The Effects of Phosphorus Starvation on Phosphate Storage, Three Storage Products and Cellular Organelles of *Chlorella pyrenoidosa*

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By
Charlotte B. Ransom
Waco, Texas

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Abstract

The ultrastructure of phosphorus starved, phosphorus restored and total nutrient cells of Chlorella pyrenoidosa was described by morphometric analysis. The rate of phosphorus incorporation into polyphosphate bodies of phosphorus starved cells was described as a volume fraction of the whole cell volume over a 210 min period.

Maximum phosphorus incorporation into polyphosphate bodies of phosphorus starved cells occurred in the first 30 min of exposure to phosphorus. After 180 min exposure, the volume fraction of the polyphosphate bodies of these cells decreased significantly.

Several different trends were observed in the volume fractions of other cellular components. The volume fraction of starch in the chloroplast was significantly larger in the total nutrient cells than in cells of any other treatment. Lipids and pyrenoid volume fractions were significantly higher in phosphorus starved and phosphorus restored cells than in total nutrient cells. Phosphorus starved cells had the smallest volume fraction of vacuoles. Upon exposure to phosphorus, the vacuolar volume fraction increased until it equalled that of total nutrient cells. No significant differences in the volume fractions of the chloroplast, nucleus or mitochondrion were observed for any treatment.

Table of Contents

List of Tables

List of Illustrations

Acknowledgments

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Literature Review

The Significance of Phosphorus for Algal Growth

Algae are part of a complex food web in aquatic systems; therefore, it is essential to understand factors that regulate their growth. Most often the immediate factors are nutrient concentrations. Since the investigations of Nauman in 1919 (from Herold, 1966), studies have consistently pointed to phosphorus and nitrogen as the limiting nutrients of algal growth (Sawyer, 1966; Weiss, 1969; Edmondson, 1970; Lange, 1970; Ryther and Dunstan, 1971; Healey, 1973; Wetzel, 1983). Further evidence indicates that phosphorus is most often the limiting nutrient for algal growth in aquatic systems (Wetzel, 1983); therefore, a knowledge of phosphorus dynamics is essential for understanding the overall productivity of biomass in a body of water.

Because phosphorus is so important, it is necessary to understand how it enters an aquatic system and is cycled there. The water entering as runoff from a drainage basin is the major external source of phosphorus. The amount of phosphorus entering a body of water by this manner is highly variable and is determined by the soil types in the drainage basin, the land use in the basin, and the total area of the basin (Keup, 1969; Vollenweider, 1969; Weibel, 1969; Loehr, Martin, and Rast, 1980). The runoff is very irregular depending on

local rainfall or snowmelts (Lind, 1979).

The Uptake of Phosphorus

Once the phosphorus has been deposited in a lake, approximately 5% is quickly taken up by various organisms, primarily algae and bacteria (Paerl and Lean, 1976; Lean and Nalewajko, 1976). The other 95% is lost to the sediments in the form of ferric phosphate and as absorption onto the surface of particles of ferric hydroxide and calcium carbonate (Andersen, 1975). Therefore, it is important for the algae to be able to store phosphorus when it is available for later use when it is not. If a lake undergoes stratification, the water at the bottom (hypolimnion) can become anerobic due to oxygen consumption through bacterial degradation of detritus (Burns and Ross, 1971). As the oxygen concentration decreases, the redox potential of the hypolimnion decreases also. At a redox potential of about +200 mv, phosphates and ferrous iron are released into the water to be recycled again (Mortimer, 1971). At turnover in the fall, the phosphorus moves up through the water column and the algae have a brief chance to sequester it again.

All algae so far studied can absorb more phosphorus from a surrounding medium than their normal metabolism can use at a given time (Ketchum, 1939; Scott, 1945; Mackereth, 1953; Kuenzler and Ketchum, 1962; and others). This phenomenon is called luxury uptake or over-plus phenomenon (Kuhl, 1974). Short chain inorganic ortho-

phosphate is the form of phosphorus absorbed in this manner by all algae (Krass, 1958; Provasoli, 1958; Overbeck, 1962a, b). Chlorella can absorb not only these short chain orthophosphates but also polyphosphates up to a chain length of 55 phosphate units (Sommer and Booth, 1938; Galloway and Krauss, 1963). Only the Cyanophyta and some bacteria appear able to utilize organic forms of phosphate such as the phosphates found in some detergents (Stewart and Alexander, 1971).

