DIRECT SELECTION OF L-ARABINOSE NEGATIVE MUTANTS OF ESCHERICHIA COLI STRAIN B/r[1]

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GROSS and ENGLESBERG (1959), using three and four factor transduction experiments with phage P1bt, found that the mutational sites of 17 L-arabinose nonutilizing mutants of *Escherichia coli* were arranged in a linear sequence. The 17 mutants were divided into three distinct functional groups on the basis of biochemical analyses. Complete correspondence was found between the order of the mutational sites of these mutants and their functional grouping. ENGLESBERG (1961) further defined the three groups of mutants by enzymatic analyses. It appears that two of the genetic loci (A and B) are responsible for the structures of the L-arabinose isomerase and L-ribulokinase enzymes respectively. The third locus (C) appears to be an operator gene.

A fourth functional group (D) of L-arabinose nonutilizing mutants has recently been discovered. Mutants in this group (*ara-53*, *ara-139*) are deficient in the enzyme L-ribulose-5-phosphate-4-epimerase, and accumulate L-ribulose-5-phosphate. The growth of these mutants is strongly inhibited by L-arabinose and mutants that are relatively resistant to L-arabinose appear in their progeny with a high frequency. When one of these relatively resistant mutants was analyzed, it exhibited the characteristics of a typical Group B mutant (ENGLESBERG *et al.* 1962).

It was postulated (ENGLESBERG *et al.* 1962) on the basis of these results that the L-arabinose inhibition of the Group D mutants may be directly or indirectly related to the accumulation of L-ribulose-5-phosphate and that relative resistance could be gained by any mutational event which prevented the accumulation of this compound. This would be accomplished by a mutation in the A or C locus as well as in the B locus.

This paper will show that the sensitivity of the *ara-53* mutant to L-arabinose is relieved by the occurrence of a mutation in any of the three known loci (A, B or C), so that this mutant provides a simple and direct selective system for obtaining L-arabinose negative A, B and C mutants. It will also be shown that (1) these resistant mutants are double L-arabinose negative mutants, i.e., each has a mutant site in either the A, B or C locus ("new" mutant site) in addition to the *ara-53* mutant site, (2) the A, B or C mutation can be ordered within the appropriate

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locus in the arabinose region, and that (3) the *ara-53* mutant site, which is common to all double mutants, can easily be eliminated to allow both selection for wild-type revertants and the mapping of adjacent "new" mutant sites.

MATERIALS AND METHODS

All media, phage P1bt, the 17 L-arabinose nonutilizing mutants (reference mutants), transduction procedures, test for lysogeny, growth experiments, and keto-sugar analysis are described by GROSS and ENGELSBERG (1959). The details concerning the L-arabinose sensitive mutant employed in this work (*ara-53*) have previously been described (ENGLESBERG *et al.* 1962).

The procedure for phage production as described by GROSS and ENGLESBERG (1959) was modified as follows: minimal tris glucose agar medium was supplemented with L-broth (200 ml of L-broth: 1000 ml of minimal tris glucose). Phage used in transduction experiments were grown twice on the desired bacterial donor strains to eliminate unabsorbed wild-type phage. When undiluted wild-type phage was used as seed, the lysates contained enough unabsorbed wild-type phage to significantly increase some frequencies of *ara*$^+$ transduction. The bacteriophage could be harvested after 4½ hours incubation and usually had a titer of 3–4 $\times$ 10^{10} plaque-forming particles/ml.

