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Control of Membrane Lipids in *Mycoplasma gallisepticum*: Effect on Lipid Order

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Adaptation of *Mycoplasma gallisepticum*, a sterol-requiring *Mycoplasma* sp., to growth in a serum-free medium supplemented with cholesterol in decreasing concentrations and with various saturated or unsaturated fatty acids enabled us to control both the cholesterol levels and the membrane fatty acid composition. An estimate of the membrane physical state from fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene indicated that the membrane lipids of native *M. gallisepticum* were highly ordered. Elongation of the saturated fatty acid chains from 14 to 18 carbon atoms caused only a small increase in the membrane lipid ordering, whereas the introduction of a *cis* double bond reduced it significantly. Lipid-phase transitions were observed in low-cholesterol-adapted organisms, whose membrane lipids were still highly ordered at the growth temperature.

Most species of *Mycoplasma* are not able to synthesize the cholesterol and long-chain fatty acids necessary for their growth (2, 39, 40, 41). Consequently, variations in the lipid composition of a medium can affect the membrane composition, the growth conditions, the morphology, and the osmotic and mechanical fragility properties of these organisms (22, 27, 28). Control of the exogenous lipids supplied to a medium allows more reproducible growth conditions and makes it possible to study the effects of these lipid components on the functioning of the organisms.

Unusual properties of *Mycoplasma gallisepticum*, such as its shape, the existence of blebinfrableb terminal structures corresponding to the sites of DNA replication (17-19, 25), the high membrane protein content (15, 23), and its resistance to osmotic lysis (26, 27), have been the focus of interest. Until now, in contrast to *Mycoplasma mycoides* subsp. *capri* and *Mycoplasma capricolum*, attempts to adapt *M. gallisepticum* to growth with very low concentrations of cholesterol have been unsuccessful (33). This paper describes the adaptation of *M. gallisepticum* to growth in a serum-free medium containing various long-chain fatty acids and low concentrations of cholesterol. We describe modifications in the physical state of the membrane lipids resulting from alterations in the fatty acid composition and cholesterol content.

MATERIALS AND METHODS

Organisms and growth conditions. *M. gallisepticum* strains A5969 and PG31 were grown statically at 37°C in MBB medium which contained (per liter) 21 g of *Mycoplasma* broth base (BBL Microbiology Systems, Cockeysville, Md.), 50 ml of 25% fresh yeast extract solution, 100 ml of horse serum (GIBCO Laboratories, Grand Island, N.Y.), 10 g of dextrose, 300,000 U of penicillin G (sodium salt), and 0.12 g of thallium acetate; this medium was adjusted to pH 7.5 (9). Once satisfactory growth was obtained, the organisms were transferred to a modified Edward medium (28) containing (per liter) 0.02 g of DNA, 12 g of heart infusion broth (Difco Laboratories, Detroit, Mich.), 5 g of peptone (Difco), 5 g of yeast extract (Difco), 2.5 g of NaCl, 7.2 g of Na_2HPO_4, 5 g of dextrose, 0.2 g of thallium acetate, 300,000 U of penicillin G (sodium salt), and 50 ml of horse serum. This medium was adjusted to pH 8.2 with 1 M NaOH and sterilized by membrane filtration (pore size, 0.22 μm; type GS; Millipore Corp., Bedford, Mass.). The horse serum fraction was subsequently replaced by 0.5% fatty acid-poor bovine serum albumin (Miles Laboratories, Inc., Kankakee, Ill.), 10 μg of cholesterol per ml, 5 μg of oleic acid (sodium salt) per ml, and 5 μg of palmitic acid (sodium salt) per ml. Cholesterol and oleic and palmitic acids were mixed in the following way before they were added to the growth medium. Typically, for a 100-ml final volume, a solution containing 0.4 ml each of oleic acid and palmitic acid (sodium salts; 1.25 mg/ml in water) was preheated to 60°C; 0.2 ml of an ethanolic solution of cholesterol (5 mg/ml) was then added, and after gentle agitation the resulting mixture was allowed to stand at 60°C for 2 min. This mixture was finally transferred into the unsupplemented medium. Strains A5969 and PG31 grown in this medium were transferred to media supplemented with various fatty acids (10 μg/ml; sodium salts), and the cholesterol concentration was progressively decreased. For the labeling of membrane cholesterol, 20 μCi of [^{3}H]cholesterol (9.5 μCi/mmol; Amersham) was added to each

1 liter of growth medium. Growth was determined by measuring the absorbance of each culture at 640 nm.

