THE TRYPTOPHAN OPERON OF *SALMONELLA TYPHIMURIUM*. FINE STRUCTURE ANALYSIS BY DELETION MAPPING AND ABORTIVE TRANSDUCTION[1]

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THE general model proposed by JACOB and MONOD (1961) to explain the control of inducible and repressible enzymes in bacteria has received considerable support to date (AMES and MARTIN 1964). According to this hypothesis, the genetic elements controlling sequential biochemical reactions are clustered on a genetic map into units of regulation called operons. An operon consists of a group of structural genes under the control of a single "operator", located at one end of the cluster. For a specific biochemical pathway the functional state of the operator is determined by its interaction with a repressor substance composed of the product of a topographically independent regulator gene and a substrate or product of the pathway. From their extensive studies of the histidine operon of *Salmonella typhimurium*, AMES and HARTMAN (1962, 1963) expanded the original model to account for the order of the structural genes in an operon and the phenomenon of polarity.

The clustering of genetic elements controlling the biosynthesis of the amino acid tryptophan in *S. typhimurium* was first demonstrated by DEMEREC and HARTMAN (1956). Mutants blocked at different steps in the pathway were classified by growth responses and cross feeding tests for accumulation of intermediates into four closely linked groups: *tryA-tryB-tryC-tryD*. Their map order, with respect to a co-transducible *cysB* marker, was determined by three-point tests to be *cysB*, *tryA-tryB-tryD-tryC* (DEMEREC and HARTMAN 1956; BALBINDER 1962). Similar studies with *Escherichia coli* (YANOFSKY and LENNOX 1959) showed that both the biosynthetic pathway and the map order of the genes involved are identical in both organisms. Recent investigations with both *E. coli* (MATSUSHIRO *et al.* 1965) and *S. typhimurium* (BAUERLE and MARGOLIN 1965) have shown that the tryptophan structural genes constitute an operon with the "operator" located near or within the structural gene for anthranilate synthetase, the first enzyme in the sequence.

A detailed genetic and biochemical correlated study of the structure of the tryptophan operon along the lines followed by AMES and HARTMAN in their work with the histidine system seemed desirable as a first step in investigating the regulation of the tryptophan enzymes. Such a study has been made possible by

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the elucidation of the tryptophan biosynthetic sequence (see MATSUSHIRO *et al.* [1965] for references) and is the subject of this report.

From an analysis of 190 tryptophan requiring auxotrophs of *S. typhimurium* we have been able to construct a map of the tryptophan operon of this organism which accounts for every enzymatic step in the pathway. This map differs from the early report (DEMEREC and HARTMAN 1956) in that it shows the existence of two genes, *tryB* and *tryE*, instead of only one, *tryB*, in the region between *tryA* and *tryD*. *tryB* is the structural gene for the second-step enzyme, anthranilic PRPP-PR transferase (PR transferase) while *tryE* seems to code for an enzyme with a dual function: phosphoribosyl anthranilic isomerase (PRA isomerase) and indoleglycerol phosphate synthetase (InGP synthetase), catalyzing sequentially the third and fourth steps. Mention will be made in the course of this presentation of mutants showing regulatory abnormalities. A preliminary report on this work has been presented (BALBINDER, BLUME and TAMAKI 1965).

MATERIALS AND METHODS

Strains: A list of the mutants employed in this work, indicating their parent strain and the mutagen employed in their isolation, is given in Table 1. Bacterial stocks as well as working strains were prepared and kept according to the technique described by HARTMAN, LOPER and SERMAN (1960).

Phage Stocks: Transducing phage PLT-22 (ZINDER and LEDERBERG 1952) was employed routinely in mapping. Phage suspensions were prepared, assayed and stored as described by HARTMAN (1956).

Media: Nutrient broth (Difco) served as a routine complex medium. As a solid medium for transduction tests the minimal medium of the Cold Spring Harbor Laboratories (HARTMAN

TABLE 1

Origins of tryptophan mutants in Salmonella typhimurium *strains*

Parent strain	Mutagen	Mutants (isolation stock number)
LT-2 (ZINDER and LEDERBERG 1952)	Spontaneous	6, 9, 10–14, 16, 17, 20, 97, 98, 101
	2-Aminopurine	71, 73–77, 79
	Nitrous acid	81, 82, 158, 162, 164, 167, 170, 171, 176, 178, 185, 187, 194, 196
	Ultraviolet light	1–4, 78, 268–275, 277–289
	X rays	84–96, 102–104, 107, 109–111, 199–239, 501
	Fast neutrons	115, 119–122, 124–130, 132–135, 141, 148
LT-7 (mut+)*	Spontaneous	24, 28, 30–32, 34–65
	Ultraviolet light	7, 8
	Unknown	26
LT-7 mutator (mut)	Spontaneous	29, 33, 66–70
ST 519 (PLOUGH)	Unknown	27
ST 533 (PLOUGH)	Spontaneous	25
Unknown	Unknown	509

* Most of these strains contain the proline segment of strain LT-2 in an LT-7 background (isolated by T. MIYAKE). In addition to the strains listed, the deletion mutants 8T1, 5T1 and 3T1 of P. MARGOLIN (1965) were employed in mapping.

