POSSIBLE MECHANISMS CONTROLLING THE INTRACELLULAR LEVEL OF INORGANIC POLYPHOSPHATE DURING SYNCHRONOUS GROWTH OF CHLORELLA PYRENOIDOSA

by

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TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

The presence of an inorganic phosphate polymer, polyphosphate, in a variety of bacteria, algae, and higher plants has aroused interest as to its metabolic role in such organisms. Polyphosphate has been shown to serve as a source of phosphorus for nucleic acid and phosphoprotein synthesis when microorganisms are placed under environmental stress[1-5]. Whether the energy of its anhydride linkages can be utilized in other synthetic reactions has not been demonstrated.

The intracellular level of acid-insoluble polyphosphate in synchronously growing Chlorella pyrenoidosa has been shown to fluctuate dramatically[6-8]. Polyphosphate reaches its peak concentration (about 35 percent of the total cellular phosphorus) early in cellular development, then decreases to a minimum of 18 percent by the end of the period of nuclear division.

The mechanism of control of the synthesis and utilization of acid-insoluble polyphosphate during cellular growth has been subject to recent investigation[6,7,9-11] and is the basis of the present studies. Resulting from kinetic studies with polyphosphate kinase from Escherichia coli which catalyzes the reaction: $xADP + (P_i)_x \rightleftharpoons xATP$, Kornberg[9] has proposed that the relative concentrations of ATP and ADP within the cell could control whether net production or utilization of $(P_i)_x$ occurred, synthesis only being possible under conditions creating a high ATP/ADP ratio. The ATP which serves as the energy source in the phosphorylation of the polyphosphate primer would be supplied by the energy-generating systems of the cell, primarily

oxidative and photosynthetic phosphorylation. During cellular growth and division of the unicellular green alga, Chlorella pyrenoidosa, the mechanism of control of the synthesis and utilization of polyphosphate may be linked to the activity of these energy-generating systems in relation to the energy-requiring systems of the cell.

From experimental evidence from this laboratory[10,15] as well as from related observations from other laboratories[12-14], it has been proposed that during the early developmental stages of the cell (0-4 hr) high photosynthetic[12] and respiratory rates[10,13] and active photophosphorylation[14] elevate the ATP/ADP ratio to a value conducive to net synthesis of polyphosphate[9,15]. Immediately following these events the photosynthetic[12] and respiratory rates[10,13] drop, depressing the ATP/ADP ratio and slowing the synthesis of polyphosphate[15]. During the 6th to 11th hr of development accelerated nucleic acid and protein synthesis[16] further depresses the ATP/ADP ratio and utilization of polyphosphate accelerates[15]. Mitosis, cross wall deposition and daughter cell release during the 8th to the 13th hr further accelerate the utilization of polyphosphate and return it to its initial cellular level[15].

As an index of activity of the energy-generating systems, endogenous respiration rates and photosynthetic rates were measured during synchronous growth of a high temperature strain of Chlorella pyrenoidosa. The observed photosynthetic rates, confirming those of Sorokin[12], are not reported. However, there were differences between respiratory rates observed in the present studies and those reported by other workers[12, 13,17,18]. Fluctuations in $(P_i)_x$ levels and photosynthetic and

respiratory rates suggested fluctuations in the intracellular levels of ATP and ADP and prompted studies of the ATP/ADP ratio during synchronous growth.

The results have been divided into two manuscripts, both of which have been submitted for publication.

POSSIBLE MECHANISMS CONTROLLING THE INTRACELLULAR LEVEL OF INORGANIC POLYPHOSPHATE DURING SYNCHRONOUS GROWTH OF CHLORELLA PYRENOIDOSA

I. ENDOGENOUS RESPIRATION

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INTRODUCTION

It was previously reported[1,2,13] that during synchronous growth of a high temperature strain of Chlorella pyrenoidosa that the uptake of inorganic phosphate was continuous and logarithmic. The intracellular level of inorganic acid-insoluble polyphosphate, $(P_i)_x$, however, fluctuated dramatically during the course of cellular development. During early growth the $(P_i)_x$ level increased reaching levels of 30 to 35 percent of the total cellular phosphorus for a brief period prior to nuclear division but dropped to levels of 15 to 18 percent by the end of this event.

Determination of the Michaelis constants for the enzyme (polyphosphate kinase) from Escherichia coli catalyzing the reaction: x ADP + $(P_i)_x \rightleftharpoons$ x ATP led Kornberg[7] to the hypothesis that the relative concentrations of ATP and ADP within the cell could control whether net

production or utilization of $(P_i)_x$ occurred, synthesis only being possible under conditions creating a very high ATP/ADP ratio.

The energy-generating systems of C. pyrenoidosa which could supply ATP for $(P_i)_x$ synthesis would be primarily oxidative and photosynthetic phosphorylation. During the course of cellular development, the ATP/ADP ratio which may control the net synthesis or utilization of $(P_i)_x$ may be linked to the activity of these energy-generating systems in relationship to the energy-requiring systems of the cell. From experimental evidence reported in the present paper as well as from related observations from other laboratories, it is proposed that during the early developmental stages of the cells high photosynthetic[14] and respiratory rates, and active photophosphorylation[9] elevate the ATP/ADP ratio to a value conducive to net synthesis of $(P_i)_x$. Immediately following these events the photosynthetic and respiratory rates decrease, depressing the ATP/ADP ratio, slowing the synthesis of $(P_i)_x$. During the following period of development, accelerated nucleic acid synthesis [4,5,8] and the exponential nature of protein synthesis[12] further depress the ATP/ADP ratio and net utilization of $(P_i)_x$ begins and accelerates. Mitosis, cross wall deposition, and daughter cell release further accelerate the utilization of ATP and $(P_i)_x$, returning $(P_i)_x$ to its initial level.

