FINE STRUCTURAL STUDIES ON SOME MARINE ALGAE FROM
THE PACIFIC COAST OF BRITISH COLUMBIA AND WASHINGTON

by

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Date April 21, 1971
ABSTRACT

Fine structural studies on some marine algae from the Pacific Coast of British Columbia and Washington

In a fine-structure study of Phaeostrophion irregularare (Dictyosiphonales) most characteristics of the organelles were found to be similar to those previously reported for other brown algae. However, the pyrenoid which is present in the cells of the sporeling is absent from the thallus which grows directly from it. This is significant because it draws attention to the possible implications of observing a limited number of heterogeneous tissue types, such as spores, sporeling, and mature thallus, in comparative studies. Phaeostrophion irregularare retains its intermediate position in the phylogenetic classification of the brown algae. It possesses some characteristics of the more advanced brown algae, such as the absence of a pyrenoid in the adult thallus and the absence of a physical association of the chloroplast and nucleus. Also it possesses more primitive characteristics such as a strictly perinuclear Golgi apparatus and a diffuse distribution of plasmodesmata.

The red algae Porphyra perforata and Bangia fuscopurpurea, both of the order Bangiales, subclass Bangiophycidae, were also studied. In the former, the thallus, bipolar sporeling, and conchocelis phase were considered, and in the latter, the thallus only. Cells of all
the tissues that were studied have very similar fine structural characteristics. It was noted also, that the fine structural features of these tissues were similar to other Bangiophycidae and the Florideophycidae. A fairly constant association was noted between the mitochondria and the forming face of the Golgi bodies. Most cells contained numerous lomasome-like bodies throughout the cytoplasm.

Two types of cell division were observed in the *Porphyra* thallus cell. The possible function of several organelles is discussed. In addition to these features seen in the thallus, cells of the young conchocelis have phycobilisomes in the chloroplasts and all cells have a typical floridean pit connection, confirming earlier light microscope observations. This characteristic had previously been considered fairly definitive for the Florideophycidae. It is suggested that with these data and other published results the status of subclass for the Bangiophycidae and Florideophycidae may have to be reviewed.
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Studies on the cytology of marine algae are of potential importance. Such studies will aid in our understanding the relationships between various algal groups. It is a fundamental axiom of taxonomy that the more information upon which a classification is based the more natural it will be. The taxonomy of algae is of some considerable interest. It is in the large and diverse group of algae that biochemical characteristics were first put to significant use. There is also some indication that studies on the fine structure of the algal cell may be of taxonomic significance.

A second valuable aspect of cytological studies of marine algae is that whenever a new organism is studied a considerable amount of information is added to our knowledge of organelle structure and function. This assumes considerable significance in a field such as electron microscopy. Too often, sweeping generalizations on organelle structure and function are made, based on observations from a very limited number of organisms.

This study was initiated to provide ultrastructural information on some local species of marine algae. These and previously published findings were used to examine hypotheses of taxonomic relationships in the Phaeophyceae and Rhodophyceae (Fig.1) and hypotheses relating to organelle structure and function in Porphyra perforata.
Figure 1 Chart showing possible interrelationships of the orders within the Phaeophyceae and Rhodophyceae

A Phenetic relationships within the Phaeophyceae.

B Phenetic relationships within the Rhodophyceae.
Figure 1  Chart showing possible interrelationships of the orders within the Phaeophyceae and Rhodophyceae.
PART ONE

Phaeophyceae

Some observations on the fine structure of the marine brown alga Phaeostrophion irregulare¹

Introduction

The fine structure of a small number of Phaeophyceae and other related groups has been investigated during the past few years and an interesting feature has been reported. According to several authors (Gibbs, 1962a; Greenwood, 1964; Bouck, 1965; Manton, 1966; Cole, Bourne, and Lin, 1968; Cole and Lin, 1968; and Cole, 1969, 1970) there is a physical association of the nucleus, chloroplast, pyrenoid, and other organelles in various brown algae and other related species. Evans (1966, 1968) has presented evidence that the presence or absence of the pyrenoid is of phylogenetic importance.

Previous publications have covered only a limited number of species and it is obvious that more information on the ultrastructure of an increased number of Phaeophyceae is required to determine the true phylogenetic significance of these findings. Only one member of the Dictyosiphonales, Dictyosiphon foeniculaceus (Evans, 1966), has been reported in the literature. Consequently a study of the fine structure of another species of the same order, Phaeostrophion irregulare, was initiated.

¹ This part of the thesis is based on an article by V.L. Bourne and K. Cole which appeared in the Canadian Journal of Botany 46: 1369-1375 (1968). The text of the original article has been brought up to date by including subsequent findings where appropriate.
Materials and Methods

The brown alga *Phaeostrophion irregulare* S.et G., a member of the family Punctariaceae in the order Dictyosiphonales, was originally described in 1924 (Setchell and Gardner, 1924), but details of its life history and ecology have only recently been elucidated (Mathieson, 1967). It is similar to several other species in this order in having a direct type of life cycle. Meiosis is probably suppressed in the unilocular sporangium although this has not been determined cytologically.

*Phaeostrophion irregulare* was collected during low tide in the winter months from 1965 to 1967 at Point No Point (Glacier Point), Vancouver Island, B.C. The mature thallus consists of a few straplike blades attached to a basal holdfast (Fig. 2). The blades are often irregularly torn and vary in length from 1.0 to 25.0 cm and in width from 0.5 to 4.5 cm. A cross section of the blade reveals both cortical and medullary layers (Fig. 3).

Cultures of the filamentous stage were initiated by washing the blades containing mature unilocular and plurilocular sporangia in sterile seawater, then placing them in fresh sterile seawater to release the spores. The spores were pipetted into another dish containing Erdschreiber solution (Starr, 1964) and maintained in a culture room at 10°C, under Sylvania cool white fluorescent tubes (F48 T12-CW) for 12 hours per day at an intensity of approximately 100 f.c. Some additional blades were collected in the field and maintained in the living condition in seawater at 5°C under the same
light conditions for several months before fixation.

For light microscopy the blades were fixed directly in 3:1 ethanol-acetic acid for 24 hr. They were then washed in 70% ethanol and embedded in paraffin wax through a graded ethanol-tertiary butyl alcohol series. Sections were cut on a Spencer rotary microtome and stained with bismarck brown.

For study by electron microscopy, blades approximately 5 cm long were fixed on the beach in either Dalton's solution (Dalton, 1955) or in 6% glutaraldehyde in 1/15 M phosphate buffer at pH 7.2 for 2-48 hr., washed thoroughly in buffer, and then postfixed in 1% or 2% osmium tetroxide in the same buffer for 2-24 hr. Cultured sporelings (developing plethysmothalli) and stored thalli were fixed in the same manner. The fixed samples were washed in buffer, dehydrated in a graded ethanol series, and then infiltrated with propylene oxide followed by an epoxy resin mixture which was polymerized in an oven at 60°C for 48 hr. The plastic resin mixture consisted of Maraglas 655, 65%; Cardolite NC 513, 20%; dibutyl phthalate, 15%; and benzyl-dimethylamine catalyst, an extra 2 ml per 100 ml resin (Freeman and Spurlock, 1962). Both tangential and cross sections of the blade were cut on an LKB Ulrotome I by use of a glass knife. They were stained in lead citrate (Reynolds, 1963) and subsequently examined with Hitachi HU-11A and HS-7S electron microscopes.
Observations

Chloroplasts

Several small chloroplasts, measuring approximately 6 µ x 2 µ in longitudinal section, are located in each cell of the blade of *Phaeostrophion irregulare* (Figs. 4, 7). The chloroplast fine structure characteristics of this alga are very similar to those previously reported for other brown algae. Bands of three thylakoids or discs traverse the whole length of each chloroplast and a peripheral band usually completely encircles it underneath the chloroplast double membrane. Each band is approximately 58 µ wide and 4.5 µ long. The thylakoids are about 11 µ thick and are not closely appressed. An exchange of discs between adjacent bands often occurs in a regular pattern so that the number of thylakoids per band tends to remain at three (Fig. 7), rather than to vary from two to four as reported for *Chorda filum* (Bouck, 1965), *Fucus serratus*, and *Pelvetia canaliculata* (Evans, 1968). This phenomenon has been observed previously in *Pylaiella littoralis* (Evans, 1966).

A constant three-thylakoid banding with no exchange of discs has been recorded in some culture sporelings of *Leathesia difformis* (Cole, Bourne, and Lin, 1968).

Membrane-free areas containing fibers presently believed to be deoxyribonucleic acid (DNA) (Bouck, 1965; Bisalputra and Bisalputra, 1967) are formed at each end of the chloroplast between the peripheral band and the termination of the longitudinal bands. Osmiophilic droplets are occasionally located between the bands (Figs. 4, 7).

An out-pocketing of stroma appeared on one side of a few
chloroplasts, leaving a large non-lamellar area between the thylakoids and the chloroplast-limiting membrane (Fig. 7). Outpockets have recently been seen on the chloroplasts of other brown algae, *Leathesia difformis* (Cole and Lin, 1968), *Eudesme virescens* (Cole, 1969), and *Scytosiphon lomentaria* (Cole, 1970). Something similar was shown by Dodge (1968) in the dinoflagellate *Aureodinium pigmentosum*, although the non-lamellar area of this organism was subterminal lateral rather than median lateral as in *Phaeostrophion*. This feature in *Phaeostrophion* does not quite resemble the rudimentary pyrenoid of some fucoids (Evans, 1968) in which there is a folding back of the chloroplast envelope to delimit the pyrenoid.

There was an interesting alteration in the internal morphology of a number of chloroplasts in material which had been stored for a long period at 5°C under artificial illumination. In some, the bands tended to pack together in several areas forming larger bands consisting of 12 to 18 tightly associated discs (Fig. 9). In other parts of the same chloroplast the thylakoids separated and formed irregular patterns (Fig. 9). Chloroplasts have also been observed without the wide bands but with the separation of thylakoids in an intermediate region (Fig. 11).

This type of packing and separation of discs has not been reported previously in the brown algae. Intermediate stacks of 40 to 60 long discs have been noted in the chloroplasts of some cells of cultured *Leathesia difformis* (Cole, Bourne, and Lin, 1968), and have been shown to be a stage in chloroplast division (Cole and Lin, 1968). However, these differed from the wide bands in *Phaeostrophion* since they represented...
the formation of extra bands between the regular ones. Also, all the thylakoids of *L. difformis* remained in a loosely bound arrangement whereas those of *P. irregularare* became tightly associated in some areas. Because of these basic differences, it is unlikely that the cytological changes reported in *P. irregularare* are a manifestation of cell division.

**Pyrenoid**

Pyrenoids were observed in cells of *Phaeostrophion* sporelings (Fig. 6), but not in the thallus (Figs. 4, 5). They have a granular, uniformly dense internal matrix lacking thylakoids, typical of those in several other species of brown algae (Gibbs, 1962b; Bouck, 1965; Evans, 1966; Cole and Lin, 1968; Cole, 1969, 1970).

In presenting electron microscopic evidence that pyrenoids occur in some orders of brown algae but not in others, Evans (1966) recorded the occurrence of pyrenoids in thallus cells of one of the Dictyosiphonales, *Dictyosiphon foeniculaceus*, although he did not include an electron micrograph to illustrate it. In the present study no typical pyrenoids were noted in the thallus cells of *Phaeostrophion*. However, a pyrenoid has been noted in the mature thallus cells of a third genus of the same order, *Punctaria* sp. (Cole, unpublished data). Pyrenoids have also been observed in some members of the related order Scytosiphonales (Cole, 1970).

**Nucleus**

Some nuclear ultrastructural characteristics of *Phaeostrophion* are very similar to those reported for *Chorda filum* and *Giffordia* sp.
Bouck, 1965). The uniformly granular nucleus is approximately 3-5 μm in diameter (Fig. 12), and is encompassed by a double membrane which is permeated by pores. It contains one or more nucleoli, about 1.0 μm in diameter, which are more densely granular than the surrounding nucleoplasm. There is no evidence that the outer nuclear membrane extends and envelops the chloroplast in the cells of the mature blade. However, this feature has been reported in the thallus of Chorda filum (Bouck, 1965), motile zoospores of Pylaiella littoralis and Ectocarpus confervoides (Evans, 1966), cultured sporelings of Leathesia difformis (Cole, Bourne and Lin, 1968), Eudesme (Cole, 1969) and some Scytosiphonales (Cole, 1970). Although all stages of development have not been fully investigated as yet, there also does not appear to be any connection between the nuclear envelope and the chloroplasts in the unilocular sporangium of Phaeostrophion.

