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TRANSMISSION ELECTRON MICROSCOPY OF APICAL CELLS OF SPHACELARIA SPP. (SPHACELARIALES, PHAEOPHYCEAE)

W. F. PRUD'HOMME VAN REINE & W. STAR

Rijksherbarium, Leiden, The Netherlands

SUMMARY

The ultrastructure of apical cells of six species of *Sphacelaria* (*S. arctica, S. cirrosa, S. nana, S. racemosa, S. radicans,* and *S. rigidula*) is studied here. In most details such as ultrastructure of chloroplasts, mitochondria, microbodies, nuclei and centrioles all *Sphacelaria* species studied are similar. Only in sections of *S. rigidula,* however, do pyrenoid-like structures occur.

INTRODUCTION

The ultrastructure of Sphacelariales has not been studied very often. All these studies deal with *Sphacelaria* species only. Bisalputra and collegues published several papers in 1969 and 1970 in which they carefully described all details of the chloroplasts of a *Sphacelaria* species. Galatis, Katsaros & Mitrakos (1977) described the fine structure of vegetative cells of *Sphacelaria tribuloides*. Evans (1966) studied the pyrenoid of zoids of *S. cirrosa* ecad *bipinnata* (as *S. bipinnata*), and Parker (1970) described cellulosic microfibrils in the cell wall of a *Sphacelaria* species. Bouck (1965) was the first author to describe general details of fine structure in cells of brown algae. His diagram of a hypothetical brown alga cell can be found in several modern handbooks. Results of subsequent studies of ultrastructure of *Phaeophyceae* usually confirm Bouck's observations.

METHODS AND MATERIALS

Methods used for preparing *Sphacelaria* filaments for ultrastructural studies were the same as described by Lokhorst (1978) for *Ulothrix*. The filaments were obtained from unialgal cultures (table 1) grown in liquid media as described elsewhere (Prud'homme van Reine, in press).

RESULTS AND DISCUSSION

Cell wall

Near the apex of the apical cell the cell wall is thinnest (plate 1, a). It consists of a very narrow electron-dense outer layer of 20–40 nm, a vesicularly layer of variable thickness

(35–120 nm) in which often some reticulate fibrils can be found, and a thicker fibrillary layer (plate 1, b). This fibrillary layer usually consists of an electron-dense outer part and an inner part with lower electron-density (plate 2, a). At some distance from the apex of the apical cells the narrow electron-dense layer usually breaks up and the contents of the vesicles in the vesicularly layer come free. These contents can be found on mature cell walls as an amorphous layer (plate 3, a) or as a somewhat flocky or a pilose fringe (plate 3, b–d). In older walls this amorphous layer erodes away. In the figures of Galatis et al. (1977) of *S. tribuloides* the walls are too old to possess these outer layers, but the electron-dense outer fibrillary layer is distinct in their figures. Usually the fibrils are arranged in parallel layers. Their orientation may be criss-cross, but in *S. arctica* the fibrils in the outer electron-dense fibrillary layers are usually transversely orientated, so they encircle the filaments. In the less dense inner fibrillary layer of *S. arctica* the fibrils are usually longitudinal. In new layers of the cell wall, which are formed at the inner side, in *S. arctica* the fibrils are mainly transverse again (compare plate 2, a and 2, b). In other species studied the structure and orientation of the walls differ in many details. We will discuss this in a separate forthcoming paper.

In transverse and longitudinal walls usually a electron-dense 'middle lamella' can be observed, together with one or more fibrillary layers (plate 2, b; 4, b). In the transverse walls usually many plasmodesmata occur (plate 4, b). Where the transverse walls meet the outer wall they are much thickened and their fibrillary layers run over a variable distance along the inner layers of the outer wall (plate 2, b). Longitudinal walls affix in a similar way to the primary transverse walls, often disconnecting a number of plasmodesmata (plate 4, b).

These results can be compared to the observations of Evans and Holligan (1972) on cell walls of *Dictyota dichotoma*. They found a cuticula which was possibly composed of alginic acid and cellulose, a middle layer composed of a mixture of sulphated polysaccharides and alginic acid, continuous with the 'middle lamella' and an innermost layer mainly composed of alginic acid. Parker (1970) observed fibrils of cellulose found in the outer cell wall of a *Sphacelaria* species. From the quantity in his figure 1 it is apparent that these cellulosic microfibrils cannot be hiding in the narrow and eroding outer layers of the cell wall of the *Sphacelaria*, but probably in the outer fibrillary layer of this wall. Parker (1968, 1969) also detected a slight trace of silica (0,001–0,86% of the dry weight) in the cell walls of several *Phaeophyceae*. These concentrations of silica may be responsible for the traces made by the knife during preparation of the ultra thin slices (see also Bisalputra & Bisalputra, 1969, f. 3).

Chloroplasts and pyrenoids

A detailed description of the chloroplasts of a *Sphacelaria* species has been given by Bisalputra & Bisalputra (1969). Later Bisalputra & Burton (1969, 1970) described the association between the chloroplast DNA and the photosynthetic lamellae and Bisalputra & Bisalputra (1970) studied the replication and segregation of the genophore during division of the chloroplast. This process of division is beautifully illustrated in a later paper (Bisalputra 1974, f. 4.12). Galatis & al. (1977) found that the chloroplasts of *S. tribuloides* did not differ from Bisalputra's descriptions, and our observations in six other *Sphacelaria* species are also in full agreement (plate 4, a).

Treharne et al. (1969) observed the number of thylakoids in cells of *Chlorella* grown at low light intensities being much larger than in similar cells grown at higher light intensities. We often observed a higher number of photosynthetic lamellae in chloroplasts grown at low light intensities (compare plate 2, a to 4, a). In transverse sections of chloroplasts possessing a small number of photosynthetic lamellae conspicuous pockets of stroma can be observed (plate 5, a). In these preparations, however, chloroplasts possessing many photosynthetic lamellae can also be observed (plate 3, a; 5, a). Bifurcation of the photosynthetic lamellae (plate 11, b; 12, c) as well as lamellae consisting of one thylakoid only (plate 1, b) have occasionally been observed by us.

Pyrenoids have been observed by Evans (1966, f. 5), who detected solitary stalked pyrenoids on chloroplasts of zoids of *S. cirrosa* ecad *bipinnata* and by Hori (1972, f. 2) who found a few small pyrenoids in vegetative filaments of *S. variabilis* (?). The pyrenoid of *Sphacelaria bipinnata* differed from all other studied pyrenoids of *Phaeophyceae* by lacking a cap or sack of metabolite. In vegetative filaments of other *Sphacelaria* spp. and other Sphacelariales pyrenoids have never been detected (Hori & Ueda, 1975; Asensi et al., 1977). Small pyrenoids, which are less projecting than usual in the *Phaeophyceae*, have been described by Evans (1968) from the chloroplasts of eggs of Fucales and also figured (Evans & Holligan, 1972, f. 10) for an egg of *Dictyota dichotoma*. Magne (1978) expressed his doubts about the pyrenoideous nature of these inclusions of the chloroplasts. Other inclusions in the chloroplasts of *Dictyota dichotoma* are precursors of physodes (Evans & Holligan, 1972; G. Feldmann & Guglielmi, 1972).

Absence of pyrenoids in a certain stage of the life-history, and presence in another stage, has also been observed in *Phaeostrophion irregulare* (Dictyosiphonales), where, according to Bourne & Cole (1968), only germlings possess pyrenoids and mature plants do not. In *Laminaria* Bisalputra et al. (1971) observed pyrenoids in gametophytes, but not in sporophytes. In *S. rigidula* we once observed a pyrenoid-like projection of a chloroplast (plate 6, a) and more often inclusions of unknown origin, enclosed by double membranes (plate 5, c; 6, a, b; 7, a). Tubular inclusions could be observed in these inclusions (plate 6, a, b; 7, a). Outpockets of the chloroplasts (plate 6, c) and a mitochondrion which seems to be located inside the choroplast envelope (plate 6, d) could also be observed in preparations of the same fragment of a filament of *S. rigidula*. The number of double membranes around the inclusions of the chloroplasts is variable and often the inclusions, still surrounded by the two double membranes of the chloroplasts, are projecting into the cytoplasm (plate 6, b). Occasionally some membranes only partly surround the inclusions (plate 5, c; 6, a). We are not sure about the pyrenoideous nature of these inclusions. Pyrenoids with tubular inclusions are known (Delépine et al., 1976; Asensi et al., 1977), certainly, but we think the inclusions in *S. rigidula* may be comparable to the concentric arrangement of thylakoids as observed in chloroplast of *Ectocarpus* species by Oliveira & Bisalputra (1977b) or to the cytoplasmatic inclusions of ageing cells also observed by them (Oliveira & Bisalputra, 1977c). A mitochondrial nature of the inclusions in the chloroplasts of *S. rigidula* is another possible suggestion (compare f. 70 of Tandler & Hoppel (1972) with our plates 6, a and 7, a). On the other hand the pyrenoid-like projection in plate 6, a is very similar to the figure published by Hori (l.c.) and the other pyrenoid-like inclusions are quite similar to the figures of small pyrenoids of Fucales (Evans, l.c.).

Gibbs (1962) observed a second double membrane outside the chloroplast envelope surrounding the chloroplasts of a number of algae. This double-membraned outer envelope was named chloroplast endoplasmatic reticulum or chloroplast ER by Bouck (1965) and was considered as characteristic of the *Phaeophyceae* and some other algal groups. Bisalputra (1974), however, stated that in brown algae chloroplast ER is best developed when the number of chloroplasts per cell is relatively low. He questioned if, in cases such as *Sphacelaria* where the number of chloroplasts is high, the ER elements which do not completely enclose the chloroplasts, can still be classified as chloroplast ER.

The figure by Evans (1966, f. 5) suggests a chloroplast ER completely surrounding the chloroplast. However, continuity between the chloroplast ER and the chloroplast envelope, as figured by Oliveira & Bisalputra (1977b, f. 1) for *Ectocarpus* species can also be suggested for Evans's figure. We also observed this continuity (plate 5, b, arrow).

