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CHOLESTEROL IN MYCOPLASMA MEMBRANES

COMPOSITION, ULTRASTRUCTURE AND BIOLOGICAL PROPERTIES OF MEMBRANES FROM *MYCOPLASMA MYCOIDES* VAR. *CAPRI* CELLS ADAPTED TO GROW WITH LOW CHOLESTEROL CONCENTRATIONS

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SUMMARY

1. Serial passages of the sterol-requiring *Mycoplasma mycoides* var. *capri* in a serum-free medium supplemented with decreasing concentrations of cholesterol resulted in the adaptation of the organisms to grow with no added cholesterol. The cells of the adapted strain were osmotically more fragile than cells of the native strain and were more permeable to erythritol.

2. The cell membrane of the adapted strain contained low amounts of cholesterol (up to 3% of the total membrane lipid as against 22–25% in the native strain) but its polar lipids were more saturated than those of the native strain, as reflected by the preferential incorporation of [^{14}C]palmitic acid when added to the growth medium together with [^{14}C]oleic acid.

3. The native strain was capable of growing at temperatures as low as 25 °C, whereas the adapted strain could not. The lowering of the growth temperature of of the native strain resulted in a decrease in the cholesterol content of the membrane (from 24–15% of the total lipid) but had no effect on the fatty acid composition of the membrane polar lipids. On the other hand, aging of the culture increased the ratio of saturated to unsaturated fatty acids in both adapted and native strains, and decreased the cholesterol content of the native strain.

4. Freeze-etching of cells of the native and adapted strains, kept at 37 °C prior to freezing, showed a random distribution of particles on the fracture faces of the cell membranes. Keeping the cells at 4 °C prior to freezing caused the aggregation of particles on the fracture faces of the adapted strain but had no effect on the distribution of particles in the native strain.

5. Our data support the thesis that cholesterol functions as a regulator of membrane fluidity and that changes in the fatty acid composition of membrane lipids may act to compensate for the lack of cholesterol.

INTRODUCTION

Although cholesterol is an important component of many biological membranes, its role is not yet fully understood. Mycoplasmas may serve as most convenient

tools for the study of the role of cholesterol because it is an essential membrane component in all *Mycoplasma* species. Being unable to synthesize cholesterol, the mycoplasmas depend on its external supply from the growth medium[1]. This dependence may be utilized to change, within certain limits, the amount of cholesterol in the membrane and to analyze the effects of such variations on membrane properties. The closely related *Acholeplasma* species do not require cholesterol for growth, but are nevertheless able to incorporate it into their membrane, though in much smaller quantities than the sterol-requiring *Mycoplasma* species. The maximum amounts of cholesterol recorded in *Acholeplasma laidlawii* membranes did not exceed 8–10% of the total membrane lipids[2,3], while in *Mycoplasma* species cholesterol may comprise up to 40% of the total lipid[3,4]. Nevertheless, the incorporation of the relatively small amounts of cholesterol into *A. laidlawii* membranes was shown to be sufficient to decrease considerably the permeability of the cells to glycerol and erythritol and to reduce the energy content of the phase transition of membrane lipids[2,5].

To further pursue this line of investigation we found it of advantage to develop a model system for studying the role of cholesterol in membranes of the sterol-requiring mycoplasmas, organisms for which high concentrations of cholesterol are vital for growth. For this purpose *Mycoplasma mycoides* var. *capri* was adapted to grow in a medium without added cholesterol. The present communication describes the effects of this adaptation on the chemical composition and ultrastructure of the cell membrane and on some biological properties of the cells, such as osmotic fragility and permeability to nonionic solutes. The accompanying communication[6] deals with the effects of cholesterol depletion on the physical state of membrane lipids and on some enzymic and transport activities localized in the membrane.

MATERIALS AND METHODS

Organism and growth conditions

M. mycoides var. *capri* (strain PG3) was grown in 500 ml volumes of a modified Edward medium[7] in which PPLO serum fraction was replaced by 0.5% fatty-acid-poor bovine serum albumin (Calbiochem, San Diego, Calif.) and 1–10 μg/ml of cholesterol, palmitic acid and oleic or elaidic acid. For the labeling of membrane lipids 0.5 μCi of [1-^{14}C]oleic acid (59.7 Ci/mole), 0.5 μCi of [1-^{14}C]palmitic acid (55 Ci/mole) or 1 μCi of [4-^{14}C]cholesterol (55.8 Ci/mole) were added to each l of the growth medium. Growth was determined by measuring the absorbance of the culture at 640 nm or by titrating the excess acid formed during growth with a standard solution of 0.01 M NaOH. The organisms were harvested after 15–20 h of incubation at 37 °C and were washed twice in 0.25 M NaCl.