Golgi complex

The Golgi complex of Phaeostrophion is similar in most respects to that described for Giffordia sp. and Chorda filum (Bouck, 1965), Leathesia difformis (Cole and Lin, 1968), Eudesme virescens (Cole, 1969) and Petalonia debilis (Cole, 1970). The "forming" face is closely associated with the nuclear membrane and the "maturing" face is directed toward the cytoplasm or vacuole (Fig. 12). It consists of many flattened cisternae, some with a slight swelling at either end when viewed in cross section. The Golgi body seems to be produced by the coalescence of smaller vesicles which have formed from blebs on the
outer nuclear membrane. This is also much the same process of formation reported previously (Bouck, 1965). As the Golgi cisternae of Phaeostrophion increase in size towards the maturing face, fibrous contents of light electron density, similar in appearance to those observed in Fucus vesiculosus (McCully, 1968) can be clearly seen within them (Fig. 12). As the Golgi complex was seldom seen near the wall it is not known if the fibrous material observed in it was directly involved in cell wall formation. The Golgi complex has been shown, using autoradiographic and other techniques, to be involved in cell wall production in other species (Barton, 1968; Wooding, 1968; Bailey and Bisalputra, 1969; Brown, 1969).

**Oil Bodies**

Large dense vesicles with very little internal structure, which are probably oil bodies, are found in most vacuolated and nonvacuolated cells of Phaeostrophion irregulare. They range in size from 0.5 µ to 5 µ and the number per cell varies. They can become so numerous that they exclude much of the cytoplasm or vacuolar material (Figs. 5, 8). Most oil bodies are free in the cytoplasm or vacuole and when appressed to a chloroplast or nucleus there seems to be no communication between the contents of these vesicles and the other organelles (Fig. 10). Since they are so extremely dense it is difficult to determine whether they have a limiting membrane. In general, they are very similar to what are referred to as the physodes in other species observed with the electron microscope: Egregia menziesii (Bisalputra, 1966), Fucus vesiculosus (McCully, 1968), Asperococcus fistulosus, Leathesia
difformis, and Nereocystis luetkeana (Cole, unpublished results). Using centrifugation and electron microscopy Neushul and Liddle (1968) and Liddle and Neushul (1969) separated the oil droplets and physodes in Zonaria eggs and noted that the physodes were not as electron dense as the oil droplets. The oil is probably a storage product.

Cell Wall and associated structures

The cell wall of Phaeostrophion thallus is composed of two structurally different layers, an outer primary alginic layer (Siegel, 1962) containing randomly oriented microfibrils with many spaces and inclusions, and an inner secondary cellulose-like layer (Siegel, 1962; Cole 1964) made up of microfibrils of parallel orientation (Fig. 4). This type of cell wall construction has been observed previously in the brown algae, Macrocystis pyrifera (Ziegler, 1963), Fucus vesiculosus (McCully, 1965) and Ectocarpus acutus (Bailey and Bisalputra, 1969). The cell wall of the Phaeostrophion sporeling has not yet been fully studied due to technical difficulties encountered with this phase of the life cycle.

A few structures with a spherical to ovoid shape and ranging in size from 1 µ to 2 µ were attached to the inner part of the cell wall (Fig. 14). They extended into a portion of the lumen that had previously been occupied by the cytoplasm, and were separated from the cell contents by the plasmalemma. Some appeared to be firmly attached to the cell wall material by thin fibers which were very similar to
the cellulosic fibers of the wall. These attached bodies had an external limiting membrane. Some showed little internal structure except for a fine granularity, while others appeared extremely dense. Similar objects have been observed attached to the inner wall of orange epicarp (Thomson, 1967) and *Fucus vesiculosus* (McCully, 1968).

A large accumulation of small circular vesicles was occasionally observed between the plasmalemma and the cell wall in the thallus (Fig. 18). Each was bounded by a single membrane and measured approximately 0.07 to 0.30 μ. The whole aggregation fitted into a depression in the cell wall. This resembled a buildup of vesicles seen next to the inner wall in orange epicarp (Thomson, 1967) and pine resin canal cells (Wooding and Northcote, 1965). Further studies are required before definite statements can be made about the origin and significance of any of these structures seen between the cell wall and the plasmalemma.

Plasmodesmata occur in *Phaeostrophion*, connecting adjacent cortical and medullary cells of the blade through pores in the cell wall (Figs. 15,16). They are not confined to a well-defined primary pit field as in *Egregia menziesii* (Bisalputra, 1966) and *Dictyota flabellata* (Dawes, Scott and Bowler, 1961), but are spread over a more diffuse area. The plasmodesmata of *Leathesia* (Cole and Lin, 1968), and some Scytosiphonales (Cole, 1970) also are spread over a diffuse area. An electron-transparent area separates the plasmodesma from the pore opening (Fig. 15) and in some cases fibrillar projections extend from the unit membrane to the surrounding cell wall.
material. Bisalputra (1966) mentioned that similar spaces in *Egregia* resulted from plasmolytic shrinkage but did not comment on the connecting fibrils which were also evident in his micrographs. It is more likely that the electron-transparent area represents a deposit of callose similar to that which surrounds the sieve pores of *Macrocystis pyrifera* (Ziegler, 1963) and *Laminaria* (Ziegler and Ruck, 1967).

**Other Cytoplasmic Bodies**

Centriolar bodies resembling those described in *Fucus vesiculosus* (Bouck, 1965), *Colpomenia peregrina* (Evans, 1966) and *Petalonia debilis* (Cole, 1970) were observed near the central part of one cell (Fig. 13). Since the cell was in a mature part of the thallus and was not a sporangium it is concluded that these are centrioles and not the basal bodies of flagella. The overall diameter of each is approximately 300 \( \mu \). It is composed of nine longitudinally oriented fibers, each fiber consisting of three subfibers. This is a common centriolar pattern in the brown algae previously studied (Bouck, 1965; Evans 1966).

Mitochondria are not plentiful in the thallus cells of *P. irregulare*. In section, they vary in shape from round to elliptic and measure from 0.25 \( \mu \) to 0.75 \( \mu \) in width and 0.80 to 1.5 \( \mu \) in length (Fig. 17). These mitochondria have characteristic brown algal tubular cristae which are continuous with the inner mitochondrial membrane (Bouck, 1965).

Lamellar structures measuring approximately 1.2 \( \mu \) by 1.7 \( \mu \)
in cross section were observed in the cytoplasm and vacuoles of several cells of the thallus (Fig. 19). Each consisted of many concentric rings or layers of paired lamellae 30 μm apart. A space of 10 μm separated the two lamellae each of which was 6 μm thick. Bodies closely resembling these lamellar structures have been observed in *Fucus vesiculosus* (McCully, 1968) and in *Leathesia difformis*, *Nereocystis luetkeana*, *Petalonia debilis* (Cole and Lin, 1970) and *Punctaria* sp. (Cole, unpublished data). These bodies also bear some morphological similarity to the "lamellasome" described in *Anacystis nidulans* (Echlin, 1964) except that there was no separate vesicles in the middle of these structures in the blue-green alga. Although the lamellasome in *Anacystis nidulans* was shown to be derived from the photosynthetic lamellae, nothing conclusive can be stated about its function or derivation in *Phaeostrophion* at the present time.

**Discussion**

On the basis of pigmentation, storage products, flagellation, and cell wall components, the brown algae are now established as constituting a distinct class, the Phaeophyceae (Scagel, 1966). Certain characteristics including flagellation, clearly distinguish the Fucales from other orders in this class and fine-structure studies seem to be corroborating this distinction. However, some controversy has arisen about the status of several other orders within the group. The Dictyosiphonales, for example, is not well delimited from the Ectocarpales on general morphological grounds (Russell, 1964). Consequently it would be useful to know if there are some differences in the fine-structure of species within these orders which could be
of taxonomic significance. From results of the current electron microscopic investigation on *Phaeostrophion irregularare* (Dictyosiphonales) it is obvious that this species has many features typical of other brown algae studied to date. Certain variations which were observed might be of importance phylogenetically. However it is clear that before any definite statements can be made, the entire life cycle of many more species in the Dictyosiphonales and Ectocarpales must be studied.

The taxonomic significance of pyrenoids in certain orders and families of Phaeophyceae has been hypothesized by Evans (1966, 1968). From his observations he has suggested that pyrenoids are present in orders such as the Ectocarpales, which are regarded as primitive on other grounds, and absent or rudimentary in the more advanced ones, such as the Laminariales and Fucales. However, his studies were almost exclusively restricted to certain life cycle stages of only one or two species in each order. Pyrenoids have since been recorded in the field material of *Leathesia difformis* (Chordariales) (Cole and Lin, 1968), *Eudesme virescens* (Chordariales) (Cole, 1969) and *Colpomenia sinuosa* and *Petalonia debilis* (Scytosiphonales) (Cole, 1970). These are all considered as "lower orders" within the Phaeophyceae. It is obvious from information obtained in the present ultrastructural study of *Phaeostrophion* that caution must be taken in drawing conclusions from limited data. For example, within the Dictyosiphonales the thallus cells of *Dictyosiphon foeniculaceus* (Evans, 1966) and *Punctaria* sp. (Cole, unpublished data) have pyrenoids while those of *Phaeostrophion*
do not. On the other hand, the sporeling cells of *Phaeostrophion*
do have well-defined pyrenoids characteristic of most brown algae.
The possibility exists that the out-pocketing on the side of a few
chloroplasts in *Phaeostrophion* (Fig. 7) could represent a vestigial
pyrenoid in the adult tissues, thus placing this alga in an
intermediate evolutionary position regarding this structure. An
outpocket was also noted in field material of *Leathesia difformis*
(Cole and Lin, 1968), *Eudesme virescens* (Cole, 1969) and *Scytosiphon
lomentaria* (Cole, 1970). However, the rudimentary pyrenoid reported
by Evans (1968) in fucalean eggs is more obviously delimited than the
one in *Phaeostrophion* (Fig. 7). Dodge (1968) has suggested that a
similar out-pocketing in the chloroplast of the dinoflagellate
*Aureodinium* may be either a developing pyrenoid or just an area where
the lamellae are still forming. It is impossible to make any firm
statement regarding the nature of this characteristic in *Phaeostrophion*
at present.

Since the sporelings of *Phaeostrophion* produce the thallus
directly, at least two points should be investigated: the stage during
thallus development when the pyrenoid is no longer produced, and the
reason for its elimination from the differentiated tissue. It is
possible that there is some change in the type of carbohydrate storage
products during maturation which may be correlated with the disappearance
of the pyrenoid. Simple analytical techniques could be used to
demonstrate whether there is a change from insoluble polysaccharide
storage in the germling to a soluble form in the blade but this type
of study is made more difficult by the numerous problems of culturing Phaeostrophion.

It has been stated that the chloroplast endoplasmic reticulum may be a typical fine-structural feature of the Phaeophyta and Chrysophyta and direct connections between it and the nuclear membrane have been noted in several species of brown algae (Bouck, 1965; Evans, 1966; Kirk and Tilney-Bassett, 1967; Cole, Bourne and Lin, 1968; Cole, 1969, 1970). However, no interrelationship between the chloroplast envelope and the nuclear membrane has been observed in Fucus and Giffordia (Bouck, 1965) or the Phaeostrophion thallus material. Current studies in this laboratory indicate that there is an association of nucleus and chloroplast which is limited to particular phases of the life cycle of some Laminariales (Cole, unpublished data). Consequently, if this characteristic is variable within a species during its life cycle one must be very certain to observe all stages before using it taxonomically.

Another ultrastructural characteristic which may be significant in phylogenetic studies of the brown algae has been proposed. From observations on one species within each of the orders Ectocarpales, Laminariales, and Fucales, Bouck (1965) suggested that the association of the Golgi apparatus with a portion of the endoplasmic reticulum as well as the nuclear membrane in the phylogenetically more advanced fucalean species could be of evolutionary significance, since it is consistently perinuclear in the more primitive ones. The Golgi bodies have been observed to be strictly perinuclear in members of
two other "lower orders", the Chordariales, *Leathesia* (Cole and Lin, 1968) and *Eudesme* (Cole, 1969) and the Scytosiphonales, *Colpomenia*, *Petalonia* and *Scytosiphon* (Cole, 1970). This would also seem to be the case for *Phaeostrophion*, which has been placed lower than the Fucales in the evolutionary scale and in which the Golgi complex is associated with only the nuclear envelope.

Centrioles have been noted in light-microscopic studies of brown algae, but only infrequently or uncertainly, e.g. *Nereocystis luetkeana* (Kemp and Cole, 1961), *Fucus sp.* (Evans, 1962), *Halidrys silquosa* (Roberts, 1966) and *Eudesme virescens* (Cole, 1967). As a result of recent ultrastructural studies on *Himanthalia lorea* (Berkaloff, 1963), *Fucus vesiculosus* (Bouck, 1965), *Colpomenia peregrina* (Evans, 1966), and *Petalonia debilis* (Cole, 1970), as well as the current one on *Phaeostrophion*, it is becoming more evident that centrioles are of general occurrence in cells of the Phaeophyceae.

Deviations from the usual three-thylakoid banding in the Phaeophyceae, which were observed in cells of stored *Phaeostrophion* blades, most likely resulted from the artificial culture conditions rather than from imperfect fixation since typical chloroplasts were also observed in the same tissue (Fig. 9). The deviation of *Phaeostrophion* might indicate early stages in degeneration. This could offer potential material for the study of senescence. Cole, Bourne and Lin (1968) have suggested that the illumination could possibly have been responsible for the production of extensive thylakoid stacking in cultured sporeling cells of *Leathesia difformis*, another brown alga. This thylakoid stacking in *Leathesia* has since been shown to be part
of chloroplast division (Cole and Lin, 1968). While the abnormal stacking phenomenon in *Leathesia* differed from that observed in *Phaeostrophion*, it is interesting to note that both species were maintained with the same medium and light and very similar temperature conditions. The fact that the *Leathesia* chloroplasts were in actively growing sporeling cells while those of *Phaeostrophion* were in mature cells of thallus tissue may explain the difference in type of thylakoid stacking between the two species.

