Proc. Nat. Acad. Sci. USA
Vol. 69, No. 11, pp. 3105–3109, November 1972

Temperature-Sensitive Mutants of *Escherichia coli* Requiring Saturated and Unsaturated Fatty Acids for Growth: Isolation and Properties

(radiation suicide/[^{3}H]acetate/lipids)

MARK E. HARDER*, IFOR R. BEACHAM*, JOHN E. CRONAN, JR.†, KATHRYN BEACHAM*, JOY L. HONEGGER*, AND DAVID F. SILBERT*‡

*Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri 63110; and †Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06510

Communicated by Roy Vagelos, August 14, 1972

ABSTRACT **A procedure is described for selection of temperature-sensitive mutants affecting fatty-acid synthesis based upon radiation suicide of wild-type organisms by tritiated acetate selectively incorporated into fatty acids. At 37°, two of the mutants extensively incorporate fatty-acid supplements provided in the medium, and grow for extended periods only when a *trans*-unsaturated or a combination of saturated and *cis*-unsaturated fatty acids is available. *In vivo* fatty-acid synthesis, measured by [^{14}C]acetate incorporation, is temperature-sensitive in these strains relative to protein synthesis and other nonlipid macromolecular syntheses using acetate. The biochemical nature of these mutations has not been identified.**

Saturated, unsaturated, and β-hydroxy fatty acids of *Escherichia coli* are synthesized in part by a common pathway. From the known sequence of reactions, one might expect to isolate mutants blocked in total fatty-acid synthesis or selectively defective in the synthesis of one or more classes of fatty acid. Unsaturated fatty-acid auxotrophs have been isolated by exogenous replacement of the essential fatty acid and penicillin enrichment (1–3). Recovery of such mutants is low, because under conditions of fatty-acid starvation (used in penicillin enrichment) the unsaturated fatty-acid auxotrophs continue to grow for one generation, and then begin to die (2). Furthermore, this approach, which relies on replacement, has not yielded any other class of fatty-acid biosynthetic (*fab*) mutants. Mutations affecting the synthesis of these other types of fatty acids may not be critical to cells or, on the other hand, might be lethal. For either reason they would not ordinarily be isolated. Recently, conditional mutants for phospholipid biosynthesis were isolated because they failed to assimilate a radioactive, and specific, phospholipid precursor and, thus, were protected from radiation death undergone by cells with an intact biosynthetic pathway (4). In this paper, we report conditions for selectively labeling cellular fatty acids with tritiated acetate of high specific activity and isolating conditional mutants affecting fatty-acid synthesis. Such mutants survive better than wild-type cells because they do not concentrate the radioisotope. Properties of two of these mutants are described.

MATERIALS AND METHODS

Strains and Media. All bacterial strains were K-12 strains of *E. coli*. The mutants obtained in this study were derived from AB1623, a glutamate-requiring strain defective in citrate synthetase, which was provided by Dr. H. L. Kornberg and has been described (5). LA1-6 and LA2-22 are two independently isolated mutants thermosensitive for fatty-acid biosynthesis; LA1-6-2 and LA2-22-6 are wild-type transductants of these mutants. In the symbols adopted for classifying mutants, *L* indicates lipid, *A* identifies the genetic background (AB1623), the *numeral before* the hyphen refers to the particular mutant selection, and the *number after* the hyphen is the particular isolate obtained. C600 is F^- and requires leucine and threonine for growth.

Minimal growth medium was medium 63 (6) containing 4 mg/ml glycerol, 5 mM potassium glutamate, 0.5 μg/ml thiamine, and 1 μg/ml yeast extract. Five solid media were used: minimal agar is 1.5% agar containing minimal medium; CA agar contains the same ingredients plus 0.2% casamino acids; TYB plates are medium 63 containing 1% tryptone, 10 μg/ml yeast extract, 1 μg/ml thiamine, 1 mM potassium glutamate, and 0.1% Brij 58; NBF_3 agar is nutrient broth agar supplemented with 0.1% Brij 58 and with palmitate, oleate, D,L-β-hydroxymyristate, all at 0.3 mM; NBF_5 contains the same ingredients plus laurate and myristate, each at 0.3 mM.

