FINE STRUCTURE AND ORGANELLE ASSOCIATIONS IN BROWN ALGAE

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ABSTRACT

The structural interrelationships among several membrane systems in the cells of brown algae have been examined by electron microscopy. In the brown algae the chloroplasts are surrounded by two envelopes, the outer of which in some cases is continuous with the nuclear envelope. The pyrenoid, when present, protrudes from the chloroplast, is also surrounded by the two chloroplast envelopes, and, in addition, is capped by a third dilated envelope or "pyrenoid sac." The regular apposition of the membranes around the pyrenoid contrasts with their looser appearance over the remainder of the chloroplast. The Golgi apparatus is closely associated with the nuclear envelope in all brown algae examined, but in the Fucales this association may extend to portions of the cytoplasmic endoplasmic reticulum as well. Evidence is presented for the derivation of vesicles, characteristic of those found in the formative region of the Golgi apparatus, from portions of the underlying nuclear envelope. The possibility that a structural channeling system for carbohydrate reserves and secretory precursors may be present in brown algae is considered. Other features of the brown algal cell, such as crystal-containing bodies, the variety of darkly staining vacuoles, centrioles, and mitochondria, are examined briefly, and compared with similar structures in other plant cells.

An envelopment of the chloroplast by an extension of the nuclear envelope has been demonstrated in several groups of algae (Gibbs, 1962 *b*), but seems to be absent in higher plants and even in other divisions of algae. The functional significance of such an envelopment is as yet unknown, but a further elaboration of the fine structure of algae possessing this characteristic promised to aid in clarifying the nature of the envelope and its relationship to other cell organelles. Because the brown algae (Phaeophyta) have a nuclear envelope-chloroplast association which may also involve the Golgi complex and the pyrenoid, they appear especially suitable for structural analysis. In the present study an attempt has been made to examine some of the hitherto poorly known general details of fine structure in the cells of brown algae, and to focus particular attention on the structural interrelationships among the various organelles. The results suggest that stored and extruded metabolites may be at least partially transported through a well defined structural system in these cells.

MATERIALS AND METHODS

Fucus vesiculosus (Fucales), *Chorda filum* (Laminariales), and *Giffordia* sp. (Ectocarpales) were collected at Woodmont Beach, Woodmont, Connecticut. Whole plants were immersed and then cut either in a solution of phosphate-buffered 6.5 per cent glutaraldehyde at pH 6.9 (Sabatini, Bensch, and Barrnett, 1963) or in 10 per cent acrolein (acrylic aldehyde) buffered in the same manner. After 2½ hours at room temperature in glutaraldehyde or ¾ hour in acrolein, the pieces of algae were transferred to buffer (4 changes) and then to 2 per cent phosphate-buffered osmium tetroxide at 6°C for 12 hours. The

plants were then rinsed rapidly in distilled water, dehydrated gradually with methyl alcohol, and embedded in Epon (Luft, 1961) without a propylene oxide intermediate step (*cf* Bouck, 1964). The transfer from absolute methyl alcohol to the Epon mixture was done gradually and the tissue remained at room temperature for 24 hours to insure adequate infiltration. The blocks were polymerized at 60°C. Sections were cut with a diamond knife on a Porter-Blum ultramicrotome and either stained in Millonig's (1961) lead stain alone or double stained with uranyl acetate in 50 per cent alcohol (Watson, 1958) followed by lead staining. A JEM 6C electron microscope was utilized for examining and photographing the sectioned material.

RESULTS

Chloroplasts

The three genera selected for examination represent members of the anatomically simplest (Ectocarpales) to the most complex (Laminariales and Fucales) of the brown algae. The chloroplasts of *Giffordia* (Ectocarpales), *Chorda* (Laminariales) and *Fucus* (Fucales) consist of a limiting envelope, composed of two membranes separated by an electron-transparent region, and a granular matrix occupied in part by a series of globuli and discs. The discs are organized into bands usually composed of three discs, each of which is separated by a highly uniform space (Figs. 6 and 7). Occasionally, discs from one band may cross the matrix to join an adjacent band. Bands of discs either terminate near the ends of the oval-shaped chloroplasts or join a peripheral band which follows the contours of the chloroplast (Berkaloff, 1961). The area just within this terminal band and at the terminus (or beginning) of the other chloroplast bands is usually less electron-opaque (see also Leyon and von Wettstein, 1954; von Wettstein, 1954) and appears to contain fine fibers (Fig. 8) similar to the 25-A DNA fibers found in the chloroplast of *Chlamydomonas* (Ris and Plaut, 1962). The usual number of discs per band is three, although four and two are not uncommon. Discs in chloroplasts from cells near the meristematic region of *Fucus* are often single or paired (Fig. 11). Electron-opaque "globuli" are scattered throughout the matrix, but starch is absent.

