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Biosynthesis and Excretion of Vitamin B_6 by *Escherichia coli* during the Lag and Acceleration Phases of Growth

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The biosynthesis and excretion of vitamin B_6 has been followed during the initial growth phases of *Escherichia coli*. The determination of vitamin B_6 has been carried out by the biological method using *Saccharomyces carlsbergensis* 4228 (ATCC 9080), *Lactobacillus casei* (ATCC 7469) and *Streptococcus faecalis* R (ATCC 8043) as test organisms. The biosynthesis of vitamin B_6 takes place at a significant rate already during the lag phase and rises to a maximum during the acceleration phase. After this the rate of biosynthesis slowly decreases to the initial level at the end of the exponential growth phase. No excretion of vitamin B_6 was observed under the experimental conditions during any of these growth periods.

In two previous communications [1,2] it was reported that *Escherichia coli* excretes certain vitamins of the B-complex in quite remarkable amounts especially during the earlier growth phases. Very little is known about the rate of biosynthesis of the B vitamins which function as coenzymes during the lag and exponential phases of bacterial growth. The present investigations were undertaken to obtain information on the rate of biosynthesis and on the excretion of vitamin B_6 by *E. coli* especially during the first phases of cell growth. The results reported here show that this organism synthesizes unusually large amounts of vitamin B_6 during the lag phase between cell divisions.

RESULTS

In the experiments the inoculum was prepared by harvesting the cells during the exponential growth phase in order to obtain rapidly growing cells and thus to shorten the lag phase. As can be seen from Fig. 1, the rate of synthesis of vitamin B_6 increases during the lag phase which in this experiment lasted 30 min. At the beginning of the lag phase, the vitamin B_6 content of the cells was 70 mμg per mg (dry weight of the cells) but the contents increased to 105 mμg per mg during the 30 min, the increase being approximately 30 % of the

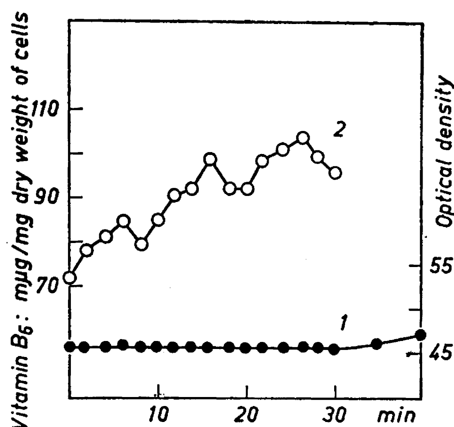

Fig. 1. Formation of vitamin B_6 by *Escherichia coli* cells during the lag phase of growth.
1. Growth curve (right-hand scale).
2. Vitamin B_6 content of cells.

original content. The test organism in this experiment was *S. carlsbergensis* 4228.

The results of the experiments on the synthesis and excretion of vitamin B_6 during the acceleration and exponential phases, and also during the early retardation phase, are shown in Fig. 2. *E. coli* did not excrete vitamin B_6 into the medium during any of these growth phases. Remarkable variations are noted in the synthesis of vitamin B_6 during the different periods of growth. The rate of formation of vitamin B_6 is high already in the lag phase and it

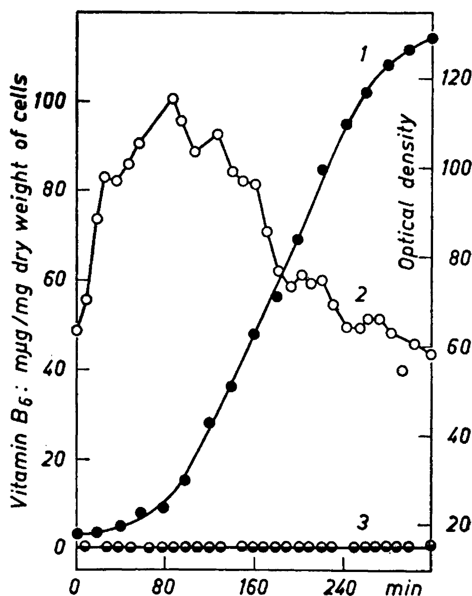

Fig. 2. Formation and excretion of vitamin B_6 by *Escherichia coli* cells during the growth cycle.
1. Growth curve (right-hand scale).
2. Vitamin B_6 content of cells.
3. Excretion of vitamin B_6 into the growth medium.