Mutants 268 through 289, and 501 (a total of 21) were obtained by ourselves. The remainder (a total of 169) were generously provided by DR. M. DEMEREC.

1956) was employed. In some experiments this was enriched with 1.25% (v/v) Difco nutrient broth (enriched minimal medium). This was satisfactory for experiments in which complete transduction resulting in prototrophic recombinants was scored. For abortive transduction tests best results were obtained with the minimal medium of VOGEL and BONNER (1956) supplemented with 0.005% acid hydrolyzed casein (SMITH and YANOFSKY 1962). In all cases, media were supplemented with the addition of 0.2% glucose as a carbon source and solidified with the addition of 1.5% (w/v) of Difco Bacto-agar.

To obtain crude extracts for enzyme determinations mutants were grown in Vogel and Bonner's liquid medium, prepared as indicated above, with the addition of growth limiting levels (2 μg/ml in some instances or 5 μg/ml in others) of anthranilic acid, indole or tryptophan as required.

Transduction experiments: "Deletion" mapping was carried out in three steps of increasing resolution.

1. Spot tests: The use of spot tests for rough mapping as well as the characterization of abortive transduction has been described by AMES and HARTMAN (1962). Recipient bacteria were grown to saturation in aerated broth cultures, centrifuged, washed once with sterile saline solution and finally resuspended in a volume of sterile saline equal to that of the original broth culture. From these washed suspensions, 0.1 ml aliquots (about 4×10^8 bacteria) were spread on the surface of minimal (or enriched minimal) agar plates by means of a glass spreader. Each plate was then spotted with 6 drops of phage suspension (about 10^{10} plaque forming units/ml), each phage lysate having been grown on different tryptophan mutant strains. The plates were incubated at 37°C for 48 hr at which time the number of colonies within each spot was counted. Spots showing no colonies, or no more colonies than arose by back mutation in the unspotted background, were scored as negative and the cross repeated by the next method.

2. Half-plate tests: Bacterial recipients were treated as above but resuspended after the saline wash in 1/10th of their original volume. They were then spread on agar plates and spotted with 10 times as much phage as in the spot test. The phage was then spread over the bacteria to insure mixing on only half of the plate, the other half remaining as the uninfected control. Plates were incubated 48 hr and colonies counted as before. Those bacterial crosses which gave no recombinants under these conditions were then repeated on full plates.

3. Full-plate tests: A saturated overnight culture of the recipient strain was divided into equal parts. One half was infected with transducing phage (multiplicity of infection between 5 and 10). The other half was used as a control and a volume of T2 buffer equal to the volume of phage suspension added to the experimental sample was added to it. Both samples were then centrifuged separately and the resulting pellets suspended in sterile saline to 1/10th of the original volume. Platings of 0.1, 0.2 and 0.5 ml/plate were made on several plates and spread as described above. Incubation and scoring were as described. Negative crosses were repeated two or three times. At least three plates were made per cross. Control platings for phage sterility were made routinely. Crosses giving no recombinants after this third step were considered as negative.

Abortive transduction test for complementation: The spot test technique described above was used. Abortive colonies (microcolonies) were scored after 48 hr incubation by examination under a low power binocular dissecting microscope using transmitted light (OZEKI 1956).

Accumulation studies: Mutant strains were grown overnight (about 24 hr) in properly supplemented liquid medium (described under media), the bacteria were collected by centrifugation and the supernatant was sampled for accumulation of different intermediates. Indole was determined by the method of YANOFSKY (1955). Indoleglycerol was detected with the $FeCl_3$ reagent of YANOFSKY (1956) and by formation of a blue color upon treatment with the Bratton-Marshall reagents (BRENNER 1955). The chromatographic procedure for the identification of anthranilic acid and 1-(0-carboxyphenylamino)-1-deoxyribulose (CDR) is that of SMITH and YANOFSKY (1960).

Enzyme assays: Bacterial cultures were grown as described for the accumulation tests. The bacteria were collected by centrifugation, washed twice in cold saline, resuspended in a small volume of 0.1 M Tris buffer, pH 7.8 and disrupted with a Bronwill Biosonik probe sonifier. The

TABLE 2

Characteristics of tryptophanless point mutants

Group (locus)	No. of mutants in group	Representative mutant	Accumulation products	Growth requirements	Enzyme activities					Step blocked (See Figure 1)
					TSase A	TSase B	InGP Syn.	PR Trans.	AA Syn.	
tryA	37	*try-8*	None	AA, Ind, Try	+	+	+	+	—	1
tryB	35	*try-14*	AA	Ind, Try	+	+	+	—	+	2
tryE	14	*try-45*	AA	Ind, Try	+	+	—	+	+	3&4
tryE	25	*try-268*	AA, CDR	Ind, Try	+	+	—	+	+	4
tryD	46	*try-10*	InG, Ind	Try	+	—	+	+	+	5 (B,AB)
tryC	20	*try-3*	InG	Ind, Try	—	+	+	+	+	5 (A,AB)
Total	177									

AA = Anthranilic acid; Ind = Indole; CDR = 1-(0-carboxyphenylamino)-1-deoxyribulose; InG = Indole glycerol; Try = Tryptophan; AA Syn. = Anthranilate synthetase; PR Trans. = PR transferase; InGP Syn. = InGP synthetase; TSase A = Tryptophan synthetase, component A; TSase B = Tryptophan synthetase component B.

cell debris was centrifuged at 41,300 × *g* in a Servall RC-2 refrigerated centrifuge for 30 minutes. The supernatant was stored in the freezer (—4°C) and used as our crude extract.

