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Effect of Inhibition of Protein Synthesis on Lipid Metabolism in *Lactobacillus plantarum*

LORETTA Y. ARBOGAST[1] AND THOMAS O. HENDERSON*
Department of Biological Chemistry University of Illinois at the Medical Center, Chicago, Illinois 60612

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In *Lactobacillus plantarum* 17-5, lipid synthesis appears to be correlated with protein synthesis. Inhibition of protein synthesis by chloramphenicol (50 μg/ml) caused the nearly simultaneous inhibition of incorporation of radioactive oleic acid into polar lipids before the cessation of growth. In addition, de novo fatty acid synthesis, as determined by the incorporation of radioactive acetate into cellular lipids, was also inhibited. Removal of the antibiotic resulted in the resumption of growth, protein synthesis, and polar lipid synthesis. Inhibition of protein synthesis by leucine deprivation also produced a marked reduction in the incorporation of radioactive oleic acid into the total polar lipids at about the same time that growth stopped (30 to 60 min after the removal of leucine). However, the different classes of lipids behaved differently. For example, the incorporation of oleic acid into cardiolipin was inhibited immediately upon removal of leucine from the cultures, whereas incorporation into phosphatidylglycerol was maintained at near normal rates for 60 min after the removal of leucine and then ceased. In contrast, the accumulation of radioactive oleic acid in a neutral lipid identified as diglyceride occurred to a much greater extent in leucine-deprived cultures than in control (+ leucine) cultures. Upon addition of leucine to leucine-deprived cultures, the rates of synthesis of phosphatidylglycerol and cardiolipin returned to normal; the amount of radioactivity in the diglyceride fraction decreased to normal levels concomitantly with increased phospholipid synthesis.

Despite much earlier interest in the lipids of *Lactobacillus plantarum* and other lactobacilli (11), there have been only a few studies on the complex lipids of these organisms, and then only with respect to identifying and quantitating the polar lipids. Furthermore, the results of those few studies are often in conflict with one another. Thorne and co-workers, for example, reported the presence of phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol (PG), cardiolipin (CL), and *O*-lysylphosphatidylglycerol in *Lactobacillus casei* (23, 24). Other workers, although reporting the presence of CL, PG, and (in some strains) *O*-lysylphosphatidylglycerol, found no phosphatidylethanolamine or phosphatidylcholine (5, 8, 12, 13) in any *Lactobacillus* species examined, including *L. casei* and *L. plantarum*. Recent observations by D. W. McNulty (personal communication) indicate that, indeed, some lactobacilli do contain phosphatidylethanolamine and, perhaps, phosphatidylserine.

In addition to the phospholipids, glycosyldiglycerides have been isolated from lactobacilli as relatively minor consitituents of the cellular lipids (19, 25).

None of the above studies (5, 8, 12, 13, 19, 23-25) concerned themselves with possible changes in polar lipid composition as a function of any variables such as culture age and phase of growth, medium composition, or physiological stress. In particular, there have been no reports on possible alterations in lipid metabolism of lactobacilli under conditions of inhibited protein synthesis. Since the polar lipids of gram-positive bacteria are generally associated with the cell membrane, a close relationship between the synthesis of polar lipids and proteins might be expected to occur, reflecting the association of lipid and protein in the membrane. It seems plausible to assume that a deficiency in synthesis of one component would cause an inhibition of the synthesis of the other, with subsequent inhibition of growth.

Several investigators have reported an apparent requirement for lipid synthesis concomitant with the synthesis of certain proteins, or the

[1]Present address: The Wistar Institute, Philadelphia, Pa. 19104.

incorporation of proteins into membranes by mitochondria (2) and *Escherichia coli* (6, 10). Furthermore, Sokawa et al. (22) (confirmed by Cronan and Vagelos [4]) found that lipid synthesis in *E. coli* is coupled to protein synthesis and is under genetic control similar to that established for the stringent coupling of ribonucleic acid (RNA) and protein synthesis.

Glaser et al. (7) studied macromolecular synthesis in a temperature-sensitive *E. coli* mutant defective in glycerol-3-phosphate acyltransferase. Their results demonstrated that at the restrictive temperature (greater than 37 C), not only did phospholipid synthesis stop, but deoxyribonucleic acid (DNA), RNA, and protein synthesis stopped as well, suggesting that all of these synthetic processes are coupled in some yet undetermined manner.

In a companion report (3), we present our observations on the changes in the polar lipid composition of *L. plantarum* as a function of culture age, as well as some observations on the membrane-associated enzyme that catalyzes the conversion of PG to CL. In this report, we present observations on the effect on lipid synthesis of inhibition of protein synthesis in *L. plantarum* and describe an apparent correlation between the synthesis of fatty acids, polar lipids, and proteins in this organism.

(Portions of this work were presented at the 72nd Annual Meeting of the American Society for Microbiology, 23–28 April 1972, Philadelphia, Pa. This work is based upon a dissertation presented by L.Y.A. to the Graduate College of the University of Illinois at the Medical Center, Chicago, in partial fulfillment of the requirements for the Ph.D. degree.)

MATERIALS AND METHODS

Chemicals. All radioactive chemicals were purchased from New England Nuclear Corp., Boston, Mass.; L-leucine was purchased from Sigma Chemical Co., St. Louis, Mo.; and chloramphenicol was a gift of H. J. Jeffay, Department of Biological Chemistry, University of Illinois at the Medical Center, Chicago, and had been purchased from Sigma Chemical Co.

