

Changes in Composition, Biosynthesis, and Physical State of Membrane Lipids Occurring upon Aging of *Mycoplasma hominis* Cultures

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During the progression of *Mycoplasma hominis* cultures from the early logarithmic phase to the stationary phase of growth, the total phospholipid content of the cell membranes decreased. Measurement of the amount of the various phospholipids during the growth cycle showed that a decrease in the phosphatidylglycerol (PG) content, accompanied by an increase in the phosphatidic acid content, occurred upon aging of the culture. Pulse labeling experiments revealed that the PG, once formed, is relatively stable, undergoing no detectable turnover in growing cultures of *M. hominis*. No changes in the fatty acid composition of the membrane phospholipids were observed on aging of the culture, with palmitic acid predominating throughout the growth cycle. The preferential incorporation of palmitate into the phospholipid fraction is apparently caused by the higher activity of the membrane-bound acyl-coenzyme A (CoA): α -glycerophosphate transacylase with palmityl-CoA rather than with oleyl-CoA as substrate. The activity of the soluble acyl-CoA synthetase was the same whether palmitate or oleate served as substrate. *M. hominis* membrane preparations contained a PG-synthetase system catalyzing the incorporation of L- α -glycerol-3-phosphate into PG. The activity of the PG synthetase system was markedly dependent on the age of the culture, being highest in cells from the early exponential phase of growth while declining sharply thereafter, and thus probably responsible, in part, for the decrease in PG content upon aging of the cells. Electron paramagnetic resonance spectra of a spin-labeled fatty acid incorporated in *M. hominis* membranes revealed a marked decrease in the freedom of motion of the spin label on aging of the culture. It is proposed that this decrease is due primarily to the decrease in the lipid-to-protein ratio of the membranes and has a marked effect on the activity of membrane-associated enzymes and transport systems.

The effect of the age of mycoplasma cultures on the activity of membrane-associated enzymes and transport systems has been previously reported (19, 24, 25). The recent findings on the marked influence of the physical state of membrane lipids on these activities (5, 21) prompted us to investigate the changes in the composition, intermediary metabolism, and physical state of membrane lipids during the growth cycle of mycoplasma. Our observations that the cell membrane of *Mycoplasma hominis* can be easily isolated (26) and that the lipid composition of this membrane is relatively simple, composed primarily of phosphatidylglycerol (27), seem to offer a useful system for such studies. In this communication, data are provided on the variations in *M. hominis* membrane lipids and the changes in the physical

state of the membrane lipids during the growth cycle of the organisms. The partial characterization of the enzymatic activities involved in lipid biosynthesis of *M. hominis* is also presented, and the possible role of these enzymes in controlling the lipid composition is discussed.

MATERIALS AND METHODS

Organisms and growth conditions. *M. hominis* (ATCC 15056), *Acholeplasma laidlawii* (oral strain), and *Mycoplasma mycoides* var. *capri* (PG-3) were grown in 1- to 2-liter volumes of Edward medium (17) supplemented with 2% PPLO serum fraction (Difco) and palmitic and oleic acids (5 μ g/ml of each). For the growth of *M. hominis*, the medium was also supplemented with 20 mM L-arginine, and its pH was adjusted to 6.5. The cells were grown in a medium containing either 2 μ Ci of [1- 14 C]palmitic acid per liter (59.0 mCi/mmol), 5 μ Ci of [9,10- 3 H]palmitic acid

per liter (500 mCi/mmol), or 20 μ Ci of [14 C]glycerol per liter (19.5 mCi/mmol) for the labeling of membrane lipids. The radioactive compounds were the products of the Radiochemical Centre, Amersham, England. After 16 to 40 h of incubation at 37 C, growth was estimated by measuring the absorbancy of the culture at 640 nm or by titrating the excess ammonia formed with a standard solution of 0.01 N HCl. The organisms were harvested by centrifugation at $12,000 \times g$ for 15 min and washed once with 0.25 M NaCl.

Preparation of cellular fractions. Cell membranes were obtained by osmotic lysis of the organisms (26). The membranes were collected by centrifugation at $34,000 \times g$ for 30 min, separated from the cytoplasmic fluid, washed twice, and suspended in β -buffer (15) diluted 1:20 with deionized water (dilute β -buffer). For isopycnic density gradient analyses, the membranes (1 to 2 mg of membrane protein per ml) were further treated with ribonuclease and deoxyribonuclease (50 μ g/ml of each, Sigma Chemical Co., St. Louis, Mo.) for 30 min at 37 C, and then washed once and suspended in dilute β -buffer.

Analytical procedures. The amount of protein in the cellular fractions was determined by the method of Lowry et al. (13) with crystalline bovine albumin as standard. Total phosphorus in the lipid fractions was determined by the method of Ames (1) after digestion of the sample with an ethanolic solution of $Mg(NO_3)_2$. Radioactivity in membrane or lipid preparations was determined in a Packard Tri-Carb liquid scintillation spectrometer using a dioxane-toluene based scintillation solvent (29). Density gradient analysis of membrane preparations was performed as described (29) using a linear sucrose gradient of 30 to 50%.

