

Regulation of Fatty Acid Synthesis During the Cessation of Phospholipid Biosynthesis in *Escherichia coli*

WILLIAM D. NUNN,* DAVID L. KELLY, AND MICHELLE Y. STUMFALL

Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92717

Received for publication 14 June 1977

In 1975, Cronan et al. (*J. Biol. Chem.* 250:5835-5840) reported that free fatty acids accumulated during glycerol starvation of an *Escherichia coli* glycerol auxotroph. On the basis of labeling experiments showing significant incorporation of [¹⁴C]acetate into the fatty acid fraction of glycerol-starved cells, these authors concluded that fatty acid synthesis proceeded normally in the absence of phospholipid synthesis. Since these findings might have been due to an increase in the intracellular specific activity of the [1-¹⁴C]acetyl coenzyme A pool of the glycerol-starved cells, we reexamined the effect of glycerol starvation on fatty acid synthesis. We found that (i) the incorporation of ³H₂O and/or [2,3-¹⁴C]succinate into the fatty acid fraction of glycerol auxotrophs is severely reduced during starvation, (ii) the incorporation of [1-¹⁴C]acetate into the lipid fraction of an acetate-requiring glycerol auxotroph is inhibited by 95% during glycerol starvation, and (iii) the accumulation of fatty acids, as measured by microtitration, in glycerol-starved cells is less than 10% that of glycerol-supplemented cells. These results indicate that fatty acid synthesis is inhibited in the absence of phospholipid synthesis in *E. coli*.

Glycerol starvation of *Escherichia coli* glycerol auxotrophs results in the inhibition of membrane phospholipid synthesis (2, 4-6). Studies in two laboratories have been concerned with determining whether glycerol deprivation specifically affects the rate of fatty acid synthesis (4, 6). Mindich (6) found that fatty acid synthesis was severely curtailed by starvation and that only trace amounts of free fatty acids accumulated under these conditions. These findings suggested that fatty acid and phospholipid syntheses are coordinately controlled during glycerol starvation. Cronan et al. (4) proposed that the lack of fatty acid accumulation during starvation might be due to the degradation of free fatty acids and/or the inhibition of fatty acid synthesis. To test their hypothesis, these investigators repeated the starvation experiments with glycerol auxotrophs, which also lacked the ability to degrade fatty acids (4). Labeling experiments with [¹⁴C]acetate suggested that these strains were able to synthesize and accumulate free fatty acids during glycerol starvation (4). These workers interpreted their results as indicating that fatty acid synthesis can proceed normally in the absence of phospholipid synthesis (4).

The conclusion of Cronan et al. (4) is based on measurements of the rate of [¹⁴C]acetate incorporation into fatty acids. Their experimental rationale, however, did not control for vari-

ations in the specific radioactivity of the fatty acid precursor. Thus, a severe decrease in fatty acid synthesis could be masked by a great increase in the specific radioactivity of the fatty acid precursor. For instance, the incorporation of [¹⁴C]acetate into both total lipids (4) and proteins increases during glycerol starvation (Nunn, unpublished data). However, this increase occurs while protein synthesis as measured by labeled amino acid incorporation is declining (2). In addition, other cellular processes (e.g., growth and deoxyribonucleic acid and ribonucleic acid syntheses) also decline (2, 4) while the synthesis of fatty acids as measured by [¹⁴C]acetate incorporation continues at a rate comparable to that of glycerol-supplemented cells (4).

Since these observations suggested that the increase in [¹⁴C]acetate incorporation into fatty acids reported by Cronan et al. (4) might be due to an increase in the specific activity of the intracellular [¹⁴C]acetyl coenzyme A (CoA) pool of glycerol-deprived cells, we decided to reexamine the effect of glycerol starvation on the rate of fatty acid synthesis. The results in this paper show that fatty acid synthesis is inhibited when glycerol auxotrophs are deprived of glycerol. These results support the findings of Mindich (6) and suggest that fatty acid and phospholipid syntheses are coordinately controlled.