Media. Difco leucine assay medium was used in leucine deprivation studies and antibiotic studies. Antibiotic studies were also performed by using a second medium of the following composition per liter (pH 6.8) (medium I): 1 g of anhydrous sodium acetate, 5 g of Difco yeast extract, 10 g of Difco tryptone, 10 g of glucose, and 5 ml of a salt solution containing 10 g of $MgSO_4 \cdot 7\ H_2O$, 0.38 g of $MnSO_4 \cdot 1\ H_2O$, 1 g of KCl, 0.27 g of $FeSO_4$, and 23 ml of 85% H_3PO_4 per 500 ml. In acetate incorporation studies, the sodium acetate concentration of this medium was reduced to 0.3 g/liter (medium II).

Organism. *L. plantarum* 17-5 (ATCC 8014), used for all studies, was grown aerobically at 33 C. Turbidity of diluted samples was measured at 440 nm as an indication of growth.

Protein synthesis. Protein synthesis was determined by the method of Lowry et al. (15), using bovine serum albumin as a standard, or by measuring the incorporation of [U-^{14}C]leucine into the cold 10% trichloroacetic acid-precipitable fraction. Two-milliliter samples of culture were removed at various time intervals and mixed with 2 ml of cold 20% trichloroacetic acid. The precipitate was collected by centrifugation at 3,000 × *g* for 10 min, washed once with cold 10% trichloroacetic acid, and suspended in 1 ml of 1 N NaOH for protein determination by the method of Lowry et al. (15). Cells grown in the presence of [^{14}C]-leucine were collected by filtration on membrane filters (HAWP 02500, pore size 0.45 μm; Millipore Corp., Bedford, Mass.) and washed with 10 ml of cold 10% trichloroacetic acid. The filter disks were dried and then transferred to counting vials, and 10 ml of toluene scintillation fluid containing 100 g of 2,5-diphenyloxazole and 1.25 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene per liter of toluene was added to each vial. Radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer, model 3310.

Nucleic acid synthesis. The synthesis of cellular DNA or RNA was measured by determining the extent of incorporation of [2-^{14}C]thymidine or [2-^{14}C]-uracil, respectively. Two-milliliter samples of cultures taken at various times were mixed with 2 ml of cold 20% trichloroacetic acid. The precipitate was collected on membrane filters and washed with cold 10% trichloroacetic acid; radioactivity was determined as described for protein synthesis above.

Complex lipid synthesis. Complex lipid synthesis was measured by following the incorporation of [^{14}C]- or [^{3}H]-oleic acid into the cellular lipids. Dispersions of [^{14}C]- or [^{3}H]-oleic acid were prepared by sonicating 50 mg of oleic acid and 250 mg of Triton X-100 in 25 ml of water. One milliliter of this solution was added to 500 ml of cultures of logarithmically growing cells. The specific activity of the radioactive oleic acid in the medium was 200 μCi/mg (4 μg/ml final concentration). *L. plantarum* does not oxidize long-chain fatty acids, but rather incorporates them directly into cellular polar lipids. Two-milliliter samples of culture were removed at various time periods and extracted in a single-phase solvent system with 7.5 ml of methanol-chloroform (2:1, vol/vol). After 4 h, 2.5 ml of chloroform was added and the sample was mixed vigorously. After standing for several hours, the sample formed two phases. The aqueous layer was removed and the organic phase was washed with 2 ml of water that had been equilibrated with methanol-chloroform (2:1, vol/vol), and then the aqueous phase was again removed. The organic phase was evaporated to dryness under nitrogen and dissolved in 0.5 ml of benzene, and the lipids were separated by thin-layer chromatography as described below.

Lipid synthesis in cultures containing [1-^{14}C]-sodium acetate (specific activity, 1.33 μCi/mg) was measured as the radioactivity incorporated into the total fatty acid fraction after saponification. Samples (0.5 ml) of cultures grown in medium containing [^{14}C]acetate were removed at specific time

intervals and saponified as described elsewhere (9). Radioactivity of the fatty acids was determined as described previously (9).

Thin-layer chromatography. Silica gel thin-layer chromatography was carried out with commercially prepared Silica Gel G thin-layer plates (0.25-mm thickness; E. Merck). The solvent system used for one-dimensional chromatography was chloroform-methanol-water (65:25:4). Free fatty acids are well separated from complex lipids by this solvent. The appropriate area of silica gel containing the complex liquid fraction was scraped into vials and the radioactivity was measured by liquid scintillation spectrometry. Two-dimensional thin-layer chromatography was used to separate the individual polar lipids. Chloroform-methanol-water (65:25:4) (26) was the solvent system for the first dimension and chloroform-methanol-acetic acid:water (65:25:8:4) (21) for the second. Lipids were located by autoradiography using GAF HR-1000 Monopak non-screen X-ray film (George W. Brady and Co., Chicago, Ill.) and quantitated by scraping the appropriate areas for scintillation counting.

