c1

FINE STRUCTURAL CHANGES OF TRIBONEMA MINUS HAZEN DURING DESICCATION

by

EMMY NAKAHARA
B.Sc., University of Toronto, 1970

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in the Department of
Botany

We accept this thesis as conforming
to the required standard

The University of British Columbia
August, 1972

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Botany

The University of British Columbia
Vancouver 8, Canada

Date August 18, 1972

ABSTRACT

Fine structural changes in vegetative cells of Tribonema minus during air-drying were examined. The nucleus became contracted into a dense sphere and chromatin condensation occurred. There was reduction in width of the cell wall, perinuclear and perichondrial spaces. Increased width and staining of the plasma membrane and inner mitochondrial membrane was observed. Concomitantly, increased homogeneity of the mitochondrial and chloroplast matrices occurred. Mitochondrial and chloroplast genophores became indistinguishable from their matrix. Increased water loss was characterized by the fusion of the three-thylakoid bands within the chloroplasts. Lipid was synthesized in the chloroplast and transported through the chloroplast endoplasmic reticulum to accumulating areas continuous with it. The product appeared to be the means by which the cell stored water and metabolites during desiccation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

page

ACKNOWLEDGMENTS

To Dr. Thana Bisalputra for his guidance, encouragement and enthusiasm.

To Dr. J.R. Stein and Dr. I.E.P. Taylor for their assistance in preparation of this thesis.

To Dr. D.J. Hibberd* for identifying the species examined.

To my family and friends for their interest and concern.

*Curator of Freshwater Algae
The Culture Centre of Algae and Protozoa
Cambridge, England.

INTRODUCTION

Algae have been reported to occur in aerial, freshwater, marine and soil habitats susceptible to desiccation (3,7,11,12, 20,21,31,32,33,38,39,42,57,67,91,104). Resistance to water loss appears to vary with the phyciological state of the organism and its immediate stress condition (32,41,43,55,73,74).

Special algal cells such as akinetes, aplanospores, cycts and hypnospores have been observed to be produced to escape and/or endure temporary adverse conditions (31,67,91). Some species appeared to retain their vegetative morphology although modifications such as thickened walls, fatty pellicle or cuticle, mucilage and lipid storage granules occurred (13,31,32,43,91). The effects of complex interrelated reactions on biological systems attributed to desiccation have not been sharply distinguished from those produced wholly or in part by another such as growth, aging or nutritional depletion (32,43,55,73, 74,91).

A comprehensive review of the Xanthophyta was presented by Massalski (71) and the fine structure of several Xanthophycean vegetative cells and zoospores have been examined (17,18,22,23, 35,36,47,48,56,63,70,71,72,76). Petersen (91) reported that Tribonema bombycinum (Ag.) Derb. et Sol. occurred in shallow bodies of freshwater known to undergo extreme fluctuation of water stress. Because cysts were not found in his investigation, vegetative stages were believed to be resistant to drought. Hawlitschka (52) reported akinetes and aplanospores were formed

by the same species. Thus, a question arose. How do vegetative cells of Tribonema become modified for "desiccation resistance"?

In this thesis, adaptations to water stress are reviewed and the fine structural changes which occur during desiccation of vegetative cells of Tribonema minus Hazen are described. The small size of this species allowed the examination of several cells in one section making it possible to standardize the age of the stain solutions and the staining time for the stages examined. The information may provide the bases for chemical, physiological and histochemical studies on the effects of water loss from cells and its relationship to species resistance.

LITERATURE REVIEW

WATER

Investigations of water as the medium from which life began and in which it continues have prompted extensive discussions and speculations (6,14,15,24). Presently, the diverse properties and functions of liquid water are explained by a "flickering cluster" model which describes water existing in a continual flux between single and groups of hydrogen bonded water molecules (80).

Water is considered to play three major roles within a cell: as a metabolite, a medium and an integral structural unit (6). As a solvent, water is responsible for the distribution of nutrients within a system and also provides the necessary aqueous medium for enzyme activity and metabolic processed such as protein synthesis (6,14). Relatively free nonhydrogen bonded molecules flowing through cell walls and protoplasmic membranes can act as substrates in enzyme catalyzed reactions such as photosynthesis. Moreover, the small size of the water molecules enables them to fill small interstices in macromolecules (14).

Advanced studies in the past few years have considered Szent-Györgyi's concept of "structured water" and its effect on macromolecules (6,37,53,61). Hazelwood, Nichols and Chamberlain (53) suggest that water in protein macromolecules exists in two distinct phases - the major phase consisting of water molecules which have lost considerable motional freedom relative to free water and the minor phase containing water molecules which have less motional freedom than in the major phase, but more than in solids.

Evidence supports the occurrence of ordered "bound" water of which the degree of polarization and deformation varies upon specific binding interactions at the interfaces of macromolecules (14,81,82). Within a confined space, organized macromolecules are believed to assemble with bound enzymes (103) to form interconnected membranes which segregate into discreet functional units defined by its particular environment (114). Thus, cellular water in plant cells exists in heterogenous aqueous regions: within membrane structures; as one or more layers of "bound" water of macromolecular surfaces; between closely paired systems of membranes e.g. perinuclear, perichondrial spaces, between associated thylakoids and the intercisternae of endoplasmic reticulum and Golgi apparatus; enclosed within organelles; and in the hyaloplasm between various organelles.

DESICCATION EFFECTS

Desiccation affects the continuum of water existing within a living cell and alteration of the physical and/or chemical property of the solvent would induce changes to occur in the cytoplasm (66,107). In particular, water stress has been reported to cause a decrease in free energy and to lower water potential specifying the need for a more highly ordered form of water (14). Crafts (14) summarized the effects of desiccation on water structure as follows:

1. increased molecular structuring bringing about thickening of multilayers at interfaces,
2. increased frictional resistance to movement resulting from increased viscosity (immobilization),and

3. strengthening of menisci and other surface film as a result of increased surface tension.

At macromolecular surfaces, where water normally occurs, it has been proposed that there is a tendency for molecules of water to be held tenaciously to the evaporation surface forming layers of ordered water (14). Furthermore, as water is lost from hydrophobic lipoprotein and phospholipid regions, it was suggested that there may be an accentuated squeezing out of water by the interaction of hydrocarbon tails becoming at least partly confluent (15). Modifications such as these are believed to restrict the movement of water and water-soluble solutes.

Physiological examinations reveal that during water loss in higher plants there is an increase in ribonucleic acids and free amino acids suggesting hydrolysis occurs (1,100,113). Water stress causes inhibition of photosynthesis (115), cellulose synthesis (85), phosphorylation (53,88), protein synthesis (58, 92,93) and glycolysis (100). Thus, desiccation favours the activity of a particular enzyme or group of enzymes (54) which in turn affect the properties of the protoplasm.

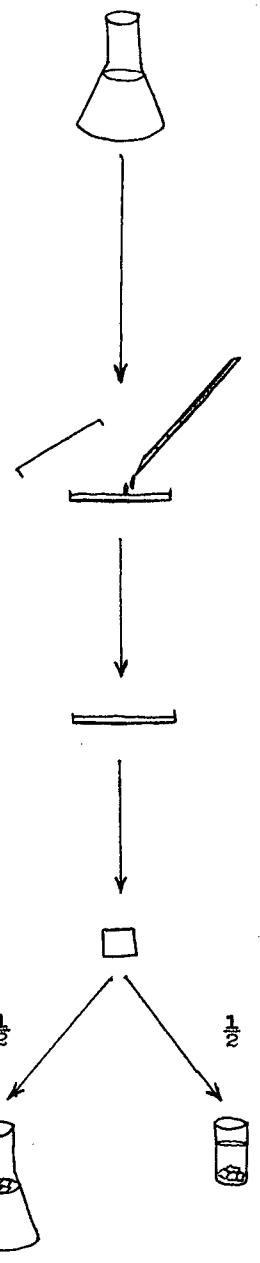
Increases in concentration of sugar in the cytoplasm during desiccation of fungi (111) and higher plants (89) were noted. Also, inositol, a polyhydroxycyclic saturated hydrocarbon, has been reported to be an effective protective substance during water loss from bacterial cells (112). Parker (89) pointed out that six-carbon sugars are chemically similar to inositol having numerous hydroxy groups and often occur in saturated rings. This was correlated to the starch$\rightleftharpoons$sugar shifts in plants exposed

to drought or cold. In both cases, hydroxy groups were believed to replace bound water within and on protein macromolecules by hydrogen-bonding thus protecting drought resistant enzymes.

MATERIALS AND METHODS

Tribonema minus Hazen was obtained from the Culture Collection of Algae, Indiana University (Collection Number 639, 106). Nonaxenic cultures were maintained in 250 ml Erlenmeyer flasks containing 200 ml of Bristol's modified liquid medium (see appendix) for one week. At the end of this period, the cultures were in their exponential growth phase. After vigorous agitation of a culture flask, 5 ml aliquots of the culture were removed with sterile 5 ml glass pipettes (see Fig. 1). One ml samples were transferred to petri plates containing *ca* 9 ml solid 1.0% (w/v) agar of nutrient medium. To insure an even distribution of cells, only 3 ml of the culture were pipetted unto the plates. Seven plates were prepared in this manner. A control plate of agar medium was prepared. The plates were then left uncovered, exposed to the air, in a 20± 2°C chamber illuminated with fluorescent light on a 16:8 light period.

The extent of desiccation was defined as relative hydration (RH; see Table 1). Vegetative cells in liquid culture (100% RH) were of a hydration state similar to those growing in shallow freshwater ponds. Cells which had been air-dried for 36 hours (0% RH) were considered to be in a dehydrated state similar to those cells which have been exposed to drought. Ultrastructural comparisons of 100, 60, 50, 40, 30, 20, 10 and 0% RH stages were carried out and the changes examined.



Nonaxenic culture of Tribonema minus Hazen was grown 1 week to exponential growth phase.

Culture vessel agitated, 5 ml of T. minus were pipetted out.

1 ml samples were transferred unto 9 ml of 1.0% (w/v) nutrient agar plates.

7 experimental plates and a control plate (no alga) uncovered; at 20 C illuminated with fluorescent light (16:8).

Samples of each stage were taken at all relative hydration stages (RH).

Samples prepared for electron microscope study (Table 1).

Samples (0.25 cm^2) were floated on the surface of 200 ml liquid medium and placed at 20 C; 16:8.

Figure 1. Outline of procedure for sampling desiccation stages.

Table 1. Weight Loss During Desiccation (in g): Weight loss from the system of the inoculum, agar and petri plate was measured. Relative hydration was defined as $\frac{\text{exposed weight}}{\text{initial weight}} \times 100$. The initial weight was the total weight of 1.0 ml T. minus liquid culture, agar and petri plate. The exposed weight was the measured weight after each exposure (sample) time. All weight measurements were made of the plates with their respective covers which were kept beside them in the growth chamber during desiccation.

Stage (% RH)	100	60	50	40	30	20	10	0	Control
Plate	-	17.624	17.086	17.594	16.813	17.623	16.814	17.596	17.259
Plate + agar	-	25.930	25.845	26.408	25.575	26.415	25.768	26.262	26.347
Initial weight	-	26.972	26.871	27.616	26.644	27.436	26.837	27.360	*26.347
Exposed weight	-	23.214	21.914	21.566	19.571	19.488	17.734	17.711	17.360
Sample time (hr)	0	28.80	30.83	31.42	32.25	32.33	33.42	36.00	36.00
Relative hydration (%)	100	59.80	49.34	39.63	28.05	19.01	9.18	1.18	-

* no culture was added.

Samples (0.25 cm^2) of each desiccation stage were floated on the surface of 200 ml of liquid medium in 250 ml flasks. Visual evidence of resumed growth was observed in each case. At the same time, samples for ultrathin sectioning in preparation for electron microscopy were fixed for 1 hr in 2.5% (v/v) glutaraldehyde in 0.1M sodium cacodylate buffer (pH 6.8) at room temperature. During water loss, sucrose was added to the buffer (up to 5.0% (w/v) sucrose for 0% RH cells) to reduce osmotic shock. After three 10 min rinses in buffer, the material was post-fixed with 1.0% (w/v) OsO_4 in cacodylate buffer for 1 hr. After washing thoroughly with distilled water, the material was dehydrated in a graded ethanol series then infiltrated and embedded in Spurr's medium (105). Following polymerization in a vacuum oven at 65 C for 11 hr, sections were cut with a du Pont diamond knife on a Reichert Omu 3 ultramicrotome. The sections were post stained with saturated uranyl acetate in 70% (w/v) methanol and lead citrate (95). Cells adjacent to the apical cells were examined primarily. Six cells of each stage were measured. Observations were made with a Zeiss EM 9A electron microscope.

RESULTS AND OBSERVATIONS

THE ULTRASTRUCTURE OF A VEGETATIVE CELL

The unbranched filaments of Tribonema minus were composed of cylindrical cells (2-3 μm wide x 5-7 μm long) in which the H-pieces of the wall were conjoined until their disarticulation during growth and reproduction. Surrounding a central nucleus, the cytoplasm contained two to four peripheral chloroplasts, endoplasmic reticulum, a single Golgi apparatus, mitochondria and vacuoles (Fig.2). The morphology of the vegetative cells of various states of hydration similar to the 100% RH cells are indicated.

CELL WALL AND PLASMA MEMBRANE: Cells of 100% RH had tapering outer cup-like cell walls (OW) 2300 ±300 Å wide which overlapped the shorter inner walls (IW) produced after nuclear division (Figs.2,3). Wall formation appeared to involve the accumulation of wall material within the cell wall membrane (CM) ca 100Å wide exterior to, but separated by a 110Å space from the 100Å wide plasma membrane (Fig.3).

NUCLEUS: Nuclei were typical of those found in eukaryotic cells (28). Slightly elongated interphase nuclei (N) of cells between 100 and 20% RH were 1.5-2.0 μm x 2.0-2.5 μm (Figs.2,4-9). The outer nuclear membrane (ON), 70Å wide, was often associated with ribosomes (R) and extended into the cytoplasm as rough endoplasmic reticulum (RER, Figs.5,6,8). Vesiculation of the outer nuclear membrane occurred along the flattened faces of the in the vicinity of the forming face of the Golgi (G, Figs. 4,5,8,

28-30). In comparison, the inner nuclear membrane (IN), ca 70Å wide, was associated with chromatin and was separated from the outer nuclear membrane by a perinuclear space (PN) 200 - 240Å wide (Figs.4-9).

Nuclear pore complexes (NP) 400Å wide interrupted the continuity of the nuclear membrane (Fig.9). The circular pore appeared surrounded by an annulus 100Å wide which encircled a medium dense pore area 200Å in diameter.

