# The Synthesis of Certain B$_6$ Enzymes during the Lag Phase

# of Growth of *Escherichia coli*

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1. Using *Escherichia coli* as test organism, the synthesis of gluta-mic acid and arginine decarboxylases, serine, threonine, and homose-rine deaminases, cysteine desulphhydrase, tryptophanase, and tryp-tophan synthetase was studied during the lag phase of growth.

2. The cells required for inoculation were grown in a medium rich in amino acids and vitamins. Cells in the lag phase of growth that had been transferred to a simple glucose and mineral salt medium were analyzed for enzyme content.

3. No changes were observed in the amounts of glutamic acid and arginine decarboxylases, of serine, threonine, and homoserine deami-nases, of cysteine desulphhydrase or of tryptophanase in the cells.

4. The synthesis of tryptophan synthetase started immediately after the cell transfer and the amount of the enzyme increased line-arly during the lag phase to four to eight times the original amount.

In spite of the fact that the first period of bacterial growth, the lag phase, is known to be a metabolically active one, and thus no rest phase, the synthesis of enzymes during this phase has not apparently been studied to any greater extent. It is true that in most growth experiments samples have been taken during the period when growth begins, but mostly these samples have been too few in number to give any clear picture of the phase in question and, furthermore, these samples may not always have been taken during the lag phase. Moreover, what literature there is on this growth phase is confusing because, in many cases, the investigators are not agreed upon the exact dura-tion of the lag phase [1].

McGarthy [2] studied the amounts of glucose dehydrogenase and succinic acid dehydrogenase present in cells of *Bact. lactis aerogenes* during the lag phase but he did not observe any changes in the enzyme contents. Rowbury and Woods [3], who investigated the enzymes that synthesize methionine in *Escheri-chia coli* during the lag phase, found that the ability of cell extract to syn-thesize methionine increased during the lag phase. Corini and Maas [4] showed that the content of ornithine transcarbamylase in the cell increased between cell divisions.

In previous experiments [5,6] we have found that the amount of vitamin $B_6$ and certain transaminases increase during the lag phase of growth of *E. coli*. In the present investigation we have studied the synthesis by *E. coli* cells of some other $B_6$ enzymes during the lag phase. The enzymes we chose to study are the decarboxylases, the serine, threonine, and homoserine deaminases, the cysteine desulphhydrases, tryptophanase and tryptophan synthetase.

## EXPERIMENTAL

*Growth organism and culture methods.* The strain of *Escherichia coli* used in this investigation was isolated as described in a previous paper [33]. Also the media employed have been described in a previous paper [6].

*Procedure.* Before the experiment *E. coli* cells were transferred from the nutrient agar slant on which they had been maintained to 5 ml of an inoculum medium. The cells were incubated 6−9 h at 37°C in this medium and then transferred aseptically to 1 000 ml of fresh, cold inoculum medium. After incubation for 12−13 h at 37°C the cells were centrifugated at some 2 500 rpm in a Wifug centrifuge for 15 min and washed twice with saline. Immediately after being washed, the cells were transferred in saline to 1 000 ml of growth medium into which they were dispersed with an electric stirrer. The first sample was taken 5 min after this transfer. Later samples were taken at intervals of 5−7 min and placed directly in ice water where the temperature of the medium sank to about 5°C in 1−1 1/2 min. Each sample contained 0.18−0.24 mg (dry weight) of cells per millilitre.

Generally the lag phase lasted 30−50 min. The turbidities of the suspensions were measured with a Klett-Summerson colorimeter employing red filter No. 62. Thirty-five-millilitre volumes of the ice-cold medium were pipetted into tubes of a Servall RC-2 centrifuge and centrifuged 10 min at 10 000 rpm. After this the cells were washed twice with cold saline and centrifuged between washings. During the centrifugation the temperature of the samples was 0°C.

*Preparation of enzyme extracts.* For the determinations of the *desulphhydrase* and *deaminase* activities the cells were frozen in a small amount of distilled water at −40°C for half an hour and warmed to 37°C. This procedure was repeated. The milky cell suspension obtained was used in the experiments as the enzyme preparation. In a few experiments a toluene powder was employed; this was prepared by a method similar to that described below for the preparation of acetone powder.

