REGULATION OF THE ENZYMES OF THE TRYPTOPHAN PATHWAY IN *ESCHERICHIA COLI*[1]

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IN 1963 GIBSON and JACKMAN identified chorismic acid as the branch point intermediate in aromatic amino acid biosynthesis. Using this substance in addition to the previously identified intermediates of the tryptophan pathway it is now possible to study details of the regulation of synthesis of individual enzymes. Most of the genetic and biochemical details of the pathway in *Escherichia coli* shown in Figure 1 have been elucidated by YANOFSKY (review 1960). Studies of *Salmonella typhimurium* have shown that the biosynthetic pathway in that organism is identical with that of *E. coli* (DEMEREC and HARTMAN 1956), and the genes involved seem to occupy the same relative positions on the linkage map (BALBINDER 1962; SANDERSON and DEMEREC 1965).

As in the biosynthetic pathways of other amino acids, the enzymes of the tryptophan pathway are repressed during growth in the presence of the end product. Repression of the tryptophan enzymes is influenced by at least one regulatory locus, *R try* ("tryptophan repressor"), near *thr* on the *E. coli* chromosome (COHEN and JACOB 1959). This regulatory locus is situated far from the clustered structural genes of the pathway.

Recent investigations have shown that the tryptophan structural genes constitute an "operon". The functioning of genes in the *try* operon is influenced by an "operator" located in or near the structural gene for anthranilate synthetase (MATSUSHIRO, SATO, KIDA, ITO and IMAMOTO 1962, 1965). This *try* operator is thought to be analogous to the *lac* operator in *E. coli* or the *his* operator in *S. typhimurium*. Direct mutation of the tryptophan operator to the O^c (constitutive) condition has not been observed in *E. coli* strains, however.

In 1961 JACOB and MONOD proposed their general model for the control of inducible and repressible enzymes in bacteria. Considerable support for this hypothesis has since been obtained (reviewed by AMES and MARTIN 1964). The properties of "polarity" mutants, causing a reduced output from genes on the side of the mutational site distal to the operator, led AMES and HARTMAN (1963) to amplify the hypothesis of JACOB and MONOD. They suggested that the function of genes in an operon is "modulated" so that distal genes are translated less frequently than proximal ones. This implies that the rate of translation may be a selective factor for the preferential order of genes within an operon.

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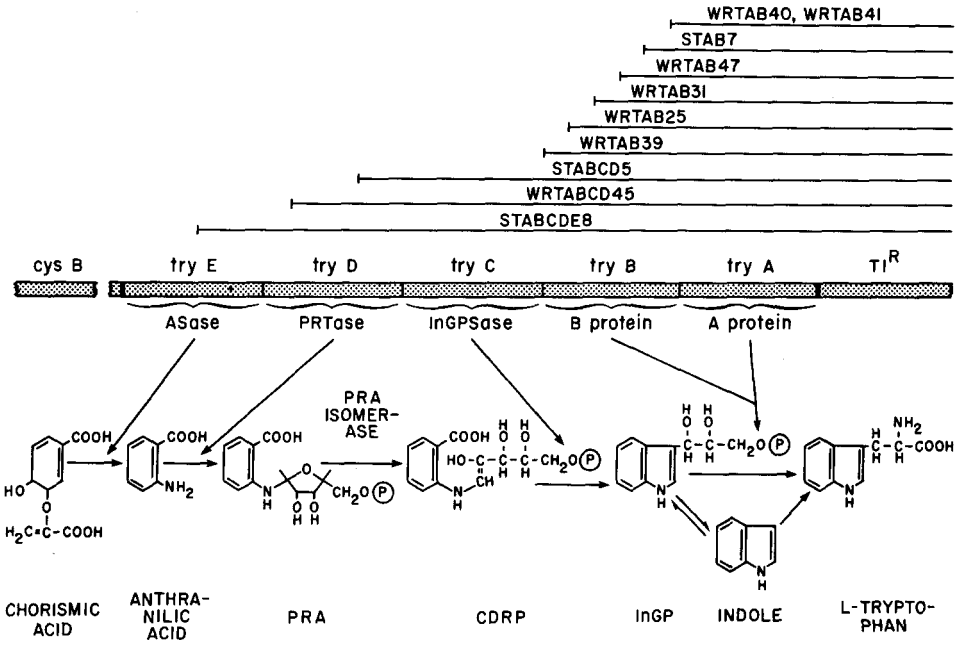

FIGURE 1.—A summary of the tryptophan pathway and the corresponding genes and enzymes in *E. coli*. *T1R*, receptor site for bacteriophage T1; *cysB*, cysteine requirement; PRA, N-5′-phosphoribosyl-anthranilate; CDRP, 1-(o-carboxyphenylamino)-1-deoxyribulose-5-phosphate; InGP, indole-3-glycerolphosphate. PRA isomerase activity may be intrinsic to the InGPSase molecule (J. DEMOSS, personal communication). All the loci shown can be transduced by a single P1 phage. Lines above the gene symbols represent the extent of the T1 resistant deletion mutants used.