Nucleoli (NU) were densely stained areas associated with chromatin (Figs.2,5,7-9). Visible in oblique sections, the nucleoli extended over an area ca 700Å across consisting of a pars granulosa (PG), pars fibrosa (PF), pars chromosoma (PC) and a central "vacuole" (Nv) devoid of fibrils (Fig.7).

MITOCHONDRIA: Mitochondria maintained a definite spacial relationship, closely associated to the chloroplasts, the Golgi apparatus and the nucleus. The double membrane bound mitochondria (M) of 100 to 20% RH cells were polymorphic. They appeared as large spherical organelles 2 x 3 µm (Fig.2) and elongate or disc-shaped (Figs.12,14,15,28,29,31).

The outer mitochondrial membrane (OM) was ca 50Å wide and separated by an 80Å perichondrial space (PCS) from the wider, ca 60Å, intensely stained inner membrane (IM). The later invaginated to form the tubular cristae (CR, Figs.12-15).

CHLOROPLASTS: Peripheral chloroplasts (CH) were bound by a double membrane envelope. Cells of 100 to 20% RH had outer (OC) and inner (IC) chloroplast membranes, 70Å wide, separated from

each other by an irregular space ca 90Å wide (Fig.19). Examination of the cross (Figs.17,26,27) and longitudinal sections (Figs.16,18-25) revealed internal membrane systems comprised of three closely associated thylakoids. Chloroplast bands were ca 820Å wide and included three thylakoids each 245Å wide (80Å wide membrane surrounding an 85Å wide loculus) which were separated by a space ca 40Å. The peripheral bands (PL) and each of the usually five central bands (CL) appeared to be separated by a 580Å stroma space (Figs.21,22,24).

The chloroplast stroma (S) took up stain more heavily than the cytoplasmic matrix in cells of 100 to 20% RH. Distinct lipid droplets (L) were found in the stroma between the thylakoide bands (Figs.16-18,20,24,26). Electron transparent regions of the chloroplast stroma containing chloroplast genophores (CG) were clearly visible (Figs.16,20-23).

Extensions of the outer nuclear membrane encircled the chloroplast forming the chloroplast endoplasmic reticulum (9; CER, Fig.9). The CER was closely adpressed to or continuous with the chloroplast membrane (Figs.18,19), extending from the chloroplast and associated with polyribosomes forming RER (Figs. 4,23), or connected to a system of lipid storage areas (LS, Figs. 18,24-27).

ENDOPLASMIC RETICULUM AND RIBOSOMES: Ribosomes occurred freely in the cytoplasm of 100 to 0% RH cells (Figs.5,6,8-11). In addition, polyribosomes (PR, Fig.6) were attached to membranes of the RER (Figs.10,11,23) or to tubules extending from the outer

nuclear membrane (Figs.6,8). On the other hand, association of ribosomes on vesicles along the flattened nuclear face formed the initial phase of dictyosome formation (Figs.8,28,29). In oblique planes of sectioning, ER appeared as sheets of cisternae (Fig.23).

GOLGI APPARATUS: A mentioned previously, vesiculation (NV) of the nuclear membrane of 100 to 20% RH cells occurred at the proximal face of the Golgi apparatus (Px). This activity appeared as the primary factor in maintaining the Golgi apparatus which was 1 μm in diameter (G, Figs.2,4,5,8,28-30). However, vesicles formed from the extended portions of the nuclear membrane were added to the Golgi cisternae (Fig.29). Seven Golgi cisternae were present in 100 to 30% RH cells (Figs.4,8,28).

Differentiation of the Golgi cisternae (Figs.28-30) involved

1. the enlargement of intercisternal space along the circumference of the central Golgi plates from 150 to 300Å,
2. the loss of ribosomes towards the distal face (Di),
3. increased stainability of the cisternal membrane towards the mature face, and
4. vesiculation along the mature face producing single membrane bound (80 - 100Å thick) Golgi vesicles (GV) and coated Golgi vesicles (CGV).

Some vesicles produced by the Golgi apparatus joined to form plates of storage and/or transport systems (*, Fig.28).

VACUOLES: Vacuoles (V) of cells of 100% RH were ca 1.3 μm in diameter and centrally located (Fig.17). Bound by a single membrane (80Å wide), the vacuoles appeared to contain vesicles and lipid globules.

CENTRIOLES AND MICROTUBULES: A pair of centrioles (CN) perpendicular to each other occurred near the nucleus where the wall pieces overlapped in 100 and 60% RH cells (Figs.33,34). Centrioles (2000Å in diameter) consisted of a ring of nine triplets of microtubules (200A in diameter) arranged like a cartwheel (Fig.33). The proximal ends of the centrioles showed dense condensations projecting from the A-tubules toward the centre to form a series of "feet" meeting centrally in a dense mass (44). The interconnection of tubules resembling a 9-pointed star described at the distal ends of the basal bodies (71) were not seen. Surrounding each centriole was a relatively clear area called the centrosphere (CS) which contained few ribosomes and fibrous inclusions (45, Fig.33).

Microtubules (MT) 200Å in diameter were of various lengths appearing parallel to or radiating from the centriole area. In cells of 100 to 20% RH, microtubules were found between Golgi vesicles and near the cell surface (Figs.8,9,34,35).

CHANGES DURING DESICCATION

CELL WALL AND PLASMA MEMBRANE: During desiccation, the cell wall decreased in width from less than 2000Å of 100% RH cells to 1600 ±100Å in 0% RH cells (Fig.27). The inner and outer cell walls of 100% RH cells did not take up stain (Fig.3), but those walls of 0% RH cells appeared stained in multilayers as seen by alternating dark and opaque layers (Figs.11,27).

The width of the plasma membrane increased from 100Å in 100% RH cells (Fig.3) to 160Å in 10% RH cells (Fig.26), and to ca 200Å in 0% RH cells (Fig.27). Increased stainability of the membrane occurred directly with increased water loss.

NUCLEUS: Nuclei of 10 and 0% RH cells appeared circular with a diameter of 1.5 μm (Figs.10,11) as compared to elongate in cells of >20% RH. The perinuclear space decreased from ca 200A in 100% RH cells to ca 150A wide in 10 and 0% RH cells (Figs.10,11).

Cells of 60 to 20% RH were observed to have undergone chromatin condensation (CC) with increased staining along the inner nuclear membrane (Figs.5-9). The nucleolar regions of 10 and 0% RH cells were unrecognizable and the chromatin during the latter stages of desiccation was highly condensed and deeply stained (Figs.10,11). In addition, water loss was accompanied by increased density of the nucleoplasm.

The nuclear pores maintained their typical structure (40) throughout desiccation. The pore region of cells of 0% RH appeared occupied by a fibrous component (Fig.11).

MITOCHONDRIA: Water loss caused a decrease in the perichondrial space from 80Å in 100 and 60% RH cells (Figs.3,13) to 50Å in 20% RH cells (Fig.15). The inner mitochondrial membrane increased in width from ca 60Å in 60% RH cells (Figs.12,13) to 80Å after 20% RH (Figs.14,15). Also, the mitochondrial matrix (MM) appeared to stain more intensely in 20% RH cells than in 60% RH cells. The genophore region became gradually indistinguishable with increased desiccation (MG, Fig.15).

CHLOROPLASTS: The extent of association between the thylakoids determined the width of the bands. With increased water loss, there was a tighter association of the thylakoids. Cells of 40% RH had bands 580Å wide (Fig.22). Cells of 10 and 0% RH had bands ca 500Å wide separated by ca 330Å stroma space (Figs. 26,27). However, the definite spacial arrangement of the peripheral and central bands was retained. Upon desiccation, there was a definite lack of stain uptake by the thylakoids (Fig.27).

With increased water stress, the lipid droplets became less distinct in 10 and 0% RH cells and the homogenous stroma was very lightly stained (Figs.26,27). The genophore region was no longer distinguihable in 0% RH cells (Fig.27).

GOLGI APPARATUS: There was continued vesiculation of the cisternae along the distal face of the Golgi apparatus, but the number of cisternae was reduced from seven in 100 to 30% RH cells to four in 20% RH cells (Fig.29). A Golgi apparatus was not detected in cells of 10 and 0% RH although vesicles were present

(Figs.26,27). However, the exact origin of the vesicles could not be determined by structure alone.

VACUOLES: Vacuoles became reduced in size from ca 1.3 μm in diameter at 100% RH (Fig.17) to 0.7 μm in diameter by 20% RH (Fig.32). No vacuoles were found in 10 or 0% RH cells.

CENTRIOLES AND MICROTUBULES: The presence of centrioles were not detected in all stages because serial sections were not taken. The morphological coninuity of the centrioles of T. minus is believed to have occurred similar to that observed in animal cells (45). Microtubules were not seen in 10 or 0% RH cells.

DISCUSSION

CELL WALL AND PLASMA MEMBRANE

The H-pieces of the cell wall of Tribonema minus appeared to be composed of a homogenous opaque substance. The cup-shaped walls fitted together like "dove-tails" (109, Fig.2). Tiffany, in 1924 (109), found by solubility, hydrolysis and optical tests that the cell walls of species of Tribonema contain pectic acid, pectose and cellulose. The variation was believed to be a matter of age, the older filaments containing less pectic compounds and a greater amount of cellulose. Haas and Hill (50) defined pectose, a galacturonic acid derivative in higher plants, as the insoluble precursor of true pectins and pectic acid as the demethylated form of pectin. More recent work by Nicolai and Preston (83) has proven that cellulose, which is mainly glucose, exists in cell walls of Tribonema sp.

Although cellulosic cell walls are uncommon in the Xanthophyceae (60), cellulose has been reported to occur in some filamentous species (60,68,87,97). Multilayers of cellulose in T. minus was evidenced by the staining pattern of dark and light layers in cells which had been air-dried. Similar layered formations occurring not in the wall, but in the protein-rich cuticle of two Chlorophyceae - Cladophora rupestris (L.) Kütz. and Chaetomorpha melagonium (Weber et Mohr) Kütz. - are believed to reflect different chemical compositions of the heavily and lightly stained areas (51). It was suggested that the dark

staining microfibrillar portion was a protein fraction and the other, carbohydrate.

The cellulose in the cell wall of T. minus may be coupled by another fraction, possibly protein, which can undergo changes in structural configuration due to water loss. Constriction of the cell wall may indicate increased structuring of water on the macromolecular surfaces composing the cell wall which was followed by the restriction of further movement of cytoplasmic water and water-soluble solutes similar to that proposed to occur in higher plants during drought (14).

Increase in width of the plasma membrane from 100A at 100% RH to ca 200A at 0% RH and the increased stain uptake due to water loss suggest the constriction of membrane subunits and perhaps the addition of a protective substance to the plasma membrane to limit any further loss of water from the cytoplasm (Figs.3,19,24,26,27).

NUCLEUS

The condensation of chromatin during desiccation was similar to that seen in spermiogenesis of animal cells during which chromatin fibers shortened and condensed into coarse, irregular dense granules which later became compacted into a dense homogenous mass (65). Du Praw (29) has interpreted similar chromatin aggregates as various coiled stages of DNA fibers with histones and non-histone proteins. Furthermore, the

activity of DNA transcription has been related to the coiling i.e. only those portions of uncoiled, naked DNA which appears electron transparent are active. If this analogy is continued, the interphase nucleus (Figs.4-9) was actively transcribing between 100 and 20% RH after which there was increased DNA coiling in 10 and 0% RH cells (Figs.10,11) such that transcription was blocked, RNA production ceased and the remaining RNA in the nucleus was released. The overall effect was reflected in the decreased perinuclear space and reduced vesiculation towards Golgi formation..

MITOCHONDRIA

The morphology of mitochondria in 100 to 20% RH cells was similar to that reported in Xanthophyceae (17,35,56,63,71,72,76) and other eukaryotes (see reviews 28,64,103). However, polymorphism of mitochondria occurred in T. minus reflecting changes in the activity of the mitochondrial and the cytoplasmic DNA as discussed by Du Praw (29).

The inner membrane is believed to be composed of multienzyme systems associated in globular complexes such that lipids form lipoprotein complexes and also patches of lipid bilayers extending between aggregates of protein molecules (103). The results show that desiccation causes an increase in the width and staining of the inner mitochondrial membrane from ca 60Å in cells of 60% RH (Figs.12,13) to 80Å in cells of 20% RH (Figs.14, 15). As water was lost, the hydrophobic bonded lipoprotein and

phospholipid regions appear to have undergone an accentuated water loss and therefore, may have become at least partly confluent (14).

Baxter (2) suggested that the more "soluble" enzymatically active proteins is synthesized at extra-mitochondrial sites under the genetic control of nuclear DNA. Thus, the outer mitochondrial membrane must act as a differentiating membrane regulating the entrance and output of metabolites and products. Furthermore, the perichondrial space would be a temporary storage and perhaps actively transporting area being the intermediate site between two very different areas of activity. The decrease in the perichondrial space from 80Å of 60% RH cells (Fig.13) to 50Å of 20% RH cells (Fig.15) due to water loss implies reduced activity of this region.

The mitochondrial matrix stained more intensely than the cytoplasmic matrix during desiccation. Electron transparent genophore areas (78,79) containing the mitochondrial DNA occurred. With desiccation of cells of 100 to 20% RH, there was increased density of the mitochondrial matrix (Figs.12-15). This increased stainability and homogeneity of the matrix occurred at the same time the genophore became indistinguishable.

CHLOROPLASTS

Chloroplasts of T. minus were similar to those described by Massalski and Leedale for T. vulgare Pascher (72). The photosynthetic membranes occurred as peripheral and central bands composed of three-thylakoid lamellae common for Xanthophyceae (17,18,35,56,71,72,76), Chrysophyceae and Dinophyceae (25, see review 46) and Bacillariophyceae and Phaeophyceae (27,34,46).

Increased water loss caused a decrease in the width of the bands from 820Å at 100% RH, 580Å at 40% RH to 500Å by 0% RH. The close association of the thylakoids within the bands may have been due to the exclusion of water from the interband region caused by hydrophobic bonding as discussed by Kirk for higher plants (59). The difficulty in distinguishing the occurrence and frequency of fusion of thylakoids in two filamentous Xanthophytes is reviewed by Massalski and Leedale (72) and may be clarified by the freeze etching method of examination.

The inability of the chloroplast thylakoids in cell of 0% RH to take up stain suggests changes occurred in the molecular configuration of the membrane components. Nevertheless, the peripheral and central spatial relationship of the bands was maintained (Fig.27) as found in a drought resistant moss, Antitrichia californica Sull. (69). This spatial regulation by the substance of the stroma reflects the possibility of a structure similar to water.

