STUDIES ON THE BROWN ALGA ECTOCARPUS IN CULTURE:
SENESCENCE - AN ULTRASTRUCTURAL STUDY

by

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A study of cell differentiation and senescence on the brown alga *Ectocarpus* sp. was carried out using standard techniques of light- and electron microscopy as well as cytochemistry. The first 6-8 cells in each filament of *Ectocarpus* are characterized by a large round and well-organized nucleus, a dense cytoplasm rich in ribosomes, endoplasmic reticulum, dictyosomes, mitochondria, and chloroplasts with conspicuous stalked pyrenoids. Few small vacuoles are present in the cytoplasm. New features not previously reported for the brown algae are also described. These include the presence of pore-like interruptions and bridge-like filaments in the cisternae of dictyosomes and chloroplast thylakoids, as well as the formation of concentric bodies of chloroplast origin. Microbody-like organelles are also reported; cytochemical studies have shown catalase activity associated with them. Peroxidase activity is reported in the cell wall, while acid phosphatase activity is found in association with both the endoplasmic reticulum and the Golgi elements, as well as inside the vacuoles. Adenosine triphosphatase is present in mitochondria, chloroplast thylakoids, and plasma membrane. These cells, therefore, can be described as meristematic or the immediate consequence of the differentiation of the meristematic cells.

The processes of autophagy and vacuolation are studied in detail and found to be particularly significant during the stages of cell differentiation which lead to the aging of *Ectocarpus* sporophytic cells. Increases in vacuolation and autophagy are paralleled by an increase in acid phosphatase activity. The ultimate results of these processes are: a reduction in cytoplasmic matrix, a general deterioration of cytoplasmic organelles, and the formation of residual bodies which overcrowd the cytoplasm. Other
features are: the irregularity of the nuclear boundary, the disorganization of the E.R. system through vesiculation, the increasing difficulty in detecting mitochondria, the development of large stacks of chloroplast thylakoids as well as numerous patches of electron dense metabolites distinct from the plastoglobuli, and the formation of conspicuous cell wall ingrowths. Assays of enzyme systems other than acid phosphatase show their distribution to be similar to those reported for young cells.

In the final stages of senescence the cells become typically necrotic; no enzymes can be localized, except acid phosphatase whose reaction products are no longer compartmentalized but have become distributed all over the cell cavity. The nucleus and dictyosomes have disintegrated. Only small remnants of the E.R. system remain; Mitochondria and chloroplasts have lost their internal organization, no cytoplasmic matrix can be detected. At the very end even the cell wall shows signs of disorganization. It is concluded that these cells represent the final stage of autolysis.
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>MATERIAL AND METHODS</td>
<td>6</td>
</tr>
<tr>
<td>OBSERVATIONS</td>
<td>11</td>
</tr>
<tr>
<td>PART I - LIGHT MICROSCOPE OBSERVATIONS</td>
<td>11</td>
</tr>
<tr>
<td>PART II - ELECTRON MICROSCOPE OBSERVATIONS</td>
<td>13</td>
</tr>
<tr>
<td>A) ULTRASTRUCTURAL FEATURES OF YOUNG CELLS (CELL TYPE #1)</td>
<td>13</td>
</tr>
<tr>
<td>B) ULTRASTRUCTURAL FEATURES OF THE TRANSITIONAL &quot;1-2&quot; CELLS</td>
<td>23</td>
</tr>
<tr>
<td>C) THE SENESCENT CELLS - ULTRASTRUCTURAL FEATURES OF THE &quot;CELL TYPE #2&quot;</td>
<td>26</td>
</tr>
<tr>
<td>D) THE SENESCENT CELLS - ULTRASTRUCTURAL FEATURES OF TRANSITIONAL &quot;2-3&quot; CELLS</td>
<td>30</td>
</tr>
<tr>
<td>E) THE SENESCENT CELLS - ULTRASTRUCTURAL FEATURES OF THE &quot;CELL TYPE #3&quot;</td>
<td>33</td>
</tr>
<tr>
<td>F) ENZYME LOCALIZATION</td>
<td>35</td>
</tr>
<tr>
<td>G) ULTRASTRUCTURAL IDENTIFICATION OF AGING PIGMENT</td>
<td>37</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>39</td>
</tr>
<tr>
<td>PART I - THE YOUNG CELLS (CELL TYPE #1)</td>
<td>39</td>
</tr>
<tr>
<td>PART II - TRANSITIONAL &quot;1-2&quot; CELLS</td>
<td>49</td>
</tr>
<tr>
<td>PART III - THE &quot;CELL TYPE #2&quot;</td>
<td>53</td>
</tr>
<tr>
<td>PART IV - TRANSITIONAL &quot;2-3&quot; CELLS</td>
<td>63</td>
</tr>
<tr>
<td>PART V - THE &quot;CELL TYPE #3&quot;</td>
<td>66</td>
</tr>
<tr>
<td>PART VI - ENZYME LOCALIZATION DURING DIFFERENTIATION AND SENESCENCE</td>
<td>70</td>
</tr>
<tr>
<td>CONCLUSION</td>
<td>76</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>78</td>
</tr>
<tr>
<td>KEY OF SYMBOLS AND PLATE EXPLANATION</td>
<td>107</td>
</tr>
<tr>
<td>KEY OF SYMBOLS</td>
<td>108</td>
</tr>
<tr>
<td>PLATE EXPLANATION</td>
<td>109</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Light microscope observation of an <em>in situ</em> embedded filament (prostrate system)</td>
</tr>
<tr>
<td>2</td>
<td>Light microscope observation of an <em>in situ</em> embedded filament (erect system)</td>
</tr>
<tr>
<td>3</td>
<td>Light microscope observation of an <em>in situ</em> embedded filament (prostrate system)</td>
</tr>
<tr>
<td>4</td>
<td>Light microscope micrograph of a chloroplast</td>
</tr>
<tr>
<td>5</td>
<td>Light microscope micrograph of portion of a filament (living material)</td>
</tr>
<tr>
<td>6</td>
<td>Chloroplast morphology (living material)</td>
</tr>
<tr>
<td>7</td>
<td>Chloroplast morphology (living material)</td>
</tr>
<tr>
<td>8</td>
<td>Pyrenoid division (living material)</td>
</tr>
<tr>
<td>9</td>
<td>Chloroplast and pyrenoid morphology (living material)</td>
</tr>
<tr>
<td>10</td>
<td>Cell wall ingrowths (living material)</td>
</tr>
<tr>
<td>11</td>
<td>Cell wall ingrowths (living material)</td>
</tr>
<tr>
<td>12</td>
<td>Cytochemical identification of DNA containing structures (light microscope micrograph)</td>
</tr>
<tr>
<td>13</td>
<td>Cytochemical identification of DNA containing structures (light microscope micrograph)</td>
</tr>
<tr>
<td>14</td>
<td>Cytochemical identification of DNA containing structures (light microscope micrograph)</td>
</tr>
<tr>
<td>15</td>
<td>Cytochemical identification of RNA containing structures (light microscope micrograph)</td>
</tr>
<tr>
<td>16</td>
<td>Cytochemical identification of RNA containing structures (light microscope micrograph)</td>
</tr>
<tr>
<td>17</td>
<td>Cytochemical identification of RNA containing structures (light microscope micrograph)</td>
</tr>
<tr>
<td>18</td>
<td>Cytochemical identification of proteins (light microscope micrograph)</td>
</tr>
</tbody>
</table>
FIGURE

19  Cytochemical identification of proteins
    ( light microscope micrograph )
20  Cytochemical identification of proteins
    ( light microscope micrograph )
21  Cytochemical identification of insoluble
    carbohydrates ( light microscope micrograph )
22  Cytochemical identification of insoluble
    carbohydrates ( light microscope micrograph )
23  Cytochemical identification of lipids
    ( light microscope micrograph )
24  Cytochemical identification of lipids
    ( light microscope micrograph )
25  Cytochemical identification of lipids
    ( light microscope micrograph )
26  Schorml's reaction for the identification of
    lipofuscin ( light microscope micrograph )
27  Toluidine blue O staining ( light microscope
    micrograph )
28a  Electron micrograph of a young cell ( prostrate
    system )
28b  Plasmalemmasomes
29  Nuclear pores
30  Fibrillar aspect of chromatin
31  Electron micrograph of a young cell ( erect
    system )
32  Electron micrograph of a young cell ( erect
    system )
33  Electron micrograph of a young cell ( prostrate
    system )
34  Nuclear envelope- dictyosome association
35  Endoplasmic reticulum - dictyosome association
36  Freeze-etch replica of the nuclear envelope
37  Freeze-etch replica of the nuclear envelope
FIGURE
37a Plasmalemmasomes (freeze-etch replica)
38 Portion of the nucleus and perinuclear region of a young cell
39 Perinuclear space inclusions
40 Perinuclear space inclusions
41 Perinuclear space inclusions
42 Dictyosome morphology
43a Freeze-etch preparation of a chloroplast
43b Detail of figure 43a
44 Endoplasmic reticulum morphology
45 Section passing through the perinuclear region of a young cell
46 Freeze-etch replica of a chloroplast
47a Osmiophilic structured bodies (origin)
47b Osmiophilic structured bodies (origin)
48 Chloroplast-mitochondrion association
49 Tangential section through the region of the chloroplast E.R.
50 Chloroplast E.R. inclusions
51 Osmiophilic structured body (morphology)
52 Osmiophilic structured body (morphology)
53 Osmiophilic structured body-pyrenoid association
54 Discharge of osmiophilic structured bodies into the paramural space
55 Nuclear envelope-dictyosome association
56 Cross section through the periphery of a young cell (concentration of mitochondria)
57 Dictyosome morphology (cisternae interruptions)
58 Dictyosome morphology (cisternae interruptions)
59 Dictyosome morphology (bridge-like filaments)
60 Dictyosome-pyrenoid association
61 Dictyosome-mitochondrion association
62 Dictyosome-vacuole association
FIGURE
63  Dictyosome-pyrenoid association
64  Dictyosome morphology (lomasomes)
65  Dictyosome morphology (lomasome origin)
66  Detail of a dictyosome derived vesicle
67  Mitochondrion morphology
68  Mitochondrion morphology (cristae inclusions)
69  Nucleus-mitochondrion association
70  Chloroplast-mitochondrion association (freeze-etch preparation)
71  Mitochondrion-pyrenoid association
72  Mitochondrion-mitochondrion association
73  Detail of the thylakoid system
74  Thylakoid architecture (freeze-etch preparation)
75  Detail of a chloroplast
76  Detail of a chloroplast
77  Detail of the thylakoid system
78  Pore-like interruptions in the thylakoids
79  Thylakoid architecture
80  Freeze-etch preparation of thylakoids
81  Freeze-etch replica of a portion of a chloroplast
82  Chloroplast morphology (pattern of thylakoid arrangement)
83  Dictyosome morphology (cisternae interruptions)
84  Mitochondria morphology (genophore)
85  Whorled pattern of thylakoid arrangement
86  Concentric pattern of thylakoid arrangement
87  Abscission of plastid-derived concentric bodies
88  Concentric body
89  Acid phosphatase activity associated with a concentric body
90  Acid phosphatase activity (control preparation)
91  Detail of a concentric body
92  Detail of a chloroplast (bridge-like elements)
FIGURE
93  Detail of a chloroplast and pyrenoid
94  Chloroplast morphology (ribosomes)
95  Freeze-etch replica of a chloroplast (general morphology)
96  Thylakoid associated fibrillar elements
97  Plastoglobulus (fibrillar composition)
98  Plastoglobulus (fibrillar composition)
99  Plastoglobuli (fibrillar composition)
100 Endoplasmic reticulum-vacuole association
101 Chloroplast division (Laminaria type)
102 Chloroplast division (Sphacelaria type)
103 Chloroplast division (Sphacelaria type)
104 Proplastid-like formation
105 Chloroplast division (potential aspect)
106 Chloroplast division (potential aspect)
107 Multiconstricted chloroplast
108 Chloroplast and pyrenoid morphology
109 Pyrenoid division
110 Pyrenoid morphology
111 Plasmodesmata
112 Pyrenoid-vacuole association
113 Cell wall morphology (young cell)
114 Pyrenoid division
115 Cytoplasm morphology (young cell)
116 Vacuole development
117 Cell wall (freeze-etch preparation of a young cell)
118 Provacuoles (freeze-etch preparation)
119 Autophagocytosis (role of the E.R.)
120 Autophagocytosis (role of the E.R.)
121 Autophagocytosis (role of the E.R.)
122 Vacuole development
123 Vacuole development
FIGURE

124 Autophagocytosis (role of the tonoplast)
125 Acid phosphatase (vacuole)
126 Acid phosphatase (vacuole)
127 Vacuole development
128 Morphology of a aging cell
129 Acid phosphatase (dictyosome)
130 Acid phosphatase (endoplasmic reticulum)
131 Acid phosphatase (control preparation)
132 Transitional "1-2" cells; detail of a chloroplast
133 Permanganate fixation; detail of a chloroplast
134 "Cell type #2"; permanganate fixation
135 Transitional"1-2" cell; general morphology
136 Transitional "1-2" cell; general morphology
137 Transitional "1-2" cell; cytoplasmic inclusions
138 Transitional "1-2" cell; acid phosphatase activity
139 Transitional "1-2" cell; acid phosphatase activity
140 Transitional "1-2" cell; acid phosphatase activity (control preparation)
141 Transitional "1-2" cell; detail of the dictyosome region
142 Transitional "1-2" cell; cell wall morphology
143 "Cell type #2"; general ultrastructure of a prostrate system cell
144 "Cell type #2"; cell wall morphology
145 Transitional "2-3" cell; general morphology
146 Transition zone between a "cell type #3" and a "cell type #2"
147 Transitional "2-3" cell; cytoplasm
FIGURE

148 "Cell type #2"; general ultrastructure of an erect system cell
149 "Cell type #2"; detail of a chloroplast
150 "Cell type #2"; detail of the cytoplasm
151 "Cell type #2"; general ultrastructure of a prostrate system cell
152 "Cell type #2"; cell wall morphology
153 "Cell type #2"; nucleus and chloroplast morphology
154 "Cell type #2"; chloroplast morphology
155 "Cell type #2"; detail of a chloroplast
156 "Cell type #2"; detail of a chloroplast
157 "Cell type #2"; detail of a chloroplast
158 "Cell type #2"; cell wall morphology
159 "Cell type #2"; detail of a chloroplast (freeze-etch preparation)
160 "Cell type #2"; general morphology
161 "Cell type #2"; cell wall ingrowth
162 "Cell type #2"; cell wall ingrowths
163 Transitional "1-2" cell; cell wall ingrowth
164 Transitional "2-3" cell; cytoplasm morphology
165 Transitional "2-3" cell; disruption of the tonoplast
166 Transitional "2-3" cell; chloroplast
167 Transitional "2-3" cell; chloroplast
168 Transitional "2-3" cell; pyrenoid
169 Transitional "2-3" cell; chloroplast
170 Transitional "2-3" cell; nucleus
171 Transitional "1-2" cell; cell wall ingrowth
172 Transitional "2-3" cell; chloroplast
173 Transitional "2-3" cell; mitochondrion
174 Transitional "2-3" cell; mitochondrion
175 Transitional "2-3" cell; mitochondrion
FIGURE

176 Transitional " 2-3 " cell; concentric body
177 Transitional " 2-3 " cell; chloroplast
178 Transitional " 2-3 " cell; detail of a chloroplast
179 Transitional " 2-3 " cell; detail of a chloroplast
180 Transitional " 2-3 " cell; chloroplast
181 Transitional " 2-3 " cell; detail of the cytoplasm
182 Transitional " 2-3 " cell; detail of a chloroplast
183 Transitional " 2-3 " cell; pyrenoids
184 " Cell type #3"; cell wall morphology
185 Transitional " 2-3 " cell; chloroplast
186 Transitional " 2-3 " cell; chloroplast
187 " Cell type #3 "; general ultrastructure of a prostrate system cell
188 " Cell type #3 "; general ultrastructure of a prostrate system cell
189 " Cell type #3 "; general ultrastructure of an erect system cell
190 " Cell type #3 "; chloroplast
191 " Cell type #3 "; acid phosphatase activity
192 " Cell type #3 "; detail of thylakoid arrangement
193 " Cell type #3 "; thylakoid disorganization
194 " Cell type #3 "; thylakoid disorganization
195 " Cell type #3 "; thylakoid disorganization
196 " Cell type #3 "; mitochondria and cytoplasm morphology
197 " Cell type #3 "; mitochondria morphology
198 " Cell type #3 "; thylakoid disorganization
199 " Cell type #3 "; plastoglobuli accumulation
FIGURE

200 "Cell type #3 "; chloroplast morphology
201 "Cell type #3 "; detail of the cytoplasm
202 "Cell type #3 "; mitochondrion
203 "Cell type #3 "; acid phosphatase activity
   (control preparation)
204 "Cell type #3 "; acid phosphatase activity
205 "Cell type #3 "; acid phosphatase activity
   (control preparation)
206 "Cell type #3 "; acid phosphatase activity
207 "Cell type #3 "; cell wall disorganization
208 "Cell type #3 "; cell wall disorganization
209 "Cell type #1 "; catalase activity in
   a microbody-like organelle
210 "Cell type #1 "; catalase activity in
   a microbody-like organelle
211 "Cell type #1 "; catalase (control preparation)
212 "Cell type #1 "; catalase (mitochondrial
   deposition of reaction product)
213 "Cell type #2 "; catalase activity
214 "Cell type #1 "; peroxidase activity in the
   cell wall
215 "Cell type #1 "; microbody morphology
216 "Cell type #1 "; peroxidase activity in the
   cell wall
217 "Cell type #3 "; acid phosphatase activity
218 "Cell type #1 "; peroxidase activity (control
   preparation)
219 Transitional "1-2" cell; ATPase activity
   in association with the mitochondrion
220 "Cell type #3 "; acid phosphatase activity
221 "Cell type #2 "; peroxidase activity
222 "Cell type #2 "; peroxidase activity (control
   preparation)
FIGURE

223 "Cell type #1"; ATPase activity (Na⁺-K⁺-Mg²⁺- activated system)

224 "Cell type #1"; ATPase activity (Na⁺-K⁺-Mg²⁺- activated system)

225 "Cell type #1"; ATPase activity (Na⁺-K⁺-Mg²⁺- activated system)

226 "Cell type #1"; ATPase activity (Mg²⁺- activated system)

227 "Cell type #1"; ATPase activity (Mg²⁺- activated system)

228 ATPase activity (Na⁺-K⁺-Mg²⁺- activated system); control preparation

229 Transitional "1-2" cell; ATPase activity (Na⁺-K⁺-Mg²⁺- activated system)

230 ATPase activity (Na⁺-K⁺-Mg²⁺- activated system); control preparation

231 Transitional "1-2" cell; lipofuscin (Fontana's reaction)

232 ATPase activity (Mg²⁺- activated system); control preparation

233 "Cell type #2"; lipofuscin (Fontana's reaction)

234 Lipofuscin (control preparation)

235 Lipofuscin (Schorml's reaction)
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INTRODUCTION

I. Morphology of Young Vegetative Cells


From these studies the basic ultrastructural features of brown algal cells were established. It also became apparent that some cellular characteristics such as the position of the Golgi bodies, the presence or absence of pyrenoids, the pyrenoid morphology, and the arrangement of plasmodesmata could serve as useful criteria in establishing relationships among major groups of this class of algae (Evans, 1966, 1968; Cole and Lin, 1968; Cole, 1970; Scagel, 1966). Chloroplasts and pyrenoids have been more intensively studied than other organelles. The nucleus (Neushul and Dahl, 1972a; Leedale, 1970; Neushul and Walker, 1971) and plasmalemmasomes (Cole and Lin, 1970) also have attracted the attention of phycologists. However, only few representatives of the Phaeophyta have been studied in detail (Bouck, 1965; Bourne and Cole, 1968; Cole and Lin, 1968; Cole, 1969, 1970; McCully,
The Ectocarpales comprise plants with the simplest forms among the brown algae (Fritsch, 1945; Smith, 1955); hence, they hold an important position in brown algal phylogenetic schemes. Previous research on members of the Ectocarpales has included studies on: Ectocarpus mitchellae (Longest, 1946); Pylaiella littoralis (Gibbs, 1962a, 1962b) Giffordia sp. (Bouck, 1965); Ectocarpus confervoides and Pylaiella littoralis (Evans, 1966); representatives of the Myrionemaceae (Loiseaux, 1967); Ectocarpus acutus (Bailey and Bisalputra, 1969); Eudesme virescens (Cole, 1969—the genus Eudesme is considered by some investigators to belong to the order Chordariales, see Scagel, 1966, for a review); Ectocarpus breviarticulatus, Giffordia indica, Botrytella micromora (Hori, 1971); Ectocarpus crouanii (Magne, 1971); Giffordia mitchellae, Hapterophycus sp., Pylaiella littoralis (Hori, 1972).

Studies on the genus Ectocarpus were concerned with analysis of a restricted number of features. Longest (1946) studied the external features of the flagella of the zooids. Evans (1966) described the characteristics of the pyrenoid in zoospores and emphasized the absence of thylakoids or thylakoid-derived structures from the pyrenoid matrix. Hori (1971) made similar observations using vegetative cells. Bailey and Bisalputra (1969) used both freeze-etching and ultrathin sectioning techniques to study the cell wall morphology in Ectocarpus acutus and concluded that the wall possesses a double layered organization. Cytochemical techniques at the ultrastructural level revealed the presence of polysaccharide-like substances in association with lipid materials in plasto-globuli (Magne, 1971).

The aim of the first part of this thesis is to provide a more detailed description of the ultrastructure
of *Ectocarpus* and to compare its salient features with those of other brown algae.

II. Cellular Senescence

In recent years, the biology of the brown algae has received increasing attention. Information on the mechanism of cell wall regeneration (Fulcher and McCully, 1971), of fertilization (Pollock, 1970), rhizoid formation (Quatrano, 1972), and cellular polarity (Neushul and Dahl, 1972b) have become available. Nevertheless, so far I am aware, no ultrastructural study has been carried out on the aging of brown algae.

