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# *THE EFFECTS OF DELETIONS, POINT MUTATIONS, REVERSIONS AND SUPPRESSOR MUTATIONS ON THE TWO COMPONENTS OF THE TRYPTOPHAN SYNTHETASE OF ESCHERICHIA COLI**

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*Introduction.*—In previous investigations with the tryptophan synthetase (TSase) of *Neurospora crassa*[1] and *Escherichia coli*[2] it was observed that gene mutations affecting the formation of this enzyme may result in the production of a protein (designated CRM) which is immunologically similar to the enzyme. In other mutants lacking TSase, in both organisms, this protein was not detected. Subsequent studies have shown that the TSase of *E. coli*[3, 4] and *N. crassa*[5] catalyzes two reactions (2 and 3) in addition to the previously studied reaction, (1).

(1) indole + *L*-serine → *L*-tryptophan
(2) indoleglycerol phosphate ⇌ indole + triose phosphate
(3) indoleglycerol phosphate + *L*-serine → *L*-tryptophan + triose phosphate

It was also concluded that reaction (3) is not the sum of reactions (1) and (2) since free indole is not formed during the course of this reaction. It appears likely therefore that reaction (3) constitutes the actual physiological mechanism of tryptophan synthesis in these organisms. Investigations on the identity of the CRM's formed by several of the TSase-lacking mutants of *E. coli* which were previously studied revealed that these proteins were enzymatically active in reaction (2), but not in the other reactions.[6] Thus the CRM's in these strains appear to be altered proteins which have retained both an enzymatic activity and an immunological identity characteristic of wild type TSase. More recent studies[4] with the TSase of *E. coli* have led to the finding that this enzyme system consists of two separable protein components (designated A and B), both of which are required for the catalysis of any of the above reactions at maximal rates. Each of the components, when tested separately, appears to have slight activity in one of the three reactions—component A in reaction (2), and component B in reaction (1). However, in neither case is the activity more than ten per cent of that obtained with a mixture of the two components.

In view of this new information on the two-component nature of *E. coli* TSase it was of interest to examine the various strains of *E. coli* in which TSase activity has been affected by mutation. The present report is concerned with the results of this investigation.

*Materials and Methods.*—All the tryptophan auxotrophs studied except the three "deletion" types were isolated by the penicillin method following ultraviolet irradiation of a culture of *Escherichia coli* strain K-12. The growth requirements and accumulation behavior of these strains are summarized in Table 1. Strain T-3 is blocked at an early step in tryptophan biosynthesis; when grown on limiting amounts of anthranilic acid it forms large amounts of normal components A and B. The A and B mutants are revertible auxotrophs with defects in the formation and/or activity of the A or B protein of TSase. The strains designated B/1T7, B/1TN, and C13T are tryptophan auxotrophs of *E. coli* strain B; they acquired their tryptophan requirement simultaneously with mutation to resistance to bacteriophage T-1. These strains appear to lack different segments of the gene cluster controlling tryptophan synthesis.[7] In all three mutants the deleted loci include the A and B genes, i.e., the genes concerned with the formation of proteins A and B.

Revertants, partial revertants, and suppressed mutants were obtained from several A and B mutants following ultraviolet irradiation and selection for ability to grow in the absence of a tryptophan supplement. Partial revertants and suppressed mutants were initially distinguished from revertants on the basis of their slow growth in the absence of, and fast growth in the presence of tryptophan, and by their accumulation of either indoleglycerol or indole. Suppressed mutants were distinguished from revertants and partial revertants by transduction tests; the original mutant types were recovered from suppressed mutants but not from revertants.

*Mutational site mapping:* The relative linear order of the mutationally altered sites in the A and B point mutants was established using transduction with phage Plkc.[7] In these experiments the various A and B point mutants were employed as donors or recipients and the frequency of appearance of tryptophan independent colonies was determined for each donor-recipient pair. To eliminate variables such as lysis of recipient cells and efficiency of adsorption, transduction from *his*$^-$ to *his*$^+$ was scored in the same experiments; that is, the donor strains were *his*$^+$*tryp*$^-$ and the recipients were *his*$^-$*tryp*$^-$, and the number of tryptophan-independent colonies observed in plated aliquots expressed as a fraction of the number of transductions to histidine independence. Joint transduction of *his* and *tryp* does not occur.