How is phosphorus taken up by an algal cell? The exact mechanism is unknown but several factors that influence this uptake have been identified. Phosphorus is taken up by active transport, and therefore is ATP dependent (Herold, 1966; Kuhl, 1974). Using Chlorella, several studies have shown that the rate of phosphorus uptake is twice as great in light as in darkness (Kuhl, 1962; Azad and Brochardt, 1970). Low CO_2 concentrations greatly increase the rate of uptake, and oxygen is not required (Wintermans, 1955). The ATP necessary for uptake comes from cyclic photophosphorylation in light (Ullrich and Simonis, 1969; Van Rensen, 1969) and from oxidative phosphorylation in darkness (Kulh, 1968). Many experiments have shown that phosphorus is always taken up by starved cells when energy generated by photophosphorylation cannot be used by other energy requiring reactions (Herold, 1966; Kuhl, 1974). Two conditions that could cause ATP requiring processes to be less than saturated are low CO_2 concentration or a block of meta-

bolic pathways.

Another factor influencing the rate of uptake of phosphorus is the concentration of certain monovalent cations. The cations involved are: K^+, Na^+, NH_4^+, Li^+, and Rb^+. Their presence increases the rate of uptake of phosphorus (Peverty, Adamec and Parthasarathy, 1978). Potassium is essential for any uptake in algae (Kuhl, 1962, 1974). One possible explanation is that potassium is a component of a proposed Na/K pump on the cell membrane of some Chlorophyta (Raven, 1967, 1968). The universality of this pump, however, has not been established (Kuhl, 1974). The concentration of two divalent cations, Mg^{++} and Ca^{++}, has been shown to affect the rate of phosphorus uptake in bacteria (Widra, 1959) and the Cyanophyta (Sicko-Goad and Stoermer, 1979) but not for other algae (Peverty, et al., 1978).

The Storage of Phosphorus

When luxury consumption occurs, the phosphorus is stored by many taxa in the form of polyphosphate bodies. The presence of these polyphosphate bodies has been demonstrated in yeast as long ago as 1888 by Liebermann (from Herold, 1966). Liebermann called these bodies "volutin granules." In pioneering studies by Waime in 1946 and Schmidt, Hecht, and Tannhauser also in 1946, these bodies in yeast were found to correspond with fractions of polyphosphate extracted from the cells. A variety of microorganisms including fungi, bacteria, Cyanophyta and Chlorophyta have polyphosphate bodies (Kech

and Stich, 1957; Herold, 1966).

Polyphosphate bodies can be seen by both light (with Albert's stain) and electron microscopy. The chemical nature of these bodies consists of three forms of inorganic phosphate: cyclic condensed phosphates (Thilo, 1962), linear condensed phosphate (Thilo, 1962), and cross-linked condensed phosphates (Herold, 1966). Of these three groups, only the linear condensed phosphates have been shown conclusively to be a common component of polyphosphate bodies in algae (Herold, 1966).

X-ray analysis has demonstrated that the chemical nature of the polyphosphate body is indeed a polymer of orthophosphate with phosphoanhydride linkages (Sicko-Goad and Stoermer, 1979; Tillberg, Rowley and Barnard, 1979, 1980). These bonds are thought to be thermodynamically equal to the "energy-rich" phosphate of ATP (Yoshida, 1955).

For what do algae use this stored phosphate? There is no direct evidence that the energy stored in polyphosphate bodies can be used as an energy source if no other such source is present (Kuhl, 1974). Evidence indicates that the stored phosphorus can be utilized as a phosphorus source when external phosphorus concentrations are low (Kuhl, 1968; Markarova and Baslarskaya, 1969).

The polyphosphate bodies are located in the vacuoles of the Chlorophyta (Sicko-Goad and Stoermer, 1979; Tillberg, et al., 1980) and are associated with the tonoplast (Peverty, et al., 1978). In

the Cyanophyta, the polyphosphate bodies do not appear to be associated with any particular structure (Jensen, Sicko-Goad and Ayala, 1977). The polyphosphate bodies are spherical bodies of electron opaque linear polyphosphates (Tillberg, et al., 1980). The spherical shape of the polyphosphate body is thought to be due to the charge on each molecule and the association with the tonoplast to be due to an anion/cation attraction between the polyphosphate anion and the cation charge on the tonoplast (Kuhl, 1974).

