THE FINE STRUCTURE OF THE LOCI tryC AND tryD OF SALMONELLA TYPHIMURIUM. I. DETERMINATION OF THE ORDER OF MUTATIONAL SITES BY THREE-POINT TRANSDUCTION TESTS[1]

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Received December 1, 1961

DEMEREC and HARTMAN (1956) have shown that four loci controlling successive steps in the biosynthesis of tryptophan in *Salmonella typhimurium* are arranged linearly and sequentially on the bacterial chromosome, and are closely linked in transduction tests. YANOFSKY and LENNOX (1959) found that this is also true in *Escherichia coli*, and recently ANAGNASTOPOULOS and CRAWFORD (1961) showed that a tryptophan cluster exists in *Bacillus subtilis*.

Mutants belonging to each of the four loci *tryA*, *tryB*, *tryC*, and *tryD* of Salmonella have been characterized by their ability to utilize certain intermediates in the tryptophan pathway, as well as by their accumulation products (BRENNER 1955; DEMEREC and HARTMAN 1956). From the results of three-point tests in which a *cysB* mutant, linked in transducing fragments to the tryptophan cluster, served as an unselected marker, DEMEREC and HARTMAN concluded that the order of the five loci was *tryD*, *tryC*, *tryB*, *tryA*, *cysB*. YANOFSKY and LENNOX (1959) obtained a very similar sequence with the corresponding auxotrophs of *E. coli*, except that *tryC* and *tryD* appeared in reverse order.

Recent biochemical and genetic studies with *E. coli* (see YANOFSKY 1960 for review) have shown that the last step in tryptophan synthesis takes place through the conversion of indole glycerol phosphate to tryptophan, and that the reaction is carried out by the enzyme tryptophan synthetase. In *E. coli* this enzyme was found to consist of two dissociable components, A and B, each controlled by one of two adjacent loci. As far as has been determined, there is perfect correspondence between the nutritional requirements and the accumulation products of the *E. coli* A and B mutants and the Salmonella *tryC* and *tryD* mutants. Furthermore, YURA (in DEMEREC *et al.* 1956) found that *tryD* mutants of Salmonella were deficient in tryptophan synthetase activity. These facts indicate the likelihood that the *tryC* and *tryD* loci of Salmonella determine the specificities

[1] Aided by a grant to DR. M. DEMEREC from the American Cancer Society.

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of two separable components of tryptophan synthetase, A and B respectively, in this organism.

The studies reported here were primarily concerned with the ordering of mutational sites within the loci *tryC* and *tryD* of Salmonella by means of three-point transduction tests. Preliminary data bearing on this question had already been obtained by DEMEREC and HARTMAN (1956). The availability of additional mutant strains and of stocks carrying *C* or *D* markers in combination with others very closely linked such as *tryA* and *tryB*, has made possible more extensive testing and a more accurate determination both of the position of each mutant site within a locus and of the relative order of the *tryC* and *tryD* loci. As will be seen, the new data show that this order is identical with the one determined by YANOFSKY and LENNOX (1959) for the corresponding loci in *E. coli*.

The results of complementation tests by means of abortive transduction (OZEKI 1956) between all available *tryC* and *tryD* mutants are also included.

MATERIALS AND METHODS

Bacterial strains: The methods for isolating and classifying tryptophanless mutants are described by DEMEREC and HARTMAN (1956). To date, 23 mutants belonging to the loci *tryC* and *tryD* have been mapped and analyzed. On the basis of nutritional requirements and ability to feed *tryB* and *tryA* mutants (whose requirements can be satisfied respectively by indole and by indole or anthranilic acid) 17 of the 23 were classified as *tryD* and six as *tryC*. One additional mutant, *try-43*, which according to its nutritional requirements was first classified as *tryD*, was later found in recombination experiments to be a multisite mutant, probably the result of a large deletion covering loci *tryC*, *tryD*, and at least part of *tryB*. The growth requirements and feeding properties of the 24 mutants (including *try-43*), as well as their derivations, are summarized in Table 1. All mutants belong to the collection of DR. M. DEMEREC. In addition to the single-mutant strains listed in Table 1, several doubly marked strains were utilized. With the exceptions of the double mutants *tryB-4 cysB-12* and *tryD-10 tryB-4*, which were the ones employed by DEMEREC, GOLDMAN and LAHR (1958), and *tryD-10 cysB-12*, which was used by DEMEREC and HARTMAN (1956), these strains were obtained specifically for these three-point tests. Their origins are described below.

Several of the mutant markers (see Table 1) were isolated in the genetic background of strain LT-7 carrying a mutator gene *mut* (MIYAKE 1960). Most of them showed a very high spontaneous reversion rate in the presence of *mut* and were consequently transferred by transduction into the background of strain LT-2, carrying the normal allele mut^+. In these cases a recipient strain of genotype *cysB-12* or *tryB-4 cysB-12* was transduced with the desired marker and selection was made on tryptophan agar. The method followed in obtaining sensitive recombinants for a desired marker after transduction is described below.