*Extract preparation:* Cells were grown at 37° with shaking on a minimal medium containing 0.2 per cent glucose and sufficient anthranilic acid, indole, or tryptophan to give near-maximal growth. A supplement of 0.1–0.5 per cent acid hydrolyzed casein was included in the growth medium of mutants B-1 through B-6 and the deletion mutants to increase the yield of these bacteria. Extracts were prepared by sonic oscillation.

*Enzyme assays:* A detailed description of the methods used to assay TSase components A and B in reaction (1), (2), and (3) has appeared elsewhere.[4] In all cases the incubation mixture contained, in addition to the substrates, a threefold excess of the component which was not being assayed. As shown previously,

TABLE 1

EXAMINATION OF DELETION AND POINT MUTANTS

| Strain | Growth Response* | Accumulation | Reaction | Enzymatic Assay: Component A S.A.§ | Component A Per Cent† | Component B S.A.§ | Component B Per Cent† | Immunological Assay: A-CRM, S.A.§ | Immunological Assay: B-CRM, S.A.§ |
|---|---|---|---|---|---|---|---|---|---|
| Wild type K-12 | Min. | ... | In → Tryp | 2.9 | 100 | 2.7 | 100 | | .. |
| | | | InGP → In | 0.1 | 3.5 | 0.07 | 2.6 | ... | .. |
| | | | InGP → Tryp | 1.1 | 38 | 0.9 | 33 | ... | .. |
| T-3 | An, In, Tryp | ... | In → Tryp | 18.7 | 100 | 13.3 | 100 | ... | .. |
| | | | InGP → In | 0.6 | 3.2 | 0.5 | 3.8 | ... | .. |
| | | | InGP → Tryp | 9.0 | 48 | 6.1 | 46 | ... | .. |
| B/IT-7 | Tryp | ... | In → Tryp | 0 | ... | 0 | ... | 0 | 0 |
| C-13-T | Tryp | An | InGP → In | 0 | ... | 0 | ... | ... | .. |
| B/IT-N | Tryp | InG | InGP → Tryp | 0 | ... | 0 | ... | ... | .. |
| A-1 | In, Tryp | InG | In → Tryp | 1.9 | ... | 5.0 | 100 | ...‡ | .. |
| | | | InGP → In | 0 | ... | 0.09 | 1.8 | ... | .. |
| | | | InGP → Tryp | 0 | ... | 2.1 | 42 | 1.01 | .. |
| A-2 | In, Tryp | InG | In → Tryp | 0 | ... | 28.7 | 100 | 0 | .. |
| | | | InGP → In | 0 | ... | 0.9 | 3.1 | ... | .. |
| | | | InGP → Tryp | 0 | ... | 14.1 | 49 | ... | .. |
| A-3 | In, Tryp | InG | In → Tryp | 10 | ... | 9.7 | 100 | ...‡ | .. |
| | | | InGP → In | 0 | ... | 0.13 | 1.3 | ... | .. |
| | | | InGP → Tryp | 0 | ... | 3.1 | 32 | 5.3 | .. |
| A-4 | In, Tryp | InG | In → Tryp | 0 | ... | 24.7 | 100 | 0 | .. |
| | | | InGP → In | 0 | ... | 0.74 | 3 | ... | .. |
| | | | InGP → Tryp | 0 | ... | 11.8 | 48 | ... | .. |
| B-1 | Tryp | In | In → Tryp | 32 | 100 | 0 | ... | ... | 35 |
| | | | InGP → In | 1.1 | 3.3 | 1.3 | ... | ... | .. |
| | | | InGP → Tryp | 11 | 33 | 0 | ... | ... | .. |
| B-2 | Tryp | In | In → Tryp | 41 | 100 | 0 | ... | ... | 20 |
| | | | InGP → In | 1.8 | 4.4 | 1.2 | ... | ... | .. |
| | | | InGP → Tryp | 16 | 39 | 0 | ... | ... | .. |
| B-3 | Tryp | In | In → Tryp | 47 | 100 | 0 | ... | ... | 20 |
| | | | InGP → In | 1.6 | 3.4 | 0.6 | ... | ... | .. |
| | | | InGP → Tryp | 20 | 43 | 0 | ... | ... | .. |
| B-4 | Tryp | InG | In → Tryp | 6.6 | 100 | 0 | ... | ... | 0 |
| | | | InGP → In | 0.18 | 2.7 | 0 | ... | ... | .. |
| | | | InGP → Tryp | 2.8 | 42 | 0 | ... | ... | .. |
| B-5 | Tryp | InG | In → Tryp | 11 | 100 | 0 | ... | ... | 0 |
| | | | InGP → In | 0.24 | 2.2 | 0 | ... | ... | .. |
| | | | InGP → Tryp | 4.9 | 45 | 0 | ... | ... | .. |
| B-6 | Tryp | InG | In → Tryp | 6.4 | 100 | 0 | ... | ... | 0 |
| | | | InGP → In | 0.17 | 2.7 | 0 | ... | ... | .. |
| | | | InGP → Tryp | 2.7 | 42 | 0 | ... | ... | .. |

