# Preferential Uptake of Lipids by Mycoplasma Membranes from Human Plasma Low-Density Lipoproteins†

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ABSTRACT: The binding and transfer of low-density lipoprotein (LDL) constituents to mycoplasma membranes were examined. *Mycoplasma capricolum* was found to bind more $^{125}$I-labeled LDL than *Acholeplasma laidlawii*. Free and esterified cholesterol uptake was 3–4 times higher in *M. capricolum* than in *A. laidlawii*. Cholesterol transfer to the membranes of both organisms far exceeded the amounts of cholesterol expected according to LDL protein found associated with the membranes. Trypsin digestion of the membranes prior to incubation with LDL decreased the binding of LDL and the transfer of free cholesterol to *M. capricolum* membranes but did not affect these processes with *A. laidlawii* membranes. These findings suggest the existence of protease-sensitive receptors on *M. capricolum* cell surface responsible for tighter contact with LDL. Analysis of LDL after incubation with large amounts of *M. capricolum* membranes revealed a loss of 60% of the lipoprotein-free cholesterol, 25.9% of the esterified cholesterol, and 20.7% of phospholipids without appreciable loss of protein. Rate zonal ultracentrifugation showed that the LDL separated as a single symmetrical peak, denser than the control. Electron microscopy showed that the lipoprotein particles retained their spherical shape but became about 10% smaller in diameter. These observations lead us to conclude that both free and esterified cholesterols are transferable from LDL to the membranes by a simple exchange process which does not involve prolonged contact or fusion of the particle with the membrane and does not result in degradation of the lipoprotein particle. We propose that similar mechanisms of free and esterified cholesterol transfer may also operate in vivo and contribute to the process of cholesterol exit from the plasma.

Several pathways for exit of cholesterol from the plasma compartment have been described in the last decade. The best studied pathway involves interaction of plasma lipoprotein with specific cellular receptors. Brown and Goldstein were the first to demonstrate the existence of receptors responsible for LDL catabolism (Goldstein & Brown, 1977; Brown et al., 1981). More recently, receptors for apoE-containing lipoproteins (Havel et al., 1980; Sherrill et al., 1980; Mahley et al., 1981) and for modified LDL (Basu et al., 1976; Brown et al., 1980; Shechter et al., 1981) have been demonstrated. The common feature of the receptor pathways is internalization of the receptor–lipoprotein complex followed by irreversible degradation of the cholesterol-containing lipoprotein particle.

Several years ago, we described another potential pathway for transport of cholesterol from human plasma lipoproteins to cells while using mycoplasma cells and membranes as models (Slutzky et al., 1976, 1977; Rottem et al., 1978). In these studies we demonstrated preferential transport of free cholesterol from human plasma low density lipoprotein (LDL) and high density lipoprotein (HDL) either to growing *Mycoplasma hominis* and *Acholeplasma laidlawii* cells (Slutzky et al., 1976) or to *A. laidlawii* membranes (Slutzky et al., 1977). More recently, Mitschelen et al. (1981) have confirmed our observations and pointed out that as much as 90% of the LDL-free cholesterol can be transported to *A. laidlawii* membranes. These observations suggest that free cholesterol may leave the plasma compartment without irreversible catabolism of lipoproteins, if similar phenomena occur in vivo.

Already in our first report (Slutzky et al., 1976), we have observed that significant amounts of cholesterol esters are also transported from LDL to *M. hominis* but not to *A. laidlawii*. It seemed, therefore, that transport of cholesterol from lipoproteins to mycoplasma membranes is not limited to the free form of the molecule and that interactions of lipoproteins with mycoplasma membranes may differ among species. *Mycoplasma capricolum* is a mycoplasma species that is especially suitable for the study of the mechanism of cholesterol transfer from lipoprotein. Unlike *A. laidlawii*, it requires cholesterol for growth, and the free and esterified cholesterol content in its membrane can be manipulated by changing the source and amounts of cholesterol in the growth medium (Razin et al., 1980; Efrati et al., 1980). Moreover, binding sites for cholesterol are apparently present in *M. capricolum* but not in *A. laidlawii* membranes (Efrati et al., 1981). These facts encouraged us to study in detail the mechanisms involved in transfer of free and esterified cholesterol from human plasma LDL to *M. capricolum* membranes. In particular, we have attempted to determine the effects of such transfer on the LDL particle. The results of the present study indeed indicate that all LDL lipids are potentially transferable to *M. capricolum* membranes, that the lipid-depleted lipoprotein remains relatively intact, and that interactions of the lipoproteins with binding sites on the membranes are associated with cholesterol transfer.