Assessment of osmotic fragility and permeability

Osmotic fragility of the washed cells was determined as described before[7]. Results were expressed as per cent lysis in 0.05 M NaCl calculated according to the formula: [(absorbance in 0.25 M NaCl − absorbance in 0.05 M NaCl)/ (absorbance in 0.25 M NaCl] · 100. Permeability of *M. mycoides* var. *capri* cells to erythritol was determined by measuring the initial swelling rates of cells suspended in 500 mM erythritol as described by De Gier *et al.*[8].

Isolation of cell membranes

Cell membranes were isolated by osmotic lysis of the organisms[9]. The membranes were collected by centrifugation at $34000 \times g$ for 30 min, washed twice in deionized water and resuspended in β-buffer[10] diluted 1:20 with deionized water (dilute β-buffer).

Lipid extraction

Lipids were extracted from freeze-dried cells or membranes by two successive extractions with chloroform–methanol (2:1, v/v), the first at 45 °C for 2 h and the second at room temperature overnight. The extracts were combined and washed according to Folch *et al.*[11], dried under a stream of N_2 and redissolved in 1 ml of chloroform.

Separation of neutral from polar lipids

Neutral lipids were separated from polar lipids by silicic acid chromatography. The lipid extracts were applied to columns (6 mm × 100 mm) of activated silicic acid (100 mesh, Mallinckrodt, St. Louis, Mo.) prewashed with chloroform. Elution was carried out successively with 50 ml chloroform and 50 ml chloroform–methanol (1:1, v/v) to separate the neutral from the polar lipids[12]. The lipid fractions were dried under a stream of nitrogen and redissolved in 0.5–1.0 ml of chloroform–methanol (2:1, v/v). Thin-layer chromatography of the neutral and polar lipids was carried out as described previously[4].

Gas–liquid chromatography

Methyl esters of the fatty acids of membrane polar lipids were prepared by heating the lipid samples for 18 h in anhydrous methanol containing 10% (w/w) HCl at 72 °C in a sealed pyrex ampule. The resultant methyl esters were extracted with light petroleum (b.p. 40–60 °C) and subjected to gas–liquid chromatography in a Packard model 840 instrument equipped with a polar column (200 cm × 0.3 cm, 15% of diethylene glycol adipate on chromosorb W). Fatty acids were identified by their retention time relative to that of standard methyl ester mixtures (Supelco. Inc., Bellefonte, Pa.).

Analytical methods

Protein was determined according to Lowry *et al.*[13]. Total cholesterol was determined by the $FeCl_3$ method[14]. Free cholesterol was separated from cholesterol esters by precipitation with digitonin[15]. Electrophoretic analysis of membrane proteins was carried out in polyacrylamide gels containing 0.1% sodium dodecylsulfate[16]. Total phosphorus in the lipid fraction was determined by the method of Ames[17] after digestion of the sample with an ethanolic solution of $Mg(NO_3)_2$. Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer using toluene-dioxane scintillation liquor[9].

Freeze-etching and electron microscopy

M. mycoides var. *capri* cells or membrane preparations were prepared for freeze-etching by suspending them either in dilute β-buffer or in dilute β-buffer containing 20% glycerol. Small droplets of these preparations were placed on 3-mm gold

specimen holders and were immediately frozen in liquid Freon 22 cooled by liquid air. The specimens were freeze-cleaved, etched and shadowed with platinum–carbon in a BA 360M Balzers' freeze-etching apparatus (Balzers, Fürstentum Liechtenstein) according to the method of Moor and Mühlethaler[18]. Etching of cleaved specimens in dilute β-buffer was done at -100 °C for 30 s. The platinum–carbon replicas were floated off on distilled water, cleaned once with 70% H_2SO_4 and then with 14% $HClO_4$, rinsed 3 times with dionized water, picked up on electron microscopic grids, and examined in a Phillips 300 electron microscope. To determine the state of dispersion of particles on the fracture face, 60–100 fracture faces of each sample were examined. Particles were considered aggregated when over one third of the area of the fracture face was free of particles, and considered dispersed when less than one third of the area was free of particles.

