Enzyme Synthesis in Synchronous Cultures of Bacteria

A Literature Survey

Presented to

the Faculty of the Department of Biology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

of

Master of Science in Biology

Plan II

---

by

Pat H. Simpson

May, 1969

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ABSTRACT

Enzyme activity was studied as a function of the cell division cycle in synchronized populations of Escherichia coli and Proteus vulgaris.

The timing of ribonuclease during synchronous growth begins and doubles at a specific time during the cell division cycle. The momentary increase in the flow of information, ribonuclease activity, was found to differ in E. coli compared to that observed in P. vulgaris.

Cell division is correlated with cell mass as measured by wet weight and ribonuclease activity. The increase in cell mass and periodicity of ribonuclease seems to be one of a probable number of decisive events in the sequence of biochemical reactions leading to cell division.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

The molecular basis for the storage, transfer, and utilization of genetic information has been intensively investigated in recent years. This area of research is commonly referred to as molecular biology and is closely associated with microbial genetics. The outstanding achievement of molecular biology has been the analysis of protein synthesis, in which the nucleic acids, deoxyribonucleic acid (DNA) and two of the three types of ribonucleic acid (RNA), have been assigned definite roles (14). DNA controls both replication of more DNA and transcription of RNA. RNA (messenger RNA) relays the message of DNA to protein synthesizing sites where the proteins are constructed. Thus, protein structure is determined by the sequence of deoxy-nucleotides in the gene. Protein biosynthesis is dependent upon first the transcription of this information in DNA to a complementary sequence of bases in RNA (mRNA) and second upon the translation of this information into protein at protein synthesizing sites. The flow of information in cellular growth can be illustrated as shown in figure 1 (9).

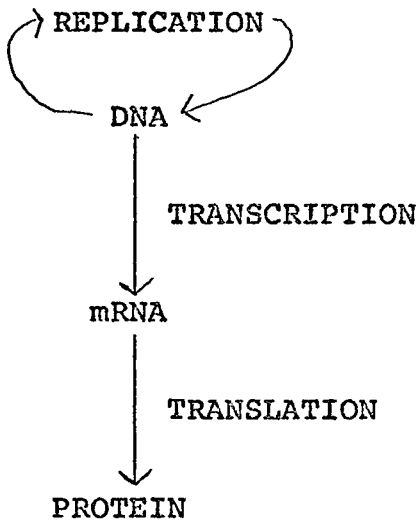


Fig. 1. Informational transfer in the synthesis of macromolecules (9).

In recent years many molecular biologists have deemed as a major objective the understanding of the mechanisms which regulate the rate of synthesis of specific proteins during the cell cycle. The study of enzyme synthesis in synchronous cultures of bacteria has been particularly successful in this area of inquiry (2). In an ideal synchronous culture all the bacteria are simultaneously at the same stage of the division cycle, all divide at the same time for many generations, and all are undergoing simultaneously the same biochemical reactions. By studying a synchronous population of cells, one can study what is happening in each individual cell (3,9,14).

The rate of synthesis of an enzyme was shown to vary with the number of copies of the gene determining its structure which are present by Jacob *et al.* (11) and again by Donachie (4). Donachie concluded from his work on *Neurospora crassa* that if the feedback from enzyme activity to enzyme synthesis is excluded, one may consider the gene to be a rate-determining catalyst in the sequence of events leading to the synthesis of an enzyme, and that there exists a linear dependence of enzyme synthesis on gene concentration. Since Yoshikawa and Sueoka (23) working on *Bacillus subtilis* and Nagata (19) and Cairns (1) working on *Escherichia coli* showed the genome to be replicated in a sequential non-random fashion, one may

conclude that there will be a specific time in the division cycle of each cell of these organisms when the number of copies of a given gene will double in concentration. These observations pose the question whether in bacterial cells the overall process of transcription and translation of genetic information is ordered. If true, then in synchronously dividing cells a step-wise production of a given enzyme would be expected.

To test this hypothesis, Masters et al. (17) used synchronous cultures of E. coli and B. subtilis to look for the expected step-wise production of enzymes. E. coli exhibited a step-wise increase in induced sucrase and derepressed aspartate transcarbamylase (ATC-ase), however induced β-galactosidase increased continuously. B. subtilis exhibited a step-wise increase in sucrase, histidase, and ATC-ase. Masters et al. noted that the enzymes studied doubled in their activity during different times of the cell division cycle and might be correlated with the replication times of the corresponding genes.

