Proc. Natl. Acad. Sci. USA
Vol. 75, No. 7, pp. 3377–3381, July 1978
Genetics

Transport of long-chain fatty acids by *Escherichia coli:* Mapping and characterization of mutants in the *fadL* gene*

(β-oxidation/lipid synthesis/enzyme activity/genetic mapping)

WILLIAM D. NUNN AND ROBERT W. SIMONS

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

Communicated by Paul Boyer, May 8, 1978

ABSTRACT **A new locus (*fadL*) that is required for the utilization of long-chain fatty acids has been mapped and partially characterized in an *Escherichia coli* mutant. The *fadL* locus has been mapped at 50 min on the chromosome. A mutant bearing a defect in this locus cannot utilize long-chain fatty acids as a sole carbon source. Derivatives of this mutant that can grow on decanoate (termed *fadR*) are capable of growth on medium-chain but not long-chain fatty acids. It is believed that the *fadL* mutant is defective in the transport of long-chain fatty acids into the cell for the following reasons: (*i*) *fadR fadL* strains can oxidize *in vivo* decanoate but not oleate; (*ii*) neither *fadL* nor *fadR fadL* strains can incorporate oleate into their membrane lipids; (*iii*) the activity of the acyl-CoA synthetase (EC 6.2.1.3) in *fadR fadL* strains is comparable to the acyl-CoA synthetase activity in *fadR fadL*$^+$ strains; and (*iv*) *in vitro* extracts from *fadR fadL* strains can oxidize oleate at rates comparable to those from *fadR fadL*$^+$ strains. If the above hypothesis is correct, the uptake of long-chain fatty acids by *E. coli* requires at least two gene products.**

Wild-type *Escherichia coli* K-12 can grow in media containing long-chain fatty acids as a sole carbon source (1, 2). The synthesis of at least five enzymes involved in fatty acid degradation (fad) are coordinately induced when this organism is grown in media containing the long-chain fatty acids (1). The structural genes that code for these fad enzymes are located at a minimum of three separate sites on the *E. coli* chromosome. Mutants that harbor a defect in one of these structural genes, the *fadD* gene, lack acyl-CoA synthetase [acid:CoA ligase (AMP-forming), EC 6.2.1.3] and cannot be induced to synthesize the other fad enzymes (1). These results led Overath and coworkers (1) to suggest that acyl-CoAs induce the synthesis of the fad enzymes.

Fatty acids with a chain length of C_{11} or less can serve as a substrate for the fad enzymes but cannot induce the synthesis of the fad enzymes (1, 2). Therefore, only fatty acids longer than C_{11} can be used as a sole carbon source by the wild type (1, 2). Spontaneous mutants that are constitutive for the synthesis of the fad enzymes can be isolated by plating the wild type on decanoate (1, 2). The genotype of these spontaneous decanoate-growing mutants has been referred to as *fadR*. Klein *et al.* (2) have shown that the double mutant *fadR fadD* synthesizes all of the fad enzymes except acyl-CoA synthetase. This mutant cannot utilize fatty acids of any chain length as a carbon source (2). In addition, the *fadR fadD* strain is unable to accumulate exogenous fatty acids into its cytosol or incorporate exogenous fatty acids into its phospholipids (2). These results led Klein *et al.* (2) to suggest that acyl-CoA synthetase is required for the transport of exogenous fatty acids into *E. coli*. Hill and Angelmaier (3) reported a *fadD*$^+$ strain that was unable to accumulate exogenous oleic acid, suggesting a second locus involved in the transport of fatty acids.

In this paper, we submit evidence that another locus is indeed required for the utilization of long-chain fatty acids in *E. coli*. Mutants carrying a defect in this locus, *fadL*, cannot grow on fatty acids of any chain length. However, *fadR fadL* mutants are capable of growth on medium-chain fatty acids but not on long-chain fatty acids. We believe that the locus codes for a product that is required for the transport of long-chain fatty acids into the cell.

MATERIALS AND METHODS

Materials. [1-^{14}C]Oleic acid and [1-^{14}C]decanoic acid were purchased from New England Nuclear Corp., Boston, MA. Fatty acids, NAD, FAD, bovine serum albumin, and ATP were purchased from Sigma Chemical Co., St. Louis, MO. CoA was purchased from Sigma and from P-L Biochemicals, Milwaukee, WI.