In conclusion, *Phaeostrophion irregulare* retains its intermediate position in the phylogenetic classification of the brown algae. It possesses some characteristics of the more advanced brown algae, such as the absence of a pyrenoid in the adult thallus and the absence of a physical association of the chloroplast and nucleus. Also it possesses more primitive characteristics, a strictly perinuclear Golgi apparatus and a diffuse distribution of plasmodesmata.

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Figure 2  Photograph of mature plants of *P. irregulare* showing the narrow, short stipes; the small disc-like holdfast (H), and the irregularly torn blades.  X 1/3

Figure 3  Cross section of a young maturing blade as seen by light microscopy, showing the developing medullary cells (MC), and unilocular sporangia (US).  X 1,000

Figure 4  Electron micrograph of a surface cell which may be a unilocular initial. Note the numerous small chloroplasts (Ch), oil bodies (Ph), cell wall (CW), and the small vacuole (V).  X 4,000

Figure 5  View of a portion of a cortical cell of the blade showing the numerous oil bodies, vacuole, and cell wall consisting of an outer primary layer (1° CW) and inclusions (arrows) and an inner secondary layer (2° CW).  X 7,000

Figure 6  The pyrenoid (Py) and part of a chloroplast in a sporeling cell.  X 34,000
Figure 7  A chloroplast in a cortical cell with a lateral out-pocketing (OP) or non-lamellar area. Note the location of the correlated thylakoid interchange (arrows) and the osmiophilic droplet (O). X 22,000

Figure 8  Part of a medullary vacuolated cell containing numerous oil bodies (Ph). X 5,000

Figure 9  Chloroplast from plant kept under artificial illumination for extended period. Note the clustering of lamellar bands into larger groups and separation of bands into single thylakoids (arrows). X 10,000

Figure 10  Oil body adjacent to a chloroplast. The chloroplast envelope (CE) separates the two (arrows). X 36,000
Figure 11  Chloroplast of vacuolated cell seen in Figure 8. Note especially the lamellar separations (arrows). X 32,000

Figure 12  Nucleus (N) with nucleolus (NL) and nuclear envelope (NE) with pores (NP). Blebs (arrows) on the outer nuclear membrane are forming small circular vesicles. These coalesce to form the Golgi apparatus (G), which consists of the forming face (ff) and maturing face (mf). X 31,000

Figure 13  Centriolar bodies. Note the three subunits per subfiber (arrows). X 25,000

Figure 14  Spheroid body with a limiting membrane (LM) between the plasmalemma and cell wall. Note the connecting fibrils (arrows) between the body and the cell wall material X 40,000
Figure 15  Portion of a pore area in a cortical cell wall.  
Note each plasmodesma (Pl), unit membrane, and the 
electron-transparent area, which may be callose (double 
arrow) between it and the cell wall material. Fibrils 
(arrows) extend from the plasmodesma to the wall 
material. X 67,000

Figure 16  Longitudinal section of plasmodesmata showing 
continuity through the wall. X 70,000

Figure 17  Two typical mitochondria (M). The inner membrane is 
continuous with the tubular cristae (arrow). X 38,000

Figure 18  Portion of a vacuolated cell showing an accumulation 
of small vesicles (arrows) between the plasmalemma and 
the cell wall. X 15,000

Figure 19  Lamellar body located in a vacuole showing the paired 
lamellae (arrows) and inner vesicles (IV). X 40,000
PART TWO

Rhodophyceae

A comparison of cellular fine structure of the vegetative thalli of *Porphyra perforata* and *Bangia fuscopurpurea* and certain phases in the life history of *Porphyra perforata*

Introduction

It has been generally accepted that the Bangiophycidae (Bangioidae) are simple rhodophycean forms which exhibit diffuse growth, relatively unspecialized sex organs, carposporangia formed by direct division of the zygote and have no pit connections or aggregation of filaments. On the other hand most of the Florideophycidae (Florideae) are more complex Rhodophyceae and usually consist of aggregated filaments with pit connections, highly differentiated sex organs, and carposporangia formed on filamentous gonimoblasts derived from the zygote (Fritsch, 1945). These criteria were used to separate the two subclasses macroscopically and by light microscopy (Fritsch, 1945). However, with the application of electron microscopic techniques we may be approaching the time when the separation of the two subclasses, Bangiophycidae and Florideophycidae, will have to be re-evaluated.

There is an increasing interest in the ultrastructure of the red algae. For example, within the Bangiophycidae there have been reports on *Bangia* (Honsell, 1963), *Porphyridium* (Brody and Vatter, 1959; Speer, Dougherty, and Jones, 1964; Gantt and Conti, 1965, 1966a and b; Gantt, Edwards, and Conti, 1968); *Compsopogon* (Nichols, Ridgway, and
Bold, 1966), Smithora (McBride and Cole, 1969, 1971), Rhodella (Evans, 1970), and Porphyra (Yokomura, 1967; Kito and Akiyama, 1968; Lee and Fultz, 1970; Bourne, Conway, and Cole, 1970; and Kazama and Fuller, 1970). However many authors considered only one or two organelles in most species, or in the case of Porphyra only one stage in the life cycle.

The basic life cycle of the Bangiales consists of a macroscopic form alternating with a filamentous microscopic form, both being capable of asexual reduplication by neutral or monospores. Numerous studies have been made on the life history of various Porphyra species. Some workers have completed the life cycle in culture (Kurogi, 1953; Tseng and Chang, 1955; Graves, 1955; Hollenberg, 1958; Kornmann, 1961; Conway, 1964b; and Chen, Edelstein, Ogata, and McLachlan, 1970). A specific example of the basic life cycle of P. perforata is given by Hollenberg (1958) where the mature thallus produces spores which develop into the filamentous conchocelis phase. This latter phase in turn produces spores which develop into the mature thallus. Although Hollenberg (1958) did not observe the production of neutral spores in summer material, he did not exclude the possibility that they existed at another time. Several researchers have shown that a few species produce aplanospores that develop into bipolar sporelings (Conway, 1964b, 1966), and neutral spores that reduplicate the bipolar sporelings (Conway, 1964b, 1966), whereas others have demonstrated the role of monospores in the conchocelis phase (Conway, 1964b; Krishnamurthy, 1969; Chen, Edelstein, Ogata, and McLachlan, 1970).
The purpose of this study was to extend the ultrastructural knowledge of the Bangiophycidae by examining and comparing the thallus cells of two local species, *Porphyra perforata* and *Bangia fuscopurpurea*, and comparing the cells of several phases of the life history of *P. perforata*. This information would be used in comparison with published data on other red algae, to determine any phylogenetical significance of the ultrastructure.

**Materials and Methods**

*Porphyra* and *Bangia* are included in the family Bangiaceae, order Bangiales, subclass Bangiophycidae. *Porphyra perforata* J.G. Agardh grows on rocks in the intertidal region of the Pacific coast of North America. The mature blade is monostromatic, has one chloroplast per cell and is highly variable in size and shape ranging from 2 x 10 cm to 15 x 30 cm (Figs 22, 23). The blade is held to the substratum by a holdfast consisting of rhizoidal processes extending from the basal cells. The color varies from a deep purplered to dark brown. *Bangia fuscopurpurea* (Dillwyn) Lyngbye grows in much the same area and has similar coloring to *P. perforata*. However the external morphology is somewhat different. The mature thallus of *B. fuscopurpurea* is a multiseriate filament, with the basal cells extending rhizoidal processes downward to form a holdfast.

Mature blades of *P. perforata* were collected from Amphitrite Point, Ucluelet; Whiffin Spit and Point No Point (Glacier Point), Vancouver Island; Brockton Point, Stanley Park and Point Grey,
Vancouver. General collections were made for fixation during afternoon low tides in the springs of 1968, 1969, and 1970. Filaments of B. fuscopurpurea were collected from a piling at Rosario Beach, Washington, U.S.A. in July 1968. The collection sites of P. perforata and B. fuscopurpurea are indicated in Figure 20. Collections for culturing P. perforata were made during November 1969 and January to April 1970. They were kept cool and brought to the University of British Columbia laboratories where they were placed in petri dishes containing a sea water culture medium. The culture medium consisted of a 1:1 mixture of enriched sea water (sterile sea water to make 1 litre plus: NaNO₃ - 200 mg., Na₂HPO₄·7H₂O - 20 mg., KNO₃ - 50 mg., EDTA - 1 mg., Tris buffer - 500 mg., soil water - 50 ml., Vitamin B₁₂ - 1 mg.,) and sterile sea water. After spore release, the thalli were removed and the media changed frequently during the subsequent growth period to reduce contamination. Cultures were maintained in a Sherer culture chamber under GE F.20.T.12 cool white fluorescent tubes at a level of illumination of about 1000 lux or 93 f.c. The light regime was 8 hours of light and 16 hours of darkness at a constant temperature of 10°C. The growth of the germlings was observed for a period of several months. The culturing for this study was done in collaboration with Dr. Elsie Conway.

All materials were fixed for 2-4 hours in 4% glutaraldehyde in 1/15 M phosphate buffer, pH 7.4 at 5°C and brought to room temperature. They were then washed in buffer, post-fixed in 1% OsO₄/phosphate for one hour, dehydrated in an alcohol-propylene oxide series, and
Figure 20  Map of the collection sites of algae used in this study

1  Ucluelet, Vancouver Island
2  Point No Point (Glacier Point), Vancouver Island
3  Whiffin Spit, Vancouver Island
4  Point Grey, Vancouver
5  Brockton Point, Stanley Park, Vancouver
6  Rosario Beach, Washington
subsequently embedded in an epoxy resin. The Maraglas epoxy (Spurlock, Kattine and Freeman, 1963) was polymerized at 60°C for 48 hours. Sections were cut with glass knives on an LKB Ultrotome I and a Reichert OM-U2, stained with lead citrate (Reynolds, 1963) and uranyl acetate (Watson, 1958) and subsequently examined with Hitachi HS-7S, HU-11A, Zeiss EM-9A, RCA EMU-3H and Philips EM300 electron microscopes. Specimens for surface study were fixed in 1% aqueous OsO₄, dehydrated and coated with Au/Pd 60:40 under vacuum. They were then observed in a Cambridge Stereoscan scanning electron microscope at 20 kv.

Some confusion might arise regarding the terms used to designate the spores produced by Porphyra during different stages of its life history. Consequently the terminology used in this study is defined:

α spores - are slightly smaller than the vegetative cells and are deeply pigmented. They are usually produced in packets of 8 - 32 by repeated division of a vegetative cell in the mature macroscopic thallus. They produce the conchocelis phase (Conway, 1964a), and are equivalent to the type III spores of Drew (1956).

β spores - are very much smaller than the vegetative cells and are almost colorless. They are produced on the macroscopic thallus much like the α spores, but with more divisions (Conway, 1964a). The β spores are produced 32-128 per packet and are also equivalent to the type III spores of Drew (1956). They are commonly referred to as spermatia.
aplanospores - are approximately the same size as the vegetative cells and are produced on the leafy thallus by differentiation of a whole cell. These are equivalent to the type II spores of Drew (1956) and develop into bipolar sporelings.

neutral spores - are produced by differentiation of whole cells in the bipolar sporelings and develop into another generation of bipolar sporelings.

monospores - are produced terminally on the conchocelis filaments by differentiation of whole cells and grow into another generation of conchocelis filaments.

conchospores - are produced on specialized branches of the conchocelis phase and develop into bipolar sporelings.

Observations

The life history of Porphyra perforata observed in this laboratory (Fig. 21) was basically similar to that described by Hollenberg (1958) for the same species although the development of the conchospore into the thallus was not obtained. The production and development of neutral and monospores, which Hollenberg (1958) did not see in his material, were observed and found to be similar to that of other Porphyra species (Drew, 1949, 1954; Kurogi, 1953; Tseng and Chang, 1955; Graves, 1955; Kornmann, 1961; Conway, 1964a and b; Krishnamurthy, 1959, 1969; Chen, Edelstein, Ogata, and McLachlan, 1970) and Bangia (Sommerfeld and Nichols, 1970). Thalli of P. perforata collected during October, 1969,
Figure 21  Diagram of the tentative life cycle of

Porphyra perforata

---  portion of the life cycle observed in this study

- - - - - -  portion of the life cycle not observed in this study but inferred from previous publications (see p.33, text)

fertilization  ?  fertilization and the role of spores in this process have not yet been conclusively demonstrated
Figure 21  Diagram of the tentative life cycle of Porphyra perforata
released aplanospores from small patches scattered over most of the blade. These spores underwent bipolar germination (Figs. 57, 58). The young bipolar sporelings quickly developed rhizoidal cells (Figs. 58, 59) and grew into small blades about 2 cm across. Many cells in the sporelings formed neutral spores, reduplicating the bipolar sporeling. Thalli collected during January 1970 released both large $\alpha$ spores and smaller $\beta$ spores or spermatia (Fig. 24), from small patches scattered throughout the blade. The $\beta$ spores (Fig. 80) when released and germinated alone, degenerated within two days. These results are similar to those obtained with P. umbilicalis (Krishnamurthy 1959, Conway 1964b). The released $\alpha$ spores (Fig. 80) germinated into the conchocelis phase (Fig. 81). After about 2-3 weeks, branched filaments appeared in culture. Many of the branches terminated in monospores. Upon release the monospores produced a new generation of conchocelis filaments. This process repeated itself for several months yielding a very profuse growth of filaments, as in P. umbilicalis (Conway, 1964b). After approximately five weeks the conchocelis filaments developed some side branches composed of almost isodiametric cells (Figs. 95, 102), which were probably the conchospore branches. These cells had a stellate chloroplast with a central pyrenoid similar to that in the vegetative cells of the macroscopic thallus.