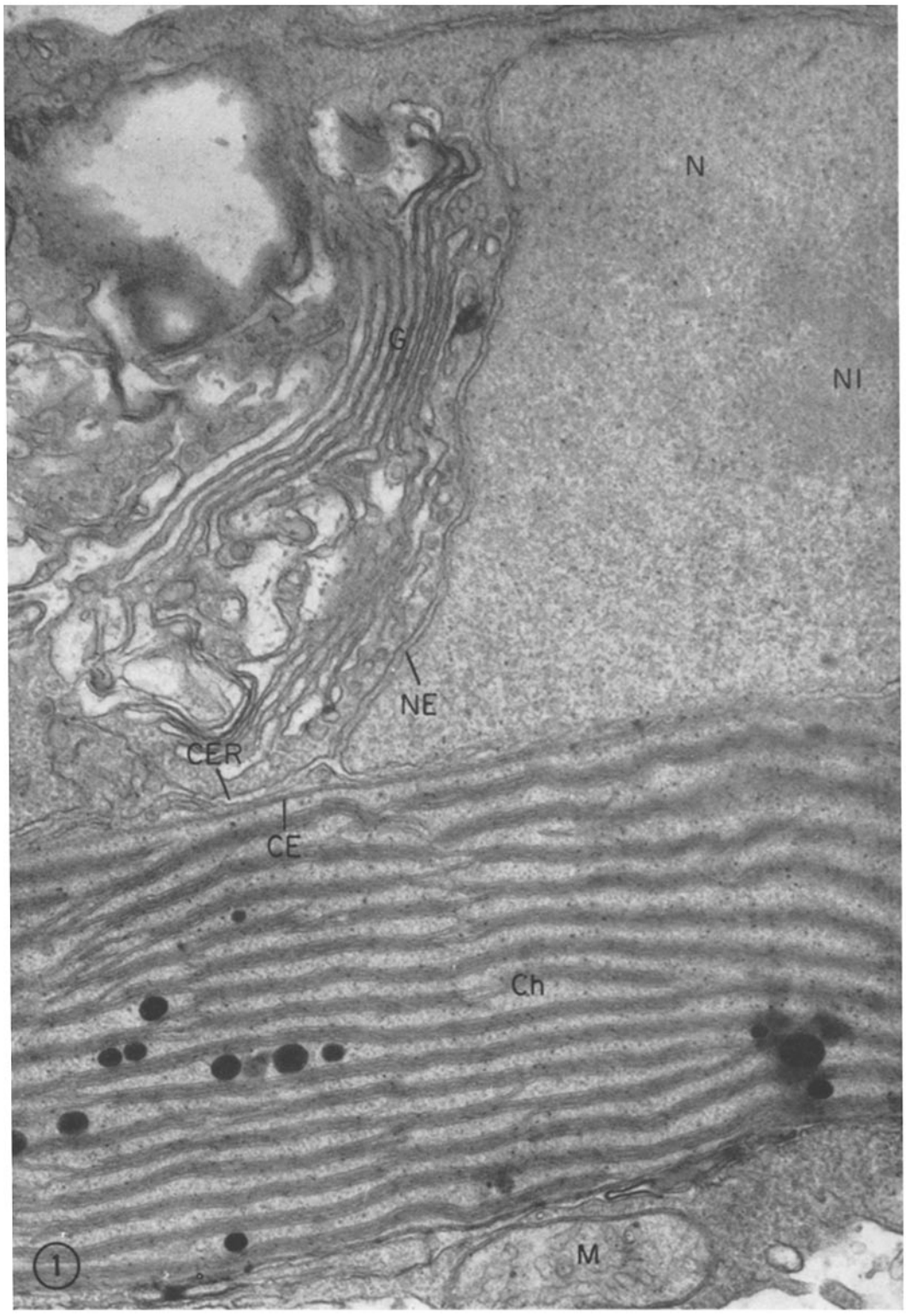
Outside the limiting chloroplast envelope is seen a second double-membraned envelope which, when followed in *Chorda filum*, is found to be continuous with the nuclear envelope (Figs. 1 and 3). This structure is similar to Gibbs "outer envelope of the chloroplast," and, for simplicity, will be termed the "chloroplast endoplasmic reticulum (ER)" in this paper.

On the surface of the chloroplast not adjacent to the nucleus, the chloroplast ER is usually applied to the surface of the chloroplast envelope proper, while on the side of the chloroplast adjacent to the nucleus the chloroplast ER usually departs from the chloroplast envelope. As noted by Gibbs (1962 *b*), the space thus formed between the two envelopes is frequently occupied by tubules. In some sections, similar tubules are seen as projections from the chloroplast ER, and thus,

Key to Labels

B, chloroplast band
Ch, chloroplast
CE, chloroplast envelope
CER, chloroplast endoplasmic reticulum
ER, endoplasmic reticulum
F, formative face of Golgi apparatus
G, Golgi apparatus
M, mitochondrion
Ma, maturation face of Golgi apparatus
N, nucleus
NE, nuclear envelope
NL, nucleolus
P, pyrenoid
PS, pyrenoid sac
T, tubule
Va, vacuole
V, vesicle

FIGURE 1 Portion of an outer assimilatory cell in *Chorda filum*. Note the large Golgi apparatus (*G*) in close association with the nuclear envelope (*NE*). The latter envelope is seen to be continuous with the chloroplast ER (*CER*), but distinct and separate from the chloroplast envelope (*CE*). *Ch*, chloroplast; *M*, mitochondrion; *N*, nucleus; *Nl*, nucleolus. × 33,000.


N
G
NI
NE
CER
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Ch
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1

seemingly unattached tubules may, in part, arise through evaginations from the chloroplast ER (Fig. 7).

The chloroplast ER is present also in *Giffordia* (Fig. 6) and *Fucus* (Fig. 11), but in neither genus have direct connections with the nuclear envelope been observed. In both plants, there are many chloroplasts per cell, as distinguished from *Chorda* in which there are one or two large chloroplasts per cell. In the latter case, because of the small size of the cell the nucleus is usually forced up against the chloroplast, while in *Fucus* or *Giffordia* there may be much greater distances separating the chloroplasts and nucleus. In *Giffordia*, individual chloroplasts can often be seen linked to one another through the chloroplast ER (Fig. 9). It seems probable in *Giffordia* and *Fucus* either that the chloroplast ER is attached to the nuclear envelope through a ramifying strand which would probably not be caught in any one section, or that the connection exists only for short periods.

Pyrenoid

A pyrenoid is present in both *Giffordia* (Figs. 6 and 9) and *Chorda* (Fig. 2), but not in *Fucus*. This structure appears as an outpocketing or "diverticulum" (Simon, 1954; Manton, 1957; Gibbs, 1962 *a*; Giraud, 1962) from the chloroplast. In *Chorda* two pyrenoids may form on a single chloroplast, but in *Giffordia* one pyrenoid per chloroplast is usual. The matrix of the chloroplast is continuous with, but usually less electron-opaque than the contents of the pyrenoid. This pyrenoid matrix often appears coarsely granular or fibrous and only very rarely contains chloroplast discs. The area of chloroplast just below the attachment of the pyrenoid likewise is usually free of bands or discs.

