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Sterols in Membranes: Growth Characteristics and Membrane Properties of *Mycoplasma capricolum* Cultured on Cholesterol and Lanosterol†

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ABSTRACT: *Mycoplasma capricolum*, a procaryotic sterol auxotroph, shows optimum growth on cholesterol and substantial growth on lanosterol. The effect of the two sterols on membrane fluidity, their ability to support growth on a broad range of fatty acid combinations, and a possible synergism of low amounts of cholesterol and high amounts of lanosterol were studied. When the cholesterol content of *M. capricolum* membranes rose from 14 to 28 mol % of the total lipid, their microviscosities increased from $\bar{\eta}$ = 3.3 to 4.5, whereas membranes containing 14–23 mol % lanosterol showed a constant microviscosity value of $\bar{\eta}$ = 3.1. Arrhenius plots of the microviscosity values of membranes rich in cholesterol were linear over a 40 °C range, but those rich in lanosterol exhibited prominent discontinuities at 20 and 25 °C. Cholesterol allowed the cells to grow on media containing a wide variety of fatty acid supplements, whereas lanosterol supported growth only with certain fatty acid combinations. Finally, low levels of cholesterol unable to support the growth of *M. capricolum* produced a synergistic effect on growth when combined with lanosterol. The results demonstrate the superiority of cholesterol compared to lanosterol as a membrane sterol and suggest a possible role for cholesterol in addition to or other than to regulate bulk membrane fluidity.

Recent studies in this laboratory have disclosed marked differences in the membrane behavior of cholesterol and lanosterol, a trimethyl-substituted precursor of cholesterol. Incorporated into model membranes such as phosphatidylcholine vesicles, lanosterol, in contrast to cholesterol, neither raises the microviscosity nor reduces the exit of vesicle-entrapped glucose (Lala et al., 1978). Carbon-13 nuclear magnetic resonance studies have shed light on the physical basis for these differences; lanosterol is much less immobilized in lecithin lipid bilayers than cholesterol (Yeagle et al., 1977). In eucaryotic sterol auxotrophs lanosterol has also been shown to be incompetent as a substitute for cholesterol (Clark & Bloch, 1959; Chang et al., 1977). Unexpectedly, however, *Mycoplasma capricolum*, a sterol-requiring procaryote, was found to grow moderately well on lanosterol, without modifying it, as well as on cholesterol (Odriozola et al., 1978). This relatively broad sterol specificity offers the opportunity for evaluating the possibility of multiple sterol functions in membranes and the importance of membrane physical state as a determinant of mycoplasma growth. To this end, we have (a) compared the relative ability of cholesterol and lanosterol to modulate membrane fluidity and (b) examined the effect of supplementing lanosterol-containing growth media with low levels of cholesterol. We find that quantities of cholesterol, inadequate by themselves, will, when combined with lanosterol, support mycoplasma growth in a synergistic manner. We also

† From the James Bryant Conant Laboratories, Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138. *Received September 19, 1979.* This work was supported by a grant from the Camille and Henry Dreyfus Foundation to C.E.D. and grants from the National Institutes of Health, the National Science Foundation, and the Eugene P. Higgins Fund of Harvard University to K.B.

0006-2960/80/0419-1467$01.00/0

compare the lipid patterns and membrane microviscosities of mycoplasma cells maintained on the two sterols singly or in combination and the effects of various fatty acid supplements on mycoplasma growth.

Materials and Methods

Chemicals. Cholesterol (Sigma) was recrystallized from ethanol and dried in vacuo. Lanosterol was purified according to Bloch & Urech (1958). Palmitic, stearic, oleic, and linoleic acids were purchased from Supelco; myristic, arachidic, and elaidic acids were from Sigma. Essentially fatty acid free bovine albumin, prepared from Fraction V albumin, was purchased from Sigma.