Isolation of cell membranes. Cells were collected by centrifuging 200- to 400-ml cultures at 7,000 × *g* for 15 min. The low degree of packing of the cell pellet resulted in minimization of the mechanical stress needed for cell suspension and an 80% recovery of cells. The pellets were washed once with a cold 0.25 M NaCl solution and suspended in Tris buffer (0.05 M Tris, 0.15 M NaCl, 0.01 M mercaptoethanol, pH 7.5). Cell lysis was achieved by the alternate freeze-thaw procedure (20). Three to four freeze-thaw cycles, each followed by a brief sonication in a disintegrator (10 s; 50 W; room temperature; Ultrasonics, Plainview, N.Y.), reduced the absorbance of the suspension by more than 70%. Membrane preparations were collected by centrifugation at 50,000 × *g* for 30 min, washed once in distilled water, and suspended (approximately 1 mg of membrane protein per ml) in diluted (1:20) β buffer (0.15 M NaCl, 0.01 M 2-mercaptoethanol, 0.05 M Tris, pH 74. [24]).

Fluorescence polarization experiments. Membrane suspensions were labeled with 1,6-diphenyl-1,3,5-hexatriene (Aldrich Chemical Co., Milwaukee, Wis.) by the procedure of Shinitzky and Inbar (37). Briefly, 10-μl stock solutions of 2 mM 1,6-diphenyl-1,3,5-hexatriene in tetrahydrofuran were injected with rapid stirring into 10 ml of diluted β buffer at room temperature. After the control suspension was bubbled with N_2 until no tetrahydrofuran odor remained, it showed negligible fluorescence. Membrane suspensions (100 to 200 μg of protein per ml) were incubated for 45 to 90 min at 38°C until a stable level of fluorescence intensity was reached. Fluorescence polarization measurements were made with an Aminco-Bowman spectrofluorimeter equipped with a Glan-Thompson prism on the excitation beam and Polaroid HN38X filters mounted as described by Chen and Bowman (5) on the emission pathway. An excitation wavelength of 365 nm was obtained by using a xenon-mercury lamp, and the emitted light was measured at 450 nm through a Corning J-73 cut-off filter. Each cuvette was held in the thermostatted cell compartment, connected to a Nesslab programmable temperature circulatory bath. Temperature was monitored with a thermolinear probe (Yellow Springs Instruments, Yellow Springs, Ohio) immersed in the sample. Corrections for scattered-light contribution were made by the method of Shinitzky et al. (36). Results were also corrected for the unequal transmission of differently polarized light and are expressed as the lipid order parameter S_r (11), according to the following equation: $S_r = [(5/2)r_\infty]^{1/2}$, where r_∞ is the limiting anisotropy value obtained from nanosecond anisotropy decay experiments (4, 7, 10, 12). For 1,6-diphenyl-1,3,5-hexatriene, r_∞ could be calculated from steady-state anisotropy measurements (r_s) by the following equation (11): $r_\infty = (9/8)r_s - 1/20$.

Analytical procedures. Protein was determined by the method of Lowry et al. (16). Lipids were extracted from whole cells or membrane suspensions by the method of Bligh and Dyer (3). Neutral lipids were separated from polar lipids by thin-layer chromatography on precoated silica gel plates (Adsorbosil 5; Applied Science Laboratories, State College, Pa.), using chloroform–methanol–water–acetic acid (65:25:4:1, vol/vol) as the developing solvent. Zones corresponding to polar lipids were scraped off, and methyl esters of fatty acids were prepared as previously described (13).