The Effects of Phosphorus Starvation

How does phosphorus starvation affect polyphosphate bodies? Phosphorus starvation results in low concentration of polyphosphate in the algal cell; therefore, very small polyphosphate bodies, or none at all if starvation is extreme, are found (Herold,1966; Kuhl, 1974; and Sicko-Goad and Stoermer, 1979).

How are the three other storage products affected by phosphorus starvation? The three storage products are: starch in the chloroplast, lipid droplets in the chloroplast, and the pyrenoid. Starch is stored by Chlorella as starch grains in the chloroplast and as a starch sheath around the pyrenoid. Starch in the chloroplast is composed of 7% amylose and 93% amylopectin (Olaitan and Northcote, 1962). Lipid droplets are found mainly within the chloroplast in Chlorella. Lipids in Chlorella pyrenoidosa are mostly composed of 18 carbon units with one, two or no double bonds (Klenk, Knipprath,

Eberhagen, and Kook, 1963). Kuhl (1974) interpreted evidence to indicate that reduced respiration, found in phosphorus starved cells (Bergmann, 1955; Daniel, 1956) causes a buildup of both lipids and starch. Therefore, I measured these two storage products to detect any volume change under conditions of phosphorus starvation.

The last storage product considered was the pyrenoid. Pyrenoid-containing species are found in every class of algae except Cyanophyta. In Chlorella the pyrenoid is not bound by a membrane of its own and is a differentiated region of the chloroplast covered by a starch sheath (Bisalputra, 1974). The pyrenoid occurs with a build up of reserve products and probably is involved in the storage of proteins (Griffith, 1970). Since the pyrenoid stores products of cellular metabolism, I was interested to see if any detectable change in volume of the pyrenoid occurred under conditions of phosphorus starvation.

Three cellular organelles were also examined for their response to phosphorus starvation. The three organelles were the chloroplast, the nucleus and the mitochondrion.

The chloroplast of Chlorella is composed of 2, 6, or many fused thylakoids with grana of variable size (Gibbs, 1962). In Chlorella pyrenoidosa the single chloroplast is cup-shaped and takes up a large volume of the cell (Bold and Wynne, 1978). Phosphorus starvation results in decreased photosynthetic rates that can be restored to normal levels with the addition of phosphorus. However, in severely

starved cells the photosynthetic rate is permanently depressed (Kuhl, 1974). It was important, therefore, to examine the general appearance and the volume of the chloroplast under conditions of phosphorus starvation and restoration.

The nucleus in Chlorella is a double membrane bound organelle and has the same function as in all eukaryotes (Feldhen, 1972). Therefore, total volume of the nucleus was measured to determine any changes under conditions of phosphorus starvation and restoration. No data on this topic have been reported.

The mitochondrion of Chlorella is thought to be several lobes of a single structure in the cytoplasm (Atkinson, John, and Gunning, 1974). The mitochondrion is the site of oxidative phosphorylation, Krebs cycle and other functions normally associated with mitochondria of eukaryotic organisms. Phosphorus starvation causes lower rates of cellular respiration (Bergmann, 1955; Daniel, 1956); therefore, it is important to determine if the volume fraction of the mitochondrion changes during conditions of phosphorus starvation and restoration.

Introduction

The purpose of this research was to determine ultrastructural changes that occur in Chlorella pyrenoidosa with phosphorus starvation and restoration. The percent of the total cellular volume occupied by four storage products and three cellular organelles was examined. The cellular organelles considered were the chloroplast, the nucleus, and the mitochondrion. The storage products were polyphosphate bodies, starch and lipid droplets in the chloroplast, and the pyrenoid.

Phosphorus is often the limiting nutrient for algal growth in aquatic systems (Wetzel, 1983). Since algae are part of a complex food web in these systems, factors that regulate their growth are important to understand, and possibly manipulate to the advantage of the entire ecosystem.

All algae so far studied can absorb more phosphorus from a surrounding medium than their normal metabolism can use at that time (Ketchum, 1939; Scott, 1945; Mackereth, 1953; Kuenzler and Ketchum, 1962; and others). This phenomenon is called luxury uptake or over-plus phenomenon (Kuhl, 1974).