The present paper describes the periodism in endogenous respiration rate and its possible relationship to the intracellular levels of $(P_i)_x$ during cellular growth and division of a high temperature strain of C. pyrenoidosa.

MATERIALS AND METHODS

Organism and culture conditions.--The high temperature strain 7-11-05 of Chlorella pyrenoidosa described by Sorokin and Myers[15] was used in these studies. Synchrony was induced by intermittent illumination by the procedure of Schmidt and King[13] as modified by Baker and Schmidt[1]. Approximately 900 ml of synchronized daughter cell suspension (45×10^6 cells/ml) was transferred to a flat Plexiglass chamber with the following inside dimensions: 15.9" width, 0.3" thickness, 15.4" height with the bottom 3" tapering to a point. The chamber was placed in a constant temperature water bath at 38.5°, and the cell suspension was aerated with 3 percent CO_2-in-air.

The first synchronous growth cycle was initiated by illumination from a bank of eight 40 watt fluorescent lamps on each side of the water bath. After the cells completed one synchronous growth cycle (13-13.5 hours), the cells were diluted in the light with fresh culture medium (pre-equilibrated at 38.5° with 3 percent CO_2-in-air) to the original cell concentration existing prior to cellular division. This dilution prevented low light intensity or low nutrient concentration from limiting the growth rate of the cells during the second generation of synchronous growth.

Biochemical and biophysical measurements.--Samples were harvested at hourly intervals throughout the two consecutive synchronous growth cycles. Cellular dry weight and total cellular phosphorus per ml of culture were determined by the method of Schmidt[12] and Fiske and Subbarow[3], respectively. Cell number per ml was determined using a

Levy-Hausser hemacytometer. The number of nuclei per cell was determined after using the staining procedure of Schaechter and DeLamater[11] as modified by Spencer et al[17].

For respiratory measurements, approximately the same amount of total cellular phosphorus (or total cellular dry weight) was harvested at each sample period. The cells were filtered from the culture medium on Millipore filters 44 mm in diameter having a pore size of 0.8 mμ. The cells were washed off the filters with fresh culture medium and made to a volume of seven or 14 ml. An aliquot of this cell suspension was taken for the determination of total cellular phosphorus per ml. 2.3 ml (approximately 130 μg total cellular-P) of the same concentrated cell suspension was added to two or four replicate 17 ml Warburg flasks containing 0.2 ml of 20 percent KOH in the center well. The flasks were then equilibrated for five minutes in a Warburg bath maintained at 38.5°. Oxygen uptake was measured by the direct Warburg method as described by Umbreit et al[18]. Readings were taken every three minutes for a half hour period. The total preparatory time from the moment of harvest to the first manometric reading was 13 minutes. Respiration rates were expressed as μl O_2 absorbed per μg total cellular phosphorus per minute.

In a study devised to stimulate culture conditions of previous workers[16], the lights were turned off at the 9th hour of synchronous growth, the time of cell division, and respiratory rates were measured hourly during a six hour period thereafter.

RESULTS AND DISCUSSION

The total cellular phosphorus of the high temperature strain of C. pyrenoidosa increased logarithmically during two consecutive synchronous growth cycles in continuous light (Fig. 1). The increase in cellular dry weight was also a logarithmic function with the same slope as that for total cellular phosphorus (Fig. 1). The rate of increase in total cellular phosphorus was virtually the same in both synchronous growth cycles. Sorokin[14] observed logarithmic increases in cellular dry weight and cell volume for the same organism during the first cycle of synchronous growth; however, these functions did not have parallel slopes.

Since total cellular phosphorus and cellular dry weight are parallel functions, oxygen uptake per unit time can be expressed on the basis of either of these two cellular parameters and still reflect the same trends in respiration rate during synchronous growth. Because the method for the determination of total cellular phosphorus is much more sensitive than that for cellular dry weight, respiration rates were expressed on a cellular phosphorus basis. Oxygen uptake was observed to be linear during the 30 minute period of measurement at different stages of cellular development (Fig. 2). Apparently certain endogenous substrates become limiting after 35 to 40 minutes of measurement because negative deviations from linearity were observed often after this time period; therefore, respiratory measurements were always restricted to a 30 minute period.

The endogenous respiration rate through two consecutive cycles of synchronous growth is shown in Figure 3. In each of the two synchronous growth cycles, the respiration rate rose rapidly during early daughter

Fig. 1. Relationship between total cellular phosphorus and cellular dry weight during synchronous growth of C. pyrenoidosa. o—o—o, total cellular phosphorus; Δ—Δ—Δ, cellular dry weight.

