L-ARABINOSE NEGATIVE MUTANTS OF THE L-RIBULOKINASE STRUCTURAL GENE AFFECTING THE LEVELS OF L-ARABINOSE ISOMERASE IN ESCHERICHIA COLI[1]

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TWENTY-two L-arabinose nonutilizing mutants of *Escherichia coli* B/r have been ordered with regard to each other and a closely linked leucine marker by three-point tests using transduction of *E. coli* by phage P1bt (GROSS and ENGLESBERG 1959; ENGLESBERG, ANDERSON, WEINBERG, LEE, HOFFEE, HUTTENHAUER and BOYER 1962; BOYER, ENGLESBERG and WEINBERG 1962). These mutants have been characterized enzymatically, into four functional groups, or genes *A*, *B*, *C*, and *D*, responsible for the synthesis of enzymes in the pathway of L-arabinose utilization (Figure 1) (ENGLESBERG 1961; ENGLESBERG *et al.* 1962). Gene *E* which controls the L-arabinose permease is unlinked to this region, as shown by transduction analysis (ISAACSON and ENGLESBERG, unpublished data).

ENGLESBERG (1961) and LEE and ENGLESBERG (1962) have shown that genes *A* and *B* are the structural genes for the enzymes L-arabinose isomerase and L-ribulokinase respectively. Gene *D* mutants are deficient in the enzyme L-ribulose 5-phosphate 4-epimerase and probably represent the structural gene for this enzyme (ENGLESBERG *et al.* 1962). Gene *C* mutants are deficient in all three en-

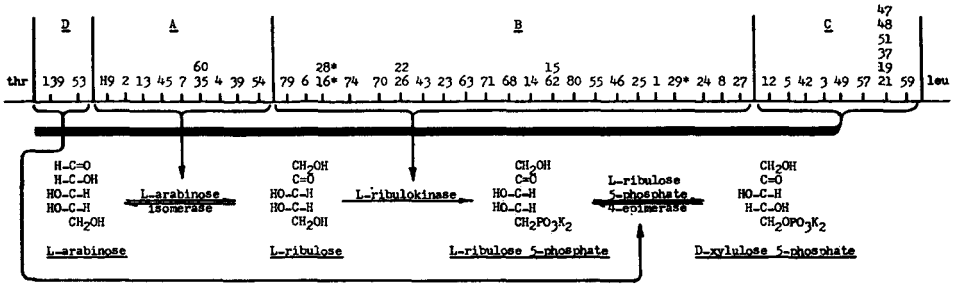

FIGURE 1.—The L-arabinose gene-enzyme complex. See text and references listed for evidence substantiating the order and functional grouping of most of the mutant sites. The order and enzymatic characterization of *ara*-35, 37, 39, 42, 45, 47, 48, 49, 51, 54, 57, 59, and 60 were established by the first author (unpublished data).

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zymes mentioned above and in the L-arabinose permease (ENGLESBERG 1961; ENGLESBERG *et al.* 1962; NOVOTNY and ENGLESBERG, unpublished data) and probably represent a new type of regulatory gene whose product is required for the induction of the L-arabinose pathway (HELLING and WEINBERG 1963).

Eight mutants in the *B* gene that were tested, besides being deficient in L-ribulokinase, produce either increased or decreased inducible levels of L-arabinose isomerase. A further analysis of three of these mutants showed that the epimerase and isomerase levels of each mutant vary in the same direction (ENGLESBERG *et al.* 1962) and the differences in isomerase levels are due to differences in rate of synthesis of an enzyme which is undistinguishable from the one produced by the wild type (LEE and ENGLESBERG 1962).

The purpose of this study was to determine if there is a relationship between the location of mutant sites in the *B* gene and their effect on the level of L-arabinose isomerase activity. This question, of physiological "subhomogeneity" of the *B* gene, was investigated by ordering additional L-arabinose negative mutants, establishing the relative distance between the mutant sites and comparing the location and the level of isomerase produced by each mutant. A brief summary of this work has been published (CRIBBS and ENGLESBERG 1963).

MATERIAL AND METHODS

The media, phage P1bt, bacterial strains, transduction and selection procedures, test for lysogeny, growth experiments and keto-sugar analysis are described by GROSS and ENGLESBERG 1959. Phage lysates were prepared using the modifications described by BOYER *et al.* 1962.

Seventeen additional L-arabinose negative ribulokinaseless mutants (*B* gene mutants) were independently isolated from the *thr leu ara*+ strain of *Escherichia coli* (*ara*+ = ability to utilize L-arabinose; *thr*, *leu* = threonine and leucine dependence, respectively). Two of these mutants, *ara*-70 and *ara*-80, were induced by X rays while the remainder (*ara*-22, 25, 26, 27, 28, 29, 43, 46, 55, 62, 68, 70, 71 and 74) were UV induced. The *thr leu* markers of each *ara* mutant strain were transduced to *thr*+ *leu*+. These *thr*+ *leu*+ *ara* strains served as donors in the transduction experiments (GROSS and ENGLESBERG 1959).

