STREPTOMYCIN RESISTANCE IN ESCHERICHIA COLI ANALYZED BY TRANSDUCTION[1]

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TWO main categories of mutation affecting bacterial susceptibility to the action of streptomycin have been described and studied, streptomycin resistance and streptomycin dependence (MILLER and BOHNHOFF 1947; DEMEREC 1948, 1950, 1951; DEMEREC *et al.* 1949; NEWCOMBE and HAWIRKO 1949; NEWCOMBE 1952; USHIBA and WATANABE 1954; WATANABE 1954, 1955). Resistance to a high concentration of streptomycin develops either in a single mutational step or by multiple steps. Genetic differences between mutants of the single-step type and the multistep type have been demonstrated by means of transformation experiments with *Diplococcus pneumoniae* (HOTCHKISS 1952; HASHIMOTO 1955a, 1957), recombination experiments with *Escherichia coli*, strain K-12 (USHIBA *et al.* 1957), and transduction experiments with *Salmonella typhimurium* (WATANABE and WATANABE, in press, a,b). Streptomycin-dependent mutants can be obtained in a single mutational step. They may differ from one another with regard to concentration of the drug required, ability to satisfy the streptomycin requirement with certain related chemicals, and some other characteristics (DEMEREC *et al.* 1949; SZYBALSKI and COCITO-VANDERMEULEN 1958). Genetic analysis by recombination experiments with *E. coli* K-12 has shown that independently originating dependent mutants are the result of mutations within a single gene locus or in very closely linked loci (DEMEREC *et al.* 1949; NEWCOMBE and NYHOLM 1950; SZYBALSKI and COCITO-VANDERMEULEN 1958), and that single-step high resistance is also inherited as if controlled by alleles of the same gene locus (NEWCOMBE and NYHOLM 1950). In transduction experiments with *E. coli* a similar observation was made (LENNOX 1955). These results indicate the presence of a particular gene locus concerned with single-step streptomycin resistance and dependence, although a number of other loci may be responsible for multistep resistance.

The study described here attempted to answer two questions: (1) whether or not streptomycin-dependent and single-step highly resistant mutants of independent origin do result from mutations at the same gene locus; and (2) if so, whether the mutations occur at different sites of the locus—that is, whether nonidentical allelism can be demonstrated in this case as it has been by DEMEREC

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and his collaborators in the case of auxotrophic mutants of *S. typhimurium* (DEMEREC *et al.* 1955; DEMEREC and Z. DEMEREC 1956; DEMEREC and Z. HARTMAN 1956; DEMEREC 1956a,b; HARTMAN 1956).

It proved unexpectedly difficult to obtain recombination between streptomycin-dependent mutants, which suggested that they might be the result of multisite changes. As such, they would not be expected to revert to wild type. Mutants having the wild type phenotype were in fact easily obtained, however, and so the next step in the study was analysis of their genotypes to ascertain whether they were true revertants, due to back mutation at the same locus, or the result of change in some other part of the genome. Reports of genetical analyses of such "revertants" by transformation experiments with *D. pneumoniae* (HASHIMOTO 1955b and unpublished) and by recombination experiments with *E. coli* (NEWCOMBE and NYHOLM 1950) indicated that at least some of them still carried in their genomes the original streptomycin-dependent mutation.

MATERIALS AND METHODS

Bacterial strains: All strains used were *E. coli* strain B/r and its mutants. The original strain B/r will be referred to simply as *str-s* because of its sensitivity to streptomycin. All streptomycin-dependent and -resistant strains were obtained by single-step selection of spontaneous mutants from *str-s*, and are designated by the abbreviations *str-d* and *str-r*, respectively, followed by numbers indicating their separate mutational origins (e.g., *str-d-1*, *str-d-2*). Streptomycin-dependent strains came from DEMEREC's stocks (obtained by selection with 50 μg/ml of streptomycin), and *str-r* strains were isolated during the experiment by selection with 500 μg of streptomycin per ml. Responses to streptomycin of the strains mainly used are shown in Table 1.