Growth of Organism and Membrane Preparation. M. capricolum (California Kid strain 14, ATCC 27342) was cultured on lipid-depleted modified Edward medium (Razin & Rottem, 1976) prepared as described in the preceding paper (Dahl et al., 1980). PPLO–serum fraction was replaced by 4 mg/mL bovine albumin, sterol, 5 μg/mL saturated fatty (SFA),[1] and 6.5 μg/mL unsaturated fatty acid (UFA) as indicated under Results. Growth studies were performed in 100 mL of media, and mass doubling times were calculated as previously described (Dahl et al., 1980). Mass doubling times on cholesterol-enriched media routinely fell between 2 and 3 h whereas those for lanosterol-supported growth varied between 6 and 10 h. Variation in growth rates on lanosterol was dependent on the lot of bovine albumin used in the growth media.[2] Data in Tables I–IV were obtained by using lot 48C-7171 whereas data in Figure 2 were obtained with lot 49C-7100. Cells were harvested and membranes were prepared as described in the preceding paper (Dahl et al., 1980).

Lipid Extraction and Analysis. Lipids were extracted according to Folch et al. (1957) and analyzed by gas–liquid chromatography as described in the preceding paper (Dahl et al., 1980).

Microviscosity. Membrane microviscosities were determined in an Elscint microviscosimeter, Model MV-1A, using 1,6-diphenyl-1,3,5-hexatriene (DPH) as a fluorescent probe. Membranes (1 mg of protein/mL) in deionized water were mixed with an equal volume of 1 μM DPH in 10 mM NaCl and incubated at 37 °C for 30 min. *P* values (polarization) were recorded at 37 °C or at the temperatures indicated under Results, and microviscosity ($\bar{\eta}$) was calculated according to Shinitzky & Inbar (1974).

Protein Content. Membrane protein was determined by the method of Lowry et al. (1951).

Results

Effect of Sterol Concentration on Growth Characteristics, Membrane Lipid Composition, and Microviscosity. It has been reported that some *Mycoplasma* species can be "adapted" to grow on low levels of cholesterol by multiple serial transfers. Such cells contain a drastically reduced level of cholesterol in the cell membrane (Rottem et al., 1973a). In a similar study, we examined the membrane properties of our native strain of *M. capricolum* grown on low sterol levels but without prior adaptation. As shown in Table I, varying the cholesterol supply in the growth medium from 10 to 1 μg/mL had only a slight effect on the growth rates, but absorbances of cultures in the late log phase were reduced when the external cholesterol concentration was lowered to 1 μg/mL. It is known that amounts of cholesterol in this range are essential for supporting detectable growth of cholesterol-requiring *Mycoplasmas* (Rodwell et al., 1970; Rottem et al., 1973a). On the other hand, lowering the external lanosterol concentration from 10 to 2 μg/mL affected neither the growth rate nor the late log absorbance of the cultures. With both cholesterol and lanosterol, the lower the sterol concentration in the growth medium the lower the sterol content of the cell membranes. The values ranged from 28 to 13 mol % for cholesterol-enriched and 23 to 14 mol % for lanosterol-enriched membranes. Cholesterol-poor membranes also had markedly lower microviscosity ($\bar{\eta}$) values, whereas a decrease in membrane lanosterol content did not change microviscosity. It should be noted that the low $\bar{\eta}$ values for lanosterol-enriched membranes ($\bar{\eta}$ = 3.10) were of the same magnitude as those shown by cells grown at limiting cholesterol concentrations ($\bar{\eta}$ = 3.34). In both cholesterol- and lanosterol-containing membranes, a decrease in membrane sterol content decreased the relative proportion of SFA to UFA but only very slightly. Lanosterol-grown cells had a somewhat higher SFA content than cholesterol-grown cells, as observed previously (Dahl et al., 1980).

Table I: Effect of Sterol Concentration on the Growth Characteristics, Fatty Acid Distribution, and Microviscosity of *M. capricolum* Membranes[a]

sterol added to growth medium (μg/mL)		mass doubling time (h)	A_{640} of late log culture	mol % sterol of total lipid	SFA/UFA ratio	$\bar{\eta}$ at 37 °C
cholesterol	lanosterol					
10		2.0	0.40	28.0	0.88	4.53
5		2.1	0.45	23.9	0.85	4.09
1		2.5	0.19	13.5	0.79	3.34
	10	6.2	0.26	22.6	1.00	3.16
	5	6.2	0.25	14.7	0.87	3.10
	2	6.6	0.20	14.6	0.87	3.10

[a] The cells were cultured at 37 °C on a medium containing 5 μg/mL palmitate, 6.5 μg/mL elaidate, and the amounts of sterol indicated. In all cases the added fatty acids accounted for >95% of the fatty acids found.