## Materials and Methods

*Organisms and Membrane Preparation. Acholeplasma laidlawii* (oral strain) and *Mycoplasma capricolum* (California kid, ATCC 27343) were grown in a modified Edward medium (Razin & Rottem, 1976) supplemented with 0.5% (w/v) glucose, 0.2% (w/v) bovine serum albumin (Sigma), and 20 μg/mL elaidic acid. For growth of *M. capricolum* 0.2–0.3% (v/v) horse serum was added. The low serum concentration which enabled only suboptimal growth of *M. capricolum* resulted in cells with low cholesterol content, while the *A. laidlawii* cells cultivated in the absence of serum had no cholesterol in their membranes. The organisms were harvested after 20 h of incubation at 37 °C. The sedimented organisms were washed twice and resuspended in 0.25 M

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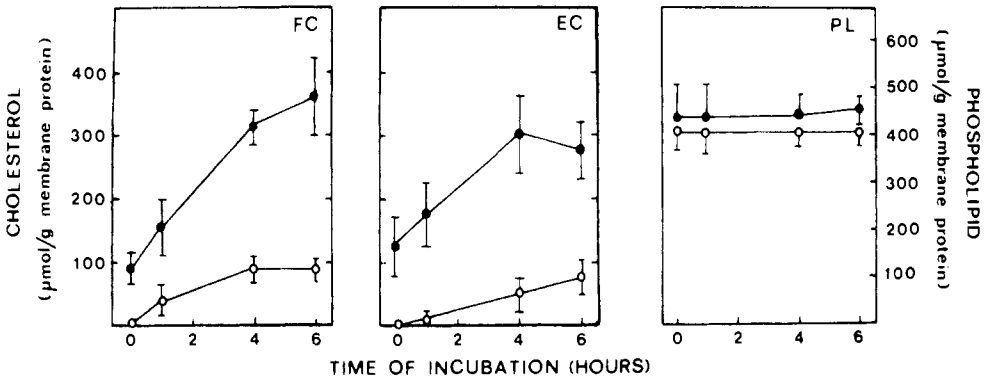


FIGURE 1: Kinetics of cholesterol and phospholipid uptake from LDL by isolated mycoplasma membranes. Isolated mycoplasma membranes were incubated with human plasma LDL at 37 °C in 0.05 M phosphate buffer, pH 7.0, at a protein ratio of LDL:membrane of 1.0. Aliquots of the mixture were withdrawn at time intervals (0, 2, 4, and 6 h of incubation) and transferred to crushed ice, and the membranes were isolated and washed by centrifugation at 4 °C as described under Materials and Methods. Free and esterified cholesterol and phospholipid content of the membranes were determined after lipid extraction (see Materials and Methods). Data are the mean of five different experiments using membranes from *M. capricolum* (●) or *A. laidlawii* (○). Abbreviations: FC, free cholesterol; EC, esterified cholesterol; PL, phospholipids.

NaCl. Cell membranes were isolated by osmotic lysis of the organisms, as described in detail by Razin & Rottem (1976). In brief, cell suspensions in 0.25 M NaCl were transferred to 100 volumes of deionized water preheated at 37 °C. Pancreatic deoxyribonuclease (20 μg/mL) was added to the lysed cell suspension to facilitate isolation of *M. capricolum* membranes free of DNA. The membranes were collected after 15 min of incubation at 37 °C by centrifugation at 38000*g* for 30 min. The membranes were then 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.

*Preparation of Lipoprotein.* Human serum low-density lipoprotein (LDL) was prepared by ultracentrifugation from 14-h fasting human plasma. The LDL was isolated at salt density interval 1.020–1.055 g/mL by the tube slicing technique (Havel et al., 1955). The LDL was washed once in a KBr solution of $d$ = 1.055 g/mL and dialyzed against three changes of 0.9% NaCl and 0.01% ethylenediaminetetraacetic acid (EDTA), pH 7.4 solution. The average composition of LDL preparation was 32.2% (w/w) protein, 32.0% cholesterol ester ($M_r$ 650), 11.0% free cholesterol, and 24.7% phospholipids ($M_r$ 750). In some experiments LDL was iodinated with $^{125}I$ (Bilheimer et al., 1972). About 95% of the $^{125}I$ was found to be associated with the lipoprotein apoprotein moiety. Before use, the lipoprotein was dialyzed for 24 h against 200 volumes of 100 mM sodium phosphate buffer, pH 7.2, and 20 mM MgCl. The lipoprotein fraction was filtered through a 0.45-μm pore size Millipore filter and stored at 4 °C until used.