For the *decarboxylase, tryptophan synthetase,* and *tryptophanase* activity determinations the cells were thoroughly mixed with 30 ml of −20°C acetone for 10−30 min and centrifuged down. The acetone remaining in the sediment was evaporated in a vacuum at room temperature. The acetone powder obtained was autolyzed for decarboxylase determination overnight at 2°C in a M/10 buffer which was the same as the substrate buffer used in the experiment. For the tryptophan synthetase and tryptophanase determinations the powder was autolyzed in M/5 buffer with reduced glutathione for half an hour at room temperature.

*Determination of enzyme activities.* For the *desulphhydrase* and *deaminase* activity determinations, 0.50-ml volumes of enzyme extract prepared as described were pipetted into 15 × 110 mm tubes. This volume of extract contained approximately 1.00−1.30 mg (dry weight) of cells. The reaction was started by adding a 0.50-ml volume of substrate solution containing 20 μmoles of L-amino acids (40 μmoles of DL-amino acids) in 0.1 M buffer. A borate buffer of pH 9.5 was employed in the determinations of serine and threonine deaminase activities and a phosphate buffer of pH 7.4 in the determination of homoserine deaminase and cysteine desulphhydrase activities. When pyridoxal phosphate was used as activator, the amount added to the reaction system was 20 μg. The reaction was stopped by adding 0.2 ml of 10 N sulphuric acid and heating in a water bath for 2 min. The keto acids formed were determined by the method of Friedemann and Haugen [7]. The absorption of the hydrazone was measured with a Klett-Summerson colorimeter employing filter No. 42. In all experiments the reaction was stopped while the activity increase was still linear.

*Table 1.* Decarboxylase activities in *Escherichia coli* U 5—41.

| Amino acid | Activity ($\mu$l CO$_2$/h). |
|---|---|
| L-Glutamic acid | 232 |
| L-Threonine | 4 |
| L-Cysteine | 8 |
| DL-Serine | 2 |
| L-Tyrosine | 0 |
| L-Dopa | 0 |
| DL-Ornithine | 0 |
| L-Aspartic acid | 0 |
| L-Leucine | 0 |
| L-Arginine | 12 |
| DL-Lysine | 4 |
| L-Valine | 0 |
| DL-Tryptophan | 0 |
| L-Histidine | 0 |

The decarboxylase activity was measured with a Warburg apparatus. For this purpose 1.0 ml of the acetone-dried cell suspension containing 0.056—0.059 mg (dry weight) of cells was pipetted into the main compartment of the Warburg vessel. A 1.0-ml volume of 0.2 M buffer containing 6—10 $\mu$moles of L-amino acid was pipetted into the side arm. The following buffers were used: acetate-buffer (pH 4.5) for histidine and glutamic acid; phosphate buffer (pH 6.0) for lysine, leucine, and valine; phosphate-citrate-buffer (pH 5.2) for arginine, threonine, and cysteine; a phosphate-citrate-buffer of pH 5.5 for ornithine, tyrosine, serine, aspartic acid, and tryptophan; and a phosphate-buffer of pH 6.8 for 3.4-dihydroxyphenylalanine.

The tryptophanase and tryptophan synthetase activities were measured in 15 × 110 mm test tubes by the methods of Gunsalus *et al.*[8] and Yanofsky[9]. No loss of indole was
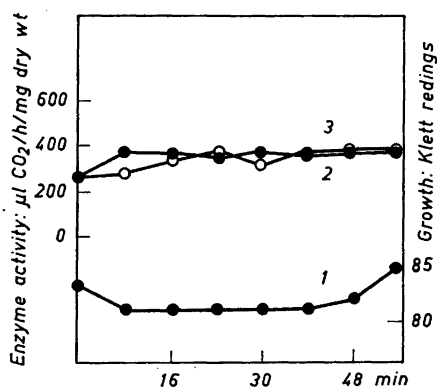