Bacteria, Media, and Growth Conditions. All strains (Table 1) were derivatives of *E. coli* K-12 except WG1012, which is a hybrid of the B and K-12 strains (4). Strains WN48 and WN49 are *fadR* derivatives of *E. coli* K-12 and E-15, respectively. Strains WN48 and WN49 were isolated in this laboratory by selection on a medium containing 0.1% decanoate as the sole carbon source. Strain WN50 (*purF, dsdA*) is a *fadL* strain derived from strain AB2557 (*purF, aroC, dsdA*) by transduction to *aroC*$^+$ with phage grown on strain E-15 (*fadL*). A recombinant, called WN50, that inherited *fadL* was purified and used in our genetic studies. Strain K-1 (*fad*-5, previously *old*-5) has decreased levels of acyl-CoA dehydrogenase, crotonase, β-hydroxyacyl-CoA dehydrogenase, and thiolase (6). Strain K-19 has an apparent lesion in an electron transport flavoprotein for acyl-CoA dehydrogenase (2).

Bacteria were routinely incubated in a New Brunswick gyratory water bath-shaker at 37°. The bacteria were usually cultured in standard medium consisting of medium E (7) supplemented with Brij 58 (4 mg/ml). Carbon sources were sterilized separately and added to the culture medium prior to inoculation. Acetate and glucose were provided at 0.4% in the growth medium. Fatty acids were suspended in 10% Brij 58, neutralized with KOH, sterilized, and added at the following concentrations: butyrate, 14 mM; valerate, 11 mM; hexanoate, 9 mM; octanoate, 7 mM; decanoate, 6 mM; laurate, 5 mM; myristate, 4 mM; palmitate, 3.5 mM; and oleate, 3.0 mM. Supplements were added at the following final concentrations: amino acids, 0.01%; and adenine, 0.002%. D-Serine was added

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviation: fad, fatty acid degradation.

* A preliminary report of material presented in this paper was made at the Conference on Microbial Membranes sponsored by the American Society for Microbiology, Feb. 26–Mar. 1, 1978.

Table 1. Bacterial strains

Strain	Relevant characteristics*	Source or construction
AB2557	F⁻,*aroC,purF,dsdA,strA*8	G. Eggertsson strain
AT2535	F⁻,*purF,his*-1,*str*-9	A. Taylor strain
E-15	F⁺,*fadL*	A. Garen strain
K-1	F⁻,*fad*-5	P. Overath strain (1)
K-12	Hfr	J. Lederberg strain
K-19	F⁻,*fadE*62	P. Overath strain (1)
K-27	F⁻,*fadD*88	P. Overath strain (2)
old 3008	F⁻,*fadL*	F. Hill strain (3)
old 3012	F⁻,*fadD*	F. Hill strain (3)
WN48	Hfr,*fadR* derivative of K-12	See text
WN49	F⁺,*fadR* derivative of E-15	See text
WN50	F⁻,*purF,fadL,dsdA,strA*8	See text
WG1012	F⁻,*pdxB*5	W. Dempsey strain (4)
KL14, KL16, KL983, KL96, PK191	Hfr strains, see Low (5) for genotype; see Fig. 1 for origins of transfer	K. B. Low strains (5)

*The allele numbers are those of the *E. coli* Genetic Stock Center, Yale University, New Haven, CT.

at 0.05%. Cell growth was monitored at 540 nm in a Klett-Summerson colorimeter.

Assay of β-Oxidation. *In vivo* fatty acid oxidation was assayed by determining the amount of $^{14}CO_2$ formed from 1-^{14}C-labeled fatty acids as described (8, 9). *In vitro* fatty acid oxidation was assayed as described by Weeks *et al.* (9).

Acyl-CoA Synthetase Assay and Protein Determination. Cell extracts were prepared as described by Gelman and Cronan (10). Protein concentrations were determined by a microbiuret procedure (11). Acyl-CoA synthetase was assayed in the presence of hydroxylamine as described by Kornberg and Pricer (12) and modified by Overath *et al.* (1).

