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Author:

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ULTRASTRUCTURAL AND CHLOROPHYLL FLUORESCENCE RESPONSES OF INTERTIDAL MARINE ALGAE TO DESICCATION

by

James Stanley Wiltens

B.A. University California, Berkeley, 1972

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
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Approval

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ABSTRACT

Ultrastructural and Chlorophyll Fluorescence Responses of Intertidal Marine Algae to Desiccation

Fluorescence induction of chlorophyll a was used as an indicator of the state of the photosynthetic apparatus during drying and rehydration. Desiccation causes a progressive inactivation of photosynthesis which is manifested by changes in transient fluorescence. This inactivation is similar in both tolerant and sensitive marine algae. The order of inactivation is: 1) Uncoupling of phosphorylation, a loss of photon transfer between photosystem II and I (alpha transitions), with a possible decrease in Calvin-Benson cycle activity, (2) loss of electron transport between photosystem II and I, (3) cessation of water-splitting, (4) alteration of the physical state of chlorophyll.

Rehydration of tolerant plants results in a rapid return of electron transport, which is followed within thirty seconds by water splitting.

Prolonged storage in the dried state was studied as well as the effects of osmotic desiccation.

A correlation is seen between tolerance and the intertidal site of the algae. Tolerant plants are always found in the high- or mid- intertidal zone.

Ultrastructural studies show a number of differences as well as similarities in the effects of desiccation on tolerant and sensitive marine algae:

1. Plasmolysis is minimal in tolerant algae presumably due to the absence of large vacuoles.

2. Drying may result in the formation of vesicles in both tolerant and sensitive algae. The appearence of vesicles is correlated with the appearence of disrupted mitochondria and the disappearence of endoplasmic reticulum and dictyosomes.

3. Mitochondrial cristae lose their staining properties in sensitive algae after drying. Tolerant algae still have visible cristae even after drying.

4. In sensitive algae the chloroplast envelope is often ruptured by drying; while in tolerant algae the envelope is maintained intact.

5. Drying may result in reversible shrinkage in thylakoid width and membrane thickness in Porphyra sanjuanensis.

6. In sensitive algae the nuclear membrane was often found to be disrupted after drying, and the nuclear material was no longer diffuse but appeared condensed.

7. Rehydration of lethally dried material results in cellular disintegration.

8. Rehydration of tolerant species promotes the reappearence of endoplasmic reticulum and dictyosomes as well as a loss of the numerous vesicles produced by drying.

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I would like to thank my major supervisor, Dr. W. Vidaver, for providing numerous opportunities and encouragement throughout my graduate career. I am also grateful to Dr. L. Srivastava and Dr. L. Druehl for their helpful comments during the preparation of this thesis.

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List of Symbols Used in the Figures

C --------- Chloroplast

CW -------- Cell wall

D --------- Dictyosome

ER -------- Endoplasmic reticulum

L --------- Lamellae traversing pyrenoid

M --------- Mitochondria

N --------- Nucleus

Nu -------- Nucleolus

p --------- Pyrenoid

S --------- Starch

T --------- Thylakoids

V --------- Vesicle

Va -------- Vacuole

Viable ---- Resumes photosynthetic activity on rehydration.

Not viable- Photosynthetic activity is irreversibly inactivated.

CHAPTER I

INTRODUCTION

Photosynthesis can be inactivated by desiccation. This inactivation may be permanent or reversible, dependent on whether the plant is tolerant or sensitive to dehydration. Resistant plants enter a 'suspended state' during which measurable metabolic activities such as respiration and photosynthesis cease. Tolerant species respond to rehydration with a resumption of activity whereas sensitive plants are permanently inactivated and non-viable. Some tolerant plants remain viable for long periods when dehydrated such as Grimmia which has been kept over H_2SO_4 for up to 22 weeks (Schroder 1886). Sensitive species of moss such as Brachythecium and Fontinalis could not survive dehydration for even three days (Lee and Stewart 1971), whereas some vascular plants succumb when the relative humidity drops below 98% (Levitt, Sullivan and Krull 1960). It is not yet clear how desiccation inactivates photosynthesis nor why some plants are sensitive and others tolerant.

Desiccation resistance has long been known to be a factor in zonation patterns where a moisture stress is involved. Viability tests after drying show that there are

differences in the ability of algae to survive dehydration (Biebl 1952, Muenscher 1915; Ogata and Matsui 1965). The vertical distribution of algae in the intertidal zone may be determined by the length of exposure the alga can withstand at low tide (Doty 1946, Zanveld 1937). Mosses (Hosokawa and Kubota 1957) and lichens (Lee and Stewart 1971, Rogers 1971) also display a distribution pattern dependent on their desiccation tolerance. In the higher plants there is a variation in the water loss necessary to produce death (Hofler, Migsche and Rotenberg 1941), but only a few species, such as Myrothamus (Hoffmann 1968) and Chamaegigas (Hickel 1967), can enter what Genkel and Pronina (1968) refer to as an "anabiotic state" in which respiration and photosynthesis are suspended. An anabiotic state enables a plant to survive drought conditions.

At the sub-cellular level the effects of desiccation have been studied for a number of processes. In the alga Fucus, progressive drying results in a decline in respiration (Kanwisher 1957), while some mosses (Genkel and Pronina 1968) and higher plants (Brix 1962; Domien 1949) may exhibit a respiratory increase with a limited amount of drying. Studies on a number of algae (Gessner 1971; Imada et al 1970), mosses (Lee and Stewart 1971), and vascular plants (Brix 1962; Graziani and Livne 1974; Pieters and Zima 1975; Schneider and

Childers 1941) show a steady decline in photosynthetic oxygen production with water loss; there are however plants in which photosynthesis is stimulated by moderate dehydration (Johnson et al 1974; Rabinowitch 1945). In the tolerant alga Porphyra perforata, Fork and Hiyama (1973) found that cytochrome f reduction was reversibly inhibited by drying, which may account for photosynthetic inactivation. Analyzing the effects of desiccation on photosynthesis in higher plants is complicated by the problem of stomatal closure induced by drying. Closure of the stomates reduces CO_2 availability and inhibits photosynthesis indirectly (Brix 1962; Graziani and Livne 1974; Vaadia, Raney and Hagan 1961). Cell free studies with chloroplasts from higher plants show that oxygen production (Boyer and Bowen 1970) as well as photophosphorylative and photoreductive capacity are reduced under dehydrating conditions (Nir and Poljakoff-Mayber 1967; Santarius 1969). Santarius (1969), studying isolated chloroplasts, came to the conclusion that high electrolyte concentrations, as a result of drying, may be responsible for inactivation of photophosphorylative and electron transport processes.

Ultrastructural alterations have also been noted in the membranes of dried plant material. Nir et al (1967) and Petrovich (1973) report that sections from flaccid or

osmotically stressed leaves show clear distortions of the chloroplast intergranal lamellae, and it has been known for some time that hypertonic conditions, as might be caused by drying, result in thylakoid conformational changes (Gross and Packer 1967; Murakami and Packer 1970). Other cellular changes include vesiculation of the cytoplasm, nuclear disruption and loss of osmium uptake by mitochondrial cristae (Cohenn et al 1974; Nir, Klein and Poljakoff- Mayber 1969).

A number of theories have been advanced to explain desiccation tolerance. The simplest theory is that mechanical stresses caused by dehydration are responsible for membrane damage and are avoided in resistant plants by small cell size, cell filling colloidal substances and a lack of plasmodesmata (Iljin 1927, 1930, 1931, 1935). It has also been proposed that drying leads to protein denaturation by causing disulphide linkages (Gaff 1966), with the implication that proteins in tolerant plants are protected from desiccation induced denaturation. A number of glycosylated fungal proteins have been shown to be desiccation tolerant due to the carbohydrate moiety (Dabyshire 1974). Santarius (1969) found that high sugar concentrations can protect photosynthetic activity in desiccated chloroplasts, and he points out that the pentoses he uses may form stable sugar-protein complexes. In mosses, Bewley (1972a, 1972b, 1973) and Gwozdz and Bewley (1975) find

that tolerant species can conserve their protein synthesizing apparatus during drying, a characteristic not found in sensitive species.

It is the purpose of this study to analyze at photochemical and ultrastructural levels the effect of drying and rehydration on photosynthesis in desiccation tolerant and sensitive marine algae. The plants used were mainly Porphyra species, although comparisons were made with other algal types. Porphyra is an excellent material for this particular analysis as it has a flat thallus which is one cell layer thick, and the genus contains both sensitive and tolerant species.

Much of this work is based on data obtained from chlorophyll a fluorescence induction. Fluorescence induction has the unique property of giving information about photosynthesis at the molecular level during drying; most other techniques are unsuitable for desiccation studies as they require an aqueous media in which to work. Also used were manometry for respiration measurements, the Pt-electrode for oxygen determinations during rehydration, and electron microscopy for an analysis of ultrastrusctural changes induced by drying and rehydration.

Our results show that desiccation causes a progressive inactivation of photosynthesis. This inactivation is similar in both tolerant and sensitive species. The order of inactivation is: uncoupling of phosphorylation, a loss of photon transfer between photosystem II and I (alpha transitions) with a possible decrease in Calvin-Benson cycle activity ⟶ loss of electron transport between photosystem II and I (PSII and I) ⟶ cessation of water splitting ⟶ alteration of the physical state of the chlorophyll.

Upon rehydration in tolerant plants there is a rapid return of electron transport, followed within 30 seconds by water splitting.

At the ultrastructural level a number of differences and similarities were noted between tolerant and sensitive algae. The sensitive algae were often found to have plasmodesmata and large vacuoles; characteristics which may contribute to a mechanical strain during drying. Dehydration results in the formation of vesicles in the cytoplasm of both tolerant and sensitive algae. Mitochondrial cristae do not readily stain with osmium in sensitive algae, while in tolerant algae they appear normal. Dehydration of a tolerant alga results in reversible shrinkage of thylakoids.

CHAPTER II

MATERIALS AND METHODS

The following algae were used in this study: Porphyra perforata J. Agardh., Porphyra sanjuanensis Krish., Porphyra schizophylla Hollenberg, Porphyra miniata (C. Agardh) C. Agardh., Nitophyllum notti Norris and Wynne., Polyneura latissima Harvey and Kylin., Porphyridium cruentum Nageli, Prasiola meridionalis Setchell and Gardner., Ulva scagelii Chihara, Enteromorpha linza (L.) J. Agardh., Petalonia fascia (O.F. Mull.) Kuntze. All of the marine samples ,except Porphyridium, were collected at Stanley Park near Brockton Point or Whytecliff Park, Vancouver, British Columbia. Porphyra species were identified by Dr. T. Mumford (University British Columbia) from pressed specimens. Ulva scagelii was identified by C. Tanner (Univ. British Columbia). The other algae were identified using keys by Scagel (1966) or Widdowson (1974a, 1974b).

Samples were transported to the lab in polyurethane bags containing sea water kept at 11 ± 3 C. The algae were then rinsed and maintained in millipore filtered sea water at 11 C, 16 hours dark, 8 hours light. Specimens were discarded if not used in three to four days. Porphyridium cultures from

the algal collection at the University of British Columbia were maintained in artifical sea water under continuous illumination according to Jones et al (1963).

Relative fluorescence yield as a function of time was recorded with an apparatus similar to that described by Franck et al (1969). A broad band of blue light was obtained with a projection lamp (G.E. model DLS-95) in combination with blue filters (Corning, CS 4-92, 6mm). Fluorescence was detected through a 4mm far-red cut off filter (Corning CS 7-69) with an EMI photomultiplier (9558B) held at 600 volts. The length of illumination was regulated with a camera shutter (Prontor Press). Standard illuminating intensity, as measured at the surface of the sample, was 3×10^4 erg/cm^2,sec. To maintain the photomultiplier in a stable range, neutral density filters (Balzers no. 5, 26.4%) were inserted between the sample and the photomultiplier when the oscilloscope (Tektronix 5103N or Hewlett Packard 132A) went off-scale at 1 volt per division. Bifurcated fiber optics (Schreiber and Vidaver 1973) directed excitation light to the sample and conducted emitted fluorescence to the photomultiplier.

Field measurements of fluorescence were done with a newly designed device (Schreiber, Groberman and Vidaver 1975) in conjunction with a portable oscilloscope (Tektronix 214).

Simultaneous fluorescence and oxygen measurements were made with a Haxo-Blinks type Pt-electrode as modified by Vidaver (1965).

Samples were dried by one of three methods. (1) The thallus was stretched over a plastic-ring and held in place with a neoprene ring. The specimen was then placed in a holding chamber, containing a window through which fluorescence measurements could be made. Drying was accomplished by a stream of drierite-cotton filtered air being directed through an inlet over the sample. (2) Samples were also desiccated by being left in the open air on a bench in the laboratory. (3) The last method was to suspend the plants from a rack at 1.1 degrees centigrade in an incubator. All three techniques were found to give essentially the same results.

An artificial thallus of Porphyridium cruentum cells was formed by filtering approximately 7×10^6 cells on to Whatmann # 1 paper supported on a plastic ring, which was subsequently treated like an algal thallus.

Osmotic desiccation was done with concentrated sea water. Fresh sea water was evaporated over a burner to 2, 3, and 4 times the normal salinity. A refractometer (American Optical Co. cat. 10402) was used to check the salinity of the

solutions. Osmolarity was determined by the freezing point depression method using an Osmette (Precision systems). Samples were submerged 15 minutes in the desiccant prior to fluorescence readings.

Water content determinations were analysed by the formula: (desiccated sample - oven dry weight / saturated sample - oven dry) x100. Oven dry weight was determined by drying the plants at 100 C for 24 hours. The saturated sample was defined as a plant which had been removed from sea water and blotted of excess water.

Water and inhibitors were added to the samples via a syringe inserted into the holding chamber. A saturated solution of 3-(3,4-dichlorophenyl), 1- dimethylurea (DCMU) was made with millipore filtered sea water.

Respiration was measured by the Warburg technique, utilizing a Gilson respirometer (Model G20). To each Warburg vessel was added 3 g of drierite in the side arm reaction flask and 0.5 ml of 6 N KOH was pipetted into the center well. Flasks were then sealed in aluminum foil and submerged in a water bath at 10 ± 1 C. Readings were taken every ten minutes after an initial equilibration. Rehydration was accomplished by disconnecting the flasks and adding 10 ml of millipore filtered sea water.

Specimens for electron microscopy were either minced in 11 C sea water or chopped while dry. Samples were then immersed in 4% glutaraldehyde made up with 0.13 M sodium cacodylate buffer mixed with half sea water, pH 6.6, and held on ice for two hours. Fixed material was rinsed thoroughly in sodium cacodylate buffer then post-fixed in cold OsO_4 and allowed to come to room temperature over two hours. The tissue was then rinsed in buffer and dehydrated through a graded ethanol series. Embedment was done in Spurrs low viscosity medium (Polysciences kit) according to Spurr (1969) or araldite according to Luft (1961). Sections were cut with a glass knife on a Reichert (OM U2) ultramicrotome and transfered to formvar-carbon coated grids. Sections were then stained with lead citrate (Venable and Coggeshall 1965). Samples were observed with a Zeiss EM-9A or a Philips EM 300 microscope. All specimens used for measurements were fixed and embedded using the same technique. Measurements were made at a high print magnification with a magnifying micrometer.

CHAPTER III

Effects of Dehydration and Rehydration on Photosynthesis

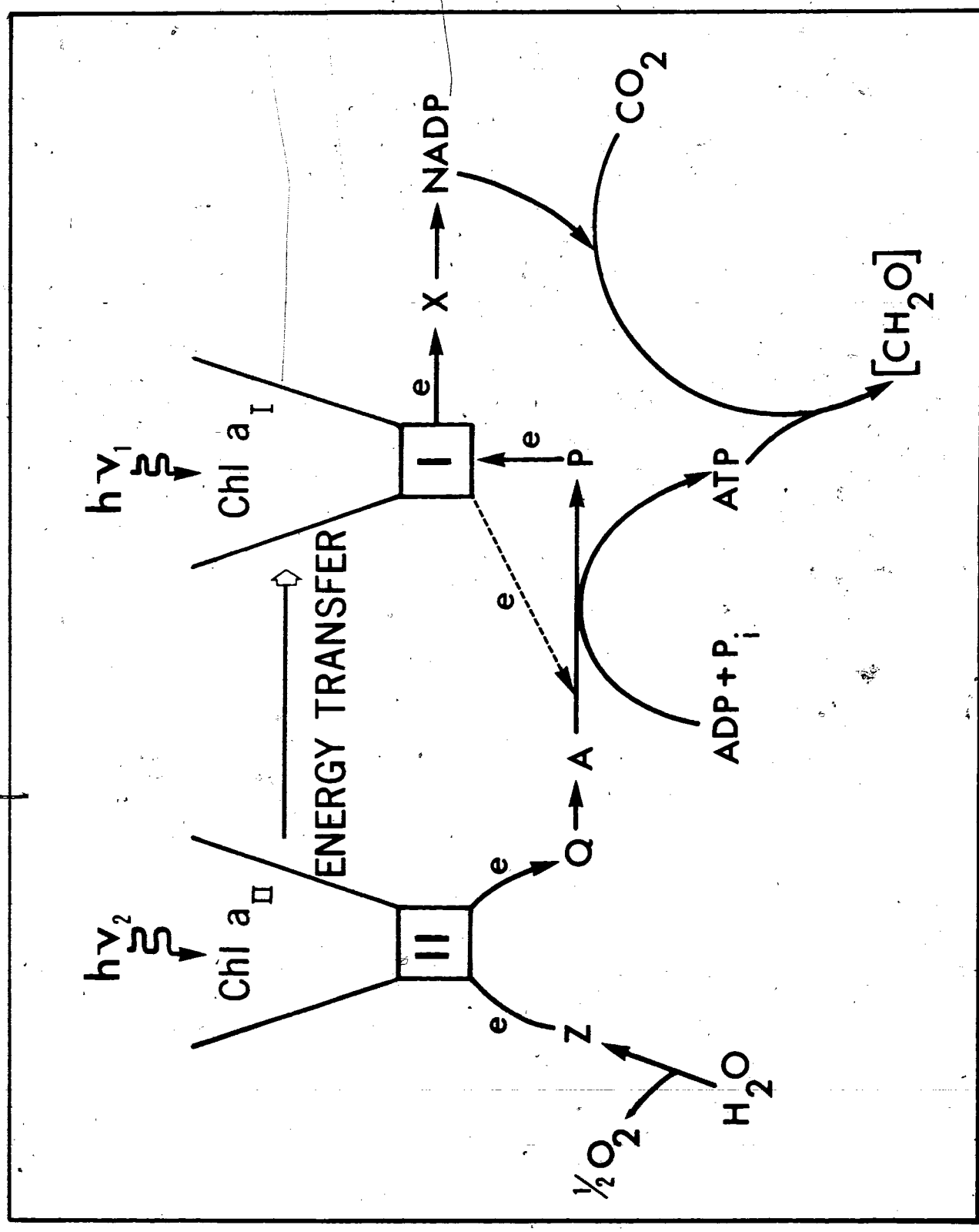
Introduction

To serve as a frame of reference in the following discussion a simplified scheme of the light and dark reactions of photosynthesis has been included (Fig. 1). The two funnels represent the bulk or antennae pigments which transfer absorbed energy, hv 1 or hv 2, to reaction center II or I. There is also a possible transfer of energy from chl a II to chl a I (alpha transfer). Z is the primary electron acceptor from water, while Q, A, and P represent an electron transport chain between photosystem II and I (PS II and PS I). X is the primary acceptor after PS I. The products of photosynthesis, ATP and reduced NADP, are shown entering the Calvin-Benson Cycle.

FLUORESCENCE INDUCTION STUDIES: Theory and Interpretation of Fluorescence Transients

In a following section, induction of chlorophyll a fluorescence was used as an indicator of the state of the

Figure 1. Series formulation of photosynthesis, simplified model. The funnels represent the light-trapping pigments, and hv2 and hv1 are quanta absorbed in PSII and PSI. P680 is the reaction center of PSII and P700 of PSI. Z and Q are primary electron donor and acceptor of PSII while P and X are primary donor and acceptor for PSI. The arrow between funnels indicates the alpha-transition of Bonaventura and Myers (1969). Electrons are represented by e.


hν1
Chl a I
hν2
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I
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NADP
CO2
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ATP
A
ADP + Pi
Q
Z
H2O
½O2

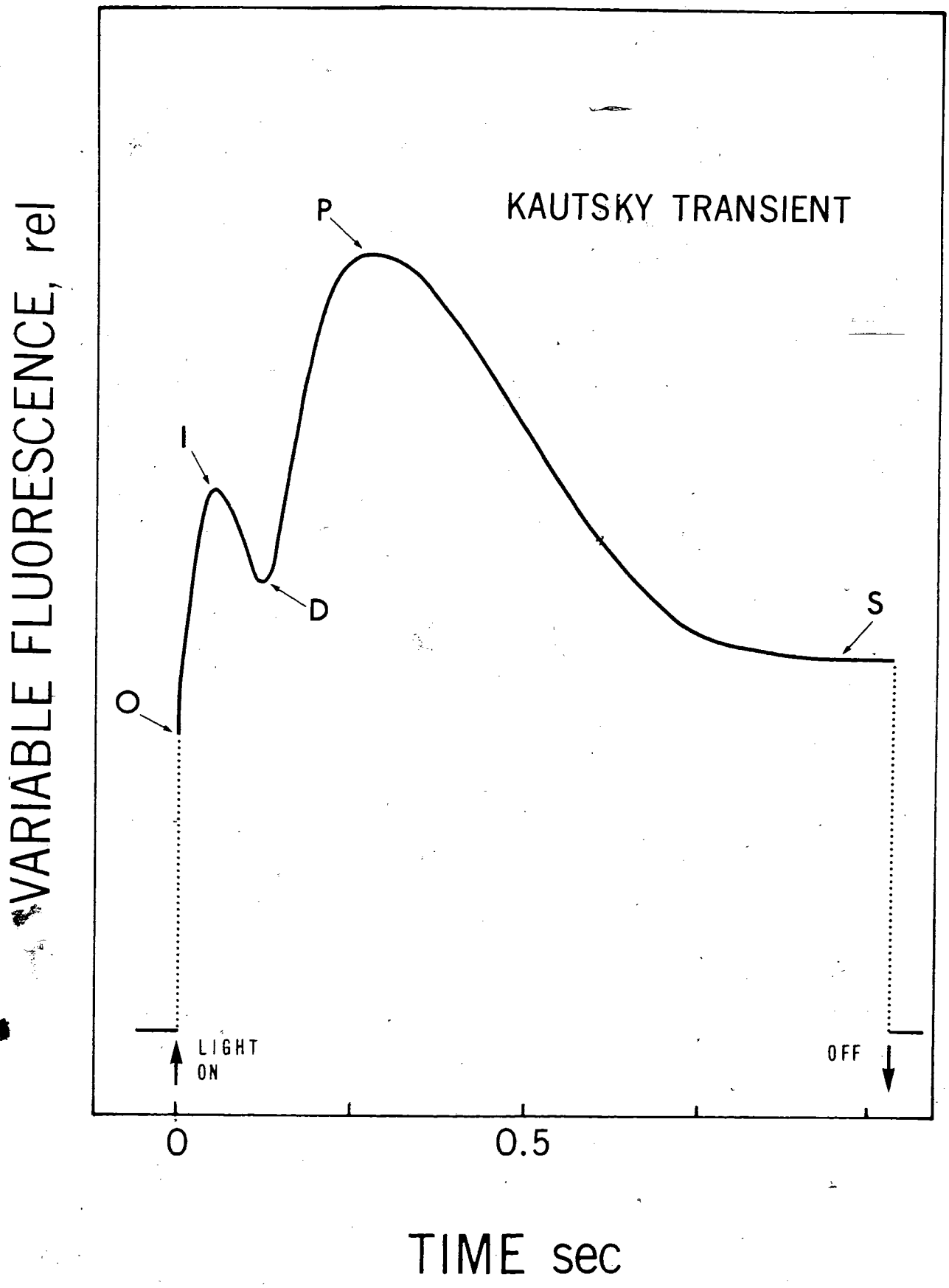
photosynthetic apparatus. The variable fluorescence curves produced, known as Kautsky transients, can give detailed information about photosynthesis at the photochemical level. Since desiccation induces certain changes in the transients, dependent on the degree of drying, it is a method of ascertaining how drying causes an eventual cessation of photosynthesis. But before a meaningful analysis of the effect of desiccation and rehydration on transients can be made it is necessary to consider fluorescence induction and what portions of the Kautsky transient (Fig. 2) represent.