Continuity between the chloroplast ER and the nuclear envelope has often been observed in brown algae. Galatis et al. observed it for *S. tribuloides*, but in the species we studied this continuity was not found, not even when nucleus and chloroplast are very close to each other (plate 8, a). Photographs we made of transverse sections of older filaments, however, suggest this continuity may occur in older cells (not figured). According to Neushul & Dahl (1972) the continuity of chloroplast ER and nuclear envelope in *Zonaria farlowii* exists only when the nucleus is very active. Our plate 8, b suggests an active nucleus, but no such continuity.

Tubular inclusions in the space between the chloroplast envelope and the chloroplast ER can often be observed (plate 4, a, c; 5, b; 6, c, d; 10, c). These tubular inclusions derive from blebs formed by the chloroplast ER (plate 4, c; 10, c).

Nucleus and centrioles

Nuclei of *Sphacelaria*, like those of other brown algae, are surrounded by a nuclear envelope which has continuities to the endoplastmatic reticulum or ER (plate 9, a). Many pores can be observed in this nuclear envelope and the outer surface often gives rise to blebs, especially in the neighbourhood of dictyosomes (plate 9, a). The active nucleus is often very irregular in outline (plate 8, b), a phenomenon also observed by Neushul & Dahl (1972) for *Zonaria farlowii*, and by Galatis et al. (1977) for *Sphacelaria tribuloides*. Centrioles have been observed near the active nucleus (plate 7, b & c) or between the two halves of a dividing nucleus (plate 9, c & d). The nuclear envelope forms a centriolar depression, in which pores are absent or infrequent (plate 7, b & c). Centrioles in vegetative cells of *Phaeophyceae* have been described by Berkaloff (1963) for *Himanthalia*, by Bouck (1965) for *Fucus vesiculosus*, by Evans (1966) for *Colpomenia peregrina*, by Bourne & Cole (1968) for *Phaeostrophion irregulare*, by Bisalputra et al. (1971) for *Laminaria*, by Neushul & Dahl (1972) for *Zonaria farlowii* and for *Dictyopteris zonarioides*, by Magne (1976) for *Bachelotia antillarum*, and by LaClaire and West (1978) for *Cutleria hancockii*. Thus they have been found in many families of the *Phaeophyceae* and may be common in all of them.

The nucleus contains a prominent nucleolus whose stroma is more electron opaque than the rest of the stroma of the nucleus. Occasionally concentrations of the chromatin can be found within the nucleus (plate 8, a; 9, c).

Golgi apparatus and endoplasmatic reticulum (ER)

The cytoplasm of the *Sphacelaria* species are traversed by many cisternae of the ER (plate 8, b). In a few micrographs unusual parallel arrangements are observed (plate 10, a & b), similar to the arrangements figures by Oliveira & Bisalputra (1973, f. 2) for *Ectocarpus* spec. There is a direct continuity between the perinuclear space and the cisternal spaces of the ER (plate 9, a). Most cells possess several dictyosomes, which are often perinuclear (plate 9, a, arrow). Dictyosomes can also be found near cisternae of the ER (plate 9, a; 11, a). The nuclear envelope or the ER blebs small vesicles which accumulate on the formative face of the dictyosomes (plate 9, a; 11, a). The relation of the golgi-vesicles formed by the dictyosomes to other inclusions of the cytoplasm will be discussed later.

Mitochondria

Numerous mitochondria which are elongate or more irregular in shape (plate 7, a; 8, c; 9, a; 10, a, c, d; 11, b) are found in the cytoplasm. They are usually frequent around the nucleus (plate 8, b; 9, a), near the periphery of the cells (plate 8, c) and around chloroplasts (plate 10, c; 11, b). The tubular cristae of the mitochondria, common in all *Phaeophyceae,* have been described in detail by Bouck (1965).

Microbodies

The microbodies described by Galatis et al. for *S. tribuloides* have been found by us in other *Sphacelaria* species (plate 1, b; 10, c, d; 11, d). Similar microbodies have been found by Bouck (1965) for *Fucus vesiculosus* and by Bisalputra et al. (1971) for *Laminaria*. Oliveira & Bisalputra (1977a, f. 3) published a micrograph on which a similar body can be observed. According to Buvat (1971) microbodies are often peroxisomes which contain catalase, but no microchemical research on microbodies of *Phaeophyceae* to endorse this is known to us.

Vacuoles

Many kinds of vacuoles, physodes and other subcellular bodies have been described in electron micrographs of *Phaeophyceae* (for a survey see Ragan, 1976, tab. 2). Combining the observations made by McCully (1968), Evans & Holligan (1972), Feldmann & Guglielmi (1972), Oliveira & Bisalputra (1973), Rawlence (1973), Pellegrini (1974a, 1974b), Magne (1976), and Ragan (1976), the following vacuoles or vesiclelike inclusions can be expected in the cells of *Phaeophyceae:*

1) Electron-dense unstructured alginate vesicles and lipid- or oil-bodies.

2) Lamellar structures which may be very osmiophilic (osmiophilic structured bodies or OSB's) and which are probably physodes (polyphenol vesicles or tannine containing vesicles) and less osmiophilic lamellar structures which are probably vacuoles containing abscissions of chloroplasts.

3) Multivesicular bodies which have partly been formed by ER-cisternae or by the chloroplast ER, partly by the vesicles derived from dictyosomes. Vacuoles containing several small vesicles of various origin and modified mitochondria may also be counted as multivesicular bodies. In several publications these multivesicular bodies have been described, at least in part, as iridescent bodies containing polysaccharides.

4) Polysaccharide-containing vesicles (Fucoidan vesicles) which are very light on the micrographs, and which probably also arise from the dictyosomes.
5) Irregular vacuoles with various contents.

In our micrographs we observed several of these subcellular bodies:
ad 1) Droplike unstructured electron-dense vesicles (osmiophilic unstructured bodies) have often been observed (plate 9, b). Often their staining is not uniform. Vesicles have been observed which were less electron-dense near their periphery (plate 7, a; 8, d), or with a less electron-dense centre (plate 11, b) as well as vesicles with a very vague outline (plate 3, a). Occasionally a vesicle seems to arise from a chloroplast (plate 8, d). They may be found outside the plasmalemma and attached to the cell wall (plate 5, a).
These dark vesicles can often be found in the distal part of the apical cells (plate 12, a).
ad 2) Osmiophilic lamellar structures (physodes) may become so dense, that they cannot be discerned from the unstructured electron-dense vesicles (plate 11, c). The structure of the physodes is often very complicated (plate 12, b, c). According to Feldmann & Guglielmi the structure of a part of the physodes is not laminate but granulo-reticulate. Such physodes have also been observed by us (plate 2, a; 11, c; 12, b, c; 13, a). Physodes can be found in the perinuclear cytoplasma (plate 8, b, though not very clear) and in the distal part of the apical cells (plate 12, a). Occasionally they can be found associated with a chloroplast (plate 12, b). Less osmiophilic lamellar structures (chloroplast abscissions) have often been found (plate 13, b). Remnants of chloroplasts in a deceased apical cell are similar in structure (plate 13, c). Physodes and unstructured vesicles are often associated with these less osmiophilic lamellar structures (plate 7, a; 11, c; 12, b; 13, a).
ad 3) Multivesicular bodies can frequently be found in the neighbourhood of dictyosomes (plate 11, a). The vesicles found near the plasmalemma may also be called multivesicular bodies (plate 8, c; 13, d). They may secrete their contents into the paramural space by reverse pinocytosis through the plasmalemma. Whaley et al. (1971) suggested that small golgi vesicles become enveloped by segments of smooth membrane, and that these fused golgi-derived vesicles impart lysosomal activity. Pellegrini (1974a, 1974b), using cytoenzymatic methods, found lysosomes associated with multivesicular bodies in *Cystoseira stricta*. Some multivesicular bodies observed in our micrographs are probably pockets of cytoplasm enclosed by ER (plate 13, e) or they are modified mitochondria (plate 14, a).
ad 4) Fucoidan-vesicles are less distinct in our micrographs. They might be pictured in plate 5, b & 11, a.
ad 5) Vacuoles with various contents can usually be found in the cytoplasm (plate 3, b; 4, a; 10, d; 14, b), often especially in the proximal region of apical cells (plate 12, a). Occasionally they seem to provoke lysis of subcellular bodies (plate 11, d; 14, d).

Plasmalemma

In *S. tribuloides* Galatis et al. found numerous finger-like ingrowths of the plasmalemma along the longitudinal walls and labyrinthine folded in plasmalemma on other places. In our micrographs we did not find numerous finger-like ingrowths but the plasmalemma did distinctly proliferate as well (plate 1, b; 2, a; 3, a; 4, a; 5, a; 6, b; 8, c). Mitochondria can usually be found nearby, but not as closely associated to the plasmalemma proliferations as in *S. tribuloides*. In the space between plasmalemma and cell wall (the paramural space) many granules, vesicles and convoluted membranes can be observed (plate 4, a).

As described by Oliveira & Bisalputra (1973) for *Ectocarpus* sp. the paramural bodies of *Sphacelaria* spp. are probably partly plasmalemmasomes and partly lomasomes. The latter bodies derived from dictyosomic activities.

Unidentified inclusions of the cytoplasm

On one occasion glycogen-like granules have been found in a vegetative cell of *S. rigidula* (plate 14, c).

CONCLUSIONS

The ultrastructure of *Sphacelaria* spp. is fairly uniform in all species studied. The cell walls, however, show differences which may be used as characters for the delimitation of taxa. In particular the structure of these walls, the orientation of the fibrils and the different patterns formed by erosion must be studied in detail for that purpose. In *S. rigidula* pyrenoid-like structures and glycogen-like granules are detected which do not occur in the other species studied. Further study of these structures is desirable.

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TABLE I. CULTURES

Sequence of data: Culture number, species, locality, annotations, date of collection, culture conditions (see table 2), date of fixation.