MATERIALS AND METHODS

Bacteria, media, and growth conditions. The bacterial strains used in this study were all derivatives of *E. coli* K-12. The genotypes of these strains are given in Table 1. Strain DL9 is an acetate-requiring strain that possesses defects in β -oxidation (*fadE*) and in the utilization of endogenous *sn*-glycerol 3-phosphate (*plsB*). This strain owes its acetate requirement to a lesion in the *aceF10* gene. The *aceF10* mutation codes for a defective pyruvate dehydrogenase (8). The defect in the *plsB* gene results in an *sn*-glycerol 3-phosphate acyltransferase with a K_m value for *sn*-glycerol 3-phosphate that is 10-fold higher than normal (3, 5). Since the endogenous supply in this strain is insufficient for normal phospholipid synthesis, it must be supplied with an exogenous source of glycerol (or *sn*-glycerol 3-phosphate) to allow growth (2). DL9 was constructed as follows. MS1, a *thr-1* derivative of LW1, was obtained by ultraviolet mutagenesis. MS2 is an *leu-6* strain derived from MS1 by transduction to *thr-1*⁻ with phage grown on X478. Finally, DL9 was derived from MS2 by transduction to *leu-6*⁻ by phage grown on Ac10. Cultures of DL9 possess less than 1% of the β -oxidation activity observed in comparable cultures of the *fad*⁻ strain BB26-36.

Bacteria were routinely incubated in a New Brunswick gyratory water bath shaker at 37°C. The bacteria were usually cultured in a standard medium consisting of medium E (13) supplemented with 0.4% sodium succinate and 0.05% casein hydrolysate (vitamin-free). In some experiments, the bacteria were cultured in standard medium with only succinate or glucose as the carbon source. When appropriate, glycerol was added to standard medium to a final concentration of 0.01%. Cell growth was monitored at 540 nm in a Klett-Summerson colorimeter. One Klett unit equals about 5×10^6 cells per ml. All experiments were initiated when the density of an exponentially growing culture reached 2.5×10^8 to 3.0×10^8 cells per ml.

Assay of lipid synthesis. The synthesis of lipids was followed by measuring the incorporation of

labeled acetate, succinate, or $^3\text{H}_2\text{O}$. Samples (1 ml) of cultures were removed at various intervals after glycerol starvation and labeled for 5 min with 10 μCi of [$1\text{-}^{14}\text{C}$]acetate (57 $\mu\text{Ci}/\mu\text{mol}$) per ml. In other experiments, cells were labeled with 17.2 mM [$2,3\text{-}^{14}\text{C}$]succinic acid (0.6 $\mu\text{Ci}/\mu\text{mol}$). After 5 min of incubation, 6 ml of chloroform-methanol (1:2, vol/vol) was added to each tube, and the lipids were treated as described by Nunn and Cronan (1, 7). The incorporation of labeled isotope was linear for at least 30 min. In some experiments, the labeled samples were filtered on membrane filters (Millipore Corp.) and washed several times before being extracted with chloroform-methanol. The radioactivity in total lipids was quantitated as described previously (9). $^3\text{H}_2\text{O}$ was used to measure the accumulation of lipids during glycerol starvation.

Analysis of lipids. All analyses were done by thin-layer chromatography. Neutral lipids were separated from phospholipids as described by Cronan et al. (4). The identification of various lipids was established by simultaneous chromatography of known standards.

Estimation of total free fatty acids accumulated during starvation. LW1 cells were grown in 2 liters of the standard medium containing 0.01% glycerol to 2.5×10^8 cells per ml. The cells were collected by centrifugation, washed once with medium E, and resuspended in 2 liters of the standard medium. The culture was divided into two equal portions. One portion was supplemented with glycerol (100 $\mu\text{g}/\text{ml}$), and the other portion was left unsupplemented. The two cultures were then incubated at 37°C, and 300-ml portions were removed at 30, 60, and 90 min. The samples were centrifuged and the lipids were extracted from the packed cells by the procedure of Ames (1). Free fatty acids were separated from phospholipids by thin-layer chromatography. The phospholipid fraction was treated with 2.3 N KOH in 50% ethanol and heated for 3 to 4 h at 60 to 80°C. After evaporation of the alcohol on a boiling-water bath, an appropriate amount of 6 N HCl was added to the solution. The fatty acids from the phospholipid fraction were then extracted with