During the first seconds of illumination complex fluorescence transients occur. The fluorescence yield can be described by the following equation:

$$F = \frac{Kf}{Kh + Kp\ Q + Kf + Kt} I$$

Where F, Kh, Kp, Q, Kf, Kt, and I stand for: yield of fluorescence, rate of heat loss, rate of photochemistry, concentration of oxidized quencher, rate of fluorescence, rate of alpha transfer, and absorbed intensity respectively (modified from Munday 1969 and personal communication U. Schreiber). The level of fluorescence is generally dependent

Figure 2. Typical variable fluorescence produced by illumination of photosynthesizing plants, Kautsky effect. Nomenclature O-I-D-P-S from Munday and Govindjee (1969). O=initial variable fluorescence, I= first peak, D=dip, P=second peak, S= second dip. The dotted line represents the rise from base-line to variable fluorescence.


KAUTSKY TRANSIENT
P
I
D
S
O
VARIABLE FLUORESCENCE, rel
LIGHT ON
OFF
0
0.5
TIME sec

on the redox state of a quencher substance, Q. Portions of the transient time curve have been designated O, I, D, P, S, (Fig. 2) and can be correlated with the activity of the different parts of the photosynthetic process.

The slope of the O-I rise is intensity- dependent and purely photochemical (Delosme 1967). Inhibitors of electron transport remove the D-P-S transient but not the O-I rise (Mohanty, Papageorgiou and Govindjee 1971). Electron donors such as dithonite, which act near PSII, do not affect the O-I transient (Schreiber, Bauer and Franck 1971). These results suggest that the O-I rise is associated with PSII prior to intersystem electron transport. Blue light pre-illumination, exciting preferentially PSII, increases the O-level in anaerobic samples (Schreiber, Bauer and Franck 1971), an effect that would be expected if a quencher were reduced by preillumination. The O-I rise is thought to represent the photoreduction of Q by PSII (Duysens and Sweers 1963; Kautsky, Appel and Amann 1960).

The I-D dip has been shown to represent PSI mediated fluorescence quenching. Using anaerobic samples and flashing light containing PSI and II actinic beams it has been found that the I-D decline is hastened by PSI light (Munday and Govindjee 1969). An action spectrum of the I-D decay clearly

shows PSI as responsible for the dip (Schreiber and Vidaver 1975). The interpretation of the I-D dip is that PSI oxidizes the quencher Q more rapidly than PS II can reduce it resulting in a decline in fluorescence due to increased quenching by Q (Munday and Goyindjee 1969).

The D-P portion of the transient is thought to be related to water splitting as the D-P rise diminishes considerably when oxygen production is inhibited by either mild heat treatment (Schreiber, Bauer and Franck 1971) or high pressures (Schreiber and Vidaver 1973). It is suggested that the exhaustion of the oxidized form of the PSI acceptor, X, allows Q to be more fully reduced (Lavergne 1974; Munday and Govindjee 1969). The high fluorescence at the peak P is then due to the quencher being in a non-quenching form due to the reductive activity of PSII.

The P-S decline is the most complicated region of the transients to analyse as it is possibly the result of several processes. Difference spectra and ratios of fluorescence intensity at different portions of the fluorescence transient have prompted Mohanty et al (1971) to suggest that the P-S decline is the result of an increase in the efficiency of energy transfer between PSI and PSII . Bonaventura and Myers (1969) working on Chlorella and Murata (1970) with Porphyra

came to the same conclusion that "Light 1" and "Light 2" states exist which can regulate the amount of energy directed to either PSI or PSII.

The P-S decline may also be related to photophosphorylation. Low concentrations of substances which uncouple phosphorylation, such as atebrin and FCCP, slow down the P-S decline, or at high concentrations abolish it completely (Mohanty, Papageorgiou and Govindjee 1971; Papageorgiou and Govindjee 1968a).

It has also been suggested that the P-S decline is related to the Calvin-Benson cycle. In this hypothesis it is held that the D-P rise is due to the reduction of NADP+ and that the P-S decline is due to the activation of the Calvin-Benson cycle, which would oxidize $NADPH_2$. Munday (1969) has shown that methyl viologen, which accepts electrons from PSI, will remove the peak, P, which agrees with the theory of D-P being related to exhaustion of PSI acceptors and P-S to activation of the Calvin-Benson cycle.

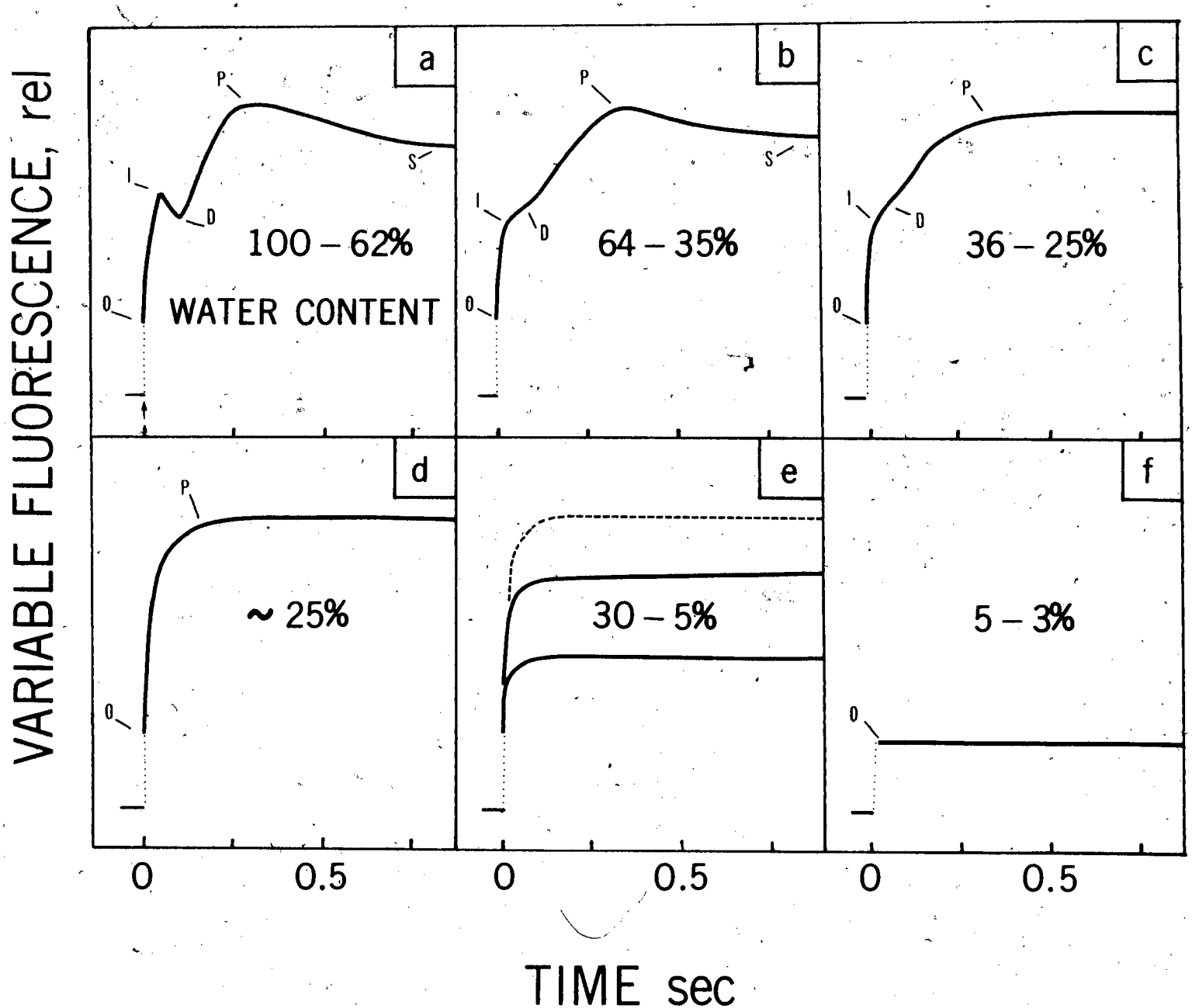
RESULTS AND DISCUSSION

Part 1: THE EFFECTS OF DESICCATION ON FLUORESCENCE TRANSIENTS IN SENSITIVE AND TOLERANT ALGAE

DESICCATION AND THE P-S DECLINE: The first effect of drying on fluorescence induction was to erase the P-S decline, in Porphyra sanjuanensis (Fig. 3c) and also in Ulva scagelii (Fig. 4b). Other algal types including Enteromorpha linza, Porphyra perforata, Porphyra schizophylla and Porphyridium cruentum gave similar results.

There are several possible interpretations for the disappearence of the P-S decline. (1) If P-S is due to alpha transitions then it may be that drying prevents energy transfer between photosystems I and II. A loss of this energy transfer would be expected to decrease the quantum yield of photosynthesis due to less efficient energy use. (2) Another possibility is that the P-S decline is related to phosphorylation and that drying acts like an uncoupler in its initial stages. Previous research on isolated chloroplasts from higher plants has shown that photophosphorylation is highly sensitive to even mild desiccation, being one of the first reactions inactivated (Nir and Poljakoff-Mayber 1967; Santarius and Ernst 1967; Santaurius 1969). (3) The

Figure 3. Effect of dehydration on fluorescence induction in Porphyra sanjuanensis. The transients are found to occur in ranges of water content which overlap. Curves were selected from 150 tests to demonstrate characteristic changes occuring with drying: (a) control, (b) smoothing of the I-D dip, (c) loss of the P-S decline, (d) loss of the I-D characteristic, (e) a progressive decrease of the O-P rise, the dashed line representing the original heigth, the same as in d, (f) loss of variable fluorescence. The dotted line represents the initial rapid rise from base-line fluorescence to variable fluorescence.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e
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P
I
D
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WATER CONTENT
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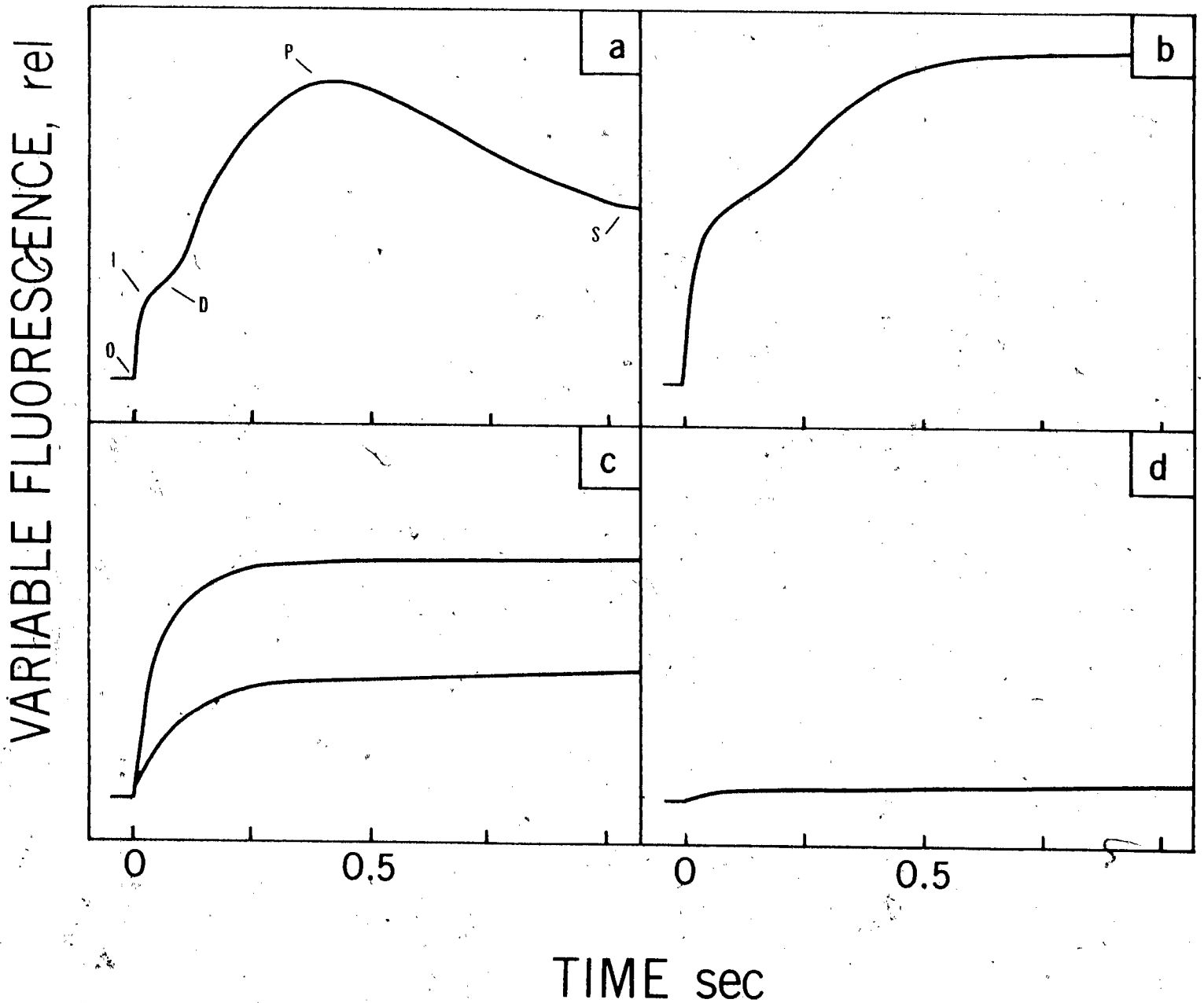
36 – 25%
~ 25%
30 – 5%
5 – 3%
0
0.5
TIME sec

Figure 4. Effect of dehydration on fluorescence induction in Ulva scagelii. The curves show only variable fluorescence, the rapid rise having been deleted. (a)control, (b) 20 min drying, (c)progressive decline of O-P, 30-50 min drying, (d)70 min drying.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Calvin-Benson cycle has also been implicated with the P-S decline. It may be that the decrease in phosphorylating activity, previously mentioned, is responsible for a decrease in Calvin cycle activity which would subsequently affect the P-S decline. This is a questionable hypothesis since phlorizin, a phosphorylation inhibitor affecting the terminal esterfication step, does not change the P-S decline (Papageorgiou and Govindjee 1968a, 1968b), but would be expected to inhibit the Calvin cycle. It may be that water stress inactivates some Calvin cycle enzyme. It is known that some enzymes are inactivated with water deficits of only 10 or 20% (Bardizik 1971). Plaut (1971) found that some enzymes of the Calvin cycle are inhibited in intact chloroplasts at low osmotic potentials. Inactivation of just one Calvin cycle enzyme would be sufficient to stop carbon fixation. The P-S decline in Porphyra sanjuanensis is found to disappear when the water content drops to about 35% (Fig. 3c) and yet Imada et al (1970), using CO_2 analysis on Porphyra tenera, found that carbon fixation continued on down to 20 to 25% water content. It is possible that a water deficit slows the rate of carbon fixation such that reduced NADP accumulates and no P-S decline occurs. The disappearence of the P-S decline may be due to one or a combination of factors.

DESICCATION AND THE I-D DIP: Following close behind a change in the P-S decline is a modification of the I-D dip. The I-D dip changes from a steep downward spike (Fig. 3a) to a rounded plateau (Fig. 3b) which disappears completely with further desiccation. In Ulva a similar disappearence of the I-D characteristic occurs (see Fig. 4a, b, c).

The eventual disappearence of the I-D dip with drying could represent a loss of PS I quenching activity, but work by Fork and Hiyama (1973) has shown that reversible light induced redox reactions of P700 still take place, even in dried Porphyra. We suggest that the electron transport chain between PS II and I is inactivated by drying thereby preventing PS I from quenching; which would account for the loss of the I-D dip.

Another possible explanation of the disappearence of the I-D dip, though less likely, is that PS II activity is increased by drying, reducing Q more rapidly than PS I can oxidize it. This possibility seems unlikely as isolated chloroplasts show decreased PS II activity when exposed to water stress as measured by ferricyanide reduction (Boyer and Bowen 1970; Nir and Poljakoff-Mayber 1967). Mosses also exhibit decreased oxygen production when desiccated (Lee and Stewart 1971), which would not be expected if PSII activity were enhanced by drying.

The gradual disappearence of the I-D decline represents a progressive inactivation of electron transport between PS II and I. With the disappearence of the I-D dip it may be assumed that oxygen production ceases as well, since there is no avenue for electron transport. The loss of the I-D characteristic occurs around 25 % water content (Fig. 3d). Imada et al (1970), using CO_2 gas analysis on Porphyra tenera, found that photosynthesis ceased at between 20 and 25% of the original water content. It is proposed that the disappearence of the I-D dip could well indicate the point at which photosynthetic activity is suspended, due to the inactivation of electron transport.

DESICCATION AND THE BLENDING OF THE O-I WITH THE D-P RISE: The disappearence of the I-D dip leaves a new transient in which the D-P portion of the curve has blended with the O-I rise (Fig. 3d). As the sample is desiccated from approximately 24 to 5% of its original water content this transient progressively decreases in height until a flat line at the O level is reached (Fig. 3f).

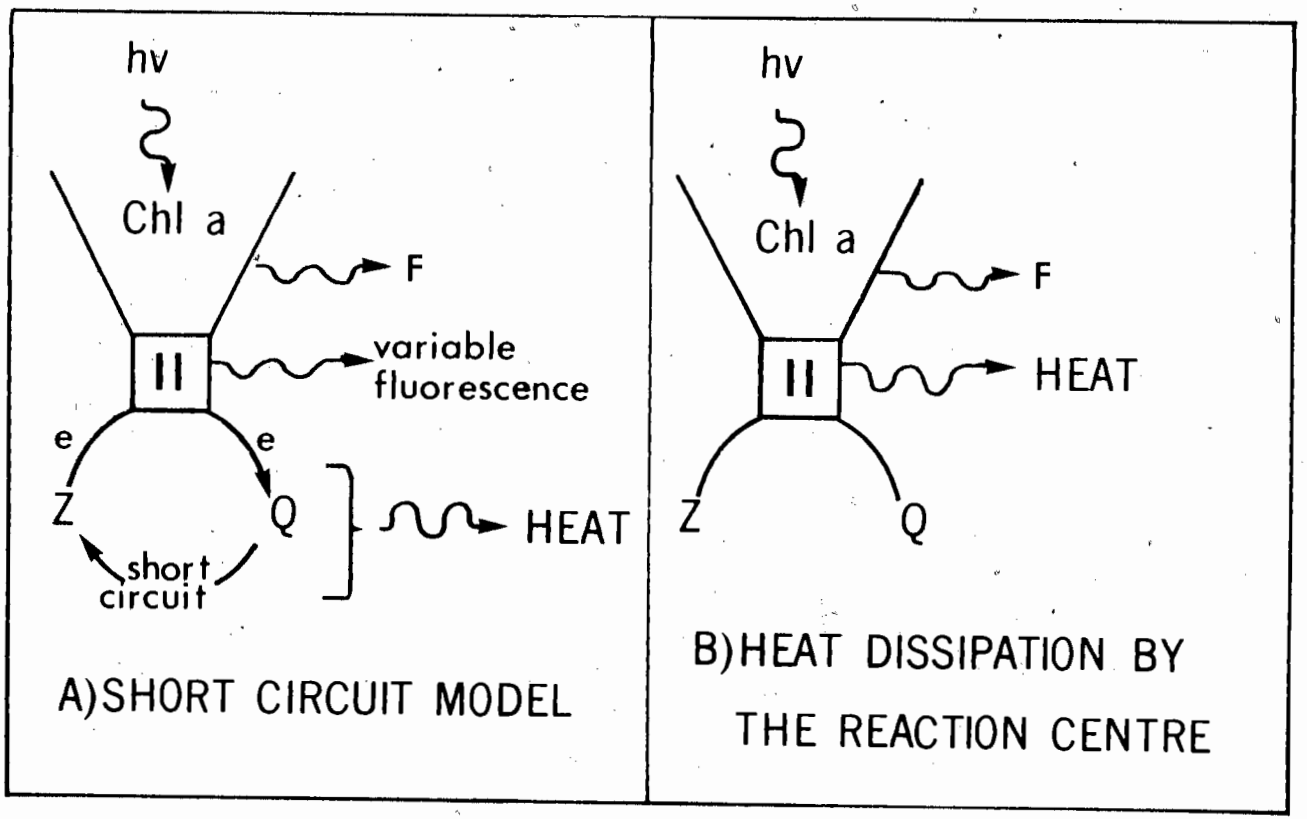
The new transient obtained on drying will be refered to as O-P (Fig. 3d). The O-P curve is thought to be representative of the reduction of Q by PSII. If the previous interpretation of the loss of the I-D dip is correct then there

will be no way in which PSI can oxidize Q_A and Q_A will eventually be fully reduced. The O-P transient is the result of Q being reduced until at the maximum, P, Q is entirely reduced.

As drying continues the height of the O-P transient decreases (Fig. 3e) until a flat O level is attained around 3 to 5% water content (Fig. 3f). It was noted that in most cases only the area above the O level was decreased by drying and that fluorescence below the O level was relatively unaffected. It would seem that there are two forms of fluorescing chlorophyll: one fairly static and the other dynamic. The dynamic one is associated with variable fluorescence and is probably the reaction center of PSII. The static fluorescence is assumed to originate in the bulk or antennae pigments.

A hypothetical explanation for the O-P decrease with drying is as follows: drying increases quenching activity of variable fluorescence as seen in the decrease of the height of the O-P transient (Fig. 3e). But from previous results it is known that intersystem electron transport has ceased, ruling out PSI-mediated quenching. It is proposed that a short circuit occurs in which electrons passed to Q are recycled back to Z (Fig. 5a). In this model PSII reaction centers are still

Figure 5. Hypothetical models to account for the decrease in the height of the O-P transient with drying. Notation same as in fig. 2.


hv
Chl a
F
variable
fluorescence
II
e
e
Z
Q
short
circuit
HEAT
A)SHORT CIRCUIT MODEL
hv
Chl a
F
II
HEAT
Z
Q
B)HEAT DISSIPATION BY
THE REACTION CENTRE

functional in that they dissipate absorbed energy. The short-circuit maintains Q in an oxidized state which quenches strongly. The energy absorbed is ultimately dissipatated as heat without doing any useful work. Fork and Hiyama (1973) suggest that the reaction center, PSII, is shut down by drying. They base their reasoning on the fact that cytochrome f cannot be reduced in dried Porphyra, but they ignore the possibility that an earlier inactivation in the transport chain could prevent them from seeing PSII activity. The fact that the O level fluorescence does not increase significantly after drying strongly suggests that energy is still being utilized by PSII, which is probably dissipated as heat. Another possible interpretation of the O-P decrease is that the reaction center of PS II has switched energy transfer from photochemistry and fluorescence to heat dissipation (Fig. 5b). This differs from the cyclic model in that there is no electron flow promoted by the reaction center.

Part 2: REHYDRATION IN TOLERANT ALGAE: Porphyra perforata, porphyra schizophylla, Porphyra sanjuanensis.

