SORUS ABSICISSON FROM LAMINAE OF
NEREOCYSTIS LUETEKANA (MERT.) POST. AND RUP.

BY

DAVID CABOT WALKER

B.A., University of California, Santa Barbara, 1968
B.A., University of California, Santa Barbara, 1970
M.A., University of California, Santa Barbara, 1972

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
in
THE FACULTY OF GRADUATE STUDIES
(Department of Botany)

We accept the thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA
February, 1980

© David C. Walker, 1980
In presenting this thesis in partial fulfilment of the requirements for
an advanced degree at the University of British Columbia, I agree that
the Library shall make it freely available for reference and study.
I further agree that permission for extensive copying of this thesis
for scholarly purposes may be granted by the Head of my Department or
by his representatives. It is understood that copying or publication
of this thesis for financial gain shall not be allowed without my
written permission.

Department of Botany

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date March 26, 1980
ABSTRACT

Advisor: Dr. Thana Bisalputra

The release of mature sori from the laminae of Nereocystis was first reported about the turn of the century. Although that report was confirmed repeatedly and the process has been called abscission, it has never been examined at any more than the gross morphological level.

This work describes the process morphologically at both the light and electron microscope levels. This was done with the intention of discovering if, indeed, work with the vascular plants or if it was even comparable. It was hoped that some new insights might be gained into the reproductive mechanisms of Nereocystis as well. Assuming that soral margins were comparable to abscission zones made it essential to know the morphology of surrounding vegetative and included soral tissues during the process of sorus release.

A gradual gradient of the developmental stages which produced the sorus is found in sections across young soral margins. Using the events of meristoderm cell periclinal division, paraphysis initial elongation and zoosporangial initial inception as markers the sorus margin can be divided into three regions. Periclinal division of the meristoderm cells begins the sorus margin and Region I. Paraphysis initial elongation indicates the boundary between Regions I and II. The appearance of zoosporangial initials and sorus lumen
demarks the border between Regions II and III. Region III ends at the sorus proper.

Sorus release consists of two phases. Release begins with necrosis and eventual erosion of the paraphysis initials, zoosporangial initials and subtending cells at the borderline between Regions II and III. Phase two commences with the schizogenous dissolution of middle lamellae between underlying cortical cells. Beginning at both surfaces sorus release is completed with dissolution of the matrix of the central medulla. This localized weakening of sorus margin tissues and separation is influenced mechanically by water motion and is interpreted as a Laminariales morphological equivalent to vascular plant abscission.

Observations on transition and expansion zones of young thalli and the process of mucilage duct inception in these led to a better understanding of the abscission process. Mucilage duct system inception involves the burial of meristematic cells accompanied by the schizogenous appearance of a duct lumen filled by the secretory initials. Confinement of this inception process to the meristoderm indicates that it is homologous to sorus inception, only in a different context and with different end products. This is supported by the discovery that zoosporangial initials begin in a secretory capacity filling schizogenous sorus lumen and that, lacking only divisions, paraphysis initials are meristematic. It is apparent that abscission is then a modification of the events
of zoosporangial inception in a meristematic setting which leads to abscission by schizogeny.

Several insights into the reproductive mechanisms of *Nereocystis* have also resulted. The mucilage duct system, through the inception process, maintains a relationship with the surface at its potential venting points and may assist in flushing spores from the sorus surface. Paraphyses play a primary role in the maintenance of the outer cell wall and appear to be regulated by the zoosporangia. Finally, an unanticipated role of the abscission process was found to be the releasing of the outer cell wall of the sorus at its perimeters to facilitate exuviation and ultimately spore release.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>xx</td>
</tr>
<tr>
<td>FIGURE CAPTION KEYS</td>
<td>xxi</td>
</tr>
<tr>
<td>I GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II MATERIALS AND METHODS</td>
<td>4</td>
</tr>
<tr>
<td>1. Materials</td>
<td>4</td>
</tr>
<tr>
<td>2. Light Microscopy</td>
<td>6</td>
</tr>
<tr>
<td>3. Transmission Electron Microscopy</td>
<td>6</td>
</tr>
<tr>
<td>4. Scanning Electron Microscopy</td>
<td>7</td>
</tr>
<tr>
<td>5. Histology</td>
<td>7</td>
</tr>
<tr>
<td>6. Negative Staining and Shadowing</td>
<td>8</td>
</tr>
<tr>
<td>III CHAPTER 1. MERISTODERM DIFFERENTIATION</td>
<td>17</td>
</tr>
<tr>
<td>1. Introduction</td>
<td>17</td>
</tr>
<tr>
<td>2. Observations</td>
<td>18</td>
</tr>
<tr>
<td>2.1. Transition Zone Meristoderm Cells</td>
<td>18</td>
</tr>
<tr>
<td>2.1.1. Anatomy</td>
<td>19</td>
</tr>
<tr>
<td>2.1.2. Histology</td>
<td>20</td>
</tr>
<tr>
<td>2.1.3. Ultrastructure</td>
<td>21</td>
</tr>
<tr>
<td>2.2. Expansion Zone Meristoderm Cells</td>
<td>47</td>
</tr>
<tr>
<td>2.2.1. Anatomy</td>
<td>47</td>
</tr>
<tr>
<td>2.2.2. Histology</td>
<td>49</td>
</tr>
<tr>
<td>2.2.3. Ultrastructure</td>
<td>50</td>
</tr>
<tr>
<td>2.3. Meristoderm cells adjacent to youngest sori</td>
<td>74</td>
</tr>
<tr>
<td>2.3.1. Anatomy</td>
<td>74</td>
</tr>
<tr>
<td>2.3.2. Histology</td>
<td>75</td>
</tr>
<tr>
<td>2.3.3. Ultrastructure</td>
<td>76</td>
</tr>
<tr>
<td>2.4. Meristoderm Cells adjacent to Mature Sori</td>
<td>95</td>
</tr>
<tr>
<td>2.4.1. Anatomy</td>
<td>95</td>
</tr>
<tr>
<td>2.4.2. Histology</td>
<td>96</td>
</tr>
<tr>
<td>2.4.3. Ultrastructure</td>
<td>97</td>
</tr>
<tr>
<td>3. Discussion</td>
<td>118</td>
</tr>
<tr>
<td>3.1. Cytological Characteristics of Meristoderm in Transition and Expansion Zones</td>
<td>119</td>
</tr>
<tr>
<td>3.2. Characteristics of Meristoderm Cells Adjacent to the Youngest Sori</td>
<td>135</td>
</tr>
<tr>
<td>3.3. Characteristics of Meristoderm Cells Adjacent to the Oldest Sori</td>
<td>145</td>
</tr>
<tr>
<td>3.4. Summary of Cytomorphogenesies in the Meristoderm</td>
<td>152</td>
</tr>
<tr>
<td>IV CHAPTER 2. MUCILAGE DUCT SYSTEM</td>
<td>160</td>
</tr>
<tr>
<td>1. Introduction</td>
<td>160</td>
</tr>
<tr>
<td>2. Observations</td>
<td>161</td>
</tr>
<tr>
<td>2.1. Mucilage Duct Inception</td>
<td>162</td>
</tr>
<tr>
<td>2.2. Mature Mucilage Ducts and Secretory Cells</td>
<td>163</td>
</tr>
</tbody>
</table>
2.3. Secretory Cells .......................................................... 165
2.4. Possible Duct Venting Points ......................................... 165
2.5. Physical Disruptions ..................................................... 166
3. Discussion ........................................................................ 168
  3.1. Mucilage Duct Differentiation ......................................... 168
  3.2. Potential Duct Venting Points and Physical Disruptions ........ 172
  3.3. Mucilage Duct System Structure and Function .................... 173

V CHAPTER 3. PARAPHYSES AND SORUS DIFFERENTIATION .......... 191
  1. Introduction .................................................................. 191
  2. Observations ................................................................. 192
    2.1. Paraphysis and Sorus Inception .................................... 192
    2.2. Paraphyses of the Post-inception/Pre-meiotic Sorus ........ 194
      2.2.1. Histology ........................................................... 195
        2.2.1.1. The Outer Cell Wall of the Pre-meiotic Sorus .......... 195
        2.2.1.2. The Extracellular Lumen of the Sorus ............... 196
        2.2.1.3. The Paraphyses, Subtending Cells and Zoosporangia .. 196
      2.2.2. Ultrastructure of the Paraphyses ............................. 197
      2.2.3. The Subtending Cells ............................................ 199
      2.2.4. The Extracellular Space and Zoosporangial Initials ... 199
    2.3. Paraphyses of the Meiotic Sorus ................................ 200
      2.3.1. Histology ........................................................... 201
        2.3.1.1. The Outer Cell Wall ....................................... 201
        2.3.1.2. The Extracellular Sorus Lumen ........................... 201
        2.3.1.3. The Paraphyses, Subtending Cells and Zoosporangia .. 202
      2.3.2. Ultrastructure of the Meiotic Sorus ......................... 202
        2.3.2.1. The Paraphyses .............................................. 202
        2.3.2.2. Subtending Cells .......................................... 206
    2.4. The Mature Sorus ..................................................... 206
      2.4.1. Histology ........................................................... 207
        2.4.1.1. The Outer Cell Wall and the Extracellular Lumen .... 207
        2.4.1.2. The Paraphyses, Subtending Cells, and Zoosporangia .. 207
      2.4.2. Ultrastructural Aspects of the Mature Sorus ................. 208
        2.4.2.1. The Outer Wall and the Paraphyses .................... 208
        2.4.2.2. The Paraphyses ............................................. 209
    2.5. Sori After Exuviation and Zoospore Release .................... 210
      2.5.1. Histology of the Released Sorus ................................ 211
      2.5.2. Ultrastructure ..................................................... 212
        2.5.2.1. Paraphyses of the Post-exuviation/Pre-spore Release Sorus .. 212
        2.5.2.2. Paraphyses of the Post-exuviation and Post-spore Release Sorus .. 213
3.3.2. Zoospore Release and Behavior ..................419
3.3.3. The Motile Zoospore Structure and Function ...........................................422
LIST OF FIGURES

MATERIALS AND METHODS FIGURES, THALLUS AND TISSUE SAMPLING

Plate I .......................................................... 9
Figure 1. The whole thallus. ................................. 10
Plate II .......................................................... 10
Figure 2. Intercalary meristem of a young Nereocystis thallus. ................. 12
Figure 3. Proximal portion of an excised fertile lamina. ......................... 12
Figure 4. Midregion of an excised fertile lamina. .............................. 12
Plate III ....................................................... 14
Figure 5. Young sorus in mature tissues of a lamina. .......................... 14
Figure 6. Scanning electron micrograph of a piece of tissue excised as above. ................. 14
Figure 7. A thin strip of tissue. .................................. 14
Figure 8. Vegetative end of a light microscope section. ....................... 14
Figure 9. Sorus margin area of an LM section. ................................ 14
Figure 10. Soral end of an LM section. .................................. 14
Figure 11. Section from vegetative end of a thin tissue strip for EM. .......... 15
Figure 12. Section across sorus margin region of a thin tissue strip for EM. ...... 15
Figure 13. Section from the soral end of a thin strip. .......................... 15

CHAPTER I FIGURES, TRANSITION ZONE ...................... 26
Plate I .......................................................... 27
Figure 1. Diagram of a whole thallus. .................................. 27
Plate II .......................................................... 30
Figure 2. Meristoderm of the transition zone. ................................ 30
Figure 3. Single TBO stained transition zone meristoderm cell. ............... 30
Figure 4. Single TBO stained transition meristoderm cell. ..................... 30
Figure 5. Single TBO stained transition zone meristoderm cell. ............... 30
Figure 6. ABB stained transition zone meristoderm cell. ........................ 30
Figure 7. ABB stained transition zone meristoderm cells. ..................... 31
Plate III .......................................................... 33
Figure 8. Transmission electron micrograph of transition zone meristoderm cells. .................. 33
Figure 9. Lip of a primitive furrow. .................................. 33
Plate IV .......................................................... 35
Figure 10. Bottom of a primitive furrow. .................................. 35
Figure 11. Internal periclinal cell wall. .......... 35
Plate V ............................................ 37
Figure 12. The centrally located nucleus. ........ 37
Plate VI ............................................ 39
Figure 13. A meristoderm cell chloroplast. ....... 39
Figure 14. A dictyosome of the golgi apparatus. ... 39
Plate VII ........................................... 41
Figure 15. Apical cytoplasm of a meristoderm cell. 41
Plate VIII ........................................... 43
Figure 16. Simple vesiculation of the apical cytoplasm. ........ 43
Figure 17. Complex apical cytoplasm vesiculation. .. 43
Figure 18. A presumptive lipid droplet. ............ 43
Figure 19. A nucleolus and associated homogeneous region. ........................................ 43
Plate IX ............................................. 45
Figure 20. Basal cytoplasm of a meristoderm cell. ... 45

CHAPTER I FIGURES, EXPANSION ZONE .................. 54
Plate X ............................................. 55
Figure 1. Young thallus expansion zone meristoderm and cortex. ...................................... 55
Figure 2. TBO stained expansion zone meristoderm cells. ............................................... 55
Figure 3. TBO stained expansion zone meristoderm cells. ............................................... 55
Figure 4. More distal expansion zone meristoderm. .................................................. 55
Figure 5. ABB stained expansion zone meristoderm cells. ........................................... 56
Figure 6. Cytoplasmic continuities visible with ABB staining. ...................................... 56
Plate XI .............................................. 58
Figure 7. Meristoderm cells of the expansion zone. .................................................. 58
Plate XII ............................................. 60
Figure 8. Golgi apparati of expansion zone meristoderm cells. ....................................... 60
Figure 9. Polymorphic nucleus in an expansion zone meristoderm cell. ................................ 60
Figure 10. Another polymorphic nucleus. ................................................................. 60
Plate XIII ............................................ 62
Figure 11. Post mitotic expansion zone meristoderm cells. ........................................... 62
Figure 12. Detail of intact outer cell wall. ......... 62
Plate XIV ............................................. 64
Figure 13. Cytokinesis of a meristoderm cell. ...... 64
Figure 14. New cell plate and cortical initial. ...... 64
Plate XV .............................................. 66
Figure 15. Binucleate/pre-cytokinetic meristoderm cell. ............................................. 66
Figure 16. A dictyosome of the golgi apparatus. ... 66
Figure 2. AB/SO stained cells adjacent to oldest sorus. ................. 104
Figure 3. ABB/PAS stained cells adjacent to oldest sorus. .................. 104
Figure 4. Osmicated and TBO stained cells adjacent to the oldest sorus. .... 104
Plate XXVI ............................................106
Figure 5. Outer cell wall and apical cytoplasm of Type I cells .................. 106
Figure 6. Outer cell wall and some apical cytoplasm of Type II cells. .......... 106
Plate XXVII ............................................108
Figure 7. Nuclear features common to Types I and II cells. .................... 108
Figure 8. Chloroplast from Type I cell. .................................... 108
Figure 9. Chloroplast from Type II cells. .................................... 108
Plate XXVIII .............................................110
Figure 10. A dictyosome on the basal hemisphere of a Type I cell nucleus. ........ 110
Figure 11. Golgi vesicles and vacuolar region of a Type I cell. .................... 110
Figure 12. Dictyosome from Type II cell. .................................... 110
Plate XXIX .............................................112
Figure 13. Apical cytoplasm of a type II cell. .................................... 112
Figure 14. A granular/lamellar structure from a Type II cell basal cytoplasm. .... 112
Figure 15. Type I cell mitochondria and microbody. .................................... 112
Plate XXX .............................................114
Figure 16. Apical cytoplasm of a Type I cell. .................................... 114
Figure 17. Apical cytoplasm of Type II cell. .................................... 114
Figure 18. Apical cytoplasm of Type II cell. .................................... 114
Plate XXXI .............................................116
Figure 19. Basal cytoplasm of Type I cells. .................................... 116
Figure 20. Basal cytoplasm of Type II cells. .................................... 116

CHAPTER 2 FIGURES, MUCILAGE DUCT SYSTEM ........................................ 176
Plate I ............................................. 177
Figure 1. One quadrant of a lamina cross section. ................................. 177
Figure 2. Meristoderm cells from inception region. ................................. 177
Figure 3. Earliest recognized mucilage duct stage of development. ............... 178
Figure 4. Subsequent progeny cell divisions. .................................... 178
Figure 5. Continued progeny cell divisions. .................................... 178
Figure 6. A full mucilage duct. ............................................. 178
Figure 7. Mucilage duct stained with ABB for proteins. ........................... 178
Plate II ............................................. 180
Figure 8. Surface section of lamina cortex. .................................... 180
Figure 9. Oblique surface section of lamina. .................................... 180
Figure 10. Most superficial extensions of mucilage duct lumen. ................. 180
Figure 11. Cup of secretory cells. 180
Figure 12. Cross section of an apparently completely
lined mucilage duct. 180
Plate III 182
Figure 13. Young undisturbed sorus material. 182
Figure 14. Mature undisturbed sorus material. 182
Figure 15. Cross section of physically agitated
young sorus. 182
Figure 16. Blistered young agitated soral material. 182
Plate IV 184
Figure 17. Cross section of physically disturbed
young lamina. 184
Figure 18. Agitated mature zoosporangial material. 184
Figure 19. Old abscessed sorus tissues. 184
Plate V 186
Figure 20. Secretory cell of a mature mucilage duct
system. 186
Plate VI 188
Figure 21. A diagrammatic summary. 188
Figure 21a. The meristoderm cell source. 188
Figure 21b. Immediate progeny cell divisions. 188
Figure 21c. Subsequent divisions. 189
Figure 21d. Mature relationship of secretory cells,
duct lumen and the meristoderm. 189
Figure 21e. Mucilage duct system and sorus
inception. 189
Figure 21f. Mucilage duct system, mature sorus
undisturbed relationship. 189

CHAPTER 3 FIGURES, SORUS AND PARAPHYES DEVELOPMENT 237
Plate I 238
Figure 1.a. Diagram of the whole thallus. 238
Figure 1.b. Cross section of the youngest sorus of a
lamina. 238
Figure 1.c. Cross section of an intermediate aged
sorus. 238
Figure 1.d. The mature sorus. 238
Plate II 240
Figure 2. The margin of a youngest sorus. 240
Figure 3. Youngest sorus margin. 240
Figure 4. Youngest sorus margin still closer to the
sorus. 240
Plate III 242
Figure 5. The subtending cell products. 242
Figure 6. The youngest visible sorus. 242
Figure 7. AB/SO staining of youngest visible sorus. 242
Figure 8. ABB/PAS staining of youngest visible
sorus. 242
Plate IV 245
Figure 9. Paraphyses of a youngest sorus. 245
Figure 10. Nucleus of paraphyses of youngest sorus. 245
Figure 11. A dictyosome adjacent to the nucleus. 245
Figure 12. Two dictyosomes located basally on the nuclear envelope. 245
Plate V  .................................... 247
Figure 13. A chloroplast from youngest paraphyses. 247
Figure 14. Apical cytoplasm of a paraphysis from a youngest sorus. 247
Figure 15. Exocytotic vesicle on apical region of the paraphysis plasma membrane. 247
Figure 16. Secretory paraphysis apical cytoplasm. 247
Plate VI ........................................ 249
Figure 17. Zoosporangia from a youngest sorus. 249
Figure 18. AB/SO stained youngest sorus zoosporangia. 249
Figure 19. Basal hemisphere of a zoosporangial initial nucleus. 249
Figure 20. Golgi vesicles of these zoosporangial initials. 249
Plate VII ....................................... 251
Figure 21. Intermediate aged sorus at meiosis. 251
Figure 22. Intermediate aged sorus. 251
Figure 23. Intermediate aged sorus disrupted. 251
Figure 24. Intermediate aged sorus TBO stained. 251
Figure 25. AB/SO stained intermediate aged sorus. 252
Figure 26. ABB/PAS staining of intermediate aged sorus. 252
Plate VIII ..................................... 254
Figure 27. Intermediate aged sorus paraphysis prior to meiosis. 254
Figure 28. Intermediate aged post-meiotic sorus paraphysis. 254
Plate IX ....................................... 256
Figure 29. Cell wall and cytoplasm of a pre-meiotic sorus paraphysis. 256
Figure 30. Outer cell wall over dead paraphysis. 256
Figure 31. Paraphysis outer cell wall and apical cytoplasm from a post-meiotic sorus. 256
Plate X ......................................... 258
Figure 32. Golgi apparatus and nucleus of a premeiotic sorus paraphysis. 258
Figure 33. Dictyosomes of a paraphysis from a post-meiotic sorus. 258
Figure 34. Paraphyses of meiotic sorus. 258
Figure 35. Paraphysis basal cytoplasm from a meiotic sorus. 258
Figure 36. Paraphysis basal cytoplasm, intermediate sorus. 259
Figure 37. Paraphysis basal cytoplasm, intermediate sorus. 259
Plate XI  Paraphysis from a meiotic sorus.  261
Figure 38. Basal periclinal wall separating paraphysis from subtending cell.  261
Figure 39. Paraphysis cytoplasm, intermediate sorus.  261