Isolation of Mutants. The mutants LA1-6 and LA2-22, which are described in this paper, were isolated in two separate experiments from cultures mutagenized with ethyl methanesulfonate (7) and nitrosoguanidine (8), respectively. In both cases, the population of mutants in fatty-acid synthesis was enriched by the following tritium suicide procedure. Mutagenized cells were grown for several generations at 30° in minimal medium supplemented with K-acetate, and isoleucine, leucine, and valine (see legend to Table 1). Brij 58 (1 mg/ml) and oleate, palmitate, and D,L-β-hydroxymyristate (all at 0.2 mM) were also present. A portion of this culture was harvested in late-log phase and suspended at 5×10^7 cells per ml in the same medium lacking fatty acids. Shortly after this culture resumed exponential growth, it was divided into two portions, which were incubated at 40° for 20 min, centrifuged, washed, and resuspended in minimal medium supplemented with branched-chain amino acids and 1 mg/ml Brij 58. Undiluted tritiated Na-acetate was added at a final concentration of 1 mM to one subculture; and the other culture, a sham suicide, was supplemented with 1 mM nonradioactive Na-acetate. Both cultures were incubated at 40° for one generation of growth, harvested on Millipore filters, and washed twice with cold medium 63 and once with distilled

‡ To whom reprint requests should be addressed.

TABLE 1. *Distribution of acetate radioisotope in AB1623*

Cell fraction	Distribution of radioactivity (%)		
	from [^{14}C]-acetate		from [^{3}H]-acetate
	−Leu	+Leu	+Leu
Lipid	80	95	88
Nonlipid	20	5	12

Cells were grown at 40° in minimal medium plus 1 mM K-acetate. If present as supplement, L-leucine was added at 0.4 mM together with 1.25 mM L-isoleucine and 1.0 mM L-valine. Exponentially growing cells were labeled with [^{14}C]acetate or [^{3}H]acetate (Silbert, D. F., Ulbright, T. & Honegger, J. L., manuscript submitted for publication), and then harvested and washed once with medium 63. Cell fractions were obtained by acid hydrolysis and ether extraction, and radioactivity was determined by scintillation counting (9). Distributions of [^{14}C]-acetate label are average values from measurements made on more than one culture with differences (in the actual percentages obtained) between similar cultures of no more than 3%. Distribution of [^{3}H]acetate was obtained with newly synthesized radioisotope. After prolonged storage at 4°, decomposition occurs and alters this distribution slightly (unpublished results).

water. Cells were resuspended in 2.0 ml of medium 63 and stored at 4°. Periodically, aliquots were removed, serially diluted, and plated for survivors at 30° on NBF_3 for the first suicide, and NBF_5 for the second suicide. Survivors were tested for growth at 30° and 40° on CA or TYB plates. Colonies that grew at 30° but not at 40° were repurified on minimal agar, or minimal agar plus fatty acids if a fatty-acid requirement was evident. In some cases, survivors were replica-plated onto TYB plates and TYB plates supplemented with oleate, palmitate, and D,L-β-hydroxymyristate and incubated at 30° and at 40° to detect any strains that might require fatty acids for growth at either temperature.

To eliminate mutants that are not specifically defective in acetate fixation, incorporation of [^{14}C]acetate and [^{3}H]glutamate was measured in cells growing under identical conditions to those used for suicide labeling.

Materials. Labeled compounds were New England Nuclear products. Sodium-[^{3}H]acetate (5.1 Ci/mmol) was prepared by exchange between tritiated water and sodium acetate. Brij 58 was the gift of Atlas Chemical Co. All nonpolar fatty acids were obtained from Hormel Inst., Austin, Minn. and were >99% pure. D,L-β-hydroxymyristic acid was synthesized by Dr. Robert M. Bell.