RESULTS

Adaptation of M. mycoides var. capri to growth in a cholesterol-poor medium

25 serial transfers of the sterol-requiring *M. mycoides* var. *capri* in a modified Edward medium containing bovine serum albumin, palmitic and oleic acids, and decreasing concentrations of cholesterol sufficed to adapt the organism to grow in the above-mentioned medium with no added cholesterol. Similar attempts to adapt *Mycoplasma hominis* and *Mycoplasma gallisepticum* to grow in the cholesterol-poor medium have failed so far. A single transfer of the adapted strain in a cholesterol-containing medium caused a loss of its ability to grow without cholesterol and serial passages were again needed for adaptation, ruling out the selection of mutants as the mechanism of adaptation. In all subsequent experiments the non-adapted native strain was grown in the medium supplemented with 10 μg cholesterol per ml while the adapted strain was grown in the same medium with no added cholesterol or with very little cholesterol (0.1–1 μg/ml). Since growth of the adapted strain with no added cholesterol was sometimes poor (depending probably on the batch of the basal medium) transfers of this strain were routinely carried out in the medium supplemented with 0.12 μg cholesterol/ml.

Effects of the adaptation on some biological properties of the cells

Temperature of growth. Table I shows that the native strain grew relatively well at a temperature as low as 25 °C, while the adapted strain grew well at 37 °C, less so at 30 °C and very poorly at 25 °C.

Osmotic fragility. The cells of the adapted strain were found to be very fragile, frequently undergoing lysis even in the growth medium. Therefore, their harvest, washing, and handling ought to be done with great care. Fig. 1 shows that throughout the temperature range of 10–40 °C the cells of the adapted strain were more fragile than those of the native strain, in particular at lower temperatures where the fragility of the native strain decreased, as is known for other mycoplasmas[19].

Permeability to erythritol. Cells of both the native and adapted strains behaved as ideal osmometers as determined by their osmotic swelling in sucrose solutions of different concentrations. The permeability of the native and adapted cells to erythritol as measured by the initial swelling rates of the cells suspended in isotonic erythritol is shown in Fig. 2. The swelling rate of the cholesterol-containing cells of the native

TABLE I

THE EFFECT OF TEMPERATURE ON THE GROWTH OF *M. MYCOIDES* VAR. *CAPRI* IN MEDIA CONTAINING VARIOUS CONCENTRATIONS OF CHOLESTEROL

The cells were grown in a modified Edward medium containing palmitic and oleic acids (5 μg/ml of each). Incubation time at 37 °C was 16 h, at 30 °C, 24 h, and at 25 °C, 48 h, when maximum growth yields were obtained. Growth was determined by measuring the absorbance of the cultures at 640 nm and by acid accumulation in the culture medium.

Temperature of growth (°C)	*Cells grown with 10 μg/ml cholesterol*		*Cells grown with 0.5 μg/ml cholesterol*		*Cells grown with no cholesterol*	
	Absorbance	*pH*	*Absorbance*	*pH*	*Absorbance*	*pH*
37	0.55	6.20	0.50	6.40	0.42	6.60
30	0.50	6.20	0.34	6.95	0.22	7.20
25	0.48	6.40	0.18	7.20	0.11	7.65

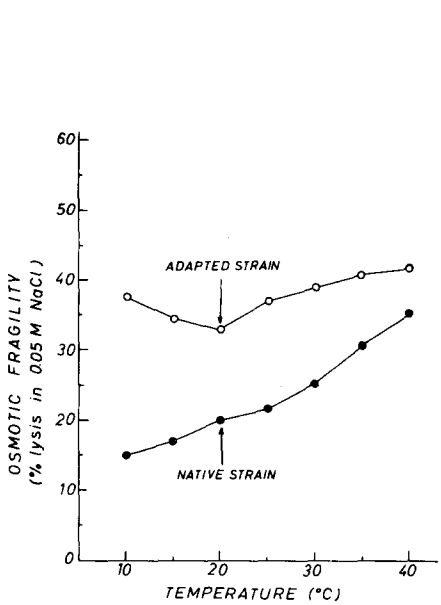

Fig. 1. Osmotic fragility of native and adapted *M. mycoides* var. *capri* strains at various temperatures.

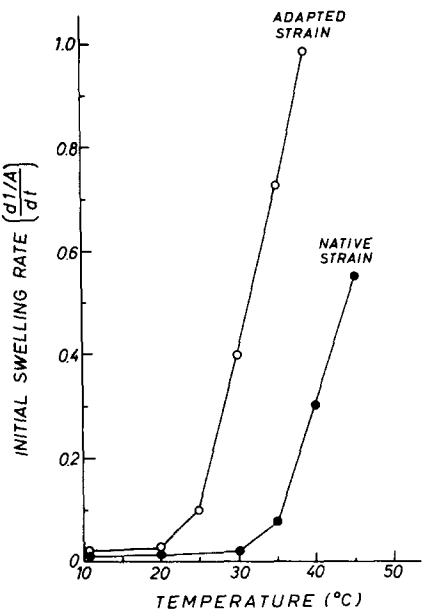

Fig. 2. Initial swelling rates of native and adapted *M. mycoides* var. *capri* cells suspended in isotonic erythritol solution at various temperatures.

strain was much lower than that of the adapted strain, indicating a lower permeability to erythritol.