RESULTS

Isolation of L-arabinose resistant mutants: Nine independently isolated L-arabinose resistant mutants were selected from an *ara-53* population in the following manner: nine small tubes of nutrient broth (0.5 ml/tube) were inoculated directly from a slant of the *thr*$^-$ *leu*$^-$ (L-threonine and L-leucine dependent) *ara-53* strain (approximately 100 cells/tube) and incubated overnight at 37°C; a loopful of culture from each tube was then streaked on an EMB (eosin methylene blue) 1.0% L-arabinose agar plate and incubated 36 hours at 37°C; one large pink colony was then picked from each plate (*ara-53* colonies are very small because the presence of L-arabinose inhibits their growth), purified, and slanted on a nutrient agar slant. The nine L-arabinose negative mutants were designated as *thr- ara-53 ara-127 leu*$^-$, *thr*$^-$ *ara-53 ara-128 leu*$^-$, etc., as they were isolated. Each of the resistant mutants were converted to *thr*$^+$ *leu*$^+$ by transduction (GROSS and ENGLESBERG 1959) so that each resistant mutant existed in a *thr*$^+$ *leu*$^+$ and a *thr*$^-$ *leu*$^-$ form.

Studies of growth, keto-sugar accumulation and enzymatic activities: If the L-arabinose resistant colonies that appear in the inhibited *ara-53* background of growth on an EMB L-arabinose agar plate result from a mutation occurring in either the A, B or C locus, one would expect these colonies to have the functional characteristics of typical Group A, B or C mutants isolated from a wild-type population. Analysis of the nine L-arabinose resistant mutants (Table 1) indicated that four are typical Group C mutants, i.e., they are deficient in L-arabinose isomerase and L-ribulokinase, accumulate little keto-sugar, and are not inhibited by L-arabinose. Four other mutants are classified as Group B mutants. They are

TABLE 1

Enzymatic activities, studies of growth and keto-sugar accumulations. The isomerase and kinase activities of the wild-type E. coli *B/r were determined by* ENGLESBERG *(1961)*

Strain	Inhibition index*	Keto-sugar accumulation (μmoles/ml)	Isomerase activity†	Kinase activity†	Functional classification
Wild-type B/r			9.3	2.8	
ara-53 ara-138	6.0	0.07	0.2	4.8	A
ara-53 ara-127	5.0	1.00	28.7	..‡	B
ara-53 ara-131	6.0	0.20	2.1	0.8	B
ara-53 ara-133	4.0	0.87	19.4	..‡	B
ara-53 ara-136	4.0	1.10	25.9	..‡	B
ara-53 ara-128	1.0	0.15	0.5	..‡	C
ara-53 ara-130	1.0	0.10	1.4	..‡	C
ara-53 ara-132	1.5	0.10	0.7	0.2	C
ara-53 ara-135	0.5	0.16	0.9	0.3	C

* The inhibition index is equal to the OD×100 in casein hydrolyzate medium minus the OD×100 in casein hydrolyzate plus L-arabinose medium at 2.5 hours of incubation (ENGLESBERG 1961).
† The isomerase and kinase activities are expressed as μmoles ribulose produced or phosphorylated/hr/mg protein.
‡ Less than 0.02 μmoles/hr/mg protein.

deficient in L-ribulokinase activity, have varying levels of L-arabinose isomerase activity, accumulate keto-sugar at varying levels, and their growth is slightly inhibited[2] by L-arabinose. Only one of the nine mutants examined is a typical Group A mutant. Its growth is slightly inhibited[2] by L-arabinose, it accumulates less keto-sugar than Group C mutants, and it has no L-arabinose isomerase activity. Thus, on the basis of these criteria, at least the three functional groups of L-arabinose nonutilizing mutants described by GROSS and ENGLESBERG (1959) and ENGLESBERG (1961) can be obtained from an *ara-53* population.

Characterization of the L-arabinose resistant mutants as doubly L-arabinose negative: During the course of transducing the *thr*$^+$ *leu*$^+$ markers into the *thr*$^-$ *leu*$^-$ L-arabinose resistant mutants isolated from *ara-53* it was observed that among the *thr*$^+$ *leu*$^+$ L-arabinose negative recombinants a good proportion were inhibited by L-arabinose in the same manner as *ara-53* (as determined by the growth response on EMB 1.0% L-arabinose agar plates). For instance, in one case, 75 percent (36/48) of the *thr*$^+$ *leu*$^+$ L-arabinose negative recombinants were uninhibited by L-arabinose and 25 percent (12/48) were inhibited. In crosses described later, it was also possible to obtain L-arabinose negative recombinants that no longer contained the *ara-53* mutant site (determined by genetic tests). Thus, the enzymatic deficiencies of the resistant mutants in isomerase (A locus), kinase (B locus), or in both isomerase and kinase (C locus), together with the segregation of the *ara-53* and *ara*"new" mutant sites from each other, demonstrate that each L-arabinose resistant mutant isolated from the *ara-53* population has two mutant sites affecting the utilization of L-arabinose.