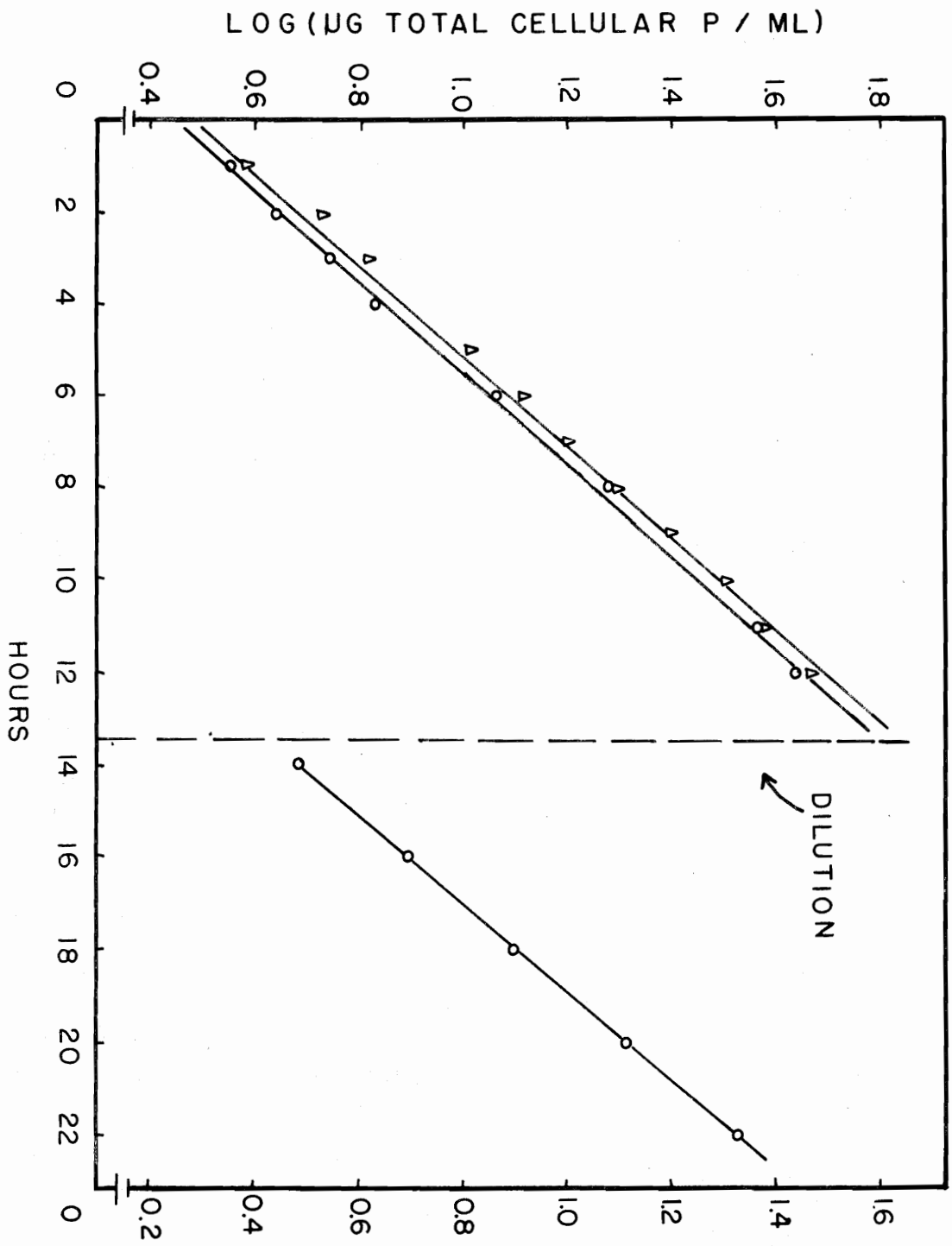

LOG (µG TOTAL CELLULAR P / ML)
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
HOURS
0
2
4
6
8
10
12
14
16
18
20
22
0
DILUTION
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
LOG (10 X MG CELLULAR DRY WEIGHT / ML)

Fig. 2. Endogenous respiration rates at different stages of cellular development of C. pyrenoidosa. o—o—o, 4th hour; •—•—•, 10th hour; Δ—Δ—Δ, 13th hour.