Notwithstanding the usefulness of these concepts in accounting for observations in the *lac* operon of *E. coli* and the *his* operon of *S. typhimurium*, the results of ENGLESBERG and his coworkers with the *ara* operon of *E. coli* B are somewhat at variance. The regulator gene in the arabinose system may produce in "inducer" of gene function rather than a "repressor" as postulated for the *lac* and *his* systems (HELLING and WINBERG 1963). In this operon, moreover, there are point mutations that increase the activity of distal loci as well as those that decrease this activity (LEE and ENGLESBERG 1963).

In this paper we present evidence concerning the coordinate formation of the tryptophan synthetic enzymes in *E. coli* K-12. We also have made observations concerning modulation in strains with mutational defects.

MATERIALS AND METHODS

Organisms: A number of tryptophan auxotrophs of *E. coli* K-12 were employed in this study. All CT mutants, A2T65, TN1, A101, B62, B63, and B101, were isolated in this laboratory after ultraviolet (UV) irradiation and penicillin selection. Other mutants were kindly provided by C. YANOFSKY; most have been described previously (YANOFSKY and LENNOX 1959; YANOFSKY and CRAWFORD 1959). A description of the phage T1 resistant *try* deletions and the methods for selecting and characterizing them has been published (CRAWFORD and JOHNSON 1964). A mutant lack-

ing tryptophanase (TPase) was obtained from M. RILEY. This strain as donor was conjugated with a *his⁻cysB⁻* streptomycin resistant recipient. From the progeny a strain which was *his⁺cysB⁻* TPase⁻ and streptomycin resistant was isolated.

Isolation of derepressed mutants: Derepressed mutants (R4, etc.) were isolated by 5-methyltryptophan selection according to COHEN and JACOB (1959). Overnight cultures of *cysB*⁻TPase⁻ in L-broth (LENNOX 1955) supplemented with 20 μg/ml of L-cysteine were subcultured into fresh L-broth and aerated until the cell density reached 2×10^8/ml. The cells were centrifuged, resuspended in an equal volume of the minimal medium of VOGEL and BONNER (1956) and UV irradiated to 0.1% survival. These cells were plated directly on solidified minimal medium containing 100 μg/ml of 5-methyltryptophan and 20 μg/ml of L-cysteine. After two days of incubation at 37°C colonies of several sizes were observed. Large colonies were selected, purified, and grown in 1 ml of L-broth overnight. Among the large colonies there are at least two mutant types; one is the derepressed mutant producing high levels of tryptophan enzymes, and the other is a 5-methyltryptophan resistant mutant with normal levels of tryptophan enzymes. The derepressed mutant can be easily identified by assaying unbroken cells for the presence of elevated levels of tryptophan synthetase activity. A number of test tubes, each containing 8 to 10 ml of minimal medium supplemented with 0.2% glucose and 20 μg/ml of L-cysteine, were inoculated with one drop of the L-broth culture. The tubes were incubated for 15 hours with shaking at 37°C, after which the cultures were centrifuged. The following reaction mixture was added to the cells: 0.4 μmole indole; 80 μmoles DL-serine; 100 μmoles Tris-chloride buffer, pH 7.8; 20 μg pyridoxal phosphate; 0.03 ml saturated NaCl solution; and distilled water to a final volume of 0.8 ml. Cells were incubated in this mixture at 37°C for 15 minutes, then 1 ml of indole reagent (SMITH and YANOFSKY 1962) was added. The derepressed mutants gave a yellow color, while others gave a red color due to presence of indole.

Preparation of cell-free extracts: Unless otherwise stated all organisms were grown in minimal medium with 0.2% glucose and amino acids as required. Cultures were agitated on a rotary shaker at 37°C for 15 to 16 hours. Growth was estimated by turbidity measurement in a Klett-Summerson colorimeter. Cells were harvested by centrifugation, washed once in 0.1 M Tris-chloride buffer at pH 7.8 and resuspended in twice their wet weight of the same buffer. After disruption for 5 to 8 minutes in a 10 kc sonic oscillator (Raytheon), cell debris was removed by centrifugation at 30,000 × *g* for 30 minutes.

Enzyme assays: Anthranilate synthetase (ASase): The reaction mixture contained 1 μmole chorismate, 4 μmoles MgSO4; 10 μmoles L-glutamine; 50 μmoles Tris-chloride buffer at pH 7.5; extract and distilled water to a final volume of 2 ml. The reaction was started by the addition of the cell-free extract to the reaction mixture prewarmed to 37°C. Activity was followed by observing the increase in fluorescence at 390 mμ (activation at 314 mμ) in a recording spectrofluorimeter (WEBER and YOUNG 1964). Chorismate was prepared according to the procedure of GIBSON (1964). Barium chorismate was converted to magnesium chorismate by $MgSO_4$ treatment prior to its use.