At first sight these results might appear to give evidence that transcription and translation of the bacterial genome are ordered. And in fact, Masters et al. suggested that enzyme studies might be used to independently map genes on the genome. However, this prediction did

not fit the β-galactosidase system in which case induction was shown to occur at any time during the cell cycle and increased continuously (5). Investigation of this problem led Kuempel et al. (13) to propose in 1965 that the activity of a particular gene is regulated by the concentration of specific repressors and that step-wise increases in enzyme amounts are generated by variation in repressor concentration during the cell cycle. By their experiments involving timing of changes between autogenous synthesis of enzymes and timing of changes in potential for those same enzymes in synchronous culture of bacteria. Kuempel et al. (13) were led to conclude that transcription and translation in bacterial cells are not necessarily ordered. Autogenous synthesis refers to the synthesis of enzymes occurring under the influence of the control mechanisms present in the normally growing cell, while the potential for synthesis refers to the rate of synthesis of enzymes under induced or derepressed conditions. Kuempel et al. (13) reasoned that since each gene is believed to replicate at a definite time in the cell duplication cycle, and since doubling the gene is supposed to double the potential for synthesis of the corresponding enzyme, then autogenous synthesis of enzymes should show a time burst corresponding to the doubling of potential. However, autogenous changes and potential changes were different for both ATC-ase of E. coli and

sucrase of B. subtilis, showing that change in potential did not immediately cause change in production of autogenous synthesis of enzymes. Figure 2 shows the results of one of their experiments.

Donachie (5) then proposed in 1965 that the discontinuous enzyme synthesis in synchronous cultures of bacteria could be explained partly by the instability of the enzymes themselves. Investigating both the decay rates and levels of enzyme activity over several cell generation cycles in synchronous cultures of B. subtilis, Donachie (5) noted that ATC-ase and ornithine transcarbamylase (OTC-ase) both exhibited discontinuous steps while alkaline phosphatase (AP-ase) did not. Donachie (5) pointed out that both ATC-ase and OTC-ase activity decays rapidly while AP-ase is a fairly stable enzyme. While admitting that Kuempel et al.'s theory that the results could be explained by variation in the degree of repression, Donachie (5) pointed out that speed of enzyme decay would also be tenable.

In 1966 Masters and Pardee (18) investigated further into what mechanisms determine the timing of the discontinuous enzyme synthesis. They ran experiments on synchronous cultures of B. subtilis in which L-histidine was supplied as the sole carbon source. DNA synthesis