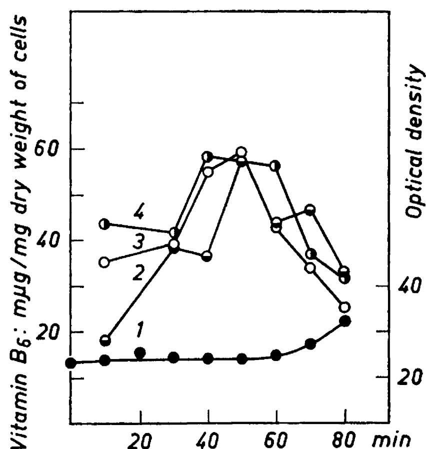

Fig. 3. Formation of pyridoxine, pyridoxamine and pyridoxal by *Escherichia coli* cells during the lag and early acceleration phases of growth.

1. Growth curve (right-hand scale).
2. Pyridoxal content of cells. *Lactobacillus casei* (ATCC 7469) used as test organism.
3. Pyridoxamine content of cells. *Streptococcus faecalis R* (ATCC 8043) used as test organism.
4. Pyridoxine, pyridoxal and pyridoxamine content of cells. *Saccharomyces carlsbergensis* (ATCC 9080) used as test organism.

reaches its maximal value during the acceleration phase. This peak was reached after the content had increased to 150 % of the original. During the exponential phase the rate of synthesis decreased steadily, and during the retardation phase the cells again contained the same amount of vitamin B_6 as at the beginning of the lag phase (50 mμg of vitamin B_6 per mg of dry weight). The results shown in this figure were obtained by using *S. carlsbergensis* 4228 as test organism.

Fig. 3 shows the results of experiments where the synthesis and excretion of vitamin B_6 in *E. coli* was followed using *Str. faecalis* R and *Lb. casei* in addition to *S. carlsbergensis* 4228 as test organisms. As the results show, the rate of synthesis of vitamin B_6 increased until the maximum content was reached during the acceleration phase, in other words, 50 min after the experiment was started. It is of interest to note that with all the test organisms used the content of vitamin B_6 was a maximum at about the same stage of the acceleration phase. No excretion of vitamin B_6 was established in these experiments. All the experiments described above were repeated at least three times.

DISCUSSION

In this paper Monod's definitions of the phases of bacterial growth have been followed [5]. The results of the experiments show that the formation of vitamin B_6 started already at the beginning of the lag phase and that generally the rate of formation rose to a maximum in the middle of the acceleration phase. As is well known, the vitamin B_6 enzymes (*e.g.* transaminases, deaminases, decarboxylases) participate in the metabolism of amino acids. The fact that cells synthesize amino acids and proteins before their division explains why the biosynthesis of vitamin B_6 should take place early in the lag phase and why the rate of biosynthesis is so high during the acceleration phase. The rate of synthesis of vitamin B_6 decreased throughout the exponential growth phase. This can probably be attributed to a more rapid formation of the vitamin B_6

enzyme systems during the acceleration growth phase than during the other phases. If this were true, the decrease in the rate of formation of vitamin B_6 would be understandable. No excretion of the vitamin into the medium was observed and therefore it can be assumed that the whole amount of vitamin B_6 synthesized was used quantitatively by the cells in the formation of vitamin B_6 enzyme systems. It should be noted that the results shown in Figs. 1 and 2 were obtained using *S. carlsbergensis* 4228 as the test organism. Therefore it cannot be said with certainty what member of the vitamin B_6 complex is formed first. However, the results of the experiments shown in Fig. 3 indicate that pyridoxal is formed in larger quantities than the other members of the vitamin B_6 complex. This is supported by the finding that the activity of vitamin B_6 was highest when *Lb. casei* was used as test organism. Snell [6] has found that of the three known forms of vitamin B_6, only pyridoxal is highly active in supporting growth of *Lb. casei*, whereas pyridoxamine and pyridoxine have negligible activity.