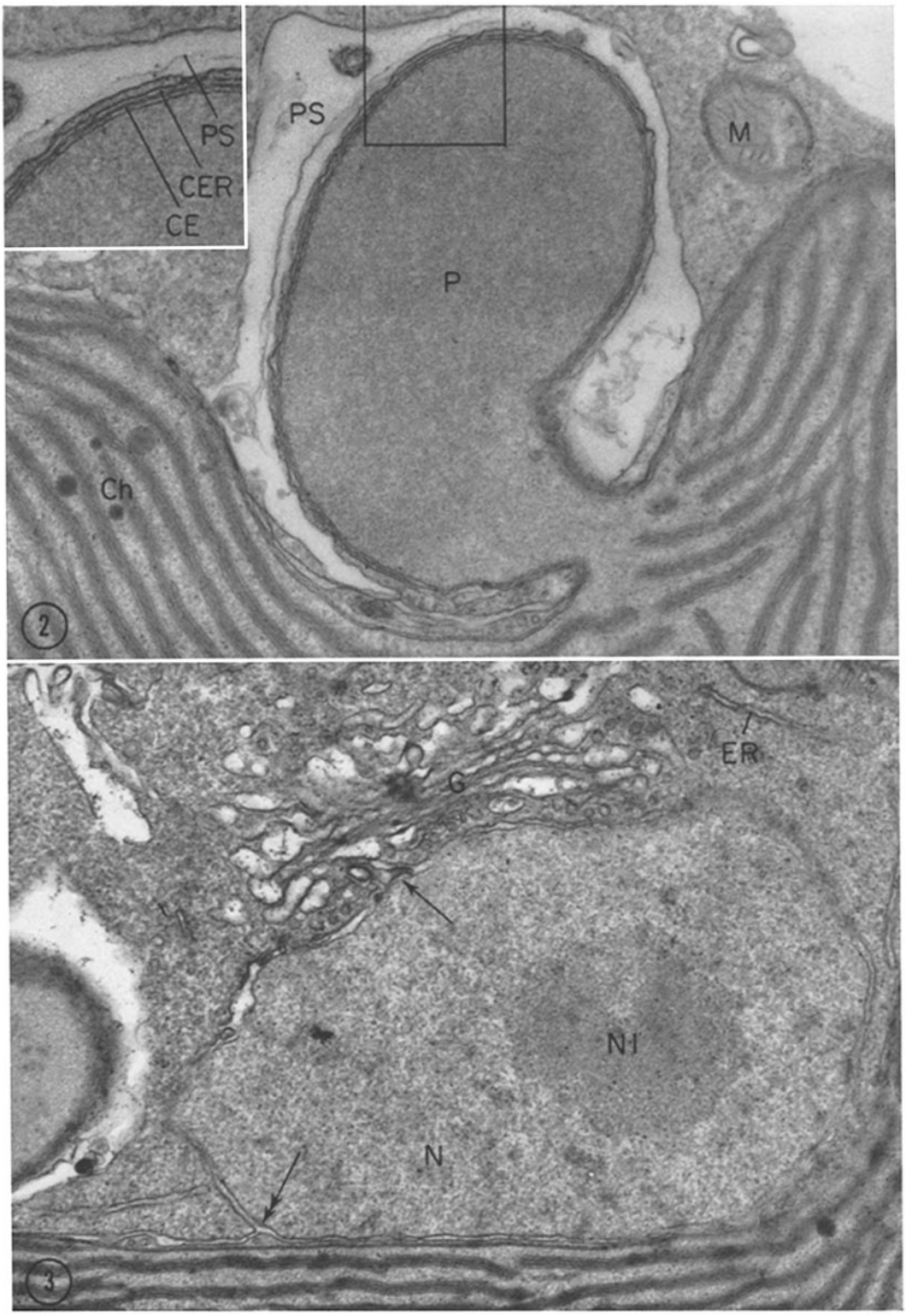
Surrounding the pyrenoid are three separate double membranes or envelopes (Fig. 2). The innermost of these envelopes marks the continuation of the chloroplast envelope around the margin of the pyrenoid. Outside this chloroplast envelope lies the chloroplast ER which comprises the middle of the three envelopes and is continuous with the chloroplast ER surrounding the rest of the chloroplast. However, in contrast to the rather loose association of chloroplast ER to the chloroplast envelope in adjacent regions, around the pyrenoid the two envelopes become closely and very regularly appressed. Finally, the outermost envelope of the pyrenoid is composed of two membranes and may be greatly dilated. This outer envelope terminates in the region of the neck and appears as a cap or sac extending over the pyrenoid (Fig. 2). There is apparently no continuity of this sac envelope with other membranes within the cell. It has been suggested (*cf* Fritsch, 1945) that pyrenoids may become detached from the chloroplast and exist independently within the cytoplasm. In the examined sections, it is difficult to distinguish truly detached pyrenoids from those sectioned in a plane other than the median. However, the frequency of sections showing attached pyrenoids would suggest that detached pyrenoids are not common in those genera examined.

Nucleus

The nucleus contains a prominent nucleolus and is bounded by the usual envelope containing pores. The outer membrane of the envelope

FIGURE 2 A section through the pyrenoid (*P*) of *Chorda filum*. Note that the matrices of the pyrenoid (*P*) and chloroplast (*Ch*) are continuous. An electron-transparent pyrenoid sac (*PS*) is seen extending around the pyrenoid, but not over the remainder of the chloroplast. In the inset, the various envelopes (double membranes) of the pyrenoid are shown. The chloroplast envelope (*CE*) limits the pyrenoid, the chloroplast ER (*CER*) constitutes the middle pair of membranes, and the pyrenoid sac (*PS*) is formed from the outermost pair of membranes. *M*, mitochondrion. × 27,000. Inset, × 54,000.

FIGURE 3 A section through an outer assimilatory cell of *Chorda filum* showing the nucleus (*N*) in close association with the chloroplast and the Golgi apparatus (*G*). A portion of the nuclear envelope can be seen extending into the Golgi apparatus (at single arrow). A bifurcation of the nuclear envelope which is continuous with the chloroplast ER is seen at the double arrow. × 25,000.


PS
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appears in many cases highly irregular and convoluted while the inner membrane seems fairly regular. The irregular outer surface often appears to be giving rise to blebs, especially in the region of the Golgi apparatus. Continuity of the nuclear envelope with the endoplasmic reticulum may be seen in many regions, and an extension of the nuclear envelope around the chloroplasts is especially well seen in *Chorda* where the nucleus is closely associated with the surface of the chloroplasts (Fig. 1). Direct continuity of the outermost envelope of the chloroplast with the nuclear envelope has been established with certainty only in *Chorda filum*.

