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POLYGLUCOSE CONTENT IN THE CELL AND THE RATE OF GLUCOSE CONSUMPTION DURING SYNCHRONOUS GROWTH OF *Escherichia coli*

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Summary The changes in the intracellular polyglucose content and in the rate of glucose consumption in the medium during synchronous growth of Escherichia coli have been investigated. The polyglucose content reaches its maximum by the beginning of cell division and is then reduced, showing another rise in the middle of the cell cycle. During incubation of concentrated cells the glucose consumption occurs at a constant rate almost throughout the cell cycle but is significantly decreased at the beginning of cell division. This is paralleled with a rise in intracellular cyclic AMP concentration.

INTRODUCTION One of the most important features of intracellular metabolism in the course of the cell cycle is the energy supply of the cells. However, little information is available on glycolysis and oxidative phosphorylation at different stages of the cell cycle of microorganisms. A detailed work by Meyenburg (1) describes the glycolytic system of a synchronous culture of the yeast Saccharomyces cerevisiae. In bacteria, however, no systematic study of the glycolytic system during the cell cycle has been made so far. The rate of utilization of various substrates of energy metabolism has been shown to be constant at certain stages of the E.coli cell cycle (2). Yet these studies do not involve the very important part of the cell cycle immediately preceding cell division.

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The aim of present work was to determine the energy sources in the cell at all stages of the cell cycle by tracing the changes in the intracellular polyglucose content and in the rate of glucose consumption in the medium during the synchronous growth of E.coli cells.

MATERIALS AND METHODS (a) Cell synchronization The bacterial cells of E.coli K-12 (wild type) and of E.coli B/r CRS were grown in medium M-9. The cells of E.coli B/r were synchronized according to Poole (3) by cell separation at the early stages of the cell cycle. For cell concentration the nucleopore filters (r = 6 cm, pore diameter = 0.42) were used. The cells of E.coli K-12 were synchronized by a modified Poole's procedure in order to obtain larger amounts of the synchronous biomass. The modification principle consisted in fractionation of a rapidly cooled culture; in this case the cells were concentrated by centrifugation for 30 min at 4000 g, 0°C.
(b) Glucose consumption, cyclic AMP and polyglucose contents To measure the rate of glucose consumption samples of the synchronous culture (20 ml) were filtered through the nucleopore filters, 3.4 cm in diameter. The filters with the cells were placed into Petri dishes filled with medium M-9 (2 ml). After incubation for 10 min at 37°C the cells were separated from the medium and aliquots were taken for glucose assay. Glucose content was determined according to Saifer et al. (4).
The polyglucose content was determined by the Dietzler's procedure (5). The cell suspension (20 ml) was filtered for 20 sec and fixed with 6 ml of 7% $HClO_4$ after centrifugation for 10 min at 4000 g.
Cyclic AMP was assayed in the supernatant as described elsewhere (6).
The precipitate was poured over with 2 ml of 2 N H_2SO_4, hydrolyzed in soldered ampules for 3 hrs at 100°C and neutralized with 0.4 ml of 10 N NaOH. Glucose was then determined as described above.
(c) Cell number 0.1 ml of diluted bacterial culture was placed into dishes containing meat-peptone agar pH 7.0 and the colonies grown after an overnight incubation at 37°C were counted. The dishes with not less than 200 colonies were used for further analysis.

RESULTS AND DISCUSSION Figure 1 shows the changes in the optical density of the cell suspension, in the number of cells, in intracellular polyglucose content and in the rate of glucose consumption during the growth of the synchronous culture of E.coli K-12. The optical density curve is suggestive of an exponential growth of the biomass. The cell number in the suspension varies periodically. During the first 60 min of incubation it remains constant; the first, 2-fold rise in the