When luxury uptake occurs, many organisms store phosphorus in the form of polyphosphate bodies. The presence of these polyphosphate

bodies have been demonstrated in yeast as long ago as 1888 by Liebermann (from Herold, 1966). Liebermann called these bodies "volutin granules." In pioneering studies of yeast by Waime in 1946 and Schmidt, Hecht and Tannhauser also in 1946, these bodies were found to correspond with fractions of polyphosphate extracted from the cells (Herold, 1966). X-ray analysis has demonstrated that the chemical nature of the polyphosphate body is indeed a polymer of orthophosphate with phosphoanhydride linkages (Sicko-Goad and Stoermer, 1979; Tillberg, et al., 1979, 1980). Evidence indicates that this stored phosphorus can be utilized as a phosphorus source when the phosphorus in the medium is low or absent (Kuhl, 1968; Markarova and Baslarskaya, 1969).

Polyphosphate bodies are spherical and consist of electron opaque linear polyphosphate (Tillberg, et al., 1980). They are located in the vacuoles of the Chlorophyta (Sicko-Goad and Stoermer, 1979; Tillberg et al., 1980) and are associated with the tonoplast (Peverty, et al., 1978).

The storage of starch in the chloroplast in response to varying phosphorus conditions was also examined. Because starch is a product of photosynthesis that can be utilized as an energy source by the algae, it is interesting to see if the volume fraction of starch in the chloroplast changes under conditions of phosphorus starvation and restoration. Lipid droplets in the chloroplast were examined for the same reason.

Many aspects of cellular metabolism including the rate of photosynthesis (Kuhl, 1968) and the rate of respiration (Daniel, 1956) are affected by phosphorus starvation, and thus it is important to look at possible volume fraction changes in the chloroplast, mitochondrion, and nucleus.

Materials and Methods

Algae Growth Conditions

A unialgal culture of Chlorella pyrenoidosa was obtained from the Culture Collection of Algae, University of Indiana, Bloomington, Indiana, at the Department of Botany, University of Texas, Austin, Texas. The stock cultures were grown in a complete nutrient solution in 250 ml Erlenmeyer flasks under a light/dark cycle of 18 hr light and 8 hr dark at 26°C. The quantity of illumination was 400 fc from fluorescent bulbs. The complete nutrient solution had the following composition:

NH_4NO_3	.15 gm/l
KCl	.25 gm/l
$MgSO_4 \cdot 7H_2O$	.25 gm/l
$CaCl_2 \cdot 2H_2O$	.15 gm/l
EDTA	.05 gm/l
$ZnSO_4 \cdot 7H_2O$	.02 gm/l
H_3BO_3	.01 gm/l
$CaCl_2$ (anhydrous)	.005 gm/l
$MnCl_2 \cdot 4H_2O$	.005 gm/l
$FeSO_4 \cdot 7H_2O$	.005 gm/l
$CaCl_2 \cdot 6H_2O$	.015 gm/l
$CaSO_4 \cdot 5H_2O$	.015 gm/l

$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$	.001 gm/l
KH_2PO_4	.04 gm/l
K_2HPO_4	.07 gm/l
Enough KOH to raise pH to 6.9	

The experimental cell cultures were taken from stock cultures that had passed through log phase growth and stabilized. The growth phase was determined by flourometry readings conducted each day. Twenty mls of stock solution were centrifuged at 14,000 g for 15 min at 10°C. The supernatant was poured off. The cells were suspended in glass distilled water and centrifuged again in the same manner. The cells were aseptically transferred to flasks containing nutrient solutions lacking only KH_2PO_4 and K_2HPO_4. These experimental cultures were kept in the same conditions as the stock cultures. The experimental cultures were harvested at 10 days and transferred to a second phosphorus-free nutrient solution by the same centrifugation techniques used previously. Three days later phosphorus in the form of KH_2PO_4 (.04 gm/l) and K_2HPO_4 (.07 gm/l) was added to the deficient cultures.

Experimental procedures

Immediately before phosphorus was added to the starved cultures, five 20 ml samples were taken from each of three phosphorus deficient cultures and from one total nutrient culture. The samples were centrifuged at 14,000 g at 10°C for 15 min. Two percent glutaraldehyde in

0.035 M sodium cacodylate buffer (pH=6.9) was added to each sample. For each change of solution, the cells were centrifuged, supernatant removed and new solution added to resuspend the cells. After the glutaraldehyde, the cells were placed in 0.035 M sodium cacodylate buffer (pH=6.9) for 30 min. Upon removal of this buffer, the cells were placed in 2% buffered osmium tetroxide for 2 hrs. The cells were then transferred to a solution of 0.035 M sodium cacodylate buffer for 30 min. The cells were dehydrated in a graded ethanol series and embedded in Spurr's epoxy (Spurr, 1969).