TABLE 1

Growth of str-d *and* str-r *strains on nutrient agar containing various concentrations of streptomycin. After 24 hours of incubation, growth was scored from — (none) to +++ (very heavy)*

Strain	Concentration of streptomycin (μg/ml)							
	0	1	5	20	100	500	1000	5000
str-s	+++	+	—	—	—	—	—	—
str-d-1	—	+	++	+++	+++	++	++	+
-4	—	++	++	+++	+++	—	—	—
-5	—	++	+++	+++	+++	±	—	—
-62	—	+	++	+++	+++	++	+	—
-67	—	++	+++	+++	+++	—	—	—
-74	—	++	+++	+++	+++	—	—	—
-77	—	++	+++	+++	+++	±	—	—
-78	—	±	++	+++	+++	++	±	—
str-r-2	+++	+++	+++	+++	+++	+++	++	++
-3	+++	+++	+++	+++	+++	+++	++	++

Nondependent mutants were isolated from dependent strains by selection on medium lacking streptomycin.

Phage: The strain used chiefly in these experiments was P1bt, a turbid-plaque isolate from phage P1b derived from LENNOX's strain P1kc. The original P1 strain was isolated by BERTANI (1951a).

Preparation of phage lysates: Lysates were prepared on confluent lysis plates (see SWANSTROM and ADAMS 1951) with modified HERSHEY tris glucose medium. Prewarmed, undried tris agar (one percent agar) plates containing about 40 ml per plate were overlaid with 2.5 ml soft tris agar (0.65% agar) containing about 2×10^8 log phase bacteria from an aerated liquid tris glucose culture and about 5×10^5 phage particles. As soon as possible after hardening, the plates were incubated for about 6–7 hours. After confluent lysis, the bacteria remaining on the plates were killed with chloroform vapor; the soft agar layer was scraped and washed off with 2.5–5 ml of prewarmed eluting fluid and centrifuged at low speed. The supernatant was shaken with 1/10 volume of chloroform and bubbled with air to remove the chloroform. After storage in a cold room overnight, the lysate was centrifuged again to remove the remaining agar, and treated once more with chloroform.

The preparations were titrated, using tris agar and a phage-sensitive strain as indicator. Their sterility was verified by plating a sample on nutrient agar. Titers were usually between 5×10^9 and 3×10^{10} per ml. The lysates were stored in a cold room, usually with a small amount of chloroform, and were fairly stable for at least several months. A sample tested after six months of storage showed a decrease in titer to one fifth the original.

When lysates were made from *str-d* strains, 100 μg/ml of streptomycin was added to the medium, since it was observed that *str-d* strains require 5 to 50 times more streptomycin in the tris medium than in nutrient broth medium. Sometimes it was difficult to get high titers in phage lysates because of variations in growth among different *str-d* strains, and in the case of *str-d-4* it was necessary to add more $CaCl_2$ (about 10^{-2} M).

Transduction technique: An aerated culture of the recipient bacteria in L broth containing 2.5×10^{-3} M $CaCl_2$ was employed in transduction experiments just before it reached full growth ($1–2 \times 10^9$ bacteria per ml). In the case of *str-d* strains, streptomycin was added (10 μg/ml) and after suitable growth the culture was centrifuged and the bacteria were resuspended in L broth or tris medium without glucose.

The technique was as follows. In some experiments, equal volumes of $1–2 \times 10^9$/ml recipient bacterial culture and 1×10^{10}/ml donor phage lysate were mixed; in other experiments the proportion was 1/10 volume of $0.5–2 \times 10^{10}$/ml bacterial culture and 5×10^9/ml phage lysate. The mixture was kept at 37°C for 30 minutes to allow adsorption of the phage. Under these conditions (phage multiplicities between one and ten), it was found that more than 80 percent of the phage particles were adsorbed to bacteria, and that bacteria survival was from 35 to 55 percent. In trials made with *leu* (leucine requirement)

as a marker, the number of transductants was 50 to 80 percent of the expected number calculated on the basis of results obtained in an experiment using lower multiplicity and assuming that frequency of transduction increases lineally with number of phage particles employed. With still higher multiplicity (about 70), bacterial survival was ten percent and the number of transductants was 3.5 percent the expected number.