Effects of the adaptation on the chemical composition of the cell membrane

Cholesterol content. Table II shows the marked decrease in the cholesterol content of the cell membrane occurring on the adaptation of *M. mycoides* var. *capri*

TABLE II

CHOLESTEROL CONTENT OF *M. MYCOIDES* VAR. *CAPRI* MEMBRANES FROM CELLS GROWN WITH VARIOUS CONCENTRATIONS OF CHOLESTEROL

The cells were grown in a modified Edward medium supplemented with palmitic and oleic acids (5 μg/ml of each) and harvested at the late logarithmic phase of growth.

Cholesterol added to medium (μg/ml)	*Cholesterol in cell membrane*	
	μg/mg membrane protein	*% of total lipid*
10	132.0	22.0
0.5	31.6	5.3
0	14.4	2.6

TABLE III

CHOLESTEROL CONTENT OF *M. MYCOIDES* VAR. *CAPRI* MEMBRANES FROM CELLS OF THE NATIVE STRAIN GROWN AT VARIOUS TEMPERATURES

Temperature of growth (°C)	*Incubation period (h)*	*Absorbance of culture (640 nm)*	*Cholesterol content of membranes (μg/μg lipid phosphorus)*
37	16	0.24	11.4
32	25	0.26	9.5
27	48	0.25	7.2

to grow with little or no cholesterol. As was previously shown[3] the cholesterol taken up from the growth medium was exclusively located in the cell membrane and could be recovered as free cholesterol. The native strain grown with 10 μg cholesterol/ml contained about 8–9 times more cholesterol than the adapted strain grown in the same medium, but with no cholesterol added. Yet, it is apparent from Table II that the adapted strain still contained detectable amounts of cholesterol. Since *M. mycoides* var. *capri*, like all other mycoplasmas, is incapable of synthesizing cholesterol[20], it is conceivable that the small amounts of cholesterol detected in the adapted strain originate from the basal Edward medium.

The growth temperature had a marked effect on the cholesterol content of the native strain. Table III shows the marked decrease in the ratio of cholesterol to membrane phospholipids on lowering the growth temperature. A similar effect was found upon aging (Table IV). The decrease was more pronounced when expressed as μg cholesterol per mg cell protein than when expressed as μg cholesterol per μg lipid phosphorus. This may indicate a decrease in the phospholipid content or a decrease in the membrane content of the cells on aging.

Fatty acid composition. The relative amounts of the major phospholipids and glycolipids were not found to differ significantly in the native and adapted strains. However, when the organisms were grown with radioactive fatty acids, the neutral

lipid fraction of the adapted strain was labeled about 10 times more intensively than that of the native strain (30% as against 2.7% of the total radioactivity in membrane lipids). About 15% of the radioactivity of the neutral lipid of the adapted strain was recovered in the free fatty acid fraction and almost all the rest in the diglyceride fraction. Since *M. mycoides* var. *mycoides* is incapable of synthesizing any of the long-chain fatty acids[20] the growth medium had to be routinely supplemented with a mixture of palmitic acid and either oleic or elaidic acid. The adaptation of the organisms to grow with no added cholesterol resulted in a 2-fold increase in the incorporation of [^{14}C]-palmitic acid residues into membrane polar lipids. The preferential incorporation of palmitate was reflected in the fatty acid composition of the membrane polar lipids shown in Table V. The saturated to unsaturated fatty acid ratio increased from 1.15 in the native strain to 1.56 in the adapted strain grown with equivalent concentrations (5 μg/ml) of palmitic and oleic acids. The higher degree of saturation of membrane polar lipids of the adapted strain was even more pronounced when the organisms were grown in a medium supplemented with 1 μg/ml of palmitic acid and 10 μg/ml of either oleic or elaidic acid (Table VI). Tables V and

TABLE IV

CHOLESTEROL CONTENT OF THE NATIVE *M. MYCOIDES* VAR. *CAPRI* STRAIN HARVESTED AT DIFFERENT AGES OF CULTURE

The cells were grown in a modified Edward medium supplemented with palmitic and oleic acids (5 μg/ml of each) and 10 μg/ml cholesterol.