Plate XII  263
Figure 40. Section from a sorus with zoosporangia in cytokinesis.  263
Figure 41. ABB stained sorus.  263
Figure 42. Paraphyses and outer cell wall of a sorus in cytokinesis.  263
Figure 43. A region of advanced paraphysis, outer wall disjunction.  264

Plate XIII  266
Figure 44. Paraphysis tip at separation from the outer wall.  266

Plate XIV  268
Figure 45. Paraphysis apical cytoplasm during sorus cytokinesis and exuviation.  268
Figure 46. Paraphysis cytoplasm, mature sorus.  268
Figure 47. Paraphysis cytoplasm, mature sorus.  268
Figure 48. Paraphysis cytoplasm, mature sorus.  268

Plate XV  270
Figure 49. Paraphysis tip after exuviation, before zoospore release.  270

Plate XVI  272
Figure 50. Sorus after exuviation and dehiscence of most zoosporangia.  272
Figure 51. TBO stained released sorus, retrieved from a holding tank.  272
Figure 52. ABB/PAS stained, released sorus retrieved as above.  272
Figure 53. AB/SA stained sorus as above.  273

Plate XVII  275
Figure 54. AB/SA stained sorus retrieved as above.  275

Plate XVIII  277
Figure 55. Mature, post-exuviation sorus subsequent to release of most zoospores.  277

Plate XIX  279
Figure 56. Paraphysis apical cytoplasm from a released sorus.  277

Plate XX  279
Figure 57. Live young sorus tissue cut and freeze-dried.  279
Figure 58. Critical point dried intermediate aged sorus.  279
Figure 59. Fracture face through Spurr's embedded intermediate aged sorus.  279
Figure 60. Detail of paraphyses prepared as in figure 59.  279

Plate XXI  281
Figure 61. Critical point dried almost mature sorus. .................................................. 281
Figure 62. Freeze dried almost mature sorus. ................................................................. 281
Figure 63. Critical point dried mature zoosporangial sorus. .......................................... 281
Figure 64. Mature sorus, freeze dried zoosporangia and paraphyses tips coated with mucilage. 281
Figure 65. The inside surface of the outer wall of a mature sorus. ................................ 281
Figure 66. The outer surface of the outer wall of a mature sorus. ................................ 282
Plate XXI ................................................................. 284
Figure 67. Young sorus of a hybrid thallus from Pelagophycus and Macrocystis. ............... 284
Figure 68. More mature sorus from same thallus as figure 67. ...................................... 284
Figure 69. Sorus material on one side of excised lamina. ............................................ 284
Figure 70. Sorus material on other side of excised lamina. .......................................... 285

CHAPTER 4 FIGURES, SORUS ABSCISSION ................................................................. 332
Plate I ................................................................................................................... 333
Figure 1. SEM of an intermediate sorus margin. ............................................................... 333
Figure 2. The margin of an abscissing mature sorus with SEM. ...................................... 333
Figure 3. Methacrylate section of a young sorus margin TBO stained. .............................. 333
Figure 4. The margin of an intermediate aged sorus .......................................................... 333
Figure 5. The margin of a mature sorus. ......................................................................... 334
Plate II ................................................................................................................... 336
Figure 6. Paraphysis initial from a youngest sorus margin .................................................. 336
Figure 7. Elongating paraphysis apical cytoplasm from a youngest margin ......................... 336
Figure 8. Intermediate sorus margin paraphyses ............................................................... 336
Figure 9. Intermediate sorus margin paraphysis apical cytoplasm and cell wall .................. 336
Figure 10. Intermediate sorus margin paraphysis apical cytoplasm ................................... 337
Figure 11. Electron dense lining of the inner surface of the anticlinal walls ................... 337
Plate III ................................................................................................................... 339
Figure 12. Mature sorus margin region I in methacrylate, ABB/PAS stained. .................... 339
Figure 13. Mature sorus margin Region II in methacrylate, ABB/PAS stained .................... 339
Figure 14. Mature sorus margin Region III in methacrylate ............................................ 339
Figure 15. Mature sorus margin Region I meristoderm cells ........................................... 339
Figure 16. Mature sorus margin Region II paraphyses. ................................................................. 340
Figure 17. Region III paraphyses and aborted zoosporangial initials. ........................................ 340
Figure 18. A paraphysis initial from Region I. ....... 340
Figure 19. Paraphyses of Region I. ....................... 340
Plate IV .................................................................. 343
Figure 20. Mature sorus margin paraphysis in region III. .............................................................. 343
Figure 21. Mature sorus margin paraphysis in Region III. ............................................................. 343
Plate V .................................................................. 345
Figure 22. Separation of internal tissues at the mature sorus margin. ........................................... 345
Figure 23. Mature sorus margin separation in the cortical layer. ................................................... 345
Figure 24. Margin of a mature abscissed sorus. .......................................................... 345
Plate VI .................................................................. 348
Figure 25. The margin of a hole left in a lamina after sorus abscission. .......................................... 348
Figure 26. Section in lamina plane at the inner cortex level in vegetative tissues of post abscission margin. ........................................................... 348
Figure 27. Abscission margins ......................................................... 348
Plate VII .................................................................. 350
Figure 28. Unwounded laminar cortical and medullary tissues. ...................................................... 350
Figure 29. A just cut edge of a vegetative lamina. ................. 350
Figure 30. A one week old cut edge of lamina. .............................................................. 350
Plate VIII .................................................................. 352
Figure 31a. Diagram of the sorus margin prior to abscission. .......................................................... 352
Figure 31b. Meristoderm erosion. ................................. 352
Figure 31c. Sorts exuviation and separation. .............................................................. 352

APPENDIX 1 FIGURES, ZOOSPOROBGENESIS .................................................................................. 435
Plate I ........................................................................ 436
Figure 1. Post-inception zoosporangial initial. .......... 436
Figure 2. ABB/PAS stained youngest sorus tissue. .... 436
Figure 3. A dictyosome of the golgi apparatus. ......... 436
Figure 4. A pair of centrioles. .................................. 437
Figure 5. Mitochondrial morphology suggestive of division. ......................................................... 437
Figure 6. A coated vesicle. .................................... 437
Plate II ........................................................................ 439
Figure 7. Zoosporangium approaching meiosis. ....... 439
Figure 8. ABB/PAS stained meiotic sorus. .............. 439
Figure 9. A dictyosome not adjacent to the nucleus. 439
Figure 10. The perinuclear cytoplasm. ................... 440
Figure 11. The perinuclear cytoplasm. ................... 440
Figure 12. A pair of centrioles in a nuclear pocket.

Plate III

Figure 13. Spurr's embedded post meiotic zoosporangial sorus.

Figure 14. Small nuclei distributed throughout the sporangium.

Figure 15. Favorable section showing the regular chloroplast distribution.

Figure 16. The peripheral cytoplasm.

Figure 17. The peripheral ER.

Plate IV

Figure 18. Nucleus during the karyokineses or meiosis.

Figure 19. Detail of nuclear pole during karyokinesis.

Figure 20. A post karyokinetic zoosporangium.

Figure 21. Microbody-like structures.

Figure 22. An osmiophilic vesicle.

Figure 23. A cluster of osmiophilic vesicles.

Figure 24. A post-karyokinetic nucleus.

Plate V

Figure 25. ABB/PAS stained sorus material at cytokinesis.

Figure 26. Nomarski optics of live mature, releasing zoosporangia.

Figure 27. Post-meiotic-pre-cytokinetic zoosporangium.

Figure 28. An early stage of golgi vesicle exocytosis.

Figure 29. Zoosporangial protoplast during cytokinesis.

Figure 30. A dictyosome during zoosporangial protoplast cytokinesis.

Plate VI

Figure 31. Typical organization of a zoospore.

Figure 32. A zoospore prior to release.

Figure 33. Cross section of a naked flagellum.

Figure 34. Oblique section of a flagellum.

Figure 35. Flagellar insertion.

Figure 36. Transverse cross section of both basal bodies.

Plate VII

Figure 37. Zoospore eyespot structure.

Figure 38. The zoospore unit.

Figure 39. Golgi apparatus of the post-cytokinetic contained zoospore.

Figure 40. The dictyosome formative face.

Figure 41. A pre-release zoospore.

Figure 42. Zoospore chloroplast during cytokinesis.
Figure 43. Nuclear envelope/ER association. .........455
Figure 44. Apical tip of a mature zoosporangium. ...455
Plate VIII .................................................................457
Figure 45. Recently released live zoospores. ..........457
Figure 46. The free swimming zoospore is pyriform. 457
Figure 47. Platinum shadowed zoospore. .............457
Figure 48. The tip of the anterior flagellum. ..........457
Figure 49. A platinum shadowed mastigoneme. ....457
Figure 50. Whole free swimming zoospore. ..........457
Figure 51. The eyespot granules. .........................458
Figure 52. Negative stained flagellar apparatus. ...458
Figure 53. A free zoospore. .................................458
Figure 54. A free zoospore. .................................458
Figure 55. A free zoospore. .................................458
Plate IX .................................................................460
Figure 56. Zoospore detained in the zoosporangium. 460
Figure 57. A zoosporangium which failed to dehisce. 460
Figure 58. Portion of a still contained zoospore. ...460
Figure 59. A vesicle contained plaque. ..................461
Plate X .................................................................463
Figure 60. Diagrammed zoospore model. ...............463
ACKNOWLEDGEMENTS

I would like to thank my family for their support, balance and patience. I would like to thank Thana Bisalputra for his support, patience and financial assistance during the course of this study. I am also indebted to M. Neushul who set me on this road and to E.Y. Chi, T. Mumford, M.J. Duncan and G. Hermanson who have contributed immeasurably along the way. I would like to also thank Dr. Foreman for his support and use of research facilities on several occasions as well. Thanks to my committee, Dr. Beamish, Dr. Bisalputra, Dr. Foreman, Dr. Hughes, Dr. Scagel and Dr. Stein for their criticism and support. Thanks also to L. Veto and J. Clappisson for their excellent technical assistance. This work has been supported by N.R.C. Grant A 2288 to Dr. T. Bisalputra.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AW</td>
<td>Anticlinal Wall</td>
</tr>
<tr>
<td>Ax</td>
<td>Axoneme</td>
</tr>
<tr>
<td>ac</td>
<td>apical cytoplasm</td>
</tr>
<tr>
<td>amm</td>
<td>appressed mitochondrial membranes</td>
</tr>
<tr>
<td>AFL</td>
<td>Anterior Flagellum</td>
</tr>
<tr>
<td>B</td>
<td>Blister</td>
</tr>
<tr>
<td>bc</td>
<td>basal cytoplasm</td>
</tr>
<tr>
<td>BP</td>
<td>Basal Plate</td>
</tr>
<tr>
<td>C</td>
<td>Cortex</td>
</tr>
<tr>
<td>Ce</td>
<td>Centriole</td>
</tr>
<tr>
<td>CE</td>
<td>Chloroplast Envelope</td>
</tr>
<tr>
<td>CER</td>
<td>Chloroplast Endoplasmic Reticulum</td>
</tr>
<tr>
<td>Ch</td>
<td>Chloroplast</td>
</tr>
<tr>
<td>CI</td>
<td>Cortical Initial</td>
</tr>
<tr>
<td>C</td>
<td>Cell Plate</td>
</tr>
<tr>
<td>cy</td>
<td>cytoplasm</td>
</tr>
<tr>
<td>D</td>
<td>Dictyosome</td>
</tr>
<tr>
<td>DC</td>
<td>Dictyosome Cisternae</td>
</tr>
<tr>
<td>dc</td>
<td>dense cytoplasm</td>
</tr>
<tr>
<td>df</td>
<td>disorganized fibrils</td>
</tr>
<tr>
<td>EC</td>
<td>Epidermal Cell</td>
</tr>
<tr>
<td>Ep</td>
<td>Epiphyte</td>
</tr>
<tr>
<td>er</td>
<td>endoplasmic reticulum</td>
</tr>
<tr>
<td>ES</td>
<td>Eye Spot</td>
</tr>
<tr>
<td>EZ</td>
<td>Expansion Zone</td>
</tr>
<tr>
<td>F</td>
<td>Fibrillar Zone</td>
</tr>
<tr>
<td>ff</td>
<td>formative face</td>
</tr>
<tr>
<td>Fl</td>
<td>Flagellum</td>
</tr>
<tr>
<td>G</td>
<td>Granular Zone</td>
</tr>
<tr>
<td>Ge</td>
<td>Genophore</td>
</tr>
<tr>
<td>Gm</td>
<td>Granular material</td>
</tr>
<tr>
<td>GV</td>
<td>Golgi Vesicle</td>
</tr>
<tr>
<td>H</td>
<td>Holdfast</td>
</tr>
<tr>
<td>hC</td>
<td>hypertrophied Cisternae</td>
</tr>
<tr>
<td>Hcr</td>
<td>Heterochromatin</td>
</tr>
<tr>
<td>her</td>
<td>hypertrophied endoplasmic reticulum</td>
</tr>
<tr>
<td>IPW</td>
<td>Inner Fericlinal Wall</td>
</tr>
<tr>
<td>L</td>
<td>Lamina</td>
</tr>
<tr>
<td>M</td>
<td>Mitochondria</td>
</tr>
<tr>
<td>Ma</td>
<td>Margin</td>
</tr>
<tr>
<td>Mb</td>
<td>Microbody</td>
</tr>
<tr>
<td>MC</td>
<td>Membrane Configuration</td>
</tr>
</tbody>
</table>
Md---Medulla
MD---Mucilage Duct System
MDW---Mucilage Duct Wall
Me---Meristoderm
mf---maturing face
ML---Membrane Layers
MU---Mucilage
N---Nucleus
NE---Nuclear Envelope
NP---Nuclear Pore
Nu---Nucleolus
OM---Osmiophilic Material
OV---Osmiophilic Vesicle
OW---Outer Wall
F---Paraphysis
pc---peripheral cytoplasm
FF---Primitive Furrow
FP---Posterior Flagellum
FI---Paraphysis Initial
PM---Plasma Membrane
Fn---Pneumatocyst
IVR---Pro-vacuolar Region
Py---Pyrenoid
S---Sorus
SbC---Subtending Cell
SC---Secretory Cell
SL---Sorus Lumen
St---Stipe
SV---Secretory Cell Vacuole
T---Tonoplast, Tubules
t---tubular extension
Th---Thylakoids
TZ---Transition Zone
vi---vesicular invagination
Z---Zoospore
ZS---Zoosporangium
ZSI---Zoosporangial Initial
ZSM---Zoosporangial Mother Cell
I GENERAL INTRODUCTION

The gross morphological events of sorus production and release by *Nereocystis luetkeana* (Mert.) Post. and Rup. are common knowledge amongst phycologists to the extent that sorus release has been called abscission (Bold and Wynne 1978). Yet before this study little was known about the mechanisms of sorus release and little more of the mechanisms of sorus development.

In light of the paucity of morphological information concerning lamina meristoderm differentiation and the few ultrastructural works dealing only with zoosporangia it seemed necessary to have some context in which to consider the tissues to either side of the soral margins, vegetative meristoderm and sorus proper. In the long course of this study it also became evident that mucilaginous material frequently was involved making it impossible to ignore the mucilage duct system. Furthermore, when it became apparent that pre-zoosporangial sori were difficult to obtain it was necessary to extend the investigation proximally to the source, the intercalary meristem. Although a narrower approach may have been shorter, it is believed that this broader, albeit longer, effort has two distinct advantages. First, in light of the paucity of information about the laminariales meristoderm and its activities a restricted consideration of only the events occurring at the soral margins seems premature. Secondly, I believe this thesis opens numerous new vistas in terms of the
morphology of the Laminariales and raises many interesting new possibilities for subsequent investigations.

The observations made in this study are presented in five units. The first represents a survey of the meristoderm of mostly fertile laminae of *Nereocystis* from the transition zone of the intercalary meristem into the region of lamina expansion and then adjacent to youngest and oldest sori with the aim of providing a cytomorphogenetic context in which the events of zoospore differentiation occur. The second chapter contains considerations of the mucilage duct system. It is presented ahead of any considerations of sori because mucilage duct system inception and differentiation phenomena were long ago associated with the meristoderm of the intercalary meristem (Guignard 1882, Skottsberg 1907). This sequence is further justified by the reports that the mucilage duct system is derived from the meristoderm prior to sorus production (Guignard 1882, Oliver 1887, MacMillan 1899, Skottsberg 1907).

The observation that the sori are likewise derived from the meristoderm was made even before those concerning the mucilage duct system (Thuret 1850). To complement the observations from the vegetative side of the sorus margin (Chapter 1) the third chapter consists of observations from the soral side. It includes consideration of the mechanisms of sorus inception and subsequent development extending even beyond release from the lamina. It's emphasis is centered around the paraphyses and other structural elements peripheral
to the zoosporangia themselves.

The process of zoosporogenesis in *Nereocystis* had not received ultrastructural attention and since the zoosporangia are the structures about which the processes of sorus morphogeneses occur they required consideration. However, since zoosporogenesis is not the central concern of this study it is included as an appendix.

Finally, with the context of the meristoderm, a clearer understanding of the processes of mucilage duct and sorus inception and an outline of paraphysis, subtending cell and zoosporangial cytomorphogenesis it is possible to interpret events observed in the margins of youngest, intermediate and oldest soral margins of *Nereocystis* (Chapter 4). It is possible to integrate these separate but interrelated sets of observations in order to define the probable modes of *Nereocystis* spore dispersal in the water column as well as compare the process of sorus release to similar phenomena elsewhere in the plant kingdom.
II MATERIALS AND METHODS

1. Materials

Thalli of *Nereocystis* (mert.)Post. and Rup. used for this study were collected from various points in Stanley Park, Vancouver B.C. Collections were also made from thalli at Bath Island (research station of R. Foreman); Chesterman Beach, Vancouver Island, B.C.; the southern edge of Pachena Bay, Vancouver Island, B.C.; and at the submerged reef out from the Nanaimo Fisheries Research Station. Collections and fixations were begun in the spring with the appearance of young sporophytes and continued into the winter as long as usable thalli persisted. Most of the tissues used in this study were fixed in the field rather than attempting to maintain portions of thalli. At the Nanaimo Fisheries Research Station it was possible to hold some plants and excised blades for a short period in a tank which was attached to a floating dock.