Vegetative cells of the thallus and bipolar sporelings of Porphyra perforata, and the thallus cells of Bangia fuscopurpurea.

The vegetative cells of P. perforata thallus and bipolar
sporelings and of \textit{B. fuscopurpurea} thalli are basically similar with
a large stellate chloroplast and central pyrenoid, a small nucleus,
mitochondria, Golgi bodies, starch-like storage products and a
loose gelatinous cell wall. The general ultrastructure of these
cells in most respects resembles that already reported for both
subclasses of the Rhodophyceae, \textit{Porphyridium} (Brody and Vatter, 1959;
Speer, Dougherty, and Jones, 1964; and Gantt and Conti, 1965), \textit{Lomentaria}
(Bouck, 1962), \textit{Polysiphonia}, \textit{Nemalion} and \textit{Kylinia} (Gibbs, 1962a and b),
Bangia (Honsell, 1963), \textit{Laurencia} (Bisalputra, Rusanowski, and Walker,
1967), \textit{Batrachospermum} (Brown and Weier, 1968, 1970), \textit{Porphyra}
\textit{vesoensis} (Kito and Akiyama, 1968), \textit{Pseudogloiophloea} (Ramus, 1969a and b) and

The most conspicuous organelle is the highly lobate central
chloroplast (Figs. 25, 26, 61, 67, 76). It is enclosed by a continuous
double membrane (Figs. 26, 62, 67, 76). The thylakoids, 20 \(\mu\) thick
are arranged parallel to one another, separated by about 100 \(\mu\)
(Figs. 28, 30, 63, 67). The overall shape of the chloroplasts of
\textit{Porphyra} and \textit{Bangia} resembles that of \textit{Porphyridium} (Brody and Vatter,
1959; Gantt and Conti, 1965; and Gantt, Edwards, and Conti, 1968) and
\textit{Smithora} (McBride and Cole, 1969). Finger-like projections of the
chloroplast extend through the cytoplasm. There is no peripheral
thylakoid, and in some lobes a group of thylakoids terminates before
reaching the chloroplast limiting membrane (Figs. 30, 34, 64, 76). A
similar phenomenon has been seen in \textit{Porphyridium} (Gantt and Conti, 1965),
and \textit{Smithora} (McBride and Cole, 1969). However, many Florideophycidae
such as \textit{Batrachospermum} (Brown and Weier, 1968, 1970) do possess a
peripheral thylakoid paralleling the limiting envelope. No loose thylakoid stacking such as that reported for Smithora (McBride and Cole, 1969) was observed in Porphyra. Some interconnections between thylakoids (Figs. 28,76) were noted as in Porphyridium (Gantt and Conti, 1965) and Smithora (McBride and Cole, 1969). Phycobilisomes were not obvious on the thylakoids of Porphyra. This resembles Smithora (McBride and Cole, 1969), and contrasts sharply with the chloroplast lamellae of Porphyridium where the phycobilisomes are very distinct (Gantt and Conti, 1965,1966a; and Gantt, Edwards, and Conti, 1968). Small electron transparent areas containing clumped fibrils 1 to 10 μm thick are located between the photosynthetic lamellae throughout the chloroplast (Figs. 28,63). These are similar to the DNA localizations in Laurencia (Bisalputra and Bisalputra,1967) and Smithora (McBride and Cole, 1969). Numerous osmiophilic droplets occur throughout the chloroplast.

The pyrenoid of Porphyra and Bangia is quite similar to that reported for other Bangiophycidae, Porphyridium (Brody and Vatter,1959; Gantt and Conti,1965) and Smithora (McBride and Cole, 1969). It is also quite similar to that reported for some Florideophycidae, Nemalion and Kylinia (Gibbs 1962a and b). It is located in the central portion of the chloroplast and is traversed by several thylakoids which are continuous with those in the chloroplast (Figs. 25,26,34,63, 66,77). Small thylakoid cisternae in the pyrenoid are slightly distended. Many of the pyrenoid lamellae are parallel (Fig. 25), a feature which corroborates Krishnamurthy's (1959) light microscope observations on the internal pyrenoid structure.
In the *Porphyra* bipolar sporelings a pyrenoid was noted within several of the chloroplast arms in some vegetative cells. This suggests that the pyrenoid was very lobate, extending through the chloroplast or that there was more than one pyrenoid present in the chloroplast. Since this is unlike the central pyrenoid in the thallus cells, it may have been a cultural aberration. On the other hand, there may have been more than one chloroplast present in these cells. This was difficult to verify by light microscopy. The large numbers of osmiophilic droplets which occur in the chloroplast of the bipolar sporeling cells (Figs. 62, 63) may also be due to culture conditions.

Floridean starch granules occur singly or in groups outside the chloroplast membrane (Figs. 30, 32, 34, 63, 64). These bodies are sausage-shaped and approximately 0.1 x 0.1 x 0.4 μ in size. They were never observed inside the chloroplast and it is assumed that they are formed outside the chloroplast limiting membrane. This feature has also been recorded in other red algae, *Porphyridium* (Brody and Vatter, 1959; Speer, Dougherty, and Jones, 1964; and Gantt and Conti, 1965), *Lomentaria* (Bouck, 1962), *Kylinia*, *Polysiphonia* and *Nemalion* (Gibbs, 1962a and b), *Pseudogloiocephloeae* (Ramus, 1969a) and *Smithora* (McBride and Cole, 1969).

Elements of the endoplasmic reticulum (ER) are located throughout the cytoplasm and are especially prevalent in some areas (Figs. 34, 46, 47, 50, 56, 60, 69, 71, 76). The ER often occurs in the form of a single cisterna near and parallel to the plasmalemma (Figs. 46, 47), as in *Porphyridium* (Gantt and Conti, 1965). It may also form several cisternae which extend throughout the cytoplasm (Fig. 56) very much
like *Lomentaria* (Bouck, 1962). Since the E R is continuous for some distance through a section it is considered to be lamellar rather than tubular. The membranes are about 80 Å thick and the cisternae are 200 Å wide. Some cisternae become slightly distended (Fig. 56). The E R is much more extensive in *Porphyra* than in either *Porphyridium* (Gantt and Conti, 1965; Gantt, Edwards, and Conti, 1968) or *Smithora* (McBride and Cole, 1969). The ribosomes do not appear to be attached to the E R membranes. A more densely staining membrane believed to be E R extends throughout some cells (Fig. 50). This has also been noted in *Smithora* (McBride and Cole, 1969).

The mitochondria (Figs. 28-31, 69, 76) resemble those reported for *Smithora* (McBride and Cole, 1969) and *Pseudogloiophloea* (Ramus, 1969a). They are approximately 0.5 to 1 μ in section are round to oval shaped, and the cristae are continuous with the inner membrane. No long branched mitochondria like those of *Porphyridium* (Gantt and Conti, 1965) nor any ring shaped mitochondria as recorded occasionally in *Smithora* (McBride and Cole, 1969) were seen in *Porphyra* or *Bangia*. Very few lamellar cristae were noted, in contrast to *Lomentaria* (Bouck, 1962). The mitochondria were often seen near the nucleus (Figs. 29, 31).

The Golgi apparatus is generally small (Figs. 28-31, 64, 69), consisting of several flattened cisternae. Towards the maturing face the extremities of each cisterna are swollen and contain fibrous material. Several free vesicles containing the same material were noted beyond the maturing face. The forming face of the Golgi apparatus is very often close to a mitochondrion (Figs. 28-31, 64, 69) as in
Corallina (Bailey and Bisalputra, 1970) and Batrachospermum (Brown and Weier, 1970). The Golgi bodies and associated vesicles are morphologically similar to those reported for Lomentaria (Bouck, 1962), Porphyridium (Gantt and Conti, 1965; Gantt, Edwards, and Conti, 1968) and Smithora (McBride and Cole, 1969).

The plasmalemma is a typical single membrane and portions of it in each cell are highly convoluted (Figs. 27, 37, 40, 55, 60, 67). Many wall fibers extend in between the various infoldings and a few are continuous with the plasma membrane (Figs. 46, 49, 50, 55). Convolutions in the plasmalemma have also been observed in Lomentaria (Bouck, 1962), and Porphyra yezoensis (Kito and Akiyama, 1968) and surface activities have been demonstrated in the red alga Laurencia (Bisalputra, Rusanowski, and Walker, 1967).

Nuclear shape and position appear to be influenced by the large chloroplast. The chloroplast tends to compress the nucleus into a peripheral location. The nucleus is at times highly lobate (Figs. 34, 68, 78), a condition previously observed by light microscopy, and discussed by Dixon (1966, p. 177). This suggests the possibility of a very active nucleus. No close association was noted between the nucleus and the pyrenoid and chloroplast as in Rhodella (Evans, 1970). The nucleoplasm is granular and surrounded by a very porous nuclear envelope (Figs. 34, 68). The single large nucleolus with areas of lower electron density appeared to have no constant position within the nucleus (Figs. 25, 33, 34, 36, 38, 68, 78). It was not necessarily oriented towards the chloroplast as in Porphyridium (Gantt and Conti,
1965). The nucleolar shape and position resembled more closely that of *Smithora* (McBride and Cole, 1969). Nuclear structure is very similar to that of other members of the Bangiophycidae, *Porphyridium* (Gantt and Conti 1965, Gantt, Edwards, and Conti 1968) and *Smithora* (McBride and Cole, 1969).

Nuclear division precedes cytokinesis and the daughter nuclei separate and are usually positioned at opposite ends of the cell well in advance of protoplasmic division in *Porphyra perforata* (Figs. 35, 36). This characteristic has also been noted in light microscope observations on other *Porphyra* species (Krishnamurthy, 1959; Pringle and Austin, 1970), as well as *Porphyridium* (Gantt and Conti, 1965). Centriolar bodies were not observed in *P. perforata* thallus and bipolar sporeling or *B. fuscopurpurea* thallus. Cytokinesis in the thallus of *Porphyra perforata* collected in the spring is generally oriented longitudinally, i.e. parallel to the long dimension of the cell, an observation also recorded by Krishnamurthy (1959) and Conway (1964b) in vegetative and sporing areas of *P. umbilicalis*. Drew (1954), however, suggested that in some *Porphyra* species the initial plane of division in vegetative cell and monospore production is longitudinal and that for carpospore (α spore) production is transverse.

Cytokinesis in the blade of *P. perforata* appears to be of two types. The first is very much like that seen in *Porphyridium* (Gantt and Conti, 1965). It commences with the initiation of a cross wall at the cell periphery. This wall is about the same thickness as the surrounding walls (Fig. 35). The wall growth continues towards the center of the cell, the thickness remaining the same (Fig. 36). The
chloroplast and pyrenoid are the last major organelles to divide, actually being caught in the opening left by the forming cross wall.

The second, more common type of division observed in this material, commences with the division of the chloroplast and pyrenoid followed by the invagination of the plasmalemma deep into the cell (Fig. 37). This invagination process continues until the plasmalemma crosses the cell completely. Unlike the previous method of cell division no wall material is laid down during this activity. The two cells are separated by only the two plasma membranes. There is then an increased Golgi and/or plasmalemma activity on both sides with the formation of small fiber-containing vesicles. This is followed by a deposit of wall fibers at various locations between the two plasma membranes until a wall is completed between the two cells (Fig. 38). It is not yet possible to tell if the wall formed at this point remains thin or thickens later. Similar cell division was also seen in Bangia (Figs. 66, 74).

It is difficult to provide an explanation for the two types of cytokinesis. The second type seems to be more general in the Porphyra species which have previously been studied cytologically. Krishnamurthy (1959) noted that a thicker wall formed between daughter cells in the vegetative area than between those in the spore producing regions. Relatively thin cell walls characterize α spore development in the current study (Fig. 43).

The production of the α spores in Porphyra is by repeated division of the spore mother cell, and cell size decreases with each division. The α spores contain cell organelles similar to those in
the vegetative cells of the thallus (Figs. 43, 44). The cell walls between the developing spores are usually much thinner than the surrounding mother cell wall. Near the cell surface, there are a large number of small vesicles which are packed with a fibrous material similar to the wall (Fig. 44). The function of these vesicles is not known, but the fibrous contents suggest that they may contribute towards the production of the spore wall.