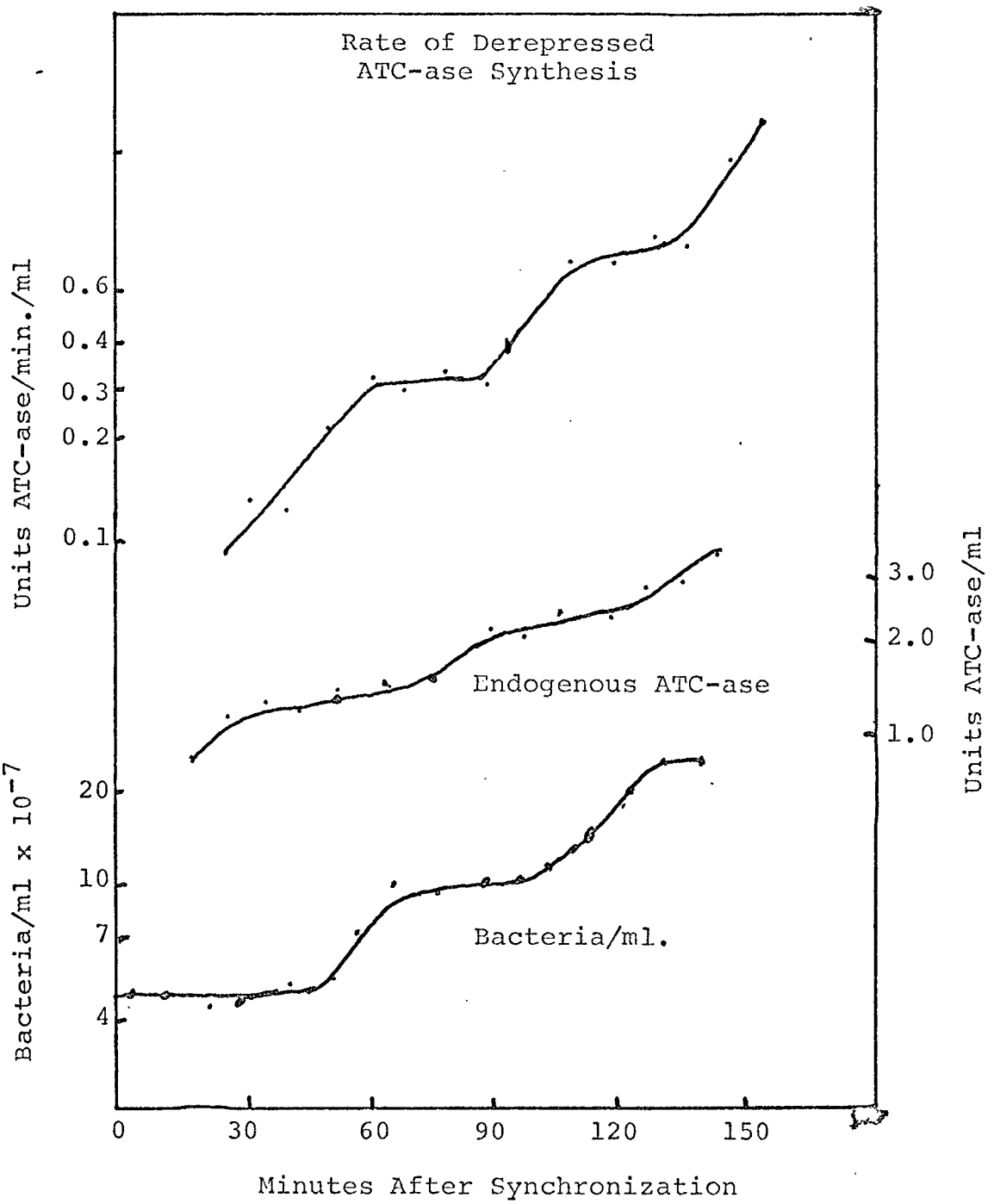


Fig. 2. The autogenous activity and the rate of the derepressed synthesis of ATC-ase in a synchronous culture of CS 101-G-1. Semi-log plot (13).

was periodic and occupied about 50 minutes of a cell cycle of 120 minutes. Even though DNA synthesis did not occur in the last 70 minutes of a cell cycle, periodic enzyme synthesis continued. This indicated that the synthetic cycles did not depend on continued gene replication. Conclusive evidence for this was obtained in another experiment in which two different strains of B. subtilis were used. DNA replication was prevented for two hours with fluorouracil deoxyribose (FUdR) in one strain of B. subtilis while the other strain was not affected by this drug. Using the drug, the synthesis of OTC-ase was followed in synchronous cultures of the two strains. In both cases enzyme synthesis continued to be periodic and the synthetic periods in the two cultures remained in phase. These experiments also gave evidence that genes are always potentially active and transcription, while it may be ordered (22), may also occur at times other than as the DNA replicates.

If oscillations in feedback repression circuits cause the discontinuous enzyme synthesis, then, Masters and Pardee reasoned, it should be possible to upset the timing of enzyme steps by experimental interference with repressor-levels. To test this idea, the synthesis of ATC-ase in a synchronous culture of B. subtilis was repressed by the addition of uracil. ATC-ase synthesis stopped and did not recommence until uracil was removed, at which time synthesis immediately began

again. OTC-ase synthesis, which normally occurs simultaneously with ATC-ase synthesis, was not affected by the uracil. This experiment shows that the addition and removal of uracil at different times can result in the displacement of ATC-ase steps relative to one another and to OTC-ase steps. Thus, release of repression can result in a specific alteration in the timing of subsequent autogenous enzyme steps. These results led Masters and Pardee (18) to conclude that the repressor level can determine not only whether or not periodic enzyme synthesis occurs, but also the time in the cell cycle at which the enzyme steps are initiated. Similar conclusions were drawn by Ferretti et al. (6,7), following their work on anaerobic photosynthetic growth of synchronous populations of Rhodopseudomonas spheroides. The previous suggestion by Donachie (5) that the instability of certain enzymes could result in step-wise increase in activity is obviously not the only factor to consider. However, enzyme instability would tend to contribute to the discontinuous enzyme amount (18). Why, then, if repressor level controls the timing of the steps of periodic enzyme synthesis, is there an apparent correspondence between order of enzyme synthesis and order of genetic replication? Goodwin (8) has proposed

a model in which enzyme synthesis is entrained to the period of gene replication by the synthesis of a small amount of mRNA at the time of gene duplication. Periodic enzyme synthesis would continue even in the absence of DNA replication because the concentration of mRNA, enzyme, and enzyme product undergo continuous oscillations, all of the same period but of differing amplitudes about their steady-state values.

One should be aware of these observations when drawing a conclusion from results based on periodic enzyme synthesis. In 1968 Helmstetter (10) and Pato and Glaser (20) estimated the origin, sequence, and direction of chromosome replication based upon the sequence of periodic enzyme synthesis. While periodic enzyme synthesis normally follows the sequence of chromosome replication, this would not necessarily be the case. Changes in repressor levels could reverse the process and result in the wrong conclusions. Cultural conditions must be carefully controlled if a temporal map is to approximate the genetic order of the enzymes involved (7).