Senescence has been defined in many different ways. Strehler (1962) defined it as changes which result in a decreased survival capacity on the part of the individual organism. Leopold (1964) interpreted it as the degenerative processes which terminated the functional life of an organ or an organism. Osborne (1967) defined senescence as a general and increasing failure of many synthetic reactions which are the normal forerunners of cell death. Woolhouse (1967) thought of senescence as a continuation of cell differentiation; while Rockstein (1967) that it was the result of reproducible time-related alterations in structure and function in an organism which result in the decreasing capacity of that organism to survive and thus would result ultimately in its death. McLean (1968) referred to senescence as those events which occur between maturity and death. Whatever the position taken, there is general agreement that senescence ultimately leads to death of cells or organisms.

Aging and senescence have been used as synonymous terms (Thung and Hollander, 1967). Rockstein (1967), however, thinks of senescence as a more restricted part
of a broader phenomenon called aging. This, according to the author, includes: 1) the initial period of development, 2) the middle life stage of growth and maturation, and 3) the period of senescence. Carr and Pate (1967) also restrict the term senescence to those changes which are clearly degenerative, while they think of aging as the sum of total changes in the whole plant. In this work senescence and aging will be considered interchangeable terms.

Senescence theories and mechanisms are as abundant as are definitions. The subject, however, was reviewed recently (Hahn, 1971; Medvedev, 1972).

There is now a considerable amount of information dealing with biochemical and physiological aspects of senescence (e.g. Varner, 1961; Wangerman, 1965; Woolhouse, 1967; Wareing and Phillips, 1970; Sax, 1962), but at the ultrastructural level data are still sparse. Information dealing with higher plants was recently reviewed by Butler and Simon (1971). In comparison, there is little information on the algae. The aging phenomena of unicellular algae has been studied in *Spongiochloris typica* (McLean, 1968), *Ochromonas* (Schuster et al., 1968), and *Euglena granulata* (Walne et al., 1970; and Palisano and Walne, 1972). Fabbri and Palandri (1969) and Palandri (1972) studied aging in the coenocytic filaments of *Halimeda tuna*.

To contribute to our knowledge of the process of senescence, the ultrastructural changes of *Ectocarpus* cells during the overall process of aging were studied.

The selection of *Ectocarpus* for such a study has proved to have certain advantages. The simple filamentous form with prominent apical growth is particularly suitable in that it is possible to follow all stages of cellular differentiation and disorganization in a sequential order.

The information obtained in this work will provide background for future experimental work on the factors
governing senescence. Among those factors, the problem of controlling senescence using growth regulators and related substances could be quite interesting to consider. Growth regulators were shown to influence senescence in higher plants (e.g., Butler and Simon, 1971; Udvardy and Parkas, 1972). Recent reports have disclosed the existence of substances with similar properties in the brown algae (e.g., Jennings, 1968; Buggeln and Craigie, 1971; Augier and Harada, 1972; Hussain and Boney, 1973). Therefore, the study of their effects on the brown algae might provide interesting information on the aging mechanism in general.

This work also attempts to establish similarities and differences that might exist between the aging process in organisms as different as a brown alga, a protist, a higher plant or an animal. These attempts will eventually help to judge assertions like that of Palisano and Walne (1972), who defended the existence of a basic common mechanism of senescence from the lower to the higher forms of life.
Ectocarpus sp. was obtained from the Culture Collection of Algae, Indiana University (culture LB 1433).

Cultures were maintained in Chihara's marine medium (Chihara, 1968) in a growth chamber, at 14°C and illuminated by fluorescent lamps for 16 hours per day, at approximately 400 ft.c. The alga was grown in "Nalgene" polypropylene petri dishes (#5500). This facilitated fixation and embedding of the material (Bisalputra et al., 1971). To ensure optimum growth the medium was changed every 5 days. Stock cultures were handled in a similar way except they were kept in large pyrex culture jars, and the culture medium changed every 3 weeks. For dark treated material cultures were grown for 3 months under normal culture conditions, then transferred to total darkness for 1 to 5 days and 35 days.

Light microscopy. Living filaments were studied directly on Nalgene petri dishes or individual plants were placed in a drop of culture medium on a microscope slide. Fixed material was observed after treatment in one of the following procedures: a) 4% (v/v solution of glutaraldehyde in cacodylate buffer (0.1 M Sodium Cacodylate, pH 7.0), to which 0.25 M of sucrose was added;

b) 10% (v/v acrolein in 0.025 M phosphate buffer (pH 6.8, 24 hours at 0°C). The material was post-fixed in 1% HgCl₂ (24 hours, at 0°C; McCully, 1966);

c) 4% (v/v phosphate buffered (0.05 M pH 7.0) paraformaldehyde.

Except when specified, all fixations were carried out at room temperature, from 3 to 28 hours. Fixations for dark treated material were carried out in total darkness. The fixed material was embedded in Spurr's medium, which is similar to that described for electron microscopy and
sectioned at a thickness of approximately 1 micron. Sectioning was routinely done using an ultramicrotome equipped with glass knives. Ribbons of 5-6 sections were transferred with a platinum loop to glass slides and dried at 60°C on an electric hot plate. Slides bearing sections were either directly placed in the staining solutions or deplasticized prior to staining (Rosenquist et al., 1971). The staining procedures were as summarized in the following page.

After staining the sections were washed, air dried, and mounted in one drop of "Permount" mounting medium.

Observations were made with a Zeiss photomicroscope using phase contrast and bright field illuminations. Photographs were taken using Ilford FP4 film.

Electron microscopy. The material was fixed and processed according to one of the following methods:

1) Glutaraldahyde-osmium fixation. The material was fixed for 2½-3 hours at room temperature, in a fixative solution containing 4% (v/v) glutaraldahyde and 0.25M sucrose, in 0.1M sodium cacodylate buffer (pH 7.0). The samples were transferred through a series of buffer solutions containing decreasing concentrations of sucrose, until finally sucrose was completely eliminated from the rinsing solutions. The material was post-fixed for a further 2½ hours in a 1.5% (v/v) OsO₄, similarly buffered, but without sucrose. It was rinsed in the buffer solution and dehydrated in graded alcohol series. Spurr's low viscosity embedding medium (Spurr, 1969) was used as the embedding vehicle according to the procedure described by Bisalputra et al. (1971). This method was found to give the best results in preserving the cellular architecture of young cells and cells in advanced stages of senescence.

Slight modifications to the above procedure were introduced by carrying the fixation overnight, using a
## SUMMARY OF LIGHT MICROSCOPE STAIN METHODS

<table>
<thead>
<tr>
<th>STAINS</th>
<th>REACTION</th>
<th>OTHER DETAILS</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Proteins</strong></td>
<td>Aniline blue-black</td>
<td>bright blue colour at protein sites</td>
<td>Fisher, 1968</td>
</tr>
<tr>
<td><strong>B. Insoluble carbohydrates</strong></td>
<td>Periodic acid-Schiff (P.A.S.) reaction</td>
<td>red colour at reaction sites</td>
<td>DNPH blockage of aldehydes</td>
</tr>
<tr>
<td><strong>C. Nucleic acids</strong></td>
<td>Methyl green-pyronin</td>
<td>DNA-containing structures stain blue-green</td>
<td>RNA-containing structures stain red</td>
</tr>
<tr>
<td><strong>D. Lipids</strong></td>
<td>Sudan Black B</td>
<td>black blue colour at reaction sites</td>
<td>procedure carried out in formaldehyde fixed material</td>
</tr>
<tr>
<td><strong>E. Polyphenols</strong></td>
<td>Toluidine blue O</td>
<td>green to turquoise colour at reaction sites</td>
<td>material fixed according to McCully (1966)</td>
</tr>
<tr>
<td><strong>F. Age Pigment</strong></td>
<td>Schorml reaction deep blue colour at reaction sites</td>
<td></td>
<td>Hendy, 1971</td>
</tr>
</tbody>
</table>
5% (v/v) solution of glutaraldehyde. The decrease in sucrose concentrations was done during the post-osmication steps. This variation was found to give best results in handling the "cell type #2" preservation, which has proved to be very difficult to preserve.

2) **Permanganate fixation.** This was carried out using 1% to 5% solutions of KMnO₄ either in distilled water or in sodium cacodylate buffer (pH 7.0) without sucrose. A wide range of fixation times were tried but the images obtained were uniformly poor, especially with respect to the older cells. These data agree with those of Barton (1966) who also found permanganate fixation inadequate for studies of senescence.

3) **Enzyme localization at the ultrastructural level.** The material used in these experiments was briefly fixed (20 minutes, at room temperature) in a 4% solution of purified glutaraldehyde (Electron Microscope Sciences, Washington) in 0.1M cacodylate buffer (pH 7.0), to which sucrose was added to a final concentration of 7.5% (w/v). After brief rinsing, the material was incubated in the appropriate incubation medium. The material was then quickly rinsed and fixed for a further 2 hours in glutaraldehyde. Subsequent processing was as described before under 1.

The composition of the incubation media were:

a) **Acid phosphatase.** Tris-maleate, 60 mM (pH 5.0); lead nitrate, 3 mM; Beta-glycerophosphate, 11.5 mM; sucrose, 220 mM (Gomori, 1952). The material was then incubated for periods of time ranging from 30 to 60 minutes, at a temperature of 37°C. Control material was incubated either in the standard incubation medium with 10 mM of sodium fluoride or from which Beta-glycerophosphate was removed.

b) **Catalase.** 10 mg diaminobenzidine (DAB); 5 ml of 0.05M propanediol buffer; 1 ml of 3% (v/v) H₂O₂. The final pH of the incubation medium was 9.0 (Frederick and Newcomb, 1969).
The incubation was carried out at 37°C for 60 minutes. Control material was incubated without \( \text{H}_2\text{O}_2 \), or in the presence of 0.01M KCN. In another test the material was boiled for 5 minutes in propanediol buffer prior to incubation in standard medium.

c) Peroxidase. Media for incubation and controls were as indicated for catalase, but the final pH was adjusted to 7.6. The incubation was carried out at room temperature for 60 minutes.

d) Adenosine triphosphatase (ATPase). Two standard media were employed to study ATPase localization (adapted from Henrikson, 1971). Medium A. Tris-maleate, 80 mM (pH 7.2); 10 mM MgSO\(_4\); 3 mM ATP; 3 mM lead nitrate; 220 mM sucrose. The incubation was carried out for 1 hour at 37°C. Control material was incubated in standard medium without ATP.

Medium B. Tris-maleate buffer, 80 mM (pH 7.2); 10 mM MgSO\(_4\); 5 mM ATP; 100 mM NaCl; 30 mM KCl; 3 mM lead nitrate; 220 mM sucrose. The incubation was done for 1 hour, at 37°C. Control material was incubated in standard medium without ATP or boiled prior to pre-fixation in standard medium.

4) Ultrastructural identification of the age pigment. These studies were done according to the modifications introduced by Hendy (1971) for the ultrastructural identification of lipofuscin-like materials. The material was fixed in a 4% (v/v) solution of formaldehyde, in 0.05M phosphate buffer (pH 7.0), for 24 hours, 1 week or 2 weeks periods. After incubation in either the Fontana's (25 ml of a 10% v/v aqueous silver nitrate solution, ammonia (s.g. = 0.880) added drop by drop, 25 ml distilled water) or Schorml's (3% ferric chloride, freshly prepared 1% potassium ferricyanide, 1:1, v/v) solutions, some of the material was post-fixed in buffered 1.5% (v/v) OsO\(_4\). Incubation procedures were as described by Hendy (1971) and
subsequent handling of the material for electron microscopy was as described before. Material fixed according to the above procedure, but incubated in neither the Fontana's nor the Schorml's solutions was used as control to determine the similarities in morphology and localization of the labelled inclusions.

5) Freeze-etch. The material was pre-fixed for 1 hour in a 4% (v/v) solution of glutaraldehyde, in 0.1M cacodylate buffer (pH 7.0), then transferred to 25% (v/v) glycerol in distilled water for periods of time ranging from 2 to 24 hours. The material was then quenched in Freon 22, prior to freezing in liquid nitrogen. The frozen material was fractured at -100 C and etched for 30 to 60 seconds using a Balzers BA 360M device.

All preparations were viewed with a Zeiss EM 9A electron microscope. The ultrathin sectioning was done using either a duPont diamond knife or glass knives on a L.K.B. ultratome I or a Reichert OMU3 ultramicrotome. Both stained (Reynolds, 1963) and unstained sections were used in these studies.
OBSERVATIONS

PART I--LIGHT MICROSCOPIC OBSERVATIONS

Studies of living filaments revealed features which were useful in interpreting the electron microscope results. The filaments were made up of a variable number of cells. The cells, rectangular in shape, measured an average of $2.5 \times 10^{-3} \times 2.0 \times 10^{-3} \text{ cm}$ in the case of the erect system and $2.0 \times 10^{-3} \times 1.7 \times 10^{-3} \text{ cm}$ in the case of the prostrate system. Other observations can be summarized as follows: 1) chloroplasts were found to be polymorphic (figures 4, 6, and 7); 2) different stages of pyrenoid division were observed (figure 8); this seems to occur by the fission of the pyrenoid body; and 3) conspicuous wall ingrowths were apparent, both along the side walls as well as at the cross walls (figures 10 and 11).

Observations of erect and prostrate filaments of the sporophytic generation of *Ectocarpus* sp., after glutaraldehyde-osmium fixation, indicate that the various cells in each filament do not respond equally to such treatment. Apical cells are very light in appearance, while the cells below them become progressively darker (figure 1). Towards the basal portion of the filaments the cells again appear to be less dense (figures 2 and 3). In the great majority of the cases studied, the transition between the darker cells and the less dense basal ones was found to be abrupt (figure 3). In a few cases, however, transitional stages were detected. In the region where light cells followed dark ones, the cross walls between cells always protruded into the light cells (figure 3). Light microscope observations of living filaments revealed similar features, showing in addition, that the internal organization of the cells on either side of the cross wall was different.
It is, therefore, apparent that at least 3 major types of cells may be recognized in a filament of *Ectocarpus*.

1) the apical cell and its immediate derivatives; 2) the cells which react intensely with OsO₄; and 3) the cells situated near the basal portion of the filament. It is also safe to assume the existence of transitional stages within the filaments.

In order to learn more about the cytoplasmic differences that might exist in these different types of cells, several cytochemical tests were carried out. Results of these investigations are summarized in the next page. To simplify the description, the following notations will be adopted throughout this work: 1) "cell type #1" refers to the group of cells represented by the apical cell and its immediate neighbors; 2) "cell type #2" refers to the cells which react intensely with OsO₄; 3) "cell type #3" refers to those cells which do not react so deeply with OsO₄ and are located near the basal end of the filaments; 4) intermediate stages between the 'type #1' and 'type #2' and between the 'type #2' and the 'type #3' will be recognized by the notations "transitional 1-2" and "transitional 2-3" respectively. To accurately relate the cells to the proper stage of development; ribbons of 2-3 cells were cut for E.M. examination and only after was the light microscope study undertaken.
<table>
<thead>
<tr>
<th>Cell Type</th>
<th>RNA ( RNA extracted )</th>
<th>DNA ( DNA extracted )</th>
<th>Proteins</th>
<th>Lipids</th>
<th>Insoluble Carbohydrates</th>
<th>Lipofuscin</th>
<th>Polyphenols</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Nucleus small, round to profile. Chromatin reaction particularly distinct. Nucleus unaltered. Figure 12.</td>
<td>Cytoplasm intensely stained. Nucleus with a clear positive reaction (arrow). Nucleus outline visible. Figure 15.</td>
<td>Intense reaction almost throughout the cytoplasm, more so in pyrenoids. Figure 18.</td>
<td>Few reacting spots. Figure 19.</td>
<td>P.A.S. reaction well visible in the cell wall. Figure 22.</td>
<td>Almost undetectable</td>
<td>Very few reaction sites.</td>
</tr>
<tr>
<td>Transitional &quot;1-2&quot; cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>Nucleus small and irregular in profile, but still conspicuously stained. Nucleus unaltered. Figure 13.</td>
<td>Stained area restricted to the perinuclear region. Nucleus unaltered and very irregular in profile. Figure 14.</td>
<td>Staining restricted to some cytoplasmic spots, pyrenoids well stained. Figure 19.</td>
<td>Reaction found apparent near the periphery. Reaction sites throughout the cytoplasm. Figure 21.</td>
<td>P.A.S. reaction well apparent in the cell wall.</td>
<td>Conspicuous reaction sites throughout the cytoplasm. Figure 24.</td>
<td>Reaction almost or totally absent. Figure 27.</td>
</tr>
<tr>
<td>63</td>
<td>Nuclei could not be detected. Figure 16.</td>
<td>Cytoplasm almost unaltered. Figure 17.</td>
<td>Very faint or negative staining throughout the cell. Figure 18.</td>
<td>Reaction spots few and sparse. Figure 19.</td>
<td>P.A.S. reaction well apparent in the cell wall.</td>
<td>Reaction almost or totally absent. Figure 20.</td>
<td>Little or almost undetectable reaction.</td>
</tr>
</tbody>
</table>
PART II.—ELECTRON MICROSCOPE OBSERVATIONS

Electron microscope observations substantiate evidence from light microscopy for the existence of 3 main types of cells within the filaments of Ectocarpus, and whose characteristics are described in the following pages.

A) ULTRASTRUCTURAL FEATURES OF YOUNG CELLS (CELL TYPE #1)

The general morphology of these cells in both the erect and prostrate systems of Ectocarpus was found to be similar (figures 28a, 31, 32, and 33). Therefore, the following descriptions apply to cells of both systems.

Nucleus. The nucleus (figures 28a, 31, 32, and 33) is bound by a double membrane envelope interrupted by numerous pores. Freeze-etching studies of the nuclear envelope reveal the existence of surfaces with few particles on them, while others are multiparticulate (figure 36). Nuclear pores are easily identified in freeze-etching preparations, but their morphology varies according to the exposed surface. This is particularly apparent in figure 36 where the face 'A' pores have a crater-like appearance and the ones on face 'B' appear as circular depressions. The pores, measuring approximately 80 nm in diameter, are round in appearance and reveal a defined substructure (figures 29, and 36). They seem to possess a hollow central core from which spokes radiate toward the edge of the pore (figure 29, arrowheads). The pore rim appears to be made up of several globular units, whose number is difficult to determine with accuracy. The nuclear pores in figure 37 are not uniformly distributed throughout the nuclear membrane, but tend to occur in certain areas and not in others.

Sections of the interphase nucleus of young
vegetative cells of *Ectocarpus* sp. usually contain one conspicuous nucleolus (figures 31, 32, 33, and 38), which seems to consist of two distinct components (figures 32, and 38). One component is fibrillar, measuring approximately 8 nm in diameter. The other component is granular, with each unit measuring approximately 15 nm in diameter. The nucleolus usually occupies a central position in the nucleoplasm, but in some cases its association with the inner membrane of the nuclear envelope is apparent (figure 38). In this case, the region of the nuclear envelope closely associated with the nucleolus seems to be devoid of nuclear pores.

Chromatin is not conspicuous, although its distribution throughout the nucleoplasm as well as its relationship to the inner membrane of the nuclear envelope is obvious in some micrographs (figures 28a, and 32). At high magnification the fibrillar nature of the chromatin is visible (figure 30). Each fibrillar unit measures approximately 10 nm in diameter.

The perinuclear space is abundantly filled with granular-fibrillar material (e.g. figures 30, 32, 38, 55, and 57). In addition, small vesicle-like structures (figures 38, arrow, 39, 40, 41, arrowheads), whose relationship to the nuclear envelope membranes is shown in figures 41 (arrowheads) and 45 (pns), are also found inside the perinuclear space. More elaborated structures are found in close association with the outer membrane of the nuclear envelope (figures 38, empty arrowhead, and 42, arrowhead). Besides the above mentioned features the association between the outer membrane of the nuclear envelope and the forming face of the dictyosomes is a consistent aspect of *Ectocarpus* cells (e.g. figure 34).

Endoplasmic reticulum (*E.R.*). Most of the endoplasmic reticulum present in these cells seems to be of the
"rough" type (figures 28a, 32, and 44). Cisternal elements are fenestrated (figures 32, black arrow, 38, big arrowheads, and 44, arrowhead). Fenestrae-like formations, are also detectable in freeze-etching preparations (figure 43a, arrowhead). Some fenestrae display a crater-like appearance (figure 43b, arrowhead).

Crystalline inclusions are observed inside dilated portions of the E.R. cisternae (figure 44, i).

E.R. elements are distributed throughout the cytoplasm (figures 28a, 31, 32, and 33). In a few micrographs the E.R. elements are observed to display concentric arrangements (figure 44). In others, an orientation of the E.R. in relation to the nuclear envelope is apparent (figures 32, and 38). Continuity between the E.R. cisternal space and the perinuclear space is observed (figure 38). The E.R. cisternal space is occupied by granular-fibrillar material (figures 32, 38, and 45) and vesicular structures (figure 45) similar in appearance to those found inside the perinuclear space. In a few micrographs, multivesicular-like structures are observed at the junction of the perinuclear and E.R. cisternal spaces (figure 38, empty arrowhead).

Endoplasmic reticulum membranes are also observed to establish spatial relationships and/or direct communication with several other organelles, i.e. associations between the endoplasmic reticulum and the outer membrane of the mitochondrial envelope (figures 35, arrowhead, and 72, arrow), and with the chloroplast (figures 44, 47a, b, 48), thereby forming a special section of the E.R. commonly known as the 'chloroplast E.R.' (Bouck, 1965).

The chloroplast E.R. follows the chloroplast envelope around the projecting pyrenoid (figure 28a). Tubular-vesicular structures arise from the chloroplast E.R. membrane facing the chloroplast envelope (figures 32, 46,
and 48). These structures can be observed in tangential view as a very complex network of tubules (figure 49). Intracisternal structures similar to those described inside the E.R. cisternal space are found inside the cisternae of the chloroplast E.R. (figure 50).

Dark osmiophilic bodies with a complex internal structure, referred in this work as "osmiophilic structured bodies" (OSB), are also found in association with the chloroplast E.R. (figures 45, 51, and 52). They can occupy the area shared by both the chloroplast E.R. and the nuclear envelope (figure 52), or they may occur in close association with pyrenoids (figure 53). High magnification studies show these bodies to have two major components: 1) a very dark osmiophilic and unstructured material (figures 51, and 53); and 2) a myelin-like component (figures 51, 53, and 54). The arrangement of the two components in relation to each other is variable, and accounts for the heterogeneity of forms displayed by these bodies (figures 45, 51, 52, 53, 54, and 55). In some cases, the origin of the "OSB" can be traced directly from the chloroplast E.R. or from a complex system of membranes arising by further elaboration of the chloroplast E.R. (figures 45, 47a, 47b). In other cases, the "OSB" are found in the cytoplasm apart from the chloroplast E.R. (figure 56). In some instances they are apparently discharged into the paramural space (figure 54).