The assay procedures for the A and B components of tryptophan synthetase (TSase) and InGP synthetase, as well as the methods of preparation of indoleglycerol phosphate (InGP) and CDR-5-phosphate (CDRP) are those of SMITH and YANOFSKY (1962). Anthranilate synthetase was assayed by measuring spectrophotometrically the formation of anthranilic acid from chorismic acid and glutamine. The assay procedure, as well as the method for obtaining chorismic acid from *Aerobacter aerogenes* strain 62-1, are those described by GIBSON and GIBSON (1964).

PR transferase was assayed by a method suggested by DEMOSS (personal communication) measuring the amount of InGP formed in the presence of excess amounts of PRA isomerase and InGP synthetase. We utilized for this purpose extracts of a mutant of *S. typhimurium*, *tryB-14*, which lacks PR transferase but has PRA isomerase and InGP synthetase. Each tube of assay mixture contained 20 μmoles of phosphate buffer at pH 8.2, 1.0 μmoles $MgCl_2$, 0.22 μmoles of anthranilic acid, 0.5 μmoles of 5-phosphoribosyl-1-pyrosphosphate (PRPP), enough extract of *tryB-14* to convert 0.8 μmoles CDRP to InGP, enzyme and water to a final volume of 1.0 ml. The reaction mixture was incubated at 37°C for 20 minutes and InGP measured according to SMITH and YANOFSKY (1962).

No assays were carried out for PRA isomerase.

RESULTS

All 190 mutants have been divided into six different phenotypic groups by correlating three specific properties: (1) accumulated intermediates, (2) growth responses, and (3) missing enzymatic activities. The results of this analysis are presented in Table 2. The sequence of biochemical reactions leading from the branch point compound in aromatic amino acid biosynthesis, chorismic acid (GIBSON and GIBSON 1964), to tryptophan is given in Figure 1.

Mutants shown in Table 2 as belonging to groups *tryB* and *tryE* would have been classified into a single group, *tryB*, by the criteria utilized in the early work (DEMEREC and HARTMAN 1956). The availability of new tools, such as assay procedures for the enzymes between anthranilic acid and InGP, and methods for the detection of CDR in culture filtrates, has allowed us to recognize three distinct phenotypes. As will be shown later, these phenotypic classes were found to constitute two separate complementation groups. As indicated in Table 2 we are designating those mutants lacking PR transferase activity as *tryB* and those lacking InGP synthetase activity as *tryE*. Among the latter, two phenotypes can be discerned: some accumulate CDR (*tryE-268* in Table 2) and some do not (*tryE-45* in Table 2). In our experiments, all *tryE* and *tryB* mutants were found to accumulate anthranilic acid.

The correlations between the three properties compared were, as a rule, those shown in Table 2. These are consistent with the expected behaviour of mutants blocked at given steps in the pathway. Consequently, it was unnecessary to perform enzymatic assays for every mutant analyzed. This was done with a representative sample from each group to establish the soundness of the criterion, the remaining mutants being classified by growth responses and accumulation tests. The groupings were further confirmed by complementation tests using abortive transduction.