In a formula describing a transduction experiment, the genotype of the recipient bacteria appears first, followed by the sign (x) and the genotype of the donor bacteria. Thus: *str-d-77* (x) *str-s*.

Methods of selecting transductants: After adsorption, 0.1 ml samples of the mixture were plated. When selection was to be made without streptomycin, the samples were spread on the surface of nutrient agar. When selection was to be made with streptomycin, on the other hand, each sample was mixed with 20 ml of melted nutrient agar in a Petri dish, and incubated at 37°C as soon as possible after hardening. Usually after three hours incubation, to allow the bacteria to undergo several divisions, two ml of nutrient soft agar containing 550 μg/ml of streptomycin was added, and the plate was reincubated. The final concentration after complete diffusion of the streptomycin was 50 μg/ml. Transductants were scored after three or four days of incubation.

Media: Nutrient agar: NaCl, 1 gm; dehydrated nutrient agar, 23 gm; H_2O, 1 liter.

Soft nutrient agar: NaCl, 5 gm; dehydrated nutrient agar, 11 gm; H_2O, 1 liter.

Enriched minimal agar: K_2HPO_4, 10.5 gm; KH_2PO_4, 4.5 gm; sodium citrate $5H_2O$, 0.47 gm; $MgSO_4$ anhydrous, 0.05 gm; $(NH_4)_2SO_4$, 1.0 gm; dehydrated nutrient broth, 0.01%; glucose, 0.2%; agar, 1.5%; H_2O, 1 liter.

L broth: tryptone, 1%; yeast extract, 0.5%; NaCl, 0.5%; glucose, 0.1%; pH, 7.0. (LENNOX 1955.)

Modified tris glucose medium: HERSHEY's tris glucose medium (1955) modified to contain 2.5×10^{-3} M $CaCl_2$.

Eluting fluid: NaCl, 0.3%; peptone, 0.1%.

Streptomycin: streptomycin sulfate.

EXPERIMENTAL RESULTS

Transduction of str-d, str-r, *and* str-s: It was noticed early in these studies that bacteria which acquire either *str-r* or *str-d* by transduction have to undergo several divisions before becoming resistant to streptomycin; that is, several divisions are required for phenotypic expression. Table 2 gives the results of one experiment designed to discover whether streptomycin dependence could be transferred by transduction, and to determine the most suitable conditions for detecting *str-d* transductants. The recipient bacteria were sensitive to streptomycin, and the donor bacteria carried the *str-d-77* marker. Plates containing the infected recipient cells were incubated for 0, 1, 3, 5, or 7 hours before streptomycin was added. The results show that *str-s* was transduced to *str-d-77*, and

TABLE 2

Delayed appearance of str-d *transductants. Number of* str-d *transductants obtained when streptomycin was added at different times after incubation of the bacteria*

	Streptomycin added after (hours)				
	0	1	3	5	7
str-s (x) *str-d-77*	0	3	53	29	0
Control	0	0	1	1.5	0

TABLE 3

Transduction experiments with str-d, str-r, *and* str-s *markers*

Recipient	Donor	Colonies per plate*	
		Treated	Control
str-s	*str-d-4*	58	1
"	*-5*	142.3	1
"	*-62*	18	1
"	*-67*	29	1
"	*-74*	380	1
"	*-77*	223.1	0.9
"	*str-r-2*	50.5	0.5
"	*-3*	116	0.5
str-d-1	*str-s*	12	3
-4	"	36	9
-5	"	241	26
-67	"	264	22
-77	"	307	35
-78	"	266	141

* Average from two to ten plates.

that the largest number of transductants was recovered after a 3-hour incubation. Presumably the shorter incubation period was not sufficient for phenotypic expression of the dependence, and longer periods allowed the background growth of sensitive bacteria to become so heavy that a considerable proportion of transductant cells were not able to form colonies. A similar method was found effective for the transfer of resistance from *str-r* donors to sensitive recipients (see Table 3).

