Korean Dairy Techno. 14(2) : 195~206, 1996

Cholesterol Uptake by *Lactobacillus acidophilus*: Its Fate and Factors Influencing the Uptake

Dong-Ouk Noh
Department of animal Science, Oklahoma State University

*Lactobacillus acidophilus*에 의한 콜레스테롤의 흡착

노 동 욱
오클라호마 주립대학교 축산대학

ABSTART

Cholesterol assimilated by *Lactobacillus acidophilus* ATCC 43121 was not metabolically degraded in that most of it was recovered with the cells. Cells grown in the presence of cholesterol micelles and bile salts were more resistant to lysis by sonication than those grown in their absence, suggesting a possible alteration of cellular membranes. Cholesterol assimilation occurred during growth at pH 6.0, the amount of which was more than that by cells grown without pH control. Cholesterol assimilated by cells was recovered in the membrane fractions of cells both grown at pH 6.0 and without pH control. The effect of unsaturated fatty acids on cholesterol assimilation was not clear, since there was no significant ($P>0.05$) difference in the amount taken up from micelles prepared using L-α-phosphatidylcholine, dioleoyl or L-α-phosphatidylcholine, distearoyl. Without Tween 80, little, if any, cell growth or cholesterol uptake was observed. In the presence of 0.05% Tween 80, cholesterol uptake increased dramatically as did growth. However, as the amount of Tween 80 increased beyond 0.05%, cholesterol uptake decreased while the amount of growth remained the same.

I. INTRODUCTION

Atherosclerosis is associated with high levels of LDL cholesterol in the blood [3, 16, 31]. It is the major cause of coronary heart disease, a leading cause of death in the United States [15, 18, 24]. Thus, reducing the LDL cholesterol level in hypercholesterolemic persons is considered important in the control of atherosclerosis [18, 19].

The absorption of cholesterol (both dietary and biliary) from the gut is an important source of cholesterol for humans. Connor et al. [5] reported a positive relationship between dietary cholesterol intake and serum cholesterol levels. Therefore, the serum cholesterol levels can be, at least to some extent, controlled through dietary modification [12].

The growth of certain lactic acid bacteria

having the ability to take up cholesterol in the small intestines has the potential to aid in the control of serum cholesterol levels in humans, since the small intestine is the primary site of cholesterol absorption in human body [4, 8]. Mann and Spoerry [21] reported that the consumption of milk fermented with strains of *Lactobacillus* reduced serum cholesterol levels in Maasai warriors. Since then, the potential hypocholesterolemic effects of cultured products has been shown by other researchers [1, 14, 20, 36].

Lactobacillus acidophilus, a normal inhabitant of small intestine, has potential for producing a hypocholesterolemic effect. According to Mott et al. [23], germ-free pigs exhibited reduced serum cholesterol levels after they were monocontaminated with *L. acidophilus* and allowed to devlop normal flora. Zacconi et al. [40] also observed reduced serum cholesterol levels in axenic mice contaminated with *L. acidophilus*.

Gilliland et al. [10] reported the assimilation of cholesterol into cells of *L. acidophilus* during growth in laboratory media. Somkuti and Johnson [35] also reported that about 80% of cholesterol removed from the growth medium during growth of *Propionibacterium freudenreichii* was recovered with the cells.

In the studies with *Mycoplasmas* species, which require exogenous cholesterol to grow, cholesterol taken up by the cells was closely associated with the membrane [7, 22]. Razin [26] reported that cholesterol increased the tensile strength of mycoplasma membrane, and permitted survival and growth of these organisms without the protection of cell walls.

Safonova et al. [32] reported the presence of saturated fatty acids inhibited cholesterol uptake by rat small intestine epithelial cells. They observed increased cholesterol uptake in the presence of oleic acid, a monounsaturated fatty acid. Razin et al. [28] also reported a higher rate constant for cholesterol uptake in oleate-enriched cells than in palmitate-enriched cells of *Acholeplasma laidlawii*.

The objectives of this study were: (1) to measure the effect of phospholipids having different fatty acid components on the cholesterol uptake by *L. acidophilus* ATCC 43121, (2) to measure the effect of different amounts of Tween 80 on the cholesterol uptake by *L. acidophilus* ATCC 43121, and (3) to measure the incorporation of cholesterol in the cell membrane fraction of *L. acidophilus* ATCC 43121.