RESULTS

Selective Radioisotope Labeling of Cellular Lipid. When AB1623, a citrate synthetase-negative strain requiring glutamate for growth, is grown in minimal medium supplemented with glutamate and [^{14}C]acetate, 80% of the incorporated radioisotope is found in lipid (Table 1). Radioisotope incorporation becomes even more selective (95% in lipid) when leucine is present in the growth medium. Aminoacid analysis of hydrolyzed protein from AB1623 labeled with [^{14}C]acetate shows that those amino acids that normally derive part of their carbon content from acetate contain negligible radioactivity (data not shown). Studies with citrate synthetase-positive strains of *E. coli* K-12 indicate that the citrate synthetase defect is required for exclusion of ^{14}C from amino acids and other nonlipids (data not shown; also see ref. 10 for studies with *E. coli* B).

Two of the three hydrogens on the methyl carbon of acetyl CoA are released during use of this precursor for fatty-acid chain elongation (11). Thus, the ratio of lipid to nonlipid radioactivity derived from [^{3}H]acetate should be about one-third that obtained from [^{14}C]acetate. The distribution of radioactivity from [^{3}H]acetate shown in Table 1 agrees with this prediction (95/5 = 19/1; 88/12 ≅ 7/1).

Isolation of Temperature-Sensitive (ts) Mutants Affecting Fatty-Acid Biosynthesis. Two cultures, one mutagenized with ethyl methanesulfonate and the other with nitrosoguanidine, were labeled with [^{3}H]acetate under identical conditions. Both cultures lost viability faster during storage than corresponding nonradioactive cultures, but the second selection experiment displayed a smaller loss of viability than the first and yielded most of the 39 temperature-sensitive clones isolated. When comparing the two experiments, differences in methods of mutagenesis, extents of labeling (0.15 dpm/cell in the first selection compared with 0.11 dpm/cell in the second), and methods of rescuing survivors should be noted.

The criterion of temperature sensitivity rules out nonlethal mutations that, by virtue of an inability to accumulate [^{3}H]acetate, survive the suicide selection. Mutants in which lipid and nonlipid syntheses are equally impaired by elevated temperature, however, would survive the suicide. To rule out

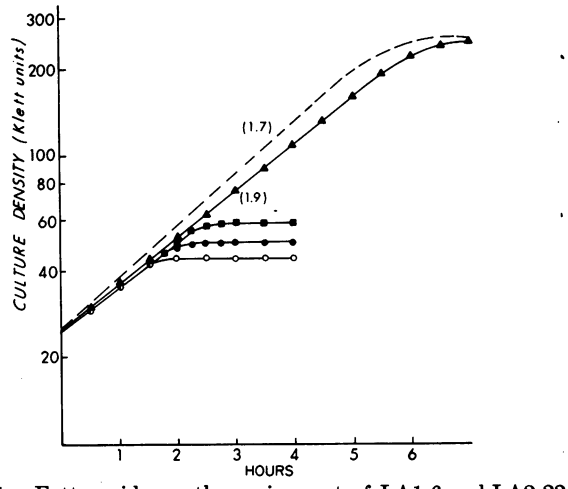

FIG. 1. Fatty-acid growth requirement of LA1-6 and LA2-22 at 37°. Cells were grown overnight at 30° in minimal medium supplemented with Brij 58 (0.4 mg/ml) and K-oleate (0.4 mM). Cells were harvested, washed, and resuspended at about 2.5 × 10^{8}/ml in fresh medium containing the above ingredients *without* K-oleate. The cell suspension was divided into portions to which various fatty-acid supplements were added (0.4 mM when present alone and 0.3 mM if added in combination). Then, all the cultures were placed in a gyrotory water-bath shaker maintained at 37.0 ± 0.2°. Growth was monitored in a Klett-Summerson Colorimeter. *Dashed line*, growth curve for AB1623 in unsupplemented or fatty-acid supplemented cultures. *Solid lines*, growth curves for LA1-6 or LA2-22: without supplement, O; with *cis*-Δ^{9}-16:1, ●; with 16:0, ■; with *trans*-Δ^{9}-16:1 or with 16:0 plus *cis*-Δ^{9}-16:1, ▲. Numbers in parentheses are generation times in hr.