It is possible to detect transduction to *str-s* in *str-d* recipients. In this case the phage infected bacteria are plated on medium lacking streptomycin, where non-transductant (*str-d*) cells can undergo only a few divisions but transductants, as well as "revertants," are able to form colonies. The number of divisions undergone by *str-d* bacteria in the absence of streptomycin (residual growth; BERTANI 1951b), is characteristic for each independently originating *str-d* mutant. The data of Table 3 show that *str-s* transductants were detected when any one of six *str-d* strains was the recipient. Similar experiments with *str-d-3*, *-62*, *-66*, *-69*, *-70*, *-71*, *-72*, *-73*, and *-74* as recipients, however, gave negative results. Ap-

parently the residual growth of these strains is not sufficient to permit the expression of *str-s*. Even in some of the positive experiments the number of *str-s* transductants was small, presumably because of inadequate residual growth of the recipient bacteria. Best results were obtained with *str-d-5* and *str-d-77*, and these two strains were the ones mainly used in subsequent experiments.

Transduction between str-d *mutants:* If *str-d* mutants of independent origin were the result of mutations at different, unlinked loci that could not be carried in the same transducing fragment, then transduction experiments between two such *str-d* mutants would be expected to produce as many *str-s* recombinants as experiments between the same *str-d* recipient and an *str-s* donor. On the other hand, if two *str-d* mutants were identical alleles, no recombinants would appear; and if they were either nonidentical alleles or the result of mutations at two closely linked loci, a few recombinants would be expected, the number depending on the chromosomal distance between the two markers.

Extensive experiments with *str-d-4*, *-5*, *-62*, *-67*, *-74*, and *-77* produced no detectable recombinants, thus eliminating the possibility that these mutants represent loci carried in different transducing fragments. The available method, however, was not sensitive enough to detect recombinants occurring in small numbers, as might be expected in experiments involving nonidentical alleles. Thus the results of these tests are not definitive but make it seem probable that one locus determines streptomycin dependence.

Transduction between str-d *and* str-r *mutants:* Results to be expected in experiments with *str-d* as recipient and *str-r* as donor would be quite different, depending on whether these markers are closely linked (carried in one transducing fragment) or are not closely linked. A donor marker can be recovered only in the case of close linkage, so that if *str-d* and *str-r* were not linked the results of such experiments would be similar to those obtained with the wild type (*str-s*) instead of *str-r* as donor. Table 4 shows the results of experiments in which *str-d-77* was the recipient and either *str-r-3*, *str-r-2*, or wild type the donor. Of 1826 tested transductants from an experiment in which the wild type was donor, 1825 were

TABLE 4

Results of transduction experiments with str-d-77 *as recipient and* str-r *or* str-s *mutant as donor*

	Donor			Control
	str-r-3	*str-r-2*	Wild type	
Number of experiments	3	2	1	2
Number of plates	53	40	10	40
Transductant colonies:				
Total	7658	4642	2231	1135
Tested	7113	4460	1826	930
Highly resistant	7053	4212	1	71
Slightly resistant	57	232	0	846
Sensitive: Total	3	16	1825	13
Wild type	3	4	not tested	0

sensitive, presumably wild type, and only one was resistant, presumably the result of a "reversion" in a recipient cell. In the experiments with *str-r* as donor, on the other hand, only a minute fraction of transductant colonies (three out of 7113 and four out of 4460) were wild type; the great majority were resistant, representing the donor type. Therefore, the results show conclusively that *str-d-77*, *str-r-2*, and *str-r-3* are closely linked.