Nomenclature: The system of nomenclature for the designation of bacterial mutants is the one given in Microbial Genetics Bulletin, No. 6 (1958). Since in

TABLE 1

Growth requirements and feeding responses of tryC *and* tryD *mutants*

Locus	Mutant	Strain	Origin	Growth requirement			Feeding	
				Anth.	Ind.	Try.	A-8	B-4
C	71	LT-2	2-AP	—	+—	+	—	—
	3	LT-2	UV	—	+	+	—	—
	48	LT-7*	Sp.	—	+—	+	—	—
	20	LT-2	Sp.	—	+—	+	—	—
	33	LT-7†	Sp.(*mut*)	—	+	+	—	—
	44	LT-7*	Sp.	—	+—	+	—	—
D	1	LT-2	UV	—	—	+	+	+
	10	LT-2	Sp.	—	—	+	+	+
	11	LT-2	Sp.	—	—	+	+	+
	35	LT-7*	Sp.	—	—	+	+	+
	73	LT-2	2-AP	—	—	+	+	+
	76	LT-2	2-AP	—	—	+	+	+
	79	LT-2	2-AP	—	—	+	+	+
	9	LT-2	Sp.	—	—	+	—	—
	6	LT-2	Sp.	—	—	+	—	—
	7	LT-7	UV	—	—	+	+	+
	66	LT-7†	Sp.(*mut*)	—	—	+	+	+
	70	LT-7†	Sp.(*mut*)	—	—	+	+	+
	29	LT-7†	Sp.(*mut*)	—	—	+	+	+
	42	LT-7*	Sp.	—	—	+	+	+
	55	LT-7*	Sp.	—	—	+	—	—
	69	LT-7†	Sp.(*mut*)	—	—	+	+	+
	78	LT-2	UV	—	—	+	—	—
	43 Del.	LT-7*	Sp.	—	—	+	—	—

* LT-7 strain containing a proline segment from strain LT-2.
† Marker transferred by transduction from LT-7 *mut* to strain LT-2, which carries the *mut*+ allele.
The strains used as testers in feeding tests were the mutants *tryB-4*, which utilize indole, and *tryA-8*, which utilizes both indole and anthranilic acid.
+ =Normal growth, as determined by colony size. +— =Slow growth, as determined by colony size. — =No growth.
Symbols: Sp=spontaneous; UV=ultraviolet radiation; 2-AP=2-aminopurine.

this paper we are dealing almost exclusively with tryptophan mutants, we will refer to them for the sake of convenience simply by their locus designation and isolation number, whenever this does not interfere with clarity.

Phage stocks: Transducing phage PLT-22 was used routinely in recombination experiments. A mutant of PLT-22, H4, was employed for transferring desired markers into different genetic backgrounds and for synthesizing double-mutant strains. H4 lysogenizes less efficiently than PLT-22, and is useful when sensitive transductants are desired. A virulent mutant of PLT-22, H5, was used in streaking to test for lysogenicity. Phage suspensions were prepared, assayed, and stored as described by HARTMAN (1956).

Media: Nutrient broth (Difco) served as a routine complex medium. The minimal salts-glucose medium of the Cold Spring Harbor laboratory (see HARTMAN 1956) was employed, with the addition of proper supplements when necessary (20 μg/ml). Enriched minimal medium (EM medium) consisted of the salts-glucose medium plus 1.25% (v/v) liquid Difco nutrient broth. This also was supplemented with specific nutrients when required.

Transduction experiments: Bacteria of mutant strains desired as recipients were grown overnight in nutrient broth at 37°C with forced aeration (2–4 × 10^9 colony forming units/ml); overnight cultures were used in all the experiments reported. Samples of the bacterial cultures were placed in Wasserman tubes with equal volumes of properly diluted phage suspension (multiplicity of infection between 5 and 10). After incubation of the mixture at 37°C for five minutes to allow adsorption, samples of 0.2 ml per plate (about 2–4 × 10^8 infected bacteria) were uniformly spread on appropriately selective EM agar. At least three simultaneous platings were made from each mixture, and in some experiments four or six platings. After about 36 hours incubation, the bacteria were replica-plated (LEDERBERG and LEDERBERG 1952) simultaneously on minimal agar and on fresh plates of the original selective agar. The replica plates were incubated overnight and scored. Control platings made were with bacteria infected with homologous phage and also with uninfected bacteria plus T2 buffer. Final values were corrected for the occurrence of spontaneous reversions as observed on the control plates.