the latter possibility, we performed [^{14}C]acetate–[^{3}H]glutamate double-label experiments. We expected that when the incorporation of ^{14}C and ^{3}H in *fab* mutants and the wild type were compared at 40°, the *fab* mutants would show a selective decrease in [^{14}C]acetate labeling. Of the 29 temperature-sensitive strains tested, 13 gave ^{14}C/^{3}H ratios between 2 and 65% of the wild-type ratio. LA1-6 and LA2-22 ratios were 15 and 5% of the wild-type values, respectively.

Growth Properties and Fatty-Acid Composition of Two ts Mutants Affected in Fatty-Acid Biosynthesis. LA1-6 and LA2-22 are temperature-sensitive mutants that can be restored to normal growth at 37° by supplementation of the medium with various combinations of saturated and unsaturated fatty acids. When the mutant strains were shifted from 30° to 37°, growth continued for almost one generation in the absence of supplement (Fig. 1). Addition of certain saturated or *cis*-unsaturated fatty acids alone enhances the extent of growth relative to the unsupplemented culture, but growth is rescued completely only when the appropriate *trans*-unsaturated fatty acid or combination of saturated and *cis*-unsaturated fatty acids is available. The detailed specificity of the fatty-acid structural requirement is the subject of another study. However, all supplements, even those that do not support prolonged growth, are incorporated into the phospholipid (Fig. 2). Wild-type strains also can incorporate these fatty-acid supplements, but the fatty-acid composition of their phospholipid is not as profoundly influenced by the supplements as that of the mutant organisms (compare the fatty-acid compositions of phospholipid from AB1623, LA1-6, and LA2-22 supplemented with *trans*-Δ^9-16:1 in Fig. 2). The more extensive use of fatty-acid supplement by the mutant strains is consistent with a decreased synthetic capacity and the dependence of these strains on exogenous fatty acid.

A small proportion of the fatty acid present in the phospholipid from mutant cells grown at 37° is synthesized. For example, the contribution of synthesis to the fatty-acid content of phosphatidylethanolamine isolated from mutant cells grown for 2.5 generations in the presence of *trans*-Δ^9-16:1 (see Fig. 2) was 28 and 41% for LA1-6 and LA2-22, respectively. Although growth can be restored by supplementation at 37°, it is not at 40°. The residual synthesis at 37° may be essential for making acyl chains that have not been provided or cannot be used from an exogenous source.

