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# 클로렐라의 同調培養法에 의한 細胞分裂의 生理學的 硏究 (第 2 報)

## ——細胞의 生活史를 통한 細胞構成成分의 生合成能에 미치는 포도당 및 2-thiobarbituric acid의 영향에 관하여——

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# Physiological studies on cell division by the technique of synchronous culture of chlorella (II)

## ——On the effect of glucose and 2-thiobiarbturic acid on the life cycle and the changes in biosynthetic activities of the cell constituents during the life cycle——

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## ABSTRACT

The effect of glucose and 2-thiobarbituric acid on the biosynthesis of cell constituents such as protein, carbohydrate, DNA, RNA, phospholipid and PCA-soluble phosphate compounds in *Chlorella* duing the life cycle was measured, and the changes in the content of these main cellular components of the algal cell were analyzed in connection with the nuclear and cytoplasmic division.

In the normal autotrophic synchronous culture the contents of protein, RNA, and DNA in the cell showed a chracteristic changes according to the progress of cell development, increasing more or less throughout all the life cycle. The synthesis of protein is more prominent in the division period and that of DNA is more active in the ripening period, while the synthesis of RNA is more rapid in the growing and ripening periods than other developmental stages.

The period of division cycle was little affected by glucose in the medium, although the synchrony of the growth and cellular division was disturbed and the n value increased. The contents of protein, carbohydrate, RNA and DNA of the cell were increased by the glucose treatment throughout all the life cycle. On the other hand, both of cellular growth and division were retarded severely and the n value was decreased by the 2-thiobarbituric acid treatment. The synthesis of protein, carbohydrate, DNA, RNA and phospholipid of the cell was also retarded by 2-thiobarbituric acid.

In the autotrophic, mixotrophic and 2-thiobarbituric acid-treated cultures, each having different mode of nuclear and cytoplasmic division, a common general scheme occurring in the cell during the life cycle may be drawn as follows. The ratio of RNA to protein attains maximum value in the $L_1$-cell stage prior to the nuclear division and thereafter decreases during the periods of ripening and division. The ratio of PCA-soluble phosphate compounds

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to protein increased from the beginning of the culture to $L_4$-cell stage successively and thereafter decreased gradually during the division period, while the ratio of protein to DNA kept almost constant up to the division period and thereafter increased during the division period. Therefore, it is presumed that the increase in the ratio of RNA to protein is to be an inducer of nuclear division and that the cytoplasmic division is induced by the increase in the ratio of protein to DNA.

## INTRODUCTION

Since the technique of synchronous culture of *Chlorella* cells has been developed by Tamiya *et al.*(1953), the study of biochemical changes during the life cycle of the algae was greatly enhanced by many workers. Changes in the contents of nucleic acid bases (Iwamura and Myers, 1959), amino acids (Kanazawa, 1954), fatty acids (Otsuka and Morimura, 1955), sterols (Otsuka, 1953), chlorophyll and carotenoids(Lee and Kim, 1964), vitamins (Morimura, 1959), polyphosphates (Miyachi and Miyachi, 1961), glucosamines (Mihara, 1961) and keto acids(Kanazawa, 1967) in *Chlorella* cells at different growing stages were reported. However, the change in the cell constituents as a whole is rarely studied in connection with nuclear and cytoplasmic division.

On the other hand, Higashiyama (1967) reported that cell division of *Chlorella* was stimulated by glucose even though this sugar evoked gigantism. Tamiya *et al.*(1962) studied the effects of various antimetabolites upon the life cycle of *Chlorella* and found that 2-thiobarbituric acid exerts, at certain concentration applied, no or only a slight inhibition upon growth, while it retards or halts the process of cell division.

The purpose of the present study is to observe the effect of glucose and 2-thiobarbituric acid, as division-stimulating and inhibiting agents respectively, on the biosynthesis of cell constituents such as protein, carbohydrate, deoxyribonucleic acid(DNA), ribonucleic acid(RNA), phospholipid and perchloric acid(PCA)-soluble phosphate compounds, and to analyze the change in the content of these main cellular components during the life cycle of *Chlorella* with regards to nuclear and cytoplasmic division.

## MATERIALS AND METHODS

**Synchronous culture.** The experimental organism was *Chlorella ellipsoidea*, and the culture methods employed were virtually the same as those reported previously (Lee and Lee, 1969), except the inoculum of synchronous culture using Da-cells instead of Dn-cells. The synchronous culture, which was started from the stage of Da-cell, was performed using so-called "DLD'-method" of Tamiya *et al.* (1961) and the method of obtaining the homologous population of Da-cells was the same as those reported by Morimura (1959).