Genetics. The procedures for bacterial conjugation, phage P1 transduction, and mutant selection were as described by Miller (13) and Cronan and coworkers (14).

RESULTS

Growth Studies. The first indication that there might be a novel locus involved in the utilization of long-chain fatty acids originated from growth studies with *fadR* derivatives of *E. coli* K-12 and E-15. We observed that strain WN49, a *fadR* derivative of strain E-15, was capable of growth on medium-chain (decanoate and laurate) fatty acids but not on long-chain (myristate, palmitate, and oleate) fatty acids as sole carbon sources (Table 2). In contrast, strain WN48, a *fadR* derivative of *E. coli* K-12, could grow on both medium and long-chain fatty acids (Table 2). The behavior of strain WN48 is typical of previously reported *fadR* strains (1, 2). The parent of strain WN48, *E. coli* K-12, was able to grow on long-chain fatty acids whereas the parent of strain WN49, strain E-15, was not. Because strain E-15 was capable of growing on acetate, these results suggested that this strain must be defective in some step in long-chain fatty acid metabolism.

Table 2. Growth behavior on various carbon sources

Carbon source	Growth K-12	WN48	E-15	WN49
Acetate	+	+	+	+
Butyrate	—	—	—	—
Valerate	—	—	—	—
Hexanoate	—	—	—	—
Octanoate	—	—	—	—
Decanoate	—	+	—	+
Laurate	+	+	—	+
Myristate	+	+	—	—
Palmitate	+	+	—	—
Oleate	+	+	—	—

Table 3. Oxidation of fatty acids by *E. coli* grown on various carbon sources

Strain	Carbon source	Rate of $^{14}CO_2$ release nmol/min/mg protein [1-^{14}C]Oleate	[1-^{14}C]Decanoate
K-12	Acetate	5.1	3.4
	Acetate + oleate	14.2	9.2
WN48	Acetate	10.2	11.9
	Acetate + oleate	20.5	13.8
	Acetate + decanoate	22.2	8.8
E-15	Acetate	0.2	2.1
	Acetate + oleate	0.2	2.7
WN49	Acetate	0.1	18.3
	Acetate + oleate	0.2	15.4
	Acetate + decanoate	0.2	14.1

***In Vivo* β-Oxidation Studies.** In order to confirm that strains E-15 and WN49 were defective in some step in the utilization of long-chain fatty acids, the ability of these strains to oxidize [1-^{14}]oleate and [1-^{14}C]decanoate was tested. The results in Table 3 show that, unlike strains K-12 and WN48, neither E-15 nor WN49 could oxidize [1-^{14}C]oleate. However, strain WN49 was able to oxidize [1-^{14}C]decanoate. When [1-^{14}]oleate was present in the growth medium as a suspension in 1.5% bovine serum albumin rather than in 1% Brij 58, no differences in the rates of release of $^{14}CO_2$ were seen.

Levels of Acyl-CoA Synthetase Activity. In addition to being incapable of oxidizing [1-^{14}C]oleate, strains E-15 and WN49 could not incorporate oleate into their phospholipids (Table 4). These results led us to suspect that the first enzyme in fatty acid degradation, the acyl-CoA synthetase, might be defective in

Table 4. Lipid synthesis and acyl-CoA synthetase activity

Strains	Carbon source	Incorporation,* nmol/min/mg protein	Acyl-CoA synthetase activity,† nmol/min/mg protein
K-12	Acetate	—	0.52
	Acetate + oleate	1.21	2.22
WN48	Acetate	—	1.92
	Acetate + oleate	1.49	1.87
E-15	Acetate	—	0.36
	Acetate + oleate	<0.01	0.45
WN49	Acetate	—	1.98
	Acetate + oleate	<0.01	1.49

*Incorporation of [1-^{14}C]oleate in the lipids of intact cells. The radioactivity in phospholipids was quantitated as described by Nunn *et al.* (8).