*Fig. 1.* Glutamic acid decarboxylase activity of *Escherichia coli* cells during the lag phase of growth, 1 = growth curve (right-hand scale), 2 = glutamic acid decarboxylase activity with added pyridoxal-5-phosphate, and 3 = glutamic acid decarboxylase activity without added pyridoxal-5-phosphate.
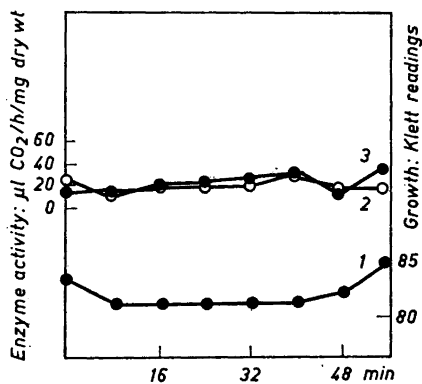
*Fig. 2.* Arginine decarboxylase activity during the lag phase of growth by *Escherichia coli.* 1 = growth curve (right-hand scale), 2 = arginine decarboxylase activity of cells without added pyridoxal-5-phosphate, and 3 = arginine decarboxylase activity with added pyridoxal-5-phosphate.
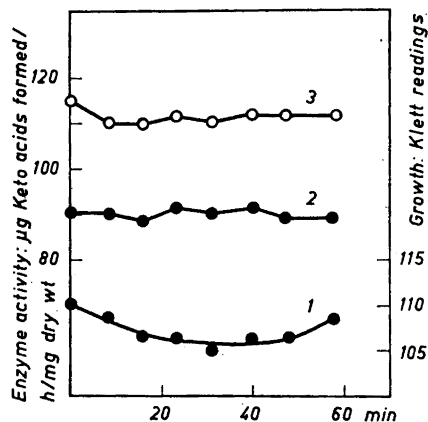
Fig. 3. Serine deaminase activity during the lag phase of growth of *Escherichia coli*. 1 = growth curve (right-hand scale), 2 = serine deaminase activity without pyridoxal-5-phosphate, and 3 = serine deaminase activity with added pyridoxal-5-phosphate.
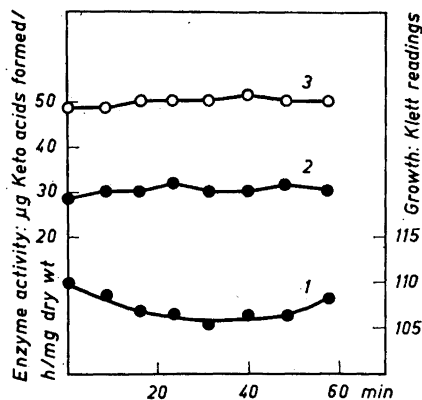
Fig. 4. Threonine deaminase activity by *Escherichia coli* cells during the lag phase of growth. 1 = growth curve (right-hand scale), 2 = threonine deaminase activity without added activator, and 3 = threonine deaminase activity with added pyridoxal-5-phosphate

observed in the determination of tryptophan synthetase during the time of testing when the reaction system lacked serine.

*Chemicals.* L-Cysteine hydrochloride, DL-serine, DL-threonine, L-histidine, and L-tryptophan were obtained from Hoffmann-La Roche; DL-serine, L-cysteine, DL-valine, L-leucine, L-glutamic acid, DL-lysine, L-arginine, and L-tyrosine from E. Merck AG. DL-Aspartic acid was obtained from Fluka. In paper-chromatographic tests each of the amino acids gave only one spot. Pyridoxal-5-phosphate was obtained from Hoffmann-La Roche. All the other chemicals used were of reagent grade.

## RESULTS

The main object of the study was to determine what kinds of amino acid decarboxylases *Escherichia coli* possesses. The results are shown in Table 1.

As can be seen from the table, only glutamic acid was decarboxylated in any marked degree. The glutamic acid and arginine decarboxylase activities were measured in samples taken during the lag phase but the results (shown in Figs. 1 and 2) demonstrate that no variations occurred in the amounts of these enzymes. Addition of pyridoxal-5-phosphate when the activities of the enzymes were measured hardly increased the activities. The activity of glutamic acid decarboxylase varied within the range of $350-400$ $\mu$l $CO_2$/mg dry weight/h and that of arginine decarboxylase within the range of $20-30$ $\mu$l $CO_2$/mg dry weight/h. The lag phase lasted $40-45$ min in these experiments.