Ⅱ. MATERIALS AND METHODS

1. Source and Maintenance of Culture

L. acidophilus ATCC 43121 (formerly strain RP 32) was from our laboratory stock culture collection. It was originally isolated from intestinal contents of a pig [10]. The culture was maintained by subculturing in lactobacilli MRS broth (Difco Laboratories, Detroit, MI) using 1% inocula and incubation at 37℃ for 18 hours. The culture was stored at 5℃ between transfers. It was subcultured at least three times just before experimental use.

2. Preparation of MRS-THIO Broth

MRS-THIO broth was prepared by dissolving 55g lactobacilli MRS broth (Difco), 2g thioglycolic acid (sodium salt, Sigma Chemical Co., St. Louis, MO) in 1,000ml of distilled water. It was further supplemented, when needed, with 2.16g (0.004M) taurocholic acid (sodium salt, Sigma Chemical Co.) or 3g (0.

3%) oxgall (Difco) per liter. The broth was autoclaved for 15 minutes at 121℃. The broth media were prepared on the day of experimental use.

3. Measurement of Cholesterol Assimilation

One ml of cholesterol-phosphatidylcholine micelles prepared according to Razin et al. [27] was added to the tubes containing 9 ml MRS-THIO broth. Egg yolk lecithin (Type Ⅲ-E, Sigma Chemical Co.) was used to prepare the micelles. Following mixing, 2 ml was transferred to a clean test tube and stored in the refrigerator at 5℃. This was used as an uninoculated control. The remaining broth was inoculated (1%) with a freshly prepared MRS broth culture of *L. acidophilus* ATCC 43121 and incubated at 37℃ for the desired time. After incubation, cells were removed by centrifugation at 12,000 × g and 4℃ for 10 minutes.

The *o*-phthalaldehyde method described by Rudel and Morris [30] was used to determine the amount of cholesterol in the spent broth and the uninoculated control. In most experiments, the amount assimilated (μg/ml) by the cells was calculated by subtracting the amount in the spent broth from that in the uninoculated control. However, in initial experiments, the cells were resuspended in distilled water to the original volume of the culture and assayed for cholesterol to determine the amount assimilated.

4. Resistance of Cells to Sonic Disruption

A freshly prepared MRS broth culture of *L. acidophilus* ATCC 43121 was inoculated (1%) into MRS-THIO broth, and into MRS-THIO broth containing 0.3% oxgall (Difco) and 1% cholesterol-phosphatidylcholine micelles. The inoculated media were incubated at 37℃ for 18hours. Cells were recovered by centrifugation at 12,000 × g and 4℃ for 10 minutes, and resuspended in distilled water to a population of approximately 4×10^9/ml. Ten ml of cell suspension was transferred to a small beaker which was placed in an ice-water bath. The cells were sonicated for 15 minutes with Sonic Dismembrator (Fisher Scientific, Pittsburgh, PA) adjusted to a maximum output. The numbers of intact cells from each medium before and after sonication were counted by direct microscopic count [25].

5. Measurement of Cholesterol Assimilation during Growth at pH 6.0

MRS-THIO broth (500ml) supplemented with 0.004M sodium taurocholate was prepared and placed into a small fermentor of about 1*l* capacity equipped with an autoclavable combination pH electrode. The fermentor also was equipped with a port for the addition of neutralizer and a line to permit continuous sparging with nitrogen gas. Then, 0.5ml of 0.2% aqueous methylene blue was added to the broth as an oxidation-reduction indicator. The entire fermentor containing the broth was autoclaved at 121℃ for 15 minutes. After cooling, 50ml of cholesterol micelles prepared using egg yolk lecithin (Type Ⅲ-E) were added. The fermentor, then, was placed in a 37℃ water bath. The flask containing the neutralizer, 5% sodium carbonate in 5% ammonium hydroxide [11], was connected to the fermentor. The automatic pH Controller (Model 5997, Horizon Ecology Co., Chicago, IL) was adjusted to maintain the pH of the

broth at 6.0. After mixing for 2 minutes, 10ml was withdrawn aseptically from the fermentor and placed into a sterilized test tube to serve as the uninoculated control. Then, 5ml of a freshly prepared MRS broth culture of *L. acidophilus* ATCC 43121 were added to the fermentor. Nitrogen gas was sparged through the broth (from bottom to top) continuously at about 11ml/min throughout the incubation period. After the incubation, 10ml of culture was withdrawn aseptically from the fermentor, centrifuged and the spent broth was assayed for cholesterol [30].