**Measurment of developmental stage.** The process of cell development was followed by measuring the number of cells per liter medium using a hemocytometer, the statistical distribution of cell size by means of an ocular micrometer, and the packed volume of cells per liter using a hematocrtit as in the previous paper (Lee and Lee, 1969).

**Fractionation of cell material.** At various intervals during the synchronous culture some portions of the cell material were taken out, and after washing 2 times with M/500 $K_2SO_4$ solution they were fractionated by the method

of Schmidt and Thanhauser(1945) as follows. The cell material was successively extracted with cold 5% PCA(twice for 30 min. and 15 min.) and hot ethanol-ether(3 : 1) mixture (3-4 times). The residual non-lipid and acid-insoluble material was incubated with 0.5 N KOH at 37°C for 16-20 hours to hydrolyze RNA, and to the supernatant obtained by centrifugation an equivolume of 5% PCA was added after neutralization. From the precipitated deoxyribonucleoprotein the RNA fraction was removed, and the residue was further extracted with 5% PCA in boiling bath for 15 minutes to remove the DNA fraction from the residual protein fraction.

**Assay.** The amounts of phosphate in the fractions of PCA-soluble, lipid and DNA were determined spectrophotometrically after the hydrolysis of phosphate compounds to orthophosphate followed by the treatment with ammonium molybdate. The amounts of DNA and RNA were expressed in relative optical density at 260 mμ. From the PCA-soluble fraction, acid-hydrolysates of the residual protein fraction and the RNA fraction the quantity of amino acid was measured by ninhydrin reaction(Troll and Cannan, 1953) for free amino acids, alkali-stable protein and alkali labile protein. The amount of carbohydrate in the PCA-soluble, ethanol-ether soluble and alkali-insoluble residual fractions was determined spectrophotometrically by anthrone method (Scott *et al.*, 1953).

## RESULTS AND DISCUSSION

### 1. Some characteristics in biosynthesis of macromolecules of *Chlorella* cell in the course of the autotrophic life cycle.

The amounts of various compounds in each fraction of *Chlorella* cell during the synchronous autotrophic, mixotrophic and 2-thiobarbituric acid-treated culture are summarized in Table 1. In the autotrophic synchronous culture the process of cell development measured by the average cell volume, cell number and packed volume per liter medium is represented in Fig. 1.

In the normal autotrophic synchronous culture of this experiment the ripening period from the stages of $L_1$-cell to $L_4$-cell, during which nuclear divisions occur, ranged from 15 to 22 hours of the culture, and the division period from the stages of $L_4$-cell to Dn-cell took 22—31 hours. It is considered due to the difference in starting cells of the synchronous culture that the period of the life cycle of *Chlorella* is shortened more or less in the present experiment compared with that of the previous paper(Lee and Lee, 1969).

The change in the amounts of protein, carbohydrate, DNA, RNA and other phosphate

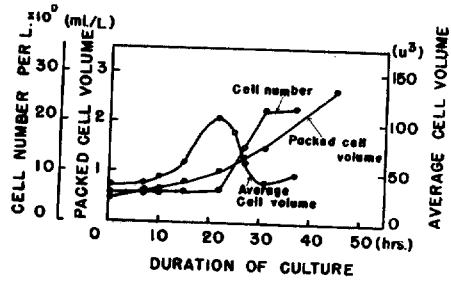


**Fig. 1.** Changes in the average cell volume, cell number and packed volume per liter medium during the autotrophic synchronous culture.

compounds in the algal cell during the normal autotrophic life cycle is shown in Fig. 2. Although the contents of protein, carbohydrate, RNA, and DNA of the algal cell increased more or less throughout the life cycle, they exhibited a characteristic change according to the progress of the cell development. Biosynthesis of protein is more prominent in division

**Table 1.** Amounts of various compounds per liter medium in each fractions of *Chlorella* cell during the synchronized normal autotrophic, mixotrophic and 2-thiobarbituric acid treated cultures (Unit: $\mu$M)