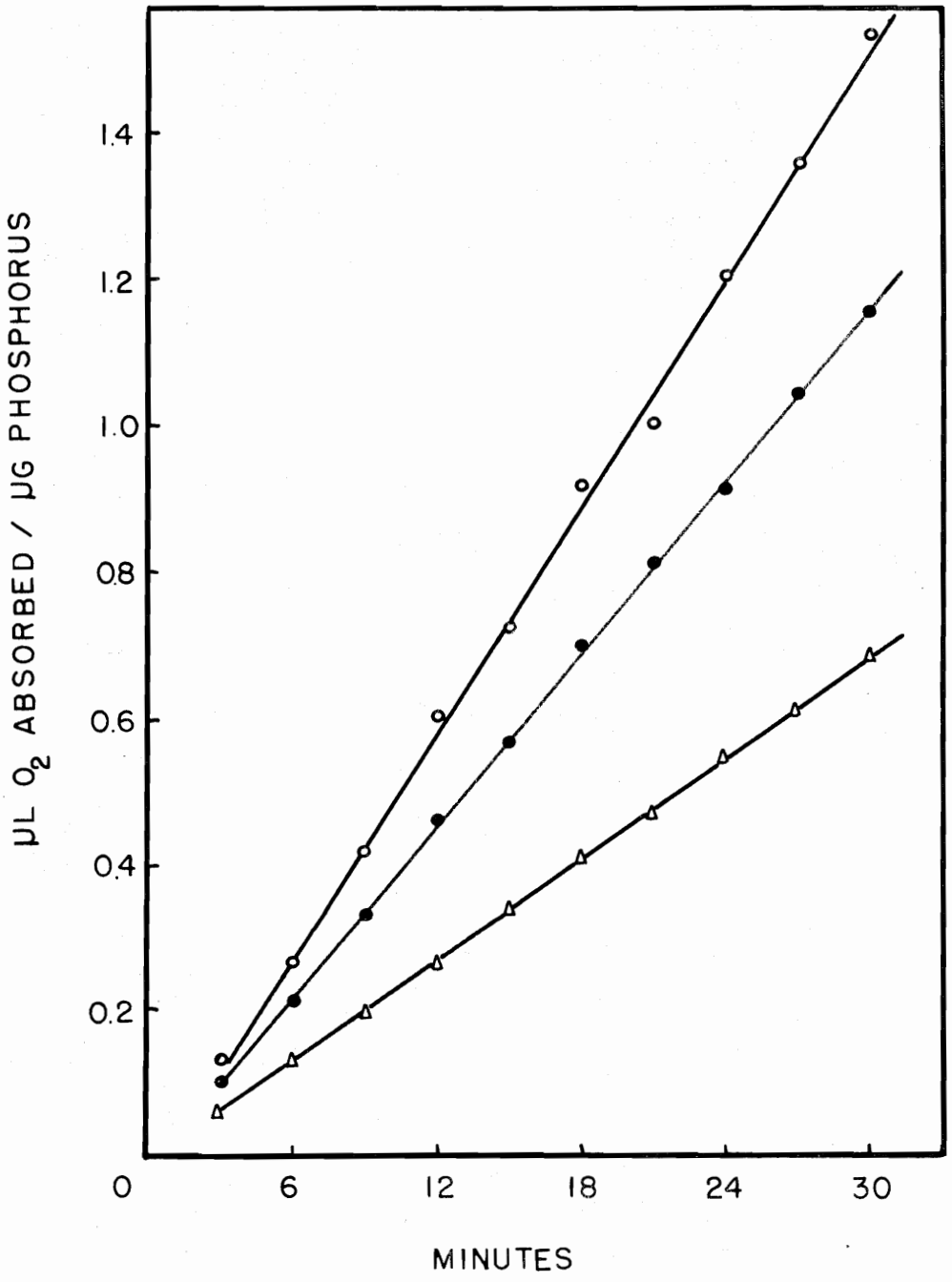

μL O2 ABSORBED / μG PHOSPHORUS
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
6
12
18
24
30
MINUTES

Fig. 3. Relationship of endogenous respiration rate to cell number during successive cycles of synchronous growth of C. pyrenoidosa.
●—●—●, endogenous respiration rate; o—o—o, cell number.

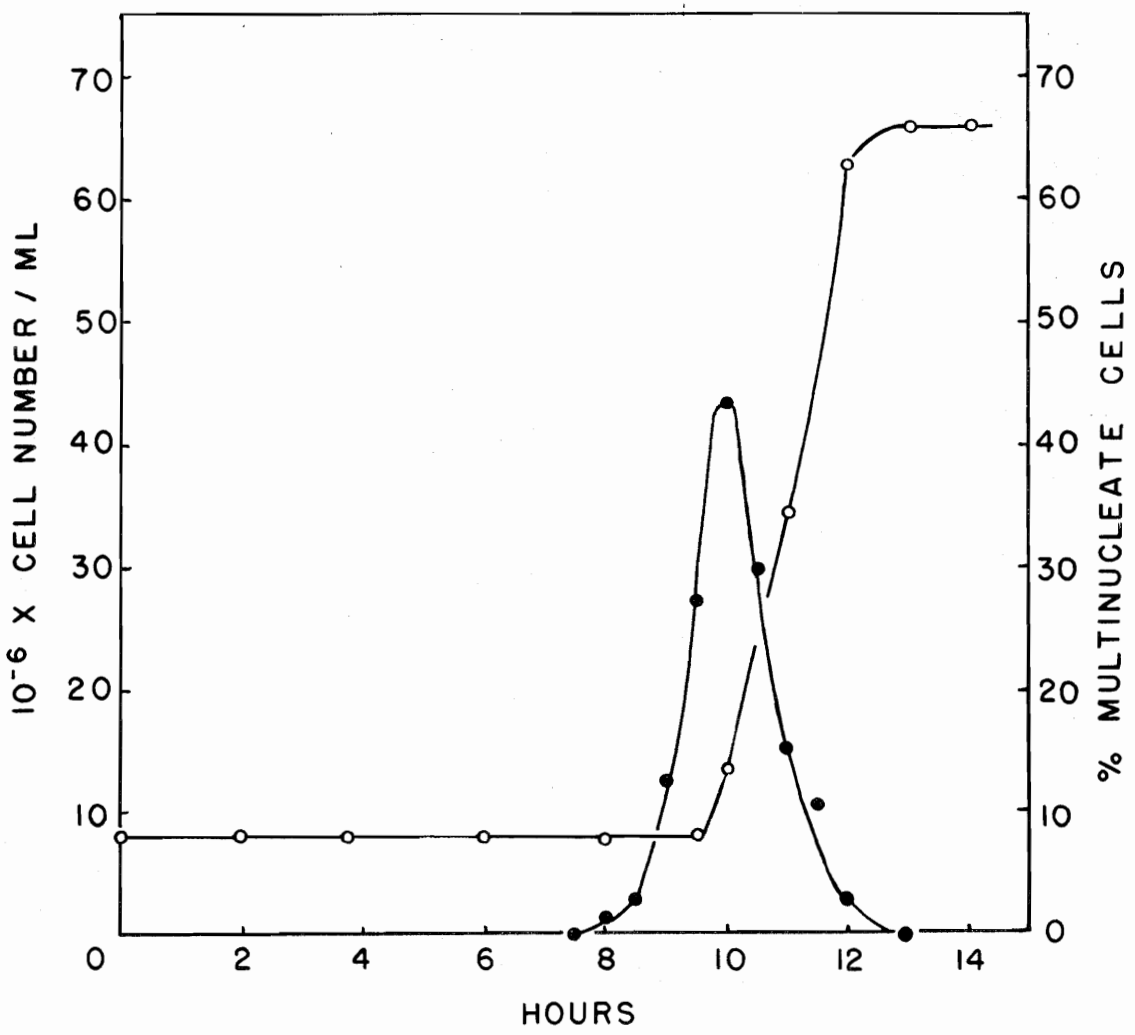
10^{-1} X µL O_2 ABSORBED / µG P / MINUTE
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
8
10
12
14
16
18
20
22
HOURS
DILUTION
10^{-6} X CELL NUMBER / ML
600
500
400
300
200
100
0