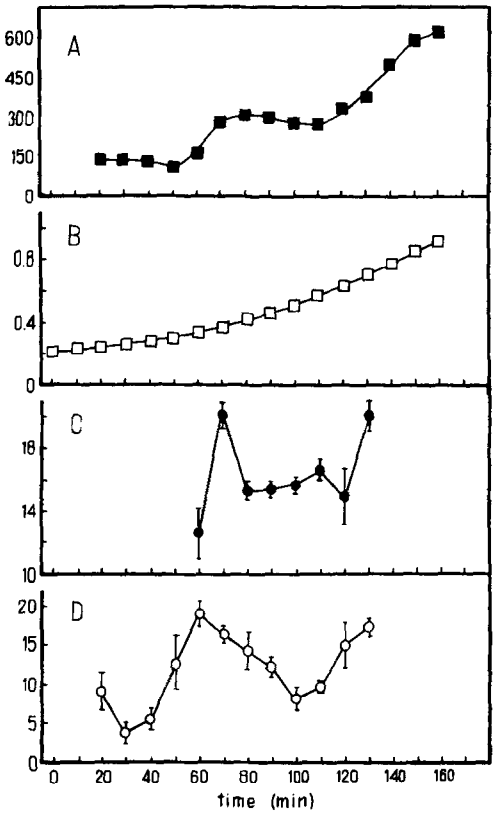

Figure 1 Glucose concentration in the medium after 10 min incubation and intracellular polyglucose content during synchronous growth of E.coli.
Abscissa - time elapsed from the beginning of incubation at 37°C; ■—■ - number of cells in 1 ml of cell suspension times the factor 10^{-6} (A); □—□ - optical density of suspension at 540 nm in a cuvette of 0.5 cm diameter (B); ●—● -glucose concentration in the medium after 10 min incubation (C), mM; ○—○ - polyglucose content in nm of glucose per 1 ml of bacterial suspension (D). The accidental error value is indicated for a confidence level of 95%. The initial glucose concentration in the medium - 22 mM.

cell number is observed by the 70-80th min and the second, a less synchronous one - after 2.3-2.8 hrs of incubation.

The polyglucose content starts increasing after 30 min during the first interphase and reaches a maximum by the beginning of cell division. In the course of cell division and immediately after it the polyglucose content is decreased, showing another rise in the middle of the second cell cycle. Similar changes occur during the growth of E.coli B/r CRS cells synchronized by centrifugation without pre-cooling. This

suggests that the changes in the polyglucose content observed in the synchronous culture do not depend on its pre-cooling to 0°C. The decrease of the glucose content in the medium during cell incubation is approximately the same almost throughout the cell cycle. It is only at the beginning of cell division that a sharp decrease of the rate of glucose consumption (almost down to zero) is observed. Therefore glucose transport from the medium to the cell is practically absent over this period of time.

The intracellular cyclic AMP content rises from 5.5±0.33 to 16.0±0.43 pmol/20 ml of bacterial suspension during the first division. These data confirm the assumption that glucose transport inhibits cyclic AMP synthesis (7, 8, 9).

Our observation that the rate of glucose consumption is constant almost throughout the cell cycle agrees well with the earlier published data (2). However, in the paper cited the part of the cycle immediately preceding cell division has not been investigated. Our results show that this part of the cell cycle is characterized by a sharply reduced rate of glucose consumption.

Since all metabolic processes are active during cell division, it is suggested that the cell derives glucose from the polyglucose breakdown to meet the energy requirements at that time. Fig. 1C illustrates the changes in glucose concentration in the medium during incubation of the concentrated cells taken from 20 ml of the synchronous suspension. The maximum drop of the concentration is observed at 3-4 mM. This corresponds to the level of glucose consumption equal to 30-40 nmole/min/ml of synchronized suspension.

The changes in the polyglucose content during the cell cycle amount to 12-15 nmole/ml of cell suspension. It can be conclu-

ded that the energy required for intracellular metabolism may only be supplied by the polyglucose breakdown for several minutes. Such a situation appears to occur at the beginning of cell division. Obviously the biological significance of the phase shift in using the substrates of energy metabolism is to provide for a steady supply of energy and substrates for various biosyntheses in the growing cell. During cell division the glucose transport to the cells ceases and, consequently, the energy expenditure associated with this process is eliminated. At the same time the activation of the polyglucose breakdown required for the maintenance of intracellular metabolism takes place.

It is also possible that the periodical changes in the polyglucose content are conditioned by oscillations in the glycolytic system in accordance with Sel'kov's models of the "biological clock" (10).

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