Fatty acids and sterols are some of the photosynthetic products of the Xanthophyceae (13,75). In T. minus, lipid droplets were present between lamellae in the dense chloroplast matrix (Figs.17,18,20,24,26). With increased water loss, the lipid droplets became less distinct due to reduced stainability of this region, especially in the low RH states (10,0%). The cause of the loss in stain uptake may have been due to the saturation of double bonds of the lipid storage product (102). Concomitantly, the chloroplast genophore such as described by Bisalputra and Bisalputra (8) and Ris and Plaut (96) became unrecognizable.

Benson and Shibuya (5) propose that lipids of algae are largely surfactant (i.e. having surface active properties) which function both as structural elements and as photosynthetic metabolites. Lipids, like carbohydrates, release hydrogen and oxygen molecules during metabolism; thus. the continuation of lipid formation and accumulation implies the binding of water to new metabolites and the recombination of such into synthetic processes as noted by Crafts for higher plants (14). It appears that during desiccation, lipid synthesizing enzymes are favoured. To test this theory, one could study the quantitative changes in the lipid synthesizing enzymes by extraction and labelling, or examine the result of blocking lipid synthesizing routes during desiccation.

The similarity in stining property of the stored contents and the chloroplast stroma of T. minus suggest that the chloroplast is the production centre. The continuity of cytoplasmic membranes would enhance the flow of information allowing rapid transformations, yet retain the particular cisternal integrity as proposed for fungi by Bracker and Grove (10).

THE "VACUOME" SYSTEM

A continuous membrane system of endoplasmic reticulum was postulated (86). De Duve (19) defined similar membrane systems as the "vacuome" system encompassing two phases: the Endoplasmic Phase and the Exoplasmic Phase. The functionally synthesizing Endoplasmic Phase included the nuclear membrane, nuclear vesicles, rough endoplasmic reticulum and the forming face of the Golgi. The second Exoplasmic Phase functioned to transport, store, anabolize and metabolize cell products. This latter phase involved the smooth endoplasmic reticulum, maturing Golgi face, lysosome, vacuoles and secretory granules.

Although the interrelationship is unclear the continual nature of membrane systems within a cell is evident (10,19,86). A discussion of the interconnection of the structurally distinct stages may illucidate the cytological changes seen during water stress.

Initially the blebbing and extension of the outer nuclear membrane of T. minus resulted in the formation of nuclear vesicles and endoplasmic reticulum, respectively (Figs.4-11). Similar

relationships were reported in other Xanthophyceae (35,36), Bacillariophyceae (26,27,108), Chrysophyceae (103) and Phaeophyceae (9). The association of polyribosomes on the surface of nuclear vesicles (Fig.28) and the tubules and plates of endoplasmic reticulum (Figs.5-8) suggest protein synthesis occurred as discussed by Schjeide for animal cells (98).

Along flattened faces of the nucleus, the nuclear vesicles fused to form stacks of Golgi cisternae (77, Figs.4,5). The proximal face of the Golgi was similar to that of the rough endoplasmic reticulum. Towards the opposite pole, ribosomes were lost and membrane transformation occurred (49). At the margins of the cisternae during maturation and also along the maturing face, secretory vesicles were produced (Figs.28-31).

Morré, Mollenhauer and Bracker (78) proposed that membrane transformation involved the metabolism of membrane lipids and additions of sugars. Earlier, Perry and Kelley (90) stated that new protein for the secretory products and for the formation of membranes appeared to be synthesized on the polyribosomes and associated messenger RNA of the RER. Functions, other than the general scheme of concentrating and transporting, were ascribed to Golgi apparati to explain the movement of proteins being secreted through this system of membranes (62). The additional functions involved the synthesis of polysaccharides and (less established) providing a location for protein maturation for export.

Golgi vesicles served to compartmentalize materials for transport to the cell surface (Figs.29,31,35,36) as previously reported for higher plant and animal cells (114). The limiting membranes of the vesicles were morphologically similar (80-100Å thick) to the plasma membrane which suggests the capacity of the former to fuse with the latter as in other plants (49,94,101). During chromatin condensation, the reduced number of Golgi cisternae from seven in 100% RH cells to four in 20% RH cells implies a relationship to the apparent block in DNA transcription and RNA production.

Some Golgi vesicles may become promary lysosmes which contain hydrolytic enzymes capable of fusing with vacuoles (Fig.17) which lose water within the cytoplasm causing hydrolysis of its contents as in other organisms (30,84). Therefore, the multivesicular bodies (Figs.31,32) were probably intermediate digestive stages. It appears that hydrolytic enzymes are active during desiccation similar to that occurring in higher plants (1,100,113).

Endoplasmic reticulum existed in close association with the nucleus and chloroplast as reported for the Xanthophyceae (35,36, 47,56,71). Extensions of the outer nuclear envelope formed RER (Fig.23) or lost the polyribosomes becoming smooth endoplasmic reticulum (SER, 16). Membranes closely adpressed to the chloroplast outer membrane in T. minus were SER, but ribosomes were attached to that portion distant from it forming RER (Fig.14) The RER functioned to synthesize protein and store it; whereas, the SER modified previously accumulated proteins and synthesized lipid or carbohydrate (114).

The chloroplast endoplasmic reticulum (CER) appeared to be a transport system continuous with large reservoirs of lipid (Figs.18,24-26). During extreme desiccation (10 and 0% RH), there was further expansion of this storage area providing a peripheral layer of lipid (Figs.26,27).

The loss in water caused shrinkage in the cell and the membranes of the "vacuome" system became closely associated, but remained distinct (Figs.24-26).

CENTRIOLES AND MICROTUBULES

The centrioles of T. minus appeared similar to those reported previously for the Xanthophyceae (17,18,35,56,71,72,76). The pair of centrioles were observed to lie typically at right angles to each other (44,45) near the cell surface (Figs.33,35).

Microtubules of T. minus extended radially from the centrioles maintaining a close association with the centrioles in the cartwheel region. The occurrence of microtubules emanating from centrioles and located among Golgi vesicles suggests that the microtubules and centrioles function as part of a conducting system as discussed by Tilney (110).

During desiccation, microtubules were evident up to 20% RH, but were not detected in the cytoplasm in 10 and 0% RH. The loss of microtubules is similar to their inability to withstand stress conditions such as hydrostatic pressure and low temperature (110).

SUMMARY

Desiccation of the vegetative cells of Tribonema minus was characterized by the following:

1. Condensation of chromatin.
2. Contraction of the nucleus into a dense sphere.
3. Reduction in width of the cell wall, perinuclear and perichondrial space.
4. Reduction in size and apparent fusion of chloroplast three-thylakoid bands. Reduced "interband" space.
5. Increased width and staining of plasma membrane and inner mitochondrial membrane.
6. Increased homogeneity of the mitochondrial and chloroplast matrices.
7. Mitochondrial and chloroplast genophores became indistinguishable from the matrices.
8. Production of a lipid component in the chloroplast and its accumulation in storage areas continuous with the chloroplast endoplasmic reticulum.

During water stress, increased lipid sysnthesis appeared to be the means by which T. minus conserved water and produced new metabolites.

For further understanding of this process, it is necessary to

1. Determine the composition of the lipid substance.
2. Examine the effects of various rates of drying by extraction and electron microscopy.

3. Compare the structure of air-dried cells to those grown axenically in culture as reported by Hawlitschka (52) and Belcher and Fogg (4) at various osmotic pressures.

4. Examine cells grown at defined humidities observing the differences in
   a) rate of respiration,
   b) rate of photosynthesis,
   c) differential enzyme activity, and
   d) comparison of mitochondrial and chloroplast DNA to nuclear DNA activity.

Correlation of the information obtained may explain the changes due to water loss and the subsequent ability of T. minus to resist desiccation.

1. Barnett, N.M. and Naylor, A.W. 1966. Amino acid and protein metabolism in Bermuda grass during water stress. Plant Physiol. 41:1222-1230.

2. Baxter, R. 1971. Origin and continuity of mitochondria. In Reinert, J. and Ursprung, H. (eds.) Origin and Continuity of Cell Organelles p.46-64. Springer-Verlag, New York.

3. Belcher, J.H. 1970. The resistance to desiccation and heat of the asexual cysts of some freshwater Prasinophyceae. Br. Phycol. J. 5:173-177.

4. Belcher, J.H. and Fogg, G.E. 1958. Studies on the growth of Xanthophyceae in pure culture. III Tribonema aequale Pascher. Arch. Mikrobiol. 30:17-22.

5. Benson, A.A. and Shibuya, I. 1962. Surfactant lipids. In Lewin, R.A. (ed.) Physiology and Biochemistry of Algae p.371-383. Academic Press Inc., New York.

6. Berendsen, H.J.C. and Migchelsen, C. 1965. Hydration structure of fibrous macromolecules. Ann. New York Acad. Sci. 125:365-379.

7. Biebl, R. 1962. Seaweeds. In Lewin, R.A. (ed.) Physiology and Biochemistry of Algae p.799-815. Academic Press Inc., New York.

8. Bisalputra, T. and Bisalputra, A.A. 1969. The ultrastructure of chloroplast of a brown alga Sphacelaria sp. I Plastic DNA configuration. The chloroplast genophore. J. Ultrastr. Res. 29:151-170.

9. Bouck, Y.B. 1965. Fine structure and organelle association in the brown algae. J. Cell Biol. 26:523-537.

10. Bracker, C.E. and Grove, S.N. 1971. Continuity between cytoplasmic endomembranes and mitochondrial membranes in fungi. Protoplasma 73:15-34.

11. Bristol, B.M. 1920. On the algal flora of some desiccated english soils. Ann. Bot. Lond. 34:35-80.

12. Cameron, R.E. and Blank, G.B. 1966. Desert algae: soil crusts and diaphanous substrata as algal habitats. Jet Propulsion Laboratory, Technical Report 32-971. p.1-41. Pasadena, California.

13. Collyer, D.M. and Fogg, G.E. 1955. Studies on fat accumulation by algae. J. Exp. Bot. 6:256-275.

14. Crafts, A.S. 1968a. Water structure and water in the plant body. In Kozlowski, T.T. (ed.) Water Deficits and Plant Growth 1:23-47. Academic Press Inc., New York.

15. Crafts, A.S. 1968b. Water deficits and physiological processes. In Kozlowski, T.T. (ed.) Water Deficits and Plant Growth 2:85-133. Academic Press Inc., New York.

16. Dallner, G., Siekevitz, P. and Palade, G.E. 1966. Biogenesis of endoplasmic reticulum membranes. I Structural and chemical differentiation in developing rat hepatocyte. J. Cell Biol. 30:73-96.

17. Deason, T.R. 1971a. The fine structure of sporogenesis in the xanthophycean alga Pseudobumilleriopsis pyrenoidosa. J. Phycol. 7:101-107.

18. Deason, T.R. 1971b. The origin of flagellar hairs in the xanthophycean alga Pseudobumilleriopsis pyrenoidosa. Trans. Amer. Micros. Soc. 90:441-448.

19. De Duve, C. 1969. The lysosomes in retrospect. In Dingle, J.T. and Fell, H.B. (eds.) Lysosomes in Biology and Pathology 1:3-40. North-Holland Publ. Co., Amsterdam.

20. Demeter, O. 1956. Über modifikationen bei Cyanophyceen. Arch. Mikrobiol. 24:105-133.

21. De Puymaly, A. 1924. Sur le vacuome des algues vertes adaptées à la vie aérienne. C. R. Acad. Sci. 178:958-960.

22. Descomps, S. 1963a. Contribution à l'étude infrastructurale des Vaucheries (Xanthophycees, Chromophytes). C. R. Acad. Sci. 256:1333-1335.

23. Descomps, S. 1963b. Observations sur l'infrastructure de l'enveloppe des chloroplastes de Vaucheria (Xanthophycees). C. R. Acad. Sci. 257:727-729.

24. Dick, D.A.T. 1966. Cell Water p.1-146. Butterworth Inc., Washington, D.C.

25. Dodge, J.D. 1968. The fine structure of chloroplasts and pyrenoids in some marine dinoflagellates. J. Cell Sci. 3:41-48.

26. Drum, R.W. 1963. The cytoplasmic fine structure of the diatom Nitzschia palea. J. Cell Biol. 18:429-440.

27. Drum, R.W. and Pankratz, H.S. 1964. Pyrenoids, raphes and other fine structure in diatoms. Amer. J. Bot. 51:405-418.

28. Du Praw, E.J. 1968. Cell and Molecular Biology p.128-129. Academic Press Inc., New York.

29. Du Praw, E.J. 1970. DNA and Chromosomes p.172-185. Holt, Rinehart and Winston Inc., New York.

30. Essner, E. and Novikoff, A. 1962. Cytological studies on two functional hepatomes: interrelations of endoplasmic reticulum, Golgi apparatus and lysosomes. J. Cell Biol. 15:289-312.

31. Evans, J.H. 1958. The survival of freshwater algae during dry periods. I An investigation of the algae of five small ponds. J. Ecol. 46:149-167.

32. Evans, J.H. 1959a. The survival of freshwater algae during dry periods. II Drying experiments. J. Ecol. 47:55-71.

33. Evans, J.H. 1959b. The survival of freshwater algae during dry periods. III Stratification of algae in pond margin litter and mud. J. Ecol. 47:72-81.

34. Evans, L.V. 1966. Distribution of pyrenoids among some brown algae. J. Cell Sci. 1:449-454.

35. Falk, H. 1967. Zum Feinbau von Botrydium granulatum Grev. (Xanthophyceae). Arch. Mikrobiol. 61:347-362.

36. Falk, H. and Kleinig, H. 1968. Feinbau und Carotinoide von Tribonema (Xanthophyceae). Arch. Mikrobiol. 61:347-362.

37. Falk, M., Poole, A.G. and Goymour, C.G. 1970. Infrared study of the state of water in the hydration shell of DNA. Can. J. Chem. 48:1530-1542.

38. Flint, E.A. 1958. Biological studies of some tussock-grassland soils. IX Algae: preliminary observations. New Zealand J. Agr. Res. 1:991-997.

39. Fogg, G.E. 1969. Survival of algae under adverse conditions. Sym. Soc. Exp. Biol. 23:123-142.

40. Franke, W.W. 1970. On the universality of nuclear pore complex structure. Z. Zellforsch. 105:405-429.

41. Fraymouth, J. 1928. On moisture relations of terrestial algae. III The respiration of certain lower plants including terrestial algae with special reference to the influence of drought. Ann. Bot. 42:75-100.

42. Fritsch, F.E. 1922. The moisture relations of terrestial algae. I Some general observations and experiments. Ann. Bot. 36:1-20.

43. Fritsch, F.E. and Haines, F.M. 1923. The moisture relations of terrestial algae. II The changes during exposure to drought and treatment with hypertonic solution. Ann. Bot. 37:683-728.

44. Fritz-Niggli, H. and Suda, T. 1972. Formation and significance of centrioles: a study and new interpretation of the meiosis of Drosophila. Cytobiologie 5:12-41.