Age of culture (h)	*Absorbance of culture (640 nm)*	*Cholesterol content of cells*	
		μg/mg cell protein	*μg/μg lipid phosphorus*
18	0.22	108.6	9.4
20	0.35	51.0	6.2
22	0.54	31.5	5.4

TABLE V

FATTY ACID COMPOSITION OF *M. MYCOIDES* VAR. *CAPRI* POLAR LIPIDS FROM CELLS GROWN WITH VARIOUS CONCENTRATIONS OF CHOLESTEROL

The medium was supplemented with palmitic and oleic acids (5 μg/ml of each).

Fatty acid	*Cholesterol concentration in medium:*	*10 μg/ml*	*1 μg/ml*	*0 μg/ml*
12:0		0.8	0.7	1.0
14:0		0.9	1.0	0.6
16:0		27.5	39.4	43.8
18:0		23.9	20.6	17.1
18:1		37.4	30.0	30.0
18:2		8.4	10.9	9.1
Satd/unsatd ratio		1.15	1.49	1.56

TABLE VI

FATTY ACID COMPOSITION OF *M. MYCOIDES* VAR. *CAPRI* POLAR LIPIDS FROM CELLS GROWN WITH VARIOUS CONCENTRATIONS OF CHOLESTEROL

The medium was supplemented with palmitic acid (1 μg/ml) and either oleic or elaidic acid (10 μg/ml).

Fatty acid	*Cells grown with oleic acid*		*Cells grown with elaidic acid*	
	Cholesterol 10 μg/ml	*No cholesterol added*	*Cholesterol 10 μg/ml*	*No cholesterol added*
14:0	0.9	0.5	0.5	1.1
16:0	23.9	36.3	20.8	30.1
16:1	0.8	0.5	0.5	1.0
18:0	18.2	20.9	11.3	11.7
18:1	41.2	38.6	59.6	50.1
18:2	10.1	4.1	7.2	5.7
Satd/unsatd ratio	0.86	1.38	0.49	0.79

TABLE VII

CHOLESTEROL CONTENT OF *M. MYCOIDES* VAR. *CAPRI* MEMBRANES FROM CELLS GROWN WITH CHOLESTEROL (10 μg/ml) AND VARIOUS FATTY ACIDS

Fatty acids added to medium (μg/ml)	*Cholesterol content of cell membrane*	
	μg/mg membrane protein	*μg/μg lipid phosphorus*
Oleic acid (5) + palmitic acid (5)	112.8	6.5
Oleic acid (10) + palmitic acid (1)	152.4	8.3
Elaidic acid (10) + palmitic acid (1)	140.4	7.7

VI also show the increase in the degree of unsaturation of the polar lipids of the native strain grown with 10 μg cholesterol per ml when the fatty acid mixture was changed to 1 μg/ml palmitic acid and 10 μg/ml of either oleic or elaidic acid. This change was accompanied by an increase in the amount of cholesterol incorporated into the membrane (Table VII).

Lowering of the growth temperature of the native strain from 37 to 25 °C did not cause any significant change in the fatty acid composition of the membrane polar lipids. On the other hand, aging of the culture increased the ratio of saturated to unsaturated fatty acids in the adapted strain (Table VIII). A similar variation, but to a smaller extent, was also found on aging of the native strain.

Protein composition and serological behavior. Only minor quantitative differences in the electrophoretic patterns of membrane proteins were observed on examination of the membranes of the native and adapted strains (Fig. 3). Growth of the adapted strain was inhibited by an antiserum prepared against the native strain. The growth-inhibition zone was about the same for both strains when tested under the same conditions by the method of Clyde[21].

TABLE VIII

FATTY ACID COMPOSITION OF THE POLAR LIPIDS OF THE ADAPTED *M. MYCOIDES* VAR. *CAPRI* STRAIN HARVESTED AT DIFFERENT AGES OF CULTURE

The cells were grown in a modified Edward medium supplemented with palmitic and oleic acids (5 μg/ml of each).

Age of culture (h)	*Absorbance of culture (640 nm)*	*Fatty acid (% of total)*				
		Palmitic acid	*Stearic acid*	*Oleic acid*	*Linoleic acid*	*Satd/unsatd ratio*
16	0.19	25.1	21.1	38.7	6.3	0.98
18	0.26	27.5	26.1	36.0	6.0	1.26
20	0.40	32.7	25.5	30.4	7.7	1.51

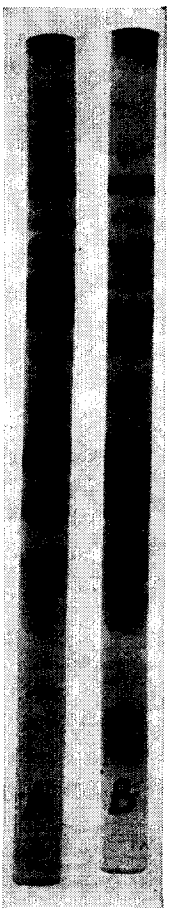

Fig. 3. Electrophoretic patterns of membrane proteins of *M. mycoides* var. *capri* in polyacrylamide gels containing sodium dodecyl sulfate. (A) membranes of the adapted strain; (B) membranes of the native strain.