Abortive-transduction test for complementation: Complementation was determined by the spot-test technique of HARTMAN, HARTMAN and SERMAN (1960). Recipient bacteria were spread evenly on minimal agar (about 10^8 per plate) and spotted with a drop of a suspension of transducing phage (about 5 × 10^9 phage particles per ml). The number of phage spots was limited to four per plate. The plates were incubated at 37°C and examined every 24 hours for two or three days under a low-power binocular dissecting microscope by transmitted light for the presence of minute colonies characteristic of abortive transduction (OZEKI 1956).

Origin of double-mutant strains: Special strains carrying a *tryD* or *tryC* mutant allele linked to an outside nonselective marker (*tryB-4* or *tryA-8*) were obtained by transduction with phage H4. After selection for the desired recombinants, sensitive colonies were recovered by single-colony isolation from a nutrient agar plate that had been spread with cells from a broth culture or liquid suspension. A number of these single colonies were isolated, cultured overnight in one ml of broth, and then tested for lysogenicity, either by spotting on soft agar seeded with a sensitive indicator strain or by streaking against the virulent mutant H5. Sensitive isolates were retested by spotting on seeded soft agar.

The *tryD tryB-4* strains carrying the *D* markers *D-9*, *D-7*, *D-66*, *D-29*, and *D-42* were obtained by transducing as recipient the double mutant *tryB-4 cysB-12* with H4 phage grown on each of the *tryD* strains and selecting for $cysB^+$ progeny on tryptophan plates. The *tryD-10 tryA-8* strain was obtained by transferring the *tryA-8* marker to the double-mutant recipient *tryD-10 cysB-12* and selecting recombinants on tryptophan. All available *tryC* mutants (see Table 1) were coupled to *tryA-8* by introducing each *C* marker into the *tryD-10 tryA-8* genotype (see above) and selecting on indole agar. After replica plating on properly supplemented agar, the double mutants of genotypes *D B-4* or *C A-8* were distinguished from the *D* B^+ or *C* A^+ recombinants by feeding tests, where possible, and also by transduction spot tests on EM agar with phage carrying the

markers *tryB-4* or *tryA-8* as required. In all cases the identification of the genotype of each strain was subsequently confirmed by genetic tests.

RESULTS

Characterization of the mutant strains: All the *tryC* mutants isolated so far can grow on indole-supplemented medium, whereas the *tryD* mutants respond only to tryptophan supplementation. As shown in Table 1, some of the *tryD* mutants feed indole-utilizing mutants, indicating indole accumulation. Other *tryD* mutants cannot feed the same indole utilizers, and presumably accumulate indole glycerol rather than indole. As judged by colony size on indole agar, some of the *tryC* mutants grow normally on indole but others grow slowly. Mutants corresponding to these four types have also been found in *E. coli* (YANOFSKY and CRAWFORD 1959; YANOFSKY 1960) and their properties have been correlated with the presence or absence of altered enzyme component (CRM), which has immunological properties similar to the normal enzyme but shows little or no enzymatic activity. In the auxotrophs corresponding to our *tryC* mutants, the altered protein is component A; in those corresponding to our *tryD* mutants, the alteration is in component B. Biochemical and immunological studies of the Salmonella mutants are in progress.

Determination of site order: The procedure is outlined in Figure 1. The position of each mutational site was determined by observing the frequency of appear-

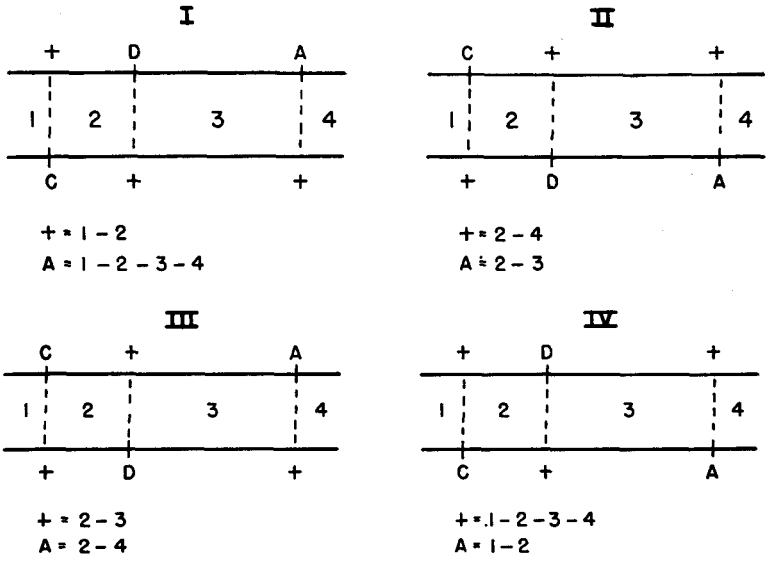

FIGURE 1.—Diagrams illustrating the method of determining the positions of mutational sites. *C* here represents the outside marker, *D* the inside. *A* is the unselected linked marker, in our experiments either *B-4* or *A-8* (see text). In each diagram, the top line represents the donor fragment (introduced by phage), the bottom line the homologous section of the recipient chromosome.

ance of a linked, unselected marker among recombinants resulting from transduction between the unknown and a tester marker. For convenience, we will refer to the double-mutant strains as tester strains, and to the *C* or *D* markers in those strains as tester markers. Mapping of the *D* locus was begun first, with *tryB-4* as the unselected marker and indole agar as the selective medium. In mapping *tryC* mutants, however, since they are able to grow on indole, *tryB-4* was replaced by *tryA-8*, which can utilize anthranilic acid whereas *tryC* mutants cannot.