Golgi Apparatus

The Golgi apparatus (dictyosome) is composed of plates often of unusually large diameter. The Golgi structures are localized in the immediate perinuclear region in *Giffordia* (Fig. 4) and *Chorda* (Fig. 5), while in *Fucus* (Fig. 10) they appear smaller and are more widely scattered throughout the cell. In agreement with the conception of Mollenhauer and Whaley (1963), the Golgi apparatus appears to be composed of two surfaces. A "forming" face characterized by separate vesicles on one surface can be distinguished from a "maturing" face of broad and often dilated cisternae on the other surface (*e.g.*, Fig. 5). In its middle area, the Golgi apparatus forms a series of flattened plates. Vesicles appear to be coalescing in the presumably older portion of the formative face, but are free spheres near the nuclear envelope or the endoplasmic reticulum. Similar spheres can also be seen as blebs from the outer of the two nuclear membranes in *Chorda* and *Giffordia* (Fig. 4), while in *Fucus* the formative face is always associated with either the nuclear envelope or a portion of the endoplasmic reticulum (Fig. 10). In the latter case, blebs may be formed from the cytoplasmic ER cisternae, but not from the membranes of the chloroplast ER.

Mitochondria

Mitochondria are limited by the usual double-membraned envelope. Characteristically, the invaginations of the inner membrane which form the cristae are tubular or villus-like rather than plate-like (Figs. 1, 2, and 14). Electron-opaque globuli (*cf* Peachey, 1964) appear to be present in small quantities in those genera examined. The shape of the mitochondria is highly variable, and there is no apparent association of the mitochondria with other organelles. In *Chorda* the mitochondria tend to lie along the margin of the cell, while in *Fucus* and *Giffordia* they are randomly scattered throughout.

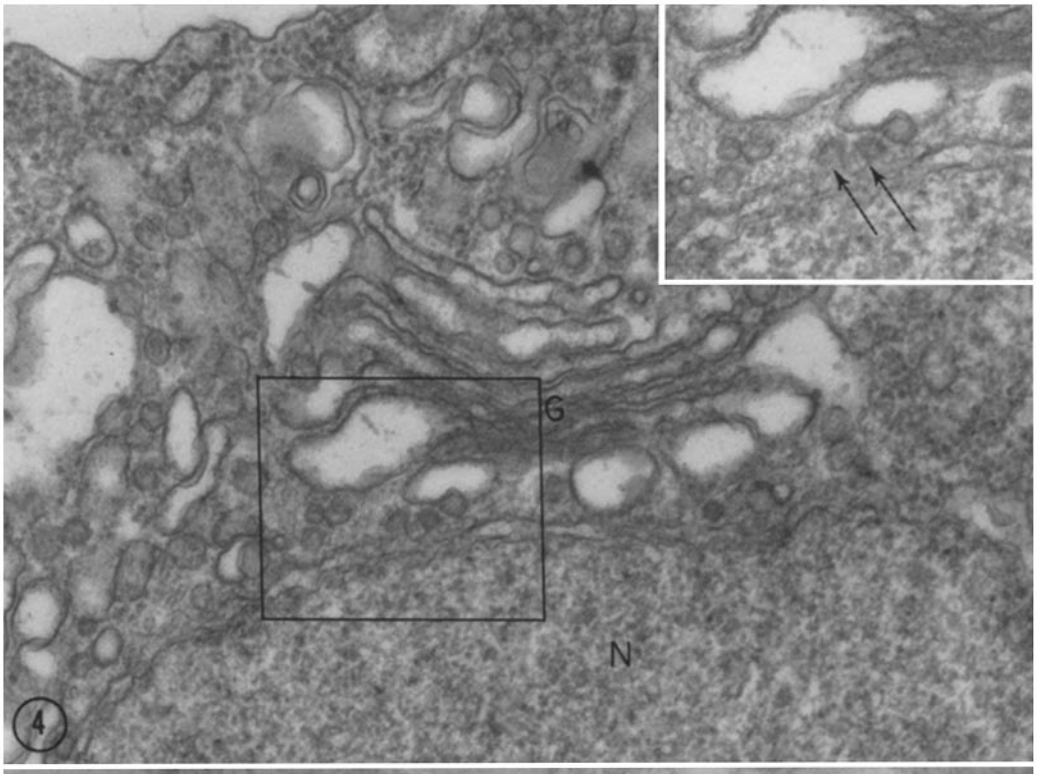
Other Cytoplasmic Structures

Paired rod-like centrioles with the usual nine skewed triplet fibers (Fig. 12) are generally found in a depression in the nuclear surface (*cf* also Berkaloff, 1963). Spindle fiber–like elements radiate from the lower portion of the centriole along the surface of the nucleus, but their ultimate endings are obscure (Fig. 13). The microtubules found in close association with the wall of higher plants (Ledbetter and Porter, 1963) are apparently not present or not preserved in a similar region in these cells.

Single membrane-limited bodies containing highly oriented internal lattices with spacings of about 175 A have been occasionally observed in *Fucus* (Fig. 15) and *Giffordia*. They resemble the

FIGURE 4 Portion of a cell of *Giffordia* showing the relation of nucleus (*N*) and Golgi apparatus (*G*). A blebbing of the outer of the two nuclear membranes is apparent at arrows in the inset. × 39,000. Inset, × 50,000.