No variations were observed in the amounts of serine and threonine deaminases in *E. coli* cells during the lag phase of growth. Fig. 3 shows the amount of serine deaminase in the cells; the activity was within the range of $88-92$
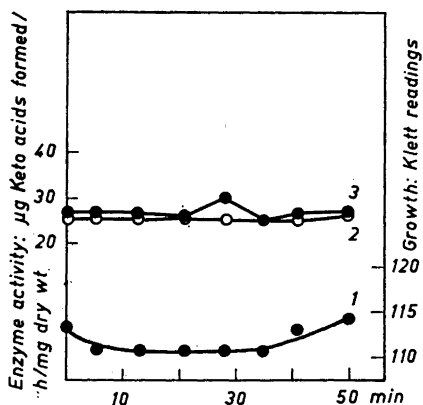
*Fig. 5.* Cysteine desulphhydrase activity during the lag phase of growth of *Escherichia coli*. 1 = growth curve (right-hand scale), 2 = cysteine desulphhydrase activity without added activator, and 3 = cysteine desulphhydrase activity with added pyridoxal-5-phosphate.
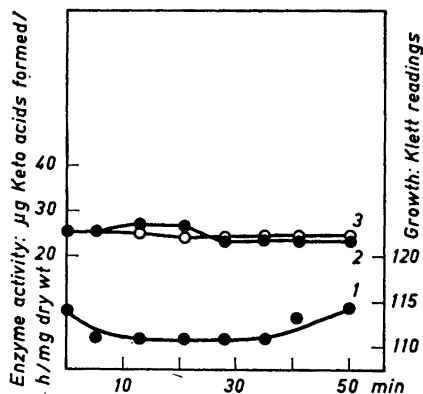
*Fig. 6.* Homoserine deaminase activity during the lag phase by *Escherichia coli* cells. 1 = growth curve (right-hand scale), 2 = homoserine deaminase activity of cells without added pyridoxal-5-phosphate, and 3 = homoserine deaminase activity with added activator.

μg of keto acid produced per mg (dry weight) of cells during 80 min. In contrast to the decarboxylases, the serine deaminase was strongly activated to the same extent by pyridoxal-5-phosphate throughout the lag phase. The values which were obtained when the activator had been added to the reaction systems for the enzyme determinations, and which correspond to those mentioned above, were 110—115 μg of keto acid produced per mg dry weight in 80 min. The lag phase lasted 40—45 min. The results obtained with threonine deaminase shown in Fig. 4 correspond to those obtained with serine deaminase. Without activator the activity was 28—32 μg of keto acid produced per mg of dry weight in 80 min. The lag phase lasted 40—45 min in these experiments.

No variations were observed in the amounts of cysteine desulphhydrase and homoserine deaminase during the lag phase. As can be seen from Fig. 5, the activity of the cysteine desulphhydrase remained within the range of 25—27 μg of keto acid produced per mg of dry weight during 240 min and was not affected to any marked extent by the addition of pyridoxal-5-phosphate. Such an addition did not either increase the activity of homoserine deaminase, as shown by Fig. 6; during the lag phase this activity remained within the range of 24—26 μg of keto acid produced/mg (dry weight) of cells/240 min. In these experiments the lag phase lasted about 40 min.

The amount of tryptophanase likewise remained constant throughout the lag phase. The activity varied within the limits of 6.2—6.5 μg of indole produced/mg dry weight/140 min, as seen from Fig. 7. Pyridoxal-5-phosphate did not increase the activity during the lag phase, which lasted 40—45 min.

Fig. 8 demonstrates that a marked increase in tryptophan synthetase (tryptophan desmolase) took place in the *E. coli* cells during the lag phase.
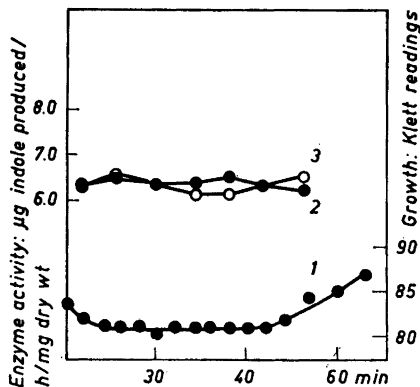
*Fig. 7.* Tryptophanase activity of *Escherichia coli* cells during the lag phase of growth. 1 = growth curve (right-hand scale), 2 = tryptophanase activity without pyridoxal-5-phosphate, and 3 = tryptophanase activity with added pyridoxal-5-phosphate.
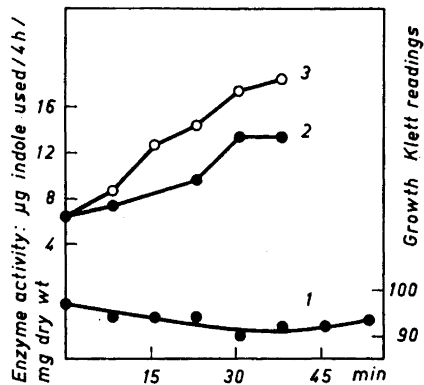
*Fig. 8.* Tryptophan synthetase activity during the lag phase of growth by *Escherichia coli*. 1 = growth curve (right-hand scale), 2 = tryptophan synthetase activity without added activator, and 3 = tryptophan synthetase with added pyridoxal-5-phosphate.