cell development and reached a maximum about one-third of the way through each growth cycle. The rate then declined steadily reaching a minimum immediately prior to the period of nuclear division when the nucleus undergoes three divisions to yield eight daughter nuclei. At the onset of nuclear division the rate again accelerated reaching a second maximum when the culture contained the greatest number of multinucleate cells (Fig. 3 and 4). As cell division (daughter cell release) was initiated, the respiration rate again decreased reaching a second minimum at the completion of this event. Recently Ried et al[10] have reported similar periodism in the endogenous respiration rate of a low temperature strain of C. pyrenoidosa during the first cycle of synchronous growth.

Because this periodism in endogenous respiration rate (high temperature strain) is recurrent through two consecutive cycles of synchronous growth in the absence of the synchronization treatment, it is very probable that the observed periodism is representative of normal cellular development. The importance of evaluating periodism observed for any metabolic event for more than one synchronous growth cycle following the synchronization treatment was discussed by Baker and Schmidt[2]. These workers have shown that certain shifts in the metabolism of nucleic acids and acid-insoluble polyphosphates were induced in the first synchronous growth cycle of C. pyrenoidosa by the synchronization treatment. These induced shifts were apparent because they were not recurrent in the second and third cycles of synchronous growth.

The greater number of daughter cells released during cell division in the first synchronous growth cycle (11.8 fold increase) as compared to

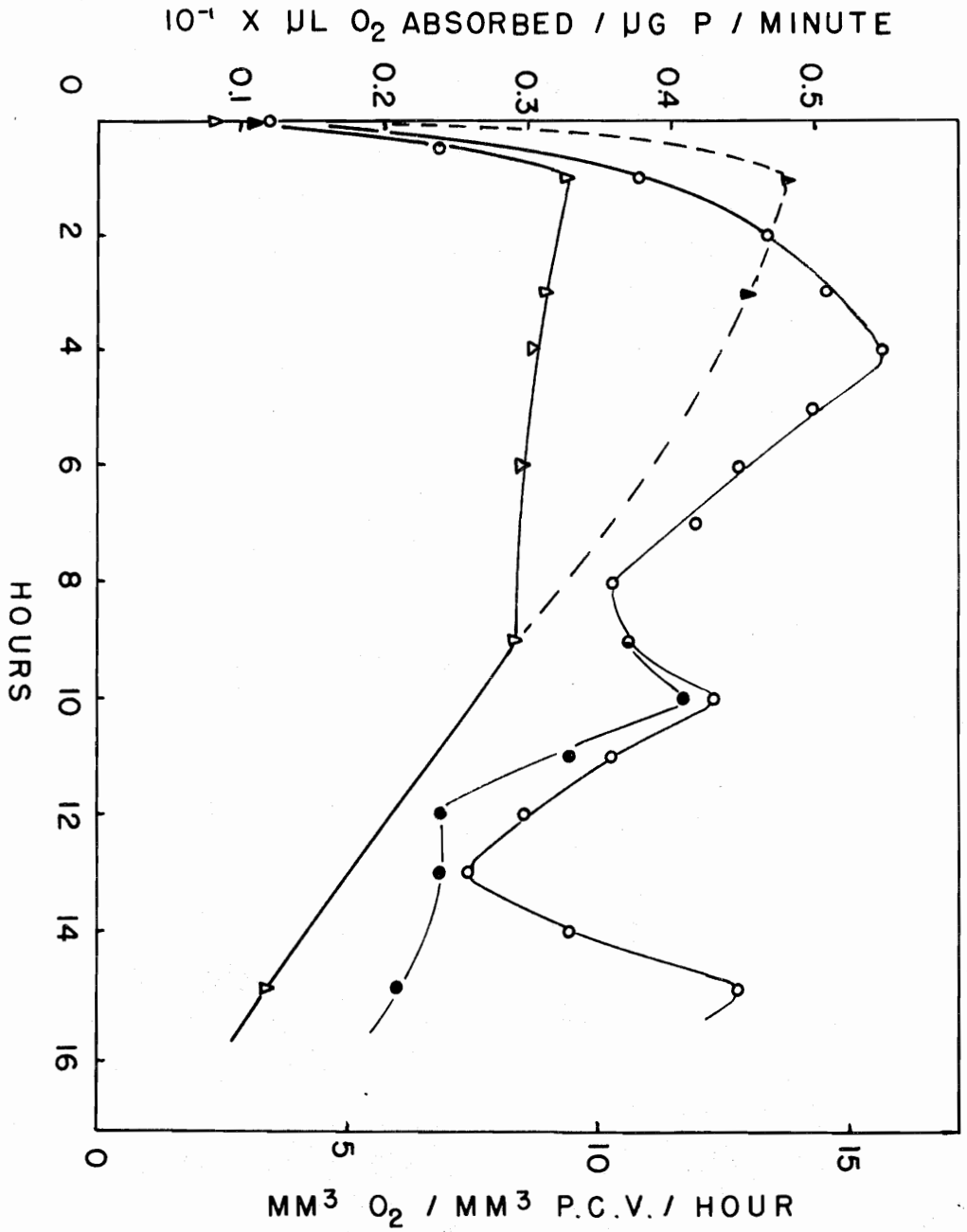
Fig. 4. Relationship of nuclear division to generation time during synchronous growth of C. pyrenoidosa. ●—●—●, % multinucleate cells; o—o—o, cell number.


