

# BIOCHEMICAL ASPECTS OF THE CELL GROWTH OF *ESCHERICHIA COLI* AS STUDIED BY THE METHOD OF SYNCHRONOUS CULTURE

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It has been beyond the reach of the conventional culture technique in microbiology to obtain information concerning the dynamic growth mechanism of a single cell of a microorganism, since the system used for the growth experiments has been, of necessity, with populations composed of cells of varying ages. The synchronous culture technique recently developed by several workers has afforded a clue to understanding the growth mechanism of individual cells of microorganisms. Several papers dealing with metabolic activities of synchronously growing cells have accumulated. Tamiya *et al.* (1953) investigated the photosynthetic and respiratory activities during the life cycle of *Chlorella ellipsoidea*. Nucleic acid metabolism of *Chlorella* (Iwamura, 1955), a mutant strain (B) of *Escherichia coli* (Barner and Cohen, 1955), and *Amoeba proteus* (James, 1954) were also investigated by this procedure. Synchronously germinating spores of *Aspergillus niger* (Yanagita, 1956a) and synchronously budding yeast cells (Ogur *et al.*, 1953) were studied with respect to their nucleic acid metabolism. The unique quantitative cytological studies of Prescott (1955) disclosed a relationship between cytoplasmic and nuclear syntheses in *Amoeba proteus*.

The present investigation aims to obtain a composite picture of biochemical changes occurring during the life span of actively growing *E. coli*. Changes in respiratory activity, total and protein nitrogen contents, and nucleic acid contents in the growing cell were compared.

## MATERIALS AND METHODS

*Escherichia coli* strain B was cultured synchronously in a synthetic medium (glucose-ammonium chloride-salt medium) by the method of Maruyama and Yanagita (1956). Cells in the logarithmic phase were harvested by centrifugation from 1-L cultures containing approximately  $10^8$  cells per ml. Larger (mature) cells were collected separately by the method of fractional

filtration and inoculated into fresh medium at a concentration of about  $10^8$  cells per ml. The culture was shaken at 37 C. Experiments were started after a 15-min preincubation period, during which nearly all of the larger cells finished fission, resulting in the formation of immature daughter cells. For the measurement of nitrogen and nucleic acid contents in the cells, aliquots were taken from the culture every 10 min. Growth of the cells was stopped by the addition of 2 N  $H_2SO_4$  to shift the pH of the medium from 7.4 to 4.0 followed by chilling in an ice bath. Each aliquot of cells was washed three times with 0.8 per cent saline by centrifugation and subjected to chemical analyses.

*Total nitrogen.* The total nitrogen content of cells was determined after the method of Johnson (Umbreit *et al.*, 1949).

*Protein nitrogen.* The washed cells were dissolved in 0.8 N NaOH at 37 C for 25 min. The solution was neutralized with 8 N HCl, and the nitrogen content of the hot 5 per cent trichloroacetic acid (TCA)-insoluble fraction was determined. The values for the TCA-insoluble fraction thus obtained were corrected by multiplying by the factor 1.3, which was obtained in a preliminary experiment. It was shown that 30 per cent of Hammarsten casein (Merck), when treated with alkali in the same manner, turned soluble in the hot TCA. Assuming that the casein was equivalent to the cell protein in this regard, the factor 1.3 was applied to the estimation of protein nitrogen in the cells.

*Nucleic acids.* The cold TCA-soluble fraction of the cell material obtained by the method of Schmidt and Thannhauser (Volkin and Cohn, 1954) was taken as a ribonucleic acid (RNA) fraction. The cold TCA-insoluble residue, on the other hand, was washed with alcohol and cold 1 N perchloric acid and extracted with hot 0.5 N perchloric acid (Ogur and Rosen, 1950). The soluble extract was taken as a desoxyribonucleic acid (DNA) fraction. RNA was determined colorimetrically (at 660  $m\mu$ ) by the orcinol

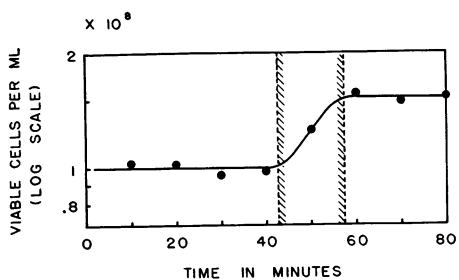


Figure 1. The viable growth curve of *Escherichia coli* strain B obtained by the synchronous culture method. A pair of vertical broken lines indicate the time of the beginning and end of cell division.

reaction and DNA spectrophotometrically by the dichromatic method (at 260 and 300  $m\mu$ ).

*Respiration.* Oxygen uptake and carbon dioxide production were measured using two Warburg flasks with and without KOH, respectively.