45. Fulton, C. 1971. Centrioles. In Reinert, J. and Ursprung, H. (eds.) Origin and Continuity of Cell Organelles p.170-221. Springer-Verlag, New York.

46. Gibbs, S.P. 1962. The ultrastructure of the chloroplasts of algae. J. Ultrastr. Res. 7:418-435.

47. Greenwood, A.D. 1959. Observations on the structure of the zoospores of Vaucheria. II. J. Exp. Bot. 10:55-68.

48. Greenwood, A.D., Manton, I. and Clarke, B. 1957. Observations on the structure of the zoospores of Vaucheria. J. Exp. Bot. 8:71-86.

49. Grove, S.N., Bracker, C.E. and Morré, D.J. 1968. Cytomembrane differentiation in the endoplasmic reticulum-Golgi apparatus-vesicles complex. Science 161:171-173.

50. Haas, P. and Hill, T.G. 1928. Chemistry of Plant Products 1:192-205. Longmans, Green and Co., London.

51. Hanic, L.A. and Craigie, J.S. 1969. Studies on the algal cuticle. J. Phycol. 5:89-102.

52. Hawlitschka, E. 1932. Die Heterokonten-Gattung Tribonema. Pflanzenforschung 15:1-36.

53. Hazelwood, C.F., Nichols, B.L. and Chamberlain, N.F. 1969. Evidence for the existence of a minimum of two phases of ordered water in skeletal muscle. Nature 222:747-750.

54. Henkel, P.A. 1964. Physiology of plants under drought. Ann. Rev. Plant Physiol. 15:363-386.

55. Hess, U. 1962. Über die hydraturabhängige Entwicklung und die Austrocknungresistenz von Cyanophyceen. Arch. Mikrobiol. 44:189-218.

56. Hibberd, D.J. and Leedale, G.F. 1971. Cytology and ultrastructure of the Xanthophyceae. II The zoospore and vegetative cell of coccoid forms with special reference to Ophiocytium majus Naegeli. Br. Phycol. J. 6:1-23.

57. Jenik, J. and Lawson, G.W. 1967. Observations on water loss of seaweeds in relation to microclimate on a tropical shore (Ghana). J. Phycol. 3:113-116.

58. Kessler, B. 1959. Nucleic acids as factors in drought resistance of plants. Proc. 9th Intern. Bot. Congr., Montreal 2:190.

59. Kirk, J.T.O. 1971. Chloroplast structure and biogenesis. Ann. Rev. Biochem. 40:161-196.

60. Klein, R.A. and Conquist, A. 1967. A consideration of the evolutionary and taxonomic significance of some biochemical, micromorphological, and physiological characters in the thallophytes. Quarterly Rev. Biol. 42:105-296.

61. Kuntz, I.D. Jr., Brassfield, T.S., Law, G.D. and Purcell, G.V. 1969. Hydration of macromolecules. Science 163: 1329-1331.

62. Leblond, C.P. 1965. General conclusions. In Leblond, C.P. and Warren, K.B. (eds.) The Use of Radioautography in Investigating Protein Synthesis 4:321-339. Academic Press Inc., New York.

63. Lefort, M. 1962. Contributions à l'étude inframicroscopique du Tribonema aequale Pascher. C. R. Acad. Sci. 254: 3022-3024.

64. Lehninger, A.L. 1965. The Mitochondrion p.205-233. W.A. Benjamin Inc., New York.

65. Lentz, T.L. 1971. Cell Fine Structure p.244-249. W.B. Saunders Co., Philadelphia.

66. Levitt, J. 1956. The Hardiness of Plants p.141-189. Academic Press Inc., New York.

67. Lund, J.W.G. 1962. Soil algae. In Lewin, R.A. (ed.) Physiology and Biochemistry of Algae p.759-770. Academic Press Inc., New York.

68. Maeda, M., Kuroda, K., Iriki, Y., Chihara, M., Nisizawa, K. and Miwa, T. 1966. Chemical nature of major cell wall constituents of Vaucheria and Dichotomosiphon with special reference to their phylogenetic positions. Bot. Mag. 79:634-643.

69. Mahmoud, M.I. 1965. Protoplasmatics and drought resistance in mosses. Ph.D. Thesis. 103 pages. University of California, Davis, California.

70. Manton, I., Clarke, B., Greenwood, A.D. and Flint, E.A. 1952. Further observations on the structure of plant cilia by a combination of visual and electron microscopy. J. Exp. Bot. 3:204-215.

71. Massalski, A. 1969. Cytological studies on filamentous Xanthophyceae. Ph.D. Thesis. 165 pages. University of Leeds, Leeds.

72. Massalski, A. and Leedale, G.F. 1969. Cytology and ultrastructure of the Xanthophyceae. I Comparative morphology of the zoospores of Bumilleria sicula Borzi and Tribonema vulgare Pascher. Br. Phycol. J. 4:159-180.

73. McLean, R.J. 1967. Desiccation and heat resistance of the green alga Spongiochloris typica in culture. Can. J. Bot. 45:1933-1938.

74. McLean, R.J. 1968. Physiological changes of Spongiochloris typica in culture. J. Phycol. 4:73-75.

75. Miller, J.D.A. 1962. Fats and steroids. In Lewin, R.A. (ed.) Physiology and Biochemistry of Algae p.357-370. Academic Press Inc., New York.

76. Moestrup, Ø. 1970. On the fine structure of the spermatozoids of Vaucheria sescuplicaria and on the later stages in spermatogenesis. J. Mar. Biol. Ass. U.K. 50:513-523.

77. Morre, D.J., Mollenhauer, H.H. and Bracker, C.E. 1971. Origin and continuity of Golgi apparatus. In Reinert, J. and Ursprung, H. (eds.) Origin and Continuity of Cell Organelles p.82-126. Springer-Verlag, New York.

78. Nass, M.M.K. 1971. Properties and biological significance of mitochondrial DNA. In Harris, P.J. (ed.) Biological Ultrastructure p.41-63. Oregon State Univ. Press, Corvallis.

79. Nass, M.M.K., Nass, S. and Afzelius, B.A. 1965. The general occurrence of mitochondrial DNA. Exp. Cell Res. 37: 516-539.

80. Nemethy, G. and Scheraga, H.A. 1962a. Structure of water and hydrophobic bonding in proteins. I A model for the thermodynamic properties of liquid water. J. Chem. Phys. 36:3382-3400.

81. Nemethy, G. and Scheraga, H.A. 1962b. Structure of water and hydrophobic bonding in proteins. III The thermodynamic properties of hydrophobic bonds in proteins. J. Phys. Chem. 66:1773-1789.

82. Nemethy, G. and Scheraga, H.A. 1962c. Structure of water and hydrophobic bonding in proteins. II Model for the thermodynamic properties of aqueous solutions of hydrocarbons. J. Chem. Phys. 36:3401-3417.

83. Nicolai, E. and Preston, R.D. 1952. Cell wall studies in the Chlorophyceae. I A general survey of submicroscopic structure in filamentous species. Proc. Roy. Soc. Lond. 140:244-274.

84. Novikoff, A.B. and Shin, W.Y. 1964. The endoplasmic reticulum in the Golgi zone and its relations to microbodies. Golgi apparatus and autophagic vacuoles in rat liver cells. J. Mikroscop. 3:187-206.

85. Ordin, L. 1960. Effect of water stress on cell wall metabolism of Avena coleoptile tissue. Plant Physiol. 35:443-450.

86. Palade, G. 1956. The endoplasmic reticulum. J. Biophys. Biochem. Cytol. 2:85-98.

87. Parker, B.C., Preston, R.D. and Fogg, G.E. 1963. Studies on the structure and chemical composition of the cell walls of Vaucheriaceae and Saprolegniaceae. Proc. Roy. Soc. Lond. 158:435-445.

88. Parker, J. 1956. Drought resistance in woody plants. Bot. Rev. 22:241-289.

89. Parker, J. 1968. Drought resistance mechanisms. In Kozlowski, T.T. (ed.) Water Deficits and Plant Growth 1:195-234. Academic Press Inc., New York.

90. Perry, R.P. and Kelley, D.E. 1968. Messenger RNA. Protein complexes and newly synthesized ribosomal subunits: analysis of free particles and components of polyribosomes. J. Mol. Biol. 35:37-59.

91. Petersen, J.B. 1935. Studies on the biology and taxonomy of soil algae. Dansk Bot. Ark. 8:1-183.

92. Petrie, A.H.K. and Wood, J.G. 1938a. Studies on the nitrogen metabolism of plants. I The relation between the content of proteins, amino acids and water in the leaves. Ann. Bot. 2:33-60.

93. Petrie, A.H.K. and Wood, J.G. 1938b. Studies on the nitrogen metabolism of plants. III On the effect of water content on the relationship between proteins and amino acids. Ann. Bot. 2:887-898.

94. Porter, K.R., Kenyon, K. and Badenhausen, S. 1967. Specialization of the unit membrane. Protoplasma 63: 262-274.

95. Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17:208-211.

96. Ris, H. and Plaut, W. 1962. Ultrastructure of DNA-containing areas in the chloroplast of Chlamydomonas. J. Cell Biol. 13:383-391.

97. Rogers, H.J. and Perkins, H.R. 1968. Cell Walls and Membranes p.114-134. E. and F.N. Spon Ltd., London.

98. Schjeide, O.A. 1970. Organelle synthesis and assembly. In Schjeide, O.A. and de Vellis, J. (eds.) Cell Differentiation p.357-425. Van Nostrand Reinhold Co., New York.

99. Schuster, F.L., Hershenov, B. and Aaronson, S. 1968. Ultrastructure observations on aging of stationary cultures and feeding in Ochromonas. J. Protozool. 15:335-346.

100. Shah, C.B. and Loomis, R.S. 1965. Ribonucleic acid and protein metabolism in sugar beet during drought. Physiol. Plantarum 18:240-254.

101. Sjostrand, F.S. 1963. A comparison of plasma membrane. cytomembranes, and mitochondrial membranes with respect to ultrastructural features. J. Ultrastr. Res. 9: 561-580.

102. Sjostrand, F.S. 1967. Electron Microscopy of Cells and Tissues 1:382-392. Academic Press Inc., New York.

103. Sjostrand, F.S. and Barajas, L. 1970. A new model for mitochondrial membranes based on structural and on biochemical information. J. Ultrastr. Res. 32:293-306.

104. Smith, G.M. 1950. The Freshwater Algae of the United States p.12-26. McGraw-Hill Co., New York.

105. Spurr, A.R. 1968. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastr. Res. 26:31-43.

106. Starr, R.C. 1964. Culture collection of algae. Amer. J. Bot. 51:1013-1044.

107. Stocker, O. 1956. Die Durreresistenz. In Ruhland, W. (ed.) Handbuch der Pflanzenphysiologie 3:696-741. Springer, Berlin.

108. Stoermer, E.F., Pankratz, H.S. and Bowen, C.C. 1965. Fine structure of the diatom Amphipleura pellucida. II Cytoplasmic fine structure and frustule formation. Amer. J. Bot. 52:1067-1078.

109. Tiffany, L.H. 1924. A physiological study of growth and reproduction among certain green algae. Ohio J. Sci. 24:65-98.

110. Tilney, L.G. 1971. Origin and continuity of microtubules. In Reinert, J. and Ursprung, H. (eds.) Origin and Continuity of Cell Organelles p.222-260. Springer-Verlag, New York.

111. Todd, G.W. and Levitt, J. 1951. Bound water in Aspergillus niger. Plant Physiol. 26:331-336.

112. Webb, S.J. 1965. Bound Water in Biological Integrity p.46-47. Charles C Thomas, Springfield, Illinois.

113. West, S.H. 1962. Protein, nucleotide and ribonucleic acid metabolism in corn during germination under water stress. Plant Physiol. 37:565-571.

114. Whaley, W.G., Dauwalder, M. and Kephart, J.E. 1971. Assembly, continuity and exchanges in certain cytoplasmic membrane systems. In Reinert, J. and Ursprung, H. (eds.) Origin and Continuity of Cell Organelles p.1-45. Springer-Verlag, New York.

115. Woodhams, D.H. and Kozlowski, T.T. 1954. Effect of soil moisture stress on carbohydrate development and growth in plants. Amer. J. Bot. 41:316-320.

PLATES AND EXPLANATIONS

LEGEND

C = chromatin
CC = chromatin condensation
CER = chloroplast endoplasmic reticulum
CG = chloroplast genophore
CGV = coated Golgi vesicle
CH = chloroplast
CL = chloroplast central lamellae
CR = mitochondrial crista
CS = centrosphere
CW = cell wall
D = dense centre of entriole
Di = distal face of Golgi apparatus
F = feet from A-tubule of centriole
G = Golgi apparatus
GC = Golgi cisternae
GV = Golgi vesicle
IC = inner chloroplast membrane
IM = inner mitochondrial membrane
IN = Inner nuclear membrane
IW = inner cell wall
L = lipid
LS = lipid storage area
N = nucleus
NP = nuclear pore
NU = nucleolus
Nv = nucleolar "vacuole"
NV = nuclear vesicle
M = mitochondrion
MG = mitochondrial genophore
MM = mitochondrial matrix
MT = microtubules
OC = outer chloroplast membrane
ON = outer nuclear membrane
OM = outer mitochondrial membrane
OW = outer cell wall
PC = pars chromosoma
PCS = perichondrial space
PF = pars fibrosa
PG = pars granulosa
PL = peripheral lamellae
PN = perinuclear space
PM = plasma membrane
PR = polyribosome
Px = proximal face of Golgi apparatus
RER = rough endoplasmic reticulum
S = chloroplast stroma
V = vacuole
WM = cell wall membrane

PLATE I

Figure 2. Longitudinal section of a 100% RH cell of Tribonema minus Hazen. H-pieces of the cell wall were conjoined. A central nucleus was surrounded by cytoplasm containing two peripheral chloroplasts, endoplasmic reticulum, a single Golgi apparatus and mitochondrion. x 15,000

Figure 3. Cross section of the overlapping outer (OW) and inner (IW) walls of a cell of 100% RH. Note the plasma membrane (PM) closely associated to the cell wall membrane (WM). x 49,000