[2] Growth of Group A and B mutants is only slightly inhibited in casein hydrolysate medium as a result of L-arabinose inhibiting the utilization of L-threonine in the hydrolysate as a carbon and energy source (ENGLESBERG 1960; ENGLESBERG *et al.* 1962). There is no detectable difference in colony size between the Group A, B or C mutants on EMB 1.0% L-arabinose agar medium.

Three factor transduction experiments: The order of three of the "new" mutant sites, in functional Groups A, B and C, with respect to the previously ordered 17 mutant sites, were determined by three factor transduction experiments using *leu* as an unselected marker (GROSS and ENGLESBERG 1959). Since the *ara-53* mutant site is to the left of the A, B and C loci (ENGLESBERG *et al.* 1962), it did not interfere with this ordering procedure.

The results indicate (Table 2) that the *ara-138*, *ara-127* and *ara-128* mutant sites are located within the known A, B and C loci, respectively, and exhibit complete correspondence with their functional classification. Because of the small numbers of *ara*$^+$ transductants in some of the reciprocal crosses (3 and 4, 9 and 10, 13 and 14, 15 and 16, 17 and 18, Table 2) and definite tendency toward negative interference (GROSS and ENGELSBERG 1959) it was not possible to order these mutational sites exactly by this procedure. The low number of *ara*$^+$ transductants in these experiments is no doubt due to the proximity of the mutant sites involved.

Frequencies of ara$^+$ *transduction:* Closely linked mutant sites can be ordered without using three factor crosses with *leu* as an unselected marker if one examines the ratios of *ara*$^+$ transduction frequencies obtained in reciprocal crosses involving double arabinose mutants and single arabinose mutants. In every cross where a double mutant is a donor, only a double crossover is required for an

TABLE 2

Three factor transduction experiments with ara-53 ara-138, ara-53 ara-127 *and* ara-53 ara-128. *In each cross the donor is* thr$^+$ leu$^+$ *and the recipient is* thr$^-$ leu$^-$

Cross	Donor	Recipient	*ara*$^+$*leu*$^+$/*ara*$^+$	Percent
1	*ara-53 ara-138*	*ara-4*	371/832	45
2	*ara-4*	*ara-53 ara-138*	192/544	35
3	*ara-53 ara-138*	*ara-7*	14/40	35
4	*ara-7*	*ara-53 ara-138*	3/19	16
5	*ara-53 ara-138*	*ara-13*	87/253	34
6	*ara-13*	*ara-53 ara-138*	208/286	73
7	*ara-53 ara-127*	*ara-24*	218/376	58
8	*ara-24*	*ara-53 ara-127*	46/205	22
9	*ara-53 ara-127*	*ara-1*	30/62	48
10	*ara-1*	*ara-53 ara-127*	19/55	35
11	*ara-53 ara-127*	*ara-15*	57/222	26
12	*ara-15*	*ara-53 ara-127*	59/110	53
13	*ara-53 ara-128*	*ara-19*	26/49	53
14	*ara-19*	*ara-53 ara-128*	3/11	27
15	*ara-53 ara-128*	*ara-21*	23/56	41
16	*ara-21*	*ara-53 ara-128*	8/15	53
17	*ara-53 ara-128*	*ara-3*	198/516	34
18	*ara-3*	*ara-53 ara-128*	114/273	42
19	*ara-53 ara-128*	*ara-5*	109/386	24
20	*ara-5*	*ara-53 ara-128*	57/132	43
21	*ara-53 ara-128*	*ara-12*	48/242	19
22	*ara-12*	*ara-53 ara-128*	30/58	52