#### RESULTS

*Growth curves of synchronous culture.* Viable counts (Yanagita, 1956b) of cells which were run in parallel to the following experiments showed a stepwise increase characteristic of synchronous multiplication (figure 1). The generation time as established by repeated experiments was 50

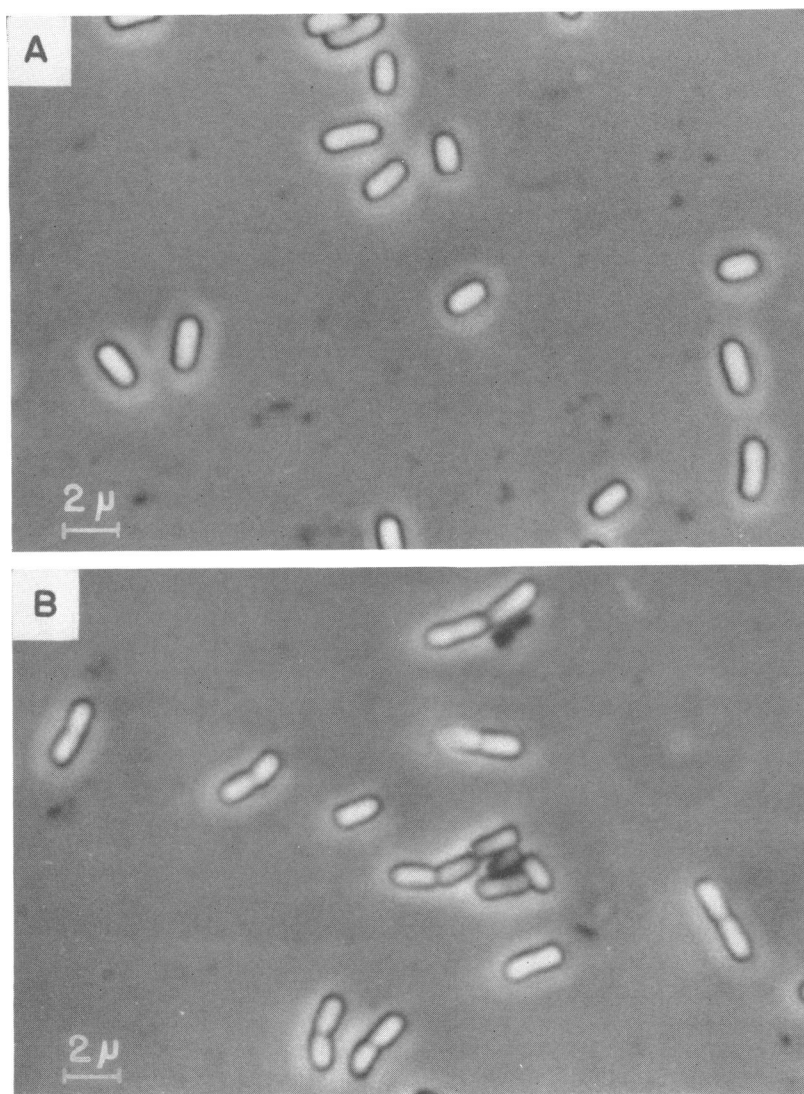


Figure 2. Phase-contrast photomicrographs of synchronized cells of *Escherichia coli* strain B. A: Cells at 5 min. B: Cells at 40 min. Cells were stained by the tannic acid-crystal violet method.

min, as described previously. Fission of cells required a time span of about 15 min. Photomicrographs taken at 5 and 40 min are presented in figure 2. Short rods were predominant at time zero and a longitudinal augmentation of cell size followed. Septa were apparently visible in some of the cells at the time of the onset of cell division. In the following presentations of figures, growth curves will not be reproduced; instead a pair of vertical broken lines will be presented to indicate the time of the beginning and end of cell division.

**Total and protein nitrogen contents.** Figure 3 shows a set of typical data comprising the change in total and protein nitrogen contents of growing cells. The total nitrogen increased almost exponentially with time until it reached an amount about twice the initial value, when the cells were completely prepared for the commencement of cell division. The protein nitrogen, on the other hand, remained almost constant initially and doubled at a later stage of cell growth. Nonprotein nitrogen, as estimated by the subtraction of protein nitrogen from total nitrogen, increased gradually and attained a maximum value prior to the time of active protein synthesis. Analysis by paper chromatography failed to reveal free amino acids in the cells ( $5 \times 10^9$  cells) at the time of maximum nonprotein nitrogen.