Effect of Cholesterol or Lanosterol on the Temperature Dependence of Membrane Microviscosity. The effect of temperature on the physical state of the membrane lipid bilayer as determined by the fluorescent probe DPH is presented in Figure 1. The Arrhenius plot of the microviscosity of membranes from cells raised on lanosterol and a palmitate-elaidate mixture exhibits prominent discontinuities at 20 and 25 °C. For cholesterol-containing membranes this relationship is strictly linear.

Effect of Exogenous Fatty Acids on Growth Characteristics, Lipid Composition, and Membrane Microviscosity of M. capricolum Cultured on Cholesterol or Lanosterol. Physical studies with model membranes and the data in Table I indicate that lanosterol, unlike cholesterol, cannot condense phospholipid fatty acyl chains nor modulate membrane fluidity. It therefore seemed likely that the fatty acid requirement of *M. capricolum*, a fatty acid auxotroph, may be stricter for cells cultured on lanosterol instead of cholesterol. This was tested by systematically varying the structures of fatty acids added to the media and measuring growth. In combination with cholesterol, elaidate alone or elaidate with either myristate,

[1] Abbreviations used: SFA, saturated fatty acid; UFA, unsaturated fatty acid; DPH, 1,6-diphenyl-1,3,5-hexatriene; $\bar{\eta}$, microviscosity; *A*, absorbance.

[2] In some instances the amount of bovine albumin in the medium was found to influence the growth rate, the maximum absorbance, and the trace amounts of membrane cholesterol (around 2% of total sterol) of cells grown on lanosterol. Better growth and lower residual membrane cholesterol (<1% of total sterol) were obtained when the bovine albumin content of medium containing 10 μg/mL lanosterol was decreased from 4 to 1 mg/mL. Cholesterol (10 μg/mL) produced the same growth response at either bovine albumin concentration.

Table II: Growth of *M. capricolum* in Medium Containing Cholesterol or Lanosterol and Various Fatty Acids[a]

sterol added to growth medium	SFA added to growth medium	A_{640nm} of late log culture: UFA added to growth medium $18:1_t$	$18:1_c$	$18:2_{c,c}$
cholesterol	none	0.43	*b*	*b*
	14:0	0.38	0.38	*b*
	16:0	0.42	0.48	0.43
	18:0	0.48	0.47	*b*
	20:0	0.42	*b*	*b*
lanosterol	none	<0.07	*b*	*b*
	14:0	<0.07	<0.05	*b*
	16:0	0.25	0.20	<0.07
	18:0	0.16	0.18	*b*
	20:0	0.26	*b*	*b*

[a] Lipid-depleted modified Edward media were supplemented with 10 μg/mL sterol and with 5 μg/mL SFA and 6.5 μg/mL UFA or 11.5 μg/mL elaidate alone. [b] Not tested.

palmitate, stearate, or arachidate, oleate with myristate, palmitate, or stearate, or linoleate with palmitate produces very similar growth characteristics (Table II). The rates of growth on cholesterol and all fatty acid combinations varied only in a very narrow range. With lanosterol as the sterol source some fatty acid combinations supported growth but others did not. Elaidate with palmitate, stearate, or arachidate and oleate with palmitate and stearate elicited similar growth responses (Table II). However, elaidate alone, myristate–elaidate, myristate–oleate, or palmitate–linoleate failed to support significant growth, in sharp contrast to the results obtained with cholesterol. Membranes prepared from cultures grown on elaidate and saturated fatty acids of varying chain length as described above were further characterized by sterol and fatty acid analyses and by measuring microviscosities (Table III). The sterol content of cholesterol-enriched membranes ranged between 25 and 33 mol % and from 21 to 23 mol % for lanosterol-enriched membranes. Much greater variations were seen in the SFA/UFA ratios. They decreased substantially with increasing SFA chain length (Table III). This trend was observed for both membrane types but was more pronounced for lanosterol-grown cells. Moreover, changes in the chain length of the SFA in combination with elaidate made little difference in the respective microviscosities (Table III). The values ranged from $\bar{\eta}$ = 4.68 to 5.1 and from $\bar{\eta}$ = 2.73 to 3.27 for cholesterol- and lanosterol-enriched membranes, respectively.