70
60
50
40
30
20
10
0
2
4
6
8
10
12
14
HOURS
10^{-6} X CELL NUMBER / ML
% MULTINUCLEATE CELLS

the second growth cycle (8.2 fold increase) was reported earlier by Johnson and Schmidt[6]. Apparently the dark period prior to the first synchronous growth cycle stimulates nuclear division because many of the cells divide into 16 daughter cells in the first cycle as compared to only eight in the second and third cycles of continuous synchronous growth following the synchronization treatment.

The periodism in endogenous respiration rate described in the present paper differs markedly from that reported earlier for the same organism by Sorokin and Myers[16] (Fig. 5). They observed that the endogenous respiration rate (expressed as mm^3 O_2/mm^3 packed cell volume/hr.) rose extremely rapidly and reached a maximum after one hour of cellular development. The rate then decreased very gradually until the time of cell division (9th hr.) when the growing culture was placed in the dark. At the onset of darkness, the rate appeared to decrease more sharply. It should be pointed out, however, that about five hours elapsed from the time lights were turned off until the next respiratory measurement was made. In a similar study in our laboratory, the lights were turned off at the same stage of synchronous growth, but respiratory rates were measured at hourly intervals thereafter (Fig. 5). The rise in respiration rate, after darkening the culture, was almost equal to that of the culture remaining under continuous illumination. However, a rapid decline in respiration rate was seen in the darkened culture after the peak at the 10th hour. It was also noted in this same study that the total cellular phosphorus of the cells continued to increase logarithmically up to two hours after darkening the culture. It seems probable that Sorokin and Myers[16] missed this second peak in respiration rate because of infrequent sampling rather than because of darkening the culture.

Fig. 5. Comparison between the endogenous respiration rates observed by Curnutt and Schmidt (present paper) and those obtained by Sorokin and Myers[16] during synchronous growth of a high temperature strain (7-11-05) of C. pyrenoidosa. o—o—o, endogenous respiration rates of cells cultured in continuous light (Curnutt and Schmidt); ●—●—●, endogenous respiration rate of cells placed in darkness at the 9th hour of development (Curnutt and Schmidt); Δ—Δ—Δ, endogenous respiration rates of cells cultured in continuous light until the 9th hour of development when the lights were turned off (redrawn from Sorokin and Myers); ▲—▲—▲, endogenous respiration rates obtained by extrapolation from observed rates (redrawn from Sorokin and Myers).


10⁻¹ X µL O2 ABSORBED / µG P / MINUTE
0.1
0.2
0.3
0.4
0.5
HOURS
0
2
4
6
8
10
12
14
16
0
5
10
15
MM3 O2 / MM3 P.C.V. / HOUR

Apparently these workers placed their cultures in the dark prior to cell division to maintain synchrony. Johnson and Schmidt[6] and Baker and Schmidt[2] have shown, however, that darkening a synchronized culture of Chlorella before it completes one synchronous growth cycle is unnecessary for maintaining a high degree of synchrony for the completion of that and several following synchronous growth cycles. Furthermore, darkening a culture undoubtedly places stresses on the cells which are reflected in a changing metabolic pattern which cannot be legitimately compared to that of cells cultured in continuous light.

The apparent difference in results obtained by this laboratory and by that of Sorokin and Myers[16] may be attributed to (a) the frequency of sampling during synchronous growth, and (b) the time consumed in preparation of the samples for respiration measurements. In the present study samples were harvested at hourly intervals during synchronous growth. Sampling times varied from two to four hours in the experiments of Sorokin and Myers[16]. This in itself may account for discrepancies such as those described earlier with respect to the observation of the second peak in respiration rate during synchronous growth.

The time lapse from the moment of sampling from the culture chamber until the first respiration measurement was shown by Sorokin and Myers[16] to be an important factor which influences the observed respiration rates. Lower respiration rates were generally observed as preparatory time increased. They observed that cells early in cellular development (1 to 3 hour cells) have the highest starting respiration rates, but these rates undergo a rapid decline soon after the cells are darkened. Cells

advanced in their development (6 to 8 hour cells) exhibited lower initial respiration rates, but these rates remained stable for almost three hours in darkness. The failure of Sorokin and Myers[16] to see the sharp peak in respiration rate at the fourth hour of cellular development probably results from the depletion of certain endogenous oxidizable substrates during the long (60 min.) preparatory procedure. The respiratory measurements made after the short (13 min.) preparatory procedure described in the present paper would seem, therefore, to more nearly represent the normal endogenous respiratory behavior of the cell during development.

The observation that the maximum intracellular level of $(P_i)_x$ accumulation (4th hour) corresponds exactly to the major peaks in endogenous respiration rate and photosynthetic rate gives support to the hypothesis that the ATP/ADP ratio regulates the intracellular level of $(P_i)_x$ during cellular development.

SUMMARY

The endogenous respiration rate was measured during two consecutive cycles of synchronous growth of a high temperature strain of C. pyrenoidosa. The respiration rate rose rapidly during early daughter cell development and reached a maximum about one-third of the way through each growth cycle. The rate then declined steadily reaching a minimum prior to the period of nuclear division. At the onset of nuclear division the rate again accelerated and reached a maximum when the culture contained the greatest number of multinucleate cells. The respiration rate, however,

decreased during cell division reaching a minimum at the completion of this event.

A rapid procedure for harvesting and preparing the cells for respiratory measurements is described. This procedure and the frequency of sampling during synchronous growth appears to explain the differences in the periodism in endogenous respiration rate reported in the present study and those reported by previous workers.