TABLE 1. Bacterial strains

Strain	Sex	Genotype ^a	Source
BB26-36	HfrC	<i>plsB26 glpD3 glpR2 phoA8 tonA22 rel-1 glpK14</i>	R. Bell
LW1	HfrC	<i>fadE</i> ^b derivative of BB26-36	L. Weisberg (3)
BB20-14	HfrC	<i>gpsA20 glpD3 glpR2 glpK14 phoA8 tonA22 rel-1</i>	R. Bell (2)
LW3	HfrC	<i>fadE</i> ^b derivative of BB20-14	L. Weisberg (3)
X478	F ⁻	<i>proC32 purE42 metE70 lysA23 thi-1 leu-6 trpE38 lacZ36 ml-1 xyl-5 ara-14 azi-6 tonA23 tsx-67 str-109</i>	R. Curtiss
Ac10	F ⁺	<i>aceF10 mel-1 supE57 supF58</i>	U. Henning (5)
MS1	HfrC	<i>thr-1</i> derivative of LW1	See text
MS2	HfrC	<i>leu-6</i> derivative of MS1	See text
DL9	HfrC	<i>aceF10</i> derivative of MS2	See text

^a The genetic symbols are those of Taylor and Trotter (12). The allele numbers are those of the Coli Genetic Stock Center, Yale University.

^b To ensure that LW1 and LW3 maintained their β -oxidation defect, we routinely tested their ability to grow on fatty acids as a sole carbon source and to oxidize $1\text{-}^{14}\text{C}$ -labeled fatty acids. These strains could not grow on fatty acids as a sole carbon source and possessed less than 1% of the β -oxidation activity observed in comparable cultures of *fad*⁺ strains BB26-36 and BB20-14.

petroleum ether as described by Polakis et al. (9). The free fatty acids and the fatty acids hydrolyzed from the phospholipids were quantitatively estimated by microtitration with 5 mM NaOH, using cresol red indicator as described by Ray et al. (11).

RESULTS AND DISCUSSION

Effect of glycerol starvation on the rate of incorporation of $[1-^{14}\text{C}]$ acetate into the lipids of an *E. coli* auxotroph. Our experiments were performed with the glycerol auxotroph used by Cronan et al. (4). Strain LW1 possesses a defect in β -oxidation (*fadE*) and in the utilization of endogenous *sn*-glycerol 3-phosphate (4). To minimize the changes that might occur in the endogenous acetyl CoA pool, we measured the rate of incorporation of $[1-^{14}\text{C}]$ acetate into the lipid fraction of glycerol-starved cells that were supplemented with a high concentration of unlabeled acetate (5.1 mM). The specific radioactivity of acetate in this culture, during a 5-min labeling period, was approximately 2 mCi/mmol. We also measured the rate of incorporation of $[1-^{14}\text{C}]$ acetate into the lipid fraction of glycerol-starved cells that were not supplemented with cold acetate (the specific radioactivity of acetate in this culture, during a 5-min labeling period, was 57 mCi/mmol). The latter experiment was similar to that reported by Cronan et al. (4). If the amount of $[1-^{14}\text{C}]$ acetate incorporated into the lipid fraction of glycerol-starved cells is due to fatty acid synthesis per se, the culture supplemented with 5.1 mM unlabeled acetate should incorporate $[1-^{14}\text{C}]$ acetate into lipids with similar kinetics to the culture lacking unlabeled acetate (albeit at different rates).