**Golgi complex.** The Golgi complex, an exclusively perinuclear organelle, is represented by groups of 2, 3, or more dictyosomes (figures 28a, 31, and 33). Each dictyosome is made up of 6 to 10 stacked cisternae, of which those closest to the nuclear envelope are conspicuously fenestrated (figures 57, and 58, arrowheads). The outer cisterna is usually hypertrophied in appearance (figures 28a, 33, 58, 59, and 61). Observations of dictyosomal cisternae at high magnification reveal the presence of two
sizes of interruptions or channels. One type has an average diameter of 25 nm (figures 57, and 83; arrowheads labeled 'a'). The others average 5 nm in diameter (figures 57, 83, arrowheads labeled 'b').

Direct continuity between adjacent cisternae is frequently observed (figures 33, 42, and 62), and the presence of intra- as well as intercisternal bridge-like filaments is also apparent (figure 59, arrowhead).

As in the case of other brown algae (Bouck, 1965; Bourne and Cole, 1968; Cole, 1969, 1970; Bisalputra et al., 1971) a close association between the outer membrane of the nuclear envelope and the formative face of the dictyosomes is evident in Ectocarpus sp. This relationship is interpreted as being indicative of the transfer of material from the perinuclear space to the dictyosomes by means of vesicles arising from the outer membrane of the nuclear envelope (figures 61, and 83). Direct continuity between the perinuclear space and the innermost cisternae of the dictyosomes occurs in some cases (figure 34, arrowhead).

Besides the ubiquitous association of dictyosomes with the nuclear envelope, associations also occur between dictyosomes and other cell organelles; for instance, with mitochondria (figure 61), with pyrenoids (figures 60, and 63), and with large vacuoles. In this case, incorporation of dictyosome-derived vesicles into vacuoles is apparent (figure 62, big arrowhead). Evidence for the release of dictyosome-derived material into the paramural space is obtained when comparing the inclusions (labelled 'L') inside the dictyosome cisterna (figure 64) with similar inclusions inside the paramural space (figure 49). This type of inclusion seems to arise from invagination of the dictyosome cisternal membrane and often shows a high degree of morphological elaboration (figure 65). Occasionally, tube-like inclusions are also found inside dictyosome cisternae (figure 66). A close association between osmiophilic
structured bodies (OSB) and dictyosomes is sometimes observed (figure 55).

**Mitochondria.** Mitochondria are usually found scattered throughout the cytoplasm (figures 28a, 31, 32, and 33), although in some cases they tend to accumulate close to the cell periphery (figure 56). Images suggesting mitochondrial division are also detected (figure 62, small arrowhead). Ultrastructurally these organelles (figures 38, 68, 84) do not appear to be different from those already described in other brown algal studies (Bouck, 1965; Bisalputra and Bisalputra, 1967; Bourne and Cole, 1968; Cole and Lin, 1968; McCully, 1968; Cole, 1969; Liddle and Neushul, 1969). Since they are polymorphic, their profile may be quite elaborated (figure 67). Distinct genophore areas may be observed (figures 38, and 84, arrowheads). Inclusions are noticed inside the cristae (figures 68, and inset). At high magnification, the inclusions appear to be circular with a hollow central core, with filaments radiating from the rims towards the cristae membranes.

Mitochondria are found in association with almost all the cell organelles. Mitochondria-dictyosome and mitochondria-endoplasmic reticulum associations have already been reported. Other associations are with the nucleus (figure 69), the chloroplast (figures 48, and 70), the pyrenoid (figure 71), and with themselves (figure 72, arrowheads).

**Chloroplasts.** Chloroplasts are bounded by a double membrane envelope, outside of which lies the chloroplast E.R. (figures 28a, 32, 33, 46, 47a, 47b, and 48). Direct continuity between the cisternal space of the two above mentioned membrane systems is detectable (figures 47a, 48, arrowheads).

Fenestra-like interruptions can be observed along the chloroplast envelope membranes both in longitudinal as
well as in tangential view (figures 76, arrowhead 'c', and 84, arrowhead 'b', respectively).

The photosynthetic lamellae consist of bands of 3 closely associated thylakoids (figures 76, and 79). Each thylakoid measures approximately 11 nm in width. The space between thylakoids of the same band as well as between neighboring bands has a high degree of uniformity, except for those regions where plastoglobuli intervened or where exchanges of thylakoids between adjacent bands occur. Occasional exceptions to this regularity are observed (figure 75). Bifurcation of thylakoids is observed (figure 85, arrowhead). Within the thylakoids interruptions are seen in longitudinal (figures 73, big arrowhead, and 76, arrowheads 'a') as well as in tangential views (figures 78, arrowheads, and inset; 84, arrowhead 'a'). In tangential view the interruptions seem to be pore-like in appearance. In some areas, thylakoid bands are interrupted by large portions of stroma material (figures 46, and 92 'F').

When freeze-etched thylakoids of *Ectocarpus* reveal two types of fracture surfaces. These (figures 80, and 81) have either many particles (surfaces 'B') or only a few (surfaces 'A').

The peripheral band of thylakoids follows the contour of the inner membrane of the chloroplast envelope, enclosing the centrally located bands (figures 28a, 31, 32, 33, and 46). In some cases, the peripheral band is not strictly peripheral, but is continuous with the centrally placed ones (figure 82, arrowheads). The central bands normally traverse the chloroplast without interruption from tip to tip or terminate just short of the tips (figures 28a, 32, 33, and 48).

In some cases, thylakoids have a highly folded arrangement (figure 85) or may be arranged concentrically (figure 86). The whorled arrangement of thylakoids is
apparently related to the abscission of certain plastid portions (figures 87, and 88). Abscissed portions seem to be incorporated into vacuoles (figure 91). Acid phosphatase activity was found associated with these formations (figure 89), but was absent from the control preparations (figure 90).

Fibrillar bridge-like elements are observed inside and between thylakoids (figures 73, 76, 79, small arrowheads). Fibrillar elements were most noticeable in sections cut tangentially to the thylakoids (figure 96, white arrowheads). The fibrillar material is not restricted to the areas mentioned above, but is also observed in the space between the peripheral thylakoid band and the chloroplast envelope (figure 92, arrowheads). The presence of fibrils inside the chloroplasts was further confirmed by freeze-etching studies (figures 43a, 74, and 95, arrowheads). The dimensions of the fibrillar elements were found to range from 30 to 5 nm in diameter, with most of the measurements averaging 3.7 to 4.2 nm.

In the stroma, ribosome-like particles and plastoglobuli are detected (figures 32, and 94). Plastoglobuli possess a definite infrastructure composed of fibrils embedded in an amorphous and less electron dense matrix (figures 97, 98, and 99). The plastoglobular fibrils show a clear relationship to the thylakoid membranes (figures 97, and 98, arrowheads). In figure 99 direct continuity between the fibrillar phases of two adjacent plastoglobuli is observed.

DNA-containing regions at the tips of chloroplasts (figures 47a, and 48, 'g') are, as shown by Bisalputra and Bisalputra (1969), part of the genophore of these organelles.

In *Ectocarpus* sp., as in other algae (Evans, 1966; Bisalputra and Bisalputra, 1970), chloroplast division is not synchronous with cell division. As a result, various stages
of chloroplast multiplication are observed in the active cells of the filaments. Processes of chloroplast multiplication were described in the brown algae (e.g. von Wettstein, 1954; Cole and Lin, 1968; Cole, 1970; Bisalputra and Bisalputra, 1970). In the case of *Ectocarpus* sp., more than one type of chloroplast division seems to take place in the sporophytic vegetative cells of both the erect and prostrate systems. Figures 102 and 103 depict two stages of a process of division. This process of chloroplast division resembles the chloroplast division of *Sphacelaria* (Bisalputra and Bisalputra, 1970). In figure 103 the stage of division seems to correspond to the onset of the formation of the peripheral lamellar bridge (arrowhead). Figure 101 shows a division by longitudinal constriction. A similar event has been recorded in *Laminaria* gametophytes (Bisalputra et al., 1971). Figures 105 and 106 are non-consecutive serial sections of another aspect resembling chloroplast division. Apparently, this process involves the progressive constriction of thylakoids without the formation of a peripheral lamellar bridge. The process closely resembles that occurring in *Egregia* chloroplasts (Bisalputra, unpublished data). Figure 107 suggests the possibility that a chloroplast is undergoing division at two constriction sites. Multiconstricting chloroplasts were described in *Fucus* by von Wettstein (1954). In dark treated material conspicuous chloroplast blebbing somewhat resembling proplastid multiplication is observed (figure 104). The bleb-like structure contains only stroma and is delimited by both the chloroplast envelope and the chloroplast E.R.. Since this phenomenon is only found in dark treated material, it is more likely to be a reaction of the organelles to dark treatment.

**Pyrenoid.** In *Ectocarpus*, generally only one pyrenoid is seen projecting from the chloroplast on the side facing the
nucleus (figure 28a). However, the presence of two or more pyrenoids per chloroplast is not uncommon (figure 108, see also 6, and 9). Most likely this is related to the phenomenon of pyrenoid division (figure 8). Gibbs (1962a) has shown that the pyrenoid matrix of *Pylaiella* consists of tightly packed fibrils 6.5-7.0 nm in diameter. Similar fibrillar elements are seen in the matrix of *Ectocarpus* pyrenoids (figure 109). A very close relationship is observed between pyrenoid filaments and thylakoids in some micrographs (figures 93, and 108), particularly in dark treated material (figure 93).

In addition to the chloroplast envelope and the chloroplast E.R., a third membrane system designated as the "pyrenoid sac" or "pyrenoid cap" is found outside the chloroplast E.R.. This membrane system is restricted to the area around the pyrenoid body (figure 28a). The relationship existent among these three membrane systems is better observed in figure 110. Bouck (1965) and Cole (1969) reported that no connection exists between the outer membrane of the pyrenoid sac and any other cytoplasmic organelle. In *Ectocarpus*, however, direct communications seem to occur between the pyrenoid and dictyosomes (figures 60, and 63), and the pyrenoid and the vacuole (figure 112).

Pyrenoid division is not synchronous with chloroplast division (figures 8, 9; see also Evans, 1966). Images of pyrenoid division are frequent (figures 109, and 114); in those situations the pyrenoid sac is noticeably absent (see also Manton, 1966a).

The cell wall and associated structures. The cell wall is composed of fibrils arranged in two distinct zones separated by an abrupt boundary (figure 142). The inner layer (il) is made up of roughly parallel microfibrils and is very compact. The outer layer (ol) is formed by loosely associated microfibrils arranged in a reticulate pattern. The bilayer...
organization of the cell wall is also evident in freeze-etched preparations (figure 117). In this picture inclusions of unidentified origin are observed inside the inner layer of the cell wall. Osmiophilic material can be seen inside the paramural space (figure 47). Other types of inclusions occupy the paramural space. The origin of some of these can be traced from the plasmalemma itself (figure 28b, arrowhead). These structures can attain a high degree of morphological complexity (figures 28a, 28b, 102, pm). Following the classification of Marchant and Robards (1968), these structures ought to be designated as "plasmalemmasomes." They are easily recognizable in freeze-etching preparations (figure 37a). However, as was previously considered, one has the impression that some of the paramural structures may have been derived from dictyosomal activity (c.f. figure 64--"L" with figure 49--"L"). These structures should, therefore, be designated "lomasomes" (Marchant and Robards, 1968). Plasmodesmata are observed in the cell walls of adjacent cells (figure 111), but they are not organized into pit-like areas.

B) ULTRASTRUCTURAL FEATURES OF THE "TRANSITIONAL 1-2" CELLS

Vacuole formation and processes of autophagy. One of the main processes for the transformation of the young cell (cell type #1) architecture into that characteristic of the "cell type #2" is related to vacuole formation and autophagy. In this section these events are analysed in detail.

1) Vacuole origin. In Ectocarpus sp. sporophytic vegetative cells, both endoplasmic reticulum and dictyosomes contribute to the development of the vacuolar system. This is indicated by the existence of direct continuity and/or close
association between elements of the endoplasmic reticulum and vacuoles (figure 100, arrowheads), as well as E.R.-derived provacuolar profiles, which show a tendency to fuse with each other and with fully developed vacuoles (figures 116, arrowhead, 148, arrows). The role of dictyosomes in vacuole development is depicted by the incorporation of dictyosomal vesicles into vacuolar structures (figure 62, big arrowhead).

2) Autophagic activity. An analysis at the ultrastructural level of how cytoplasmic areas and/or organelles are isolated from the remainder of the cytoplasm, become transformed, and gain access to vacuoles is rendered difficult due to the fact that one is studying static pictures of a highly dynamic phenomenon. Bearing this in mind, and after examination of multiple micrographs, it seems that autophagy in Ectocarpus sp. can take place by more than one mechanism.

One of the mechanisms seems to involve the isolation of cytoplasmic portions by the endoplasmic reticulum (figures 119, 120, 121). The isolated area is, hence, surrounded by a double membrane system. Continuity between these concentric E.R. and normal cisternal elements is apparent at an early stage of isolation (figures 120, 121). The isolated regions are gradually transformed into typical vacuoles (figures 122, 123, and 127).

In other cases, access to the vacuolar space is gained by a different mechanism, which seems to involve the invagination of the tonoplast in the region where the material to be isolated contacts or approaches the vacuole boundary (figure 145, arrowheads). This process appears to be responsible for the incorporation of abscissed plastid parts into vacuoles (figures 145, upper arrowhead, see also figure 88). Invagination of tonoplast with a subsequent intake of cytoplasmic material is also seen in figure 124.
This mechanism differs from the E.R.-dependent mechanism in that the isolated material was not segregated from the cytoplasm prior to its uptake by the invaginating tonoplast.

3) **Autophagic activity**—evidence from cytochemistry of acid phosphatase. Although acid phosphatase activity was detected in association with cisternal E.R., cisternae of dictyosomes, and provacuole-like structures (figures 129, and 130); recently isolated cytoplasmic areas show no acid phosphatase activity. Control material fails to show any signs of reaction products in association with the above mentioned organelles (figure 131). As the process of degradation of isolated material proceeds, its appearance becomes more and more altered and membranous remains become visible inside the vacuoles (figure 49). Acid phosphatase activity was found to be associated with these formations (figures 125, and 126), but absent from controls (figure 131). Further vacuolation and degradation of isolated material leads to loss of recognizability of organelles and cytoplasmic remnants inside the vacuoles. Instead, two types of inclusions are observed: 1) a homogeneous granular substance (figures 135, 136, 'a'), and 2) a polymorphic myelin-like figure (figures 135, 136, 'b'). The granular type of inclusion is found alone or in association with the myelin-like component (figures 135, 136, and 137, 'a'). The myelin-like form, however, does not occur by itself.

With greater vacuolation and cellular digestion, the cytoplasm of the cells becomes more and more altered (figures 135, 136), approaching finally (figure 128) the cellular organization of "cell type #2" (figures 148, 151), which is described below. The increase in acid phosphatase activity parallels vacuolation and degradation of cytoplasmic materials (figures 138, and 139). Reaction products are absent from control sections (figure 140).
4) Other cellular alterations. Other changes in cellular ultrastructure contribute to the establishment of the "cell type #2" morphology. These include: 1) a change in thylakoid orientation into patches of large thylakoid stacks (figure 132); 2) the occurrence of electron dense metabolites at the cell wall periphery and among the fibrillar elements of the cell wall (figure 142); and 3) the appearance of localized cell wall ingrowths (figure 163, cwi), a phenomenon that coincides with the observation of high activity at the Golgi complex level (figure 141). This problem will be dealt with in detail later.

C) THE SENESCENT CELLS - ULTRASTRUCTURAL FEATURES OF THE "CELL TYPE #2"

Since all biological phenomena represent dynamic and continuous situations the determination of a boundary between the "transitional 1-2" cells and the "cell type #2" is difficult. To solve this difficulty it is established that whenever chloroplasts show increasing signs of production of highly electron dense metabolites together with altered thylakoid arrangements (figures 143, 148, 149, 151, 152, 154, and 156), the cell is considered as belonging to the "cell type #2".

The ultrastructural morphology of these cells was found to be similar in the erect and the prostrate systems (figures 143, 148, and 151). Therefore the following descriptions apply to cells of both systems.

Nucleus. The nucleus remains clearly discernible at this stage of cellular development. The nuclear boundary has become irregular (figures 143, and 148). Nuclear pores are difficult to detect. Chromatin-like material can be seen in some cases either throughout the nucleoplasm or in association with the inner membrane of the nuclear envelope.
The nucleolus, when apparent, is comprised of both fibrillar and granular elements (figure 153). The association between the outer membrane of the nuclear envelope and elements of the endoplasmic reticulum and dictyosomes are still maintained (figure 143).

**Endoplasmic reticulum.** The E.R. system becomes less prominent in these cells than in the "cell type #1" (figures 143, 148, 151). This is due, at least in part, to the disorganization of the E.R., which is caused by the vesiculation of its elements (figure 143, 151, regions labelled "E.R."). These vesicles then become incorporated into vacuoles (figure 151). In these areas, ribosomes of any form are difficult to detect. They are, however, observed in less altered cytoplasmic regions (figure 153).

**Golgi complex.** The Golgi complex is still well represented at this stage of cellular differentiation (figures 143, 148). Its functional association with the outer membrane of the nuclear envelope is evident. However, the usual hypertrophied appearance of the outermost cisterna of the dictyosomes, so characteristic of the "cell type #1", is no longer observed.

**Mitochondria.** Mitochondria are very difficult to detect at this stage of aging. More favourable sections allow one to account for scarcely more than a double membrane envelope and cristae (figure 153).

**Chloroplasts.** Of all cell organelles, chloroplasts exhibit the most striking alterations which have been employed as a criterion for identification of the "cell type #2".

The appearance of patches of highly electron dense metabolites is visible at many sites inside the chloroplasts (figures 143, 148, 151, 152, arrows). The intraplastid metabolites, which start as small scattered formations (figure 143), become bigger and more numerous as senescence proceeds (figures 148, 151). The regions where the
metabolite accumulates present conspicuous patterns of thylakoid arrangement (figures 149, 154, 156, 157). The relationship between the metabolite and the special constant arrangement of thylakoids suggests involvement of thylakoids in the production of the metabolite. This relationship is also apparent in freeze-etched material (figure 159, arrowhead). Permanganate fixation could preserve neither the intraplastid regions where the metabolite is found nor the plastoglobuli (figure 133, arrows). This implies a possible lipid nature to the intraplastid metabolite; a possibility further confirmed by its affinity for Sudan Black B. The intraplastid metabolite seems to be released into the cytoplasm (figures 155, and 157), contributing to the osmiophilic appearance of the cytoplasm. Plastoglobuli are easily distinguished from the osmiophilic metabolite described above. There is no relationship between the two materials, because: 1) both structures are present at the same time and are morphologically different (figures 143, 148, 151); 2) an increase in the number of metabolite containing regions occurs with advancing senescence, while the number of plastoglobuli remains, at this stage of aging, nearly constant; and 3) plastoglobuli bear a different type of relationship with thylakoids.

Pyrenoids. Pyrenoids are easily detectable (figures 143, 148). The fibrillar nature of the pyrenoid matrix is no longer apparent, nor are the boundary membranes.

Cytoplasm morphology. Figures 143, 148, 150, 151, 160 depict the condition of the cytoplasm in "cell type #2". The development and origin of cytoplasmic inclusions has already been presented.

It can be summarized that three main types of cytoplasmic inclusions are present. Firstly, myelin-like inclusions (figures 148, 150), at least partially preserved after permanganate fixation. The preserved portion represents
the non-myelinic component (figure 134, arrowhead '2'). The second is a granular type of inclusion (figures 148, 150) which is preserved by permanganate fixation (figure 134, arrowhead '1'). Both types of inclusions have been shown to have lysosomal origins. A third type of inclusion is expected to be present in the cytoplasm of these cells. This type of inclusion would correspond to the intraplastid metabolite, since its discharge into the cytoplasm has been demonstrated. Its presence in the cytoplasm is further substantiated by comparing the behaviour of the different types of inclusions after permanganate fixation. Of the 3 types of inclusions reported, only one shows a response similar to that of the intraplastid metabolite, i.e., non-preservation using the permanganate fixation (c.f. figure 133, white arrow with figure 134, arrowhead '3').

Cell wall. The cell wall as a whole seems to have increased in thickness (figure 160). However, neither its thickness nor structural complexity are uniform, since conspicuous cell wall ingrowths are observed. The cell wall ingrowths are made up of metabolites trapped between the old layers of the cell wall and the newly formed ones underneath (figure 171). This phenomenon begins early in the transition "1-2" period, and attains its highest degree of elaboration in "cell type #2". Figures 56 and 63 (cwi) show a very early stage in formation of the ingrowths. At this stage, metabolites, probably of vacuolar origin (figure 153, arrows), begin to accumulate in the paramural space. Figure 151 shows (at arrowheads) new cell wall material laid down in the space between the paramural metabolites and the plasma membrane. Deposition of new cell wall material continues until a conspicuous wall is formed, isolating the metabolites from the plasma membrane (figures 153, 158, '2'). New metabolites can now be discharged into the paramural space between the new cell wall material and the plasma membrane.
giving to the conjunct a layered pattern (figures 152, 158). Deposition of new wall material alternately with discharge of metabolites can produce striking patterns of cell wall ingrowths (figure 161). In the cross walls it can be observed that ingrowths may match similar structures in adjacent cells (figures 158, and 162). Plasmodesmata were seen to run through the newly laid wall material (figure 158, arrowheads). These ingrowths are characteristic of this stage of senescence and are easily detected at the light microscope level in living cells (figures 10, 11, arrowheads), as well as in fixed material (figure 26, arrowhead). Cytochemical tests carried out at the light microscope level have shown that the cell wall ingrowths stain a greenish color after toluidine blue 0 and deep blue after the Schorml's reaction. Cell wall ingrowths formation appears to coincide with an increase in activity of the Golgi apparatus, as indicated by the loaded appearance of the Golgi-derived vesicles (figure 141).

Discharge of metabolites into the external medium also seems to occur (figure 144).

