THE EFFECT OF ALGAL INHIBITORS ON HIGHER PLANT TISSUES

by

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Abstract

Commonly-occurring algae in Colorado reservoirs and ponds were studied with regard to inhibitor production and their effects on higher plants. Seven algal genera that are abundant in Colorado irrigation waters were found to have significant effects on higher plants. The genera were Aphanizomenon, Microcystis, Nitzschia, Hydrodictyon, Scenedesmus, Lyngbya and Anabaena. A rapid, sensitive bioassay system, using cultured radish cotyledons was developed to test the inhibitor effects. Growth was decreased significantly when compared to controls and in some cases resulted in necrotic areas on the leaf or death of the entire cotyledon. Cytological investigations with the electron microscope also revealed subcellular changes including retardation of protein body degradation, lack of starch formation, disruption of proper photosynthetic lamellar (thylakoid) formation in chloroplasts, decrease in cell size, and retardation of vascular tissue development. Depending on the specific alga, the inhibitors have multiple effects on higher plant tissues and in a natural system could stress the plant, thus increasing their susceptibility to pathogens.

Introduction

Representatives from several algal groups produce some kind of toxin or inhibitor that affects animals (Gorham, 1964; Sawyer, 1968; Shilo, 1967), and consequently have received the major share of research. Conversely, studies involving algal inhibitors and their effects on non-animal systems are in their infancy. With the exception of one report (Harris, 1974) all investigations have been concerned with algal or bacterial growth inhibition. It was found that certain representatives of the Order Volvocales, in the green algae, produce two kinds of inhibitors--autoinhibitors and heteroinhibitors. Autoinhibitors decrease the growth of the inhibitor-producing alga, whereas heteroinhibitors affect the growth of other algae (Harris, 1970, 1971a, 1971b, 1971c, 1972, 1974; Harris and Caldwell, 1974; Harris and Parekh, 1974). Apparently both types of inhibitor affect the light reaction of photosynthesis in green algae (Harris, 1971c, 1974a) and in addition may be antibiotic to bacteria (Chrost, 1975a, 1975b; Harris, 1970, 1971a). These limited but important observations may provide an alternate hypothesis to our ideas concerning phytoplankton blooms and their sudden disappearance (Harris and Parekh, 1974; Rice, 1974). For instance, the inhibitor-producing algal species may decrease the population of another, allowing the inhibitor producer to become the dominant in a particular lake or reservoir. Our past ideas have centered around nutrient availability and interspecies competition for these nutrients. An extension of this hypothesis involves the use of inhibitors to control nuisance algae and is being hailed as a possible biological control mechanism in these instances (Harris and James, 1974).

The adoption of biological control mechanisms should also consider inhibitory effects on other organisms including higher plants. Since higher plants, both aquatic and terrestrial, have similar metabolic pathways to algae, it should be expected that they would also respond to the same inhibitors. If irrigation water is obtained from ponds, lakes or other sources that support algal blooms, enough inhibitors could be present to affect growth of seedlings or mature plants, possibly affecting crop yield. Additional inhibitor production could also occur in stationary water in irrigation rows to further aggravate the plant.

Since most of the green algae that apparently produce inhibitors are common "bloom" organisms in Colorado and because large areas of crop land are under irrigation, the possible effects of algal inhibitors are pioneering studies. The results could be of significance to farmers, plant pathologists, fisheries biologists and aquatic ecologists. For example, crop plants may be stressed by algal inhibitors and could be rendered susceptible to a variety of pathogens, adding a new parameter to epidemiological studies that has previously been unrecognized. A more indirect application could be in lakes that support an excellent fish habitat consisting of vascular plants. The inhibitors may affect or decimate these aquatic plants and destroy the desirable habitat. A specific instance where this may be occurring is Pyramid Lake in Nevada (Galat, pers. comm.) and studies on the lake's bloom organism, Nodularia, are proceeding on this hypothesis.

It is the intent of this study to screen algae from several groups and establish an initial list of inhibitor producing algae. In addition,

the effects of inhibitors on the ultrastructure of higher plants are described in a controlled bioassay system. Specific observations on chloroplasts were made since algal inhibitors apparently affect this plant organelle; specifically the pigment system of the light reaction.

MATERIALS AND METHODS

COLLECTION SITES

Collections of test organisms were made from Fossil Creek Reservoir, the St. Vrain River, South Platte River, Red Feather Lakes, various farm ponds, and irrigation ditches in the State of Colorado. Each locality, with the exception of Red Feather Lakes, was chosen because the water is used for irrigation purposes and contained "blooms" of algae.

ISOLATION OF ALGAE AND CULTURING

Algae were isolated into unialgal and axenic cultures by the micropipette technique, streaking and spotting agar plates. The media for growth were Bold's Basal Medium, Allen's Medium or Chu's No. 10 medium (Starr, 1974). Algae were grown at 25°C under 16-8 hr. light-dark cycles in Percival controlled environmental chambers.

TEST ORGANISMS

The algal material used for this study was obtained from two sources. Two green algae, Hydrodictyon reticulatum and Scenedesmus quadricauda, two blue-green algae, Aphanizomenon flos-aquae and Microcystis aerusinosa, and the diatom, Nitzschia palea, were collected from either lakes, ponds, or irrigation waters under natural conditions. This method of collection was necessary to collect sufficient quantities of each genera because either the culturing methods are not worked out completely or the quantities needed are not obtainable by culturing. Those genera that were easily

cultured were grown in culture vessels within growth chambers. They were kept at a constant temperature of 20°C and light of 250 to 300 ft-c with a light-dark regime of 16-8 hours respectively. Anabaena and Lyngbya were the two genera of blue-green algae cultured this way.

Higher plant tissue was obtained by germinating radish seeds (Raphanus sativus) of the Scarlet Globe variety.

PREPARATION OF PLANT MATERIAL

Depending on the availability, five to twenty grams of each alga were extracted in boiling 80% ethanol for 10 minutes. This treatment rapidly kills the cells, stops enzymatic activity, and releases most of the cellular contents. Although some denaturing of proteins and polypeptides may occur, it is believed that this damage is minimal and due to the rapidity of the extraction, preferable over other extraction methods. The rough extract was then filtered and the filtrate used in the radish cotyledon bioassay to determine the presence of growth regulating compounds.

Higher plant tissue was derived from seeds of the Scarlet Globe variety of radish (Raphanus sativus). Cotyledons were obtained by germinating the radish seeds and allowing them to grow at 25°C, in the dark, for 60 to 70 hours on moist paper toweling in Pyrex baking dishes. The inner cotyledon of the seedling was excised and used as the higher plant bioassay indicator tissue due to its sensitivity to growth regulating compounds (Bentley-Mowat and Reid, 1968).

CHROMATOGRAPHY

The algal extract was line loaded on strips of Whatman 3mm chromatography paper measuring 20 X 1.5 inches. Approximately 50 microliters of extract was loaded onto each strip. The extract was then separated

using a 1-butanol:glacial acetic acid:water solvent in a 2:1:1 ratio by descending chromatography. The running time for complete separation ranged between 15 and 16 hours, after which the strips were removed and allowed to dry for 72 hours at room temperature. Each strip was then divided into twenty 1 X 1.5 inch sections and each section placed into separate 60 X 15 mm petri dishes for bioassay.