The control data in Table 4 show the proportions of highly resistant, slightly resistant, and sensitive "revertants" when *str-d-77* bacteria were grown without transducing phage. It is evident that by far the largest number of the revertants (about 91%) were resistant to a low concentration of streptomycin (5 μg/ml) but not to a high concentration (1000 μg/ml); whereas a very few (about 1.4%) were sensitive to streptomycin, and the rest (about 7.6%) were resistant to a high concentration. In the experiments with *str-r-2* and *str-r-3*, most of the tested colonies were highly resistant to streptomycin; and, judging by the control data, a large majority of these represented transduction by the donor marker, only a small fraction being highly resistant "revertants" from *str-d-77*. All low-resistance types were presumably due to "reversions." Sensitive types could have originated either by "reversion" or by recombination between *str-d-77* and *str-r*. Sensitive "revertants" still carry the *str-d* gene, as demonstrated by results described later in this paper; and so it is possible to distinguish them from recombinants by transduction tests. Among the 19 sensitive colonies obtained in these experiments, seven were found to be wild type—and thus recombinants—whereas the other 12 were "revertants." So it is clear that recombination occurs between *str-d-77* bacteria and both *str-r-2* and *str-r-3*, but that the frequency of recombinants is very low—four in about 4000 in the case of *str-r-2* and three in about 7000 in the case of *str-r-3*.

Similar experiments were made with *str-d-5* as recipient and *str-r-2*, *str-r-3*, and *str-s* as donors. The results show conclusively that *str-d-5* is also very closely linked with the *str-r* markers. Recombinants were not obtained among 745 colonies tested in the experiments with *str-r-3* as donor, nor among 266 colonies in the *str-r-2* experiments.

Genetic analysis of str-d *"revertants":* The streptomycin-sensitive "revertants" from *str-d* were analyzed to determine whether they were due to reverse mutation at the same locus as the original change that gave rise to *str-d*, or to mutation at a suppressor locus, which modified the expression of the *str-d* mutant gene. If "revertants" were caused by reverse mutations, they could be expected to have the wild type genotype; whereas if they resulted from the action of a suppressor, they should still carry the original *str-d* gene and in addition have a new mutant gene at some other locus.

The genotype of the sensitive *str-d* "revertants" could readily be determined by transduction experiments in which they were the recipients and sensitive wild type bacteria were the donors. The infected cells were grown in medium containing a high concentration of streptomycin. In the event that the revertants had the true wild type genotype, only a few streptomycin-resistant colonies

could be expected—the result of mutations from sensitivity to resistance among the recipient bacteria. They should not exceed the number found on control plates containing sensitive "revertants" on streptomycin medium without transducing phage. On the other hand, if the "revertant" recipients carried the original *str-d* gene plus a mutation at a suppressor locus, all recombinations involving the suppressor locus would give rise to the *str-d* phenotype, and many streptomycin-resistant colonies having the properties of the original *str-d* mutant colonies would appear.

All together, 42 "revertants" were tested—30 obtained from *str-d-77* and 12 from *str-d-5*. Seventeen of them were sensitive to streptomycin and the other 25 were resistant to a low concentration. In every case, many *str-d* colonies appeared, showing conclusively that suppressor mutations had been responsible for the "revertants."