To obtain samples of the meristoderm proximal to the sori, tissues were excised by taking transverse slices from the narrow transition zone at the base of the lamina (Fig. 1 and 2) and the expansion zone (Fig. 1, 2 and 3). Tissues from these areas were employed to investigate the meristoderm at its source and to observe the mechanism of mucilage duct system inception. Individual sori were not followed through their
entire developmental sequence because of problems with maintaining large thalli in culture and frequent sampling of the same sorus by excision would disrupt the integrity of the sorus lumen. It is fortunate that fertile laminae usually bear a number of sori (Fig. 1 and 4). If a sample is taken of each sorus on an individual lamina, it is then possible to examine and reconstruct the developmental sequence in the correct chronological order.

For the examination of zoosporangial sorus development laminae with three visible sori were most often used. A rectangular piece of tissue was cut from the lateral margin of each sorus (Fig. 5). This rectangle (Fig. 6) was immediately immersed in fixative and sliced transversely to produce thinner rectangles of tissue which included sorus and vegetative tissues at either end and sorus margin in the middle (Fig. 7). For light microscopy these pieces were sectioned lengthwise so that each section would pass from vegetative tissue at one end (Fig. 8), through the sorus margin (Fig. 9) into sorus tissue (Fig. 10) far enough to be representative of that sorus. This was checked by a comparison with tissues from the sorus center. For electron microscopy, sections were taken first from the vegetative end (Fig. 7 and 11) and then the sorus end (Fig. 7 and 13) of the block. Next the block was retrimmed to allow sectioning of the sorus margin (Fig. 7 and 12).

In this manner it was possible to describe the processes of sorus differentiation and sorus abscission for Nereocystis.
An integration of data from this mode of sorus sampling and data from the transition and expansion zones has provided the observations for this thesis.

2. Light Microscopy

Some tissues were fixed in a solution of 5% formalin in sea water. However, most were fixed as described below for electron microscopy with either a glutaraldehyde or a paraformaldehyde/glutaraldehyde solution. In any case, after washing all were dehydrated in an ethanol series and then infiltrated with glycolmethacrylate (Polysciences JB-4 Embedding Kit). After polymerization sections were cut on a Sorval JE-4 microtome at 2.5 um and mounted on glass slides for subsequent staining and viewing.

3. Transmission Electron Microscopy

Tissue pieces obtained as in the first section were immersed in the fixative solution in a petri dish. Most fixations are a modified Karnovski's fixative, a mix of 1% paraformaldehyde and 4% glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.4. Fixation was for a duration of two to four hours. This was followed by three 30 min washes in the same buffer. Post fixation was carried out in 1%
osmium tetroxide for four hours at room temperature or overnight at 4°C. This was followed by dehydration in an acetone series, and subsequent infiltration with Spurr's (1966) resin. Sections were cut with a diamond for TEM on a Reichert CMU 3 ultramicrotome, stained in uranyl acetate and Sato's (1967) lead. In some cases 1 μm sections were taken of this tissue for light microscopy.

4. Scanning Electron Microscopy

Fresh material was quenched in liquid Freon 22, cooled in liquid nitrogen and then frozen in liquid nitrogen. Freezing was followed by drying in an Edward's Speedivac tissue dryer. Other material was taken after aldehyde and post fixation from acetone into amyl acetate and then critical point dried in a Parr Inst. Co. pressure bomb. Tissues were gold or gold-palladium coated in either a Mikros VE-10 vacuum evaporator or a Polaron Sputter Coating Device. All tissues were viewed in a Cambridge scanning electron microscope. In certain cases plastic embedded specimens were fractured and then coated as above for viewing in the SEM.

5. Histology

Tissues processed with the JB-4 microtome in methacrylate
were subjected to a variety of staining procedures. The Toluidine Blue O technique as outlined by Feder and O'Brien (1968) was used to localize presumed acid and/or sulfated polysaccharides through its purple to red metachromasia as employed by McCully (1966, 1968ab, 1970). In order to differentiate acid from sulfated polysaccharides the Alcian Blue/Safranin O (AB/SC) method adapted from Spicer (1960, 1962) was used. Here the blue staining of alcian blue indicated acid polysaccharides while red the presence of sulfated polysaccharides. As a check this technique was tried on Fucus and localization of acid and sulfated polysaccharides agreed with those of McCully (1966, 1968ab). Analin Blue Black (ABB) was used according to Fisher (1968) as a general protein stain for the cytoplasm by itself and following the Periodic Acid Schiff's technique (Jensen 1962) for neutral polysaccharides. To ensure that aldehydes of the fixatives were not interfering with this technique an aldehyde block was performed according to Kasten and Lala (1975). This significantly enhanced specificity.

6. Negative Staining and Shadowing

Negative staining was performed with phosphotungstic acid according to Hyatt (1975). Shadowing was done with platinum in a Balzers Freeze-etch apparatus.
MATERIALS AND METHODS FIGURES, THALLUS AND TISSUE SAMPLING
Plate I

Figure 1. The whole thallus. The thallus of *Nereocystis* is anchored to the substrate by a holdfast consisting of branched haptera. The stipe bears a hollow swollen pneumatocyst at its upper extent. The numerous laminae are attached to the apical dome of the pneumatocyst by their transition zones of the intercalary meristems. Laminae which are fertile usually bear from one to several sori in a linear sequence with the youngest sori most proximally located. Holes remain where most distal mature sori are released from the laminae.
Figure 2. Intercalary meristem of a young *Nereocystis* thallus. The transition zone is at the junction of lamina and pneumatocyst seen here as the tissue between the arrowheads. Lamina tissue distal to the transition zone tissue is still increasing in width and the distal extents of lamina splitting are seen (heavy arrows). Usually a visible shallow furrow (fine arrows) distally precedes the advancing point of lamina splitting. Scale equals 0.5 cm.

Figure 3. Proximal portion of an excised fertile lamina. The tissue from the transition zone distally to the point of parallel lamina edges marks the extent of the expansion zone. A sorus is visible in the mature vegetative tissues. Scale equals 2.5 cm.

Figure 4. Midregion of an excised fertile lamina. Three sori are visible (A, B and C). The youngest (A) is closest to the meristem and pneumatocyst. The oldest (C) is located most distally from the meristem and is delimited by a clear margin. The D marks the hole left by a previously released sorus. Sorus B is in an intermediate stage of development. Scale equals 2.5 cm.
Figure 5. Young sorus in mature tissues of a lamina. For all fixations a rectangular piece of tissue is excised along the margin of the sorus (dashed lines). Scale equals 2.5 cm.

Figure 6. Scanning electron micrograph of a piece of tissue excised as above. The soral tissue is clearly separated from vegetative by the sorus margin. These rectangles were sliced transversely into thin strips (as included in the dashed lines). Thin strips were processed for transmission electron microscopy while wide ones were processed for light microscopy. Scale equals 0.5 mm.

Figure 7. A thin strip of tissue. The dotted lines represent planes in which thin sections were taken for transmission electron microscopy across the sorus margin, vegetative and soral tissues. For light microscopy sections of wider blocks of tissue were taken along their whole length. Scale equals 1 mm.

Figure 8. Vegetative end of a light microscope section. Histologically the vegetative tissues consist of two outer layers of meristoderm cells, two inner layers of cortical cells and the central layer of medulla. Scale equals 100 μm.
Figure 9. Sorus margin area of an LM section. Note disjunction of the outer wall (arrowheads), erosion of meristoderm constituents, and thinning of the central medulla layer. Scale equals 100 um.

Figure 10. Soral end of an LM section. Histologically the meristoderm has differentiated into zoosporangia, paraphyses and subtending cells. The outer wall is loosened (arrowhead). Scale equals 100 um.

Figure 11. Section from vegetative end of a thin tissue strip for EM. Note meristoderm and cortex cells typical of vegetative tissues adjacent to the sori. Scale equals 10 um.

Figure 12. Section across sorus margin region of a thin tissue strip for EM. Note the zoosporangial and paraphysis initials and their subtending cells. Scale equals 10 um.

Figure 13. Section from the soral end of a thin strip. Components of the sorus are visible; zoosporangia, paraphyses, outer cell wall, extracellular space and subtending cells. Scale equals 10 um.
III CHAPTER 1. MERISTODERM DIFFERENTIATION

1. Introduction

The term meristoderm was applied to superficial cells in the Laminariales by Sauvageau (1918). This meristoderm directly generates more meristoderm and new cortical tissues through anticlinal and pericinal divisions (Fritsch 1945). This meristoderm is also the source of the zoosporangial sori in this order (Fritsch 1945). Knowledge of a meristoderm role in both mucilage duct and mucilage gland cell inception in the Laminariales is long standing (Guignard 1892, Oliver 1887, Skottsberg 1907, Yendo 1909). In addition evidence for a more direct involvement than previously indicated has been presented recently (Kasahara 1967, 1973, Walker and Bisalputra 1977). Although somewhat variable the meristoderm may play a role in thallus fissuring or splitting (Wells 1910), a process responsible for much of the diversity within this order (Fritsch 1945).

Although there is interest in the growth of the Laminariales the focus of interest has not been anatomical (Russell 1973). There are only a few ultrastructural studies which have dealt with any portion of the meristoderm of members of this order (Chi and Neushul 1972, sporophylls of Macrocystis; Davies, Ferrier and Johnston 1973, hapteras of Laminaria
Evans, Simpson and Callow 1973, sulfate metabolism of meristoderm cells of *Laminaria*). Although Gherardini and North (1972) have looked at embryonic sporophytes of *Macrocystis* with the EM, development was not followed to intercalary meristem differentiation. Recently Penny (Personal communication) has described mitosis in the inner cortex of *Laminaria* intercalary meristems.

In light of this paucity of information concerning subcellular aspects of the meristoderm it has been necessary to undertake a survey of the meristoderm of fertile laminae of *Nereocystis*. Observations from the basal transition zone of the lamina, the region of lamina expansion, the region adjacent to youngest sori and the region adjacent to most mature sori are reported here. These regions of meristoderm are morphologically characterized by comparison with similar observations in other brown algae. The structural aspects of the four tissue regions are compared and cytomorphogeneses are discussed in light of similar morphological studies (Fagerberg and Dawes 1977, Oliveira and Bisalputra 1973 and 1977a-d).

2. Observations

2.1. Transition Zone Meristoderm Cells
The transition zone of *Nereocystis* is considered here to lie at the junction between pneumatocyst and lamina (Fig. 1a). This is the narrowest point of the lamina and the superficial layer of meristoderm cells found here contribute to the tissues of the lamina by anticlinal and periclinal divisions (Fritsch 1945). The transition zone is all that anchors the lamina to the pneumatocyst. It was surprising to discover that this tissue in younger thalli required the greatest care in selection and preservation. Juvenile thalli exposed to air disintegrate during fixation at this point.

2.1.1. Anatomy

Meristoderm cells of this zone are loosely associated. The outer layer of wall material is often loosened and partially if not completely dislodged (Fig. 2 and 3). The innermost layer of the outer cell wall remains and is continuous with the anticlinal cell walls (Fig. 4). The internal periclinal walls, also continuous with the anticlinal walls (Fig. 4), remain intact between meristoderm cells and cortical initials as well as between cortical cells (Fig. 2 and 4-6). Anticlinal walls of adjacent meristoderm and cortical cells are dissociated (Fig. 2 and 5). Thus the meristoderm cells appear as apical meristem cells of closely associated filaments rather than as cells of a unified tissue (Fig. 2 and 6). Meristoderm cells which have just completed an anticlinal division are closely associated (Fig. 5). Otherwise there are
fairly large spaces between anticlinal walls (Fig. 2 and 7). In section these meristoderm cells are generally at least twice as long as they are wide (Fig. 6 and 7). In most cases, the new cortical cells are no wider than the meristoderm cells they subtend which reinforces the filamentous appearance (Fig. 6 and 7).

2.1.2. Histology

With Toluidine Blue O (TBO) staining for acid and sulfated polysaccharides the continuous outer layer of cell wall material stains metachromatically positive. The cell wall material immediately surrounding each meristoderm cell is even more metachromatic. This layer is very thin over the apical surface of the cell (Fig. 3). Nuclei (Fig. 6 and 7) stain turquoise and often are accompanied by densely staining reddish-metachromatic material on their basal or lateral margins (Fig. 3). Most of the relatively small whitish-green chloroplasts are in the apical cytoplasm (Fig. 3 and 4) with a mixture of blue-green granules and some vesiculation (Fig. 3-5). The ground cytoplasm is a light turquoise-blue with TBO staining. With Analin Blue-Black (ABB) staining for proteins the filamentous appearance of this tissue is accentuated (Fig. 6). The nuclei and chloroplasts are the dominant features of the cytoplasm as they stain densely (Fig. 6 and 7) in contrast to the cytoplasm. Chloroplasts in the apical cytoplasm of these cells are clearly seen (Fig. 7). Small
densely staining, polymorphic granules are also seen throughout the cytoplasm (Fig. 7).

2.1.3. Ultrastructure

Morphological and histochemical observations which revealed the delicate and somewhat loose nature of the cell walls of these transition zone meristoderm cells are confirmed by observations at the ultrastructural level. In tissues used for electron microscope (EM) fixations the outermost layer of continuous periclinal wall material has been lost during preparation (Fig. 8). All that remains of the outer wall over the apical surfaces of the cells are sparse cloud-like masses of fibrillar material (Fig. 8 and 15) and a thin layer of fibrillar wall around the plasma membrane (Fig. 15). This thin wall of the apical surface of the protoplast is continuous around the entire cell. It thickens basipetally along the anticlinal walls (Fig. 8, 11 and 12). There are frequently lateral spaces between meristoderm cells which are usually filled with less dense cell wall material (Fig. 8, 9 and 10). The variability seen in anticlinal cell wall thickness is probably the result of time elapsed since the completion of the last anticlinal division (Fig. 9). Although in median transverse section the basal periclinal walls may show variations in thickness, they nevertheless remain densely stained (Fig. 8-11). Newly formed anticlinal cell walls are frequently observed between cells on the edges of apparent
meristoderm infoldings (Fig. 9 and 10) previously identified by MacMillan (1899) as primitive furrows. Pit fields of typical plasmodesmata (Bisalputra 1966) are found in the anticlinal cell walls (Fig. 11) but mostly in the basal periclinal walls (Fig. 8 and 10).

Prominent and slightly basal nuclei effectively divide the protoplasts into a larger apical and smaller basal cytoplasm (Fig. 9 and 10). Nuclear shape is variable (Fig. 8-10 and 12). Invariably a nucleolus is present consisting of three very distinct regions (Fig. 12 and 19). There is an internal pocket delimited by a densely staining fibrillo-granular region (Fig. 12 and 19). This pars fibrosa is surrounded by a layer of presumptive granular ribonucleoproteins, the pars granulosa (Fig. 12 and 19). There are small masses of heterochromatin throughout the nucleus and along the nuclear envelope (Fig. 12). The nuclear envelope is interrupted frequently by pores. At points the perinuclear space is confluent with the intracisternal space of the chloroplast endoplasmic reticulum (CER) (Fig. 12, arrowhead 1). The CER may also show luminal confluence with the ER of the cell (Fig. 12, arrowhead 2). There is continuity, therefore, between the nuclear envelope, CER and ER membranes.

The chloroplasts which occur primarily in the apical cytoplasm are delimited by CER and a chloroplast envelope (Fig. 13). Often in section there are regions between the CER and the chloroplast envelope which contain some small vesicles
The chloroplast matrix stains lightly and contains the typical peripheral thylakoids, ring genophore and two to four central stacks of three thylakoids (Fig. 13). From the variety of morphologies seen in the apical cytoplasm it would appear that many chloroplasts are in the process of division (Fig. 8-10 and Bisalputra and Bisalputra 1970).

The dictyosomes are usually located on the basal and lateral surfaces of nuclei (Fig. 8-10 and 12). By means of serial sectioning, three regions of the membrane organization of the dictyosomes are distinguishable similar to those described elsewhere (Whaley 1975). In the central region of the cisternal membranes are closely associated (Fig. 14). Moving radially the membranes of the cisternae dissociate, producing a larger irregular lumen and in section show discontinuities (Fig. 14). These represent the reticulate or fenestrate areas of the cisternae and thus are not actual disjunctions (Morre, Mollenhauer and Bracker 1971). The distal most portions are hypertrophied and contain what appears to be cell wall precursors (Fig. 14). It is these hypertrophied portions or large vesicles which dominate the golgi apparatus of these cells (Fig. 12). Scattered about the individual dictyosomes are small vesicles (Fig. 14). Some of these vesicles appeared to originate from the nuclear envelope and presumably are incorporated into the formative face of the dictyosome (Fig. 14). Tubular extensions of hypertrophied portions of the cisternae and coated vesicles are routinely
observed.

Ultrastructurally the apical cytoplasm is most often characterized by chloroplasts, a more or less complex system of membrane bound vesicles, a number of mitochondrial profiles, the occasional microbody-like structure, and tubules of rough ER (Fig. 15). The plasma membrane in this area shows shallow invaginations and convolutions (Fig. 15, large arrowheads). The membranes of the vesicles are heavily stained and comparable to those found around the golgi apparatus (compare Fig. 15, 16 and 17 with Fig. 14). Their contents, when visible, are not identical to the fibrillar material found in hypertrophied regions of the dictyosome cisternae (Fig. 16 and 17). They appear to be larger and in some cells present extremely complex configurations (Fig. 17). Where they have not become so complex there are areas where the vesicles appear collapsed (Fig. 15, small arrowheads and "V"), with an association of the membranes. In many areas there are both single and double membrane bound inclusions (Fig. 16). The single membrane bound inclusions are invaginations of the vesicles seen in cross section (Fig. 16, vi), and the double membrane bound inclusions are produced by interdigitation of neighboring vesicles (Fig. 16, *).

The basal cytoplasm is characterized by few chloroplasts, a number of mitochondrial profiles, some rough ER, the occasional golgi vesicle and often in the longer cells a region of lighter homogenous cytoplasm (Fig. 20). Microbody-like
structures are often found in the basal cytoplasm (Fig. 20). Careful examination of the margins of the clear, homogenous areas of basal cytoplasm reveals profiles of ER surrounding the entire area (Fig. 20) and having only small apparent disjunctions (Fig. 20, large arrowheads). In Figure 20 a golgi vesicle with some membrane bound inclusions appears to have been admitted to or included in this region. Clusters of free ribosomes are distributed fairly evenly throughout the cytoplasm of these cells and serial sections reveal that many of the mitochondrial profiles are in reality just portions of the same organelle. They are typical in that they have tubular cristae (Fig. 20).
CHAPTER I FIGURES, TRANSITION ZONE
Figure 1. Diagram of a whole thallus. Representative transverse sections through each of the regions examined follow.

a. Whole thallus of *Nereocystis*. The transition region is at the junction of the pneumatocyst and laminae. The expansion zone is that portion of lamina distal to the transition zone but proximal to the point where the lamina margins become approximately parallel. The youngest sorus is that closest to the pneumatocyst while the oldest is most distal.

b. Histological diagram of a transverse section through the transition zone. Outer dark line equals the single cell layer of epidermal cells. Central black core represents the medulla and the intervening white region the cortex. In this region a transverse cross section is oval to circular.

c. Histological diagram of the expansion zone of a young lamina where blade splitting is incomplete. Black outer line equals single cell layered epidermis. Black central layer is the medulla and the intervening white is cortex. The two constrictions in the lamina represent
future regions of lamina splitting.

d. Histological diagram of a transverse section through a youngest sorus. Raised surface with perpendicular lines is sorus present on both surfaces. Outer line of sorus represents the outer cell wall which is continuous over the juvenile sorus, not a layer of cells. Outer black line adjacent to the sorus does represent the epidermis. There is no evidence of the impending sorus abscission. The single cell layered epidermis adjacent to the sorus was examined.

e. Histological diagram of a transverse section through the most mature sorus. Erosion of the sorus at its margins is complete and separation may begin. Sampling was done between sorus margins and lamina margins, closer to the sorus.
Plate II

Figure 2. Meristoderm of the transition zone. Middle lamellar dissociation is evident for anticlinal walls. Note outer layer of TBO densely stained material. Meristoderm cells appear as apical cells. Scale equals 25 μm.