The relationship of the periodism in the energy-generating systems to the energy-utilizing systems is discussed as a possible mechanism for controlling the intracellular ATP/ADP ratio which may in turn regulate the net synthesis or utilization of inorganic acid-insoluble polyphosphate during cellular growth and division.

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POSSIBLE MECHANISMS CONTROLLING THE INTRACELLULAR LEVEL OF INORGANIC POLYPHOSPHATE DURING SYNCHRONOUS GROWTH OF CHLORELLA PYRENOIDOSA

II. ATP/ADP RATIO

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INTRODUCTION

Metabolic control of the accumulation and utilization of inorganic acid-insoluble polyphosphate, $(P_i)_x$, during cellular growth has been subject to recent investigation[1-4]. During synchronous growth of a high temperature strain of Chlorella pyrenoidosa the level of $(P_i)_x$ was shown[1] to increase dramatically to 30 to 35 percent of the total cellular phosphorus prior to nuclear division and to drop to 15 to 18 percent at the end of this event. It was proposed by Kornberg[3] that the relative concentration of ATP and ADP within the cell could control the net synthesis or utilization of $(P_i)_x$, synthesis only being possible under conditions creating a high ATP/ADP ratio.

Curnutt and Schmidt[4] described a possible relationship of energy-generating systems, e.g. oxidative and photosynthetic phosphorylation, to energy-requiring systems and the synthesis and utilization of $(P_i)_x$.

It was proposed that maxima in photosynthetic and respiratory rates early in cellular development elevate the ATP/ADP ratio to values conducive to the net synthesis of $(P_i)_X$. Likewise, a later decrease in photosynthetic and respiratory rates depress the ATP/ADP ratio, slowing $(P_i)_X$ synthesis. Events such as the synthesis of nucleic acids and proteins, mitosis, cross wall deposition, and daughter cell release serve to depress the ATP/ADP ratio further and net utilization of $(P_i)_X$ begins. The present report concerns studies of the intracellular ATP/ADP ratio during synchronous growth of C. pyrenoidosa and its correlation with the preceding proposals.

MATERIALS AND METHODS

The high temperature strain of Chlorella pyrenoidosa, 7-11-05, described by Sorokin and Myers[5] was used in these studies. Synchrony was induced by intermittent illumination by the procedure of Schmidt and King[6] as modified by Baker and Schmidt[1]. Previously synchronized cells with an initial density of $12 \cdot 10^6$ cells per ml were cultured in continuous light in a 35 l Plexiglass chamber as described by Baker and Schmidt[1]. Cells were harvested every 2 hr., throughout two successive synchronous growth cycles, directly from the culture chamber into a Servall refrigerated centrifuge with a continuous-flow attachment. Sample pellets were extracted immediately with 0.9N $HClO_4$ at 0° for 1 hr. The extract was neutralized with 2N KOH and assayed for ATP and ADP content. An extraction time study showed that 1 hr. extraction with 0.9N $HClO_4$ yielded highest nucleotide levels. After the completion of one synchronous growth cycle,

cells were diluted with fresh medium to the original initial cell concentration.

ATP was determined enzymatically using the phosphoglycerate kinase system[7] by measuring the decrease in reduced DPN using an Eppendorf recording spectrophotometer. ADP was assayed similarly using the pyruvic kinase system[8]. It was found that the length of harvest time (5 to 40 min.) required for large samples did not influence ATP or ADP recovery.

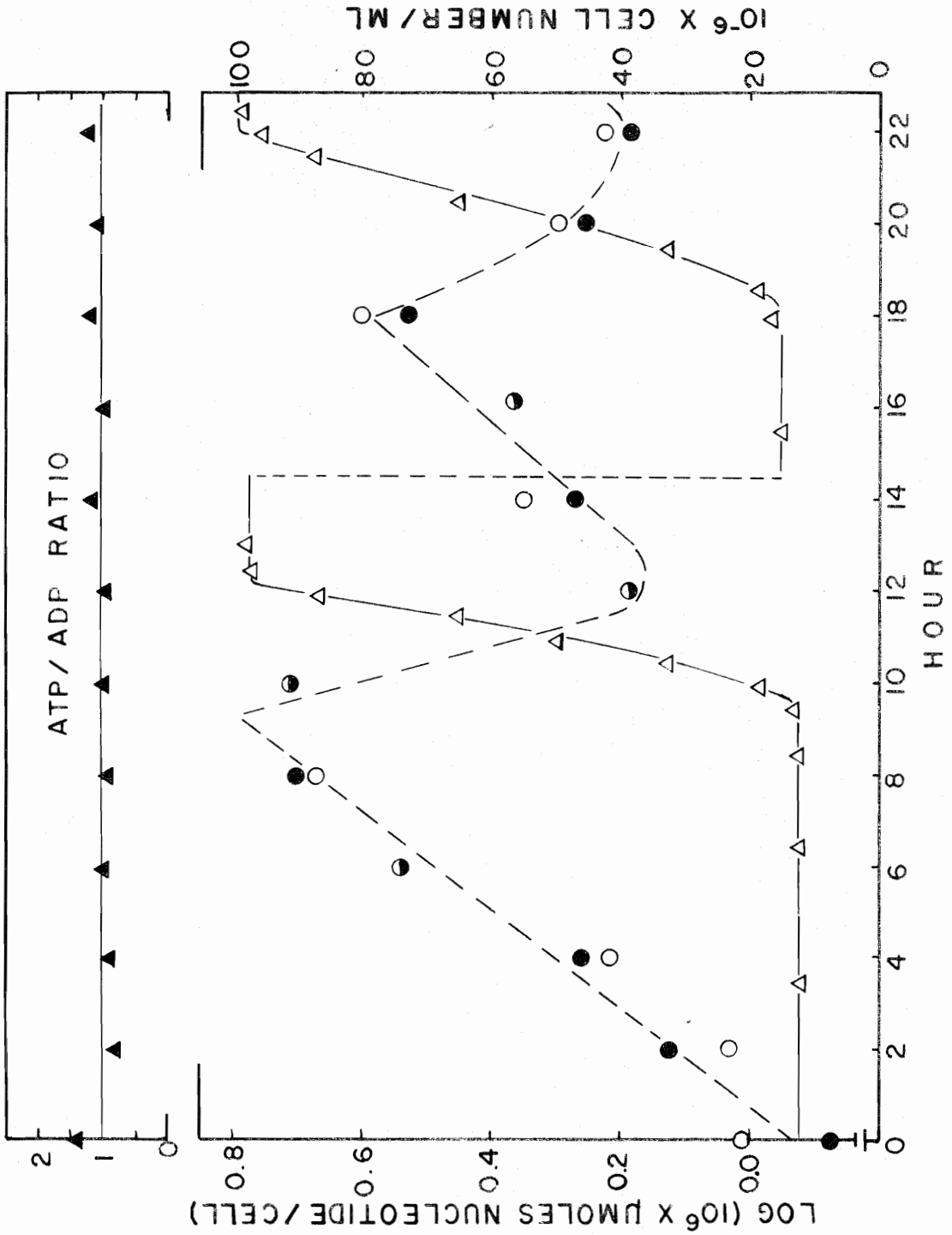
Cell concentration per ml of culture was determined using a Levy-Hausser hemacytometer. Total cellular phosphorus per ml of culture and per sample was determined by the method of Fiske and Subbarow[9]. An exponential increase in total cellular phosphorus per ml of culture was observed throughout the two synchronous growth cycles[2,4].