The cultures were labeled with 10 μCi of $[1-^{14}\text{C}]$ acetate for 5 min at various time intervals after glycerol starvation. After glycerol starvation, the carrier-free culture began, after a lag of 15 to 20 min, to incorporate $[1-^{14}\text{C}]$ acetate into its lipids at an increasing rate (Fig. 1b). The rate of $[1-^{14}\text{C}]$ acetate incorporation continued to increase in this culture (Fig. 1b) after it had stopped growing (Fig. 1a). These results are comparable to those reported by Cronan and Bell (3). In contrast, the incorporation of $[1-^{14}\text{C}]$ acetate into the lipids of the acetate-supplemented culture did not increase during glycerol starvation (Fig. 1c).

Table 2 shows that $[1-^{14}\text{C}]$ acetate is incorporated at an increasing rate into both the free fatty acid and phospholipid fraction of the carrier-free culture during glycerol starvation. In contrast to the carrier-free culture, the acetate-supplemented culture did not appreciably incorporate $[1-^{14}\text{C}]$ acetate into its free fatty acid and phospholipid fractions during glycerol dep-

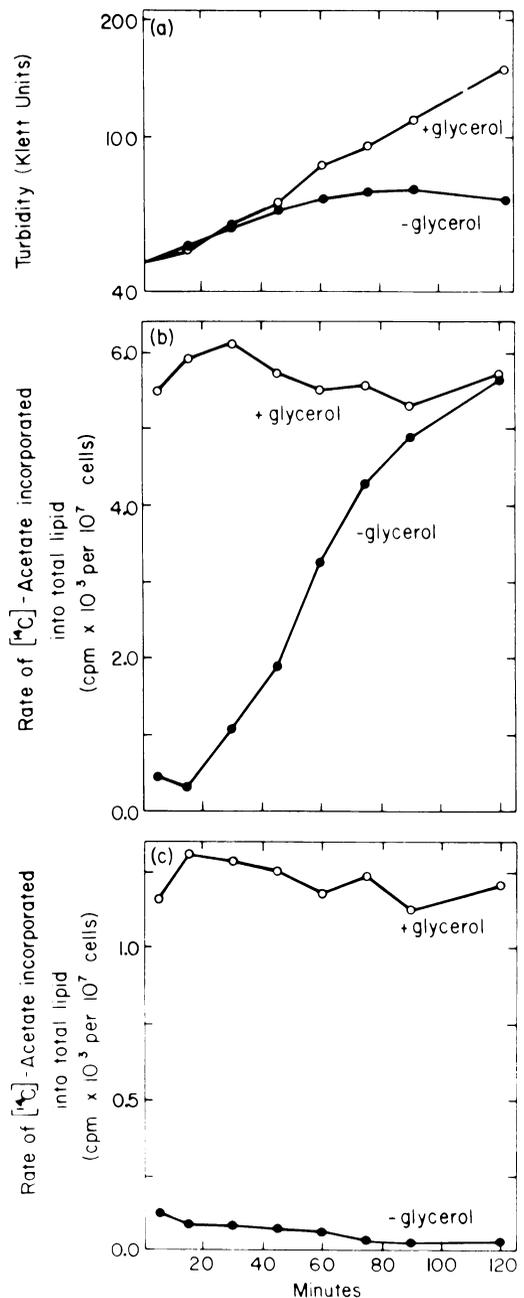


FIG. 1. Effect of glycerol starvation on growth and total lipid synthesis in strain LW1. Two cultures of LW1 were grown at 37°C in medium E (13) supplemented with 0.4% sodium succinate and 0.01% glycerol. One culture was supplemented with 5.1 mM sodium acetate, and the other was left unsupplemented. Upon reaching a density of 2.5×10^8 cells per ml, both cultures were harvested by filtration, washed, and resuspended in the same medium minus glycerol. Each of the cultures was divided into two equal portions. One portion was

TABLE 2. Rate of [^{14}C]acetate incorporation into the lipids of glycerol-starved cultures of LW1^a