In a tolerant alga, which has been desiccated to an O level response, the addition of water results in a return of variable fluorescence. A normal Kautsky-type curve is not

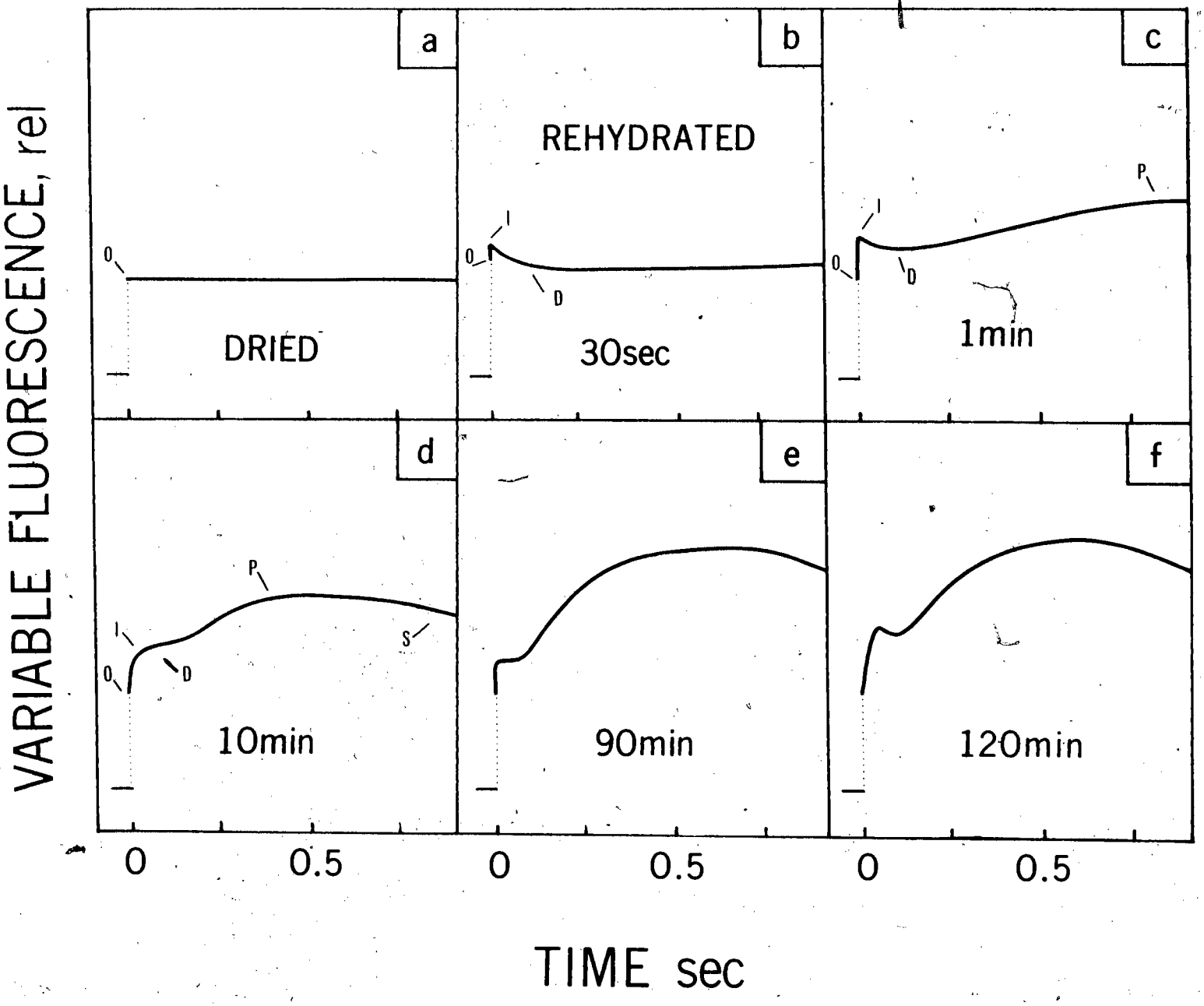
re-established immediately, rather intermediate stages of transients occur.

Within 30 seconds of rehydration a curve is obtained (Fig. 6b) in which there is a sudden rise in fluorescence followed by a decline.

The return of an O-I rise indicates that the PSII fluorescing centers are active and interactive with Q. The addition of water has increased the quantum yield of variable fluorescence, supposedly by affecting the physical state of the chlorophyll molecules.

If the decline seen during the first 30 seconds of rehydration corresponds to the I-D dip, one would expect its inhibition by DCMU. When dried Porphyra sanjuanensis was rehydrated with a saturated DCMU solution the decline did not occur (Fig. 7). From this it can be concluded that PSI activity and intersystem electron transport are re-established within the first 30 seconds of rehydration. Fork and Hiyama (1973) found that cytochrome f redox reactions, which are dependent on intersystem electron transport, were re-vitalized as rapidly as they could measure with their apparatus.

Figure 6. Effect of rehydration on fluorescence induction in Porphyra sanjuanensis. Transients were taken from a single sample with the exception of (c) which required a new sample due to the need for dark adaptation.

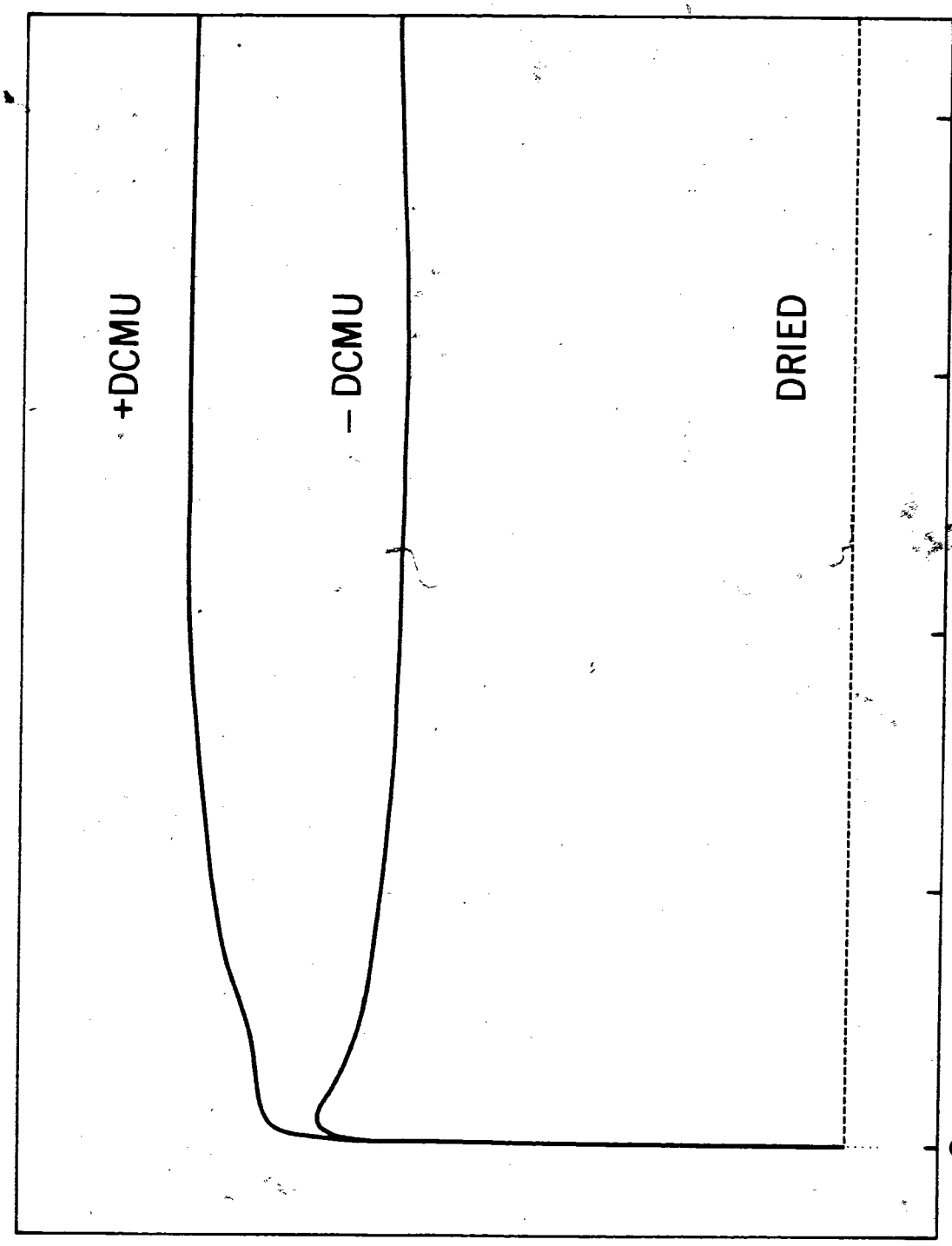

a
b
c
REHYDRATED
DRIED
30sec
1min
d
e
f
10min
90min
120min
O
I
D
P
S
VARIABLE FLUORESCENCE, rel
0
0.5
TIME sec

PSI activity seems to be in excess of the reductive capacity of PSII during the early stages of rehydration as there is no pronounced D-P transient. The transients produced after 30 seconds of rehydration are similar to transients of anaerobically adapated algae in which water splitting is blocked (Schreiber and Vidaver 1975). This suggests that water splitting is reactivated more slowly upon rehydration than is intersystem electron transport.

Viewed on a longer time-scale, even in the presence of DCMU, there is a slow fluorescence decline (Fig. 8). As the DCMU block should prevent quenching by PSI the decline must be due to some other effect. It is known that slow alpha-changes from state II to state I, which reflect changes in the distribution of light quanta between the two photosystems, are not blocked by DCMU (Bonaventura and Myers 1969). If these alpha-changes are present after one minute of rehydration there would be an expected decline in fluorescence as energy is shunted from PSII (active fluorescing center) to PSI (low quantum yield for fluorescence).

Within one or two minutes of rehydration a D-P rise is present in transients (Fig. 6c). At this point it is assumed that water splitting is occuring. Simultaneous recordings of fluorescence and oxygen evolution after 2 minutes of wetting

Figure 7. The effect of rehydration on fluorescence induction in DCMU poisoned Porphyra sanjuanensis. Tissue was observed in the dry state, after rehydration, and after rehydration in sea water + 2×10^{-4} M DCMU. Only variable fluorescence is shown. Time of rehydration 30 sec.


+DCMU
– DCMU
DRIED
0
0.5
TIME sec
VARIABLE FLUORESCENCE, rel

confirm that photosynthesis is re-established, (Fig. 9b). With longer periods of rehydration the D-P rise increases in height relative to the O-I rise (Fig. 9b, c, d) until after fifteen to twenty minutes typical fluorescence and oxygen indution transients occur.

Further rehydration results in subtle changes in the transient which already shows most of the characteristics of a fully rehydrated transient (Fig. 6d, e, f).

It is interesting to note that the sensitive alga Porphyridium cruentum cannot be dried to the point that variable fluorescence disappears and survive, but it may be desiccated until its transients are of the O-P type (Fig. 3d), and then be successfully rehydrated (Fig. 10). Similar results were obtained with Enteromorpha linza. Since O-P type transients are indicative of a lack of intersystem electron transport, photosynthesis can be reversibly inhibited even in these two sensitive algae by drying. It should be stressed that any further water loss will permanently inactivate photosynthesis in these two sensitive algae.

Part 3: PROLONGED STORAGE OF DRIED TOLERANT ALGAE

Figure 8. Fluorescence induction of DCMU poisoned *Porphyra sanjuanensis* after rehydration as compared to non-poisoned sample. Dried specimens were checked for the absence of variable fluorescence prior to rehydration. Only the variable fluorescence is shown. Rehydration 1 min. DCMU, saturated solution in sea water.

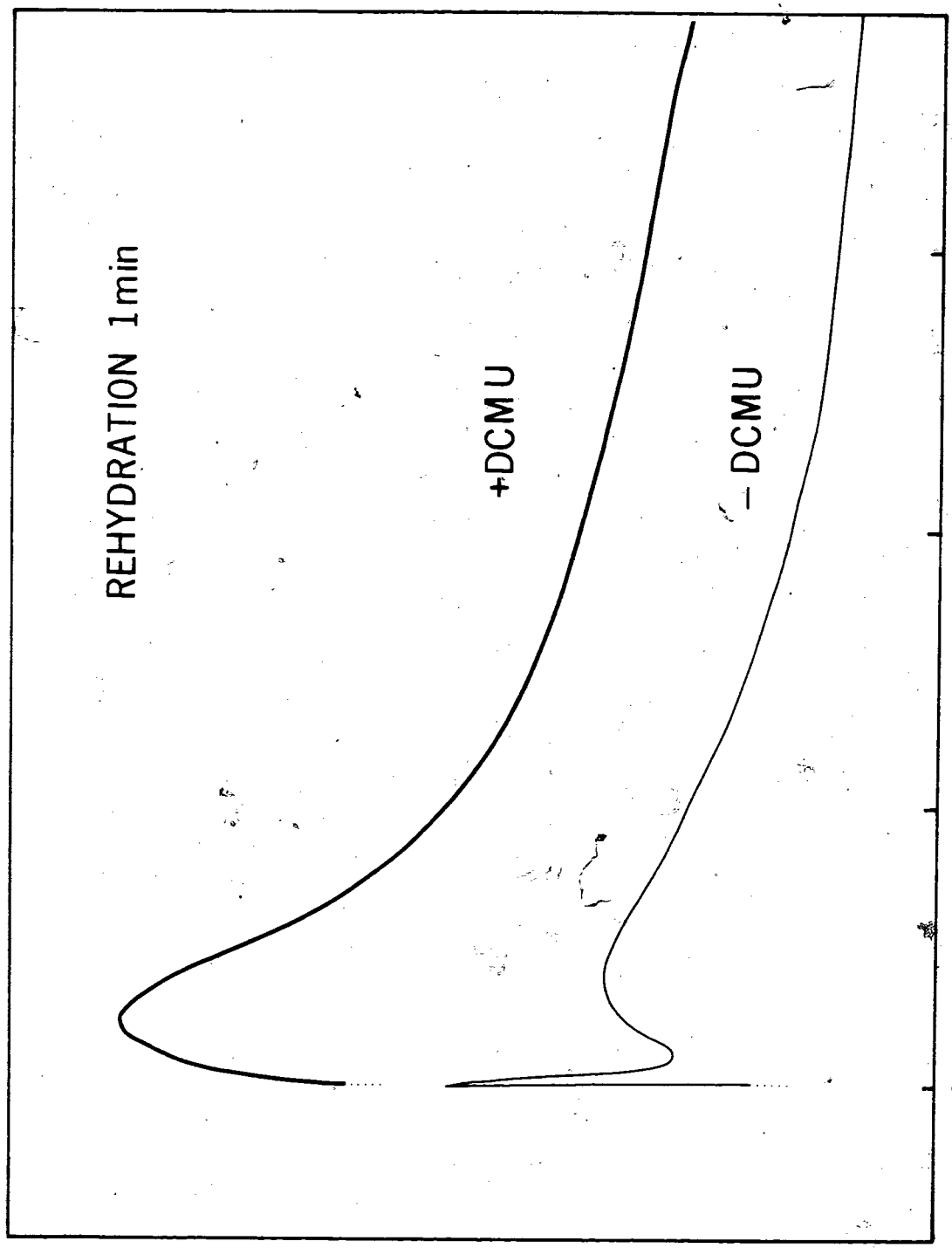

REHYDRATION 1min
+DCMU
– DCMU
0
10
TIME sec
VARIABLE FLUORESCENCE, rel

Figure 9. Simultaneous recordings of Chl a fluorescence and oxygen exchange rate in Porphyra sanjuanensis after rehydration. Samples were dried 24 hrs in the lab on racks to a loss of variable fluorescence. The control curve is restored within 15-25 min of rehydration. Note that the base-line for oxygen determinations is drifting in all but (a). Other conditions same as in figure 2.

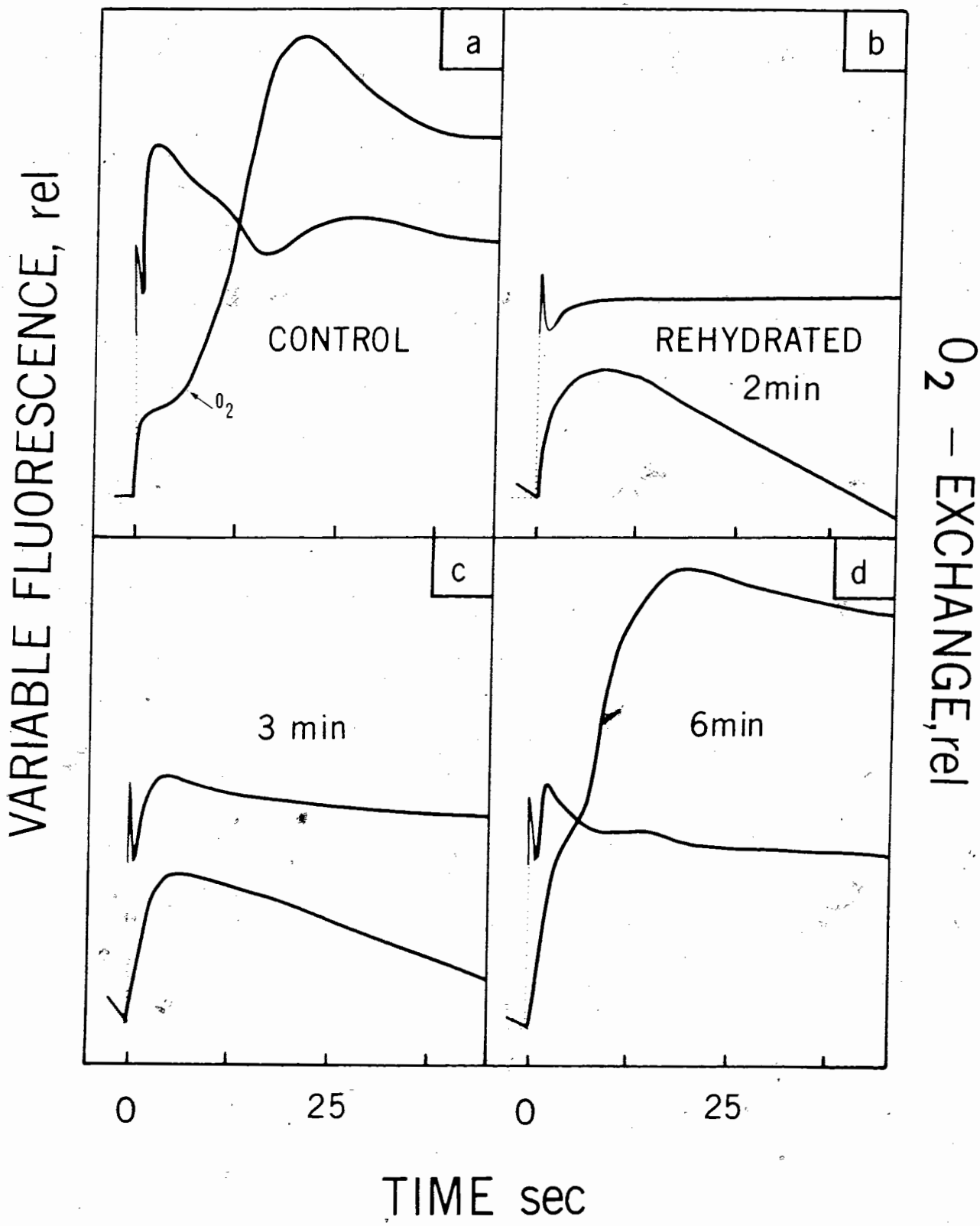
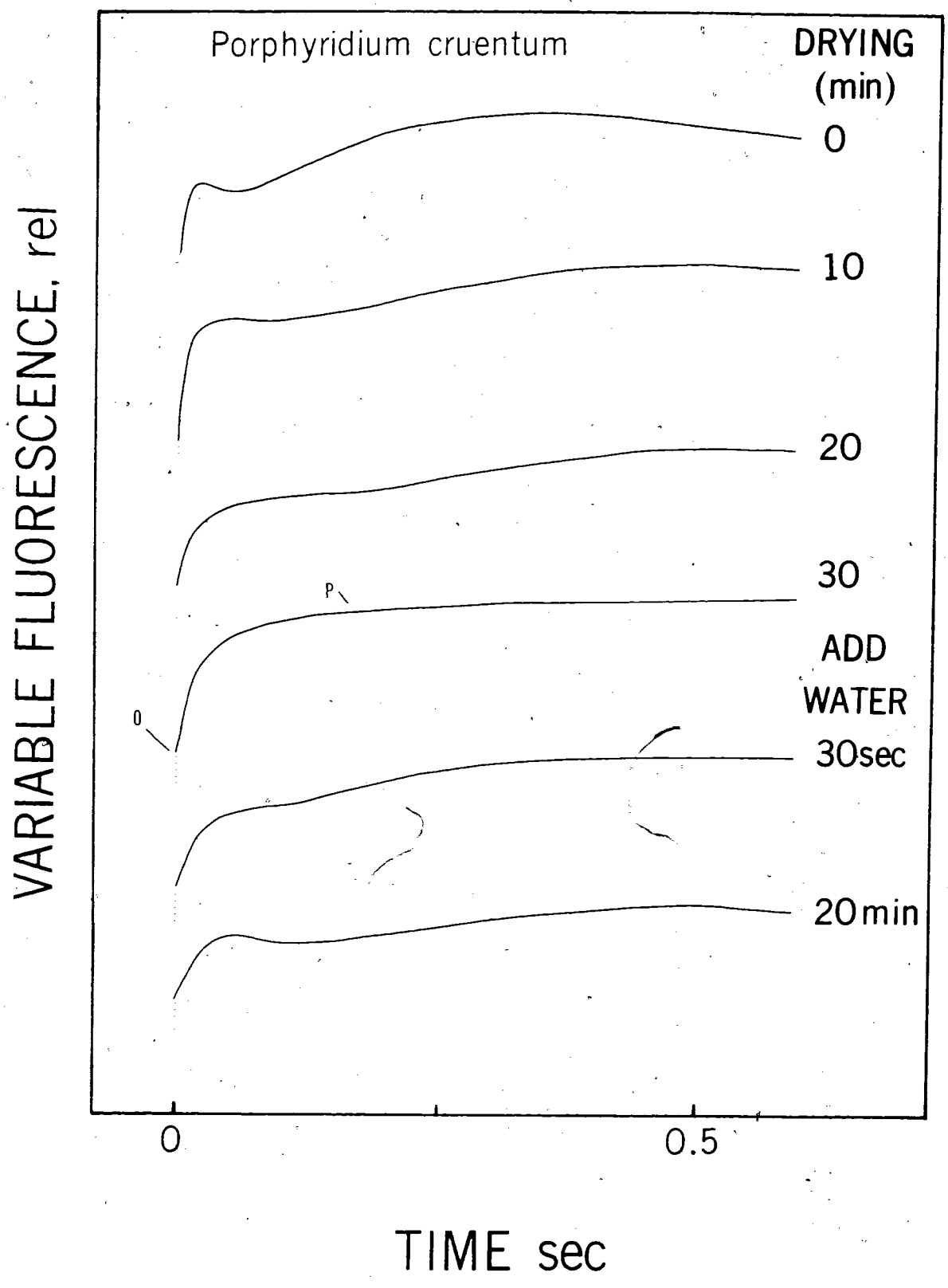

a
b
c
d
CONTROL
REHYDRATED
2min
3 min
6min
O_2
VARIABLE FLUORESCENCE, rel
O_2 – EXCHANGE, rel
0
25
0
25
TIME sec

Figure 10. Effect of drying and subsequent rehydration on fluorescence induction in Porphyridium cruentum. After 30 min of drying under a drierite-filtered air-stream the sample was rehydrated, It should be noted that the drying has not been done to the point that it completely removes variable fluorescence. Further drying, resulting in a loss of all variable fluorescence, is not reversible.