BIOASSAY

Each of the twenty sections of the chromatogram were wet using 10m MKCl. The amount of KCl solution added was 5 ml per gram of chromatography paper. It has been found that naturally-occurring salts stimulate cotyledon growth, therefore KCl is added to raise the salt level on each section above the background salt level and thereby stimulate growth evenly on all sections. After the sections were moistened, five radish cotyledons were placed on each of the twenty chromatogram sections and allowed to grow for 72 to 80 hours in light at 25°C.

Control strips were chromatographed along with the algal extracts, and their preparation was identical to the test strips. One control with 5 cotyledons was prepared for each test strip. Three test strips and controls were bioassayed simultaneously.

INHIBITORY OBSERVATIONS

Fresh weight changes were used to indicate growth of the cotyledons as a response to algal compounds. Each measurement was a mean of 5 cotyledons. Initial weights were determined by weighing 50 cotyledons and deriving a mean initial weight per cotyledon. Final weights were determined after 72 hours of growth by deriving a mean final weight from the 5 cotyledons. Initial weights were then substracted from final weights

and the resultant fresh weight change per cotyledon was used to indicate differences. These fresh weight changes were then analyzed statistically to determine if the fresh weight changes of the cotyledons subjected to the algal compounds were significantly different from the fresh weight changes of the control cotyledons.

ELECTRON MICROSCOPY

Electron microscopy of radish cotyledons was used to determine ultrastructural changes of the cotyledon cells in response to algal compounds. The cotyledons were subjected to the same bioassay as above and then prepared for electron microscopy. Preparation of the radish cotyledon tissue consisted of fixing the material in 2.5% glutaraldehyde buffered with 1M Na-cacodylate, followed by post-fixation in 2% OsO_4 in the same buffer. The tissue was then dehydrated in an ethanol series and imbedded in Epon 812. The sectioned material was then stained with uranyl acetate and lead citrate and examined with an AEI-6B electron microscope.

TISSUE CULTURE

Suspension cultures (Manasse, 1972) of sugar beet and corn cells were attempted using Linsmaier and Skoog's (1965) medium. Cells from these plants did not respond to growth in liquid medium.

STATISTICAL ANALYSES

All data were analyzed using 2-way Analysis of Variance. Confidence intervals were then determined using Dunnett's Test at the 0.05 or 95% confidence level. All data were programmed and computer analyzed by the Colorado State University Computer Center.

Results

The initial year of this study was concerned with isolation, culturing and screening of potential inhibitor-producing algal species. Volvox tertius was used as the test organism since it is apparently the most sensitive to inhibitors. Among the organisms tested, were the genera Pandorina and Platydorina, Harris' inhibitor producers but our changes were statistically insignificant and they were not considered in further studies.

Algae from three divisions were screened but only seven showed some effect on our higher plant bioassay organism. The algal species and their specific effects are summarized in Table I. Our first consideration of inhibition was weight decrease. There apparently were no statistically valid differences between the control and test organisms in Scenedesmus and Hydrodictyon. Nitzschia could not be grown in sufficient quantity so chromatography could not be employed on this species.

Lyngbya has four statistically significant inhibitory areas, Anabaena has five areas and the 1:1 mixture of Aphanizomenon-Microcystis has 16 inhibitor sites. Separately, however, Aphanizomenon and Microcystis had several growth promoting sites.

Electron microscopy revealed more than simple wet weight determinations and subsequent statistical analyses. Descriptions for the control and the effect of each alga on cotyledonary tissue will be described separately.

Control (Figs. 1-5). At the time radish cotyledons are removed from the dark, excised and begin growth on moist filter paper, the cells are relatively inactive in their metabolism and appear as

they would in the seed (Fig. 1). The cells contain large quantities of lipid, one to several large protein bodies and chloroplasts are in a proplastid state. If left in the dark (Fig. 2), protein degradation and some lipid vesicle breakdown begin. Proplastids form prolamellar bodies and will form functional thylakoids when exposed to light (Fig. 3). Chloroplasts form stacked discoid thylakoids called grana (Figs. 3, 4, and 5) and this is the major site of the light reactions of photosynthesis. Polymerization of glucose molecules results in visible starch grains in the chloroplasts and are stored until needed. Figures 3 and 4 are at two days' illumination whereas Fig. 5 is at three days' illumination. During this two and three day growth period, protein degradation and lipid vesicle breakdown take place (compare Figs. 1, 3, 4, 5). After the protein is hydrolyzed completely, the space previously occupied by the protein becomes the cell vacuole, and the membrane that formerly surrounded the protein becomes the tonoplast.

Scenedesmus Treated Cells (Figs. 6 and 7). The effects of Scenedesmus extract after three days' incubation include incomplete protein degradation and enlarged lipid vesicles, probably through coalescence of the smaller kind. Chloroplast thylakoids, while normal in appearance, are clustered together, either in the center of the chloroplast (Fig. 6) or in several distinct clusters (Fig. 7). Starch is totally absent as a storage product in the chloroplasts. Some cellular necrosis was also observed.

Aphanizomenon-Microcystis Mixture. Two effects from different inhibitory regions of the chromatograph are shown in Figures 8 and 9. In one (Fig. 8) grana are formed but are proportionately larger in diameter than those in the control. Starch is generally absent as a storage product. In the other inhibitory region (Fig. 6) some grana are formed but the chloroplasts appear to be amyloplasts. Amyloplasts are plastids that polymerize and store starch, accompanied by the lack of pigmentation and thylakoid development. This would account for the yellow-green pigmentation listed in Table I. Ribosome numbers also appeared to be decreased, although comparative counts were not made.

Hydrodictyon. The most drastic cellular changes took place in the cotyledons treated with extracts of Hydrodictyon. Protein degradation was normal but lipid degradation was greatly impeded (Figs. 10 and 11). However, the greatest change was in chloroplasts. They contained large amounts of starch, few internal membranes and in most cases degenerating grana and stroma thylakoids (Fig. 10). Other cellular organelles, such as mitochondria were normal but ribosomes were fewer and clustered into polysomes.

The remaining genera have not been examined with the electron microscope, except Nitzschia, for which the major inhibitory features are listed in Table I. It parallels the structural observations for the other genera.

Conclusions and Discussion

This is the first report of algal inhibitors affecting higher plant growth and causing cellular changes. The term inhibitor is used for convenience since weight loss did not occur to a significant degree in some instances. In a brief report, Harris (1974) noted that algal extracts from the green algal flagellate, Pandorina morum, caused reduced oxygen evolution in isolated and disrupted spinach chloroplasts. Ultrastructural changes in the thylakoids were also observed. Harris' system, however, was an unnatural one due to cellular disruption. From his studies on algae and spinach, Harris generalized that Volvocalean inhibitors affect the photosynthetic apparatus.

We attempted to bioassay some of the same algal species used by Harris but our results were not statistically valid, so we eliminated the colonial flagellates in our studies and proceeded to screen algae from different taxa. Our negative observations might be explained on the basis of possible different culture conditions for our algae, or having grown different strains of algae. Furthermore, radish cotyledons are intact and inhibitor barriers such as the plasma membrane may have prevented the entry of the inhibitors studied by Harris.

In those instances where positive results were obtained, the structure of chloroplasts was altered in several ways, indicating impairment of the photosynthetic apparatus. In addition, lipid and protein degradation also were impaired by some substances. Therefore inhibitors other than photosynthetic inhibitors are reported by us for the first time.