ara$^+$ transductant (Figure 2, A and B). In reciprocal crosses, i.e., when the double mutant is used as a recipient, a double *or* a quadruple crossover may be required for an *ara*$^+$ transductant, depending on the position of the "new" mutant site of the double mutant (Figure 2, C and D). In a cross where the double mutant is the donor and the reference mutant site is to the right of the "new" mutational site (Figure 2, A) an *ara*$^+$ transductant can be obtained by a double crossover involving regions c and d, and the frequency of *ara*$^+$ transduction can be expressed as f_A *ara*$^+ = cd$, c and d representing the probability of crossing-over in regions c and d. Similarly, the frequency of *ara*$^+$ transduction in the reciprocal cross (Figure 2, C) can be expressed as f_C *ara*$^+ = ca$. Thus, the ratio of *ara*$^+$ transduction frequency within the reciprocal cross is f_A *ara*$^+/f_C$ *ara*$^+ = cd/ca = d/a$, which should remain constant for reciprocal crosses involving a particular double mutant and reference mutants whose mutant sites are to the right of the "new" mutant site of the double mutant but not far enough away to influence region d. In crosses involving double mutants as donors and reference mutants as recipients with mutant sites to the left of the "new" mutant sites of the double mutants, only double crossovers are required for *ara*$^+$ transductants (Figure 2, B), and frequency of *ara*$^+$ transduction can be expressed as f_B *ara*$^+ = ef$. However, in the reciprocal cross (Figure 2, D), a quadruple crossover is required for an *ara*$^+$ transductant and the frequency of *ara*$^+$ transduction is f_D *ara*$^+ = aefg$. The ratio of f_B *ara*$^+/f_D$ *ara*$^+$ is $ef/aefg$, which equals $1/ag$ and should remain constant for any set of reciprocal crosses between a particular double mutant and any reference mutant whose mutant site is to the left of the "new" mutant site of the double mutant. It can be seen in Table 3 that this ratio remains fairly constant (ranging from 5.0 to 5.5) for such reciprocal crosses involving *ara-53 ara-127* and several reference mutants while similar ratios for crosses involving *ara-53 ara-128* and several reference mutants vary over a wider range (3.6 to 6.7). Although a variation is found in these ratios for crosses involving a particular double mutant and several reference mutants, there is a definite correlation between the ratios of *ara*$^+$ transduction frequencies and the order of mutant sites obtained from three factor crosses. The data presented in Table 2 showed that the *ara-127* mutant site is between *ara-15* and *ara-24*. It was not possible to order *ara-127* with respect to *ara-1* by three factor crosses as previously mentioned. However, since the ratio of *ara*$^+$ transduction frequencies for the *ara-1* and *ara-53 ara-127* reciprocal cross falls into the same class as that of the *ara-24* and *ara-53 ara-127* reciprocal cross and not in the same class as reciprocal crosses between *ara-53 ara-127* and *ara-15*, *ara-14* and *ara-23*, it appears that the *ara-127* mutant site is to the left of the *ara-1* mutant site. Similarly, the *ara-138* and *ara-128* mutant sites can be fixed with respect to all of the reference mutant sites (Table 3; Figure 1) mapped by GROSS and ENGLESBERG (1959).

Thus, the ratios of *ara*$^+$ transduction frequencies can be used to order the "new" mutant sites of double mutants that are closely linked to other mutational sites. The double mutants do not revert to *ara*$^+$ at a detectable rate so one can distinguish between identical or overlapping mutant sites and closely linked

TABLE 3

Frequencies of ara$^+$ *transduction. In each cross the donor is* thr$^+$ leu$^+$ *and the recipient is* thr$^-$ leu$^-$