67–31–4, *S. radicans*, Norway, Hordaland, Espegrend, Skogsvåg, Barholmen, upper littoral, in belts of *Pelvetia* and of *Fucus spiralis*, in shady crevices of rocks, coll. 29/5/1967, 12 °L, fix. 13–2–1978.
67–34–3, *S. nana*, Norway, Hordaland, Espegrend, Mariholmen, in upper littoral, in belt of *Fucus spiralis*, coll. 28/5/1967, 4 °L, fix. 13/2/1977.
67–35, *S. cirrosa* ecad *cirrosa*, Norway, Hordaland, Espegrend, Skogsvåg, Barholmen, dredged, 16–22 m deep, on stipes of *Laminaria hyperborea*, coll. 29/5/1967, 12 °L, fix. 22/2/1977.
67–57–1, *S. nana*, Norway, Hordaland, Hardangerfjord, Øystese, on jetty in front of the hotel, upper littoral, 3/6/1967, 4 °L, fix. 13/2/1978.
67–57–2, as 67–57–1, but grown at 4 °D.
67–58–1, *S. cirrosa* ecad *cirrosa*, unknown locality, probably from Norway, Hordaland, Hardangerfjord, Øystese, first observed 2/11/1971, 12 °L, fix. 22/2/1977.
67–65–1, *S. arctica*, Norway, Akershus, Drøbak, south of Askholmene, dredged, 4–5 m deep, on boulders, shells, and small algae, coll. 7/6/1967, 12 °L & 12 °D, fix. 16/12/1976.
68–32–2, *S. rigidula*, France, Ille-et-Vilaine, St. Lunaire, in front of the Grotte de Sirènes, between *Laminaria digitata*, 16/3/1968, 12 °L, fix. 26/1/1977.
71–11, *S. racemosa*, Scotland, St. Andrews, Hind Rock, steep northern side, in small tidal pools 10/2/1971, 12 °L, fix. 13/2/1978.

TABLE II. CONDITIONS IN CULTURE ROOMS.

Temperature (°C) and code	light intensity in Lx	covered by white paper	light/dark regime in hours	period in weeks between replenishment of media
4°L	1400–2800	no	8/16	5
4°D	88– 500	no	8/16	5
12°L	700–1400	yes	16/8	4
12°D	175– 700	no	16/8	4

ABBREVIATIONS IN PLATE I–14

AL, amorphous layer of the cell wall;
ap., apical;
B, blebs;
C, centriole;
CA, chloroplast abscission;
CD, centriolar depression of the nuclear envelope;
CE, chloroplast envelope;
CER, endoplasmatic reticulum associated with the chloroplast;
Ch, chloroplast;
Chr, chromatin;
C. s., cross-section;
CW, cell wall;
D, dictyosome;
ER, endoplasmatic reticulum;
FV, supposed fucoidan vesicle;
G, genophore;
Gl, glycogen-like granules;
GV, golgi vesicle;
IFL; inner fibrillary layer of the cell wall;
L.s., longitudinal section;
LW, longitudinal cell wall;
M, mitochondrion;
Mb, microbody;
ML, 'middle lamella';
MvB, multivesicular body;
N, nucleus;
NE, nuclear envelope;
NFL, new fibrillary layer;
NL, nucleolus;
NP, nuclear pore;
O, outpocket of the chloroplast;
OFL, outer fibrillary layer;
OG, osmiophilic globule;
OL, outer layer of the cell wall;
P, plasmodesmata;
PB, paramural body;
Ph, physode;
Pl, plasmalemma;
PLa, photosynthetic lamella;
PS, paramural space;
Py, pyrenoid-like structure;
sec., secondary;
segm., segment;
T, thylakoid;
Tu, tubular inclusions;
TW, transverse cell wall;
UV, unstructured electron-dense vesicle;
V, vacuole;
VL, vesiculary layer of the cell wall.

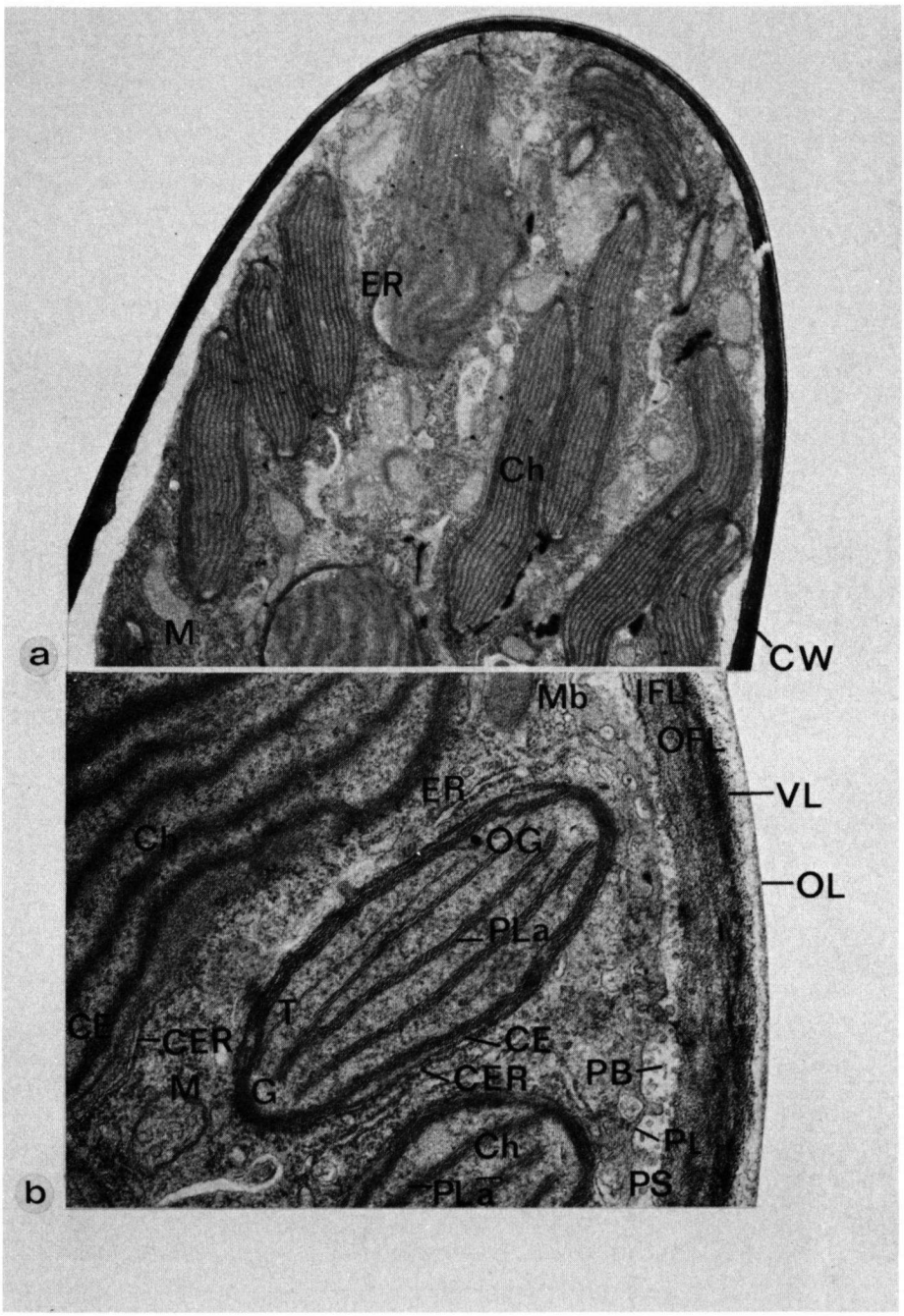

Plate 1. *S. arctica*, culture 67–65–1. L.s. of an ap. cell — a. ×9,900; — b. Detail. Note photosynthetic lamellae of the chloroplast containing 1, 2, or 3 thylakoids. Cell wall with four layers. ×29,700.