Lipid analysis. Lipids were extracted from freeze-dried membranes (2 to 4 mg of membrane protein) by two successive extractions with chloroform-methanol (2:1, vol/vol) at 45 C for 2 h. The extracts were combined, washed by the method of Folch et al. (8), and filtered through glass wool. The solvent was evaporated under nitrogen, and the dried lipid was dissolved in 1 ml of chloroform.

The chloroform-soluble lipid was applied to a column (8 by 70 mm) of activated silicic acid (100 mesh, Mallinckrodt Chemical Works, St. Louis, Mo.) prewashed with chloroform for the separation of the neutral lipids from the polar lipids. Elution was carried out by the successive application of 10 ml of chloroform to remove the neutral lipids followed by 10 ml of chloroform-methanol (1:1, vol/vol) to remove the polar lipids (30).

Gas-liquid chromatography of the fatty acid methyl esters of the *M. hominis* polar lipid fraction was performed as previously described (27) using a polar column of 15% diethylene glycol adipate on Chromosorb W.

Thin-layer chromatography (TLC) of the polar lipids was carried out on silica gel HR-coated plates prepared from a 30% slurry of silica gel HR in 0.6% Florisil (Hopkin & Williams, Chadwell Heath, Essex, England). Samples of the polar lipid fraction (25 μ liters) were applied to the plates as short bands, and the plates were developed in chloroform-methanol-

acetic acid-water (80:13:8:0.3, vol/vol). The lipid bands were detected by iodine vapor and by the molybdate spray reagent (6). In some experiments the lipid bands were scraped off the plate into scintillation vials and the radioactivity was measured as described. Water-soluble phosphate esters were prepared from the polar lipid fraction by mild alkaline hydrolysis (7). The phosphate esters were separated by chromatography on cellulose chromatogram sheets (Eastman Kodak Co., Rochester, N.Y.) developed in water-acetic acid-ethanol (100:10:12, vol/vol). The phosphate esters were detected by the molybdate-perchloric acid reagent (33) and identified by comparing their mobility to that of known standards.

Paramagnetic resonance spectrometry. Membranes were spin labeled with the *N*-oxyl-4',4'-dimethylxazolidine derivative of 5-ketostearic acid (Syva, Palo Alto, Calif.) by exchange from bovine serum albumin as previously described (22). Electron paramagnetic resonance spectra of the spin-labeled membranes were obtained with a Varian E-4 spectrometer attached to a temperature control device. The hyperfine splitting ($2T_{11}$) was measured to within ± 0.5 Gauss.

Enzyme assays. The acyl-coenzyme A (CoA) synthetase activity was determined by the hydroxamate assay (32) in a reaction mixture containing (in micromoles): CoA, 0.3; adenosine triphosphate, 30; dithioerythritol, 0.06; $MgCl_2$, 12; and potassium salt of oleic or palmitic acids, 2.4; as well as membrane or supernatant fluid containing 3 to 6 mg of protein, and 0.9 ml of a freshly prepared hydroxylamine reagent (32) in a final volume of 2.5 ml. After 30 min of incubation at 37 C, the reaction was stopped by the addition of 0.15 ml of 72% $HClO_4$. The tubes were centrifuged at $3,000 \times g$ for 5 min, and the supernatant fluid was separated from the precipitate and extracted twice with 5-ml volumes of diethyl ether-ethanol (7:3). The hydroxamates in the extract were determined by the method of Stern and Shapiro (34). With the quantity of protein employed, the rate of the acyl-CoA synthetase activity was found to be constant during the incubation period. The acyl-CoA: α -glycerophosphate transacylase activity was assayed spectrophotometrically by the method of Cronan et al. (3), by determining the release of CoA resulting from the acyltransfer from palmityl-CoA or oleyl-CoA to *L*- α -glycerol-3-phosphate. The reaction mixture (final volume of 1 ml) consisted of: tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.5), 100 μ mol; $MgCl_2$, 5 μ mol; *L*- α -glycerol-3-phosphate, 3 μ mol; dithionitrobenzene (DTNB), 1 μ mol; palmityl-CoA or oleyl-CoA, 0.05 μ mol; bovine serum albumin, 1 mg; and membrane protein, 50 to 200 μ g. The acyl-CoA derivatives were the products of Sigma Chemical Co., St. Louis, Mo., and were employed at substrate saturation levels. The release of CoA was measured by the reaction of the thiol of the free CoA with DTNB to give an increase of absorbance at 413 nm. Initial rates were derived by measuring absorbancies at 37 C at 5-s intervals in a spectrophotometer equipped with a multiple absorbance recorder and reference compensator. The rates were linear with respect to the amount of protein employed. The acyl-

transferase activity measured was corrected for endogenous thioesterase activity by subtracting the absorbancy changes in a parallel cuvette lacking L- α -glycerol-3-phosphate. Enzyme activities were calculated by assuming a millimolar extinction coefficient for DTNB of 13.6 cm^{-1} at 413 nm.