| Compound | Duration of culture(hrs.) | Treatment | | |
|---|---|---|---|---|
| | | Control | 2-thiobarbituric acid (1mM/L) | Glucose (50mM/L) |
| Amino acid (PCA-soluble) | 0 | 19.39 | 19.39 | 19.39 |
| | 15 | 26.26 | 8.32 | — |
| | 19.5 | — | — | 105.30 |
| | 22 | 71.94 | 11.57 | 54.40 |
| | 25 | 54.28 | — | — |
| | 27 | 52.14 | 14.95 | 126.75 |
| | 31 | 51.80 | 11.43 | 131.40 |
| | 45 | 61.23 | 31.39 | 327.62 |
| Protein (amino acid equivalent) | 0 | 10.50 | 10.50 | 10.50 |
| | 15 | 30.82 | 16.33 | — |
| | 19.5 | — | — | 36.49 |
| Alkali-labil | 22 | 38.22 | 13.65 | 38.25 |
| | 25 | 52.50 | — | — |
| | 27 | 33.40 | 16.39 | 41.48 |
| | 31 | 46.29 | 19.88 | 73.50 |
| | 45 | 57.90 | 27.30 | 11.24 |
| Alkali-stable | 0 | 26.83 | 26.83 | 26.83 |
| | 15 | 32.40 | 43.40 | — |
| | 19.5 | — | — | 71.25 |
| | 22 | 48.15 | 67.50 | 172.50 |
| | 25 | 148.50 | — | — |
| | 27 | 148.50 | 63.25 | 1,031.25 |
| | 31 | 1,196.25 | 207.40 | 1,296.50 |
| | 45 | 1,247.40 | 289.50 | 2,131.50 |
| Total | 0 | 37.33 | 37.33 | 37.33 |
| | 15 | 63.22 | 59.73 | — |
| | 19.5 | — | — | 107.74 |
| | 22 | 86.37 | 81.15 | 210.75 |
| | 25 | 201.00 | — | — |
| | 27 | 181.90 | 79.64 | 1,093.75 |
| | 31 | 1,242.54 | 227.28 | 1,370.00 |
| | 45 | 1,305.30 | 316.80 | 2,142.74 |
| Phosphate compounds | 0 | 16.52 | 16.52 | 16.52 |
| | 15 | 44.20 | 31.20 | — |
| | 19.5 | — | — | 86.80 |
| PCA-soluble | 22 | 146.55 | 59.80 | 212.80 |
| | 25 | 58.50 | — | — |
| | 27 | 47.20 | 45.50 | 195.00 |
| | 31 | 86.80 | 172.20 | 51.10 |
| | 45 | 72.10 | 189.90 | 49.85 |
| EtOH-ether sol. | 0 | 9.06 | 9.06 | 9.06 |
| | 15 | 17.76 | 7.20 | — |
| | 19.5 | — | — | 26.15 |
| | 22 | 24.00 | 6.00 | 24.80 |
| | 25 | 21.90 | — | — |
| | 27 | 30.30 | 5.60 | 51.00 |
| | 31 | 28.35 | 16.50 | 63.36 |
| | 45 | 40.30 | 6.88 | 131.25 |
| Carbohydrate (glucose equivalent) | 0 | 11.61 | 11.61 | 11.61 |
| | 15 | 7.88 | 15.76 | — |
| | 19.5 | — | — | 28.89 |
| PCA-soluble | 22 | 26.16 | 7.88 | 28.89 |
| | 25 | 22.32 | — | — |
| | 27 | 20.30 | 10.20 | 70.91 |
| | 31 | 21.92 | 6.47 | 82.61 |
| | 45 | 22.02 | 9.65 | 205.00 |

| | | | | |
|---|---|---|---|---|
| EtOH-ether sol. | 0 | 3.89 | 3.89 | 3.89 |
| | 15 | 7.98 | 15.45 | — |
| | 19.5 | — | — | 6.36 |
| | 22 | 7.58 | 4.04 | 7.78 |
| | 25 | 22.22 | — | — |
| | 27 | 12.27 | 1.01 | 11.11 |
| | 31 | 24.65 | 1.11 | 18.18 |
| | 45 | 15.76 | 2.37 | 17.98 |
| Alkali-insoluble | 0 | 15.25 | 15.25 | 15.25 |
| | 15 | 36.26 | 27.07 | — |
| | 19.5 | — | — | 55.56 |
| | 22 | 44.45 | 18.79 | 94.45 |
| | 25 | 43.69 | — | — |
| | 27 | 50.91 | 17.78 | 204.55 |
| | 31 | 68.18 | 31.82 | 252.02 |
| | 45 | 153.79 | 30.05 | 391.42 |
| Total | 0 | 30.76 | 30.76 | 30.76 |
| | 15 | 57.17 | 58.28 | — |
| | 19.5 | — | — | 90.86 |
| | 22 | 78.69 | 30.71 | 131.11 |
| | 25 | 88.23 | — | — |
| | 27 | 83.48 | 28.99 | 286.67 |
| | 31 | 114.75 | 39.39 | 316.46 |
| | 45 | 191.62 | 42.07 | 614.34 |
| RNA (uv-absorbancy) | 0 | 0.92 | 0.92 | 0.92 |
| | 15 | 3.03 | 1.19 | — |
| | 19.5 | — | — | 4.44 |
| | 22 | 3.90 | 1.32 | 4.46 |
| | 25 | 4.40 | — | — |
| | 27 | 4.46 | 1.44 | 7.06 |
| | 31 | 4.43 | 1.47 | 11.76 |
| | 45 | 4.70 | 1.15 | 39.54 |
| DNA UV-absorbancy | 0 | 0.19 | 0.19 | 0.19 |
| | 15 | 0.46 | 0.27 | — |
| | 19.5 | — | — | 0.91 |
| | 22 | 0.70 | 0.32 | 0.95 |
| | 25 | 0.74 | — | — |
| | 27 | 0.74 | 0.32 | 0.91 |
| | 31 | 1.01 | 0.29 | 1.08 |
| | 45 | 0.97 | 0.42 | 1.41 |
| Phosphate components | 0 | 11.74 | 11.74 | 11.74 |
| | 15 | 17.04 | 11.04 | — |
| | 19.5 | — | — | 38.90 |
| | 22 | 24.80 | 14.40 | 42.65 |
| | 25 | 30.63 | — | — |
| | 27 | 37.10 | 10.50 | 43.95 |
| | 31 | 34.50 | 16.45 | 53.15 |
| | 45 | 47.30 | 13.80 | 66.62 |