* An = Anthranilic acid; In = indole; InG = indoleglycerol; InGP = indoleglycerolphosphate. Tryp = tryptophan.
† Setting the In → Tryp activity at 100 per cent.
‡ Inhibited to the same extent as normal A in this reaction (by antibody).
§ S.A. = Specific activity.

maximal A or B activity is obtained with a threefold or greater excess of the other component. Partially purified component A and partially purified component B, each free of the other component, were employed as assay reagents. Reactions (1) and (3) were performed in a 1 ml. incubation mixture containing serine, pyridoxal phosphate, indole or indoleglycerol phosphate, 0.09 *M* tris buffer at pH 7.8, and 0.03 ml of a saturated solution of NaCl. Reaction (2) was assayed in 0.1 *M* phosphate buffer, pH 7.0, in the presence of $5 \times 10^{-3}$ *M* hydroxylamine. Indole disappearance (reaction 1) or appearance (reaction 2) was estimated colorimetrically, while tryptophan formation (reaction 3) was determined microbiologically[8] on the supernatant of a perchloric acid-treated reaction mixture. One unit of A or B activity in reaction (1), (2), or (3) is defined as the amount which catalyzes the disappearance of 0.1 $\mu$M of reactant or the appearance of 0.1 $\mu$M of product in 20 minutes at 37° C. Specific activity is expressed as units of enzyme activity per milligram

of extract protein. Protein was determined by the method of Lowry *et al.*[9]

*Immunological assays:* Antisera produced in rabbits in response to injections of partially purified *E. coli* TSase preparations (containing components A and B) have been found to have high anticomponent B activity and low anticomponent A activity. The anti-A in these sera could be removed by absorption with component A. Specific antibodies to component A were obtained by the injection of a highly purified preparation of component A, employing the adjuvant technique. No immunological cross reaction between the two components was demonstrable. One unit of anti-A or anti-B serum is defined as the amount which inhibits one unit of A or B activity in any of the three reactions. The term CRM[10] (cross-reacting material) will be used to refer to an altered protein, present in a mutant extract, which can combine with antibody to its *normal* counterpart. Altered component A will be designated A-CRM and altered component B, B-CRM. The methods used for the immunological assay of B-CRM have been described previously.[6] In the tests reported here, crude anti-B serum was employed and a threefold excess of component A was included in the reaction mixture to insure that the small amount of antibody to component A present in the anti-B serum would not contribute to the observed inhibition of activity. A-CRM assays were performed in the same manner as B-CRM assays. One unit of A or B-CRM (A or B antigenic material) is defined as the amount of protein which combines with one inhibiting unit of the appropriate antiserum.

*Results.—Deletion mutants:* Three deletion mutants, each lacking genic material corresponding to genes A and B, have been examined for component A or component B activity in the three biochemical reactions catalyzed by these proteins. As can be seen in Table 1, no trace of activity in extracts of any of the three mutants was detected. In other tests (Table 1) these strains were examined for A-CRM or B-CRM, but neither was detected. Thus the deletion mutants appear to represent strains in which the absence of genes A and B results in the loss of ability to form components A and B, and any protein or proteins immunologically similar to these components.

*Point mutations affecting components A and B:* Four mutants with defects in the formation or activity of component A have been studied to date; these strains were designated A-1 through A-4. All four of these strains yielded tryptophan-independent colonies in spontaneous reversion experiments; thus they were provisionally classified as point mutants. The mutationally altered site in each of these strains was mapped and all four sites were located in one small region of genetic material. Strains A1 and A3 form an A-protein which is effective in the indole → tryptophan reaction, but not in either of the reactions involving indoleglycerol phosphate (Table 1). Mutants A2 and A4 do not form any material which exhibits component A activity in any of the three biochemical reactions. Immunological tests with extracts of the four A mutants are consistent with these findings; they indicate that mutants A2 and A4 do not form an A-CRM while mutants A1 and A3 do. Furthermore, these tests demonstrated that the A-CRM in the latter two strains, though completely inactive in reactions (2) and (3), has the same activity to antigen ratio in reaction (1) as does normal component A. All four A mutants produce normal component B (Table 1).