Some stages of aplanospore development and release have been observed in Bangia (Figs. 76-79). These spores are about the same size as the vegetative cell. Their ultrastructural details are much like those of the vegetative cell. The spore wall is much thinner than the surrounding mother cell wall (Figs. 78, 79) and becomes separated from it as development nears completion (Fig. 79). Previous reports (reviewed by Drew, 1956; Sommerfeld and Nichols, 1970) indicate that the Bangia spore is released naked. The presence of a cell wall observed in the present study is possibly due to the fact that the spore is starting to germinate in situ. This has also been reported previously (reviewed by Drew, 1956; Cole, 1971).

Paramural bodies and associated structures such as concentric lamellar bodies and polyvesicular bodies are seen in the vegetative cells. The most conspicuous of these are the concentric lamellar bodies which are 0.5 to 1.5 \( \mu \) in diameter and have concentrically arranged membranes (Figs. 45-51, 69-73). Some consist of many single or paired thick lamellar layers (Figs. 49, 73) whereas others have much thinner, tightly packed membranes (Figs. 48, 71). They may also contain small tubules (Fig. 45). The position of these bodies
is somewhat diverse; some are at the edge of the cell (Figs. 46,49), others through the cytoplasm (Figs. 45,48,71,73), and a few are located in the chloroplast (Figs. 48,70). Lamellar bodies similar to those in the cytoplasm occasionally occur in the wall (Figs. 47,72) near the cell surface and can be adjacent to cytoplasmic lamellar bodies in the cytoplasm. Wall fibers attached to the plasmalemma near a lamellar body are shown in Figure 46. Similar configurations were seen in *Porphyridium* (Gantt and Conti, 1965,1966b) and *Batrachospermum* (Brown and Weier,1970).

Polyvesicular or lomasome-like structures about 0.7 μ in diameter are observed in the cytoplasm. These consist of one or two enveloping membranes, containing several smaller vesicles, the outermost of which may contact the cell wall (Fig. 49, 51-54). They resemble the lomasomes of Moore and McAlear (1961). They are often in close association with concentric lamellar bodies (Fig. 49), therefore their function may be related to that of the latter structures. Bouck (1962) noted a similar polyvesicular body in *Lomentaria* between the cell membrane and the cell wall, but the ontogeny of this was not known. Also, Ramus (1969a) demonstrated structures that resembled these in the red alga *Pseudogloiothecia*, both in the cytoplasm and between the plasmalemma and wall. He concluded that these lomasomes were involved in cell wall production. Lee and Fultz (1970) showed similar structures in the conchocelis phase of *Porphyra leucosticta*.

The *Porphyra* cell wall is composed of xylan microfibrils and a mannan-xylan embedding matrix (Frei and Preston, 1964). It is a
loose network of fibrils embedded in an amorphous matrix and resembles the sheath of Porphyridium (Gantt and Conti, 1965) and walls of Laurencia (Bisalputra, Rusanowski, and Walker, 1967) and Smithora (McBride and Cole, 1969). It consists of several layers. Although the fibers of both the inner and outer layers are reticulate, the more conspicuous fibers in the outer layers (Figs. 25, 33) are parallel to the cell surface whereas those in the inner layers tend to be perpendicular to the cell surface (Figs. 34, 36). Sections through cells in the holdfast region (Figs. 39-41) of Porphyra show the very thin rhizoidal processes of the cells surrounded by extremely thick walls (Fig. 39). The plasmalemma of these processes is highly convoluted (Figs. 40, 41). Scanning electron microscopy of the Porphyra field material reveals a highly folded surface of the whole thallus (Fig. 42). The material has been fixed and dried so this may not reflect the true picture in nature. Cross sections of Porphyra fixed and embedded in plastic also show an undulating surface, although it is less pronounced.

Conchocelis phase cells of Porphyra perforata

On germination, the spore (Fig. 80) forms a tube into which the entire cytoplasmic mass eventually migrates. A cross wall is then formed sealing off the empty spore case (Fig. 85) (Drew, 1949, 1954; Krishnamurthy, 1959, 1969). Fine structural studies confirm this. The spore cases (Figs. 86, 87) were devoid of any cytoplasm, and a plugged pit connection had formed between the spore case and the conchocelis cell (Fig. 86).
The *P. perforata* conchocelis phase cells are long and narrow (Fig. 85) about 20 μ by 7 μ. As in the cells of the macroscopic thallus, the chloroplast of the conchocelis phase is the most prominent organelle (Figs. 83,84,94). It is not stellate and its shape seems to be determined by the long tubular cell shape. Projections of the chloroplast extend throughout the individual cell, filling it in certain areas (Figs. 83,84) as in *P. leucosticta* (Lee and Fultz, 1970). The thylakoids are single, about 15 μ thick and separated by 100 μ. Interconnections of individual lamellae occur (Fig. 83), as in the chloroplasts of the macroscopic thallus as well as in *Smithora* (McBride and Cole, 1969). No association of thylakoids, such as those seen in *Smithora* (McBride and Cole, 1969) were noted. Although not always the case (Fig. 82), the chloroplasts often have a peripheral thylakoid (Figs. 84,92,94). This characteristic was also noted by Lee and Fultz (1970) in *P. leucosticta* conchocelis. However it should be mentioned that a peripheral thylakoid is not shown in all of their figures. Osmiophilic droplets occur between the photosynthetic lamellae and often at the periphery of the pyrenoid (Figs. 84,93). In the young pink (2-3 week) conchocelis filaments there were numerous round phycobilisomes attached to the chloroplast lamellae (Fig. 83), whereas in the old brown (6-8 week) conchocelis and the brown colored thallus none were observed (Figs. 92-94). Round phycobilisomes were also noted in *Porphyridium* (Gantt and Conti, 1965,1966a; and Gantt, Edwards, and Conti,1968). It is quite unlike *Batrachospermum* (Lichtlé and Giraud,1970) which has rod shaped phycobilisomes, and unlike the macroscopic thallus of *Porphyra* with
its apparent absence of phycobilisomes.

Phycobilisomes were observed only in the young pink conchocelis of *Porphyra perforata*. It is probable that the phycobilisomes contained more phycoerythrin and were therefore more round and easily observed than the other coin-shaped form, seen in *Porphyridium* (Gantt and Conti, 1966b; Gantt, Edwards, and Conti, 1968). As the conchocelis grew older it was subjected to a light intensity that may have been fairly high for it (100 f.c.). This possibly helped to shift the phycoerythrin/phycocyanin ratio and changed the shape of the phycobilisomes so that they resembled those in the adult thallus.

The thallus grows near the high water mark, where the light intensity is much greater than in deep water. Since the phycobilisomes are sensitive to fixation at the best of times (Gantt and Conti, 1966a) the less easily seen forms probably became almost unnoticeable. It would be interesting to know the relative amounts of the accessory pigments in *Batrachospermum virgatum* which has rod shaped phycobilisomes (Lichtlé and Giraud, 1970).

Small areas of low electron density containing a fibrillar material resembling the DNA-like patches in the field material are clumped between the photosynthetic lamellae (Figs. 83, 84). The pyrenoid (Figs. 84, 92, 93, 94) in the chloroplast is traversed by several thylakoids, continuous with those in the chloroplast, and is very similar to the pyrenoid in the macroscopic plant. The vegetative cells of the conchocelis sometimes have more than one pyrenoid (Figs. 92, 94), a phenomenon which may have been induced by culture conditions.
The nucleus, about 1-2 μ diameter (Figs. 82, 94), is usually appressed to one side of the cell, and can be partially surrounded by the chloroplast or other organelles. It is similar to the nucleus of the field material, possessing a double nuclear membrane, which is permeated by numerous prominent pores. Condensed chromatin was seen in most sections of nuclei (Fig. 82), which could correspond to heterochromatic bodies seen by light microscopy in the nuclei of germinating conchocelis in Porphyra umbilicalis (Krishnamurthy, 1959). No centriolar bodies were observed in the conchocelis phase of Porphyra perforata.

The vacuole (Fig. 86) is quite large, in some sections occupying most of the cell. It is enclosed by a single membrane. The starch bodies (Figs. 84, 86, 92, 94) are very similar in size and shape to those in the vegetative cells of the macroscopic phase and are also found only outside the chloroplast membrane. In contrast to the cells of the macroscopic phase which have a considerable amount, the endoplasmic reticulum is not very extensive in most of the cells of the conchocelis phase (Fig. 82). The mitochondria (Figs. 83, 89, 91) have tubular cristae continuous with the inner membrane and are very similar to those of the macroscopic phase.

The wall of the conchocelis vegetative cell (Figs. 82, 83, 86) is much thinner than the wall of the macroscopic thallus. The fibrils are so densely packed that it is difficult to distinguish them. In contrast, it is interesting that the walls of both the monospore and θ spore resemble much more closely the wall of the macroscopic phase (Figs. 86, 87).
The conchocelis cells possess typical florideophycidean plugged pit connections (Figs. 86, 88-91). They are similar to those reported in the conchocelis of *P. leucosticta* (Lee and Fultz, 1970) and have already been reported in *P. perforata* (Bourne, Conway, and Cole, 1970, Appendix I). The plug is bounded by a membrane, and the plasmalemma of adjacent cells is firmly attached to it, as can be seen in slightly plasmolyzed cells (Fig. 88). The plasmalemma takes on a distinctly different appearance at the point of attachment to the plug. It becomes very diffuse and loses its three-layered organization (Fig. 88). Brown and Weier (1970) showed that the plasmalemma in *Batrachospermum* was continuous from cell to cell and did not cross the face of the plug. In the wall, at the level of the middle lamella, adjacent to and surrounding the plug are a series of small vesicles (Figs. 88, 90). The origin and function of these is not known. However, since they did not appear to penetrate the wall it is unlikely that they are plasmodesmata. In older cells there was a build up of a second layer of wall material on the inside of the original wall (Fig. 91) very much like that which occurred in *Pseudogloiophloeas* (Ramus, 1969b).

Several differences were noted between the conchocelis of *Porphyra perforata* described here and the conchocelis of *P. leucosticta* (Lee and Fultz, 1970). *P. perforata* had phycobilisomes, and a very narrow, dense wall. No prominent nucleolus was observed in this study. *P. leucosticta* had a slightly wider and less dense cell wall, a prominent nucleolus, and no phycobilisomes. These may be innate species differences. On the other hand, they are more easily explained on the basis of different culture conditions and developmental stages.
P. leucosticta conchocelis was obtained from the algal culture collection at Indiana University, which has been maintained for several years, whereas P. perforata conchocelis was grown immediately from spores released by field material.

The conchospore branch cells of the conchocelis are isodiametric measuring about 12 μ across (Figs. 95,97). The stellate chloroplast, pyrenoid, nucleus, nucleolus, vacuoles and starch grains (Figs. 96-99) are very similar to those found in the macroscopic phase. The wall separating the conchospores within a branch is permeated by a typical floridean plugged pit connection (Fig. 99). These cells are surrounded by a rather unique thick cell wall, which has many projections from the surface (Figs. 96,97). It is very wrinkled in surface view, as revealed by the scanning electron microscope (Fig. 101). The appearance is most striking when it is compared with the much smoother surface of the conchocelis vegetative filament (Fig.100).

Drew and Richards (1953) and Drew (1954) have shown that the outer cell walls of P. umbilicalis conchospore branches remain intact while the cross walls break down to form a tube. The conchospores are then released from the end of the tube. The reason given for the tube formation was that the branch was buried in the surface of a shell. Chen, Edelstein, Ogata and McLachlan (1970) noted that the conchospores of P. miniata were released through a pore located between the second and terminal conchosporangium. The plugged pit connections between the conchospore branch cells may be the site of initial wall breakdown and facilitate tube formation. This process could be similar to the dissolution of pit-containing walls during formation of the
There are three possible functions of the thick surface walls of the conchospore branch cells. These are: 1) substrate attachment after release, if the spores are released with these walls intact; 2) a dispersal mechanism with the protuberances attaching to passing objects, resulting in shaking of the branch; this function would apply if the spores are released through a tube formed by the outer conchospore branch cell walls; 3) protection during overwintering of the spores. It is difficult to make a definite choice. However, the fact that the conchospores of *Porphyra* can be released naked through a tube formed by the outer conchospore branch cell walls (Drew, 1954; Chen, Edelstein, Ogata, and McLachlan, 1970) indicates a slight preference for the second interpretation.