6. Isolation of Cellular Membranes

Cells of *L. acidophilus* ATCC 43121 were grown in 10ml of MRS-THIO broth supplemented with 0.004M sodium taurocholate and cholesterol micelles (prepared using Type Ⅲ-E egg yolk lecithin) at 37℃ for 18 hours and harvested by centrifugation at 12,000 × g at 4℃ for 10 minutes. The cell pellets were washed with distilled water and membrane isolation was carried out according to the method by Thorne and Barker [37]. The washed cells and membrane fractions were assayed for cholesterol [30], ATPase activity and protein.

7. Measurement of ATPase Activity and Protein Content

Adenosine triphosphatase (ATPase) activity was assayed by measuring the amount of inorganic phosphorus released during the incubation at 37℃ for 30 minutes according to Rottem and Razin [29], and Fiske and Subbarow [9]. The specific ATPase activity was expressed as μmoles of inorganic phosphorus released per mg protein/min. The protein content was measured by the method of Bradford [2] using human albumin (Sigma Chemical Co.) as a standard.

8. Preparation of Water-soluble Cholesterol

The stock solution of water-soluble cholesterol was prepared by dissolving polyoxyethanyl-cholesteryl sebacate (Sigma Chemical Co.) with distilled water to a concentration of 20mg/ml. The solution was passed through a sterile 0.45μm membrane filter into a sterile test tube and stored at 5℃. The stock solution was diluted as necessary with sterile distilled water and added to the growth medium for the assay of cholesterol uptake.

9. Effect of Phospholipids having Different Degrees of Unsaturation on Cholesterol Uptake

In order to measure the influence of the phospholipids having different degrees of unsaturation on cholesterol assimilation, four different phospholipids were used. They were egg yolk lecithin (Type Ⅲ-E), soybean lecithin (Type Ⅲ-S), L-α-phosphatidylcholine, dioleoyl, and L-α-phosphatidylcholine, distearoyl, all from Sigma Chemical Co. Soybean lecithin (Type Ⅲ-E) contains more unsaturated fatty acid moieties than does egg yolk lecithin (Type Ⅲ-E). Cholesterol-phospholipid micelles were prepared [27] using each of the four phospholipids. The micelles were used as cholesterol sources to compare cholesterol uptake by *L. acidophilus* ATCC 43121 as described in the section on measurement of cholesterol uptake.

10. Influence of Tween 80 on Cholesterol Assimilation

Lactobacilli MRS broth was prepared from individual ingredients according to the manufacturer's (Difco) formulation with Tween 80 (polyoxyethylene sorbitan monooleate). It was supplemented with 0.2% sodium thioglycollate and 0.004 M sodium taurocholate. Then Tween 80 was added to aliquots of the broth to make MRS-THIO broth containing 0.05, 0.1, 0.15, and 0.2% Tween 80. Following autoclaving (121℃ for 15 min) and cooling, one ml portions of cholesterol micelles (prepared as described above using L-α-phosphatidylcholine, dioleoyl) were added to 9ml portions of MRS-THIO broth containing the different concentrations of Tween 80. Two ml aliquots were taken from each to serve as uninoculated controls. The remaining broth in each tube was inoculated (1%) with a freshly prepared MRS broth culture of *L. acidophilus* ATCC 43121. After the incubation at 37℃ for 18 hours, 1ml portions of the culture were diluted with 9ml of distilled water and the absorbance was measured at 620nm against a water blank with a Spectronic 21D Colorimeter (Milton Roy, Rochester, NY) to compare relative amounts of growth. Cells from the remainders of the cultures were removed by centrifugation at 12,000 × g and 4℃ for 10 minutes. Then, the cholesterol contents of spent broths and uninoculated controls were assayed [30].

11. Statistical Analyses

Analysis of variance was performed on each set of data to determine if significant differences existed among the samples. The differences and confidence levels were determined by calculating the least significant difference with SAS® [33].

Ⅲ. RESULTS

1. Cholesterol Assimilation by *Lactobacillus acidophilus* ATCC 443121

In static cultures of *L. acidophilus* ATCC 43121 grown in MRS-THIO broth supplemented with 91.7μg/ml cholesterol and 0.3% oxgall, 47.8μg/ml of the cholesterol was recovered with the cells (Table 1). The cholesterol content in resuspended cells plus that in the spent broth was approximately equal to that in the uninoculated control broth. This indicates that little, if any, of the cholesterol was degraded by the culture during growth.

