CHOLESTEROL UPTAKE BY *LACTOBACILLUS ACIDOPHILUS*: ITS FATE AND FACTORS INFLUENCING THE UPTAKE

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CHAPTER I

INTRODUCTION

Cholesterol, found in cell membranes of all animals, is essential for the synthesis of several steroid hormones as well as bile acids. However, a high level of serum cholesterol is closely related to the atherosclerosis, a disease in which the accumulation of cholesterol in the arteries forms bulky plaques, results in the inhibition of the flow of blood and causes coronary heart disease. Coronary heart disease is a major cause of deaths in the United States. However, by reducing serum cholesterol levels the number of deaths by coronary heart disease can be greatly reduced (41).

After a study with Maasai warriors showing the possible hypocholesterolemic effect by fermented milk (45), many researchers have studied the hypocholesterolemic effects of cultured or culture-containing dairy products. Consumption of dairy foods containing selected cultures of lactobacilli

resulted in the reduction of serum cholesterol levels in humans and also in animals.

Some lactobacilli take up cholesterol during growth in laboratory media. Since cholesterol is an important component of many types of cell membranes, it is possible that the cholesterol taken up by the lactobacilli is incorporated into cell membrane. In a study with mycoplasmas which do not have cell walls, the cholesterol taken up was incorporated into the cell membranes (61).

The fatty acids, especially oleic acid, play an important role in the growth of cells of *L. acidophilus*. The presence of Tween 80, a non-toxic esterified form of oleic acid, enhanced the stability of lactobacilli during frozen storage (73). They suggested that growth in the presence of oleic acid increased the fluidity of the membranes of the lactobacilli. Such alterations in the cellular membrane may increase the uptake of cholesterol by lactobacilli.

The objectives of this study were: (1) to measure the effect of different phospholipids 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 into the cell membrane of *L. acidophilus* ATCC 43121.

CHAPTER II

REVIEW OF LITERATURE

Role of Cholesterol in the Body

Cholesterol, a steroid normally found in the human body, plays an important role as a precursor of compounds such as steroid hormones, bile salts, and vitamin D (32, 46), and also acts as one of the main components of cellular membranes (46). In addition, as in human red blood cells, a high level of cholesterol in the membranes has a number of effects on the permeability, transport, and enzyme activities of the membrane (9, 10, 68, 72).

According to Dietschy and Wilson (13), in general, there are two ways to acquire cholesterol in the body: endogenous synthesis or absorption of dietary cholesterol. They reported that the synthesis and/or the absorption of most cholesterol takes place in two organs, the liver and the gastrointestinal tract. The transport of this cholesterol throughout the body is carried out by lipoproteins. There

are four lipoproteins differentiated by density: very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). Among them, the last two lipoproteins, LDL and HDL, are mainly involved in the cholesterol transport.

LDL facilitates the deposition of cholesterol in tissue cells peripheral to the liver, thus increasing the risk of coronary heart disease. HDL carries the cholesterol away from tissues and back to the liver where some of it is excreted from the body in the form of bile salts (32). Due to their comparative roles, high levels of HDL and low levels of LDL are thought to be protective factors against coronary heart disease (22).

Cholesterol in Membranes of Mycoplasmas

As mycoplasmas do not have cell walls, they are easily distinguishable from other prokaryotes and may serve as convenient tools for the study of the role of cholesterol because it is an essential membrane component in all *Mycoplasma* species (60). However, being unable to synthesize cholesterol, the mycoplasmas depend on an

external supply solely from the growth medium (58). As Kahane and Razin (35) reported, increased concentrations of cholesterol in the growth medium as a cholesterol-phosphatidylcholine dispersion resulted in better growth of *Mycoplasma hominis*.

According to Razin *et al.* (61), the phospholipids taken up by *M. capricolum* from the growth medium were incorporated into the membrane and served as additional binding sites for cholesterol. Thus, they concluded that cholesterol uptake was correlated with the uptake of exogenous phospholipid. Efrati *et al.* (15) and Melchior and Rottem (47) also reported that the cholesterol taken up by *M. capricolum* was tightly associated with the membranes.

Because of those facts, mycoplasmas have been studied widely for the role of cholesterol in the membrane. Razin (57) reported that cholesterol increased the tensile strength of the mycoplasma membrane, providing survival and growth without the protection of cell wall. It was reported that the crystallization of membrane lipids was prevented by cholesterol and the membrane lipids were maintained in a state of fluidity to provide the optimal conditions for several enzyme activities of mycoplasma membrane (67). They

found that the Arrhenius curves of the ATPase activities in the membranes of *M. mycoides* var. *capri* grown at different temperatures (10-40^{0}C) were linear and had the same slope.