Using scanning electron microscopy, a collar was seen between adjacent cells of the vegetative filaments and also adjacent conchospore branch cells in surface view (Fig. 103). No collar was noted in the region of attachment between the conchocelis filament and the conchospore branch. The collar could be an artifact, the result of the preparation techniques used for scanning electron microscopy. The severe drying under vacuum possibly caused the cells to shrink. Therefore, the support of the internal cross wall resulted in a raised collar between cells of the filaments. No collar was seen in the transmission electron micrographs (Figs. 91, 97), or in the light micrographs (Fig. 102).
Discussion

The internal morphology of vegetative cells of *Porphyra perforata* and *Bangia fuscopurpurea* have been observed in detail in the present study and it has been found that these two genera in the order Bangiales are quite similar. The fine structure of *B. fuscopurpurea* found locally appears very similar to that of *B. fuscopurpurea* found in Naples, Italy (Honsell, 1963). The chloroplasts of the thalli are stellate, possess a large central pyrenoid, are bounded by a double membrane, and lack a peripheral thylakoid. The single thylakoids occasionally penetrate the pyrenoid. Although the thylakoids appear to be free in the stroma, it is possible that some association may exist since a functional stacking has been demonstrated in *Porphyridium* by freeze etching (Neushul, 1970). There is an alternate layering of thylakoids and phycobilisomes which results in a resonance contact between layers. Close stacking, when the phycobilisomes are not present, has been shown in *Smithora* (McBride and Cole, 1969, 1971). The endoplasmic reticulum, Golgi apparatus, nuclei, mitochondria, plasmalemma and cell wall are also very similar in both genera.

In addition, both genera contain paramural bodies, such as concentric lamellar bodies and polyvesicular bodies. The major difference between the two genera is the external morphology of the macroscopic thallus.

Cells of the thallus and conchocelis phase of *Porphyra perforata* have some similarities; the pyrenoid located within the chloroplast, separate thylakoids some of which penetrate the pyrenoid, nuclear and mitochondrial morphology. However, there is some variation between
these distinctly different growth forms of the same species, the thallus and conchocelis phases, relating to cell shape, chloroplast morphology and presence of a vacuole. In addition, the thallus cells possess more endoplasmic reticulum and thicker, less dense cell walls; and lack the plugged pit connection which occurs between conchocelis cells.

Several general morphological characteristics can be used to separate the two rhodophycean subclasses Bangiophycidae and Florideophycidae. These are the number of carpospores per carposporangium, number of spermatia per spermatium, number of secondary pit connections, and number of nuclei per cell. Although diffuse or parenchymatous growth is considered to be a characteristic of the Bangiophycidae, it can be found in some members of the Delesseriaceae, order Ceramiales and Corallinaceae, order Cryptonemiales (Florideophycidae) (Fritsch, 1945; Taylor, 1957) and likewise, growth by an axial cell, a characteristic of the Florideophycidae, is seen in Compsopogon (Bangiophycidae) (Fritsch, 1945). The specialization of the female sexual apparatus has been used as a major criterion for separating the two subclasses. However, this characteristic forms an almost continuous spectrum of increasing complexity from the most primitive members of the Bangiophycidae to the most advanced members of the Florideophycidae.

It is obvious that comparative ultrastructure is as valid as comparative morphology for classification purposes. Therefore as more observations are made it becomes possible to correlate certain taxonomic groups and various ultrastructural characteristics. Perhaps as
this is done some re-evaluation of the classification could be profitable. A characteristic which seems limited to the Rhodophyceae is the association between the Golgi apparatus and a mitochondrion. This has been shown in the Bangiophycidae, Porphyra in this study, and Porphyridium (Gantt and Conti, 1965), and in the Florideophycidae, Griffithsia (Peyrière, 1969) and Corallina (Bailey and Bisalputra, 1970). Although not specifically mentioned it can be seen in micrographs of other genera, Lomentaria (Bouck, 1962), Pseudogloiophloea (Ramus, 1969b) and Batrachospermum (Brown and Weier, 1970). Another feature distinctive to the red algae is the plugged pit connection. Although it is mostly restricted to the Florideophycidae it has also been shown to occur in the conchocelis phase of the life cycle of the Bangiophycidae in an essentially identical form (Lee and Fultz, 1970; Bourne, Conway, and Cole, 1970). The thylakoids are generally similar in the Bangiophycidae and Florideophycidae. They are single, interconnect and often have attached phycobilisomes. A major difference is the single large chloroplast with pyrenoid and no peripheral thylakoid in the Bangiophycidae vs. the several small chloroplasts with peripheral thylakoids, and lack of pyrenoid in the Florideophycidae. However, these features are not completely diagnostic for either group. Some Nemalionales (Florideophycidae) such as Nemalion have a single chloroplast with a pyrenoid (Fritsch, 1945), whereas Goniotrichopsis (Bangiophycidae) has many small chloroplasts and no pyrenoid (Drew, 1951). It is not known if Nemalion lacks a peripheral thylakoid or if Goniotrichopsis has one. A peripheral thylakoid commonly absent in the
Bangiophycidae has been recorded as a regular feature of the chloroplasts of Smithora (McBride and Cole, 1969).

It is interesting not only to compare the ultrastructural features of members of the Bangiophycidae with those of the Florideophycidae, but also to note that there can be as much or more variation of characteristics between alternate phases of the life cycle of one species (Porphyra perforata) as there is between the members of two subclasses. As more characteristics are found that are common to both subgroups the distinction between the subclasses will tend to become less pronounced. If it is eventually found that the taxa Bangiophycidae and Florideophycidae are no longer natural, there is no doubt that these terms would remain in common use for some time and that the orders presently included in both subclasses would remain in the class Rhodophyceae.

It is possible that several cell structures are involved in the process of wall production in P. perforata. These include the Golgi apparatus, polyvesicular bodies, concentric lamellar bodies, endoplasmic reticulum and plasmalemma. The Golgi apparatus in Porphyra consists of a number of small flattened cisternae, forming vesicles which contain fibers similar to those of the wall. These may be involved in wall material deposition. The Golgi apparatus is most often cited in connection with cell wall production in plants (Mollenhauer, Whaley, and Leech, 1961; Wooding and Northcote, 1964; Northcote and Pickett-Heaps, 1966; Pickett-Heaps, 1967a and b; Barton, 1968; Gantt, Edwards, and Conti, 1968; Wooding, 1968; and review in Beams and Kessel, 1968). The frequent association of the forming face of the
Golgi apparatus and a mitochondrion in Porphyra (Figs. 28-31) and other red algae (Gantt and Conti, 1965; Peyrière, 1969; Bailey and Bisalputra, 1970; and Brown and Weier, 1970) suggests that the Golgi body may originate from the mitochondrial outer membrane. A direct connection between the two has been demonstrated in Corallina (Bailey and Bisalputra, 1970). An alternative explanation of the association is that the mitochondrion is supplying energy to the Golgi apparatus for the synthesis of some product. Both possibilities are equally likely.

There seems to be an increasing number of reports of polyvesicular bodies, lamellar bodies, and other lomasome-like structures similar to those seen in Porphyra, in a wide range of plant species (Marchant and Robards, 1968). They were first described in fungi (Girbardt 1958, 1961; Moore and McAlear, 1961). Following this there was a number of observations on both higher plants and algae, and studies on the Rhodophyta in particular have demonstrated their presence in Lomentaria (Bouck, 1962), Porphyridium (Gantt and Conti, 1965), Laurencia (Bisalputra, Rusanowski, and Walker, 1967), Pseudogloioiphloea (Ramus, 1969b), Smithora (McBride and Cole, 1969) and Porphyra leucosticta (Lee and Fultz, 1970).

Polyvesicular bodies similar to these observed in Porphyra have also been observed in the higher plants, Helianthus (Walker and Bisalputra, 1967), Helleborus (Echlin and Godwin, 1968) and Beta vulgaris (Esau, Cheadle, and Gill, 1966). Marchant and Robards (1968) have considered the origin and function of these bodies which apparently are formed within the cytoplasm, move to the
cell surface, and are released into the cell wall. Walker and Bisalputra (1967) also showed a sequence in Helianthus involving vesicular migration to the cell surface, with a subsequent release of materials into the wall. It is possible that material is carried to the cell surface by the polyvesicular bodies in Porphyra.

The green algae Chara and Nitella have been shown to possess complex elaborations of the plasmalemma or "charasomes" on the cell wall side (Crawley, 1965; Barton, 1965a and b). However, their function is still open to question. Barton (1968) demonstrated that radioactive glucose reaches the cell wall in Chara by way of the Golgi bodies and not these lomasome-like structures. Also using autoradiography (radioactive sugars), Pickett-Heaps (1967a and b) found that the Golgi apparatus was involved in the deposition of cell wall precursors in Triticum while at the same time he found no evidence of involvement of lomasomes etcetera in this activity (personal communication to Fowke and Setterfield, 1969). This may be taken as evidence that the Golgi bodies are involved in cell wall formation while the paramural bodies are not. However, it must be kept in mind that cell wall metabolism involves more than just deposition of simple sugars and other precursors. These paramural structures may also be involved in wall breakdown, which occurs in Porphyra, Bangia and other plants during such times as spore release. However, there is no evidence to confirm this, such as build up prior to spore release.

Although it has been speculated that the various paramural structures are involved in carbohydrate deposit in the cell wall, attempts to trace the path of carbohydrate into the wall of Porphyra
perforata failed to show any deposit of tracer material (Appendix II). The true function of the paramural bodies in P. perforata must remain unknown until further studies have been made. They are probably not artifacts as claimed by Fowke and Setterfield (1969) for the following reasons: their structure is so complex that there must have been a "phenomenally rapid growth of this membrane during fixation" (Cole and Lin, 1970); there is no large vacuole present in this cell to supply the membrane material to a fixation artifact as in Helianthus (Fowke and Setterfield, 1969); and similar structures have been shown by freeze-etching in the red alga Corallina (Bailey and Bisalputra, 1970) and in the fungus Verticillium (Griffiths, 1970). Heath and Greenwood (1970) concluded that plasmalemmasome formation in fungi was the result of plasmalemma production exceeding wall expansion.

The E R is quite discernable close to the plasmalemma in several areas of Porphyra and Bangia and it may be engaged in cell wall synthesis or modification. The E R has been implicated in cell wall production in a number of other species. Porter and Machado (1960) showed that the E R could develop into the elements of the phragmoplast in Allium root tips. Hepler and Newcomb (1964) gave some evidence in favor of the possible role of the E R in wall production. Some of their figures show clusters of fibrillar elements within cisternae of the E R of Coleus. These were believed to have been produced for eventual deposition in the wall. In Haemanthus katherinae (Hepler and Jackson, 1968) few Golgi bodies were noted but many E R lamellae were associated with
the phragmoplast during plate formation. Pickett-Heaps (1967a and b) used radiotracers to demonstrate rather conclusively that both the Golgi bodies and E R in wheat seedlings (Triticum vulgare) took up the wall precursors during production of cell plate and secondary wall thickenings.

A formation in Porphyra which must be considered along with those previously mentioned is a system of densely staining membranes which extend throughout the cytoplasm linking various concentric lamellar bodies and polyvesicular bodies (Figs. 50,51). The membranes of these conspicuous features appear thicker than the endoplasmic reticulum (E R) membranes. Since a few connections have been observed between these membranes and the E R it is quite possible that these are modified elements of the E R. In some cases the cisternae expand forming an enlarged lumen which contain concentric lamellar bodies and polyvesicular bodies (Fig. 51), while in other cases it simply extends into finger-like cytoplasmic extensions in the wall (Fig. 50).

The convoluted portions of the plasmalemma in Porphyra (Figs. 40,50) with attached wall fibers are possibly areas of fiber elaboration. Convolutions in the plasmalemma have also been observed in the red algae Lomentaria (Bouck, 1962) and Porphyra yezoensis (Kito and Akiyama, 1968) as well as in other plants, and are generally associated with activities such as transport of wall materials out of the cell (Freywyssling, 1962). Surface activities were also demonstrated in the red alga Laurencia spectabilis
Small membrane-bounded droplets at the cytoplasm-cell wall interface of this species were interpreted as material released from the cell into the wall. Cronshaw and Bouck (1965) implicated the plasmalemma in metabolic activities such as cell wall elaboration in *Avena* coleoptiles by suggesting that some thick portions of the membrane might contain enzymes on the outer surface associated with the final synthesis and orientation of wall materials.