The four A-mutants were indistinguishable in accumulation tests; indoleglycerol

was the predominant compound detected. In growth experiments they all responded to indole as well as to tryptophan. The ability of mutants A1 and A3 to grow in the presence of indole is understandable since these strains form an A-CRM which is effective in the conversion of indole to tryptophan. The response of mutants A2 and A4 to indole cannot be explained in this manner, since these strains do not form normal or altered component A. However, they do form abnormally high levels of component B (see Table 1), and the activity of this component in reaction (1) (in the absence of component A) is probably sufficient to account for their growth behavior. This activity is about 1–3 per cent of the activity exhibited in the presence of component A when assayed in tris buffer, and 5–10 per cent when assayed in phosphate buffer.

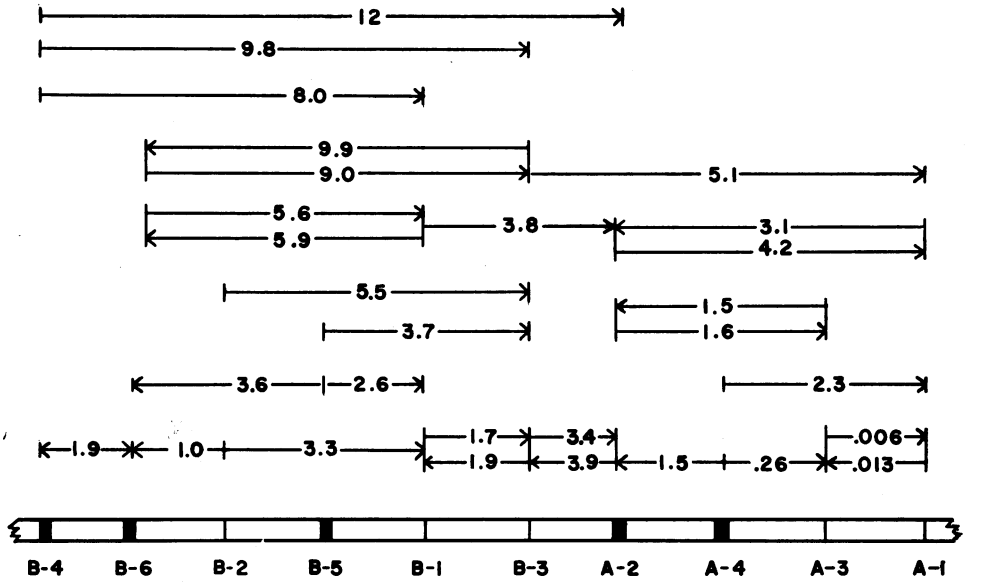


FIG. 1.—Tentative mutational site map for genes A and B. Black bars indicate the location of mutationally altered sites which lead to inability to form the A or B protein. The thin lines represent mutationally altered sites in A-CRM or B-CRM producing mutants. The arrows indicate direction of transfer.

Six auxotrophs in which component B has been affected by mutation have been examined in biochemical, immunological, and genetic tests. All six of these mutants can revert. The mutationally altered site in each of these strains has been mapped and found to lie within a small region of genetic material. Of these six B auxotrophs three accumulate indole and three accumulate indoleglycerol. The indole accumulators, on enzymatic examination, were found to form a B protein which is active in reaction (2) but ineffective in reactions (1) and (3) (Table 1). Extracts of the other three B mutants, the indoleglycerol accumulators, do not have component B activity in any of the three biochemical reactions. Immunological tests performed with extracts of the six B mutants indicate that those strains which have indoleglycerol phosphate → indole activity form a B-CRM while the other

three B mutants do not. The B-CRM formed by two of the mutants is as effective per unit B-antigenic material in reaction (2) as is normal component B (Table 1). The B-CRM in the third mutant, strain B-2, is more active enzymatically per unit B-antigenic material than is normal component B. All six of the B mutants form component A which appears normal in the three biochemical reactions.

*Mutational site map for A and B point mutants:* The results of the transductional analyses designed to map the mutationally altered sites in the A and B point mutants are summarized in Table 2, and the mutational site map based on these data is shown in Figure 1. It can be seen that the data obtained permit the arrangement of the altered mutational sites in a reasonable linear order. Agreement between duplicate experiments was very poor, however; sometimes the values obtained varied by as much as 40 per cent. Mutants A-3 and A-4 gave an unexpectedly small number of *tryp*$^+$ colonies in reciprocal transduction experiments. The other transductions with these strains indicated that much higher values should have been obtained. The explanation for this observation is not known.