D) THE SENESCENT CELLS—ULTRASTRUCTURAL FEATURES OF "TRANSITIONAL 2-3" CELLS

The degree of difference between the "cell type #2" and "cell type #3" is just as distinct as that between "cell type #1" and "cell type #2". Transitional stages between types "2" and "3" cells are, therefore, to be expected. These transitional stages, however, must be very ephemeral, since they were difficult to find and only on a few occasions were they detected in electron microscopic observations. For the most part, the transition between the type "#2" and type "#3" cells was abrupt (figure 146).

The general morphology of one of these transitional
cells is shown in figure 145. At high magnification, cell morphology does not seem to be strikingly different from that in "cell type #2", but a closer examination shows that the cytoplasm has a higher degree of autolysis (figures 147, 164). Disruption of the tonoplast is common (figure 165, arrowheads). The E.R. system is more and more difficult to detect, although small profiles are still recognizable (figure 177).

Nucleus. The nucleus is rarely observed in cells at this stage of senescence. When present, it shows unmistakable signs of disorganization (figure 170). Disorganization of the nuclear envelope (arrows) seems to occur through the vesiculation of its membranes. This process does not proceed uniformly throughout the nuclear boundary since, as can be observed in figure 170, the nuclear envelope is no longer detectable in certain places. It should be noted that the portion of the nuclear envelope still intact is that facing a dictyosome. Internally to the remains of the nuclear envelope, a disorganized granular-fibrillar material constitutes the remains of the nucleoplasm. No chromatin or nucleolus can be recognized.

Golgi complex. Similarly to the nucleus, dictyosomes are rarely detected. When present (figure 170, D), they are atypical. No steps in the disorganization of these organelles could be followed. Nuclear-envelope-dictyosome associations cease to exist at this stage of cellular senescence; no transfer of material is detectable between the two membrane systems.

Mitochondria. These organelles seem to be more numerous at this stage of cellular senescence than in "cell type #2". They are usually round in profile and small in dimensions (figures 147, 164). Cristae are conspicuous; some of them display a concentric arrangement (figures 174, 175). The matrix shows a rather homogeneous and dense appearance.
Chloroplasts. These organelles possess a morphology reminiscent of that found in "cell type #2". Extensive stacks of thylakoids usually occupy most of the chloroplast body (figures 167, 169, and 172). Occasionally, patches of intraplastid metabolites are seen in association with thylakoid stacks (figure 177, white arrow).

An increase in the number and size of plastoglobuli seems to take place (figures 166, 167, 169).

The most conspicuous changes occurring in the chloroplasts are the budding off of portions of the plastids. Different steps in the formation and release of these plastid-derived bodies are shown in figures 169, 172, 178, 179, 180, 182, 185, and 186. It is apparent from most of these pictures that thylakoids participate in the formation of such bodies. Release into the cytoplasm of plastid-derived bodies is supported by studying figures 167, 169, 176, 181 (arrowheads), where formations similar to those reported above are found either near plastids or elsewhere in the cytoplasm.

Release of these formations seems to involve a process of budding (figures 169, 172, 178, 185). Figures 180 and 182 suggest that fusion of the outer membrane delimiting these bodies with the chloroplast envelope membrane may also be important in achieving their release. The fate of these bodies in the cytoplasm could not be followed, but the sequence of events and the morphological as well as enzymatic characteristics of "cell type #3" suggest that they become degenerated along with all the other cytoplasmic inclusions. The significant result of this phenomenon is the reduction in plastid volume. The chloroplast boundary becomes extremely variable. While some micrographs still suggest the presence of both the envelope membranes and the chloroplast E.R. (figure 177), others show no indication of the chloroplast E.R., outside the usual double
membrane envelope (figures 185, 186). At other places, only one membrane of the chloroplast envelope remains (figure 182). There is a large number of vesicles associated with such a membrane, suggesting the vesiculation of the outer membrane of the chloroplast envelope.

**Pyrenoid.** Pyrenoids are observed at this stage of senescence (figures 166, 167, 169). Although they were not found in "cell type #3" (see next section), the steps involved in pyrenoid disorganization could not be fully elucidated at present, due to the scarcity with which transitional stages were found. Certain micrographs (figure 168, arrowheads), however, suggested the possibility of pyrenoids becoming isolated from chloroplasts. Other micrographs (i.e., figure 183) showed pyrenoidal structures inside vacuoles. Nevertheless, it is difficult to ascertain if such images represent true stages in pyrenoid destruction or are simply the result of peculiar planes of sectioning.

**Cell wall.** The cell wall shows most of the features already mentioned in "cell type #2".

E) THE SENESCENT CELLS—ULTRASTRUCTURAL FEATURES OF THE "CELL TYPE #3"

This cell type represents the last step in senescence and leads to total degeneration and cell death.

Nuclei and dictyosomes are never detected. The E.R. is reduced to remnants. Chloroplast and mitochondria are still recognizable, but show unmistakable signs of disorganization. The background cytoplasm is also highly disorganized (figures 187, 188, 189, 196, 201, and 202). The presence of ribosomes cannot be determined with accuracy. At very advanced stages the plasmalemma seems to have vanished (figure 206), and the disappearance of the cytoplasmic matrix is almost complete (figures 191, 206). Studies of acid phosphatase activity show a uniform distribution
of reaction products throughout the cell cavity (figures 191, 206). Reaction products are absent from the control sections (figure 203).

Mitochondria. Mitochondrial remnants are observed (figures 196, 197, 201, 202). These are round in profile and small in size, with none or very little matrix. The mitochondrial envelope is disrupted in places (figures 201, 202). Vesicular structures, many still attached to the inner membrane, represent the remains of the cristae (figures 196, 197, 202).

Chloroplasts. The morphology of the plastids varies according to the plane of sectioning (figures 187, 188, 189), and/or the degree of degeneration (figures 191, 198). As senescence proceeds, these organelles show a tendency to round up (figure 191). The stroma has almost completely disappeared (figures 190, 191), except in certain areas between thylakoids (figures 193, 194). The remains of the chloroplast envelope consist of a single membrane as described in the last section (figures 190, 198). In more advanced stages of degeneration this envelope membrane breaks down in places, leaving thylakoids in direct contact with the remains of the cytoplasm (figure 191).

The 3-thylakoid band pattern is still recognizable, but there is a tendency for the bands to clump and form stacks of up to 40 or more thylakoids (figures 192, 200). Subsequently, thylakoid breakdown begins (figures 193, 194, 195, 198). Conspicuous deposition of plastoglobuli is also observed (figures 188, 189, 195, 199). In addition, a variation in the electron opacity of plastoglobuli can be seen (figures 195, 199). Genophore-like regions are identifiable in a few degenerating plastids (figure 200, g), but pyrenoids are totally absent.

Cell wall. Plasmodesmata possess acid phosphatase activity, but enzyme activity is absent from control sections (see
figures 204, and 205, respectively).

Although the cell wall exhibits features similar to those reported in "cell type #2" (figure 188), there are signs of cell wall degeneration. In some cells, a loosening of the packing of the wall fibrillar material leads to the appearance of lacuna-like spaces (figure 184). In other cells the disorganization of the outer layer of the cell wall is apparent (figure 207). Figure 208 illustrates another step in cell wall degeneration, where only the inner layer of the wall seems to remain.

F) ENZYME LOCALIZATION

In order to gain a better understanding of the processes of differentiation and senescence, the localization of several enzyme systems was carried out at the ultrastructural level in the various cell types described. The enzyme systems studied included acid phosphatase, already reported elsewhere, catalase, peroxidase, and adenosine triphosphatase. The following are the results of the study.

Catalase. It appears that in young cells (cell type #1), catalase reactions were primarily localized in microbody-like structures (figures 209, 210), although mitochondria also showed some deposition (figure 212). Control sections of aminotriazole-treated material showed no deposition of reaction products in microbodies, but mitochondria had some (figure 211).

Microbody-like organelles are single membrane- bounded and possess a granular matrix (figures 211, 215). In some cases, a non-crystalline core is also apparent (figure 215, arrow). When the core is present, reaction products seem to be preferentially associated with it as well as with the delimiting membrane (figure 249). It is interesting to note the microbody-mitochondrion association (figures 209, 211, 213). Microbody-like structures also
seem to be present in "cell type #2" (figure 213, arrowhead), but were never detected in "cell type #3".

The membrane surrounding the pyrenoid also shows deposition of reduced products (figure 209), but similar results are obtained in control material (figure 211), therefore it is likely that this is not the result of enzyme activity but due to a reaction between DAB and OsO₄.

**Peroxidase.** In "cell type #1" peroxidase activity is primarily associated with the cell wall (figures 214, 216). Control experiments substantiate such conclusions. Figure 218 shows the effect of KCN inhibition on the material. No reaction is found. Deposition of reaction products are also found in the "transitional 1-2" cells. No deposition is detected in "cell type #3."

The presence of peroxidase in "cell type #2" cannot be ascertained with confidence, due to the existence of large amounts of osmiophilic metabolites in between the cell wall fibrillar material (figure 144). Figures 221, and 222 show peroxidase localization and control experiments in "cell type #2", respectively, thus further demonstrating the difficulties in determination of the existence of peroxidase activity.

**Adenosine triphosphatase.** Material (cell type #1) incubated in a medium containing K⁺-Na⁺-Mg²⁺-ATP shows conspicuous depositions of reaction products on the plasma membrane (figures 223, 225), on mitochondrial membranes (figure 224), and in thylakoids (figures 223, 225). Figure 228 shows the control experiment. The absence of reaction products is apparent.

Deposition of reaction products is observed in "transitional 1-2" cells (figure 229), but the enzyme activity in "cell type #2" cannot be determined with certainty due to the characteristics of these cells. Figure 230 shows a control for "transitional 1-2" cells; the
absence of reaction products is evident. ATPase activity was absent from "cell type #3".

The Mg\(^{++}\)-ATP incubated material shows in "cell type #1" reaction products within the mitochondria (figure 226), on the plasmalemma and in thylakoids (figure 227). However, the amount of deposition does not seem to be so intense as in the case of the K\(^+\)-Na\(^+\)-Mg\(^{++}\)-ATP-dependent system. Deposition of reaction products seases when the material is incubated in a medium without ATP (figure 232). Reaction products are apparent in the "transitional 1-2" cells (figure 219), but again it is difficult to confirm their presence in "cell type #2". Reaction products are missing in the cell organelles of "cell type #1".

G) ULTRASTRUCTURAL IDENTIFICATION OF AGING PIGMENT

Many inclusions found in lysosome-like structures present in Ectocarpus resemble the so-called lipofuscin material (aging pigment), which is considered to be one of the most important morphological features of the aging process in both protozoan and animal cells (e.g., Rudzinska, 1961; Strehler, 1962).

In order to test for such material in aging cells of Ectocarpus, two techniques adapted for ultrastructural identification of the pigment (Hendy, 1971) were used in the present study.

Light microscope studies showed that as the cell aged a striking increase in lipofuscin-reacting substances occurred (figure 26). At the electron microscope level the use of Fontana's modified procedure showed the labelling to take place preferentially in vacuolar inclusions (figure 231). Reactions increase significantly in "cell type #2" (figure 233). Furthermore, labelling is restricted
to vacuolar inclusions and does not occur in the plastid-driven metabolites (figure 233, arrows). Schorml's modified procedure increases the electron opacity of some vacuolar inclusions (figure 235). Figure 234 shows vacuolar inclusions after formaldehyde fixation.
DISCUSSION

PART I--THE YOUNG CELLS (CELL TYPE #1)

Cells of *Ectocarpus* within a filament exhibit progressive increases in the tendency to bind osmium from the apex downward, followed by sudden losses in the affinity for osmium towards the basal part of the filaments. Light and electron microscopic observations, supplemented by cytochemical and enzymatic studies, have revealed major and progressive differences in the morphology and metabolic conditions of cells in each filament. Three main cell types are recognized. While "cell type #1" displays ultrastructural features characteristic of non-senescent cells (c.f. Bouck, 1965; Bourne and Cole, 1968; Cole and Lin, 1968; Cole, 1969, 1970; Bisalputra et al., 1971), "cell type #2" and "cell type #3" show unmistakable signs of senescence.

The overall morphology of the nucleus of *Ectocarpus* "cell type #1" is similar to that described in other brown algae (Bouck, 1965; Bourne and Cole, 1968; Cole and Lin, 1968; Liddle and Neushul, 1969; Cole, 1970; Bisalputra et al., 1971). In the resting stage, the nucleus is typified by the inconspicuous appearance of chromatin. Chromatin becomes more apparent as cells approach nuclear division (Cole and Lin, 1968; Cole, 1969). Liddle and Neushul (1969) reported that ultracentrifugation studies of *Zonaria farlowii* oogonium indicated that two granular phases, differing in size and density, could be recognized in the nucleolus. McCully (1968) reported only one granular component in *Fucus* nucleoli. Bourne and Cole (1968) described *Phaeostrophion irregulare* nucleoli as granular structures, while the nucleoli of *Eudesme* were described as irregular dense structures (Cole, 1969). In *Ectocarpus* sp., nucleoli have two distinct components, which in morphology and dimensions closely
resemble those described for nucleoli of higher plants (Lafontaine and Chaourdinard, 1963). A similar organization has been reported in nucleoli of Laminaria meristodermatic cells (Davies et al., 1973).

The association between chromatin and the inner membrane of the nuclear envelope is observed at many sites around the nucleus. This association has been found in other material to occur with the annuli of the nuclear pores complex (Comings and Okada, 1970a; DuPraw, 1970). Nevertheless, evidence also exists that such relationships could take place at sites other than the annuli (Comings and Okada, 1970b). The fibrillar material detected in the nucleoplasm in close relationship with the inner membrane of the nuclear envelope could, according to its dimensions, be homologous to chromatin fibers of interphase nuclei (Zirkin and Kim, 1972).

The functional significance of chromatin-nuclear envelope associations has long been debatable. Several authors (e.g., Comings and Okada, 1970a, 1970b; DuPraw, 1970) have postulated that such an association could be important in determining arrangements of the chromatin fibers prior to their condensation into chromosomes; thereby influencing nuclear division. Other authors suggest that this relationship could have an effect on organization and distribution of nuclear pores (Maul et al., 1971; Thair and Wardrop, 1971; Teigler and Baerwald, 1972). The latter hypothesis could be important in explaining nuclear pore arrangements in Ectocarpus. In Ectocarpus, as well as in Dictyota (Neushul and Dahl, 1972a), freeze-etched preparations of the nuclear envelope show zones of uniformly arranged pores alternating with pore-free regions. Recent studies on brown algal nuclei (Neushul and Dahl, 1972a) have shown that the staining properties of the peripheral nucleoplasm vary with changes in nuclear
metabolism. It is possible, therefore, that the internal architecture of the nucleus influences the pattern of nuclear pores distribution. The absence of nuclear pores from the region of the nuclear envelope associated with the nucleolus is also known in other material (LaCour and Wells, 1972). Although nuclear pores are ubiquitous structures, their fine organization has not yet been completely elucidated, and several structural models have been currently proposed (e.g., LaCour and Wells, 1972; Wunderlich and Speth, 1972). Furthermore, Franke (1970) has suggested that studies on nuclear pore structure should be extended to the 2 nm level before any conclusions can be drawn. Discussion of nuclear pore organization in _Ectocarpus_ is therefore unwarranted at present.

As in the case of _Dictyota_ (Neushul and Dahl, 1972a), freeze-etch studies of the nuclear envelope reveal in _Ectocarpus_ two distinct fracture faces, one representing the outer and the other the inner membrane of the envelope. The scarcity of data, however, makes any interpretation of the freeze-etch images difficult. The problem is further complicated by conflicting opinions on whether the fracture plane occurs along the organelle-cytoplasm interface or passes through the hydrophobic regions of the membranes. Recent evidence (DaSilva and Branton, 1970; Tillack and Marchesi, 1970; Teigler and Baerwald, 1972; Monneron et al., 1972) favors the latter hypothesis. However, the possibility that fracture planes are at the cytoplasm-membrane interface, which has already been defended by Northcote and Lewis (1968), was revived by Thair and Wardrop (1971—see also Robinson and Preston, 1972).

Production of membranous material by the nuclear envelope seems to be fairly common in brown algal cells (e.g., Cole and Lin, 1968; McCully, 1968; Bouck, 1969; Walker, 1971; Neushul and Dahl, 1972a). In the present
study either vesicular or more complex membranous structures are observed, but their functions and final fates are uncertain.

Continuity between the outer membrane of the nuclear envelope and the perinuclear E.R. is frequently observed. Perinuclear E.R. has been detected in other algae (e.g., Massalski and Leedale, 1969; Palandri, 1972). Palandri (1972) has suggested that the perinuclear E.R. arrangement might indicate intense metabolic activities. This might also be the case for *Ectocarpus*, since sections with such arrangements of the E.R. usually contain crystalline deposits inside dilated portions of the E.R.

Continuity between the E.R. and the outer membrane of the mitochondrial envelope has been observed in *Ectocarpus* as well as in higher plant and animal cells (Bracker and Grove, 1971; Morre et al., 1971; Franke and Kartenbeck, 1971). Since details of the functional significance of E.R.-mitochondrial associations were fully discussed by Bracker and Grove (1971) and Franke and Kartenbeck (1971), no detailed considerations will be necessary here. They pointed out that the concept of a continuous channeling system involving all the cisternal cytomembranes should be extended to include the cisternal compartment bordered by the outer and the inner mitochondrial membranes. If such an assumption is valid, it is then legitimate to further extend that concept to include the space between the two membranes of the chloroplast envelope, since its continuity with the chloroplast E.R. cisternal space is obvious in *Ectocarpus*.

The structures designated as osmiophilic structured bodies are abundant in certain cells. Their origin is closely associated with the chloroplast E.R. In some sections it is possible to follow their release into the paramural space. However, their function is unknown. These structures could correspond to the so-called physodes of other authors.
(Feldman and Guglielmi, 1972).

Pore- and bridge-like formations similar to those reported in other plant material (Franke and Scheer, 1972; Franke et al., 1972) are found in Ectocarpus dictyosomes. In all other aspects the Ectocarpus dictyosomes are morphologically similar to those found in other brown algae (Bouck, 1965; Bourne and Cole, 1968; Cole and Lin, 1968; McCully, 1968; Cole, 1969, 1970; Bisalputra et al., 1971).

A distinct association between dictyosomes and nuclei is very much in evidence. Direct continuity between the perinuclear space and dictyosomes can be seen, but in general, the relationship between these two membrane systems is indicated by the transfer of small vesicles from the outer membrane of the nuclear envelope to the formative face of dictyosomes (Bouck, 1965; Bourne and Cole, 1968; Cole and Lin, 1968; Cole, 1969, 1970; Bisalputra et al., 1971). In addition dynamic interactions take place between dictyosomes and pyrenoids (figures 60, 63), mitochondria (figure 61) and vacuoles (figure 112). Of possible significance are the dictyosome-pyrenoid interactions, which could facilitate a direct and more efficient transfer of photosynthates to dictyosomes. It is possible to assume that such a direct transfer route could also affect metabolite composition in relation to the photosynthates transferred via the chloroplast E.R.-perinuclear space-dictyosome pathway (Bouck, 1965). Dictyosomes in Ectocarpus are exclusively perinuclear in position, a feature that according to some authors (Bouck, 1965; Cole and Lin, 1968; Cole, 1969) may be of phylogenetic significance.

The fine structure of mitochondria in active cells of Ectocarpus is similar to that reported in other brown algae (Bouck, 1965; Bourne and Cole, 1968; Cole and Lin, 1968; Cole, 1969; Liddle and Neushul, 1969).

Ectocarpus chloroplast fine structure is also
similar to that reported for other brown algae (Bouck, 1965; Bourne and Cole, 1968; Cole and Lin, 1968; Evans, 1968; Bisalputra and Bisalputra, 1969; Cole, 1969, 1970; Liddle and Neushul, 1969; Bisalputra et al., 1971), except for the presence of filaments and pores in the thylakoids. Filament-like elements are seen joining thylakoids of the same or neighboring thylakoid bands as well as in an intralocular position. So far as I am aware, this is the first report of these structures in brown algal chloroplasts. Nevertheless, Staehelin (1966), applying freeze-etching techniques, was able to report in *Chlorella*, the presence of fibrils 4 nm in diameter running from the plasmalemma through the ground cytoplasm into the chloroplast. In a later work, the same author was able to show that in *Cyanidium caldarium* similar fibrils linked photosynthetic lamellae to one another. The filaments also traversed the loculi of the thylakoids (Staehelin, 1967). In the present study, although dimensions of fibrillar elements were found to be variable, the mean of the averages closely approach those reported by Staehelin (1966, 1967). In *Ectocarpus* as well as in other brown algal chloroplasts (e.g., Bouck, 1965; Bourne and Cole, 1968; Cole and Lin, 1968; Cole, 1969) the space between thylakoids in each band and between thylakoid bands as well as the relationship of the peripheral band to the chloroplast envelope show striking uniformity. It is, therefore, possible that the fibrillar elements described above could play an important role in maintaining space regularities within the chloroplasts. Staehelin (1967) has expressed a similar point of view. This point of view would fit into a broader concept, where establishment of structural uniformity by membrane to membrane linkages may be physiologically advantageous (e.g., Franke et al., 1971a, 1971b, 1972).

Pore-like interruptions are observed in the
thylakoid membranes. These interruptions are probably not restricted to *Ectocarpus* chloroplasts, but their presence has not yet been reported. It is not known if they are permanent features of the thylakoid membranes.

Freeze-etch studies of the aspect and distribution of particles in algal thylakoid membranes were recently carried out (Neushul, 1971). Based on these studies, two types of fracture faces are recognized in the brown alga *Dictyota*. Freeze-etch images of *Ectocarpus* thylakoids seem to be similar to those in *Dictyota*. The exposed faces, as shown in earlier studies (Meyer and Winkelmann, 1969; Neushul, 1970), seem to result from splitting of the thylakoid membranes. Further comments on the significance of these findings seem unwarranted here, since the subject was thoroughly considered by Neushul (1971).

Concentric lamellar bodies of probable plastid origin were found in the cytoplasm of the red algae (e.g. Brown and Weier, 1970). Dawes and Rhamstine (1967) and Sabnis (1969) reported the presence of spherical lamellar bodies in the cytoplasm of the green alga *Caulerpa prolifera*, whose characteristics also point towards a plastid origin. Striking thylakoid patterns have been reported in plastids of *Leathesia difformis* sporelings (Cole and Lin, 1968; Cole et al., 1968), but this was found to be related to a special process of plastid division.