The enzyme activity in the cells rose from 2.4 to 9.8 $\mu$g of indole used/mg (dry weight) of cells/4 h, a fourfold increase. When pyridoxal-5-phosphate was added to the enzyme determination system a still greater increase was observed — the activity increased from 2.4 to 14.6 $\mu$g of indole/mg of dry weight/4 h, a sixfold increase. The lag phase lasted 40—45 min in this experiment. In other experiments, the results of which are not given in this paper, the increase was from four- to eightfold (12 experiments) and the lag phase varied between 25 and 35 min in length. The enzyme synthesis always started immediately after the grafting and continued linearly throughout the lag phase.

## DISCUSSION

In this investigation the synthesis of certain $B_6$ enzymes in *Escherichia coli* was studied during the lag phase of growth, which we have taken to be the first period of growth before cell division occurs. In previous works we established that the concentrations of vitamin $B_6$ and certain transaminases increase greatly in the cells during the lag phase [5,6].

It is a well-known fact that the enzyme content of cells depends on the growth conditions and on the composition of the medium in which the cells are grown before inoculation. Far less known is the fact that the enzymes are not formed at the same rate throughout the growth cycle. An extensive synthesis of enzymes important for catabolic reactions takes place during the late phases of growth. Gale [10] has divided the enzymes into two groups, those of type I active in the early, and those of type II active in the late phases of growth. Under type I he lists, for instance, arginine dehydrolases and the enzymes that

effect glycolysis, under type II decarboxylases, deaminases, and dehydrogenases. The cells employed in our experiments for inoculation were all taken towards the end of the exponential phase or during the retardation phase of growth. Immediately after the cells had been transferred again to the same inoculum medium, they began to grow without lag phase (cf. Hinshelwood [11]). Not only the growth phase of the cells but also the repression affects the amounts of enzymes in the cells. In a repression the end product inhibits the enzyme synthesis producing this product. E. g., the amount of tryptophan synthetase produced during cell growth varies with the amount of tryptophan present in the medium as shown by Moyed and Friedman [12] and by Lester and Yanofsky [13] and also by Monod and Cohen-Bazire [14]. We have found in this laboratory that tryptophan inhibits the synthesis of tryptophan synthetase already during the lag phase even if it is added to the medium before the inoculation or during the lag phase of growth [15]. The amounts of amino acid decarboxylases [16-18], tryptophanase [19,20], and serine and threonine deaminases [21,22] increase in cells growing in a medium rich in amino acids. Hence the cells may for various reasons have stored a sufficient amount of enzymes even before the lag phase of growth. Accordingly, the cells do not then synthesize these enzymes during the active growth phases even when they need them for the anabolic reactions. This may be the case with threonine deaminase which is necessary for the biosynthesis of isoleucine.

The E. coli cells used as inoculum in our experiments grew in a medium rich in amino acids and vitamins which contained Bacto-tryptone and yeast extract in addition to glucose. The results obtained in this study support previous ones in that large amounts of the enzymes essential to the catabolism of amino acids were present in the cells. The fact that the amount of tryptophan synthetase is low is apparently due to the repression. Cysteine desulphhydrase, serine deaminase, and tryptophanase are enzymes which, as far as we know at present, are required only in the catabolism of amino acids. Therefore variations in the formation of these are not likely to occur at the beginning of the growth.

Threonine deaminase and homoserine deaminase again belong to those enzymes which are essential for both anabolic and catabolic reactions of amino acids; both are required for the biosynthesis of isoleucine. Apparently the cells synthesize the amounts of threonine deaminase and homoserine deaminase necessary for catabolic reactions already in the inoculum cells. Tryptophan synthetase is a purely anabolic enzyme, a fact which explains the large amounts of it that are found in the cells during the lag phase of growth.

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