In another study, Rottem *et al.* (68) grew cells of *M. mycoides* var. *capri* in serum-free media supplemented with successively decreasing concentrations of cholesterol. Following this procedure, the organism adapted to growth with no cholesterol. They found these adaptive strains were fragile osmotically, and were more permeable to erythritol. In addition, they could not grow at 25^{0}C, while the nonadaptive strains could. They concluded that cholesterol in the membranes of mycoplasma acts as a regulator of membrane fluidity.

Relationship Between Cholesterol and Coronary Heart Disease

Atherosclerosis is associated with a high level of LDL cholesterol in the blood (4, 37, 69), and is the major cause of coronary heart disease which is the first leading cause of death in the United States (36, 41, 54, 76). Atherosclerosis is a thickening of the inner wall of the arteries due to the accumulation of esterified cholesterol,

and as a result, it inhibits the flow of blood until a clot eventually forms, obstructing an artery and causing a heart attack. Therefore, by lowering the levels of total plasma cholesterol and LDL cholesterol, the incidence of coronary heart disease can be reduced (41, 42).

For humans, the absorption of dietary and biliary cholesterol from the gut is an important source of cholesterol. About 50% of blood cholesterol, on average, is derived from this source in the western diet (7, 13, 50). Connor *et al.* (8) also reported a positive relationship between dietary cholesterol intake and serum cholesterol levels. Therefore, the blood cholesterol level can be, at least partially, controlled through dietary modifications and, in addition, drugs also can be used to reduce the cholesterol levels (24, 41, 51).

However, there is concern about using drugs for the treatment of hypercholesterolemia, since most drugs have undesirable side effects. Such side effects include constipation, heartburn, and nausea (41). In addition, such a drug (cholestyramine, a bile acid sequestrant) interrupted the normal intestinal absorption of fats and fat-soluble vitamins (53).

People with hypercholesterolemia may synthesize excess cholesterol, since they may have no controlling mechanism over endogenous synthesis of cholesterol. According to Safonova *et al.* (71), there is a positive correlation between the rate of cholesterol uptake and the intestinal cholesterol synthesis for patients with high and medium rates of cholesterol uptake. They speculated that both cholesterol uptake and synthesis were stimulated by enhanced demand for cholesterol.

To reduce serum cholesterol levels, several approaches have been suggested (23). Among them, the first is a dietary change to low fat diets, which can be easily achieved. The second one is the inhibition of cholesterol synthesis in the body, and the third one is to reduce the absorption of cholesterol from small intestine.

Effect of Cultured or Culture-containing Dairy Products on the Reduction of Serum Cholesterol Levels

Lactic acid bacteria in the small intestines can play an important role in providing nutritional and health benefits to humans (11, 16). Some of them have potential

hypocholesterolemic properties which could reduce serum cholesterol levels in humans. These bacteria, primarily *L. acidophilus* and species of *Bifidobacterium*, subsequently may exert positive benefits as residents of or while traveling through the gastrointestinal tract, since the small intestine maintains the cholesterol balance in human body (6, 18). In general, such lactic acid bacteria can be introduced through fermented milk products or food supplemented with the viable bacteria.

Because of their potential hypocholesterolemic activities, there have been many *in vitro* studies involving the lactobacilli (19, 20, 40, 78), and also *in vivo* studies (11, 19, 25, 28, 45). The benefit of using these lactic acid bacteria is based on the fact that the risk of heart disease in humans can be lowered by decreasing the serum cholesterol levels. Therefore, the ability of such cultures to reduce the amount of serum cholesterol in hypercholesterolemic humans has been the major purpose of many studies.

After having studied Maasai tribesman in Africa, Mann and Spoerry (45) found that after 21 days of consuming large amount of milk fermented with a wild strain of

Lactobacillus, there was a great reduction in their serum cholesterol levels. Men who gained more than 5 lb in body weight in 3 weeks showed an average decrease of cholesterol of 28 mg/100 ml, whereas those gaining less than 5 lb showed a decrease of 8 mg/100 ml. From this result, they suggested there was a factor in the fermented milk that lowered serum cholesterol level.

In a later study, Mann (43) also reported that when Vitamin D-enriched cow's milk fermented with commercial yogurt cultures was consumed for 12 days, there was a significant ($P < .05$) decrease in serum cholesterol levels in human subjects. There also were studies reporting the reduction in serum cholesterol in humans (29) and in the rabbits (75) after the consumption of yogurt. On the contrary, nonfermented milk did not show any reduction of cholesterol levels. Howard and Marks (31) also reported no hypocholesterolemic effect by milk.

In contrast, Kiyosawa *et al.* (38) reported that skim milk had more hypocholesterolemic effect on the male rabbits than yogurt. Other studies also showed some beneficial effects of skim milk on humans (30, 66). In those studies, it was assumed that the effect might be due to the lower

lipid content of skim milk. Therefore, it was suggested that the effective agent in yogurt was produced or enhanced in milk by microbial action of lactic acid bacteria.