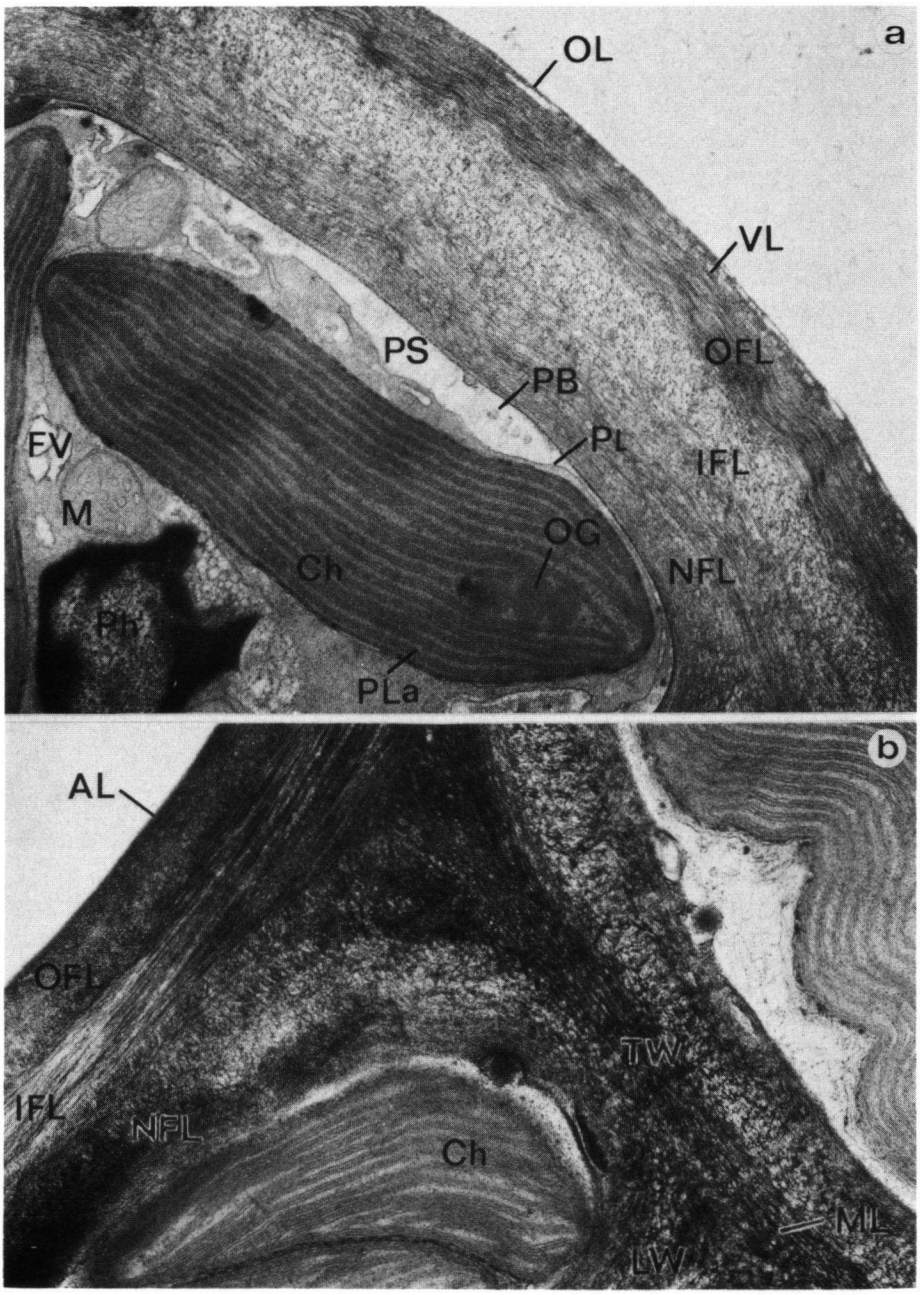

Plate 2. *S. arctica*, culture 67–65–1. ×29,700. Note cell wall with different orientation of micro-fibrils. — a. C.s. of a segment. — L.s. of ap. cell and superior sec. segm.

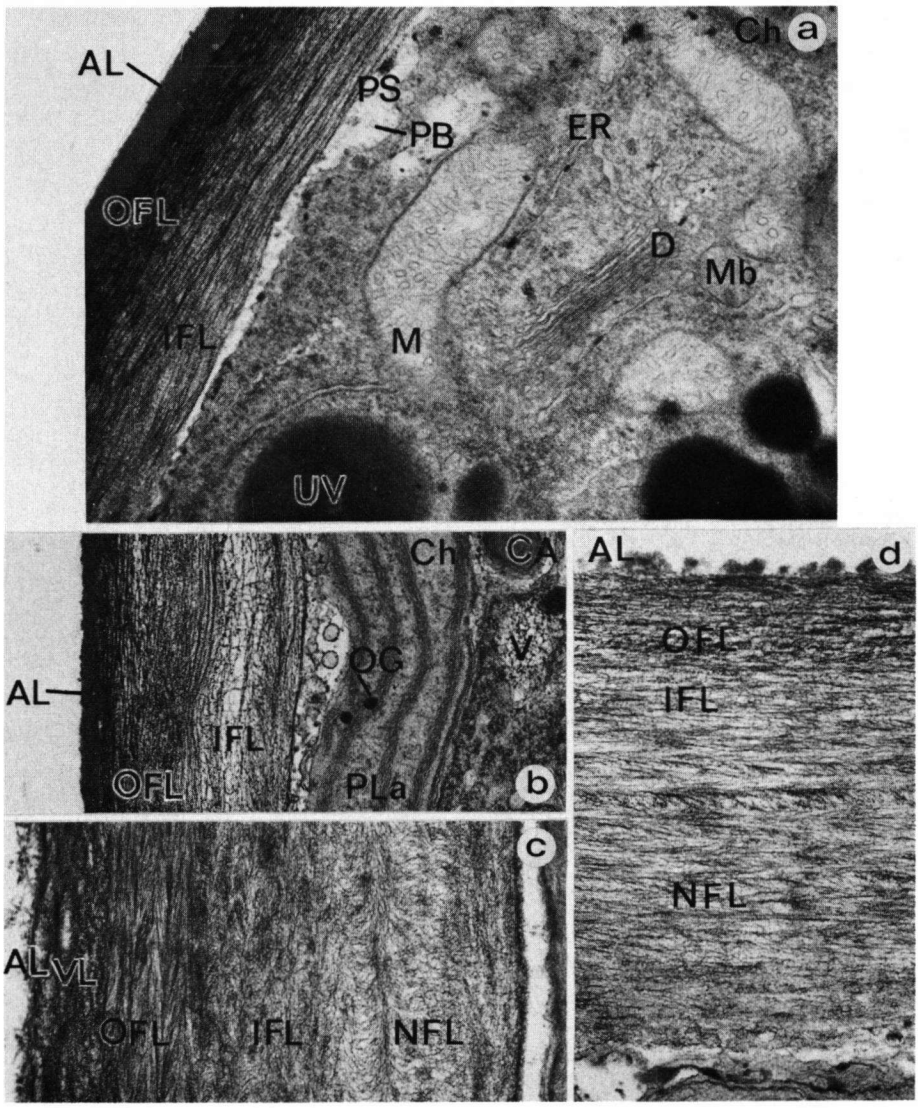

Plate 3. L.s. of cell walls ×29,700. — a. *S. nana*, culture 67–65–1. Ap. cell of young lateral. Note in cytoplasm unstructured electro-dense vesicles without distinct outer membrane. — b. *S. rigidula*, culture 68–32–2. Aged ap. cell. Cell wall with flocky amorphous layer and inconspicuous difference between the fibrillary layers. — c. *S. nana*, culture 67–34–3. Ap. cell. Flocky amorphous layer. — d. *S. cirrosa*, culture 67–58–1. Mature sec. segm. Cell wall with pilose fringe and remnants of vesiculary layer.

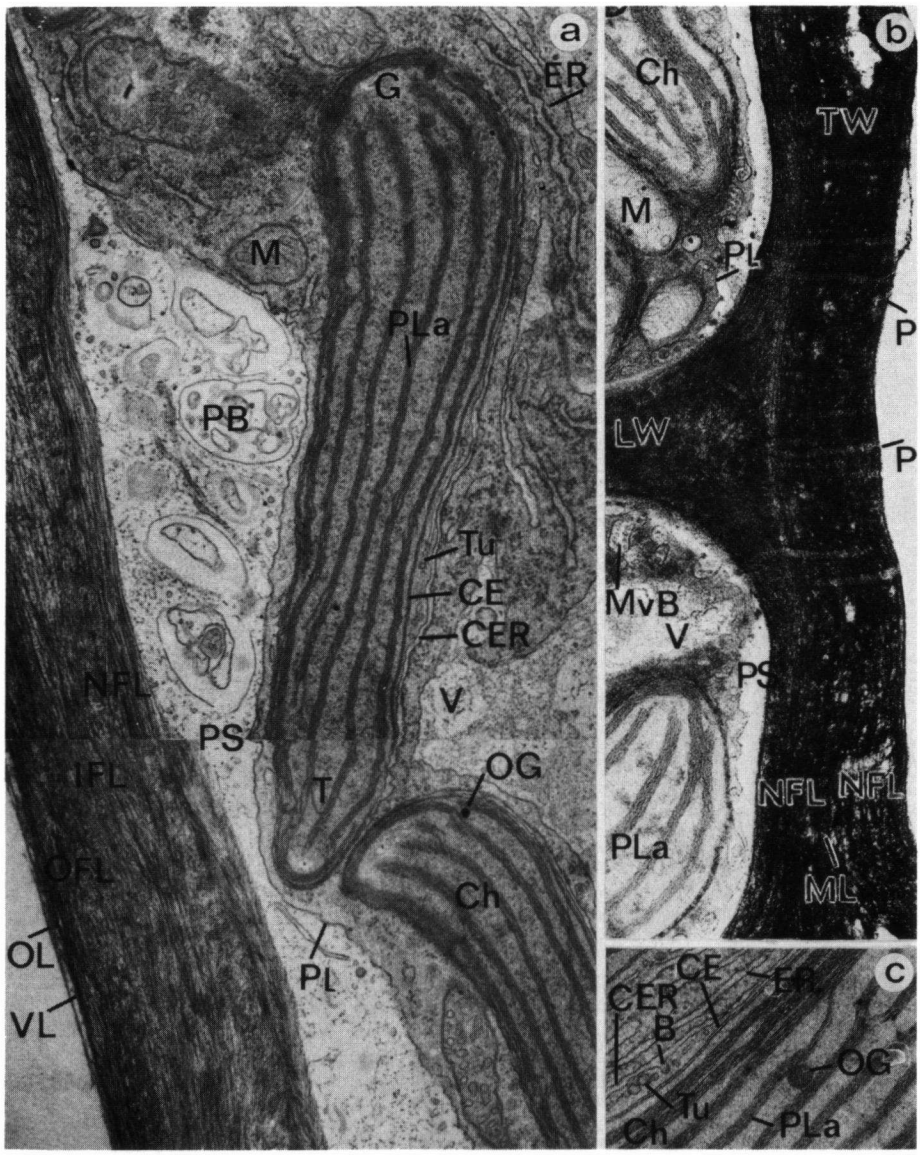

Plate 4. — a. *S. arctica*, culture 67-65-1. L.s. of ap. cell. Note the large paramural space with many paramural bodies and the different structure of the contents of the vacuole. ×20,156. — b. *S. cirrosa*, culture 67-35. L.s. of transv. cell wall between a superior sec. segm. and an inferior one. Figure has been turned for a quarter. Note plasmodesmata disconnected by the thickened attachment of a wall. ×29,700. — c. *S. radicans*, culture 67-31-4. L.s. of ap. cell. ×29,700.