Most of our results are complex and further studies are needed to elucidate the mechanisms underlying the cellular changes observed in our

bioassay system. The inconsistency between weight and cellular alteration might be explained primarily by water uptake which must occur readily and is responsible for initial growth in young plants due to lowered water potential in the cells. Therefore weight measurements may not be valid in our system, and cellular aberrations and physiological tests must be employed in the future.

Another inconsistency occurred in our results with Microcystis and Aphanizomenon. At times these produced potent inhibitors when mixed together (Figs. 6 and 7) but when extracts were taken individually a growth-promoting effect was observed. This growth promotion has been found previously in other algae (Bentley-Mowat, 1964; Bentley-Mowat and Reid, 1968; Gupta and Shukla, 1968; Gupta and Agarwal, 1973; Gupta and Gupta, 1973), specifically in the blue-green alga Phormidium. The growth promoter might be a kinetin-like substance since radish cotyledons are extremely sensitive to this group of plant hormones. The synergistic effect exhibited by mixing the two species, however, is difficult to explain. Since we did not investigate the various growth conditions under which inhibitor production occurs, this will be a consideration in our future investigations.

It is interesting to note that the same algae which produce animal toxins also affect plant tissues. Lyngbya, Anabaena, Microcystis and Aphanizomenon exhibit a variety of effects in animal systems and in our study caused a weight decrease in radish cotyledons.

Initial plans for a bioassay system had included cultured cells of crop plants, however, several negative features of such a system became evident. These included lack of tissue growth in liquid medium, tedious

measurements, slow bioassay procedures and unnatural conditions under which the plant cells would be growing. Furthermore, obtaining conditioned media in Linsmaier and Skoogs medium was impossible since algae would not grow in this medium. We therefore developed an alternate bioassay system used by plant physiologists to study growth substances. This was the radish cotyledon assay. It allowed rapid bioassay of possible inhibitors, constituted an intact system which responded readily to exogenous compounds, and contained several cytological features that could be investigated concurrently.

There are several possible applications based on our results. Although the establishment of a cause-effect relationship in the field is premature at this stage of our investigations, farmers who irrigate could be advised to avoid water containing blooms of algae. This would be especially true at the seedling stage although in later growth the plant stress caused by algal inhibitors rendering the plant susceptible to a variety of plant pathogens.

Secondly, algal blooms in prime fishing lakes may reduce the desirable aquatic vascular plant habitat. This might be the case in Pyramid Lake in Nevada which supports a yearly bloom of the blue-green alga Nodularia.

Finally, if a potent algal inhibitor is found and isolated, it may be used as a natural herbicide in either aquatic or terrestrial habitats.

Our investigations are continuing in several directions. We are continually screening additional algae, to obtain an extended list of inhibitor producers. Techniques of inhibitor identification and purification are being refined and ecological conditions under which inhibitors will be produced are being investigated. Other plant tissue systems will

be studied to determine whether inhibitors affect a broad variety of plants. The culture media for algae need to be refined so that large quantities can be grown for use in our studies.

In conclusion, algae from three divisions were found to have inhibitory effects on higher plant cells. These are blue-green algae primarily, green algae, and a diatom. The list is provided in Table I, including summarized effects.

Table I

Summary of algal inhibitor effects on radish cotyledons after three days growth

Algal extract	Visible pigmentation	Growth (significance) by weight	Lipid Degradation	Protein Degradation	Chloroplast structure	Cell Necrosis	Vascular tissue dev.
Control	dark green	normal	most	total	thylakoids and starch	none	abundant
Scenedesmus	dark green with purple patches	normal	most	total	displaced thylakoids	some	impeded
Hydrodictyon	green	normal	impeded	total	malformed	some	impeded
Nitzschia	green	slight inhibition	most	most	normal but lack starch	some	impeded
Anabaena	green	inhibited	N.O.	N.O.	N.O.	N.O.	N.O.
Lyngbya	green	inhibited	N.O.	N.O.	N.O.	N.O.	N.O.
Microcystis	green	promoted	N.O.	N.O.	N.O.	N.O.	N.O.
Aphanizomenon	green	promoted	N.O.	N.O.	N.O.	N.O.	N.O.
Aphanizomenon-Microcystis	yellow-green	extreme inhibition	total	most	amyloplast	some	impeded

N.O. = Not observed.

Literature Cited

Bentley-Mowat, J. A. 1964. A survey of results on the occurrence of auxins and gibberellins in algae. Marina Botanica 7:149-155.

Bentley-Mowat, J. A. and S. M. Reid. 1968. Investigation of the radish leaf bioassay for kinetins and demonstration of kinetin-like substances in algae. Ann. Bot. 32:23-32.

Carmichael, W. W., D. F. Briggs and P. R. Gorham. 1975. Toxicology and pharmacological action of Anabaena flow-aquae toxin. Science 184:542-544.

Chrost, R. J. 1975a. Inhibitors produced by algae as an ecological factor affecting bacteria in water ecosystems. I. Dependence between phytoplankton and bacterial development. Acta Microbial. Polonica Ser. B. 7(24):125-133.

Chrost, R. J. 1975b. Inhibitors produced by algae as an ecological factor affecting bacteria in water. II. Antibacterial activity of algae during blooms. Acta Microbial. Polonica Ser. B. 7(24):167-176.

Gorham, P. R. 1964. Toxic Algae, In Algae and Man (D. F. Jackson, ed.), Plenum Press, New York, 307 pp.

Gupta, A. B. and A. C. Shukla. 1968. Effect of algal extracts of Phormidium spp. on growth and development of rice seedlings. Hydrobiologica 34:77-84.

Gupta, A. B. and P. R. Agarwal. 1973. Extraction, isolation and bioassay of a gibberellin-like substance from Phormidium foveolarum. Ann. Bot. 37:737-741.

Gupta, A. B. and K. K. Gupta. 1973. Effect of Phormidium extract on growth and yield of Vigna catjang (Cowpea) T. 6269. Hydrobiologica 41:127-132.

Harris, D. O. 1970. An autoinhibitory substance produced by Platydorina caudata. Plant Physiol. 45:210-214.

Harris, D. O. 1971a. A model system for the study of algae growth inhibitors. Arch. Protistenk. 113:230-234.

Harris, D. O. 1971b. Growth inhibitors produced by the green algae (Volvocaceae). Arch. Mikrobiol. 76:47-50.

Harris, D. O. 1971c. Inhibition of oxygen evolution in Volvox globater by culture filtrates from Pandorina morum. Microbios 3:73-75.

Harris, D. O. 1972. Life history and growth inhibition studies in Platydorina caudata (Volvocaceae). Soc. Bot. Fr. Mem. 161-174.

Harris, D. O. and C. D. Caldwell. 1974. Possible mode of action of a photosynthetic inhibitor produced by Pandorina morum. Arch. Mikrobiol. 95:193-204.

Harris, D. O. and D. E. James. 1974. Toxic algae. Carolina Tips 37:13-14.

Harris, D. O. and M. C. Parekh. 1974. Further observations on an algicide produced by Pandorina morum, a colonial green flagellate. Microbios 9:259-265.

Jørgensen, E. G. 1956. Growth inhibiting substances formed by algae. Physiol. Plantarum 9:712-726.

Linsmaier, D. M. and F. Skoog. 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plantarum. 18:100-109.

Manasse, R. J. 1972. A new culture flask for plant tissue suspension cultures. Experientia 28:273.