*Uptake of Cholesterol and Phospholipids by Membranes.* Isolated membranes were incubated with LDL in 0.05 M phosphate buffer or in 0.05 M Tris-HCl, pH 7.0, containing 0.01 M EDTA and 0.05% sodium azide, at 37 °C for 4 h. Similar results were obtained with either buffer. At the end of the incubation period, the membranes were collected by centrifugation at 48000*g* for 20 min at 4 °C. The membranes were washed twice in 0.05 M NaCl. More washes or incubation of the doubly washed membranes with 1% albumin for 30 min at 37 °C did not change the $^{125}I$-labeled LDL radioactivity associated with the membranes, indicating that the washing procedure sufficed for removing unbound LDL from the membranes. In some experiments, before the incubation with LDL, the membranes were incubated with 50 μg/mL trypsin (Sigma, diphenylcarbamoyl chloride treated) in 0.05 M phosphate buffer, pH 7.0, for 2 h at 37 °C. The membranes were sedimented and washed twice with 0.05 M NaCl. Sodium dodecyl sulfate ($NaDodSO_4$)–polyacrylamide gel electrophoresis of the LDL apoproteins and the amounts of $Cl_3CCOOH$-soluble radioactivity in these experiments were similar to those observed after incubation of LDL with membranes not treated with trypsin. Thus, trypsin activity was not detected in the membranes.

The lipoproteins in the incubation mixtures after removal of the membranes were concentrated by ultracentrifugation at a density of 1.085 g/mL as described above. In some experiments, the LDL was isolated by ultracentrifugation for 140 min at 42000 rpm by using a 14 Ti zonal rotor and NaBr gradient of $d$ = 1.00–1.30 g/mL (Patsch et al., 1974).

*Electron Microscopy.* Negative-staining electron microscopy of LDL was performed on collodium–carbon coated grids stained with 2% potassium phosphotungstate. Electron micrographs were obtained with a Philips 300 electron microscope at 60 kV and instrument magnification of 90000×. Size distribution of LDL was determined by measuring the diameter of 100 particles in 2.5× enlarged prints.

*Analytical Procedures.* Protein was determined according to the method of Lowry et al. (1951). $NaDodSO_4$–polyacrylamide gel electrophoresis of LDL apoprotein and of membranes was performed on 15% polyacrylamide gels following the procedure of Weber & Osborn (1969). Lipids were extracted from membranes or from LDL with chloroform–methanol (2:1 v/v; Rottem & Razin, 1973). The membrane lipid extracts were analyzed for cholesterol by the colorimetric technique of Rudel & Morris (1973). The LDL cholesterol was determined by the cholesterol oxidase–cholesterol esterase technique (Patsch et al., 1976) on unextracted LDL. Lipid phosphorus was determined after digestion of the sample by an ethanolic solution of $Mg(NO_3)_2$ (Ames, 1966). This was done on membrane lipid extracts and unextracted LDL. Thin-layer chromatography of polar lipids was performed on silica gel HR (Merck) plates (Razin et al., 1980). For phosphorus quantitation, the area of the lipid was scraped off the plate, and phosphorus was determined directly on the collected silica gel material. Neutral lipids were separated on silica gel G (Merck) plates, and the amounts of free and esterified cholesterol were determined as described in detail previously (Razin et al., 1980).

## Results

*Transfer of Lipids from LDL to Membranes.* Transfer of lipids from human plasma LDL to membranes isolated from *M. capricolum* (grown in medium containing 0.2% serum) and *A. laidlawii* (grown in medium without serum) as a function of incubation time at 37 °C is shown in Figure 1. Continuous transfer of free cholesterol and cholesterol ester but almost