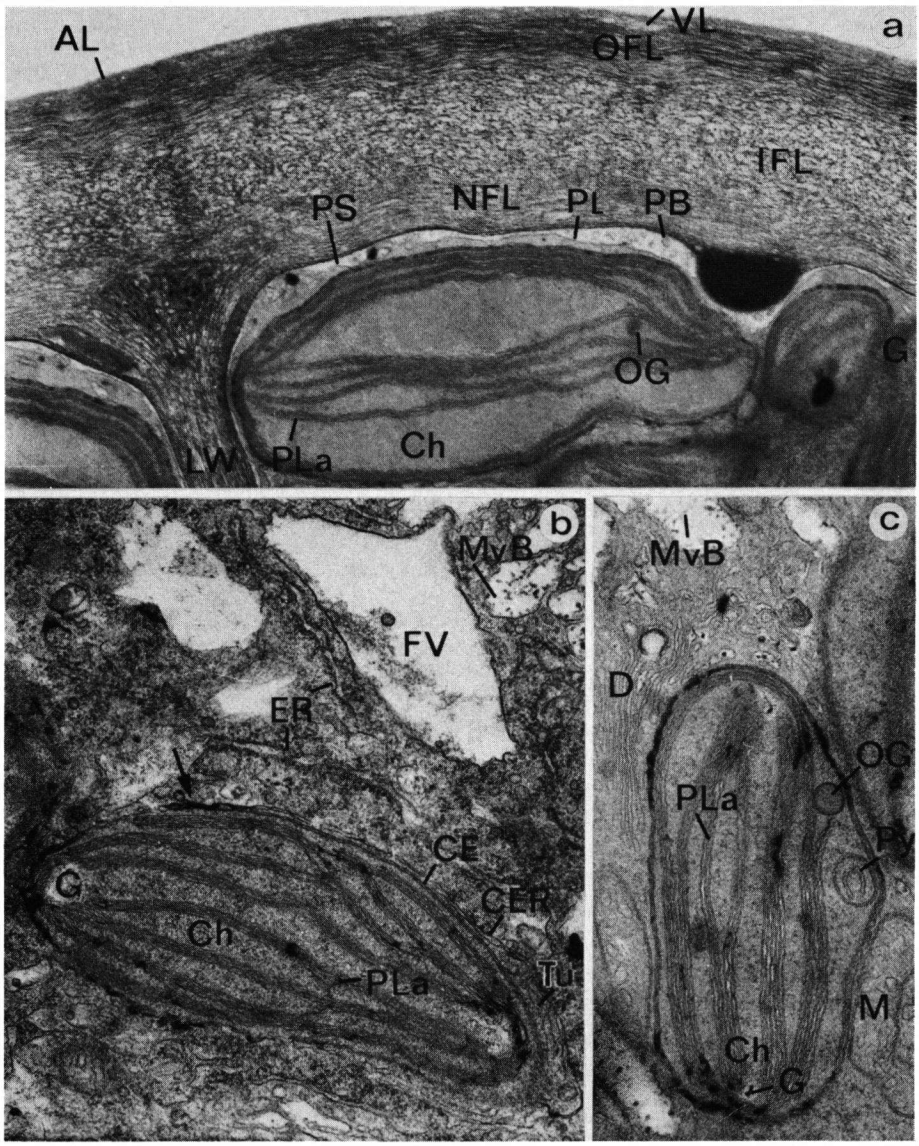

Plate 5. All ×29,700. — a. *S. arctica*, culture 67–65–1. C.s. of sec. segm. Note stroma of chloroplast not traversed by photosynthetic lamellae, cell wall with remnants of vesicular layer and unstructured electron-dense vesicle in paramural space and associated to the cell wall. — b., c. *S. rigidula*, culture 68–32–2. L.s. of ap. cell. Note chloroplast with two double membraneous envelopes and with continuity between these envelopes (arrow in 'b'). In 'c' pyrenoid-like structure in probably aged ap. cell.

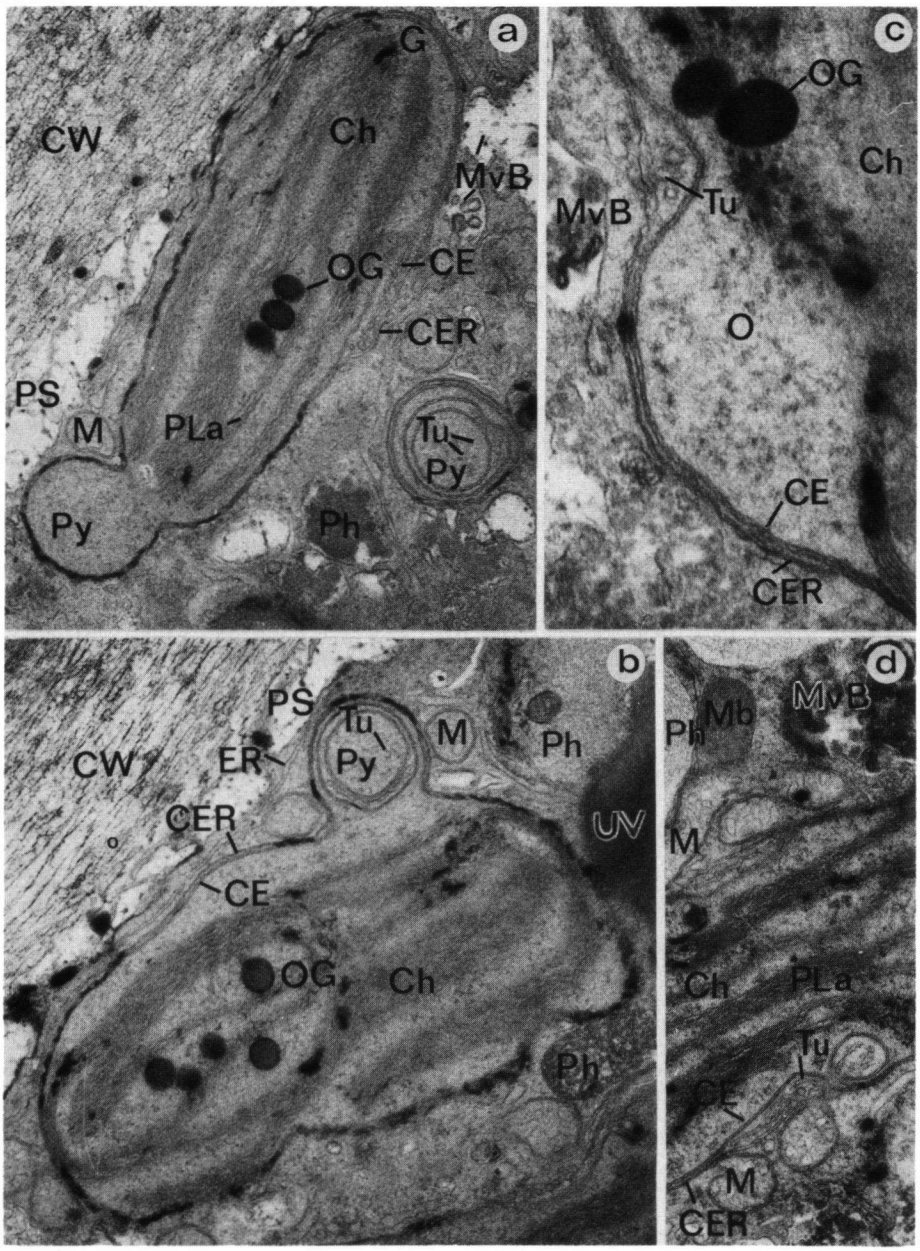

Plate 6. *S. rigidula*, culture 68–32–2. L.s. of a probably aged ap. cell. — a., b. Note pyrenoid-like structure. ×29,700. — c. Detail of an outpocket of a chloroplast. ×57,750. — d. Note mitochondria closely associated to chloroplast. ×29,700.