TABLE 2

TRANSDUCTION EXPERIMENTS WITH A AND B POINT MUTANTS

| | Recipient | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *his*$^-$ *A-1* | | | *his*$^-$ *A-2* | | | *his*$^-$ *A-3* | | |
| Donor | tryp$^+$ * | his$^+$ | tryp$^+$/his$^+$, Per Cent | tryp$^+$ | his$^+$ | tryp$^+$/his$^+$, Per Cent | tryp$^+$ | his$^+$ | tryp$^+$/his$^+$, Per Cent |
| *his*$^+$ *A-1* | 0 | *ca.* 5,000 | ... | 1,169 | 38,030 | 3.1 | 2 | 15,038 | 0.013 |
| *his*$^+$ *A-2* | 1,313 | 31,376 | 4.2 | 0 | *ca.* 5,000 | ... | 477 | 29,812 | 1.6 |
| *his*$^+$ *A-3* | 2 | 33,402 | 0.006 | 597 | 39,390 | 1.5 | 0 | *ca.* 5,000 | ... |
| *his*$^+$ *A-4* | 897 | 38,722 | 2.3 | 548 | 37,130 | 1.5 | 76 | 29,832 | 0.26 |
| *his*$^+$ *B-1* | ... | ... | ... | 109 | 2,890 | 3.8 | ... | ... | ... |
| *his*$^+$ *B-3* | 309 | 6,100 | 5.1 | 788 | 23,228 | 3.4 | ... | ... | ... |
| *his*$^+$ *B-4* | ... | ... | ... | 256 | 2,180 | 12 | ... | ... | ... |

| | Recipient | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *his*$^-$ *B-6* | | | *his*$^-$ *B-1* | | | *his*$^-$ *B-3* | | |
| Donor | tryp$^+$ | his$^+$ | tryp$^+$/his$^+$, Per Cent | tryp$^+$ | his$^+$ | tryp$^+$/his$^+$, Per Cent | tryp$^+$ | his$^+$ | tryp$^+$/his$^+$, Per Cent |
| *his*$^+$ *B-1* | 1,711 | 29,233 | 5.9 | 0 | *ca.* 5,000 | ... | 251 | 14,959 | 1.7 |
| *his*$^+$ *B-2* | 177 | 16,990 | 1.04 | 403 | 12,358 | 3.3 | 996 | 18,239 | 5.5 |
| *his*$^+$ *B-3* | 759 | 7,665 | 9.9 | 55 | 2,978 | 1.9 | 0 | *ca.* 5,000 | ... |
| *his*$^+$ *B-4* | 308 | 16,040 | 1.9 | 897 | 11,280 | 8 | 2,032 | 20,715 | 9.8 |
| *his*$^+$ *B-5* | 304 | 8,574 | 3.6 | 222 | 8,418 | 2.6 | 364 | 9,959 | 3.7 |
| *his*$^+$ *B-6* | 0 | *ca.* 5,000 | ... | 584 | 10,439 | 5.6 | 1,095 | 12,134 | 9 |
| *his*$^+$ *A-2* | ... | ... | ... | ... | ... | ... | 932 | 23,816 | 3.9 |

* The *tryp*$^+$ values listed represent actual colony counts while the *his*$^+$ values given were calculated from a ten or twenty-fold dilution which was actually plated. The values reported were corrected for any reversion in control platings.

It is interesting to note from the mutational site map that the A and B genes are very close to one another and that some mutational sites in the B gene are closer to mutational sites in the A gene than they are to other mutational sites within the B gene. Furthermore, it can be seen from the B mutational site map that mutations which lead to the two different types of protein alterations are not restricted to specific regions of the mutational site map; two sites which, when mutated, lead to loss of ability to form the B protein bracket a B-CRM producing site.

*Revertants and partial revertants:* Ultraviolet-induced revertants were isolated from a representative of each of the A and B mutant types (CRM producers and nonproducers). These strains were examined in enzymatic and immunological

tests to determine whether or not reversion restored a normal A or B protein. Two revertants obtained from strain A3, an A-CRM producer, formed what appears to be normal component A, i.e., this component catalyzes the three biochemical reactions at the same relative rates as wild-type A and is inhibited to the same extent by anti-A serum (Table 3). The growth of these revertants is not stimulated by tryptophan, and they do not accumulate indoleglycerol. The partial revertant (strain A-2 pr-6) obtained from the A mutant which does not form A-CRM, although capable of growing in the absence of tryptophan, is stimulated by this amino acid. In addition it accumulates indoleglycerol, a property which also distinguishes it from the wild-type strain and full revertants. The component A formed by this partial revertant is capable of participating in all three of the biochemical reactions in which normal component A participates. However, the relative activities of this component A in the various reactions differs somewhat from those observed with wild-type component A (see Table 3), suggesting that an abnormal A or a mixture of A's is formed by this strain. Immunological tests (Table 3) were consistent with these observations since they demonstrated that anti-A was much less effective in inhibiting the activity of this component A than that of normal component A.