**RNA and DNA contents.** The nucleic acid contents followed the courses of increase shown in figure 4. The RNA content in the cells increased during the initial period of the generation span and leveled off shortly before the onset of active protein synthesis. The DNA content seemed to remain constant during the major period of cell growth and doubled shortly before or during the division period. Such a seemingly sudden increase in the DNA content of the cells was proved to be real by repeated experiments. It should be mentioned, however, that the building blocks for the synthesis of DNA may be synthesized through a gradual course of events. The ratio RNA:DNA calculated from these two experiments is also shown in the figure as a broken line. During fission it showed a markedly low value (3.7), while during cytoplasmic synthesis it showed a higher constant value (4.8).

**Oxygen uptake and carbon dioxide production.** The oxygen uptake and carbon dioxide production of the culture increased gradually as growth proceeded (figure 5). In repeated experiments there usually was found a slight but definite

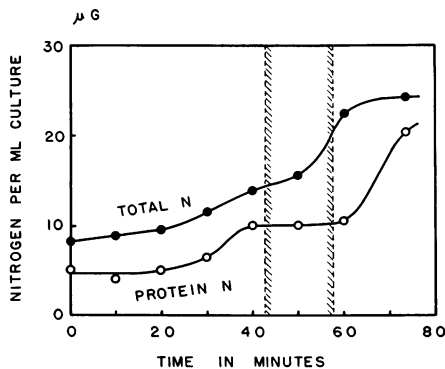


Figure 3. Increase in the total and protein nitrogen of synchronized cells. The density of the viable cells at time 0 was  $1.7 \times 10^8$  per ml.

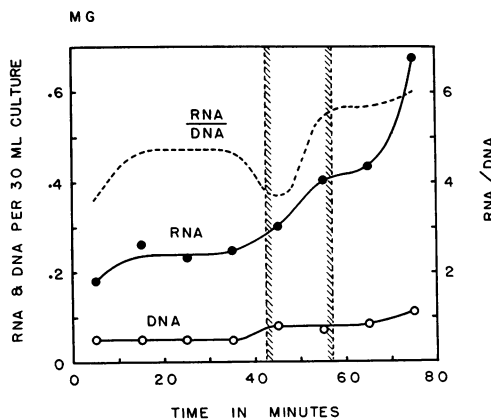


Figure 4. Synthesis of ribonucleic acid and deoxyribonucleic acid of synchronized cells. The density of the viable cells at time 0 was  $1.4 \times 10^8$  per ml.

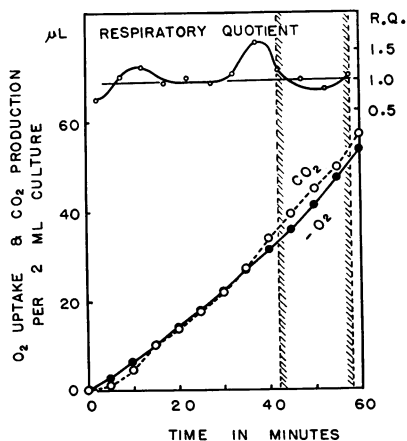


Figure 5.  $O_2$  uptake and  $CO_2$  production of the synchronized cells. The density of the viable cells at time 0 was  $1.2 \times 10^8$  per ml.

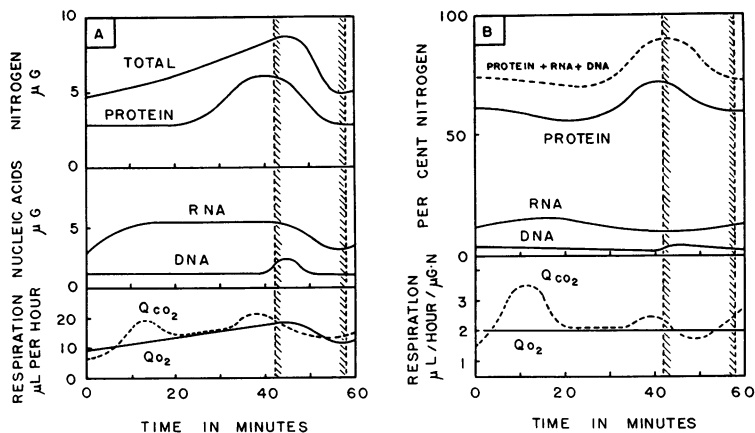


Figure 6. Diagrams schematically showing the relationships among nitrogen contents, nucleic acid syntheses, and rate of respiration of synchronized cells. *A*: Expressed in terms of  $10^8$  cells. *B*: Expressed in terms of total nitrogen. The nitrogen contents of ribonucleic acid and deoxyribonucleic acid were roughly estimated to be 15 per cent (Chargaff, 1955, Magasanik, 1955).

decrease in the rate of carbon dioxide production at about 20 min. This is clearly represented in figure 6A (bottom), in which the course of change in the rate of both oxygen uptake or carbon dioxide production per unit number of cells is shown. The rate of carbon dioxide production was found to be minimal at 0 and 20 min and maximal at 12 and 38 min, while that of oxygen uptake increased almost linearly and leveled off at the onset of cell division. The respiratory quotient calculated as the ratio, rate of  $\text{CO}_2$  production:rate of  $\text{O}_2$  uptake, changed as shown in figure 5 (top). It showed its lowest value (0.7) after the first cell division and its highest (1.6) shortly before the time of cell division.