Figure 3. Single TBO stained transition zone meristoderm cell. Note outer wall consisting of outer dense layer, intermediate light material and innermost layer continuous with anticlinal walls. See also light chloroplasts and nucleus with associated purple metachromatic material (arrowhead). Scale equals 5 μm.

Figure 4. Single TBO stained transition meristoderm cell. The innermost layer of outer wall is all that remains. Points of plasmodesmatal continuity with subtending cortical cells are visible. Scale equals 5 μm.

Figure 5. Single TEC stained transition zone meristoderm cell. Even when broad enough to divide anticlinally they are still longer than wide. Scale equals 5 μm.

Figure 6. ABB stained transition zone meristoderm cell. Cytoplasm stains lightly. Apical masses are chloroplasts. Small dense masses in cytoplasm are presumed mitochondria. Central nucleus with nucleolus is clearly visible (arrowhead). Scale equals 5 μm.
Figure 7. ABB stained transition zone meristoderm cells. After an anticlinal division progeny cell at far right has divided periclinaly to produce a cortical initial. Scale equals 5 μm.
Plate III

Figure 8. Transmission electron micrograph of transition zone meristoderm cells. Note typical complement of organelles, central nucleus with electron dense heterochromatic masses and prominent nucleolus, adjacent dictyosomes of the golgi apparatus, mitochondria and chloroplasts. Anticlinal cell walls may be compact and electron dense (arrowheads) or thinned in areas (*). Outer cell wall consists of discrete layers of fibrils (arrow) parallel to the plasma membrane, layer 1 and disorganized loose fibrils outside of that. Scale equals 1 μm.

Figure 9. Lip of a primitive furrow. Cells of recent anticlinal divisions show close apposition along their new anticlinal walls (opposing arrowheads). There are loose regions where thinning and transient schizogenies are observed (arrows). The furrow(*'s) is bordered by the free surfaces of the meristoderm cells. Scale equals 1 μm.
Plate IV

Figure 10. Bottom of a primitive furrow. Outer cell wall constituents are retained in the furrow lumen (*). Note the apical cytoplasm membrane configurations, chloroplasts, and somewhat centrally located nuclei. Basal cytoplasm varies with the time since last periclinal division. Little basal cytoplasm exists following cortical cell initial separation. Electron translucent regions (small *) characterize the basal cytoplasm. Plasmodesmatal continuities exist between adjacent meristoderm cells and cortical initials (arrowheads). Scale equals 1 μm.

Figure 11. Internal periclinal cell wall. Note the two pit fields (brackets) of several plasmodesmata in the periclinal wall. There is no wall thickening amongst plasmodesmata. Scale equals 1 μm.
Plate V

Figure 12. The centrally located nucleus. Nucleoli of the 4 to 5 um nuclei consist of a central cavity (*), granular and fibrillar zones. The nucleoplasm contains electron opaque masses of heterochromatin and is delimited by a double membrane envelope, interrupted by pores. Note luminal continuities between the nuclear envelope and chloroplast ER (arrowhead 1). Dictyosomes usually lie at the basal hemisphere of the nucleus where its outer membrane vesiculates (arrows). The maturing face cisternae and vesicles show hypertrophy. Chloroplasts and mitochondria are around the nucleus. Peripheral elements of the ER may show continuities with the chloroplast ER (arrowhead 2). Scale equals 1 um.
Figure 13. A meristoderm cell chloroplast. The chloroplast envelope and ER delimit it. Note vesiculation between the chloroplast ER and chloroplast envelope (small arrowheads). The genophore within girdle lamellae is visible. Note the DNA (large arrowhead). Thylakoids may cross over between adjacent stacks (arrows). See the presumptive small fenestrae of thylakoids (small arrowheads). Scale equals 0.5 μm.

Figure 14. A dictyosome of the golgi apparatus. The formative face lies adjacent to the nuclear envelope, which vesiculates (small arrowhead to right). Note central region of close cisternal association (between larger arrowheads), surrounded by areas of slight hypertrophy which are fenestrate (arrows). Perimeters of the cisternae at the maturing face are hypertrophied (*) and contain a reticulum of electron dense fibrillar material. Scale equals 0.5 μm.
Figure 15. Apical cytoplasm of a meristoderm cell. Most chloroplasts are here. They possess an electron translucent matrix within few thylakoids, scattered granules and no plastoglobuli. Note the peripheral ER, several mitochondrial profiles and the occasional microbody-like structures(*). Vesicles with electron dense membranes which show various morphologies, some collapsed (small arrowheads). Invaginations of plasma membrane at the free surface contain membranous configurations. Note thin innermost layer 1 of cell wall fibrils continuous over these regions (large arrowheads). External to this is a layer of apparently disorganized fibrils. Thin inner layer of fibrils is continuous with the anticlinal walls which thicken basipetally. Scale equals 1 \( \mu m \).
Figure 16. Simple vesiculation of the apical cytoplasm. Intravesicular constituents are attributed to vesicular invaginations (VI) and vesicular interdigitation seen here in two planes of section (*'s). Plasma membrane invaginations at upper left. Scale equals 0.5 um.

Figure 17. Complex apical cytoplasm vesiculation. Note tubular constituents possibly from extreme invagination (arrowheads) and membrane layering. Scale equals 0.5 um.

Figure 18. A presumptive lipid droplet. Note the lack of clear delimiting membrane and the associated ER cisternae. Scale equals 0.5 um.

Figure 19. A nucleolus and associated homogeneous region. Nucleoli show distinct granular and fibrillar regions and are distinct from heterochromatin. The homogeneous region (arrowheads) contains a reticulum of homogeneous filamentous material. Scale equals 0.5 um.
Figure 20. Basal cytoplasm of a meristoderm cell. Nucleus and dictyosome are at upper left. Provacuolar region is delimited by disjunct cisternae of endoplasmic reticulum (arrowheads). Note included golgi vesicle interdigitated (small arrow) with cisternae. Chloroplasts contain presumed plastoglobuli initials (small arrowheads). Microbody-like structures and mitochondria are present. Scale equals 0.5 um.
2.2. Expansion Zone Meristoderm Cells

Observations for this portion of the meristoderm are primarily made on those tissues distal to the transition zone where the lamina is still expanding laterally as is obvious from the lack of parallel margins (M. and M., Fig. 2). Some observations have also been made with the light microscope on the tissues between the youngest sorus visible and the point where lateral expansion of the lamina appears to cease, as evidenced by parallel margins (materials and methods, M and M, Fig. 3 and 4). Unless it is otherwise indicated observations reported here are from the former region. This emphasis was based on two observations. First, from light microscopy it is evident that there is not a great difference between meristoderm cells just above the youngest sorus and those adjacent to it. Secondly, mucilage duct system inception, which also is a product of the meristoderm occurs in the tissues just distal to the transition zone where lamina expansion is still occurring.

2.2.1. Anatomy

Although meristoderm cells still appear as apical cells of filaments. It is easy to trace the lineage of cortical cells produced by each extant meristoderm cell back into the cortex (Fig. 1a-e). This is made possible by the apparent lack of
lateral expansion of the young cortical cells, even though they have certainly increased the lengths of their anticlinal cell walls (Fig.1). Immediate products of periclinal divisions are no wider than their parent meristoderm cells, except where an anticlinal division has intervened (Fig.5) or in the case where the immediate product is a secretory initial cell of the differentiating mucilage duct system (Fig.6, * and Chapter II Fig.3). This appearance of a filamentous lineage is accentuated by ABB protein staining which leaves cell walls essentially unstained (Fig.5 and 6). The more substantial cell walls of this region, and the outer cell wall of the meristoderm cells emphasizes the cohesive appearance of this tissue (Fig.1, 2 and 3). Occasionally sheets of the outer layer of the cuter wall may be lost as in meristoderm cells of the transition zone. Except for those meristoderm cells which have just divided anticlinally there is usually a one to one relationship between meristoderm cells and the immediately subtending cortical cells (Fig.1-3, 5 and 6). At the other end of this region of meristoderm just above the youngest visible sorus and after lateral blade expansion has slowed down the meristoderm cells are more uniform in their anticlinal cell wall lengths (Fig.4). Furthermore, in section each cortical cell usually subtends at least two meristoderm cells and appears less elongate and slightly more cuboidal (Fig.6).
2.2.2. Histology

With TBO all of the cell walls stain heavily (Fig.1, 2 and 3). A thin external layer of the continuous outer cell wall which shows red metachromasia for sulfated polysaccharides is visible only when curled up during preparations (Fig. 1 next section and Chapter II). The remaining layers of this outer cell wall and the other delimiting cell walls show a reddish-purple metachromasia for acid and sulfated polysaccharides. The centrally located nucleus stains a blue-gray with TBO and contains a nucleolus (Fig. 2). More regularly than for the transition zone, these nuclei have a basally located cap of densely stained metachromatic material (Fig. 2 and 3). Granules of the same metachromasia appear in the apical cytoplasm as well, and the lighter blue-gray chloroplasts still occupy a peripheral position (Fig. 2 and 3). In this part of the cytoplasm dense blue-green granules are often seen with TBO staining indicative of phenolics (Fig. 2).

The basal cytoplasm may contain the metachromatic material mentioned above, a couple chloroplasts and often shows clear unstained areas (Fig. 2 and 3). In section all meristoderm cell walls stain blue for acid polysaccharides with AB/SO (Fig. 4) and its outermost layer is stained red indicating a concentration of sulfated polysaccharides. This coincides with TBO metachromasia of the same material. Occasionally, blue staining material, acid polysaccharides are visible in the
apical cytoplasm of these cells. In sections of ABB stained material the apical cytoplasm appears as a clear and densely staining mosaic of granules delimited by moderately stained chloroplasts to the sides and the central nucleus below (Fig. 5 and 6). Variations in this pattern are due to the plane of sectioning. The basal cytoplasm is characterized by clear areas, indicating the beginnings of vacuolation, surrounded by densely staining peripheral cytoplasm and the occasional chloroplast. The basal cytoplasm adjacent to the nucleus may show clear vesiculate areas corresponding to the dense metachromatic material seen with TBO staining.

2.2.3. Ultrastructure

There is a strong similarity between these meristoderm cells (Fig. 7 and 8) and those of the transition zone. However, there are a number of notable differences. Due to the greater mitotic frequency nuclear shape is much more irregular (Fig. 7, 9 and 10). Also indicative of their frequent mitotic activity are the numerous thin basal periclinal cell walls and small cortical initials they delimit (Fig. 11, 14 and 15). Frequently various stages of cytokinesis may be observed. Only small golgi vesicles occur at sites of cytokinesis (Fig. 13, small arrowhead). If furrowing is occurring it is apparently asymmetric (Fig. 13).

The quantity and distribution of organelles varies with
the time elapsed since the last periclinal division. Dictyosomes are usually located on the basal perimeter of the nucleus (Fig. 8). However, they may also cover the apical portion as well (Fig. 9). In agreement with light microscope observations the cell walls in this region are more substantial (Fig. 12 and 22). Frequently, the outer periclinal cell wall is almost completely preserved (Fig. 12 and 22). This wall is composed of four architecturally different layers. In the innermost layer, microfibrils are oriented primarily tangential to the membrane (Fig. 12 and 22). Layer two is a region of mixed fibrillar orientation while those of the third layer are mostly oriented perpendicular to the plasma lemma and are considerably less electron opaque (Fig. 12 and 22). The outermost layer consists of a reticulum of electron dense irregularly thickened fibrils (Fig. 22).

The nucleoplasm (Fig. 7-9 and 11) and nucleoli are essentially identical to those of the transition zone meristoderm cells (compare Figure 11 with Figure 12 of the previous section). The homogeneously fibrillar regions reported in cells of the transition zone occur here as well (Fig. 10). Luminal continuities between the ER and nuclear envelope are observed (Fig. 23). Prior to or after the completion of cytokinesis dictyosomes become located at the most distant poles of the two nuclei (Fig. 11, 14 and 15). There is more morphological variation in the individual dictyosomes of these meristoderm cells. In median sections
most dictyosomes reveal a region of central plates where the membranes of individual cisternae are closely associated (Fig. 17 here) as described in the previous section (Fig. 14). This central region is again surrounded by only slightly hypertrophied regions where the cisternae become branched, fenestrated, or reticulated (Fig. 20). In contrast to the previous section, some dictyosomes are observed where cisternae of the maturing face are not peripherally hypertrophied (Fig. 16). However, small vesicles are also present in the vicinity of the maturing faces of dictyosomes not showing hypertrophied cisternae (Fig. 16). In peripheral extensions of cisternae of the formative faces the cisternal membranes may be associated with small densely osmiophilic membrane whorls (Fig. 18 and 19). Finally, in the region of the nucleus and most often in proximity to the golgi apparatus one finds multivesiculate vesicles (Fig. 21).

With the exception of a few features the chloroplasts of these cells are the same as those of the transition zone (Fig. 7 and 21). However, infrequently they may have pyrenoid like structures (Fig. 26) and small whorls of membrane apparently originated from both chloroplast ER and chloroplast envelope membranes (Fig. 25). Careful examination reveals small osmiophilic spheres in the chloroplast matrix (Fig. 21). The apical cytoplasm of these cells is little different from that of the transition zone meristoderm cells (Fig. 22 compared with Figure 15 previous section). However, the peripheral rough ER
of this region may appear to be more prominent by virtue of slight swelling containing a lightly osmiophilic material (Fig. 22).

At the apical extent of anticlinal cell walls the plasma membrane apparently may evidence tubular extensions into or through layer 1 of the outer wall (Fig. 24).

Throughout these cells, particularly in the basal cytoplasm there are numerous profiles of mitochondria and occasionally of microbody-like structures in section. Many mitochondria show regions where there is little or no matrix and are devoid of cristae (Fig. 27). From different planes of section it is apparent that they have become cup shaped and only the rims contain the matrix and cristae. Furthermore, the concave area of the cup often contains a volume of lightly, homogeneous cytoplasm delimited by fenestrate ER (Fig. 13, 21 and 27), reminiscent of provacuolar regions in the basal cytoplasm already illustrated (Fig. 20, previous section).
CHAPTER I FIGURES, EXPANSION ZONE
Figure 1. Young thallus expansion zone meristoderm and cortex. The lineage of meristoderm cell products may be traced into the cortex (a to e). Four periclinal divisions (between a and b, b and c, c and d, and d and e) have occurred to the one anticlinal division (between c and c). Note recent meristoderm anticlinal division (arrowhead). See recently produced cortical initials (b and b). Scale equals 10 um.

Figure 2. TBO stained expansion zone meristoderm cells. Note the lightly staining chloroplasts, the centrally located nuclei and the basal mass of metachromatic material (arrowheads). Scale equals 10 um.

Figure 3. TBO stained expansion zone meristoderm cells. Modest basal vacuolation occurs in some cells. Anticlinal walls cut in surface section showing lighter staining pit fields (arrows). Scale equals 10 um.

Figure 4. More distal expansion zone meristoderm. Note broader subtending cortical cells. Wall staining is the AB of the AB/SO. Outer layer shows slight SO staining. Note outer continuous layer, space, then inner layer specific to each cell. Scale equals 10 um.
Figure 5. ABB stained expansion zone meristoderm cells.

Cells reach about 10 μm. Across (*) before dividing anticlinally (arrow). Basal vacuolation is seen in some. Scale equals 10 μm.

Figure 6. Cytoplasmic continuities visible with ABB staining.

Presumptive secretory cell initial maintains cytoplasmic density and enlarges in contrast to neighboring vacuolate cortical initials. Cytoplasmic continuities are seen at arrowheads. Note densely staining granules, chloroplasts and clear regions of apical cytoplasm. Scale equals 10 μm.
Figure 7. Meristoderm cells of the expansion zone.

Centrally located nuclei dominate the cytoplasm. Chloroplasts and mitochondria are distributed throughout the cytoplasm. The apical cytoplasm shows vesiculation. The outer wall is more substantial. Pit fields of plasmodesmata interconnect meristoderm cells to meristoderm and cortical cells. Vacuolar initial regions are prominent in cortical initials. Scale equals 1 μm.
Figure 8. Golgi apparatus of expansion zone meristoderm cells. Dictyosomes usually lie on the basal hemisphere of the nucleus (arrowheads). Note the pit field between meristoderm cell and cortical initial (arrow). Loose areas occur in anticlinal walls (*). Scale equals 5 μm.

Figure 9. Polymorphic nucleus in an expansion zone meristoderm cell. Nucleus is centrally constricted and dictyosomes are not confined to the basal hemisphere. Note nuclear envelope vesiculation at formative face of individual dictyosome (arrowhead). Nucleolus is prominent. Scale equals 1 μm.

Figure 10. Another polymorphic nucleus. Note the pear shape and associated dictyosomes. See the persistent homogeneous region (arrowheads). Scale equals 1 μm.
Plate XIII

Figure 11.  Post mitotic expansion zone meristoderm cells.

The new cell plate contains very little cell wall material.  A pit field is already visible (arrowhead).  The dictyosomes are at the apical hemisphere of the nucleus.  Cell to the right is bi-nucleate and there is no cell plate yet.  An electron dense granule lies adjacent to the apical nucleus.  Basal and apical nuclei both have nucleoli.  Scale equals 1 um.

Figure 12.  Detail of intact outer cell wall.  Four morphologically distinct layers occur.  The first (between arrowheads numbered 1) consists of fibrils oriented parallel to the plasma membrane and is continuous with those of the anticlinal walls.  The second (between arrowheads numbered 2) consists of fibrils oriented from parallel to perpendicular.  The third layer (between arrowheads numbered 3) consists of loose fibrils primarily perpendicular to the plasma membrane.  The fourth layer (arrowheads numbered 4) consists of an electron dense material of a disorganized nature.  Scale equals 0.5 um.
Plate XIV

Figure 13. Cytokinesis of a meristoderm cell. Note the anticlinal wall origin and cytoplasmic extremity of the cytokinetic furrow (bordered arrowheads). Pit fields are already present (plain arrowheads). Note the ER cisternae parallel the plate and a dense mass of cytoplasm lies in advance of the furrow (*). Scale equals 1 μm.

Figure 14. New cell plate and cortical initial. Pit fields are present in anticlinal wall with clear plasmodesmata (arrowheads). Initial fibrils of the new periclinal wall are visible in pit fields (arrow). Anticlinal/periclinal wall are visible in pit fields (arrow). Anticlinal wall junctions are indicated (bordered arrowheads). Cisternae of ER parallel new cell wall on both faces. Cortical initial nucleus contains a large nucleolus with granular and fibrillar zones. Dictyosomes and centrioles are all at the basal hemisphere adjacent to the former inner periclinal cell wall of the parent meristoderm cell. See membrane configuration between chloroplasts (*). Scale equals 1 μm.
Plate XV

Figure 15. Binucleate/pre-cytokinetic meristoderm cell. Apical cytoplasm of meristoderm half is indicated. Note dictyosomes are at the polar ends of the two nuclei. Scale equals 1 μm.

Figure 16. A dictyosome of the golgi apparatus. The formative face lies adjacent to the nucleus with the usual intervening vesicles. Note the small amount of cisternal hypertrophy at the maturing face and the numerous small vesicles. Some of these vesicles (*) may be associated with cisternae at the maturing face (arrowhead). Scale equals 0.1 μm.