period and that of DNA is more active in the ripening period, while the synthesis of RNA is more rapid in the growing and ripening periods than other periods.

On the other hand, the amounts of PCA-soluble phosphate compounds, amino acid and PCA-soluble carbohydrate in the algal cell increased significantly in the ripening period and then decreased in the division period. It is considered that such a change in the amount of PCA-soluble compounds is related to the active synthesis of protein in the division period. The increase in the amount of RNA during the growing and ripening periods is in accordance with the observation that the synchronously growing *Chlorella pyrenoidosa* exhibits prevalent synthesis of transfer RNA followed by a steady increase in the amount of ribosomal RNA and thereafter the amount of total RNA decrease

slightly during the division period.

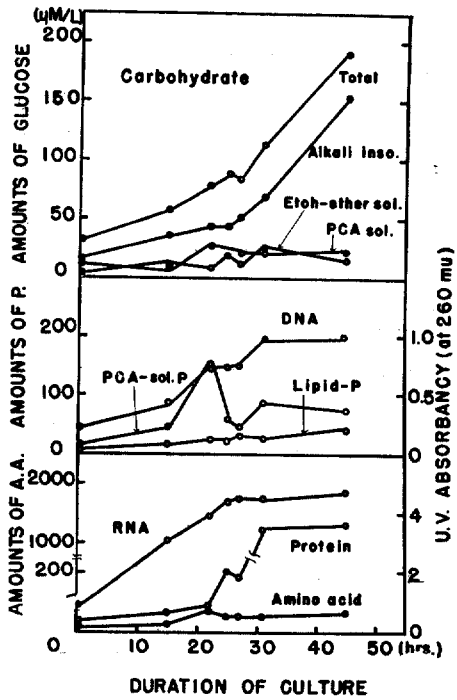


Fig. 2. Changes in the amounts of carbohydrate, protein, DNA, RNA and other phosphate compounds in *Chlorella* during the autotrophic synchronous culture.

## 2. Effects of glucose on the synchronous cellular division and the biosynthesis of the cell constituents of *Chlorella* during the life cycle.

The period of division cycle was little affected by 50 mM glucose in the medium, although the synchrony of growth and cell division was disturbed severely and the n value(daughter nuclear number arising from a single mother nucleus) was increased by mixotrophic culture. The statistical distribution of cell size during the synchronous culture in the glucose medium is represented diagramatically in Fig. 3. When the cells are grown in a basal medium a small cell becomes larger in size and ripens into a mother cell, from which four autospores are usually produced. In the glucose medium,

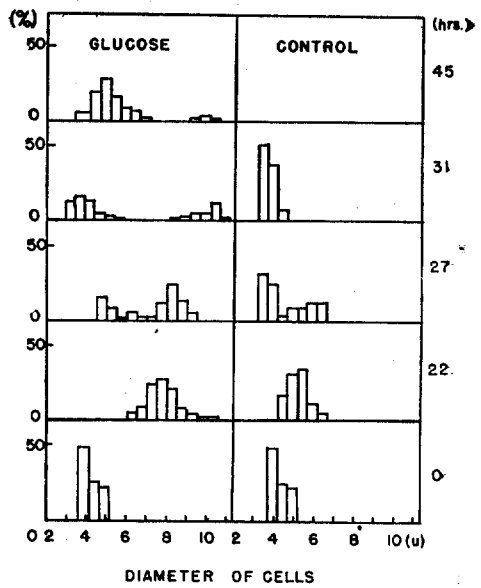


Fig. 3. Changes of the statistical distribution of cell size observed during the autotrophic and mixotrophic synchronous culture.

however, the cell enlarges more rapidly than in a bassal medium and the period of cell division is not shortened but almost equal in both cultures. Consequently, in the glucose medium of this experiment abnormally large "giant cells," which are more than 10 $\mu$ in diameter, appeared and in subsequent stage of cell development 8—16 autospores were released. The ripening period, in the mixotrophic culture of this experiment, ranged from 15 to 22 hours of the culture as in the case of the normal autotrophic culture, but the end of the division period was prolonged to 45 hours.