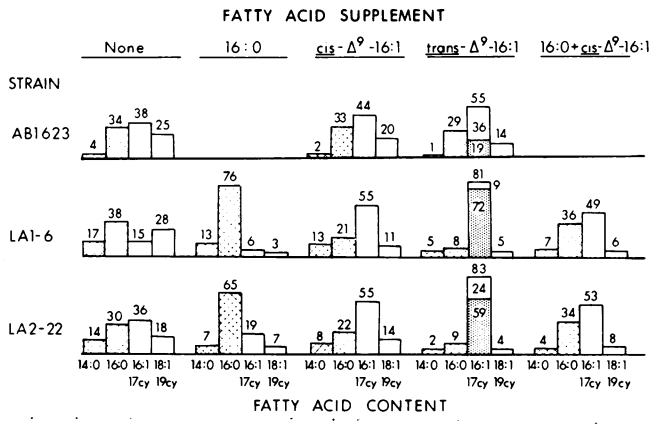

FIG. 2. Fatty-acid composition (weight %) of phosphatidylethanolamine. Cells were grown at 37° (see legend for Fig. 1) and then harvested at 160 Klett units, or if growth ceased prematurely, at the maximum OD reached. Cellular lipid was extracted, phosphatidylethanolamine isolated, and fatty-acid composition determined (12, 13). *Hatched*, *stippled*, and *clear bars* designate the content of saturated, *trans*-unsaturated, and *cis*-unsaturated fatty acids, respectively. The numbers above (and the relative heights of) the bars correspond to the weight % of each fatty acid. *Cy* is the cyclopropane derivative of the corresponding unsaturated fatty acids; we assume that the small amounts present were all derived from only *cis*-unsaturated fatty acid.

TABLE 2. *Effect of temperature on distribution of* [^{14}C]*acetate*

Strain	Lipid (nCi/mg of protein) 30°	Lipid (nCi/mg of protein) 37.5°	Nonlipid (nCi/mg of protein) 30°	Nonlipid (nCi/mg of protein) 37.5°	Lipid:nonlipid ratio 30°	Lipid:nonlipid ratio 37.5°
AB1623	28.8	49.7	15.2	19.4	1.90	2.56
LA1–6	10.8	5.2	13.9	13.4	0.78	0.39
LA2–22	28.2	2.8	15.4	9.0	1.87	0.31

AB1623, LA1-6, and LA2-22 were grown initially at 30° in minimal medium plus 1.0 mM K-acetate, 0.4 mg/ml Brij 58, and 0.2 mM K-oleate (K-oleate sustains growth of LA1-6 and LA2-22 for an extra generation compared to unsupplemented cultures). Each culture was divided into two aliquots: one was maintained at 30°; the other was placed in a gyrotory water bath adjusted to 37° (or 38°) and incubated for about 2 hr until the new growth rate was clearly established. 5-ml Aliquots of the exponentially growing cultures were pulse-labeled with 6.6 μCi of [^{14}C]acetate for 15 min (incorporation into macromolecules was essentially linear over this interval), and then chilled, and precipitated with cold Cl_3CCOOH. Washed Cl_3CCOOH pellets were hydrolyzed with 6 N HCl under reduced pressure for 16 hr. Aliquots of the acid hydrolysate were used for determination of leucine equivalents (14) and then extracted with chloroform. These conditions yielded a separation of lipid and nonlipid components that was comparable to that obtained previously with ether extraction of an acid hydrolysate neutralized to pH 5.6 (9). Radioactivity was determined (9). The amount of cell protein subject to hydrolysis was calculated with the conversion factor 10.2 leucine equivalents/mg of protein (9). AB1623 cultures were grown and pulsed at both 37° and 38°, and the results were averaged. LA1-6 and LA2-22 were grown at 37° and 38°, respectively.

Thermolability of Fatty-Acid Synthesis In Vivo. Preliminary studies with a double-label technique indicated that incorporation of [^{14}C]acetate into lipid relative to incorporation of [^{3}H]glutamate into nonlipid at 40° was much lower in the mutants as that compared to their wild-type parent. To rule out pleiotropic conditonal mutations that incidentally affect acetate transport or activation, the distribution of [^{14}C]acetate into both lipid and nonlipid cell constituents was examined. To facilitate this measurement, the very small incorporation of [^{14}C]acetate into the nonlipid cell fraction of AB1623 and its derivatives was increased by omission of leucine from the medium (Table 1). Representation of the data as a ratio of lipid to nonlipid acetate incorporation adjusts for any differences in the specific radioactivity of acetate pools in various cultures during the radioisotope pulse. A preferential effect of temperature on incorporation of [^{14}C]acetate into lipid relative to nonlipid was demonstrated in the mutant strains (Table 2).