The autogenous synthesis of ribonuclease (RNase) in synchronous cultures of E. coli and P. vulgaris has been investigated in our laboratory.

MATERIALS AND METHODS

The bacterial strains employed in these studies were *E. coli* K-12 Hfr CS 101 met$^-$ and *P. vulgaris* phase B. These strains were grown in a synthetic mineral salts medium (C- medium) at pH 7.3 with 0.5% glucose.

Synchronization was accomplished by the stationary phase method (3).

The cell growth was harvested by centrifugation in the cold and washed twice. Aliquots were withdrawn periodically for wet weights, cell counts and ribonuclease determinations.

The aliquot used in the assay for ribonuclease was pelleted by centrifugation, quick frozen in dry ice and ground with twice the cell wet weight of alumina. The appropriate dilutions of the cell extracts were made, centrifuged and the supernatant used for enzyme assays.

Ribonuclease was assayed by the method of Kalnitsky, Hummel and Dierks (12).

RESULTS

Synchronization has been accomplished by the stationary phase method (3). Figures 3 and 5 show three cycles os synchronous division for E. coli and P. vulgaris, respectively. Variability in the length of the cell division is shown to occur from one generation to the next.

The transition from generation to generation is not always sharp; it becomes difficult to decide where one generation ends and the next begins. This difficulty is overcome by defining the length of a generation as the intercept on a line drawn through two successive generations by plotting cumulative cell numbers.

The synchronization index (SD) describes quantitatively the degree of synchronous division in a population in which n_o and n equal the number of organisms just before and after burst of synchronous division, t is the time span and g the generation time.

$$SD = (n/n_o - 1)(1 - t/g)$$

The synchronization index appears to show a higher degree of synchronous division in populations of P. vulgaris than in populations of E. coli (Table I).

TABLE I

DEGREE OF SYNCHRONOUS DIVISION

ORGANISM	SYNCHRONOUS INDEX NUMBER OF SYNCHRONOUS STEP			
	1	2	3	THEORETICAL
P. vulgaris	0.75	0.98	0.68	1.0
E. coli	0.78	0.46	0.32	1.0

Synchronously dividing cells were used to examine the pattern of ribonuclease synthesis during the cell division cycle in E. coli and P. vulgaris as shown in figures 4 and 6, respectively.

During the period between one cell division and the next, a burst of RNase activity occurs. Two such bursts in enzyme activity are noted in synchronous populations of E. coli in contrast to P. vulgaris. It is suggested that two such bursts in enzyme activity as a function of the cell division cycle could be a response to DNA replication and/or a redundancy in genetic information, or both. The latter speculation (redundancy) is contrary to that observed in bacterial cells but not at all foreign to what has been observed in mammalian systems.

The increase in ribonuclease activity in P. vulgaris as shown in figure 6 increases linearly reaching a plateau of continuous synthesis prior to the next cell division cycle. The pattern is repetitive over the period of logarithmic growth, leveling off as the stationary phase is approached and cell division synchrony becomes random (Figs. 4 and 6).

Figure 4 shows that with E. coli, each of the two bursts of linear ribonuclease activity is followed by a plateau of continuous synthesis. The results suggest that when the amount of enzyme becomes constant with time, the

enzyme is in a steady state, its synthesis and degradation occurring at equal rates. The enzyme activity falls when the rate of synthesis is less than the rate of degradation (Figs. 4 and 6).

The amount of enzyme synthesized per ml corresponds to the rate of increase in wet weight per ml and cell.

It should be stressed that the number and kinds of observations is quite small and there is a clear need for more research in the field before any generalizations can be made regarding the integration of biosynthetic activity during the cell division cycle. It would be of interest to know the nature of the control mechanism that regulates ribonuclease activity in E. coli and P. vulgaris. Such studies should lead to a better knowledge of cell dynamics and biological organization.

Figure 3. Synchronous cell division of E. coli. A indicates the stepwise number of cells per ml characteristic of synchronous growth. B is the cumulative plot of the number of cells per ml. The dashed vertical lines indicate the generation time and the arrow the time aliquots were removed for cell counts, wet weight and ribonuclease determinations.