Table I: Uptake of LDL by Isolated Mycoplasma Membranes[a]

| organism | temp (°C) | LDL-protein bound (μg/mg of membrane protein) | cholesterol uptake (μg/mg of membrane protein) | | | |
|---|---|---|---|---|---|---|
| | | | actual values | | calcd values[b] | |
| | | | FC | EC | FC | EC |
| *M. capricolum*[c] | 0 | 14.5 ± 9.6 | 7.3 ± 7.8 | 26.3 ± 8.6 | 4.9 ± 3.3 | 14.5 ± 9.6 |
| | 37 | 40.3 ± 17.1 | 106.4 ± 22.1 | 107.3 ± 10.6 | 13.7 ± 5.8 | 40.3 ± 17.1 |
| *A. laidlawii*[c] | 0 | 12.8 ± 8.8 | 8.4 ± 7.1 | 6.8 ± 7.1 | 4.3 ± 3.0 | 12.8 ± 8.8 |
| | 37 | 26.8 ± 15.2 | 26.8 ± 12.0 | 33.1 ± 7.9 | 9.1 ± 5.2 | 26.8 ± 15.2 |

[a] Isolated mycoplasma membranes were incubated with human plasma LDL at an LDL:membrane protein ratio of 1:1 for 4 h at either 0 or 37 °C. The incubation mixture contained about (7–10) × $10^6$ cpm of $^{125}$I-labeled LDL/mg of protein. Free and esterified cholesterol content associated with the membranes were determined as described under Materials and Methods. The amount of LDL protein in the membrane fraction was calculated from the radioactivity values associated with the membrane and the LDL specific activity. Data are mean ± SD of five different experiments. [b] Calculated according to the amounts of $^{125}$I-labeled LDL protein bound to the membranes and based on LDL proteins:EC:FC:PL composition of 32:32:11:24 (w/w). [c] Lipid composition of *M. capricolum* membranes was the following: phospholipids, 0.43 μmol/mg of protein; cholesterol ester, 0.12 μmol/mg of protein; free cholesterol, 0.09 μmol/mg of protein. *A. laidlawii* membranes did not contain free or esterified cholesterol, and their phospholipid content was 0.41 μmol/mg of protein.

no transfer of phospholipids to the membranes is evident. For both free and esterified cholesterol the transfer to *M. capricolum* membranes exceeded that to *A. laidlawii* membranes by 3–4-fold, at all time intervals. With the ratio of LDL protein to membrane protein used in these experiments of 1:1 (w/w), leveling of cholesterol uptake was almost reached after 4–6 h of incubation. In all subsequent experiments, the 4-h incubation period was used. The possible transfer of LDL proteins was investigated by using $^{125}$I-labeled LDL (98% of radioactivity in protein) of known specific activity and was compared to transfer of free and esterified cholesterol (Table I). After incubation at 0 °C, minimal amounts of $^{125}$I-labeled LDL and of free and esterified cholesterol were found associated with the washed membranes. As no $^{125}$I radioactivity was extracted by chloroform–methanol, it was concluded that the $^{125}$I in the membranes represented LDL proteins. At 37 °C, the amount of $^{125}$I-labeled LDL found associated with the washed *M. capricolum* and *A. laidlawii* membranes approximately doubled as compared to that at 0 °C. In both mycoplasmas more free cholesterol and esterified cholesterol were taken up as compared to $^{125}$I-labeled LDL protein. For *A. laidlawii*, however, the presence of cholesterol esters associated with the membrane could be attributed to the adherence of LDL particles while in *M. capricolum* an excess of 2–3-fold cholesterol ester was found in the membrane as compared to LDL protein. In both species, transfer of free cholesterol to the membrane far exceeded that calculated on the basis of attached $^{125}$I-labeled protein (Table I).

For determination of whether or not the small amounts of LDL in the membranes are associated through interaction with membrane protein, the uptake experiment was repeated after trypsin digestion of the membranes (Figure 2). Trypsin digestion resulted in a considerable decrease of the binding of $^{125}$I-labeled LDL and of the uptake of free cholesterol by membranes from *M. capricolum*. Minimal or no effect was observed in trypsin-digested membranes from *A. laidlawii*.