Phosphoribosyl transferase (PRTase): The reaction mixture contained 10 mμmoles anthranilate, 0.5 μmoles phosphoribosyl pyrophosphate, 4 μmoles $MgSO_4$, 50 μmoles Tris-chloride buffer at pH 7.8, extract (usually 0.02 to 0.05 ml) and distilled water in a final volume of 2 ml. The reaction was followed at 37°C by observing the decrease in fluorescence at 390 mμ as compared to a control without extract.

Indoleglycerol phosphate synthetase (InGPSase): This assay was performed according to the method of SMITH and YANOFSKY (1962) modified by using 0.1 M Tris-chloride buffer at pH 7.8 rather than pH 8.8.

Tryptophan synthetase: The A and B protein subunits of this enzyme were assayed according to SMITH and YANOFSKY (1962). B-CRM (cross reacting material) and A-CRM were also estimated enzymatically. Although B-CRM has no activity in the indole to tryptophan reaction, it assists the normal A subunit in cleaving InGP to indole. A-CRM has no activity in the InGP to indole reaction but can be assayed in the indole to tryptophan reaction in the presence of the normal B subunit.

Definition of unit and specific activity: One enzyme unit is defined as the production or consumption of 0.1 μmole of product or substrate in 20 minutes at 37°C. The use of this unit enables direct comparison with most other studies of the tryptophan biosynthetic enzymes. Specific activities are expressed as units of enzyme activity per mg protein. Protein was determined by the LOWRY, ROSEBROUGH, FARR and RANDALL (1951) method.

RESULTS

Coordinate formation of tryptophan biosynthetic enzymes: Several methods were used for varying the levels of enzymes concerned with the biosynthesis of tryptophan.

1. *Repression:* A tryptophanase-less mutant was chosen for this study to avoid complications from tryptophanase, an "inducible tryptophan synthetase" (NEWTON and SNELL 1964). A strain containing normal *try* genes, *cysB*⁻TPase⁻, was grown in minimal medium plus 0.2% glucose and 20 μg/ml L-cysteine in the presence of 0, 2, 5, 10, 20, 30, 40, and 50 μg/ml L-tryptophan. Figure 2 shows the result when extracts of these cells were assayed for the five activities under the control of the *try* operon. It can be seen that all the enzymes are repressed to the same extent by growth in the presence of tryptophan. It has been shown previously that enzymes involved in chorismate synthesis are not repressed by L-tryptophan (MATSUSHIRO *et al.* 1965). We have also observed this result; that is, chorismic acid synthetase is not repressed by tryptophan.

2. *Derepression by tryptophan deprivation: E. coli cysB*⁻TPase⁻ cells were grown under repressing conditions (excess tryptophan), then resuspended in medium lacking tryptophan. Under these conditions the activity of all five enzymes increased linearly for 50 minutes. After 50 minutes the specific activities of all the enzymes began to decrease owing to repression. The specific activity of each enzyme was plotted against that of the A-protein, according to the method of AMES and GARRY (1959). Figures 3a-d present the data obtained with the enzymes of the tryptophan biosynthetic pathway. From the straight lines observed, ASase, PRTase, InGPSase, B-protein, and A-protein appear to be coordinately controlled.

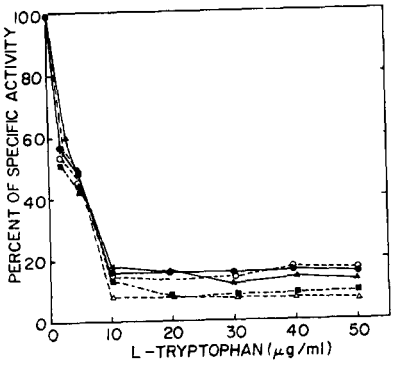

FIGURE 2.—Coordinate repression of the tryptophan biosynthetic enzymes in *E. coli* K-12. ●, A protein; ○, B protein; ■, InGPSase; ▲, PRTase; △, ASase. Data are presented as percent of the specific activity observed in wild-type *E. coli* K-12 grown in minimal medium.