Two methods each employing transduction, were used to construct a linkage map of the *B* gene. The relative order of the mutant sites was determined by analysis of reciprocal three-factor crosses between the different *ara* sites using leucine as the nonselected marker (GROSS and ENGLESBERG 1959). The relative distances between *ara* sites were determined by the ratio of *ara*+ to *leu*+ recombinants per unit volume of the transduction mixture plated on mineral arabinose, threonine, leucine plates (selection for *ara*+) and on mineral glucose, threonine plates (selection for *leu*+).

All arabinose negative mutants used in this study revert spontaneously to utilize arabinose.

The previous method used for the assay of L-arabinose isomerase activity (ENGLESBERG 1961) was modified. The reaction mixture consists of glycylglycine buffer, (pH 7.6) 75 μmoles; $MnCl_2$, 2.5 μmoles; L-arabinose, 150 μmoles; and cell extract in a final volume of 1.0 ml. The changes in the reaction mixture effected a several fold increase in the activities previously reported without affecting the relative position of the mutants with regard to levels of isomerase production. This increased activity is mainly due to substitution of Mn^{++} for Mg^{++} (the former is a more effective activator of this enzyme), change from a tris(hydroxymethyl)aminomethane buffer (Tris) to a glycylglycine buffer (Tris is slightly inhibitory), and an increase in concentration of L-arabinose (to fully saturate the system). Cysteine previously employed was found unnecessary.

Reaction mixture, without extract, in small test tubes was placed in an ice bath, enzyme extract was added, and a 0.1 ml sample removed and pipetted into 0.9 ml of 0.1 N HCl. The tubes

containing the reaction mixture were then placed in a 37°C water bath and an additional 0.1 ml sample was removed in exactly 3 minutes. Assays for ribulose were conducted on these samples directly employing the cysteine-carbazole test (DISCHE and BORENFREUND 1951). The color produced was determined exactly 20 min after the addition of reagents, using a Klett Summerson Colorimeter with a No. 54 filter. L-ribulose-o-nitrophenylhydrazone was employed as a standard and under the conditions of our assay 22.5 Klett units is equivalent to 0.01 μmole L-ribulose. One unit of specific activity is defined as the number of micromoles of ribulose produced per hr per mg of protein. Protein was determined by the method of LOWRY, ROSEBROUGH, FARR and RANDALL (1951) using crystalline bovine serum albumin (California Corporation for Biochemical Research) as a standard. Activity measured under these conditions is directly proportional to the amount of extract added up to 10 μmoles of ribulose per hr per ml of reaction mixture. Extracts were diluted in 10^{-3}M ethylenediamine tetraacetic acid (EDTA), 10^{-3} M glutathione, 0.1 percent bovine serum albumin, at pH 7.4, so that activity would be within this range.

Since it was crucial in this study to have an accurate comparison of the isomerase activity of the 17 new *ara* mutants as well as the eight previously studied mutants, extracts of all 25 mutants were prepared under identical conditions in duplicate and each extract was assayed for isomerase activity as described above. Cell free extracts were prepared as follows: An overnight 5 ml mineral casein hydrolysate (1.0 percent) mineral culture (GROSS and ENGLESBERG 1959) was added as an inoculum to 50 ml of mineral 1 percent casein hydrolysate, 0.4 percent L-arabinose medium contained in 500 ml Erlenmeyers. The flasks were placed on a shaker at 37°C and incubation was continued for 3 hours. The cultures were then spun down in the cold, washed in 25 ml of 10^{-3}M EDTA, at pH 7.4, and finally resuspended in 1.0 ml of 10^{-3}M EDTA, 10^{-3}M glutathione, at pH 7.4. The suspensions were distributed into two cellulose nitrate centrifuge tubes (10 × 80 mm), stoppered, and 12 such tubes were placed in the treatment cell of a 10 kc Raytheon sonic oscillator kept cold by the rapid passage of water maintained at 3°C. The sonic treatment was continued for 5 min, the tubes were then transferred to a high speed head of a Servall centrifuge and spun 30 min in the cold at 10,000 rpm. The supernatant was poured off and stored frozen. With the facilities we had available, 16 arabinose negative mutants were grown simultaneously, sonicated, and assayed in duplicate.

As a control each extract was assayed for isocitric dehydrogenase activity by measuring nicotinamide adenine dinucleotide phosphate (NADP) reduction spectrophotometrically at 340mμ at 30°C using a Beckman DU spectrophotometer with an automatic absorbancy recording device. The reaction mixture contained Tris buffer, pH 7.4, 300 μmoles; $MnCl_2$, 10 μmoles; NADP, 1 μmole; DL and allo sodium isocitrate, 12 μmoles, and cell extract (10 lambda–approximately 140 μg of protein) in a total volume of 3.0 ml. A blank contained all the above except substrate. The rate of NADP reduction was linear within the range used and was directly proportional to the amount of extract added. Specific activity is equal to the number of micromoles of NADP reduced per hr per mg of protein.