#### DISCUSSION

The expression of these data in terms of number of cells and nitrogen may be worthwhile to understand the situations occurring in the growing cell. Figures 6A and 6B are presented on these bases.

The total nitrogen per cell doubled, following an almost exponential course of increase, and then dropped to the initial value during the period of cell division. The total nitrogen content of a cell may roughly be considered to be a measure of its dry weight. It was observed in an individual cell of *Amoeba* that a value representing the dry weight of the cell increased almost linearly and leveled off prior to cell division (Prescott, 1955). In *E. coli*, the stationary

"predivision period" in total nitrogen was observed indistinctly.

The hot-TCA insoluble nitrogen fraction, defined as protein nitrogen, does not necessarily represent the true protein nitrogen, since some part of the polypeptide constituents of the cell may escape the measurement. In figure 6B the percentages of protein-, RNA- and DNA-nitrogens in the total nitrogen at each time interval are represented as the smooth broken curve. It is evident from this curve that during the early phase of cell growth some nitrogen fraction other than protein (hot-TCA insoluble fraction) and nucleic acids should exist in the cell. This nitrogen fraction probably constitutes the building blocks of high molecular weight proteins and nucleic acids, although the existence of free amino acids in the fraction could be excluded. The quantity of these building blocks was found to be minimal shortly before the cell division. The protein nitrogen content thus defined showed a sigmoidal course of increase. The same was also true in *Aspergillus niger* spore germination (Yanagita, 1956a). Again this was rather inconsistent with the observations made with *Amoeba*, in which a linear increase accompanied by a predivision leveling was observed (Prescott, 1955). In *Tetrahymena*, on the other hand, an almost exponential increase in protein nitrogen was found (Scherbaum and Zeuthen, 1954).

The RNA content in the cell seemed to increase initially. Important is the fact that the

active protein synthesis commenced just after the completion of the RNA doubling. The same was also true in the germinating spores of *A. niger* where the RNA synthesis apparently preceded protein synthesis. (Yanagita, 1956a). In synchronously cultured *Chlorella*, RNA and protein syntheses proceeded almost in parallel (Iwamura, 1955).

The DNA synthesis in *E. coli* started shortly before cell division and ended during the division period. It was found in synchronous cultures of *Bacillus megaterium* that the nuclear division occurred just after cell division (Hunter-Szybalska *et al.*, 1956). Thus, in bacteria, nuclear division is supposed to take place much earlier than cell division. It is thus consistent to find that the DNA synthesis in *E. coli* occurred shortly before cell division, after which nuclear division might follow. In *Chlorella*, DNA synthesis occurred prior to the autospore formation which resulted in cell division (Iwamura, 1955) and in yeast it was also found to precede the budding (Ogur *et al.*, 1953). Although the phase of DNA synthesis in *E. coli* seems to be delayed in comparison with other organisms, in common with them is the fact that the DNA is synthesized prior to nuclear division.

Interpretation of changes in the respiratory quotient over the life cycle of the *E. coli* cell awaits further investigation. It should be mentioned, however, that the manifestation of the highest RQ value shortly before the cell division was consistent with the observation in *Chlorella* (Nihei *et al.*, 1954). In this organism the value attained its maximum prior to the formation of autospores in the cell. It was reported earlier by Hershey and Bronfenbrenner (1938) that the oxygen uptake by *E. coli* in terms of nitrogen remained about unity during the whole course of culture. Noteworthy is the fact that this was found to be true even during the course of cellular growth. As shown in figure 6B, the  $Q_{O_2}$  per nitrogen remained constant over one life cycle. The  $Q_{CO_2}$  per nitrogen, however, did not remain constant.

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#### SUMMARY

Using a synchronous culture of *Escherichia coli* strain B the relationships among nitrogen metabolism, nucleic acid syntheses, and respiratory activity during the generation span of actively growing cells were investigated.

Total nitrogen in the cell increased almost exponentially. Protein nitrogen remained constant for the initial period of cell growth and doubled at the middle of the growth cycle.  $Q_{O_2}$  increased almost parallel with total nitrogen, while  $Q_{CO_2}$  showed a characteristically variable course, with two maxima. Ribonucleic acid doubled initially and leveled off prior to protein synthesis, while desoxyribonucleic acid doubled shortly before and during the cell division.

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