Substituting the lower melting oleate for elaidate in combination with palmitate gave a different pattern (Table IV). The SFA/UFA ratio increased significantly in lanosterol-enriched membranes, but no such effect occurred in cholesterol-enriched membranes. Similar results were found for both membrane types when stearate instead of palmitate was

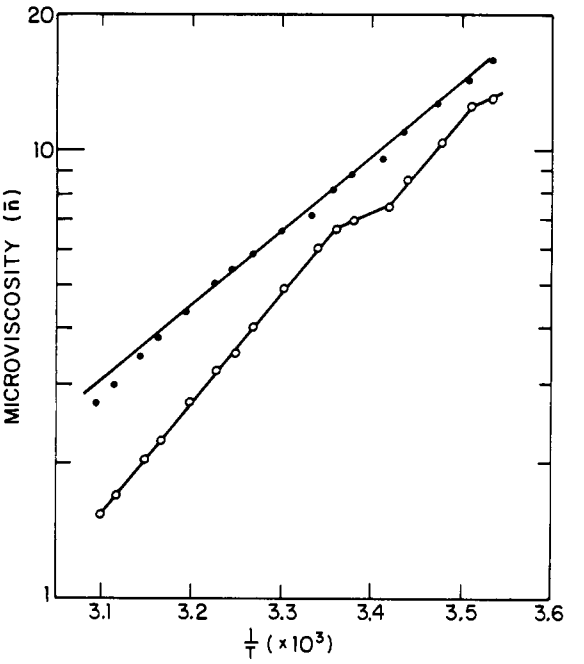

FIGURE 1: Effect of temperature on the microviscosity of the lipid bilayer of membranes from *M. capricolum* grown on 10 μg/mL cholesterol, 5 μg/mL palmitate, and 6.5 μg/mL elaidate (●) or 10 μg/mL lanosterol, 5 μg/mL palmitate, and 6.5 μg/mL elaidate (○).

combined with elaidate or oleate. Furthermore, substituting oleate for elaidate in cholesterol-grown cells lowered membrane microviscosity significantly from $\bar{\eta}$ = 4.84 to 3.84. The same substitution (oleate for elaidate) did not change the microviscosity of membranes enriched with lanosterol (Table IV). The latter result may be related in part to the increase in the SFA/UFA ratio in these membranes which did not occur in the corresponding cholesterol experiments.

Effect of Low Levels of Cholesterol on the Growth and Membrane Properties of M. capricolum Cultured on Lanosterol. In the companion paper we raised the question of whether low levels of cholesterol may be required for the growth of *M. capricolum* when lanosterol is the added source of sterol (Dahl et al., 1980). Such a requirement is difficult to prove because the growth medium retains trace amounts of cholesterol even after exhaustive delipidation. We approached this problem by culturing cells on a medium containing a constant amount of lanosterol and small but increasing concentrations of cholesterol. The delipidated medium which fails to support any measurable growth contained ~0.02 μg/mL cholesterol in these experiments. Growth curves of *M. capricolum* on limiting amounts of cholesterol with and without lanosterol are given in parts a and b of Figure 2. The inoculum for all cultures was a 1:1000 dilution of cholesterol-grown cells in the late logarithmic phase of growth. All

Table III: Effect of Saturated Fatty Acid Chain Length on the Lipid Composition and Membrane Microviscosity of *M. capricolum* Grown on Cholesterol or Lanosterol[a]

sterol added to growth media	SFA added to growth media	mol % sterol of total lipid	fatty acid composition of membrane lipids (wt % distribution): 14:0	16:0	18:0	18:1	20:0	SFA/UFA ratio	$\bar{\eta}$ at 37 °C
cholesterol	14:0	25.0	39.4	3.5	3.1	53.9	<0.1	0.85	4.68
	16:0	33.0	<0.1	40.0	5.0	55.0	<0.1	0.81	4.84
	18:0	26.8	<0.1	0.6	37.6	61.8	<0.1	0.61	5.11
	20:0	31.2	<0.1	3.2	1.8	69.0	25.9	0.44	4.99
lanosterol	16:0	20.8	<0.1	46.0	2.1	50.9	<0.1	0.96	3.27
	18:0	22.7	<0.1	6.7	32.4	60.7	<0.1	0.64	3.02
	20:0	21.4	<0.1	3.7	2.8	73.6	19.9	0.35	2.73

[a] Lipid-depleted modified Edward media contained 10 μg/mL sterol, 6.5 μg/mL elaidate, and 5 μg/mL saturated fatty acid.