RESULTS

Ordering of L-arabinose negative mutant sites by three-factor crosses: Three-factor reciprocal crosses between pairs of independently isolated *ara* mutants, using leucine as a nonselected marker, were employed to order the new arabinose mutants, with regard to each other, and the original eight ordered mutants in the *B* gene (GROSS and ENGLESBERG 1959). Typically, the *leu*$^+$ marker was incorporated into 40 to 50 percent of the *ara*$^+$ recombinants in one member of each pair of crosses and into 20 to 30 percent in the other member, yielding a difference of 10 to 30 percent between members of a pair. This difference between the frequency of "double" and "quadruple" crossing over, enables one to place most of the arabinose negative mutants in a linear order (Table 1). However, not all

TABLE 1

Three-factor crosses and recombination distances between ara *sites**

Cross	R L-arabinose negative	D mutants		Total $\frac{ara^+\ leu^+}{ara^+}$		Total $\frac{ara^+}{leu^+}$		Average distance
1	79	6	(1)	$\frac{38}{110}$	34.5%	$\frac{107}{7020}$	1.52%	1.67
	6	79	(1)	$\frac{54}{104}$	51.9%	$\frac{62}{3130}$	1.98%	
2	6	74	(4)	$\frac{30}{106}$	28.3%	$\frac{34}{12040}$	0.28%	0.32
	74	6	(4)	$\frac{142}{248}$	57.3%	$\frac{96}{28700}$	0.33%	
3	74	70	(3)	$\frac{22}{81}$	27.2%	$\frac{19}{14280}$	0.13%	0.31‡
	70	74	(2)	$\frac{126}{331}$	38.1%	$\frac{253}{74080}$	0.34%	
4	70	22	(3)	$\frac{46}{192}$	24.0%	$\frac{78}{38540}$	0.20%	0.19
	22	70	(4)	$\frac{29}{64}$	45.3%	$\frac{16}{10000}$	0.16%	
5	22	26	(3)	0	. .	$\frac{1}{36740}$	0.003%	0.004
	26	22	(3)	0	. .	$\frac{2}{35669}$	0.005%	
6	22	23	(8)	$\frac{21}{113}$	18.6%	$\frac{23}{30150}$	0.076%	0.072
	23	22	(8)	$\frac{33}{60}$	55.0%	$\frac{13}{19890}$	0.065%	
7	23	63	(7)	$\frac{21}{95}$	22.1%	$\frac{26}{8240}$	0.32%	0.33
	63	23	(7)	$\frac{97}{252}$	38.5%	$\frac{90}{26990}$	0.33%	
8	70	43	(2)	$\frac{23}{76}$	30.3%	$\frac{76}{32240}$	0.24%	0.22
	43	70	(3)	$\frac{28}{58}$	48.3%	$\frac{44}{21600}$	0.20%	
9	22	43	(5)	$\frac{7}{31}$	22.6%	$\frac{19}{62740}$	0.031%	0.031
	43	22	(5)	$\frac{0}{6}$	. .	$\frac{10}{31598}$	0.032%	
10	26	43	(2)	0	. .	$\frac{2}{21700}$	0.009%	0.02‡
	43	26	(2)	$\frac{2}{10}$	20.0%	$\frac{6}{13747}$	0.04%	

TABLE 1—Continued

Cross	R L-arabinose negative	D mutants		Total $\frac{ara^+ leu^+}{ara^+}$		Total $\frac{ara^+}{leu^+}$		Average distance
11	43	23	(3)	$\frac{0}{7}$	. .	$\frac{7}{24833}$	0.028%	0.022
	23	43	(4)	$\frac{0}{8}$	. .	$\frac{3}{21557}$	0.014%	
12	43	63	(1)	$\frac{21}{73}$	28.8%	$\frac{73}{14274}$	0.51%	0.60
	63	43	(2)	$\frac{129}{196}$	65.8%	$\frac{196}{30800}$	0.65%	
13	63	71	(5)	$\frac{19}{66}$	28.8%	$\frac{25}{26870}$	0.093%	0.091
	71	63	(3)	$\frac{31}{71}$	43.7%	$\frac{9}{10540}$	0.09%	
14	71	14	(1)	$\frac{21}{88}$	23.9%	$\frac{29}{4490}$	0.65%	0.67
	14	71	(1)	$\frac{46}{104}$	44.2%	$\frac{15}{2074}$	0.72%	
15	14	15	(1)	$\frac{29}{106}$	27.4%	$\frac{13}{4800}$	0.27%	0.23
	15	14	(1)	$\frac{40}{92}$	43.5%	$\frac{37}{16920}$	0.22%	
16	15	80	(3)	$\frac{16}{51}$	31.4%	$\frac{29}{7760}$	0.37%	0.46
	80	15	(3)	$\frac{34}{61}$	55.7%	$\frac{32}{5390}$	0.59%	
17	80	55	(1)	$\frac{15}{59}$	25.4%	$\frac{5}{3570}$	0.14%	0.13
	55	80	(1)	$\frac{23}{51}$	45.1%	$\frac{5}{3939}$	0.13%	
18	55	46	(3)	$\frac{0}{5}$	. .	$\frac{3}{32260}$	0.009%	0.015
	46	55	(4)	$\frac{7}{18}$	38.9%	$\frac{20}{120060}$	0.016%	
19	55	1	(3)	$\frac{106}{323}$	32.8%	$\frac{148}{52440}$	0.28%	0.34‡
	1	55	(3)	$\frac{109}{231}$	47.2%	$\frac{175}{41600}$	0.42%	
20	80	46	(1)	$\frac{14}{54}$	25.9%	$\frac{7}{3960}$	0.18%	0.19
	46	80	(1)	$\frac{35}{79}$	44.3%	$\frac{16}{8360}$	0.19%	