Under the essay conditions employed, *E. coli* did not produce any inhibiting or any growth promoting factors as no upward or downward drifts were noted in the microbiological determinations.

In a previous report from this laboratory [1], it was demonstrated that, when a very complex growth medium is used, *E. coli* cells excrete large amounts of vitamin B_6 into the medium,particularly during the exponential growth phase. In the present study the medium had a more simple composition. This is, no doubt, the reason why no excretion of vitamin B_6 was observed during the growth cycle.

EXPERIMENTAL

Organism and media. Escherichia coli, strain U5-41, was cultured with monthly transfers in an agar medium which contained 2.0 g of glucose, 1.4 g of KH_2PO_4, 100 mg of trisodium citrate, 200 mg of ammonium sulphate, 220 mg of Casamino acids (Difco), 20 mg of $MgSO_4 \cdot 7\,H_2O$, 10 mg of tryptophan, and 2.0 g of agar (Difco) in 100 ml of distilled water and which had previously been adjusted to pH 6.7 – 6.8 and autoclaved 10 min at 105°C.

The inoculated agar slant was incubated 24 h at 3°C and then stored in a refrigerator.

The inoculum medium contained 1.0 % of glucose, 1.0 % of trisodium citrate, 0.5 % of Bacto-tryptone (Difco), and 0.5 % of yeast extract (Difco). The pH of the medium was adjusted to 6.7 – 6.8 and the medium autoclaved 10 min at 115°C.

The growth medium of *E. coli* contained 1.0 g of glucose, 700 mg of KH_2PO_4, 50 mg of trisodium citrate, 100 mg of $(NH_4)_2SO_4$, 100 mg of NH_4Cl, 10 mg of $MgSO_4 \cdot 7H_2O$ and 20 mg of L-glutamic acid in 100 ml of distilled water. The medium was neutralized with 1 N NaOH to pH 6.7 – 6.8 and boiled shortly before the experiment was started.

Determination of vitamin B_6. The total pyridoxine, pyridoxamine and pyridoxal content was determined with *Saccharomyces carlsbergensis* 4228 (ATCC 9080) according to Atkin *et al.*[3], the pyridoxamine and pyridoxal content with *Streptococcus faecalis* R (ATCC 8043) by the method of Rabinowitz and Snell [4], and pyridoxal with *Lactobacillus casei* (ATCC 7469) according to Rabinowitz *et al.*[5]

Procedure. At the beginning of each experiment 2 l of the growth medium of *E. coli* was inoculated with a strong inoculum which was prepared as follows. *E. coli* was transferred from the agar slant to 100 ml of the inoculum medium. After overnight incubation at 37°C, the cells were harvested by centrifuging (3 500 rpm), transferred into 200 ml of the same medium, and again incubated 10 – 15 h at 37°C. The cells were then centrifuged (3 500 rpm) 15 min and washed three times with 0.9 % NaCl solution. Immediately after washing, this inoculum was placed in a refrigerator (+ 2°C) for at least 30 min. All the

cells were used as inoculum and the resulting growth medium contained approximately 0.05 mg of *E. coli* cells (calculated as dry weight) per millilitre. Before inoculation the temperature of the growth solution was raised to 37° on a water bath, and after this adjustment continually mixed with an electric stirrer. After the incubation, specimens were taken at intervals of 2 min. during the lag phase and at intervals of 10–15 min during the other growth phases. Each specimen taken from the medium was cooled in ice-water with continuous stirring. The turbidities of the cooled specimens were measured with a Klett-Summerson photometer employing a 620 mμ filter. The cells were harvested from 10 ml of the suspension by centrifuging (3 500 rpm) 15 min and the medium was completely removed with a pipette. The cells were washed three times with 0.9 % NaCl and then autoclaved together with 5 ml of 0.005 N HCl at 120°C for 3 h. After the hydrolysis, the pH was adjusted to 4.5–5.0 and the volume made up to 20 ml. When vitamin B_6 was determined with *Lb. casei* the cells were hydrolyzed in 3 ml of the 0.5 % acetate buffer, pH 4.5, to which about 1 mg each of papaine and diastase had been added, under toluene at 37°C for 24 h and the specimen to be analyzed was added aseptically before the inoculation.

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