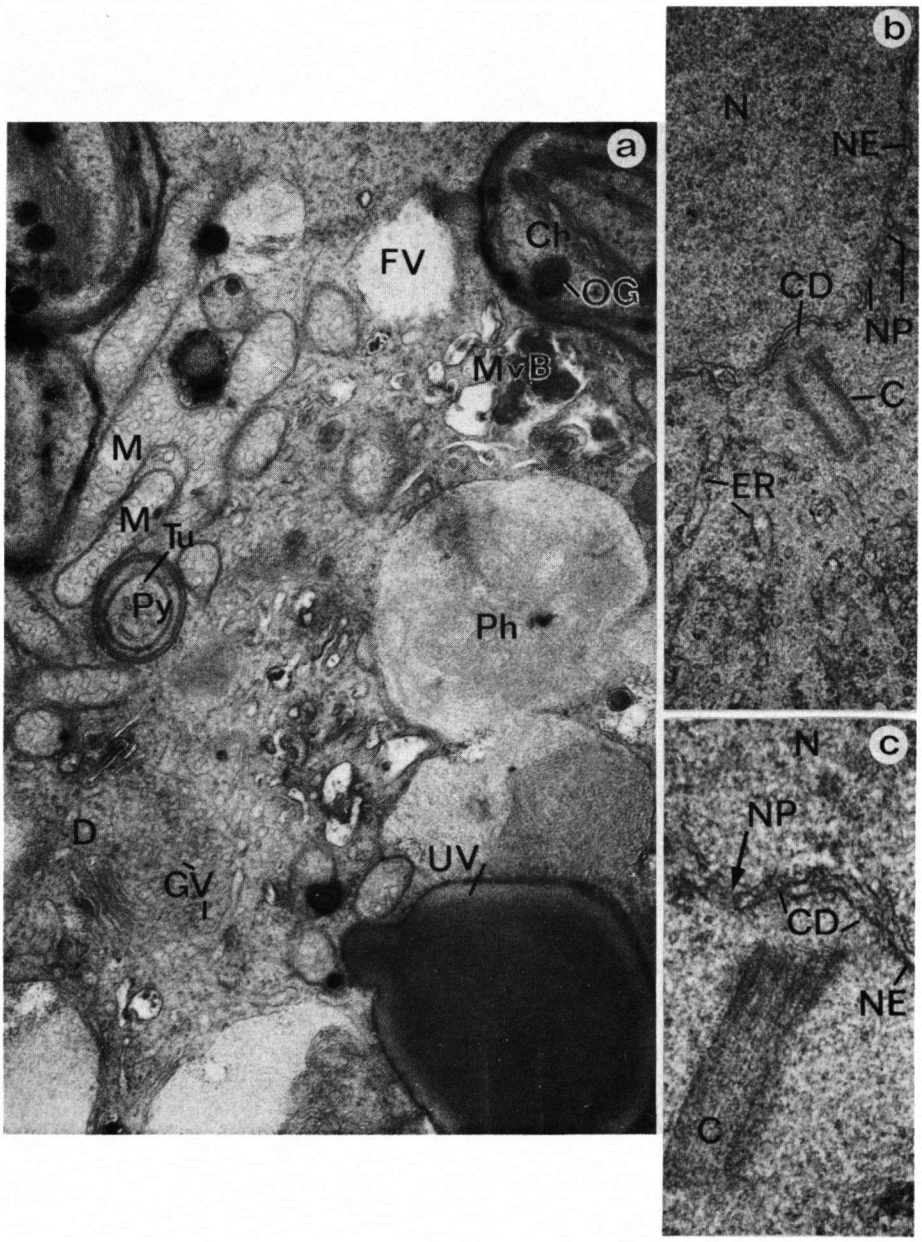

Plate 7. — a. *S. rigidula*, culture 68–32–2. L.s. of a probably aged ap. cell. Note branched mitochondrion and pyrenoid-like structure. ×29,500. — b., c. *S. artica*, culture 67–65–1. L.s. of ap. cell. Note centriole. 'a' ×29,500; 'b' ×57,750.

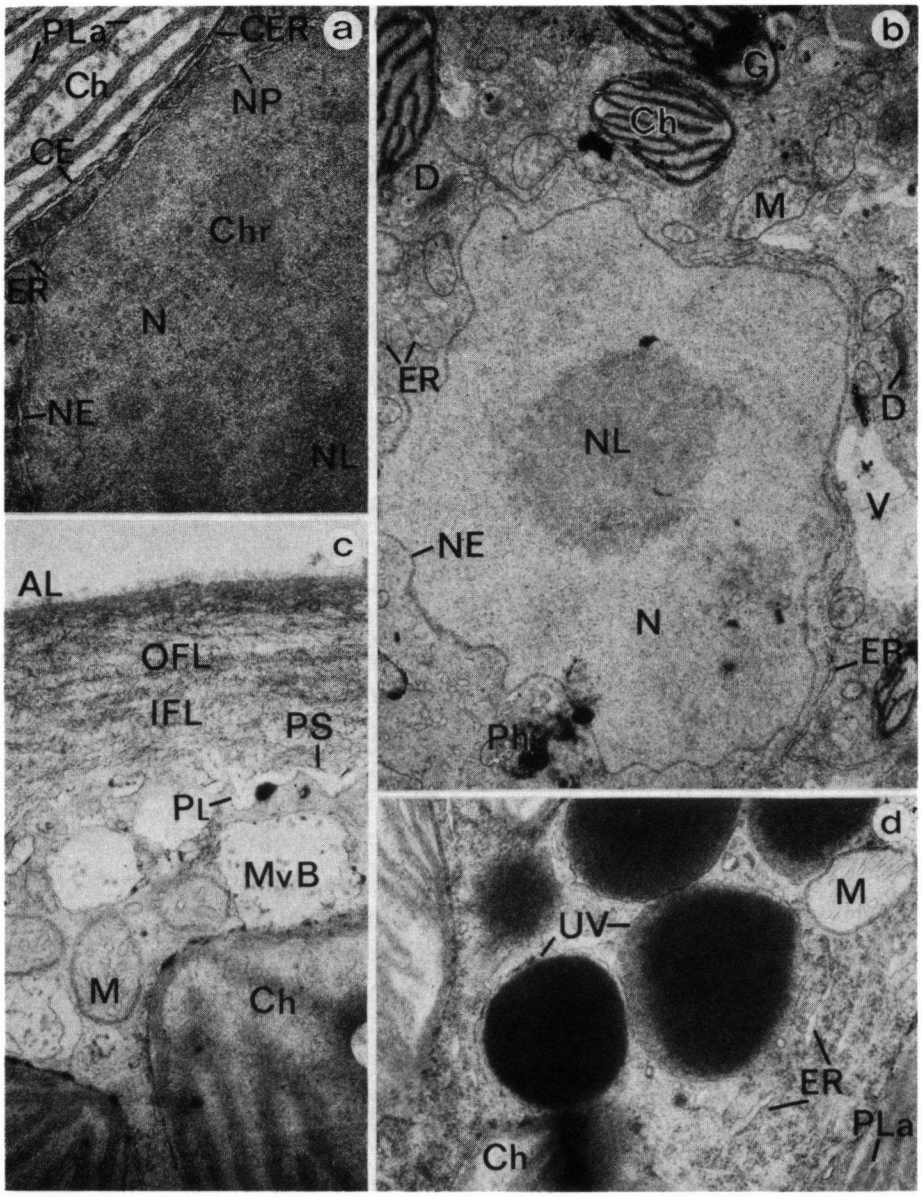

Plate 8. — a. *S. cirrosa*, culture 67–35. L.s. of superior sec. segm. No visible connection between nucleus and chloroplast. ×29,700. — b. *S. radicans*, culture 67–31–4. L.s. of ap. cell with active nucleus. ×8,085. — c. *S. racemosa*, culture 71–11. L.s. of ap. cell. ×29,700. — d. *S. nana*, culture 67–57–2. L.s. of ap. cell. Unstructured electron-dense vesicles associated with chloroplast. ×29,700.

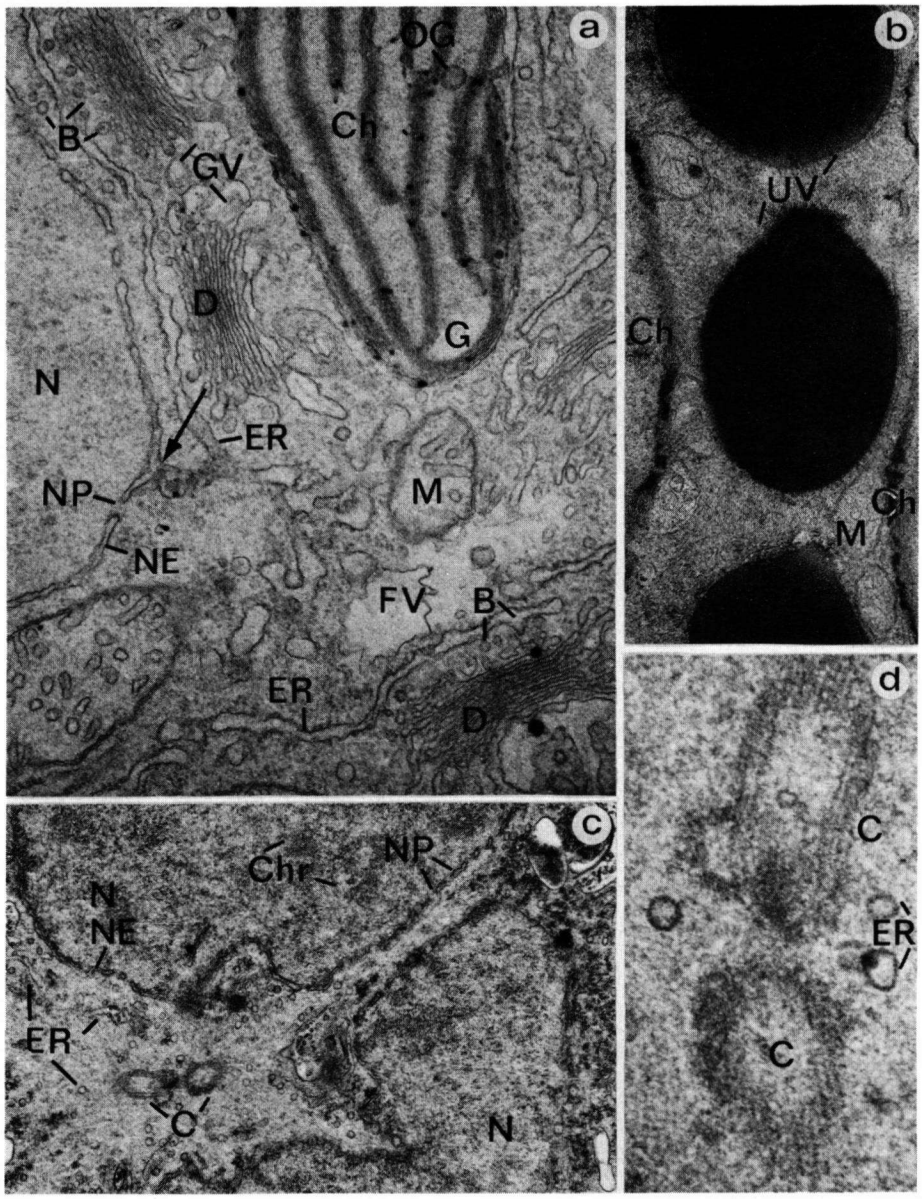

Plate 9. — a. *S. radicans*, culture 67-31-4. L.s. of ap. cell. Dictyosomes perinuclear as well as associated to ER. ×29,700. — b. *S. rigidula*, culture 68-32-2. L.s. of a probably aged ap. cell. Electron-dense vesicles. ×29,700. — c., d. *S. cirrosa*, culture 67-35. L.s. of a primary segm. with divided nucleus and centrioles. 'a' ×18,150; 'b' ×57,750.