The effect of glucose on the biosynthesis of cell constituents, such as protein, carbohydrate, DNA, RNA, amino acid and PCA-soluble phosphate compounds of *Chlorella* during the life cycle is shown in Fig. 4. The contents of protein, carbohydrate, RNA, and DNA of the cell were increased by the glucose treatment throughout the life cycle, although the increase in the amount of RNA and

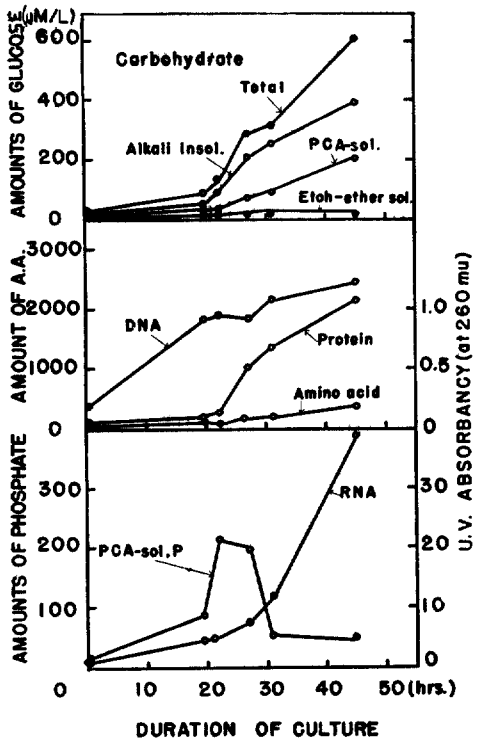


Fig. 4. Changes in the amounts of protein, DNA, RNA, carbohydrate and PCA-soluble phosphate compounds in *Chlorella* during the mixotrophic synchronous culture.

carbohydrate in the division period is more prominent than that of other compounds compared with autotrophic culture showing the disturbance in the synchrony of the cell division. On the other hand, the amount of PCA-soluble phosphate compounds in the cell increased prominently in the ripening period and then decreased in the division period as in the case of autotrophic culture.

### 3. Effect of 2-thiobarbituric acid on the synthesis of cell constituents and cell division of *Chlorella*.

Both cellular growth and division were retarded severely and the n value decreased by the treatment of 1 mM 2-thiobarbituric acid. In the 2-thiobarbituric acid-treated culture, the packed volume of the cells increased only 2-fold the inoculum within 45 hours, while it increased 5-fold within the same duration of autotrophic culture. In the 2-thiobarbituric acid-treated culture of this experiment, the ripening period, during which the nuclear division occurs, ranged 22–31 hours and the division period ranged 31–45 hours of the culture, showing the severe retardation of the algal growth and division.

As represented in Fig. 5 the synthesis of main cellular constituents such as protein, carbohydrate, DNA, RNA and phospholipid of the algal cell was severely retarded by 2-thiobarbituric acid compared with normal autotrophic culture. Especially, the increase in the content of carbohydrate, RNA and phospholipid of the cell was very little compared with the increase in the DNA and

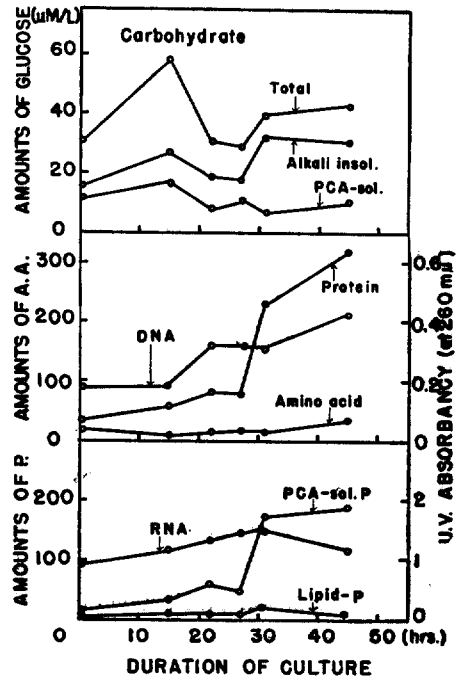


Fig. 5 Changes in the amounts of carbohydrate, DNA, RNA, protein and PCA-soluble phosphate compounds in *Chlorella* during the 2-thiobarbituric acid treated synchronous culture.

protein content. On the other hand, the content of PCA-soluble phosphate compounds of the cell increased prominently during the ripening period, and no increase in the division period was observed showing the characteristic difference from the normal process of cell development.