TABLE 1—Continued

Cross	R L-arabinose negative	D mutants		Total $\frac{ara^+ leu^+}{ara^+}$		Total $\frac{ara^+}{leu^+}$		Average distance
21	46	1	(4)	$\frac{88}{285}$	30.9%	$\frac{124}{44360}$	0.28%	0.29
	1	46	(2)	$\frac{141}{275}$	51.3%	$\frac{101}{34220}$	0.29%†	
22	1	24	(2)	$\frac{35}{125}$	28.0%	$\frac{25}{4160}$	0.60%	0.50
	24	1	(2)	$\frac{80}{144}$	55.6%	$\frac{37}{8270}$	0.45%	
23	1	29	(3)	$\frac{335}{1041}$	32.2%	$\frac{929}{25698}$	3.61%	1.89‡
	29	1	(3)	$\frac{391}{944}$	41.4%	$\frac{575}{53880}$	1.07%	
24	29	24	(1)	$\frac{76}{281}$	27.0%	$\frac{308}{12822}$	2.40%	2.96‡
	24	29	(1)	$\frac{80}{175}$	45.7%	$\frac{840}{25902}$	3.24%	
25	24	8	(1)	$\frac{19}{53}$	35.8%	$\frac{29}{3880}$	0.75%	0.54‡
	8	24	(2)	$\frac{77}{129}$	59.7%	$\frac{24}{5920}$	0.41%	
26	8	27	(3)	$\frac{30}{156}$	19.2%	$\frac{67}{9460}$	0.71%	0.59‡
	27	8	(3)	$\frac{60}{125}$	48.0%	$\frac{79}{15440}$	0.51%	

* In each cross the recipient bacteria (R) are *thr leu;* the donor phage (D), *thr*$^+$ *leu*$^+$. The numbers in parentheses indicate the number of separate experiments performed to obtain the total *ara*$^+$ *leu*$^+$/*ara*$^+$ and *ara*$^+$/*leu*$^+$ ratios. The recombination distance (*ara*$^+$/*leu*$^+$ × 100) is obtained for each mutant pair by assaying 0.4 ml of the transduction mixture for the number of *ara*$^+$ recombinants and, on separate plates, 0.4 ml of a 1:20 dilution for the number of *leu*$^+$ recombinants. The average distances represent pooled *ara*$^+$/*leu*$^+$ ratios (×100) of reciprocal crosses.

† Repetitive crosses of the same mutant pair not homogeneous.

‡ A significant difference between the *ara*$^+$/*leu*$^+$ ratios of a reciprocal cross.

mutant pairs, when reciprocally crossed with one another gave as large a difference as this.

Differences of less than 10 percent between reciprocal *ara*$^+$ *leu*$^+$/*ara*$^+$ frequencies were tested to determine if the reciprocal ratios differed enough to establish the relative order of the mutants. This was done by arranging the pooled ratios in a 2 × 2 table and computing chi-square with one degree of freedom.

Six pairs of mutants: *ara*-28 and *ara*-74; *ara*-16 and *ara*-74; *ara*-16 and *ara*-6; *ara*-62 and *ara*-14; *ara*-68 and *ara*-71; *ara*-25 and *ara*-1 cannot be ordered by three-factor crosses (Table 2). In each instance, the double and quadruple crossover frequencies approach equality, that is, they exhibit a high degree of "negative interference" (GROSS and ENGLESBERG 1959; CRIBBS and ENGLESBERG 1961). The tendency toward negative interference decreased when these mutants were

TABLE 2

Three-factor crosses and recombination distances of ara *mutants showing negative interference**