After we established that the temperature-sensitive [^{14}C]-acetate incorporation by LA1-6 and LA2-22 was due to reduced lipid synthesis, we could now use incorporation of

TABLE 3. *Effect of temperature on [^{14}C]acetate and [^{3}H]-glutamate incorporation by supplemented cultures*

Strain	Incorporation rate (nCi/min per mg of protein) [^{14}C]acetate 30°	[^{14}C]acetate 37°	[^{3}H]glutamate 30°	[^{3}H]glutamate 37°
AB1623	1.3	2.6	1.4	2.8
LA1–6	1.0	0.6, 0.5	1.2	1.7
LA2–22	1.6	0.4, 0.3	2.1	1.4

Cells were grown and prepared for labeling as described in Table 2 except that the fatty-acid supplement was palmitelaidate (0.2 mM), and leucine, isoleucine, and valine were also present in the medium (see Table 1 for concentrations). Radioisotope incorporation was measured: 5 ml of exponentially growing culture was exposed to 5 μCi each of [^{14}C]acetate and [^{3}H]glutamate; at 5, 10, and 15 min, 1-ml aliquots were removed from the labeled cultures and treated with cold Cl_3CCOOH. The precipitates were collected on 0.45-μm Millipore filters, which were then washed with Cl_3CCOOH, dried, and counted in a liquid scintillation spectrometer. Rates of incorporation determined at different cell densities were normalized (1 Klett unit at 660 nm was equivalent to 1 μg of cellular protein). Duplicate [^{14}C]acetate incorporation assays given for LA1-6 and LA2-22 at 37° were made with an interval of one cell generation between determinations. Similar duplicate measurements were made with AB1623 and, as shown here for the mutants, the results obtained were not dependent on the particular experimental time or cell density. Incorporation of [^{3}H]glutamate at 30° is slightly high for LA2-22 in this experiment. In other experiments with LA2-22 growing at the same rate as here, incorporation was 1.6 nCi/min per mg of protein. All rates of incorporation were linear between 5 and 15 min.

[^{14}C]acetate by the mutants to obtain instantaneous rates of total lipid synthesis in both fatty-acid-supplemented and unsupplemented cultures. To perform these measurements, we returned to conditions (leucine supplementation) favoring the nearly exclusive incorporation of [^{14}C]acetate into lipid. Synthesis of protein and nucleic acid was monitored simultaneously by following incorporation of [^{3}H]glutamate (Table 3). In cultures supplemented with *trans*-Δ^9-16:1, ratios of [^{14}C]acetate incorporation rate to [^{3}H]glutamate incorporation rate change from near unity for all three strains at 30° to 0.9, 0.3, and 0.2 for AB1623, LA1-6, and LA2-22 at 37°, respectively, indicating that the mutants have defects selectively affecting fatty-acid synthesis relative to protein and nucleic acid synthesis. Incorporation of [^{14}C]acetate decreases in both mutants at 37° relative to their rates at 30° and to the wild-type rate at 37°.

Table 4 examines the rate of [^{14}C]acetate incorporation, alone and relative to glutamate incorporation, at 30° and 37°, for wild-type, mutants, and temperature-resistant transductants of LA1-6 and LA2-22 in cultures *not* supplemented with fatty acid. The rates at 37° were determined in this experiment 1.5 hr after the shift-up for all cultures, and this timing corresponded to the point where mutant growth was just beginning to slow down. The results agree with those in Table 3. Furthermore, the fact that correction of temperature sensitivity by transduction also leads to restoration of normal [^{14}C]-acetate incorporation indicates that the properties of LA1-6 and LA2-22 are due to a single point mutation or, if multiple, to mutations that are genetically close together.

In the experiments discussed above, fatty-acid synthesis in the mutant strains is not totally arrested at 37°. When the relative amounts of the different classes of fatty acids synthesized in one of these expriments (see Table 2) are measured (9), β-hydroxymyristate increases from 13 to 26% of the total in LA1-6 and LA2-22 after the change in growth temperature from 30° to 37°; the proportion of this fatty acid synthesized in AB1623 (13%) is not altered by the same change in temperature. This observation demonstrates that decreased fatty-acid synthesis in the mutant is associated with a preferential conservation of synthesis of the major and characteristic fatty-acid component of cellular lipopolysaccharide.