*Properties of the Lipid-Depleted LDL. M. capricolum* membranes were used to study the effects of lipid transfer on LDL particles. So that maximal transfer could be achieved, incubation was carried out with a constant amount of LDL (2 mg of protein) and increasing amounts of membranes (2–10 mg of protein). As can be seen in Figure 3, free cholesterol and esterified cholesterol lost from LDL were found in the membranes. Some phospholipids were also lost from LDL especially when incubated with high amounts of membranes. Thin-layer chromatography of LDL phospholipids revealed that both lecithin and sphingomyelin were lost (more of the latter) and that some mycoplasma phospholipids were added to LDL. Accordingly, the lecithin to sphingomyelin molar

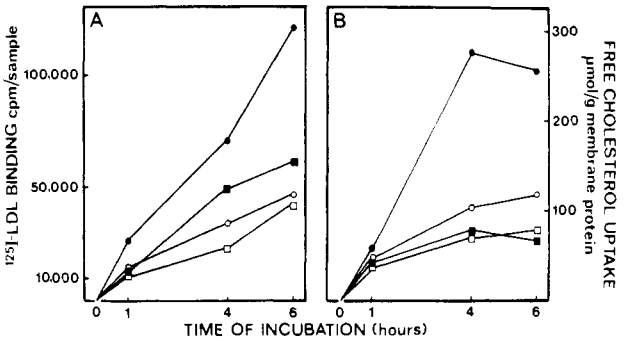


FIGURE 2: $^{125}$I-Labeled LDL binding and free cholesterol uptake by trypsin-treated mycoplasma membranes. Data are the mean of two separate experiments using untreated membranes (●) or trypsin-treated membranes (○) of *M. capricolum* and untreated membranes (■) or trypsin-treated membranes (□) of *A. laidlawii*.

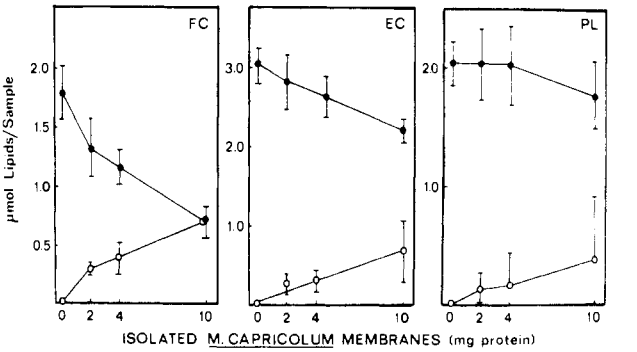


FIGURE 3: Cholesterol and phospholipid content of LDL and *M. capricolum* membranes: effects of increasing amounts of membranes. Human LDL (2 mg of protein) was incubated at 37 °C for 4 h with various amounts of membranes. The LDL and membranes were separated by centrifugation at 4 °C after the incubation. The membranes were washed, and the LDL was isolated by ultracentrifugation, as described under Materials and Methods. The figure shows the free and esterified cholesterol and phospholipid content of LDL (●) and the increase in free and esterified cholesterol and of phospholipid content of the membrane (○). Data are mean of five separate experiments.

ratio in LDL increased from 2.67 (untreated LDL, mean of two experiments) to 3.71 (LDL incubated with 10 mg of membranes). Analysis of the LDL after incubation with membranes revealed maximal loss of 60% of the lipoprotein free cholesterol, 25.9% of the cholesterol ester, and 20.7% of the phospholipids without appreciable loss of protein (Table II). As these losses occurred after incubation of LDL with membranes from *M. capricolum* grown in a medium with limited amounts of serum (0.2%), it was of interest to determine whether similar effects are taking place with membranes

Table II: Depletion of LDL Lipids by Mycoplasma Membranes[a]

| membranes (mg of protein) | loss of LDL lipids (% of native LDL lipids) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *M. capricolum*[b] (grown with 0.2% serum) | | | *M. capricolum*[c] (grown with 5% serum) | | | *A. laidlawii*[d] (grown with no serum) | | |
| | FC | EC | PL | FC | EC | PL | FC | EC | PL |
| 2 | 22.4 ± 6.9 | 14.9 ± 7.0 | 0.0 ± 0.0 | 4.7 | 0.0 | 0.0 | 8.7 | 5.0 | 0.0 |
| 4 | 34.4 ± 4.4 | 13.9 ± 6.3 | 4.0 ± 5.2 | 13.9 | 0.7 | 0.0 | 9.2 | 2.3 | 0.0 |
| 10 | 60.0 ± 1.6 | 25.9 ± 3.6 | 20.7 ± 6.3 | 23.4 | 1.1 | 0.0 | 18.4 | 7.8 | 0.0 |