2. Resistance of Cells to Sonic Disruption

Cells of *L. acidophilus* ATCC 43121 grown in broth containing oxgall and cholesterol micelles were more resistant to sonic disruption than were cells grown in broth without them (Table 2). When cells were grown in MRS-THIO broth without cholesterol micelles and oxgall, 95% of cells were disrupted in 15 minutes. However, when cells were grown in MRS broth containing 0.3% oxgall and cholesterol micelles, only 17% of cells were disrupted during the same time period.

3. Cholesterol Uptake during Growth at pH 6.0

The effect of maintaining the pH during growth at a level to prevent precipitation of any free cholic acid [17] on the cholesterol uptake by *L. acidophilus* ATCC 43121 was tested by growing the culture statically (i.e. without

Table 1. Assimilation of cholesterol by *Lactobacillus acidophilus* ATCC 43121[1]

Sample	Cholesterol amount[2] (μg/ml)
Uninoculated control broth	91.7[b]
Spent broth	42.9[a]
Resuspended cells	47.8[a]

[1] Cells were incubated for 10 hrs at 37℃ in MRS-THIO broth containing 0.3% oxgall, and 10% cholesterol micelles (prepared using Type Ⅲ-E egg yolk lecithin).

[2] All numbers are the means of 10 trials; means with different superscripts differ significantly ($P<.05$).

Table 2. Comparison of lysis by sonication of cells of *Lactobacillus acidophilus* ATCC 43121 grown in the presence and absence of cholesterol and bile salts[1]

Growth medium	Sample	DMC[2]/ml	Disruption of cells (%)
Medium A	control	4.0×10^9	
	sonicated	2.0×10^8	95%
Medium B	control	4.7×10^9	
	sonicated	3.9×10^9	17%

[1] Cells were grown in Medium A or Medium B for 18 hrs at 37℃ and sonicated for 15 min with Sonic dismembrator. All numbers are the means of 2 trials.

[2] Direct Microscopic Cell Counts

[3] Medium A: MRS-THIO broth

[4] Medium B: MRS-THIO broth containing 0.3% oxgall and cholesterol micelles.

pH control) and in the medium maintained at pH of 6.0 during growth (Table 3). The culture grown at pH 6.0 appeared to take up more cholesterol (39μg/ml) than those grown without pH control (28μg/ml), although the difference was not significant ($P>.05$).

4. Cholesterol in the Membrane Fraction of Cells Grown Statically and at pH 6.0

Cholesterol was recovered in the membrane fractions of cells grown with and without con-

Table 3. Influence of maintaining growth medium at pH 6.0 on cholesterol uptake by cells of *Lactobacillus acidophilus* ATCC 43121[1]

Growth conditions	Cholesterol uptake[2] (μg/ml)
Static	28[a]
pH 6.0	39[a]

[1] Cells were grown for 18 hrs at 37℃ statically and with pH controlled at 6.0 in MRS-THIO broth supplemented with 0.004M sodium taurocholate. Cholesterol micelles were prepared using Type Ⅲ-E egg yolk lecithin (broth contained 92μg/ml of cholesterol initially).

[2] All numbers are the means of 3 trials; means with same superscripts are not significant ($P>.05$).

trol at pH 6.0 (Table 4). The specific ATPase activities were significantly higher ($P<.05$) in the membrane fractions compared to the whole cells for the culture grown under both conditions. The whole cells of both cultures (i. e. static and pH 6.0) showed the same level of enzyme activity. The specific activity of ATPase was significantly higher ($P<.05$) in the membrane fraction of the cells grown at pH 6.0 than in the fraction from the cells grown without pH control. This suggests a greater degree of purification of the membrane from the cells grown at pH 6.0, since ATPase activity is normally associated with bacterial cellular membranes.

The amounts of cholesterol assimilated into cells and membrane were expressed as m-moles/mg protein. Cells membranes from the static culture had significantly ($P<.05$) more cholesterol than the cells grown at pH 6.0. Cell membranes from the static cultures contained significantly ($P<.05$) more cholesterol than did the statically grown whole cells. The cell membranes of the cultures grown at pH 6.0 contained numerically, but not significantly ($P<.05$), more cholesterol than did the whole cells grown at pH 6.0.