As illustrated in Figure 1, the relative positions of the two mutant sites to be ordered can be determined from the frequencies of the two recombinant classes. For example, in cross I, D^+A recombinants will be in the minority because they are the result of a quadruple exchange in regions 1–2–3–4 and D^+A^+ recombinants are due to a double exchange in regions 1–2 (only the products of even exchanges can be recovered in transduction). In cross IV, on the other hand, where the combination of markers has been reversed, D^+A recombinants are expected to be in the majority and D^+A^+ recombinants in the minority, because the former result from a double 1–2 exchange and the latter from a quadruple 1–2–3–4 exchange. In crosses II and III, one compares the frequencies of classes resulting from double exchanges in regions 2–3 and 2–4, and these will depend on the relative sizes of regions 3 and 4. If 4 is longer than 3, the results of reciprocal crosses in which the marker combinations are the same but the donor-recipient relations are reversed (i.e., crosses I-II, and III-IV) will show the same majority and minority recombinants. This will not be the case if region 3 is longer than region 4. The first situation is experimentally desirable, since it eliminates the ambiguity arising from discrepant reciprocal crosses; and that is why *tryA* and *tryB* were chosen as unselected linked markers, rather than *cysB*, for they are much closer to the *tryC* and *tryD* loci (DEMEREC and HARTMAN 1956). As can be seen from Tables 2–5 the results of reciprocal crosses are now in agreement (aside from a few exceptions, which are discussed in a separate paper (BALBINDER 1962)). In a few preliminary experiments with *cysB* as the unselected linked marker, on the other hand, reciprocal crosses produced discrepant results.

Table 2 gives the results of a number of reciprocal three-point transductions between different *tryD* and *tryC* mutants in all possible combinations. It is evident that the results of four independent crosses between any two mutants are consistent with only one possible ordering of these mutants with respect to each other. In general:

(1) For the same combination of markers, the same relation of majority to minority classes obtains in reciprocal crosses, regardless of which is the donor and which the recipient;

(2) In crosses between a given pair of mutants, the frequencies of the two recombinant classes are reversed when the coupling of the mutants to the linked unselected marker is reversed.

In crosses between *tryD* mutants and tester strains *tryD tryB-4*, the majority class is seldom below 70–80 percent and the minority class seldom above 20–30 percent, except in special cases (BALBINDER 1962). These relative proportions

TABLE 2

Frequencies of D⁺B *and* D⁺B⁺ *recombinants in crosses between different* D *mutants, with* B-4 *as the unselected linked marker; and frequencies of* C⁺A *and* C⁺A⁺ *recombinants in crosses between different* C *mutants, with* A-8 *as unselected linked marker*

Cross R×D*	Colonies scored*	*D⁺B* or *C⁺A* Percent	Exchange	*D⁺B⁺* or *C⁺A⁺* Percent	Exchange
D-10 × *D-9 B-4*	93	14.0	1–2–3–4	86.0	1–2
D-9 B-4 × *D-10*	23	8.7	2–3	91.3	2–4
D-9 × *D-10 B-4*	25	84.0	2–4	16.0	2–3
D-10 B-4 × *D-9*	18	100.0	1–2	0.0	1–2–3–4
D-10 × *D-66 B-4*	95	5.3	1–2–3–4	94.7	1–2
D-66 B-4 × *D-10*	187	13.4	2–3	86.6	2–4
D-66 × *D-10 B-4*	13	100.0	2–4	0.0	2–3
D-10 B-4 × *D-66*	24	95.8	1–2	4.2	1–2–3–4
D-9 × *D-7 B-4*	54	1.8	1–2–3–4	98.2	1–2
D-7 B-4 × *D-9*	24	37.5	2–3	62.5	2–4
D-7 × *D-9 B-4*	20	100.0	2–4	0.0	2–3
D-9 B-4 × *D-7*	58	91.4	1–2	8.6	1–2–3–4
D-9 × *D-66 B-4*	58	8.6	1–2–3–4	91.4	1–2
D-66 B-4 × *D-9*	24	33.3	2–3	66.7	2–4
D-66 × *D-9 B-4*	91	83.5	2–4	16.5	2–3
D-9 B-4 × *D-66*	6	100.0	1–2	0.0	1–2–3–4
C-71 × *C-3A-8*	1023	14.1	1–2–3–4	85.9	1–2
C-3 A-8 × *C-71*	1571	14.7	2–3	85.3	2–4
C-3 × *C-71 A-8*	141	48.2	2–4	51.8	2–3
C-71 A-8 × *C-3*	249	59.8	1–2	40.2	1–2–3–4
C-71 × *C-44 A-8*	139	9.4	1–2–3–4	90.6	1–2
C-44 A-8 × *C-71*	396	29.0	2–3	71.0	2–4
C-44 × *C-71 A-8*	105	68.6	2–4	31.4	2–3
C-71 A-8 × *C-44*	135	88.2	1–2	11.8	1–2–3–4
C-3 × *C-48 A-8*	54	16.7	1–2–3–4	83.3	1–2
C-48 A-8 × *C-3*	77	9.1	2–3	90.9	2–4
C-48 × *C-3 A-8*	329	74.5	2–4	25.5	2–3
C-3 A-8 × *C-48*	55	78.2	1–2	21.8	1–2–3–4
C-48 × *C-33 A-8*	76	19.7	1–2–3–4	80.3	1–2
C-33 A-8 × *C-48*	128	32.8	2–3	67.2	2–4
C-33 × *C-48 A-8*	70	68.6	2–4	31.4	2–3
C-48 A-8 × *C-33*	9	88.8	1–2	11.2	1–2–3–4