Concn of unlabeled acetate in medium (mM)	Time of labeling (min)	[^{14}C]acetate incorporated into lipid (cpm/10 ⁷ cells)					
		+gly PL	-gly PL	Ratio of -gly PL/+gly PL	+gly FFA	-gly FFA	Ratio of -gly FFA/+gly FFA
0	5	5,258	64	0.012	265	400	1.51
	15	5,677	44	0.008	286	300	1.05
	30	5,862	150	0.026	295	930	3.15
	45	5,490	260	0.047	277	1,640	5.92
	60	5,310	450	0.085	268	2,810	10.50
	90	5,067	670	0.132	255	4,200	16.47
	120	5,479	790	0.144	276	4,930	17.86
5.1	5	1,104	17	0.015	56	103	1.83
	15	1,249	13	0.010	63	80	1.27
	30	1,230	13	0.011	62	78	1.26
	45	1,197	12	0.010	60	76	1.26
	60	1,123	10	0.009	56	64	1.14
	90	1,070	6	0.006	54	34	0.63
	120	1,151	6	0.005	58	34	0.59

^a The growth conditions and experimental protocol were identical to those described in the legend to Fig. 1. The lipids were analyzed as described by Cronan et al. (4). FFA, Free fatty acid; PL, phospholipid; gly, glycerol.

riation (Table 2). The results with the acetate-supplemented culture (Table 2) support the findings of Bell (2) and Mindich (6) that showed that the incorporation of [^{14}C]acetate into the phospholipid fraction of glycerol auxotrophs was severely inhibited during glycerol starvation.

Since the results with the carrier-free culture (Fig. 1b and Table 2) probably reflect an increase in the intracellular specific activity of the [^{14}C]acetyl CoA pool available for lipid biosynthesis, we also measured the incorporation of [^{14}C]acetate into the lipid fraction of DL9, an acetate-requiring derivative of strain LW1. Since this strain is unable to synthesize acetate, we could control the specific radioactivity of acetyl CoA by adding [^{14}C]acetate of known specific radioactivity. The results (Fig. 2) show that the incorporation of [^{14}C]acetate into the total lipid fraction of DL9 was reduced 95% by glycerol starvation. The glycerol-

starved culture of DL9 incorporated [^{14}C]acetate predominantly into their free fatty acid fraction (Fig. 2, lower panel). However, except for 40 min after glycerol starvation, the glycerol-supplemented cultures incorporated comparable amounts of [^{14}C]acetate into their free fatty acid fraction (Fig. 2, lower panel).

Effect of glycerol starvation on the incorporation of [2,3- ^{14}C]succinate and $^3\text{H}_2\text{O}$ into the lipids of strain LW1. The above results indicate that fatty acid synthesis, as measured by [^{14}C]acetate incorporation, does not proceed normally during glycerol starvation. We substantiated these findings by measuring lipid synthesis during glycerol deprivation with two other radioactive precursors, [2,3- ^{14}C]succinate and $^3\text{H}_2\text{O}$. When [2,3- ^{14}C]succinate is used as both labeled precursor and sole carbon source, variations in the specific activity of the [^{14}C]acetyl CoA pool resulting from changes in endogenous unlabeled acetyl CoA synthesis are precluded. Furthermore, the rate of lipid labeling from [2,3- ^{14}C]succinate should more closely approximate the *in vivo* rate of synthesis than that from added [^{14}C]acetate. The incorporation of the tritium from $^3\text{H}_2\text{O}$ into fatty acids is independent of the source of acetyl CoA and unaffected by transport processes (9). Thus, the use of $^3\text{H}_2\text{O}$ as precursor probably provides the best estimate of the rate of fatty acid synthesis. During glycerol starvation of strain LW1, the incorporation of both $^3\text{H}_2\text{O}$ and [2,3- ^{14}C]succinate into lipids was reduced by 95% (Fig. 3). Comparable results were obtained

supplemented with 0.01% glycerol, and the other portion was left unsupplemented. The cultures were then further incubated at 37°C. The rate of total lipid synthesis was determined by briefly labeling 1 ml of cells from each culture with 10 μCi of [^{14}C]acetate (specific activity, 57 $\mu\text{Ci}/\mu\text{mol}$) for 5 min as described in the text. The lipids were extracted into chloroform-methanol, and the incorporated label was quantitated as described in the text. (a) Shows the growth curve of the culture that was not supplemented with acetate. The growth curve of the acetate-supplemented culture was identical to the unsupplemented culture. See figure for difference between (b) and (c).