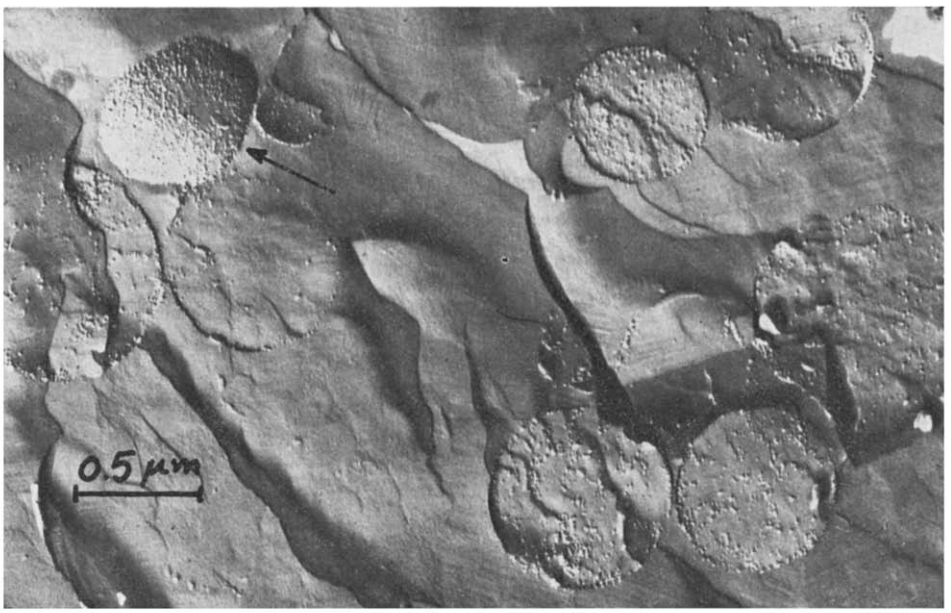

Fig. 4. Platinum–carbon replica of freeze-cleaved cells of the native *M. mycoides* var. *capri* strain suspended in 20% glycerol. The only fracture face is marked by an arrow; all other membranes were sectioned rather than fractured. Fracturing was carried out at −150 °C.

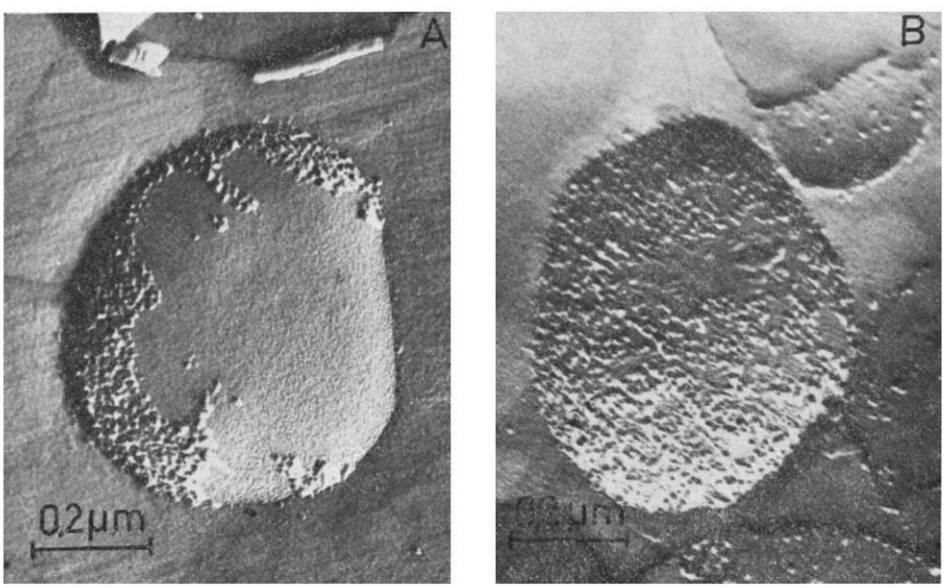

Fig. 5. Platinum–carbon replica of the convex fracture faces of freeze-cleaved *M. mycoides* var. *capri* membranes suspended in 20% glycerol and incubated at 4 °C prior to freezing. (A) membranes of the adapted strain; (B) membranes of the native strain.