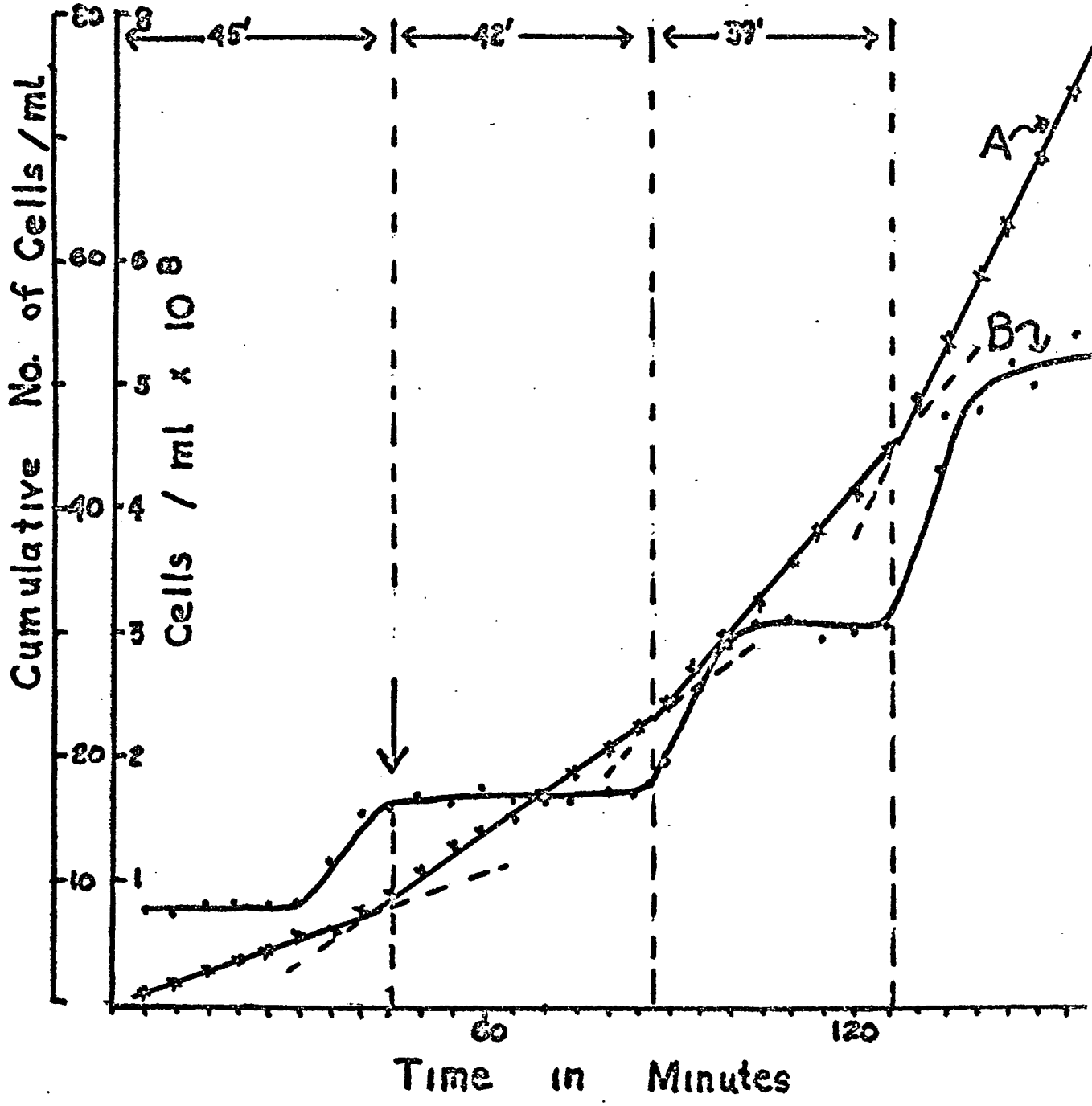

45'
42'
37'
A
B
Cumulative No. of Cells / mL
Cells / ml x 10 8
80
60
40
20
0
8
6
5
4
3
2
1
60
120
Time in Minutes

Figure 4. Variations in ribonuclease and wet weight as functions of the cell division cycle, E. coli. A indicates ug of ribonuclease per ml, B the ug of ribonuclease per cell, C the ratio of ug of ribonuclease to the ug of wet weight per cell, D the ug wet weight per ml and E the ug wet weight per cell.