Antibiotic and viability studies. Chloramphenicol was added as an ethanol solution to exponentially growing cultures to give a final concentration of 50 μg/ml. The antibiotic was removed by harvesting cells by centrifugation and washing the cells three times with cold sterile 0.9% NaCl solution. Cells were then suspended in fresh prewarmed medium and incubation continued. Cells grown in leucine-supplemented leucine assay medium were collected and washed in the same way; cells were suspended in prewarmed leucine-free assay medium and incubation continued. In certain experiments, leucine (10 μg/ml final concentration) was added back to leucine-deprived cultures 100 min after leucine deprivation.

RESULTS

Antibiotic studies. Addition of chloramphenicol to growing cultures of *L. plantarum* at a concentration of 50 μg/ml produced inhibition of lipid synthesis as indicated by the decrease in [^{14}C]oleic acid and [^{14}C]acetate incorporation as well as an inhibition of protein synthesis as measured by [^{14}C]-leucine incorporation (Fig. 1). When lipid synthesis was measured by following [^{14}C]acetate incorporation, the rate of synthesis was decreased within 15 min after the addition of chloramphenicol and ceased 15 min after the addition of the antibiotic. The inhibition of protein synthesis followed the same time course. Protein synthesis ceased 15 min after chloramphenicol addition, whereas oleate incorporation continued at the normal rate for 30 min after the addition of chloramphenicol and then stopped. In both the acetate and oleate studies, growth continued at a normal rate for 15 min after chloramphenicol addition and then continued at a diminished rate for the duration

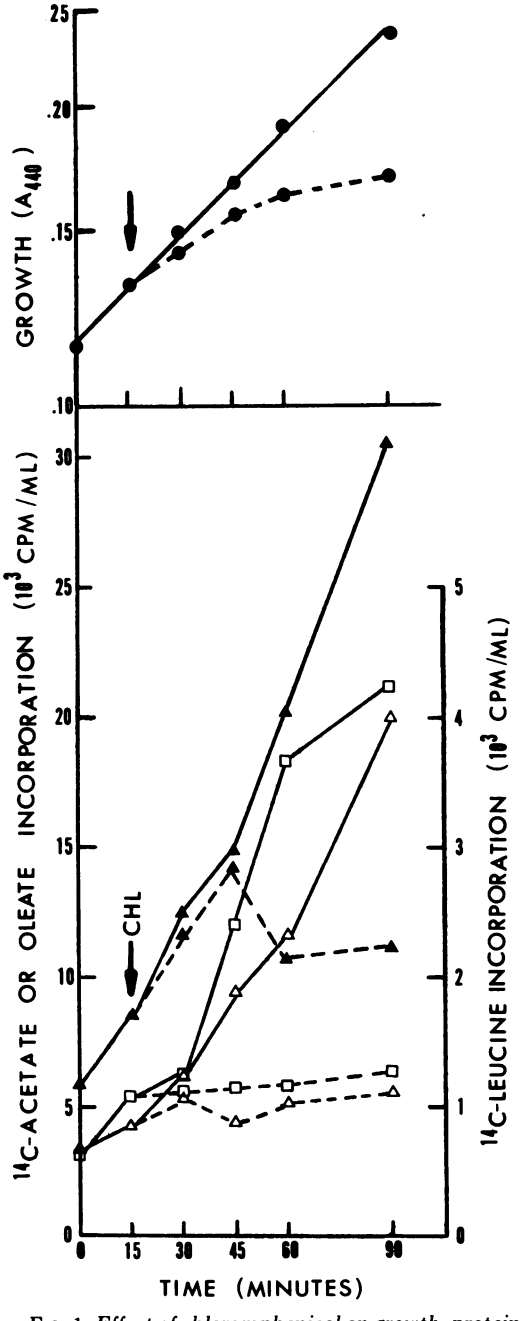

FIG. 1. *Effect of chloramphenicol on growth, protein synthesis, and polar lipid synthesis in L. plantarum. [^{14}C]-acetate (△) and [^{14}C]oleic acid (▲) incorporation into polar lipids and [^{14}C]leucine (□) incorporation into proteins were determined as described in the text. Growth was determined as the absorbance at 440 nm (●). Solid lines represent control cultures; dashed lines represent cultures to which chloramphenicol was added at 15 min (indicated by the arrow) to give a final concentration of 50 μg per ml of medium.*

of the experiment. In this particular experiment, the early inhibitory effects of chloramphenicol on lipid synthesis were more pronounced with [^{14}C]acetate than with [^{14}C]oleate.

The inducibility of the oleate transport system in *L. plantarum* was determined in the presence of chloramphenicol (Fig. 2). Chloramphenicol added at zero time produced the same type of inhibition of polar lipid synthesis and growth in cells grown in medium I containing 50 μg of oleic acid per ml as was observed with cultures grown in the absence of oleic acid. Prior growth in the presence of oleic acid did not noticeably alter the effect of chloramphenicol on oleic acid incorporation.

Leucine deprivation studies. Deprivation of leucine was also studied as a means of inhibiting protein synthesis in cultures of *L. plantarum* (Fig. 3). After culture manipulation (i.e., centrifugation, washing of culture, and suspension in fresh leucine-containing medium), a lag period ensued before the onset of protein synthesis (30 min), polar lipid synthesis (60 min), and growth (120 min) in control cultures (Fig. 3A). Leucine-deprived cultures exhibited a 20-min lag in protein synthesis after transfer from (+)leucine to (−)leucine medium, at which time protein synthesis began (Fig. 3B). However, after a total of 30 min, protein synthesis ceased. During the course of the experiment, the leucine-deprived cells did not exhibit further protein synthesis as determined colorimetrically or polar lipid synthesis as measured by oleic acid incorporation. During the initial 60-min time period after leucine deprivation, the cells did not grow; in fact, cell mass as determined by absorbance at 440 nm decreased. There then followed a small, but steady, increase in cell mass from 60 to 165 min, at which time it ceased.