Pratt, R. 1943. Studies on Chlorella vulgaris. VI. Retardation of photosynthesis by a growth-inhibiting substance from Chlorella vulgaris. Am. J. Bot. 30:32-33.

Rice, E. L. 1974. Roles of allelopathy in phytoplankton succession. In: Physiol. Ecol. (Kozolwski, T. T., ed.).

Rice, T. R. 1954. Biotic influences affecting population growth of planktonic algae. Fish. Bull. 54:227-245.

Sawyer, P. J., J. H. Gentile, and J. J. Sasner. 1968. Demonstration of a toxin from Aphanizomenon flow-aquae. Can. J. Microbiol. 14:119-1204.

Shilo, M. 1967. Formation and mode of algal toxins. Bacteriol. Rev. 31:180-193.

Figures

Ultrastructural changes that take place in a normal developmental pattern in radish cotyledons and some patterns exhibited when treated with an algal inhibitor. All figures are electron micrographs.

Figure Legends

Abbreviations: C = chloroplast, CS = cell wall, G = granum, L = lipid vesicle, M = mitochondrion, P = proplastid, PB = protein body, PrB = prolamellar body, R = ribosome, S = starch, T = thylakoid, V = vacoule.

Fig. 1. Radish cotyledon cells at time of growth initiation after excising. Note the large number of lipid vesicles and large protein body. Few proplastids are present. X20,000.

Fig. 2. Radish cotyledons grown in dark after two days growth. Protein partially degraded. Prolamellar bodies formed. Some starch present in chloroplasts. X30,000.

Fig. 3. Light grown cells after two days growth. Note thylakoid development and lipid and protein body degradation. X20,000.

Fig. 4. Higher magnification of chloroplast after two days growth. Grana formation is taking place. Note some protein remaining in periphery of vacoule. X40,000.

Fig. 5. Cells after three days growth. Protein has disappeared and formed the vacoule. Small amounts of lipid still present. X30,000.

Fig. 6. Cells after treatment and incubation with Scenedesmus quadricanda extracts (3 days). Note thylakoid displacement, protein remnants. X40,000.

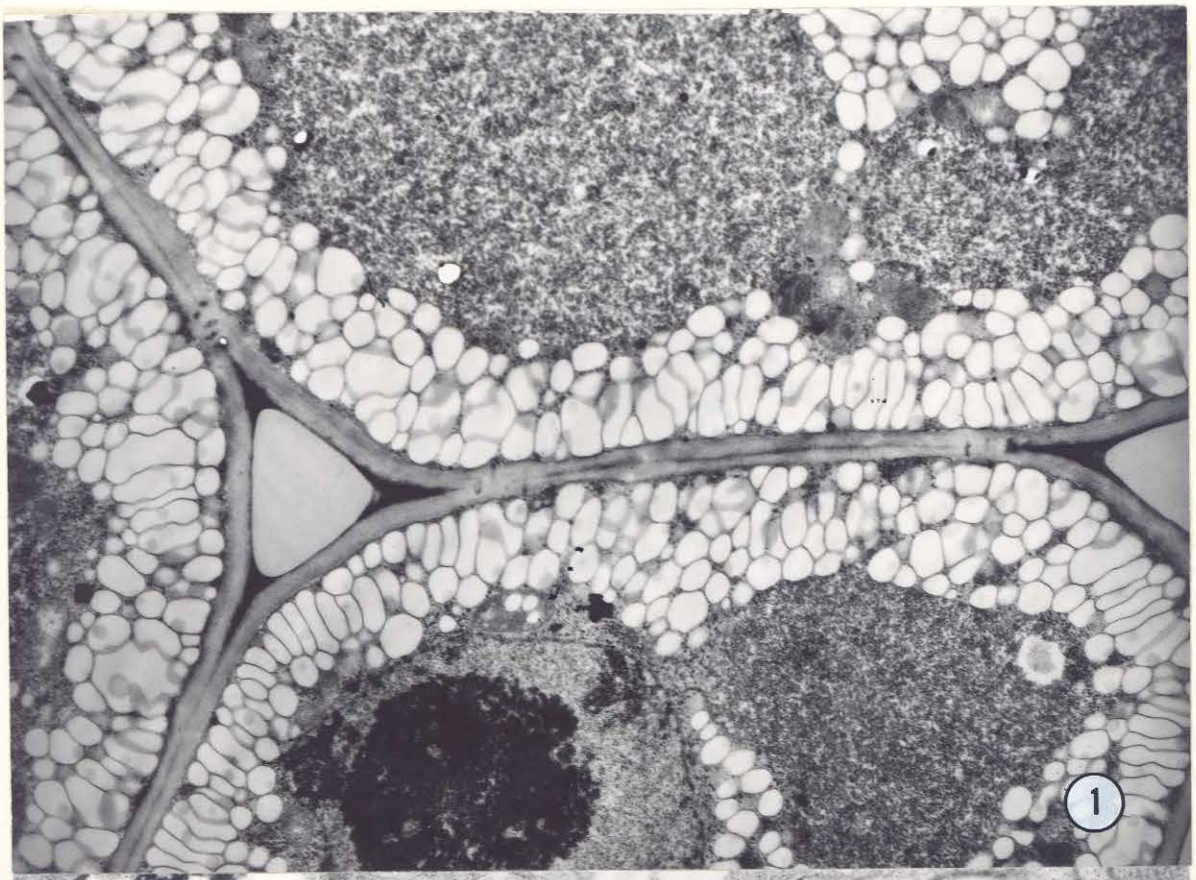
Fig. 7. Cells after Scenedesmus treatment. Note the large lipid vesicles. X20,000.

Fig. 8. Portion of cell from Microcystis extract treatment. Note the broad grana and lack of stroma thylakoids. X40,000.

Fig. 9. Portion of cell from Aphanizomenon extract treatment. Note the disruption of thylakoid organization and the storage of large amounts of starch. X30,000.

Fig. 10. Portion of cell treated with Hydrodictyon extract. Note the thylakoid disruption. X40,000.