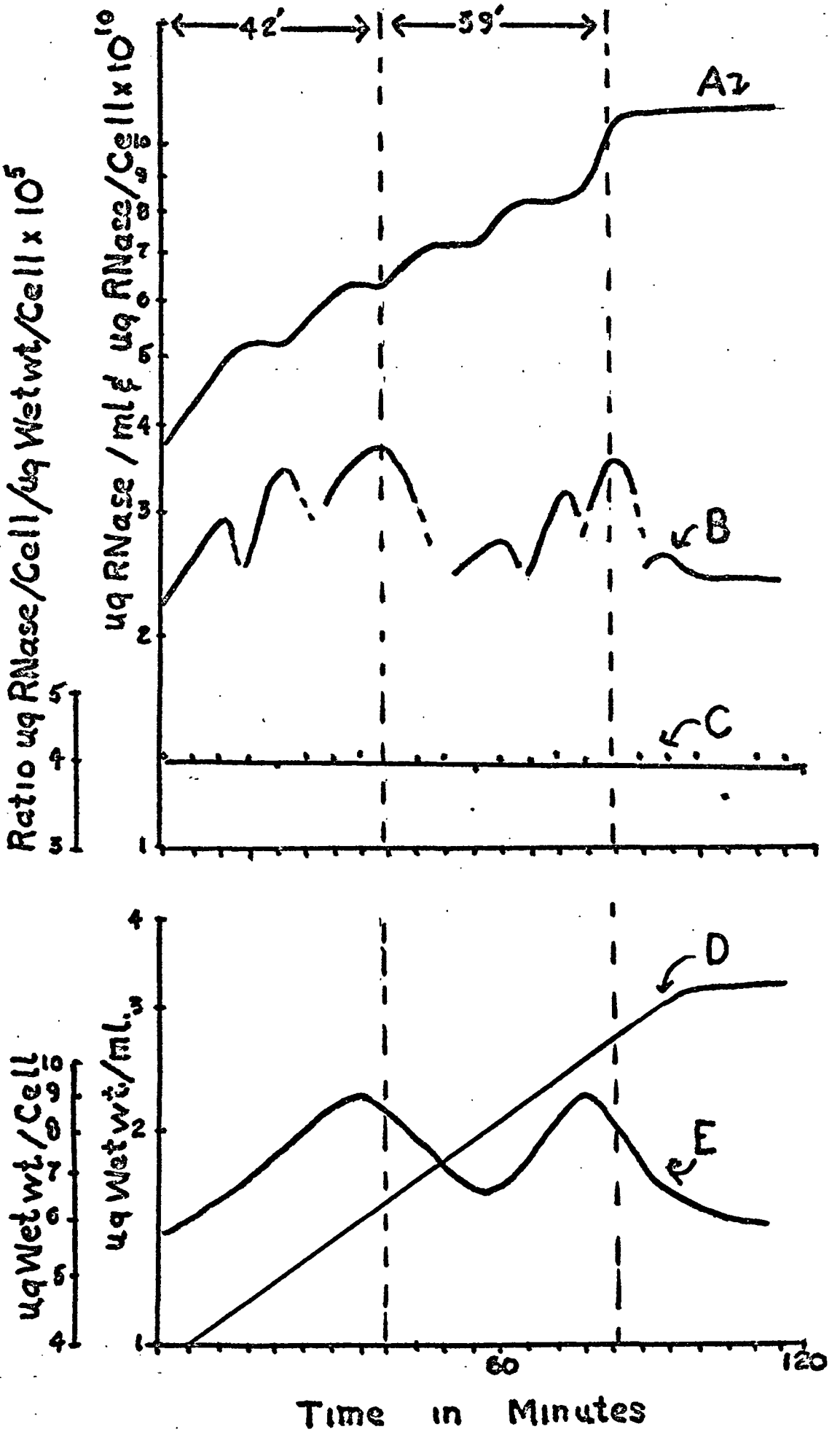

42'
59'
A
B
C
D
E
Ratio ug RNase/Cell/ug Wet wt/Cell x 10^5
ug RNase / ml & ug RNase/Cell x 10^10
ug Wet wt/Cell x 10^10
ug Wet wt/ml
60
120
Time in Minutes

Figure 5. Synchronous cell division of P. vulgaris. A indicates the stepwise number of cells per ml characteristic of synchronous growth. B is the cumulative plot of the number of cells per ml. The dashed vertical lines indicate the generation time and the arrow the time aliquots were removed for cell counts, wet weight and ribonuclease determinations.