In bovine milk, 3-hydroxy-3-methylglutarate (HMG), uric and orotic acids have been identified as inhibitors of cholesterol biosynthesis (1, 14, 27, 44, 52). However, according to Haggerty *et al.* (26), there was a significant ($P < .05$) decrease of orotic acid during yogurt fermentation, but the amount of uric acid did not change. Ferriera (17) did not observe a decrease in orotic acid content during yogurt fermentation. Thus, there might be strain differences among yogurt starter cultures in the ability to use orotic acid. Therefore, it seems reasonable that other factors, in addition to orotic acid, are responsible for hypocholesterolemic effect of yogurt (64).

In rats, the feeding of thermophilus milk for 29 days resulted in a significant ($P < .05$) decrease in blood plasma cholesterol levels as compared with those in rats fed with skim milk (56). Bazzarre *et al.* (3) reported that, for human female subjects, there was a significant ($P < .001$) decrease in total serum cholesterol following consumption of yogurt. In addition, the average HDL-cholesterol levels and

HDL:total cholesterol ratios of females were significantly (P <.0001) higher after yogurt consumption. On the contrary, they reported there were no significant differences in HDL-cholesterol levels and HDL:total cholesterol ratios among male subjects.

Since serum cholesterol is discharged from the body as biliary bile acids, the transformation of cholesterol into bile acids by the liver is an important process by which serum cholesterol may be eliminated. In their experiment, Imaizumi *et al.* (33) found that the effect of the whey prepared from cultured skim milk on the secretion of bile acids by primary cultured hepatocytes varied widely depending on the bacterial strains, rather than on the species used for the culture. Among the strains tested, whey preparations from milk fermented with *L. casei* 2230 and *Bifidobacterium longum* 2912 increased the activity of cholesterol 7α-hydroxylase, a rate-limiting enzyme for bile acid synthesis, thus resulting in the stimulation of the secretion of bile acids. Modler *et al.* (48), however, reported there was no direct relationship between bifidobacteria and the reduction of cholesterol levels.

Some reports have indicated no hypocholesterolemic effect of the lactobacilli-containing products. Jaspers et al. (34) studied the effect of consuming nonfat yogurt on total cholesterol, HDL cholesterol, LDL cholesterol, serum lipoproteins, and serum triglycerides in human male subjects having normal levels of serum cholesterol. They found that there was no significant ($P > .05$) change in cholesterol level for either the test or the control group after 21 days. Total serum cholesterol decreased until 14 days, but returned to control levels one week later. Therefore, they concluded that the hypocholesterolemic effect was transient.

Lin *et al.* (40) studied the effect of Lactinex™, a tablet containing *L. acidophilus* ATCC 4962 and *L. delbrueckii* subsp. *bulgaricus* ATCC 33409, on the concentrations of serum lipoproteins in humans. However, the results did not show any effect on serum lipoprotein levels. Howard and Marks (31) also reported there was no hypocholesterolemic effect by yogurt. The human subjects consumed 2 liters of yogurt daily for 2 weeks, but there was no significant ($P > .05$) change in serum cholesterol levels, although there was a 5.5% decrease after 2 weeks. Thompson *et al.* (77) also reported that there was no evidence for a

hypocholesterolemic effect by cultured buttermilk, yogurt, and acidophilus milk although there was no rise in cholesterol amount expected from the increased fat intake for 3 weeks. Pulusani and Rao (55) observed no hypocholesterolemic effect in rats fed thermophilus, bulgaricus and acidophilus milks.

Hypocholesterolemic Activities of *Lactobacillus acidophilus*

Lactobacilli are normal components of the intestinal microflora in humans. They contribute to the stabilization of the microflora and help maintain the resistance against pathogens (2, 21). Some reports have shown that the supplementation of diets with *L. acidophilus* reduced serum cholesterol levels in human and animal subjects (11, 19, 28).

Mott *et al.* (49) reported that germ-free pigs, monocontaminated with *L. acidophilus* and allowed to develop a normal flora, showed reduced serum cholesterol levels. They suggested that normal intestinal microflora, along with *L. acidophilus*, played a role in the reduction of serum cholesterol levels. Zacconi *et al.* (80) also reported a

reduction in serum cholesterol levels in axenic mice which were contaminated with *L. acidophilus*. According to Danielson and Gustaffson (12), germ-free rats on high cholesterol diets had higher serum cholesterol levels than conventional ones.