FIGURE 5 Section of an unstained cell of *Chorda filum* fixed in acrolein-OsO_4 and embedded in cross-linked methacrylate. The Golgi apparatus is composed of two faces. The formative face (*F*) consists of vesicles and short flattened cisternae apparently in a process of coalescence. The small vesicles are presumably derived from blebs from the nuclear envelope (see Fig. 5). The maturation face (*Ma*) appears to be composed of expanded sacs which seem to have begun their expansion at a lower region in the Golgi apparatus. Note that vesicles similar to those in the formative area may be found in other regions of the Golgi apparatus. *N*, nucleus; NE, nuclear envelope. × 40,000.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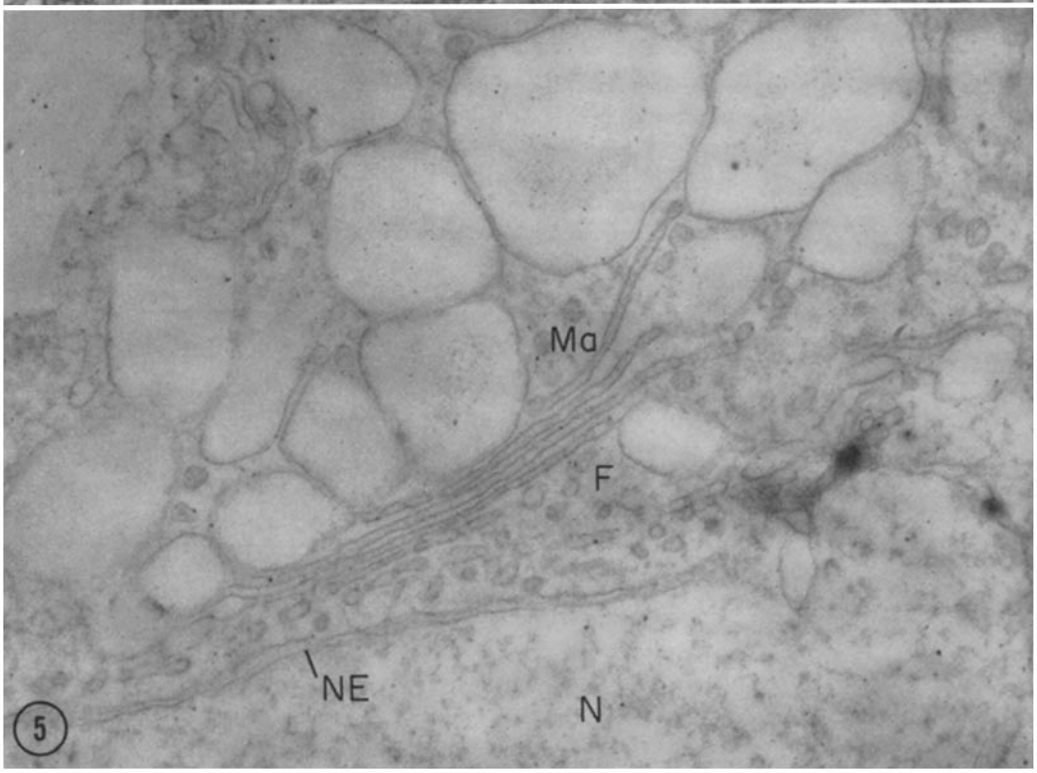
Ma
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crystal-containing bodies of fungi and higher plants (Thornton and Thimann, 1964; Cronshaw, 1964).

Vacuoles are limited by a single membrane and may contain a variety of materials varying in their electron opacity. In *Fucus* especially, the contents of the vacuoles frequently appear heavily electron-opaque (Fig. 14) and resemble the accumulations of tannins found in other plant tissues (Politis, 1961). Also in *Fucus*, spherical membrane-limited vesicles about 800 mμ in diameter are often abundant in the outer cells. In some sections, these vesicles appear to protrude from the cytoplasm into the wall region, suggesting that there is a release of material to the wall (Fig. 10). Many of the great variety of vacuoles and membrane-limited vesicles in *Fucus* (Fig. 14) undoubtedly are related to the various "physodes" of the classical light microscope literature (*e.g.* Chadefaud, 1936).

In the cell wall may be recognized fine fibrils, presumably cellulose microfibrils, in agreement with the findings of Cronshaw, Myers, and Preston (1958) and of Dawes, Scott, and Bowler (1961). The "paraplasmic" membrane observed by Berkaloff (1963) in *Himanthalia* has not been seen in any of the genera examined here.

DISCUSSION

In this examination of brown algae from the anatomically simplest to the most complex forms, a consistent and in some ways unique pattern of cell fine structure has been found. Chloroplasts are a prominent feature of the cytoplasm and contain bands usually of three uniformly separated discs. Near the ends of the chloroplasts electron-transparent areas often contain fine fibers, and in some genera pyrenoids arise from the chloroplast surface. Mitochondria are found to possess villus-like rather than plate-like cristae, and are, therefore, suggestive of the mitochondrial structure reported for protozoa (*cf* Sedar and Rudzinska, 1956). Crystal-containing bodies similar to those found in higher plants and fungi are seen in small quantities in brown algae, and thereby extend the range of these anomalous structures. Paired centrioles and associated spindle-like fibers appear structurally identical to their counterparts in animals and lower plants. In the brown algae, however, the membranes surrounding the pyrenoid, the membranes of the chloroplast, those of the nuclear envelope, and those of the Golgi apparatus have an unusual relationship with one another. Because they suggest that an integrated structural system for the transport of food reserves and secretory precursors may be present in the brown algal cell, these relationships thus form the subjects of major general interest in this report.

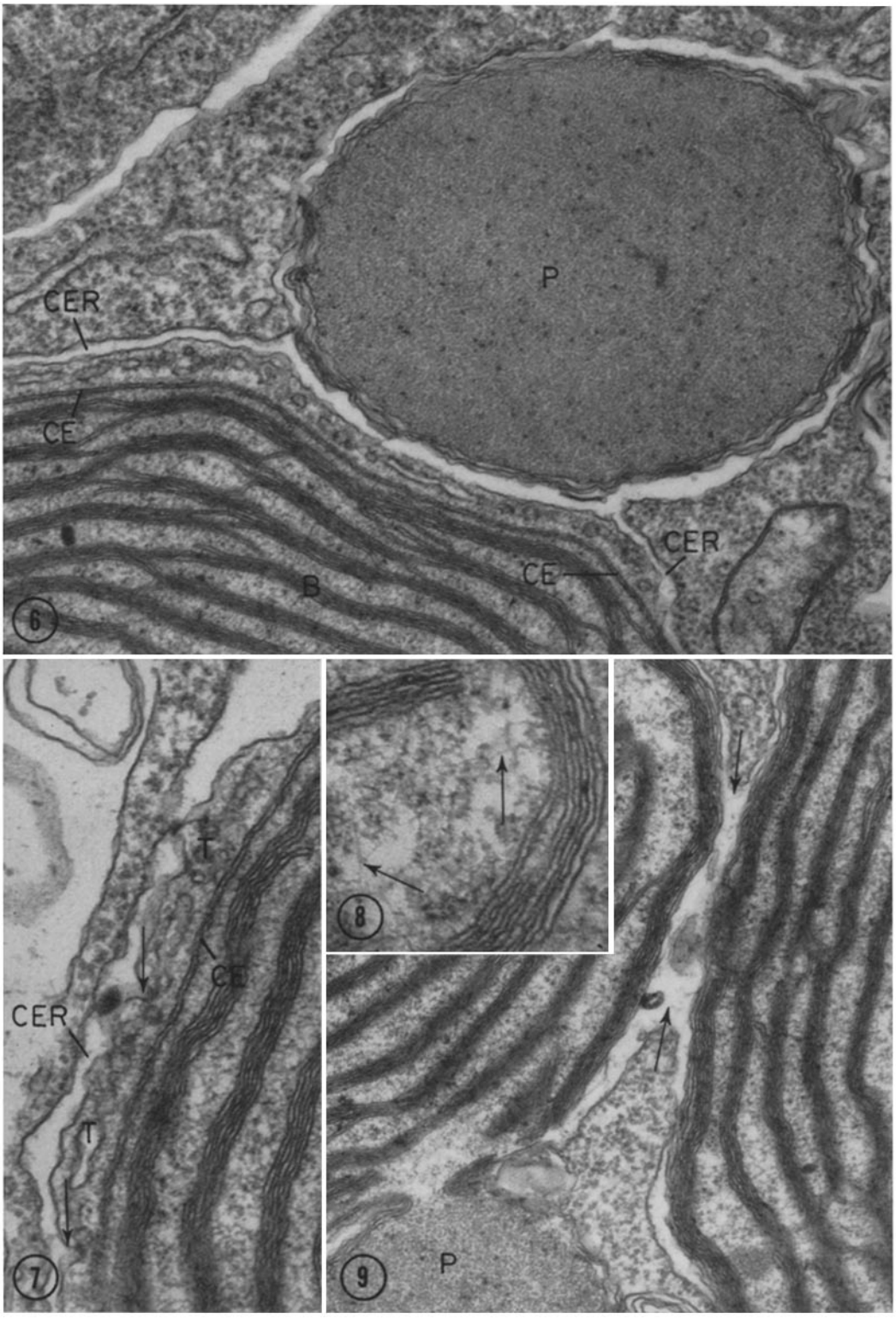
The chloroplast envelope consists of paired membranes which limit the chloroplast and follow the contours of the pyrenoid and pyrenoid neck. The outer chloroplast membrane is smooth and appears never to be continuous with other membranes within the cell. The chloroplast is thus a distinct structural entity. However, in the brown algae and a few other algal forms (*cf* Gibbs, 1962 *b*) an additional envelope may be recognized. As designated herein, this "chloroplast ER" is not continuous with any portion of the chloroplast