Figure 17. A dictyosome of another morphology. The formative face as above is adjacent to the nucleus and small vesicles lie between. Note the hypertrophy of cisternae at the maturing face and golgi vesicles. Regions of cisternal hypertrophy closest to the cisternal stack contain an even reticulum of electron dense material (*) while more distal regions (hC) and golgi vesicles contain a stretched fibrillar material. Luminal continuities exist between extreme and more proximal regions of hypertrophy (arrowheads). Scale equals 0.1 μm.
Plate XVI

Figure 18. The peripheral region of a stack of dictyosome cisternae. Note the cisternal interdigitation (large arrowheads) and small configuration of membranes (*). The nuclear envelope, formative face and intervening vesicles (small arrowheads) are indicated. Scale equals 0.1 μm.

Figure 19. Peripheral region of dictyosome cisternae. Nuclear envelope, formative face, and intervening vesicles (arrowheads) are at figure bottom. A dense membrane configuration occurs amongst the cisternae (*). Scale equals 0.1 μm.

Figure 20. Dictyosome in surface section. Cisternal centers are fenestrated (*) and further out are tubular extensions which may hypertrophy. Scale equals 0.5 μm.

Figure 21. Cytoplasmic detail. Multi-vesiculate vesicles (*) occur frequently. Chloroplasts contain small osmiophilic masses (arrowhead) and genophores. Numerous mitochondria are cup shaped with the cup bottom made of appressed mitochondria membranes and delimiting an electron translucent region of cytoplasm and ER. Scale equals 0.5 μm.
Figure 22. Apical cytoplasm in cell of expansion zone. The chloroplasts, vesicles and peripheral sheath of ER are the most outstanding features. The plasma lemma still shows invaginations over the apical dome and there may be fairly large spaces at some anticlinal junctions between cells (small *). Vesicles may be simple or occasionally contain whorls of membrane (large *). Mitochondria are ever present. The four layers of the outer cell wall are particularly clear here. The outermost layer, number 4, is electron dense and disorganized. Scale equals 1 um.

Figure 23. The apical cytoplasm. Note especially the luminal continuity between the ER system of the apical cytoplasm and the nuclear envelope (arrow). Scale equals 1 um.

Figure 24. An intercellular space at an anticlinal wall junction. Apparent microvillous extensions of plasma membrane (arrowheads) extend through the cell walls. Scale equals 0.1 um.
Figure 25. The chloroplast envelope and ER. Small vesicles occur between chloroplast ER and chloroplast envelope (arrowheads). Vesicles appear to be of chloroplast ER origin (tcp arrowhead). A mass of concentric membranes occurs within the chloroplast ER (*). Scale equals 0.1 \( \mu \text{m} \).

Figure 26. A pyrenoid-like structure. Note the narrow neck (arrowhead), lacking thylakoids and surrounded by chloroplast envelope and chloroplast ER. Scale equals 0.1 \( \mu \text{m} \).

Figure 27. A cup shaped mitochondrion. The cup consists of appressed mitochondrial membranes where cisternae (arrowhead) and matrix are excluded. The cup defines an electron translucent region of cytoplasm (*) surrounded by an apparently fenestrate ER cisterna. Scale equals 0.1 \( \mu \text{m} \).
2.3. Meristoderm cells adjacent to youngest sori

From field observations it is apparent that there is some variability as to the distance from the transition zone at which the sori first appear. Except for some cases observed at the end of the growing season, however, the sori do not seem to appear closer to the transition zone than the point where lateral expansion of the lamina has slowed, as indicated by essentially parallel margins.

2.3.1. Anatomy

The meristoderm cells in this region form a layer quite distinct from the cortex (Fig. 1). Superficially, it is difficult to determine the relationship between the meristoderm cells and the derived cortex cells. In sections, it is not uncommon to find four meristoderm cells subtended by one large cortical cell (Fig. 2). This would imply approximately 16 meristoderm cells overlying each of the cortical cells. It is apparent at a glance that the anticlinal walls of these meristoderm cells are shorter than those of either of the previous sections. The cells are still mostly longer than wide accept those showing a more recent periclinal division (Fig. 1 and 3).
2.3.2. Histology

The cell walls of these meristoderm cells stained with TBO show a dense (Fig. 1) red-purple metachromasia for acid and sulfated polysaccharides as did those in the expansion zone. There is also a thin red metachromatic external layer of the outer continuous periclinal cell wall (Fig. 1, arrow) probably a sulfated polysaccharide. The denser blue-gray nuclei are often surrounded or masked by the relatively large gray chloroplasts (Fig. 1, *). Nucleoli are not easily seen in these nuclei. The area of the apical cytoplasm stains moderately and frequently contains densely staining granules (Fig. 1). Densely stained masses of material are also seen inside vacuoles of the basal cytoplasm although they need not be metachromatic (Fig. 1). The metachromatic material on the basal face of the nucleus as shown previously is observed. The staining pattern with AB/SO is essentially the same as that described in the previous section (Fig. 2) except that blue staining material, acid polysaccharides, in the apical cytoplasm is more distinct (Fig. 2). In sections of ABB/PAS stained material the nuclei, chloroplasts and cytoplasm stain densely for proteins with the ABB (Fig. 3) except for small clear vesiculations in the apical-most part of the cytoplasm and similar but larger clear areas in the basal cytoplasm (Fig. 3). The outermost layer of the continuous outer periclinal cell wall is PAS positive for neutral polysaccharides, and there are faint extensions of this
material down the middle lamellae of the anticlinal cell walls (Fig. 3).

2.3.3. Ultrastructure

These meristoderm cells are characterised by the prominent large chloroplasts with their well developed thylakoids, the relatively smaller, largely homogenous nuclei and prominent vacuolar regions (Fig. 4). Although cell walls show comparatively less csmiophilia than cytoplasm they are more substantial and better withstand tissue processing (Fig. 4 and 5).

The nuclei are delimited by a typical nuclear envelope interrupted in section by occasional pores (Fig. 6). The homogeneously fibrillar regions and small nucleoli are present in the nucleoplasm (Fig. 6, arrowheads). Although there is a thin layer of chromatic material lining the inner membrane of the nuclear envelope, there are no large masses of heterochromatin. However, there are small, uniform clumps or chains of heterochromatin distributed throughout the nucleoplasm (Fig. 6). This gives the nucleus a denser but more even electron density (Fig. 6). The nucleus is usually crowded by other organelles. With the exception of a small column of apical cytoplasm, the apical surfaces of the nucleus may be bordered by chloroplasts (Fig. 4 and 6). This is often true of lateral surfaces as well. The basal surface of the nucleus
has at least one closely associated dictyosome (Fig. 4, 8, 12 and 14). Chloroplast organization is normal, with chloroplast ER, chloroplast envelope, peripheral thylakoids and ring genophore (Fig. 7). However, in contrast to the chloroplasts of the previous section (3.2. Meristoderm Expansion Zone) there are at least twice as many stacks of internal thylakoids and well developed plastoglobuli (Fig. 4, 7 and 14). Furthermore, whorls of membranes associated with the chloroplasts are still present and they may become quite elaborate (Fig. 9, 10 and 11). With the increase in density of the cytoplasm and crowding of organelles it becomes difficult to observe direct luminal continuities between CER and ER, CER and nuclear envelope or nuclear envelope and ER. The dictyosomes are now almost invariably located on the basal surface of the nuclei (Fig. 4). There is a marked reduction in hypertrophy of their cisternae although some may persist (Fig. 4, 8 and 14). The association between formative face and nuclear envelope is clear (Fig. 8). Vesicles of apparent golgi origin are observed throughout the cytoplasm (Fig. 8 and 9). Because of the crowding in these meristoderm cells, an association of membrane whorls with dictyosomes is not as clear cut as that described in the last section (3.2. Expansion Zone).

The apical cytoplasm has become compact and restricted. There is usually a thin layer of apical cytoplasm between the apical periclinal cell wall and chloroplasts (Fig. 4). The
cytoplasmic matrix is dense making it difficult to distinguish profiles of peripheral ER (Fig. 9). Golgi vesicles distinguished by their contents and numerous chloropast derived membrane whorls contribute to a complex morphology (Fig. 9 and 13). This apical cytoplasm may extend between the chloroplasts to the nucleus (Fig. 13) or the nucleus may be squeezed between these apical chloroplasts and further restrict the apical cytoplasm (Fig. 12). The apical cytoplasm varies considerably between cells. In some cases, it may appear as an extension of the compressed apical layer of cytoplasm described above containing dictyosome vesicles and various membrane whorls (Fig. 9). Yet in cases it may appear vacuolated through an apparent hypertrophy of ER and loss of contents (Fig. 13), while in others this hypertrophied ER is filled with a homogenous material (Fig. 12).

The basal cytoplasm becomes dominated by chloroplasts, mitochondria and various patterns of vacuolation (Fig. 14). The chloroplasts here are identical to those of the apical cytoplasm and the mitochondria appear to be orthodox and show none of the cup morphology of the previous section (2.2. Expansion Zone). These mitochondria represent elongate spherical morphologies in section indicating that they are in fact tubular (Fig. 14).

In contrast to the two previous sections (2.1. And 2.2.) there is invariably some degree of vesiculation in the basal cytoplasm (Fig. 14). Vacuolation seems to occur in an
homogeneous region of basal cytoplasm delimited by sheets of apparently fenestrated ER (Fig. 12 and 14). This agrees with the first indications of vacuolation already considered (Fig. 20, section 2.1.). Some of the structures commonly found included in or dominating this area are hypertrophied golgi vesicles; membrane whorls of various sizes and osmiophilia; osmiophilic granules; and various single and double membrane bound vesicles (Fig. 14 and 15). The large membrane whorl of Figures 14 and 15 is apparently contained within a hypertrophied cisterna of the ER delimiting this region (Fig. 15 small arrowheads). The obvious hypertrophied golgi vesicle is apparently just included within or has been allowed to pass the membranes delimiting this region (Fig. 14). Contents of the various single and double membrane bound inclusions vary in texture and osmiophilia (Fig. 14 and 15). Although not illustrated here, numerous microbody-like structures were observed in these meristoderm cells.
CHAPTER I FIGURES, YOUNGEST SORI
Plate XIX

Figure 1. TBO stained meristoderm cells adjacent to the youngest sorus. Cell walls are a metachromatic purple and an outer red layer has come off (arrow). Where nuclei are visible the apical and basal cytoplasm are dilimited. Chloroplasts(*) occur in the apical and basal cytoplasm. Apical and basal cytoplasm may contain densely staining granules (small arrowheads) and the basal cytoplasm is vacuolar (larger arrowhead). Scale equals 10 μm. Note size difference between meristoderm cells and immediately subtending cortex cells. Scale equals 10 μm.

Figure 2. AB/SO stained cells adjacent to the youngest sorus. All walls stained for alcian blue. Note especially the heavy alcianophilia of the outer cell wall and cytoplasmic granules (arrowhead). The outermost layer stained purple indicating positive safranin staining. Scale equals 10 μm.

Figure 3. AEB/PAS stained cells adjacent to the youngest sorus. Cytoplasmic organelles stain densely with ABB. Vacuolate regions are unstained except for some dense granules (small arrowheads). Apical cytoplasm may show varied vesiculation (large arrowhead). A peripheral cytoplasm containing few chloroplasts lines the vacuoles of the cortical cells. Outer layer and peaks between
cells often show PAS positive, not visible here. Scale equals 10 μm.
Figure 4. Three meristoderm cells over a single cortical cell. Osmiophilia between the cytoplasm and cell walls is marked. Nuclei still maintain a mostly central location, but chloroplasts may crowd the apical and basal cytoplasm as well as nuclei. When the apical cytoplasm is visible it is vesiculate. Dictyosomes usually occupy a basal position always adjacent to the nuclear envelope. Pit fields with plasmodesmata still interconnect adjacent meristoderm cells (arrow) and these in turn with subtending cortical cells (not illustrated here). Scale equals 1 um.

Figure 5. Anticlinal and outer cell walls. Anticlinal cell wall consists of regularly parallel fibrils continuous with the innermost layer 1 of the outer cell wall (between arrowed bars). Layer 2 and 3 are no longer distinguishable from one another and thus constitute the second layer. The outermost layer, layer 4, has thickened and is more regular, outside arrowed bars 2/3 to right. Scale equals 1 um.

Figure 6. Nuclear detail. The homogeneous region is still present (arrowheads) and a modest nucleolus occurs at the nuclear envelope, which is interrupted by pores in section. Chloroplast ER continuities with nuclei are difficult to find at best. Scale equals 0.5 um.
Figure 7. Half of a chloroplast. Chloroplasts are defined by chloroplast ER and envelope. Genophore is at the pole in cross section and plastoglobuli (arrowhead) are present in the matrix between the numerous stacks of thylakoids. Scale equals 0.1 um.

Figure 8. The golgi apparatus of a cell. The formative face lies adjacent to the nuclear envelope which evidences vesiculation (arrowhead larger). Vesicles and cisternae hypertrophy at the maturing face. Hypertrophy of fenestrate regions produces membranous tubules across the cisternal lumen (small arrowheads). Scale equals 0.1 um.

Figure 9. Apical cytoplasm. In addition to chloroplasts, golgi vesicles, profiles of ER, ground substances and layered membrane configurations (*) are primary constituents. Membrane configurations are often associated with chloroplast ER. Reticulate material occurs in the golgi vesicles and the intramural space. Scale equals 0.1 um.

Figure 10. A membrane configuration within the chloroplast ER. Scale equals 0.1 um.
Figure 11. Oblique section of a chloroplast. Note small membrane configuration within chloroplast ER. Scale equals 0.1 um.
Plate XXII

Figure 12. Vacuolar region in a meristoderm cell. Regions of homogeneous cytoplasm (*,s) are surrounded by ER. Hypertrophied and unhypertrophied regions of ER are in continuity (large arrows). Contents of hypertrophied regions of ER in contact with the region of homogeneous cytoplasm may be difficult to delimit (plain arrowheads) and (lower bordered arrowhead). Here the vacuolar region extends up towards the apical cytoplasm. Scale equals 1 μm.

Figure 13. Vacuolar region. Note here the completely electron translucent regions (large *) in contrast to remaining homogeneous material (small *). Arrows indicate continuities with non-hypertropheid regions and arrowheads delimiting membranes. This is apical to the nucleus. Scale equals 1 μm.
Figure 14. Basal cytoplasm. The golgi apparatus usually faces the basal cytoplasm. Mitochondria, chloroplasts and microbody-like structures may be present. The vacuolar region is complex. Note presumed lipid granule and concentric membrane configuration. Single membrane delimited vesicles labelled "a" are of golgi origin. Type "b" is delimited by two membranes in some portions of its perimeter and one in others. Type "c" appears double all around. Scale equals 1 um.
Figure 15. Basal cytoplasm vacuolar region. Concentric membrane configuration lies within an hypertrophied portion of ER which has expanded into the provacuolar region. Note un-enlarged cisternae bordering provacuolar region of homogeneous cytoplasm (*) and delimiting membrane of hypertrophied cisterna (small arrowheads). Double membrane portions of vesicle type "b" of Figure 14 are due to apposition of its single membrane and that of the hypertrophied ER (large arrowheads). Scale equals 0.5 um.
2.4. Meristoderm Cells Adjacent to Mature Sori

In the course of this work it became clear that the mature sori of *Nereocystis* are released from the lamina periodically, at the beginning of the lowest low tide series of each month, rather than continuously. This has been confirmed (Duncan and Walker, unpublished observations). Consequently, the oldest sori collected just prior to this release are found to be in the first stages of sorus exuviation and release from the lamina, while the sori collected after this massive release may still have another waiting interval before release. This may explain the morphological variety observed in these tissues. This morphological variation culminated in the designation of two tissue types I and II. The essential characteristic differentiating type I meristoderm cells from type II will be seen to be the golgi apparatus. In spite of this difference there are numerous features which characterize these cells and distinguish them from those meristoderm cells of the previous section 2.3. Finally, although the condition of the lamina around the oldest scus can often be tattered from mechanical disturbance the tissues may not show signs of cellular deterioration.

2.4.1. Anatomy

In essential morphological features the meristoderm cells
show little variation from those just described in section 2.3. They are more cuboidal than those adjacent to the youngest sori and some are even wider than they are tall (Fig. 4). In older tissue outer walls may be quite thick and if laminae are immobilized so that one side always faces up, there will be a clear differential in thickness of the outer continuous periclinal cell wall (Ch. III).

2.4.2. Histology

With TBO cell walls stained as heavily (Fig. 1) as those of cells opposite the youngest sorus (section 2.3., Fig. 1). Chloroplasts and nuclei again stained approximately as in those meristoderm cells opposite the youngest sorus (compare Fig. 1 with Fig. 1 of section 2.3). However, the apical cytoplasm becomes highly vacuolated and contains little cytoplasmic matrix (Fig. 1 and 4). A single large vacuole usually displaced the basal cytoplasm (Fig. 1 and 3). In LM of material prepared for EM and also stained with TBO this is clearly demonstrated (Fig. 4) often osmiophilic granules are seen within the vacuole (Fig. 4). From this material (Fig. 4) and AB/SO material (Fig. 2) the outer cell wall is seen to have a trilamellar appearance. Both the apical cytoplasm and the vacuoles contain blue staining material, acid polysaccharides, with AB/SO. ABB/PAS staining also demonstrates the density of the chloroplast and nuclear proteins as well as the obvious extensive vacuolation of the basal cytoplasm (Fig. 3). The
extensive vesiculation of the apical cytoplasm is also clearly demonstrated with ABB protein staining (Fig. 3). The external layer of the outer continuous periclinal cell wall is still PAS positive for neutral polysaccharides, with some reduction of intensity (Fig. 3).

2.4.3. Ultrastructure

Ultrastructurally these cells are distinct in that their cell walls are quite substantial, their chloroplasts invariably seem to possess pyrenoids, luminal continuities and associations amongst the various endomembrane systems are again prominent. Also there is a notable variation in the general appearance of the apical and basal cytoplasm in section. The key to variation in apical and basal cytoplasm seems to be related to golgi function which reveals two distinct morphologies.

The walls of these cells are more osmiophilic (Fig. 5) than those of section 2.3. and this property is intensified if Ruthenium Red is included in the fixatives (Fig. 6 as by Luft 1971ab). Other than being thicker and apparently more substantial the anticlinal and basal periclinal cell walls are morphologically similar to those of the previous section. The apical or outer periclinal wall is apparently quadralamellar. However, these layers are not identical to those regions previously indicated. The clearest layers are the inner and
outermost ones (Fig. 5 and 6). In section the innermost layer, adjacent to the plasma membrane, is constituted of microfibrils oriented almost exclusively parallel to the plasma membrane (Fig. 5). This layer is restricted in extent to individual cells and is continuous all around the individual cell (Fig. 4, 5 and 6). The next layer also consists of microfibrils, however, their orientation is less uniform relative to the plasma membrane and this layer possess a continuous covering over the meristoderm cells (Fig. 5). In some areas, though still a reticulum, the predominant orientation of microfibrils is horizontal, parallel to the surface of the lamina (Fig. 5 and 6). The next outer layer is very similar to this second except that the microfibrillar components are difficult to distinguish and what would be spaces in the reticulum of the microfibrils of the immediately subtending layer appear here as lesions (Fig. 5 and 6). This layer is certainly a layer of the outer wall and is not restricted to individual cells but is continuous (Fig. 5 and 6). The outermost layer is new in this tissue. It is composed of a finely fibrillar to flocculent material which extends perpendicular to the underlying layer of fibrils (Fig. 5 and 6). It does not resemble the outermost layer of any of the previous regions. It is essential to note the difference in the abundance of this material between the type I cell of figure 5 and the type II cell of figure 6. This flocculent layer is definitely less abundant on the outer surface of the type II cells (Fig. 6).
The nuclei of the meristoderm cells of this region are very homogeneous except for their nucleoli and light, homogeneous, fibrillar regions (Fig. 7) as described earlier. In both types of cells (type I and II) of this region there are abundant examples of clear luminal continuities between the nuclear envelope and the CER surrounding the plastid proper and its pyrenoid (Fig. 8 type I and 9 type II). A pair of centrioles still persists on the basal surface of the nuclear envelope (Fig. 9) in addition to dictyosomes of the golgi apparatus (Fig. 10 type I and 11 type II).