Effects of the adaptation on the ultrastructure of the membranes

Replicas of cells suspended in 20% glycerol and fractured at −150 °C without etching (Fig. 4) showed that almost 50% of the cell membranes of the native strain were sectioned rather than fractured (66 sections *versus* 72 fractures), whereas only 25% of the cell membranes of the adapted strain were sectioned (23 sections *versus* 72 fractures). The ultrastructural appearance of the fractured faces of the cell membranes of the native and adapted strains isolated at 37 °C and kept at the same temperature for 60 min prior to transfer to the freezing medium were similar, showing

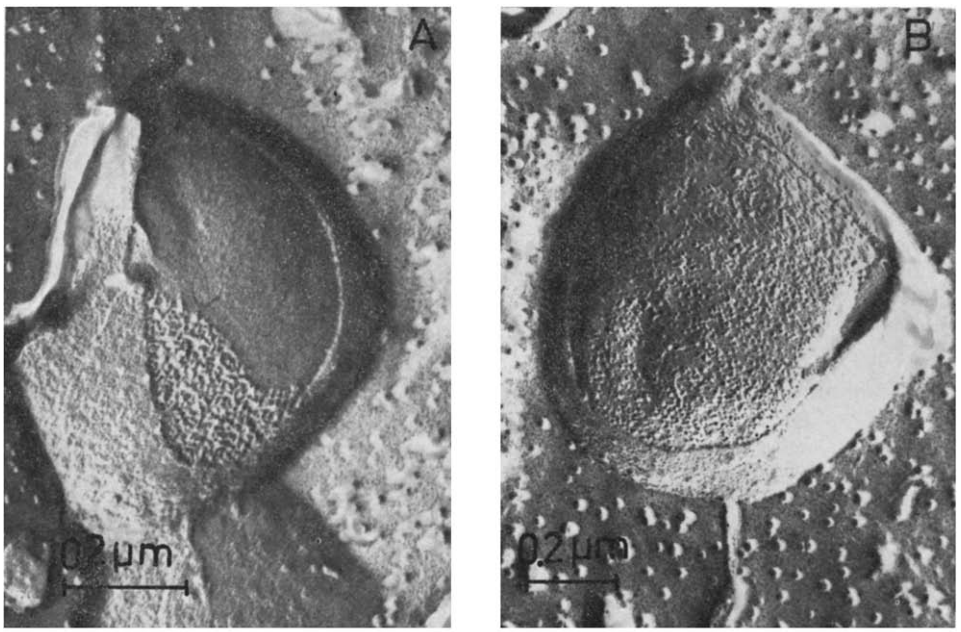

Fig. 6. Platinum–carbon replica of the convex fracture faces of freeze-cleaved and deep-etched *M. mycoides* var. *capri* membranes incubated at 4 °C prior to freezing. (A) membranes of the adapted strain; (B) membranes of the native strain.

TABLE IX

DISTRIBUTION OF PARTICLES ON THE FRACTURE FACES OF *M. MYCOIDES* VAR. *CAPRI* MEMBRANES FROM CELLS GROWN WITH DIFFERENT CHOLESTEROL CONCENTRATIONS

Distribution of particles	*Cells grown with 0.1 µg/ml cholesterol*		*Cells grown with 10 µg/ml cholesterol*	
Temp of membrane isolation:	*4 °C*	*37 °C*	*4 °C*	*37 °C*
Aggregated *	85	9	11	15
Dispersed	16	51	70	71

* Over 1/3 of the fracture face is free of particles.

a random distribution of particles on the faces (Fig. 5). Incubation of the membranes at 4 °C prior to freezing caused the aggregation of the particles on the fracture faces of the adapted strain leaving large smooth areas, but had no effect on the distribution of particles on the fracture faces of the native strain (Fig. 5 and Table IX). Similar results were obtained with freeze-etched membranes suspended in dilute β-buffer with no glycerol (Fig. 6).

DISCUSSION

The ability to adapt a sterol-requiring mycoplasma to grow with very little cholesterol permits the critical analysis of the effects of variations in the cholesterol content on membrane properties, thus providing a most useful tool for studying the role of cholesterol in biomembranes. *M. mycoides* var. *capri* appeared to be a suitable candidate for adaptation since its cholesterol requirement was shown to be less stringent than that of other *Mycoplasma* species[22]. Nevertheless, it is unclear as yet whether the adapted strain can grow in the total absence of cholesterol. The available evidence rather suggests that very small amounts of cholesterol are still required for growth and that these are apparently provided by the traces of cholesterol present in the growth medium. A drop below a certain critical level in the cholesterol content of the growth medium is probably responsible for the frequent failure to obtain satisfactory growth of the adapted strain in certain batches of the medium, unless supplemented with 0.1 μg cholesterol per ml. These observations are in accord with those of Rodwell *et al.*[23] who failed to adapt the goat mycoplasma strain Y to grow in a defined medium with no cholesterol. The addition of 0.5 to 1.0 μM (0.19–0.38 μg/ml) cholesterol was found to be essential for growth in this case.