†Oleoyl CoA synthetase activity in extracts was assayed as described by Overath *et al.* (1)

Table 5. Oxidation of oleic acid by *E. coli* extracts*

Strains	Carbon source	Rate of $^{14}CO_2$ release, pmol/min/mg protein
K-12	Acetate	1.58
	Acetate + oleate	4.69
WN48	Acetate	4.16
	Acetate + oleate	4.68
E-15	Acetate	1.26
	Acetate + oleate	0.98
WN49	Acetate	5.26
	Acetate + oleate	3.95

*Assay conditions as described by Weeks *et al.* (9). Substrate was [1-^{14}C]oleate.

activating long-chain fatty acids. However, the results in Table 4 clearly show that strain WN49 has levels of oleoyl-CoA synthetase activity that are comparable to those of strain WN48.

***In Vitro* β-Oxidation Studies.** Because it was conceivable that strains E-15 and WN49 were incapable of oxidizing long-chain fatty acids due to a defect in the specifity of one of the other fad enzymes (1, 2), we decided to determine if cell-free extracts of these strains could oxidize [1-^{14}C]oleate. The results in Table 5 show that extracts from strain WN49 oxidized [1-^{14}C]oleate at rates comparable to those of strain WN48. These data suggest that the inability of strains E-15 and WN49 to degrade long-chain fatty acids is not a consequence of a defect in one of their fad enzymes.

These studies suggest that another locus is required for the utilization of fatty acids. We will hereafter refer to such a locus as *fadL*. Strains having the normal ability to utilize long-chain fatty acids will be referred to as *fadL*$^+$.

Genetic Mapping of the *fadL* Locus by Hfr Crosses. The new genetic locus, *fadL*, has now been mapped in strain E-15. Fig. 1 shows the origins and directions of chromosome transfer of five Hfr strains (KL14, KL16, KL983, KL96, and PK191) used to determine the approximate location of the *fadL* locus by the method of Low (5). Phenocopy cultures of E15 *str*^{R} were obtained by starvation of logarithmic-phase cells in medium E for 5 hr at 37° (13). The *fadL*$^+$ *str*^{R} recombinant colonies were selected on minimal agar. These recombinants were formed only in matings with Hfr strains KL16, KL983, and PK191, implying that the *fadL* gene was located between min 47 and 52 on the genetic map (5, 15).

A cross between strains E-15 (Hfr, *purF*$^+$, *fadL*, *his*$^+$, *str*^{s}) and AT2535 (F$^-$, *purF*, *fadL*$^+$, *his*$^-$, *str*^{R}) revealed that 85% of the *purF*$^+$ *str*^{R} recombinants were *fadL* and 27% of the *his*$^+$ *str*^{R} recombinants were *fadL*. These results suggested that the *fadL* locus is close to the *purF* locus at min 50 on the genetic map (15).

Transductional Mapping of the *fadL* Locus. The results from the conjugation experiments led us to test for cotransduction of *fadL* with various loci in the region of 50 min. Phage P1 stocks grown on strain E-15 were used to transduce to prototrophy strains carrying mutations in the *purF*, *aroC*, and *dsdA* loci. Table 6 shows that 45% of the *aroC*$^+$ recombinants were also *fadL* (cross no. 3) and 31% of the *dsdA*$^+$ recombinants were *fadL* (cross no. 4). The *purF* locus was about 23% cotransducible with the *fadL* locus (crosses no. 1 and 2). When a P1 stock from a *purF*$^+$ *fadL*$^+$ strain was used to transduce strain WN50 (*purF fadL*) to either *purF*$^+$ or *fadL*$^+$, about