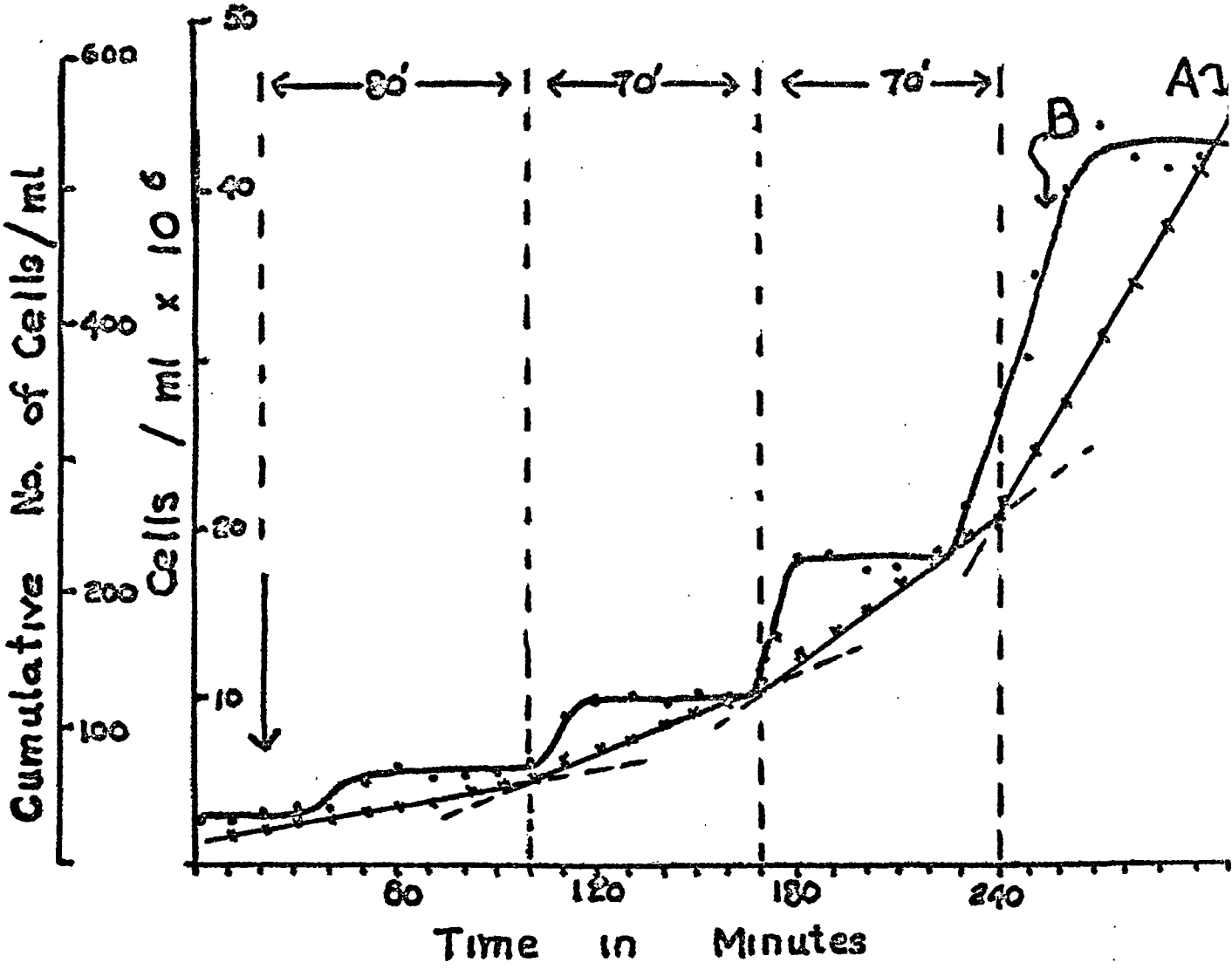
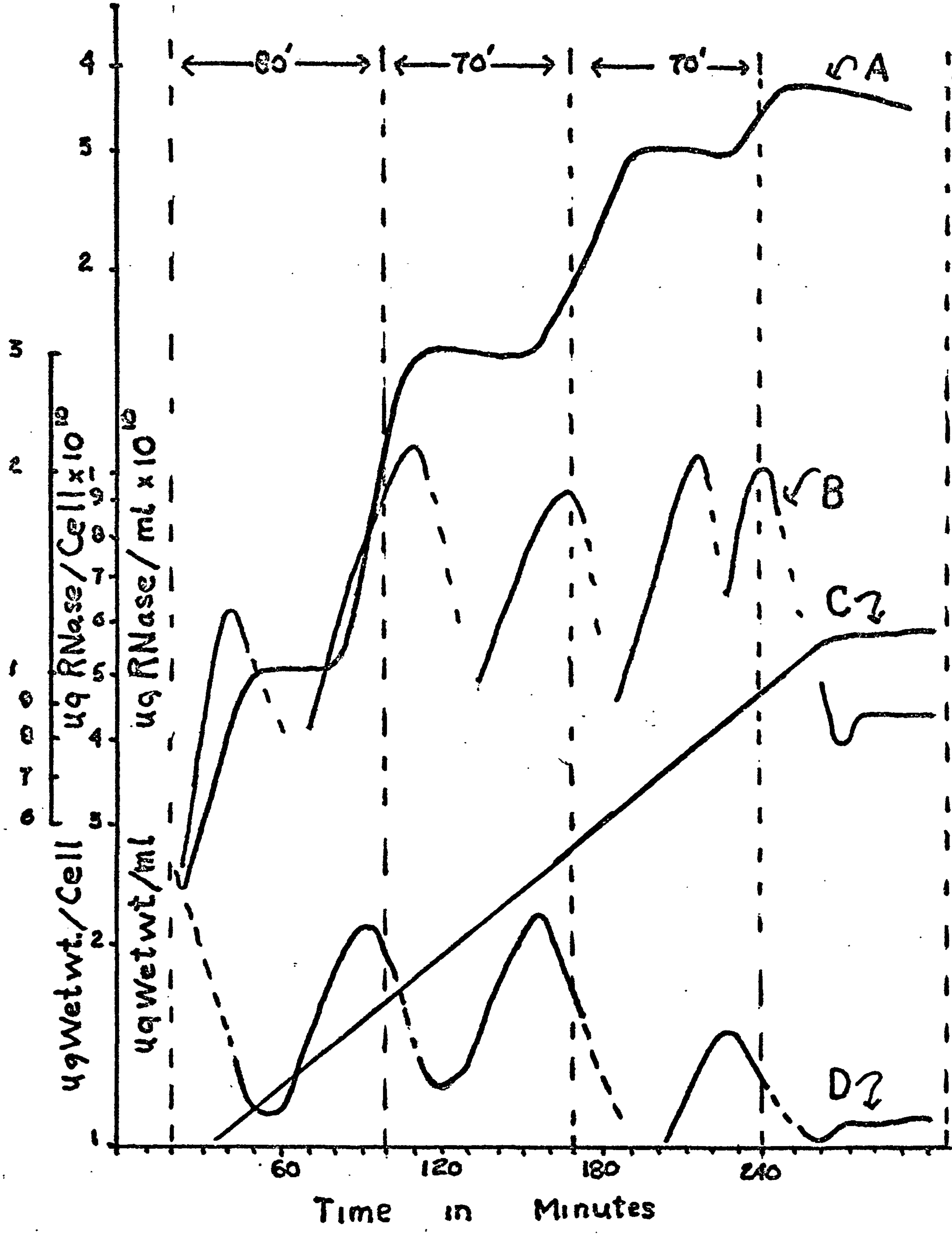