Table IV: Effect of Unsaturated Fatty Acid Configuration on the Lipid Composition and Membrane Microviscosity of *M. capricolum* Grown on Cholesterol or Lanosterol[a]

sterol added to growth media	UFA added to growth media	mol % sterol in total lipid	fatty acid composition of membrane lipids (wt % distribution)			SFA/UFA ratio	$\bar{\eta}$ at 37 °C
			16:0	18:0	18:1		
cholesterol	$18:1_t$	33.7	40.0	5.0	55.0	0.81	4.84
	$18:1_c$	36.6	45.0	2.3	52.7	0.89	3.84
lanosterol	$18:1_t$	20.8	46.9	2.1	50.9	0.96	3.27
	$18:1_c$	23.8	61.8	1.9	36.1	1.70	3.29

[a] Lipid-depleted modified Edward media contained 10 μg/mL sterol, 5 μg/mL palmitate, and 6.5 μg/mL unsaturated fatty acid.

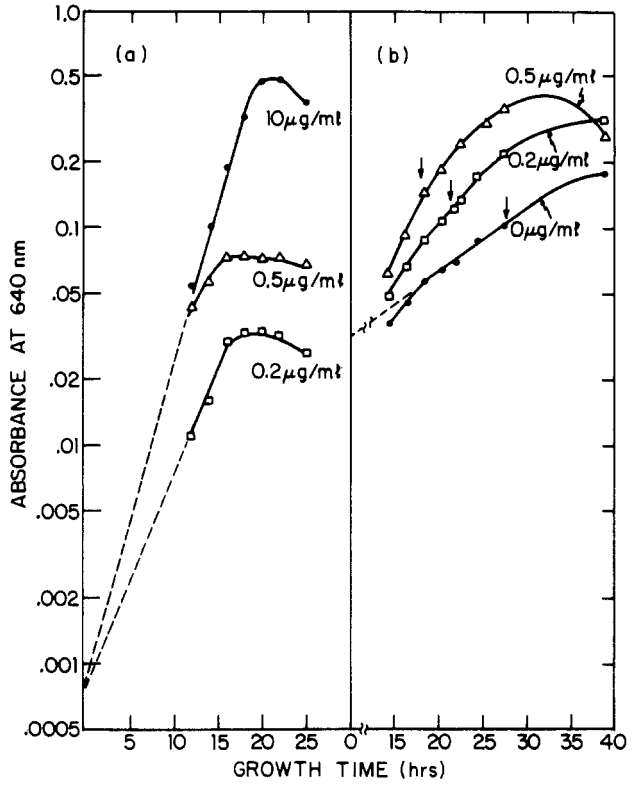

FIGURE 2: Growth curves of *M. capricolum* cultured at 37 °C on media containing (a) 5 μg/mL palmitate, 6.5 μg/mL elaidate, and the amounts of cholesterol indicated or (b) 5 μg/mL palmitate, 6.5 μg/mL elaidate, 10 μg/mL lanosterol, and the amounts of cholesterol indicated.

the growth curves for cholesterol-grown cells could be extrapolated back to an initial absorbance of $\sim 8 \times 10^{-4}$ (Figure 2a). In contrast, the growth curve for cells on lanosterol-supplemented media (with no added cholesterol) (Figure 2b), if extrapolated along a line corresponding to the exponential phase of growth, yields a considerably higher initial absorbance (3×10^{-2}). This indicated that the initial growth rate on lanosterol is faster than the rate measured in the exponential phase and suggests a synergistic effect between lanosterol and some component in the medium or the inoculum, presumably cholesterol. Indeed low levels of cholesterol not only increase the rate of growth on lanosterol but also raise the absorbance at 640 nm of late log cultures far above that seen with a corresponding dosage of cholesterol alone. For example, the maximum absorbance of cultures grown on 10 μg/mL lanosterol alone was ~0.18 *A* units and on 0.2 μg/mL cholesterol alone was 0.03. When combined, the same amounts of the two sterols afforded a maximum absorbance of ~0.30. The effects seem to be more than additive (Table V).