The apical cytoplasm is dominated by the large chloroplasts, in both cell types, to the extent that the nucleus may be crowded by them (Fig. 4 and 7). Other aspects of the apical cytoplasm may show considerable variation. Often, in the area of the apical cytoplasm of type I cells not occupied by chloroplasts there are numerous vesicles containing a fibrous material similar in appearance to the outermost flaccidulent layer of the cell wall (Fig. 5 and 16). Occasionally these vesicles are observed to be in luminal continuity with the extracellular space between the inner face of the cell wall and the plasma membrane (Fig. 16). This presumably represents exocytosis. In comparable type II specimens the apical cytoplasm that is not occupied by chloroplasts may contain large irregular single membrane-bound vesicles (Fig. 17 and 18). These most often contain an homogeneous matrix which may exhibit varying degrees of
csmiophilia (Fig. 13, 17 and 18). In section their contents sometimes suggest either an interconvertability to an apparent membranous myelin-like body or else a close association with whorls of membranous material (Fig. 13 and 14). There are invariably profiles of ER in the apical cytoplasm of all of these cells and they contain flocculent material (Fig. 5, 17 and 18). Although these densely homogeneous matrix vesicles are often located near a chloroplast (Fig. 13, 17 and 18), continuities between their limiting membranes and those of the chloroplast ER were not observed. It should be noted however, that small whorls of membrane can occur in ER associated with pyrenoids of the chloroplasts (Fig. 12). Careful examination of the delimiting membranes of small vesicles in the apical cytoplasm of these type II cells reveals that they are of golgi and not ER origin (Fig. 11, 12 and 18). The cytoplasm of this apical region is very dense, often denser than that of the pyrenoid, chloroplast, and homogeneous vesicle matrices as well (Fig. 18). Membranes of the homogeneous vesicles are easily distinguished from those of golgi vesicles which show the typical trilamellar structure more comparable to that of the plasma membrane (Fig. 18).

The chloroplasts of both types of meristoderm cells observed are typical (Fig. 5, 7, 13, 16, 18, 19 and 20). In spite of the variation in the cytoplasm of these type I and type II meristoderm cells their chloroplasts invariably possess pyrenoids. These appear as relatively homogeneous extensions
of the chloroplast matrix, devoid of thylakoids and delimitated by the chloroplast envelope and the chloroplast ER (Fig. 8, 9 and 18). The pyrenoids may or may not show a restricted basal neck (Fig. 9 and 18). On occasions their chloroplast ER may be continuous with ER (Fig. 7 and 12) or more often with the nuclear envelope (Fig. 8 and 9). The luminal continuities of these endomembrane systems (NE, ER and CER) are very clear and easily detected in this material (Fig. 7, 8 and 9).

The basal cytoplasm, other than the chloroplasts, is structurally dominated by the vacuolar system (Fig. 1, 3, 4, 19 and 20). The golgi apparatus consisting of at least one dictyosome is usually on the basal hemisphere of the nucleus and therefore often in close proximity to the vacuole. At first glance the golgi apparati of both type I and II meristoderm cells are not very extensive (Fig. 10, 11 and 12). However, in the type I cells small vesicles of apparent dictyosome origin are found protruding in against the tonoplast membrane (Fig. 10). These may apparently enlarge there and are easily identified by the two layers of membranes while still in the vacuolar region of the cells (Fig. 10 and 11). The vacuoles of these cells may also contain densely osmiophilic masses (Fig. 11). The remaining volume of the vacuole may contain scattered flocculent material (Fig. 11) while in type II meristoderm cells this is the predominant appearance of the vacuolar contents (Fig. 20). The golgi apparatus of the type II cells have small secretory vesicles (Fig. 12) around it and
in the apical cytoplasm (Fig. 17 and 18). These vesicles may show some hypertrophy in the apical cytoplasm (Fig. 11) but not to the degree seen in the type I cells.

In type I cells of this region one often finds typical microbody-like structures (Fig. 15). These are rarely observed in the later type II of this region. The closest structures to microbody-like structures are the single membrane-bound bodies described earlier (Fig. 17 and 18). Mitochondrial profiles in both types of cells of this tissue are typical in structure as in the previous region and often contain osmiophilic granules in their matrices (Fig. 14 and 15).
CHAPTER I FIGURES, OLDEST SORI
Plate XXV

Figure 1. TBO stained cells adjacent to the oldest sorus. Cell size and shape are more uniform. Vacuolation is usually basal. There may be four meristoderm cells to one subtending cell in section. Scale equals 5 μm.

Figure 2. AB/SO stained cells adjacent to oldest sorus. Closer to sorus than Figure 1. Outer wall stains most densely with AB (large arrowhead) and outer layer may show SO positive staining. Note border between inner wall layer about individual cell and darker staining continuous material of outer wall (small arrow). Apical cytoplasm may contain densely staining granules (small arrowheads). Scale equals 5 μm.

Figure 3. AEB/PAS stained cells adjacent to oldest sorus. Cells show basal vacuolation. Note apical vesiculation in favorable section (larger arrowhead) and outer layer of outer cell wall is PAS positive (small arrowheads). Scale equals 5 μm.

Figure 4. Osmicated and TBO stained cells adjacent to the oldest sorus. They are more distant from the sorus. Note almost cuboidal cell shapes, dominant chloroplasts and vacuoles with osmiophilic contents (larger arrowheads). Plasmodesmatal connection persists at pit fields (small arrowheads). Scale equals 5 μm.
Figure 5. Outer cell wall and apical cytoplasm of Type I cells. Innermost layer of parallel fibrils (1 between bars) is continuous with anticlinal cell walls. Layer 2/3 is easily distinguished from 1 but not as easily from 4 (see bar between) but 4 is distinguishable by its more regular fibril orientation, fibril density and apparent lesions. Atop layer 4 is a lush layer of perpendicular flocculent material. Note here especially the Golgi vesicle in the apical cytoplasm and compare its contents with the material in the intramural space and the outer layer of flocculent material. Scale equals 0.5 \( \mu \text{m}. \)

Figure 6. Outer cell wall and some apical cytoplasm of Type II cells. Fibrillar layers 1, 2/3 and 4 are essentially identical. However, the outermost layer of perpendicular flocculent material is sparser and eroded away. Golgi vesicles are present in apical cytoplasm but very small. Scale equals 0.5 \( \mu \text{m}. \)
Figure 7. Nuclear features common to Types I and II cells. Both nucleoli and homogeneous regions (*) are present. The envelope is occasionally interrupted by pores and often shows luminal continuity with ER and chloroplast ER (arrowheads), or one in the same with chloroplast ER. Scale equals 0.5 μm.

Figure 8. Chloroplast from Type I cell. Portion of a pyrenoid surrounded by chloroplast envelope and CER which is also nuclear envelope (arrowhead). Scale equals 0.5 μm.

Figure 9. Chloroplast from Type II cells. Pyrenoids always present. Note association between chloroplast ER and nuclear envelope as in Type I cells. A pair of centrioles persist at the nuclear envelope. Scale equals 0.5 μm.
Plate XXVIII

Figure 10. A dictyosome on the basal hemisphere of a Type I cell nucleus. Note golgi vesicles invaginating vacuole membrane. Scale equals 0.1 um.

Figure 11. Golgi vesicles and vacuolar region of a Type I cell. Note series of three golgi vesicles each surrounded by vacuole membrane to give double membrane appearance (arrows). Osmiophilic material is a usual constituent. Scale equals 1 um.

Figure 12. Dictyosome from Type II cell. Note association of formative face with nuclear envelope. See gradient of dictyosome membrane thicknesses from formative face to plasma lemma like golgi vesicles at maturing face (arrow heads). A small concentric membrane configuration is present in the chloroplast ER around a pyrenoid. Scale equals 0.1 um.
Plate XXIX

Figure 13. Apical cytoplasm of a type II cell. Note large single membrane-bound structure containing juxtaposed granular material and lamellar membrane configuration. Scale equals 0.5 um.

Figure 14. A granular/lamellar structure from a Type II cell basal cytoplasm. See juxtaposed granular matrix and concentric membrane configuration. Note also mitochondria. Scale equals 0.5 um.

Figure 15. Type I cell mitochondria and microbody. Note osmiophilic granule in the mitochondrial matrix. Scale equals 0.1 um.
Figure 16. Apical cytoplasm of a Type I cell. Note the golgi vesicle fused to the plasma membrane. Its contents resemble that of other vesicles prior to exocytosis (arrowheads). Scale equals 0.5 um.

Figure 17. Apical cytoplasm of Type II cell. Note elements of the peripheral ER system and the occasional small golgi vesicle. See the large single membrane-bound masses of electron dense granular material (*) which may be associated with the chloroplast. Scale equals 0.5 um.

Figure 18. Apical cytoplasm of Type II cell. See small golgi vesicles and peripheral ER system elements. Possible exocytosis of a golgi vesicle (larger arrowhead). See large mass of lighter electron dense granular material contained within a single membrane (small arrowhead) possibly continuous with ER (arrow). Note also the pyrenoid with constricted neck and encasement by both chloroplast envelope and ER. Scale equals 0.5 um.
Plate XXXI

Figure 19. Basal cytoplasm of Type I cells. Note the pit fields and plasmodesmal continuities in depressions of anticlinal and periclinal walls between meristoderm cells and subtending cortical cell (opposing arrowheads). Vacuoles contain osmiophilic masses, flocculent material and cytoplasm may contain the occasional membrane configuration. Scale equals 1 μm.

Figure 20. Basal cytoplasm of Type II cells. Vacuolar constituents contain primarily flocculent material and single membranes (arrowheads) may be from swollen regions of ER (small arrows). Note also plasmodesmal continuity between these cells and subtending cortical cells (lighter large arrows). Chloroplasts of these and Type I cells Figure 19 are similar in size, numbers of stacks of thylakoids and presence of plastoglobuli. Scale equals 1 μm.
3. Discussion

This work is derived from two approaches to the study of vegetative meristoderm cells of the laminae of *Nereocystis*. In the first two sections ultrastructural observations of the transition and expansion zones of laminae of young, often infertile thalli are reported. The methacrylate embedded, light microscope observations have been made on a variety of aged thalli from both zones. The emphasis of ultrastructural studies on transition and expansion zones of prefertile thalli was to insure that meristematically active material was used. This is important since it has been observed that vegetative lamina elongation may be slowed to such an extent that sorus differentiation may proceed up to the transition zone. This is most often observed during August. The reason for extending this study to include the expansion and transition zones was to ascertain the nature of the meristoderm which precedes sorus differentiation. The second approach was carried out in a comparative manner. Meristoderm adjacent to the sori of various ages was examined to define the context in which both sorus development and abscission occur. In light of the lack of information on meristematic tissues of the Laminariales (Davies, Ferrier and Johnson 1973, Penny personal communication) the observations from the expansion and transition zones are discussed first in an attempt to characterize this tissue. This is followed by a definition of the various cytomorphogenetic processes occurring from the
transition of juvenile thalli to the vegetative meristoderm cells adjacent to the oldest sori of reproductively mature thalli. This can not be a comprehensive description and must stand as a brief but realistic outline of major cytological trends evidenced in the meristoderm of *Nereocystis* laminae in both a temporal and linear sense.

3.1. Cytological Characteristics of Meristoderm in Transition and Expansion Zones

**Nuclei**

Large numbers of chromatin masses and well developed nucleoli with distinct *pars granulosa* and *pars fibrosa* are interpreted as indicative of an active nucleus. The former condition has been observed in *Laminaria* haptera meristoderm (Davies, Farrier and Johnston 1973) and in other dividing brown algal cells (Cole 1969, Neushul and Dahl 1972b). The latter nuclear condition has been observed in *Laminaria* lamina meristoderm (Davies, Farrier and Johnston 1973) and *Zonaria* apical meristoderm cells as well (Neushul and Dahl 1972b). Although evidence of mitotic activity is present for both zones the observation of numerous binucleate cells, cf irregularly shaped nuclei; of irregularly distributed dictyosomes and the
frequent observations of partially formed basal periclinal walls in cells of the expansion zone is interpreted as indicative of a higher mitotic activity. The irregular distribution of dictyosomes is the result of their association with the pairs of centrioles which migrate to the apical and basal poles prior to mitosis. This behavior has been reported in some brown algae (Loiseaux 1973, Chi and Neushul 1972, Markey and Wilce 1975, 1976a and b). Evidence for an irregular distribution of mitoses on the surface of the laminae comes from the abundance of new anticlinal cell walls at the lips of the primitive furrows.

Golgi apparatus

The golgi apparatus consisting of several dictyosomes invariably associated with the nuclear envelope is similar to what has been observed in other members of the Laminariales (Bouck 1965, Chi and Neushul 1972, Gheradini and North 1972, Toth 1974, Evans, Simpson and Callow 1973, Schnepf 1963, Davies, Farrier and Johnston 1973). This is apparently a characteristic for many orders of the Phaeophyta (Scagel 1966, Russell 1973). Only in the Sphacelariales (Galatis, Katsaros and Mitrakos 1977), Dictyotales (Neushul and Dahl 1972ab) and Fucales (Rawlence 1973, Brawley, Quatrano and Wetherbee 1977, Pellegrini 1976 and Berkaloff 1963) are the dictyosomes not exclusively associated with the nuclear envelope. The
biphasic morphology of the dictyosomes which apparently correlates with the mitotic cycle is of particular interest. Although this degree of dictyosome morphological variation was described in the hapteron meristoderm of Laminaria (Davies, Farrier and Johnston 1973) the relationship to the mitotic cycle was not recognized. Furthermore there was no mention of the abundance of small vesicles associated with the maturing face of the non-hypertrophied dictyosomes as observed here. A lack of dictyosomal hypertrophy has been correlated with both quiescence and drugged conditions in cells of Zonaria from the Dictyotales (Neushul and Dahl 1972b, Walker, Chi and Neushul 1975). Less numerous and relatively inactive dictyosomes have been associated with the cells of the quiescent zone of maize root meristems as well (Clowes 1964). In a similar vein Pellegrini (1976) reports that dicytosome cisternae of the apical cell and basal derivatives in the promeristem of Cystosiera also show significantly less hypertrophy than in the neighboring new meristoderm cells or lateral derivatives of the apical cell which are being transformed into new epidermal elements. Rawlence (1973) notes that small dictyosome vesicles contribute to forming cell plates in Ascophyllum, another member of the Fucales, and that later larger hypertrophied dictyosome vesicles contribute to the newly formed periclinal walls. The latter has been noted also in Fucus (Brawley, Quatrano and Wetherbee 1977) and may account for the continued hypertrophy of dictyosomes of the internal daughter cells of the epidermal cell divisions reported by
Pellegrini (1976) in *Cystosiera*. The report here of numerous small vesicles associated with dictyosomes and the developing cell plates both here and in the internal meristem cells of the cortex of *Laminaria* (Penny, personal communication) provide strong evidence that the non-hypertrophied morphology of the golgi apparatus of these cells does not constitute a quiescent morphology but a phase of cell plate synthesis. This interpretation immediately suggests that a reassessment of observations such as those of Pellegrini (1976) may be in order. The hypertrophied dictyosome morphology has long been associated with secretion in the mucilage duct system (Schnepf 1963) and in zoosporogenesis of the Laminariales (Chi and Neushul 1972, Chi 1973, Toth 1974). This is true for other phenomena of other members of the Phaeophyta as well, Fucales (McCully 1968, Pellegrini 1976, Evans and Callow 1973) Ectocarpales (Baker and Evans 1973ab, Loiseaux 1973, Markey and Wilce 1975, 1976ab, Lofthouse and Capon 1975), the Dictyotales (Evans and Callow 1976, Neushul and Dahl 1972a). Thus it is concluded that within the individual meristoderm cells of both the transition and expansion zones of *Nereocystis* the golgi apparatus is continually undergoing a qualitative, not quantitative alternating shift in function between secretion to the outside and internal secretion into cell plate formation with the cell cycle.

Chloroplasts
In contrast to other tissues, chloroplasts of these cells are distinctive not only by virtue of their small size (2-3 µm in length), but also in their paucity of central stacks of thylakoids (only 2 to 3). Indeed they are not unlike chloroplasts of other brown algal meristematic tissues (Berkaloff 1963, Neushul and Dahl 1972a, Evans and Holligan 1972, and Pellegrini 1976). The chloroplast endoplasmic reticulum, double membrane envelope, peripheral and central thylakoids and ring genophore are typical of brown algae (Bouck 1965, Bisalputra and Bisalputra 1970, Scagel 1966, Russell 1973). The presence of small pores of unknown function in the thylakoids first observed by Oliveira and Bisalputra (1977b) is confirmed here. The observation here of immature very small plastoglobuli in the chloroplast matrix not associated with the thylakoids differs from observations of other authors in members of the Dictyotales (Evans and Holligan 1972b). Furthermore, the description of pyrenoids here confirms the argument for their presence in the Laminariales at least at specific times (Chi 1971, Chi 1973). It is noteworthy that in accord with the general organization of the brown algal cell (Bouck 1965, Scagel 1966, Russell 1973) the chloroplast is closely linked with the nuclear envelope through the extensions of and continuities with the ER and chloroplast ER. Based on their small size, few thylakoids and minute plastoglobuli it is presumed that these chloroplasts most probably cannot support the level of synthetic activity indicated for these cells. In
fact it has been suggested, based on radioisotope data, that for members of this order the meristems of *Macrocystis* and probably *Nereocystis* are photosynthate sinks (Schmits and Srivastava 1974b, Lobban 1978ab and personal communication). The proposed route of active transport through cells of the inner cortex or outer medulla has been suggested for *Nereocystis* (Nicholson and Briggs 1972) and other members of the Laminariales (Schmitz and Lobban 1976). From the observations made with light microscopy (Smith 1939) and here with electron microscopy and pit fields and plasmodesmata are present in appropriate walls of both meristoderm and cortical cells to accommodate such a transport.

All ER elements illustrated in the generalized brown algal cell of Fouck (1965) and updated by Russell (1973), are present in the cells of these two regions of the meristoderm. Clear connections have been observed in *Nereocystis* between the nuclear envelope and portions of both the chloroplast and cytoplasmic ER. Two additional types of ER have been observed in these cells. First there is the peripheral system of tubular and cisternal ER which lies approximately parallel to the plasma membrane and which frequently interconnects the chloroplast ER of adjacent chloroplasts. A similar organization has been noticed during a phase of zoosporogenesis in *Macrocystis* (Chi and Neushul 1972, Chi 1973) and in some red algae (Evans, Callow, Percival and Fareed 1974). Evans (1974) has proposed some role in supply to the plasma membrane and a
similar hypothesis has been proposed for certain vascular plant cell systems (Morre and van der Woude 1974) Addicott personal communication) as a mode of supporting cell wall growth other than through the golgi apparatus. The prominence of this system in cells of the expansion zone and older tissues somewhat supports this interpretation. However, a role as additional link in the suggested nuclear envelope, ER, golgi system of Bouck (1965) seems equally probable at this time. The second new component of the endomembrane complex is the cisternal membranes which delimit the variously shaped regions of homogeneous cytoplasm which is devoid of ribosomes and other organelles (Section 2.1. Fig. 20) found particularly in the basal cytoplasm of meristoderm cells which have not recently undergone periclinal divisions. These areas are definitely not daughter nuclei (compare Fig. 20 section 2.1. with 11, 14 and 15 of section 2.2.) for they contain no heterochromatin or nucleoli. Small but similar regions have been observed in the invaginations of cup shaped mitochondria which are particularly common in these cells (Figs. 13, 21 and 27, section 2.2.). With this and other evidence it is concluded that these represent the initiation of the vacuole and are equivalent to so called pro-vacuolar regions (Fig. 20, section 2.1.) as reported by Khera and Tilney-Bassett (1976) and Goff (1979).