Structure of the suppressor locus: Experiments were made to determine whether the suppressor mutations involved one gene locus or more than one, and whether—in case only one was involved—recombination could be detected between mutants of independent origin. Reciprocal transduction tests were carried out with ten sensitive *str-d-77* suppressor mutants (*su-6-str*, *su-7-str*, *su-8-str*, *su-10-str*, *su-11-str*, *su-13-str*, *su-14-str*, *su-15-str*, *su-16-str*, *su-18-str*) and the wild type streptomycin-sensitive strain (Table 5). The data from the tests with wild type as recipient and suppressor (*su-str*) strains as donors provide information about the relative frequencies of transfer of the *str-d-77* marker by phage from the different strains. The data of the experiments with wild type as donor indicate the relative frequencies of transfer of the (*su-str*)$^+$ marker to the differ-

TABLE 5

Results of reciprocal transduction experiments between streptomycin-sensitive suppressor mutants (su-6-str, su-7-str, *etc.*) *derived from* str-d-77 *bacteria. (Number of streptomycin-dependent colonies found on plates containing 50 μg per ml of streptomycin)*

Recipient	Control	Donor										
		Wild type	*su-6*	*-7*	*-8*	*-10*	*-11*	*-13*	*-14*	*-15*	*-16*	*-18*
Wild type	2	1	161	326	177	161	406	490	113	139	65	243
su-6	0	80	0	3	0	0	1	0	0	0	0	3
-7	3	448	22	1	16	11	25	19	14	11	11	0
-8	0	137	1	2	0	0	3	0	0	0	0	8
-10	0	87	0	7	0	0	1	0	0	0	0	1
-11	0	182	2	12	1	1	0	3	1	0	2	14
-13	0	151	0	11	0	0	4	1	0	0	0	11
-14	0	126	0	12	0	0	0	0	0	2	0	6
-15	1	169	2	11	0	3	4	4	2	3	2	11
-16	0	52	0	5	0	1	0	0	0	0	1	7
-18	1	116	1	0	1	0	2	6	2	2	0	0

The data for wild type as recipient (top row) indicate the relative potencies of the donors in transduction of the *str-d-77* marker; and the data for wild type as donor (third column) indicate the relative potencies of the recipients in transduction of the *su-str* marker.

ent suppressor recipients. The "control" column indicates the number of spontaneous back mutations of the suppressor gene.

If two independent suppressor mutants represented mutations at two different gene loci, not closely linked, reciprocal transduction experiments between them would be expected to produce approximately the same numbers of dependent recombinants as would experiments between either one of them and a wild type donor. On the other hand, the numbers of recombinants would be smaller if the mutants were nonidentical alleles, or the result of changes at closely linked loci; and if they were identical alleles, no recombinants would be expected.

The data of the table show unmistakably that the ten suppressor mutants had originated through mutations either at one gene locus or at very closely linked loci. Since recombination between the different suppressor mutants either did not occur or was detected with very low frequency, the more likely possibility seems to be that one gene locus was involved and that the mutants were allelic. If this is so, the data demonstrate that recombination occurs between at least some alleles. No conclusions can be drawn, from these experiments, as to which combinations are unable to produce any recombinants, for the data are not adequate. Only three plates were used per experiment; and in addition the efficiency of transduction of the recipient bacteria was very low as indicated by the tests in which the wild type was donor. According to those tests, the bacterial culture of *su-7* showed the highest frequencies of transduction; and a large proportion of the tests of *su-7* with other donors produced detectible recombinants.

Two of these ten streptomycin-sensitive suppressor mutants were also tested with six "revertants" of *str-d-77* that were resistant to low concentrations of streptomycin (growing on about 5 μg per ml but not on 50 μg per ml). Results of the tests (Table 6) are similar to those obtained with the sensitive suppressor mutants. They indicate that all 16 of the tested mutants (Tables 5 and 6) carried alleles of the same suppressor locus. The larger numbers of recombinants obtained in the tests with the slightly resistant suppressor mutants were probably due to higher transduction efficiency of the recipient bacteria—indicated by their high frequencies of transduction with wild type donor.

TABLE 6

Results of transduction experiments between slightly resistant suppressor mutants (su-1, su-2, . . .) *derived from* str-d-77 *bacteria and two streptomycin-sensitive suppressor mutants*

Recipient	Control	Donor		
		Wild type	*su-7*	*su-11*
su-1	21	1030	116	51
-2	2	1086	96	3
-4	36	1570	108	281
-24	41	742	31	1
-28	55	785	39	58
-30	35	983	53	118

The third column indicates the relative potencies of the recipients in transduction of the *su-str* marker.