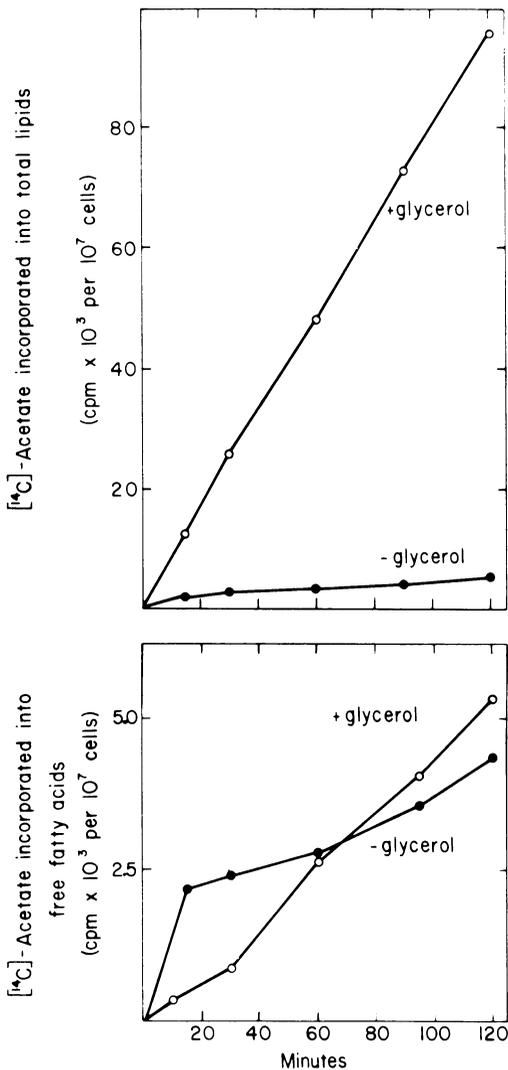


FIG. 2. Effect of glycerol starvation on the incorporation of $[1-^{14}\text{C}]$ acetate into the lipids of an acetate-requiring glycerol auxotroph. Cultures of DL9 were grown at 37°C in medium E supplemented with 0.4% succinate, 0.01% glycerol, and $400\ \mu\text{g}$ of sodium acetate per ml. When a cell density of 2.5×10^8 cells per ml was reached, the bacteria were harvested by centrifugation, washed once, and resuspended in the same medium minus glycerol. The culture was then divided into two equal portions: one portion was supplemented with 0.01% glycerol and the other portion was left unsupplemented. Each portion was supplemented with $2.5\ \mu\text{Ci}$ of $[1-^{14}\text{C}]$ acetate (final specific activity, $0.5\ \mu\text{Ci}/\mu\text{mol}$) per ml. The cultures were then incubated at 37°C and, at the indicated time intervals, 1-ml portions were added to 6 ml of chloroform-methanol (1:2, vol/vol). The lipids were extracted as described in the text.

with another glycerol auxotroph, strain LW3 (data not shown). The genotype of strain LW3 is given in Table 1.