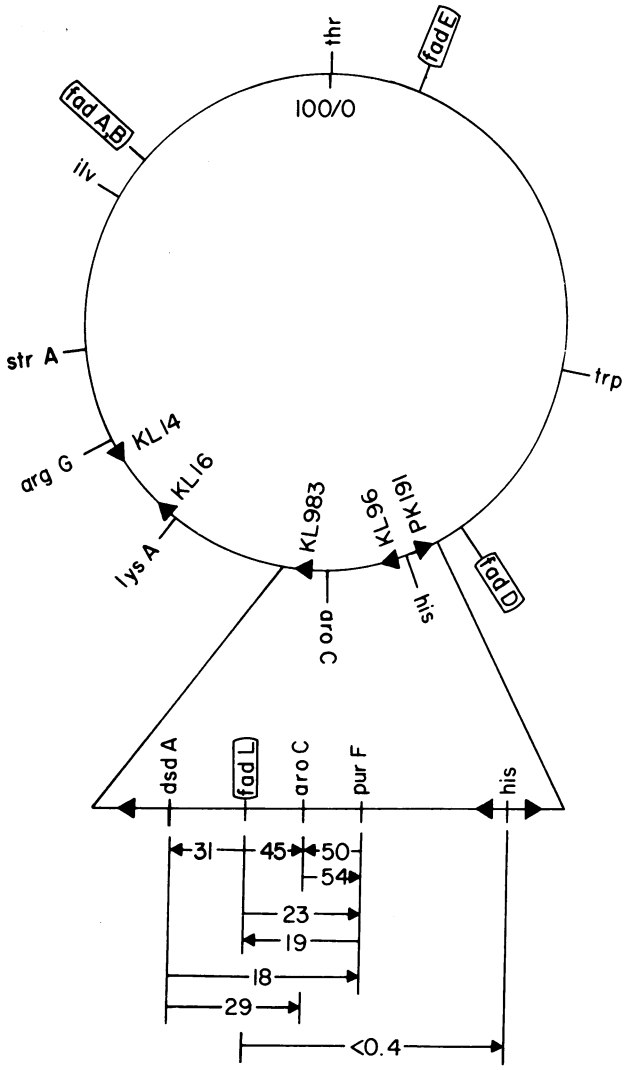

FIG. 1. Location of *fadL* on genetic map of *E. coli*. Circular map is adapted from Bachmann *et al.* (15). The origins and direction of transfer of Hfr strains are taken from Low (5). The values at the bottom are phage P1 cotransduction frequencies from Table 7, with arrowheads indicating selected markers. This enlarged portion of the map is not drawn to scale.

24% of the *purF*$^+$ recombinants were also *fadL*$^+$ and approximately 19% of the *fadL*$^+$ recombinants were *purF*$^+$ (crosses no. 6 and 7). Table 6 also shows that the *his* gene does not cotransduce with the *fadL* locus (cross no. 5). The latter finding was expected because the *his* gene maps at min 44 on the linkage map (15).

Orientation of the *fadL* Gene with Respect to the *purF*, *aroC*, and *dsdA* Loci. The known order for the above three loci is *purF aroC dsdA* (16). To order the *fadL* locus with respect to these loci, we used phage P1 stocks grown on the *fadL* strain E-15 to transduce the *purF*, *aroC*, *dsdA* strain AB2557 to either *purF*$^+$ or *aroC*$^+$ and scored the unselected markers (Table 7). The data in Table 7 suggest that the *fadL* locus lies between *aroC* and *dsdA*.

The latter conclusion was drawn from an analysis of the data from three factor crosses which yielded information on the orientation of: (*i*) *purF* with respect to *aroC* and *fadL*; (*ii*) *aroC* with respect to *purF* and *fadL*; and (*iii*) *aroC* with respect to *dsdA* and *fadL*. Among the *purF*$^+$ recombinants, the rarest class was *aroC fadL* (Table 7), which would arise by a quadruple crossover. This finding is consistent with the clockwise

Table 6. Cotransduction of markers in 50-min region

Cross	Bacterial strains and relevant markers: Donor	Recipient	Marker selected	Colonies with donor marker/ total colonies selected	Cotransduction frequency, %
1	E-15 (*fadL*)	AB2557 (*purF*)	*purF*+	183/758	24.1
2	E-15 (*fadL*)	AT2535 (*purF*)	*purF*+	37/177	20.9
3	E-15 (*fadL*)	AB2557 (*aroC*)	*aroC*+	557/1234	45.1
4	E-15 (*fadL*)	AB2557 (*dsdA*)	*dsdA*+	27/86	31.3
5	E-15 (*fadL*)	AT2535 (*his*)	*his*+	0/277	<0.4
6	WG1012 (*fadL*+)	WN50 (*purF, fadL*)	*purF*+	22/90	24.4
7	WG1012 (*purF*+)	WN50 (*purF, fadL*)	*fadL*+	83/442	18.8
8	E-15 (*purF*+)	AB2557 (*purF, aroC*)	*aroC*+	552/1047	52.7
9	E-15 (*aroC*+)	AB2557 (*purF, aroC*)	*purF*+	264/491	53.8
10	E-15 (*dsdA*+)	AB2557 (*purF, dsdA*)	*purF*+	16/86	18.6
11	E-15 (*dsdA*+)	AB2557 (*aroC, dsdA*)	*aroC*+	26/86	30.2