Methyl esters were extracted with pentane and purified by thin-layer chromatography on Adsorbosil 5, using a solution containing petroleum ether (bp, 60 to 70°C), ethyl ether, and acetic acid (80:20:1, vol/vol) as the developing solvent. The resulting methyl esters were subjected to gas-liquid chromatography in a Hewlett-Packard model 5720A apparatus (columns contained the following: 15% DEGS WHP on 80/100 mesh; 10% Silar-10C on 100/120 Gas-Chrom Q mesh; 10% Apiezon L on 80/100 CP AW mesh). Fatty acids were identified by their retention times relative to the retention times of standard methyl ester mixtures (lipid standards 189-1; Sigma Chemical Co., St. Louis, Mo.) and the BCMIX-L mixture (Applied Science Laboratories). The cholesterol contents of membrane preparations were calculated from their known specific activities after counting in Instagel (Packard Instrument Co., Inc., Rockville, Md.) fractions dissolved in sodium dodecyl sulfate. Comparisons with determinations by the Wycoff-Parsons method (43) generally coincided within 5% (15). Total phosphorus contents in the lipid samples were determined by the method of Ames (1) after digestion of the samples with an ethanolic solution containing $Mg(NO_3)_2$ and 0.5 N HCl. Electrophoresis of membrane proteins was performed by the method of Weber and Osborn (42) on 7.5% polyacrylamide gels containing 0.1% sodium dodecyl sulfate. Protein bands were stained with Coomassie blue.

RESULTS

Adaptation of *M. gallisepticum* to low cholesterol concentrations. Replacement of the horse serum fraction with mixtures containing 10 μg of cholesterol per ml and palmitic and oleic acids (sodium salts) resulted in satisfactory growth; maximal absorbance of the culture at 640 nm varied between 0.35 and 0.45 for both strain PG31 and strain A5969. Single fatty acid species (10 μg/ml) could be used to replace the oleic acid-palmitic acid mixture and still resulted in good growth (Table 1). Thus, after three to four serial transfers, supplementation by myristic, palmitic, or stearic acid resulted in comparable maximum absorbance values for strain A5969. Generally, the turbidities of the cultures were greater during lipid manipulations for strain A5969 than for strain PG31, and thus most of the subsequent studies were done with strain A5969. For the unsaturated fatty acids tested, growth was limited with palmitoleic acid to a maximum absorbance value of 0.16 to 0.17, whereas values of 0.23 to 0.27 were obtained regularly with palmitelaidic and oleic acids. All of the native organisms used were able to acidify the growth medium to pH 5.1 to 5.3 (Table 1).

Adaptation of *M. gallisepticum* to growth in

TABLE 1. *Phospholipid and cholesterol contents of M. gallisepticum A5969 membranes from native cells grown in the presence of various exogenous fatty acids*[a]

Fatty acid(s) added	Absorbance of culture at 640 nm	pH of culture	Maximum absorbance of culture	Minimum pH of culture	Contents of membranes		
					Lipid phosphorus (μmol of P_i/mg of membrane protein)	Cholesterol (μg/mg of membrane protein)	Cholesterol (μmol/μmol of lipid P_i)
$C_{14:0}$	0.26	6.62	0.38	5.20 ± 0.03 (4)[b]	0.22	102	1.18
$C_{16:0}$	0.23	6.66	0.52	5.22 ± 0.02 (5)	0.15	77.5	1.35
$C_{18:0}$	0.29	6.36	0.40	5.25 ± 0.02 (3)	0.11	46.0	1.07
$C_{16:1c}$	0.16	6.99	0.17	5.23 ± 0.03 (4)	0.15	115	2.01
$C_{16:1t}$	0.14	7.05	0.27	5.24 ± 0.03 (4)	0.17	116	1.80
$C_{18:1c}$	0.24	6.62	0.27	5.30 ± 0.06 (6)	0.12	64.1	1.39
$C_{16:0}$ + $C_{18:1c}$	0.13	7.09	0.45	5.10 ± 0.02 (6)	0.22	104	1.22
	0.19	6.80			0.18	96.3	1.35
	0.29	6.54			0.16	86.2	1.40