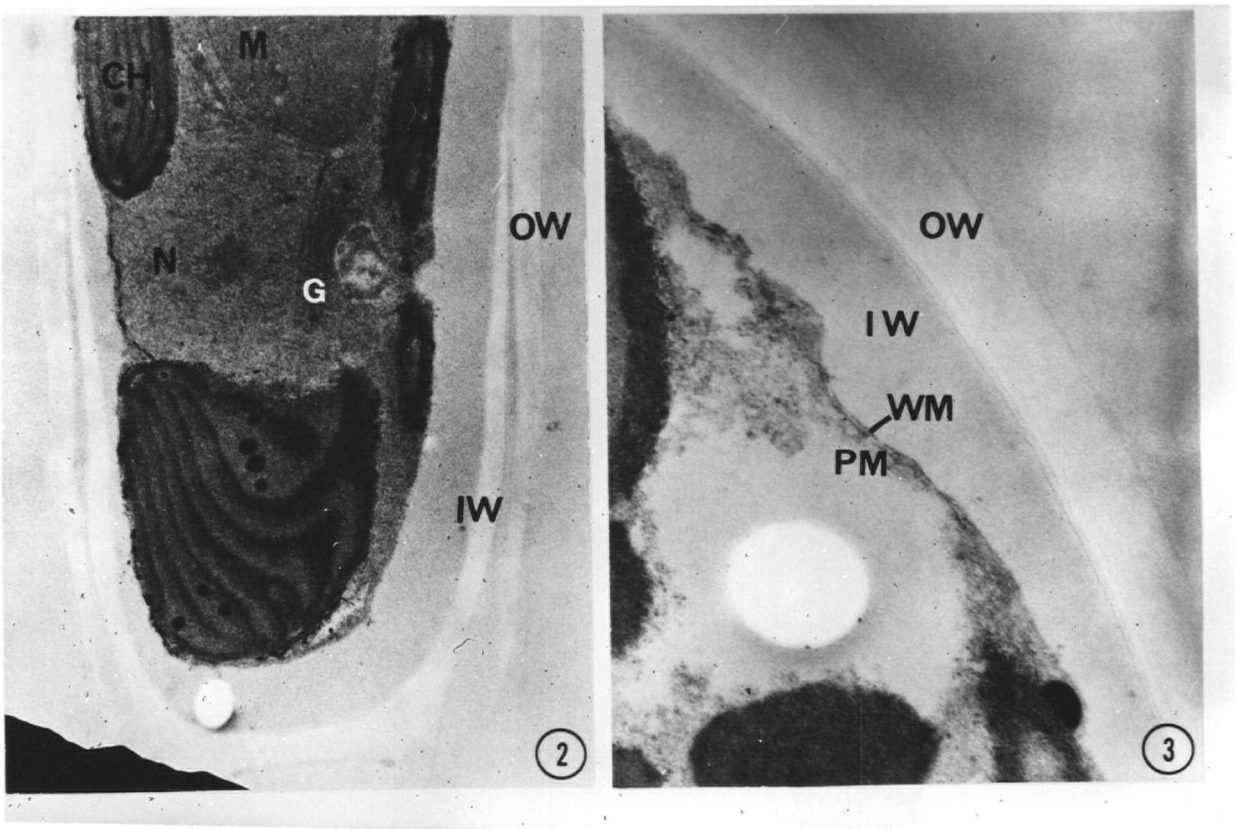

CH
M
N
G
OW
IW
2
OW
IW
WM
PM
3

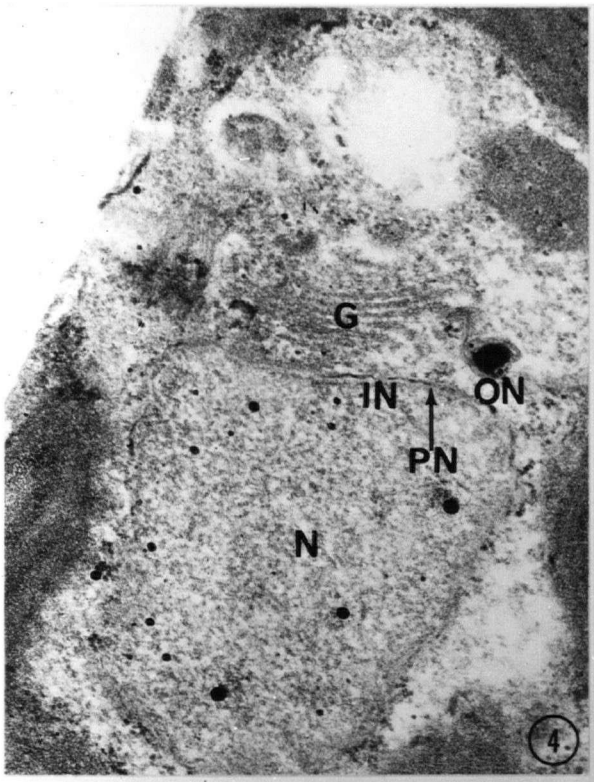
PLATE II

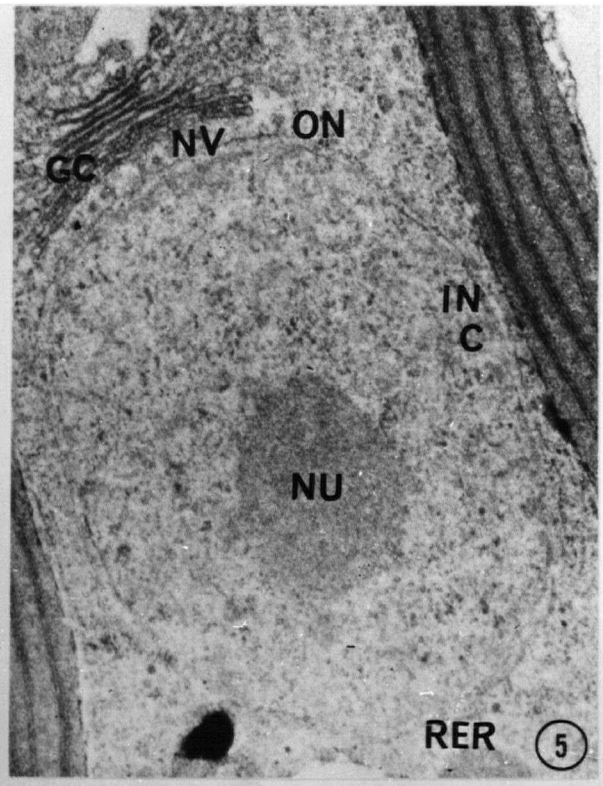
Figure 4. The interphase nucleus (N) of 100% RH cell. x 42,000

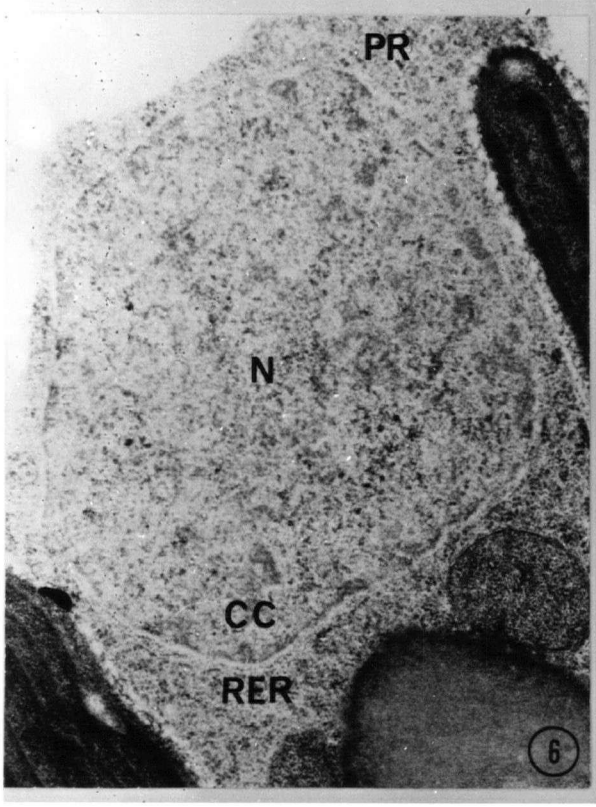
Figure 5. The nucleus of 60% RH cell. Attachment of the chromatin (C) to the inner nuclear (IN) membrane was seen. x 36,000

Figure 6. Chromatin condensation along the inner nuclear membrane (IN) in 50% RH cell. x 30,000

Figure 7. The nucleolus of 50% RH cell. x 32,000


G
IN
ON
PN
N
4


CC
NV
ON
IN
C
NU
RER
5


PR
N
CC
RER
6

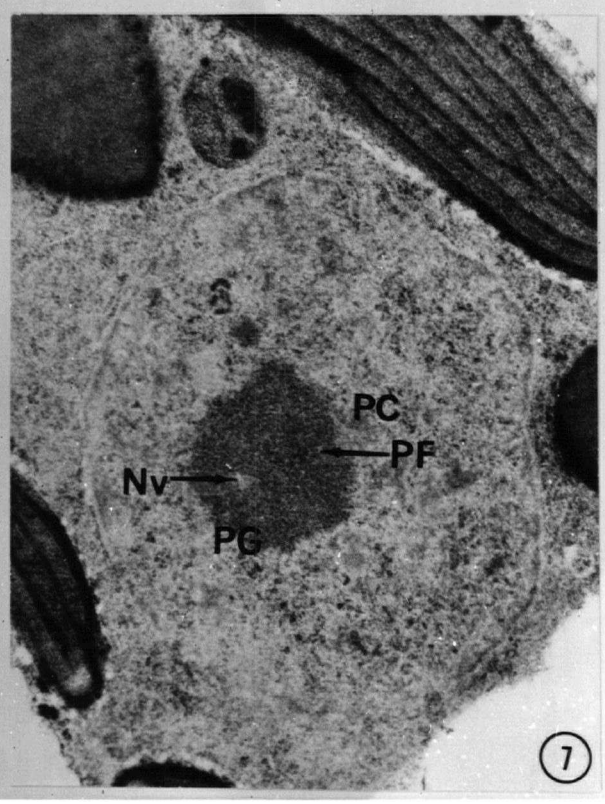

PC
PF
Nv
PG
7

PLATE III

Figure 8. The nucleus of 30% RH cell showed further chromatin condensation. Note that Golgi vesicles (GV) from the maturing distal face (Di) of the Golgi apparatus was interspersed with microtubules (MT). x 27,000

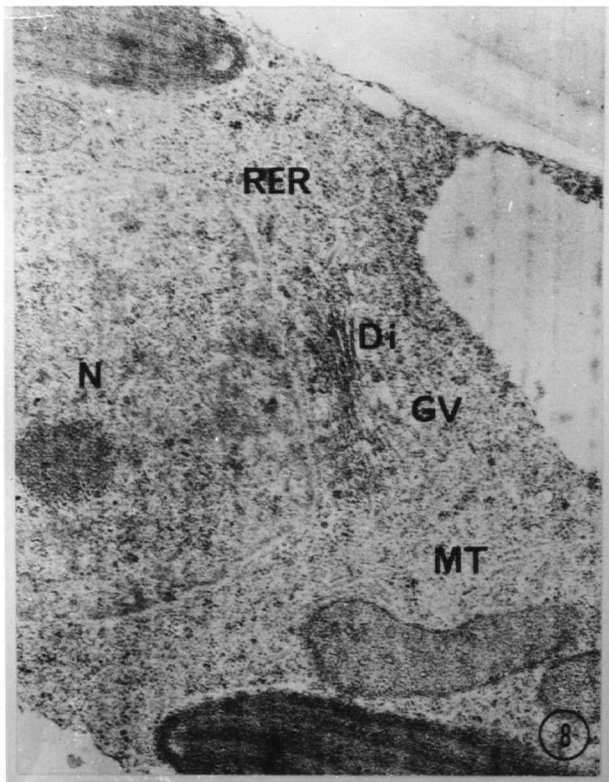
Figure 9. Increasing chromatin condensation (CC) occurred in 20% RH cell. The outer nuclear membrane extended to form the chloroplast endoplasmic reticulum (CER). x 42,000

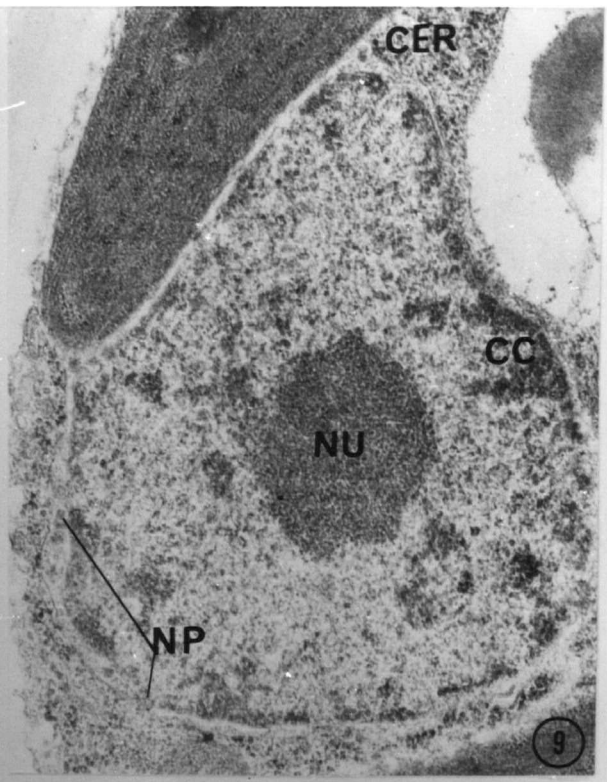
Figures 10 and 11.