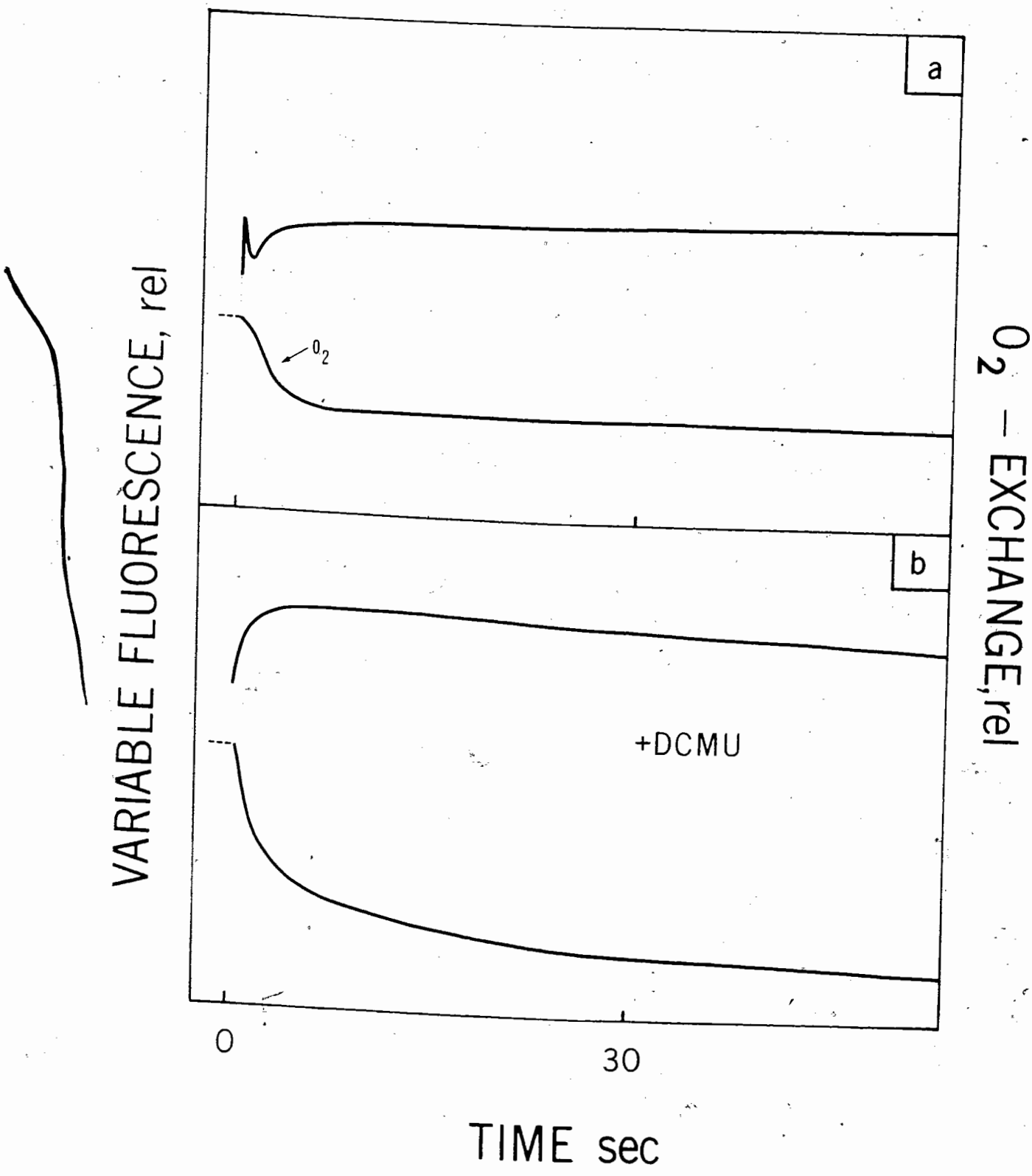

Porphyridium cruentum
DRYING (min)
0
10
20
30
P
O
ADD WATER
30sec
20min
VARIABLE FLUORESCENCE, rel
0
0.5
TIME sec

Porphyra has a limit as to how long it can remain viable in the dried state. Long range drying tests with Porphyra perforata, collected in June and held at 11 C, showed the plants to succumb, as measured by induction transients, around day 8. Samples collected in May exhibited survival up to 19 days. Observation of the growth cycle of P. perforata over a two year period showed that by the middle of June most high intertidal Porphyra was bleached and, according to induction transients, dead. At this time of year there are low spring tides during the day and relatively high temperatures for Vancouver. It may be that the resistance of the alga is decreased by harsh conditions or repetitious drying. Porphyra sanjuanensis was also studied but drying was done at room temperature in the lab. Porphyra sanjuanensis, collected in April, was viable up to 9 days. Porphyra miniata, the most sensitive Porphyra studied, could not withstand even one day of drying. The three Porphyra species studied are each limited to a certain vertical intertidal region at the site of study, which was found to be related to their desiccation resistance. P. perforata is located in the high-, P. sanjuanensis is found in the mid-, and P. miniata in the low intertidal zone.

An attempt was made to localize the photosynthetic process damaged by prolonged drying. At day 13 of drying the transient fluorescence is different than control curves (Fig.

11a), but certain characteristics are still present. The presence of an O-I rise indicates that PSII reaction centers retain their ability to reduce Q. The dip region in these transient curves was found to be DCMU sensitive (Fig. 11b). This sensitivity supports the assumption that the dip is homologous to the I-D dip, therefore PSI activity and electron transport are still present. A minimal D-P rise indicates little or no water-splitting, which was confirmed with simultaneous oxygen measurements (Fig. 11a). The failure to detect oxygen production does not necessairly mean that water splitting was not occuring. There is a poorly understood uptake phenomena associated with PSI (Chandler and Vidaver 1970; French and Fork 1963; Vidaver 1965), which in stressed plants may mask oxygen production. The presence of a minor D-P rise suggests that some water splitting is occuring in order to keep Q in a reduced state. The lack of net oxygen production in the presence of a D-P rise leads us to suspect that water splitting activity is fairly low and masked by PSI uptake phenomena. The inhibition of water-splitting may be due to the lack of a final electron acceptor due to an inactivated Calvin cycle. The absence of a P-S decline supports this possibility. The unavailability of a final electron acceptor would also stop oxygen production. Both water splitting and Calvin cycle activity are minimal in Porphyra samples stressed by prolonged drying.

Figure 11. Simultaneous recordings of Chl a fluorescence and oxygen exchange in Porphyra sanjuanensis, upon rehydration after prolonged storage in the dried state. Samples were dried for 13 days on racks in the laboratory. Rehydrated with (a)sea water, , (b)saturated solution of DCMU. Rehydration was for 25 min.


a
b
O_2
+DCMU
VARIABLE FLUORESCENCE, rel
O_2 – EXCHANGE, rel
0
30
TIME sec

Part 4: ECOLOGICAL LOCATION OF TOLERANT AND SENSITIVE ALGAE

There appears to be a strong correlation between the intertidal location of various algae and their ability to be dried to the point that all variable fluorescence is lost. From Table I it can be seen that those algae commonly associated with the high intertidal region: Prasiola meridionalis, Porphyra perforata, Porphyra sanjuanensis, and Porphyra schizophylla are also tolerant of being dried to the point that induction transients no longer occur; whereas algae found in the lower intertidal or subtidally, including Enteromorpha linza, Ulva scagelii, Polyneura latissima, Porphyra miniata and Nitophyllum notti do not possess such tolerance. Petalonia fascia at first seems to be an exception in that this sensitive alga is found in the high intertidal, but Petalonia occupies this region only during the wet winter months when there is little chance of drying. Of the plants tested all tolerant species were found in the upper- or mid-intertidal where such a characteristic would be most beneficial.

Field work on Porphyra sanjuanensis has demonstrated that dehydration in the natural environment is sufficient in some cases to reversibly inhibit variable fluorescence. The

Table 1: Tolerance of the photosynthetic apparatus to dehydration in relation to an alga's intertidal location. Viability of photosynthesis is based on the criterion that an alga could be desiccated, with a subsequent loss of variable fluorescence, and that rehydration would restore typical fluorescence transients.

Table 1

ALGAE	INTERTIDAL RANGE	VIABILITY
Prasiola meridionalis	upper (Scagel 1966)	+
Porphyra perforata	upper (Smith 1969)	+
Porphyra sanjuanensis	upper and mid (Scagel 1957)	+
Porphyra schizophylla	mid (collection site)	+
Enteromorpha linza	mid (Smith 1966)	-
Ulva scagelii	mid to lower (collection site)	-
Petalonia fascia	upper (Taylor 1966)	-
Porphyra miniata	low (collection site)	-
Polyneura latissima	subtidal (collection site)	-
Nitophyllum notti	subtidal (collection site)	-

stress observed in the lab is therefore also found in the plants habitat.

Part 5: OSMOTIC DESICCATION: Porphyra perforata and Porphyridium cruentum.

The effect of osmotic desiccation on fluorescence transients was investigated using concentrated sea water as the desiccant. Both the sensitive alga Porphyridium cruentum and the resistant alga Porphyra perforata were exposed to osmotic stress. From Figs. 12 and 13 it appears that osmotic desiccation results in characteristic changes of the transients similar to those induced by air drying (Fig. 3a-e). Porphyridium cruentum transients when exposed to concentrated sea water demonstrate the disappearence of the P-S decline followed closely by loss of the I-D dip (Fig. 12). The curve at -68 bars is similar to an O-P transient, and the data suggest that for P. cruentum photosynthesis ceases between -51 and -68 bars. This conclusion is reached due to the interpretation that the loss of the I-D characteristic signals the end of electron transport. Photosynthesis in Porphyra perforata appears to be more resistant to osmotic desiccation than P. cruentum, as even at -68 (bars (Fig. 13) the transient gives evidence of electron transport between PSI and II. Gessner (1971) has shown that Porphyra umbilicalis

Figure 12. Effect of sea water concentration on fluorescence induction in Porphyridium cruentum. The plants were submerged in 1, 2, 3, or 4x concentrated sea water for 15 min prior to measurements. Fresh samples were used in all cases.

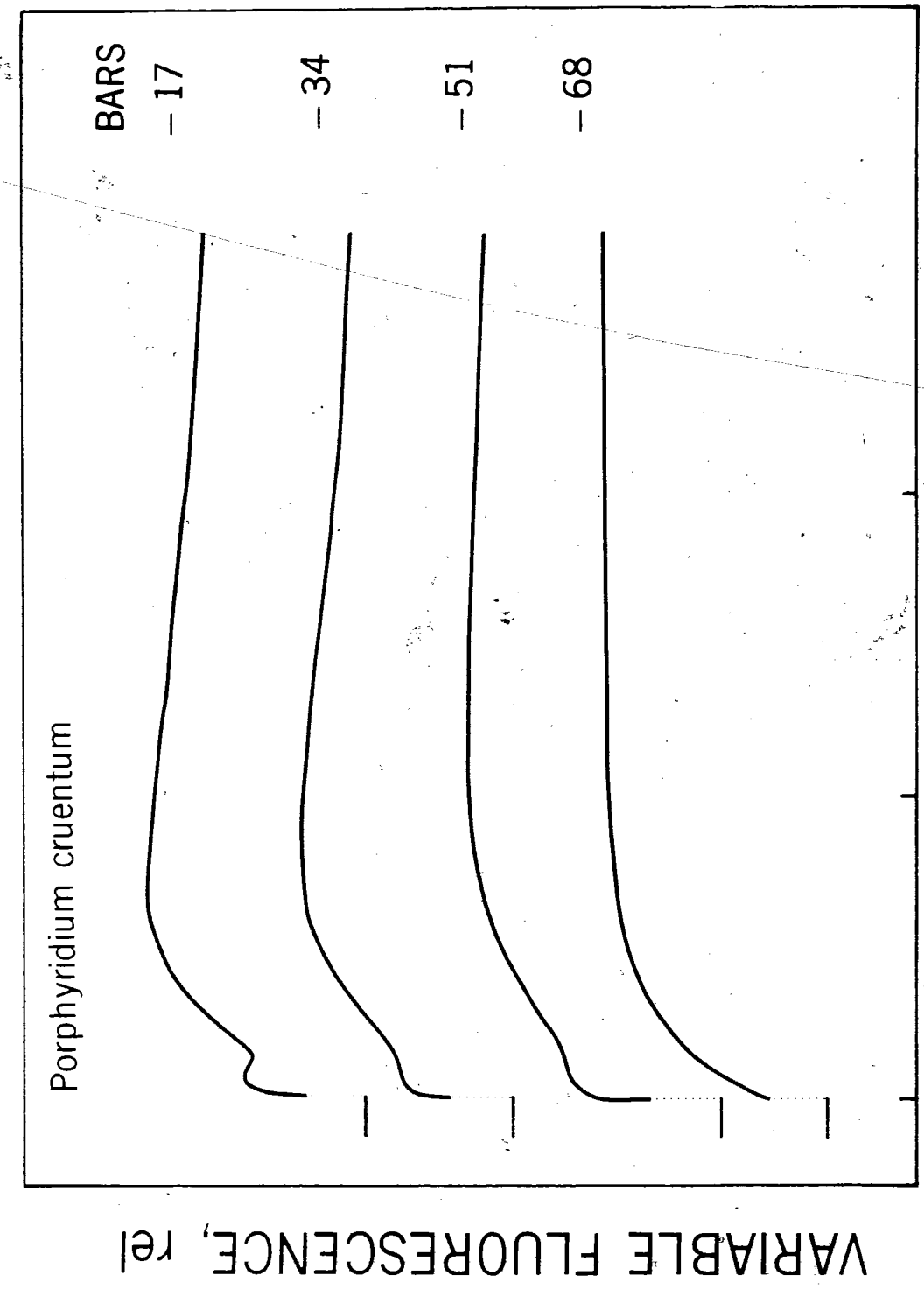
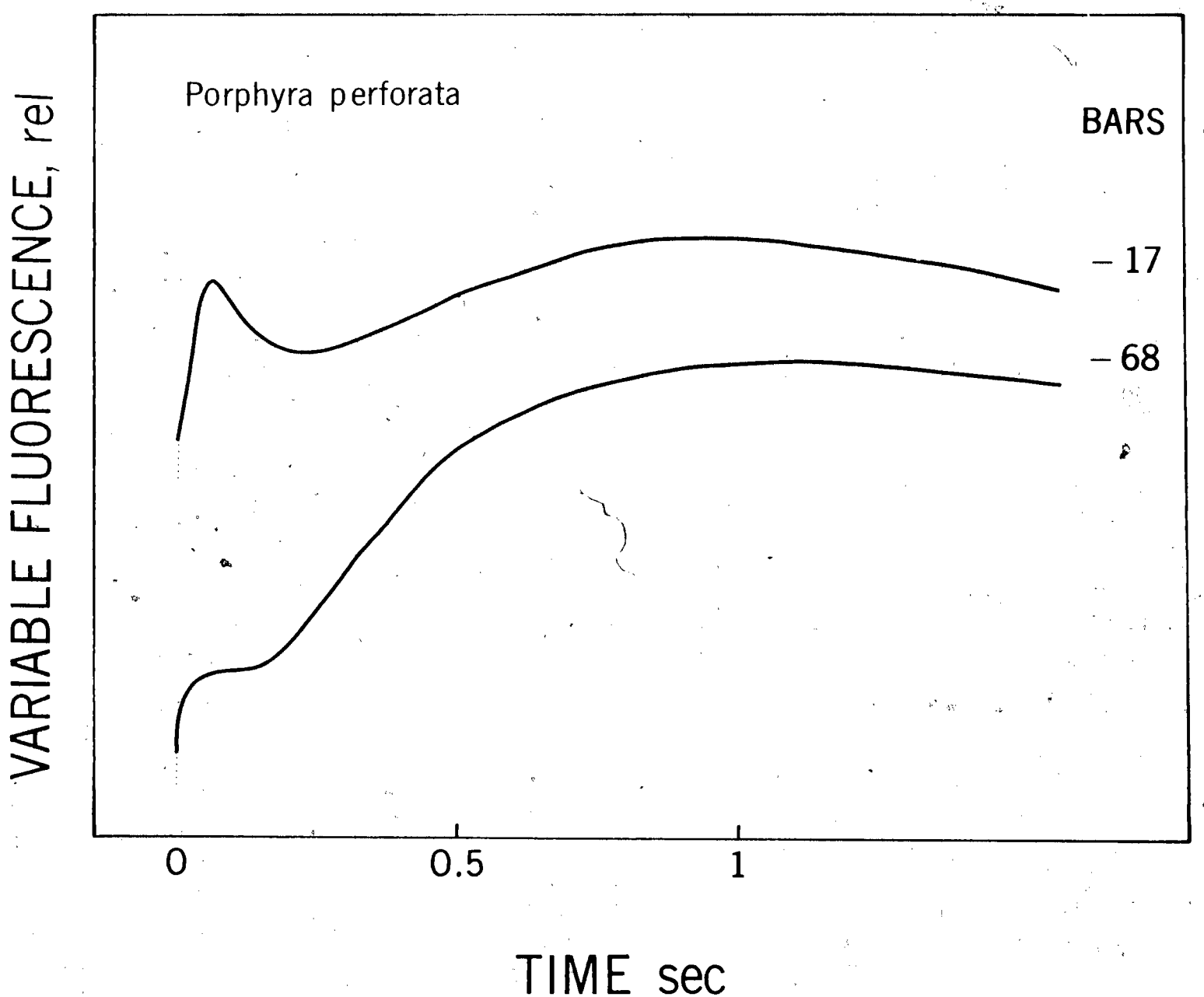

Porphyridium cruentum
BARS
– 17
– 34
– 51
– 68
0
0.5
1
TIME sec
VARIABLE FLUORESCENCE, rel

Figure 13. Effect of sea water concentration on fluorescence induction in Porphyra perforata. Intermediate osmotic pressures of -34 and -51 bars had relatively little effect on the control curve. Only the variable fluorescence portion of these curves are shown. Other condition as in fig. 12.


Porphyra perforata
BARS
-17
-68
VARIABLE FLUORESCENCE, rel
0
0.5
1
TIME sec

requires osmotic media of more than -100 bars to stop photosynthesis as measured by polarographic methods. Further osmotic desiccation to O level fluorescence transients was not attempted as sea water begins to precipitate around 5 times its normal salinity.

Part 6: RESPIRATION AT A FLUORESCENCE O LEVEL AND AFTER REHYDRATION

Samples of Porphyra perforata were dried at 11 C in an incubator. Fluorescence transients were checked and found to be of the flat O level type (Fig. 3f). Manometry was used to determine the respiration rate of Porphyra in both the dry and rehydrated condition. As can be seen from Table II, the dry sample has a negligible respiration rate within the region of apparatus error, 0.035 ± 0.028 micro liters CO_2 evolved/min, gram oven dry weight. Respiration has ceased at the dried O-fluorescence level. When these same plants were rehydrated there is a rapid recovery of activity with a respiration rate similar to control values. Respiration does not appear to be damaged by short-term drying to an O level fluorescence transient in Porphyra perforata.

Table 2: Dark respiration of the desiccation tolerant alga Porphyra perforata in the wet, dried, and rehydrated states.

Table 2

SAMPLE TREATMENT	μ liters CO_2 evolved/ min/gram oven dry wt.
Wet	7.435 ± 1.165
Dry	0.035 ± 0.028
Rehydrated	7.678 ± 1.227

CHAPTER IV

ULTRASTRUCTURAL OBSERVATIONS OF DESICCATION STRESSED ALGAE: *Porphyra perforata*, *Porphyra sanjuanensis*, *Prasiola meridionalis*, *Ulva scagelii*, *Petalonia fascia*, and *Nitophyllum notti*.

To ascertain if there are ultrastructural features which could account for desiccation tolerance, both tolerant and sensitive marine algae were compared in the wet and dried states. An alga was designated as tolerant if variable fluorescence could be reversibly inactivated by dehydration (see Chapter I). Three tolerant species were used, the red algae, *Porphyra perforata* and *Porphyra sanjuanensis*, and a green alga, *Prasiola meridionalis*. Three sensitive algae were also observed, the green alga, *Ulva scagelii*, the brown alga, *Petalonia fascia*, and the red alga *Nitophyllum notti*. Specimens were viewed in the wet, dried and in two cases the rehydrated states.

Part 1: *Porphyra perforata* and *Porphyra sanjuanensis* (Rhodophyta)

WET CONTROL:

The two *Porphyra* species, *P. perforata* and *P.*

sanjuanensis, have many features in common and shall be treated together. A single multilobed chloroplast occupies a large portion of the cell (Figs. 14, 24). Thylakoids lie singly in the stroma and tend to be more or less parallel in their arrangement with one another (Fig. 15). The intrathylakoid space is usually of a uniform dimension but occassionally this space is expanded, ballooning (Figs. 16, 25). A pyrenoid is located inside the chloroplast (Figs. 14, 25). This pyrenoid is traversed by a number of slightly distended lamellae which appear to be extensions of thylakoids (Fig. 16). Endoplasmic reticulum (E.R.) is sparsely distributed in the cytoplasm with a tendency to be located near the plasmalemma. Dictyosomes are commonly found with their forming face in proximity to a mitochondrion (Fig. 15), a finding previously reported by Bourne (1971) and Tripodi (1971). Porphyra sanjuanensis differs from P. perforata by having a number of vacuoles dispersed through the cytoplasm (Figs. 24, 25), which tend to be larger and more numerous than those found in P. perforata.

DEHYDRATED:

Ultrastructural characteristics of dried plants were similar regardless of the method or duration of drying. Samples were either dried rapidly over a drierite-filtered air

stream (100 min), or left on racks and dried for five, seven, or twenty-seven days. In all but the twenty-seven day dried samples the algae were viable upon rehydration. Dried plants retained many of the features observed in the wet state. Chloroplasts and thylakoid organization were still present (Figs. 17, 18, 21, 22). The pyrenoid and its traversing lamellae were also found intact (Fig. 18). Mitochondria and their cristae were discernable (Figs. 17, 18, 19 ,23).

The most obvious change in dried P. perforata were the numerous vesicular elements which arose in the cytoplasm (Figs.17, 18, 19, 21, 22). These vesicles are bounded by an osmiophilic line which is presumably a membrane (see Fig 20). In most cases the interior of the vesicles is electron transparent. In the seven day dried algae there are an abundance of small vesicles (Figs.19, 21). Some of the vesicles may be the remains of disrupted mitochondria. In figure 19 a mitochondria appears to be breaking down and giving rise to vesicles. Dictyosomes and E.R. are conspicuously absent from dried material (Figs. 17, 18).

Porphyra perforata which had been dried for twenty-seven days was no longer viable, yet cellular detail had been preserved similar to viable material dried for shorter periods (Fig. 24).

REHYDRATION:

Rehydration of P. sanjuanensis for one or ten minutes (Figs. 26, 27) resulted in no observabele difference from dehydrated material. After thirty minutes of rehydration a pattern begins to emerge in which two types of rehydrated cells occur. One type of cell, delta, has typical thylakoid arrangement, normal mitochondria and fewer vesicles (Fig.28). Delta cells still lack dictyosomes but have indications of E.R. reforming . The other cell type, gamma,(Fig. 29) differs from both control and dehydrated samples. This cell still has extensive vesiculization in the cytoplasm. The thylakoid membranes show excessive ballooning and lack their normal parallel nature. The pyrenoid is no longer distinct and contains large osmiophilic bound spaces, presumably the remains of the traversing lamellae. Mitochondria appear to be disintegrating.

After rehydration for seventy-five minutes the two cell types, delta and gamma, are still present. The disintegrating cell, gamma, has few intact thylakoid regions at this point (Fig. 33). In delta cells there are few vesicles and though endoplasmic reticulum and dictyosomes are still limited there is evidence of their return (Fig. 32), such a reappearence is not seen in those cells with extensively disrupted

chloroplasts. Previous work (Nir, Klein and Poljakoff-Mayber 1969) has demonstrated that the addition of water to lethally dried tissue results in extensive disruption after three hours. It may be that some cells in P. sanjuanensis are not as hardy as others and are killed by the dehydration procedure Rehydration results in cellular breakdown which is not evident in the desiccated state.

Figs. 14-16. Porphyra perforata, fresh control.

Fig. 14. Single chloroplast and it's lobes occupy much of the cell. Pyrenoids located inside the chloroplast are traversed by lamellae. Vacuolation is minimal. x 7,200.

Fig. 15. An enlarged view of the chloroplast and adjacent cytoplasmic region. Note the forming face of the dictyosome in close association with a mitochondrion (paired arrows). Thylakoids are arranged roughly parallel with one another. x 18,000.

Fig. 16. Arrows point to thylakoids with expanded intrathylakoid space. Lamellae in pyrenoid appear to be extensions of thylakoids. x 29,200.