The activity of the phosphatidylglycerol (PG)-synthetase system was determined by measuring the incorporation of uniformly labeled L-[^{14}C]- α -glycerol-3-phosphate (New England Nuclear, Boston, Mass.) into the polar lipid fraction in a system containing (in micromoles): Tris-maleate buffer (pH 7.4), 50; β -mercaptoethanol, 2; MnCl_2 , 0.1; MgCl_2 , 1; L-[^{14}C]- α -glycerol-3-phosphate (120 mCi/mmol), 0.006; cytidine diphosphatedipalmitin, 0.025; as well as 2.5 mg of Triton X-100 and 1 to 4 mg of membrane protein in a final volume of 0.5 ml. The cation requirements were determined by omitting Mn^{2+} and Mg^{2+} from the reaction mixture, and the various cations were tested in concentrations of 0.1 to 2.0 mM. The effect of detergents was determined by omitting Triton X-100 from the reaction mixture, and the various detergents were added to final concentrations of 0.1 to 2.0 mg/ml. The effect of inhibitors on the enzyme system was determined in a reaction mixture without β -mercaptoethanol. The reaction mixtures were incubated at 37 C for 30 min and the reaction was terminated by the addition of 3 ml of methanol and 5 ml of chloroform. The water-soluble compounds were extracted from the organic phase by the addition of 10 ml of 2 M KCl and vigorous shaking for 2 min. After phase separation, the upper layer was discarded and the extraction was repeated. The chloroform layer was washed once with 10 ml of deionized water, and samples of the chloroform phase were taken for radioactivity measurements and for TLC analysis as described above. With the quantity of protein employed, the rate of the activity of the PG-synthetase system was found to be constant during the incubation period.

RESULTS

Growth phases of *M. hominis*. A typical growth curve of *M. hominis* in Edward medium (17) supplemented with 2% PPLO serum fraction and 20 mM arginine is presented in Fig. 1. The increase in absorbancy of the culture upon growth was accompanied by a concomitant increase in pH values. When the medium was inoculated at an inoculum level of 0.1%, the culture reached its maximal absorbancy after approximately 22 h of incubation at 37 C, and after a short stationary phase the cells began to lyse resulting in a decrease in the absorbancy of the culture. Adjusting the pH of the medium to 6.5 throughout the growth period had only a slight effect on the growth curve; however, replacing the PPLO serum fraction (Difco) with 3% horse serum resulted in better growth, with the cultures reaching absorbancies of up to 0.7 at the end of the exponential phase of growth.

Neutral and polar lipid fractions. Since *M.*

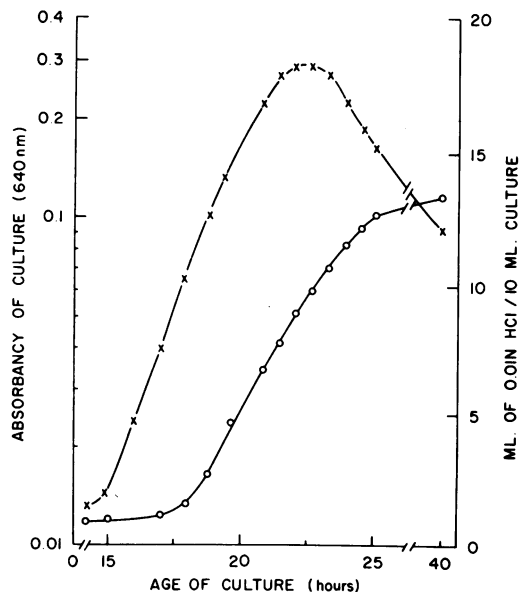


FIG. 1. Growth curve of *M. hominis* in Edward medium (17) containing 2% PPLO-serum fraction. Symbols: x, absorbancy of culture; O, ammonia production of culture.

hominis cannot synthesize long-chain fatty acids (18), its membrane lipids could be labeled by growing the cells with a radioactive fatty acid. When lipid extracts from cells grown in a medium supplemented with [^{14}C]palmitate and harvested at various phases of growth were subjected to silicic acid chromatography, only a small percentage (5 to 7.5%) of the total radioactivity could be eluted with chloroform, the rest being eluted with chloroform-methanol. Hence, the radioactivity was incorporated almost entirely into the polar lipid fraction regardless of the age of the culture. Upon saponification of the polar lipid fraction, about 98% of the radioactivity was recovered in the fatty acids.

Fatty acid composition. Table 1 shows the fatty acid composition of *M. hominis* membrane polar lipids. Palmitic acid is the major fatty acid accounting for the high saturated-to-unsaturated fatty acid ratio of the polar lipid fraction. When the cells were grown in a medium not supplemented with exogenous fatty acid, the palmitic acid content of the membrane polar lipids dropped from about 50% in early exponential-phase cells to about 40% in the decline-phase cells. However, when the medium was supplemented with 5 μg of palmitate per ml, the palmitic acid content of membrane polar lipids was unchanged throughout the growth cycle.