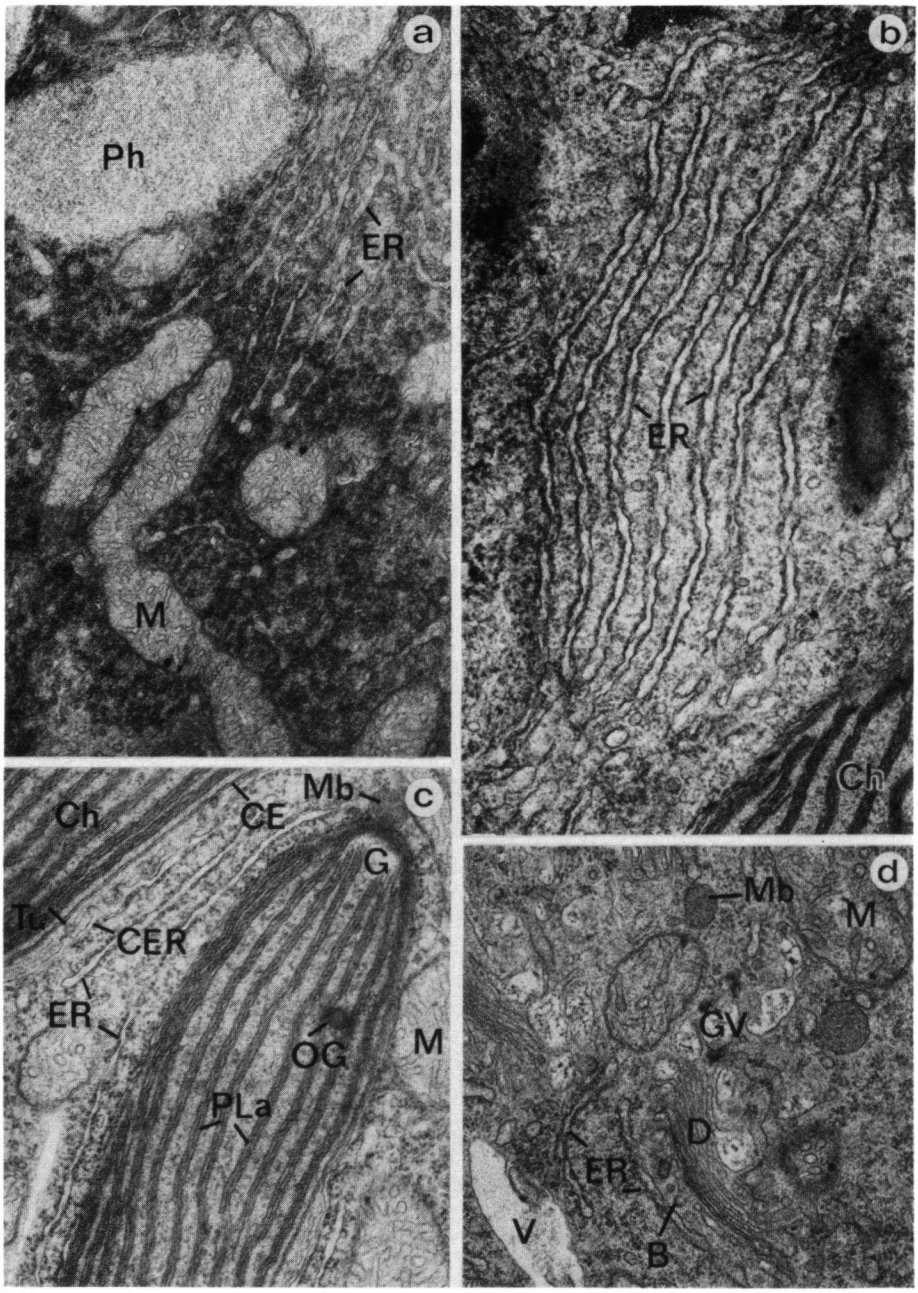

Plate 10. L.s. of ap. cells ×29,700. — a. *S. nana*, culture 67–57–2 and b. *S. radicans*, culture 67–31–4. Note parallel orientation of ER. — c., d. *S. arctica*, culture 67–65–1. Microbodies.

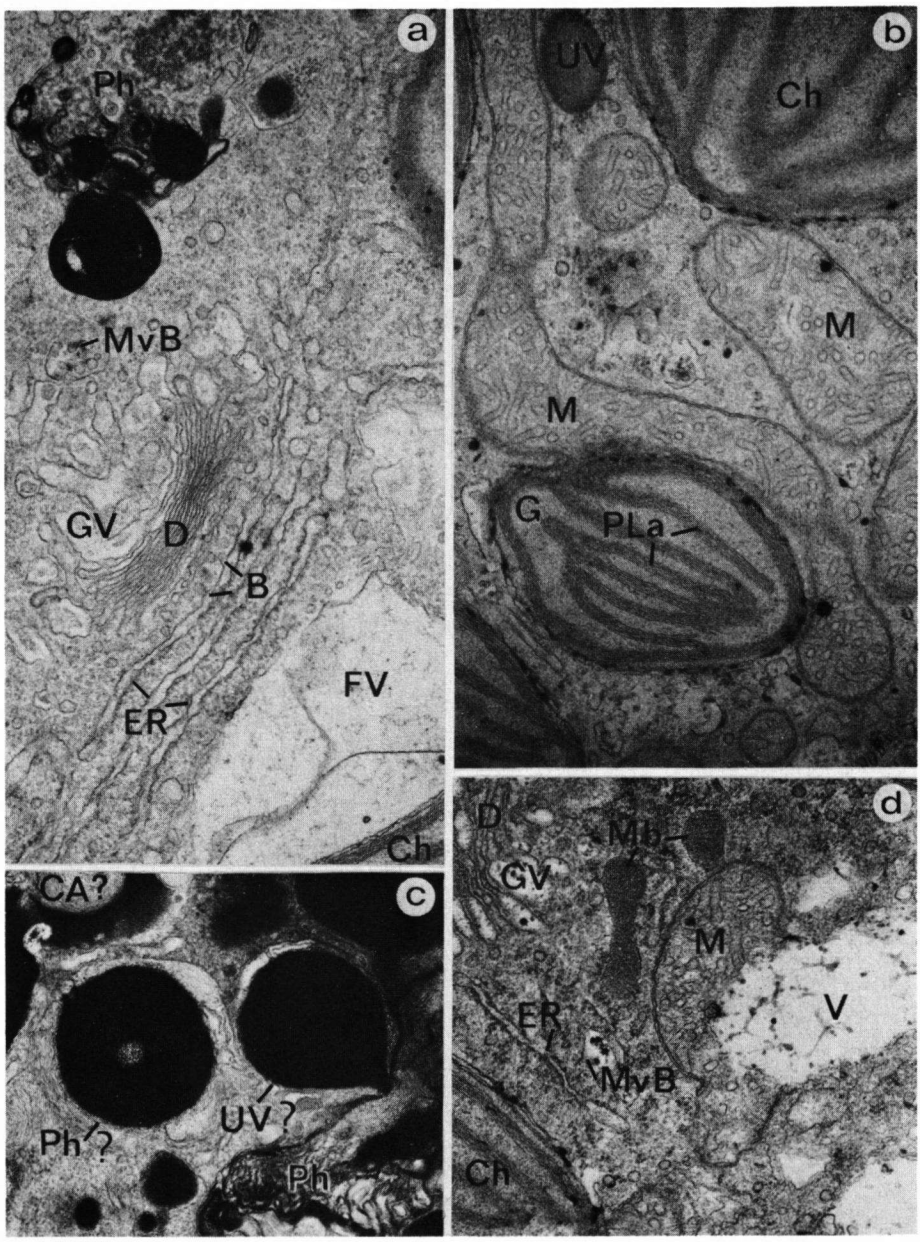

Plate 11. L.s. of ap. cells. ×29,700. — a. *S. radicans*, culture 67–31–4. — b. *S. racemosa*, culture 71–11. — c. *S. nana*, culture 67–34–3. — d. *S. rigidula*, culture 68–32–2. Vacuole provoking lysis of a mitochondrion.