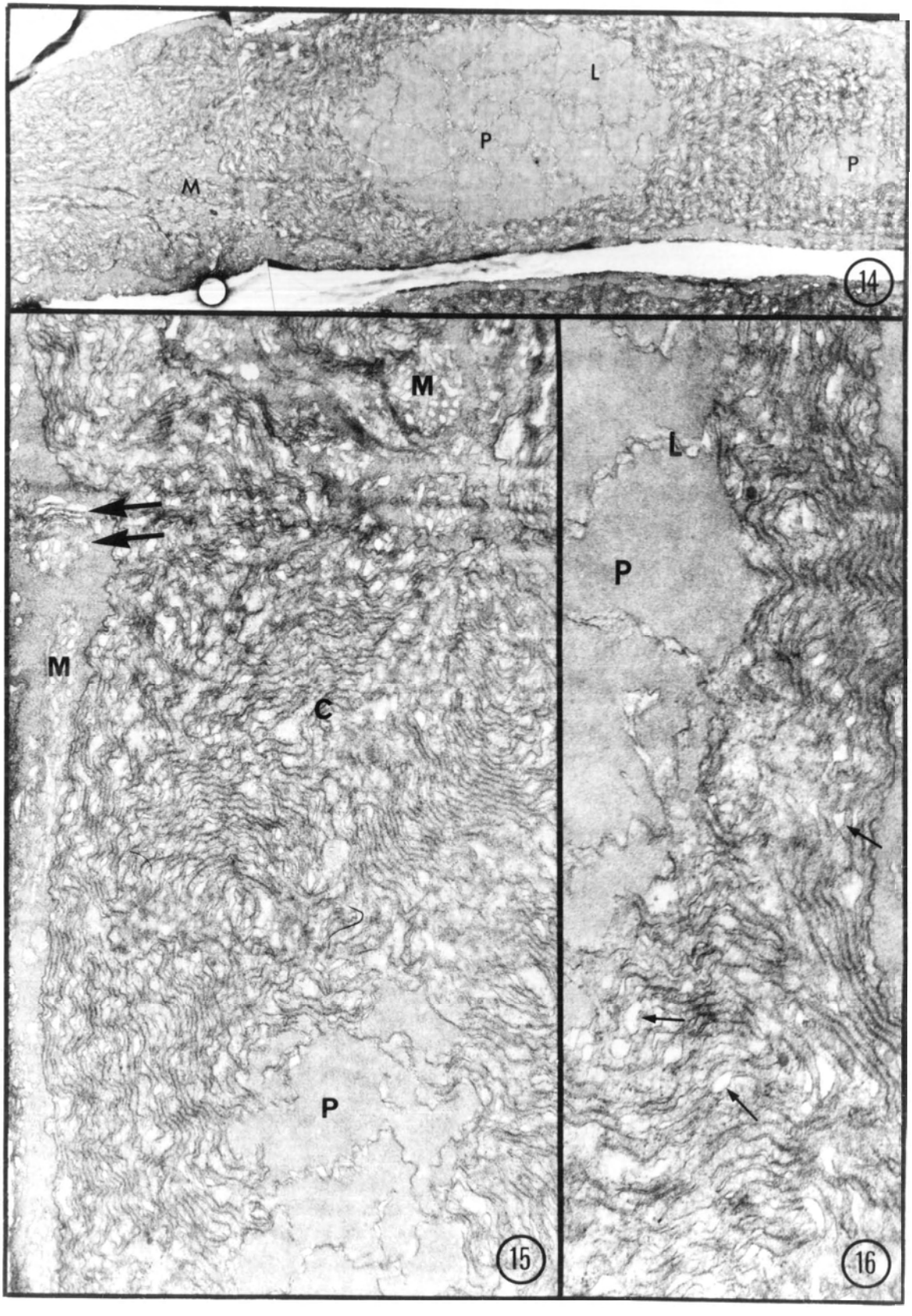
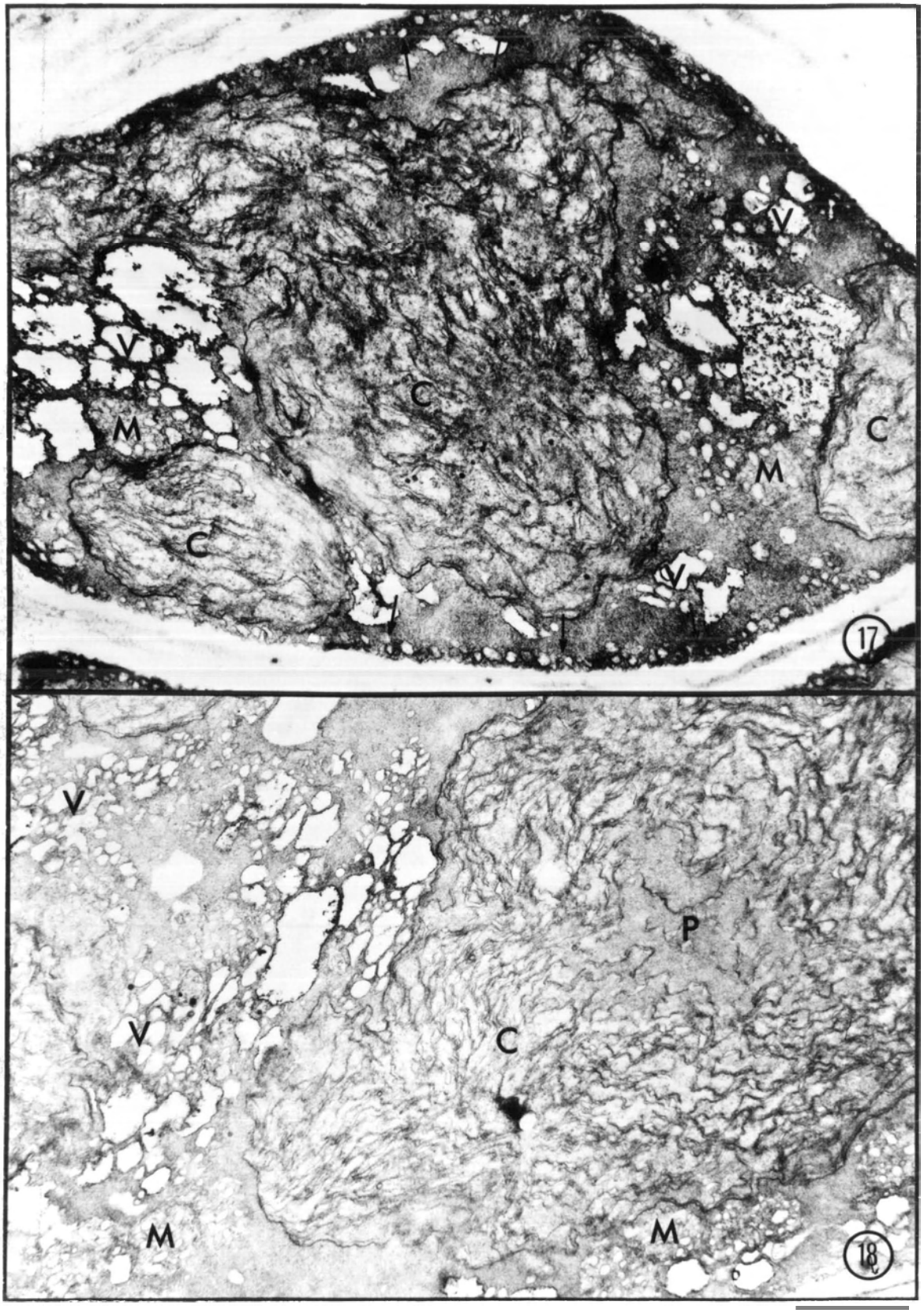

L
P
M
P
14
M
M
C
P
15
L
P
16

Fig. 17. Porphyra perforata dried for 100 m under a stream of drierite-filtered air at room temperature. Viable. Chloroplast and mitochondria are still intact. Note numerous vesicles in cytoplasm, especially the array of vesicles adjacent to the plasmalemma (arrows). x 18,900.

Fig. 18. Porphyra perforata air dried for 5 days at 11 C. Viable. Note numerous vesicles in the cytoplasm. x 14,400.

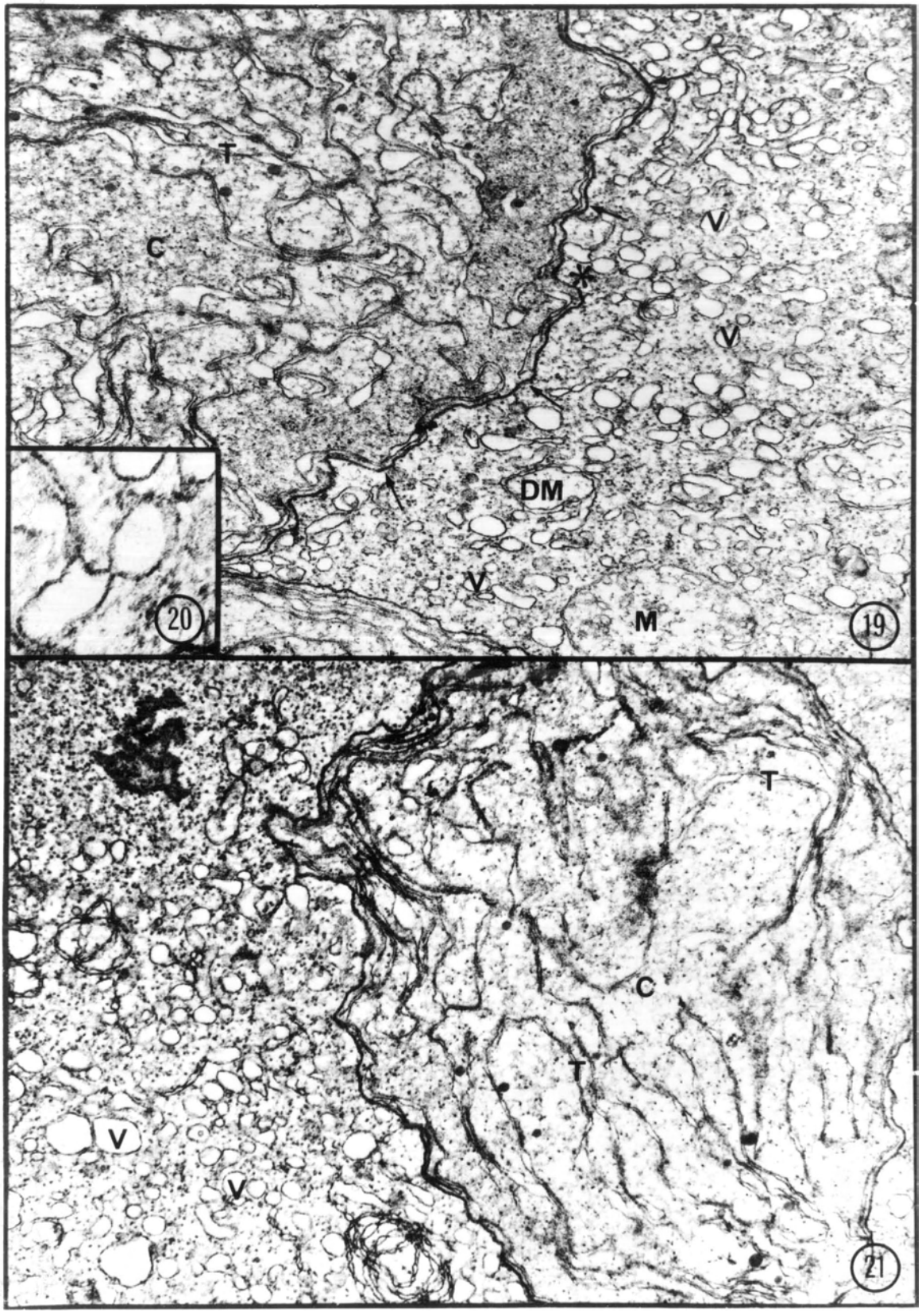

V
V
M
C
C
V
C
M
V
17
V
V
M
C
P
M
18

Figs. 19-21. Porphyra perforata air dried for 7 days at room temperature. Viable.

Fig. 19. Note the chloroplast envelope (arrows), and numerous vesicles in the cytoplasm. Disrupted mitochondria appear to be forming vesicles. x 24,100.

Fig. 20. Enlargement of area marked by * in Fig. 19 showing vesicles. x 77,300.

Fig. 21. One of the lobes of the chloroplast. Note prevalence of vesicles in cytoplasm but their absence in chloroplast. x 24,800.


T
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20
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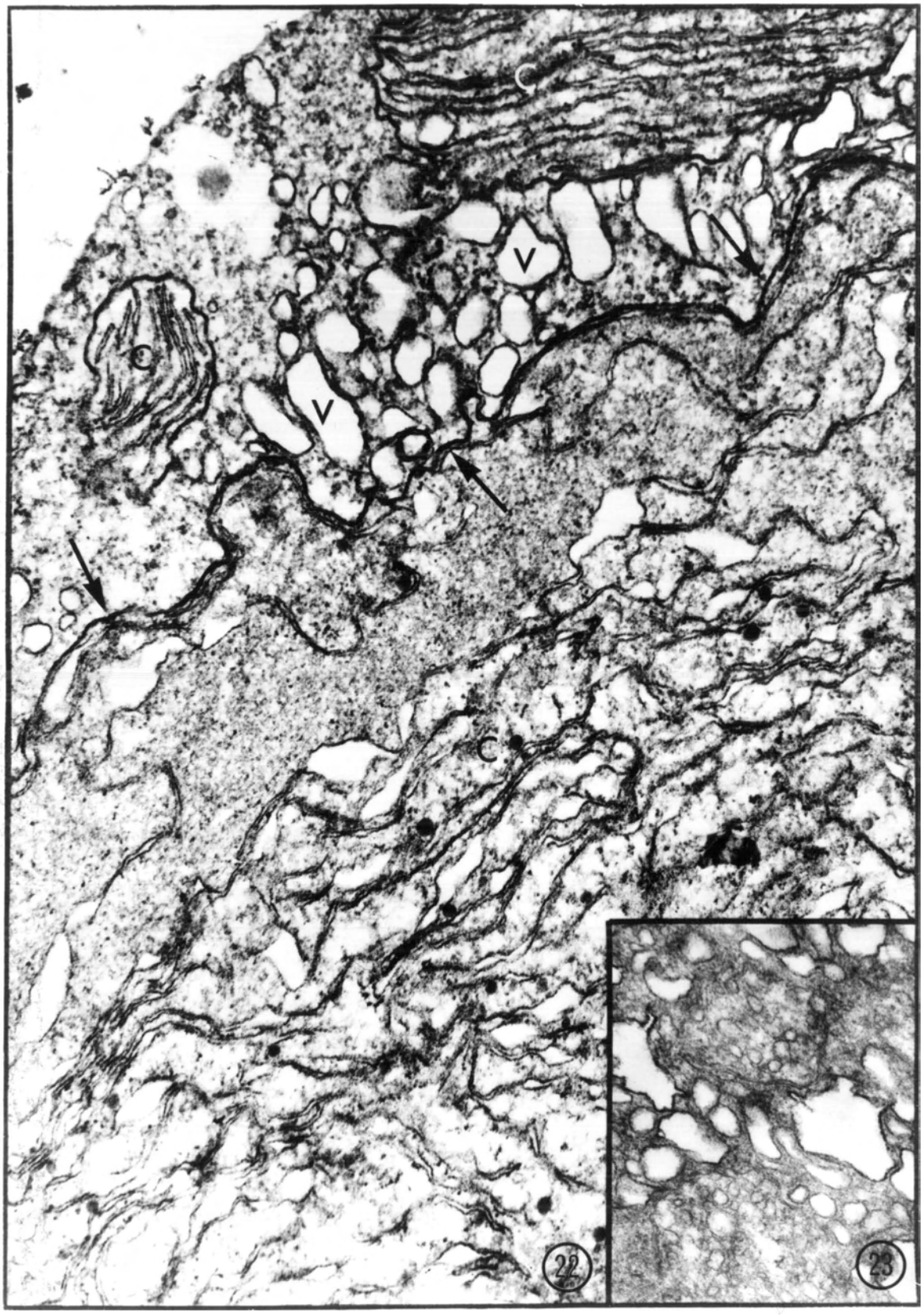
21

Figs. 22-23. Porphyra perforata air dried for 27 days. Not viable.

Fig. 22. Part of main chloroplast and two lobes are shown. Chloroplast envelope (arrows). Thylakoids are still intact. x 38,000.

Fig. 23. Mitochondria still appear to be intact. Cristae are visible. x 34,300.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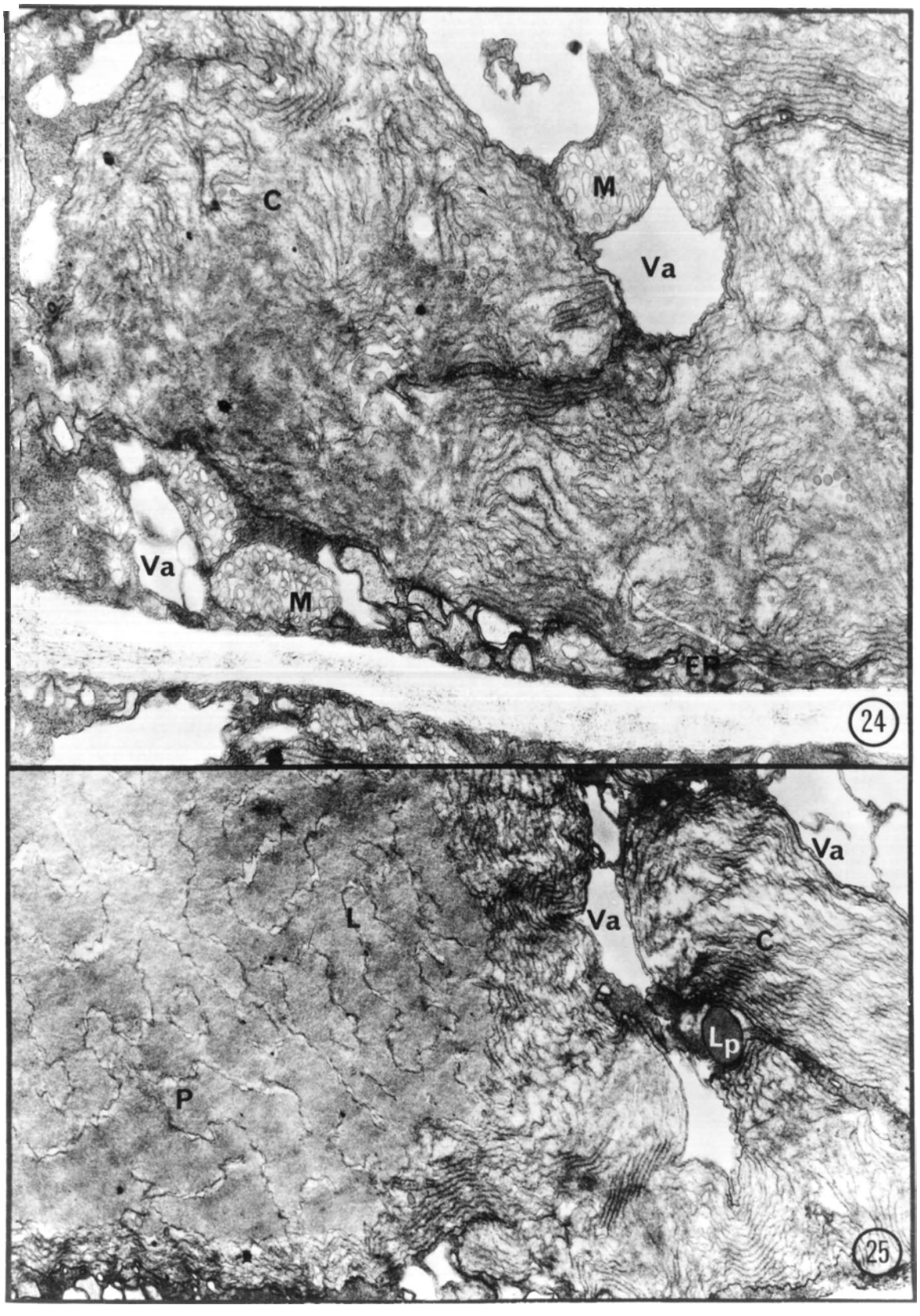
C
22
23

Figs. 24-25. Porphyra sanjuanensis, fresh control.

Fig. 24. Large single chloroplast and it's lobes occupy much of the cell. Vacuoles are larger than those found in Porphyra perforata. x 20,000.

Fig. 25. Enlarged view of pyrenoid and traversing lamellae. Part of chloroplast and some ballooning thylakoids (see text) are seen. x 22,100.


C
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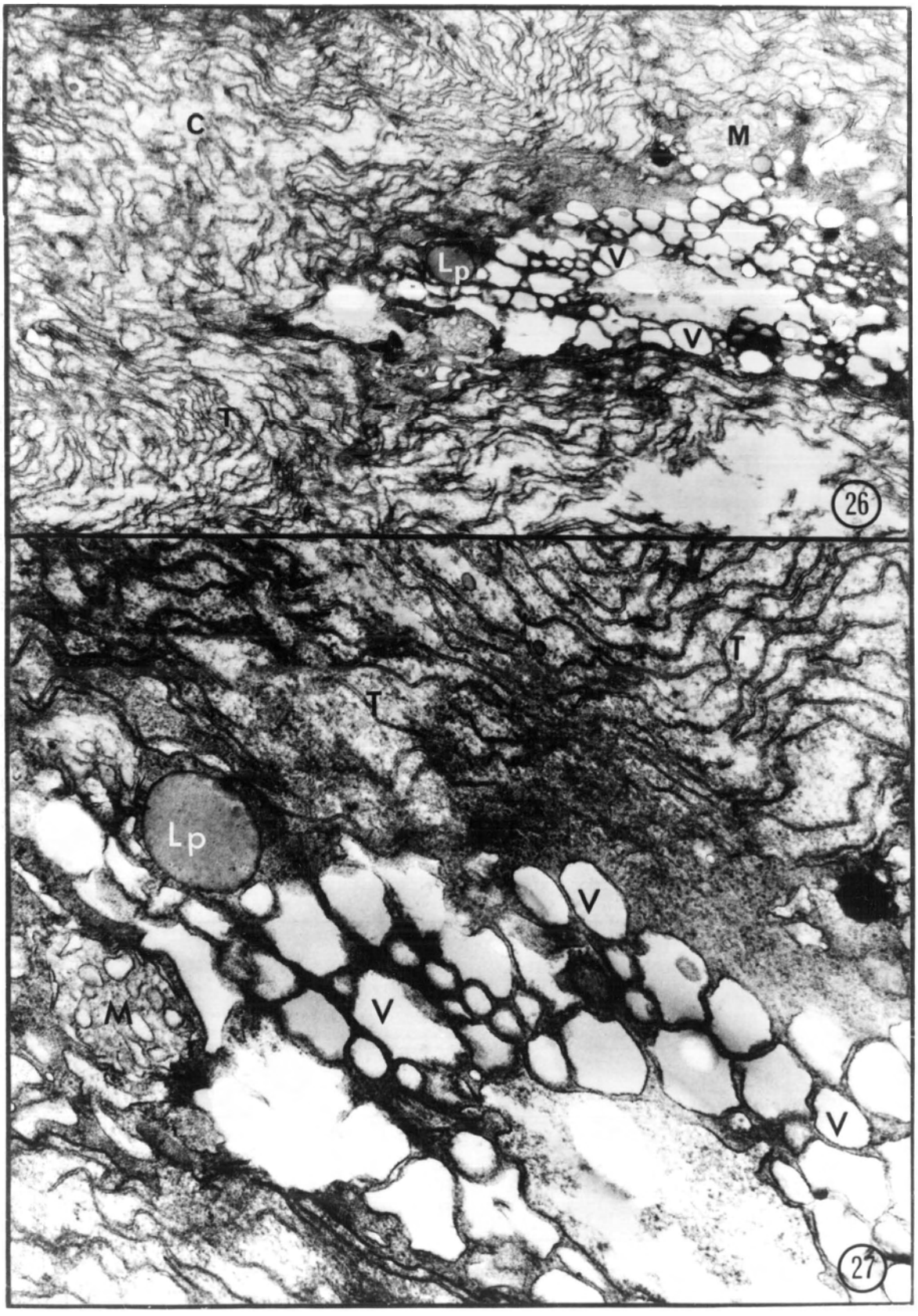
Va
M
EP
24
Va
Va
L
C
Lp
P
25

Figs. 26-27. Porphyra sanjuanensis, air dried for 24 h at room temperature and rehydrated for 10 m. Viable.

Fig. 26. Thylakoids are intact and there is no evidence of excessively expanded intrathylakoid spaces. Numerous vesicles are still present. x 16,000.