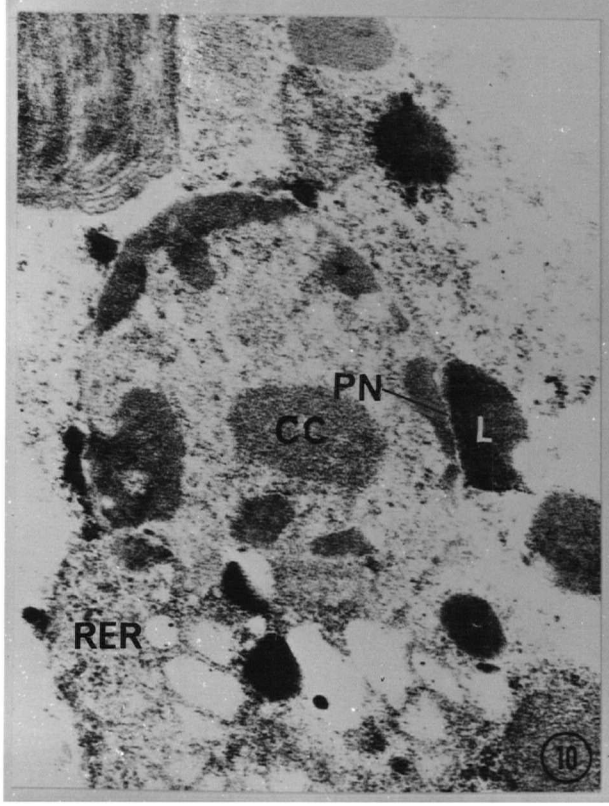
Cells of 10 and 0% RH, respectively, showed extreme chromatin condensation (CC). Association of lipid (L) around the outer nuclear membrane was seen. The cells of 0% RH had fibrous components in the pore region.

x 36,000

x 50,000


RER
Di
N
GV
MT
8


CER
CC
NU
NP
9


PN
CC
L
RER
10

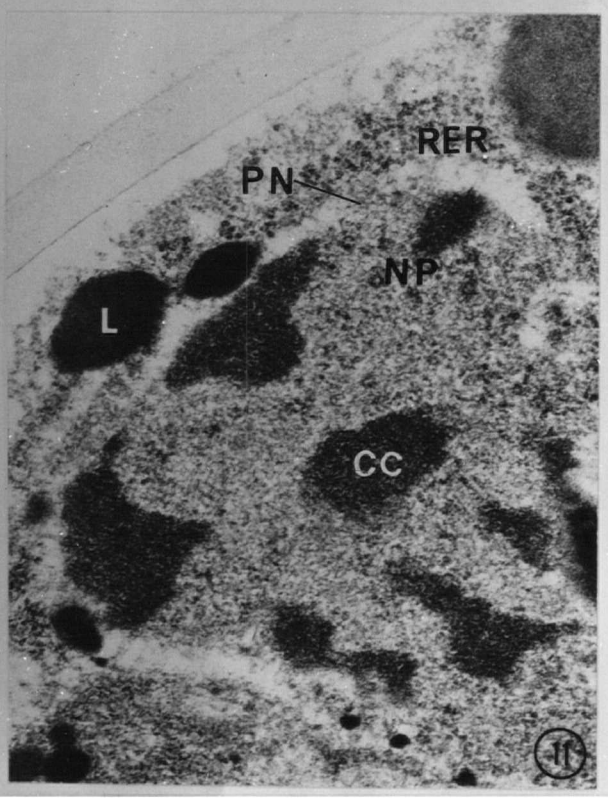

RER
PN
NP
L
CC
11

PLATE IV

Figures 12 and 13.

Mitochondria of 60% RH cells with electron transparent genophores containing DNA (MG).

x 48,000

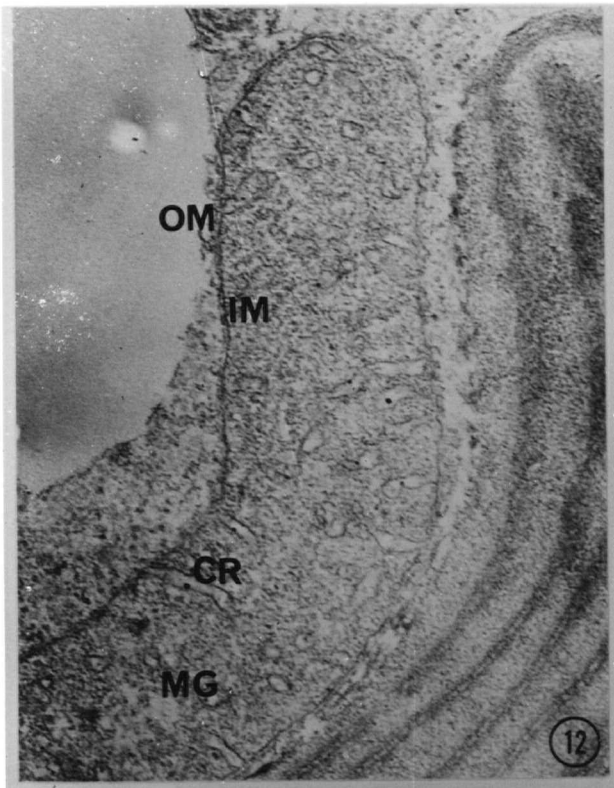
x 100,000

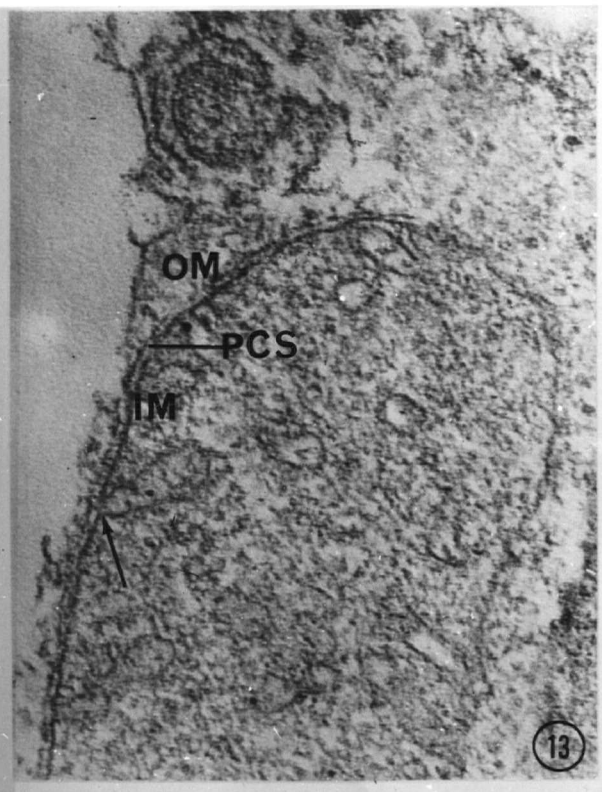
Figures 14 and 15.

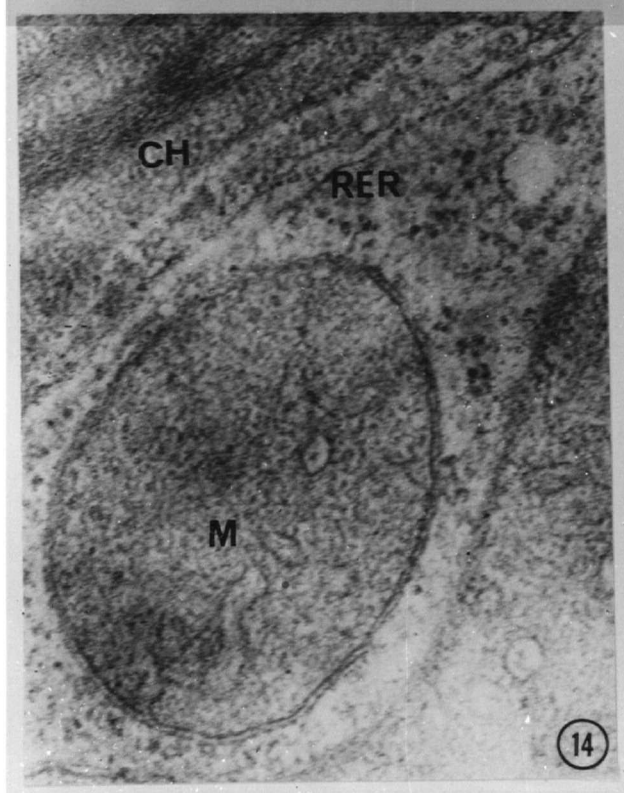
Increased density of the mitochondrial matrix (MM) in 20% RH cells was followed by the genophores becoming unrecognizable.

x 80,000

x 60,000


OM
IM
CR
MG
12


OM
PCS
IM
13


CH
RER
M
14

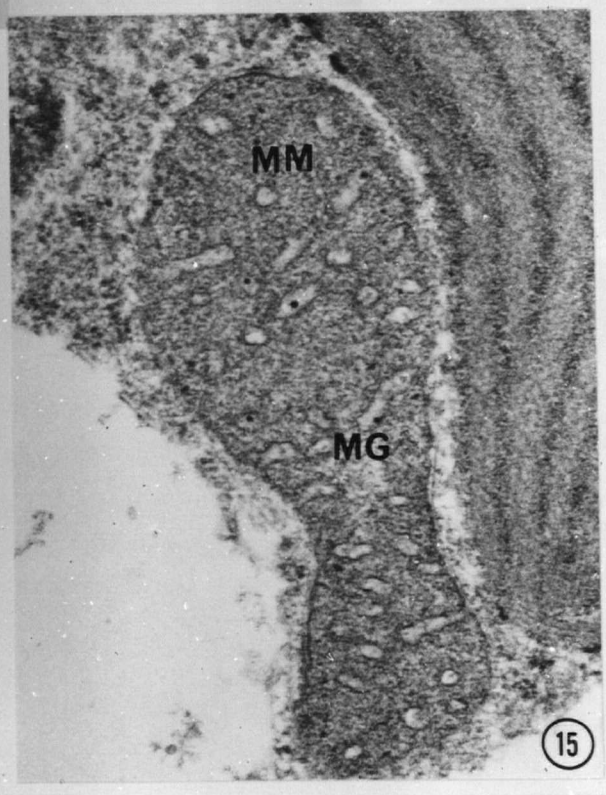

MM
MG
15

PLATE V

Figures 16 and 17.

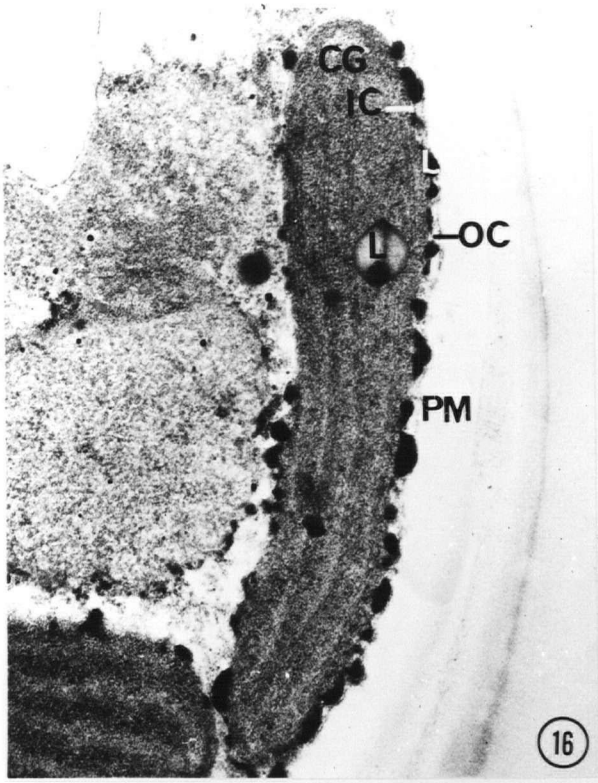
Peripheral chloroplasts of 100% RH cells occurred closely associated to the plasma membrane. Note that lipid droplets (L) were seen in the stroma (S), in the space between the two chloroplast membranes (OC and IC), and in membrane bound lipid storage areas (LS).

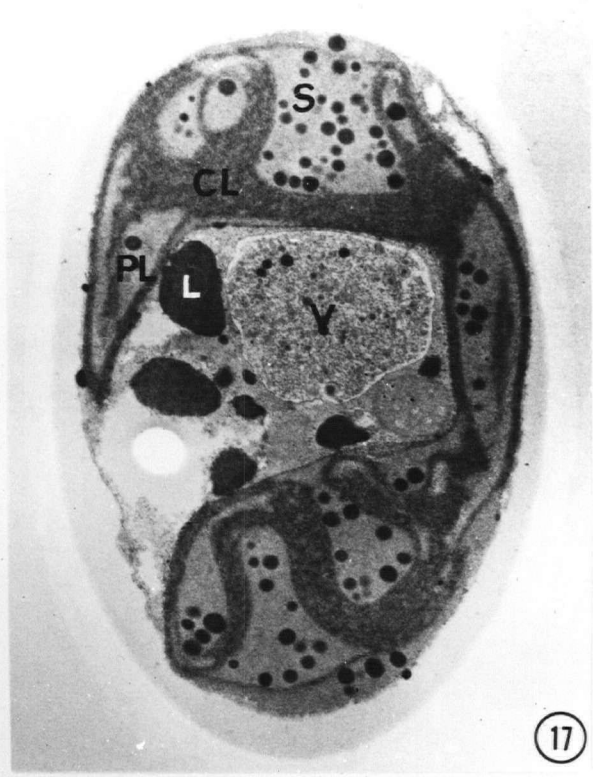
x 30,000

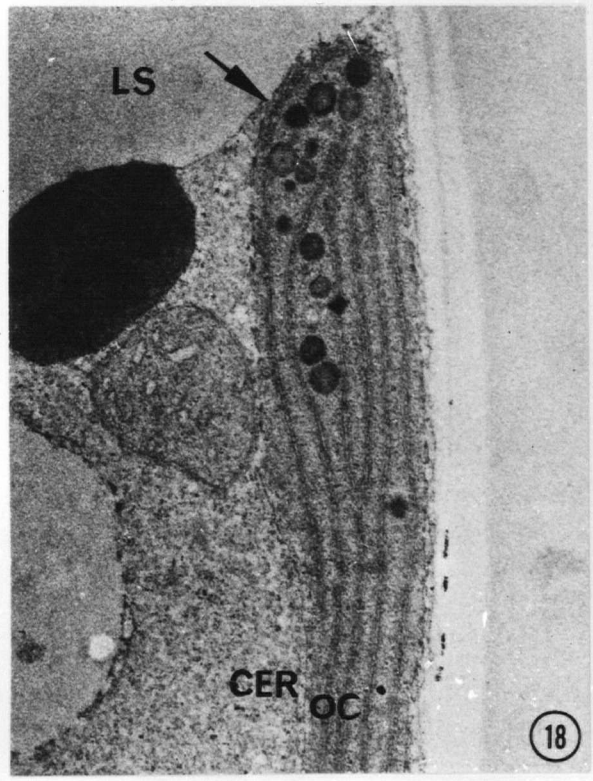
x 19,000

Figure 18. The outer chloroplast membrane of a 60% RH cell was closely associated to the chloroplast endoplasmic reticulum (CER) which appeared to be continuous with the lipid storage area. x 32,000

Figure 19. Along the length of a chloroplast of 60% RH cell, there was close association of the outer and inner chloroplast membranes. x 55,000