Cross	R L-arabinose negative	D mutants		Total $\frac{ara^+\ leu^+}{ara^+}$			Total $\frac{ara^+}{leu^+}$		Average distance
1	28	79	(1)	$\frac{58}{144}$	40.3%	+19.4	$\frac{123}{9920}$	1.24%	1.00§
	79	28	(2)	$\frac{14}{67}$	20.9%		$\frac{10}{3400}$	0.29%	
2	28	6	(4)	$\frac{298}{603}$	49.4%	+ 9.5	$\frac{285}{26560}$	1.07%	1.02
	6	28	(4)	$\frac{71}{178}$	39.9%		$\frac{82}{9580}$	0.86%	
3	28	16	(6)	0	..	..	$\frac{4}{55320}$	0.007%	0.01
	16	28	(4)	0	..		$\frac{3}{13250}$	0.02%	
4	28	74	(2)	$\frac{76}{173}$	43.9%	+ 4.4†	$\frac{22}{4400}$	0.50%	0.44
	74	28	(2)	$\frac{70}{177}$	39.5%		$\frac{77}{18110}$	0.43%	
5	28	70	(2)	$\frac{33}{98}$	33.7%	−12.4	$\frac{46}{6080}$	0.76%	1.05§
	70	28	(2)	$\frac{111}{241}$	46.1%		$\frac{246}{21700}$	1.13%	
6	16	79	(2)	$\frac{47}{85}$	55.3%	+28.7	$\frac{17}{3160}$	0.54%	0.40
	79	16	(2)	$\frac{21}{79}$	26.6%		$\frac{10}{3560}$	0.28%	
7	16	6	(4)	$\frac{129}{220}$	58.6%	+ 9.5†	$\frac{110}{16680}$	0.66%	0.58§
	6	16	(4)	$\frac{55}{112}$	49.1%		$\frac{61}{12760}$	0.48%	
8	16	74	(2)	$\frac{42}{131}$	32.1%	− 4.8†	$\frac{13}{7070}$	0.18%	0.20
	74	16	(2)	$\frac{83}{225}$	36.9%		$\frac{20}{9340}$	0.21%	
9	16	70	(1)	$\frac{22}{84}$	26.2%	−12.4	$\frac{51}{4170}$	1.22%	1.07
	70	16	(1)	$\frac{34}{88}$	38.6%		$\frac{42}{4520}$	0.93%	
10	68	63	(1)	$\frac{44}{86}$	51.2%	+17.0	$\frac{62}{12000}$	0.52%	0.60
	63	68	(2)	$\frac{27}{79}$	34.2%		$\frac{47}{6220}$	0.76%	

TABLE 2—Continued

Cross	R L-arabinose negative	D mutants		Total $\frac{ara^+ leu^+}{ara^+}$			Total $\frac{ara^+}{leu^+}$		Average distance
11	68	71	(5)	61/155	39.4%	+ 1.6†	46/36300	0.13%	0.08§
	71	68	(5)	31/82	37.8%		28/36000	0.078%	
12	68	14	(3)	186/552	33.7%	—13.4‡	152/44560	0.34%	0.32
	14	68	(2)	24/51	47.1%		37/13810	0.27%	
13	62	71	(1)	34/67	50.7%	+22.1	67/8020	0.84%	0.65§
	71	62	(1)	14/49	28.6%		24/5880	0.41%	
14	62	68	(2)	84/200	42.0%	+13.2	90/12360	0.73%	0.74
	68	62	(2)	53/184	28.8%		90/12100	0.74%	
15	62	14	(3)	59/142	41.5%	+ 2.6†	61/32220	0.19%	0.21
	14	62	(3)	42/108	38.9%		42/17000	0.25%	
16	62	15	(3)	0	. .	. .	1/31073	0.003%	0.005
	15	62	(2)	0	. .		2/19910	0.01%	
17	62	80	(1)	22/65	33.8%	—11.4	63/18700	0.34%	0.34
	80	62	(1)	42/93	45.2%		46/12981	0.35%	
18	25	55	(6)	129/245	52.6%	+29.5	41/42240	0.098%	0.095
	55	25	(4)	12/52	23.1%		28/30140	0.093%	
19	25	46	(4)	17/32	53.1%	+23.4	24/105103	0.023%	0.026
	46	25	(4)	11/37	29.7%		13/37320	0.035%	
20	25	1	(5)	161/453	35.5%	— 2.1†	238/99130	0.24%	0.24
	1	25	(3)	47/125	37.6%		63/24160	0.26%	

TABLE 2—*Continued*

Cross	R L-arabinose negative	D mutants		Total $\frac{ara^+ leu^+}{ara^+}$			Total $\frac{ara^+}{leu^+}$		Average distance
21	25	24	(4)	$\frac{29}{113}$	25.7%	−16.1	$\frac{89}{13752}$	0.65%	0.69
	24	25	(5)	$\frac{132}{316}$	41.8%		$\frac{225}{31632}$	0.71%	

* See footnote to Table 1 for explanation of headings. (+) or (—) indicates whether the reference mutant is to the right or left, respectively, of the nearby *ara* site.
† Indicates a difference in reciprocal ara^+/leu^+ ratios too small to relatively order the pair of mutants.
‡ Repetitive crosses of the same mutant pair not homogeneous.
§ A significant difference between the ara^+/leu^+ ratios of a reciprocal cross.

crossed with other more loosely linked *ara* mutants. For this reason, the approximate location of the *ara* mutants showing negative interference could be established.