[a] Human plasma LDL (2 mg of protein) was incubated at 37 °C for 4 h with increasing amounts of membranes of *M. capricolum* grown with either 0.2% serum or with 5% serum or with membranes of *A. laidlawii* grown with no serum. Data show the percentage decrease of LDL lipids as compared to the intact incubated lipoprotein and are from five experiments with 0.2% and two with 5% serum and no serum. [b] Lipid composition of membranes before incubation with LDL: free cholesterol and esterified cholesterol, 0.04 and 0.09 μmol/mg of membrane protein, respectively; phospholipids, 0.33 μmol/mg of membrane protein. [c] Lipid composition of membranes before incubation with LDL: free cholesterol and esterified cholesterol, 0.18 and 0.24 μmol/mg of membrane protein, respectively; phospholipids, 0.46 μmol/mg of membrane protein. [d] Lipid composition of membranes before incubation with LDL: no cholesterol; phospholipids, 0.42 μmol/mg of membrane protein.

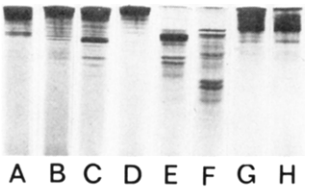


FIGURE 4: $NaDodSO_4$–polyacrylamide gel electrophoresis of LDL apoprotein after incubation with various amounts of mycoplasma membranes. (A) LDL incubated with buffer; (B) LDL after incubation with membranes (2 mg of protein) of *M. capricolum* grown with 0.2% serum; (C) LDL after incubation with membranes (10 mg of protein) of *M. capricolum* growth with 0.2% serum; (D) LDL after incubation with membranes (10 mg of protein) of *A. laidlawii* grown with no serum; (E) membranes of *M. capricolum* grown with 0.2% serum; (F) membranes of *A. laidlawii* grown without serum; (G) LDL after incubation with membranes (2 mg of protein) of *M. capricolum* grown with 5% serum; (H) LDL after incubation with membranes (10 mg of protein) of *M. capricolum* grown with 5% serum.

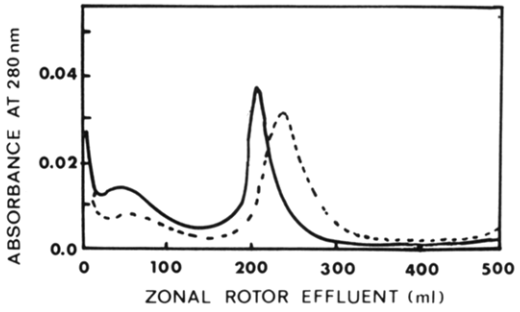


FIGURE 5: Rate zonal ultracentrifugation of human plasma LDL after incubation in buffer alone (—) or with membranes (10 mg of protein) of *M. capricolum* (- - -). Incubation was carried out for 4 h in 0.05 M phosphate buffer, pH 7.0.

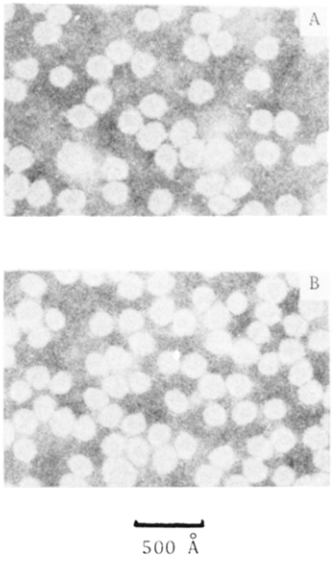


FIGURE 6: Electron micrographs of negatively stained LDL after incubation for 4 h with buffer (A) and with membranes (10 mg of protein) of *M. capricolum* (B).

of cells grown in a medium supplemented with 5% horse serum and with membranes from *A. laidlawii*, grown without serum. With *M. capricolum* grown in 5% serum, no losses of cholesterol ester and phospholipids were observed, but some free cholesterol (up to 23.4% of that present in the original LDL) was lost through transfer to the membranes. Similar results were observed with *A. laidlawii*, where there was minimal transfer of LDL cholesterol ester to the membranes (Table II).