Fig. 11. Portion of cell treated with Hydrodictyon extract. X40,000.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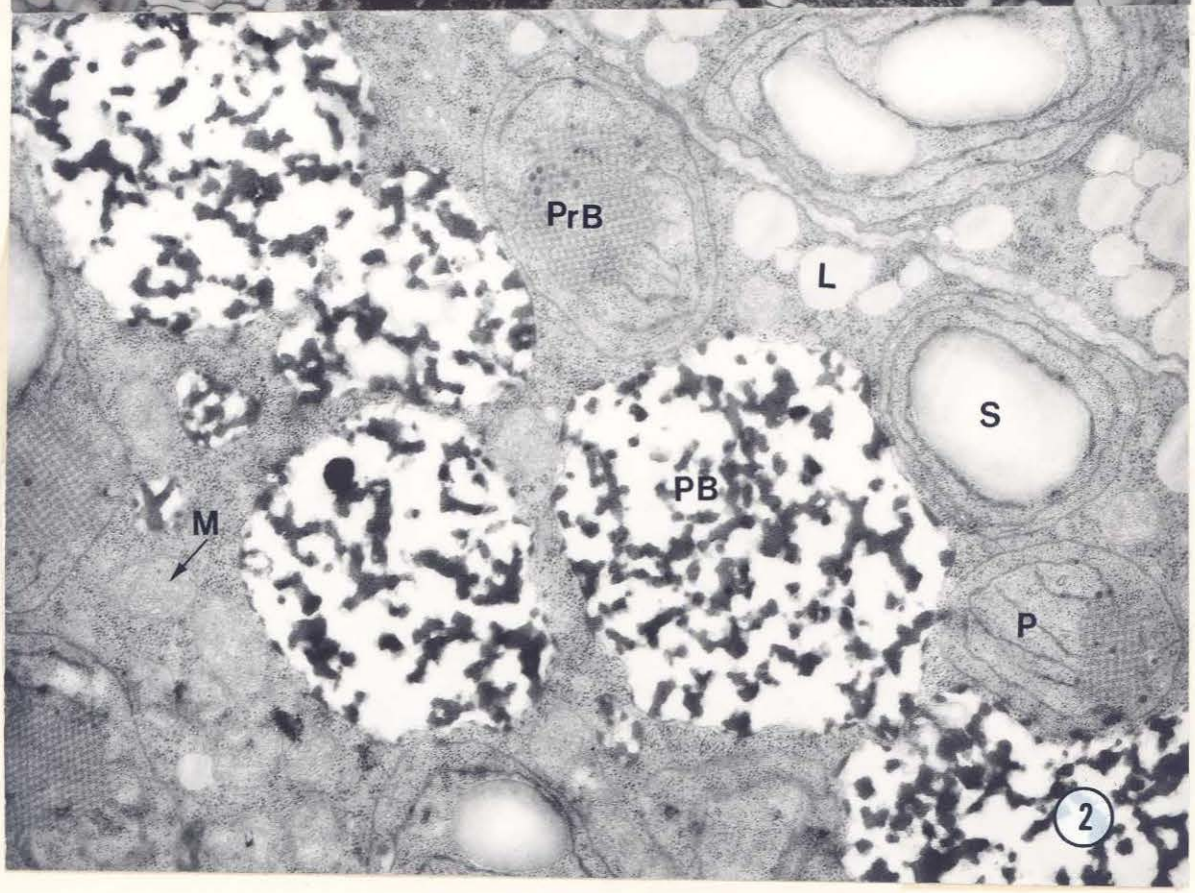
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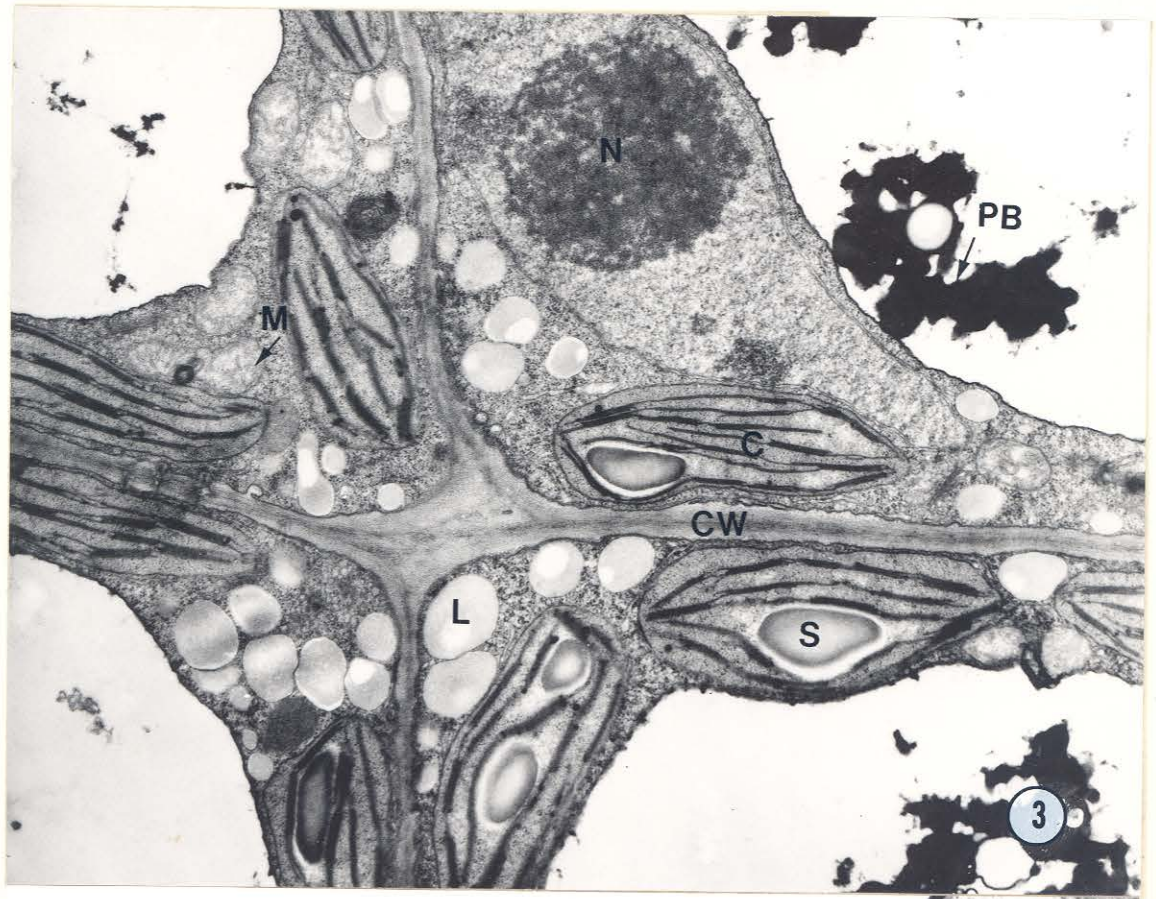