Fig. 27. Enlarged view of vesicles in Fig. 26. The vesicular membrane is clearly evident (arrows). x 51,000


C
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26
T
T
Lp
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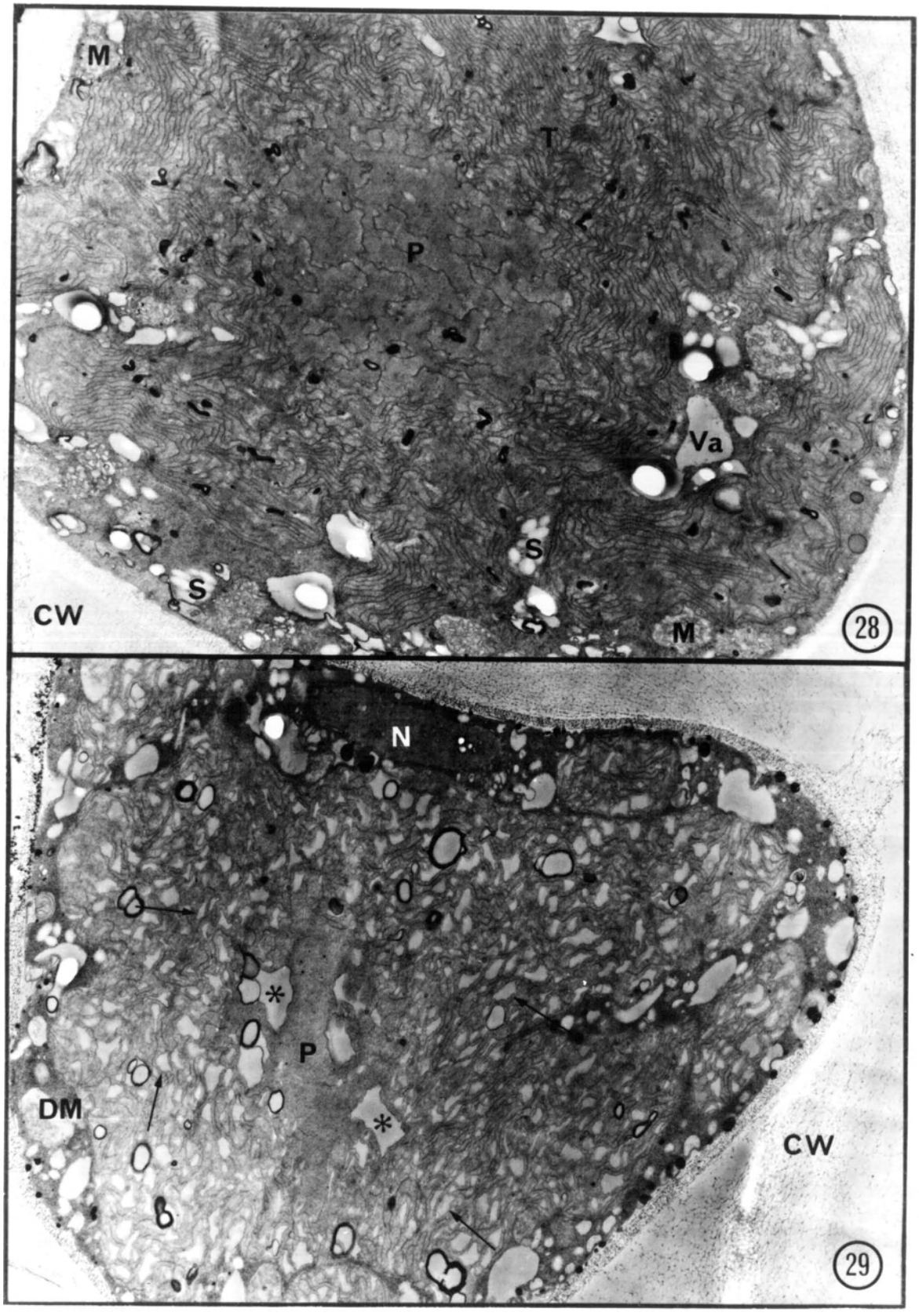
27

Figs. 28-29. Porphyra sanjuanensis air dried at room temperature for 24 h. and rehydrated for 30 m. The thallus is viable, though this may not be the case for all cells (see text).

Fig. 28. A delta cell (see text) showing apparently normal thylakoids and chloroplast. A relative lack of vesicles in the cytoplasm. Irregular black deposits may be artifact. x 12,000.

Fig. 29. A gamma cell (see text) showing numerous expanded intrathylakoid spaces (arrows), disrupted traversing lamellae in pyrenoid (*), and partially disintegrated mitochondria. x 10,100.


M
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CW
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29

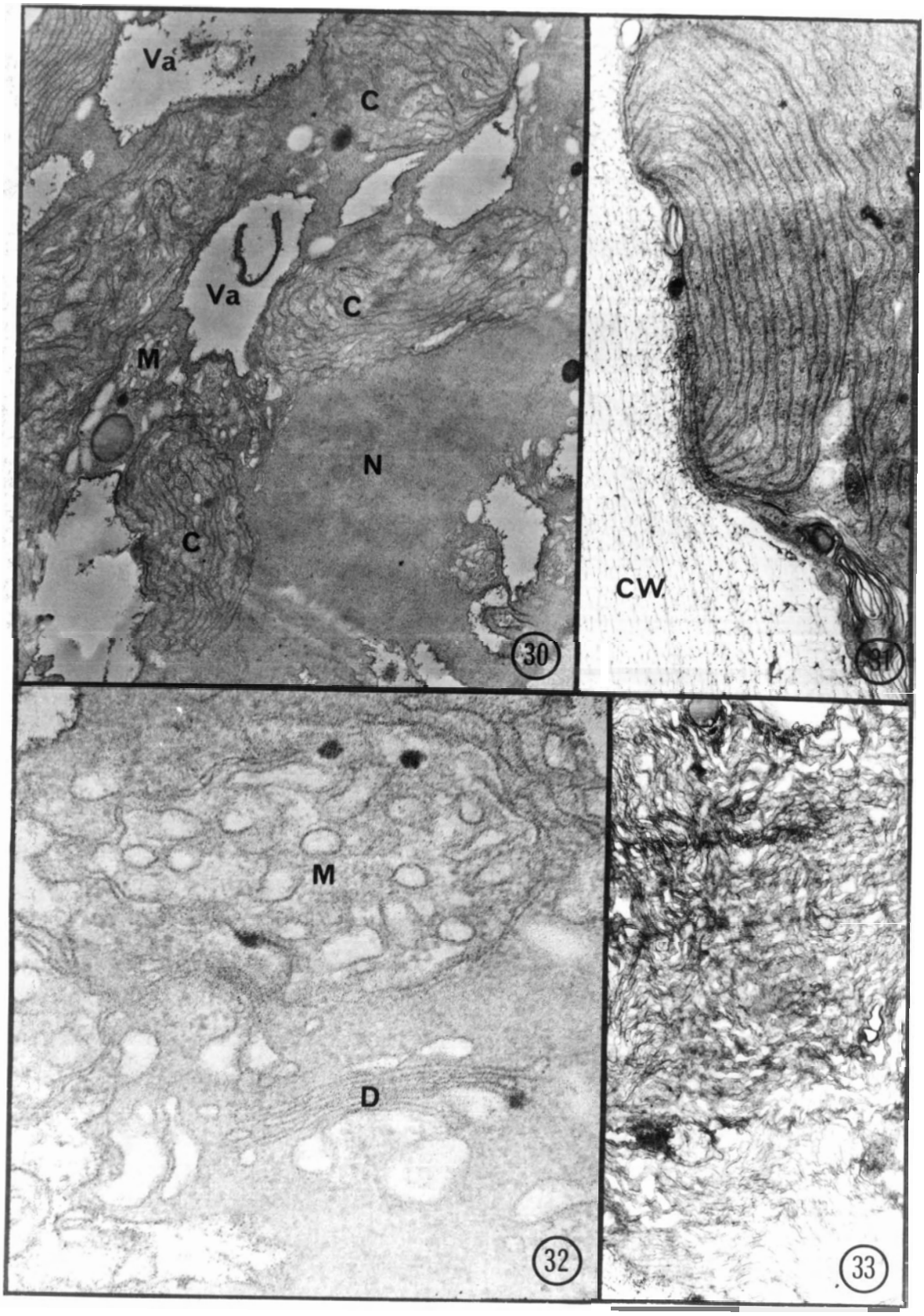
Figs. 30-33. Porphyra sanjuanensis air dried at room temperature for 24 h and rehydrated for 75 m. Viable. Delta and gamma cells (see text).

Fig. 30. A delta cell showing relative lack of small vesicles. Large vacuoles are characteristic for this species. x 25,000.

Fig. 31. A delta cell showing a chloroplast lobe. Note that the thylakoids appear normal. x 39,100.

Fig. 32. A delta cell showing the reappearence of dictyosomes. x 83,700.

Fig. 33. A gamma cell showing part of chloroplast and intense disruption of thylakoids. x 11,600.


Va
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33

Part 2: *Prasiola meridionalis* (Chlorophyta)

WET CONTROL:

Prasiola meridionalis contains a large dominant chloroplast (Fig. 34). The thylakoids in the chloroplast tend to be arranged groups of three; this is interesting in that most green algae do not have their lamellae in groups of three (Gibbs 1962; Ueda 1961), though such an arrangement is a common characteristic of the Phaeophyta (Gibbs 1962). A pyrenoid is located inside the chloroplast. This pyrenoid may or may not have starch grains located at its periphery. The pyrenoid is traversed by several lamellae. Endoplasmic reticulum and dictyosomes are sparsely distributed through the cytoplasm. The mitochondria are randomly spaced in the cytoplasm and have tubular cristae. Few vacuoles are found, but when present they occupy only a small portion of the total cell volume.

DEHYDRATED:

Desiccated *P. meridionalis* exhibits a general loss of cytoplasmic detail (Fig. 35). Reticular elements are still present but can only be distinguished at high magnification. Mitochondria no longer stain as clearly as wet controls; cristae and the outer double membranes are faint. The

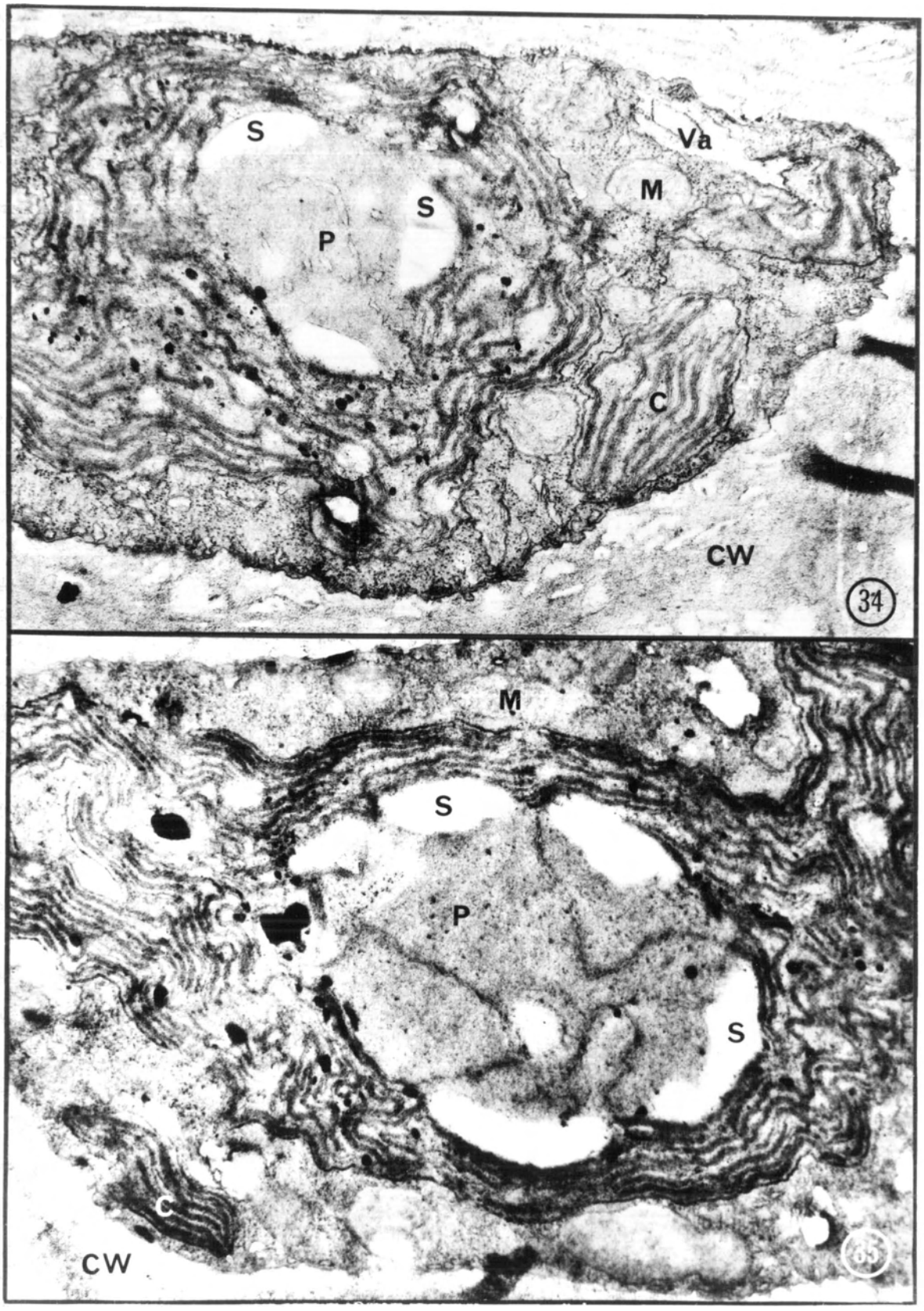
chloroplast, including the thylakoids, pyrenoid and starch sheath, do not appear to have been significantly altered by drying.

One notable difference between dried Porphyra and Prasiola is the maintainence of some reticular elements and the lack of vesiculization in Prasiola.

Figs. 34-35. *Prasiola meridionalis*.

Fig. 34. Fresh control showing cellular detail especially the lack of extensive vacuolation or E.R. x 28,500.

Fig. 35. Air dried for 24 h at room temperature. The cell structure is similar to that of fresh material, Fig. 34. x 27,700.


S
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34
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P
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C
CW
35

Part 3: *Ulva scagelii* (Chlorophyta)

WET CONTROL:

The large chloroplast in *Ulva scagelii* contains thylakoids with a characteristic stacking pattern of two, and multiples of two up to ten (Fig. 37). A pyrenoid is located inside the chloroplast. The pyrenoid usually has a single lamella traversing it. A starch sheath almost completely encircles the pyrenoid. Starch grains are also found randomly distributed in the stroma. The double nature of the nuclear membrane and the nucleolus are clearly defined (Figs. 36, 37). The highest proportion of dictyosomes and E.R. is located in a region adjacent to the nucleus (Fig. 36). Mitochondria are randomly distributed through the cytoplasm. A characteristic feature of these cells is a large vacuole offset to one side and occupying half the cell volume, smaller vacuoles are distributed in the cytoplasm.

DEHYDRATED:

U. scagelii was dried until a loss of fluorescence inducton transients occured. This dehydration proved to be lethal (see Table 1). Harsh drying with a drierite-filtered air stream gave somewhat different results from a mild drying

treatment on racks for one hour. Drierite dehydrated Ulva shows extensive plasmolysis (Fig.38). The cytoplasm has become filled with vesicles. There is almost a complete absence of such organelles as mitochondria, dictyosomes and E.R.. Thylakoid membranes can still be seen, though it is doubtful if the chloroplast envelope is intact.

Less harsh drying on racks for one hour causes less disruption (Figs. 39, 40). The degree of plasmolysis is not as severe as found in the harshly dried samples. Vesicle formation is not as prevalent. The cytoplasm has little E.R. and few dictyosomes. Extensive changes can be seen in the mitochondria as few or no cristae remain. The thylakoids sometimes show changes in which numerous electron transparent spaces form between thylakoids (Fig. 40). The nuclear membrane is no longer a smooth double lamellar system but has numerous puffed regions. The nucleolus is no longer visible (Fig. 39).

REHYDRATION:

U. scagelii which had been lethally dried with a drierite-filtered air stream for thirty minutes exhibit extensive disintegration after one hour rehydration. Two types of rehydrated cells occur. In the first cell type the

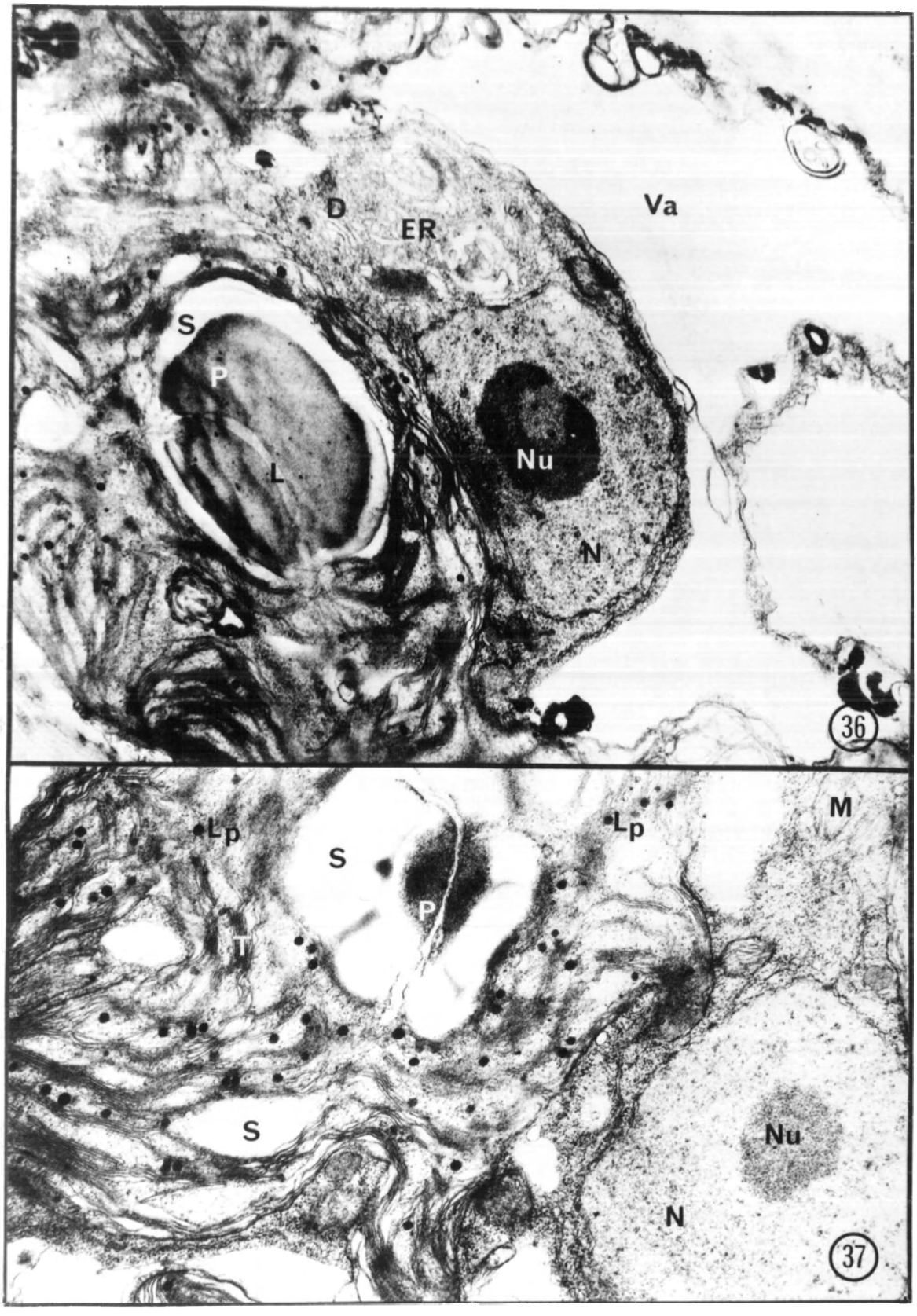
chloroplast has large regions which are not occupied by thylakoids (Fig. 41). This is not common in control or dried samples. It appears as if the chloroplast envelop has expanded. The pyrenoid is still intact as are the traversing lamellae. Vesicles are numerous in the cytoplasm. Dictyosomes and E.R. are absent. Mitochondria are in various states of disruption with few staining cristae. Some vesicles may be the vestiges of mitochondria (Fig.41). The remains of the nucleus are stained homogenously with numerous vesicles outlining where the double nuclear membrane once was. No obvious plasmolysis exists after rehydration.

The other cell type appears to be in a more advanced state of breakdown (Fig. 42). Little more than the chloroplast region is distinguishable, with diffusely stained lamellae and the remains of a starch sheath but no pyrenoid . The cytoplasm is filled with debris including membrane fragments, occassional vesicles and sparse particulate matter.

Fig. 36-37. Ulva scagelii, fresh control.

Fig. 36. Large vacuoles are present in the cell. Endoplasmic reticulum and dictyosomes are often located near the nucleus. x 16,400.

Fig. 37. The grana stacks occur in groups of two or multiples of two. X 23,400.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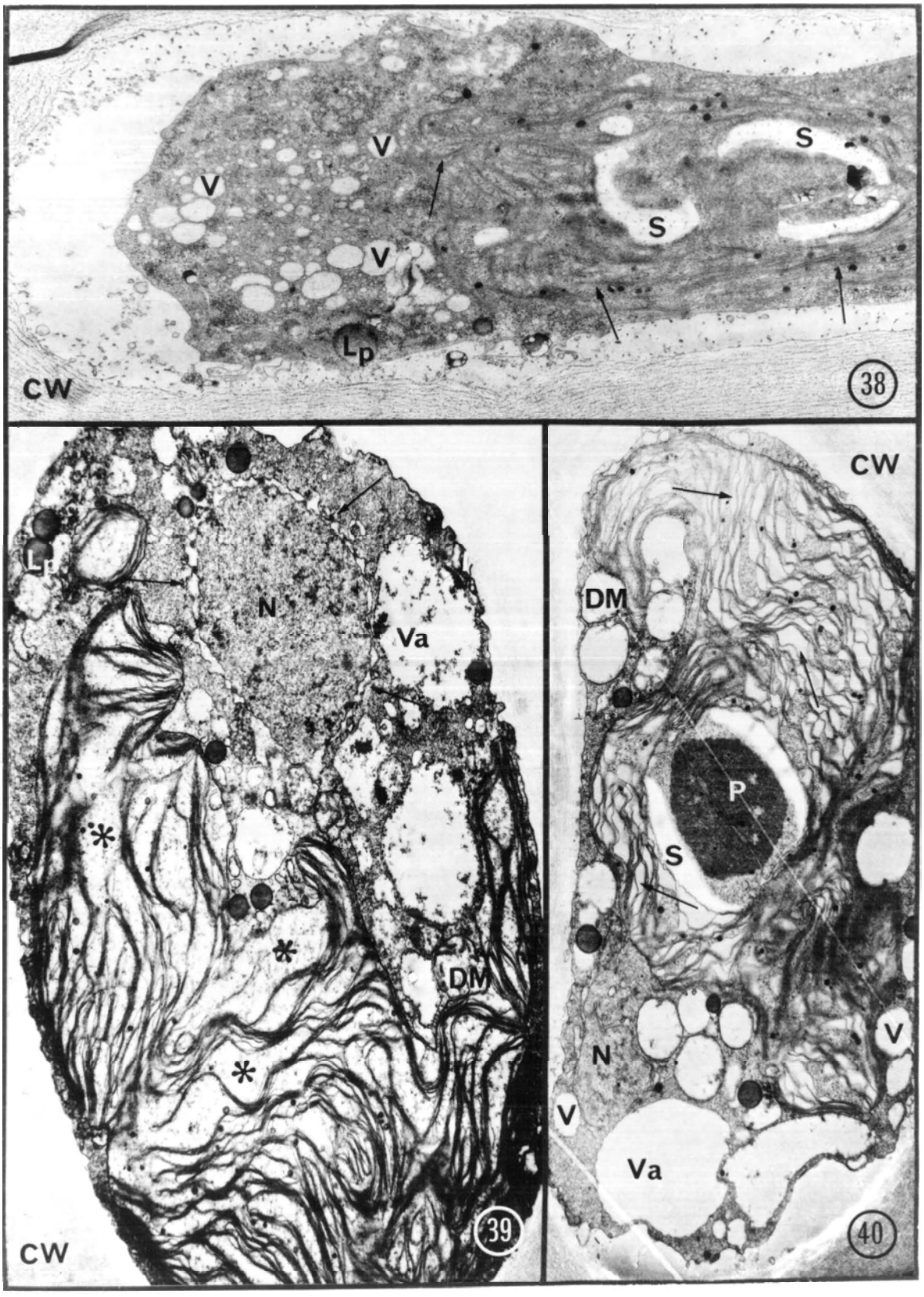
37

Figs. 38-40. Ulva scagelii, dried. Not viable.