Table V: Growth of *M. capricolum* Cultured on Cholesterol Alone or Lanosterol with Graded Amounts of Cholesterol[a]

cholesterol added to growth medium (μg/mL)	absorbance of culture at 640 nm	
	without lanosterol	with lanosterol
10	0.5	
0.5	0.07	0.40
0.2	0.03	0.30
0	0	0.18

[a] Data were taken from Figure 2.

Table VI: Effect of Low Levels of Cholesterol on the Sterol Content, Fatty Acid Distribution, and Microviscosity of *M. capricolum* Cultured on Lanosterol

sterol added to growth medium (μg/mL)		mol % sterol of total lipid		SFA/UFA ratio	$\bar{\eta}$ at 37 °C
lanosterol	cholesterol	lanosterol	cholesterol		
10	0	24.4	0.6	1.13	3.23
10	0.2	21.5	2.1	1.06	3.23
10	0.5	17.8	3.0	0.99	3.23

The cultures described in Figure 2b were harvested at the times indicated by the arrows and analyzed for sterol and fatty acid content and membrane microviscosity (Table VI). Increasing cholesterol added to the lanosterol-containing medium from 0 to 0.5 μg/mL raised the membrane cholesterol content from 0.6 to 3.0 mol % of the total lipid. The lanosterol level of the same membranes dropped slightly, from 25 to 18 mol %, and total sterol ranged between 21 and 25 mol %. The SFA/UFA ratios of the corresponding membranes fall between 0.99 and 1.1 whereas their microviscosity values remain constant at $\bar{\eta} = 3.23$. A comparison of the lanosterol to cholesterol ratio added in the growth medium and that found in the membrane indicates that there is a preferential uptake of cholesterol by growing cells. Such a phenomenon has been observed previously in insects, mammalian intestine, and L cell mouse fibroblasts with cholesterol and structurally related sterols (Nes & McKean, 1977).

Discussion

This investigation was prompted by the unexpected finding that the sterol specificity for *M. capricolum* is relatively broad and in particular that lanosterol supports moderate growth of this bacterial sterol auxotroph (Odriozola et al., 1978). Since in model membranes lanosterol does not show the condensing effects typical of cholesterol, the question arose whether the different in vitro behavior of the two sterols is also expressed physiologically.

By lowering the cholesterol concentration in the media 10-fold, the cellular cholesterol content was progressively reduced to approximately one-half. Moreover, the microviscosity ($\bar{\eta}$) values of these isolated membranes declined in parallel with their cholesterol content. Such relationships between cholesterol content and fluidity have been well documented for artificial membranes (Oldfield & Chapman, 1972; Shinitzky & Inbar, 1976) and noted for several natural membranes as well (Butler et al., 1978; Sinensky, 1978; Rottem et al., 1973b).

Within the twofold range of membrane cholesterol attained, mass doubling times of *M. capricolum* were unaffected. However, the absorbances of late log cultures were diminished, probably because in the lower range the cholesterol available from the medium was exhausted.

In parallel experiments with lanosterol, the membrane sterol content also fell when the sterol content of the medium was

lowered. In this case, however, membrane microviscosities remained constant and independent of membrane lanosterol content. It may be noted that all lanosterol-containing membranes showed the same low $\bar{\eta}$ values that are found in membranes grown on minimal amounts of cholesterol (1 μg/mL). Thus, lanosterol appears to be incompetent in altering the membrane fluidity of natural as well as of artificial membranes. Nevertheless, lanosterol supports *M. capricolum* growth. In this instance, a twofold variation of membrane sterol content affects neither the growth rate nor the absorbances of late log cells.

The failure of lanosterol to modulate bilayer fluidity by condensing phospholipids is also strikingly evident from the temperature dependence of mycoplasma membrane microviscosities containing this sterol. While cholesterol-rich membranes (30 mol %) do not display a phase transition over a 40 °C range, membranes containing lanosterol undergo abrupt fluidity changes. Similar discontinuities have also been observed in the Arrhenius plots of membranes of *M. mycoides* but only if adapted to low cholesterol levels (Rottem et al., 1973b). Whether the breaks in the Arrhenius plots of cholesterol-poor membranes and lanosterol-rich membranes are expressions of similar phenomena is not clear, but the analogy strongly suggests that lanosterol, even when present in relatively large amounts, does not prevent phase transitions or lateral phase separations in membranes. Lateral phase separations would further distinguish the organization of lanosterol-rich membranes from membranes containing cholesterol, in line with other evidence showing poor interaction of lanosterol with phospholipid fatty acyl chains (Yeagle et al., 1977; Lala et al., 1978).