After the phosphorus was added, five 20 ml samples were taken every 30 min for 210 min from each of three treatment cultures. These samples were prepared for electron microscopy in the same manner as the first samples.

All experiments were begun at 4:00 p.m. This time was chosen because it was in the center of the 18 hr light cycle under which all cells were grown and maintained. Total cell volume was presumed to remain unchanged over the 210 min duration of the experiment (Fagerberg, 1983).

Thin (60 nm) sections for electron microscopy were prepared but no post-staining was done due to possible loss of polyphosphate bodies by hydrolysis (Jensen, et al., 1977).

Morphometric Analysis

For each sample time and total nutrient culture, 10 cells were

photographed (Wiebel, 1973). At least three cells from each of the three original treatment cultures and 10 cells from the total nutrient culture were selected by viewing a section of cells between 2 grid bars and starting 2/3 of the way down on one grid bar. A transect across the area between these bars was then followed. Every cross section of a cell in this transect was photographed. Counts of cellular components were made and their mean volume fractions calculated according to methods described by Steer (1981). The significance of differences between means was assessed by a one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) test at a significance level of P 0.05 (Sokal and Rohlf, 1969).

Results

There were no significant volume fraction differences among total nutrient, phosphorus starved, and phosphorus restored cells for the chloroplast, nucleus, or mitochondrion (Table I). The differences among the total nutrient, the phosphorus starved, and the phosphorus restored cells occurred in the amount of storage products and volume fractions of vacuoles (Table I). Polyphosphate bodies were found in the vacuoles in association with the tonoplast (Fig. 1). In the phosphorus starved and early phosphorus restored cells the volume fraction of vacuoles was significantly less than in later phosphorus restored and total nutrient cells (Table I). The vacuoles of the total nutrient cells were partially filled with probable storage product thought to be a simple carbohydrate (Elenor Cox, personal communication) (Fig. 2).

The polyphosphate bodies were both smaller and occurred less often in the vacuoles of the total nutrient and the phosphorus starved cells than in the phosphorus restored cells. The total nutrient cells had the smallest volume fraction of polyphosphate bodies (Table I). The phosphorus starved cells had a significantly larger volume fraction of polyphosphate bodies than did the total nutrient cells. In the phosphorus starved cells, the volume fraction

bodies (Table I). The phosphorus starved cells had a significantly larger volume fraction of polyphosphate bodies than did the total nutrient cells. In phosphorus starved cells, the volume fraction of polyphosphate bodies rose significantly in the first 30 min of exposure to phosphorus and held steady for 150 min. After 180 min, the volume fraction of polyphosphate bodies decreased significantly and continued a similar decrease at 210 min (Table I).

Three other storage products also exhibited significant differences among treatments. The volume fraction of starch in the chloroplast was much higher in the total nutrient cells than in any other treatment cells (Table I). The volume fraction of lipid was significantly greater in the phosphorus starved and the phosphorus restored cells than in the total nutrient cells (Table I). The relative volume of the pyrenoid was also significantly greater in the phosphorus-starved cells and the phosphorus restored than in the total nutrient cells (Table I). The volume fractions of pyrenoid, lipid and starch did not show similar trends to that of the polyphosphate bodies over the 210 min of this study.

Table I. Mean (±S.D.) volume fractions of cellular organelles and storage products of *Chlorella pyrenoidosa* (n=10).

	TN[a]	0 min.[b]	30 min.[c]	60 min.	90 min.	120 min.	150 min.	180 min.	210 min.
Mitochondria	2.30± 1.14	1.90± .47	2.23± .90	1.92± .56	2.09± .58	2.27± .80	1.82± .59	2.17± .36	2.12± .53
Nucleus	13.96± 3.19	13.33± 4.77	12.14± 2.83	12.43± 3.42	12.14± 2.70	14.04± 3.11	14.12± 3.52	13.69± .86	14.00± 2.28
Chloroplast	59.51±11.38	57.49± 9.58	56.50±10.50	58.81± 7.49	58.66±10.11	59.73± 6.80	57.51±12.59	58.79± 5.56	57.15± 7.54
Vacuole	9.99± 2.14	1.16± .86[d]	3.50± 1.18[d]	9.16± 3.80	9.90± 2.89	9.93± 2.51	10.65± 2.61	9.81± 2.87	10.12± 2.15
Starch	8.77± 2.40[d]	1.71± .58	2.25± .95	1.87± .77	2.34± .80	2.11± .92	1.78± .59	2.31± .68	2.04± .48
Lipid	.66± .15[d]	1.58± .10	1.64± .53	1.62± .78	1.52± .36	1.30± .33	1.43± .40	1.29± .86	1.49± .53
Pyrenoid	1.00± .16[d]	1.90± .48	2.25± .57	2.35± .71	2.27± .85	2.01± .61	2.42± .82	2.03± 1.08	1.96± .28
Polyphosphate	.87± .23[d]	1.05± .10[d]	2.92± .81	2.98± .71	3.33± 1.60	3.24± 1.22	2.92± .98	2.45± 1.07[d]	2.24± 1.08[d]