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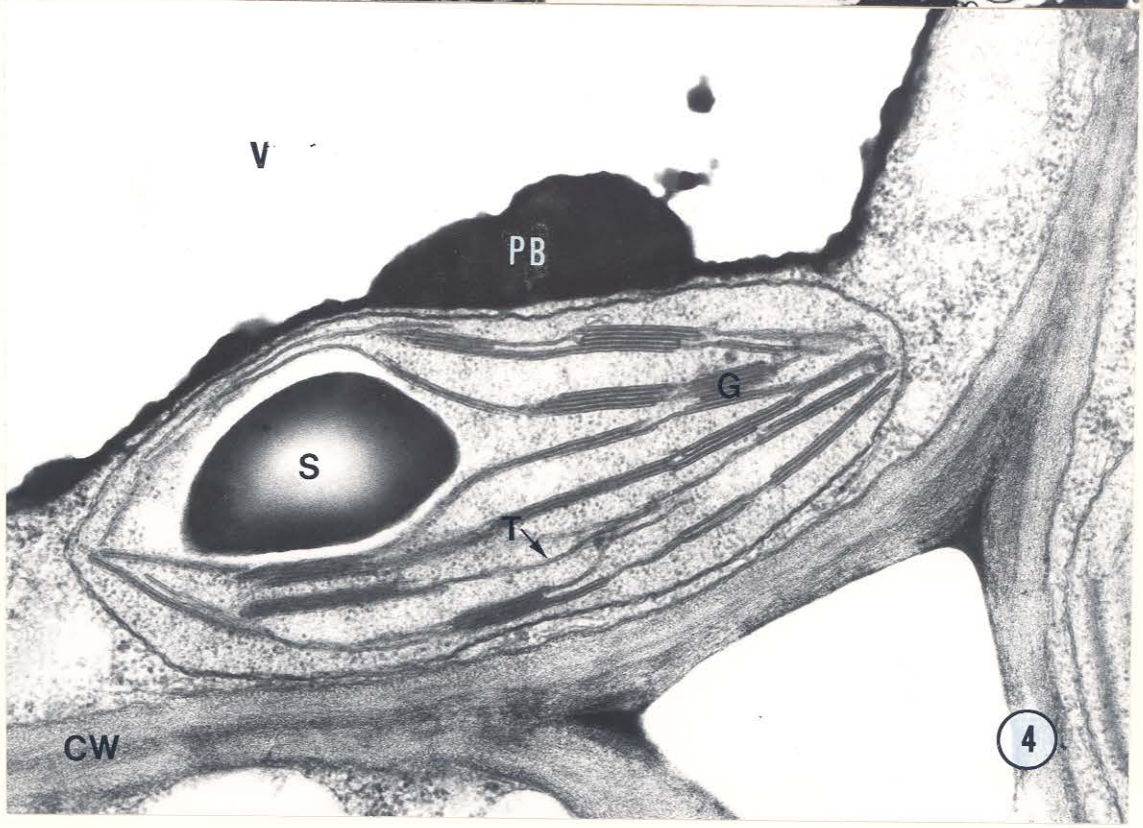
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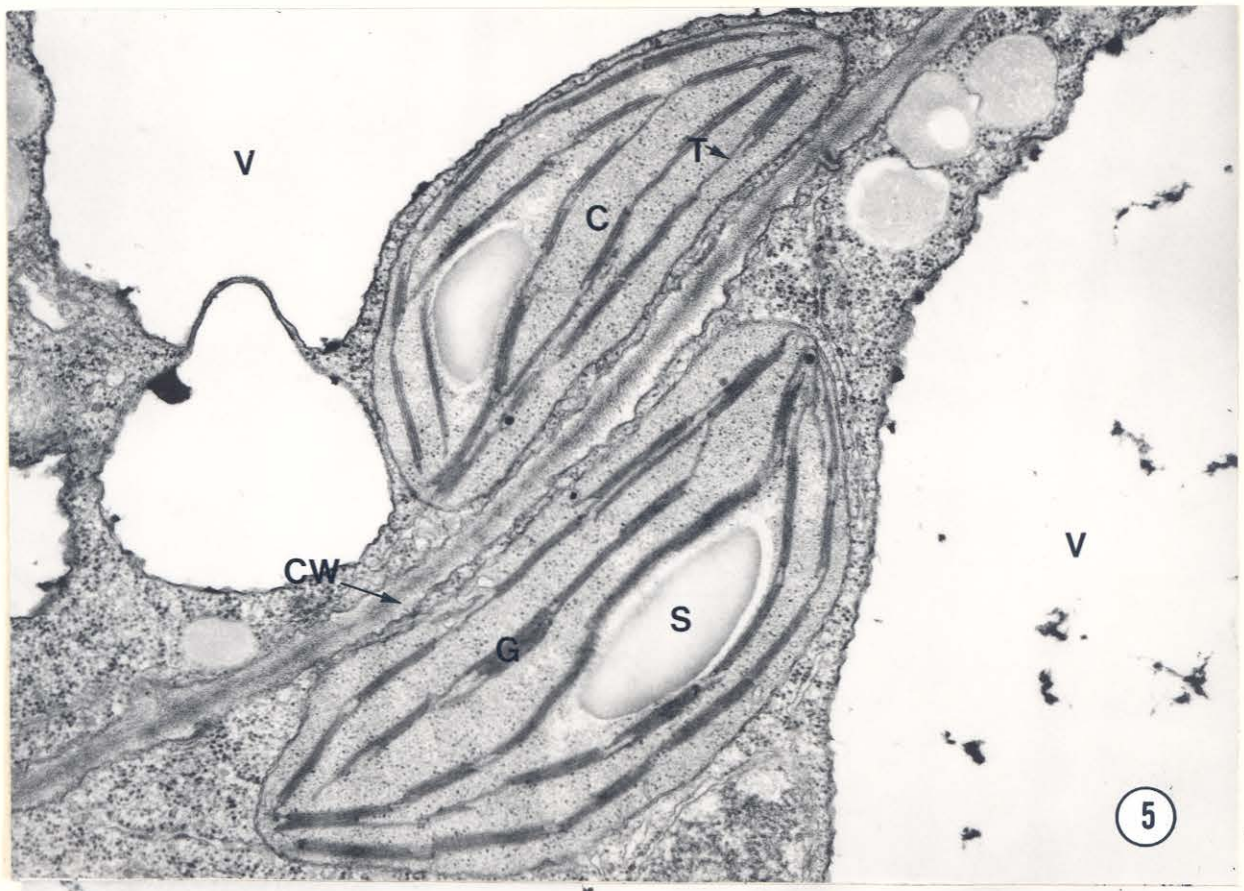
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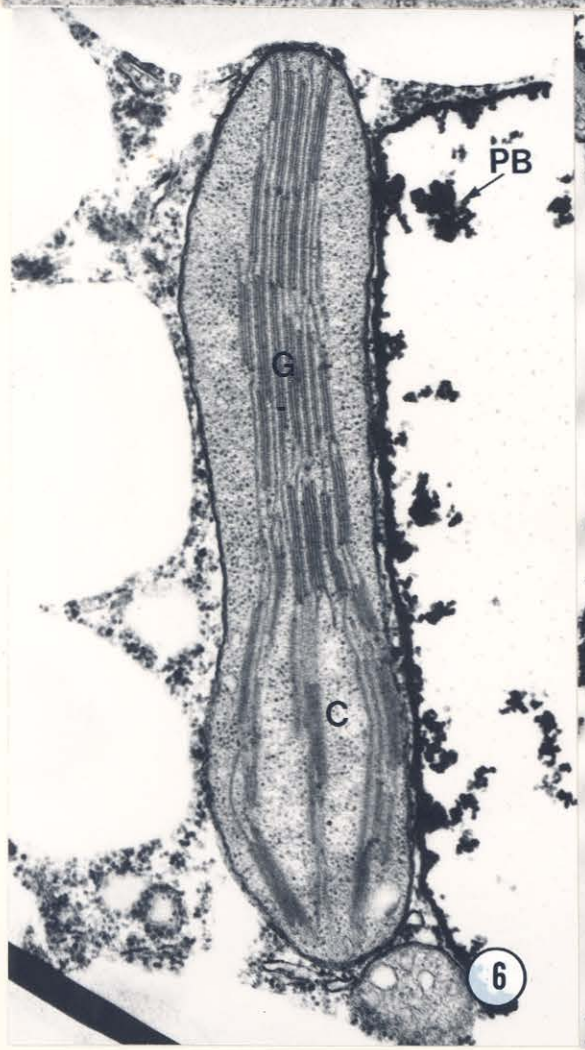