DISCUSSION

This paper is the first report of mutants of *E. coli* affecting total fatty-acid synthesis. The growth properties of these mutants demonstrate the requirement for saturated as well as *cis*-unsaturated fatty acids. A dual requirement for these two classes of fatty acids was first recognized (16) during studies on the growth of *Trichomonas*. More recently, isolation of fatty-acid biosynthetic mutants in *Saccharomyces cervisiae* (17, 18) and *Neurospora crassa* (19) has also revealed that saturated and *cis*-unsaturated fatty acids are essential. The failure to rescue growth at 40° with saturated and *cis*-unsaturated fatty-acid supplements is consistent with, though not proof of, the hypothesis that other fatty acids found in *E. coli*, such as dodecanoic, tetradecanoic, and β-hydroxymyristic acids, and the complex lipid(s) of which they are a part, are also essential for this particular organism. Attempts to demonstrate that these fatty acids can be provided effectively as exogenous supplements to prototrophs or mutant

TABLE 4. *Effect of temperature on [^{14}C]acetate incorporation by cultures not supplemented with fatty acid*

Strain	[^{14}C]Acetate incorporation (nCi/min per mg of protein) 30°	[^{14}C]Acetate incorporation (nCi/min per mg of protein) 37°	Incorporation ratio [^{14}C]acetate/[^{3}H]glutamate 30°	Incorporation ratio [^{14}C]acetate/[^{3}H]glutamate 37°
AB1623	3.9	7.0 (100)	1.9	2.0
LA1–6	3.0	0.5 (7)	1.8	0.6
LA1–6–2	5.5	7.6 (109)	2.0	2.0
LA2–22	3.2	1.8 (26)	1.7	1.2
LA2–22–6	3.6	6.6 (94)	1.7	2.2

Cultures were grown and rates of radioisotope incorporation were measured at the appropriate temperatures (see the legend to Table 3) with the following differences: no fatty-acid supplement was present, the concentration of K-acetate was 10 mM, and 2.5 μCi of [^{14}C]acetate was used per ml of culture for pulse labeling. LA1-6-2 and LA2-22-6, the temperature-resistant transductants of LA1-6 and LA2-22, respectively, were obtained by transduction with P_1 virulent phage (grown on C600) as described (15) with the following exceptions: bacteriophage were absorbed at 33–34°; bacteria were plated and maintained at this temperature for 5 hr before being shifted to 40°. LA1-6-2 and LA2-22-6 are identical to AB1623 with respect to growth requirements and generation times at 30° and 40°; at 37° (and 30°), the phosphatidylethanolamine from all three strains has the same fatty-acid composition. The numbers in parentheses are percentages of the AB1623 incorporation rate.

cells have been unsuccessful (manuscript submitted for publication and unpublished observations).

The biochemical nature of the *E. coli* mutations affecting fatty-acid biosynthesis is not known. Preliminary genetic mapping indicates that several distinct sites are represented by the small collection of presumptive *fab* mutants described in this report. In addition to true *fab* mutants, i.e., those affecting genes coding for enzymes or regulatory elements required for fatty-acid biosynthesis, the selection used here might also generate mutations reducing the level of intracellular TPNH. The temperature stability of glucose-6-phosphate dehydrogenase, gluconate-6-phosphate dehydrogenase, and TPN-specific malic enzyme, three of the enzymes producing TPNH, are the same in crude cell extracts from LA1-6, LA2-22,, and AB1623, and the specific activities are comparable to those reported for other wild-type strains of *E. coli* K-12 (20, 21). Although mutations affecting acetyl CoA synthesis from other carbon sources could have a preferential effect on fatty-acid synthesis, the temperature sensitivity of LA1-6 and LA2-22 is not corrected by acetate supplementation and, furthermore, the [^{3}H] acetate radiation suicide procedure would have been equally lethal to acetate auxotrophs and wide-type cells. The *fab* mutants obtained in this study should be valuable for examining the specificity for fatty acids in phospholipid synthesis and the formation of functional membranes, the mechanism of fatty acid biosynthesis, and the chromosomal organization of genes connected with this pathway.

