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CHOLESTEROL UPTAKE IS DEPENDENT ON MEMBRANE FLUIDITY IN MYCOPLASMAS

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Summary

The transfer of elaidate-enriched *Acholeplasma laidlawii* cells in culture from 37°C to 4°C virtually arrested exogenous cholesterol incorporation into the cell membrane. Cholesterol uptake continued, though at a slower rate, in oleate-enriched *A. laidlawii* cells undergoing similar temperature shift-down. It is concluded that the incorporation of exogenous cholesterol into the cell membrane of living mycoplasmas is rapid when the membrane lipid bilayer is in the liquid-crystalline state and very slow when the lipid bilayer is in the gel state.

Elucidation of the factors controlling incorporation of exogenous cholesterol into cell membranes is currently a subject of great interest, in light of the possible involvement of cholesterol in atherogenesis [1]. A variety of eukaryotic cells as well as the minute prokaryotes, mycoplasmas, were found to take up exogenous cholesterol into their cell membrane by a physicochemical process independent of energy metabolism [2—7]. Calorimetric evidence obtained with mixed phospholipid bilayers suggests that once cholesterol is in the lipid bilayer it interacts preferentially with the lipid species of the lowest melting temperature. Thus, when lateral separation of the phospholipids occurs, such as when the temperature is lowered, cholesterol tends to associate with lipids that are still in the liquid-crystalline state [8, 9]. It seemed of interest to investigate whether the physical state of a biological membrane influences the incorporation of exogenous cholesterol into it. Mycoplasmas are eminently suitable for studying this problem since they incorporate large quantities of cholesterol into their plasma membrane without modifying it. All the cholesterol in the mycoplasma membrane is exogenously derived, as none of these microorganisms can synthesize sterols [10]. Moreover, the fatty acid composition of the mycoplasma membrane lipids can be easily varied, enabling controlled alterations of membrane fluidity [11].

Acholeplasma laidlawii (oral strain) was grown at 37°C in 5-l volumes of a cholesterol-less Edward medium supplemented with 0.4% (w/v) fatty acid-poor bovine serum albumin, and 20 μg/ml of either elaidate or oleate [12]. When culture turbidity at 640 nm reached 0.15—0.20, the culture was divided into two parts. One was rapidly chilled to 4°C and the other was kept at 37°C. Cholesterol was added to both portions from a Tween 80-cholesterol solution [5] to give final concentrations of 20 μg cholesterol/ml and 0.01% (w/v) Tween 80. Aliquots of culture (0.5—1 l) were withdrawn at various time intervals and their absorbance was measured. The organisms were harvested, washed once in 0.25 M NaCl, and osmotically lysed in deionized water [12]. The isolated membranes were washed once in deionized water, followed by 0.05 M NaCl in 0.01 M phosphate buffer, pH 7.5, and again in deionized water. The washed membranes were analyzed for protein [13] and extracted with chloroform/methanol (2 : 1, v/v) as described previously [14]. Cholesterol was determined in the lipid extract according to Rudel and Morris [15] and lipid phosphorus was measured by the method of Ames [16]. The fatty acid composition of membrane lipids was determined by gas-liquid chromatography as described by Rottem and Razin [14]. Elaidate was found to comprise from 72 to 84% of the total fatty acids in the elaidate-enriched cells, while oleate made up from 67 to 78% of the total fatty acids in the oleate-enriched cells, depending on the batch tested.

The addition of cholesterol to the elaidate-enriched culture at 37°C was followed by a rapid uptake of cholesterol into the cell membranes. The organisms continued to grow as evidenced by increases in culture turbidity (Fig. 1), viable counts, and cell protein (not shown). The transfer of the elaidate-enriched culture from 37 to 4°C caused an immediate rise in culture turbidity, which was reversible upon returning the culture to 37°C (Fig. 1). The oleate-enriched cultures showed only a slight change in turbidity on chilling, if any. The phase transition temperature of elaidate-enriched *A. laidlawii* membranes, determined calorimetrically, was found by McElhaney [17] to range from 5 to 32°C, whereas that of the oleate-enriched membranes of this organism was