Cross	Donor	Recipient	*ara*$^+$/*leu*$^+$*	Percent	Percent donor† / Percent recipient
1	*ara-53 ara-138*	*ara-4*	988/119,394	0.83	
2	*ara-4*	*ara-53 ara-138*	531/92,080	0.58	1.4
3	*ara-53 ara-138*	*ara-7*	40/149,640	0.027	
4	*ara-7*	*ara-53 ara-138*	19/94,640	0.020	1.4
5	*ara-53 ara-138*	*ara-13*	253/87,720	0.29	
6	*ara-13*	*ara-53 ara-138*	184/222,400	0.08	3.7
7	*ara-53 ara-127*	*ara-24*	376/75,320	0.50	
8	*ara-24*	*ara-53 ara-127*	205/84,660	0.24	2.0
9	*ara-53 ara-127*	*ara-1*	34/89,340	0.04	
10	*ara-1*	*ara-53 ara-127*	55/392,860	0.02	2.0
11	*ara-53 ara-127*	*ara-15*	222/44,580	0.50	
12	*ara-15*	*ara-53 ara-127*	110/125,160	0.09	5.5
13	*ara-53 ara-127*	*ara-14*	233/62,950	0.37	
14	*ara-14*	*ara-53 ara-127*	162/241,840	0.07	5.3
15	*ara-53 ara-127*	*ara-23*	51/7740	0.65	
16	*ara-23*	*ara-53 ara-127*	181/137,400	0.13	5.0
17	*ara-53 ara-128*	*ara-19*	50/161,080	0.03	
18	*ara-19*	*ara-53 ara-128*	11/164,920	0.006	4.5
19	*ara-53 ara-128*	*ara-21*	56/89,600	0.06	
20	*ara-21*	*ara-53 ara-128*	15/175,600	0.009	6.7
21	*ara-53 ara-128*	*ara-3*	3427/504,400	0.68	
22	*ara-3*	*ara-53 ara-128*	273/166,640	0.16	4.3
23	*ara-53 ara-128*	*ara-5*	277/59,420	0.47	
24	*ara-5*	*ara-53 ara-128*	134/202,220	0.07	6.7
25	*ara-53 ara-128*	*ara-12*	242/47,200	0.51	
26	*ara-12*	*ara-53 ara-128*	58/41,600	0.14	3.6

* In the fraction *ara*$^+$/*leu*$^+$ the numerator represents the number of *ara*$^+$ transductants per volume plated; the denominator represents the number of *leu*$^+$ transductants present in an equal volume (calculated from independent selection of *leu*$^+$ transductants by plating 0.4 ml of a 1/20 dilution of the transduction mixture).

† Percent donor/percent recipient is the ratio of the *ara*$^+$ transduction frequency obtained when the double mutant was used as the donor to the *ara*$^+$ transduction frequency obtained when the double mutant was used as the recipient.

mutant sites, e.g., the *ara-128* mutant site can be distinguished from the *ara-19* and *ara-21* mutant sites.

Elimination of the ara-53 *mutant site from double mutants:* Using a double mutant as a donor and a *thr*$^+$ *ara*$^+$ *leu*$^-$ strain as a recipient, selection was made for *leu*$^+$ transductants; approximately 20 of such transductants that were also *ara*$^-$ were scored for the *ara-53* mutant site by using *ara-53* as a donor for these strains and selecting for *ara*$^+$ transductants; on the average, one out of five *leu*$^+$ transductants was found to have only the *ara*-"new" mutant site and these strains were labeled as *thr*$^+$ *ara-127 leu*$^+$, *thr*$^+$ *ara-128 leu*$^+$, etc.; a *thr*$^+$ *ara*-"new"

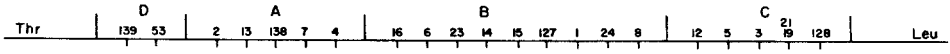

FIGURE 1.—Schematic representation of the order of L-arabinose mutational sites with respect to the mutational sites of threonine and leucine.