Viability studies. Cells did not lose their viability upon treatment with chloramphenicol for 105 min. Chloramphenicol was added to growing cultures of *L. plantarum* at 0 min and the rate of [^{14}C]oleate incorporation into polar lipids in chloramphenicol (Fig. 4). After removal of chloramphenicol and suspension of cells in fresh medium, lipid synthesis resumed immediately; however, growth did not resume until 30 min after the chloramphenicol was removed. Similar results were observed in cultures shifted from (−)leucine to (+)leucine medium as shown in Fig. 5 and described below.

Growth properties and synthesis of macromolecules. In leucine assay medium containing 10 μg of L-leucine per ml, *L. plantarum* had

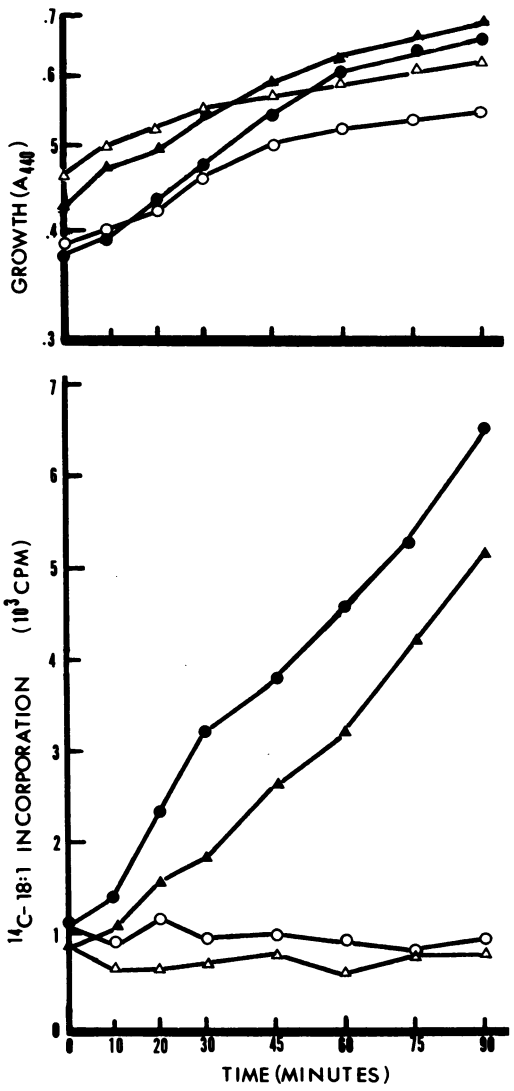

FIG. 2. *Effect of chloramphenicol on growth and [^{14}C]oleic acid incorporation into polar lipids in cultures of L. plantarum preincubated in medium with or without nonradioactive oleic acid. Those cultures subjected to incubation in medium containing 50 μg of nonradioactive oleic acid per ml for 1 h before zero time are indicated by (▲) and (△), those without by (●) and (○). Chloramphenicol was added to cultures represented by the open symbols (○, △) at zero time; control values are indicated by the closed symbols (●, ▲). Growth and polar lipids were measured as described in the text.*

a doubling time of 120 to 150 min. Upon removal of leucine from leucine assay medium, cells maintained nearly normal growth for 60 min, at which time growth essentially stopped (Fig. 5A). Lipid synthesis was normal for 30 min

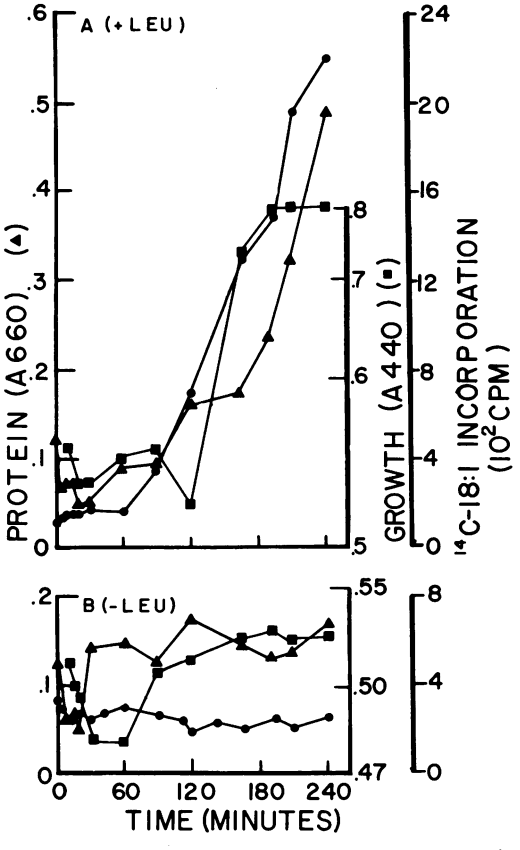

FIG. 3. *Effect of leucine deprivation on growth, polar lipid synthesis, and protein synthesis in L. plantarum. L. plantarum was grown in defined assay medium containing 10 μg of leucine per ml to an absorbance of about 0.4. At that time, the cells were collected by centrifugation, washed, and resuspended at zero time in fresh leucine assay medium as described in the text. (A) Behavior of controls resuspended in (+)leucine medium; (B) cultures resuspended in (−)leucine medium. Protein synthesis (absorbancy at 660 nm, ▲), growth (absorbancy at 440 nm, ■), and polar lipid synthesis ([14C]oleate incorporation, ●) were determined as described in the text.*