A number of mutants were found to deviate from the criteria established in

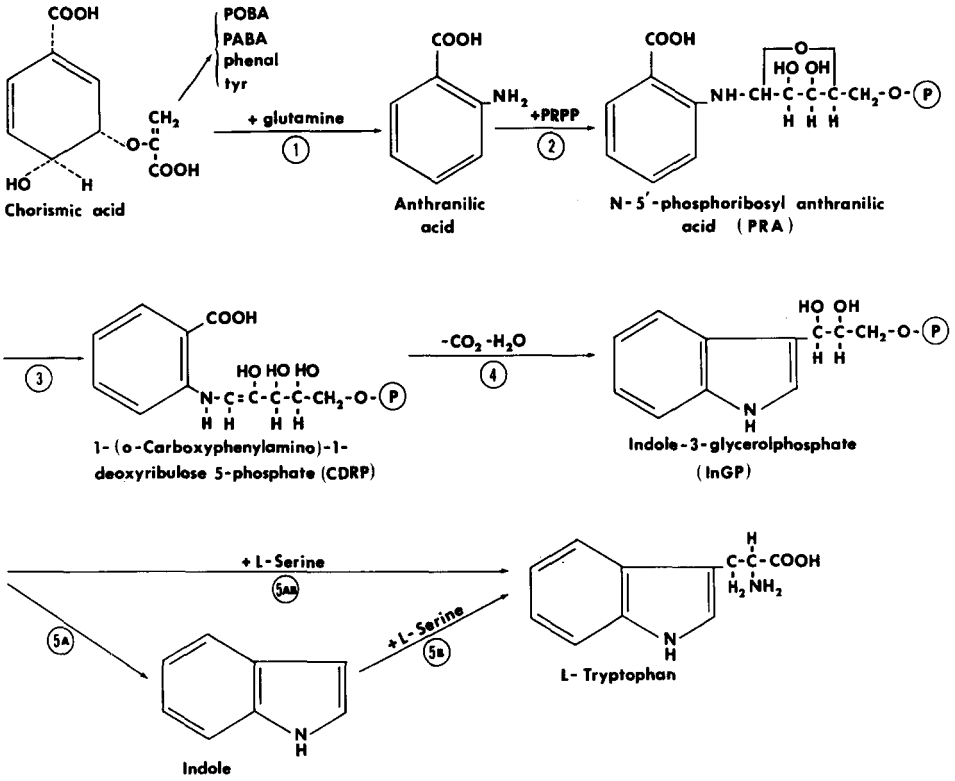

FIGURE 1.—The tryptophan biosynthetic pathway. Steps 1 through 4 are catalyzed by the following enzymes: (1) anthranilate synthetase, (2) PR transferase, (3) PRA isomerase, (4) InGP synthetase. The reactions 5A, 5B and 5AB are all carried out by tryptophan synthetase (YANOFSKY 1960).

Table 2. After extensive analysis, some proved to be multisite mutants, and others single site mutants exhibiting pleiotropic effects. We will discuss them in this order.

Multisite mutants (deletions): Thirteen of the mutants listed in Table 1 fulfilled the criteria for multisite mutations since (1) the mutational defect extended over a chromosomal segment marked by several single site mutations, and (2) they did not revert spontaneously or after treatment with diethyl sulfate, a broad spectrum mutagen believed capable of inducing both transitions and transversions (EISENSTARK and ROSNER 1964). The properties and origins of these mutants are summarized in Table 3. As can be seen in Table 3 there was, in general, good agreement between the enzymatic activities missing and the extent of the mutational lesion as determined by recombination experiments (see also Figure 2). It is likely that these mutants arose as the result of deletions. For reasons of convenience we will refer to them as such.

Some exceptional cases are evident in Table 3. These are deletions *try-130* on the one hand, and *try-43* and *try-107* on the other. In deletion *try-130*, genes

TABLE 3

Characteristics and origin of multisite deletion mutants

Multisite mutation	Loci covered	Origin	Growth requirement	Accumulation products	Enzyme activities: TSase A	TSase B	InGP Syn.	PR Trans.	AA Syn.
try-130†	*A,B,E*	Fast neutrons	Try	....	0	0	0	0	0
try-230	*E*	X Rays	Ind, Try	AA, CDR	+	+	0	+	+
try-141	*E*	Fast neutrons	Ind, Try	AA, CDR	+	+	0	+	+
try-171	*E*	Nitrous acid	Ind, Try	AA	+	+	0	+	+
try-95	*E*	X Rays	Ind, Try	AA	+	+	0	+	+
try-109	*C*	X Rays	Ind, Try	InG	0	+	+	+	+
try-196	*D,C*	Nitrous acid	Try	InG	0	0	+	+*	+*
try-164	*B,E,D*	Nitrous acid	Try	AA	N.A.	0	0	0	+*
try-167	*A,B,E,D*	Nitrous acid	Try	....	N.A.	0	0	0	0
try-43	*B,E,D,C*	Spontaneous	Try	....	0	0	0	0	0
try-107	*B,E,D,C*	X Rays	Try	....	0	0	0	0	0
try-176‡	not determined	Nitrous acid	Try, Ind	....	not determined				
try-101	*A,B,E,D,C*	Spontaneous	Try	....	0	0	0	0	0

+* = Enzyme activity assumed from accumulation products.
+ = Enzyme activity present on assay; 0 = Enzyme activity lacking on assay; N.A. = Enzyme not assayed.
† = We did not determine whether the lack of InGP synthetase activity was due to the pleiotropic effect of deletion *try-130* (see text) or to a structural alteration of the enzyme itself. For details about the relation between InGP synthetase and PRA isomerase, see text.
Horizontal lines underline enzymes expected to be missing according to the genetic data.
Abbreviations are the same as those in Table 2.
‡ = *try-176* was not analyzed extensively but we tentatively consider it a deletion because it does not revert, and it fails to accumulate pathway intermediates although it can grow on indole.
.... = No accumulation products.

tryD and *tryC* are intact. These are the structural genes for components B and A respectively of tryptophan synthetase (BALBINDER 1962). A mutant blocked before tryptophan synthetase should be capable of growth on indole. *try-130*, however, shows no detectable TSase activity and cannot grow on indole, but upon treatment with the mutagen diethyl sulfate readily produces secondary mutants capable of growth on indole. This mutant has the same characteristics as other deletion mutants such as 8T1, 5T1 and 3T1 (MARGOLIN 1965) which extend into the operon from the operator end, in that the mutation affects the expression of unaltered adjacent distal genes.

try-43 and *try-107* differ from *try-130* in that the presumably deleted segment covers the distal end of the operon, leaving the *tryA* gene and the operator intact (Figure 2). Contrary to expectations, these mutants show no anthranilate synthetase activity in crude extracts and fail to accumulate anthranilic acid in culture filtrates. They exhibit a pleiotropic effect on the function of an adjacent proximal gene unaltered by the mutation. Mutants with similar anti-polar effects have also been found within the tryptophan operon of *E. coli* by ITO and CRAWFORD (1965).