### 4. Changes in cellular constituents and their ratio of distribution in the cell with special reference to the cell division of *Chlorella*.

Changes in the ratio of distribution of cellular components in *Chlorella* cell during the life cycle in the normal autotrophic, mixotrophic and 2-thiobarbituric acid-treated cultures were listed in Table 2. The changes in the ratio of PCA-soluble component to the total compounds such as carbohydrate and proteinous compound and the concentration ratio of protein and PCA-soluble phosphate compounds to DNA are represented in Fig. 6, and the changes in the ratios of RNA, carbohydrate, lipid-P and PCA-soluble phosphate compounds to protein are shown in Fig. 7. In the normal autotrophic culture the ratios of PCA-soluble carbohydrate to total carbohydrate and of PCA-soluble amino acid to total protein and free amino acids in the cell decreased during the growing period from the beginning of the culture to the $L_1$-cell stage, but increased in the ripening period, during which the nuclear

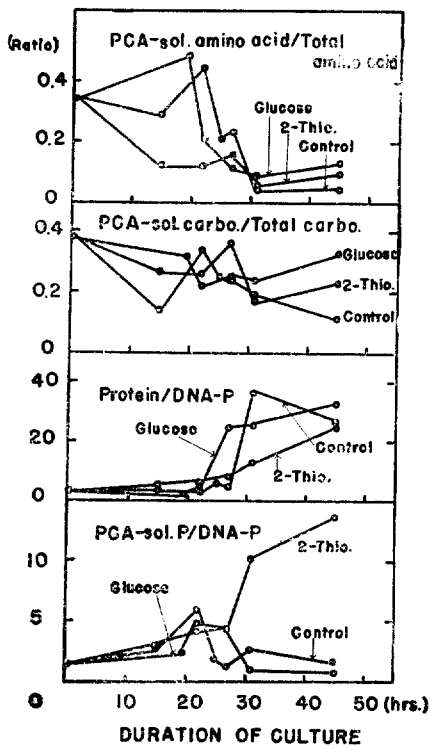


**Fig. 6.** Changes in the ratio of PCA-soluble component to total carbohydrate and total proteinous compounds and the concentration ratio of protein and PCA-soluble phosphate compound to DNA in *Chlorella* during the autotrophic, mixotrophic and 2-thibarbituric acid treated synchronous culture.

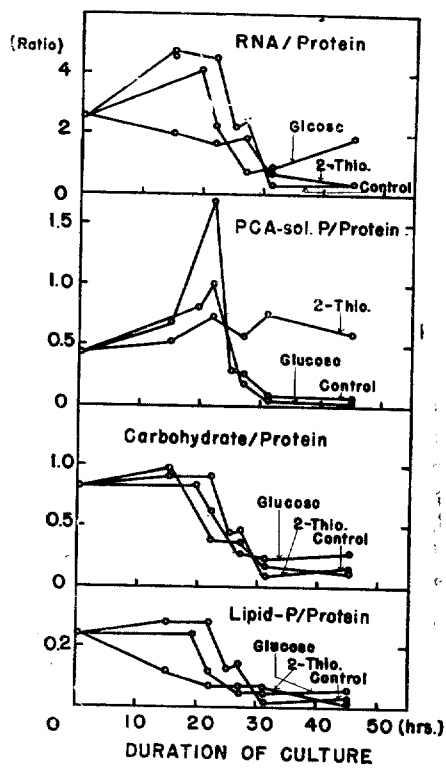


**Fig. 7.** Changes in the ratio of PCA-soluble phosphate, RNA, carbohydrate and lipid-P to protein in *Chlorella* during the autotrophic, mixotrophic and 2-thiobarbituric acid treated synchronous culture.

**Table 2.** Concentration ratio of various compounds in *Chlorella* cells during the synchronous autotrophic, mixotrophic and 2-thiobarbituric acid treated cultures.