We thank Dr. H. L. Kornberg for the gift of strain AB1623, Dr. R. A. Bradshaw for assisting us with the aminoacid analyses, Dr. R. E. O'Brien for preparing the [^{3}H]acetate of high specific activity, and Dr. P. R. Vagelos for his encouragement. This work was supported by Grants GM-16292 and HSAA 5-SO4-FR 06115-04 to D. F. S. and AI-10186 to J. C. C. M. E. H. is a recipient of NIH Training Grant GM-01311 and I. R. B., a Damon Runyon Postdoctoral Fellowship, DRF-651-T.

1. Silbert, D. F. & Vagelos, P. R. (1967) *Proc. Nat. Acad. Sci. USA* **58,** 1579–1586.
2. Henning, V., Dennert, G., Rehn, K. & Deppe, G. (1969) *J. Bacteriol.* **98,** 784–796.
3. Silbert, D. F. & Vagelos, P. R. (1972) "Biomembranes, Part A," *in Methods in Enzymology,* eds. Fleisher, S., Packer L. & Estabrook, R. W. (Academic Press, Inc., New York), in press.
4. Cronan, J. E., Jr., Ray, T. K. & Vagelos, P. R. (1970) *Proc. Nat. Acad. Sci. USA* **65,** 737–744.
5. Ashworth, J. M., Kornberg, H. L. & Nothmann, D. L. (1965) *J. Mol. Biol.* **11,** 654–657.
6. Pardee, A. B., Jacob, F. & Monod, J. (1959) *J. Mol. Biol.* **1,** 165–178.
7. Lin, E. C. C., Lerner, S. A. & Jorgensen, S. E. (1962) *Biochim. Biophys. Acta* **60,** 422–424.
8. Adelberg, E. A., Mandel, M. & Chen, G. C. C. (1965) *Biochem. Biophys. Res. Commun.* **18,** 788–795.
9. Silbert, D. F., Cohen, M. & Harder, M. E. (1972) *J. Biol. Chem.* **247,** 1699–1707.
10. Roberts, R. B., Abelson, P. H., Cowie, D. B. & Britten, R. J. (1963) *Studies of Biosynthesis in Escherichia coli* (Carnegie Inst. Wash. Publ. 607, Wash., D.C.), pp. 113–133.
11. Bressler, R. & Wakil, S. J. (1961) *J. Biol. Chem.* **236,** 1643–1651.
12. Silbert, D. F. (1970) *Biochemistry* **9,** 3631–3640.
13. Silbert, D. F., Ruch, F. & Vagelos, P. R. (1968) *J. Bacteriol.* **95,** 1658–1665.
14. Troll, W. & Cannan, R. K. (1953) *J. Biol. Chem.* **200,** 803–811.
15. Cronan, J. E., Jr., Silbert, D. F. & Wulff, D. L. (1972) *J. Bacteriol.*, in press.
16. Shorb, M. S. & Lund, P. G. (1959) *J. Protozool.* **6,** 122–130.
17. Schweizer, E. & Bolling, H. (1970) *Proc. Nat. Acad. Sci. USA* **67,** 660–666.
18. Henry, S. A. & Keith, A. D. (1971) *Chem. Phys. Lipids* **7,** 245–265.
19. Henry, S. A. & Keith, A. D. (1971) *J. Bacteriol.* **106,** 174–182.
20. Fraenkel, D. G. & Levisohn, S. R. (1967) *J. Bacteriol.* **93,** 1571–1578.
21. Sanwal, B. D. & Smando, R. (1969) *J. Biol. Chem.* **244,** 1817–182B.