TABLE 1. Fatty acid composition of the polar lipids of *M. hominis* cells harvested at different phases of growth

Age of culture (h)	Absorbancy of culture (640 nm)	Fatty acid (% of total)							
		Cells grown in medium supplemented with palmitic acid (5 µg/ml)				Cells grown in medium not supplemented with palmitic acid			
		14:0 ^a	16:0	18:0	18:1	14:0	16:0	18:0	18:1
15	0.09	1.5	57.2	18.4	18.2	3.1	51.1	18.4	14.8
18	0.20	1.5	58.2	14.6	20.7	1.3	46.1	21.1	25.7
21	0.30	0.9	61.2	15.4	19.4	2.5	37.5	26.8	27.8
40	0.20	1.1	57.1	16.4	22.8	1.9	39.0	28.6	22.8

^a The first number indicates chain length and the second indicates the number of double bonds.

Phospholipid content and the physical state of membrane lipids.

Figure 2A shows the changes in the phospholipid content of *M. hominis* membranes on aging of the culture as indicated from the changes in the radioactivity of membranes derived from cells grown in a medium supplemented with [¹⁴C]palmitate. The most pronounced change was the 2 to 3 times lower lipid-to-protein ratio in membranes obtained from cells harvested at the stationary or decline phase of growth as compared to membranes obtained from cells harvested at the exponential phase of growth. The radioactivity-to-lipid phosphorus ratio, however, was almost unchanged throughout the growth cycle. The different lipid-to-protein ratios of the membranes from cells harvested at the various phases of growth markedly affected the densities of the membrane preparations and the fluidity of their lipid moiety (Fig. 2B). Thus, membranes from cells harvested at the decline phase of growth had a pronounced higher density (1.19 g/cm³) as compared to membranes obtained from mid-exponential (1.15 g/cm³) phase cells and a lower fluidity as indicated by the decreased freedom of motion of the *N*-oxyl-4', 4'-dimethyloxazolidine derivative of 5-ketostearic acid (5NS) incorporated into the membranes. The freedom of motion of the spin-labeled probe in the membrane is related to the hyperfine splitting values, higher hyperfine splitting values being associated with a lesser freedom of motion of the probe (9).

Phospholipid composition. TLC of *M. hominis* membrane polar lipids extracted from cells grown in a medium supplemented with [¹⁴C]palmitate revealed three major radioactive spots with most of the radioactivity associated with a spot corresponding in mobility to PG. Table 2 shows that the distribution of radioactivity among these spots varied depending on the age of the culture. The variation was mainly a decrease in radioactivity of the PG

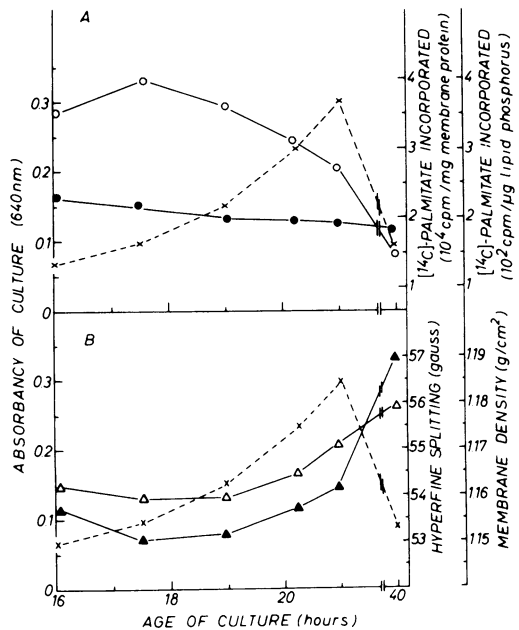


FIG. 2. Effect of the age of culture on the labeling of the *M. hominis* membranes by [¹⁴C]palmitate (A), and on the density of the membranes and the hyperfine splitting of a spin-labeled fatty acid (5NS) in the membranes (B). Symbols: x, absorbancy of culture; ○, radioactivity per membrane protein; ●, radioactivity per lipid phosphorus; Δ, hyperfine splitting; ▲, membrane density.

spot and an increase in the radioactivity of a fast-migrating lipid (FML) on aging of the culture. Figure 3 shows the results of the chromatographic analysis of the deacylated products of the polar lipid fraction obtained from stationary-phase *M. hominis* cells grown with [1-¹⁴C]glycerol, and mixed with unlabeled phosphatidic acid (PA), PG, and cardiolipin (CL). Whereas most of the labeling was found in glycerylphosphorylglycerol, the deacylated product of PG, a low but consistent labeling was

TABLE 2. Incorporation of labeled palmitic acid into the various components of the polar lipid fraction of *M. hominis* cells harvested at different phases of growth

Age of culture (h)	Absorbancy of culture (640 nm)	Radioactivity in major phospholipids (% of total) ^a		
		Lyso-PG	PG	FML
17	0.09	4.3	79.0	3.5
21	0.25	3.0	76.0	7.7
40	0.10	4.5	70.0	17.0

^a Abbreviations: Lyso-PG, lysophosphatidyl-glycerol; PG, phosphatidylglycerol; FML, fast migrating lipid.