The results presented in Table 2 also indicate that mutants located at the same or closely linked site may give either similar or different transduction results when crossed with other *ara* mutants. *ara*-16 and *ara*-28, two mutants at identical or closely linked sites, give similar results when crossed with nearby *ara* sites (Crosses 1 to 9, Table 2). *ara*-62 shows a high degree of negative interference when crossed with *ara*-14 (Cross 15, Table 2), but *ara*-15, identical or closely linked with *ara*-62, does not show any tendency toward negative interference when crossed with *ara*-14 (Cross 15, Table 1).

In several cases, it was not possible to order mutants by three-factor crosses due to the low yield of ara^+ recombinants. *ara*-43, located between *ara*-63 and *ara*-70, cannot be ordered with regard to *ara*-22, *ara*-26, and *ara*-23 owing to the low yield of ara^+ recombinants when *ara*-43 is crossed with these mutants (Crosses 8 to 12, Table 1). *ara*-46 and *ara*-55 are also too closely linked to establish a relative order, although both of these mutants can be placed between *ara*-80 and *ara*-1 (Crosses 17 to 21, Table 1).

Crosses between *ara*-22 and *ara*-26 (Cross 5, Table 1); *ara*-28 and *ara*-16; *ara*-62 and *ara*-15 (Crosses 3 and 16, Table 2) failed to yield a net number of ara^+ recombinants, i.e. the number of spontaneous reversions on the control plates of homologous phage and bacteria equaled or exceeded the number of ara^+ recombinants, indicating that these mutants may be at the same or closely linked sites.

Recombination distances between the L-*arabinose negative mutant sites:* In many cases, a number of independent transduction experiments using the same *ara* donor and *ara* recipient were carried out to obtain a significant yield of ara^+ recombinants. Variations in the ara^+/leu^+ ratios from repetitive identical crosses were analyzed by calculating and evaluating chi square. In each case, the degree of freedom was one less than the number of transduction experiments performed. Fifty-nine series were tested and only three were not homogeneous at the 5 percent level of significance (Cross 21, Table 1; Crosses 7 and 12, Table 2).

The average distance between *ara* sites was obtained by pooling the ara^+/leu^+ ratios of reciprocal crosses, since the distance between two mutants should be the

same regardless of whether the *ara* sites are in the donor or recipient. The assumption that distance data did not differ appreciably in reciprocal crosses was tested by entering the pooled ara^+/leu^+ ratios from a reciprocal cross into a 2×2 table, computing and evaluating chi square with one degree of freedom. Of 47 reciprocal crosses tested, 12 had ara^+/leu^+ ratios that differed significantly at the five percent level.

The recombination distance values served in several cases to establish the probable order of mutants which could not be precisely ordered by three-factor crosses, because of negative interference or close linkage.

As shown above, *ara*-43 cannot be located relative to *ara*-22, *ara*-26, and *ara*-23 by three-point crosses because of the low yield of ara^+ recombinants when *ara*-43 is crossed with these mutants. Recombination distances from crosses 6, 9 to 11, Table 1, indicate that *ara*-43 is 0.031 and 0.02 units from *ara*-22 and *ara*-26, respectively, and 0.022 units from *ara*-23. Since *ara*-22 is 0.072 units to the left of *ara*-23, (by three factor crosses), then *ara*-43 must be located between *ara*-22 and *ara*-23. *ara*-71 and *ara*-68, which show a high degree of negative interference, are located 0.091 and 0.60 units, respectively from *ara*-63. These results indicate *ara*-68 is to the right of *ara*-71 or more distant from *ara*-63 (Cross 13, Table 1; Cross 10, Table 2). The relative distances of *ara*-68 and *ara*-71 from *ara*-14 also indicate *ara*-71 is to the left of *ara*-68 (Cross 12, Table 2; Cross 14, Table 1).

Four additional mutants whose probable order can be resolved in a similar fashion are *ara*-55 and *ara*-46; *ara*-25 and *ara*-1. *ara*-46, closely linked to *ara*-55, is 0.026 and 0.29 units to the left of *ara*-25 and *ara*-1, respectively. *ara*-55 is 0.095 and 0.34 units to the left of *ara*-25 and *ara*-1, respectively, indicating *ara*-55 is to the left of *ara*-46 (Crosses 19, 21, Table 1; Crosses 18, 19, Table 2). By the same reasoning, *ara*-25, which gives negative interference when crossed with *ara*-1, would be more closely linked to both *ara*-46 and *ara*-55 than *ara*-1. This order is further supported by the smaller distance between *ara*-1 and *ara*-24, 0.50 units, than between *ara*-25 and 24, 0.69 units (Cross 21, Table 2; Cross 22, Table 1).