Fig. 38. Harsh drying under a stream of drierite-filtered air for 30 m. This treatment causes extensive plasmolysis. Chloroplast envelope does not appear to be intact, though thylakoids are still evident (arrows). Starch sheath presumably outlines the region previously occupied by the pyrenoid. x 12,900.

Fig. 39. Mild air drying for 1 h does not cause excessive plasmolysis Note irregularity of the nuclear membrane (arrows). The stroma region of the chloroplast appears to be diffusely stained (*) when compared with controls, Figs. 36-37. x 13,000.

Fig. 40. Mild air drying for 1 h. Some vesicles appear to contain remants of cristae, suggesting they are disintegrating mitochondria. Intrathylakoid region appears disrupted and more electron transparent than in controls. x 13,000.


V
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39
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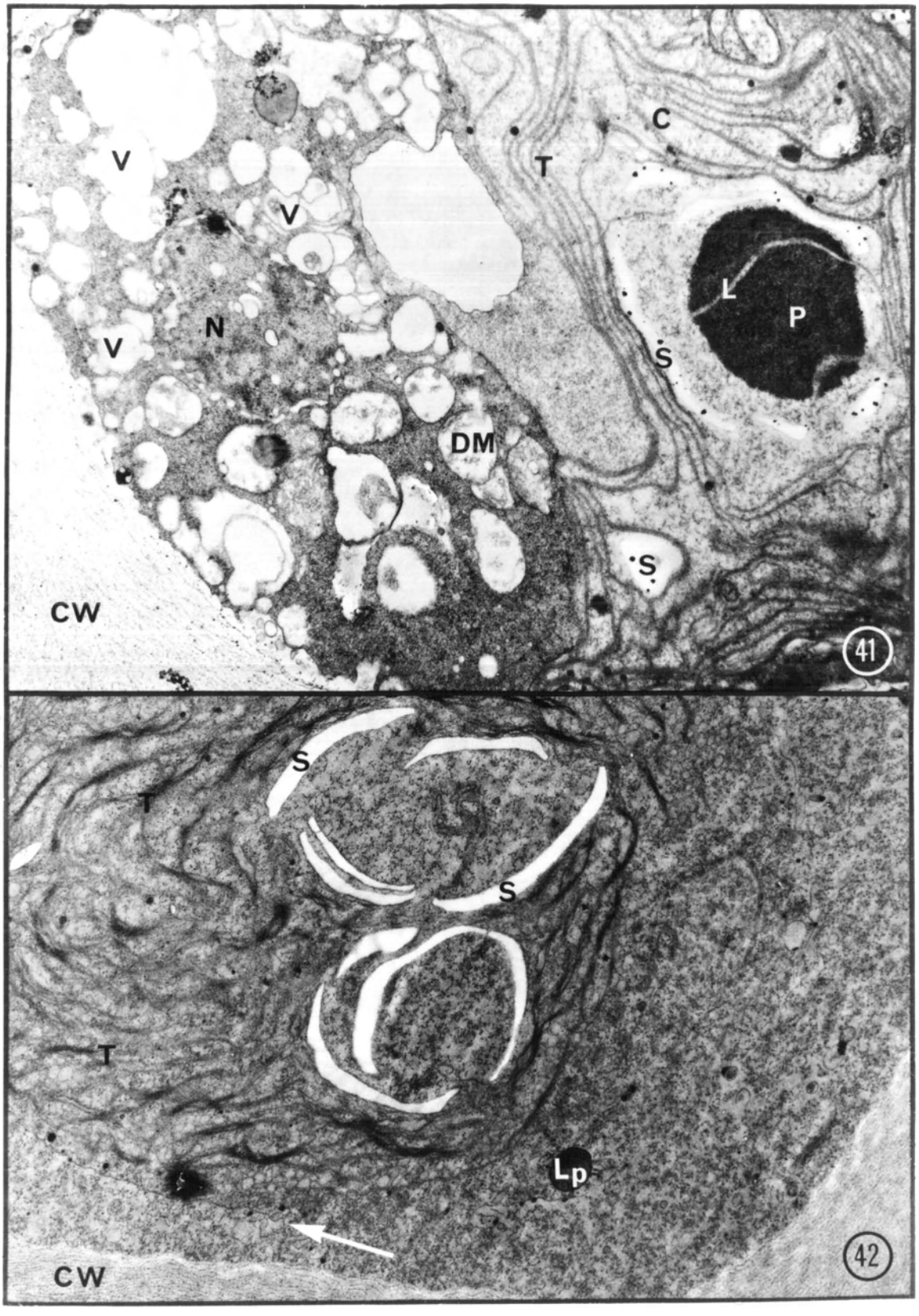
Va
40

Figs. 41-42. Ulva scagelii dried for 30 m under a stream of drierite filtered air, and then rehydrated for 1 h. Not viable.

Fig. 41. The chloroplast envelope and thylakoids still appear intact. The nuclear membrane and mitochondria are in various stages of disintegration. Numerous vesicles in the cytoplasm. x 13,700.

Fig. 42. Cell in advanced state of disintegration. Thylakoids are no longer distinct and chloroplast envelope is ruptured (arrows). Remants of pyrenoid are outlined by starch sheath. The cytoplasm contains membraneous fragments and granules which may arise from disrupted organelles. x 12,500.


V
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V
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L
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DM
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41
S
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S
S
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42

Part 4: *Petalonia fascia* (Phaeophyta)

WET CONTROL:

There are two major types of cells present in the thallus of *Petalonia fascia*, large vacuolate medullary cells (Fig. 43), and smaller non- vacuolate cortical cells (Fig. 45). These cells may contain one or more chloroplasts. The thylakoids are arranged in groups of three (Fig. 44) a commom characteristic of the Phaeophyta (Evans 1966; Gibbs 1962). E.R. is present. Mitochondria have numerous cristae. Of special interest are the plasmodesmata connecting cortical cells (Fig. 45).

DEHYDRATED:

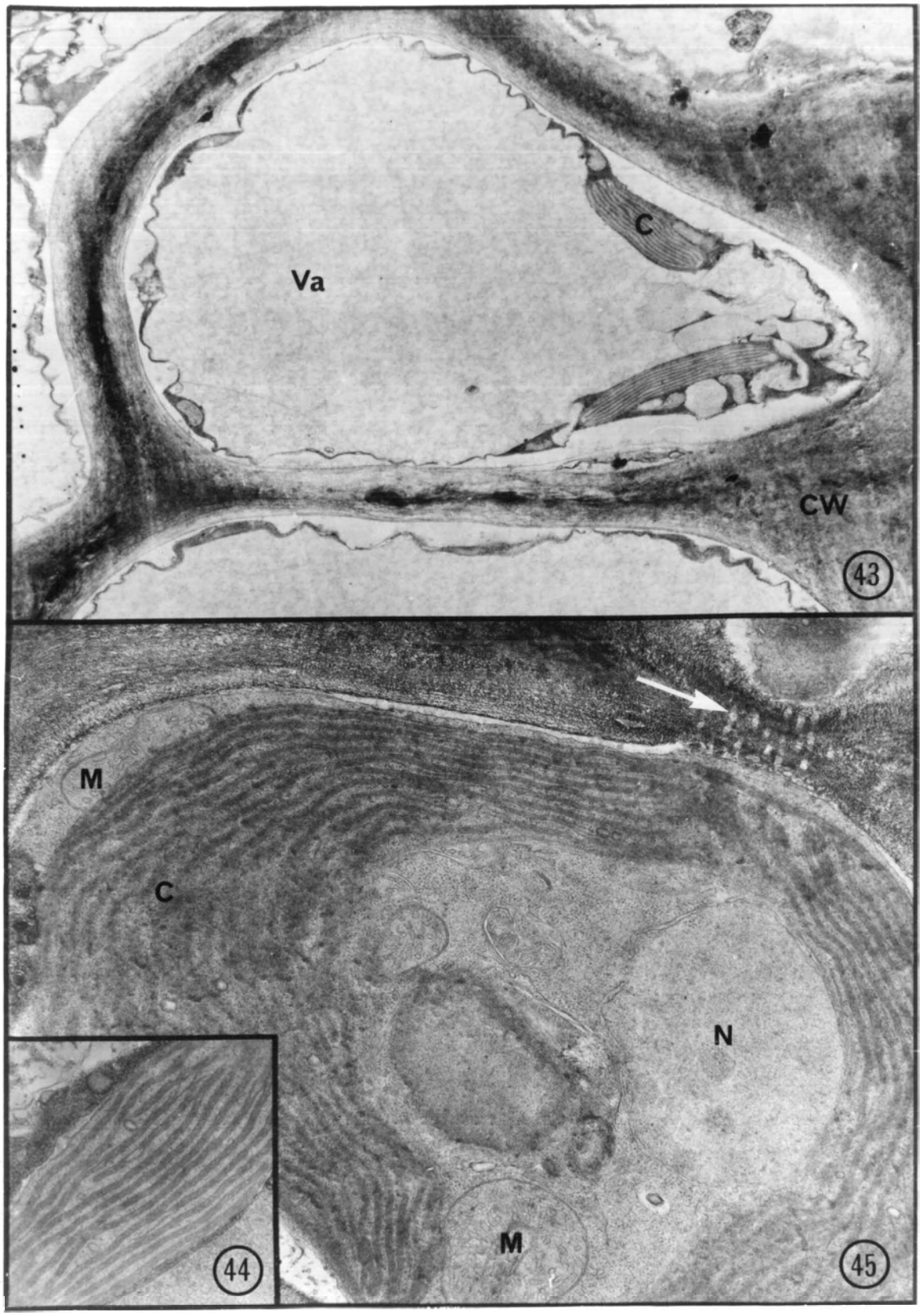
Desiccation is lethal for this alga. The large vacuolate cells are obliterated when dried. In the cortical cells the chloroplast may remain intact after drying (Figs. 46, 47), though in many cases the chloroplast envelope is ruptured. There is a modest amount of vesicle formation. No E.R. nor mitochondria are visible in the dried state. The plasmalemma has pulled away from the cell wall extensively, and shreds of material can be seen attached to the area around the plasmodesmata (Fig.46).

Figs. 43-45. Petalonia fascia, fresh control.

Fig. 43. A vacuolate medullary cell with chloroplasts located in the peripheral region. x 6,300.

Fig. 44. A single chloroplast showing the typical stacking pattern of thylakoids, viz. three thylakoids per stack. x 73, 200.

Fig. 45. A cortical cell showing a lack of vacuoles. The chloroplasts occupy most of the cell and appear to encircle the cytoplasm and other organelles. Note plasmodesmata connections between cells (arrows). x 7,300.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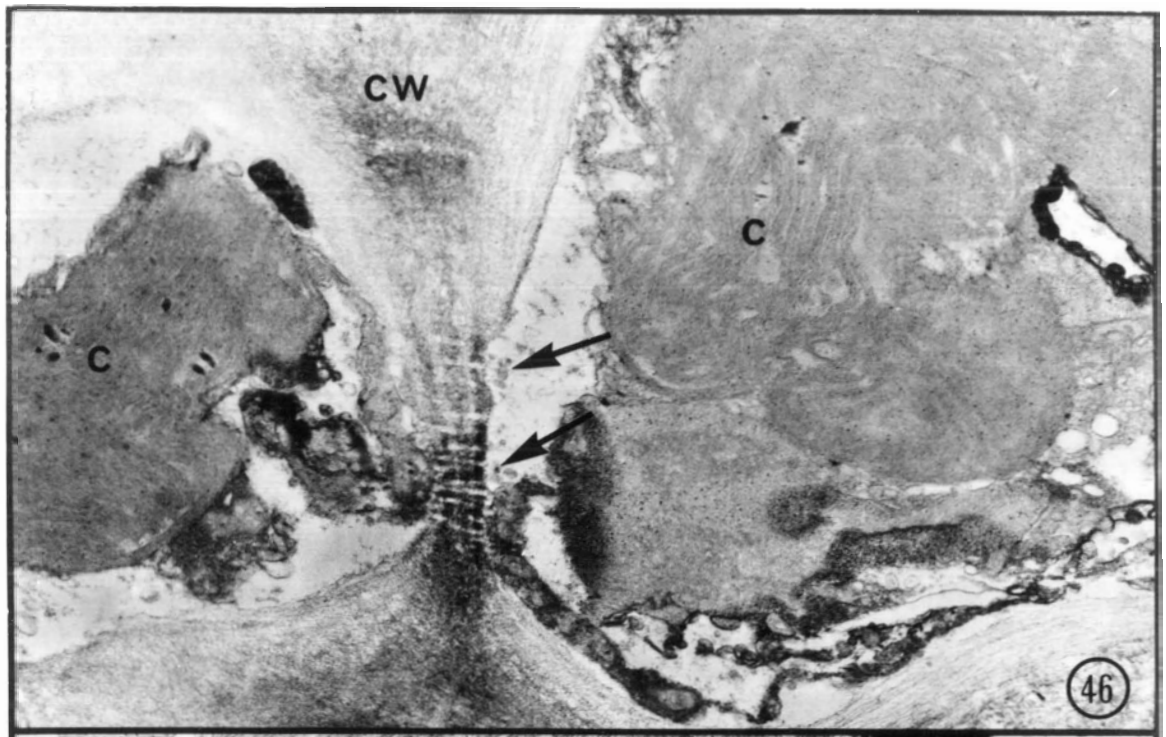
44
45

Figs. 46-47. Petalonia fascia, cortical cell air dried for 24 h. Not viable.

Fig. 46. Cells are plasmolyzed. Plasmodesmata attached to shreds of cellular material (arrows). x 23,000.

Fig. 47. Thylakoid banding pattern is maintained. Nucleus is intact though the nuclear membrane shows blebbing. x 59,400.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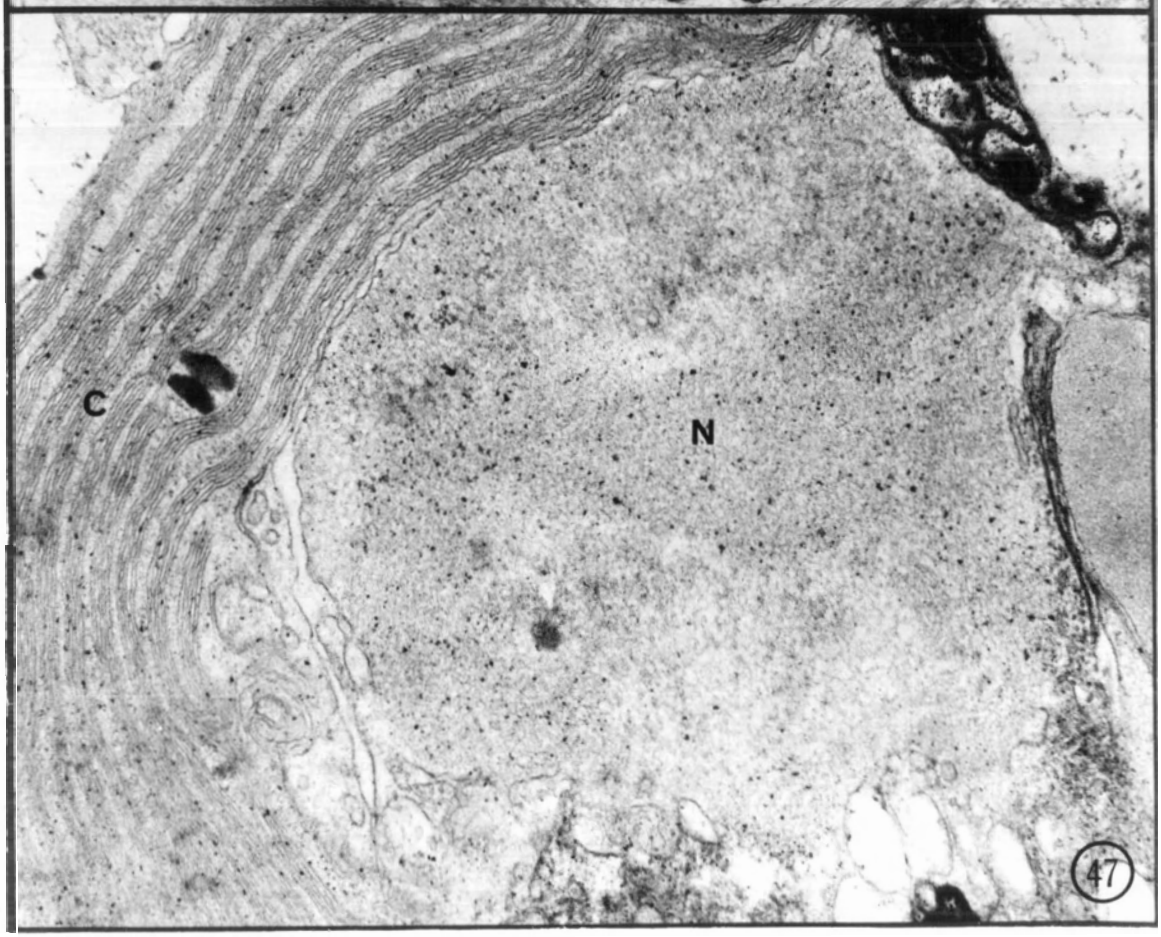
46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47

Part 5: Nitophyllum notti (Rhodophyta)

WET CONTROL:

Nitophyllum notti has numerous chloroplasts (Fig.48). The single thylakoids are commomly arranged parallel to one another (Fig.49), with occassional starch grains found in the stroma. E.R. is sparsely located throughout the cytoplasm. Numerous dictyosomes are often found with their forming face adjacent to a mitochondrion (Fig.49), an ultrastructural trait shared with Porphyra (Fig.15). The predominant feature of these cells is the central vacuole that occupies most of the cell, the cytoplasm lies along the periphery next to the cell wall (Fig. 48).

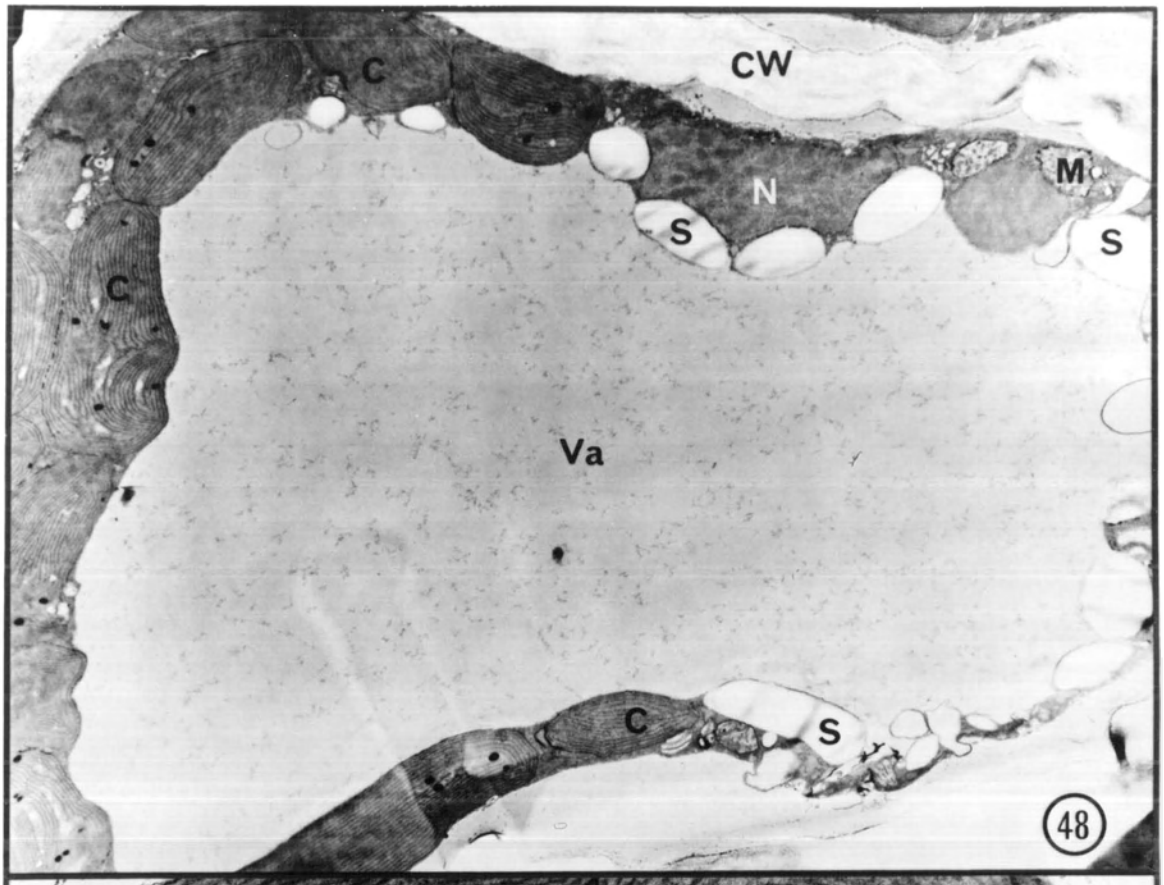
DEHYDRATED:

The effects of desiccation are dramatic. The cytoplasm pulls away from the cell wall and appears to collapse into the vacuole (Fig. 50). Mitochondria, dictyosomes E.R. and the nucleus are completely obliterated. The chloroplast envelope is torn but amazingly the thylakoids are preserved intact and still tend to be parallel with one another (Fig. 51). Starch grains are randomly distributed in the cell. There are no obvious vesicles in this material as there are in dried Porphyra species.

Figs. 48-49. Nitophyllum notti, fresh control.

Fig. 48. The cytoplasm and organelles are located at the periphery around a large central vacuole. x 6,400.

Fig. 49. An enlargement of cell periphery showing organelles and cytoplasm. x 24,100.