Mitochondria
The mitochondria of these meristoderm regions are of two general morphologies. One most common in the transition zone and older tissues, is that of an irregularly elongate tubular morphology. The other is that of a cup morphology which is most common in meristoderm cells of the expansion zone.

The former morphology although represented by numerous profiles is observed, through serial sectioning, to consist of few individual mitochondria. This morphology is closest to that generally indicated in numerous brown algal cells (Russel 1973 for review).

The latter cup morphology has been previously identified for only two other brown algae (Bisalputra 1966, meristoderm cells of *Egregia*; Walker, Chi and Neushul 1975, phenobarbital treated meristematic cells of *Zonaria*). In subsequent discussion this will be linked to the process of vacuolation.

These observations provoke both an interest in morphological variability and intracellular distribution of brown algal mitochondria. Only McCully (1968) and Rawlence (1973) have illustrated clearly peculiar intracellular distributions of brown algal mitochondria. Fibrillar inclusions have been noted within cristae of fucoid spermatozoa (Pollock and Cassell 1977) and Laminariales meiospores (Chi 1973). It would seem valuable therefore in the future to apply serial sectioning to these cells in order to determine the number and precise distribution of mitochondria in them.
Other Cytoplasmic Inclusions

The consistent occurrence of a small number of microbodies in the cells of both the transition and expansion zone, as well as in all subsequent stages of meristoderm cells suggests their consistent role in the metabolism of these cells. Subsequent variations in older cells suggest that these organelles should be investigated using enzyme localization techniques.

Two features of the cytoplasm, the apical vesicles and the relatively large proportion of ground cytoplasm, set these cells off from the older subsequent meristoderm cells. The origin of these large vesicles, judging from their membrane thickness, and general morphology can be attributed to the golgi apparatus. The tendency to interdigitate which is also observed in dictyosome cisternae (Fig. 18 and 19 section 2.2.) perhaps explains the variety of membrane complexes observed in the meristoderm cells of both regions. It is not clear at this time whether these represent golgi vesicles which have released their contents through exocytosis and returned to the cytoplasm or those which have yet to undergo exocytosis. The numerous small cavitations of the most apical portion of the plasma membrane and the recurrent phases of golgi hypertrophy are classical signs of active localized secretion (Whaley 1975, review). Outer cell wall behavior and appearance would support this interpretation as will be discussed subsequently. Although the contents of these vesicles are electron translucent the carbohydrate secretory contents may not have
been well preserved. This is supported by the light microscope histological localization of both acid and sulfated polysaccharide material. It is also supported by other histological studies of cells with active golgi apparati as well as of surface cells (Schnepf 1963, McCully 1968, Evans and Hcolligan 1972a, Evans and Callow 1973). Another possibility is that some of these vesicles represent phenolics or physode storage vesicles which may be difficult to preserve well (Evans and Holligan 1972b). The blue-green TBO staining granules of these cells imply the presence of some phenolic compounds (McCully 1968), however, their scarcity in these cells is closer in number to the osmiophilic droplets frequently observed here with TEM. This material is morphologically indistinguishable from the proposed phenolic material demonstrated in the meristematic cells of Dictyota by Evans and Hcolligan (1972b).

Cell Walls and Tissues

Having discussed the intracellular constituents it is now possible to consider the walls of these meristoderm cells and comment on the histology of this tissue in general. The following discussion will explain the observation of this author and others who have dealt with the meristoderm in this region of Nereocystis and other Laminariales (Whyte personal communication, Kaneko personal communication) that this tissue
is in fact frail in juvenile thalli and is difficult to preserve.

It is important first to consider the biochemical work of Whyte and Englar (1975) on the laminae of *Nereocystis*. He reported that the cell walls of the mature laminae of *Nereocystis* contain a beta one four linked glucan polymer which accounts for 27.8% of the dry lamina; an acid polysaccharide, presumably alginic acid which constitutes approximately 19% of the dry lamina; and a sulfated polysaccharide fraction "fucoidan", 2.8% of dry lamina, which contains a mixture of several sugars, fucose 38%, mannose 32%, galactose 20%, glucose 5%, arabinose 3% and xylose 2% (Whyte and Englar 1975). A protein cell wall fraction has also been identified in these studies. The beta one, four glucan or cellulosic component could account for the fibrillar component of these cell walls. That this material is present in the cell walls has been histologically corroborated by the use of sulfuric acid IKI staining (Jensen 1962) for cellulose. A positive response or blue staining is observed in the cell walls of *Nereocystis*. The presence of acid polysaccharides or alginic acid component of these cell walls is also confirmed with histological techniques. There is general staining of these cell walls with AB/SA for acid polysaccharides and this is also indicated by metachromasia with TBO. From the chemical properties of alginic acid it is possible to assume that this component could exist in two physical states depending upon whether it were
present as either a calcium alginate or a sodium or potassium alginate since alginic acid may behave as a gel in the presence of calcium (Mackie and Preston 1974, review). Furthermore, the fluidity of this polymer may well be affected by the ratio of polymannuronic to polyguluronic acid constituents (Mackie and Preston for review, 1974). It is thus conceivable that the alginic acid may function as a matrix surrounding the cellulosic fibrillar material as well as being perhaps fibrillar itself in light of its birefringence when sufficient calcium is present (Mackie and Preston 1974). Finally we must consider the sulfated polysaccharide component of mixed sugar moieties. It is most likely, in light of the diversity of constituent sugars in this fraction that this is at best a matrix material and most probably a transient component of the cell walls of *Nereocystis*. Sulfate metabolism has been clearly associated with the golgi apparatus and its synthetic processes in numerous animal systems (Young 1973, Whaley 1975 for review) as well as in brown algae (Evans and Holligan 1972a, Evans, Simpson and Callow 1973, Evans and Callow 1978).

Turning then to the walls of these meristoderm cells, the structural differences between the outer periclinal walls and the internal walls become very interesting. The biphasic nature of the wall, is also observed for the outer cell wall of *Laminaria* haptera meristoderm cells (Davies, Farrier and Johnston 1973). However, it is noteworthy that these authors report a cuticle like layer as defined by Hanic and Craigie.
(1969) to be present on haptera grown in quiet waters, presumably low water motion conditions (Davies, Farrier and Johnston 1973). Although it is possible to argue that such a cuticle like layer existed for meristoderm cells here, its absence in the deep, protected primitive furrows raises doubts as to its existence. Perhaps as seen here it is a characteristic of more mature tissues only. This basic bi-phasic nature of the cell wall has also been noted at the ultrastructural level for cells of Zonaria (Neushul and Dahl 1972b) where no outer cuticle is observed as well. This outer layer of electron dense material is presumed to correspond with the outer light microscope layer which stains densely for acid polysaccharides with AB/So as well as metachromatically with TBO. This dense region in favorable sections is also stained for sulfated polysaccharides at its outermost limits with AB/So. It should be noted here that the staining for sulfated polysaccharides especially in the internal cell walls shows variability. One explanation might be is that the AB/So technique is not consistent for the sulfated polysaccharides in Nereocystis. However, this technique has been applied by this author to Fucus tissues prepared in the same manner and the staining patterns observed here agreed with the distribution of sulfated polysaccharides described in Fucus by McCully (1965 and 1966). Furthermore, it has been learned that the sulfated polysaccharide components may bleed from fixed tissues being held for any length of time (Vreeland, personal communication).
Thus it is tentatively concluded here that the outer layer of electron dense material here labeled layer 4 represents primarily acid polysaccharide. This is not to exclude the possibility that this material may contain a sulfated polysaccharide component either as a static or transient constituent. It is also concluded that the inner fibrillar portion of outer wall consisting of the layers 1, 2 and 3 primarily represents three relatively distinct regions of cellulose orientation in the cell wall. Again this is not to exclude the possibility that this phase does not contain some alginic acid either in a gel or sol. It is conceivable that sulfated polysaccharides may be present in these inner fibrillar layers as a transient component as well.

The inner fibrillar phase deserves particular attention here because of its apparent instability and the distinctness of the three layers. Maser, O'Brien and McCully (1967) have presented evidence suggesting that the distinct layering seen here, may be caused by differences in microfibrillar orientation in cell walls. Layer 1, the innermost, is characterized by fibrils running parallel to the plasma membrane. Furthermore, the fibrils of this layer are continuous with similarly oriented fibrils of the anticlinal cell walls. Layer 2 is a layer of microfibrils with a mixed orientation from parallel to perpendicular. Layer 3 microfibrils are primarily oriented perpendicular to the free
surface of these cells below layer 4. The significance of these varicus layers can only be dealt with in a developmental context and therefore is considered with the subsequent two tissue sections later. However, it is possible to speak at this point about the origins of the outer non-fibrillar phase. These cells evidence a large amount of golgi activity in that the cisternae are hypertrophied and sulfated polysaccharides may be in evidence in the outer layer of the cell wall. Meristoderm cells of *Fucus* secreting sulfated polysaccharides show such morphology (McCully 1968, Evans, Simpson and Callow 1973, ). Evans, Simpson and Callow (1973) suggest that the mucilage duct sytem of *Laminaria* is the site of sulfated polysaccharide synthesis. They believe the sulfated polysaccharides are loaded into the mucilage ducts and then bleed through the anticlinal walls to the surface of the lamina as suggested by Guignard (1882) for the points identified now as potential venting points (Walker and Bisalputra 1977). However, sulfated polysaccharides are present both in the cytoplasm and on the surface of *Nereocystis* tissue before the inception of the mucilage duct system begins. Furthermore, autoradiographic studies of Evans, Simpson and Callow (1973) do not include controls for the possibility that their localization of sulfate fixation is not a wound response and in their microbeam analysis (Evans and Callow 1976) they do not take readings directly over the top of an epidermal cell to compare with over an anticlinal cell wall. Thus the probability that surface cells may produce sulfated
polysaccharides has not been eliminated. Leppard (1974) has
also implicated the endomembrane system of the brown algae in
acid polysaccharide synthesis and movement. Thus it is
concluded here that the high golgi activity here is directed
towards the production of the amorphous acid polysaccharides as
well as a sulfated polysaccharide component secreted at the
free surface and accumulated to some degree outside the third
fibrillar layer. This outer layer is assumed to be somewhat
transient as it seems to erode at the lamina surface while it
persists in the deep clefts of the primitive furrows.
Presumably the acid polysaccharide component or alginic acid is
in a more fluid, perhaps sodium or potassium alginate form.
It should be noted that the mucilage duct system is filled with
a mass of non-birefringent alginic acid and sulfated
polysaccharides and that the cell wall across which secretion of
these polysaccharides occurs, also shows a loose unstable
organization (Chapter 2).

The anticlinal and internal periclinal cell walls are all
similar in morphology with their parallel microfibrils. They
are very thin and middle lamellae are not easily distinguished.
At the corners where cells meet they are essentially so thin
that intercellular spaces are present. From histochemical
evidence these walls are primarily cellulosic and acid
polysaccharide. The lack of a visible middle lamella may
contribute to the instability of the tissue as well as the thin
walls. From the patterns of tissue separation during
dissolution (Fig. 2, Section 2.1.) and from ultrastructural observations it is apparent that the numerous primary pit fields of plasmodesmata serve as a cohesive as well as communicative devices in this young tissue.

3.2. Characteristics of Meristoderm Cells Adjacent to the Youngest Scri

Histology

The sharp contrast in cell size between surface meristoderm cells and subtending cortical cells and the relatively thick anticlinal meristoderm cell walls (compare Fig. 1 Section 2.3. with 1 of Section 2.1. and 1 of Section 2.2.) is best explained by a sequential cessation of meristoderm mitotic activity. Indeed if periclinal divisions ceased while meristodermal anticlinal divisions and lateral enlargement of both cortical and meristoderm cells continued, the appearance of this tissue makes sense. The relatively thick anticlinal walls of these meristoderm cells is evidence for the subsequent cessation of anticlinal divisions as well. The occasional meristoderm cell which has divided periclinally but does not show enlargement of the internal progeny cell is interpreted as resulting from its nearness to the sorus. Thus
it would appear that meristematic activity, periclinal and anticlinal divisions, has ceased in stages, first periclinal divisions and meristoderm cell elongation, then anticlinal divisions eventually followed by lateral cell enlargement and finally wall deposition.

Nuclei

The nuclei are no longer morphologically dominant but are crowded and often distorted by the prominent chloroplasts. The observation of a homogeneous nucleoplasm and smaller nucleoli reinforces this and agrees with previous observations from mature tissues in other genera of the Laminariales (Bouck 1965, Bisalputra 1966, Chi 1973 and Evans, Simpson and Callow 1973) as well as the epidermal cells of *Fucus* (McCully 1968) and *Ascophyllum* (Rawlence 1973). The extremely homogeneous fibrillar regions previously mentioned are present in this aged tissue as well. Only the nuclear envelope/endomembrane system association needs to be discussed here. The relationship between nuclear envelope and the perinuclear dictyosomes of the golgi apparatus is characterized by the outer nuclear membrane producing vesicles and contributing them to the formative faces of adjacent dictyosomes as in the other brown algae (Bouck 1965, Scagel 1966, Russell 1973). These nuclei are interesting since nuclear envelope continuities with the ER or the chloroplast ER (Gibbs 1962, Bouck 1965, Scagel 1966, Russel
1973) are difficult to observe in contrast to previous sections. The apparent scarcity of such continuities has also been reported for thallus and sporangial cells of Phaeostrophion irregulare by Bourne and Cole (1968). This is believed to suggest that there has been a distinct shift in the role of the nucleus in the endomembrane system.

Golgi Apparatus

Although there is no reduction in number of dictyosomes, hypertrophy of their cisternae is now only moderate and coated vesicles are no longer observed. With the obvious lack of mitotic activity, this shift in golgi apparatus morphology cannot be explained by cell plate formation. Yet some secretory function continues as is evidenced by the presence of reticulate material in the vesicles adjacent to the dictyosomes, in the apical cytoplasm and in the intramural space (Whaley 1978, Brinkley and Porter 1977). The observation of complete hypertrophy of fenestrate maturing face dictyosome cisternae is most attractive as a possible origin of the multivesiculate vesicles observed often in the basal vacuolar region. The internal vesicles and tubules are apparent products of the elongation of cisternal fenestrae into tubules in partly hypertrophied cisternae and ultimately vesicles are formed by their fragmentation in the more hypertrophied cisternae (Fig. 14 Sec. 2.1.). The numerous
vesicles, many clearly of golgi origin, in the apical cytoplasm strongly suggest a polarity of golgi secretion to the outer surface. This is supported by similar observations in the epidermal cells of Fucus (McCulley 1968) and Cystosiera (Pellegrini 1976) of the Fucales.

Chloroplasts

The chloroplasts are the dominant organelle. They are characterized by their seven to ten central stacks of three thylakoids, frequent plastoglobuli, peripheral genophores and lack of pyrenoids. They are very similar to chloroplasts of other mature vegetative brown algal cells (Bouck 1965, Bourne and Cole 1968, Cole and Lin 1968, Cole 1969, Cole 1970, Oliveira and Bisalputra 1977b). The chloroplasts are assumed then to be photosynthetically mature. They possess the usual chloroplast envelope and chloroplast endoplasmic reticulum, but they are of particular interest in that they are usually associated with membranous configurations of various sizes which are contained in the chloroplast ER. It should be recalled that less frequently occurring but identical structures have been observed in the chloroplast ER in the expansion zone (Sec. 2.2., Fig. 25). Essentially identical or similar structures have been observed in chloroplasts of Ectocarpus (Oliveira and Bisalputra 1977b) and Leathesia (Cole and Lin 1968), Cole, Bourne and Lin 1968). In the former case
these structures have been proposed to represent a form of recycling of chloroplast materials while in the latter it has been associated with the process of chloroplast division. The confinement of these structures to the lumen of the chloroplast endoplasmic reticulum and regions of vacuolation are interpreted as closer to the situation observed for *Ectocarpus*.

**Endomembrane system and Vacuolation**

The endomembrane system of these cells as it relates to the nuclear envelope and the provacuolar region is of particular interest. The nuclear envelope/golgi relationship remains intact but direct luminal continuity of the nuclear envelope with the chloroplast ER so obvious in the previous stages is no longer detectable. Although Baker and Evans (1973) noted the latter, apparent discontinuity the nuclear envelope and portions of the endoplasmic reticulum remained linked. Bourne and Cole (1968) have also noted that vegetative and sporangial cells of *Phaeostrophion irregulare* lack direct chloroplast endoplasmic reticulum/nuclear envelope continuities and no interconnecting channels of endoplasmic reticulum were described. However, without a developmental context as suggested by Bourne and Cole (1968) it is difficult to compare such observations with what has been reported here, particularly with the differences in thallus differentiation.
between *P. irregulare* and *Nereocystis*. It is possible that either proximity of chloroplast ER to nuclear envelope or brief intermittent continuities unobserved suffice to satisfy the needs for the secretory processes of these cells. In light of the photosynthetic maturity of this tissue, the lack of mitotic activity and the presumed role as both the primary producers and exporters of photosynthates to the medulary tissues (Fritsch 1945, Nicholson and Briggs 1972, Schmitz and Srivastava 1974ab, Lobban 1978) it is perhaps not surprising that its own endomembrane/secretory system is not outstanding. This does not mean that there is little evidence of metabolic activities.