The adaptation of *M. mycoides* var. *capri* to grow with very little cholesterol was accompanied by a marked increase in the saturated fatty acid content of the membrane polar lipids. The preferential incorporation of the saturated fatty acids from the growth medium may compensate, at least partially, for the lack of cholesterol as these acids will exert a similar condensing effect on the hydrocarbon chains of the membrane lipid core[24]. Nevertheless, EPR measurements[6] indicated that the membranes of the adapted strain, although containing more saturated fatty acids, were still more fluid at temperatures above 25 °C than membranes of the native, cholesterol-containing strain. It is of interest to mention in this context that changes in the cholesterol content of *A. laidlawii* membranes, introduced by growing the organisms with different concentrations of cholesterol, did not influence the fatty acid composition of its membrane lipids[2]. However, it should be stressed that, unlike *M. mycoides* var. *capri*, this organism does not require cholesterol for growth, and the mechanism controlling membrane fluidity in *A. laidlawii* may differ from that of *M. mycoides* by not being dependent on cholesterol.

The variations in membrane lipid composition observed on lowering the growth temperature of microorganisms have long been taken as evidence for the existence of a mechanism regulating the fluidity of the lipid domain in biomembranes. The marked increase in the percentage of unsaturated and cyclopropane fatty acids in *Escherichia coli* membrane lipids occurring on lowering the growth temperature is well documented[25,26]. Our results with the native *M. mycoides* var. *capri* strain do not show any significant variations in the fatty acid composition, but do show a

decrease in the cholesterol content of the membrane on lowering the growth temperature. A striking result found in the present study was the dissimilarity in the response of the native and adapted strains to low temperatures. The growth of the adapted strain was much more affected by low temperature than that of the native strain. This might be due to the inability of the adapted organisms to alter their fatty acid composition when grown at low temperatures. In the absence of cholesterol the membrane lipids of the adapted strain were found to undergo a phase transition at a temperature of about 25 °C[6]. Thus, the gelation of the membrane lipid core at temperatures around 25 °C may be responsible for the cessation of growth of the adapted strain. The presence of significant amounts of cholesterol in the membrane of the native strain prevents the thermal phase transition of the lipid core[6] and may thus be correlated with the ability of the native strain to grow at 25 °C.

The increase in permeability of the adapted cells to erythritol is in agreement with earlier observations showing that the presence of cholesterol in biological membranes and in lecithin liposomes lowers the permeability of the membranes for various substances[2,5,7,27], apparently by causing the tighter packing of the hydrocarbon chains in the membrane lipid core. Although the increase in the proportion of saturated fatty acids in the adapted strain should minimize the effects of cholesterol depletion on erythritol permeability, the differences in permeability between the native and adapted strains, as measured by swelling rates, were greater than those described in similar experiments using liposomes[2,27] and *A. laidlawii* cells[2,5]. However, determination of permeability by swelling rates may be affected by some other, yet unmeasured, factors such as membrane elasticity and cell shape, which apparently differ in the various membrane systems examined.

The increased osmotic fragility of the adapted strain resembles the increase in the fragility of erythrocytes on the partial removal of cholesterol from their cell membrane[28]. The increase in osmotic fragility could be explained either on the basis of a decreased resistance of the membrane to brittle fracturing due to a reduced molecular cohesion of membrane constituents or to differences in cell shape. Thus, *M. mycoides* var. *capri*[29] and *M. mycoides* var. *mycoides*[30] cells grown in a cholesterol-deficient medium lost their characteristic filamentous shape and became spherical. This morphological change was accompanied by a marked increase in osmotic fragility[29,30].

Freeze-etching enables one to compare the hydrophobic membrane core of the native and adapted strains. Smooth-faced areas are believed to be mainly lipid domains while the particles are apparently of protein nature[31]. Chilling the adapted *M. mycoides* var. *capri* cells to 4 °C causes the aggregation of particles leaving 2/3 of the fracture faces particle-free. This aggregation phenomenon is probably due to structural alterations in the membrane lipid core such as the crystallization of the lipid phase[32–34]. No such aggregation could be demonstrated with the cholesterol-containing native strain supporting the physical data showing that cholesterol prevents the hydrocarbon chains from crystallization[6].

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