In conclusion, it has been shown that the thallus cells of *Porphyra perforata* and *Bangia fuscopurpurea* are ultrastructurally similar. This is not unexpected since they have been classified in the same order. However, the ultrastructural differences between the two phases of the life cycle of *P. perforata* are interesting and could be of some phylogenetic significance. These are: the plugged pit connection between adjacent conchocelis cells which is absent from the thallus, phycobilisomes in the chloroplasts of the young conchocelis cell not present in the thallus cells, the ribbon-like chloroplast in the conchocelis cells and stellate chloroplast in the thallus. There is much similarity between the ultrastructure of the two rhodophycean subclasses Bangiophycidae and Florideophycidae, with no ultrastructural feature restricted to either one. A number of organelles including the Golgi apparatus, endoplasmic reticulum paramural bodies and plasmalemma may be involved in cell wall production and/or modification in *Porphyra perforata*.
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Figures 22 - 56  Vegetative thallus of Porphyra perforata

Figure 22  Blades collected from Ucluelet, Vancouver Island  
X 1/3

Figure 23  Blades collected from Point Grey, Vancouver  
X 1/3

Figure 24  A light micrograph of a portion of the blade showing  
the packets of large, densely pigmented α spores and  
adjacent packets of smaller, lighter colored β spores.  
The single cells are vegetative cells.  X 100

Figure 25  An electron micrograph of a single vegetative cell  
showing the large pyrenoid (Py) surrounded by the  
chloroplast, the nucleus (Nu), nucleolus (Nl) and  
reticulate cell wall (CW). This section is parallel  
to the blade surface.  X 7,000
Figures 26 - 29  Electron micrographs of the vegetative thallus cells of *Porphyra perforata*

Figure 26  A cross section of the blade showing similar organelles to those of Figure 25. Note the nucleus (Nu) and pyrenoid (Py) are located approximately midway between the two blade surfaces. X 2,500

Figure 27  Section through the cell surface showing the convoluted plasmalemma (Pl) and numerous vesicles (arrows) between cytoplasm and cell wall (CW). X 150,000

Figure 28  Section showing the vegetative chloroplast (Ch) with single lamellae. Note the interconnections (arrows) between numerous lamellae. Also note the DNA fibrils (double arrows) and the close association between mitochondrion (M) and Golgi apparatus (G). X 30,000

Figure 29  A portion of a vegetative cell. Note the forming face of the Golgi apparatus is directed towards the mitochondrion (M) and the maturing face oriented towards the cell wall (CW). X 30,000
Figures 30 - 32  Electron micrographs of cells in the vegetative thallus of *P. perforata*

Figure 30  Two mitochondria (M) with associated Golgi bodies (G). Note the floridean starch granules (arrows). X 36,000

Figure 31  Section showing the Golgi apparatus (G) and mitochondrion (M). Note the small vesicles between the mitochondrion and the forming face of the Golgi body. Observe the fibrous material in Golgi vesicles (GV). X 36,000

Figure 32  Two nuclei (or possibly two lobes of one nucleus) in a vegetative cell. A portion of one nucleus is directed towards the opposite end of the cell. X 16,000
Figures 33 - 36  Stages of one type of cell division in

*P. perforata*

**Figure 33**  Single vegetative nucleus (Nu), appressed to one side of the cell. Note convoluted portions of the plasmalemma (Pl).  X 5,000

**Figure 34**  Highly lobate vegetative nucleus, which may be the initial stage of nuclear division. Note the nuclear pores (NP), floridean starch (St) and the elements of the endoplasmic reticulum (ER) near the cell surface.  X 26,000

**Figure 35**  Initial stage of cytokinesis. The two daughter nuclei (Nu) are at opposite ends of the cell. The dividing wall is starting to form from the cell periphery.  X 7,000

**Figure 36**  A later stage of cell division. The cell wall (CW) extends most of the way across the cell. Note the pyrenoid and chloroplast have not yet divided and are caught in the annular opening.  X 10,000
Figures 37 and 38 Stages in another type of cell division in *P. perforata*

Figure 37 The two nuclei (Nu) in this cell are separated by the invaginated plasmalemma. In this section the plasmalemma extends most of the way across the cell (arrows). No cell wall material has been laid down yet. X 16,500

Figure 38 A later stage of this type of cell division. The plasmalemma (Pl) is convoluted in this area. A number of cell wall fibers have been laid down between the two cells. X 18,000
Figures 39 - 41 Holdfast region of *P. perforata*

Figure 39  Note the very thick layered cell wall in relation to the small rhizoidal process in the center. Wrinkles are due to section stretching.  \( \times 6,500 \)

Figure 40  Cross section of the rhizoidal process with highly convoluted plasmalemma (Pl).  \( \times 31,000 \)

Figure 41  Longitudinal section of rhizoidal process showing thick cell wall (CW) and convoluted plasmalemma.  \( \times 15,000 \)

Figure 42  Scanning electron micrograph of the surface of the vegetative thallus.  \( \times 1,200 \)
Figures 43 - 44  \( \alpha \) spore producing portion of thallus of

**P. perforata**

**Figure 43**  Section through \( \alpha \) spore producing portion of the thallus. Note the thin cell wall between developing \( \alpha \) spores. X 5,700

**Figure 44**  Numerous small fiber-filled vesicles near the cell wall (CW) of differentiating \( \alpha \) spores. X 32,000

Figures 45 - 47  Paramural bodies in the vegetative thallus of **P. perforata**

**Figure 45**  Concentric lamellar body (CLB) with tubules located inside the outer membrane. X 52,000

**Figure 46**  Portion of a vegetative cell. Note the cell wall fibers attached to the plasmalemma (Pl) particularly in the vicinity of the concentric lamellar body. X 68,000

**Figure 47**  Concentric lamellar body in the cell wall (CW). Note the endoplasmic reticulum (ER) closely paralleling the plasmalemma (Pl). X 52,000
Figures 48 - 51 Paramural bodies in *Porphyra perforata*

**Figure 48** A cell with concentric lamellar bodies (CLB) in the cytoplasm, and in the chloroplast (arrow). X 13,000

**Figure 49** A concentric lamellar body (CLB) in close proximity to a polyvesicular body (PVB). Both are located near the cell surface. X 40,000

**Figure 50** Finger-like projections of cytoplasm into the cell wall (CW). Note the membranes that extend into these projections (arrows). X 21,000

**Figure 51** Note the membranes (arrows) interconnecting the concentric lamellar bodies (CLB) and polyvesicular bodies (PVB), in the vegetative cell. X 32,000
Figures 52 - 55  Paramural bodies in *P. perforata*

**Figure 52**  Polyvesicular body with tubular inner vesicles.  
X 97,000

**Figure 53**  Polyvesicular body (PVB) located near the cell periphery.  X 35,000

**Figure 54**  Polyvesicular body in contact with the cell wall (CW).  X 56,000

**Figure 55**  A complex elaboration of the plasmalemma, located between the plasmalemma and cell wall (CW).  
X 29,000

**Figure 56**  Endoplasmic reticulum (ER) in the central portion of a cell. Note the numerous membranes.  X 90,000
Figures 57 - 60 Bipolar sporelings of *Porphyra perforata*

**Figure 57**  Light micrograph of released aplanospores.  
X 2,000

**Figure 58**  Young bipolar sporeling with rhizoidal process at basal end.  X 1,600

**Figure 59**  Older bipolar sporeling. Note the rhizoidal processes at the basal end.  X 4,700

**Figure 60**  Electron micrograph of cell from older bipolar sporeling showing the thick cell wall and convoluted plasmalemma.  X 32,000
Figures 61 - 64 Finestructural details of bipolar sporeling cells of *P. perforata*

**Figure 61** Portion of a cell showing chloroplasts with pyrenoid and single lamellae. X 33,000

**Figure 62** Chloroplast with pyrenoid and osmiophilic droplets. X 33,000

**Figure 63** Cell with thick cell wall (CW). Note the numerous osmiophilic droplets between the chloroplast lamellae and the chloroplast DNA fibrils (arrows). X 18,000

**Figure 64** Portion of a cell with chloroplasts (Ch), starch granules (St), and a Golgi apparatus (G) which is situated near a mitochondrion. X 12,000
Figures 65 - 79  Micrographs of *Bangia fuscopurpurea*

Figure 65  Light micrograph of a filament.  X 2,500

Figure 66  Electron micrograph of a cell in the filament.  
Note that one cell contains two chloroplasts (Ch) and two pyrenoids (Py). It is probably undergoing division.  X 7,600

Figure 67  Cell surface with highly convoluted plasmalemma (Pl).  X 44,500

Figure 68  Lobate nucleus (Nu) with prominent nucleolus (Nl) similar to the vegetative nucleus of *Porphyra perforata* (Figs. 33,34).  X 13,000

Figure 69  Section through a vegetative cell showing endoplasmic reticulum (ER) connected to a concentric lamellar body, and a mitochondrion (M) with a closely associated Golgi apparatus (G).  X 30,000
Figures 70 - 75  Electron micrographs of *Bangia fuscopurpurea* thallus

Figure 70  A concentric lamellar body (CLB) in a chloroplast (Ch). X 40,000

Figure 71  A round concentric lamellar body in the cytoplasm (aplanospore). X 31,000

Figure 72  Concentric lamellar body in a cell wall, X 48,000

Figure 73  An irregularly shaped concentric lamellar body in the cytoplasm. X 31,000

Figure 74  Cell division. Note the membrane (arrows) separating the two chloroplasts and pyrenoids (Py). X 16,000

Figure 75  Thick multilayered cell wall between two cells (aplanospores). X 16,000
Figures 76 - 79  Development of aplanospores in Bangia fuscopurpurea

Figure 76  Note the extensive endoplasmic reticulum (ER) in the peripheral cytoplasm and layer of cell wall material surrounding the developing spore, and the interconnections of the thylakoids (arrows). X 18,000

Figure 77  Developing aplanospore showing partial separation of the spore wall from the mother cell wall. Note the highly lobate chloroplast with its central pyrenoid. X 4,000

Figure 78  Developing aplanospore. Note that the spore cell wall consists of several layers. X 5,000

Figure 79  Aplanospore released from the spore mother cell wall but still in the lumen. Again note that the spore cell wall consists of several layers. X 5,000
Figures 80 - 103 Micrographs of the conchocelis phase of *Porphyra perforata*

Figure 80 Light micrograph of spores released from the mature thallus. The larger spore is the α spore and the smaller, the β spore. X 2,000

Figure 81 Light micrograph of a young conchocelis filament. X 3,000

Figure 82 Electron micrograph of a cross section of a conchocelis filament showing a nucleus (Nu), with some chromatin attached to the nuclear membrane, chloroplast (Ch), and endoplasmic reticulum (ER). X 27,000

Figure 83 Cross section of conchocelis filament showing a chloroplast (Ch) with phycobilisomes (Ph) attached to the thylakoids. Note that some of the thylakoids interconnect. X 27,000
Figures 84 - 87  Electron micrographs of the conchocelis phase of *P. perforata*

**Figure 84**  Conchocelis cells showing chloroplast (Ch), starch (St), pyrenoid (Py). X 6,600

**Figure 85**  Light micrograph of conchocelis filament showing the empty spore case (Sp). X 1,500

**Figure 86**  Electron micrograph of first conchocelis cell and the empty spore case (Sp). Note the vacuole (V), plugged pit connection between the first cell and the spore case and the difference between cell walls of the conchocelis filament and spore. X 23,000

**Figure 87**  Empty spore case and first cell of conchocelis. Note the thick, less dense cell wall of the spore case and the thin, very dense wall of the conchocelis. X 6,000
Figures 88 - 91

Electron micrographs of the wall between adjacent conchocelis cells of P. perforata

Figure 88
Plugged pit connection (Pg) between two vacuolated conchocelis cells. Note the attachment of the plasmalemma to the plug, the small vesicle in the wall beside the plug (arrow) and the floridean starch granules (St). X 56,000

Figure 89
Plugged pit connection (Pg) between two conchocelis cells. X 42,000

Figure 90
Cell wall adjacent to a plugged pit connection. Note the small vesicle in the wall next to the plug (arrow). X 35,000

Figure 91
Plugged pit connection (Pg) between two older conchocelis cells. Note the paramural body next to the plug and secondary thickening of the cell wall (arrows). X 15,000
Figures 92 - 94  Older conchocelis vegetative cells of *P. perforata*

Figure 92  Note the two pyrenoids (Py) in one chloroplast, and the accumulation of starch granules.  X 7,200

Figure 93  Chloroplast (Ch) and pyrenoid (Py) of older conchocelis cell.  Note the absence of phycobilisomes. X 9,000

Figure 94  Longitudinal section of conchocelis cell. Note the two pyrenoids and the nucleus (Nu).  X 7,200

Figure 95  Light micrograph of conchospore branches.  X 500
Figures 96 - 99 Electron micrographs of conchospore branch cells of *P. perforata*

**Figure 96** Conchospore branch cell showing projection of the cell wall (CW). X 9,800

**Figure 97** Conchospore branch cells showing convoluted cell wall, chloroplast and pyrenoid (Py). X 4,300

**Figure 98** Conchospore branch cell with nucleus (Nu), nucleolus (Nl), and chloroplast (Ch). X 15,000

**Figure 99** Plugged pit connection (Pg) between two conchospore branch cells. Note the large number of starch granules (St). X 15,000
Figures 100 - 103  Conchocelis phase of *P. perforata*

**Figure 100**  Scanning electron micrograph of the surface of conchocelis filaments. Note the relatively smooth cell wall.  X 7,000

**Figure 101**  Scanning electron micrograph of the surface of conchospore branch cells. Note the highly convoluted cell wall.  X 7,000

**Figure 102**  Light micrograph of the connection between the vegetative conchocelis cells and the conchospore branch cells.  X 2,000

**Figure 103**  Scanning electron micrograph of the connection between the vegetative conchocelis cells and the conchospore branch cells (arrow). Note the collar between adjacent conchocelis cells and between adjacent conchospore branch cells. Also note that there appears to be no collar between the conchocelis cell and the conchospore branch cell.  X 5,000
GENERAL CONCLUSIONS (PART I AND II)

On the basis of ultrastructure, Phaeostrophion irregulare possesses some characteristics common to the higher orders of the Phaeophyceae:

a. absence of a pyrenoid in the adult thallus
b. absence of a physical association of the chloroplast and nucleus.