| Ratio | Duration of culture(hrs.) | Treatment | | |
|---|---|---|---|---|
| | | Control | 2-thiobarbituric acid(1 mML) | Glucose (50 mM/L) |
| PCA-sol. P/DNA-P | 0 | 1.41 | 1.41 | 1.41 |
| | 15 | 2.59 | 2.83 | — |
| | 19.5 | — | — | 2.23 |
| | 22 | 5.91 | 4.15 | 4.98 |
| | 25 | 1.91 | — | — |
| | 27 | 1.27 | 4.33 | 4.37 |
| | 31 | 2.52 | 10.47 | 0.96 |
| | 45 | 1.52 | 13.76 | 0.75 |
| Carbohydrate/DNA-P | 0 | 2.62 | 2.62 | 2.62 |
| | 15 | 3.36 | 5.28 | — |
| | 19.5 | — | — | 2.34 |
| | 22 | 3.17 | 2.13 | 3.07 |
| | 25 | 2.88 | — | — |
| | 27 | 2.25 | 2.76 | 6.52 |
| | 31 | 3.33 | 2.40 | 5.95 |
| | 45 | 4.05 | 3.05 | 9.22 |
| Lipid-P/DNA-P | 0 | 0.77 | 0.77 | 0.77 |
| | 15 | 1.04 | 0.65 | — |
| | 19.5 | — | — | 0.67 |
| | 22 | 0.97 | 0.42 | 0.58 |
| | 25 | 0.71 | — | — |
| | 27 | 0.82 | 0.53 | 1.16 |
| | 31 | 0.82 | 1.00 | 1.19 |
| | 45 | 0.85 | 0.49 | 1.97 |
| Protein/DNA-P | 0 | 3.18 | 3.18 | 3.18 |
| | 15 | 3.71 | 5.41 | — |
| | 19.5 | — | — | 2.77 |
| | 22 | 3.48 | 5.64 | 4.94 |
| | 25 | 6.56 | — | — |
| | 27 | 4.90 | 7.59 | 24.43 |
| | 31 | 36.02 | 13.82 | 25.78 |
| | 45 | 27.60 | 22.96 | 32.16 |
| RNA/DNA | 0 | 4.88 | 4.88 | 4.88 |
| | 15 | 6.58 | 4.40 | — |
| | 19.5 | — | — | 4.87 |
| | 22 | 5.61 | 4.13 | 4.92 |
| | 25 | 5.99 | — | — |
| | 27 | 6.07 | 4.57 | 7.75 |
| | 31 | 4.38 | 4.99 | 11.09 |
| | 45 | 4.86 | 2.78 | 28.04 |
| Amino acid/DNA-P | 0 | 1.05 | 1.05 | 1.05 |
| | 15 | 1.55 | 0.75 | — |
| | 19.5 | — | — | 2.70 |
| | 22 | 2.90 | 0.80 | 1.28 |
| | 25 | 1.77 | — | — |
| | 27 | 1.41 | 1.41 | 2.88 |
| | 31 | 1.21 | 0.69 | 2.47 |
| | 45 | 1.29 | 2.27 | 4.92 |
| PCA-sol. P/Protein | 0 | 0.44 | 0.44 | 0.44 |
| | 15 | 0.70 | 0.52 | — |
| | 19.5 | — | — | 0.81 |
| | 22 | 1.70 | 0.74 | 1.01 |
| | 25 | 0.29 | — | — |
| | 27 | 0.26 | 0.57 | 0.18 |
| | 31 | 0.07 | 0.76 | 0.04 |
| | 45 | 0.06 | 0.60 | 0.02 |

| | | | | |
|---|---|---|---|---|
| Carbohydrate/Protein | 0 | 0.83 | 0.83 | 0.83 |
| | 15 | 0.91 | 0.98 | — |
| | 19.5 | — | — | 0.84 |
| | 22 | 0.91 | 0.38 | 0.62 |
| | 25 | 0.44 | — | — |
| | 27 | 0.46 | 0.37 | 0.26 |
| | 31 | 0.09 | 0.17 | 0.23 |
| | 45 | 0.15 | 0.13 | 0.29 |
| Lipid-P/Protein | 0 | 0.22 | 0.22 | 0.22 |
| | 15 | 0.28 | 0.12 | — |
| | 19.5 | — | — | 0.24 |
| | 22 | 0.28 | 0.07 | 0.12 |
| | 25 | 0.13 | — | — |
| | 27 | 0.14 | 0.07 | 0.05 |
| | 31 | 0.02 | 0.07 | 0.05 |
| | 45 | 0.03 | 0.02 | 0.06 |
| RNA/Protein | 0 | 2.46 | 2.46 | 2.46 |
| | 15 | 4.79 | 2.00 | — |
| | 19.5 | — | — | 4.12 |
| | 22 | 4.52 | 1.63 | 2.21 |
| | 25 | 2.20 | — | — |
| | 27 | 2.45 | 1.81 | 0.66 |
| | 31 | 0.36 | 0.65 | 0.86 |
| | 45 | 0.36 | 0.36 | 1.85 |
| PCA-sol. amino acid/Total amino acid equivalent | 0 | 0.34 | 0.34 | 0.34 |
| | 15 | 0.29 | 0.12 | — |
| | 0.49 | — | — | 0.49 |
| | 22 | 0.45 | 0.12 | 0.21 |
| | 25 | 0.21 | — | — |
| | 27 | 0.23 | 0.16 | 0.11 |
| | 31 | 0.04 | 0.05 | 0.09 |
| | 45 | 0.04 | 0.09 | 0.13 |
| PCA-sol. carbohydrate/ Total carbohydrate | 0 | 0.38 | 0.38 | 0.38 |
| | 15 | 0.14 | 0.27 | — |
| | 19.5 | — | — | 0.32 |
| | 22 | 0.34 | 0.26 | 0.22 |
| | 25 | 0.25 | — | — |
| | 27 | 0.24 | 0.36 | 0.25 |
| | 31 | 0.19 | 0.17 | 0.24 |
| | 45 | 0.11 | 0.23 | 0.33 |

division occurs, and then decreased again in the division period.