To determine how severely fatty acid synthesis is inhibited in glycerol-starved cells, we quantitated the amount of fatty acids that

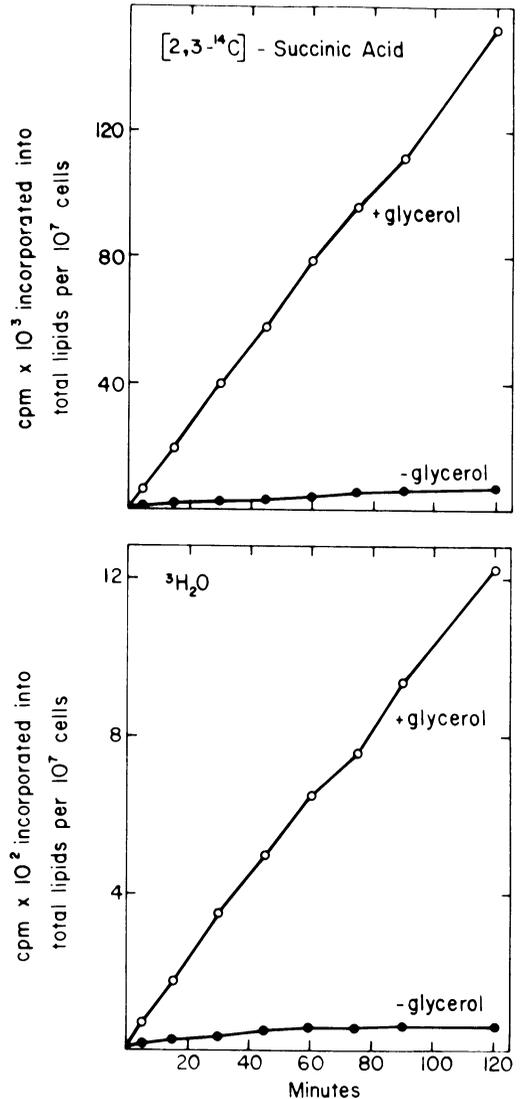


FIG. 3. Effect of glycerol starvation on the incorporation of $[2,3-^{14}\text{C}]$ succinate and $^3\text{H}_2\text{O}$ into the lipids of strain LW1. Cultures of strain LW1 were grown at 37°C in the same medium described in the legend to Fig. 1. When $[2,3-^{14}\text{C}]$ succinate was used, the cultures were supplemented with $20\ \mu\text{Ci}$ of the isotope per ml at a final specific activity of $0.6\ \mu\text{Ci}/\mu\text{mol}$. When $^3\text{H}_2\text{O}$ was used, the final specific activity of the labeled cultures was $652\ \mu\text{Ci}/\text{mmol}$. The lipids were extracted and quantitated as described in the text.

TABLE 3. Accumulation of free and esterified fatty acids in glycerol-supplemented and -starved cultures of strain LW1^a

Growth conditions	Time (min)	Fatty acids (nmol/mg of dry wt) ^b	
		Esterified	Non-esterified (free)
Plus glycerol	30	71	4.0
	60	127	7.3
	120	232	13.5
Minus glycerol	30	1.2	6.6
	60	2.1	7.0
	120	2.7	10.4

^a The fatty acids were quantitated by microtitration with NaOH as described in the text.

^b These values represent only the de novo fatty acids accumulated per milligram of dry weight during the 2-h experiment.

accumulated during glycerol starvation by microtitration. Table 3 shows that the glycerol-supplemented and -starved cultures of LW1 accumulate approximately the same small amount of free fatty acids. However, total de novo fatty acid accumulation in glycerol-starved cells was 95% less than in the glycerol-supplemented cultures (Table 3).

In summary, the results in this paper show that fatty acid and phospholipid syntheses are coordinately inhibited in glycerol auxotrophs of *E. coli* during glycerol starvation. These results support the findings of Bell (2) and Mindich (6). Although we made no effort to measure the endogenous acetyl CoA pool in glycerol-starved cells, we believe that it decreases during glycerol starvation. Such a decrease in the unlabeled endogenous pool would account for the increase in [1-¹⁴C]acetate labeling of lipids during glycerol starvation (4; Fig. 1b). Thus, when we circumvented the problems of endogenous acetyl CoA pool levels, a low level of free fatty acid accumulation was found during glycerol starvation.