RESULTS AND DISCUSSION

The intracellular ATP/ADP ratio remained approximately 1:1 throughout the synchronous growth of C. pyrenoidosa (Fig. 1). This ratio represents the relative amounts of ATP and ADP within the entire cell. Although the ATP/ADP ratio remained essentially constant, the level of ATP and ADP per cell increased logarithmically at a rate equal to those observed[4] for total cellular phosphorus and dry weight throughout cellular development (Fig. 1).

Kornberg's hypothesis that the relative concentrations of ATP and ADP control the synthesis and utilization of $(P_i)_x$ is not substantiated, but certainly not invalidated, by these studies because the presence of small pools of ATP and ADP at sites of $(P_i)_x$ synthesis where their relative

Fig. 1. The ATP/ADP ratio and the intracellular levels of these nucleotides during synchronous growth of C. pyrenoidosa. Cell number per ml, —Δ—Δ—; ATP/ADP ratio, —▲—▲—; ATP, —o—o—, and ADP —●—●—, levels per cell.


ATP/ADP RATIO
2
1
0
LOG (10^6 X μMOLES NUCLEOTIDE/CELL)
0.8
0.6
0.4
0.2
0.0
10^{-6} X CELL NUMBER/ML
100
80
60
40
20
0
HOUR
0
2
4
6
8
10
12
14
16
18
20
22

amounts might vary in accordance with the proposals of Curnutt and Schmidt[4] is not precluded by the present findings.

SUMMARY

The intracellular ATP/ADP ratio was determined enzymatically during two consecutive cycles of synchronous growth of a high temperature strain of Chlorella pyrenoidosa. The ATP/ADP ratio remained approximately 1:1 throughout synchronous growth. The levels of ATP and ADP were assayed throughout the life cycle using the phosphoglycerate kinase system and the pyruvic kinase system, respectively.

The constant ATP/ADP ratio and its relationship to a possible mechanism for the control of the net synthesis or utilization of inorganic acid-insoluble polyphosphate during cellular growth and division is discussed.

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POSSIBLE MECHANISMS CONTROLLING THE INTRACELLULAR LEVEL OF INORGANIC POLYPHOSPHATE DURING SYNCHRONOUS GROWTH OF *CHLORELLA PYRENOIDOSA*

ABSTRACT

The endogenous respiration rate and the intracellular ATP/ADP ratio were determined during synchronous growth of a high temperature strain of *Chlorella pyrenoidosa* in order to correlate their activities with a proposed mechanism for the control of intracellular levels of inorganic acid-insoluble polyphosphate, $(P_i)_x$, during cellular growth and division.

The respiration rate measured during two consecutive cycles of synchronous growth rose rapidly during early daughter cell development and reached a maximum about one-third of the way through each growth cycle. The rate then declined steadily reaching a minimum prior to the period of nuclear division. At the onset of nuclear division the rate again accelerated and reached a maximum when the culture contained the greatest number of multinucleate cells. The respiration rate decreased during cell division reaching a minimum at the completion of this event.

A rapid procedure for harvesting and preparing the cells for respiratory measurements is described.

Enzymatic determination of ATP and ADP levels showed the ATP/ADP ratio to remain essentially 1:1 throughout the synchronous growth cycle.

The relationship of the periodism in endogenous respiration rates to energy-utilizing systems, e.g. the regulation of net synthesis or

utilization of $(P_i)_x$, is discussed. Although the intracellular ATP/ADP ratio thought to determine the aforementioned synthesis or utilization of $(P_i)_x$ proved to remain 1:1 throughout cellular development, this does not preclude the presence of small pools of ATP and ADP at the sites of $(P_i)_x$ synthesis where their relative amounts might vary in accordance with the proposed control mechanism.

Further studies are needed to locate the intracellular sites of $(P_i)_x$ synthesis and to determine the relationship of these sites to the energy-generating systems of the cell.