These three deletion mutants share one common characteristic: enzyme activities are missing for which the structural genes are intact. The reverse situation, that is mutants which possess the enzymatic activities corresponding to presumably deleted structural genes, has not been found.

Point mutants with pleiotropic effects: A group of mutants within the anthranilate synthetase locus, *tryA* have low levels of all the tryptophan enzymes under conditions of maximal derepression besides lacking anthranilate synthetase activity. These mutants were discovered due to a discrepancy between their growth requirements and their accumulation products: they did not grow or grew slowly on anthranilic acid but normally on indole, and accumulated no pathway intermediates. All pleiotropic mutants, including deletions, will be the subject of a separate report (BLUME, TAMAKI and BALBINDER, in preparation).

Mapping the tryptophan operon: The map presented in Figure 2 has been constructed from (1) crosses between multisite and single site mutants, (2) crosses between multisite mutants, and (3) complementation tests by abortive transduction. In deletion mapping, the results were scored as presence or absence of any number of recombinant prototrophs in excess of the reversion frequency of the recipient strain. In abortive transduction tests, complementation was scored as presence and lack of complementation as absence of microcolonies.

We were able to utilize only 145 of our 177 point mutants in a genetic analysis. The remainder were found to be either lysogenic or resistant to infection by phage PLT-22 and therefore could not be efficiently employed either as recipients or donors in transduction crosses. The 145 mutants mapped are arranged in Table 4 in 22 groups corresponding to the 22 chromosomal regions of Figure 2. The complementation experiments will be discussed in the next section.

The map presented in Figure 2 is the only one compatible with all our genetic data. The following conclusions emerge from a consideration of Figure 2 and Table 4: (1) all mutants blocked in the same enzymatic step map as a group

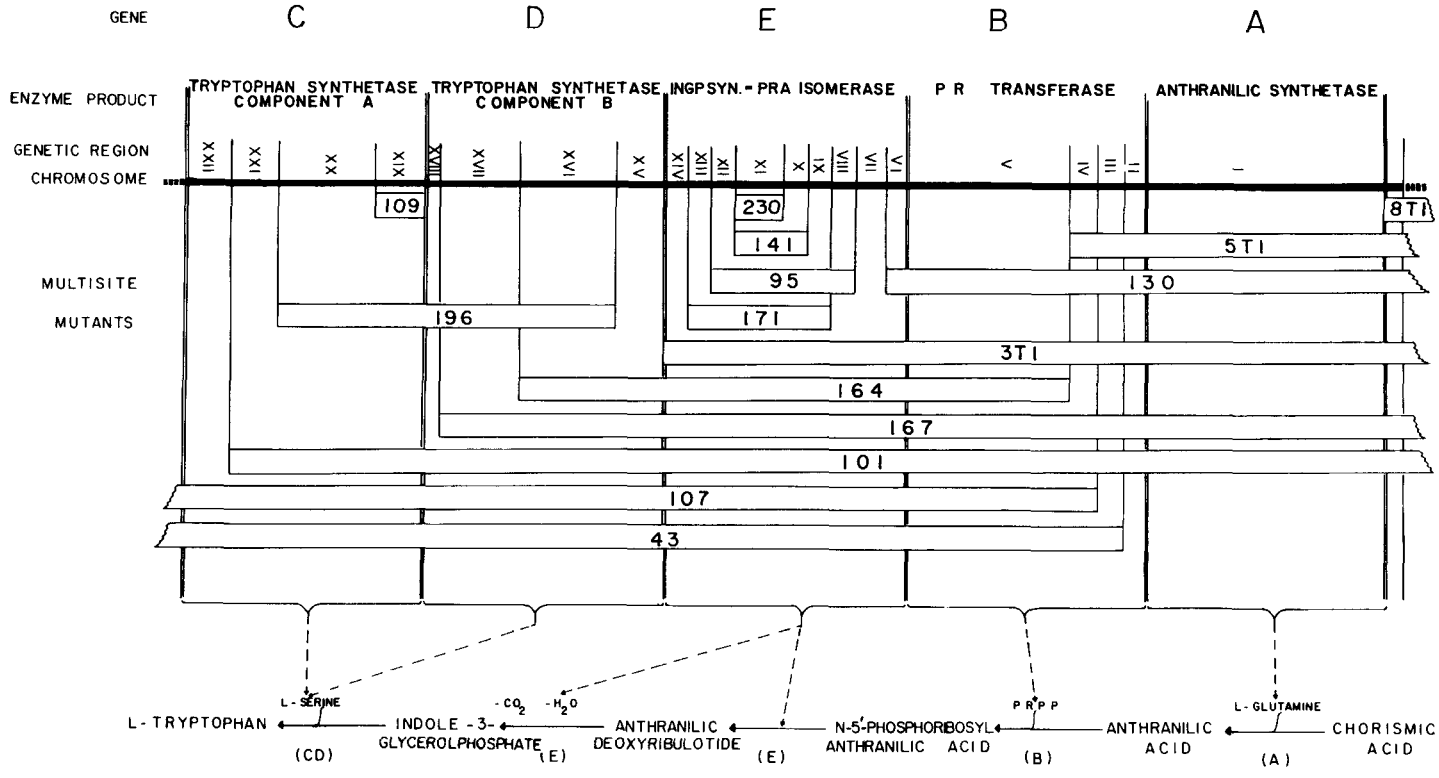

FIGURE 2.—Map of the tryptophan operon of *S. typhimurium*. The bacterial chromosome is represented by the dark horizontal line. The bars beneath the chromosome represent the multisite (deletion) mutants and indicate the physical segment missing in each. The Roman numerals indicate the genetic segments into which this region can be subdivided by a combination of deletion mapping and complementation studies. The physical sizes of these segments are not known. The differences in length between segments reflect roughly the number of single site mutants mapping within each. The operator region is adjacent to the *A* locus, on its right as the map is drawn (BAUERLE and MARGOLIN 1965).