and then stopped (Fig. 5C). Protein and RNA syntheses (Fig. 5B and D, respectively) were inhibited immediately upon removal of leucine (zero time). In contrast, DNA synthesis continued for 2 h at a near-normal rate and then ceased (Fig. 5E). Upon readdition of leucine (10 μg/ml) to leucine-deprived cultures, there was a 30-min lag in RNA synthesis, at which time it resumed. DNA synthesis showed a 60-min lag and then resumed at the normal rate. Lipid and protein synthesis resumed immediately, whereas growth did not resume normal rates until 60 min after the addition of leucine.

Behavior of individual polar lipid species. Two-dimensional thin-layer chromatography and autoradiography revealed the incorporation of [14C]oleic acid into four major polar lipids by

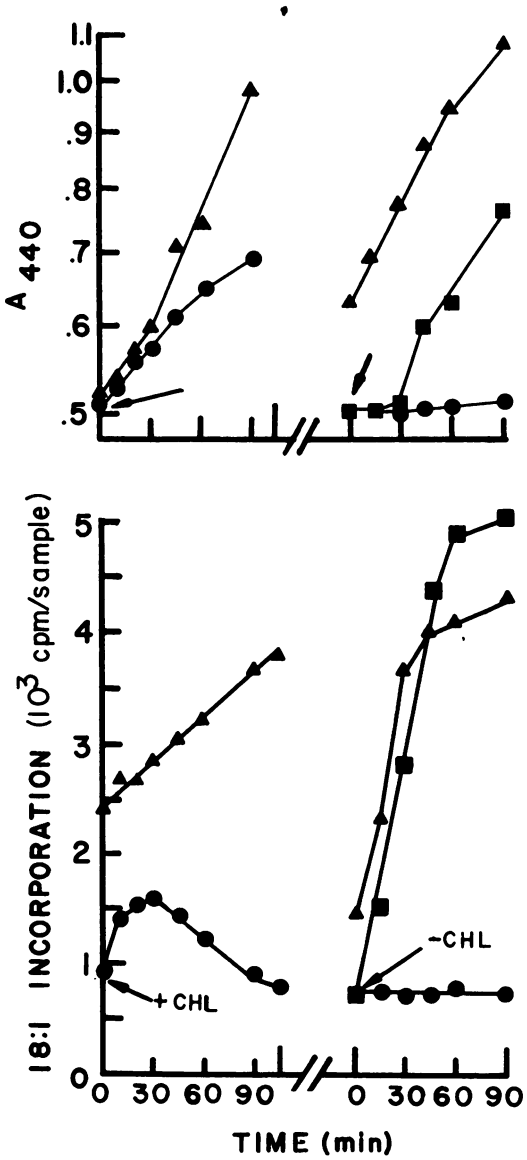

FIG. 4. *Reversibility of the chloramphenicol-induced inhibition of growth and polar lipid synthesis in L. plantarum. Control cultures are represented by (▲), and chloramphenicol-treated cultures by (●) added at zero time). At 105 min, all cultures were centrifuged, washed, and then resuspended in fresh medium. Those cultures initially treated with chloramphenicol which were resuspended in medium free of chloramphenicol are represented by (■). Growth and polar lipid synthesis were determined as described in the text.*

L. plantarum (Fig. 6). By comparison of chromatographic behavior of the intact lipids and the deacylated derivatives with standard compounds, lipids 4 and 5 were identified as CL and PG, respectively. Lipids 2 and 6 were glycolipids, probably diglycosyldiglyceride and triglycosyldiglyceride, respectively (T.O. Henderson, unpublished observations), both of which have been reported to be present in *L. plantarum* (19, 25).

The pattern of [^{14}C]oleic acid incorporation into individual polar lipid species for the experiment is shown in Fig. 7. In control cultures, the [^{14}C]oleate was initially incorporated into the phospholipids (PG and CL) and then into glycolipids. This sequential incorporation occurred to only a limited extent in leucine-deprived cultures. Although there was only limited net incorporation of [^{14}C]oleate into the polar lipids during leucine deprivation, nearly all of the exogenously supplied [^{14}C]oleate was eventually incorporated into a neutral lipid, which we determined to be diglyceride (Fig. 8). This observation was borne out by similar experiments in which radioactive glycerol incorporation was measured.

In leucine-deprived cultures, there was a marked reduction in the incorporation of [^{14}C]oleate into CL throughout the course of the experiment, whereas PG synthesis appears to proceed at the same rate as the control cultures for the first hour of leucine deprivation. After 1 h, there was no further accumulation of [^{14}C]oleate in PG. Upon addition of leucine to the leucine-deprived cultures, there was a return to a pattern of polar lipid synthesis similar to that displayed by control (+leucine) cells (Fig. 7), and the radioactivity of the diglyceride fraction decreased to levels found in control cultures (Fig. 8).