* The number of colonies scored is the total obtained on at least three plates (4×10^8 infected bacteria per plate). R = recipient, D = donor.

are not much different when 1–2 exchanges are compared with quadruples and when 2–4 exchanges are compared with 2–3 exchanges. In crosses between *tryC* mutants and *tryC tryA-8* tester strains, however, the ratio of majority to minority recombinants tends to be higher when doubles are compared with quadruples than when 2–4 and 2–3 exchanges are being compared. This was to be expected, since the distance between *C* and *A* must be greater than that between *D* and *B;*

but the differences between majority and minority classes were generally large and clear cut, and allowed for unambiguous positioning.

The results were reproducible upon repetition of the same cross and were not affected by (1) the use of different phage preparations from the same donor strain, (2) by the use of different cultures of the same recipient strain, or (3) by differences in the total number of recombinants obtained. The degree of reproducibility can be inferred from Table 3. In all tables, the data from separate experiments have been pooled.

Whenever it was possible to use a mutant both alone and linked to an unselected marker as a tester, all possible crosses to locate the site unambiguously could be carried out (Table 2). This was done for all *C* mutants, since they were available in combination with *A-8* as well as with A^+, and for some *D* mutants. When double mutants were not available, the position of the single mutant could be determined by reciprocal crosses with all tester strains, since the results of reciprocal crosses were in agreement (see Tables 3 and 4). In the same way, the positioning of all *C* mutants with respect to the *D* mutants could be undertaken on a larger scale than had been possible earlier (DEMEREC and HARTMAN 1956), by means of reciprocal crosses between the *tryC tryA-8* tester strains and the single *D* mutants. Results of several crosses are given in Table 5 (see also Table 4 in BALBINDER 1962).

The map obtained is shown in Figure 2. With the exception of *tryD-1* (which appears from recombination frequencies to be located in the *tryC* region but by physiological characteristics and complementation behavior belongs to the *tryD* locus), all *D* mutants are clearly located between *C* and *A*, giving the order

TABLE 3

Reproducibility of results in three-factor crosses involving tryD *and* tryC *mutants and the unselected linked markers* tryB-4 *and* tryA-8

Cross R×D	Frequency of D^+B/D^+ or C^+A/C^+ in individual expt.				Pooled ratio
D-1 × *D-7 B-4*	No. observed	8/77	9/323	.....	17/400
	Percent	10.4	2.8	.....	4.2
D-7 B-4 × *D-1*	No. observed	5/70	9/213	.....	14/283
	Percent	7.2	4.2	.....	4.9
D-10 × *D-7 B-4*	No. observed	4/14	0/15	7/89	11/118
	Percent	28.6	0	7.9	9.3
D-7 B-4 × *D-10*	No. observed	16/76	6/73	26/200	48/349
	Percent	21.1	8.2	13.0	13.8
C-44 × *C-3 A-8*	No. observed	90/113*	141/176	55/66	286/355
	Percent	79.6	80.0	83.3	80.6
C-3 A-8 × *C-44*	No. observed	15/18	27/33	.....	42/51
	Percent	83.3	81.8	.....	82.3
D-55 × *C-3 A-8*	No. observed	17/21	212/252	.....	229/273
	Percent	80.0	84.1	.....	83.9
D-69 × *C-71 A-8*	No. observed	15/20	236/317	.....	251/337
	Percent	75.0	74.5	.....	74.5

* Donor was *C-3 A-8 cysB-12;* selection on anthranilic-cysteine agar.