TABLE 4

Location of point mutations in specific genetic regions

Locus	Region	
A	I	8, 24, 25, 28, 46, 47, 49, 50, 52, 61, 64, 81, 82, 88, 91, 111, 120, 128, 129, 134, 148, 211, 218, 231, 237, 238, 274, 275, 278, 509
B	II	54
	III	40
	IV	17
	V	12, 14, 16, 31, 36, 39, 41, 53, 59, 63, 65, 67, 77, 121, 122, 132, 203, 207, 223
E	VI	32
	VII	45
	VIII	94, 158
	IX	4, 26, 37, 38, 97, 200, 208, 226, *30*, *235*, *501*
	X	*110*, *206*, *268*
	XI	*2*, *57*, *60*, *62*
	XII	*135*, *201*
	XIII	*74*
	XIV	*87*, *104*, *215*, *229*, *234*, *285*
D	XV	42, 55, 69, 78, 85, 115, 126, 273, 277, 280
	XVI	1, 6, 7, 9, 29, 35, 66, 70, 73, 76, 79, 92, 93, 125, 127, 187, 205, 209, 225, 233
	XVII	10, 11, 86, 96, 102, 103, 170, 204, 210, 269, 279, 281
	XVIII	*75*
C	XIX	33, 44, 84, 90, 98, 222, 270
	XX	3, 20, 48, 71, 99, 133, 212, 217, 221, 271
	XXI	213
	XXII	227
Total No. mutants mapped = 145		

Italicized mutants are CDR accumulators.

without overlapping or ambiguity, (2) the gene order parallels the sequence of enzymatic steps in the pathway.

Our results agree with the order of structural genes previously determined by three-point tests (DEMEREC and HARTMAN 1956; BALBINDER 1962) and by mapping against a different set of deletions (MARGOLIN 1965). The same gene order has been found in *E. coli* (YANOFSKY and LENNOX 1959; SMITH 1965). Our results are also in good agreement with the conclusions of MARGOLIN (1965) about the extent of the physical deletions in the mutants 8T1 and 3T1. There is some uncertainty at the moment about the left terminus of 5T1 as well as the right terminus of *try-107* as the map is drawn. MARGOLIN and BAUERLE (private communication find that 5T1 is capable of recombining with the single site mutants *tryB-17*, *tryB-40* and *tryB-54* while *try-107* does not recombine with any of them. These three *tryB* mutants are leaky and have been difficult to map for this reason.

Complementation studies: Reciprocal abortive transduction tests involving mutants of all five groups listed in Table 2 clearly show that each constitutes an independent complementation unit. For *tryA*, *tryC* and *tryD* mutants the correspondence between one phenotype, one gene and one enzymatic activity (or more properly for *tryC* and *tryD*, one gene and one enzyme subunit) is clear. The situation is more complex when we consider the *tryB* and *tryE* mutants and deserves some comment.

As mentioned earlier, among mutants classified as *tryE* because they lacked InGP synthetase activity, two distinct types could be distinguished on the basis of CDR accumulation: some were accumulators and some were not. This suggested that the nonaccumulators also lack PRA isomerase activity (see Figure 1) and the *tryE* gene may be controlling the structure of an enzyme with a dual function. Since a good assay procedure for the isomerase reaction was not available at the time, we tested this idea by complementation analysis. Some of the results of this analysis are summarized in Figure 3. Since *tryB* mutants show the same accumulation characteristics as the isomeraseless mutants (see Table 2), we included them in the analysis.

As shown in Figure 3, the distinction between *tryB* and *tryE* mutants is unequivocal: each constitutes a separate complementation group. All *tryB* mutants but only one *tryE* mutant are covered by deletion *try-130*, while deletions *try-95* and *try-171* cover most of the *tryE* mutants but do not cover any of the *tryB*'s. Thus we have two distinct cistrons by all criteria: (1) nonoverlapping on a recombination map, (2) nonoverlapping on a complementation map, and (3) having two different enzymes affected.