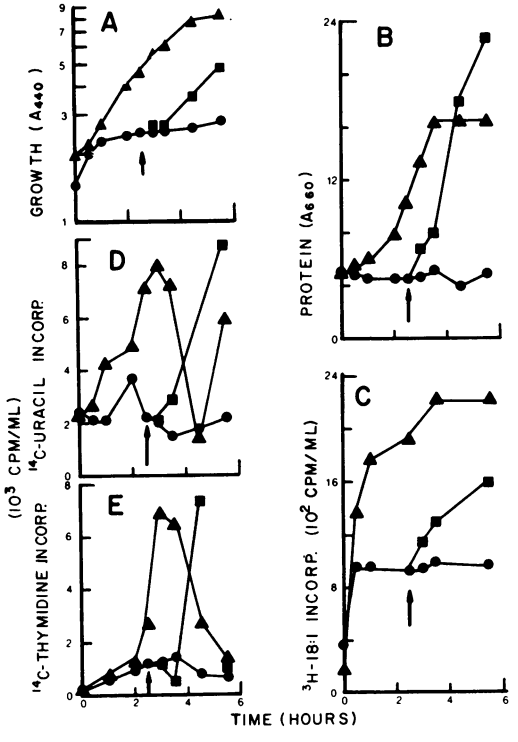

FIG. 5. *Effect of leucine deprivation and reversal of leucine deprivation on: (A) growth; (B) protein synthesis; (C) polar lipid synthesis; (D) RNA synthesis; and (E) DNA synthesis in L. plantarum. Control cultures are represented by (▲), leucine-deprived cultures by (●), and leucine-deprived cultures to which 10 μg of leucine was added per ml at 2.5 h by (■). Growth and synthesis of protein, RNA, DNA, and polar lipids were determined as described in the text. Leucine was added to leucine-deprived cultures where indicated by the arrow.*

DISCUSSION

If inhibition of protein synthesis affects synthesis of other macromolecules merely by stopping bacterial growth, the inhibition of all synthetic processes should occur at or near the time growth stops. This did not occur in the case under investigation; inhibition of protein synthesis in *L. plantarum* appeared to cause nearly simultaneous inhibition of the synthesis of polar lipids, with both effects becoming apparent before growth stopped. This suggests the operation of a regulatory mechanism that links protein and polar lipid synthesis, possibly through the close association of lipids and proteins in cellular membranes.

Although treatment of *Haemophilus parainfluenzae* with chloramphenicol did result in significant reduction of the amounts of CL and PG (18), inhibition of protein synthesis by tetracycline in *E. coli* (1) and *H. parainfluenzae* (18) did not result in significant short-term inhibition of phospholipid synthesis. Unlike the results of Kahane and Razin (14) with *Mycoplasma laidlawii*, we observed a distinct and rapid inhibition of polar lipid synthesis in *L. plantarum* upon inhibition of protein synthesis by chloramphenicol. The inhibitory effects on lipid synthesis produced by chloramphenicol appear to occur 0 to 30 min after addition of the antibiotic and at about the same time that inhibition of protein synthesis becomes manifest. Both effects are apparent before growth stops (Fig. 1 and 2).

Chloramphenicol, which apparently inhibits protein synthesis by binding to the 50S ribo-