TABLE 4

Frequencies of D⁺B *recombinants resulting from reciprocal crosses between different* D *mutants and standard tester strains* D B-4

Mutant	Used as:	Tester strains: *D-10 B-4* D^+B/D^+	*D-10 B-4* Percent	*D-9 B-4* D^+B/D^+	*D-9 B-4* Percent	*D-66 B-4* D^+B/D^+	*D-66 B-4* Percent	*D-7 B-4* D^+B/D^+	*D-7 B-4* Percent
D-1	D	51/212	24.1	15/125	12.0	11/153	7.2	14/283	4.9
	R	9/71	12.7	16/219	7.3	11/372	3.0	17/400	4.2
D-11	D	0	0	30/64	46.9	12/99	12.1	24/161	14.9
	R	0	0	40/100	40.0	2/11	18.2	0/22	0.0
D-73	D	131/146	89.7	0	0	6/38	15.8	2/42	4.8
	R	33/41	80.5	11/28	39.3	13/109	11.9	2/37	5.4
D-6	D	125/134	93.3	6/6	100.0	5/20	25.0	2/36	5.5
	R	28/34	82.4	76/91	83.5	1/14	7.2	2/29	6.9
D-70	D	220/241	91.3	35/37	94.6	5/5	100.0	9/14	64.3
	R	242/287	84.3	180/213	84.5	55/73	75.3	7/7	100.0
D-55	D	118/121	97.5	104/106	98.1	10/11	90.9	56/56	100.0
	R	57/57	100.0	178/187	95.2	62/62	100.0	45/45	100.0
D-78	D	57/62	91.9	16/17	94.1	5/6	83.3	4/5	80.0
	R	0	0	356/403	88.3	172/194	88.7	36/41	87.8

TABLE 5

Relative positions of tryC *and* tryD *loci, as indicated by frequencies of* C⁺D⁺A *recombinants in crosses between different* D *mutants and* C A-8 *testers*

D mutants	Used as:	Tester strains: *C-71 A-8* Cols. scored	*C-71 A-8* Percent *A*	*C-3 A-8* Cols. scored	*C-3 A-8* Percent *A*	*C-48 A-8* Cols. scored	*C-48 A-8* Percent *A*	*C-20 A-8* Cols. scored	*C-20 A-8* Percent *A*	*C-33 A-8* Cols. scored	*C-33 A-8* Percent *A*	*C-44 A-8* Cols. scored	*C-44 A-8* Percent *A*
1	R	561	45.5	499	18.6	584	26.4	631	25.4	364	18.4	331	10.6
	D	196	64.3	173	28.9	153	43.1	235	33.6	222	19.8	177	32.8
76	R	360	69.7	184	52.2	259	61.1	163	60.7	73	57.6	81	58.1
	D	218	84.4	138	76.1	112	91.0	73	75.4	63	71.4	33	60.6
9	R	537	56.2	284	43.7	335	58.8	263	50.2	184	41.9	150	47.3
	D	72	87.5	7	. . .	47	91.5	36	58.3	29	62.1	15	80.0
6	R	374	59.3	160	63.1	190	65.2	189	60.3	120	59.2	93	58.1
	D	484	82.4	159	82.4	217	86.6	89	82.0	69	76.8	28	78.6
7	R	60	80.0	31	71.0	35	85.7	21	81.0	5	. . .	. .	. . .
	D	399	90.0	85	82.4	191	90.1	157	96.0	168	83.3	75	88.0
55	R	362	71.5	273	83.8	330	74.9	221	73.8	64	78.1	80	76.3
	D	100	97.0	34	88.3	50	98.0	25	92.0	49	77.5	22	90.9
69	R	337	74.5	209	74.6	281	78.7	203	76.4	65	80.0	22	100.0
	D	264	94.7	108	89.8	171	92.4	95	96.8	102	91.2	44	97.7
78	R	327	59.3	411	51.5	217	68.2	232	56.5	108	45.4	92	55.4
	D	282	75.9	282	79.4	177	89.9	214	80.8	213	78.4	106	77.4

C-D-B-A cysB instead of *D-C-B-A cysB* as was reported by DEMEREC and HARTMAN (1956). The reasons for the discrepancy will be considered in the discussion. The order indicated here is the same as that found by YANOFSKY and

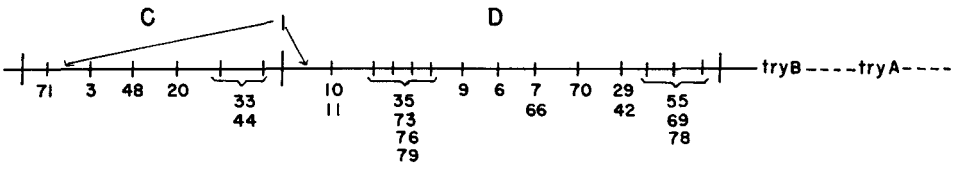

FIGURE 2.—Order of six *tryC* and 17 *tryD* mutant sites in relation to *tryB* and *tryA*. Braces group those mutants that belong in a particular region, but have not been precisely located with respect to one another. Mutants that have been found to share the same site are so indicated.