In the present study, concentric lamellar bodies were shown to have a plastid origin. Incorporation of the concentric bodies into vacuoles with associated acid phosphatase activity suggests that this process could facilitate massive transport of plastid material to the cytoplasm, where, after digestion in lysosomal-like fashion, the products can be recovered and reutilized by the cells.

Alterations of the basic pattern of thylakoid arrangement (3 thylakoids/band) were recorded in
Chorda filum (Bouck, 1965), Fucus serratus and Pelvetia canaliculata (Evans, 1968), Leathesia difformis (Cole and Lin, 1968). In Ectocarpus, deviations from the basic pattern also occur. These seem to be due either to the exchange of thylakoids between adjacent bands or to the bifurcation and merging of neighboring bands.

The close relationship between thylakoid membranes and plastoglobuli indicates the possibility of thylakoid involvement in plastoglobular production, a phenomenon well known in chromoplasts (e.g. Harris, 1970; Lichtenthaler, 1970) and plastid senescence (e.g. Lichtenthaler, 1968). In both cases, however, plastoglobuli formation seems to be the result of thylakoid breakdown. In Ectocarpus "cell type #1" no signs of thylakoid breakdown are evident, so plastoglobuli accumulation is most likely the result of an excess of lipid production over the rate of thylakoid assembly. The lipid nature of plastoglobuli is corroborated by their destruction after permanganate fixation. However, Magne (1971) has recently shown that in a wide range of plants, including Ectocarpus crouanii, insoluble polysaccharides are present at the plastoglobular sites. Therefore, the origin of plastoglobuli might be considerably more complex than simply related to thylakoid assembly and their role in chloroplast metabolism more elaborated than previously supposed.

Nowhere else has such a wide range of varieties of chloroplast division been observed in the same plant as in Ectocarpus. This could be the result of an extensive study of all the cells in sequence along the filaments of both the erect and the prostrate systems of Ectocarpus sp., rather than limiting the studies to certain cells. The other and more likely explanation is that images suggesting plastid division, in most cases reflect peculiar planes of sectioning through multilobed chloroplasts (figure 4),
where several interpretations are possible. However, figures 102 and 103 are without question visualizations of true stages of chloroplast division. This conclusion is based not only on the study of Ectocarpus material in serial sections, but actually these images match chloroplast division images in the brown alga Sphacelaria (Bisalputra and Bisalputra, 1970). It seems apparent from the present studies that an interpretation of images suggesting chloroplast division must be carefully considered and not taken to be accurate evidence without intensive studies.

The general morphology of the pyrenoid agrees with that described in other brown algae (see Griffiths, 1970, for a review).

The pyrenoid body contains fibrillar material which is highly reminiscent of that of Pylaieila littoralis pyrenoids (Gibbs, 1962a). In the present material, pyrenoids have a clearly positive protein reaction (see also Rosowski, 1970). No DNA or RNA reactions were detected in the pyrenoids of Ectocarpus, although the presence of both substances have been reported in Tetracystis excentrica (Brown and Arnott, 1970) and in other algal pyrenoids (Esser, 1967; Simon, 1954).

Pyrenoids appear in Ectocarpus as projecting stalked bodies, which usually arise from the side of the chloroplast facing the nucleus. This type of pyrenoid is suggested to have phylogenetic implications (e.g., Evans, 1966, 1968; Bourne and Cole, 1968; Hori, 1971).

In Ectocarpus sp., usually only one pyrenoid is found per chloroplast. Cases of two or more pyrenoids occurring in the same chloroplast appear to be related to the process of pyrenoid division. It is apparent from this study that sometimes a very close relationship exists between the fibrillar elements of the pyrenoid matrix and the thylakoid membranes. The meaning of this relationship requires
elucidation; the possibility exists that a physiological involvement of thylakoids in pyrenoid development might occur. This relationship is particularly apparent in the dark treated material, a treatment which seems to stimulate pyrenoid division (Manton, 1966a).

Bailey and Bisalputra (1969) have described the ultrastructure of the cell wall in *Ectocarpus acutus*. The present findings support their description.

Lomasomes and plasmalemmasomes (Marchant and Robards, 1968) were found in *Ectocarpus* sp. during sporeling development (unillustrated data) as well as in sporophytes. Although these structures seem to be well represented in algae (see Cole and Lin, 1970, for a review), their presence in the brown algae has only recently been reported in sporelings of *Petalonia debilis* (Cole and Lin, 1970). Bracker (1967) suggested 9 different functions for the lomasomes. As indicated by Cole and Lin (1970), evidence concerning the role of these structures is mostly circumstantial, and therefore, difficult to sustain without further investigation. Recent autoradiographic studies, however, implicate these structures in the deposition of matrix materials in the cell wall (Cox and Juniper, 1973).

Plasmodesmatal continuity between neighboring cells in the brown algae was shown by Bisalputra (1966). Since the publication of this work further evidence showing plasmodesmatal continuity in these algae has rapidly accumulated. The organization of these structures in the cross walls adjoining neighboring cells has been suggested as phylogenetically significant (e.g. Cole and Lin, 1968; Hori, 1971). Plasmodesmata are also observed in *Ectocarpus* sp. where the lack of organization into pit areas is apparent.

In conclusion, the ultrastructure of young cells of *Ectocarpus* is not different from that reported in other brown algae. The young cells are typically meristematic
or the immediate derivative of these. These cells are confined to the first 6-8 cells in each filament. They are characterized by a dense cytoplasm, with a well organized nucleus, mitochondria, chloroplasts, stalked pyrenoids, exclusive perinuclear location of the Golgi apparatus, and the non-organization of plasmodesmata into pit fields.

Several of these features (stalked pyrenoid morphology, perinuclear location of dictyosomes, plasmodesmata non organized in pit fields) support the hypothesis of earlier authors (e.g. Fritsch, 1945; Smith, 1955) that *Ectocarpus* is primitive among the brown algae. Nevertheless, other authors (e.g. Bisalputra et al., 1971; Chi, 1971; Neushul and Dahl, 1972a) have warned that careful use of the above mentioned characteristics as phylogenetic markers is necessary until more information regarding their behaviour and occurrence is obtained at all developmental stages.

**PART II - TRANSITIONAL "1-2" CELLS**

**Vacuolation.** Initial steps in differentiation of "cell type #2" involves the process of vacuolation. Vacuoles were reported to arise in a variety of ways, including local dilations of the E.R., special activity of the Golgi apparatus or other structures such as mitochondria, or combinations of these (e.g. Marinos, 1963; Barton, 1965; Ueda, 1966; Mesquita, 1969; Matile and Moor, 1968; Belitser, 1972; Berjack, 1972). The evidence from *Ectocarpus* indicates that vacuoles are the result of E.R. activity, although dictyosomes are also involved.

In addition to the well known role of vacuoles as reservoirs of waste products in plant cells (e.g. Robards, 1970), the discovery of a wide range of associated lysosomal
enzymes (e.g. Gahan, 1969; Matile, 1968, 1969a, 1969b) has led to the concept that vacuoles may function in plant cells as a lysosome equivalent (e.g. Matile, 1969a; Hall and Davie, 1971). In *Ectocarpus* remnants of cytoplasmic structures inside vacuoles as well as associated acid phosphatase activity are clear indicators of the role of vacuoles as lysosome equivalents.

**Autophagy.** Several mechanisms have been described in the literature to explain the origin of vacuolar inclusions; that is, to explain how cytoplasmic material becomes trapped inside vacuoles (see review by Fineran, 1971). In *Ectocarpus* sp. more than one mechanism is involved in trapping cytoplasmic regions inside vacuoles. One of the mechanisms involves the isolation of pockets of cytoplasm by the E. R. The same process of cellular autophagy was reported in other plants (e.g. Buvat, 1968; Mesquita, 1972; Villiers, 1972; Coulomb, 1973; Marty, 1973). The other mechanism of autophagy found in *Ectocarpus* involves invagination of the tonoplast with subsequent incorporation of cytoplasmic material into the vacuoles. This process is found to occur frequently in other plant cells (Coulomb and Buvat, 1968; Matile and Moor, 1968; Wardrop, 1968; Matile and Winkenbach, 1971; Belitser, 1972; Hall and Davie, 1971; Coulomb, 1973). There is also evidence suggesting that the cytoplasm might take an active part in this process of autophagy. A similar situation was described in *Praxinus excelsior* (Villiers, 1972). It is quite possible that in any mechanism of incorporation of cytoplasmic material into vacuoles both the tonoplast and the cytoplasm may take an active part in the process (Fineran, 1971).

Subsequent to accumulation of materials inside vacuoles there is a marked reaction of acid phosphatase activity. Progressive alterations of the vacuolar inclusions are observed; leading to the appearance of two types of
remnant materials. The first of these materials is heterogeneous and comprised of a granular component intermingled with a myelin-like component. The second is simply granular. Morphologically, the "osmiophilic structured bodies" and the mixed vacuole remnants are much alike, although their origins are quite distinct in both time (the "OSB" appears in almost every young cell before autophagy becomes detectable) and space (the "OSB" originates from the chloroplast E.R. while the mixed remnants are the result of autophagy and lysosomal activity). Nevertheless, the majority of electron-dense inclusions accumulated in these transitional cells can be broadly classified as "residual bodies", which clog the vacuoles. The reason for the clogging of the vacuoles is not understood. Recent work has suggested some possible explanations. Tappel (1968) finds that proteins and membranes are less digestible after being damaged by lipid peroxidation. He suggests that, indirectly, this property contributes to lysosomal engorgement. Another line of evidence shows that the hydrolase content of a cell varies due to the heterogeneity of the lysosomal enzyme population (e.g., Hayashi, 1967; Matile, 1968; Schultz and Jacques, 1971; Hanker et al., 1972). It is, therefore, quite understandable that lysosomal structures may be incapable of digesting the inclusion material, especially when their enzyme complement is not appropriate. This situation can lead to a permanent or temporary engorgement of vacuoles. Comolli et al. (1972) have also suggested that lysosome engorgement could result from changes in the lysosomal enzymes brought about by modifications in their rates of synthesis and/or degradation.

Acid phosphatase activity was found in Ectocarpus to be associated with both the E.R. and Golgi apparatus. E.R.-associated acid phosphatase was demonstrated in a number of plants (e.g., Camefort, 1966; Catesson and Czaninsky,
1968; Figier, 1968; Roland, 1969; Robards and Kidway, 1969; Zee, 1969; Poux, 1970; Coulomb and Coulomb, 1971; Figier, 1972; Gezelius, 1972), as was the dictyosome-associated acid phosphatase (e.g. Poux, 1962a, 1962b; Figier, 1968; Coulomb, 1969; Halperin, 1969; Roland, 1969; Poux, 1970; Coulomb and Coulon, 1971; Figier, 1972; Micalef, 1972; Rougier, 1972). It is not known, however, if the E.R.-associated acid phosphatase and that associated with the dictyosomes possess different hydrolytic potentialities, and therefore, if these findings bear any relationship to the clogging of the vacuolar apparatus.

**Chloroplast.** Also associated with the differentiation of the "cell type #1" morphology into that characteristic of the "cell type #2" is a tendency for chloroplast thylakoids to form large stacks. An increase in thylakoid number with aging was also reported in other plants (e.g. McLean, 1968; Dodge, 1970; Messer and Ben-Shaul, 1972).

**Cell Wall.** The existence of osmiophilic metabolites among the cell wall microfibrils and at the cell wall periphery seems to support Fogg and Boalch (1958) and Armstrong and Boalch (1960) results for the release of metabolites into the culture medium.

During these transitional stages, formation of the so-called cell wall ingrowths (cwi) seems to reach a peak. Simultaneous with ingrowths formation, both electron and light microscope results (localization of insoluble carbohydrates by the P.A.S. reaction) show intense dictyosomal activity, further supporting other authors results that dictyosomes are involved in cell wall development in the brown algae (Cole, 1969) as well as in plant material in general (e.g. Pickett-Heaps, 1967a, 1967b; Wooding, 1968; Barton, 1968).
Several authors consider intracellular accumulation of lipid material to be symptomatic of senescence in both animal (e.g., Takahashi et al., 1970; Howse and Welford, 1972) and plant cells (e.g., McLean, 1968; Schuster et al., 1968; Palisano and Walne, 1972). As one studies the *Ectocarpus* cells further away from the apical tip, it can be observed that simultaneously with an increasing tendency to bind osmium there is an increasing affinity for Sudan black B. Electron microscope studies reveal a variety of inclusions crowding the cytoplasm. After permanganate fixation some of these inclusions are not preserved, while others are only partially preserved; further suggesting that lipid material is present in many of these inclusions. The designation of these cells as senescent seems, therefore, justified. This is further corroborated by the morphology of their cell organelles.

The nuclear boundary is usually irregular, a common feature of senescing cells in both plants (Shaw and Manocha, 1965; Butler, 1967; Berjack and Villiers, 1970, 1972a, 1972b; Briarty et al., 1970; Fabbri and Palandri, 1970; Villiers, 1972) and animals (e.g., Sohal and Allison, 1971; Lipetz and Cristofalò, 1972). Nuclear pores are very difficult to detect. It is not known from the present data if this is due to an increasing difficulty in distinguishing the pores or if this is the result of a reduction in the number of pores due to the low metabolic state of these cells.

Variations in the number of nuclear pores due to the metabolic state of the cells were reported in other biological material (e.g., Afzelius, 1955; Barnes and Davies, 1959; Grasso et al., 1962; Moor and Muhlethaler, 1963; Wiener et al., 1965; Franke, 1967; Franke and Scheer, 1970; Wunderlich
Speth, 1972). The reason for the variations is not understood. However, as suggested by some authors (e.g. Thair and Wardrop, 1971; La Fountaine and La Fountaine, 1973), these variations may reflect the synthetic activity of the nucleus and simultaneously indicate the degree of exchange that takes place between the nucleus and the cytoplasm. The evidence seems also to indicate that cells having a low metabolic activity have a lower number of nuclear pores (e.g. Barnes and Davies, 1959; Grasso et al., 1962; Moor and Muhlethaler, 1963). Particularly pertinent are the results obtained by Moor and Muhlethaler, (1963), who have shown decreases in nuclear pore number to be associated with the aging process in yeast cells. The nuclear envelope ceases to produce vesicles or other membranous structures. Nucleoli, when observed, are similar in organization to those found in "cell type #1". The presence of nucleoli is corroborated by light microscope cytochemistry. Increased nucleolar size with rearrangement of the nucleolar components has been observed in senescing nuclei in other plants (Fowke and Setterfield, 1968; Chapman and Jordan, 1971; Jordan and Chapman, 1971; Jordan, 1972). No similar correlation could be done in *Ectocarpus*.

Some electron micrographs suggest the presence of chromatin. This is confirmed by light microscope data. Therefore, even when other cell organelles (e.g. E.R., chloroplasts) already display a greatly altered morphology, nuclei still give a conspicuous DNA reaction. Nuclei have been considered very resistant to disruption during senescence. (Butler, 1967; Roux and McHale, 1968; Mittelheuser and van Steveninck, 1971). Shaw and Manocha (1965) found that even after nuclei of advanced senescing cells of wheat leaves have lost most of their RNA and some protein, DNA levels remained high.

A decrease in levels of both RNA and protein is
observed in these cells (see summary of light microscope cytochemical data). The present data, however, suggest that such decreases affect primarily the pattern of distribution of these substances. A reduction in the size of RNA and protein staining areas is observed, without any apparent effect on staining intensity. (c.f. figure 15 with figure 16, and figure 18 with figure 19). Reductions in stained areas are easily understood after electron microscope studies, which show the reduction to be mainly due to increasing vacuolation and autophagy. The stability in staining intensity is interesting and indicates that in the remaining cytoplasm RNA and protein contents are comparatively high. Certain characteristics of the nucleus, i.e., nucleolar morphology, DNA content, together with high RNA and protein content indicate the capacity of these cells for active protein synthesis. This does not imply that there is no alteration in the proteins being synthesized.

Protein synthesis is a nucleic acid-directed phenomenon, although much less is known about the mechanisms involved in regulating their synthesis. There is evidence to suggest that a variation in gene expression occurs with different metabolic conditions, that is, some genes are expressed at one stage of the cell life span but not at others (e.g. Carr and Pate, 1967). This would affect the type of proteins being produced and subsequently cell development, differentiation and senescence (e.g. Heslop-Harrison, 1967; Shannon, 1968; Scandalios, 1969, Spencer and Titus, 1972). Such a possibility has led Woolhouse (1967) to think of differentiation and senescence as two stages of the same process, which is the result of a controlled gene expression (see also Carr and Pate, 1967). Alterations in the amount and type of proteins being synthesized and disruption of normal cell functions can be the result of causes other than those mentioned above. Pelc (1970) thinks of aging as the result of an accumulation of damaged DNA
copies (until no correct copies are left), rather than the result of a controlled expression of genes. The possibility also remains (Johnson and Strehler, 1972) that aging may result from loss of genes coding for ribosomal RNA. Aging-dependent protein variations may also be the result of mechanisms not directly related to protein synthesis (e.g. Simon, 1967; Cherry, 1967). Whatever the explanation, the fact remains that aging is dependent upon the presence and production of certain specific proteins (e.g. Matile and Winkenbach, 1971). An increase in the amounts of lytic enzymes during aging in both plant and animal cells was reported by different authors (Klamer and Fennell, 1963; Elliott and Bak, 1964; Blum, 1965; Sommer and Blum, 1965; Schuster et al., 1968; Grusky and Aaranson, 1969; Elens and Wattiaux, 1969; Cristofalo, 1970; Comolli, 1971; Matile and Winkenbach, 1971; Palisano and Walne, 1972). In *Ectocarpus* sp., as the cell ages, an increase in acid phosphatase activity can be detected by cytochemical means. However, increases in enzyme activity are not necessarily related to de novo synthesis of enzymes (Klamer and Fennell, 1963). The increase in enzyme activity can be due to activation of latent enzymes (e.g. Simon, 1967). More recent evidence seems to suggest that the increase could, at least in part, be the result of an aging-dependent reduction in the rate of enzyme degradation (e.g. Mainwaring, 1968, 1969; Srivastava, 1969; Comolli et al., 1972).

Walne et al., (1970) and Palisano and Walne (1972) have suggested that in plant as well as in animal cells, lipofuscin-like pigments accumulate as the result of aging. In *Ectocarpus* sp. "cell type #2" some of the cytoplasmic inclusions are morphologically reminiscent of the so-called lipofuscin inclusions of aging animal tissues (e.g. Takahashi et al, 1970; Sohal and Sharma, 1972). Samorajski et al. (1965) proposed that such lysosomal inclusions should be
considered as lipofuscin inclusions when myelin or laminated figures could be visualized. However, as recently reviewed by Hasan and Glees (1972), morphological characteristics of lipofuscin inclusions can be quite variable. Therefore, the use of fine structural characteristics as a criterion in identification of lipofuscin does not seem to be a valid one (see also Zeman, 1971). Of the different hypothesis advanced to explain the origin of lipofuscin (see Toth, 1968, and Zeman, 1971, for a review), the one relating them to lysosomes is the most widely accepted (e.g., Essner and Novikoff, 1960; Strehler and Mildvan, 1962; Koenig, 1963; Samorajski et al., 1964, 1965; Goldfisher et al., 1966; Frank and Christensen, 1968; Hirsch, 1970; Brunk and Ericsson, 1972a). Since most of "cell type #2" cytoplasmic inclusions seem to be of lysosomal origin, two techniques adapted by Hendy (1971) for the ultrastructural identification of lipofuscin were applied to the present material.

Toluidine blue staining show an increase in the amount of greenish-turquoise-stained inclusions with aging. These inclusions are, therefore, probably polyphenolic in nature (e.g., McCully, 1966; Evans and Holligan, 1972).

Fontana's silver solution (Hendy, 1971) relies on silver nitrate impregnation to identify lipofuscin material. Silver nitrate, however, is also known to impregnate phenolic compounds (e.g., Chadeau, 1936). Schorml's solution (Hendy, 1971) uses a mixture of iron chloride and potassium ferricyanide to specifically label lipofuscin inclusions. Phenolic vacuoles (e.g., physodes) respond positively to iron salts (e.g., Chadeau, 1936). However, Fritsch (1945) reported that no response could be recorded with iron chloride. Culling (1963) considered Schorml's method to be quite specific for the identification of lipofuscin. In addition, an intense Sudan black B response characterizes "cell type #2". Physodes do not react to Sudan black B (e.g., Chadeau, 1936; Evans and Holligan,
1972), but lipofuscin-like material gives, at least during certain phases of its formation, a strong response to the stain (e.g. Culling, 1963; Pearse, 1972). It seems, therefore, that the evidence points to the possibility of some of the inclusions found in aging cells of *Ectocarpus* to be lipofuscin related.

Gifford (1968) suggested that plastid-derived inclusions believed to contain both lipids and phenolic compounds accumulated inside vacuoles. Pearse (1972) also suggested the possibility of simultaneous occurrence of lipofuscin and materials resulting from transformation of phenolic compounds inside vacuoles. From the present studies, it is impossible to determine whether in *Ectocarpus* phenolic compounds exist independently or are mixed with lipofuscin metabolites.

The implications of lipofuscin accumulation and its relation to cellular metabolism have remained largely obscure. The inclusions have been suggested to represent inert waste products (e.g. Bjorkerud, 1964), but most consider them to interfere with the physiology of the cells, leading eventually to cell death (e.g. Samorajski et al., 1964, 1968; Raychaudhuri and Desai, 1971; Sohal and Sharma, 1972). Hers and van Hoof (cited in Zeman, 1971) postulated that cellular dysfunction was the result of mechanical disturbances created by increasing accumulations of residual material in the cytoplasm of the cells. Accumulation of lipofuscin inclusions, known to contain reaction products of lipid peroxidation and other free radical reactions (e.g. Tappel, 1965; Bjorkerud, 1964), was shown to produce rapid deterioration of the membrane systems of several organelles, including lysosomes (Packer et al., 1967). Membrane deterioration, especially lysosomal membranes, was suggested by Hochschild (1971) to lead to the leakage of hydrolytic enzymes into the cytoplasm; hence profoundly affecting cell metabolism. Recent evidence (Sullivan and
Debusk, 1973) seems to support this hypothesis and suggests that cellular aging may result from membrane damage and the consequent synthesis of altered proteins. Sohal and Sharma (1972) have proposed that the effects of lipofuscin accumulation on the physiology of the cell could be the result of two interrelated phenomena: 1) as lipofuscin accumulates the amount of native cytoplasm is reduced, disrupting normal cellular functions, and 2) the accumulation of lipofuscin creates a new environment around the nucleus that might influence genetic activity. This hypothesis is particularly attractive in the case of *Ectocarpus*. In *Ectocarpus*, most of the accumulation of inclusions coincides with vesiculation of the E.R. Since there is evidence to indicate that the nucleus might be important in maintaining the integrity of the E.R. (e.g. Flickinger, 1968), a possible relationship might exist in *Ectocarpus* between inclusion accumulation and E.R. vesiculation. The process of E.R. vesiculation has often been noted in other senescing plant material and has been interpreted as a sign of E.R. degeneracy (Shaw and Manocha, 1965; Bain and Mercer, 1966; Butler, 1967; Treffry et al., 1967; Villiers, 1972; Potapov and Krishnamurthy, 1972).