[a] Fatty acids are designated by the number of carbon atoms, followed by the number of double bonds present in each molecule. c and t indicate the *cis* and *trans* configurations of the double bonds, respectively. Values for absorbance and pH of culture correspond to the values at which the organisms were harvested for the membrane composition analysis. Values for maximum absorbance of culture and minimum pH of culture were determined under late-log-phase conditions. Media contained 10 μg of cholesterol per ml and either 10 μg of one fatty acid (sodium salt) per ml ($C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{16:1c}$, $C_{16:1t}$, $C_{18:1c}$) or 5 μg of $C_{16:0}$ (sodium salt) per ml and 5 μg of $C_{18:1c}$ (sodium salt) per ml.

[b] Numbers in parentheses are numbers of determinations.

the presence of reduced amounts of exogenous cholesterol was achieved by successive serial transfers; the method used was the same method used for *M. mycoides* subsp. *capri* strain PG3. The cholesterol concentration in the medium was successively reduced from 10 μg/ml to 5, 2.5, 2, 1.5, 1, and 0.9 μg/ml after satisfactory growth was obtained for each step. Best results were obtained with strain A5969, although significant growth was observed at each step for strain PG31. Adaptation to a low cholesterol concentration (0.9 μg/ml) resulted in an increase in the generation time (from 170 to 250 min) and a reduction in the maximum absorbance of the culture (Fig. 1 and Table 2). A similar observation has been reported for *M. mycoides* subsp. *capri* (13, 33). Using the same batch of basic medium, we transferred adapted *M. gallisepticum* daily, and these cultures maintained maximum absorbance values comparable to those shown in Table 2 for several weeks. Despite their extreme fragility, adapted cells frozen directly from the growth medium and kept at −40°C for 4 months could be used to start a new culture without any loss of the adaptation properties. In contrast, a single transfer in the initial medium containing 10 μg of cholesterol per ml caused the loss of the ability to grow at low cholesterol concentrations. Although less pronounced than in *M. mycoides* subsp. *capri*, a decrease in the ability to lower the pH of the medium was observed during low-cholesterol adaptation of strain A5969, supporting the hypothesis of a direct effect of cholesterol on membrane proton permeability (13). Finally, the electrophoretic profiles of both native and adapted *M. gallisepticum* cells were comparable, and the growth of adapted cells was inhibited by an antiserum prepared against the native strain.

Adaptation of *M. gallisepticum* resulted in large decreases (four to seven times) in the cholesterol contents of the membranes (in micrograms per milligram of membrane protein) and in the cholesterol/phospholipid ratios (Tables 1 and 2). Table 3 shows the fatty acid compositions of the polar lipids of native and adapted cells. When oleic and palmitic acids were provided as exogenous fatty acids, they represented as much as 90% of the membrane fatty acids, in agreement with the recent data of Rottem and Markowitz (29). When only one exogenous fatty acid was provided, it still represented the most abundant fatty acid species (in moles percent) found in the membranes; the highest degree of enrichment was obtained for elaidic acid. A large variation (from 0.2 to 3.4) in the ratio of saturated fatty acids to unsaturated fatty acids was observed in the native strain. After low-cholesterol adaptation, this ratio decreased for cells supplemented with palmitate and stearate but increased for cells supplemented with oleate, elaidate, and oleate plus palmitate.