order of *purF aroC fadL*. When *aroC*+ recombinants were examined, we found that there was no rare class apparent. These results suggested that the *aroC* locus was between *purF* and *fadL*. The position of *fadL* with respect to *aroC* and *dsdA* was determined by scoring the unselected markers among the *aroC*+ transductants. The four-crossover class in the *aroC*+ recombinants was *dsdA*+ *fadL*+. The latter finding suggested that the *aroC* and *dsdA* loci flanked the *fadL* locus. Therefore, the overall clockwise map order of *purF aroC fadL dsdA* is indicated for this region of the linkage map. The final results of the mapping experiments are shown in Fig. 1. It is of interest to note that the genes involved in fatty acid degradation that have been identified to date are widely distributed around the *E. coli* chromosome.

Recombination between *fadL* Strains. Among the known *fad* mutants (K-1, K-19, K-27, *old* 3012, E-15), only strain E-15 (*fadL*) is capable of spontaneously forming *fadR* progeny on medium containing decanoate as a sole carbon source (data not shown). Hill and Angelmaier (3) isolated a mutant that has a phenotype similar to that of strain E-15. We have found that *fadR* derivatives of their mutant, *old* 3008, can be isolated on decanoate. Crosses between strain WN40 (*fadL purF*) and *old* 3008 yielded no *fadL*+ recombinants (Table 8) whereas crosses between the other *fad* mutants and strain WN40 yield *fadL*+ recombinants. In addition, crosses between strain E-15 and *old* 3008 yielded no *fadL*+ recombinants. The *fad* lesion in *old* 3008 cotransduced with *purF* at 28% and *aroC* at 38.2%. These results suggest that *old* 3008 harbors the *fadL* defect.

Table 7. Map location of the *fadL* marker with respect to *purF*, *aroC*, and *dsdA**

Selected marker	Unselected markers	Cotransductants,† no.	Cotransduction frequency, %
purF+	*aroC*+*fadL*+	183/570	32.1
	aroC+*fadl*−	129/570	22.6
	aroC−*fadL*+	238/570	41.7
	aroC−*fadL*−	20/570	3.5
aroC+	*purF*+*fadL*+	305/1145	26.6
	purF+*fadL*−	246/1145	21.5
	purF−*fadL*+	328/1145	28.6
	purF−*fadL*−	266/1145	23.2
aroC+	*dsdA*+*fadL*+	5/89	5.6
	dsdA+*fadL*−	21/89	23.6
	dsdA−*fadL*+	39/89	43.8
	dsdA−*fadL*−	24/89	27.0

*A P1*vir* lysate of E-15 (*purF*+ *fadL, aroC*+, *dsdA*+) was used to transduce AB2557 (*purF, fadL*+, *aroC, dsdA*) to *purF*+ or *aroC*+. The *purF*+ transductants were then scored for the above unselected markers.

†Values represent the number of cotransductants/total number of selected transductants tested.

DISCUSSION

The studies presented in this paper indicate that a new locus (*fadL*) is required for the utilization of long-chain fatty acids in *E. coli*. The *fadL* mutation maps at a location separate from other known *fad* genes (15). Although the exact nature of the *fadL* mutation is unknown, the observations described here suggest that the *fadL* mutant is defective in its ability to transport long-chain fatty acids into the cell. In support of the latter conclusion, we have recently found that: (*i*) *fadR fadL* strains can transport medium-chain but not long-chain fatty acids; and (*ii*) the activities of the five key fad enzymes [acyl-CoA synthetase (Table 4), acyl-CoA dehydrogenase, enoyl-CoA hydratase, β-hydroxyacyl-CoA dehydrogenase, and thiolase] in *fadR fadL* strains are comparable to those in *fadR fadL*+ strains. These results (unpublished data) also suggest that the activity of the *fadL* gene product is the rate-limiting step during growth of *E. coli* on long-chain fatty acids.