Harrison and Peat (28) reported that human infants who were fed milk containing *L. acidophilus* had significantly ($P < .001$) lower serum cholesterol levels, greater weight gains ($P < .05$) and increased numbers of lactobacilli in their stools ($P < .001$) when compared to infants receiving sterile milk formula. Robins-Browne *et al.* (65) also observed increased numbers of *L. acidophilus* in humans after the consumption of Latinex™ containing viable cells of *L. acidophilus*. Grunewald (25) found that rats consuming skim milk fermented with *L. acidophilus* had significantly ($P < .05$) lower plasma cholesterol levels than rats fed water or control milk.

Gilliland *et al.* (19) reported that certain strains of *L. acidophilus* could remove cholesterol from a growth medium when grown under anaerobic conditions in the presence of bile. They provided the *in vitro* evidence for the assimilation of cholesterol by *L. acidophilus*. They used

strains of *L. acidophilus* isolated from the fecal flora of pigs. When the cells were grown in MRS broth supplemented with oxgall and a pleuropneumonia-like organism (PPLO) serum fraction as the cholesterol source, there was a reduction of cholesterol in the spent broth and an increase in the cells. One cholesterol-assimilating strain, *L. acidophilus* RP32, was fed to the pigs along with a cholesterol-containing diet. After 10 days, they found the amount of serum cholesterol was significantly lower in this group ($P < .05$) compared to the control group. Feeding of *L. acidophilus* P47 which lacked the ability to remove cholesterol from the laboratory growth medium had no effect on serum cholesterol concentration.

Danielson *et al.* (11) obtained several isolates of *L. acidophilus* from the feces of mature boars. Among them, they found one strain, *L. acidophilus* 16, was the best at *in vitro* assimilation of cholesterol. So, they made acidophilus yogurt with that strain, and fed it to the boars which had been previously on a high cholesterol diet for 56 days. They found that serum cholesterol level of the test group was significantly reduced ($P < .01$), compared to the control group.

Somkuti and Johnson (74) reported that the cultures of *Propionibacterium freudenreichii*, which are used in the making of Swiss cheese, also reduced the cholesterol amount by 50% or more during growth in the medium containing a PPLO serum fraction. Approximately, 80% of cholesterol removed from the growth medium was recovered with the bacterial cells.

Recently, Buck and Gilliland (5) obtained isolates of *L. acidophilus* from human intestines. They observed much variation among the isolates in the ability to assimilate cholesterol in laboratory media. Based on the results, they suggested that those isolates which assimilated the highest amounts of cholesterol and were most bile tolerant had great potential to be used as dietary adjuncts to reduce serum cholesterol levels in humans.

According to Klaver and Van der Meer (39), the decrease of cholesterol content of culture medium during growth of *L. acidophilus* was not due to the uptake of cholesterol by the cells, but resulted from the bile salt-deconjugating activity of those cells and the "coprecipitation" of cholesterol with deconjugated bile salts which have a decreased solubility at pH lower than 6.0. Reynier *et al.*

(63) also reported that the deconjugated bile salts, with reduced solubility, affected the cholesterol solubility. However, Walker and Gilliland (78) reported that there was no significant correlation between cholesterol assimilation and bile salt deconjugation by *L. acidophilus*.

Effect of Fatty Acids on Cholesterol Uptake

Safonova *et al.* (70) reported that the presence of saturated fatty acids inhibited cholesterol uptake, while oleic acid, a monounsaturated fatty acid, increased cholesterol uptake by rat small intestine epithelial cells. However, linoleic acid, a polyunsaturated fatty acid, inhibited cholesterol uptake by 50%. The cells were incubated previously in the presence of different fatty acids, harvested, washed and then incubated in the presence of cholesterol micelles. Increased cholesterol uptake was observed at concentrations up to 100μM of oleic acid, but further increase in the concentration of oleic acid resulted in a decrease of cell viability.

Razin *et al.* (62) reported that oleate-enriched *Acholeplasma laidlawii* cells had a significantly higher rate constant for uptake of cholesterol than either palmitate-

enriched or native *A. laidlawii* cells. However, they found that the amount of cholesterol in the membrane at equilibrium was not affected by the fatty acid composition of the membrane.

Later, Razin (59) reported that cholesterol uptake was rapid when the membrane lipid bilayer was in the liquid-crystalline state and slow when it was in the gel state. He found that when *A. laidlawii* cells grown in elaidate-enriched medium were transferred from 37°C to 4°C, cholesterol uptake was arrested, but the oleate-enriched cells transferred from 37°C to 4°C continued to take up the cholesterol, though at a slower rate.

It has been proposed that surfactant food additives could increase the absorption of cholesterol in the intestine, resulting in hypercholesterolemia. However, when Tween 20, a surfactant which is a polyoxyethylene sorbitol ester of lauric acid, was used in humans on a high cholesterol diet, there was no hypercholesterolemic effect (45). They concluded that the presence or absence of the surfactant did not affect the cholesterol levels in humans. However, they mentioned more study was needed for human subjects.