It also has some characteristics common to the lower orders of the Phaeophyceae:

a. strictly perinuclear Golgi apparatus
b. diffuse distribution of plasmodesmata

Consequently, this species in the order Dictyosiphonales retains its intermediate phyletic position which had previously been determined using more gross characteristics.

The ultrastructure of Porphyra perforata and Bangia fuscopurpurea both in the order Bangiales (Rhodophyceae), is very similar.

The ultrastructure of the conchocelis phase of P. perforata is basically similar to that of the vegetative thallus. However the following differences were noted:

a. the plugged pit connection between adjacent conchocelis cells is absent from the thallus
b. phycobilisomes in the chloroplasts of the young conchocelis are not present in the thallus cells
c. chloroplast shape is ribbon-like in the conchocelis and stellate in the thallus.
There is much similarity between the ultrastructure of the two rhodophycean subclasses Bangiophycidae and Florideophycidae. A number of organelles including the Golgi apparatus, endoplasmic reticulum, and paramural bodies may be involved in cell wall production and/or modification in *Porphyra perforata*. 
APPENDIX I.

On the ultrastructure of pit connections in the conchocelis phase of the red alga *Porphyra perforata* J.Ag. 2,3

As Dixon (1963) has pointed out, there is still doubt about the presence and structure of pit connections in the Bangiophycidae. Fan (1960) listed early reports of connections seen by light microscopy, and these were followed by Magne (1960), Belcher (1960), and Krishnamurthy (1969). However, Nichols, Ridgway, and Bold (1966) were unable to confirm with the electron microscope Fan's observations on *Compsopogon*. In our study of *Porphyra perforata*, pit connections in the transverse walls of conchocelis filaments have been observed both by light and electron microscopy.

Filaments of conchocelis grown in culture for 3 to 4 weeks were fixed in a glutaraldehyde-osmium fix and embedded in Maraglas. The sections were stained with uranyl acetate and lead citrate (McBride and Cole, 1969).

In the transverse walls of most cells a pit connection of about 0.5 μm diameter was obvious with the light microscope (Fig.104), but no details could be seen. Ultrastructurally, the longitudinal and outer edges of the transverse walls of the filaments were 0.25 μm thick and composed of closely packed, electron dense microfibrils. There were

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2 This article by V.L. Bourne, E. Conway and K. Cole appeared in *Phycologia* 9: 79-81 (1970). The study was supervised by E. Conway and K. Cole.

3 An article by Lee and Fultz (1970) appeared while this was in press describing a similar pit connection in the conchocelis phase of *Porphyra leucosticta*. It has been discussed earlier (p. 50).
local increases in thickness in wall areas adjacent to the pit and at the meeting points of longitudinal and transverse walls (Fig. 105). The pit, centrally located within the transverse wall, was blocked by a membrane-bounded plug. The plasmalemma of adjacent cells appeared to be closely associated with the plug membrane (Fig. 106). The core of the plug consisted of material less electron dense than that of the surrounding wall.

No cytoplasmic continuity was observed through the plug. These general ultrastructural details of the pit connections appear to be similar to those already known in the Florideophycidae (Myers, Preston, and Ripley, 1959; Bouck, 1962; Bischoff, 1965; Bisalputra, Rusanowski, and Walker, 1967; and Ramus, 1969 a and b).

Literature Cited


*Phycologia* 8: 177-186


**Literature Cited in Footnotes**

Figure 104 Light micrograph of conchocelis filaments showing the pit connection (arrows) between adjacent cells. X 2,000

Figure 105 Low magnification electron micrograph of a pit connection. X 13,000

Figure 106 High magnification electron micrograph of the pit connection in Figure 105. Note the cell wall (CW), plug (Pg), chloroplast (Ch), plasma membrane (PM), and plug membrane (arrows). X 120,000
APPENDIX II

A preliminary experiment using radiotracer techniques in an attempt to follow the path of polymerized glucose, mannose, and xylose in the macroscopic thallus of *Porphyra perforata*.

Introduction

Since Frei and Preston (1964) demonstrated that the wall of *Porphyra* consists of mannose and xylose in a particular configuration little has been done to trace the path followed by the wall precursors. This seemed unfortunate in the light of successful attempts to show the path of glucose polymers through the Golgi apparatus into the cellulosic fraction of the wall of *Chara* (Sarton, 1968) and *Triticum* (Pickett-Heaps, 1967). A number of organelles have been observed in *Porphyra* cells (page 44) that are morphologically similar to organelles in other species which are suspected of being involved in cell wall deposition and/or organization. These observations indicated that some hypothesis concerning the synthesis and deposition of wall material in *Porphyra* could be formed and tested. The hypothesis formulated was that some or all of the organelles seen in *Porphyra* are involved in the direct synthesis and/or deposition of wall material. It was tested by using electron microscopic autoradiographic techniques to localize the deposition of polymerized mannose and xylose in and around the cell. This would of course test only for synthesis and deposition of carbohydrate materials.
Therefore, a negative result would not exclude the possibility of any of the organelles being involved in deposition of protein or in the reorganization of wall material.

Materials and Methods

Porphyra blades for this experiment were collected during evening low tides and kept at 5°C overnight. The material for the glucose test was collected on January 20th, 1969; for mannose, March 3rd, 1969; and for xylose on April 5th, 1969. The blades were placed in a culture solution composed of 25 ml autoclaved sea water with 200 mg NaN03 and 20 mg Na2HPO4·7H2O added plus 1 mc of glucose-6-tritium; 1 mc of mannose-1-tritium; and 0.05 mc of D-xylose C\textsuperscript{14} all at approximately 10°C. The culture vessel was a glass bubble chamber (Fig. 107) which allowed the easy removal and reimmersion of the material in the culture solution. Samples were collected by clipping a small piece from the blade at total times of approximately 0, 3, 8, 15, 20, 30, 40, 70, 100, 190 and 350 minutes. As each piece was removed it was fixed and embedded as previously described (page 31).

The embedded material was cut and stained (see page 33). The grids were coated with Gevaert Scientia NUC 3.07 emulsion (Bourne, and Cole, 1969) and stored at 5-8°C for 1 to 10 months. Upon removal from storage the emulsion coated grids were developed for 3 minutes in Kodak D 19-b developer, fixed, washed and examined in an Hitachi HU - 11A electron microscope. Test grids were exposed to white light and developed to check the emulsion coating.
Observations and Discussion

Cross wall formation and Golgi bodies containing fibrous material were observed in the sectioned cells and it might be expected that sugars would be incorporated at these areas of metabolic activity. However, very few silver grains were observed and there was no distinct correlation of silver grains with a specific organelle. There were as many grains off the sections as on them. Test grids exposed to light and developed at the same time showed that the emulsion was coated onto the grids.

Since very little silver was reduced in the emulsion it can be questioned whether or not there was any incorporation of the labelled sugars at all. This could be tested by macerating and dissolving the whole material and then measuring the radioactivity with a liquid scintillation counter. An absence of evident incorporation could be explained by a number of factors; the sugars did not penetrate the walls, the alga was not metabolizing these sugars at those times, perhaps the test should have been run in the dark, the sugars were not polymerized enough and were therefore still soluble, or possibly the reactions that were being studied were in a closed chain that the labelled material could not enter. On the other hand, if there was incorporation it is possible that it was not detected because the storage temperature was too low or the exposure not long enough.
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Diagram of bubble chamber showing immersion of *Porphyra perforata* blades in the radioactive sugar solution. The blades are attached to the glass support rod. The rod and blades can be removed easily for sampling purposes.
Figure 107  Diagram of bubble chamber used for the radiotracer experiment.
A simplified technique for the application of nuclear emulsion in electron microscopic autoradiography

Several techniques for the application of nuclear emulsions in electron microscopic autoradiography have been developed in the last 10 years. However, most of these present inherent difficulties related either to the preparation and application of the emulsion, or to the interpretation of results. The silver evaporation technique of Silk, Hawtrey, Spence and Gear (1961) has been criticized because it has low radio sensitivity and high chemical sensitivity (Caro and van Tubergen, 1962; Koehler, Mühlethaler, and Frewyssling, 1963; Pelc, 1963; Caro, 1966). The dipping and dropping methods devised and published by a number of workers including O'Brien and George (1959); Pelc, Coombes, and Budd (1961); Przybylski, (1961); Kopriwa and LeBlond, (1962); Hay and Revel, (1963); and Salpeter and Bachmann, (1964) lead to uneven or excessively thick layers of emulsion (Caro and van Tubergen, 1962; Koehler, Mühlethaler, and Frewyssling, 1963). The semiautomatic instrument of Kopriwa (1966) is a modification of the dipping procedure and requires the use of a piece of equipment which may not be readily available in some laboratories. The use of a wire loop (George and Vogt, 1959; Revel and Hay, 1961; Kayes, Maunsbach, and Ullberg, 1962), centrifugation (Dohlman, Maunsbach, Maunsbach, and Ullberg, 1962), centrifugation (Dohlman, Maunsbach, Maunsbach, and Ullberg, 1962),

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4 This article by V.L. Bourne and K. Cole appeared in the Canadian Journal of Botany 47: 1821-1822 (1969). The study was supervised by K. Cole.
Hammarström, and Appelgren, 1964), and the agar block method
(Caro and van Tubergen, 1962), require complex observations and
manipulations under safe-light conditions.

The rotating disc method of Koehler, Mühlethaler and Frewyssling
(1963) seems the most promising of all the techniques reported to
date since it requires a minimum of equipment while maintaining easy
reproduction of thin, even emulsion coats. However, in addition to
the carbon coat applied to the sections to prevent chemical reduction
of the emulsion by the tissues (Salpeter and Bachmann, 1964), it
introduces an extra layer of formvar between the carbon-coated
sections and the emulsion. This layer could not only reduce microscopic
resolution by electron scatter (Sjöstrand, 1956) but also reduce
autoradiographic resolution by moving the emulsion layer beyond the
ideal distance from the radioactive source (Pelc, Coombes and Budd
1961, Caro and van Tubergen, 1962). Consequently a simple modification
of this technique was designed to improve and simplify it for use
in a moderately well-equipped E M laboratory.

The modified rotating disc technique (Fig. 108) was tested using
grids coated first with collodion and then with a layer of carbon.
If necessary the collodion base may be removed leaving only the carbon
support before attaching sections. Under indirect yellow-green
safelight illumination (Wratten series OA) a short strip of \( \frac{1}{2} \) in.
masking tape was attached to the center of the head of a small
centrifuge. The centrifuge was then spun at low speed to observe the
center of rotation. The rotor was stopped and the grid placed at the
center. Only extremely light pressure was needed for the grid to remain
on the tape even at high speeds. It was difficult to remove when fastened with too much pressure. The grid was then spun at about 10,000 r.p.m. Undiluted Gevaert Scientia NUC 3.07 emulsion was melted in a water bath which had been preheated to 40°C. The emulsion was picked up in a pipette and a single drop about 3 mm in diameter was released onto the center of the spinning grid. The size of the drop may not be too critical since most of the emulsion is thrown off immediately after application. The grid was spun for about \( \frac{1}{2} \) to 1 minute to allow the emulsion to dry, then removed with a pair of fine forceps and placed in a capsule.

To demonstrate the evenness of the coating, some undeveloped grids as well as others which had been developed in Kodak D-19 after exposure to white light for several minutes were observed and photographed in the electron microscope. D-19 developer was used to obtain the characteristic developed silver pattern of a tangled skein, seen in Fig. 110. If a higher resolution with a much finer grain is needed then a solvent developer such as Microdol-X (Caro and van Tubergen, 1962) or a phenidone base developer (Paweletz, 1967) may be used. Since a relatively high illumination level is required for successful completion of the operation it was necessary to test the effect of the safelight on the emulsion. Therefore a number of prepared grids were placed about 3 ft. directly in front of the yellow-green light for 20 minutes and subsequently developed and examined.

A fairly even distribution of silver halide granules was produced in all groups in the test (Figs. 109, 110). Similar results were
obtained by Koehler, Mühlethaler and Freywyssling (1963). However, this modification of their technique is an improvement, since it eliminates the extra layer of formvar over the carbon-coated sections before emulsion application. Also, it is simpler, reducing the number of operating steps and making it possible to coat a large number of grids in quick succession. The gelatin was not removed in these tests, but may be if desired.

Although Przybylski (1961), Salpeter and Bachmann (1964), and Caro (1966) indicated that the emulsions can be used safely under a yellow-green light, some workers seem to use the dark red lamp (Kopriwa and LeBlond 1962, Northcote and Pickett-Heaps 1966). Obviously, the efficiency and speed of the rotating disc method are improved if the operation is carried out under the brighter illumination. It was evident from the present tests that this is possible, since exposure to direct yellow-green light for periods of 20 minutes had no effect on the emulsion.

Literature Cited


Figure 108  Diagram showing the relationship of the emulsion, grid, and centrifuge spindle.

Figure 109  Electron micrograph showing the undeveloped silver halide granules just after application of the electron beam.  X 18,000

Figure 110  Electron micrograph showing the silver particles after exposure to light and development in D-19.  X 10,000