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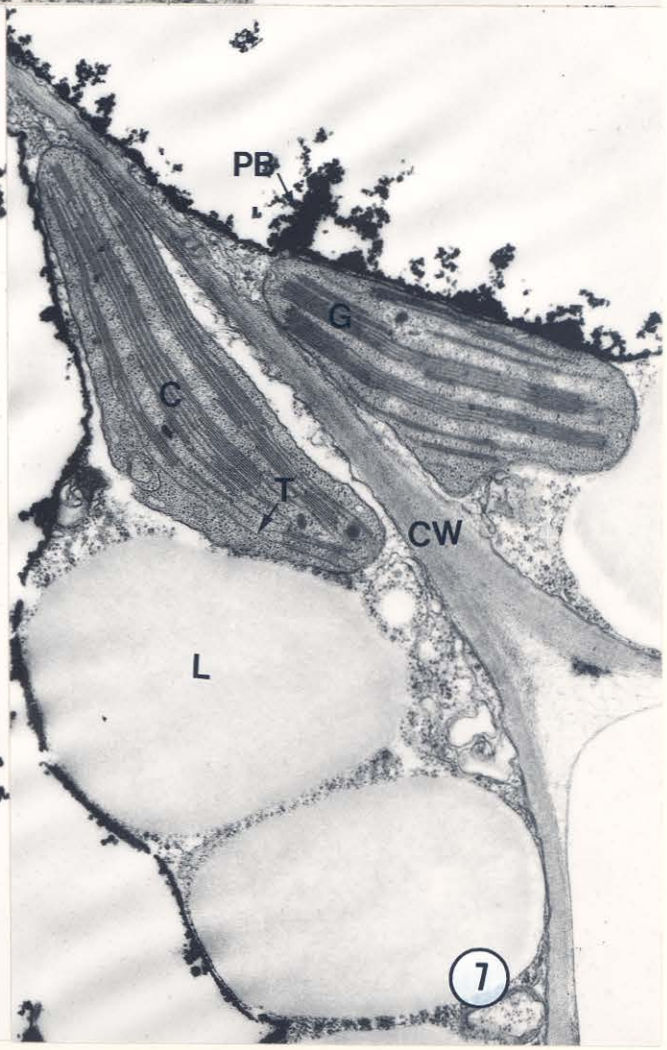
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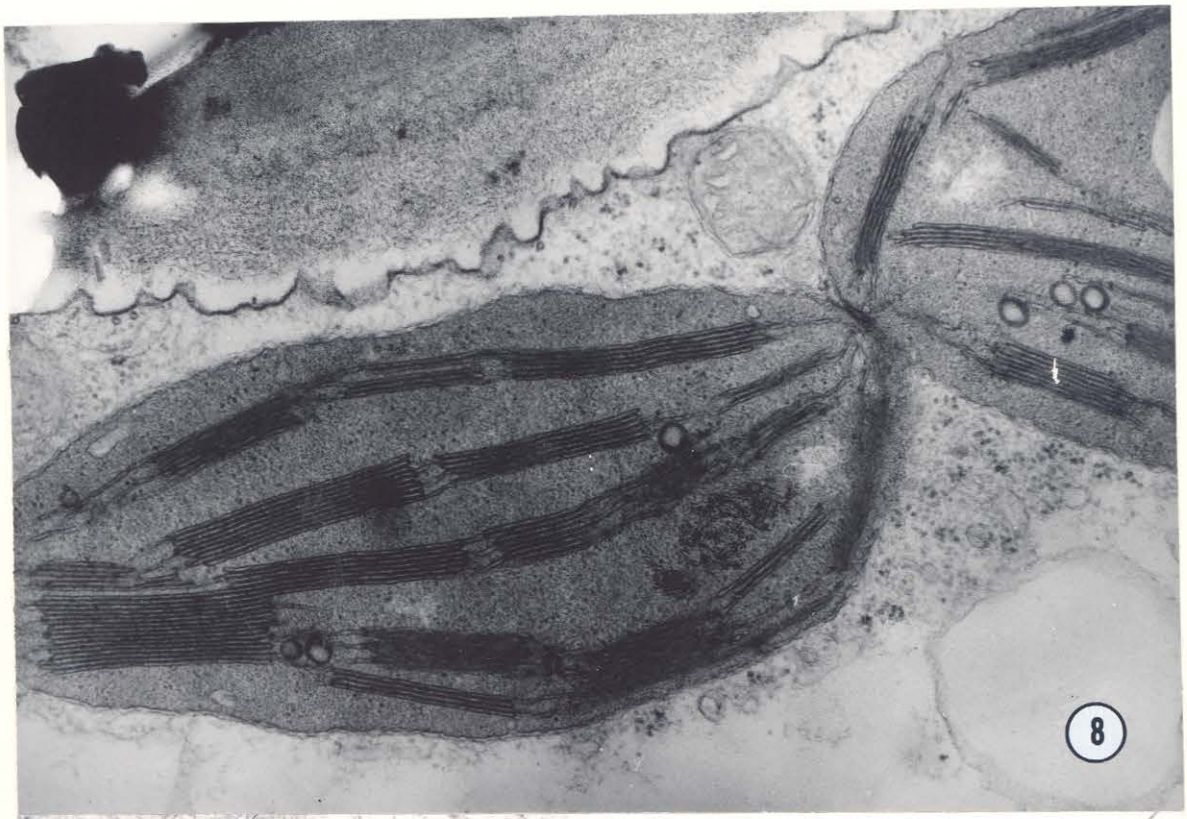
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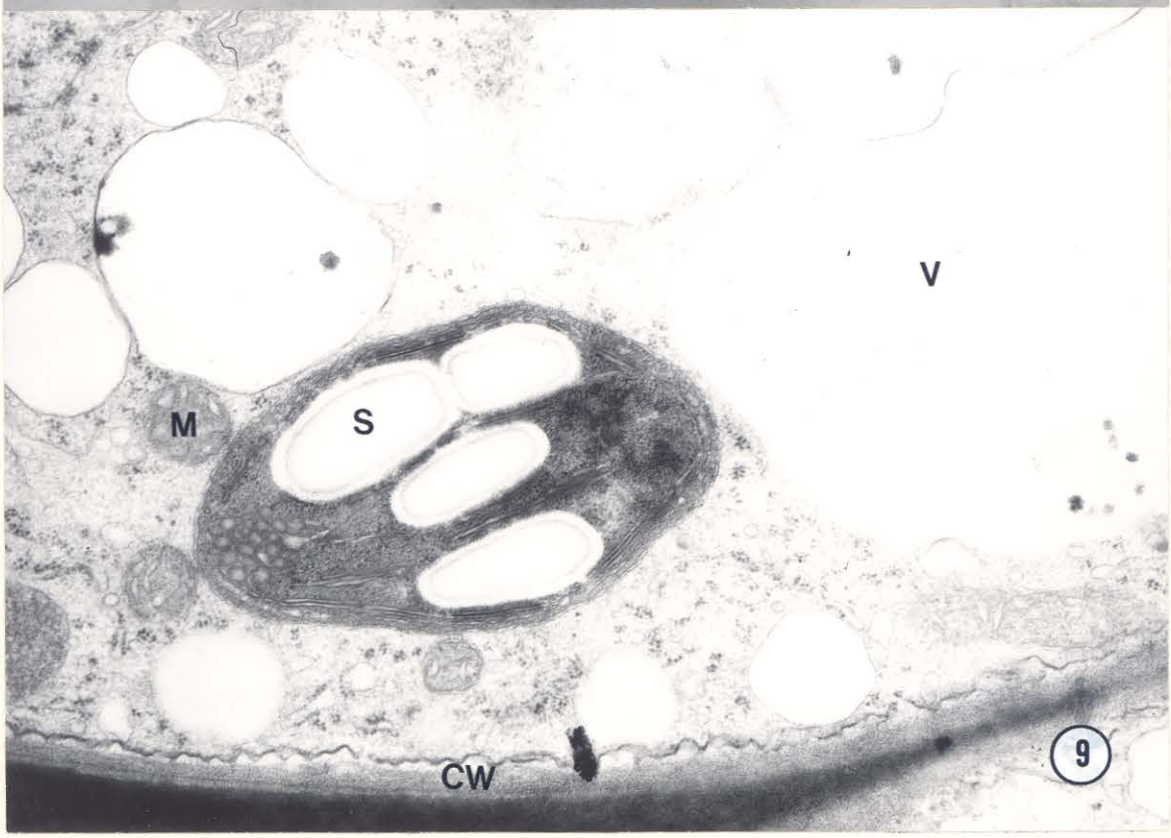