$NaDodSO_4$–polyacrylamide gel electrophoresis of LDL apoproteins after incubation with membranes is shown in Figure 4 together with the $NaDodSO_4$–polyacrylamide gel electrophoresis of the membrane protein. After the incubation lower molecular weight peptides were observed, and the possibility that some mycoplasma proteins cosedimented as minute membrane fragments with the LDL cannot be ruled out. For determination of whether some LDL proteins were degraded to peptides and amino acids, the amount of $^{125}I$ remaining in the supernatant after precipitation of the LDL with cold 10% trichloroacetic acid ($Cl_3CCOOH$) was determined immediately after the incubation period and separation of the membranes. $Cl_3CCOOH$ nonprecipitable radioactivity was 3.7% of total radioactivity in native unincubated LDL; after incubation with *M. capricolum* membranes, $Cl_3CCOOH$ nonprecipitable $^{125}I$ was 3.7%, 4.1%, and 6.2% with 2, 4, and 10 mg of membrane proteins, respectively. With *A. laidlawii* membranes equivalent to 2 and 10 mg of protein, it was 3.4% and 4.5%, respectively.

Possible effects of the incubation with membranes on the hydrated density of LDL were determined by ultracentrifugation in a zonal system after incubation of the LDL with *M. capricolum* membranes (Figure 5). After the incubation with the membranes, LDL eluted as a symmetrical peak, similar to the control lipoprotein, but appeared 10–20 mL later in the rotor effluent and was somewhat broader.

Negatively stained electron micrographs of LDL after incubation in buffer alone and in buffer containing *M. capricolum* membranes (10 mg protein) are shown in Figure 6. After incubation with membranes, the LDL retained the spherical shape of the lipoprotein particle but appeared somewhat smaller. Indeed, the mean diameter of LDL in-

cubated with buffer was 217 ± 23.6 Å (mean ± SD) and that of LDL incubated with membranes was 195 ± 17.2 Å.

## Discussion

Several studies have shown that human plasma low-density lipoprotein supplies free cholesterol to either growing mycoplasma (Slutzky et al., 1976, 1977; Rottem et al., 1978) or isolated mycoplasma membranes (Slutzky et al., 1977; Mitschelen et al., 1981). The studies with membranes were carried out with *A. laidlawii* grown in the absence of serum or other cholesterol sources. The membranes therefore were completely devoid of cholesterol. In such systems, as much as 90% of the LDL free cholesterol is transferable to the membranes (Mitschelen et al., 1981).

In the present study we show similar transfer of free cholesterol from human plasma LDL to *M. capricolum* membranes. *M. capricolum* is a sterol-requiring mycoplasma. The amounts of cholesterol in the membranes can be restricted when the organism is grown in the presence of small amounts of serum. We show that membranes isolated from *M. capricolum* and containing some cholesterol are better acceptors for LDL cholesterol than *A. laidlawii* membranes devoid of cholesterol. We found that 60% of the LDL free cholesterol was transferred to *M. capricolum* membranes after 4 h of incubation with LDL at an LDL:membrane protein ratio of 1:5 (w/w). With *A. laidlawii*, only 18.4% of the LDL free cholesterol were transferred under the same conditions to the membranes. The percentage of LDL free cholesterol transferred to *A. laidlawii* membranes reported by Mitschelen et al. (1981) with a similar LDL:membrane protein ratio was about 40% after 72 h of incubation [extrapolated from Figure 4 of Mitschelen et al. (1981)]. The reason for the differences in cholesterol uptake capacity between the two mycoplasmas is not clear. We have found a somewhat higher binding of $^{125}$I-labeled LDL to *M. capricolum* isolated membranes as compared to *A. laidlawii* (Table I). Growing *M. capricolum* cells were found by us (unpublished data) to bind about 3 times as much of $^{125}$I-labeled LDL than growing *A. laidlawii*. The contact of the LDL particles with the membranes is probably an important step for transfer of free cholesterol from the lipoprotein to the membrane.

The greater ability of *M. capricolum* to bind LDL as compared with *A. laidlawii* is probably due to the presence of binding sites to LDL in *M. capricolum* membranes. We have previously reported that protease treatment of *M. capricolum* membranes reduced the uptake of cholesterol by the membranes from phospholipid–cholesterol vesicles (Efrati et al., 1981). Protease treatment did not affect cholesterol uptake by *A. laidlawii* membranes. Our present study suggests that similar protease-sensitive binding sites may also exist for LDL in *M. capricolum* membranes. Thus, we found that pretreatment of *M. capricolum* membranes with trypsin reduced both $^{125}$I-labeled LDL binding and free cholesterol uptake. Neither phenomenon was observed with *A. laidlawii*. The nature of the LDL-binding sites in *M. capricolum* membrane needs further clarification.