[a] Total Nutrient Cells
[b] Phosphorus Starved Cells
[c] Phosphorus Restored Cells
[d] $P<.05$

Figure 1. Phosphorus restored cell of Chlorella pyrenoidosa. Structures in this micrograph: nucleus (N), polyphosphate body (P), vacuole (V), chloroplast (C). (X51,000).

0.5 µm

Fig. 1

Figure 2. Total nutrient cell. Structures in this micrograph: chloroplast (C), nucleus (N), carbohydrate (CH), and vacuole (V). (X51,000).

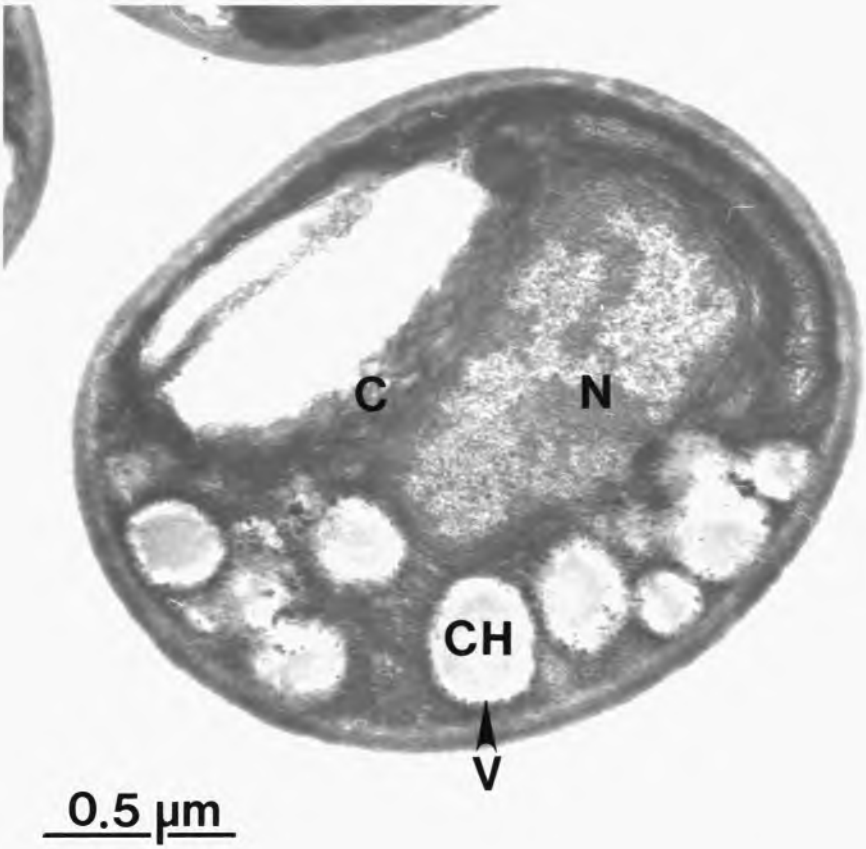

Fig. 2

Discussion

Three cellular organelles (chloroplast, nucleus, and mitochondrion) had no significant difference in their relative volumes between the total nutrient and any other treatment group. Similar results with Diatoma tenue var. elongatum were obtained for total nutrient, phosphorus starved and phosphorus restored cells (Sicko-Goad and Stoermer, 1979). No researchers have reported any difference in appearance of any of these three organelles under phosphorus deficient conditions. Although lower rates of photosynthesis, cellular respiration and the general metabolism of the cell have been observed (Herold, 1966; Kuhl, 1974), apparently no ultrastructural changes occur. Therefore, phosphorus starvation and restoration apparently do not effect these organelles. However, the volume fraction of vacuoles did differ among treatment groups. Apparently both the concentration of phosphorus as well as the length of time of exposure to phosphorus does cause ultrastructural differences in volume fractions of vacuoles.