LENNOX (1959) using a completely different method, for the homologous loci of *E. coli*.

With the exception already mentioned of *D-1*, all *tryD* mutants are grouped together as one unit and *tryC* mutants as a separate unit, as was reported earlier by DEMEREC and HARTMAN for *Salmonella* and by YANOFSKY and LENNOX for *E. coli*. In three cases (*tryD-7* and *-66*, *tryD-29* and *-42*, *tryD-10* and *-11*) two mutants did not recombine with each other although they exhibited recombination with all other mutants in reciprocal two-point and three-point tests, an indication that each pair represents changes at one site within the *D* locus. In each of the first two pairs, the members were similar with respect to spontaneous reversion frequencies, reversion patterns induced by various mutagens (BALBINDER 1962), and responses to specific suppressors; whereas the mutants of the third pair (D-*10* and *-11*) differed in their mutability as well as in their reactions to the same suppressors (DEMEREC *et al.* 1958; BALBINDER, unpublished). DEMEREC and HARTMAN (1956) had also failed to observe any recombination between *D-10* and *D-11*. The exceptional behavior of some of these mutants in recombination is reported separately (BALBINDER 1962).

As Table 2 shows, in crosses between two *D* or two *C* mutants, that is, mutants belonging to the same complementation group (see below), the frequencies of recombinant classes resulting from the same exchanges were generally alike, regardless of genotype: quadruple exchanges gave rise to D^+B^+ and D^+B recombinants in about the same proportions. This finding excludes the possibility that the results were significantly affected by multiple mating events (LENNOX 1955)—which, under these plating conditions, would have given rise to a systematic excess of wild-type recombinants—or by differential survival of recombinants on the plates. Preferential selection of different recombinants also appears unlikely on the basis of other observations. For example, when the same mutant (*D-10*) was available in two different tester strains, linked either to *B-4* or to *A-8*, and both strains were used for determining the positions of all *tryD* mutants in relation to *D-10*, the same order was obtained in each case. Also, when *tryC* mutants were mapped with respect to *C-3*, the same results were obtained regardless of whether the tester strain was the triple mutant *C-3 A-8 cysB-12*, with plating on anthranilic-cysteine agar and subsequent replication on cysteine agar, or the double mutant *C-3 A-8*, with selection on anthranilic agar and replication on minimal agar (see Table 3, cross *C-44* × *C-3 A-8*). The only exceptions seem to be the cross *C-3* × *C-71 A-8* and its reciprocal (Table 2), in which C^+A^+

recombinants were excessive as compared with C^+A-*8* recombinants arising from identical events in the cross *C-71* × *C-3 A-8* and its reciprocal. The reason for this exception is not known, but the results of all crosses between *C-3* and *C-71* are still compatible with only one relative order of these two mutants.

In crosses between *C* and *D* mutants, which were found to complement each other (see below), colonies appeared which failed to replicate on fresh plates of the original selective agar (EM plus anthranilic acid). Work now in progress shows that such colonies contain neither $C^+D^+A^+$ nor C^+D^+A-*8* recombinants, and may be unstable heterogenotes. Since order was always deduced from the relative proportions of these two recombinant types, the "nonprinting" colonies were not included in the final scoring. They were in general quite distinct in appearance from the recombinants scored, and their presence had no effect on the reproducibility of the results (see last two crosses, Table 3). In a few crosses involving specific *C* and *D* markers, an excess of the $C^+D^+A^+$ type was consistently observed. Detailed examination of the progeny of one such cross revealed a high frequency of mixed colonies containing wild-type recombinants. Results of these crosses were not used in mapping. A report covering both mixed and "nonprinting" colonies will be issued separately. The occurrence of a high frequency of mixed colonies in some crosses might explain the results obtained with *D-1*. This possibility has not yet been tested. Another explanation is considered elsewhere (BALBINDER 1962).

Complementation: Reciprocal complementation tests by observation of minute colonies due to abortive transduction (OZEKI 1956) were carried out for all 23 mutants. The results agree with and extend the preliminary findings of YURA (DEMEREC *et al.* 1956) and are consistent with the idea that *tryC* constitutes one complementation group and *tryD* another. All *tryC* mutants complement all *tryD* mutants (including *tryD-1*), and *vice versa.* Intralocus complementation was not observed among the *tryC* mutants, but a larger sample should be studied before any definite conclusions can be reached. In the *tryD* group, *D-70* appeared to give rise to abortive (minute) colonies when employed as recipient in tests with a few other *D* mutants, but in the reciprocal tests the presence of small abortive colonies could not be definitively ascertained because of heavy background growth. The background growth of some of these *tryD* mutants made detection of very small colonies difficult, so that intralocus complementation within the *D* locus may well exist.