C
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Va
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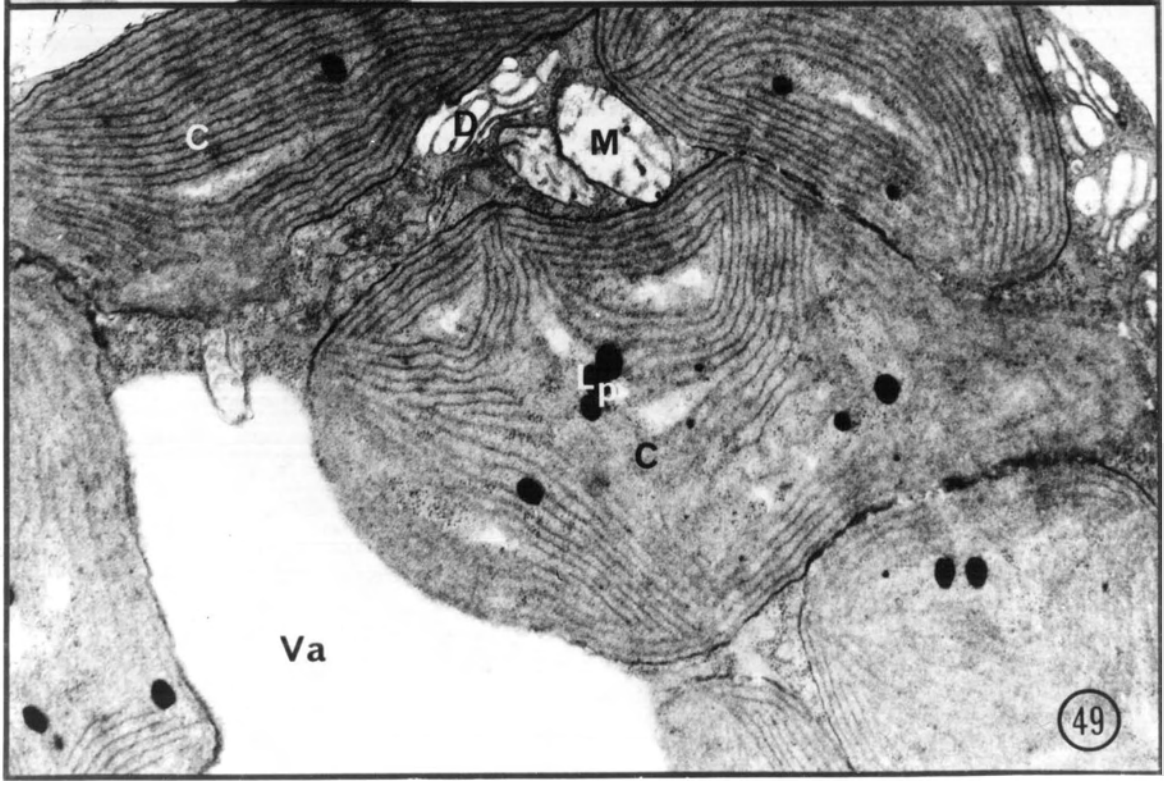
48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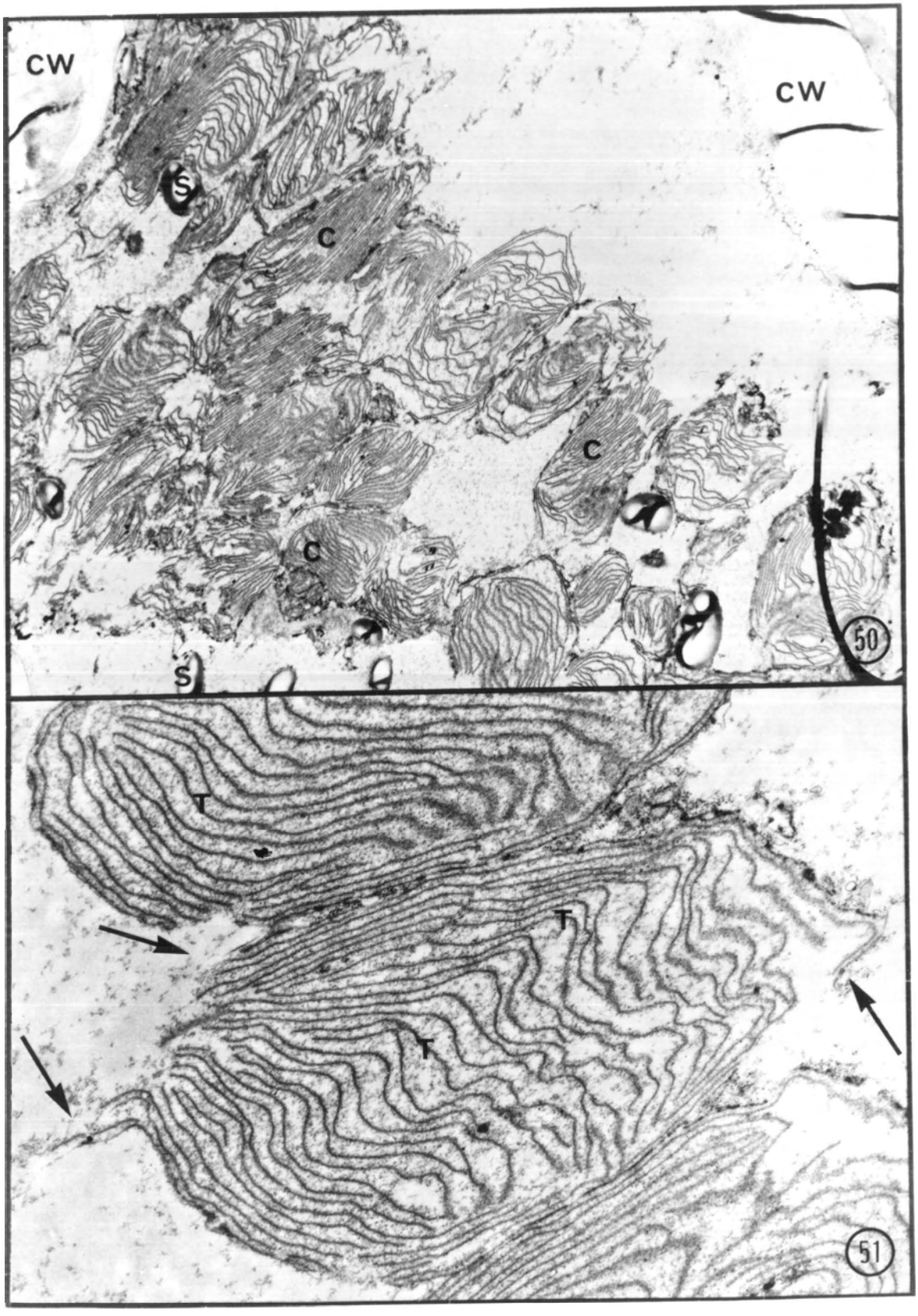
49

Fig. 50-51. Nitophyllum notti, dried for 30 m under a stream of drierite filtered air. Not viable.

Fig. 50. Chloroplasts have collapsed into the central vacuole. Other cell organelles are obliterated. x 10,800.

Fig. 51. Enlarged view of chloroplasts. Arrows indicate ruptured chloroplast envelope. Thylakoids are still intact. x 21,900.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51

DISCUSSION OF ULTRASTRUCTURAL RESULTS:

There may be some question as to the appropriateness of using aqueous fixatives on dehydrated material. Is material fixed in such a manner actually representative of the dried state? Nir et al (1969) used aqueous as well as a dry vapor method of fixation and obtained similar results on dried pea roots. The fact that dried Ulva (Fig. 38) retains its plasmolyzed nature when using an aqueous fixative, suggests that buffered glutaraldehyde does preserve the dried state. When Ulva samples were rehydrated and then fixed, plasmolysis was not evident (Fig. 41).

During drying, if the cell wall does not collapse, the protoplasm is subjected to strong negative wall pressures. These negative wall pressures may reach values of -20 (Holle 1915) to -73 atmospheres (Blum 1937). This tension could cause the plasmalemma to pull away from the cell wall as a result of plasmolysis. When plasmolysis is extensive it can result in mechanical injury such as tearing of the plasmalemma (Oppenheimer and Jacoby 1963). Large vacuoles probably act as sites of undue mechanical stress during drying and would be expected to promote plasmolysis. In the sensitive algae looked at, all have large vacuoles which occupy half or more of the cell volume; Ulva (Fig. 36), Petalonia (Fig. 43), and

Nitophyllum (Fig. 48). These three algae were also subject to some degree of plasmolysis; Ulva (Fig. 38), Petalonia (Fig. 46), and Nitophyllum (Fig. 50). In contrast the tolerant algae have small vacuoles; P. perforata (Fig. 14), P. sanjuanensis (Fig. 24), and Prasiola (Fig. 34). As would be expected due to their small vacuole size the tolerant algae did not show signs of plasmolysis when dried (Figs. 17, 35). There are instances where tolerant plants have large vacuoles but in such cases there is usually a mechanism by which the stress is minimized, such as the solidification of the vacuole (Oppenheimer and Halevy 1962; Stuart 1968).

Plasmodesmata connections between cells are potential sites of plasmalemma rupture during drying. In Petalonia the plasmalemma pulls away from the cell wall during drying and the plasmodesmata connections are torn resulting in extensive disruption of the plasmalemma (Fig. 46). The absence of plasmodesmata observed in toleraht cells was not unexpected as they impose a strain on the plasmalemma during dehydration, a strain which would not contribute to tolerance.

Dehydration results in the formation of numerous vesicles in the cytoplasm of P. perforata (Figs. 17-22) and Ulva (Fig. 38). Other researchers have seen similar vesicles formed in desiccated pea roots (Nir, Klein and Poljakoff-Mayber

1969), a tolerant moss (Tucker, Costerton and Bewley 1975), and lethally dried and rehydrated fungal sporangia (Choen et al 1974).

Vesiculization in the cytoplasm of dehydrated material did not occur in all cases. Neither the tolerant alga Prasiola (Fig. 35), nor the two sensitive algae Nitophyllum (Fig. 50) and Petalonia (Fig. 46) exhibited the presence of vesicles in the dried state. The absence of vesicles in dried Nitophyllum and Petalonia is probably due to the extensive disruption of cytoplasmic detail caused by drying, The appearence of numerous vesicles in dehydrated algae may be related to the disappearence of E.R. and dictyosomes. In Porphyra the E.R. is usually found parallel to the plasmalemma (Bourne 1971), but after drying this region is occupied by a strand of vesicles (Fig. 17). The dictyosomes of Porphyra are often in close association with mitochondria, but in dried material the dictyosomes have disappeared and vesicles are fornd near the mitochondria (Fig. 18). In Ulva the E.R. and dictyosomes are concentrated in a region near the nucleus (Fig. 36). After drying this region is filled with vesicles (Fig. 41). Prasiola maintains both dictyosomes and E.R. in the dried state and there is no sign of vesiculization. These results suggest that vesicles produced during drying are the remains of

disrupted E.R. and dictyosomes. Other researchers have shown that many membranes form vesicles when disrupted in the appropriate media (Fleischer et al. 1969; Levy et al 1967; Steck et al 1970).

It should be mentioned that in harshly treated material, such as Porphyra dried seven days or lethally dried Ulva, mitochondrial breakdown may contribute to the number of vesicles formed. In Porphyra (Fig. 19) a partially disrupted mitochondrion seems to have formed several vesicles. Dried Ulva has large vesicles which occassionally show the remnants of what appear to be cristae (Fig. 40). It seem that the mitochondrial substructure is more tolerant of drying than either E.R. or dictyosomes but it is eventually disrupted and may form vesicles.

In both the tolerant Porphyra and sensitive Ulva the E.R. and dictyosomes have formed vesicles. Presumably the vesicles no longer function as they did in the intact E.R. or dictyosomal system. In order to maintain normal cellular processes the tolerant alga, Porphyra, must re-establish both E.R. and dictyosomes after rehydration. There are two ways in which this could take place: (1)The organelles are reformed from the vesicles, or (2)a de novo synthesis takes place. In rehydrated Porphyra vacuolation persists for up to thirty minutes with some signs of E.R. present near the plasmalemma.

Not until seventy-five minutes do signs of dictyosomes become evident (Fig. 32). The time interval necessary to re-establish dictyosomes and E.R. suggest that the organelles are being re-synthesized in the tolerant alga Porphyra. In Ulva rehydration does not re-establish the E.R. or dictyosomal elements but rather accelerates the deteoriation of all cytoplasmic details.

The preservation of dictyosomes and E.R., and the absence of vesicles in dried Prasiola suggest that this plant is even more tolerant to drying than Porphyra. Staining dried membranes results in less distinct images but scrutiny of micrographs at high magnifications demonstrates that E.R. is still present in the dried state. The reticular membranes of Prasiola may have a different composition making them less prone to desiccation damage.

Nir et al (1969) made the observation that dehydrated pea root mitochondria no longer retain visible cristae when fixed with glutaraldehyde-osmium. Using a different fixation technique (Nir, Poljakoff-Mayber and Klein 1970) it was found that cristae are still present in the dried state. This led to the conclusion that drying causes changes in the cristae membrane such that they no longer react with

osmium. Work on water stressed-corn mitochondria has shown that mitochondrial membrane permeability has been altered. No tolerant species have previously been studied. Both tolerant species, Prasiola and P. perforata, retain visible cristae in the dried state (see Figs. 17, 19, 35). Dehydrated sensitives do not maintain visible cristae: Ulva (Fig. 38-40), Petalonia (Fig. 46). There may be a difference in the membrane composition between cristae of tolerant and sensitive marine algae as suggested by their staining properties in the dried state.

In the three tolerant algae, P. perforata, P. sanjuanensis and Prasiola the chloroplast structure was not grossly altered by drying, but subtle changes of thylakoid dimensions may occur. The thylakoid width and membrane thickness of P. sanjuanensis was measured in wet, dried, and rehydrated states. From table 3 it can be seen that drying resulted in a contraction of the thylakoid width from 203 Å to 171 Å. Individual thylakoid membranes decreased in thickness from 82 Å to 58 Å. Rehydration for seventy-five minutes restored approximately the original dimensions of the thylakoids. Murakami and Packer (1970) reported shrinkage of the thylakoid diameter when hypertonic dessicants were used and predicted a decrease in membrane thickness with water loss. On the other hand Colbow and Jones (1974) using a lecithin bilayer

system and X-ray diffraction found that water loss caused an increase in the thickness of their artifical membrane. Colbow and Jones (1974) results are in contradiction to those obtained for Porphyra, but they did not use electrolytes in their media which may account for the differences seen. Träuble and Eibl (1974) have shown that divalent cations cause a slight contraction of a negatively charged lipid bilayer.

Chloroplasts of sensitive algae exhibit several differences after dehydration. In Ulva and Petalonia the chloroplast envelope may or may not remain intact, while Nitophyllum chloroplasts are invariably ruptured after drying. The stroma of Ulva and Nitophyllum has been altered by drying, changing from a homogenously stained material in controls to clumped particulate matter in dried specimens. Petalonia maintains a uniformly stained stroma in dehydrated material when intact chloroplasts are found (Fig. 47). Even though the chloroplast envelope of Nitophyllum has been ruptured the thylakoids are still distinct parallel lamellae. In dried Ulva there are often cells in which the locular space between thylakoid membranes has expanded to form an electron transparent space. Nir and Poljakoff-Mayber (1967) mentioned similar distortions of intergranal lamellae in flaccid Swiss chard leaves.

Table 3: Measurements of thylakoid diameter and membrane thickness after drying and rehydration in Porphyra sanjuanensis.

Table 3

TREATMENT	THYLAKOID WIDTH Å	STANDARD DEVIATION OF THE MEAN ±
Wet control	203	14
Dried	171	5
Rehydrated	212	30

	MEMBRANE THICKNESS Å	
Wet control	82	10
Dried	58	6
Rehydrated	98	20

Rehydration acclelerates chloroplast breakdown in lethally dried cells. Ulva thylakoids stain with less intensity after rehydration (Fig. 41) or appear broken and indistinct (Fig. 42). Though P. sanjuanensis is considered to be a tolerant species, there are cells which, after rehydration, show signs of chloroplast disruption. The locluar space expands excessively and along a considerable portion of the thylakoid. Rehydration probably activates lytic substances which have been released by dehydration and promote chloroplast breakdown.

SUMMARY OF ULTRASTRUCTURAL RESULTS:

There are differences as well as similarities in the effects of desiccation on tolerant and sensitive algae. Following is a list of some of the changes seen:

1. Plasmolysis is minimal in tolerant algae supposedly due to the absence of large vacuoles.

2. Drying may result in vesicle formation in both tolerant and sensitive algae. The appearence of vesicles is correlated with the disappearence of E.R., dictyosomes and possibly disrupted mitochondria.

3. Mitochondrial cristae lose their staining properties in sensitive algae after drying. Tolerant algae still have visible cristae even after drying.

4. In sensitive species the chloroplast envelope is often ruptured by drying; while in tolerant algae the envelope is maintained intact.

5. Drying may result in reversible shrinkage of thylakoid width and membrane thickness in P. sanjuanensis.

6. In sensitive algae the nuclear membrane was often found to be disrupted after drying, and the nuclear material was no longer diffuse but appeared condensed.

7. Rehydration of lethally dried material results in cellular disintegration.

8. Rehydration of tolerant species results in a reappearence of E.R. and dictyosomes as well as a loss of the numerous vesicles produced by drying.

CHAPTER V

Conclusions and Summary:

Photosynthesis in desiccation tolerant and sensitive marine algae was analyzed during drying and rehydration. Previous attempts to monitor dehydrating samples have been hampered by the lack of appropriate methods. The unique application of fluorescence induction techniques to this problem has made it possible to follow changes at the photochemical level in the photosynthetic apparatus during drying and rehydration.

Desiccation caused a progressive step by step inactivation of photosynthesis in both sensitive and tolerant algae. The order of inactivation proceeds as follows: (1) First there is a loss of energy transfer between photosystem II and I (alpha transition), a decrease in photophosphorylating activity, and a reduction of Calvin-Benson cycle activity. (2) Next, and very significant, there is a loss of electron transport between photosystem II and I (PS II and I). At this stage, photosynthetic activity is analogous to that found in a DCMU-poisoned system. PS II is still a functional unit but its activity is restricted to the reduction of Q. With the inhibition of electron transport there is no longer a sink for

electrons released from water, and photosynthetic oxygen production is negligible. (3) Further drying causes a cessation of water- splitting. (4) Finally there is an alteration of the physical state of the chlorophyll molecules.

An important effect of dehydration is to concentrate electrolytes in the cell. These electrolytes may in turn be responsible for some of the stages of photosynthetic inactivation. High concentrations of NaCl are known to increase the quantum yield of fluorescence in DCMU-poisoned cells (Clement-Metral and Lefort-Tran 1974; Murata 1971a, 1971b); ions presumably interact with the photosynthetic apparatus and affect efficiency. Concentrated solutions of NaCl also suppress the efficiency of NADP reduction while slightly enhancing the activity of the Hill reaction (Murata 1971a). This strongly suggests that high NaCl concentrations interfere with electron transport between PS II and I. Increasing electrolyte concentration in drying plants could account for the stepwise loss of photosynthetic functions.

Desiccation with its accompanying increase in electrolyte concentrations, can induce conformational changes in thylakoid membranes. Drying occurs in P. sanjuanensis with a reversible shrinkage of thylakoid membranes (Table 3). This shrinkage may be due to an increased salt concentration rather

than water loss alone. Murakami and Packer (1970) showed that sucrose, when used as an osmotic desiccant, does not affect thylakoid membrane thickness *in vitro*. If water loss were responsible for the decrease sucrose should cause a shrinkage but it doesn't. Clement-Metral and Lefort-Tran (1974), on the other hand, demonstrated that thylakoid membranes decrease in thickness when exposed to 4 M NaCl. This leads to the conclusion that dehydration-concentrated salts are responsible for membrane shrinkage.

Träuble and Eibl (1974) showed that lipid bilayers are sensitive to cation concentration. Some cations can cause expansion and others contraction of an artificial membrane by shifting the transition temperature of the fluid to ordered phase. These researchers found that going from a fluid to an ordered state decreased the area occupied by a bilayer. It is tempting to associate the membrane shrinkage seen in dehydrated *Porphyra* with a shift towards a more ordered state in the lipids of the membrane.

The structural change, thylakoid membrane shrinkage, produced as a result of drying could account for the progressive inactivation of photosynthesis. A relation between function and conformation has been accepted for chloroplasts for some time (Crofts, Deamer and Packer 1967; Dilley and

Vernon 1964; Gross and Packer 1967; Itoh and Shibata 1963). Alteration of conformation by drying could presumably upset the membranes function.

If PS II and I are distinct structures then conceivably photosynthesis would be dependent on the location and orientation of these structures in relation to one another; a relation which could be modified by drying. Researchers using detergents (Briantais 1967, 1969) and mechanical techniques (Sane et al 1970) have found that PS I and II activity can be separated. Koenig et. al. (1972) have characterized PS I and II subunits as intrinsic chlorophyll-protein complexes I and III respectively; although all photosystem activity is not associated with these complexes (Hiller, Pilger and Genge 1973). This implies that the two photosystems may exist as separate units in vivo. Freeze-etch work on thylakoids has shown the presence of small particles with PS I activity and larger particles with PSII activity (Arntzen, Dilley and Crane 1969). This does not mean that all photosystem activity is necessarily restricted to these particles, but it does demonstate, in this case, some physical separation of photosystems. These particles are not static in their membrane orientation but show mobility in response to light (Torres-Pereira et al 1974) and cations (Wang and Packer 1973). This mobility may reflect an overall orientation of PS I and II

subunits related to their function. Shrinkage of the thylakoid membrane as a result of drying could interfere with the interaction of the photosystem units, subsequently affecting their function.

Another possible alteration at the structural level may contribute to inactivation. Extrinsic proteins are associated with the external surface of thylakoids (Anderson 1975). These extrinsic proteins are easily removed, such as the phosphorylation coupling factor CF1 or carboxydismutase (Strotmann, Hesse and Edelmann 1973). If these proteins are released from the membrane by drying it could account for a decrease in photosynthetic actvity.

Upon rehydration photosynthesis is re-activated in tolerant algae. The sequence of reactivation is: (1) There is a restoration of the physical environment occupied by the chlorophyll molecules to the undesiccated state. (2) There is a return of electron transport in which PS I activity predominates over PS II for a period of approximately one minute. (3) Water-splitting starts within two minutes or less. (4) A slow stabilization, taking approximately 60 minutes, occurs. At the end of this period a typical response is obtained, and complete recovery of photosynthetic function is presumed.

Why is the photosynthetic apparatus of one species functional upon rehydration and another not? Obviously there must be 'differences' between sensitive and tolerant algae. These differences may exist at a gross structural level, a subtle molecular level, or be a combination of both.

One difference in the photosynthetic apparatus of sensitive and tolerant alge may be located at the site of water-splitting. Fluorescence transients demonstrate that water-splitting is one of the last photosynthetic processes inactivated by drying. The sensitive algae Porphyridium cruentum and Enteromorpha linza, dried to an O-P fluorescence transient (water splitting still present, electron transport between PS II and I stopped), and immediately rehydrated regained their photosynthetic activity. Neither of these sensitive algae tolerate further drying which inactivates water splitting. Thus tolerant algae withstand inactivation of the water-splitting process and sensitives cannot. This experiment implies that the water-splitting system is different in sensitive and tolerant algae. Since the chloroplasts were studied in vivo there of course may be other factors which account for the lack of re-activation in sensitives. Isolation of PS II subunits is probably necessary to determine if there are actual differences in sensitivity of the water-splitting enzyme systems.

One obvious difference at the structural level which may contribute to sensitivity is vacuolation. Sensitive algae tend to have large vacuoles occupying half or more of the cell volume. As the vacuoles shrink during drying they impose a mechanical stress on the protoplasmic contents. In Nitophyllum notti and medullary cells of Petalonia fascia, both species containing large vacuoles, the mitochondrial and chloroplast enevelopes are ruptured when dried. This undoubtly contributes to the inactivation of these organelles. The absence of large vacuoles in tolerant algae reduces the amount of mechanical strain on desiccation.

In tolerant algae the plasmalemma is usuallly maintained intact in the dried state. Sensitive algae often show evidence of plasmalemma disruption, as seen in dried Ulva, Nitophyllum and Petalonia. This rupture probably affects cytoplasmic and organellar activity. A loss of cytoplasmic contents and the release of lytic substances should significantly alter metabolic processes. Rupture of the plasmalemma may be due to mechanical stress, such as caused by large vacuoles and plasmodesmata, both present in sensitive and reduced or absent in tolerant algae.

The appearence of numerous vesicles in dehydrated material (Figs. 17, 18, 19, 36, 37) may be related to the

disappearence of E.R. and dictyosomes. This phenomena can occur in both tolerant and sensitive algae. Rehydration promotes a reappearence of E.R. and dictyosomes and a disappearence of the numerous vesicles only in tolerant algae. Whether the reticular system reforms from the vesicles or if a new synthesis occurs is an interesting question.

Mitochondrial cristae of sensitive algae do not stain readily in the dried state; whereas in tolerant algae the cristae stain normally. Nir et al (1970) reported a lack of stain uptake by cristae of dry corn mitochondria. This led to the suggestion that drying altered the membranes such that they no longer reacted with osmium. Miller et al (1971) subsequently confirmed that mitochondrial membrane properties are changed on dehydration. In the dry tolerant algae Porphyra perforata and Prasiola meridionalis the cristae are still visible. This, coupled with the finding that respiration is rapidly re-activated to control levels in P. perforata, suggests that no irreversible changes occur in the mitochondrial membranes of tolerant algae. The lipid or protein content of the cristal membranes of sensitive algae probably differ from that found in tolerant species.

A physio-ecological perspective shows a strong correlation between tolerance and vertical distribution of

intertidal algae. All tolerant species were found in the high- or mid- intertidal where such an attribute is adaptive. Sensitive alge were always located in the mid- or lower-intertidal region. It should be mentioned that the assigning of tolerant or sensitive does not take into account resistance to desiccation, ie. water savers such as Fucus. A tolerant plant is defined as one capable of anabiosis.

The work reported on here has considered the question of desiccation tolerance in intertidal marine algae on a physiological and ultrastructural basis. Further investigations along these lines could provide more information about the nature of desiccation resistance, and could be extended to similar questions regarding other plant types such as terrestial xerophytes and semi-arid crop plants. Tentative work in this lab has already shown that the response of conifers and lichens to freeze-desiccation and rehydration is similar to that found in tolerant marine algae. There may also be parallels found in the tolerance of some algae and the anabiotic response of certain insects (Asahina, Aoki and Shinozaki 1954) and intertidal invertebrates (Kanwisher 1955).

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