CG
IC
L
OC
L
PM
16


S
CL
PL
L
V
17


LS
CER
OC
18

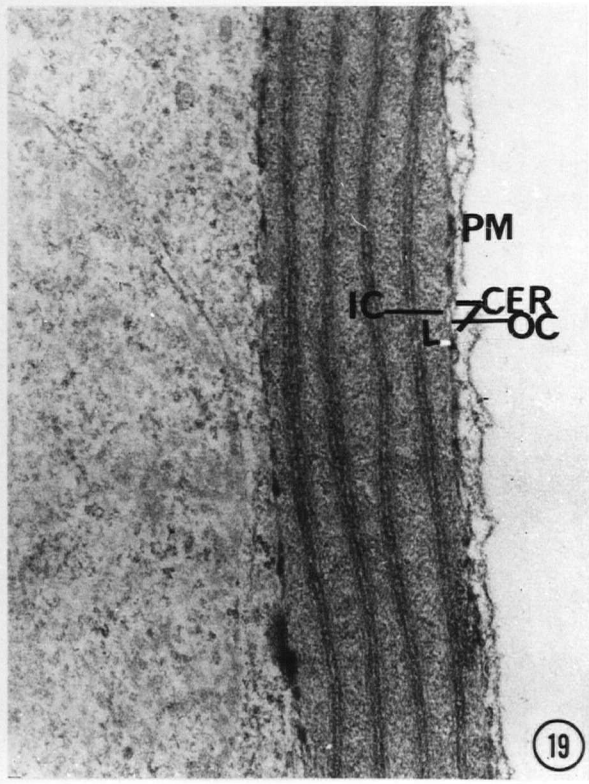

PM
IC
CER
L
OC
19

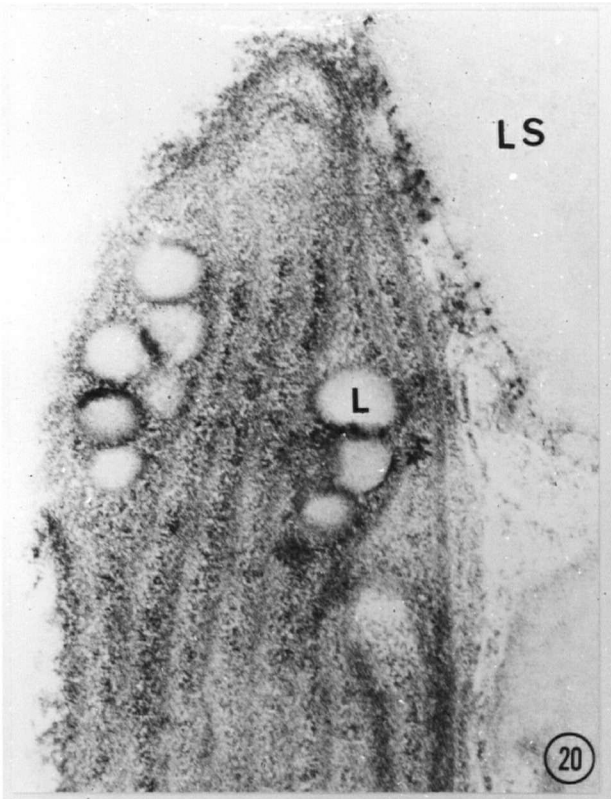
PLATE VI

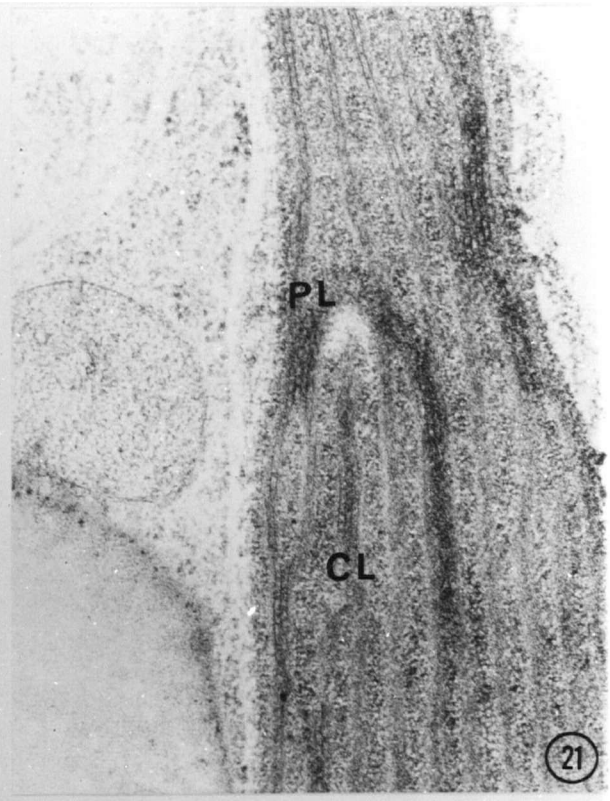
Figure 20. Chloroplast of a cell of 50% RH with a lipid producing area of the stroma in close proximity to the lipid storage area (LS). x 53,000

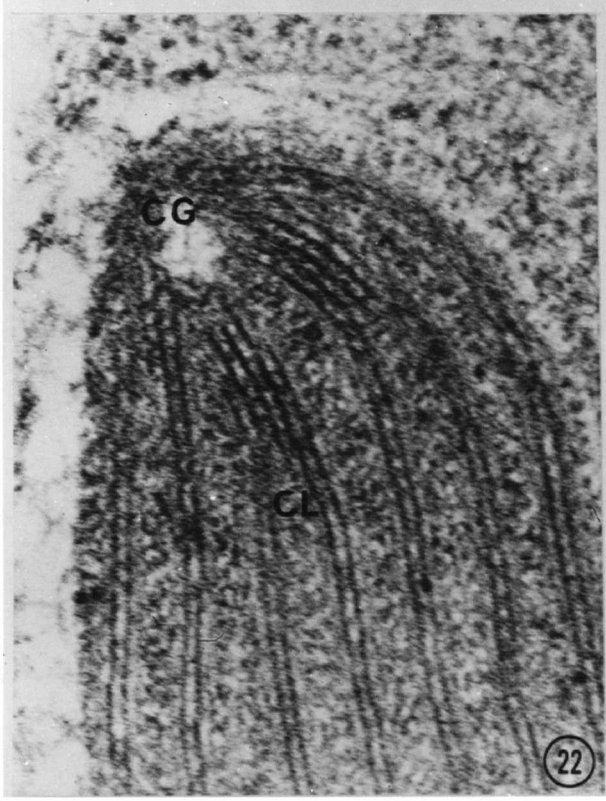
Figure 21. Oblique section through a chloroplast of a 50% RH cell showed peripheral (PL) and central (CL) lamellae associating with other bands along its length. x 55,000

Figure 22. Chloroplast of a 40% RH cell with closely associated or fused thylakoids. x 120,000

Figure 23. Chloroplast endoplasmic reticulum of a cell of 30% RH extended into the cytoplasm and appeared connected to the rough endoplasmic reticulum. x 36,000


LS
L
20


PL
CL
21


CG
CL
22

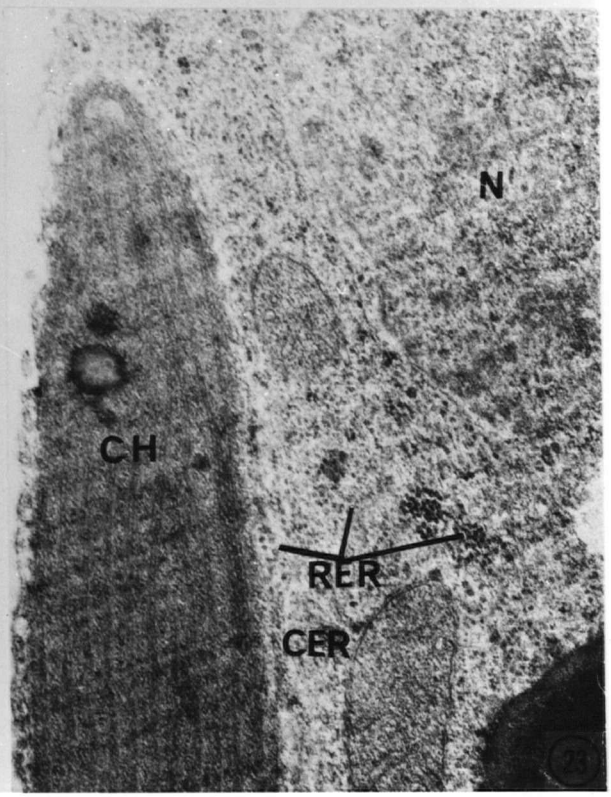

N
CH
RER
CER

PLATE VII

Figures 24 and 25.

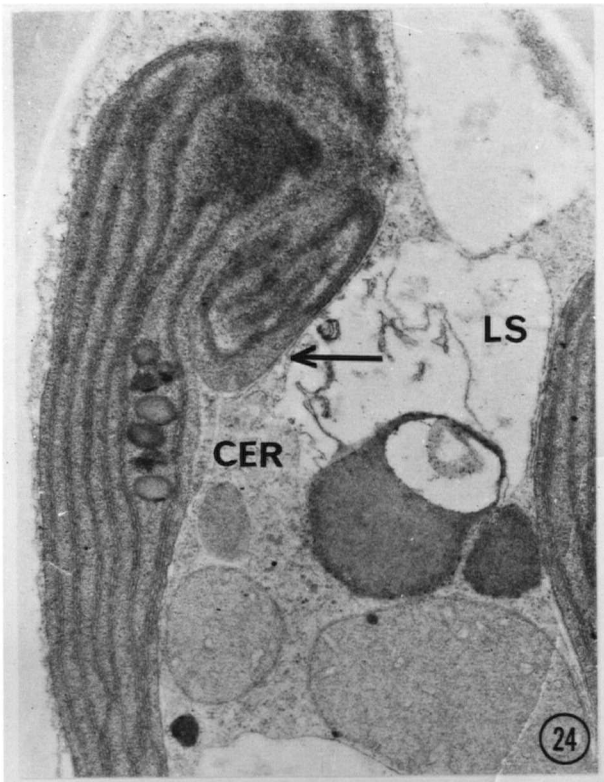
The chloroplast endoplasmic reticulum of a 20% RH cell appeared continuous (arrow) with the membrane surrounding the lipid storage area.

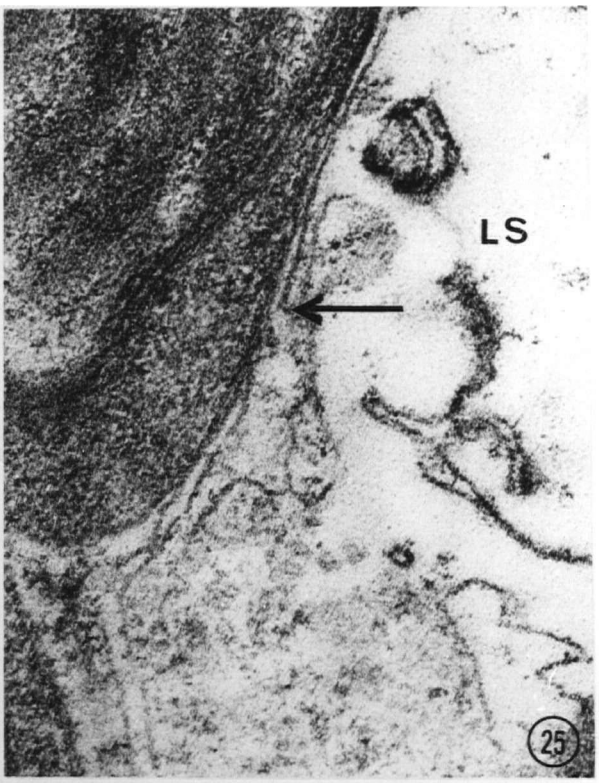
x 24,000

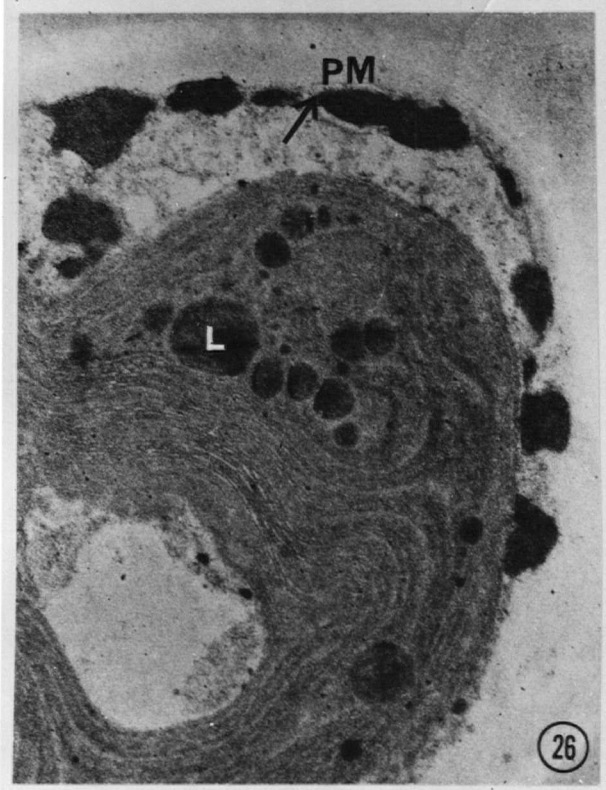
x 59,000

Figure 26. Lipid storage areas of 10% RH cell were closely appressed to the plasma membrane, but remained distinct from it. x 15,000

Figure 27. Peripheral and central lamellae of air-dried cells, 0% RH, were "negatively" stained. Note lack of stain uptake. x 30,000


LS
CER
24


LS
25


PM
L
26

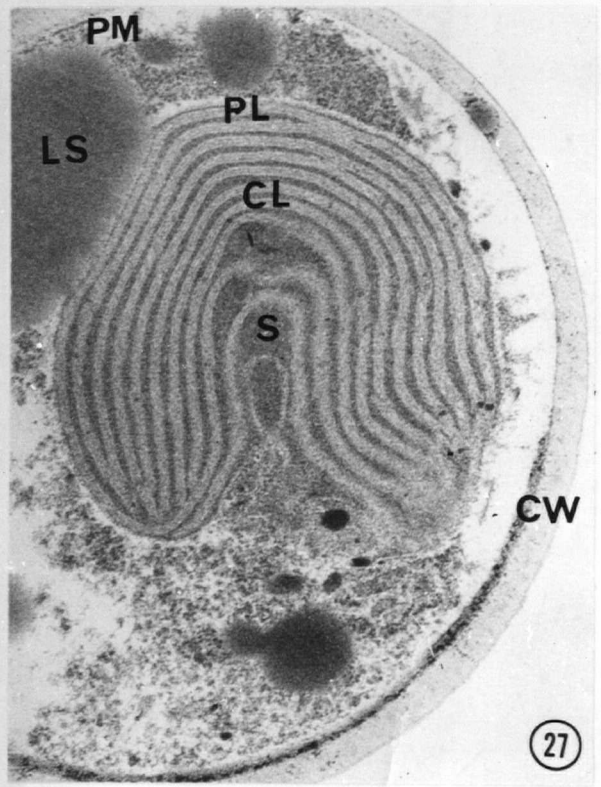
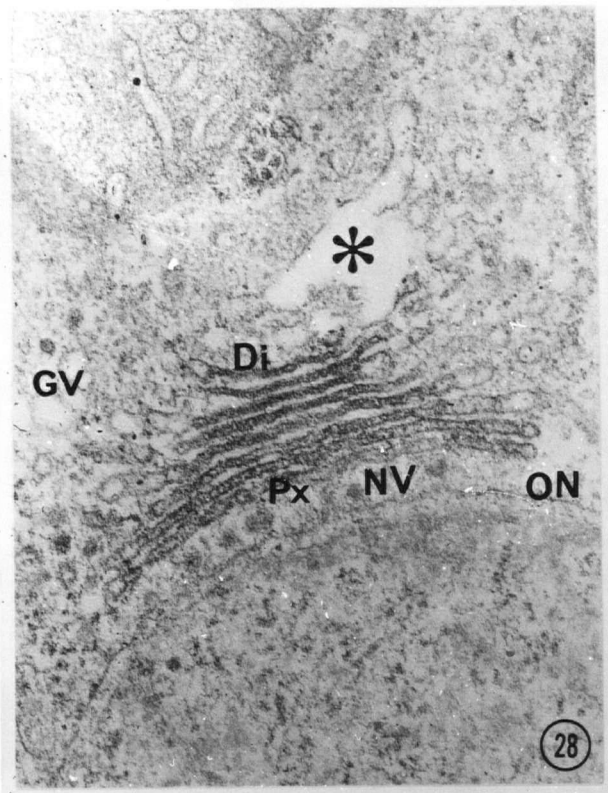