Cumulative No. of Cells/ml
600
400
200
100
Cells / ml x 10⁶
50
40
20
10
80'
70'
70'
B
A
60
120
180
240
Time in Minutes

Figure 6. Variations in ribonuclease and wet weight as functions of the cell division cycle, P. vulgaris. A indicates ug of ribonuclease per ml, B the ug of ribonuclease per cell, C the ug wet weight per ml and D the ug wet weight per cell.


80'
70'
70'
A
B
C
D
uq RNase/Cell x 10^10
uq RNase/ml x 10^10
uqWetwt./Cell
uqWetwt/ml
60
120
180
240
Time in Minutes

DISCUSSION

Growth of a bacterial population involves a doubling of the macromolecules of the cell prior to cell division. The question may be raised as to what extent synthesis of the macromolecules of the cell is an ordered process and how and to what extent are these biochemical reactions sequenced. The use of synchronous cultures provides an ideal system for examining such questions. Therefore, it was the intent of this paper to investigate enzyme systems as a potential toward elucidating cellular regulation, control and temporal organization of living systems.

The synthesis of a number of the few enzymes analyzed has been shown to be discontinous, regular and sequential during the cell division cycle in synchronously dividing populations of bacteria. It has been suggested that if DNA replication is initiated at the same time for all synchronized cells and that if gene replication is an ordered sequential non-random process, then transcription and translation should also be ordered, sequential and non-random (2).

The timing of ribonuclease during synchronous growth

begins at a specific time and doubles only at a specific time during the cell division cycle (Figs. 4 and 6). Whether or not such oscillations are an intricate part of the dynamics of organized cells is not understood. On the other hand, cell division is correlated with cell mass as measured by wet weight and ribonuclease activity. Similarly, cessation of synchronous cell division is accompanied by like changes in cell wet weight and cell ribonuclease activity. Pronounced damping of periodic enzyme oscillations follows variance in generation cycle and on further successive generations becomes effectively randomized, as does the cell population. The increase in wet weight, cell mass and periodicity of ribonuclease seems to be one of a probable number of decisive events in the sequence of biochemical reactions leading to cell division.

The timing in change of enzyme appears to be periodic and may well be a gene dose response. But just how changes in enzyme activity are related to DNA replication and the cell division cycle cannot be ascertained from these experiments. Further studies relating to genome and cell division synchrony and transcription are necessary before such questions can be answered.

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