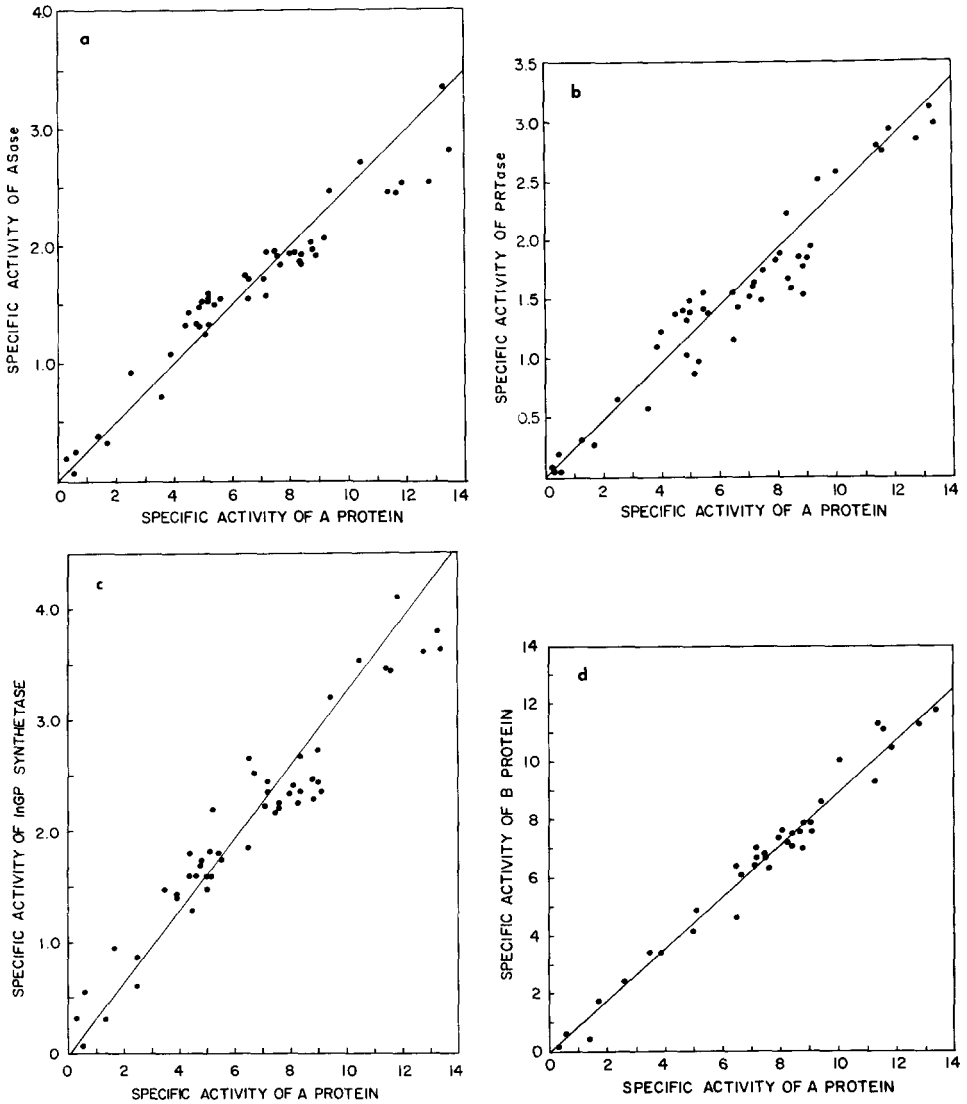

FIGURE 3 (a-d.)—Coordinate variation of ASase, PRTase, InGPSase, and B protein with respect to A protein. Different enzyme levels were obtained as follows. Strain *cysB*-TPase⁻ was grown in minimal medium plus 0.2% glucose, 20 μg/ml L-cysteine ,20 μg/ml L-tryptophan and 0.1% acid-hydrolyzed casein at 37° for 4 hours on a rotary shaker. The exponentially growing culture was cooled rapidly and centrifuged in the cold. Cells were washed once with cold minimal medium and resuspended in the same medium. The washed cells were then transferred to prewarmed minimal medium containing 0.2% glucose, 20 μg/ml L-cysteine and 0.1% acid-hydrolyzed casein. A 1.5 liter culture was shaken in a 4 liter flask at 37°C. At various times during the subsequent 50 minutes, 50 ml aliquots of the bacterial culture were harvested, cooled rapidly, and centrifuged in the cold. Extracts were prepared from the bacteria, and protein and enzyme activities were assayed as described in METHODS. Data from three independent experiments are presented in the figures. FIGURE 3a. Specific activity of ASase plotted against specific activity of A protein. FIGURE 3b. Specific activity of PRTase plotted against specific activity of A protein. FIGURE 3c. Specific activity of InGPSase plotted against specific activity of A protein. FIGURE 3d. Specific activity of B protein plotted against specific activity of A protein.

TABLE 1

Derepression of tryptophan biosynthetic enzymes by 3-methylanthranilate

3-methylanthranilate (μg/ml)	Enzymes				
	ASase	PRTase	InGPSase	B Protein	A Protein
0	0.34(100)	0.31(100)	0.61(100)	2.10(100)	2.60(100)
5	0.52(134)	0.43(138)	0.86(141)	2.95(140)	3.79(145)
10	0.65(190)	0.54(174)	1.16(190)	4.10(195)	5.00(192)

Wild-type *E. coli* K-12 was grown in minimal medium containing 0.2% glucose with 3-methylanthranilate added as indicated. Extracts were prepared and assayed as described in METHODS. Figures show the specific activity of the enzymes indicated. Figures in parentheses show the percent of activity relative to that observed with cells grown in minimal medium.