Fluorescence polarization experiments. (i) Native cells. Very high steady-state anisot-

ropy values were obtained at 37°C for native cells; these values ranged from 0.253 for oleic acid-supplemented cells to 0.274 for stearate-supplemented cells. These values are significantly higher than the values described for most mammalian membranes (37) and are about twice as high as the values obtained for *M. mycoides* subsp. *capri* grown in similar media (14, 31). As judged from the values calculated for the lipid order parameter S_r, the membrane lipids of native strain A5969 were highly ordered (Fig. 2 through 5). This was true for oleate-supplemented cells despite the significant reduction in S_r which resulted from the introduction of the *cis* double bond (Fig. 3). Increasing the chain length from 14 to 18 carbon atoms had only a small effect on the lipid ordering of membranes enriched with single saturated fatty acid species ($C_{14:0} < C_{16:0} < C_{18:0}$) (Fig. 2). No clear lipid-phase transitions could be detected in the range of temperatures studied, which agreed well with the high cholesterol/phospholipid ratios found in these preparations.

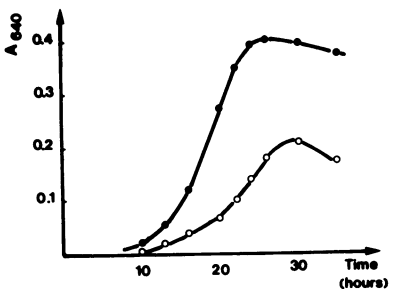

FIG. 1. *Growth curves of native and low-cholesterol-adapted strain A5969. Cells were grown on medium supplemented with oleic and palmitic acids which contained 10 μg of cholesterol per ml (●) or 0.9 μg of cholesterol (○) per ml. A_{640}, Absorbance at 640 nm.*

(ii) Cells adapted to low cholesterol concentrations. Changes in the slope of S_r versus temperature, indicative of the occurrence of liquid-gel lipid-phase transitions, were observed for the three membrane compositions studied. Transition temperatures were 28, 23, and 25°C for cells enriched with palmitate (Fig. 5), oleate (Fig. 3), and palmitate plus oleate (Fig. 4), respectively. However, at the growth temperature, S_r was larger than 0.72, indicating that the degree of order of membrane lipids was quite high in adapted cells. We also observed that differences in S_r values as a function of the fatty acid composition were more marked in low-cholesterol-adapted cells than in native cells. Finally, low-cholesterol adaptation resulted in an increase (particularly marked for oleate- and palmitate-supplemented cells) in the degree of lipid order for temperatures below the phase transitions.

DISCUSSION

We present evidence here that *M. gallisepticum* can be adapted to growth in the presence of reduced amounts of exogenous cholesterol. In agreement with the recent data of Rottem and Markowitz (29), growth of the native strain can be sustained by replacing the horse serum fraction with a mixture of cholesterol and various fatty acids. The extent of membrane incorporation of the fatty acid(s) added to the growth medium was comparable to the extent of incorporation obtained under similar conditions with *M. mycoides* subsp. *capri* (13, 33) and with *Acholeplasma laidlawii* grown in the absence of cerulenin or avidin (8, 22, 30). High cholesterol

TABLE 2. *Phospholipid and cholesterol contents of M. gallisepticum A5969 membranes from cells adapted to grow in the presence of low amounts of cholesterol*[a]

Amt of cholesterol added (μg/ml)	Fatty acid(s) added	Absorbance of culture at 640 nm	pH of culture	Maximum absorbance of culture	Minimum pH of culture	Contents of membranes: Lipid phosphorus (μmol of P_i/mg of membrane protein)	Contents of membranes: Cholesterol (μg/mg of membrane protein)	Contents of membranes: Cholesterol (μmol/μmol of lipid P_i)
0.9	$C_{16:0}$	0.20	6.84	0.26	6.47 ± 0.02 (6)[b]	0.23	18.0	0.21
0.9	$C_{18:1c}$	0.12	6.84	0.17	5.97 ± 0.14 (6)	0.18	18.2	0.26
0.9	$C_{18:1t}$	0.18	6.96	0.20	5.70 ± 0.05 (4)	0.21	16.5	0.20
0.9	$C_{16:0}$ + $C_{18:1c}$	0.09	7.34	0.22	5.84 ± 0.03 (5)	0.22	21.3	0.25
		0.16	6.83			0.17	13.6	0.21
		0.18	6.69			0.17	14.6	0.22

[a] Media contained 0.9 μg of exogenous cholesterol per ml and either 10 μg of one fatty acid (sodium salt) per ml ($C_{16:0}$, $C_{18:1c}$, $C_{18:1t}$) or 5 μg of $C_{16:0}$ (sodium salt) per ml and 5 μg of $C_{18:1c}$ (sodium salt) per ml.