In agreement with our previous reports (Slutzky et al., 1976, 1977), we find here that the transfer of free cholesterol from LDL to the membranes far exceeds the amount of lipoprotein protein bound to the membrane and therefore cannot be explained by nonspecific adsorption of lipoprotein particles to the membranes. The mechanism of free cholesterol transfer to the membranes has not been elucidated in the present study. One possibility is that transfer of free cholesterol occurs during a transient contact of the LDL with binding sites present in the membrane, as discussed above. Free cholesterol transfer, however, may also occur through the water phase, as reported for cholesterol exchange between phospholipid vesicles (McLean & Phillips, 1981; Backer & Dawidowicz, 1981). With either mechanism, net transfer of free cholesterol seems to take place from a particle with a high cholesterol to phospholipid ratio to a particle or membrane with a low cholesterol to phospholipid ratio (Cooper, 1978). In support of these observations, we found, in fact, a significantly smaller transfer of free cholesterol from LDL to membranes isolated from *M. capricolum* grown with 5% serum and containing a much higher content of cholesterol.

Previous studies on the transfer of cholesterol from LDL to mycoplasma membranes (Slutzky et al., 1976, 1977; Mitschelen et al., 1981) or animal cells (Cooper, 1978; Rothblat et al., 1978) emphasized the free cholesterol moiety of the lipoprotein. In the present study we have focused on other LDL lipid and protein constituents as well. An outstanding finding of the study was transfer of one-fourth to one-third of the LDL cholesterol ester, but not of apoB, to *M. capricolum* membranes grown with limited amounts of serum. Phospholipids were also lost from LDL. This loss of phospholipids, however, was not apparent with relatively small amounts of membranes and was less pronounced than cholesterol ester transfer. Thus, apparently LDL donates small amounts of cholesterol ester without loss of phospholipids or protein when the lipoprotein comes into contact with a cholesterol-poor membrane.

It is usually assumed that cholesterol esters (a core-lipid constituent of lipoproteins) enter tissues only when the lipoprotein particle is internalized and irreversibly catabolized (Brown et al., 1981). Several reports, however, have described preferential loss of cholesterol esters from lipoproteins without catabolism of the particle. Fielding (1978) demonstrated loss of cholesterol esters from chylomicrons to either heart or endothelial cells (Fielding et al., 1979). This finding was recently confirmed in our laboratory while following transfer of a cholesterol ester analogue, cholesteryl linoleyl ether (Friedman et al., 1981; Chajek-Shaul et al., 1981). In these studies, it has been shown that the presence of lipoprotein lipase is a prerequisite for the transfer of the cholesterol ether molecule to cells (Friedman et al., 1981; Chajek-Shaul et al., 1981). Our study demonstrates loss of cholesterol esters from LDL in a system devoid of lipoprotein lipase. We therefore conclude that cholesterol esters may move from LDL by a surface membrane transfer process (see above) without prior interaction with an enzyme.

After the prolonged incubation with mycoplasma membranes, the LDL retained many of the original properties of the lipoprotein. The LDL floated at appropriate gravity (1.019–1.063 g/mL), exhibited a symmetrical peak in a zonal ultracentrifugation system, and retained its spherical shape. The 10% decrease in diameter of the LDL particles agrees with the loss of 20–30% of the cholesterol esters (based on volume–diameter consideration). The mechanism for preferential loss of cholesterol esters from LDL described here may serve as a metabolic path by which "large" LDL form "smaller" particles, without disintegration of the lipoprotein. In vivo the operation of a similar path may explain the observations that during passage of LDL through the human liver, the lipoprotein loses some cholesterol esters but not apoB (Sniderman et al., 1978). The potential physiological importance of a pathway for preferential loss of cholesterol esters from lipoproteins (without irreversible catabolic interaction) cannot be overemphasized. By calculation, LDL and HDL catabolism accounts for only 50–70% of the lipoprotein cholesterol esters

cleared from human plasma in a day (Stein & Stein, 1980). The pathway discussed here may provide one of the mechanisms for transport of the other 30–50% of the plasma cholesterol esters from circulary lipoproteins to cells. Hepatic and endocrine cells (e.g., adrenal and gonades) may serve in the intact animals as acceptors for both free cholesterol and cholesterol esters. These cells utilize and secrete large amounts of cholesterol—or metabolic products of cholesterol—as part of their metabolic activity, a process that renders the cell membranes relatively unsaturated with cholesterol.

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