The disorganization of the E.R. is also expected to affect the ribosomal population of these cells. Shaw and Manocha (1965) reported that disappearance of the E.R. was simultaneous with that of ribosomes. Fukazawa and Higuchi (1966) provided evidence for the existence of a correlation between a reduction in the ribosomal population and a reduction in the E.R. However, Mittelheuser and van Steveninck (1971) have shown that ribosomes possess a greater stability than other cell organelles. Berjack and Villiers (1972b) reported that even in stages of extreme disorganization monosomes could be found randomly scattered throughout the cytoplasm (see also Opik, 1966; Berjack and Villiers, 1970; Mia, 1972). On the contrary, Palandri (1972) showed that
although the E.R. is still well represented, most of the ribosomal population has disappeared from senescing cells of *Halimeda tuna*. In *Ectocarpus*, small clusters of particles are still found here and there during early stages of "cell type #3". However, their ribosomal nature could not be determined and so the pattern of ribosomal behaviour is difficult to establish.

The nucleus-dictyosome association is still very apparent at this stage of senescence and production of dictyosome-derived vesicles from the maturing face of dictyosomes is also detectable. These vesicles seem to be loaded with metabolites, but their fate is difficult to ascertain. This is not only due to the characteristics of the background cytoplasm, but also to the vesiculation of the E.R. that makes it difficult to distinguish either type of vesicle.

Cell wall thickening is associated with aging in different plant materials (e.g., James, 1966; Fabbri and Palandri, 1968, 1969; McLean, 1968; Palandri, 1972). In *Ectocarpus* senescence is also accompanied by wall thickening with localized formation of cell wall ingrowths. These cell wall ingrowths are made of two different components: 1) metabolites, probably of vacuolar origin, and 2) cell wall materials which isolate the metabolites from the plasma membrane. The metabolites closely resemble some of the material accumulated in the paramural space of senescing root cells of *Glechoma hederacea* (Bowes, 1972). This author has also shown the metabolites to be vacuolar in origin and possible lomasomal in nature. In *Ectocarpus* the vacuolar origin of the cell wall ingrowth metabolites is at least partially apparent, but their morphological characteristics hardly fit into a lomasome concept.

One can say that this process represents in *Ectocarpus* a mechanism for the cell to get rid of excess waste metabolites. However, this hypothesis is circumstantial
and fails to account for the reason(s) why the phenomenon does not occur uniformly all over the cell wall, but it is particularly evident at places.

Cell wall ingrowths allow for the easy recognition of this cell type under the light microscope. Similar structures seem to exist in other brown algae (Chadefaud, 1936), but have never been described at the electron microscope level. Cytochemical tests carried out at the light microscope level showed these structures to give positive reactions for both phenolic compounds (greenish with toluidine blue 0—McCully, 1966) and lipofuscin (deep blue with Schorml's—Hendy, 1971). Their phenolic nature was also recorded by Chadefaud (1936). This double staining response could be rather significant. Indeed, this could be interpreted as evidence for the occurrence in the same inclusion of both phenolic and lipofuscin-like substances. It is interesting that as localized ingrowths enlarge at the cross wall level, symplast continuity between cells does not seem to be disrupted. This observation could be particularly pertinent for the advance of senescence, since as proposed by Simon (1967), the rate of senescence could be dependent upon the existence of a sink (in Ectocarpus "cell type #1" could act as a sink) for materials exported from aging parts of the plants.

An analysis of the mitochondrial population as well as mitochondrial features in "cell type #2" seems unwarranted here, since these organelles are very difficult to detect. At first one gains the impression that the mitochondrial population might have drastically decreased at this stage of senescence. Two facts argue against such an hypothesis: 1) these cells possess a very crowded cytoplasm with all sorts of inclusions which make the mitochondria very difficult to distinguish (see also Schuster et al., 1968), and 2) more advanced stages of senescence have large mitochondrial
populations, which become visible when the number of inclusions in the ground cytoplasm have decreased, revealing more easily the remaining structures.

Conspicuous stacks of thylakoids are visible in "cell type #2" chloroplasts. A similar phenomenon was noticed in other aging plant material (e.g. McLean, 1968; Dodge, 1970; Messer and Ben-Shaul, 1972), and has been considered as a sign of reduction of plastid activity (McLean, 1968). In *Ectocarpus* a very close relationship exists between this type of thylakoid arrangement and the formation of metabolites of high electron density, whose response to the osmium and permanganate fixations as well as to the Sudan Black B indicates a possible lipid content. The appearance of large amounts of lipid material in plastids was hypothesized by Harnischfeger (1972) as a sign of metabolic conditions which result in damage of the electron transport chain and therefore in reduced plastid activity.

Phenolic compounds (or phenolic compound precursors) were suggested to be plastid derived (e.g. Gifford, 1968; Evans and Holligan, 1972; Davies et al., 1973). The possibility that the *Ectocarpus* plastid metabolite is phenolic in nature is not supported by the silver nitrate reaction (Chadefaud, 1936), since it fails to label the intraplastid metabolite (figure 233, arrows). It is also interesting to note that non-labelling of intraplastid metabolites or similar material found in the cytoplasm support the concept that the labelling is most likely restricted to inclusions of lysosomal origin. The possibility also exists that intraplastid metabolites are the result of fixation artifacts. Other evidence seems to indicate, however, that intraplastid inclusions are significant in senescence. Firstly, these inclusions are associated with a special arrangement of the thylakoid membranes. Secondly, in permanganate fixed material as well as freeze-etching similar preparations
bearing the same relationship to thylakoids are also detectable.

Discharge of plastid metabolites into the cytoplasm is apparent and therefore contributes to the establishment of the ground cytoplasm characteristics of "cell type #2".

PART IV - "TRANSITIONAL 2-3" CELLS

As senescence continues the cytoplasm becomes increasingly autolytic to the point where very few or almost no inclusions are present. At the light microscope level the transparency of the cells becomes apparent.

The striking differences existing between "cell type #2" and "cell type #3" make it reasonable to assume the existence of intermediate stages. These transitional stages were seldom detected and in most cases the transition was abrupt.

Loss of vacuolar compartmentation is conspicuous in plant cell senescence (e.g. Shaw and Manocha, 1965; Butler, 1967; Roux and McHale, 1968; Halperin, 1969; De Vecchi, 1971; Habeshaw and Heyes, 1971; Matile and Winkenbach, 1971; Stearns and Wagenaar, 1971; Berjack and Villiers, 1972a, 1972b). Gahan (1965) and Barton (1966), among others, have suggested that the rupture of the tonoplast marked the beginning of the sequence of events leading to the massive autolytic condition of the cells. The autolytic condition is most likely due to the massive release of toxic substances (e.g. Shaw and Manocha, 1965; Robards, 1970) and lytic enzymes (e.g. Matile, 1969a), which are known to be present in the cell sap.

Other authors (e.g. Shaw and Manocha, 1965; Butler, 1967) maintained that the vacuole content might diffuse through the tonoplast. The latter possibility seems to be supported by recent cytochemical evidence (Brunk and
Ericsson, 1972b). Diffusion through the tonoplast of cell sap substances may thus trigger an irreversible chain of events ultimately leading to cell death; and the rupture of the tonoplast will be a consequence rather than the cause. The causes of modification in tonoplast permeability are as yet undetermined, although different possibilities have been advanced in the literature. An analysis of them in relation to Ectocarpus results will be presented.

Accumulation of lipofuscin inclusions such as those in Ectocarpus has been suggested to cause damage to lysosome membranes, affecting permeability and resulting in leakage of hydrolytic contents into the cytoplasm (Gabrielescu, 1970; Hochschild, 1971; Brunk and Brun, 1972). Berjack and Villiers (1970) thoroughly discussed the possibility that incorporation into vacuoles of dictyosome-derived vesicles carrying carbohydrate metabolites ultimately causes bursting of lysosomal membranes. The possibility also exists that lytic enzymes of extralysosomal origin may play a role in "cell type #3" formation (Lin and Fishman, 1972). Whatever the true explanation, the evidence suggests that once a massive autolysis is initiated, transformation of the cell content must proceed with extreme speed since in most of the cases studied the transition between the "cell type #2" morphology and that characteristic of "cell type #3" is sudden (see also Berjack and Villiers, 1970, 1972b; Woolhouse, 1967).

All steps involved in nuclear disorganization could not be followed, but available information shows many similarities with the same situation in other senescing plants (e.g. Shaw and Manocha, 1965; Barton, 1966; Wooding, 1966).

The number of mitochondria seemed to have increased. One must, however, be cautious in interpreting these
observations. Firstly because of the difficulty in studying "cell type #2" mitochondria, and secondly, multiplication of mitochondria at this stage of senescence would be difficult to explain. Despite this, an increase in the mitochondrial population was reported in aging cells of *Halimeda tuna* (Palandri, 1972) and aging slices of potato tubers (Lee and Chasson, 1966). These observations are exceptions rather than the rule. Indeed, in most of the cases studied, aging in plant cells has not been correlated with increases in the mitochondrial population (see Butler and Simon, 1971).

At this stage of senescence, the mitochondrial envelope remains intact and the matrix is darker. The cristae are less numerous than in "cell type "1" and occasionally assume concentric arrangements, a configuration also detected in aging wheat leaves (Shaw and Manocha, 1965). Similar features have been interpreted as an indication of mitochondrial senescence in other plant material (see Butler and Simon, 1971, for a review).

It is known that a decline in respiratory efficiency usually accompanies senescence (e.g. Drapper and Simon, 1971). Lund et al., (1958) have reported that alteration of the mitochondrial architecture reflects decreases in respiratory efficiency. Data for *Ectocarpus* are purely morphological and not backed up by experimental evidence. Moreover, a drop in respiratory efficiency could have occurred long before initiation of any alteration in mitochondria (see Drapper and Simon, 1971). The problem is, nevertheless, interesting to consider. Varner (1961) has suggested that changes in respiratory metabolism, particularly in oxidative phosphorylation, could be a major cause of senescence, due to the many cellular activities directly or indirectly dependent upon it.

Of all the cell organelles, chloroplasts undergo the
most striking changes. In some cases plastid morphology still resembles that characteristic of "cell type #2". As production of intraplastid metabolites cease, changes in thylakoid organization become more apparent. There is also an apparent increase in the number of plastoglobuli. Of interest is the budding of stroma containing structures from the plastid body. Morphologically similar phenomena were reported in other plant material, but not in connection with senescence (Gullvag, 1968; Schotz et al., 1971). Although the significance of the plastid budding phenomenon might not be completely understood, it seems reasonable to assume that it plays a major role in the reduction of plastid volume (see also Dodge, 1970; Fabbri and Palandri, 1970). Indeed, a decrease in plastid size seems to occur in _Ectocarpus_, particularly in "cell type #3" (see also Fritsch, 1945). A decrease in plastid size is rather common in senescent plant material (Ikeda and Ueda, 1964; Barton, 1966; Ljubesic, 1968; Dodge, 1970, De Vecchi, 1971; Stearns and Wagenaar, 1971).

Since pyrenoids were never found in "cell type #3", it seems safe to assume that they might have disappeared during these transitional stages. However, as already pointed out, images suggesting pyrenoid breakdown were very difficult to interpret and the elucidation of the mechanism of pyrenoid disorganization must await further research.

**PART V--THE "CELL TYPE #3"**

It is clear that transitional "2-3" cells possess a very altered cellular architecture. The ground cytoplasm shows extreme autolysis; the mitochondria and plastids are senescent; the tonoplast has broken down in places; the nucleus is difficult to detect and when present highly disorganized; the dictyosomes are rarely detected and
abnormal in appearance. All these features are unmistakable signs of cell necrosis. Therefore, "cell type #3" should be considered as a phase in the final process of cell autolysis and not separated from the "transitional 2-3" cells.

The autolytic appearance of the background cytoplasm in "cell type #3" correlates directly with the distribution of acid phosphatase activity. Reaction product is seen all over the cytoplasm, a sign that the compartmentation of lysosomes no longer exists and the cell cavity represents a single large lysosomal unit. All the other enzymes tested seem to have ceased to function and the light microscope data give further proof of cell degeneration.

Nuclei and dictyosomes were never observed in "cell type #3". This is not surprising since the nuclei, as previously shown, begin to disorganize during the "transitional 2-3" stages. The E.R., if present, is reduced to very altered and almost unidentifiable membrane profiles. The plasmalemma is discontinuous, the tonoplast no longer recognizable; all inclusions, with the exception of mitochondria and plastids remains have disappeared.

Mitochondrial profiles are still numerous, but the mitochondrial envelope is disrupted in several places. The cristae and the matrix of the mitochondria are no longer detectable. These features are similar to those found in degenerating mitochondria in other senescent plants (e.g. De Vecchi, 1971; Butler and Simon, 1971).

Chloroplasts are most often delimited by a single membrane, a remnant of the chloroplast envelope. They are almost devoid of stroma, and for the most part only large stacks of lamellae remain. As disorganization proceeds the lamellar system becomes greatly reduced and the envelope membrane is also absent. The final steps in disorganization of *Ectocarpus* plastids are, therefore, similar to those reported for other plants (e.g. Shaw and Manocha, 1965;
In association with thylakoid breakdown conspicuous depositions of plastoglobuli are detectable (e.g. Ikeda and Ueda, 1964; Barton, 1966; Fabbri and Palandri, 1970; Dodge, 1970; Butler and Simon, 1971; Stearns and Wagenaar, 1971). In some cases, one gains the impression that the plastoglobular population of degenerating chloroplasts is heterogeneous in their electron opacity. A similar situation was reported by Fabbri and Palandri (1970) in *Ricinus communis* senescing cotyledons. Stearns and Wagenaar (1971) also reported similar phenomena in senescing chloroplasts of autumn leaves and suggested that variations in electron opacity of plastoglobuli were due to their role as waste baskets for breakdown products. Butler (1967) concluded that although plastoglobuli in senescing chloroplasts were the result of an accumulation of membrane breakdown products, they probably also acted as storage bodies for insoluble lipid materials not necessarily associated with membrane breakdown.

Plastids seem to be the most resistant organelles in senescing *Ectocarpus* cells. It is interesting to note that the same situation was found in necrotic cells of *Ascophyllum nodosum* (Rawlence, 1972).

Dissolution of the cell wall represents in *Ectocarpus* the last stage in cellular destruction. Cell wall breakdown occurs in other plants (e.g. Clowes and Juniper, 1968; Halperin, 1969; Bal and Payne, 1972).

Bal and Payne (1972) found a correlation between cell wall breakdown and E.R. development (see also Clowes and Juniper, 1968). However, when wall breakdown occurs in *Ectocarpus* the cell is reduced to an almost virtually empty cavity. Acid phosphatase is present inside the cells but deposition of reaction products is not conspicuous in the cell wall. De Jong (1966) reported that glutaraldehyde fixation, although not affecting intracellular acid phosphatase activity, did block the cell wall reactions that were
otherwise evident in unfixed cells. Other reports (e.g. Halperin, 1969), show that acid phosphatase activity is localized in cell walls, but even in these cases no functional relationship between its presence and cell wall breakdown could be demonstrated. Horton and Osborne (1967) have shown that as senescence proceeds an increase in cellulase activity is also observed. Cell wall breakdown was related to high levels of cellulase activity in cultured carrot cells (Halperin, 1969). No attempts were made to study other enzyme activities in Ectocarpus cells, and so the enzymatic machinery behind cell wall disorganization remains unknown.

The process of disintegration of the cell wall suggests the presence of two different components: 1) a fibrillar component, and 2) a cement-like (matrix) material which seems to be the first to disorganize. Cronshaw et al. (1958), and Myers and Preston (1959a, 1959b) reported that cell walls of marine algae were apparently composed of a network of fibrils embedded in an amorphous matrix. Recent evidence supports the occurrence of a similar construction pattern for the cell wall of Fucus (Fulcher and McCully, 1971). The fibrillar component of the wall is probably cellulosic (Percival, 1968), but it might also be a carboxylated polysaccharide, probably alginic acid, which is known to assume a microfibrillar configuration (McCully, 1968). The nature of the cementing substance or amorphous matrix is not entirely known, but McCully (1966, 1968) has shown it to be possibly a sulfated polysaccharide. Preston (1969), Lamport (1965), and Thompson and Preston (1969) suggested that the orientation of the microfibrils and the stabilization of the cell wall structure could be on the responsibility of proteins.
PART VI—ENZYME LOCALIZATION DURING DIFFERENTIATION AND SENESCENCE

The last part of this discussion will be reserved for an analysis of enzymatic data. Since detailed considerations regarding acid phosphatase activity and lipofuscin identification have already been discussed, the present considerations will be concerned with the analysis of adenosine triphosphatase, catalase, and peroxidase results. Adenosine triphosphatase (ATPase). Recently a number of papers have appeared which deal with plant ATPases (e.g. Coulomb and Coulomb, 1972; Cronshaw and Gilder, 1972; Hodges et al., 1972; Hall and Davie, 1971; Lai and Thompson, 1972a, 1972b; Maier and Maier, 1972; Sundberg et al., 1973). The reports seem to be conflicting. Coulomb and Coulomb (1972) and Maier and Maier (1972), for instance, reported Mg\(^{++}\)-dependent ATPase at the plasma membrane level. Other authors reported that ATPase activity is stimulated by monovalent ions, but inhibited (under certain conditions) by divalent ions (Atkinson and Polya, 1967). A pH-dependent K\(^{+}\)-stimulated ATPase was detected in the plasma membrane of the green alga Mougeotia (Sundberg et al., 1973). Further evidence was presented for the occurrence of monovalent ions stimulated ATPases at the level of the plasmalemma, although the system was said to be different from the K\(^{+}\)-Na\(^{+}\)-ATPase activated systems (e.g. Hodges et al., 1972; see also Ratner and Jacoby, 1973). Lai and Thompson (1972a) provided evidence for the existence of a K\(^{+}\)-Na\(^{+}\)-stimulated ATPase associated with the plasmalemma, as did Bowling et al. (1972). Bowling et al. (1972) concluded, however, that the plant ATPase they studied had different properties from the K\(^{+}\)-Na\(^{+}\)-ATPase system of animal cells.

ATPases are associated with some of the most important functions of the cell (e.g. Heyden, 1969). A study
of ATPase localization in Ectocarpus, therefore, would allow a correlation between the architecture of the cell and its physiological status. The results for Ectocarpus have shown that the pattern of reaction product deposition was the same for the Mg\textsuperscript{2+} and K\textsuperscript{+}-Na\textsuperscript{+}-Mg\textsuperscript{2+}-ATPase systems. However, monovalent ions (Na\textsuperscript{+}, K\textsuperscript{+}) seem to have enhanced the deposition of reaction products at both the plasmalemma and the thylakoid levels. Involvement of monovalent ions dependent ATPases in stimulation of ion transport has been stressed in plant cells (e.g. Hall and Davie, 1971; Coulomb and Coulomb, 1972; Maier and Maier, 1972). Ratner and Jacoby (1973) found that in their material monovalent salt effects on ATPase were not cation specific, and therefore not related to the cell potential for cation absorption. Whether or not the enhancement of reaction product deposition in Ectocarpus reflects cation specificity remains to be determined. However, other authors have shown that Mg\textsuperscript{2+} is required in order for plant ATPases to be stimulated by monovalent ions (Fisher and Hodges, 1969; Fisher et al., 1970; Leonard and Hanson, 1972).

The reaction of the mitochondria is probably due to the Mg\textsuperscript{2+}-activated system only, without being affected by monovalent ions. Indeed, deposition of reaction products is conspicuous in both systems assayed. Novikoff et al. (1958) also reported that Mg\textsuperscript{2+} ions preferentially stimulated the ATPase system of mitochondrial fractions.

The above considerations hold for both "cell type #1" and "cell type #2". In "cell type #3", however, none of the systems assayed led to deposition of reaction products. The apparent lack of ATPases in these cells leads one to conclude that they are no longer functional. The absence of ATPase at the plasma membrane implies deeply altered physiological properties in this membrane system, although at the ultrastructural level the membrane may still display
an intact appearance (figure 191). This is in agreement with reports showing that as senescence proceeds changes in permeability are observed in cells (e.g. Eilam, 1965; Sacher, 1967; Fergusson and Simon, 1973).

*Catalase.* Biochemical, cytochemical, and ultrastructural studies have, during the past few years, provided enough evidence for the identification in plants of a class of organelles usually described as "microbodies" (Molhenhauer et al., 1966; Frederick et al., 1968; Frederick and Newcomb, 1969a) or under more specific conditions as peroxysomes (Frederick and Newcomb, 1969b; Marty, 1969, 1970; Trelease et al., 1971) and glyoxyxomes (Vigil, 1970; Beevers, 1970; Gerhardt and Beevers, 1970; Gruber et al., 1970; Trelease et al., 1971).

Cytoplasmic organelles whose ultrastructural features (single membrane boundary, granular matrix, occasional core-like structures) resemble those of the so-called microbodies are found in all *Ectocarpus* sporophytic cells, with the exception of "cell type #3". These microbody-like structures are identified cytochemically by their response to diaminobenzidine (DAB) incubation. Under these conditions heavy deposition of reaction products is associated with the microbodies. Control sections, mainly those incubated in aminotriazole medium, show the reaction to be due to the presence of catalase (Margoliash and Novogrodsky, 1958; Margoliash et al., 1960) which is the enzyme marker for microbodies (Tolbert, 1971). This is corroborated by the parameters of incubation, i.e., optimum pH in the alkaline range, incubation temperature (37°C) (e.g. Czaninski and Catesson, 1970, 1971; Poux, 1972a, 1972b, 1972c).