[b] Numbers in parentheses are numbers of determinations.

TABLE 3. *Extent of incorporation of exogenous fatty acids into polar lipids of native and low-cholesterol-adapted strain A5969*

Cells	Absorbance of culture at 640 nm[a]	Addition(s) to medium[b]	Concn of the following fatty acids in polar lipids (mol/100 mol):									Ratio of saturated fatty acids to unsaturated fatty acids
			$C_{12:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	
Native	0.16	$C_{14:0}$		47.5	0.1	12.6	1.0	0.2	13.4	20.1	5.2	2.81
	0.19	$C_{16:0}$		0.5	0.1	63.1	0.7	0.1	13.2	14.0	8.0	3.39
	0.24	$C_{18:0}$	0.1	1.0	0.2	7.9	4.2	0.2	61.5	13.5	11	2.47
	0.26	$C_{18:1c}$	1.1	1.5	0.2	14.2	1.2	0.7	21.0	57.5	2.2	0.65
	0.28	$C_{18:1t}$		0.6	0.1	8.4	0.7	0.4	8.5	76.8[c]	3.3	0.22
	0.21	$C_{16:0} + C_{18:1c}$		0.4	0.2	49.6	0.1	0.6	7.1	40.4	1.6	1.38
Adapted	0.22	$C_{16:0}$	0.1	0.4		54.8	0.5	0.6	15.4	21.7	6.5	2.48
	0.17	$C_{18:0}$	1.0	1.3	0.7	6.0	1.1	1.2	56.4	28.3	4.0	1.99
	0.15	$C_{18:1c}$	0.7	2.5	0.4	24.4	0.5	1.2	21.9	47.1	1.8	1.03
	0.18	$C_{18:1t}$	0.2	1.5	0.2	16.3	0.2	0.7	12.6	66.4[d]	1.6	0.46
	0.17	$C_{16:0} + C_{18:1c}$		0.5	0.1	53.3	0.2	0.6	8.3	36.1	0.6	1.70

[a] Absorbance at which the organisms were harvested.

[b] The first number indicates the length of the carbon chain; a 0 as the second number indicates a saturated fatty acid and a 1 as the second number indicates one double bond. c and t indicate the *cis* and *trans* configurations of the double bonds, respectively.

[c] Elaidic acid ($C_{18:1t}$) represented 92% of the total $C_{18:1}$.

[d] Elaidic acid represents 89% of the total $C_{18:1}$.

contents and low phospholipid contents (decreasing upon aging of the cultures) were also found in these membranes. This is in agreement with data obtained with horse serum-supplemented media (15, 32).

