 vol of ethanol stock (20 g/liter) to sterile media held at 100°C. The cholesterol content of membranes was determined by the cholesterol oxidase method (1). Protein was measured by the method of Lowry et al. (13) as described by Layne (12). Values reported represent averages of two membrane preparations.

[b] Cells were harvested by centrifugation (5,000 × *g*, 5 min) and washed twice with 0.1 M Tris-hydrochloride (pH 7.5) Total membranes were prepared by the method of DiRienzc and Inouye (8).

[c] Cytoplasmic membranes and outer membranes were prepared by the method of Osborn et al. (17), using sucrose gradient centrifugation.

TABLE 2. *Effect of cholesterol and growth temperature on membrane fluidity*[a]

Growth temp (°C)	Cholesterol supplement	Polarization[b] (preparations)
25	−	0.247 ± 0.001 (2)
25	+	0.277 ± 0.002 (2)
37	−	0.276 ± 0.001 (3)
37	+	0.348 ± 0.001 (3)

[a] Cells were grown and total membrane was isolated as described in footnote *a*, Table 1. Polarization was determined using the fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene, as previously described (11).

[b] Average ± standard deviation (number of membrane preparations examined). Triplicate measurements of polarization were performed on each membrane preparation. These measurements were performed at 37°C, regardless of growth temperature.

grown at 37°C than in cells grown at 25°C, consistent with the higher level of cholesterol incorporation at 37°C.

If homeoviscous adaptation in *E. coli* is a response to changes in bulk membrane fluidity, one would expect that the incorporation of exogenous cholesterol would induce large changes in fatty acid composition analogous to a decrease in growth temperature. The observed differences in bulk fluidity (Table 2) indicate that the cells were unable to make sufficient changes in fatty acid composition to compensate for the effects resulting from cholesterol incorporation. Indeed, the fatty acid composition of *E. coli* remained unaffected after the incorporation of cholesterol, despite the observed large changes in bulk fluidity (Table 3). Further, cholesterol did not interfere with the normal ability of cells to alter their membrane fatty acid composition in response to changes in growth temperature. Since mammalian cells (4, 20) and *M. mycoides* (21) alter their fatty acid composition in response to sterol content, our results with *E. coli* provide further evidence that multiple mechanisms exist for the regulation of membrane fatty acid composition.

TABLE 3. *Effects of temperature and cholesterol on the fatty acid composition of strain TB4*[a]

Growth Temp (°C)	Supplemental cholesterol (20 mg/liter)	Fatty acid composition (%)[b]				
		14:0	16:0	16:1	Δ17	18:1
37	−	4.8	35.9	29.7	0.6	28
37	+	4.2	35.5	31.5	0.4	28
25	−	2.7	25.1	34.2	0.4	37
25	+	3.2	25.3	33.0	0.2	38

[a] Cells were grown as described in Table 1, footnote *a*. Lipids were extracted and fatty acid composition was determined as previously described (10). Values represent averages of duplicate determinations.

[b] Fatty acids: 14:0, myristic; 16:0, palmitic; 16:1, palmitoleic; Δ17, *cis*-9,10-methylene hexadecanoic; 18:1, vaccenic.

Membrane composition is determined both by the acyltransferase enzymes and by the enzymes of fatty acid synthesis. Our results indicate that bulk membrane fluidity per se is not involved in the regulation of membrane fatty acid composition in *E. coli*. This result is consistent with the enzymes of fatty acid synthesis being the primary determinants of lipid composition. The incorporation of cholesterol into membranes would not be expected to affect such soluble enzymes. Although the acyltransferase enzymes do not appear to be affected by bulk membrane fluidity, the temperature-dependent changes in acyl-chain specificity may involve more specific types of interactions between these enzymes and membrane phospholipids, which are unaffected by cholesterol.

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