3. *Derepression by an analogue of anthranilic acid:* LESTER and YANOFSKY (1961) showed that growth in the presence of 3-methyl-anthranilate stimulates the formation of tryptophan synthetase. This compound appears to inhibit the conversion of phosphoribosyl anthranilate to indoleglycerol phosphate, decreasing the rate of endogenous tryptophan production. If the tryptophan enzymes are synthesized in a coordinate fashion, one can expect that both early and late enzymes of this pathway will be derepressed. In accordance with this expectation, when wild-type *E. coli* K-12 is grown in minimal medium containing 5 or 10 μg/ml of 3-methylanthranilate the activities of all the tryptophan enzymes are higher than those of the normal cell (Table 1).

4. *Derepressed mutants:* COHEN and JACOB (1959) demonstrated that mutations which affect the regulator gene of the tryptophan biosynthetic pathway (*R try*) cause a nonrepressible overproduction of tryptophan synthetase as well as the second enzyme in this pathway, PRTase. We have isolated a number of derepressed mutants from a TPase⁻ strain (see METHODS) and determined the repressibility of the enzymes of the tryptophan pathway. Figure 4 shows the

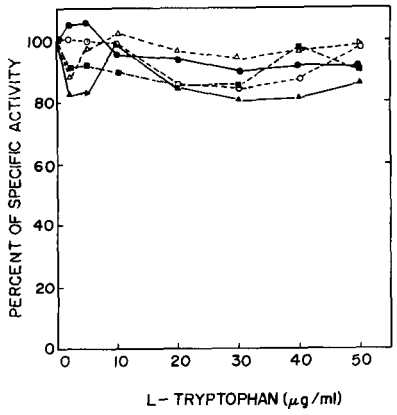

FIGURE 4.—Failure of tryptophan to repress the enzymes of its own synthesis in strain *cysB*⁻ R4TPase⁻. This experiment was performed exactly like that of Figure 2. ●, A protein; ○, B protein; ■, InGPSase; ▲, PRTase; △, ASase. The specific activities corresponding to 100% in this experiment were: A protein, 19; B protein, 18; InGPSase, 5.5; PRTase, 4.5; ASase, 4.4.

TABLE 2

Inhibition of anthranilate synthetase by L-*tryptophan*

Strain	Experiment	L-tryptophan	Enzyme activity (units)	Inhibition (percent)
wild*	1	none	5.30	0
		5×10^{-5}M	0.55	90
	2	none	5.20	0
		5×10^{-6}M	4.15	20
		5×10^{-5}M	0.57	89
cysB-R4	1	none	3.95	0
		5×10^{-5}M	0.46	89
	2	none	4.14	0
		5×10^{-6}M	3.38	20
		5×10^{-5}M	0.39	91

* An A-CRM-less strain, A4, was used in this experiment as a source of wild-type enzyme.
Assays were performed as described in METHODS, with the addition of the indicated amount of L-tryptophan to the reaction mixture.

result observed with extracts of one of our mutants. It can be seen that none of the enzymes are repressible by the addition of L-tryptophan. This mutant's enzyme levels are 10 times greater than those of the wild type grown in minimal medium. None of our mutants are of the "operator constitutive" (O^c) type, because their nonrepressibility is not co-transducible with the tryptophan genes. In the case of our strain *cysB*-R4TPase$^-$, as with COHEN and JACOB's *R try*, the nonrepressible character is closely linked to *thr* in transduction experiments. These mutants differ from the "feedback resistant" mutants reported by MOYED (1960) because the first enzyme, ASase, is still as sensitive to the end product, L-tryptophan, as is the wild-type enzyme (Table 2).

All the above results support the hypothesis that the synthesis of the tryptophan enzymes is under the control of a common repressor or inducer.

Enzyme levels in various tryptophan auxotrophs: The *E. coli try* gene-cluster shows several of the features of an operon including, as we have shown, coordinate control of gene products. We therefore sought to examine a variety of auxotrophs to see if mutation in a particular gene could alter the functioning of other genes in the operon.