FIGURE 6 Portion of a cell of *Giffordia* either not sectioned through the neck of the pyrenoid (*P*) or sectioned through a pyrenoid not attached to the chloroplast. The chloroplast ER (*CER*) clearly can be traced around the pyrenoid. *B*, chloroplast band; *CE*, chloroplast envelope. × 30,000.

FIGURE 7 Portion of a cell of *Giffordia* illustrating the relationships of chloroplast ER (*CER*) to the chloroplast envelope (*CE*). The region between these two envelopes often contains "tubules" (*T*). These may arise as projections from the chloroplast ER (arrows). × 40,000.

FIGURE 8 A region near the end of the chloroplast which is usually less electron-opaque than other parts of the chloroplast matrix. Note appearance of fine fibers (arrows). × 70,000.

FIGURE 9 Two chloroplasts of *Giffordia* which are connected by chloroplast ER (arrows). The pyrenoid matrix (*P*) is more electron-opaque than, though apparently continuous with, the chloroplast matrix. × 27,000.


P
CER
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7
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9
P

envelope, but does form a loosely fitting sac surrounding the chloroplast. Four features of this sac are worthy of notice and may provide clues to its function: (1) around the pyrenoid and in contrast to the remainder of the chloroplast surface the chloroplast ER is very regularly and closely appressed to the pyrenoid (*i.e.*, chloroplast) envelope; (2) the space between the chloroplast envelope and the chloroplast ER frequently contains projections or villi from the chloroplast ER; (3) the chloroplast ER may be continuous with the nuclear envelope; and (4) the chloroplast ER lacks ribosomes on its inner surface. Considering that the ER may serve as a channeling or conveyor system in other kinds of cells (*e.g.*, Porter, 1961; Caro and Palade, 1964), it seems not unlikely that the chloroplast ER is gathering up photosynthesates diffused or carried through the chloroplast envelope and that the villi may aid in this process. It appears that some of these gathered chloroplast products may ultimately be fashioned into secretory materials, since the chloroplast ER may be continuous with the nuclear envelope or the cytoplasmic ER which, in turn, may pass off materials to the Golgi apparatus (see below).

Around the pyrenoid a different mechanism is indicated. The regular apposition of chloroplast ER and chloroplast envelope as well as the positioning of a pyrenoid sac just external to these envelopes suggests that materials synthesized within the pyrenoid are passed *across* rather than along the intervening membranes. Such a hypothesis would be consistent with the finding in other groups of algae that carbohydrate reserves are usually located just external to the pyrenoid. It remains, however, for suitable histochemical techniques to demonstrate that the pyrenoid sac contains the soluble reserves characteristic of brown algae.

The chloroplast ER, through temporary or permanent connection with the nuclear envelope, maintains contact with the regions blebbing vesicles to the Golgi apparatus. In the brown algae, the Golgi apparatus is consistently oriented adjacent to the nuclear envelope except in the order Fucales in which the Golgi apparatus may be associated with a portion of the cytoplasmic ER as well as the nuclear envelope. The connection between Golgi apparatus and nuclear envelope appears to be indirect. That is vesicles derived from the nuclear membrane are first seen as free spheres and are then incorporated into the formative region of the Golgi apparatus.