The *tryE* group shows a very different picture. Separation of the CDR accumulators and nonaccumulators into distinct complementation groups was not observed. The data fit better the notion that *tryE* constitutes a single gene locus which exhibits complex intra-cistronic complementation. This interpretation, however is not the only possible one in view of two rather remarkable facts. First, as can be seen in Table 4, all CDR accumulators tend to be clustered on the left side of the *tryE* map (regions IX-XIV, Figure 2) while the nonaccumulators tend to be clustered on the right side (regions VI-IX). A similar clustering has also been found with the corresponding mutants of *E. coli* (SMITH 1965 and private communication). Second, if both the PRA isomerase and InGP synthetase activities are associated with a single protein, it would be difficult to account for (a) the fact that small deletions in the CDR accumulator region (*try-230* and *try-141*) seem to retain PRA isomerase activity, and (b) some of the complementation results between these two deletion mutants and CDR nonaccumulators which are shown in Figure 3. The alternative interpretation, that *tryE* actually includes two adjacent structural genes each coding for a separate polypeptide, cannot be discarded. On this interpretation, a complex of the two proteins would be essential for InGP synthetase activity but only one protein would be sufficient for the isomerase reaction. This problem is under investigation.

It should be pointed out that one possible type of mutant, lacking only PRA isomerase, could not be distinguished from mutants lacking PRA isomerase and

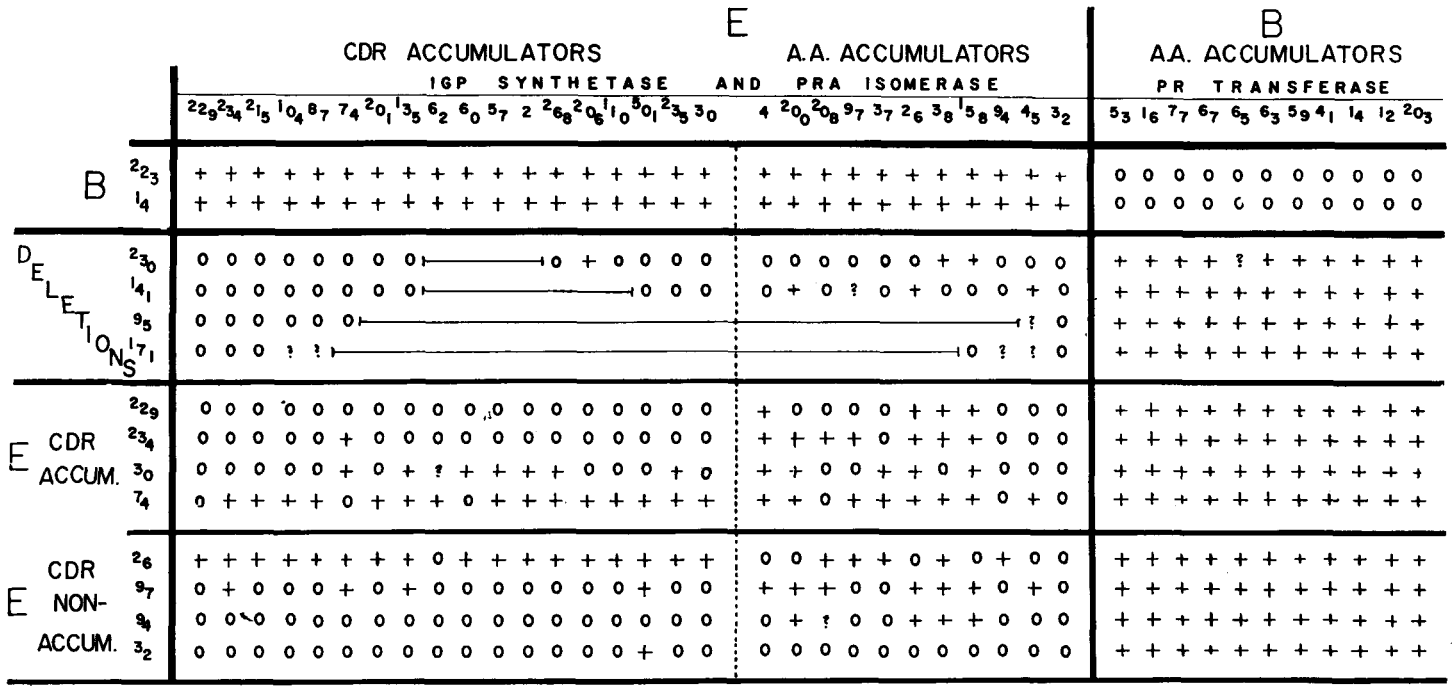

FIGURE 3.—Complementation between *tryB* and *tryE* mutants by abortive transduction. The table is a summary of tests performed as reciprocal transductions between pairs of mutants. + = abortive transduction observed; 0 = abortive transduction not observed; ? = results difficult to interpret. Rows 1 and 2 give the results of reciprocal transductions between the PR transferase negative mutants *tryB-223* and *tryB-14*, used both as recipients and donors, and all the mutants listed on the upper part of the table. Rows 3 to 6 give the results of similar tests between the latter and the InGP synthetase-negative deletions *tryE-230*, *tryE-141*, *tryE-95* and *tryE-171*. The horizontal bars indicate the sites covered by each deletion (see also Figure 2 and Table 4). The shorter deletions (*tryE-230* and *tryE-141*) which fall entirely within the CDR accumulator region are themselves CDR accumulators, while the longer deletions (*tryE-95* and *tryE-171*) which extend into the CDR nonaccumulator region are themselves CDR nonaccumulators (see Table 3). The remainder of the table shows results of reciprocal transductions between single-site mutants. These are grouped on the table by their accumulation properties and arranged approximately in their map order.