5. Influence of phospholipids having Different Degrees of Unsaturation on Cholesterol Uptake

The relative amounts of unsaturated fatty acids in the phosphatidylcholine used to prepare the cholesterol-phospholipid micelles influenced the amount of cholesterol assimilated by *L. acidophilus* ATCC 443121 (Table 5). Significantly more ($P<.05$) cholesterol was taken up from the micelles prepared using L-α-phosphatidylcholine, dioleoyl and L-α-phosphatidylcholine, distearoyl than from those prepared using egg yolk and soybean lecithins. However, significantly more ($P<.05$) growth occurred in broths containing the micelles prepared using L-α-phosphatidylcholine, dioleoyl and L-α-phosphatidylcholine, distearoyl than in the broths containing micelles prepared using the egg yolk and soybean lecithins.

Table 4. Choleterol in cells and membranes of cultures of *Lactobacillus acidophilus* ATCC 43121 grown at pH 6.0 and without pH control[1]

Growth conditions	Fraction	Cholesterol[2] (mmoles/mg protein)	ATPase[3] (Specific activity)
STATIC	Washed cells	0.485[a]	0.17[a]
	Membranes	0.912[b]	0.28[b]
pH 6.0	Washed cells	0.283[a]	0.17[a]
	Membranes	0.316[a]	0.41[c]

[1] Cells were grown at 37℃ for 18 hrs statically and at pH 6.0 in MRS-THIO broth supplemented with 0.004M sodium taurocholate and cholesterol micelles (prepared with Type Ⅲ-E egg yolk lecithin).

[2] All numbers are the means of 3 trials; means with different superscripts differ significantly ($P<.05$).

[3] The specific ATPase activity is expressed as μmoles/min/mg protein. All numbers are the means of 3 trials; means with different superscripts differ significantly ($P<.05$).

Table 5. Influence of phospholipids containing different degrees of unsaturation on growth and cholesterol uptake by *Lactobacillus acidophilus* ATCC 43121[1]

Phospholipids[2]	Growth[3] (A_{620nm})	Cholesterol uptake[4] (μg/ml)
Ⅲ-E	0.168[a]	21[a]
Ⅲ-S	0.160[a]	33[ab]
Dioleoyl	0.224[b]	47[b]
Distearoyl	0.235[b]	41[b]

[1] Cells were incubated at 37℃ for 12 hours in MRS-THIO broth containing 0.1% Tween 80 and 0.004M sodium taurocholate.

[2] Ⅲ-E = egg yolk lecithin; III-S = soybean lecithin; dioleoyl = L-α-phosphatidylcholine containing oleic acid; distearoyl = L-α-phosphatidylcholine containing stearic acid

[3] All numbers are the means of 3 trials; means with different superscripts differ significantly ($P<.05$).

[4] All numbers are the means of 3 trials; means with different superscripts differ significantly ($P<.05$).

Table 6. Influence of Tween 80 on growth and uptake of cholesterol from two sources by *Lactobacillus acidophilus* ATCC 43121[1]

Cholesterol source[2]	Tween 80 (%)	A_{620nm}[3]	Cholesterol uptake[4] (μg/ml)
Cholesterol-	0	0.087[b]	8[a]
phospholipid	0.05	0.155[c]	55[cd]
micelles	0.10	0.160[c]	42[bc]
	0.15	0.160[c]	32[b]
	0.20	0.155[c]	22[b]
Water-	0	0.021[a]	7[a]
soluble	0.05	0.151[c]	117[f]
cholesterol	0.10	0.158[c]	82[de]
	0.15	0.151[c]	72[d]
	0.20	0.155[c]	66[d]

[1] Cultures were incubated at 37℃ for 18 hrs in MRS-THIO broth supplemented with 0.004M sodium taurocholate and the indicated amounts of Tween 80.

[2] Cholesterol-phospholipid micelles prepared using phosphatidylcholin, dioleoyl (final cholesterol concentration in broth = 101μg/ml); water-soluble cholesterol = polyoxyethanyl-cholesteryl sebacate (final cholesterol concentration in broth = 134μg/ml).

[3] All numbers are the means of 3 trials; means with different superscripts differ significantly ($P<.05$).

[4] All numbers are the means of 3 trials; means with different superscripts differ significantly ($P<.05$).