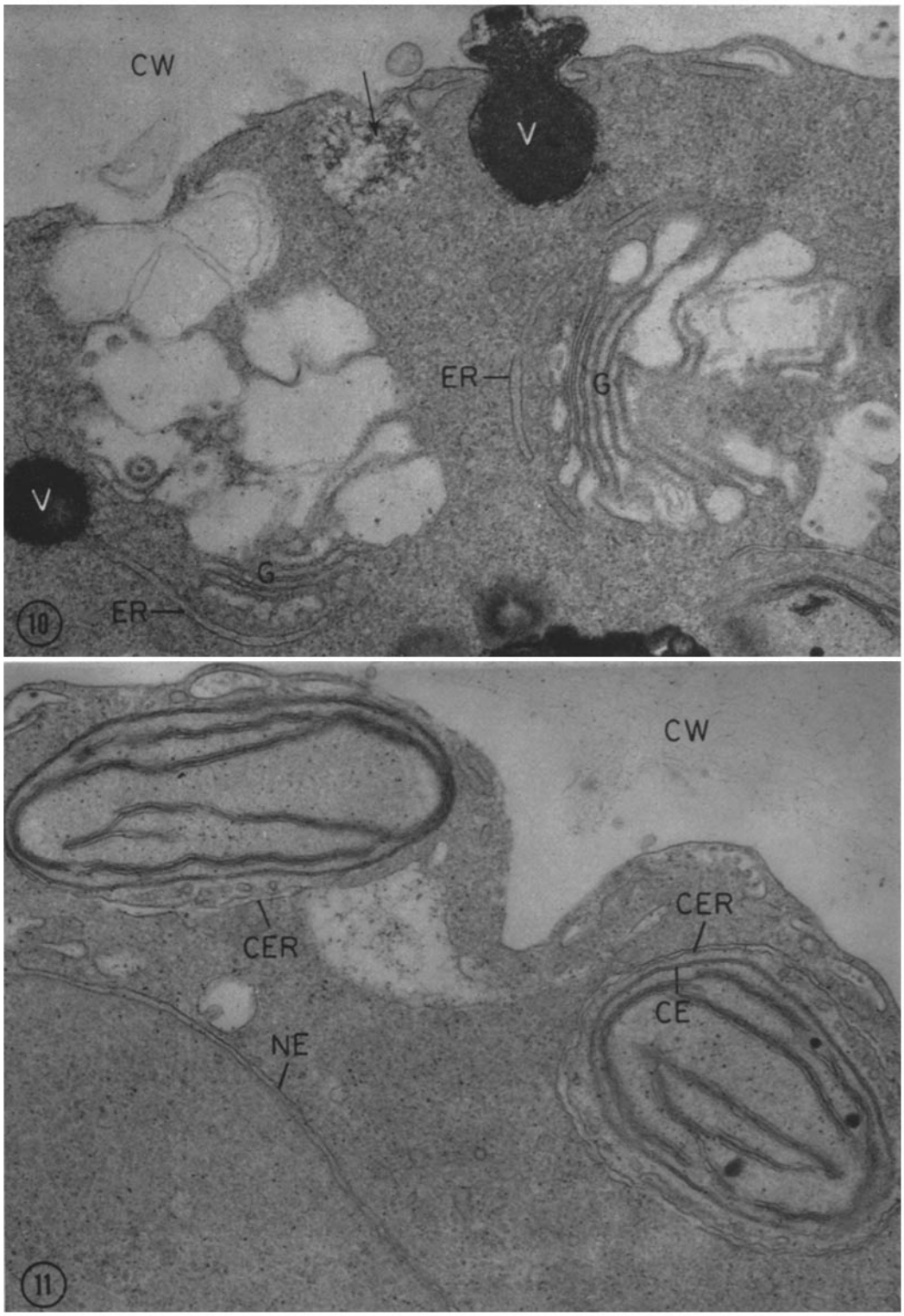
The dynamic nature of the Golgi apparatus has been frequently noted, and, in general, materials incorporated into the formative region are believed to undergo condensations and elaborations to become the secretory vacuoles of the mature face. These secretory vacuoles have been shown in other plants to be released at the cell surface (*e.g.* Bouck, 1962; Mollenhauer and Whaley, 1962), and such a mechanism seems applicable in the brown algae as well. The nature of the secretory products is as yet unknown, but presumably some wall polysaccharides are secreted in this manner.

The relationship of the Golgi apparatus to the cytoplasmic ER in the Fucales, first noted by Berkaloff (1963) in *Himanthalia*, is confirmed in *Fucus* in the present study. This tendency of the Golgi apparatus to be associated with a portion of the ER in the phylogenetically more advanced brown algae may be of some evolutionary significance. The formation of Golgi plates from a "special portion of ER" has been demonstrated in animal cells (*e.g.* Zeigel and Dalton, 1962; Friend, 1964), and the condition in the Fucales may reflect a trend in this direction.

In a fungus, Moore and McAlear (1963) note a relationship between the Golgi apparatus and the nuclear envelope, while Manton (1960) has suggested that a tubular form of ER may be asso-

FIGURE 10 A section through two Golgi bodies (*G*) in *Fucus* showing the association of the formative face with a portion of the ER. Membrane-bounded electron-opaque vesicles (*V*) appear protruding on the wall (*CW*). At the arrow is a vesicle which may have released its contents. × 25,000.

FIGURE 11 A portion of a cell of *Fucus vesiculosus* sectioned near the apical growing region. Chloroplasts appear to have an outer chloroplast ER (*CER*) envelope. *CE*, chloroplast envelope; *CW*, cell wall; *NE*, nuclear envelope. × 21,000.


CW
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ciated with the Golgi apparatus in the hornwort, *Anthoceros*. However, in higher plants, no convincing evidence of an association of the Golgi apparatus with the ER or nuclear envelope has yet been recorded (*e.g.* Whaley, Mollenhauer, and Kephart, 1959), and there the mechanisms of acquisitions by the Golgi apparatus remain obscure.

In summary, in the brown algae it would seem that materials manufactured within the pyrenoid are passed across the surrounding ER system into a collecting sac and perhaps stored there for internal use. Conceivably, other materials synthesized within the chloroplast are passed through the surrounding ER system, sequestered by the Golgi apparatus, and ultimately extruded from the cell. Thus, the expansion of the nuclear envelope and the chloroplast ER system to include the Golgi apparatus, at least in brown algae, seems appropriate. It provides a structural channeling system from the site of carbon fixation and sugar synthesis to the probable site of the sequestering, possible polymerization, and secretion of a polysaccharide (Fig. 16).