The difference in volume fraction of polyphosphate bodies in total nutrient and phosphate starved cells may have been due to cell death and recycling of the released organic phosphates by bacteria present in the phosphorus starved cultures. In phosphorus starved

cells, the change in volume fractions of polyphosphate bodies indicated that the phosphorus was taken up and stored as polyphosphate bodies in the first 30 min following exposure to phosphorus. This very rapid uptake agrees with results of inorganic phosphorus uptake by phosphorus deficient cells of Ankistrodesmus braunii (Ullrich, 1972). In fact, Ankistrodesmus braunii took up all the phosphorus it could within 10 min (Ullrich, 1972). The vacuolar volume fraction also increased as the volume fraction of polyphosphate bodies increased in phosphorus starved cells.

The high vacuolar volume fraction in the total nutrient cells suggests that more vacuolar volume is needed when products of cellular metabolism need to be stored. The vacuoles of the total nutrient cells are filled with a substance tentatively identified as carbohydrate (Elenor R. Cox, personal communication). The data suggest that as vacuoles are needed for phosphate or any type of storage they are formed or expanded.

Quantitative measurements of phosphorus deficient cells exposed to phosphorus for more than 3 hrs may not be an accurate description of the initial amount of phosphorus taken up by the cells. Phosphorus mobilization from the polyphosphate bodies to the general contents of the cell may be underway by that time. Most researchers have exposed phosphorus starved cells to phosphorus for 4 hrs or longer and then prepared the cells for whatever photography or chemical analysis that was to be done (Sicko-Goad and Stoermer,

1979; Tillberg, et al. 1979; many others). The rates of photosynthesis, cellular respiration and other energy-requiring processes are reduced by phosphorus starvation but recover within several hours after phosphorus addition (Herold, 1966; Kuhl, 1974). Therefore, an underestimation of initial phosphorus uptake and storage would occur because it is more energy efficient for the cell to utilize the phosphorus already within the cell than to bring in more by active transport once cellular metabolism has recovered to some extent. The fact that cells from total nutrient cultures have little polyphosphate supports this explanation. Also, evidence that phosphorus can only be taken up by starved cells when energy generated by photophosphorylation and cellular respiration cannot be used by other energy requiring reactions is further support for this argument (Herold, 1966; Kuhl, 1974).

The volume fraction of starch was significantly higher in total nutrient cells than in phosphorus starved and phosphorus restored ones. Kuhl (1962), however, found an increase in stored starch in phosphorus deficient cells. An explanation for this difference may be the period of phosphorus starvation which was shorter for the cells used in Kuhl's study (1962). The Chlorella cells in my phosphorus deficient cultures may have been in poor physiological condition as evidenced by decreasing cell numbers. This severe phosphorus starvation may account for differences in results.

The volume fractions of lipid and pyrenoid followed the same

pattern. Both were significantly higher in phosphorus starved and phosphorus restored than in total nutrient cells. I believe that this reflects the lower rate of cellular respiration (Kuhl, 1974) and therefore a build up of lipids and the pyrenoid. The higher levels of lipids in phosphorus starved cells have been found in other algae and in Chlorella by biochemical techniques (Bergmann, 1955; Daniel, 1956). No studies on changes in the pyrenoid due to phosphorus starvation have been reported. The dark matrix of the pyrenoid is believed to be mainly proteinaceous material (Griffith, 1970). The storage of proteins may be caused by decreased metabolic activities that require proteins, such as reproduction and cell growth. Cell growth and reproduction have been shown to decrease or stop entirely under conditions of phosphorus starvation (Kuhl, 1974).

The volume fractions of cell organelles and storage products of the total nutrient cells comprize approximately 85% of the total cellular volume. This is a very high proportion. Reports of volume fractions of cellular organelles of Sargassum suggest approximately 75% of the total volume of epidermis cells is cellular organelles (Fagerberg & Dawes, 1977). The total nutrient cell represented in Fig. 2 is a good example of the general cellular appearance. These cells are very compact and have small volume fractions of hyaloplasm.

I believe that phosphorus starvation greatly slows the overall metabolism of Chlorella cells. Upon exposure to orthophosphates,

uptake is very rapid. The cells take approximately 180 min to begin to utilize the stored phosphate for cellular metabolism.

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