# CORRELATION OF CHOLESTEROL TO PHOSPHOLIPID CONTENT IN MEMBRANES OF GROWING MYCOPLASMAS*

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## 1. Introduction

The mechanism by which cells control the amount of exogenous cholesterol incorporated into their plasma membrane is still undefined. Mycoplasmas may serve as excellent models for studying this problem since they cannot synthesize cholesterol and depend on the growth medium for its supply [1]. That a control mechanism for cholesterol uptake does operate in mycoplasmas is quite obvious since their membrane cholesterol content shows significant differences even when the organisms are grown with the same amount of exogenous cholesterol. Thus, the cholesterol content in *Acholeplasma laidlawii* membranes did not exceed 10% of the total membrane lipids while that in *Mycoplasma hominis* reached almost 40% when grown with excessive amounts of exogenous cholesterol [2, 3].

Do the differences in the cholesterol uptake capacity of the various mycoplasmas result from differences in membrane phospholipid or protein composition, or are they caused by differences in the molecular organization of these components in the membrane? Our recent studies [4,5] on the uptake of cholesterol by isolated mycoplasma membranes indicated that the lipid domain of the membrane is the main element responsible for cholesterol uptake, while membrane proteins contribute little to cholesterol binding. Yet, these studies also indicated that the control mechanism of cholesterol uptake operates faultily once the membrane is isolated from the cell [6]. It seemed necessary, therefore, to study the factors controlling cholesterol uptake in actively growing mycoplasmas. Use has been made of our recent finding (this paper and unpublished observations of S. Rottem and A. Greenberg) that the ratio of membrane lipid to protein decreases markedly on aging of mycoplasma cultures, and increases on the addition of chloramphenicol to actively growing cultures [6].

The data presented in this report show that the marked changes in the protein content of mycoplasma membranes occurring on aging or on chloramphenicol treatment have little influence on the amount of cholesterol incorporated into the membranes and thus the ratio of cholesterol to membrane protein is liable to great variations. On the other hand, the ratio of cholesterol to membrane phospholipids or total lipids was found to be essentially constant under the experimental conditions employed, though the value markedly differed for the various mycoplasmas. It is suggested that the cholesterol content in membranes of growing mycoplasmas is largely dependent on the amount and composition of the phospholipids and probably of other membrane lipids.

## 2. Materials and methods

*Acholeplasma laidlawii* (oral strain) and *Mycoplasma mycoides* var. *capri* (PG-3) were grown in a modified Edward medium [7] adjusted to pH 8.5 and supplemented with 2% (v/v) of PPLO Serum Fraction (Difco) which served as a cholesterol source. *Mycoplasma hominis* (ATCC 15056) was grown in the same medium adjusted to pH 6.5 and supplemented with 20 mM L-arginine in addition to the PPLO Serum

* Number V in the series: Cholesterol in Mycoplasma Membranes.

Fraction. Small volumes (0.25 to 1 ml) of logarithmic cultures were used to inoculate 3 liter volumes of the growth media incubated at 37°C. When absorbance at 640 nm reached about 0.20 each of the cultures was divided into three equal parts: one received 20 μg chloramphenicol per ml, the other 100 μg chloramphenicol per ml, and the third part was kept as a control with no chloramphenicol added. Incubation was continued at 37°C; samples (500 ml) were withdrawn at various time intervals and the organisms were harvested by centrifugation at 12 000 *g* for 15 min and washed once in 0.25 M NaCl. The washed organisms were osmotically lysed [8] and the isolated membranes were washed once in deionized water, then in 0.05 M NaCl in 0.01 M phosphate buffer, pH 7.5, and again in deionized water.

The washed membranes, suspended in deionized water, were subjected to the following analyses: 1) total protein content, measured according to Lowry et al. [9], 2) density, determined by centrifugation on linear sucrose gradients of 30% to 60% [10], 3) total lipid content, determined colorimetrically by the method of Saito and Sato [11] on chloroform-methanol (2:1) extracts of the membranes, 4) lipid phosphorus, determined by the method of Ames [12] after digestion of the extracted lipid fraction with an ethanolic solution of $Mg(NO_3)_2$, and 5) cholesterol content, determined on the extracted lipid fraction by the colorimetric technique of Rudel and Morris [13].

## 3. Results

The progress of the three mycoplasma cultures from the early logarithmic to the stationary phase of growth was accompanied by a marked decrease in the total membrane lipid to protein ratio, as well as in the ratio of membrane phospholipid to protein (fig. 1). Accordingly, the density of the membranes was found to increase with the age of the culture. Thus, the density of *M. hominis* membranes increased from 1.162 g/cm$^3$ at the early logarithmic phase to 1.182 g/cm$^3$ at the late logarithmic phase, and that of *A. laidlawii* membranes from 1.157 g/cm$^3$ to 1.177 g/cm$^3$. Fig. 2 shows that the cholesterol to protein ratio also decreased with age, but the cholesterol to phospholipid ratio remained essentially constant throughout the logarithmic phase of growth. Fig. 2 also demonstrates