InGP synthetase on the basis of accumulation tests alone. It is possible that a mutant of this type is present in our group of 14 *tryE* non-CDR accumulators. The existence of mutants of this type would not alter our general picture of *tryE* as a single complex locus.

Preliminary results with the loci *tryA*, *tryC* and *tryD* indicate that *tryD* shows intracistronic complementation but *tryA* and *tryC* do not. A more detailed account of complementation analysis in this operon will be published separately (BALBINDER, BLUME and BALBINDER, in preparation).

DISCUSSION

By using overlapping deletions we have been able to arrange 145 tryptophan-less mutants of *S. typhimurium* on a linear map composed of 22 regions of known order. Complementation tests by abortive transduction provided an additional method of high resolving power, particularly in the analysis of the *tryB* and *tryE* loci. The four small deletions in *tryE*, (*tryE-141*, *tryE-230*, *tryE-95* and *tryE-171*) were invaluable for this purpose since we were able to obtain results free of the ambiguities of intracistronic complementation. The present map accounts for all the steps in the tryptophan pathway and defines the number of structural elements associated with this pathway in *S. typhimurium*. The number of structural genes is at least five and not more than six if the *tryE* locus as we have defined it includes in effect two adjacent structural genes, each coding for a different polypeptide.

Our data on the *tryE* locus allow for two alternative interpretations: (1) only one cistron is involved in determining the structure of an enzyme with a dual function, PRA isomerase and InGP synthetase, or (2) there are two distinct cistrons and two distinct polypeptides, but their relations are such that while one is sufficient to carry out one of the reactions (PRA isomerase) both are needed, perhaps as a complex, for the second reaction (InGP synthetase). There are precedents in the literature for both interpretations. In the pathway for histidine biosynthesis the locus *hisB* codes for an enzyme which catalyzes two heterologous and non-sequential reactions: imidazole glycerol phosphate dehydrase and 1-histidinol phosphate phosphatase (LOPER *et al.* 1964). Mutants within this locus lack only dehydrase activity or both dehydrase and phosphatase. There is an obvious parallel between the *hisB* and at least some of our *tryE* mutants: CDR accumulators lack InGP synthetase while retaining PRA isomerase, while others (i.e. *tryE-45*) seem to have lost both activities. The one polypeptide-two activities hypothesis could account for the properties of the small deletion mutants *tryE-141* and *tryE-230*, which seem to have retained PRA isomerase as evidenced by their ability to accumulate CDR, if we assume that part of the enzyme is dispensable for PRA isomerase activity but a high degree of structural integrity is necessary for InGP synthetase. Perhaps a parallel to this situation is also found with the *hisB* enzyme since VASINGTON (quoted by LOPER *et al.* 1964) finds in one mutant a protein with phosphatase activity which has a molecular weight of about half that of the normal enzyme. A further interesting fact which lends

plausibility to this interpretation is the report by DEMOSS and WEGMAN (1965) that is *Neurospora crassa* one gene locus, *tryp-1* controls both PRA isomerase and InGP synthetase. On the other hand, the same report presents evidence showing that several of the enzymatic activities in the early part of the tryptophan pathway in Neurospora are catalyzed by an enzyme aggregate made up of the products of independent genes, thus establishing a precedent for the existence of enzyme complexes in this pathway.

Although the tryptophan operon fits the general model of JACOB and MONOD, the existence of structural mutants with novel pleiotropic effects such as the unexpected "reverse polarity" observed in the deletion mutants *try-43* and *try-107* introduces a new element into the picture. A more detailed discussion of pleiotropic mutants is left for a future publication (BLUME, TAMAKI and BALBINDER, in preparation).

Table 3 shows the origin of our multisite mutants. It can be seen that a number of them (38.5%) arose following treatment with nitrous acid, a mutagen believed to act by inducing single base transitions (FREESE 1959). However, TESSMAN (1962) has reported that it could induce deletions in phage T4 and therefore has a second mechanism of action. Reports of nitrous acid induced deletions in bacteria have been published (DEMEREC 1962; EISENSTARK and ROSNER 1964).

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SUMMARY

A total of 190 tryptophanless auxotrophs of *S. typhimurium* were characterized phenotypically by a combination of growth response tests, accumulation of intermediates and missing enzymatic activities. Thirteen of the mutants were found, in genetic tests, to be multisite in nature, probably deletions. They were employed, together with deletions 8T1, 5T1 and 3T1 in constructing a map of the tryptophan operon against 145 single site mutants blocked at different steps in the pathway. Reciprocal complementation tests by abortive transduction were also used in mapping. The map obtained consists of at least five, and not more than six structural genes arranged in the same order as the reaction sequence in the pathway. The ambiguity in determining the exact number of structural loci arises from the properties of mutants lacking InGP synthetase and assigned tentatively to one gene, *tryE*. Preliminary evidence indicates that the *tryE* locus may code for an enzyme with a dual function, which can catalyze two sequential steps in the pathway: PRA isomerase and InGP synthetase; but the possibility that a complex of two distinct polypeptides determined by adjacent genes is needed for these reactions has not been excluded. *tryE* and *tryB* are two distinct structural genes which in earlier reports had been considered as one locus, *tryB*. Mention is made in this report of the pleiotropic effects exhibited by some single site and some multisite mutants.

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