Nevertheless, the results agree with a grouping of mutants according to nutritional properties as well as by map position. Similar results have been obtained with regard to the corresponding mutants in *E. coli* (YANOFSKY 1960). The complementation data support the idea that the tryptophan synthetase in Salmonella as in *E. coli*, consists of two dissociable components.

DISCUSSION

The results of three-point transduction tests demonstrate that a series of closely linked mutational sites involved in the control of the enzyme tryptophan synthe-

tase in *Salmonella typhimurium* are arranged in linear order, and that there is an exact correspondence (with the exception of the mutant *D-1*) between the grouping of mutants on the map and their physiological and complementation properties. A similar correspondence has been shown in other systems by similar tests (GROSS and ENGLESBERG 1959; CLOWES 1958) as well as in the entire 4-loci tryptophan cluster of Salmonella (DEMEREC and HARTMAN 1956).

As was pointed out by GROSS and ENGLESBERG, one of the advantages of three-point tests is that results are independent of variations in the absolute yield of recombinants and depend rather on relatively large differences in the proportions of recombinants carrying unselected markers. This fact was amply confirmed in our experiments. Although total numbers of recombinants varied in repetitions of the same cross, the relative proportions of the two recombinant classes carrying unselected markers were quite constant. The method employed in this work was sensitive enough to permit the unambiguous positioning of most mutant sites as well as the detection and characterization of exceptional behavior of other mutants in recombination (BALBINDER 1962). No detectable differences were observed between intrastrain and interstrain (LT-2 or LT-7) crosses.

The preliminary results of DEMEREC and HARTMAN (1956) in examining the order of six *tryD* mutants are in very good agreement with those reported in this paper, although they were obtained by somewhat different methods. The mutants in question are (in their present order) *D-1*, (*D-10*, *D-11*), *D-9*, *D-6*, *D-7*. The only difference between this order and that reported by DEMEREC and HARTMAN is in the position of *D-7*, which they located between (*D-10*, *D-11*) and *D-9*. The discrepancy is probably due to the fact that those authors based their determination on indirect estimates of crossover values rather than on direct experimental observations.

As has been mentioned, the relative order of the *tryC* and *tryD* loci indicated by our results is identical with that found for the corresponding loci of *E. coli* (YANOFSKY and LENNOX 1959) and the reverse of that reported in the earlier work of DEMEREC and HARTMAN (1956). The present results are considered to give a more accurate picture of the relation between these two loci, for they are based on the analysis of a larger number of *tryC* and *tryD* mutants than in the earlier work, for which only one *tryC* mutant (*C-3*) was available. At that time the order of *C* with respect to *D* was deduced from three-point transductions between *C-3* and *D-10*, with *cysB-12* as the unselected linked marker. As it now appears, some sources of error were inherent in this method, since (1) *D-10* can behave in an exceptional fashion in recombination (BALBINDER 1962), and (2) *cysB-12* provides a less sensitive testing system as an unselected linked marker, than closer markers such as *B-4* or *A-8*, because there is disagreement between reciprocal crosses.

The changed order of *C* and *D* does not affect the original conclusion of DEMEREC and HARTMAN that the arrangement of *try* loci on a linkage map coincides with the sequence of reactions in the tryptophan biosynthetic pathway in Salmonella, for the *C* and *D* loci probably control two separable components of a single enzyme and are thus involved in a single enzymatic step.

SUMMARY

The order of 17 mutants of locus *tryD* and six mutants of locus *tryC* of *Salmonella typhimurium* has been determined in three-point transduction experiments, from the assortment of the unselected marker *tryB-4* or *tryA-8* in recombination between pairs of *tryC* or *tryD* mutants. The experiments showed that mutations at 14 sites of the *tryD* locus and six sites of the *tryC* locus are responsible for the 23 mutants.

These two loci control the last step in the biosynthesis of tryptophan. Complementation tests involving abortive transduction showed that the 17 *tryD* mutants constitute one complementation group and the six *tryC* mutants another. Intralocus complementation within each of the loci cannot be excluded on the basis of present evidence, however. The relative order of the *try* loci has been found to be *tryC-D-B-A*(*cysB*), instead of *tryD-C-B-A*(*cysB*). In general, the genetic picture regarding these two loci in Salmonella agrees very well with that in *E. coli*. Thus it is very likely that, as in *E. coli*, the tryptophan synthetase consists of two dissociable components.

ACKNOWLEDGMENT

The author wishes to express his gratitude to DR. M. DEMEREC and his associates at the Department of Genetics, Carnegie Institution of Washington, for their advice, encouragement and help during the course of this investigation.

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