A consistent and close association was found in *Ectocarpus* between microbodies and mitochondria. Association with chloroplasts, although detectable, was not so evident.
The mitochondrion-microbody association is not restricted to the *Ectocarpus* material (e.g., Hilliard et al., 1971; Poux, 1971, 1972b; Tourte, 1972). This association also occurs in yeast and *Tetrahymena* cells, where its occurrence is related to the distribution of glyoxylate cycle enzymes (Tolbert, 1971). It is impossible, however, without biochemical evidence, to make any conclusion about the significance of such an association in *Ectocarpus* sp.

Reaction products were also found in mitochondria. The explanation for mitochondrial deposition of reaction products is still debatable. Since reaction products are present even after incubation in aminotriazole, one may rule out the possibility of catalase involvement, although catalase has been said to be present in mitochondria (Herzog and Fahimi, 1972). Diaminobenzidine is known to be oxidized by hemoproteins other than catalase, for instance, peroxidase and cytochrome oxidase (Seligman et al., 1968; Todd and Vigil, 1972). Peroxidase is indicated as a possible cause of mitochondrial reactions (Rothman, 1968; Childs and Miller, 1971; Gerhardt and Berger, 1971). In *Ectocarpus*, however, after incubation under conditions favouring optimum localization of peroxidase, no obvious mitochondrial reactions were found. The data of Plesnicar et al. (1967) showed the absence of any kind of peroxidatic activity in plant mitochondria. The possibility then remains that mitochondrial deposition of reaction products is very likely due either to cytochrome oxidase activity (Seligman and Karnovsky, 1968; Gerhardt and Berger, 1971; Kataoka, 1971) or, as indicated by Beard and Novikoff (1969), Novikoff and Goldfisher (1969), Novikoff (1970), Novikoff et al. (1971), and Poux (1972a), to cytochrome c activity.

**Peroxidase.** An increase in peroxidase activity was reported to be characteristic of the ageing process (e.g., Galston and Davies, 1969). In *Ectocarpus* "cell type #3" peroxidase
activity is not detectable. Although it is possible that in "cell type #2" there is peroxidase activity, the overall characteristics of these cells make it very difficult to evaluate the pattern and intensity of the reaction. Therefore, any comparison with data available for "cell type #1" or the transitional stages immediately following is not possible.

Peroxidase activities in plants have been localized, using the DAB/H$_2$O$_2$ procedure, in many parts of the cells: cell wall (e.g. Ridge and Osborne, 1970; Hall and Sexton, 1972; Poux, 1972a, 1972b, 1972c); dictyosomes (e.g. Poux 1969, 1972a; Hall and Sexton, 1972); vacuoles (e.g. Coulomb, 1971; Hall and Sexton, 1972; Hanzely and Vigil, 1972; Poux, 1972a, 1972b, 1972c); ribosomes (e.g. Poux, 1972a, 1972b, 1972c); E.R., perinuclear spaces and plasma membranes (e.g. Poux, 1972a). In *Ectocarpus*, deposition of reaction products is found in the paramural space and in the cell wall. That deposition of reaction products is due to peroxidase activity can be inferred from the experimental conditions (e.g. Poux, 1972a, 1972b, 1972c) as well as from the following control experiments: 1) aminotriazole incubation, which does not inhibit deposition of reaction products, and 2) potassium cyanide incubation which inhibits deposition of reaction products (Strum and Karnovsky, 1970; Poux, 1972a).

The absence of reaction products in other intracellular locations does not necessarily prove its absence. Existing evidence shows that peroxidases, although ubiquitous in distribution, possess an unprecedented degree of heterogeneity (e.g. Shannon, 1968; Delincee and Radola, 1970; Radola and Drawert, 1970). Rucker and Radola (1971) have shown that, in tobacco tissue cultures, a number of peroxidase isoenzymes occur which could be separated into 3 groups on the basis of their isoelectric points. Hall and Sexton (1972) have studied the effect of pH on peroxidase activity and found a wide variation in pH-dependent responses. The evidence
shows, therefore, that different isoperoxidases might require individual incubation parameters to be visualized, as a recent study seems to indicate (Poux, 1972a).

The present data suggest that peroxidase activity in *Ectocarpus* is mainly a cell wall associated phenomenon. Although recent years have seen increasing interest in the biochemical and physiological aspects of peroxidases, little is known about their real functions. Ridge and Osborne (1970) have suggested that wall peroxidases might facilitate hydroxylation of proline in the wall proteins, hence affecting cell wall extensibility (Ridge and Osborne, 1971). Any role of peroxidases in cell wall formation in *Ectocarpus* must, however, wait for further knowledge in wall composition in this alga.
CONCLUSION

The present study has two objectives. The first intends to provide a more detailed knowledge of the cellular morphology of non-senescing vegetative cells of the sporophytic generation of *Ectocarpus*. Hopefully this will lead to a better understanding of the similarities and the differences existent among the brown algae and between these and other organisms in the plant kingdom. The second objective of the present work is concerned with the study of the senescing process as it occurs in *Ectocarpus* under culture conditions. Culture conditions are known to affect the metabolism of the organisms (e.g. Carol et al., 1972) and so the interpretation of the results cannot be taken as duplication of field events. Nevertheless, it provide a useful and appropriate mean of understanding important changes in development and senescence in a reproducible way.

In studying the aging process of any organism the identification of a single initial change could provide a lead for a better understanding of the events of senescence. Nevertheless, as pointed out by Butler (1967), initial damage to the cell could be direct result of a number of factors acting alone and in concert, and therefore even a small change would have an accumulative effect. Identification of a single initial alteration at the ultrastructural level is a very difficult task. Therefore, any attempt to localize chronologically and spatially senescence-associated phenomena must be regarded as an approximation to the real sequence. Bearing this in mind, a summary of organelle characteristics and modifications occurring in *Ectocarpus* from "cell type #1" to cell death is presented in the following page.
<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Nucleus</th>
<th>Endoplasmic reticulum</th>
<th>Golgi</th>
<th>Mitochondria</th>
<th>Chloroplast</th>
<th>Pyrenoid</th>
<th>Cytosol</th>
<th>Cell Wall</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Found in profiles; nuclear pores numerous and distinct; high activity of the nuclear membrane; production of vesicles and other membrane structures; nucleolar conspicuous.</td>
<td>Mostly distal and round; frequently displaying conspicuous perinuclear arrangements.</td>
<td>Represented by several dictyosomes, with hypertrophied appearance. Dictyosomes are nucleated and numerous.</td>
<td>Polymorphic; 3 thylakoids per bundle; double membrane envelope outside which lies the chloroplast R.B. Production of concentric bodies; plasmid division frequent.</td>
<td>Stained and conspicuous; matrix filaments; external boundary resolved into 3 membrane systems; chloroplast envelope, chloroplast R.B., and pyrenoid sac.</td>
<td>Rich in ribosomes; few spaces vacuoles tonoplast intact.</td>
<td>Distinct biseriate arrangement; plastid, vacuolization and inclusions present in the peripheral space.</td>
<td></td>
</tr>
<tr>
<td>Transitional 2-3 cells</td>
<td>Mostly as in &quot;cell type 1&quot;, except for a reduction in activity of nuclear membranes.</td>
<td>Mostly as in &quot;cell type 1&quot;, but the perinuclear arrangement no longer observed.</td>
<td>As in &quot;cell type 1&quot;, but as cells approach &quot;cell type 2&quot;, they become more difficult to distinguish from the cytoplasm.</td>
<td>Mostly as in &quot;cell type 1&quot;; immune with plastid division rare; large stacks of thylakoids appear as cells approach &quot;cell type 2&quot;.</td>
<td>Mostly unchanged, but external boundary becomes progressively more difficult to distinguish.</td>
<td>Rapid increase in vacuolization and hypertrophy; accumulation of different kinds of inclusions, some probably lipids in nature. Ribosomes visible in some areas; tonoplast intact.</td>
<td>Similar to &quot;cell type 2&quot;; but with production of localized aggregates.</td>
<td></td>
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<tr>
<td>Transitional 3-4 cells</td>
<td>In early stages the nuclear profile is nearly round, becoming irregular at a more advanced phase; nuclear pores difficult to detect; nucleoli visible.</td>
<td>Disorganization; vesicles detected in some areas.</td>
<td>Present and active as judged by the production of vesicles from the reticulum facing a cluster of larger, more numerous, and more heterogeneous vesicles.</td>
<td>Thyloplasts in stacks, display striking organization, with some associated with electron dense metabolites.</td>
<td>Matrix homogenous; external boundary difficult to resolve.</td>
<td>Crowded with different kinds of inclusions; ribosomes very difficult to detect.</td>
<td>Consistency well appreciated; present; metabolites inside well and at well periphery well apparent.</td>
<td></td>
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<tr>
<td>2</td>
<td>Rarely observed and very disorganized.</td>
<td>Short profiles not always easy to recognize.</td>
<td>Only rarely observed and abnormal in appearance.</td>
<td>Thyloplasts in stacks, but production of metabolites have ceased. Budding of stroma containing bodies; dissolution of chloroplast boundary.</td>
<td>Mechanism of pyrenoid destruction possibly operational.</td>
<td>Towoplast disrupted; high degree of autolysis; reduction in the number of inclusions.</td>
<td>As in &quot;cell type 2&quot;.</td>
<td></td>
</tr>
<tr>
<td>3 (less advanced stages)</td>
<td>Not observed.</td>
<td>If present must be reduced to very small and short profiles.</td>
<td>Not observed.</td>
<td>Envelope disrupted; matrix almost absent; large stacks of thylakoids present; signs of thylakoid breakdown visible.</td>
<td>Not observed.</td>
<td>Highly disorganized; almost all inclusions; tonoplast indiscernible.</td>
<td>Thick with inclusions still recognizable.</td>
<td></td>
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<tr>
<td>3 (final stages)</td>
<td>Not observed.</td>
<td>Not observed.</td>
<td>Cannot be detected.</td>
<td>Very altered remains only.</td>
<td>Not observed.</td>
<td>Almost absent; plasmalemma disrupted.</td>
<td>Disorganization of cell wall apparent.</td>
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</table>
From the data it can be said that increases in vacuolation and autophagy as well as chloroplast modifications seem to be the first detectable signs of aging in *Ectocarpus*. A full comparison of the order of events in relation to the data provided by senescence studies in other plants is unwarranted here; not only because of difficulties in establishing with precision the actual order of events, but also because, as discussed by Roux and McHale (1968), variations in the order of appearance of alterations might be species dependent.

It is apparent, however, from this work that the pattern of cellular disorganization in *Ectocarpus* has many points in common with the events reported during senescence in other plants.

The possible presence of lipofuscin metabolites during the aging of *Ectocarpus* also shows that a common denominator of aging might exist from algae to man as suggested by Palisano and Walne (1972) during their study of senescence in *Euglena granulata*. Other changes associated with aging in other plant and animal cells support such a suggestion: i.e. lobing of the nucleus (Lipetz and Cristofalo, 1972; Munnell and Getty, 1968), mitochondria with denser matrix and fewer cristae (Lipetz and Cristofalo, 1972; Weinbach et al., 1967), increases in cellular autophagy (Sohal and Allison, 1971; Lipetz and Cristofalo, 1972).

In conclusion, it can be said that no single technical approach will elucidate such a complex phenomenon as is the case of senescence. Each technical approach is able to provide partial information. This information must then be compared and correlated with other data, obtained by different means, and from a large number of test organisms. Only afterwards can the complex process of senescence as well as life in general be better understood.


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processes?" Geriatrics 23(10): 97-105.


KEY OF SYMBOLS
AND
PLATE EXPLANATION
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tr>
<td>ac</td>
<td>apical cell</td>
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<tr>
<td>ce</td>
<td>chloroplast envelope</td>
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<tr>
<td>cer</td>
<td>chloroplast endoplasmic reticulum</td>
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<td>Ch</td>
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<td>cell wall ingrowth</td>
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<tr>
<td>D</td>
<td>dictyosome</td>
</tr>
<tr>
<td>Dv</td>
<td>dictyosome vesicle</td>
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<tr>
<td>ER</td>
<td>endoplasmic reticulum</td>
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<tr>
<td>er</td>
<td>endoplasmic reticulum</td>
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<tr>
<td>g</td>
<td>genophore</td>
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<td>il</td>
<td>inner layer of the cell wall</td>
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<tr>
<td>L</td>
<td>lomasome</td>
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<tr>
<td>M</td>
<td>mitochondrion</td>
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<tr>
<td>m</td>
<td>microbody</td>
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<td>N</td>
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<tr>
<td>nl</td>
<td>nucleolus</td>
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<tr>
<td>ol</td>
<td>outer layer of the cell wall</td>
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<tr>
<td>OSB</td>
<td>osmiophilic structured body</td>
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<tr>
<td>Pg</td>
<td>plastoglobuli</td>
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<tr>
<td>pm</td>
<td>paramural space</td>
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<tr>
<td>Py</td>
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<td>Pys</td>
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<tr>
<td>r</td>
<td>ribosomes</td>
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<td>v</td>
<td>vacuole</td>
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Figure 1  Phase contrast observation of an in situ embedded filament (prostrate system). A progressive increase in the of affinity for osmium is observed from the apical cell (a.c.) downwards. X 750

Figure 2  Phase contrast observation of an in situ embedded filament (erect system). A sudden loss in affinity for osmium occurs in cells of the older portion of filaments. X 1,000

Figure 3  In situ embedded filament (prostrate system). The loss of affinity for osmium is sudden (cells located at the older end of the filament). Notice that the cell wall between the dark and the light cell is convex. X 1,000

Figure 4  Multilobed chloroplast. The pyrenoid is identified by an arrowhead. Phase contrast observation of living material. X 1,500

Figure 5  Bright field observation of living material. The transition between two different cell types (older portion of a filament) is identified by the cell wall convexity. X 1,000

Figure 6  Phase contrast observation of living material. Possible phases of chloroplast division (arrowheads). X 1,500
Figure 7  Phase contrast observation of living material. Chloroplast morphology. X 800

Figure 8  Phase contrast observation of living material. Two different stages of pyrenoid division are observed (arrowheads). X 900

Figure 9  Phase contrast observation of living material. A chloroplast with 3 pyrenoids is depicted (arrowheads). X 1,500

Figure 10  Phase contrast observation of living material. At arrowheads localized ingrowths of the cell wall are shown. X 1,000

Figure 11  Phase contrast observation of living material. A cell wall ingrowth is seen at arrowhead. X 750

Figures 12, 13, & 14  Cytochemical identification of DNA-containing structures, after RNA extraction.

Figure 12  "Cell type #1". Intense staining of the nucleus, especially apparent at arrowheads. X 1,200

Figure 13  "Cell type #2". Nucleus perfectly stained. Nucleolus unstained (arrowhead). X 1,200

Figure 14  "Cell type #3". Absence of DNA staining characterizes this cell type. X 1,200
Figures 15, 16, & 17 Cytochemical identification of RNA-containing structures, after DNA extraction.

Figure 15 "Cell type #1". Nucleus unstained (N). Intense staining is found all over the cytoplasm. X 1,200

Figure 16 "Cell type #2". Nucleus unstained (N). Staining restricted to the perinuclear region. X 1,200

Figure 17 "Cell type #3". The almost absence of staining characterizes this cell type. X 1,000

Figure 18 Cytochemical identification of protein staining regions. "Cell type #1". Intense staining is seen almost all over the cytoplasm, particularly at the chloroplasts (Ch) and pyrenoids (Py) levels. X 800

Figure 19. "Cell type #2". Chloroplasts (Ch) and pyrenoids (Py) show intense staining. Cytoplasmic staining is restricted to certain areas. X 800

Figure 20. "Cell type #3". The almost absence of staining from the cytoplasm of these cells is characteristic. X 1,200


Figure 21. Cell belonging to the "transitional 1-2" stages. Staining is observed in the perinuclear region (Golgi area), paramural space (arrowhead), and cell wall (cw). X 1,200

Figure 22. "Cell type #1". Section through the cell wall showing intense staining properties. X 1,000

Figures 23, 24 & 25. Cytochemical study of lipid distribution (Sudan Black B).

Figure 23. "Cell type #1". Very few
sites with affinity for Sudan.
X 1,000

Figure 24 "Cell type #2. Conspicuous staining all over the cytoplasm. X 1,000

Figure 25 "Cell type #3". An almost absence of staining characterizes this cell type. X 1,000

Figure 26 "Cell type #2". Lipofuscin-like pigment distribution after treatment with the Schorml's reagent is shown in this picture. X 750

Figure 27 "Cell type #2". Toluidine blue O. Some of the cytoplasmic inclusions show a green to turquoise colour indicative of polyphenolic content. X 750
Figure 28a  General ultrastructure of a young cell (cell type #1) belonging to the prostrate system of Ectocarpus sp. X 18,000

Figure 28b  Plasmalemmasomes. The relationship of one of these structures to the plasmalemma is shown at arrowhead. X 22,000

Figure 29  Section tangential to the nuclear envelope showing nuclear pores. Observe that from a hollow central core (arrowheads) spokes radiate toward the outer rim. X 100,000

Figure 30  Fibrillar aspect of chromatin. X 90,000
Note: To best detect the intranuclear fibrillar system this picture must be observed in the direction indicated by the arrowhead.
Figures 31 & 32  General ultrastructural morphology of erect system cells. "Cell type #1". X 18,000
Figure 33  General ultrastructural morphology of a young prostrate system cell. X 18,000

Figure 34  Direct continuity between the nuclear envelope and the dictyosome is depicted at arrowhead. X 16,000

Figure 35  Association between the endoplasmic reticulum (E.R.) and the mitochondrion (M) is shown at arrowhead. X 30,000
Figure 36  Freeze-etch replica of the nuclear envelope
A many-particulate (A) and a sparsely
particulate (B) surfaces are recognized.
X 60,000

Figure 37  Freeze-etch replica of the nuclear envelope
Pore containing areas are permeated by
pore free regions. X 30,000

Figure 37a  Freeze-etch replica of the periphery of a
young cell. Plasmalemmasomes are observed
in the paramural space (pm). X 26,000
Figure 38  Portion of the nucleus and perinuclear region of a young cell. The close association between the nucleolus (nl) and the inner membrane of the nuclear envelope is apparent. Direct continuity of the perinuclear space with the E.R. is observed. At other place (empty arrowhead) a multivesicular structure occupies a position shared by both the E.R. and the perinuclear space. Other features are E.R. fenestrae (arrowheads) and mitochondria (M). The small arrowhead points to a mitochondrion DNA-containing area. X 55,000

Figures 39 & 40  Vesicular-like inclusions are seen inside the perinuclear space (arrowheads). X 18,000

Figure 41  The relationship of the perinuclear inclusions to the nuclear envelope membranes is shown at arrowheads. X 40,000

Figure 42  A vesicular structure (arrowhead) is observed in association with the outer membrane of the nuclear envelope. A dictyosome (D) is also depicted. X 25,000
Figure 43a  Freeze-etch preparation of a chloroplast, "g" indicates the position of the genophore. Thylakoid associated fibrils are depicted at small arrowheads and at nearby positions. A fenestra-like structure is shown in the chloroplast E.R. (big arrowhead). X 40,000

Figure 43b  Detail of the area labelled "A" in figure 43a. Both the chloroplast envelope (ce) and (probably) the chloroplast E.R. (cer) are shown. A pore-like structure is observed at arrowhead. X 30,000

Figure 44  Endoplasmic reticulum morphology. A crystalline-like inclusion is seen inside the E.R. (i). The arrowhead points to a fenestra-like structure. X 60,000
Figure 45  Similarity in the origin and morphology of the perinuclear space (pns) and endoplasmic reticulum (er) inclusions is shown in this picture. A "Osmiophilic Structured Body" (OSB) is seen in association with the chloroplast (Ch). X 90,000

Figure 46  Freeze-etch preparation of a chloroplast. The pattern of thylakoid arrangement and the position of the chloroplast envelope (ce) in relation to the chloroplast E.R. (cer) are shown. Other features include the presence of a thylakoid free area (F), and of vesicular structures in the narrow cytoplasmic region found in between the "ce" and the "cer" (big arrowhead). X 36,000
Figure 47a A portion of a chloroplast is depicted; "g" indicates the position of the genophore. The existence of direct continuity between the chloroplast E.R. (cer) cisternal space and the chloroplast envelope (ce) is shown at arrowhead. Also apparent is an initial step in the formation of a "Osmiophilic Structured Body" (OSB). Metabolites of high electron opacity are seen to be discharged in the paramural space (pm). X 42,000

Figure 47b A step in the process of formation of a "OSB" is depicted. The relationship of the "OSB" to the chloroplast E.R. (cer) is apparent. X 40,000

Figure 48 Portion of a chloroplast showing a genophore-like region (g). Direct luminal continuity between the chloroplast E.R. (cer) cisternal space and the chloroplast envelope (ce) is apparent (arrowhead). The close association between the chloroplast and the mitochondrion (M) is also shown. X 42,000

Figure 49 An almost tangential section through the region of the chloroplast E.R. (cer) shows the intricate pattern of arrangement of the "cer" derived tubular structures. Lomasome-like structures (L) are present in the paramural space. Membrane residues are seen inside the vacuoles (V). X 24,000
Figure 50  Vesicular-like inclusions are present inside the chloroplast E.R. cisternal space (cer). X 36,000

Figure 51  Osmiophilic structured body morphology. Notice that a non structured component is intermingled with a myelin-like one. X 60,000

Figure 52  An osmiophilic structured body (osb) is observed in the space shared by the chloroplast E.R. (cer) and the perinuclear space (pns). X 30,000

Figure 53  The association between a osmiophilic structured body (osb) and the pyrenoid (Py) is shown. A portion of the pyrenoid sac (Pys) is also depicted. X 36,000

Figure 54  The discharge of osmiophilic structured bodies (osb) into the paramural space (pm) is shown. X 36,000

Figure 55  The nuclear envelope-dictyosome relationship is shown. Also depicted is the association of the dictyosome (D) with a osmiophilic structured body (osb). X 50,000
Figure 56 Portion of a young cell (cell type #1) showing peripheral localization of mitochondria. Osmiophilic structured bodies (osb) are found either in association with the chloroplast (Ch) or they lie in the cytoplasm without any apparent relationship to these organelles. An early stage in the process of formation of a cell wall ingrowth (cwi) is also depicted. X 30,000
Figure 57  Dictyosome morphology. Interruptions can be recognized in the cisternae of the dictyosome. These seem to be of two types: 1) a narrow tubular-like interruption (arrowheads labelled b), and 2) a larger fenestra-like interruption (arrowheads labelled a). X 80,000

Figure 58  Dictyosome morphology. Interruptions are recognizable in the cisternae of the dictyosome (arrowheads). These interruptions seem to be more abundant in the innermost cisternae. The outermost cisterna displays a hypertrophied appearance (D). X 70,000
Figure 59. Dictyosome morphology. Bridge-like elements are observed in between the cisternae of the dictyosome as well as in an intracisternal position (arrowheads). X 90,000.