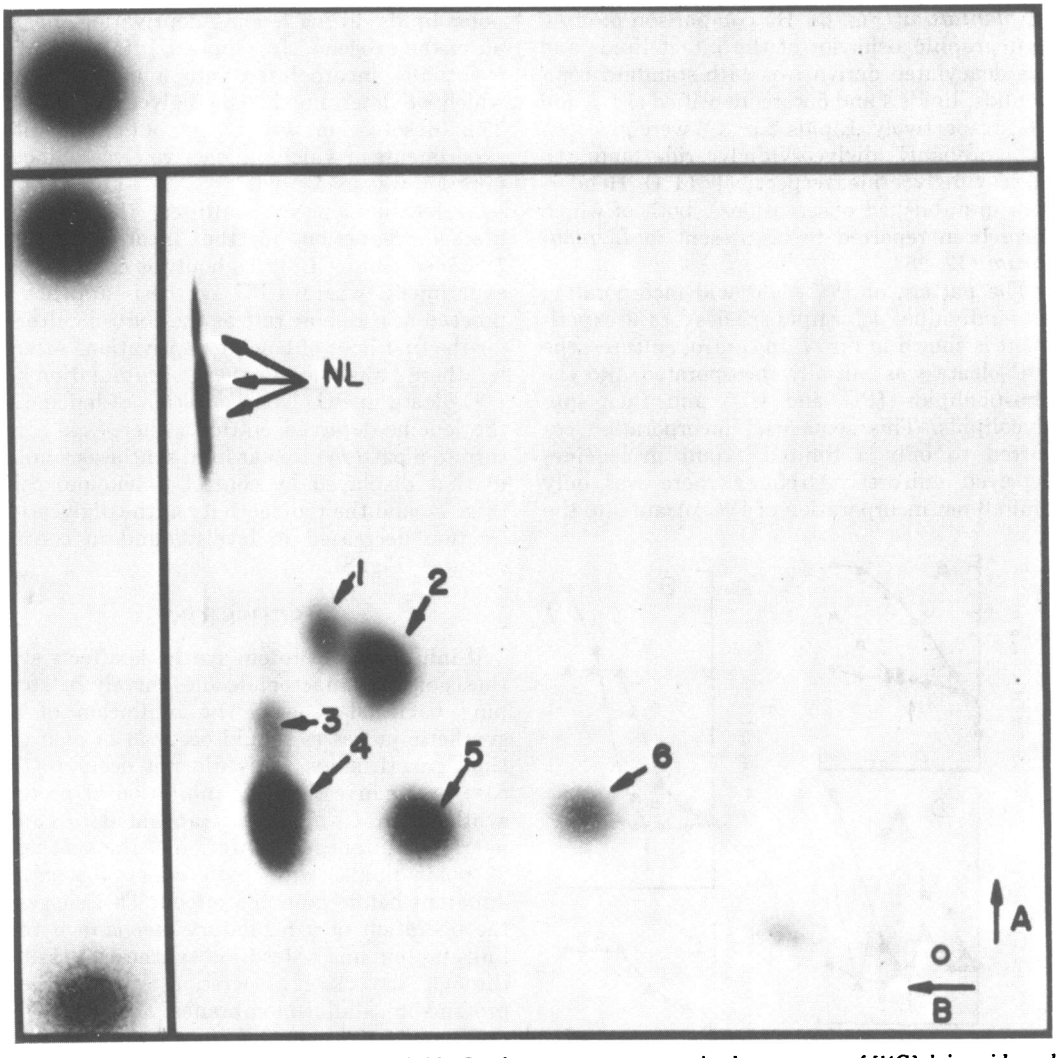

FIG. 6. *Autoradiogram of L. plantarum lipids. L. plantarum was grown in the presence of [^{14}C]oleic acid, and the lipids were extracted and chromatographed in two dimensions on Silica Gel G (20 by 20 cm) analytical plates as described in the text. The lipids are: NL, neutral lipids; 1, unidentified glycolipid; 2, glcyolipid I (tentatively identified as a diglycosyldiglyceride); 3, unidentified phospholipid; 4, cardiolipin; 5, phosphatidylglycerol; 6, glcyolipid II (tentatively identified as a triglycosyldiglyceride). The origin is indicated by the symbol (O). A and B indicate the first and second directions of development, respectively, and were carried out in $CHCl_3$-CH_3OH-H_2O (65:25:4) (26) and $CHCl_3$-CH_3OH -HAc-H_2O (65:25:8:4) (21), respectively.*

some and interfering with peptide chain elongation, almost completely abolishes polar lipid synthesis as measured by incorporation of exogenous radioactive oleic acid into cellular polar lipids (Fig. 1B). It is also an effective inhibitor of in vivo formation of polar lipids from radioactive acetate (Fig. 1A). The observation that acetate incorporation is inhibited before oleic acid incorporation may indicate that different regulatory mechanisms are involved or that one enzyme is more sensitive to a regulatory molecule than the other.

Similar inhibition of protein and polar lipid synthesis occurred when streptomycin was added to growing cultures of *L. plantarum*, although the extent of inhibition of both [^{14}C]oleic acid and [^{14}C]acetate incorporation was greater in chloramphenicol-treated cells than in streptomycin-treated cultures (L.Y. Arbogast and T.O. Henderson, Abstr. 72nd Annu. Meet. Am. Soc. Microbiol. 1972, G30, p. 35).

The results of the study using cells to incorporate [^{14}C]oleic acid into cellular lipids or to be

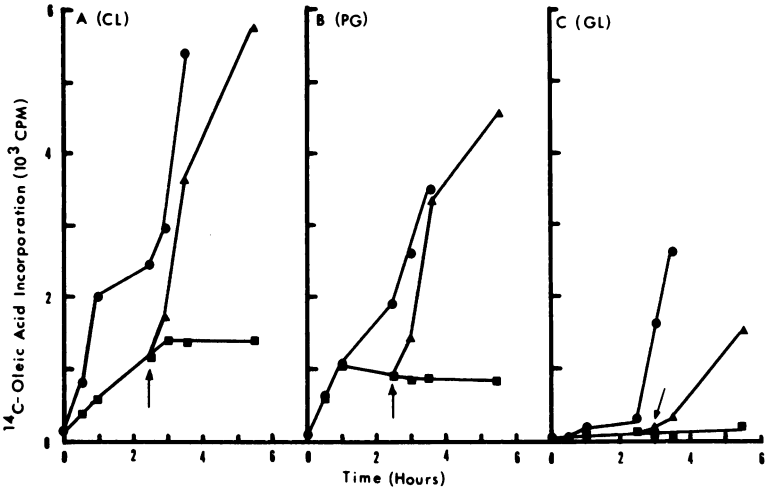

FIG. 7. *Effect of leucine deprivation and reversal of leucine deprivation on the incorporation of [^{14}C]oleic acid into individual lipid species. (A) incorporation into cardiolipin; (B) incorporation into phosphatidylglycerol; and (C) incorporation into glycolipids I plus II. In all cases, control cultures are represented by (●), leucine-deprived cultures by (■), and leucine-deprived cultures to which 10 μg of leucine was added per ml at 2.5 h by (▲) (time of leucine addition indicated by the arrow).*