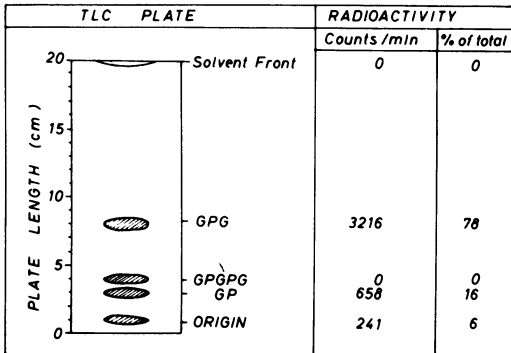


FIG. 3. Thin-layer chromatography of the water-soluble phosphate esters obtained by mild alkaline hydrolysis of the polar lipid fraction of *M. hominis* cells grown with [¹⁻¹⁴C]glycerol and mixed with unlabeled PA, PG, and CL. Solvent system: water-acetic acid-ethanol (100:10:12). Visualization of spots by the molybdate-perchloric acid reagent (3). Abbreviations: GP, glycerophosphate; GPG, glycerylphosphorylglycerol; GPGPG, glycerylphosphorylglycerol.

associated with the deacylated product of PA, the glycerophosphate spot. No labeling was found in the deacylated product of CL (glycerylphosphorylglycerol), suggesting that, unlike in other microorganisms (2, 4, 16), a shift from PG to PA rather than from PG to CL occurs upon aging of *M. hominis* cells. The identification of the FML as PA gained further support from the analysis of the deacylated products of FML isolated from the polar lipid fraction of *M. hominis* cells grown with [¹⁻¹⁴C]glycerol. Over 90% of the radioactivity was associated with the glycerophosphate fraction.

Figure 4 shows the results of a "pulse-chase" experiment where the cells were grown for 10 h with [¹⁻¹⁴C]palmitate, washed, and suspended

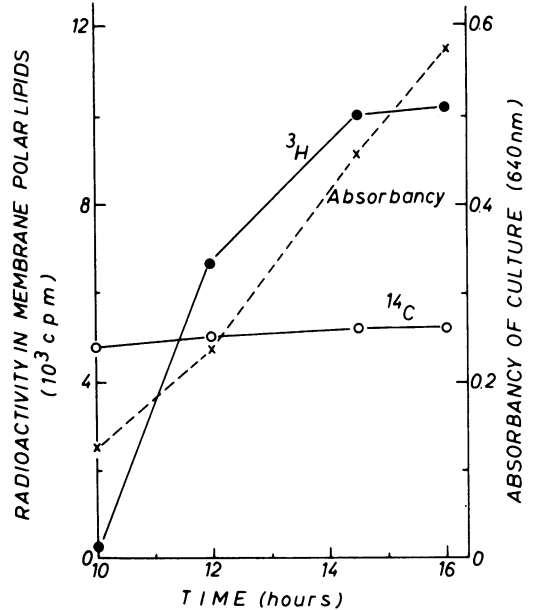


FIG. 4. Turnover of ¹⁴C-labeled lipids in *M. hominis* membranes. Cells were grown in 500 ml of Edward medium containing 1 μCi of [¹⁻¹⁴C]palmitate. After 10 h of incubation the cells were sedimented, washed, and suspended in a fresh medium containing 5 μCi of [9,10-³H]palmitate. Growth was continued and at the time intervals indicated 100-ml samples were withdrawn and the lipids were extracted and analyzed as described in Materials and Methods.

in a fresh medium containing [9,10-³H]palmitate, and their growth was continued to the end of the exponential growth phase. As is apparent from Fig. 4, the level of ¹⁴C in membrane polar lipids was almost unchanged throughout the growth period. Furthermore, TLC analysis of the polar lipid fractions revealed that, whereas 85% of the ¹⁴C was found throughout the experiment in the PG fraction, the PA fraction was mainly ³H-labeled.

Activation of free fatty acids. *M. hominis* cells were found to possess an acyl-CoA synthetase activity catalyzing the formation of acyl-CoA from fatty acids. About 95% of the activity was localized in the cytoplasmic fluid and had a very broad pH optimum (6.5 to 8.5). Table 3 shows that the acyl-CoA synthetase activity was high in cells harvested at the early exponential phase of growth and decreased somewhat in cells harvested at the stationary phase of growth (23 h). The enzyme activated either palmitate or oleate, but the rate of activation of palmitate was 1.5 times higher than that of oleate. No change in the ratio of rates of palmityl-CoA synthetase to oleyl-CoA

synthetase activities could be found on aging of the culture (Table 3).