TABLE 3

ENZYMATIC AND IMMUNOLOGICAL EXAMINATION OF EXTRACTS OF REVERTANTS AND PARTIAL REVERTANTS

| | | Enzymatic Assay | | | | Immunological Assay | |
|---|---|---|---|---|---|---|---|
| | | Component A | | Component B | | | |
| Strain | Reaction | S.A.† | Per Cent‡ | S.A.† | Per Cent‡ | A Inhibited Per Cent of Control§ | B Inhibited Per Cent of Control§ |
| Wild type K-12 | In → Tryp | 2.9 | 100 | 2.7 | 100 | | |
| | InGP → In | 0.1 | 3.5 | 0.07 | 2.6 | | |
| | InGP → Tryp | 1.1 | 38 | 0.9 | 33 | | |
| A-2 pr-6 | In → Tryp | 0.3 | 100 | 1.5 | 100 | 23 | |
| | InGP → In | 0.05 | 17 | 0.044 | 2.9 | .. | |
| | InGP → Tryp | 0.33 | 110 | 0.6 | 40 | .. | |
| A-3 R-2 | In → Tryp | 1.7 | 100 | 1.45 | 100 | 91 | |
| | InGP → In | 0.05 | 2.9 | 0.04 | 2.9 | .. | |
| | InGP → Tryp | 0.7 | 41 | 0.61 | 42 | .. | |
| A-3 R-9 | In → Tryp | 1.6 | 100 | 1.42 | 100 | 95 | |
| | InGP → In | 0.05 | 3.1 | 0.04 | 2.8 | .. | |
| | InGP → Tryp | 0.71 | 44 | 0.63 | 44 | .. | |
| B-1 pr-3 | In → Tryp | 44 | 100 | 0.33 | 100 | | 0* |
| | InGP → In | 1.3 | 2.9 | 1.37 | 415 | | .. |
| | InGP → Tryp | 16.7 | 38 | 0.33 | 100 | | .. |
| B-4 R-8 | In → Tryp | 2.7 | 100 | 2.14 | 100 | | 94 |
| | InGP → In | 0.06 | 2.2 | 0.06 | 2.8 | | .. |
| | InGP → Tryp | 1.2 | 44 | 0.68 | 32 | | .. |

* This extract had a B-CRM specific activity of 53.3.
† Specific activity.
‡ Setting the In → Tryp activity at 100 per cent.
§ Control was inhibition of wild type A or B by homologous antiserum.

Of the B revertants examined, strain B-4 R-8 produced a component B which was normal, as judged by enzymatic and immunological criteria (Table 3). Strain B-1 pr-3, a partial revertant derived from a B-CRM producer, formed a B-protein which could participate in all three reactions, although its activity in two of the reactions, (1) and (3), was considerably less than normal B, per unit B-antigenic material. Its component B had a normal activity:antigen ratio in reaction (2), however.

The revertants and partial revertants described in this section do not necessarily constitute the principal types obtainable from the various A and B mutants studied.

The strains examined were ones which had previously been characterized genetically and were employed in the present study for this reason.

*Suppressed A mutants:* Ultraviolet induced suppressed mutants derived from the two types of A and B mutants, CRM producers and nonproducers, were also examined (Table 4). Each of these suppressed mutants accumulated indoleglycerol or indole and its growth was stimulated by tryptophan. Enzymatic tests indicated that the suppressed A mutants formed normal component B and the suppressed B mutants formed normal component A. Suppression of the A- mutant which did not produce an A-CRM yielded a strain (A-2 su-2) which formed an A-protein which was indistinguishable from normal A in the three biochemical reactions and was similar in its sensitivity to anti-A serum (Table 4). However, as can be seen in Table 4, this strain produced much less component A than component B, and this abnormal behavior may be indicative of an A-protein alteration. The component A formed by the suppressed mutant derived from the A-CRM producer, strain A-3 su-3, has abnormal activity ratios and is less active per unit A-antigen in the indoleglycerol phosphate reactions than is normal component A. It appears, therefore, that a mixture of A-proteins or an altered A-protein which differs from the A-3 component A is formed by this suppressed mutant.