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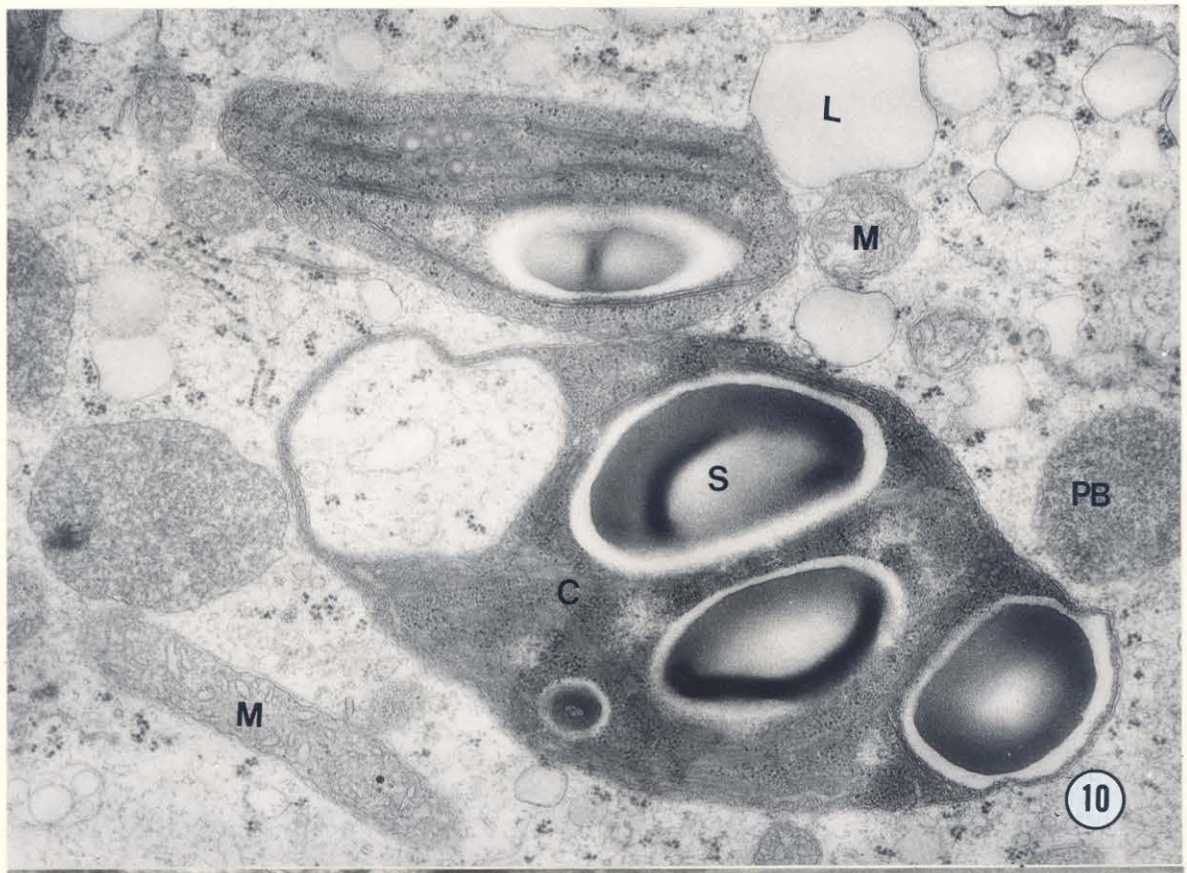
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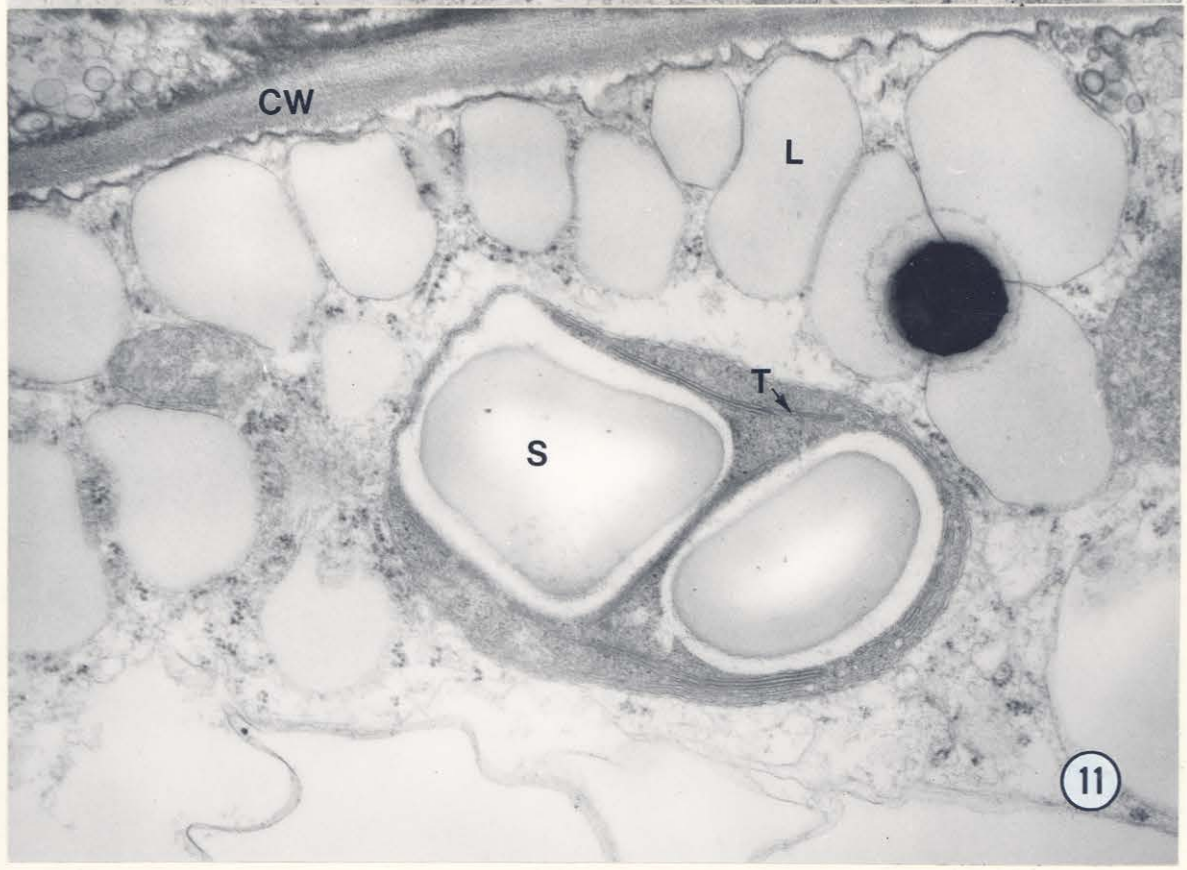
CW
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