Williams *et al.* (79) reported that oleic acid could replace the need of biotin by lactobacilli. Tween 80 is an excellent and a non-toxic source of oleic acid for use in the culture media. Smittle *et al.* (73) reported that sodium oleate was the active component of Tween 80 providing the stability of lactobacilli during freezing in liquid nitrogen.

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CHAPTER III

CHOLESTEROL ASSIMILATION INTO CELLULAR MEMBRANE OF *LACTOBACILLUS ACIDOPHILUS* ATCC 43121

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ABSTRACT

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, though not significant ($P > .05$), 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 ($P > .05$) difference in the amount taken up from micelles prepared using L-α-phosphatidylcholine, dioleoyl or L-α-phosphatidylcholine, distearoyl. Without Tween 80 (as source of oleic acid), 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.

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).

Lacobacillus 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 develop 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 into the cell membrane fraction of *L. acidophilus* ATCC 43121.

MATERIALS AND METHODS

Source and Maintenance of Culture

L. acidophilus ATCC 43121 (formerly strain RP32) 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°C for 18 hours. The culture was stored at 5°C between transfers. It was subcultured at least three times just before experimental use.

Preparation of MRS-THIO Broth

MRS-THIO broth was prepared by dissolving 55 g lactobacilli MRS broth (Difco), 2 g (0.2%) thioglycolic acid (sodium salt, Sigma Chemical Co., St. Louis, MO) in 1,000 ml of distilled water. It was further supplemented, when needed, with 2.16 g (0.004 M) taurocholic acid (sodium salt, Sigma Chemical Co.) or 3 g (0.3%) oxgall (Difco) per liter. The broth was autoclaved for 15 minutes at 121°C. The broth media were prepared on the day of experimental use.

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°C. 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°C for the desired time. After incubation, cells were removed by centrifugation at 12,000 x *g* and 4°C 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.

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°C for 18 hours. Cells were recovered by centrifugation at 12,000 x *g* and 4°C for 10 minutes, and resuspended in distilled water to a population of approximately 4 x 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).

Measurement of Cholesterol Assimilation
During Growth at pH 6.0

MRS-THIO broth (500 ml) supplemented with 0.004 M sodium taurocholate was prepared and placed into a small fermentor of about 1 liter 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, 0.5 ml 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^{0}C for 15 minutes. After cooling, 50 ml of cholesterol micelles prepared using egg yolk lecithin (Type III-E) were added. The fermentor, then, was placed in a 37^{0}C 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, 10 ml was withdrawn aseptically from the fermentor and placed into a sterilized test tube to serve as the uninoculated control. Then, 5 ml 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 11 ml/min throughout the incubation period. After the incubation, 10 ml of culture was withdrawn aseptically

from the fermentor, centrifuged and the spent broth was assayed for cholesterol (30).

Isolation of Cellular Membranes

Cells of *L. acidophilus* ATCC 43121 were grown in 10 ml of MRS-THIO broth supplemented with 0.004 M sodium taurocholate and cholesterol micelles (prepared using Type III-E egg yolk lecithin) at 37°C for 18 hours and harvested by centrifugation at 12,000 x *g* at 4°C 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.

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°C 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.

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 20 mg/ml. The solution was passed through a sterile 0.45 μm membrane filter into a sterile test tube and stored at 5^{0}C. The stock solution was diluted as necessary with sterile distilled water and added to the growth medium for the assay of cholesterol uptake.

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.

Influence of Tween 80 on Cholesterol Assimilation

Lactobacilli MRS broth was prepared from individual ingredients according to the manufacturer's (Difco) formulation without 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^{0}C for 15 min) and cooling, one ml portions of cholesterol micelles (prepared as described above using L-α-phosphatidylcholine, dioleoyl) were added to 9 ml 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°C for 18 hours, 1 ml portions of the cultures were diluted with 9 ml of distilled water and the absorbance was measured at 620 nm 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 x *g* and 4°C for 10 minutes. Then, the cholesterol contents of spent broths and uninoculated controls were assayed (30).

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).

RESULTS

Cholesterol Assimilation by *Lactobacillus acidophilus* ATCC 43121

In static cultures of *L. acidophilus* ATCC 43121 grown in MRS-THIO broth supplemented with 91.7 μg/ml cholesterol and 0.3% oxgall, 47.8 μg/ml of the cholesterol was recovered with the cells (Table I). 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.

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 II). 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

TABLE I

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°C 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 ($P < .05$). S.E. = 4.47; 27 df.

TABLE II

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[3]	control	4.0×10^9	
	sonicated	2.0×10^8	95%
Medium B[4]	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°C 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.

micelles, only 17% of cells were disrupted during the same time period.

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

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 control at pH 6.0 (Table IV). 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

TABLE III

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^{0}C 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 92 μg/ml of cholesterol initially).