A number of different tryptophan auxotrophs were grown under conditions of derepression (5 μg/ml L-tryptophan). Extracts of these cells were assayed for the enzymes of the tryptophan pathway. In Table 3 the specific activity of enzymes in the auxotrophic strains is compared to enzyme levels in the derepressed mutant *cysB*-R4TPase$^-$ grown in minimal medium. In most cases the value recorded is an average of two preparations. Certain ASase-less mutants have a reduced capacity to synthesize the other enzymes, as observed earlier (MATSUSHIRO *et al.* 1965; SOMERVILLE and YANOFSKY 1965). The extent of this reduction may be very slight, as in CT11, moderately severe, as in T15, or drastic, as in T26N or the double mutant A2T65. In addition, there are instances of mutations within genes other than the ASase locus that decrease the levels of enzymes formed by

TABLE 3

The specific activity of tryptophan biosynthetic enzymes in various auxotrophic strains

Strain	Block	Enzymes				
		ASase	PRTase	InGPSase	B protein	A protein
K-12	Wild	(0.42)	(0.36)	(0.59)	(2.26)	(2.70)
cysB-R4TPase⁻	*R try*	100 (4.42)	100 (4.40)	100 (5.59)	100 (21.6)	100 (26.8)
A2T65	ASase, A protein	0	2	3	1	0
T26N	ASase	0	1	5	6	5
T15	ASase	0	2	12	13	19
CT11	ASase	0	84	52	48	87
T80	PRTase	28	0	36	35	80
T58	PRTase	58	0	44	24	65
T16	PRTase	46	0	123	105	94
CT4	InGPSase	94	68	0	34	39
CT5	InGPSase	99	68	0	41	58
CT6	InGPSase	89	88	0	51	78
CT7	InGPSase	148	104	0	47	66
CT8	InGPSase	121	101	0	53	76
T20	InGPSase	217	164	0	43	75
T24	InGPSase	135	122	0	76	75
T4-3	InGPSase	175	193	0	148	218
TN1	InGPSase	176	143	0	169	121
B8	B protein	343	322	337	*	200
B2	B protein	997	959	272	*	432
B3	B protein	894	827	299	*	330
B51	B protein	984	827	290	*	337
B62	B protein	783	845	308	*	500
B63	B protein	785	474	315	*	400
B4	B protein	469	420	217	0	22
CT4	B protein	669	402	237	0	73
B5	B protein	809	786	177	0	88
B6	B protein	600	795	179	0	35
B101	B protein	803	897	261	0	47
A1	A protein	135	123	77	41	†
A3	A protein	127	96	77	64	†
A101	A protein	147	99	102	70	†
A2	A protein	505	445	420	192	0
A47	A protein	512	400	330	160	0

* B-CRM protein.
† A-CRM protein.
Cells were cultured in minimal medium containing 0.2% glucose and 5 μg/ml L-tryptophan. A supplement of 0.01% acid-hydrolyzed casein was included in the growth medium of all *B* mutants to increase the yield of bacteria. The cells were harvested in the stationary phase after 15 hours of growth. The enzyme levels are expressed relative to those found in the derepressed mutant, *cysB*-R4TPase⁻. The specific activities of *cysB*-R4TPase⁻ and the wild type are presented in parentheses for reference.

the genes on the side of the mutation distal to the operator. About half of the InGPSase-less mutants show such a polarity effect to a slight but significant extent. MATSUSHIRO *et al.* (1965) reported one instance of a polarity effect

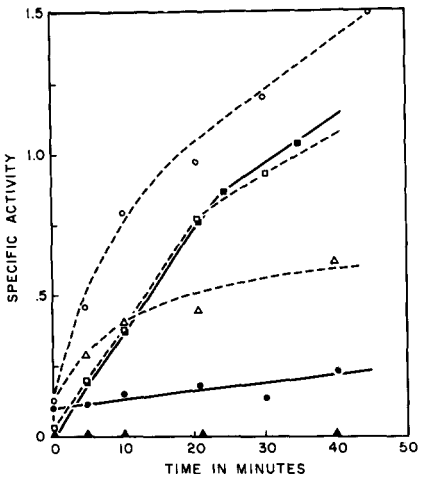

FIGURE 5.—Differential rate of enzyme synthesis of A protein by a B-CRM-less mutant (B4) and by a B-CRM mutant (B1). The experimental method was identical with that described for Figures 3a-d except that the final culture medium contained 0.1 μg/ml L-tryptophan and no cysteine. Enzyme activities and B-CRM were assayed as described in METHODS. The initial rates of synthesis of all enzymes and B-CRM were slower than that of wild-type cells. This delay during initial derepression probably reflects the presence of tryptophan or its precursors in the cells. ○, A protein in strain B1; ●, A protein in strain B4; △, B-CRM in strain B1; ▲, B-CRM in strain B4; □, ASase in strain B1; ■, ASase in strain B4.

involving a *tryB* mutant, W4627. In our experiments with mutants in the *tryB* gene there is a striking difference in the level of A protein formed by B-CRM and B-CRM-less mutants, in spite of the fact that the levels of the other enzymes seem almost the same. This result was confirmed by an experiment in which the differential rate of synthesis of A protein by a B-CRM-less mutant was compared to that of a B-CRM mutant. ASase was also followed as a control. Figure 5 shows the difference in the level of A protein formed during release of repression by strain B4 (CRM-less) and strain B1 (CRM containing). From these results we suggest that CRM-less mutants of the *B* gene have a polarity effect.