Because the detectable effects of lanosterol on membranes are minimal, we speculated that the range of fatty acids compatible with lanosterol as growth factors for *M. capricolum* might be limited. This restriction is indeed observed when lanosterol is supplied to this organism in combination with certain fatty acid pairs, e.g., myristate–elaidate, myristate–oleate, or palmitate–linoleate. Such combinations do not support significant growth when lanosterol is the sterol source but are fully active for cells presented with nonlimiting cholesterol. Moreover, elaidate alone will serve as a fatty acid source for cells growing on cholesterol but not on lanosterol. Thus, cholesterol remains a competent sterol for optimum membrane function even when the fatty acid supply varied widely, a property not shared by lanosterol. In the presence of lanosterol, fatty acid composition rather than sterol content appears to be the overriding factor in controlling the physical state of the membrane compatible with growth.

Rodwell (1971) has reported that *Mycoplasma* strain Y, when presented with elaidate and a long-chain SFA, preferentially incorporates the trans olefin. Our results corroborate this finding for *M. capricolum* whether the sterol source is cholesterol or lanosterol. Under these conditions mycoplasmas adjust to nutritional changes by selectively incorporating certain fatty acids and as a result maintain a constant membrane microviscosity. On the other hand, when oleate replaces elaidate, in combination with palmitate or stearate, membranes supplied with cholesterol have lower microviscosity values; they fail to compensate by increasing the SFA content. Yet the growth characteristics of the cell are not affected. An analogous experiment substituting oleate for elaidate in the medium of cells growing on lanosterol resulted in a significant increase in SFA content with no concomitant change in membrane microviscosity. This apparent inconsistency, i.e., the ability of mycoplasmas to incorporate fatty acids selectively in one case but not in another, may be explained as follows.

When membranes are rich in cholesterol, phospholipid acyl chain–sterol interactions may be the predominant forces that control the bulk physical state of the membrane. These interactions per se appear to be more important for growth than the absolute microviscosity values of the resultant membrane. In the presence of cholesterol, variations in membrane microviscosity are tolerated by *M. capricolum* without adverse effects on growth. Plausible interactions with long-chain fatty acids occur at both the α and β face of cholesterol, but, as has been suggested, cholesterol may interact preferentially with a conformationally more rigid long-chain SFA on the α face and an intrinsically more flexible UFA on the β face (Huang, 1977). If this is the case, the SFA/UFA ratios need not change when oleate replaces elaidate. On the other hand, when lanosterol is the membrane sterol, van der Waals' interactions between fatty acyl chains and the sterol nucleus are minimized by the bulky methyl groups at C-4 and C-14 (Dahl et al., 1980), and hence fatty acyl–fatty acyl interactions will predominate. Indeed, when oleate is substituted for elaidate, lanosterol-grown cells increase their SFA content, facilitating and favoring the stronger van der Waals' interactions, i.e., between two relatively rigid hydrocarbon chains in the extended trans conformation.

The above discussion emphasizes the ability of cholesterol and the incompetence of lanosterol to influence the physical state of membranes. Structural reasons for this difference are presented in the preceding paper (Dahl et al., 1980). Clearly, modulation of bulk membrane fluidity is one important factor responsible for the superior growth rates and yields of *M. capricolum* raised on cholesterol as compared to lanosterol. Nevertheless, the fact remains that lanosterol supports substantial mycoplasma growth. Necessarily therefore the question arises as to whether membrane sterols can play a role other than to regulate *bulk* membrane fluidity. As previously suggested, a more primitive function of sterols in membranes may be to separate phospholipid head groups (Yeagle et al., 1977; Odriozola et al., 1978). For such purposes the bulk and the rigidity of the sterol ring system, which lanosterol shares with cholesterol, may be sufficient.