6. Influence of Tween 80 on Uptake of Two Sources of Cholesterol

Water-soluble cholesterol was taken up more than the cholesterol-phospholipid micelles (Table 6). Without Tween 80, little, if any, cholesterol from either source was taken up by

L. acidophilus ATCC 43121 and little growth was observed. In the presence of Tween 80, cell growth was not significantly different ($P>.05$) among various amounts of Tween 80. In the presence of 0.05% Tween 80, cholesterol uptake was the most ($P<.05$) for both cholesterol sources. However, as the amount of Tween 80 increased beyond 0.05%, the amount of cholesterol uptake decreased.

Ⅳ. DISCUSSION

Hypocholesterolemic activity of *L. acidophilus* has been reported in several studies [6, 10, 13]. According to Gilliland et al. [10], cholesterol removed from laboratory media during growth of *L. acidophilus* was assimilated by the cells. Klaver and Van der Meer [17] reported that the presumed assimilation of cholesterol by *L. acidophilus* was due to the "coprecipitation" of cholesterol along with free bile acids resulting from deconjugation of the bile acids by the lactobacilli during growth. They based this conclusion largely on the fact that in their experiments no cholesterol was removed from the broth medium when cells were harvested from a culture that had been maintained at pH 6.0 during growth, a pH at which free bile acids would remain in solution. We, however, obtained uptake of cholesterol by *L. acidophilus* ATCC 43121 during growth at pH 6.0. This indicates that the cholesterol did not merely coprecipitate with free bile acids as indicated by Klaver and Van der Meer [17]. They relied on flushing the head space of their fermentor with nitrogen to maintain anaerobic conditions. We used sodium thioglycollate in the medium coupled with sparging nitrogen gas through the medium to maintain anaerobic conditions. This could account for the differences observed. Additionally, in the present study, cholesterol was recovered in the cell membrane of *L. acidophilus* grown in a medium containing cholesterol. Furthermore, we have shown in a previous study [38] that there is no relationship between the ability of *L. acidophilus* to deconjugate bile acids and assimilate cholesterol.

Incorporation of cholesterol into the membranes of mycoplasmas also has been reported [22, 27]. Cholesterol in cell membranes of mycoplasmas protects the cells by increasing the tensile strength of the membranes [26]. Cells of *L. acidophilus* ATCC 43121 grown in the presence of oxgall and cholesterol micelles showed increased resistance to lysis by sonication compared to cells grown in control broth. These results suggest that cholesterol may have altered the cellular membrane of the lactobacilli so that they were more resistant to sonic disruption. In addition, in a preliminary experiment (data not shown), cells of *L. acidophilus* grown in the presence of oxgall and cholesterol micelles did not all stain Gram positive, whereas those grown without cholesterol micelles did. This result further suggests changes in the cells of lactobacilli as a result of growth in the presence of cholesterol and bile salts.

According to Williams et al. [39], oleic acid is an important growth factor for lactobacilli. Smittle et al. [34] reported that growth of *L. acidophilus* in media containing Tween 80 resulted in cells that survived freezing much better than did cells grown in its absence. Oleic acid in the Tween 80 was identified as a component responsible for the improved resistance of the cells to frozen storage. Growth in its presence modified the fatty acid composition of the cells. This was significantly re-

lated to the increased survival of the cells during freezing.

Cholesterol uptake by *L. acidophilus* ATCC 43121 in the present study was affected by the presence or absence of Tween 80. Without Tween 80, little, if any, cholesterol uptake was observed. This was likely due to reduced growth in its absence. Of the concentrations tested, the presence of 0.05% Tween 80 supported the highest level of cholesterol uptake; the amount taken up decreased as the concentration of Tween 80 increased beyond 0.05%. The differences in cholesterol assimilation in the media containing 0.05~0.2% Tween 80 were not due to differences in amounts of growth. These findings suggest that *L. acidophilus* has an optimum level of Tween 80 or oleic acid required to maximize cholesterol assimilation. Smittle et al. [34] reported that the optimum level of oleic acid in the growth medium varied among strains of *L. bulgaricus*. A similar relationship among strains of *L. acidophilus* may exist with respect to the influence of oleic acid on cholesterol uptake.

Cells grown at pH 6.0 showed more cholesterol uptake than those grown statically, though the difference was not significant ($P > .05$). However, the membranes of static cultures contained more cholesterol per mg protein than did those from the pH 6.0 culture. The difference between the ability of static and pH 6.0 cultures to assimilate cholesterol into the membranes might be due to differences in the membranes resulting from two growth conditions. More work is needed to identify the composition of the membranes from cells grown under both conditions.