The data of Table 3 also show that all *B* mutants and A-CRM-less mutants have unusually high specific activities of ASase and PRTase. This phenomenon may reflect the action of TPase, which converts tryptophan to indole. Because indole is not a repressor, cells unable to reconvert it to tryptophan may be provoked into a greater derepression of the tryptophan biosynthetic enzymes under conditions of tryptophan limitation (YANOFSKY 1960). All *B* gene mutants except B8 are unable to grow on the indole. (Although strains B8 and all A-CRM-less mutants can utilize indole for growth, their ability to convert this compound to tryptophan is feeble compared to wild type, A-CRM forming mutants, or mutants in earlier steps.) One tryptophanase-less *tryB* mutant, B51TPase$^-$, was synthesized and assayed after growth on a limiting amount of tryptophan (4 μg/ml). Although the yield of B51TPase$^-$ cells was markedly increased over that of B51TPase$^+$ cells grown in the same medium, the A protein specific activity of the tryptophanase-less strain was found to be only 20 units per mg, one fifth that of

TABLE 4

The derepressed enzyme levels of deletion mutants of the tryptophan biosynthetic pathway

Strain	Enzymes				
	ASase	PRTase	InGPSase	B protein	A protein
cysB-R4TPase⁻	100	100	100	100	100
STABCDE8	0	0	0	0	0
STABCD5	39	0	0	0	0
WRTABCD45	46	0	0	0	0
WRTAB39	197	80	12	0	0
STAB7*his*⁻	168	167	10	0	0
WRTAB31	154	123	18	0	0
WRTAB40	229	204	13	0	0
WRTAB25	296	222	8	0	0
WRTAB41 *his*⁻*pro*⁻	500	391	21	0	0
WRTAB47 *his*⁻	602	456	6	0	0

All deletion mutants were grown in minimal medium plus 0.2% glucose, 5 μg/ml L-tryptophan and 0.01% acid-hydrolyzed casein for 15 hours at 37°C. Supplements of 20 μg/ml of L-histidine and L-proline were added to the growth medium of histidine and proline requiring strains.

the tryptophanase containing mutant. This result substantiates the hypothesis that derepression is more effective in cells whose ability to form tryptophan from indole is impaired.

All *B* mutants with the exception of B8 have reduced levels of InGPSase, formed by the *C* gene which is immediately *proximal* to the *B* gene. We doubted that this reduced level of InGPSase could be the result of interference by the A protein during the assay, for the A protein alone has very little InGP to indole activity. To examine this point, however, several T1 resistant *try* deletion mutants were grown under derepressing conditions and examined for the level of enzymes. Table 4 gives the results for seven deletions ending in the *B* gene and some longer deletions. The extent of the deletions is indicated in Figure 1. It can be seen that all *AB* deletions have decreased levels of InGPSase. This decrease cannot be the result of an assay artifact caused by the A protein. Because other mutants (T16, STABCD5, and WRTABCD45) show the same effect on the production of the enzyme formed by the gene on the proximal side of the mutation, this phenomenon must not be a specific property of the *B* mutants. It would appear that these are truly defects in the translation of genetic information.

DISCUSSION

The results presented above show clearly that the enzymes specific for tryptophan biosynthesis in *E. coli* are coordinately regulated in wild-type cells. These results are in agreement with other published studies on the regulation of clustered genes of related function in enteric bacteria (reviewed by AMES and MARTIN 1964). By analogy with the *lac* operon in *E. coli* and the *his* operon in *S. typhimurium*, our results are compatible with the hypothesis that the tryptophan enzymes might all be coded on one large messenger RNA molecule. Very

recent work by IMAMOTO, MORIKAWA, and SATO (J. Mol. Biol., in press) indicates that such messenger RNA molecules for the entire *try* region may actually exist. Their results also indicate that tryptophan messenger RNA molecules are synthesized from the operator end.

The *try* mutants we examined in Tables 3 and 4 differ considerably in derepressed enzyme levels. AMES and HARTMAN (1963) found polarity effects (a 50 to 90% reduction in the productivity of genes distal to the mutational site) in about half the *his*⁻ mutants of *S. typhimurium* examined. The effect was noted in derepressed as well as repressed cells. The most notable examples of polarity among the *try*⁻ mutants of *E. coli* are in the *B* gene. There a clear pattern emerges; all CRM-less mutants have low A protein levels, whereas all CRM-forming mutants have normal enzyme levels. From these results we would propose that all mutants showing polarity effects within the *try* operon of *E. coli* K-12 are CRM-less, and perhaps the result of nonsense mutations. It is interesting to note that one of the *C* gene mutants which produces a normal level of the A and B proteins, T4–3, possesses an InGPSase CRM (O. SMITH, personal communication). Additional work on the products of the *A*, *B*, and *C* genes will be needed to test the generality of the hypothesis, however.