It has been reported that glycerol auxotrophs of *Bacillus subtilis* and *Staphylococcus aureus* accumulate free fatty acids during glycerol starvation (6, 10). These studies (6, 10) primarily used [1-¹⁴C]acetate to measure free fatty acid synthesis. However, variations in the specific radioactivity of the fatty acid precursor were avoided because these studies were performed with high concentrations of acetate (6, 10), and similar results were obtained with labeled glucose (6). Therefore, it appears that gram-positive bacteria differ from *E. coli* in their regulation of free fatty acid synthesis.

At this time, we do not know how glycerol starvation and/or the inhibition of phospholipid synthesis regulates fatty acid synthesis in *E. coli*. Since the strains used in this study are defective in β -oxidation, the lack of free fatty acid accumulation cannot be attributed to rapid catabolism of fatty acids.

ACKNOWLEDGMENTS

We thank John E. Cronan, Jr., and E. P. Kennedy for their helpful discussions during the progress of this work. We also thank Calvin S. McLaughlin and Rowland Davis for critical reading of the manuscript.

This investigation was supported by Public Health Service grant GM-22466-1A from the National Institute of General Medical Sciences, grant 813 from the California Division of the American Cancer Society, and a grant from the American Heart Association, Orange County Chapter.

LITERATURE CITED

- Ames, G. F. 1968. Lipids of *Salmonella typhimurium* and *Escherichia coli*: structure and metabolism. *J. Bacteriol.* 95:833-843.
- Bell, R. M. 1974. Mutants of *Escherichia coli* defective in membrane phospholipid synthesis: macromolecular synthesis in an sn-glycerol 3-phosphate acyltransferase K_m mutant. *J. Bacteriol.* 117:1065-1076.
- Cronan, J. E., Jr., and R. M. Bell. 1974. Mutants of *Escherichia coli* defective in membrane phospholipid synthesis: mapping of sn-glycerol 3-phosphate acyltransferase K_m mutants. *J. Bacteriol.* 120:227-233.
- Cronan, J. E., Jr., L. J. Weisberg, and R. G. Allen. 1975. Regulation of membrane lipid synthesis in *Escherichia coli*: accumulation of free fatty acids of abnormal length during inhibition of phospholipid synthesis. *J. Biol. Chem.* 250:5835-5840.
- Hsu, C. C., and C. F. Fox. 1970. Induction of the lactose transport system in a lipid-synthesis-defective mutant of *Escherichia coli*. *J. Bacteriol.* 103:410-416.
- Mindich, L. 1972. Control of fatty acid synthesis in bacteria. *J. Bacteriol.* 110:96-102.
- Nunn, W. D., and J. E. Cronan, Jr. 1974. *rel* gene control of lipid synthesis in *Escherichia coli*: evidence for eliminating fatty acid synthesis as the sole regulatory site. *J. Biol. Chem.* 249:3994-3996.
- Nunn, W. D., L. A. Meade, and B. E. Tropp. 1975. Lipid synthesis in stringent *Escherichia coli*: an artifact in acetate labeling of phospholipids during a shutdown in growth rate. *J. Bacteriol.* 121:396-399.
- Polakis, S. E., R. B. Guchhait, and M. D. Lane. 1973. Stringent control of fatty acid synthesis in *Escherichia coli*: possible regulation of acetyl coenzyme A carboxylase by ppGpp. *J. Biol. Chem.* 248:7957-7966.
- Ray, P. H., T. T. Lillich, and D. C. White. 1972. Consequences of glycerol deprivation on the synthesis of membrane components in a glycerol auxotroph of *Staphylococcus aureus*. *J. Bacteriol.* 112:413-420.
- Ray, T. K., V. P. Skipski, M. Barclay, E. Essner, and F. M. Archibald. 1969. Lipid composition of rat liver plasma membranes. *J. Biol. Chem.* 244:5528-5536.
- Taylor, A. L., and C. D. Trotter. 1972. Linkage map of *Escherichia coli* strain K-12. *Bacteriol. Rev.* 36:504-524.
- Vogel, H. J., and D. M. Bonner. 1956. Acetylornithinase of *E. coli*: partial purification and some properties. *J. Biol. Chem.* 218:97-106.