TABLE 4

ENZYMATIC AND IMMUNOLOGICAL EXAMINATION OF SUPPRESSED MUTANTS

| | | Enzymatic Assay | | | | Immunological Assay | |
|---|---|---|---|---|---|---|---|
| | | Component A | | Component B | | | |
| Strain | Reaction | S.A.† | Per Cent‡ | S.A.† | Per Cent‡ | A Inhibited Per Cent of Control§ | B Inhibited Per Cent of Control§ |
| A-2 su-2 | In → Tryp | 0.29 | 100 | 13.5 | 100 | *ca.* 50–80 | .. |
| | InGP → In | *ca.* 0.01 | 3.4 | 0.46 | 3.4 | ... | .. |
| | InGP → Tryp | 0.12 | 41 | 6.6 | 49 | ... | .. |
| A-3 su-3 | In → Tryp | 43.4 | 100 | 37.8 | 100 | 89 | .. |
| | InGP → In | *ca.* 0.005 | 0.01 | 0.5 | 1.3 (?) | .. | .. |
| | InGP → Tryp | 0.19 | 0.44 | 15.8 | 42 | .. | .. |
| B-1 su-1 | In → Tryp | 53.6 | 100 | 0.36 | 100 | .. | 0* |
| | InGP → In | 1.7 | 3.2 | 1.7 | 470 | .. | .. |
| | InGP → Tryp | 23.9 | 45 | 0.28 | 78 | .. | .. |
| B-4 su-4 | In → Tryp | 5.2 | 100 | 0.79 | 100 | .. | 97 |
| | InGP → In | 0.1 | 1.9 | 0.013 | 1.6 | .. | .. |
| | InGP → Tryp | 1.9 | 37 | 0.36 | 46 | .. | .. |

* Also had a B-CRM specific activity of 59.8.
† Specific activity.
‡ Setting the In → Tryp activity at 100 per cent.
§ Control was inhibition of wild type A or B by homologous antiserum.

A suppressed mutant (strain B-1 su-1) derived from a B-mutant which does not form B-CRM was found to produce what appears to be normal component B, although, as was the case with the corresponding suppressed A-mutant, the level of B present was unusually low when compared with the level of component A which was formed. The component B formed by the suppressed mutant (B-4 su-4) derived from the B-CRM producer had abnormal activity ratios in the various reactions and was less active per unit B-antigenic material in the reactions yielding tryptophan than was normal component B. Thus this suppressed mutant probably forms a mixture of B-proteins, or a component B which differs from both the normal B and the mutant B.

*Discussion.*—The results obtained with the deletion mutants and the A and B point mutants indicate that specific genic regions control the formation of the A and B proteins, and that point mutations affecting one of these proteins have

no effect on the formation of the other. In the point mutants which were examined two types of mutational effects on each of the components of TSase were detected, one leading to the loss of ability to form any protein recognizable enzymatically or immunologically as component A (or B), and the other leading to the formation of an altered protein with one or more properties similar to those of the normal component. The mutations leading to these two types of defects do not appear to be entirely restricted to different segments of the gene since in one case a B-CRM mutational site was found to be between two "loss" mutational sites. The possibility remains, however, that a protein or protein fragment is produced in some of the non-CRM formers but is not identifiable enzymatically or immunologically as component A or B.

The altered proteins formed by the A and B mutants were examined in enzymatic tests, and it was found that without exception, each was effective (in the presence of the normal second component) in one of the three reactions. It is probably significant that the activity associated with each of the altered proteins is the activity which the *second* (unaltered) component possesses to a slight degree by itself. That is, normal component B catalyzes the indole → tryptophan reaction (but not the other two) to a limited extent alone. With altered A present it catalyzes this reaction at the wild-type (maximal) rate but is still ineffective in the other two reactions. Similarly, the altered B-normal A combination catalyzes the indoleglycerol phosphate→indole reaction at the wild-type rate, while normal A alone can catalyze only this reaction, and at a very low rate. It is perhaps also significant in this regard that in every CRM-forming strain but one, mutant B-2, the altered A or B protein has a normal activity:antigen ratio in the effective reaction. These observations may be taken to indicate that component A is the principal active member in reaction (2) and component B in reaction (1) but until additional information is available on the nature of the interaction between the two proteins, no definitive conclusions can be drawn.