A most unexpected finding that occurred often in the present study was poor production from the gene immediately *proximal* to the mutational site. This effect appeared with certain point mutants, but was invariable when T1 resistant deletion mutants were examined. AMES and HARTMAN (1963) did not observe this phenomenon with point mutants in the *his* operon of *S. typhimurium*; they did not report its occurrence in several deleted strains examined, though the deletions they used did not extend through the distal end of the operon as ours do. It is noteworthy that the magnitude of this *short range anti-polar effect* seems independent of the amount of genetic material remaining in the deleted gene. Strain WRTAB40, extending but a short distance into the *B* gene, produces no more InGPSase than strain WRTAB39 which fails to recombine with all known *B* gene point mutants. We would prefer not to suggest a mechanism for this effect until it has been studied under conditions of repression as well as derepression and until it has been sought with internal deletions of the operon as well as terminal ones.

Concerning modulation within the *try* operon, WILSON and CRAWFORD (1965) have concluded that the products of the *A* and *B* cistrons normally are produced in equimolar amounts. This is not surprising, for these products are the two subunits of a single enzyme, tryptophan synthetase. It will be more interesting to compare the molar ratios of the products of the *C*, *D*, and *E* genes with tryptophan synthetase. The only relevant information available to us concerns the *E* gene product, ASase. This enzyme has been purified by BAKER and CRAWFORD (manuscript in preparation). It appears to have a molecular weight near 200,000 and a specific activity of about 550 units per mg. Using these figures, and the corresponding ones for the A protein (molecular weight 29,500; 5200 units per mg), the molar ratio ASase: A protein in wild type and strain R4 is 1:4.4. ASase is composed of subunits, but their size and number remains unknown. Under one

circumstance, that of four like subunits, the molar ratios of the *E*, *B*, and *A* gene products would approximate unity. Only if the ASase molecule contains more than four identical subunits would the output of the *E* gene exceed that of the *A* and *B* genes. In the light of present evidence, then, modulation in the sense of AMES and HARTMAN (1963) seems unlikely within the *try* operon of *E. coli*.

The repressor gene mutants described in this study are in every way like the *R try* mutants described by COHEN and JACOB (1959). The mutational alteration in these strains maps near the *thr* locus, not near the *try* operon. The mutants excrete tryptophan during growth on minimal medium. The ASase of one of these mutants is normally sensitive to end-product inhibition. Certain mutants within the *E* gene having an ASase insensitive to tryptophan inhibition are known (MOYED 1960; SOMERVILLE and YANOFSKY 1965). It would be interesting to quantitate the rate of tryptophan excretion that would be observed with the *R try* and ASase mutations present separately and together.

Somewhat greater derepression of the tryptophan pathway was observed in strain *cysB*$^-$R4TPase$^-$ than has been reported for COHEN and JACOB's *R1try*. Although we did not construct a merodiploid for the *thr* region with our strain, in experiments using a colicinogenic episome bearing a normal *try* operon (FREDERICQ 1963) we constructed a merodiploid of the genotype *cysB*$^-$*try*$^+$ R4TPase$^-$/F′ colVcolB*cysB*$^+$*try*$^+$. For brevity we have not presented the results of enzyme assays in this strain; all enzymes of the *try* pathway were found in twice the amount present in the parental R4 strain, however. The presence of the episome in wild-type *E. coli* K-12 causes a similar doubling in the (lower) levels of enzyme produced in the presence of the wild *R try* gene. Our findings are most easily interpreted in terms of a paucity or malfunction of the cytoplasmic *R try* gene product. We have no evidence suggesting that the *try* operon or its operator site are abnormal in strain R4.

BAUERLE and MARGOLIN (1965), working with *S. typhimurium* mutants, have postulated the presence of two "promoters" (sites of initiation of gene expression) within the *try* operon. The positions of these promoters in our terminology would be at the *tryE* end of the operon and between the *tryD* and *tryC* genes. None of our mutants suggest that gene expression distal to these sites can be lost as a unit. Unfortunately, however, we have not been able to study deletions extending into the operon from the *tryE* end as BAUERLE and MARGOLIN have done with Salmonella.

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SUMMARY

Five proteins involved specifically in the synthesis of tryptophan are coordinately regulated in wild-type *E. coli*. The productivity of the structural genes for these proteins can be influenced by the state of a regulatory locus, *R try*, located far from the *try* operon. A survey of mutants located in one or another of

these structural genes revealed some polarity effects. Those mutants exhibiting the polarity phenomenon within the *tryB* gene were invariably CRM-less (lacking crossreacting material); CRM-forming mutations were invariably without polarity effects. A "short range anti-polar effect" observed with some point mutants and all the deletions studied causes a marked depression in the product of the nearest unmutated gene on the operator side of the mutational defect. Present evidence does not indicate the occurrence of modulation within the normal *try* operon.

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