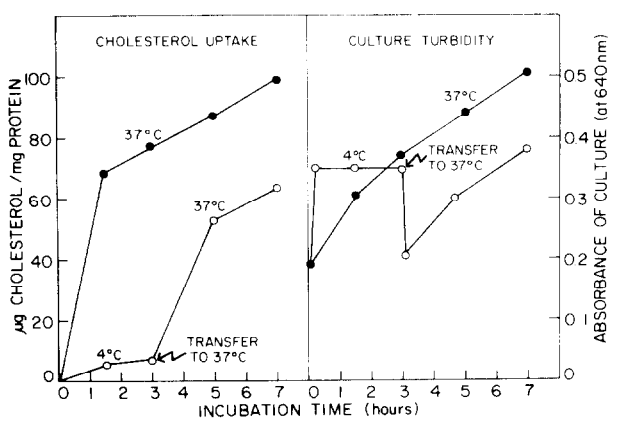

Fig. 1. Effects of temperature shifts on the absorbance and cholesterol uptake capacity of elaidate-enriched *A. laidlawii* cells in culture. Cholesterol was added when absorbance of culture reached 0.2 (zero time). Closed circles, culture kept at 37°C throughout the experiment; open circles, culture undergoing temperature shifts. For other experimental details see text.

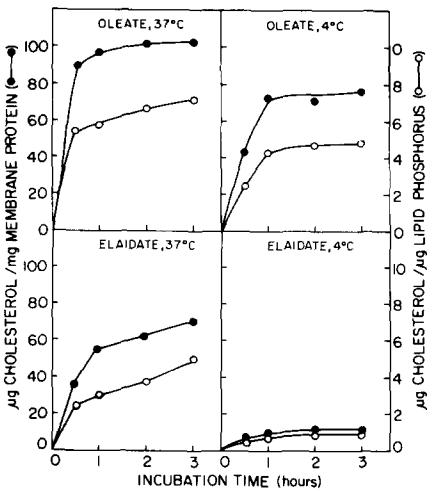

Fig. 2. Effect of temperature on cholesterol uptake by oleate- and elaidate-enriched *A. laidlawii* cells in culture. The cultures were divided into two parts when culture absorbance at 640 nm reached 0.18. One part was kept at 37°C and the other was transferred to 4°C. Cholesterol was added (zero time) and culture samples were withdrawn at the time intervals shown in figure for analysis of membrane protein, cholesterol and lipid phosphorus.

−22°C to −4°C. Hence, the marked turbidity change observed after chilling of the elaidate-enriched cells is an expression of the phase transition of membrane lipids from the liquid-crystalline to the gel state [18].

Fig. 1 also shows that cholesterol uptake by the chilled elaidate-enriched cells was very low. Transfer of the chilled culture to 37°C enhanced cholesterol uptake considerably. The picture was different with the oleate-enriched cultures. As seen in Fig. 2, cholesterol uptake by these cells was significant at 4°C also, though it was slower and did not reach the same level as at 37°C. In addition, uptake by the oleate-enriched organisms at 37°C was faster and reached equilibrium sooner than with the elaidate-enriched organisms incubated at this temperature. The reason for the lower level of cholesterol in the oleate-enriched cells kept at 4°C is not known. A possible explanation can be based on a recent finding (Clejan, S., Bittman, R. and Rottem, S., personal communication) that the transbilayer movement ("flip-flop") of cholesterol in membranes of growing *Mycoplasma capricolum* is rapid at 37°C and very slow at 4°C. The fact that in oleate-enriched cells significant uptake of cholesterol also occurs at 4°C indicates that growth is not essential for cholesterol uptake. In fact, membranes isolated from oleate-enriched *A. laidlawii* cells took up cholesterol at a faster rate than membranes from palmitate-enriched cells [19], a result which is in accord with the present data obtained with intact cells.

In conclusion, the data presented in this report show that incorporation of exogenous cholesterol into the cell membrane of living mycoplasmas depends on membrane fluidity. Cholesterol uptake is rapid when the membrane lipid bilayer is in the liquid-crystalline state and very slow when the lipid is in the gel state.

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