Not all the difficulties encountered in ordering the mutants by three-factor crosses can be resolved by the use of recombination distances. The results of three-factor crosses and the distance data of *ara*-16 and *ara*-28 with *ara*-6 and *ara*-74 did not permit an unambiguous order of *ara*-16 and *ara*-28 to be established (Crosses 1 to 9, Table 2). In some instances, the distance data are not statistically reliable (see above) and cannot be used alone to establish the genetic order of the *ara* sites. In other cases, the distance data for a given mutant are not in good agreement with the distance data obtained with other mutants in that area of the chromosome. The most extreme case is *ara*-29, which gives abnormally high distances when crossed with *ara*-1 and *ara*-24 (Crosses 23 and 24, Table 1).

Enzymatic analysis: All the 17 new mutants, which we have shown to map within the *B* gene or adjacent to mutant sites within the *B* gene, are deficient in L-ribulokinase activity and have varying levels of L-arabinose isomerase activity

which places them functionally within the *B* gene. The activity of L-arabinose isomerase of these 17 mutants varied from a low of 5 units to a high of 300 units (Table 3). *ara*-14 remains the lowest isomerase producer with a specific activity of three. Thus there is a 100 fold difference in L-arabinose isomerase activity among the 25 mutants in the L-ribulokinase structural gene. The remaining mutants have isomerase activities distributed between these high and low values. On the other hand, there was a maximum variation of only 2.5 in isocitric dehydrogenase activity as measured with the same extracts (Table 3). An inspection of the genetic map indicates no localization of L-ribulokinase mutant sites affecting low or high L-arabinose isomerase activities nor is there any recognizable pattern in mapped distance or position of these sites (Figure 2).

TABLE 3

Enzymatic characterization of 25 B-*gene* L-*arabinose negative mutants and the wild type*

Bacterial strain	L-ribulokinase*	L-arabinose isomerase†			Isocitric dehydrogenase‡		
		1	2	Average	1	2	Average
Wild type	2.82§	73.5	65.5	70	15.5	29.8	23
ara mutant							
79	0.38	240	230	235	26.3	20.7	24
6	0.07§	48.4	52.8	51	34.3	37.8	36
16	0.1§	178	133	156	35.6	42.5	39
28	—	108	70	89	26.0	31.0	29
74	—	51.0	43.5	47	32.6	29.0	31
70	0.19	151	169	160	25.5	26.4	26
22	0.13	150	191	171	22.0	17.0	20
26	0.05	216	244	230	31.0	31.0	31
43	—	177	172	175	40.0	35.0	38
23	0.84	176	245	211	14.7	21.6	18
63	—	152	162	157	24	10.4	17
71	—	256	343	300	22.5	28.4	26
68	0.10	15.5	16.2	16	31.7	24.3	28
14	trace	3.0	3.1	3	42.0	42.0	42
15	0.15§	181	254	218	34.1	38.1	36
62	—	248	250	249	24.0	25.0	25
80	0.53	24.6	26.4	26	28.8	34.0	31
55	—	7.8	8.2	8	21.3	19.0	20
46	—	139	115	127	29.8	25.3	28
25	—	7.6	6.3	7	23.2	24.0	24
1	0.04§	24.9	24.9	25	38.4	43.1	41
29	—	13.1	13.8	14	23.2	20.4	22
24	0.50§	216	217	217	34.8	35.6	35
8	—	74.3	74.8	75	36.5	29.2	33
27	—	4.7	4.4	5	32.2	28.6	30

* μmoles of ribulose phosphorylated/hr/mg protein — = no detectable activity (less than 0.04 μmoles/hr/mg protein).
† μmoles of ribulose/hr/mg protein. Figures under columns 1 and 2 are isomerase assays performed on duplicate extracts which were sonicated and assayed separately.
‡ μmole NADP reduced/hr/mg P. Figures in columns 1 and 2 are assays of the same extracts employed in estimating isomerase activity.
§ Average of kinase levels determined by ENGLESBERG (1961).