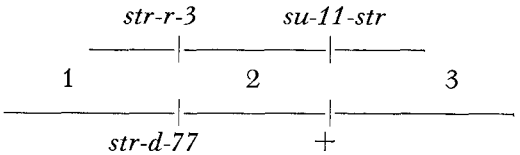
Linkage among str-d, str-r, *and* su-str *loci:* The experiments discussed so far showed that a mutation at a suppressor locus (*su-str*) was responsible for "revertants" among *str-d* bacteria, but they threw no light on the question of whether or not the *su-str* and *str-d* loci are close enough together to be carried in one transducing fragment. Experiments were designed especially to answer that question.

First a transduction test was made between recipient bacteria carrying both streptomycin dependence and a suppressor mutation, *str-d-77 su-11-str*, and donor bacteria carrying streptomycin resistance and no suppressor, *str-r-3* (*su-str*)$^+$. Analysis of the colonies that appeared on transduction plates showed that three classes were represented: (1) resistant to a high concentration of streptomycin; (2) resistant to a low concentration of streptomycin; and (3) dependent on streptomycin. The genotypes of class (1) and class (2) colonies were determined by transduction tests with *str-d-77* (*su-str*)$^+$ as recipient and bacterial cultures from individual colonies as donors. Eight class (1) colonies were tested, and all were *str-r* (*su-str*)$^+$; whereas five class (2) colonies tested were all *str-r su-str*. Thus, in the transduction experiment

str-d-77 su-11-str (x) *str-r-3* (*su-str*)$^+$

the donor genotype was recovered, *str-r* (*su-str*)$^+$, as well as two recombinants, *str-r su-str* and *str-d* (*su-str*)$^+$. Since a donor genotype representing two markers can be recovered only if both markers are carried in the same transducing fragment, these results show that the *str* and *su-str* loci are closely linked. They also reveal that *su-11-str* acts on *str-r-3* as well as on *str-d-77*. Bacteria of the genetic constitution *str-r-3 su-11-str* are resistant only to a low concentration of streptomycin.

In order to discover the approximate distance between the *str* and *su-str* loci, 420 colonies from an experiment *str-d-77* (*su-str*)$^+$ (x) *str-r-3 su-str*, selected on medium containing no streptomycin, were picked at random, and their genotypes were determined by tests of their ability to grow on plates containing no streptomycin, 50 μg of streptomycin, or 1000 μg of streptomycin. Eighty-three of the colonies, or 19.7 percent, were *str-r su-str*$^+$ (class A); 286, or 68.0 percent, were *str-r su-str* (class B); 50, or 11.9 percent, were *str-d su-str* (class C); and one was probably of mutational origin. This experiment can be illustrated by the following diagram:



The lower line represents the recipient bacterial chromosome and the upper line the transducing fragment. The numbers are inserted to designate the sections of chromosome lying between and beyond the loci concerned. Class A recombinants are produced by recombinations involving regions 1 and 2; class B

involve regions 1 and 3; and class C, regions 2 and 3. The fact that class B was considerably larger than either A or C indicates very close linkage between the *str* and *su-str* loci.

Specificity of su-str: Evidence already presented shows that *su-11-str* affects both *str-d-77* (dependence) and *str-r-3* (resistance). In addition, tests of *su-11-str* with *str-d-5* and *str-d-67* mutants indicated that these genes were affected in the same way as *str-d-77*. Therefore *su-11-str* is not allele specific.

It is interesting to note that *su-11-str* with either *str-d-5*, *str-d-67*, or *str-d-77* produced a streptomycin-sensitive phenotype, whereas with *str-r-3* it produced a phenotype resistant to low concentrations of streptomycin.