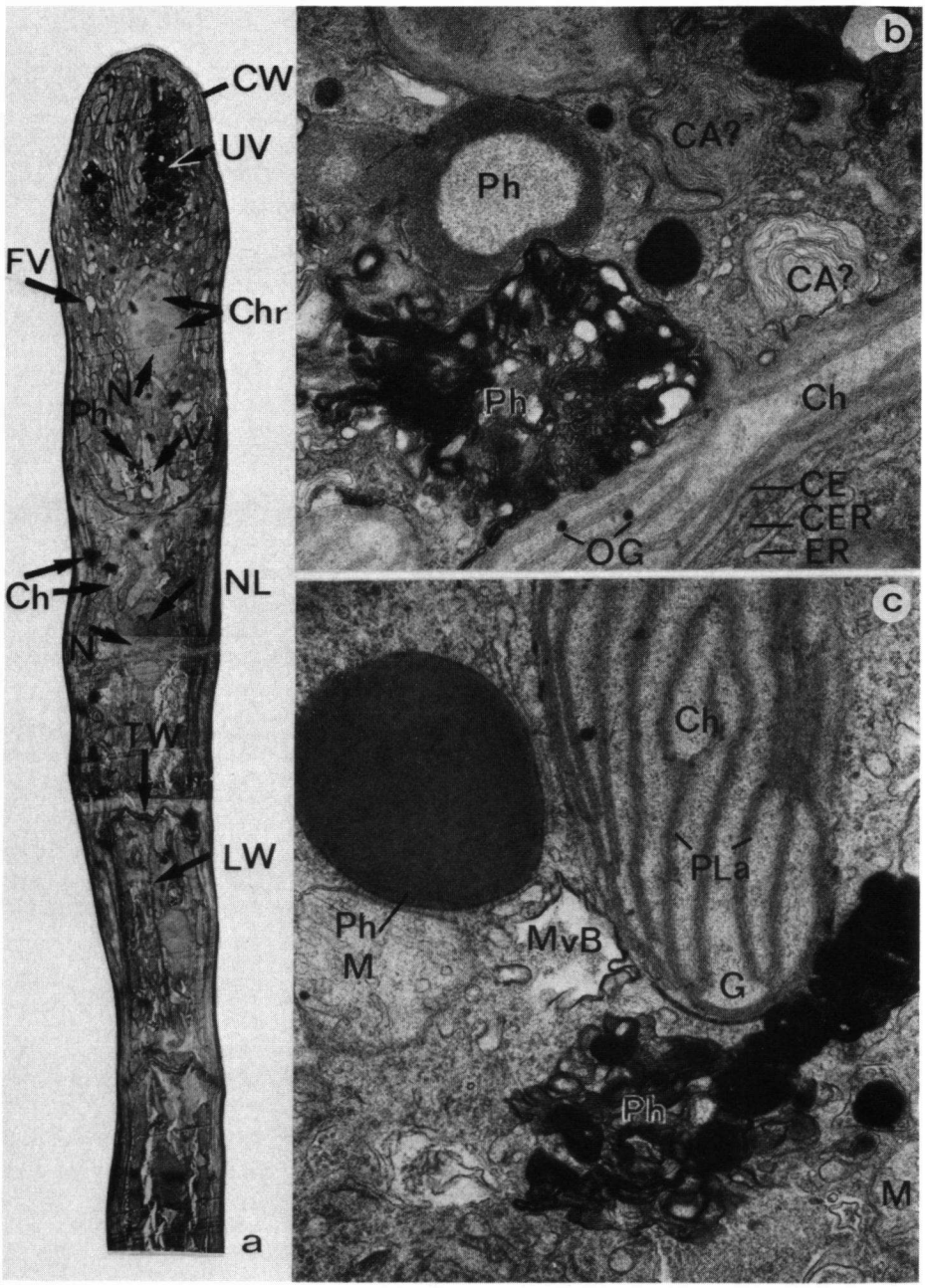

Plate 12. — a., b. *S. nana*, culture 67-34-3. 'a'. L.s. of filaments. ×1750. 'b'. L.s. of ap. cell with physodes. ×29,700. — c. *S. radicans*, culture 67-31-4. L.s. of ap. cell with vesicles. ×29,700.

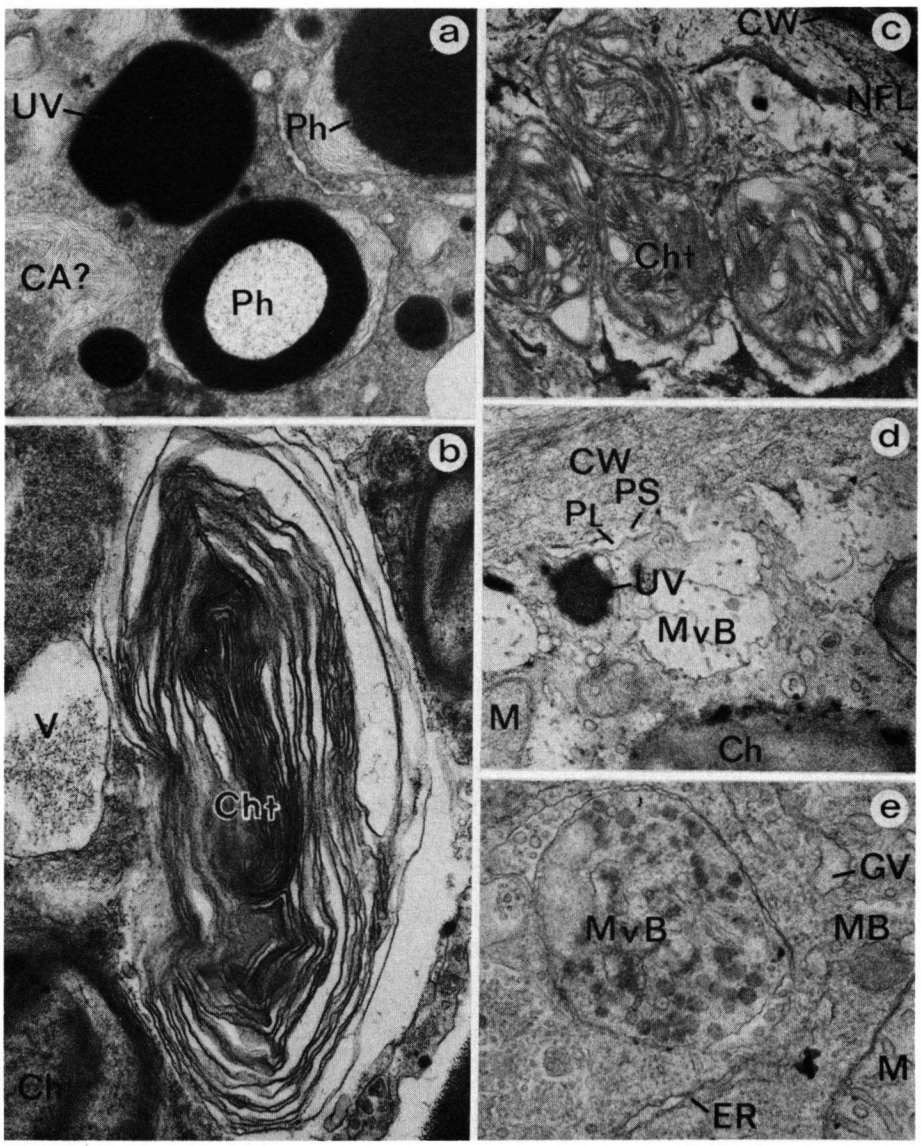

Plate 13. — a. *S. nana*, culture 67-34-3. L.s. of ap. cell with physodes. ×29,700. — b. *S. cirrosa*, culture 67-35. L.s. of primary segm. with deceased chloroplast. ×29,700. — c. *S. rigidula*, culture 68-32-2. L.s. of deceased ap. cell with remnants of chloroplasts. ×11,715. — d. *S. racemosa*, culture 71-11. L.s. of ap. cell. ×29,700. — e. *S. radicans*, culture 67-31-4. L.s. of ap. cell, with multivesicular body. ×29.700.

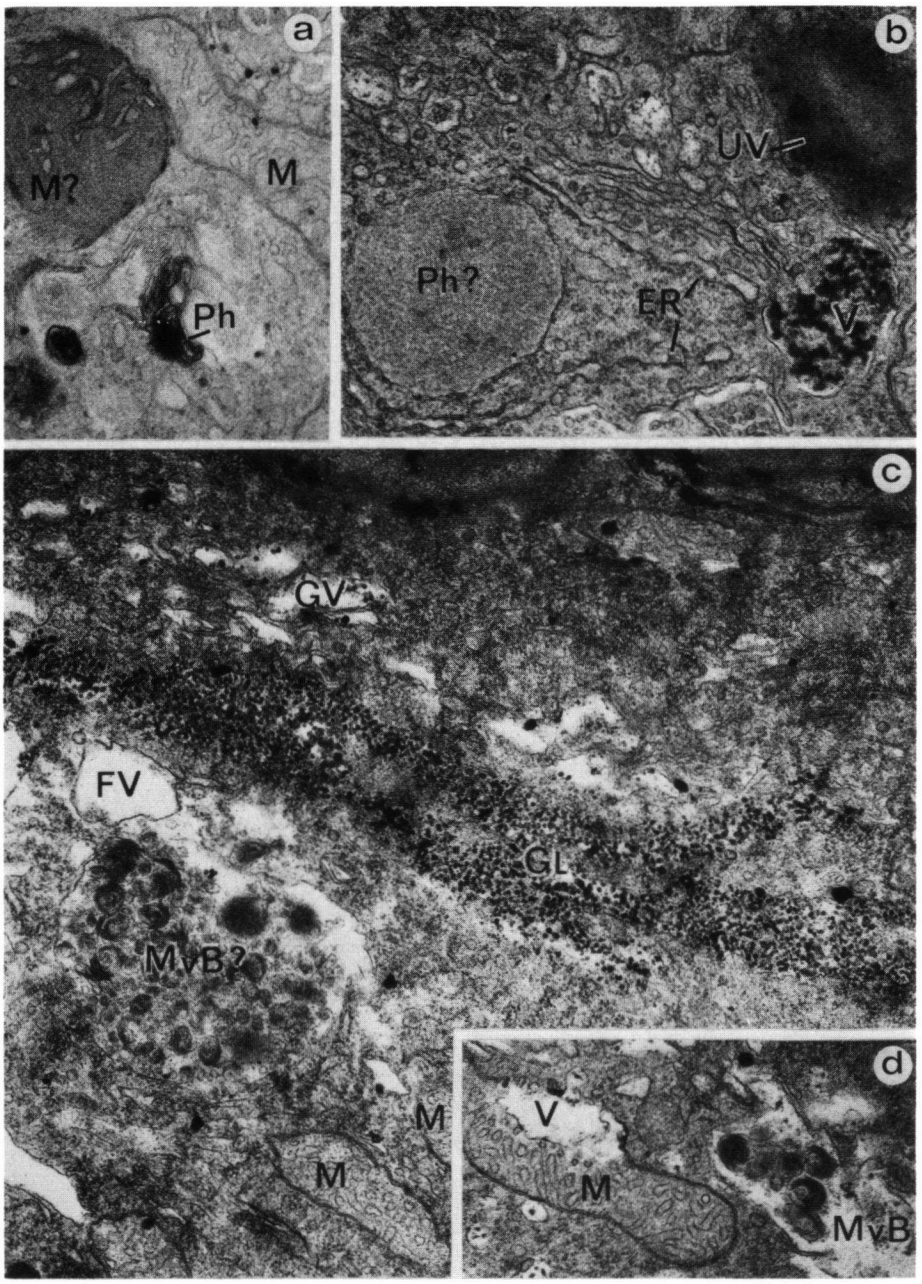

Plate 14. L.s. of ap. cells. ×29,700. — a., b. *S. radicans*, culture 67-31-4. In 'a' supposed modified mitochondrion. — c., d. *S. rigidula*, culture 68-32-2. In 'c' with glycogen-like granules, in 'd' with vacuole provoking lysis of a mitochondrion.