Acylation of L- α -glycerol-3-phosphate.

Acyl-CoA: α -glycerophosphate transacylase activity was found in *M. hominis* and in *A. laidlawii* cells, but the activity in *A. laidlawii* was 3 to 4 times greater than that in *M. hominis* (Table 4). The activity of both organisms was localized in the cell membrane (Table 4) and, since whole cells showed no significant activity, it seems that the enzymatic system is localized on the inner surface of the membrane. Table 5 shows that, whereas the acyl-CoA: α -glycerophosphate transacylase activity of *A. laidlawii* membranes showed a higher rate with oleyl-CoA than with palmityl-CoA as substrate, *M. hominis* membranes showed a much higher rate with palmityl-CoA. The optimal pH for activity also differed in the two organisms and, whereas membranes of *M. hominis* showed maximal activity at acidic pH values, the optimal pH for the activity of *A. laidlawii* membranes was above 7.5 (Fig. 5). Due to the low activity of *M. hominis* membranes at pH values of 8.0 and above, the enzyme activities at the more alkaline pH range could not be measured accurately.

Synthesis of phosphatidylglycerol. A cell-free system containing *M. hominis* membranes

TABLE 3. Activation of palmitate and oleate by the cytoplasmic fluid of *M. hominis* cells harvested at different phases of growth

Age of culture (h)	Absorbance of culture (640 nm)	Acyl-CoA synthetase activity ^a	
		Palmitate	Oleate
17	0.07	1.2	0.8
19	0.13	1.3	0.9
23	0.30	1.0	0.6

^a Expressed as nanomoles of hydroxamate formed per milligram of protein per minute.

TABLE 4. Acyl-CoA: α -glycerophosphate transacylase activity of whole cells, isolated membranes, and cytoplasmic fluids of *M. hominis* and *A. laidlawii*

Preparation	Sp act ^a	
	<i>M. hominis</i>	<i>A. laidlawii</i>
Whole cells	0.5	0.9
Membrane fraction	5.8	22.2
Cytoplasmic fluid fraction	2.0	0.8

^a Expressed as nanomoles of CoA released per milligram of protein per minute in a reaction mixture containing palmityl-CoA.

TABLE 5. Acyl-CoA: α -glycerophosphate transacylase activity of *M. hominis* and *A. laidlawii* membranes with palmityl-CoA and oleyl-CoA as substrates

Organism	Sp act ^a	
	Palmityl-CoA	Oleyl-CoA
<i>M. hominis</i>	4.1	0.7
<i>A. laidlawii</i>	15.4	18.4

^a Expressed as nanomoles of CoA released per milligram of protein per minute.

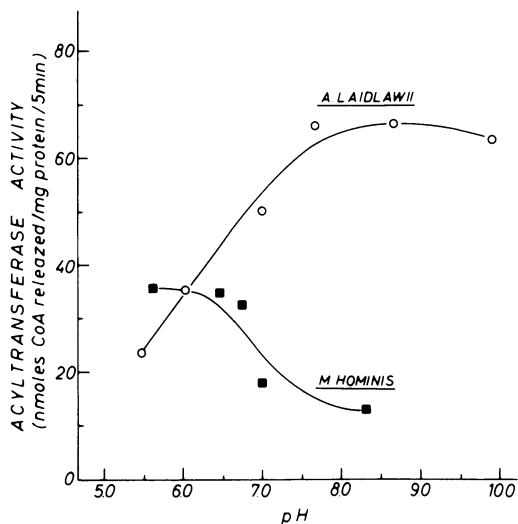


FIG. 5. Effect of pH on the acyl-CoA: α -glycerophosphate transacylase activity of *M. hominis* and *A. laidlawii* membranes with palmityl-CoA as substrate.

incorporated L-[¹⁴C]- α -glycerol-3-phosphate into the complex lipid fraction. No significant incorporation was detected when the membrane fraction was replaced by the cytoplasmic fluid fraction, indicating that in *M. hominis* the enzymes associated with complex lipid biosynthesis are localized in the cell membrane. TLC analysis of the products obtained by the incorporation of L-[¹⁴C]- α -glycerol-3-phosphate (Fig. 6) shows that the activity of the PG-synthetase system of *M. hominis* was markedly affected by the age of the culture. With membranes from cells harvested at the early exponential phase of growth, the activity was highest, decreasing sharply toward the end of the exponential phase of growth. No sharp pH optimum was noted for the *M. hominis* PG-synthetase activity and the activity was more or less constant at pH values ranging from 7.0 to 8.5 in 0.05 M Tris-maleate buffer and declined steeply at pH values below 6.2 and above 9.0.