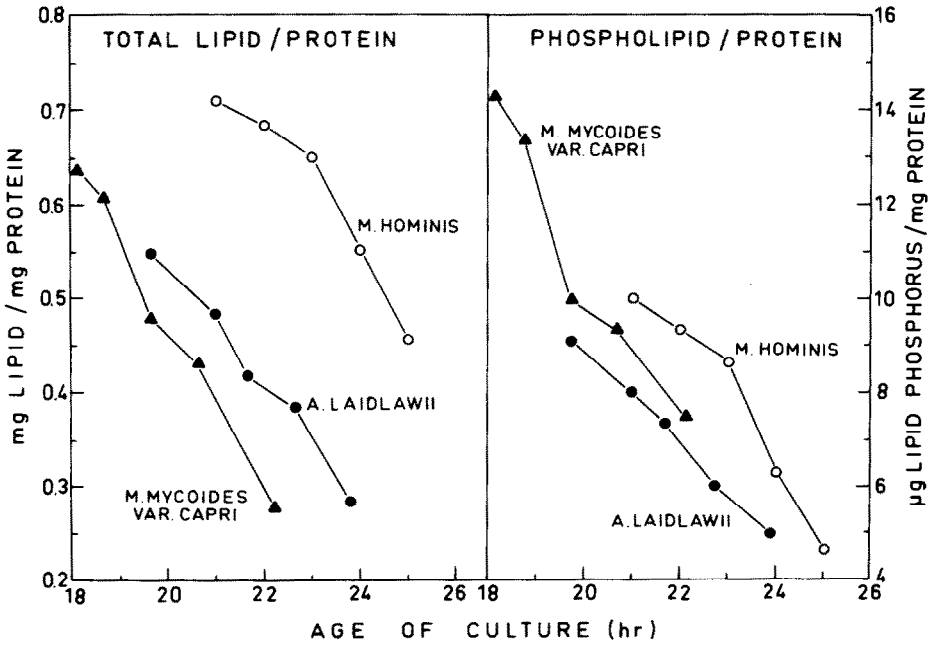


Fig. 1. Changes in the ratio of total membrane lipid to protein and phospholipid to protein in membranes of mycoplasmas harvested at various time intervals from the early to the late logarithmic phase of growth.

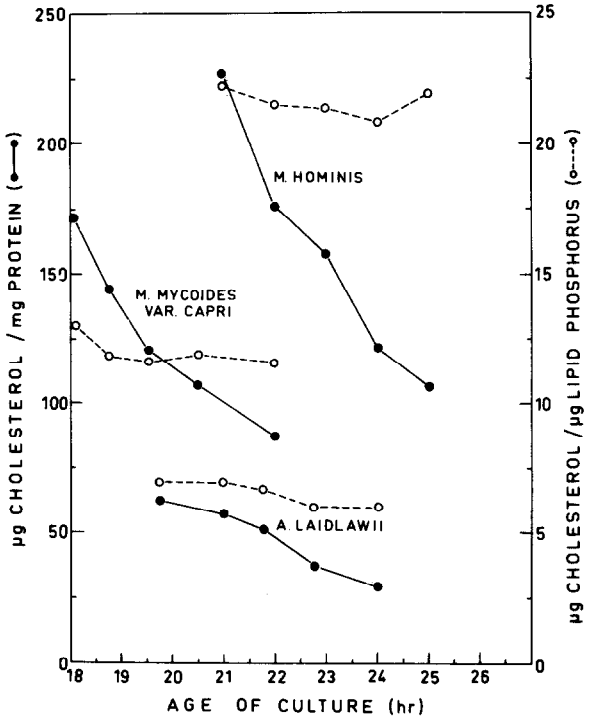


Fig. 2. Ratio of cholesterol to protein and phospholipid in membranes of mycoplasmas harvested at various time intervals from the early to the late logarithmic phase of growth.

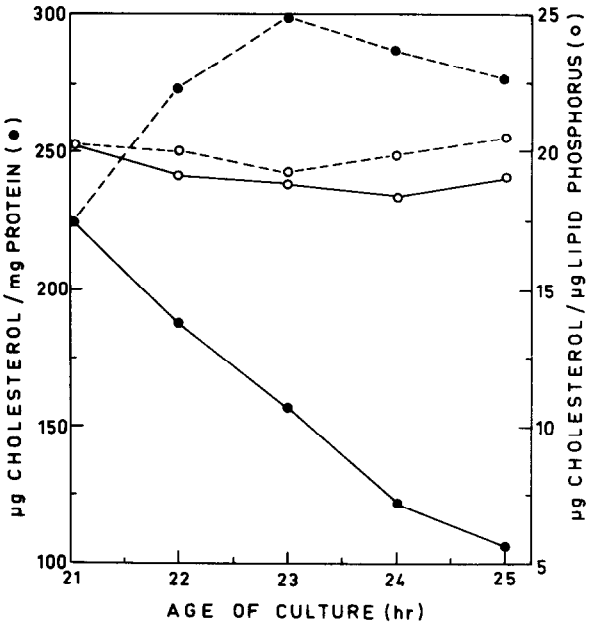


Fig. 3. Ratio of cholesterol to protein (●) and phospholipids (○) in membranes of *Mycoplasma hominis* cells treated with 20 μg chloramphenicol/mg (broken lines) compared with membranes of untreated cells (solid lines). Chloramphenicol was added at the early logarithmic phase of growth (21 hr culture).