Our studies with a mutant isolated by Hill and Angelmaier (3) confirm and extend their belief that another genetic locus, besides the *fadD* locus, is required for the transport of oleic acids in *E. coli*. We have found that their mutant, *old* 3008, has a defect in the *fadL* locus.

The *fadL* strains are novel because they are the only known *fad* mutants from which spontaneous constitutive (*fadR*) progeny can be derived by plating on medium containing de-

Table 8. Recombination between *fad* mutants*

Strain	Defect	Transductants of WN50† *fad*+	Transductants of WN50† *purF*+	Ratio of frequencies, *fad*+/*purF*+
E-15	*fadL*	<0.01	1.43	<0.007
old 3008	*fadL*	<0.01	1.05	<0.009
K-27	*fadD*	0.66	1.30	0.51
old 3012	*fadD*	0.83	2.34	0.35
K-1	*fad*-5	0.61	1.21	0.50
K-19	*fadE*62	0.37	0.98	0.38

*Strain WN50 was transduced by phage grown on respective strains. Transductants were selected for either ability to utilize oleate as sole carbon source or ability to grow in the absence of supplemental adenine.

†Values represent frequencies of transductants per 10^6 cells plated.

canoate as the sole carbon source. This unique characteristic has enabled us to screen a large number of *fad* mutants for the specific isolation of those carrying the *fadL* lesion. Several of these *fadL* mutants can grow on long-chain fatty acids at 30° but not as 42° (unpublished data). These preliminary findings suggest that the *fadL* gene may be the structural gene for a protein that facilitates the entry of long-chain fatty acids through the cytoplasmic membrane.

We thank Rowland Davis, Wendell M. Stanley, Jr., Calvin S. McLaughlin, and Daniel L. Wulff for helpful discussions and comments on the manuscript. We also thank Paula Hennen and Stanley Maloy for technical assistance and Joyce Long for excellent secretarial service. This investigation was supported by U.S. Public Health Service Grant GM 22466-1A from the National Institute of General Medical Sciences and a grant from the American Heart Association, Orange County Chapter.

1. Overath, P., Pauli, G. & Schairer, H. U. (1969) *Eur. J. Biochem.* **7,** 559–574.
2. Klein, K., Steinberg, R., Fiethen, B. & Overath, P. (1971) *Eur. J. Biochem.* **19,** 442–450.
3. Hill, F. F. & Angelmaier, D. (1972) *Mol. Gen. Genet.* **117,** 143–152.
4. Dempsey, W. B. (1969) *J. Bacteriol.* **100,** 295–300.
5. Low, K. B. (1973) *J. Bacteriol.* **113,** 798–812.
6. O'Brien, W. J. & Frerman, F. E. (1977) *J. Bacteriol.* **132,** 532–540.
7. Vogel, H. J. & Bonner, D. M. (1956) *J. Biol. Chem.* **218,** 97–106.
8. Nunn, W. D., Cheng, P., Deutsch, R., Tang, C. T. & Tropp, B. E. (1977) *J. Bacteriol.* **130,** 620–628.
9. Weeks, G., Shapiro, M., Burns, R. O. & Wakil, S. J. (1969) *J. Bacteriol.* **97,** 827–836.
10. Gelmann, E. P. & Cronan, J. E., Jr. (1972). *J. Bacteriol.* **112,** 381–387.
11. Munkres, K. D. & Richards, F. M. (1965) *Arch. Biochem. Biophys.* **109,** 466–479.
12. Kornberg, A. & Pricer, W. E., Jr. (1953) *J. Biol. Chem.* **204,** 329–343.
13. Miller, J. (1972), *Experiments in Molecular Genetics.* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
14. Cronan, J. E., Jr., Birge, C. H. and Vagelos, P. R. (1969). *J. Bacteriol.* **100,** 601–604.
15. Bachmann, B. J., Low, K. B. & Taylor, A. L. (1976) *Bacteriol. Rev.* **40,** 116–167.
16. McFall, E. (1967) *Genetics* **55,** 91–99.