One further significant observation may help to clarify the role of sterols in membranes. Introduction of small amounts of cholesterol into lanosterol-rich membranes produces a synergistic response on growth without raising the bulk membrane fluidity. This points to the existence of localized cholesterol-specific interactions in the membrane. Such regional effects may be more important in optimizing membrane functions than bulk physical state. The fact that sterol-requiring mycoplasmas can be grown successfully on very low cholesterol levels but never in the complete absence of cholesterol also supports this hypothesis (Rodwell et al., 1970; Rottem et al., 1973a).

In conclusion, all the data presented emphasize the superiority of the cholesterol molecule as a sterol constituent in *M. capricolum* membranes. Cholesterol is far more competent than lanosterol in lending rigidity to cell membranes, in keeping cell membranes in a homogeneous physical state over a wide range of temperatures, and in allowing the cell ample flexibility to grow in the presence of a variety of fatty acids. Low levels of membrane cholesterol enhance cell growth substantially when lanosterol is the bulk sterol, but the question of whether *M. capricolum* has an absolute requirement for cholesterol remains unsettled.

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Phosphorylation of Nuclear Poly(adenylic acid) Polymerase by Protein Kinase: Mechanism of Enhanced Poly(adenylic acid) Synthesis†

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ABSTRACT: Previous studies have shown that nuclear poly(A) polymerase (EC 2.7.7.19) is a phosphoprotein and that phosphorylation of the enzyme in vitro enhances its activity [Rose, K. M., & Jacob, S. T. (1979) *J. Biol. Chem. 254*, 10256–10261]. The present investigation was undertaken to elucidate the mechanism by which phosphorylation of poly(A) polymerase by exogenous protein kinase in vitro alters its catalytic activity. Phosphorylated poly(A) polymerase has an elevated primer requirement and synthesizes a greater number of poly(A) chains relative to control enzyme. Phosphorylation does not significantly alter the product size. Reactions catalyzed by the activated enzyme display rapid, linear kinetics whereas poly(A) synthesis by the nonphosphorylated enzyme exhibits a lag phase at early time points of the incubation. Over prolonged periods of incubation, the net synthesis of poly(A) remains unaffected by phosphorylation of the enzyme. These results indicate that phosphorylation of poly(A) polymerase in vitro does not alter the extent but augments the rate of poly(A) synthesis as a result of increased affinity of enzyme for its polynucleotide primer. These data suggest that the rate of polyadenylation of mRNA in vivo may be regulated by posttranslational modification of poly(A) polymerase.

The nuclear DNA of eucaryotes is complexed with an array of proteins, both basic (histones) and acidic (nonhistones) in nature. Recent observations suggest a role for histones in packaging the DNA into nucleosome structures [see Kornberg (1977) and Felsenfeld (1978)]. In contrast, the function of most of the nonhistone proteins remains obscure. In spite of this fact, much evidence has been accumulated correlating the phosphorylation of nonhistone proteins with elevated rates of RNA synthesis and increased gene expression in vivo [see Kleinsmith (1974)]. In vitro investigations have indicated that DNA-dependent RNA polymerases I (Hirsch & Martelo, 1976) and II (Kranias et al., 1977) can be phosphorylated with resultant increases in RNA synthesis, suggesting that phosphorylation of these two nonhistone chromatin proteins in vivo may regulate the rate of RNA synthesis.

Over the past several years we have been involved in characterizing the enzyme poly(A) polymerase, EC 2.7.7.19 [see Jacob & Rose (1978)]. In the nucleus, this enzyme is tightly bound to the chromatin matrix (Rose et al., 1977a,b), where it most probably catalyzes the initial addition of poly(A) to the mRNA precursors. Although the exact function of poly(A) has not been completely elucidated, poly(A) addition can apparently modulate gene expression by affecting the processing of the nuclear precursors (Derman & Darnell, 1974), the transport of the mRNA into the cytoplasm (Darnell et al., 1971), and/or the stability of the mRNA (Marbaix et al., 1975; Levy et al., 1975). Recently, we have ascertained that nuclear poly(A) polymerase is a phosphoprotein and that the degree of phosphorylation is under biological control, with the enzyme from a rapidly growing hepatoma more highly phosphorylated than its counterpart from normal liver (Rose & Jacob, 1979). These results have suggested that in vivo phosphorylation of the nonhistone chromatin protein, poly(A) polymerase, may augment gene expression via increased polyadenylation. In the present investigation we have explored the mechanism by which phosphorylation of poly(A) polym-

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0006-2960/80/0419-1472$01.00/0