PM
PL
LS
CL
S
CW
27

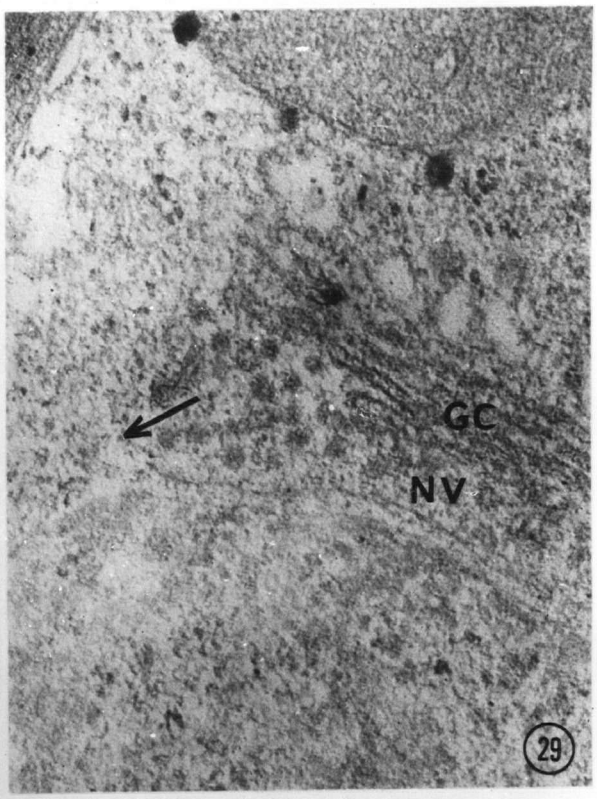
PLATE VIII

Figure 28. Golgi apparatus of a 60% RH cell with a large accumulating system (*) at its distal face (Di). x 66,500

Figure 29. Cell of 20% RH had nuclear vesicles (NV) which appeared directed towards the Golgi apparatus. x 76,000

Figure 30. Cell of 20% RH had Golgi vesicles blebbing off the mature face of the Golgi apparatus near the cell surface. x 60,000


*
Di
GV
Px
NV
ON
28


GC
NV
29

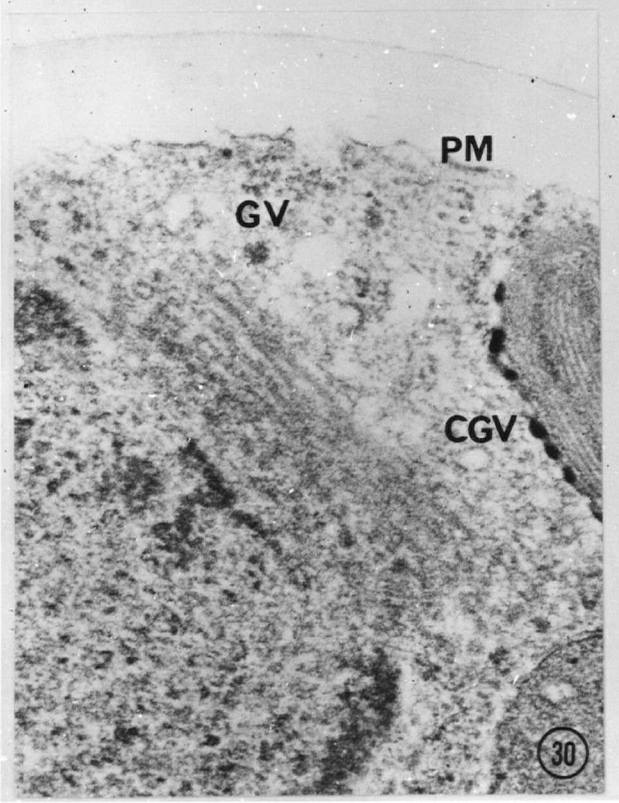

PM
GV
CGV
30

PLATE IX

Figures 31 and 32.

Cells of 20% RH had small vacuoles (V) containing vesicular and membranous inclusions.

x 30,000

x 95,000

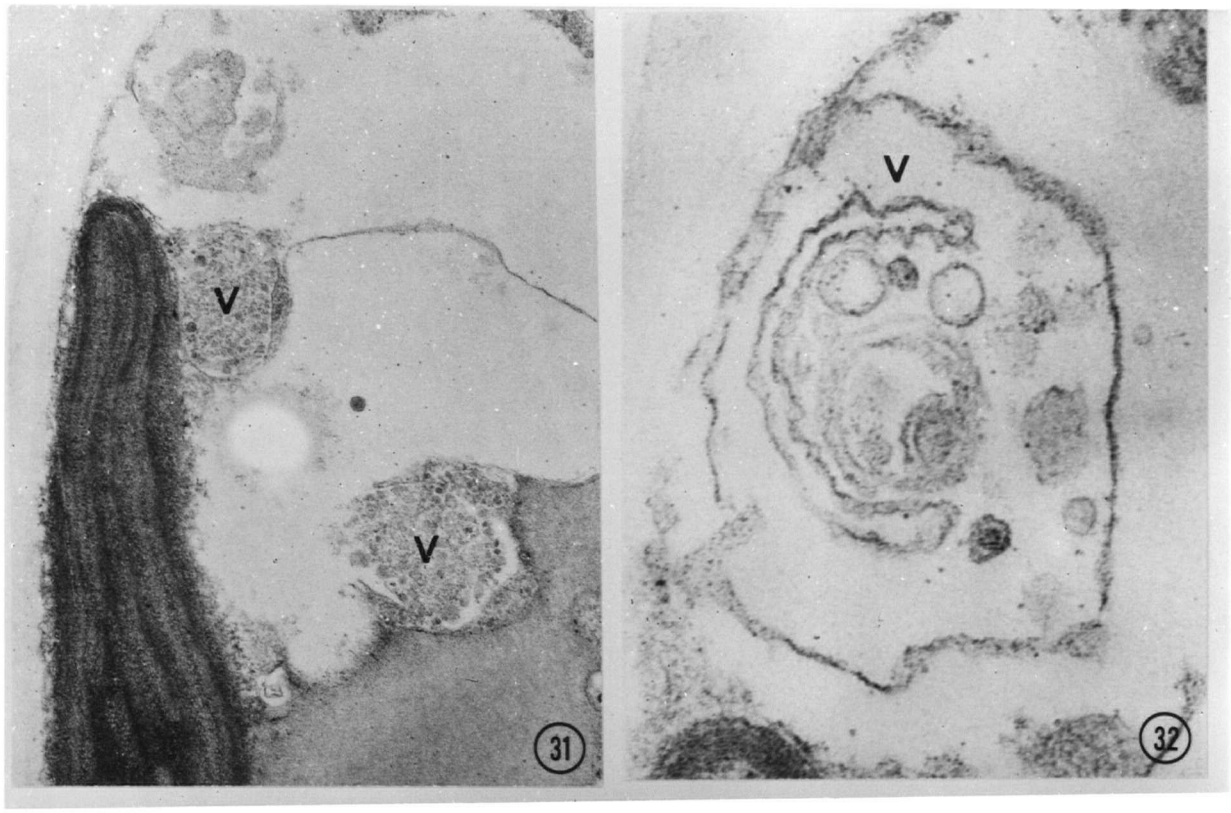
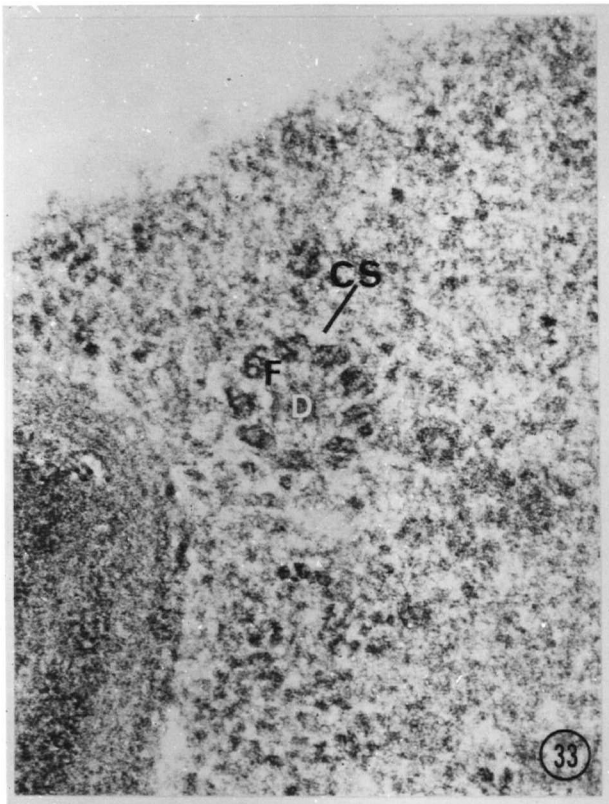
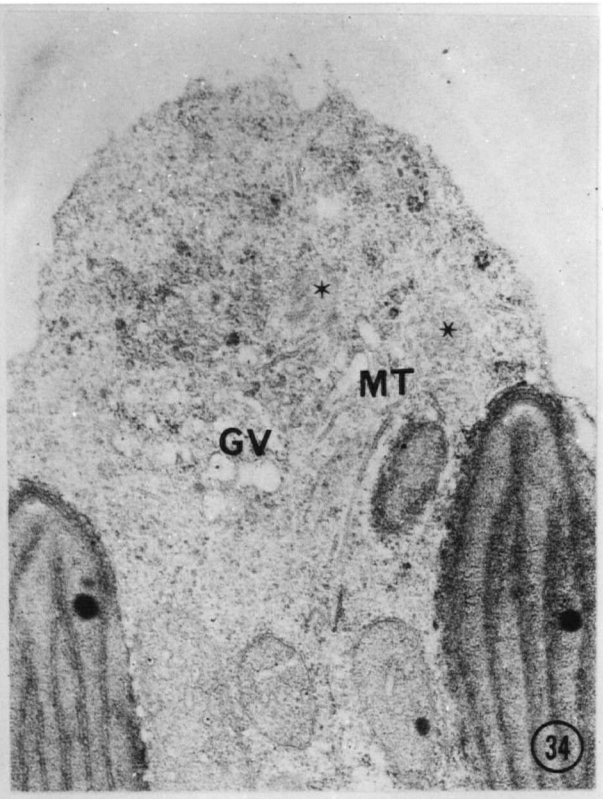

V
V
31
V
32

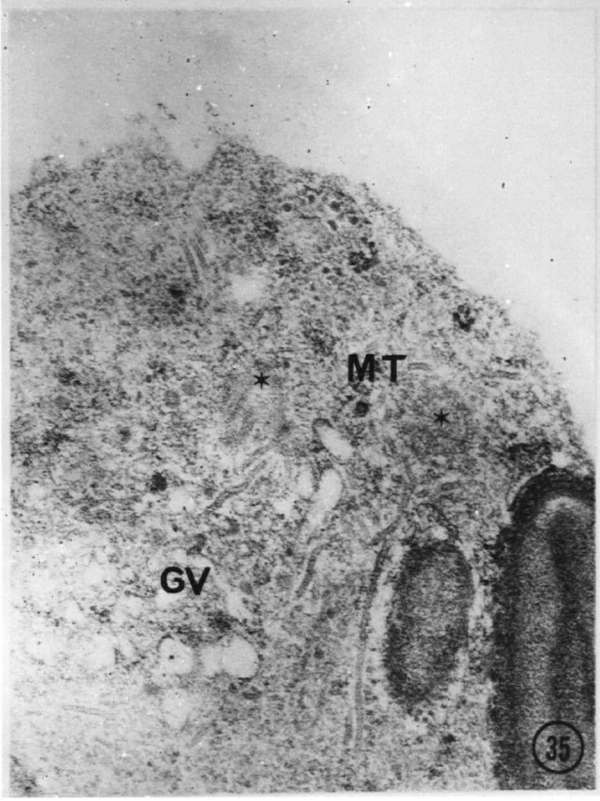
PLATE X

Figure 33. A pair of centrioles in a 100% RH cell.
x 102,000

Figures 34 and 35.
Cell of 60% RH with microtubules (MT) radiating from and running parallel to the centrioles(*). Note that the microtubules occurred near the surface and between Golgi vesicles (GV).
x 36,000
x 47,000


CS
F
D
33


MT
GV
34


MT
GV
35

APPENDIX

Modified Bristol's Medium

Add 10 ml of each of Bristol's stock solutions to 940 ml of distilled water and 1 ml of Hutner's trace element solution.

Bristol's Stock Solutions (Bold, H.C. 1949. Bull Torrey Club 76:101-108.)

Make six 400 ml stock solutions.

stock solution	quantity
$NaNO_3$	10.0g
$CaCl_2$	1.0g
$MgSO_4.7H_2O$	3.0g
K_2HPO_4	3.0g
KH_2PO_4	7.0g
$NaCl_2$	1.0g

Hutner's Trace Element Solution (Hutner, S.H., Provasoli, L., Schatz, A., and Hawkins, C.P. 1950. Proc. Amer. Phil. Soc. 94:152-170.)

Add salts to 75 ml distilled water. After each addition, adjust to pH 5.5. Boil, cool slightly and adjust to pH 6.5 with KOH pellets. Dilute to 100 ml.

material	quantity
EDTA	5.00g
$ZnSO_4.7H_2O$	2.20g
H_3PO_3	1.00g
$CaCl_2$	0.50g
$MnCl_2.4H_2O$	0.50g
$FeSO_4.7H_2O$	0.50g
$CoCl_2.6H_2O$	0.15g
$CuSO_4.5H_2O$	0.15g
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.10g