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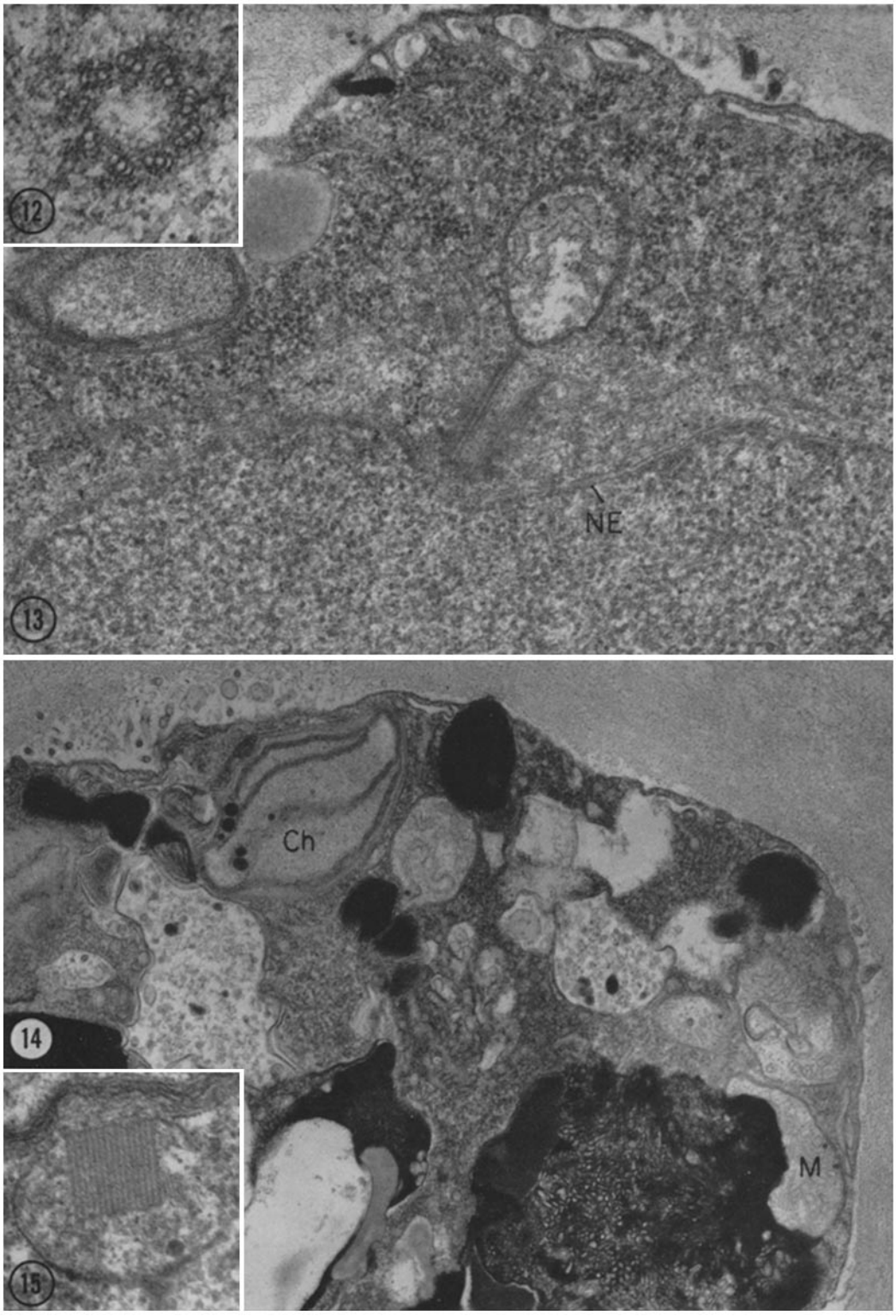
(*For Bibliography, See p. 536*)

FIGURE 12 Transverse section through a centriole of *Fucus* showing the typical nine skewed triplet fibers. × 85,000.

FIGURE 13 A section through the long axis of a centriole of *Fucus*. Note the depression in the nuclear envelope (*NE*) in which the rod-like centriole is placed. Spindle fiber-like elements radiate from the lower portion of the centriole over the nucleus as well as towards the cell surface. × 39,000.

FIGURE 14 A cell of *Fucus* illustrating some of the variety of vacuoles and membrane-limited vesicles. A vacuole at lower right appears to be filled with electron-opaque tubules. Some of these vacuoles probably correspond to the "physodes" of light microscopists. *Ch*, chloroplast; *M*, mitochondrion. × 19,000.

FIGURE 15 Single membrane-limited body of *Fucus* showing a lattice of regularly spaced, electron-opaque lines or dots. Spacings are about 175 A. × 56,000.


12
13
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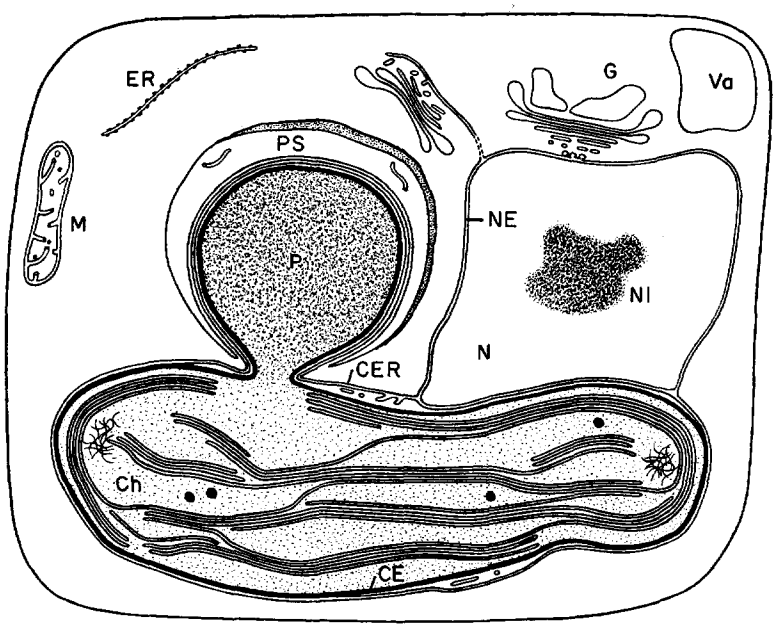

FIGURE 16 Diagram of a hypothetical brown alga cell illustrating some of the organelle associations found to occur within the group. The chloroplast envelope (*CE*) limits the chloroplast (*Ch*) and pyrenoid (*P*). On the cytoplasmic side of the chloroplast envelope lies the chloroplast ER (*CER*) which is seen, at least in *Chorda filum*, to be continuous with the nuclear envelope (*NE*) and also follows the contours of the pyrenoid. Golgi bodies (*G*) are closely associated with the nuclear envelope surface or a portion of the ER. It is believed that vesicles originating from either the nuclear envelope or the ER are incorporated into the formative region of the Golgi apparatus. The pyrenoid sac (*PS*) forms a distinct and often dilated envelope which may contain reserve carbohydrates synthesized and/or polymerized within the pyrenoid. *M*, mitochondrion; *N*, nucleus; *Nl*, nucleolus.

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