In summary, *L. acidophilus* ATCC 43121 took up the cholesterol during growth and assimilated at least part of it into cellular membranes. Growth of the culture in media containing cholesterol and oxgall produced cells that did not stain Gram positive and were more resistant to lysis by sonication than were cells grown in their absence. These observations suggest membrane modification.

Based on results obtained using cholesterol micelles prepared using phosphatidylcholines having different amounts of saturated versus unsaturated fatty acids, the influence of unsaturated fatty acids on the assimilation is not clear. However, Tween 80 which contains oleic acid, an important growth factor for lactic acid bacteria, influenced the cholesterol uptake. The effect was not totally related to the influence of Tween 80 on growth. Based on prior studies showing the influence of Tween 80 on cellular lipid composition [34], this further suggests involvement of the membrane in cholesterol assimilation by *L. acidophilus*.

V. REFERENCES

1. Bazzarre, T. L., S. L. Wu and J. A. Yuhas. 1963. Total and HDL-cholesterol concentrations following yogurt and calcium supplementation. Nutr. Rep. 28:1225.
2. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248.
3. Brown, M. S. and J. L. Goldstein. 1984. How LDL receptors influence cholesterol and atherosclerosis. Sci. Am. 251:58.
4. Chen, H., E. Born, S. N. Mathur, F. C. Johlin, Jr. and F. J. Field. 1992. Sphingomyelin content of intestinal cell membranes regulates cholesterol absorption: Evi-

dence for pancreatic and intestinal cell sphingomyelinase activity. Biochem. J. 286:771.
5. Connor, W. E., D. B. Stone and R. E. Hodges. 1964. The interrelated effects of dietary cholesterol and fat upon human serum lipid levels. J. Clin. Invest. 43:1691.
6. Danielson, A. D., E. R. Peo, K. M. Shahani, A. J. Lewis, P. J. Whalen and M. A. Amer. 1989. Anticholesteremic property of *Lactobacillus acidophilus* yogurt fed to mature boars. J. Anim. Sci. 67:966.
7. Efrati, H., S. Rottem and S. Razin. 1981. Lipid and protein membrane components associated with cholesterol uptake by mycoplasmas. Biochim. Biophys. Acta 641:386.
8. Field, F. J., N. T. P. Kam and S. N. Mathur. 1990. Regulation of cholesterol metabolism in the intestine. Gastroenterology 99:539.
9. Fiske, C. H. and Y. Subbarow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375.
10. Gilliland, S. E., C. R. Nelson and C. Maxwell. 1985. Assimilation of cholesterol of *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 49:377.
11. Gilliland, S. E. and C. N. Rich. 1990. Stability during frozen and subsequent refrigerated storage of *Lactobacillus acidophilus* grown at different pH. J. Dairy Sci. 73:1187.
12. Grundy, S. M. and M. A. Denke. 1990. Dietary influences on serum lipids and lipoproteins. J. Lipid Res. 31:1149.
13. Harrison, V. C. and G. Peat. 1975. Serum cholesterol and bowel flora in the newborn. Am. J. Clin. Nutr. 28:1351.
14. Hepner, G., R. Fried, S. ST. Jeor, L. Fusetti and R. Morin. 1979. Hypocholesterolemic effect of yogurt and milk. Am. J. Clin. Nutr. 32:19.
15. Kannel, W. B., W. P. Castelli and T. Gordon. 1979. Cholesterol in the prediction of atherosclerotic disease. New perspectives based on the Framingham study. Ann. Intern. Med. 90:85.
16. Kern, F. 1991. Normal plasma cholesterol in an 88-year old man who eats 25 eggs a day. Mechanisms of adaptation. New England J. Med. 324:896.
17. Klaver, F. A. M. and R. Van der Meer. 1993. The assumed assimilation of cholesterol by lactobacilli and *Bifidobacterium bifidum* is due to their salt-deconjugating activity. Appl. Environ. Microbiol. 59:1120.
18. Lipid Research Clinics Program. 1984a. The lipid research clinics coronary primary prevention trial results. I. Reduction in incidence of coronary heart disease. J. Am. Med. Assoc. 251:351.
19. Lipid Research Clinics Program. 1984b. The lipid research clinics coronary primary prevention trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. J. Am. Med. Assoc. 251:365.
20. Mann, G. V. 1977. A factor in yogurt which lowers cholesteremia in man. Atherosclerosis. 26:335.
21. Mann, G. V. and A. Spoerry. 1974. Studies of a surfactant and cholesteremia in the Maasai. Am. J. Clin. Nutr. 27:464.
22. Melchior, D. L. and S. Rottem. 1981. The organization of cholesterol esters in *Mycoplasma capricolum* membranes. Eur. J. Biochem. 117:147.
23. Mott, G. E., R. W. Moore, H. Redmond and R. Reiser. 1973. Lowering of serum