Table 1
Effect of age and chloramphenicol treatment on the gross composition of *Mycoplasma mycoides* var. *capri* membranes

| Culture | Age of culture (hr) | Absorbance of culture at 640 nm | Total membrane protein (mg) | Total membrane lipid (mg/mg protein) | Phospholipids (μg lipid phosphorus/ mg protein) |
|---|---|---|---|---|---|
| Untreated culture | 18.00 | 0.235 | 6.2 | 0.63 | 14.0 |
| | 18.45 | 0.290 | 8.0 | 0.62 | 13.0 |
| | 19.35 | 0.410 | 11.1 | 0.47 | 10.0 |
| | 20.35 | 0.630 | 13.4 | 0.43 | 9.5 |
| | 22.05 | 0.950 | 30.2 | 0.27 | 7.5 |
| Culture treated with 20 μg/ml chloramphenicol (added at 18.00 hr) | 18.00 | 0.235 | 6.2 | 0.63 | 13.0 |
| | 18.45 | 0.250 | 6.3 | 0.58 | 12.8 |
| | 19.35 | 0.285 | 6.8 | 1.20 | 18.0 |
| | 20.35 | 0.285 | 6.8 | 1.16 | 17.6 |
| | 22.05 | 0.320 | 7.0 | 1.06 | 18.7 |

Table 2
Effect of age and chloramphenicol treatment on the cholesterol content of *Mycoplasma mycoides* var. *capri* membranes

| Culture | Age of culture (hr) | Cholesterol | | |
|---|---|---|---|---|
| | | μg/mg protein | μg/μg lipid | μg/μg lipid phosphorus |
| Untreated culture | 18.00 | 172 | 270 | 13.0 |
| | 18.45 | 144 | 280 | 12.5 |
| | 19.35 | 120 | 229 | 11.5 |
| | 20.35 | 109 | 213 | 12.0 |
| | 22.05 | 87 | 223 | 11.5 |
| Culture treated with 20 μg/ml chloramphenicol (added at 18.00 hr) | 18.00 | 172 | 270 | 13.0 |
| | 18.45 | 154 | 280 | 13.2 |
| | 19.35 | 248 | 236 | 13.5 |
| | 20.35 | 245 | 209 | 12.5 |
| | 22.05 | 232 | 229 | 13.2 |

the great differences in the cholesterol content of the various mycoplasmas; the highest being found in *M. hominis* and the lowest in *A. laidlawii.*

The addition of chloramphenicol to mycoplasma cultures at the early logarithmic phase of growth stopped membrane protein synthesis most rapidly. A concentration of 20 μg chloramphenicol/ml was as effective as 100 μg/ml. The data in tables 1 and 2 obtained with *M. mycoides* var. *capri* can be regarded as representative since very similar results were obtained with the other two mycoplasmas. Table 1 shows that while membrane protein synthesis was inhibited rather abruptly by chloramphenicol, phospholipid synthesis continued for some time resulting in a marked increase of the phospholipid to protein ratio (table 1). Likewise, the ratio of cholesterol to protein increased during the first two hours of chloramphenicol treatment. Again, as with aging cultures, the ratio of cholesterol to phospholipid or to total membrane lipids remained essentially constant (table 2 and fig. 3).

## 4. Discussion

Our data suggest that the amount of cholesterol incorporated into the cell membrane of any specific mycoplasma is largely determined by its phospholipid or total lipid content. Yet, the great differences in the cholesterol content of membranes from different mycoplasmas cannot be explained on this basis. Although the ratio of phospholipid to protein was found to be somewhat higher in membranes of the cholesterol-rich mycoplasmas (fig. 1), the differences in this ratio are too small to account for the marked differences in the cholesterol content of the various mycoplasmas. The ratio of cholesterol to phospholipid was, indeed, much higher in the cholesterol-rich membranes (fig. 2). Hence, qualitative rather than quantitative differences in membranes phospholipids may be responsible for the markedly different cholesterol binding capacity of the various mycoplasmas. Moreover, the presence of lipids other than phospholipids in the membrane may also affect the cholesterol binding capacity. Thus, in the cholesterol-poor *A. laidlawii* membrane a large proportion (up to 45%) of the total lipids consists of glycolipids [14], but in the cholesterol-rich *M. hominis* membranes no glycolipids are present [2].

While the results of our study appear to support the thesis that the type and quantity of the membrane polar lipids govern the amount of cholesterol incorporated, the significance of differences in the molecular organization of membrane proteins and lipids on cholesterol uptake must not be overlooked. Our current studies aim at the elucidation of the contribution of each of these factors to the cholesterol uptake capacity of the different mycoplasma membranes.

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