

# TRYPTOPHAN BIOSYNTHESIS IN *SALMONELLA TYPHIMURIUM*\*

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Recent studies have demonstrated that tryptophan is synthesized in *Escherichia coli* from anthranilic acid via indole.<sup>1</sup> A study has been made of tryptophan auxotrophs of *Salmonella typhimurium*, and the results, reported below, indicate that the same pathway of tryptophan biosynthesis is followed in this strain.

Ten tryptophan-requiring mutants of *S. typhimurium*, strain LT2, kindly provided by Dr. M. Demerec, have been investigated. The nutritional properties of the strains were established by inoculating *ca.* 10<sup>8</sup> cells into 5 ml. of A medium<sup>2</sup> with added supplements and incubating the cultures for 18 hours at 37° C. Accumulations were studied by centrifuging 48-hour-aerated cultures of the strains in A medium supplemented with 5 µg/ml L-tryptophan, followed by the application of various procedures to the supernatant fluids. Anthranilic acid was identified by its characteristic blue fluorescence, by its ultraviolet absorption spectrum with a maximum at 3100 Å, by the red-purple color (absorption maximum at 5550 Å) developed with the Bratton-Marshall reagents<sup>3</sup> and prevention of color development by prior acetylation, and by paper chromatography. Compound B is an as yet unidentified substance characterized by an ultraviolet absorption maximum at 2780 Å, a blue color (absorption maximum at 6250 Å) with the Bratton-Marshall reagents unaffected by prior acetylation, insolubility in ether, no color reaction with *p*-dimethylaminobenzaldehyde, and conversion to indole by steam distillation in the presence of 0.1 N NaOH.<sup>4</sup> Indole was identified by its solubility in ether, the characteristic rose color developed with *p*-dimethylaminobenzaldehyde, and a red color reaction (absorption maximum at 5350 Å) with the Bratton-Marshall reagents which is unaffected by prior acetylation.

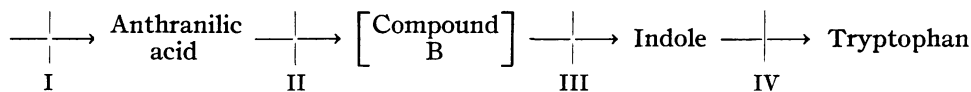
The results are given in Table 1, from which it can be seen that the mutants can be divided into four distinct groups. Demerec and his collaborators<sup>5</sup> have shown that these mutants can be separated into four classes, depending on the frequencies

TABLE 1  
GROWTH REQUIREMENTS AND ACCUMULATIONS OF TRYPTOPHAN  
AUXOTROPHS OF *S. Typhimurium*

try-MUTANTS	GROWTH IN A MEDIUM PLUS 20 µG/ML SUPPLEMENTS				ACCUMULATIONS	GROUP
	None	Anthra-nilic Acid	Indole	L-Trypto-phan		
-8	-	+	+	+	None detected	I
-2; -4	-	-	+	+	Anthranilic acid	II
-3	-	-	+	+	Compound B	III
-1; -6; -7; -9; -10; -11	-	-	-	+	Mixture of compound B and indole	IV

of transductions; transductions between members of the same class occur with a smaller frequency than do those between members of different classes. The indication is that all the members of one transduction class possess mutations in the same gene locus (nonidentical alleles), while different classes are characterized by

mutations in separate loci. A comparison of the present findings with those of Demerec has shown that the four phenotypic groups are identical with the four transduction classes, which suggests that each group is characterized by a block in one of the steps of tryptophan biosynthesis and that each block is produced by mutation in one gene locus. The data are therefore consistent with the following biosynthetic pathway in which each step is controlled by a single gene:



The precise role of compound B as an intermediate has not yet been determined. It possesses no growth-promoting activity for mutants blocked in earlier stages and may, in fact, be produced as a side reaction of the normal intermediate. This problem is at present under investigation.

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<sup>3</sup> A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 537, 1939.

<sup>4</sup> S. Brenner (unpublished).

<sup>5</sup> M. Demerec, personal communication; *Carnegie Inst. Wash. Year Book*, **53**, 227, 1954.

## AMINO ACID ADSORPTION AND PROTEIN SYNTHESIS IN *ESCHERICHIA COLI*\*

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Two quite distinct theories of protein synthesis are currently popular. One holds that amino acids are linked into small peptides which then serve as building blocks for the proteins. The other postulates that amino acids are individually adsorbed on a large template molecule and are then linked together into the polypeptide chains. This paper reports the results of studies of amino acid incorporation by growing *Escherichia coli*. The results furnish experimental evidence in favor of the template theory.

When *E. coli* cells (grown in a medium<sup>1</sup> containing glucose, salts, and ammonia) are washed and treated with cold 5 per cent trichloroacetic acid (TCA), a number of amino acids are extracted. These amino acids are transient intermediates of synthesis. When C<sup>14</sup>-glucose is added to the medium, their specific radioactivity