[2]All numbers are the means of 3 trials; means with same superscripts are not significant (P >.05). S.E. = 6.36; 4 df.

TABLE IV

CHOLESTEROL 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 Whole Cells	.485[a]	0.17[a]
	Membranes	.912[b]	0.28[b]
pH 6.0	Washed Whole Cells	.283[a]	0.17[a]
	Membranes	.316[a]	0.41[c]

[1]Cells were grown at 37°C for 18 hours 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 ($P < .05$). S.E. = 0.16; 8 df.

[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 ($P < .05$). S.E. = 0.035; 8 df.

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 membranes were expressed as mmoles/mg protein. Cell 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.

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 43121 (Table V). 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.

Influence of Tween 80 on Uptake of Two Sources of Cholesterol

Water-soluble cholesterol was taken up more than the cholesterol-phospholipid micelles (Table VI). 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%,

TABLE V

INFLUENCE OF PHOSPHOLIPIDS CONTAINING DIFFERENT DEGREES OF UNSATURATION ON GROWTH AND CHOLESTEROL UPTAKE BY *LACTOBACILLUS ACIDOPHILUS* ATCC 43121[1]

Phospholipid[2]	Growth[3] (A_{620nm})	Cholesterol Uptake[4] ($\mu g/ml$)
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°C 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 ($P < .05$). S.E. = 0.019; 8 df.

[4]All numbers are the means of 3 trials; means with different superscripts differ significantly ($P < .05$). S.E. = 7.34; 8 df.

TABLE VI

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)
	0	0.087^{b}	8^{a}
Cholesterol-	0.05	0.155^{c}	55^{cd}
Phospholipid	0.10	0.160^{c}	42^{bc}
Micelles	0.15	0.160^{c}	32^{b}
	0.20	0.155^{c}	22^{b}
	0	0.021^{a}	7^{a}
Water-	0.05	0.151^{c}	117^{f}
soluble	0.10	0.158^{c}	82^{de}
Cholesterol	0.15	0.151^{c}	72^{d}
	0.20	0.155^{c}	66^{d}

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

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

[3]All numbers are the means of 3 trials; means with different superscripts differ significantly ($P < .05$). S.E. = 0.014; 20 df

[4]All numbers are the means of 3 trials; means with different superscripts differ significantly ($P < .05$). S.E. = 11.77; 20 df.

the amount of cholesterol uptake decreased.

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 related 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*.

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APPENDIX A

DATA FROM TREATMENTS

TABLE VII

DATA FROM ASSIMILATION OF CHOLESTEROL BY *LACTOBACILLUS ACIDOPHILUS* ATCC 43121[a]

	cholesterol amount (μg/ml)		
No. of trials	control	spent broth	cells
1	91.0	51.4	52.2
2	84.0	31.5	53.0
3	93.2	34.8	60.2
4	101.7	38.8	52.1
5	99.1	32.4	59.5
6	102.5	52.8	25.1
7	97.6	40.2	51.1
8	71.8	46.7	32.8
9	84.6	45.0	38.8
10	91.0	55.0	53.6
$\bar{X}$	91.7	42.9	47.8

[a]Cells were grown for 10 hrs at 37°C in MRS-THIO broth containing 0.3% oxgall and 10% cholesterol micelles.

TABLE VIII

DATA FROM COMPARISON OF LYSIS BY SONICATION OF CELLS OF *LACTOBACILLUS ACIDOPHILUS* ATCC 43121 GROWN IN THE PRESENCE AND ABSENCE OF CHOLESTEROL AND BILE SALTS

Growth Medium	Sample	DMC[1]/ml			% disruption
		trial 1	trial 2	$\bar{X}$	
Medium A[2]	Control	4.1×10^9	3.9×10^9	4.0×10^9	
	Sonicated	1.1×10^8	2.8×10^8	2.0×10^8	95%
Medium B[3]	Control	5.3×10^9	4.0×10^9	4.7×10^9	
	Sonicated	4.5×10^9	3.2×10^9	3.9×10^9	17%

[1]Direct Microscopic Cell Counts

[2]Medium A: MRS-THIO broth

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

TABLE IX

DATA FROM CHOLESTEROL UPTAKE BY STATIC AND pH 6.0 CELLS OF *LACTOBACILLUS ACIDOPHILUS* ATCC 43121[a]

Growth Conditions	Exp.1	Exp.2	Exp.3	$\bar{X}$
Static	36	28	21	28
pH6.0	30	44	44	39

[a]Cholesterol amount is expressed as μg/ml.