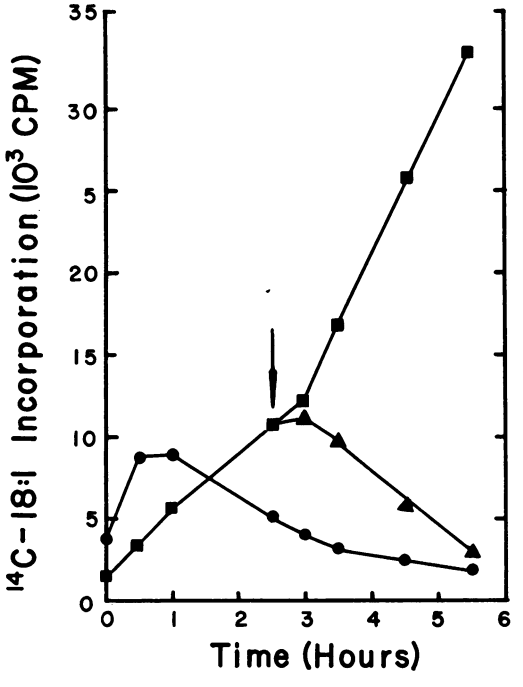

FIG. 8. *Accumulation of [^{14}C]oleic acid in diglycerides under conditions described for Fig. 7 (these data were taken from those same experiments). The symbols are as in Fig. 7.*

inhibited by chloramphenicol (Fig. 2), thus ruling out the possibility that the effect was actually the result of inhibition of synthesis of an inducible oleic acid transport system.

Polar lipid synthesis was also drastically reduced by depriving cells of leucine (Fig. 3), providing additional evidence that the inhibition of polar lipid synthesis may be a specific effect of general inhibition of protein synthesis.

To eliminate the possibility that antibiotic treatment or leucine deprivation leads to irreversible damage in the cells, therefore causing an apparent biosynthetic inhibition, the behavior of cells upon release of inhibition was examined. Release of inhibition was accomplished by removal of antibiotic from the medium (Fig. 4) or addition of leucine to leucine-deprived cultures (Fig. 5 and 7). Cells responded in each case with a resumption of growth and normal synthetic patterns.

On the basis of the observations reported here, it appears that normal polar lipid synthesis in *L. plantarum* requires the maintenance of protein synthesis. Similar results have been reported by Sokawa et al. (22), who found that the rate of lipid synthesis in stringent auxotrophs of *E. coli* was reduced when leucine or threonine was removed from the growth medium. Mindich (16), studying *Bacillus subtilis* B-42 (a glycerol-requiring auxotroph), also found a limitation of lipid synthesis in the presence of chloramphenicol to 30% of the rate of untreated cultures and an eventual termination of synthesis 60 min after addition of the antibiotic. Mindich further reported (17) the reversible inhibition of fatty acid and phospholipid synthesis after deprivation for tryptophan and histidine and after chloramphenicol treat-

ment in *B. subtilis* B-42, although fatty acid synthesis continued at reduced rates. A recent report by Glaser et al. (7) also described the tight coupling of macromolecular syntheses. Cessation of protein, DNA, and RNA synthesis in a temperature-sensitive mutant of *E. coli*, defective in glycerol-3-phosphate acyltransferase, paralleled the cessation of phospholipid synthesis at the restrictive temperature.

A correlation of lipid and protein synthesis would be observed if the enzymes of lipid synthesis were extremely labile and therefore required continuous replacement by de novo protein synthesis. Perturbing protein synthesis would then markedly affect the rate of polar lipid synthesis. An alternate possibility is that there is a requirement for the presence of a specific membrane protein(s) to act as an acceptor for nascent polar lipids. In the absence of the synthesis of such a protein, the presence of low levels of nonmembranous polar lipids possibly inhibit de novo synthesis of the polar lipids or their precursor (e.g., fatty acids).

The data presented in Fig. 7 give insight into the fate of the individual polar lipids during leucine deprivation. The observation that [^{14}C]oleic acid is incorporated into PG in leucine-deprived cultures at the same rate as in control cultures during the first 60 min of leucine deprivation indicates that the synthesis of this compound is probably not coupled directly to protein synthesis. Furthermore, the observation that, during the time period investigated, exogenous [^{14}C]oleic acid is incorporated to a marked extent into diglyceride by leucine-deprived cultures indicates, at a minimum, that the process by which performed fatty acids are taken into the cells and activated to the acyl carrier protein or coenzyme A derivative is probably not inhibited by inhibition of protein synthesis.

These observations, coupled with the marked inhibition of [^{14}C]oleic incorporation into CL soon after leucine deprivation, could be explained by the absence of a protein "acceptor" for CL, resulting in an accumulation of free CL. Burritt and Henderson (3) observed that the presence of nanomolar amounts of CL inhibits the membrane-bound CL synthetase of *L. plantarum* in vitro. Short and White (20) reported similar in vitro inhibition of *S. aureus* CL synthetase by addition of CL. Burritt and Henderson (3) also found that membranes of *L. plantarum* possess a phospholipase that converts PG to diglyceride under conditions in which CL is not hydrolyzed. The inhibition of CL synthetase as well as the absence of acceptor protein for PG could result in the accumulation of PG and its subsequent conversion to diglyceride by the phospholipase. In this way, the inhibition of protein synthesis could give the appearance of directly inhibiting net polar lipid synthesis such as described in this report, when, actually, polar lipid synthesis is occurring, but the rate of synthesis is offset by the concomitant breakdown of the polar lipids.

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