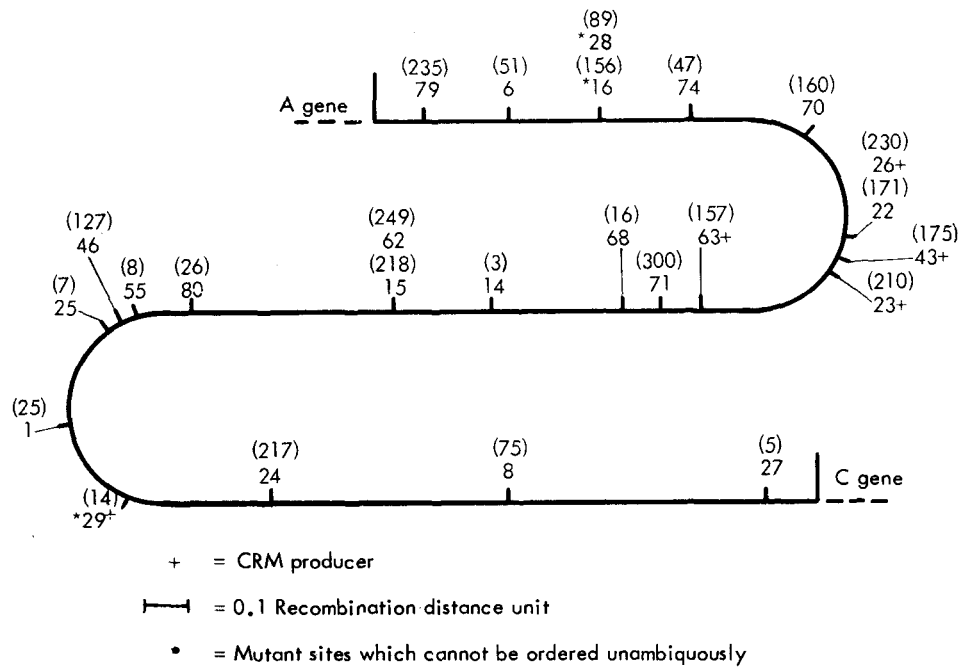

FIGURE 2.—Isomerase levels, order, and relative recombination distances between *ara-* sites of the L-ribulokinase structural gene, gene *B*. Numbers in parenthesis indicate the L-arabinose isomerase specific activity of the *ara* mutants listed below it.

DISCUSSION

The results of the transduction experiments show that most of the arabinose negative mutants could be linearly ordered by analysis of three-factor crosses. Certain mutants could not be ordered on the basis of these results because of close linkage or negative interference. In most cases, recombination distances over short regions of the chromosome were in good agreement and the distance measurements were used to order some of the mutants where this could not be done by analysis of three-factor crosses. With other mutants, the results of three-factor crosses and recombination distances were too ambiguous to establish a genetic order.

The genetic and enzymatic studies give no indication of a relationship between distribution of arabinose negative sites in the *B* gene and the level of L-arabinose isomerase produced by these mutants. In some cases mutants which are very closely linked differ significantly in their level of isomerase activity. As an example, *ara*-55, *ara*-46 and *ara*-25, three closely linked mutants, show approximately an 18 fold difference in isomerase activity. For this reason, it is doubtful that genetic and enzymatic analysis of additional arabinose negative mutants would reveal any correlation between location of the mutant site in the *B* gene and level of isomerase production. The different recombination patterns of some mutants that are closely linked or at identical sites indicate that the recombination

properties of a specific mutant may depend on the type of mutational change that has occurred rather than the location of the mutation in the *B* gene. Unique recombination properties of specific mutant alleles have also been noted in transduction studies with Salmonella (DEMEREC, GOLDMAN and LAHR 1958; BALBINDER 1962; MARGOLIN 1963) and with Pneumococcus (HOTCHKISS and EVANS 1958). In addition to specific recombination properties, the type of mutational damage may also be the major factor which determines the activity level of L-arabinose isomerase, the product of the adjacent structural *A* gene.

A study of these *B* gene mutants by LEE and ENGLESBERG (1963) has shown that mutations at these sites effect a coordinated increase or decrease in inducible levels of L-arabinose isomerase, L-ribulose 5-phosphate 4-epimerase, and L-ribulokinase CRM, demonstrating that this *A*, *B*, *D*, gene complex acts as a "genetic unit of coordinate expression" (JACOB and MONOD 1961). Dual effects leading only to decreased inducible levels of enzyme have been shown in other systems (JACOB and MONOD 1961; AMES and HARTMAN 1963). The data obtained by LEE and ENGLESBERG (1962, 1963) have led them to propose that the dual effect is probably the direct result of a change in the DNA code of a structural gene, a code which programs the structure of an enzyme and sets limits to the rate of synthesis of this and other enzymes specified by a genetic unit of coordinate expression.

SUMMARY

Genetic and enzymatic analysis of 17 additional L-arabinose nonutilizing mutants of *Escherichia coli* B/r, together with eight previously analyzed mutants of the *B* gene, show no relationship between the location of a mutant site in the *B* gene and its effect on the level of inducible L-arabinose isomerase, the product of the adjacent structural *A* gene. The results of reciprocal three-factor crosses were used to establish the linear genetic order of most arabinose negative mutants. Other mutants could not be ordered by this method because of high negative interference or close linkage. Recombination distances were used to establish the probable order of most of these mutants.

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