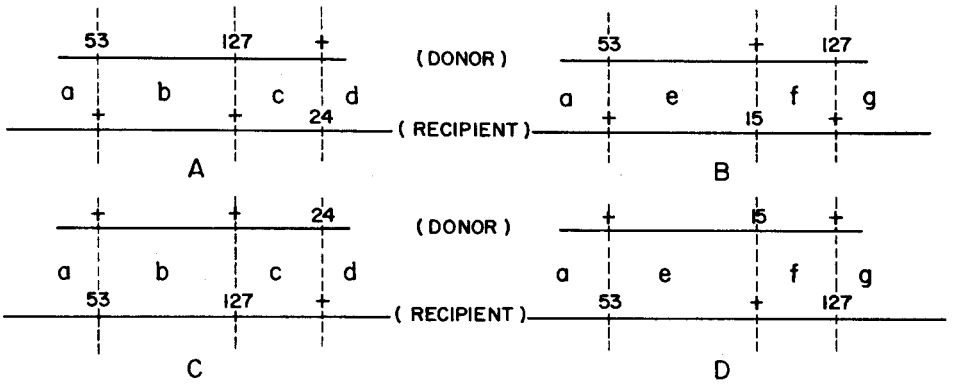

FIGURE 2.—A and B schematically represent the regions of possible crossing-over involved when a double L-arabinose negative mutant is the donor and single L-arabinose negative mutants are the recipients. C and D are the schematic representations of the regions of possible crossing-over involved in the reciprocal crosses where the single L-arabinose negative mutants are the donors. A, B and C would require double crossovers for *ara*+ transductants while D would require a quadruple crossover for *ara*+ transductants.

leu$^+$ that was nonlysogenic was then used as a donor and a *thr*$^-$ *ara*$^+$ *leu*$^-$ strain as a recipient and selection was carried out for *thr*$^+$ transductants; the *thr*$^+$ transductants that were also *leu*$^-$ *ara*$^-$ and nonlysogenic were used as recipient strains in transduction experiments.

Since the *ara-53* mutant site can be easily eliminated it is now possible to use three factor transduction experiments with *leu* as an unselected marker (GROSS and ENGLESBERG 1959) and frequencies of *ara*$^+$ transduction (described above) to determine the order of adjacent "new" mutant sites. Equally important, selection for *ara*$^+$ reversion is also possible, which makes the system more versatile, e.g., for studies of the effect of mutagens on forward and reverse mutation rates (FREESE 1959).

Transduction experiments with ara-"*new*" *single mutants:* The transduction experiments in Tables 2 and 3 were repeated with *ara-127* and *ara-128* as single mutants. As expected in such crosses, the ratios of *ara*$^+$ transduction frequencies within reciprocal crosses are close to one whether the reference mutant site is to the left or to the right of the *ara*-"new" mutant site (Table 4). Data from three factor transduction experiments using the *ara*-"new" single mutants does not conflict with the order of mutant sites obtained previously using the double mutants.

DISCUSSION

The results presented show that the sensitivity of the *ara-53* mutant to L-arabinose can be relieved by a mutation in the A or C locus as well as the B locus. The sensitivity to L-arabinose of the functional Group D mutants (ENGLESBERG *et al.* 1962) therefore provides a direct method for selecting L-arabinose negative A, B and C mutants. Two similar direct systems for selecting bacterial mutants have previously been reported. DAVIS (1952) describes a mutant of *Escherichia*

TABLE 4

Transduction experiments with ara-127 *and* ara-128. *In each cross the donor is* thr$^+$ leu$^+$ *and the recipient is either* thr$^-$ leu$^-$ *or* thr$^+$ leu$^-$