DISCUSSION

Results obtained in these transduction experiments with strain B/r of *E. coli* support a previous conclusion, based on the evidence of recombination experiments with strain K-12 (DEMEREC *et al.* 1949; NEWCOMBE and NYHOLM 1950), that resistance to high concentrations of streptomycin and dependence on streptomycin are controlled by alleles of the same gene locus. Genetic control of low, multistep resistance in *E. coli* has not yet been investigated, and consequently we do not know the relation between the gene governing one-step resistance and the gene or genes governing multistep resistance.

The fact that no recombinants were observed in transduction experiments with six streptomycin-dependent mutants indicates either that these *str-d* mutations occurred at very closely adjacent sites or, more probably, that they are multisite changes. It should be pointed out, however, that the technique for detecting recombinants in the streptomycin-resistance system is considerably less sensitive than the technique employed with auxotrophs.

Similar tests involving one *str-d* mutant as recipient and two *str-r* strains as donors produced very few recombinants and thus indicated a very close association also between *str-d* and *str-r* mutational sites.

It is well known that streptomycin-dependent bacteria may mutate to nondependent types, which are either sensitive or resistant to streptomycin. This mutability from dependence to nondependence has been put to extensive use in testing the comparative mutagenicity of various agents (DEMEREC 1951; DEMEREC, BERTANI, and FLINT 1951; DEMEREC and FLINT 1951). The recent development of a method for applying transduction techniques to *E. coli* made it possible to analyze the nondependent mutants genetically, and to determine whether the "reversions" are due to back mutation at the *str-d* locus or to changes in some other part of the genome. The results have been reported in this paper. They reveal that the "revertants" do not originate through true reversions at the *str-d* locus but are the consequence of independent mutations at another locus, which suppress the activity of *str-d*. These suppressor mutations are evidently not specific, but affect the expression of both *str-d* and *str-r* genes.

Sixteen suppressor mutants—all that were tested—proved to be allelic, an indication that only one gene locus is involved in suppression. Furthermore, it

appears that the suppressor locus and the locus governing resistance and dependence are close enough to each other to be transduced together.

Thus the results of these studies as a whole reveal that the genetic control of high streptomycin resistance and dependence in *E. coli* resides within a short region of chromosome, which also contains a genetic mechanism for control of the degree of resistance; and that reversions from dependence, although not due to changes in the *str-d* gene, are the result of mutations from the wild type at one single locus which is closely associated with the *str-d* locus.

SUMMARY

Genetic analyses have been made of streptomycin dependence (*str-d*), resistance (*str-r*), and sensitivity (*str-s*), as well as "reversion" from dependence, in *Escherichia coli* strain B/r by means of transduction experiments utilizing P1 phage. The results can be summarized as follows: (1) The *str-d* and *str-r* markers are transferable by transduction to *str-s* bacteria. (2) The *str-s* marker can be transferred to *str-d* bacteria. (3) In reciprocal experiments with six *str-d* mutants of independent origin, no recombinants appeared, an indication that all of them resulted from mutations at a single gene locus. (4) Experiments with two *str-d* mutants as recipients and two *str-r* mutants as donors indicated that *str-d* and *str-r* are nonidentical alleles, occupying very closely linked sites; for only a small number of streptomycin-sensitive recombinants appeared. (5) Analysis of 42 "revertants" derived from two *str-d* strains proved that all of them still carried the mutant *str-d* gene. In other words, mutation at another (suppressor) locus is responsible for "reversion" to nondependence. (6) Ten streptomycin-sensitive and six slightly resistant "revertants" were tested by reciprocal transduction for allelism of the suppressor mutations. The data indicate that all these *su-str* mutants resulted from an allelic series of changes at one locus. Several nonidentical alleles have been identified. (7) There is evidence of close linkage between the suppressor locus and the locus governing resistance and dependence. (8) There is evidence that the suppressor mutations are not allele specific. They affect the expression of both *str-d* and *str-r*.

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