Adaptation of strain A5969 to growth in the presence of 0.9 to 1.0 μg of exogenous cholesterol per ml was accompanied by a four- to sevenfold decrease in the cholesterol content of the membranes, and the cholesterol/phospholipid ratio obtained (~0.2) was equal to or even slightly lower than the cholesterol/phospholipid ratio described for the adapted *M. capriocolum* strain (6). The absolute values obtained (expressed as micrograms of cholesterol per milligram of membrane protein) are also comparable to the absolute values obtained by Rottem et al. (33) for *M. mycoides* subsp. *capri* grown in exogenous cholesterol-free medium. It is difficult to explain why previous attempts to adapt *M. gallisepticum* to low cholesterol levels failed, but the reasons why we succeeded are probably related to the slightly different growth media which we utilized and to the way cholesterol and fatty acids were added in our experiments. A comparison of native and adapted cells collected at comparable stages of growth does indicate that no systematic changes in the phospholipid contents of membranes occur. On the other hand, as previously reported for *M. mycoides* subsp. *capri* (33), cells supplemented with oleate, elaidate, and oleate plus palmitate increased their saturated fatty acid/unsaturated fatty acid ratios upon adaptation to low cholesterol. Although changes in membrane lipid composition that occur when cultures age make comparisons difficult (13), the additional observation that in stearate- and palmitate-supplemented cultures adaptation had the opposite effect (i.e., an increase in the unsaturated fatty acid species) supports the proposal of Rottem et al. (33) that these modifications tend to oppose the decrease in cholesterol content. Thus, for cells supplemented with unsaturated fatty acids, the increase in saturated species may partially compensate for the condensing effect of cholesterol. For cells supplemented with saturated fatty acids, cholesterol exerts a "fluidizing" effect, and the decrease in cholesterol content is accompanied by an increase in the percentage of unsaturated fatty acids.

The process of estimating the physical state of membrane lipids from changes in steady-state fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene is well documented (35). Although absolute values of "microviscosity," which can be calculated from steady-state depolarization data, should be interpreted with caution (4, 7, 12), theoretical considerations (11) and experimental results (12) have demonstrated that steady-state measurements of fluorescence anisotropy provide a good approximation of the membrane lipid order parameter. This particularly holds true for r_s values higher than 0.15 (10), which was the case in our experiments, in which the lowest value recorded (at 37°C) was 0.225 (low-cholesterol oleate-supplemented cells). Therefore, our data indicate that, com-

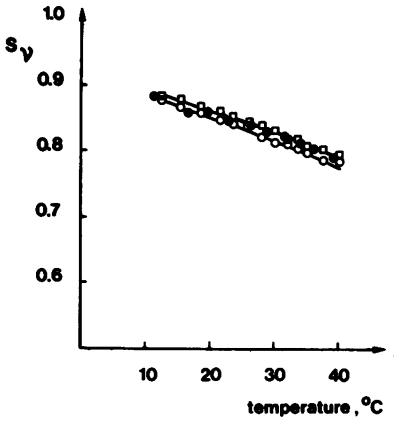

FIG. 2. *Temperature dependence of the lipid order parameter S_ν in native strain A5969 grown in the presence of the following saturated fatty acids: $C_{14:0}$ (absorbance at 640 nm, 0.26)* (○); *$C_{16:0}$ (absorbance at 640 nm, 0.20)* (●); *and $C_{18:0}$ (absorbance at 640 nm, 0.18)* (□).

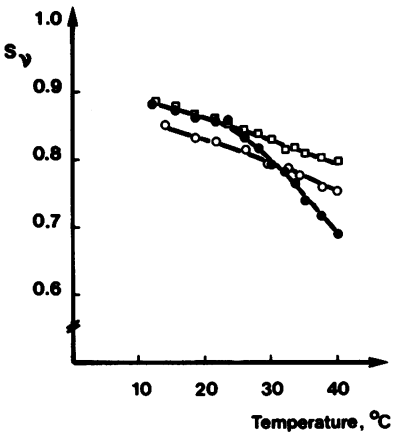

FIG. 3. *Effects of a cis double bond and reduced amounts of cholesterol on the lipid order parameter in membranes enriched with C_{18} acids. Native cells were grown in the presence of 10 μg of cholesterol per ml and either 10 μg of stearate per ml* (□) *(absorbance at 640 nm, (0.28) or 10 μg of oleate per ml* (○) *(absorbance at 640 nm, (0.24). Adapted cells* (●) *were grown with 0.9 μg of cholesterol per ml and 10 μg of oleate per ml (absorbance at 640 nm, 0.14).*