Figure 60. The existence of a very close association between the dictyosome (D) and pyrenoid (Py) is observed. X 30,000.

Figure 61. Dictyosome (D)-mitochondria (M) association is depicted. X 30,000.
Figure 62  Incorporation of dictyosome-derived vesicles (big arrowheads) into vacuoles (V). Three possible cases of mitochondrial division are shown at small arrowheads. X 36,000

Figure 63  Dictyosome (D)-pyrenoid (Py) association. X 20,000

Figure 64  A stage in the formation of a lomasome-like structure (L) is seen inside an hypertrophied dictyosome cisterna (c.f. with figure 49, showing a potential lomasome-like structure 'L' inside the paramural space). X 24,000

Figure 65  Multimembranous inclusion occupies the outermost cisterna of a dictyosome. The mode of formation of this structure through the invagination of the cisterna peripheral membrane is depicted at the arrowhead. X 80,000

Figure 66  Tubular-like inclusions are seen inside a dictyosome-derived vesicle (Dv). X 80,000
Figure 67 Mitochondrion morphology. X 20,000

Figure 68 Detail of the mitochondrion cristae inclusions are shown at arrowhead and in the inset. X 50,000
Inset X 120,000

Figure 69 Mitochondrion (M)-nucleus (N) association. X 24,000

Figure 70 Freeze-etch preparation showing the association of a mitochondrion (M) with a chloroplast (Ch). X 22,000

Figure 71 Mitochondrion (M)-pyrenoid (Py) association. X 24,000

Figure 72 Mitochondrion-mitochondrion association (arrowheads). At arrow the mitochondrion- E.R. association is depicted. X 30,000

Figure 73 High magnification observation of thylakoids. Big arrowhead points to an interruption. Small arrowheads indicate the presence of bridge-like elements. X 120,000

Figure 74 Freeze-etch preparation of a portion of the thylakoid system of a chloroplast. Arrowheads indicate the presence of bridge-like elements. X 120,000
Figure 75  Deviations from the normal pattern of thylakoid arrangement are shown. X 60,000

Figure 76  Thylakoid interruptions are seen at arrowheads "a". Arrowheads "b" point to bridge elements. Arrowheads "c" indicate pore-like interruptions in the chloroplast envelope. X 80,000

Figure 77  High magnification observation of the arrowhead labelled "a" in figure 78. X 100,000

Figure 78  Section tangential to the thylakoid membranes showing the presence of pore-like interruptions (arrowheads). X 50,000

Figure 79  High magnification observation of the thylakoid architecture. Arrowheads point to bridge-like elements. X 90,000

Figure 80  Freeze-etch preparation of thylakoids showing the particulate nature of the exposed surfaces. A many particulate (B) and a sparsely particulate (A) surfaces are recognized. X 60,000
Figure 81 Freeze-etch preparation of a chloroplast. The particulate nature of exposed surfaces is depicted. A many particulate (B) and a sparsely particulate (A) surfaces are observed. X 60,000

Figure 82 Chloroplast architecture. The pattern of bifurcation and merging of neighboring thylakoid bands is depicted. Notice that the peripheral band of thylakoids is not continuous, instead is made up of portions of the centrally placed bands (arrowheads). X 40,000
Figure 83  Tangential section through a dictyosome. Interruptions: 1) large (arrowheads "a"), 2) narrow (arrowheads "b") are observed in one of the cisternae. X 80,000

Figure 84  Mitochondrial architecture. The mitochondrial genophore is indicated by arrowheads. In the chloroplast (Ch), pore-like interruptions are detected in the thylakoids (arrowheads "a") and in the chloroplast envelope (arrowhead "b"). X 75,000
Figures 85, 86, 87, and 88  Different stages in the formation and detachment from chloroplasts of concentric bodies.

Figure 85  the arrowhead points to the place where bifurcation of one thylakoid has occurred. X 45,000

Figure 86  Concentric arrangement of thylakoids is observed inside the chloroplast. X 40,000

Figure 87  Concentric body becomes detached from the plastid (arrowhead). X 36,000

Figure 88  Concentric body lies free in the cytoplasm. X 26,000

Figure 89  Acid phosphatase activity is found associated with concentric bodies once they are present inside vacuoles. X 30,000

Figure 90  Acid phosphatase activity (control preparation). Notice the absence of reaction product. X 30,000

Figure 91  Concentric body inside vacuole. X 30,000
Figure 92  Portion of a chloroplast showing a thylakoid free region (F). Bridge-like elements are found between the outermost thylakoid of the peripheral band and the chloroplast envelope. X 80,000

Figure 93  The close relationship between the pyrenoid matrix fibrils and the surrounding thylakoid membranes is observed (material held for 5 days in total darkness). X 60,000
92

93
Figure 94  Section tangential to the thylakoid membranes. Ribosome-like particles (r) are seen in close association with thylakoids. X 50,000

Figure 95  Freeze-etch preparation of a chloroplast. Fibrillar elements are seen in association with the thylakoids (arrowheads). A plastoglobulus (Pg) is also depicted. X 36,000

Figure 96  Section parallel to the thylakoid membranes. Fibrillar elements are seen to run in between thylakoids (white arrows). X 90,000
Figures 97, 98, and 99. Plastoglobuli architecture. Notice the fibrillar organization of these structures. At places the fibrillar elements that composed these bodies seem to be linked to the thylakoid membranes (arrowheads). One of the micrographs (figure 99) shows the fibrillar phase of one plastoglobulus to be continuous with the fibrillar phase of another.

Figures 97, and 98 X 100,000

Figure 99 X 80,000

Figure 100 E.R.-vacuole continuity is depicted at arrowheads. X 25,000

Figure 101 Laminaria type of chloroplast division. The constricting region of the chloroplast is indicated by an arrowhead. X 20,000

Figures 102, and 103 Sphacelaria type of chloroplast division.

Figure 102 Plasmalemmasome-like (pm) structures are also depicted. X 12,000

Figure 103 Arrowhead points to the place of the peripheral lamellar bridge formation. X 18,000
Figure 104  Bleb-like structure produced by a chloroplast of dark treated material (5 days in total darkness). X 60,000
Figures 105, and 106. Non consecutive serial sections showing a constricting chloroplast.

Figure 105  X 16,000

Figure 106  X 26,000

Figure 107 Chloroplast with two distinct constriction sites (big arrowheads). Genophore is indicated by small arrowheads. X 24,000

Figure 108 Chloroplast with two pyrenoids. The pyrenoid on the right side of the micrograph shows a close relationship to thylakoids. X 24,000
Figure 109 This figure depicts a stage in the process of pyrenoid division; notice both the chloroplast envelope (ce) and the chloroplast E.R. (cer). X 100,000

Figure 110 Cross section of a pyrenoid depicting the relationship existent among the different membrane systems which surround the pyrenoid. X 40,000

Figure 111 Cross wall adjoining two consecutive cells. Notice that the plasmodesmata are not organized into a pit-like area. X 30,000

Figure 112 Direct continuity between the pyrenoid sac space (Pys) and the vacuole (v). X 22,000
Figure 113  Cell wall organization. Two regions are recognized in the cell wall: 1) an inner layer (i) showing a parallel arrangement of its fibrillar elements, 2) an outer layer (o) reticulate in appearance. X 60,000

Figure 114  Longitudinal section passing through the region of division of a pyrenoid. X 50,000

Figure 115  At arrowheads, one process of autophagocytosis, involving the invagination of the tonoplast, is depicted. Provacuole-like structures are indicated by arrows. Their origin from the E.R. is apparent. X 36,000

Figure 116  Detail of a portion of the cytoplasm showing provacuolar structures and their tendency to merge with themselves and with vacuoles (arrowheads). X 40,000
Figure 117 Freeze-etch preparation of the cell wall, showing the bilayered organization. X 30,000

Figure 118 Freeze-etch preparation of a region of the cytoplasm showing provacuolar structures, and their tendency to fuse with each other (arrows). X 40,000

Figure 119 The involvement of E.R. elements in the isolation of cytoplasmic territories is shown. Notice at arrow the bifurcation of the E.R. X 40,000

Figures 120, and 121 Aspects of the isolation of cytoplasmic territories involving the participation of the E.R. X 60,000

Figures 122, and 123 Two different stages in the process of transformation of E.R. membranes and isolated materials into typical vacuoles are shown. X 60,000

Figure 124 The trapping of cytoplasmic material by tonoplast invagination is shown. X 45,000
Figures 125, and 126 Acid phosphatase activity is associated with vacuoles. X 30,000

Figure 127 Vacuole showing remnants of disorganized membrane inclusions. X 30,000

Figure 128 Inclusions crowding the cytoplasm of a transitional "1-2" cell. X 6,000

Figures 129, and 130 Acid phosphatase activity in dictyosomes (figure 129) and E.R. (figure 130).

Figure 129 X 10,000

Figure 130 X 20,000

Figure 131 Acid phosphatase activity (control preparation). Notice the absence of reaction product. X 12,000

Figure 132 Transitional "1-2" cell. Notice the pattern of thylakoid arrangement. X 25,000

Figure 133 Notice that neither the plastoglobuli (Pg) nor the region of intraplasmid metabolite (big arrowhead) are preserved by permanganate fixation. X 20,000

Figure 134 Permanganate effects on the preservation of cytoplasmic inclusions. Notice that while some inclusions are preserved "1", others are only partially "2", and still others are not preserved at all "3". X 17,500
Figures 135, and 136: Transitional "1-2" cells.

Two types of vacuolar inclusions seem to be present in these cells. One type (a) is granular in appearance, the other is myelin-like (b). X 36,000
Figure 137  High magnification observation of vacuolar inclusions found in transitional "1-2" cells. A mixed, myelin-like, inclusion (B) and granular inclusions (A) are depicted. X 80,000

Figures 138, and 139  Transitional "1-2" cells. Distribution of acid phosphatase activity X 12,000

Figure 140  Transitional "1-2" cell. Acid phosphatase activity (control preparation). Notice the absence of reaction product. X 10,000

Figure 141  Transitional "1-2" cell. Aspect of the Golgi region. Dictyosome-derived vesicles (Dv) are filled with metabolites. X 20,000
Figure 142  Transitional "1-2" cell. Cell wall. Notice the presence of electron dense metabolites among the cell wall microfibrils and at the wall periphery. X 36,000

Figure 143  "Cell type #2". General ultrastructure of a prostrate system cell. At black arrows several chloroplast regions involved in metabolite production are indicated. White arrows point to a highly vesiculated E.R. region. X 18,000

Figure 144  "Cell type #2". Cell wall. Notice the presence of conspicuous patches of metabolites among the cell wall microfibrils and at the wall periphery. X 6,000

Figure 145  Transitional "2-3" cell. General morphology of an erect system cell. X 8,000

Figure 146  This picture displays the abrupt transition between "cell type #2" and "cell type #3" morphologies. X 8,000

Figure 147  Transition "2-3" cells. General aspect of the background cytoplasm. X 26,000
Figure 148 "Cell type #2". General ultrastructure of an erect system cell. At arrows several chloroplast regions involved in metabolite production are indicated. X 14,000

Figure 149 High magnification observation of a chloroplast region involved in metabolite production. X 60,000

Figure 150 High magnification observation of the background cytoplasm of a "cell type #2". X 60,000
Figure 151  "Cell type #2". General ultrastructure of a prostrate system cell. Several chloroplast regions engaged in metabolite production are indicated (arrows). A region of E.R. disorganization is also depicted. X 20,000

Figure 152  "Cell type #2". Cell wall ingrowths are observed. The alternate disposition of metabolite (1, 3, 5) and wall material (2, 4) are indicated. X 40,000
Figure 153  "Cell type #2". The nucleus (N) and the nucleolus (nl) are depicted. Mitochondria (M) are also observed. A cell wall ingrowth is seen and its double constitution indicated ( labels 1, and 2 ). Black arrows indicate the similarity existent between the cell wall inclusion material and the vacuole inclusion material. X 50,000

Figures 154, and 156  High magnification observation of chloroplast zones of metabolite production.

Figure 154  X 70,000

Figure 156  X 36,000

Figure 155  Release of intraplastid metabolite into the cytoplasm. X 18,000
Figure 157  Release of intraplastid metabolites into the cytoplasm. X 32,000

Figure 158  Portion of a cross wall separating two "cell type #2" neighboring cells. The layering constitution of one of the cell wall ingrowths is indicated. At arrowheads plasmodesmata are indicated. X 40,000

Figure 159  Freeze-etch preparation of portion of a chloroplast depicting a region of metabolite production (arrowhead). X 34,000

Figure 160  "Cell type #2". General ultrastructure of an erect system cell. The morphology of the cell wall is also depicted. X 10,000

Figures 161, and 162  Cell wall ingrowths morphology.

Figure 161  X 12,000

Figure 162  X 20,000

Figure 163  Transitional "1-2" cell. An early stage in the formation of a cell wall ingrowth is shown. X 30,000
Figure 164  Transitional "2-3" cell. General aspect of the background cytoplasm. X 12,000

Figure 165  Transitional "2-3" cell. Tonoplast is absent at certain places (arrowheads). X 50,000

Figure 166  Transitional "2-3" cell. Chloroplast morphology. Notice at arrowhead the protrusion from the chloroplast body of a stroma containing structure. Notice also that this structure is distinct from the pyrenoid (Py), but similar to other structures seen in the cytoplasm of other cells (c.f. figures 167, 169, arrowheads). X 30,000

Figures 167, and 169  Transitional "2-3" cells. Chloroplast and pyrenoid morphology. Notice the lamellar nature of the region labelled "A". Plastid-derived bodies are detected in the cytoplasm (arrowheads). X 22,000

Figure 168  Transitional "2-3" cell. The chloroplast envelope (ce) is clearly defined around the pyrenoid body (Py). At the arrowhead the abscission of the pyrenoid from the chloroplast seems to be occurring. X 36,000
Figure 170  Transitional "2-3" cell. Notice that the nucleus is in the process of disorganization. The nuclear envelope is no longer recognizable at places, while in others seems to be degenerating (arrows). The portion of the nuclear envelope longer remaining intact seems to be the one facing the dictyosome (D). X 50,000

Figure 171  Transitional "1-2" cell. A phase in the formation of a cell wall ingrowth (cwi) is depicted. Deposition of new wall material is seen at arrowheads. X 38,000

Figure 172  Transitional "2-3" cell. Chloroplast morphology. Notice the lamellar nature of the area labelled "A" and the phenomenon of abscission of plastid derived structures "B". X 50,000
Figures 173, 174, and 175  Transitional "2-3" cells. Mitochondrial morphology. Notice the circular arrangement of the mitochondrial cristae (figures 174, and 175).
X 30,000

Figure 176  Plastid-derived body. X 36,000

Figure 177  Transitional "2-3" cell. The relationship of metabolite to the chloroplast lamellae is still apparent in this picture (white arrowhead). X 36,000

Figure 178  Transitional "2-3" cell. Abscission of chloroplast-derived structures from the plastid body. X 42,000

Figure 179  Transitional "2-3" cell. The involvement of thylakoids in the isolation of stroma regions is depicted (arrowheads). X 50,000
Figure 180  Transitional "2-3" cell. Chloroplast morphology. The isolation of stroma regions from the main plastid body is shown. X 20,000

Figure 181  Transitional "2-3" cell. Chloroplast derived bodies (arrowheads) are seen in the cytoplasm. Mitochondria are seen at "A". X 16,000

Figure 182  Transitional "2-3" cell. A portion of a plastid showing an area of isolation of stroma material is depicted. At arrowhead the membrane of one of the isolated areas has fused with the chloroplast envelope membrane, which is single membrane in constitution. X 40,000

Figure 183  Transitional "2-3" cell. Pyrenoid-like structures (Py) are observed inside vacuoles. X 24,000

Figure 184  "Cell type #3". Aspect of the cell wall. X 60,000
Figures 185, and 186  Transitional " 2-3 " cells.
Serial sections showing the release of plastid derived bodies into the cytoplasm.
X 36,000
Figures 187, 188, and 189 "Cell type #3". General ultrastructural morphology.

Figures 187, and 188 Prostrate system cells. X 6,000

Figure 189 Erect system cell. X 6,000

Figure 190 "Cell type #3". Chloroplast morphology. X 30,000

Figure 191 "Cell type #3". Acid phosphatase activity (erect system cell). Notice that reaction product is distributed throughout the cell cavity. X 20,000
Figure 192  "Cell type #3". Detail of a thylakoid stack. X 70,000

Figures 193, and 194  "Cell type #3". Portions of chloroplasts showing signs of thylakoid disorganization. X 30,000

Figure 195  "Cell type #3". Portion of a chloroplast showing extensive disorganization of the thylakoid system. X 24,000

Figure 196  "Cell type #3". Aspect of the disorganization of the cytoplasm and mitochondria (M). X 36,000

Figure 197  "Cell type #3". Mitochondrial (M) remnants showing different stages of disorganization. X 36,000
Figure 198  "Cell type #3". Chloroplast showing widespread signs of thylakoid disorganization. X 36,000

Figure 199  "Cell type #3". Accumulation of plastoglobuli inside the chloroplast. X 26,000

Figure 200  "Cell type #3". Detail of thylakoid arrangement. A genophore-like region (g) is apparent. X 30,000

Figure 201  "Cell type #3". Aspect of disorganization of cytoplasm and mitochondria (M). X 28,000

Figure 202  "Cell type #3". Mitochondrial remnant. X 42,000
Figure 203  "Cell type #3". Acid phosphatase activity (control preparation). Notice the absence of reaction product. X 12,000

Figure 204  "Cell type #3". Acid phosphatase activity. Reaction product is apparent in plasmodesmata and cytoplasm. X 36,000

Figure 205  "Cell type #3". Acid phosphatase activity (control preparation). Notice that no reaction product is found associated with plasmodesmata. X 30,000

Figure 206  "Cell type #3". Acid phosphatase activity. Reaction product is distributed throughout the cell cavity. X 12,000

Figures 207, and 208  "Cell type #3". Aspects of disorganization of the cell wall.

Figure 207  X 30,000

Figure 208  X 45,000
Figures 209, and 210  "Cell type #1". Material incubated for determination of catalase activity. Microbody-like organelles (m) show deposition of reaction product.

Figure 209  X 30,000

Figure 210  Arrow points to core-like structure.  X 36,000

Figure 211  "Cell type #1". Aminotriazole incubation (catalase control). No reaction product is observed in the microbodies (m).  X 22,000

Figure 212  "Cell type #1". Material incubated for determination of catalase activity. Reaction product is observed in mitochondria (M).  X 30,000

Figure 213  "Cell type #2". Material incubated for determination of catalase activity. Arrowhead points to zone of deposition of reaction products.  X 30,000

Figure 214  "Cell type #1". Peroxidase activity is observed in the cell wall.  X 16,000

Figure 215  "Cell type #1". A microbody showing a core-like structure (arrow) is depicted.  X 36,000

Figure 216  "Cell type #1". Peroxidase activity is apparent in the cell wall (cw) and paramural space.  X 13,000
Figures 217, and 220. "Cell type #3". Acid phosphatase activity. Reaction product is distributed throughout the cell cavity. X 26,000

Figure 218. "Cell type #1". Peroxidase activity; control preparation (KCN). Notice the absence of reaction product. X 12,000

Figure 219. Transitional "1-2" cell. ATPase activity (Mg\(^{++}\)-dependent system). Notice deposition of reaction product in the mitochondrion (arrow). X 18,000

Figure 221. "Cell type #2". Material incubated for localization of peroxidase activity. X 30,000

Figure 222. "Cell type #2". Peroxidase activity (control preparation). X 30,000
Figures 223, and 225: "Cell type #1". ATPase activity (Na\(^+\)-K\(^+\)-Mg\(^{++}\) dependent system). Deposition of reaction product is conspicuous at the plasmalemma and in association with thylakoids.

Figure 223  X 16,000

Figure 225  X 36,000

Figure 224: "Cell type #1". ATPase activity (Na\(^+\)-K\(^+\)-Mg\(^{++}\) dependent system). Deposition of reaction product is observed in the mitochondrion (M).  
X 32,000

Figure 226: "Cell type #1". ATPase activity (Mg\(^{++}\) dependent system). Deposition of reaction product is apparent in the mitochondrion.  
X 40,000

Figure 227: "Cell type #1". ATPase activity (Mg\(^{++}\) dependent system). Reaction product is observed in association with the plasmalemma and thylakoids. The reaction, however, does not seem so intense as in the case of the Na\(^+\)-K\(^+\)-Mg\(^{++}\) dependent system.  
X 12,000

Figure 228: ATPase activity (Na\(^+\)-K\(^+\)-Mg\(^{++}\) activated system; control preparation). Notice the absence of reaction product.  
X 14,000
Figures 229, and 230 ATPase activity (Na\(^+\)-K\(^+\)-Mg\(^{++}\)-activated system).

Figure 229 Deposition of reaction product is observed in association with the plasmalemma and thylakoids. X 14,000

Figure 230 Control preparation (incubation medium minus ATP). Notice the absence of reaction product. X 12,000
Figures 231, and 233 Identification of lipofuscin-like material (Hendy, 1971, modification of the Fontana's technique). Notice that labelling is primarily found in vacuolar inclusions.

Figure 231 X 20,000

Figure 233 Notice that zones of intraplasmid metabolite formation are free of labelling (arrows), and so are similar inclusions present in the cytoplasm. X 20,000

Figure 232 ATPase activity (Mg$^{++}$-activated system; control preparation). Notice the absence of reaction product. X 18,000

Figure 234 Lipofuscin (control preparation). X 18,000

Figure 235 Schorml's modification (Hendy, 1971) for the ultrastructural identification of lipofuscin. X 18,000