On the other hand, the ratio of RNA, lipid-P and carbohydrate to protein increased in the growing period from the beginning of the culture to $L_1$-cell stage, prior to nuclear division, but decreased more or less, or unchanged during the ripening period, and then decreased abruptly during the division period. The ratio of protein to DNA is almost unchanged up to the ripening period, and then increased prominently during the division period. In contrast to this, the ratio of PCA-soluble P to DNA increased from the beginning of the culture to the $L_4$-cell stage successively and then decreased abruptly.

In the autotrophic, mixotrophic and 2-thiobarbituric acid-treated synchronous culutre, each having different mode of nuclear and cytoplasmic division, a common general scheme occuring in the cell in relation to the cell division may be drawn as follows. The ratio of RNA to protein attains the maximum value prior to the nuclear division, and then decreases during the periods of nuclear and cytoplasmic division. The ratio of PCA-soluble phosphate to protein attains the maximum value prior to cytoplasmic division and then decreases gradually during the division period, and the ratio of protein to DNA increases abruptly during the division period. Therefore, it is presumed that the increase in the ratio

of RNA to protein is to be an inducer of nuclear division. The increase in the ratio of protein to DNA during the division period of this experiment is in well accordance with the observation of Gadgil *et al.*, (1967) that the process of cell division of *Pasteurella pestis* is delayed owing to an increase in the ratio of DNA to protein by temperature. Accordingly, it is presumed that the increase in the ratio of protein to DNA during the division stage is due to the decrease in the ratio of PCA soluble phosphate to protein after the $L_4$-cell stage and that the cytoplasmic division is induced by the increase of the ratio of protein to DNA.

## 摘 要

클로렐라의 生活史를 통한 細胞의 蛋白質, 炭水化物, 核酸, 燐脂質 및 酸可溶性 燐酸化合物 등의 生合成에 미치는 포도당 및 2-thiobarbituric acid 의 영향을 測定하고, 生活史에 따른 이들 主要細胞構成成分의 含量의 變化를 특히 核分裂 및 細胞質分裂과 關聯시켜 解析하였다.

基本培地에서의 正常的인 同調培養 過程에서 細胞의 蛋白質, DNA 및 RNA의 含量은 生活史의 모든 時期에서 다소 增加하기는 하였으나 細胞의 發育에 따라 特徵的 變化를 나타내었다. 蛋白質의 生合成은 分裂期에 가장 왕성하였고 DNA 의 生合成은 成熟期에 가장 왕성하였는데 RNA는 特히 生長期와 成熟期에 현저히 증가하였다.

포도당 배지에서는 細胞分裂의 同調性이 다소 교란되고 n 値도 增加하였으나 分裂週期에는 별 영향이 없었다. 포도당 배지에서 자란 細胞의 蛋白質, 炭水化物, RNA 및 DNA 含量은 正常培地에서 자란 細胞에 비하여 모든 發育時期에서 增加하였다. 그러나 2-thiobarbituric acid 處理區에서는 細胞의 生長과 分裂이 현저히 지연되었고 n 値도 減少하였을 뿐만 아니라 體物質의 生合成能도 正常培地에서 자라는 細胞에 비하여 현저히 감소되었다.

각기 核分裂과 細胞質分裂의 樣狀을 달리하는 이들 세 培養區에서 共通的으로 찾아 볼 수 있는 一般的인 事實은 蛋白質에 대한 RNA의 比率이 核分裂직전의 $L_1$-cell 에서 極大가 된 다음에 成熟期와 分裂期에는 감소된다는 것과 蛋白質에 대한 酸可溶性 燐酸化合物의 比率이 배양초부터 $L_4$-cell 에 이르기까지 계속 증가한다음에 分裂期에 감소된다는 점 그리고 DNA에 대한 蛋白質의 비율이 細胞質分裂直前부터 增加하기 시작한다는 것 등이다. 따라서 蛋白質에 대한 RNA의 比率의 增加가 核分裂을 誘發하는 것으로 생각되며 DNA에 대한 蛋白質의 比率의 增加는 細胞質分裂을 誘發하는 것으로 생각되었다.

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