TABLE X

DATA FROM CHOLESTEROL AMOUNT ASSIMILATED INTO CELL MEMBRANES OF STATIC AND pH 6.0 CULTUES OF *LACTOBACILLUS ACIDOPHILUS* ATCC 43121[a]

Growth Conditions	Fractions	Exp.1	Exp.2	Exp.3	$\bar{X}$
Static	Whole Cell	0.549	0.518	0.389	0.485
	Membrane	1.076	1.180	0.479	0.912
pH6.0	Whole Cell	0.311	0.253	0.285	0.283
	Membrane	0.380	0.230	0.339	0.316

[a]Cholesterol amount is expressed as mmoles/mg protein.

TABLE XI

DATA FROM THE ATPase ACTIVITY OF CELLS AND MEMBRANES OF STATIC AND pH 6.0 CULTURES OF *LACTOBACILLUS ACIDOPHILUS* ATCC 43121[a]

Growth Conditions	Fractions	Exp.1	Exp.2	Exp.3	$\bar{X}$
Static	Cells	0.17	0.13	0.20	0.17
	Membranes	1.46	0.29	0.26	0.67
pH6.0	Cells	0.13	0.20	0.19	0.17
	Membranes	0.33	0.44	0.45	0.41

[a]ATPase activity is expressed as μmoles/min/mg protein.

TABLE XII

DATA FROM INFLUENCE OF PHOSPHOLIPIDS CONTAINING DIFFERENT DEGREES OF UNSATURATION ON GROWTH OF *LACOBACILLUS ACIDOPHILUS* ATCC 43121[a]

Phosphatidyl-choline	Exp.1	Exp.2	Exp.3	$\bar{X}$
III-E	0.158	0.176	0.171	0.168
III-E	0.145	0.154	0.177	0.160
Dioleoyl	0.193	0.263	0.216	0.224
Distearoyl	0.265	0.213	0.226	0.235

[a]Growth is expressed as the absorbance at 620nm (of a 1:10 dilution of the culture) after the incubation for 18 hr at 37°C in MRS-THIO broth containing and 0.004M sodium taurocholate.

TABLE XIII

DATA FROM INFLUENCE OF PHOSPHOLIPIDS CONTAINING DIFFERENT DEGREES OF UNSATURATION ON CHOLESTEROL UPTAKE BY *LACTOBACILLUS ACIDOPHILUS* ATCC 43121[a]

Phosphatidyl-choline	Exp.1	Exp.2	Exp.3	$\bar{X}$
III-E	18	27	17	21
III-S	36	21	43	33
Dioleoyl	43	37	60	47
Distearoyl	46	42	36	41

[a]Cholesterol amount is expressed as μg/ml.

TABLE XIV

DATA FROM INFLUENCE OF TWEEN 80 ON THE GROWTH OF *LACTOBACILLUS ACIDOPHILUS* ATCC 43121[a]

Tween 80 (%)	Exp.1	Exp.2	Exp.3	$\bar{X}$
0	0.085	0.129	0.046	0.087
0.05	0.151	0.159	0.156	0.155
0.10	0.150	0.152	0.178	0.160
0.15	0.150	0.166	0.163	0.160
0.20	0.154	0.147	0.163	0.155

[a]Growth is expressed as the absorance at 620nm (of a 1:10 dilution of the culture) after the incubation at 37°C for 18 hours in MRS-THIO broth containing 0.004M sodium taurocholate. The MRS-THIO broth was supplemented with egg yolk lecithin.

TABLE XV

DATA FROM INFLUENCE OF TWEEN 80 ON THE GROWTH OF *LACTOBACILLUS ACIDOPHILUS* ATCC 43121 IN THE PRESENCE OF WATER-SOLUBLE CHOLESTEROL[a]

Tween 80 (%)	Exp.1	Exp.2	Exp.3	$\bar{X}$
0	0.021	0.025	0.018	0.021
0.05	0.174	0.144	0.135	0.151
0.10	0.159	0.162	0.153	0.158
0.15	0.159	0.151	0.142	0.151
0.2	0.169	0.159	0.138	0.155

[a]Growth is expressed as the absorbance at 620nm after the incubation at 37^{0}C for 18 hours in MRS-THIO broth containing 0.004M sodium taurocholate.

TABLE XVI

DATA FROM INFLUENCE OF TWEEN 80 ON CHOLESTEROL UPTAKE BY *LACTOBACILLUS ACIDOPHILUS* ATCC 43121[a]

Tween 80 (%)	Exp.1	Exp.2	Exp.3	$\bar{X}$
0	14	5	6	8
0.05	47	43	76	55
0.10	46	36	44	42
0.15	30	26	39	32
0.20	24	21	78	22

[a]Cholesterol amount is expressed as μg/ml. The MRS-THIO broth is supplemented with cholesterol-phospholipid micelles prepared with egg yolk lecithin.