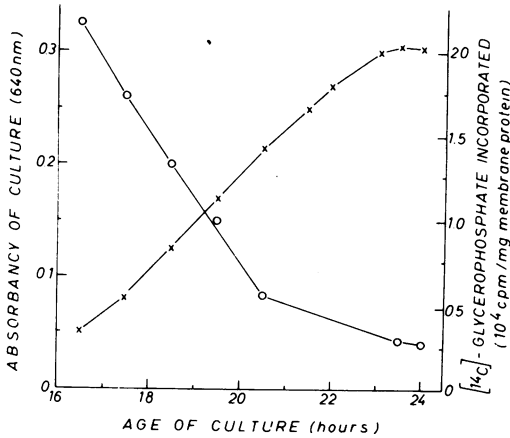


FIG. 6. Effect of the age of the culture on the incorporation of L-[¹⁴C]α-glycerol-3-phosphate into complex lipids by *M. hominis* membranes. Symbols: x, absorbancy of culture; O, radiactivity.

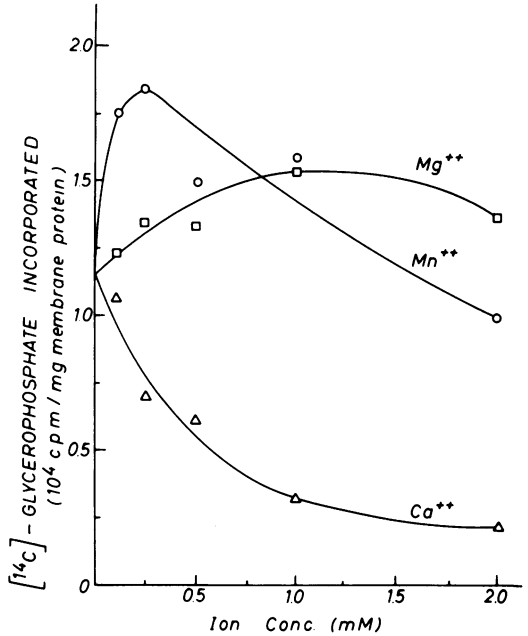


FIG. 7. Effect of divalent cations on the incorporation of L-[¹⁴C]α-glycerol-3-phosphate into complex lipids by *M. hominis* membranes.

Figure 7 shows the effect of divalent cations on the activity of the PG-synthetase system of *M. hominis*. A low Mn²⁺ concentration (0.1 to 0.25 mM) markedly stimulated the activity. This requirement could be partially met by Mg²⁺. However, Ca²⁺ ions markedly inhibited the activity, probably due to competition with Mg²⁺ ions present in mycoplasma membranes (10). The effects of some potent inhibitors on the activity of the PG-synthetase system of *M. hominis* are presented in Table 6. Ethylenediaminetetraacetic acid, 2,4-dinitrofluorobenzene, and *p*-chloromercuribenzoate were the most effective inhibitors while NaF was less effective. Dinitrophenol, sodium azide, and diisopropyl-fluorophosphate at concentrations of up to 10⁻² M had no effect on the activity. The dual effect of some detergents on the activity of the PG-synthetase system is shown in Fig. 8. Low concentrations (0.1 to 0.5 mg/ml) of the non-ionic detergent, Triton X-100, and, to a lesser degree, of sodium deoxycholate stimulated the activity, whereas at higher concentrations, sufficient for partial membrane solubilization, and inhibitory effect was detected. The ionic detergent, sodium dodecyl sulfate, rapidly inhibited the activity of the PG-synthetase system at concentrations much below those required for membrane solubilization.

DISCUSSION

The limited biosynthetic capacity of *M. hominis* makes this organism, as other mycoplasmas (18), dependent on the external supply of saturated and unsaturated fatty acids for growth. However, *M. hominis* preferentially makes use of saturated fatty acids for polar lipid

TABLE 6. Effect of inhibitors on the incorporation of L-[¹⁴C]α-glycerol-3-phosphate into complex lipids by *M. hominis* membranes

Inhibitor ^a	Concn of inhibitor (M)	Inhibition ^b (%)
Ethylenediaminetetraacetic acid	10 ⁻²	99
	10 ⁻³	91
<i>p</i> -Chloromercuribenzoate	10 ⁻³	33
	10 ⁻⁴	3
2,4-Dinitrofluorobenzene	10 ⁻²	85
	10 ⁻³	4
Sodium fluoride	10 ⁻²	35
	10 ⁻³	4

^a Membranes were suspended in deionized water instead of β-buffer. The inhibitors were added to the reaction mixture 5 min before addition of the substrate.