cholesterol by intestinal bacteria in cholesterol-fed piglets. Lipids 8:428.

24. Pekkanen, J., S. Linn, G. Heiss, C. M. Suchindran, A. Leon, B. M. Rifkind and H. A. Tyroler. 1990. Ten-year mortality from cardiovascular disease in relation to cholesterol level among men with and without pre-existing cardiovascular disease. N. Engl. J. Med. 322:1700.
25. Push, D. J., F. F. Busta, W. A. Moats and A. E. Schulze. Direct microscopic count. *In* Compendium of methods for the microbiological examination of foods (M.L. Spec, ed.) pp.132-151. American Public Health Association, Washington, DC.
26. Razin, S. 1967. The cell membrane of mycoplasma. Ann. N.Y. Acad. Sci. 143: 115.
27. Razin, S., S. Kutner, H. Efrati and S. Rottem. 1980. Phospholipid and cholesterol uptake by mycoplasma cells and membranes. Biochim. Biophys. Acta 598:628.
28. Razin, S., M. Wormser and N. L. Gershfeld. 1974. Cholesterol in mycoplasma membrane. Ⅱ. Components of *Acholeplasma laidlawii* cell membranes responsible for cholesterol binding. Biochim. Biophys. Acta. 352:385.
29. Rottem, S. and S. Razin. 1966. Adenosine triphosphatase activity of mycoplasma membranes. J. Bacteriol. 92:714.
30. Rudel, L. L. and M. D. Morris. 1973. Determination of cholesterol using o-phthalaldehyde. J. Lipid Res. 14:364.
31. Rudel, L. L., J. S. Parks, F. L. Johnson and J. Babiak. 1986. Low density lipoproteins in atherosclerosis. J. Lipid Res. 27:465.
32. Safonova, I. G., D. D. Sviridov, J. -L. Nano, P. Rampal and V. S. Repin. 1994. Regulation of cholesterol uptake in the rat intestinal cell line. Biochim. Biophys. Acta 1210:181
33. SAS® Institute Inc. 1985. SAS® procedures guide for Personal computers, version 6 ed. Cary, NC:SAS® Institute Inc.
34. Smittle, R. B., S. E. Gilliland, M. L. Speck and W. M. Walter, Jr. 1974. Relationship of cellular fatty acid composition to survival of *Lactobacillus bulgaricus* in liquid nitrogen. Appl. Microbiol. 27:738.
35. Somkuti, G. A. and T. L. Johnson. 1990. Cholesterol uptake by *Propionibacterium freudenreichii*. Curr. Microbiol. 20:305.
36. Thakur, C. P. and A. N. Jha. 1981. Influence of milk, yoghurt and calcium on cholesterol-induced atherosclerosis in rabbits. Atherosclerosis 39:211.
37. Thorne, K. J. I. and D. C. Barker. 1972. The occurrence of bactoprenol in the mesosome and plasma membranes of *Lactobacillus casei* and *Lactobacillus plantarum*. J. Gen. Microbiol. 70:87.
38. Walker, D. K. and S. E. Gilliland. 1993. Relationships among bile tolerance, bile salt deconjugation, and assimilation of cholesterol by *Lactobacillus acidophilus*. J. Dairy Sci. 76:956.
39. Williams, W. L., H. P. Broquist and E. E. Snell. 1947. Oleic acid and related compounds as growth factors for lactic acid bacteria. J. Biol. Chem. 170:619.
40. Zacconi, C., V. Bottazzi, A. Rebecchi, E. Bosi, P. G. Sarra and L. Tagliaferri. 1992. Serum cholesterol levels in axenic mice colonized with *Enterococcus faecium* and *Lactobacillus acidophilus*. Microbiologica 15: 413.