TABLE XVII

DATA FROM INFLUENCE OF TWEEN 80 ON WATER-SOLUBLE CHOLESTEROL UPTAKE BY *LACTOBACILLUS ACIDOPHILUS* ATCC 43121[a]

Tween 80 (%)	Exp.1	Exp.2	Exp.3	$\bar{X}$
0	1	12	7	7
0.05	106	125	121	117
0.10	77	52	118	82
0.15	52	84	79	72
0.20	54	76	68	66

[a]Cholesterol amount is expressed as μg/ml.

APPENDIX B

STATISTICAL ANALYSES

TABLE XVIII

ANALYSIS OF VARIANCE TABLE[1] - ASSIMILATION OF CHOLESTEROL BY *LACTOBACILLUS ACIDOPHILUS* ATCC 43121

Source	DF	Sum of squares	Mean square	F Value
Model	2	14415.26867	7207.63433	
Error	27	2701.89300	100.07011	
Total	29	17117.16167		

[1]Data from Table VII
OSL <.05
$OSL_{0.05}$ = 9.18

TABLE XIX

ANALYSIS OF VARIANCE TABLE[1] - CHOLESTEROL UPTAKE BY STATIC AND PH 6.0 CELLS OF *LACTOBACILLUS ACIDOPHILUS* ATCC 43121

Source	DF	Sum of squares	Mean square	F Value
Model	1	181.5000000	181.5000000	2.98
Error	4	243.3333333	60.83333333	
Total	5	424.8333333		

[1]Data from Table IX
OSL <.05
$LSD_{.05}$ = 17.681

TABLE XX

ANALYSIS OF VARIANCE TABLE[1] - CHOLESTEROL AMOUNT ASSIMILATED INTO CELL MEMBRANES OF STATIC AND PH 6.0 CULTURES OF *LACTOBACILLUS ACIDOPHILUS* ATCC 43121

Source	DF	Sum of squares	Mean square	F Value
Model	3	0.75151092	0.25050364	6.38
Error	8	0.31431800	0.03928975	
Total	11	1.06582892		

[1]Data from Table X
OSL <.05
$LSD_{.05}$ = 0.3732

TABLE XXI

ANALYSIS OF VARIANCE TABLE[1] - ATPase ACTIVITY OF CELLS AND MEMBRANES OF STATIC AND PH 6.0 CULTURES OF *LACTOBACILLUS ACIDOPHILUS* ATCC 43121

Source	DF	Sum of squares	Mean square	F Value
Model	3	0.11426667	0.03808889	20.59
Error	8	0.01480000	0.00185000	
Total	11	0.12906667		

[1]Data from Table XI
OSL <.05
$LSD_{.05}$ = 0.081

TABLE XXII

ANALYSIS OF VARIANCE TABLE[1] - INFLUENCE OF PHOSPHOLIPIDS CONTAINING DIFFERENT DEGREES OF UNSATURATION ON GROWTH OF *LACTOBACILLUS ACIDOPHILUS* ATCC 43121

Source	DF	Sum of squares	Mean square	F Value
Model	3	0.01301492	0.00433831	7.38
Error	8	0.00470133	0.00058767	
Total	11	0.01771625		

[1]Data from Table XII
OSL <.05
$LSD_{.05}$ = 0.0456

TABLE XXIII

ANALYSIS OF VARIANCE TABLE[1] - INFLUENCE OF PHOSPHOLIPIDS CONTAINING DIFFERANT DEGREES OF UNSATURATION ON CHOLESTEROL UPTAKE BY *LACTOBACILLUS ACIDOPHILUS* ATCC 43121

Source	DF	Sum of squares	Mean square	F Value
Model	3	1150.333333	383.444444	4.73
Error	8	648.666667	81.083333	
Total	11	1799.000000		

[1]Data from Table XIII
OSL <.05
$LSD_{.05}$ = 16.954

TABLE XXIV

ANALYSIS OF VARIANCE TABLE[1] - INFLUENCE OF TWEEN 80 ON THE GROWTH OF *LACTOBACILLUS ACIDOPHILUS* ATCC 43121

Source	DF	Sum of squares	Mean square	F Value
Model	9	0.05619920	0.00624436	21.57
Error	20	0.00578867	0.00028943	
Total	29	0.06198787		

[1]Data from Table XIV and XV
OSL <.05
$LSD_{.05}$ = 0.029

TABLE XXV

ANALYSIS OF VARIANCE TABLE[1] - INFLUENCE OF TWEEN 80 ON CHOLESTEROL UPTAKE BY *LACTOBACILLUS ACIDOPHILUS* ATCC 43121

Source	DF	Sum of squares	Mean square	F Value
Model	9	33333.63333	3703.73704	17.77
Error	20	4169.33333	208.46667	
Total	29	37502.96667		

[1]Data from Table XVI and XVII
OSL <.05
$LSD_{.05}$ = 24.591

VITA

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