The amounts of A and B protein in the various mutants studied deserves comment; in practically every instance more of these proteins were formed by a mutant than is formed by the wild-type strain. Other studies with *E. coli*[11] have shown that the formation of the enzymes involved in the biosynthesis of tryptophan is subject to repression by excess tryptophan.[12] The medium employed in growing the various mutant strains examined in the present study was supplemented with growth-limiting levels of anthranilic acid, indole, or tryptophan; apparently under these conditions the mutants overproduce the A and B proteins.

The studies performed with revertants, partial revertants, and suppressed mutants demonstrate that restoration of the ability to grow in the absence of tryptophan is correlated with the appearance of an A or B activity which was not present in the mutant. In those strains derived from A mutants it has also been found that the restored activity is stable to acidic conditions which precipitate most *E. coli* proteins, but not component A. Whether the suppressed mutants and partial revertants obtained from CRM-producing mutants form a mixture of altered and active protein or a new species of altered protein is not known, but it is clear that in these strains the restored activity is associated with a considerable excess of immunologically active material. This does not appear to be due to changes in the immunological reactivity of the protein or proteins, since the ability to participate

in one of the three biochemical reactions is usually near normal on a per unit antigen basis. In the suppressed mutants obtained from the A and B non-CRM formers, the restored A or B protein appears normal in enzymatic and immunological properties, but the level of activity restored is unusually low (compared to the unaltered component), indicating that some defect in A or B protein formation persists in these strains.

In several recent studies with *N. crassa* it has been observed that heterocaryons formed between two mutants lacking the same enzymatic activity may produce an active protein.[13-16] Some instances of heterocaryon complementations of this type might result from interactions in two component systems similar to the one described in this paper if the two proteins were required for the catalysis of one reaction, and if each of the mutants contributed the ability to form a different unaltered protein to the heterocaryon. In several instances, however, it has been shown that mixtures of extracts of complementing mutants are enzymatically inactive in the relevant reaction. The possibility exists that in some of these systems the complementing mutants bear defects in nonidentical polypeptide chains which are normally associated in a single protein molecule. In such cases, the normal and altered polypeptide chains contributed to the heterocaryon by the two mutants might be synthesized separately or might be subjected to conditions which favor dissociation and reassociation, thereby permitting the formation of a normal protein. A model for interactions of this type is provided by the recent elegant demonstration by Singer and Itano[18] that the human hemoglobin molecule consists of acid-dissociable nonidentical halves.

Recent discussions based on the Watson-Crick model of DNA have led to the suggestion that nucleotide sequences in DNA segments determine amino acid sequences in specific proteins, and that mutations may result from nucleotide substitutions in DNA.[19] Of considerable interest in this connection are the fine structure and chemical mapping studies of Benzer[20] and Benzer and Freese,[21] and the demonstration by Ingram and co-workers that single amino acid differences distinguish some altered forms of human hemoglobin from the normal form.[22, 23] Considering the results reported here, mutations leading to loss of ability to form the A or B protein could represent "nonsense" mutations,[19] i.e., nucleotide changes in which a new sequence does not specify an amino acid and thus only an incomplete or no polypeptide chain can be formed. Those mutations resulting in the formation of an A or B CRM would then represent instances in which a nucleotide change leads to a different coding sequence and thus a single amino acid substitution in the A or B protein. Whether this interpretation will prove to be correct awaits more detailed genetic and biochemical analysis.

*Summary.*—The effects of deletions, point mutations, suppressor mutations and reversions on the two components of the TSase of *E. coli* were examined. It was found that deletion mutants lacking the A and B genes also lack the A and B proteins. Point mutants at the A locus and point mutants at the B locus formed either an altered form of the corresponding protein or none at all. In each instance where an altered protein was produced it was enzymatically active in one of the three reactions in which the normal component participates. Full revertants obtained from A and B mutants formed what appears to be normal components while partial revertants and some suppressed mutants produced either a mixture of normal and

abnormal proteins or a new species of active protein. Suppressed mutants derived from non-CRM formers produced relatively little of one of the two components suggesting that some defect in A or B protein formation exists in these strains.

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## *ON THE LIMIT OF SOLUTIONS OF DIFFERENTIAL-DIFFERENCE EQUATIONS AS THE RETARDATION APPROACHES ZERO*

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1. *Introduction.*—In various parts of mathematical physics, we encounter equations of the form

$$\epsilon L_m(u) + L_n(u) = 0 \tag{1.1}$$

where $L_m(u)$ is a differential operator of order $m$, $L_n(u)$ is a differential operator of