Donor	Recipient	*ara*$^+$ *leu*$^+$/*ara*$^+$	Percent	*ara*$^+$/*leu*$^+$*	Percent	Percent donor† / Percent recipient
ara-127	*ara-24*	162/326	50	326/55,000	0.59	
ara-24	*ara-127*	42/163	26	163/22,520	0.72	0.8
ara-127	*ara-1*	11/39	28	39/45,200	0.08	
ara-1	*ara-127*	13/58	22	5/16,200	0.03	2.6
ara-127	*ara-15*	130/523	25	523/87,880	0.60	
ara-15	*ara-127*	259/543	48	955/84,120	1.13	0.5
ara-127	*ara-14*	75/215	32	215/26,940	0.80	
ara-14	*ara-127*	214/490	44	807/51,440	1.56	0.5
ara-128	*ara-19*	15/30	50	30/53,800	0.056	
ara-19	*ara-128*	7/15	47	15/80,000	0.02	2.3
ara-128	*ara-21*	20/41	49	41/55,800	0.07	
ara-21	*ara-128*	28/89	31	89/241,780	0.037	1.9
ara-128	*ara-3*	76/209	36	209/18,400	1.13	
ara-3	*ara-128*	218/421	52	1081/87,920	1.24	0.9
ara-128	*ara-5*	40/220	18	588/42,400	1.39	
ara-5	*ara-128*	162/265	61	455/36,920	1.23	1.1
ara-128	*ara-12*	24/168	14	563/49,600	1.14	
ara-12	*ara-128*	105/170	70	1596/53,600	2.98	0.5

* In the fraction *ara*$^+$/*leu*$^+$ the numerator represents the number of *ara*$^+$ transductants per volume plated; the denominator represents the number of *leu*$^+$ transductants present in an equal volume (calculated from independent selection of *leu*$^+$ transductants by plating 0.4 ml of a 1/20 dilution of the transduction mixture).

† Percent donor/percent recipient is the ratio of the *ara*$^+$ transduction frequency obtained when the double mutant was used as the donor to the *ara*$^+$ transduction frequency obtained when the double mutant was used as the recipient.

coli that accumulates 5-dehydroshikimic acid, which competitively interferes with the utilization of shikimic acid. This mutant provides a direct method for obtaining mutants unable to synthesize 5-dehydroshikimic acid. The second system involves a galactose-1-phosphate uridyl transferase negative mutant of *Escherichia coli* which accumulates galactose-1-phosphate and a UDPgalactose-4-epimerase negative mutant of *Salmonella enteritidis* which accumulates UDP-galactose (KURAHASHI and WAHBE 1958; KALKAR, KURAHASHI and JORDAN 1959; YARMOLINSKY, WIESMEYER, KALKAR and JORDAN 1959; LEDERBERG 1960; NIKAIDO 1961). Galactose nonutilizing, galactose nonsensitive clones isolated from either the transferase or epimerase negative mutant population lack the enzyme galactokinase as well as the transferase or epimerase.

It was difficult, in several crosses, to obtain large numbers of *ara*$^+$ transductants. Since this is necessary for ordering mutant sites by three factor crosses as described by GROSS and ENGELSBERG (1959), the complete order of mutant sites could not be obtained by this method. In addition, crosses involving *ara-53 ara-128* with several reference mutants exhibit negative interference with regard to the unselected *leu*$^+$ marker as reported by GROSS and ENGLESBERG (1959) and CRIBBS and ENGLESBERG (1961). However, the ratios of *ara*$^+$ transduction frequencies within reciprocal crosses appear to offer a solution for such problems.

The negative interference does not render the ratios of *ara*+ transduction ineffective, although it might account for the variation of ratios.

It is interesting to note that the dual effect of the spontaneous mutations (the *ara*-"new" mutations) in the A or B locus with respect to enzymatic activities is the same as that of the UV-induced mutations examined by ENGLESBERG (1961).

SUMMARY

The sensitivity to L-arabinose of an L-arabinose nonutilizing mutant of *Escherichia coli* strain B/r (*ara-53*) can be relieved by the occurrence of a mutation in either of three known genetic loci (A, B or C) controlling the utilization of L-arabinose, which permits direct selection for L-arabinose negative A, B and C mutants. The functional characteristics of these A, B and C mutants correspond with the order of their mutant sites, as has been previously found for the A, B and C L-arabinose negative mutants isolated from a wild-type population by the nonselective techniques available.

The *ara-53* mutational site can be easily eliminated from the double mutants to allow ordering of adjacent mutational sites and selection for *ara*+ revertants.

The presence of two mutational sites, each affecting the utilization of L-arabinose, in these double L-arabinose negative mutants can be useful in ordering mutant sites.

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