pared with other biological membranes, the membrane lipids of native strain A5969 are highly ordered. To date, higher r_s values have been reported only in rat intestinal microvillus membranes (34). This high degree of order, which implies a reduced molecular motion of the acyl chains of phospholipids, is probably largely responsible for the difficulty encountered in the disruption of native cells by osmotic or mechanical shock. Because the S_ν values of *M. mycoides* subsp. *capri* membranes of similar cholesterol content and fatty acid composition are significantly lower than the S_ν values which we obtained in the present experiments (14, 31), we can suggest by analogy with data obtained for rat microvillus membranes that the high protein content of strain A5969 membranes is responsible, at least in part, for the high degree of order of the lipids in these membranes. Increasing the chain length of saturated fatty acids from 14 to 18 carbon atoms has only a limited effect on the

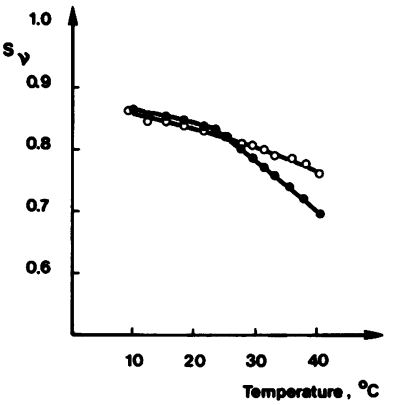

FIG. 4. *Effect of low-cholesterol adaptation on cells enriched with oleate and palmitate. Values were obtained for membrane preparations derived from cells harvested at identical absorbance values (absorbance at 640 nm, 0.15). Symbols:* ○, *native cells;* ●, *cells grown in the presence of 0.9 μg of exogenous cholesterol per ml.*

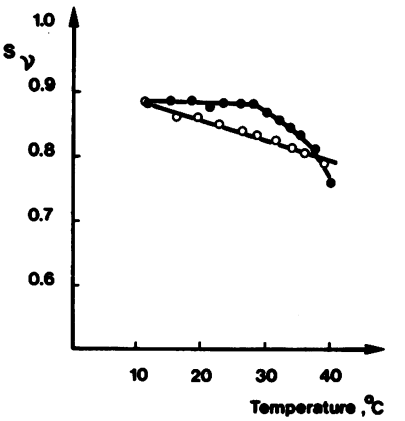

FIG. 5. *Effect of low-cholesterol adaptation on palmitate-enriched cells. Symbols:* ○, *native cells (absorbance at 640 nm, 0.20);* ●, *cells grown in the presence of 0.9 μg of exogenous cholesterol per ml (absorbance at 640 nm, 0.16).*

degree of ordering of the membrane lipids; this can be explained by a parallel increase in the amount of di-unsaturated fatty acid incorporated. Introduction of a *cis* double bond affects this chain packing more markedly and significantly reduces S_r. In native cells showing comparable cholesterol/phospholipid ratios, S_r increases in the following order: $C_{18:1c} < C_{16:0} + C_{18:1c} < C_{14:0} < C_{16:0} < C_{18:0}$, a sequence which correlates well with the sequence determined for nonelectrolyte permeability characteristics of *A. laidlawii* cells. This provides additional support for the hypothesis that changes in membrane lipid order directly affect the permeation rate of nonelectrolytes (21).

Adaptation of strain A5969 to low cholesterol resulted in the appearance of lipid-phase transitions within the range of temperatures studied. This observation is in agreement with the recognized effects of cholesterol on lipid-phase transitions in both artificial and biological membranes and also indicates that despite the very high protein/lipid ratio, most of the membrane lipids are not involved in an "immobilized boundary layer" around membrane proteins. Cholesterol also increases lipid ordering for temperatures higher than the liquid-gel transitions of oleate-enriched cells and palmitate-oleate-enriched cells, whereas it decreases lipid ordering for lower temperatures. However, this decrease in S_r is limited, which further demonstrates that cholesterol is not the only factor responsible for the high degree of ordering in the membranes of the native strain. Finally, the existence of lipid-phase transitions in adapted cells probably explains their increased sensitivity to mechanical stress and particularly the lysis which often occurs during harvesting of these cells.

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