^b As compared to the incorporation with no inhibitor.

synthesis which accounts for the high palmitic acid content of membrane polar lipids (27). Lacking acyl carrier protein (23), the incorporation of exogenous fatty acids by *M. hominis* must involve the formation of acyl-CoA derivatives, and subsequently the transfer of the acyl residue to α-glycerophosphate (11, 12). Our notion that the transacylase activity is responsible for the preferential incorporation of palmitate into *M. hominis* membrane polar lipids

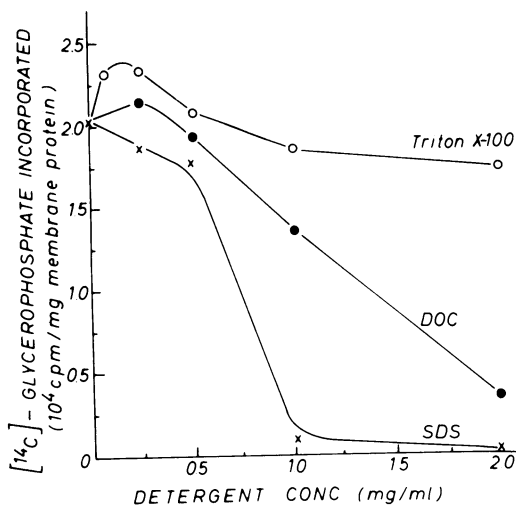


FIG. 8. Effect of detergents on the incorporation of L-[¹⁴C]α-glycerol-3-phosphate into complex lipids by *M. hominis* membranes. Abbreviations: DOC, sodium deoxycholate; SDS, sodium dodecylsulfate.

gains support from our observations that the membrane-bound acyl-CoA:α-glycerophosphate transacylase activity was higher with palmityl-CoA than with oleyl-CoA, whereas the acyl-CoA synthetase activity of *M. hominis* was almost as high with palmitate as substrate as with oleate. Hence, *A. laidlawii* cells, known to incorporate both palmitate and oleate into their complex lipids (20), were shown to possess a transacylase with almost the same activity with palmityl-CoA or oleyl-CoA as substrates (Table 5). The fact that oleyl-CoA is actively formed by *M. hominis* but poorly utilized in the transacylase reaction may be due to a different affinity of the transacylase system to acyl-CoA derivatives (4). A low affinity of the enzyme for oleyl-CoA would thus require the presence of high concentrations of oleyl-CoA in the cells. The membrane-bound thioesterase activity recently found in several mycoplasma species (S. Rottem, unpublished data) may, therefore, play an important role in controlling the acyl-CoA levels in the cells. Unlike the pronounced changes in the fatty acid composition of *A. laidlawii* membrane lipids over the growth cycle, primarily the changes in the unsaturated fatty acid content (22), no meaningful changes were detected in *M. hominis* membranes and palmitic acid predominated throughout the growth cycle (Table 1).

The radioactivity of membrane preparations isolated from [¹⁴C]palmitate-grown cells can be taken as a measure of the phospholipid content of *M. hominis* membranes from cells harvested

at various phases of growth since three major criteria were met: (i) throughout the growth cycle, the percentage of radioactivity in the polar lipid fraction underwent no detectable changes; (ii) the palmitic acid content of the polar lipid fraction was unchanged, and (iii) the turnover of the palmitic acid residues incorporated into the membrane was found to be very low. From the radioactivity-to-protein ratios in membrane preparations of cells harvested at the various phases of growth, it is apparent that a decrease in the lipid-to-protein ratio occurred upon aging of the culture, as compared to an increase in the membrane lipid content recorded in other microbial systems (2,14,31). The fact that *M. hominis* membrane polar lipids showed a very low rate of turnover throughout the growth cycle may suggest that this decrease is due primarily to a decrease in the rate of phospholipid biosynthesis rather than to the breakdown of membrane lipids. In view of the marked influence of membrane proteins on the physical state of membrane lipids (28), it is most likely that the decrease in the fluidity of *M. hominis* membrane lipids when the cultures progressed from the early exponential to the stationary phase of growth is due mainly to the decrease in the lipid-to-protein ratio of the membranes on aging. The almost constant fatty acid composition of *M. hominis* membrane polar lipids (Table 1) as well as the constant cholesterol-to-lipid ratio during the *M. hominis* growth cycle (S. Razin, personal communication) exclude the possibility that changes in membrane lipid constituents play a role in decreasing the fluidity of *M. hominis* membrane lipids on aging of the culture. It seems plausible that the increase in the viscosity of membrane lipids on aging may result in the marked decrease in the activity of membrane-associated enzymes and transport systems (19,24,25).

The decrease in PG content of membrane polar lipids on aging of the culture is in accord with changes in the lipid composition of other microbial systems (2,4,16), except that the most common trend appears to be the accumulation of CL in the stationary phase of growth at the expense of PG (2, 16). In *M. hominis*, PA, but not CL, is accumulated in the membrane. When compared to the high rate of PG turnover in a variety of microbial systems (2,4,36), the PG in growing *M. hominis* cells showed a very low turnover. The low turnover and the fact that phospholipase D activity could not be detected in *M. hominis* preparations (A. Greenberg, unpublished data) strongly suggest that the PA of *M. hominis* membranes is not a breakdown

product of PG but is synthesized de novo and is accumulated in the membrane due to a block in PG synthesis. Hence, the high PA content of cells harvested in the late exponential or stationary phase of growth is the result of the steep decline in the activity of the PG-synthetase system of *M. hominis* membrane preparations on aging of the culture (Fig. 6).

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