The provacuolar regions described in the basal cytoplasm of cells in the transition and expansion zones persist. From the observations of Oliveira and Bisalputra (1977b) it is demonstrated that in *Ectocarpus* the chloroplast membrane configurations are derived from the thylakoids and ultimately become incorporated in the vacuole. Although clear origin from the thylakoids and separation from the chloroplast were demonstrated the actual mechanism of transfer to the vacuole remains unclear. From the observations reported here it is clear that such membrane configurations, although not clearly of thylakoid origin, are formed within the chloroplast ER and appear in the hypertrophied ER of the provacuolar regions. Therefore, it would seem that the observations of Oliveira and Bisalputra (1977b) are both confirmed and extended. This in
addition to hypertrophy of the provacuolar ER with granular material and the apparent invasion of occasional golgi vesicles defines the process of vacuolation in these meristoderm cells. With this understanding it is possible to explain the vacuolate regions in more mature tissues. Vacuolation begins in the mitotically active transition and expansion zone with the delimiting of a basal portion of cytoplasm by a fenestrate sphere of ER. It is suggested that mitochondria are directly involved as is evidenced by the interfacing of smaller regions of fenestrate ER with cup shaped mitochondria. It is significant to note that Bisalputra (1966) also found cup shaped mitochondria in the meristoderm cells of *Egregia*. Furthermore, Walker, Chi and Neushul (1975) reported strikingly similar mitochondrial/ER relationships to occur with great frequency in *Zonaria farlowii* apical meristoderm cells treated with phenobarbital. This is important for two reasons. The most obvious is that phenobarbital treatment arrested meristematic activity and it is well known that the process of differentiation of plant cells from the meristematic to vegetative condition usually entails vacuolation. This is further supported by the observation that cup shaped mitochondria are present in the *Zonaria* meristoderm cells prior to phenobarbital treatment although at a significantly lower frequency. This is in accord with the observation of a zone of vacuolation in the basal portion of normal *Zonaria* meristem cells (Neushul and Dahl 1972a). There is a lack of agreement amongst botanists as to the precise mode of provacuole
formation in plants. Arguments range from a plasma membrane origin to a golgi origin or an ER origin (see reviews of Matile 1975, and Khera and Tilney-Bassett 1976). Oliveira and Bisalputra (1977a) have proposed that vacuole initiation may be principally achieved in *Ectocarpus* by the fusion of provacuolar elements derived from the ER. The observations presented here seem to support a mode of vacuolation similar to that reported by Khera and Tilney-Bassett (1976). At this time it is not possible to state just how vacuolation occurs in the subtending cortical elements, but work in that direction is in progress. Furthermore, differences between what has been observed here and the observations of Khera and Tilney-Bassett (1976) in *Pelargonium* meristematic tissues may be due to the phylogenetic distance of these two organisms. Observations from meristoderm cells adjacent to the youngest sori demonstrate that hypertrophy in regions of the spherically delimiting fenestrate ER of the provacuolar region is all that is required to produce the vacuoles of these cells. In the early stages illustrated here hypertrophy can be achieved by the arrival of membrane configuration masses (Fig. 15 and 16, Sec. 2.3.), by means of chloroplast ER/ER continuities, or the accumulation of granular materials as observed (Fig. 12 Sec. 2.3.). The final factor in vacuolation of these cells is the apparent ability of golgi vesicles to invade the provacuolar region. This will also further the interpretation of the more complex vacuole morphologies of the next developmental stage.
Mitochondria

Mitochondrial morphology has resumed a rod-shaped morphology as evidenced by elongated and circular profiles throughout the cytoplasm. The degree of development of cristae attest to the continued active nature of these organelles. The absence of the cup-shaped mitochondria is indicative of the cessation of mitotic activity and its concomitant necessity for vacuolar reformation in these cells. McCully (1968) with Fucus as well as Galatis, Katsaros and Mitrakos (1977) with Sphacelaria have related the peculiar distributions of mitochondria and plasma membrane invaginations with active transport from the environment. Although the apical plasma membrane of these cells is certainly convoluted, there is no evidence of a mitochondrial apical cap nor of the repeated associations of plasma membrane and mitochondria. It is likely, however, that these cells may be involved in the uptake of material from the environment (Evans, Simpson and Callow 1973, Schmitz and Srivastava 1974ab, Nicholson and Briggs 1972). Perhaps their smaller size and greater numbers makes such conditions as found in Sphacelaria (Galatis, Katsaros and Mitrakos 1977) unnecessary.

Microbody-like structures and other Inclusions

Microbodies are still present and prominent. However, without cytochemical enzymatic localizations little can be said
of their function at this time.

The outer periclinal wall

Although there are no new constituents in the outer periclinal walls of these cells there are significant organizational changes from previous two regions. The number of distinct layers of the cell wall in the previous two stages of development, four, are now reduced to three. Layers two and three of the previous stages (Sections 2.1. and 2.2.) are now indistinguishable by virtue of their compactness and are henceforth referred to as layer 2/3. Layer one of parallel fibers has become thickened and more distinct while the increased thickness of layer four and the more horizontally fibrillar nature of its constituents are distinct. There is little doubt that the layering in this outer wall becomes more stable.

Anticlinal and Internal Periclinal Cell Walls

As with layer 1 of the outer wall, anticlinal cell walls have thickened and their fibrils are oriented parallel to the plasma membrane. The evenness of thickness implies that outer wall layer I and the anticlinal walls have grown uniformly about the cells. The only inequities occur at pit fields. The extracellular intramural space here is interpreted as an
artifact both in these cells and those of the previous sections except for one region. The intramural space about the plasmodesmata of new walls is believed to be essential for the formation of pits. This portion of the plasma membrane surrounding plasmodesmatal continuities either does not contain cell wall microfibrillar producing enzymes or the physical separation of the plasma membrane in these areas from the primary wall makes thickening other than through secretory activity not possible or slower. It is proposed that the thickening of the anticlinal walls, internal periclinal walls and cell wall layer 1 of the outer wall is the result of activities of enzyme systems on the plasma membrane and that the golgi activity, now reduced and primarily apical in direction, is of a maintenance role. The absence of coated vesicles about the golgi apparatus of these cells makes it tempting to speculate that they may be involved in the process of keeping cell walls of the apical portions at least loose enough to allow for cellular enlargement similar to other plant systems such as pollen tubes and fungal hyphal tips (Cresti, Pacini, Ciampolini and Sarfatti 1977, Bowles and Northcote 1976, Gooday 1975, Kiermayer and Dobberstein 1973, Willison and Brown 1978, Morre and van der Woude 1974, review).

3.3. Characteristics of Meristoderm Cells Adjacent to the Oldest Sori
Anatomy

Morphologically these meristoderm cells do not show marked differences from those of the preceding section, except for the deepened simple pits in the walls and the more cuboidal shape. These phenomena can be explained by continued cell wall thickening and further enlargement of the internal subtending cortical cells. However, mitoses in the meristoderm have ceased. Even if small progeny cells subtend the meristoderm cells, they are as vacuolate as the rest without any ultrastructural evidence of meristematic activity. Although cells of this stage can clearly be put into two types it would seem that this is primarily due to differences in golgi apparatus function and in other respects type one and two are in fact morphologically very similar and distinctly set apart from cells of the previous section.

Nuclei

The nuclei of both types of these meristoderm cells show no significant differences from each other. It is of interest to note the presence of centrioles adjacent to these nuclei of presumably fully differentiated and determinant cells. This lends support to the view that centrioles are maintained throughout any cell's existence and do not re-assemble de novo with need, either for mitoses or meioses as some have suggested (Toth 1974, Markey and Wilce 1976a). The nuclei of
type I and II cells are consistent in that luminal continuities between the nuclear envelopes and both their chloroplast ER and ER systems are clear and occur frequently as was seen earlier in the transition and expansion zones. It is interesting that these luminal continuities are present even though organelles are as crowded as in the preceding stage. Based on the model of Bouck (1965) and supporting evidence of others (Evans 1974, Leppard 1975) it seems safe to assume that there has been another shift in metabolic activities signaled by this establishment of nuclear envelope continuities with components of the ER systems.

Golgi apparatus

The golgi apparatus accounts for much of the distinction between type one and type two cells. In type one cells the apparent hypertrophy and concomitant intrusion of golgi derived vesicles into the vacuolar region stands in contrast to the smaller golgi derived vesicles of type two cells and certainly creates a more complex morphology in the vacuolar region. Furthermore, the flocculent contents of these type one golgi vesicles which come to crowd the apical cytoplasm where they secrete their contents to the free surface create a morphologically distinctive apical cytoplasm and most probably explain the disparity between flocculent layers of the outer
cell walls of type one and two meristoderm cells. The work of Evans and Callow (1976), Evans, Callow and Coughlan (1977), Hogsett and Quatrano 1977) with brown algal secretory processes strongly points both biochemically and morphologically to a transient sulfated polysaccharide which is derived in the golgi apparatus and crosses the cell wall to dissipate from the surface. The histological and morphological observations made here would support this observation as well. Therefore, it is supposed that type two cells represent cells whose outer exfoliating layer of presumed sulfated polysaccharides has undergone a marked reduction. The interdigitation of golgi vesicles and vacuolar components is interesting in light of the argument for such a relationship in higher plants from physiological and biochemical data as a mode of indirect influence of the vacuole upon other cellular compartments (Butcher, Wagner and Siegelman 1977). In spite of the differences between these two cell types the dictyosomes themselves are very much alike in their possession of few cisternae and diminutive sizes. In this respect they stand out from all three previous sections. Even in the more active type one cells not only do cisternal numbers per dictyosome differ but coated vesicles are not associated with these type one meristoderm cell dictyosomes.

Chloroplasts
The chloroplasts of both type one and two cells remain dominant inclusions of the cytoplasm as they did in the previous section. Two features stand out consistently in both type one and type two cells. The first is luminal continuities of CER with both ER and nuclear envelope specifically at the loci of the numerous pyrenoids which constitute the second feature. It is conceivable that the absence of a pyrenoid sack as described by Bouck (1965) and Evans (1966 and 1968) is compensated for here by the invariable presence of these specific continuities with the nuclear envelope and/or ER. It is finally of interest to note that the membrane configurations so commonly included in the chloroplast ER of the previous section are no longer present or reduced here.

Endomembranes and Vacuolation

The chloroplast ER, nuclear envelope and some of the ER have already been discussed. It is clear now that in both types of these cells the chloroplast ER, ER, nuclear envelope, golgi system is as clearly discerned as it was in the transition and expansion zone cells. In addition this membrane complex includes the pyrenoids. Although differing quantitatively the golgi apparati of both cell types have been secretory and have been reconnected to the endomembrane system in a secretory role. This interpretation agrees with other
works dealing with components of this endomembrane system in other brown algae (Schnepf 1961, Leppard 1975, Evans and Callow 1976, Hogsett and Quatrano 1977). The nature of the secretory activity in the type one cells is believed to be directly related to the presence of the fine flocculent in the outermost layer of the outer cell wall as discussed already. The metachromasia of this flocculent material with TBO, suggests that this material represents either a sulfated or an acid polysaccharide component. The transient nature of this material is attested to by the significant reduction it undergoes at the cell surface in the type two meristoderm cells with their lesser golgi activity. This proposed golgi origin of the sulfated polysaccharide transient carbohydrate is supported by the work of Evans, Simpson and Callow (1973) in *Laminaria* and by Matthews, Evans and Callow (1976) in *Fucus*. The relationship of golgi function to fucose and polysaccharide synthesis and secretion in higher plants and in animal cells as well (Young 1973) is well established. It has been suggested that in these cells the vacuole now consists of the variously hypertrophied portions of ER as well as the complicating addition of numerous golgi vesicles destined for exocytosis. Thus the system is as complex as in the previous stage of development if not more so. In the case of the type two cells secretory activity and perhaps photosynthate export activity have decreased. The vacuolar system is more uniform yet it still does not consist of a simple tonoplast delimited lumen considered to be the case in most mature higher plant cells but
rather is a complex of interdigitating and adjacent membrane bound vesicles. It is noteworthy that there is apparently no longer a central region of homogeneous ground cytoplasm.

Mitochondria

The overall mitochondrial morphology present in the previous section is preserved here in both cell types, however, there may be a decline in cristae for the type two cells mitochondria suggesting an altered if not declining metabolic level.

Microbody-like structures and other Cytoplasmic Inclusions

Typical spherical single membrane bound microbody-like structures occur in the type one cells. In the type two cells another structure is in evidence. These structures are single membrane bound and contain a granular matrix, the variability in electron density, irregular shapes and the occasional presence of membranous configurations differs markedly from anything in the type one cells. Both their origins and function remain unknown. However, a possible relationship with the microbodies is worth pursuing in light of observations such as those of Heath (1977) with fungal microbody morphogenesis.
Cell Walls

The layer four of the cells in previous developmental stages is now well established and there is an additional outer layer of material which, as has been discussed, appears to consist of transient material by virtue of its abundance being correlated with golgi activity. The internal anticlinal and periclinal walls are if anything thicker which is interpreted as evidence of a continued deposition of parallel fibrils, most likely cellulosic in nature.

3.4. Summary of Cytomorphogenesies in the Meristoderm

In 1965 Bouck, based on information from previous works concerning the endomembrane system of members of the brown algae and his own, proposed a model for a generalized brown algal cell. This model was updated by Russell (1973). From the observations reported here and already discussed it is possible to construct a similar model for the meristoderm which will serve several purposes. First it may be used to compare and contrast with current models. Secondly it may provide a developmental context for and some thread of continuity to the phenomena observed in the remaining four chapters. The nucleus is still of central importance as it was in the original model (Bouck 1965). It begins as a mitotically active nucleus showing masses of dense chromatic material.
presumably prophase or telophase chromosomes and prominent nucleolus with distinctive granular and fibrillar zones. The nucleus continues to back and perhaps mediate dictyosome replication in accordance with the replication and segregation of the pairs of centrioles. Nuclear envelope continuities between chloroplast ER and ER are present and clear. The heretofore undescribed homogeneous fibrillar regions are present. Some of these features persist through the developmental stages of the meristoderm cells while others do not. With the transition from a meristematically active cell to a photosynthetically mature one the mitotic activity ceases and the nucleoplasm becomes more homogeneous, nucleoli become less prominent but persist as do the homogeneous fibrillar regions. Curiously, although the nuclei are more closely crowded by the other organelles the prominent nuclear envelope ceases to maintain the continuities with ER and chloroplast ER. Nuclear morphology is more regular and the golgi apparatus with occasional exceptions due to crowding comes to lie in the basal region of the peri-nuclear cytoplasm. In light of physiological data concerning the role of the meristoderm of kelps in providing photosynthates to the meristomatic sinks it has been suggested that this diminution of the nucleocentric endomembrane system may reflect an increased export rate for photosynthates to internal tissues. In the transition from these cells to those which are older and adjacent to the oldest sori there is little change in the contents of the nucleus. However, the key shift is in the nuclear envelope/endomembrane
association. The continuities between nuclear envelope, ER and the chloroplast ER become very distinct and in particular usually involve the chloroplast ER around the pyrenoids. This feature is equally clear in both types of these oldest meristoderm cells.

The organelle most closely related to the nucleus is the golgi apparatus which consists of at least a few dictyosomes. The strict perinuclear locus of this organelle persists throughout the duration of development examined. In the meristematically active cells of the transition and expansion zones it would appear that dictyosome replication and secretory activity is as closely tied to the cell cycle as are the centrioles. The dictyosomes begin with a biphasic secretory morphology believed here to correlate with the processes of cell plate formation and secretion at the apical cell walls of the meristoderm cells. This latter secretion both adds to and maintains the outer cell wall through the secretion. It is noteworthy as will be seen in subsequent chapters, that there are coated vesicles associated with the dictyosomes in the apically directed secretory activity phase. This will hold true in subsequent cases. In the transition from meristematic to mature lamina adjacent to the youngest sori the dictyosomes shift to a more modest apically directed secretory morphology, which interestingly correlates with the stabilization of the outer cell walls and tissues in general. Coated vesicles are no longer present. Much of the morphological differences
between the two types of the oldest meristoderm cells adjacent to the mature sori is attributed to the varied golgi apparatus morphology. In the one type golgi vesicles hypertrophy to invaginate and intermingle with vacuolar elements. Exocytosis is obvious and correlates with the presence of a finely flocculent outermost layer. This layer erodes with the markedly reduced activity of the second type of oldest meristoderm cells. Both the vacuolar morphology as well as that of the apical cytoplasm is somewhat simplified by the fewer and diminutive golgi vesicles.

The endomembrane system of these cells is centered around the nucleus as previously suggested. The peripheral system of ER, and the provacuolar membrane elements, observed here, add a new dimension to the model of Bouck (1965). The mode of origin of the provacuoles suggested by observations in the transition zone and expansion zone meristoderm cells is interesting in light of earlier reports of cup-shaped mitochondria (Bisalputra 1966, Walker, Chi and Neushul 1975) as well as mitochondrial ER associations in brown algae. It certainly deserves further consideration. Provacuolar systems such as that illustrated in these transition and expansion zone cells show how the morphological complexities of the basal cytoplasmic vacuolar regions develop. The localized hypertrophy of the cisternal elements into the central region and invagination of those elements by dictyosomes vesicles confuses the basic organization. ER continuities with the
Cisternal elements provide an easy route to the vacuolar regions of the membranous masses accumulating inside the chloroplast ER of the plastids of the meristoderm cells adjacent to the youngest sori. Cytochemical investigations of this system may prove to be enlightening. It would appear that this vacuolar system reaches a peak of morphological complexity about the time of the type one meristoderm cells adjacent to the oldest sori. It is believed that this may then shift into the state of the type two cells of this region simply through a general hypertrophy of the cisternal elements. Thus it is apparent that the endomembrane system of Bouck (1965) must be extended. It will be interesting to explore the fate of these provacuolar regions in the cortical initials as well. The peripheral system of ER may apparently interconnect with the chloroplasts which usually occupy a peripheral position in the cytoplasm. It is an attractive system in terms of its being an intracellular conducting system for supplying the plasma membrane with resources for the various cell surface phenomena and deserves further investigation.

The chloroplasts, though discrete organelles, are inseparable from the endomembrane system of all stages. Their increasing numbers of stacks of thylakoids is not surprising as is the increase in numbers of plastoglobuli (Lichtenthaler and Feveling 1967, Freeman, Platt-Aloia, Mudd and Thompson 1978). What is quite interesting, is the universal appearance of pyrenoids in the older meristoderm cells adjacent to the oldest
sori. This tendency is reinforced in other processes of the
meristoderm as will be seen in subsequent chapters. Although
mitochondria are universally present in the meristoderm cells
it is only in those cells of the transition zone and expansion
zone that morphology shows marked variation. It would be
immensely valuable to have more work done with these
meristematic cells, particularly with respect to a possible
mitochondrial involvement in provacuolar differentiation. In
a similar vein, the peak of microbody-like structure size and
numbers which occurs in the photosynthetically very active
meristoderm cells adjacent to the youngest sori deserves
careful cytochemical and biochemical work correlated with
similar data from other points in meristoderm activity such as
in the process of zoosporogenesis (Appendix 1).

Finally, the outer cell wall deserves some attention for
its nature and morphogeneses reflect intracellular activities
of the golgi apparatus. As a truly continuous structure which
in a sense transcends individual cellular limits, the outer
cell wall is particularly interesting. This wall is unstable
in the beginning which would seem a logical consequence of the
processes of both cellular enlargement and divisions. These
processes necessitate a delicate balance between rigidity and
extensibility. This balance is easily upset by the process of
tissue preservation. Immediate advantage of this unstable
outer cell wall is that continuous exfoliation probably retards
colonization and invasion by bacteria and epiphytes from these
developmentally critical tissues. The selective advantage of this protective mechanism is obvious.

With the cessation of divisions and the attainment of photosynthetic maturity, the outer cell wall is stabilized. At this time an additional outer layer of apparently golgi derived transient material appears and persists as long as the golgi activity continues. This functionally replaces the previous exfoliative nature of the outer cell wall. Furthermore, with the termination of divisions the innermost layer, layer one of the outer wall, which is continuous with the anticlinal walls, begins to thicken with them. In fact, this inner layer should not be considered a proper component of the outer cell wall. This view receives further support in subsequent chapters.

Thus, through time and space from the transition zone, the meristoderm generates new internal as well as more meristodermal tissues. With the cessation of mitoses and cell enlargement these cells shift to a primary producing role for both their own maintenance as well as exporting photosynthates to other tissues. This shift is reflected by discontinuities in the endomembrane system. This is followed by a temporary return to secretion and correlates with a resumption of the previous endomembrane continuities and with the appearance of a new outermost layer of the outer cell wall. Finally, the disappearance of the new outermost layer correlates with the cessation of golgi activity at the beginning of lamina
senescence.
IV CHAPTER 2. MUCILAGE DUCT SYSTEM

1. Introduction

Observations made during the course of examining the development of zoosporangial sori of *Nereocystis luetkeana* led to a re-examination of the mucilage duct system and its ontogeny. Sori which were releasing zoospores at the time of collection were characterized by a distinctly slimy texture. Mechanical agitation employed during methacrylate infiltration of the tissues resulted in disruptions of its organization and further release of mucilage. The mucilage duct system characteristic of some Laminariales has been fairly well defined anatomically and histologically (Fritsch 1945). However, in a few species its presence has been disputed (Kasahara 1973), and there is little information concerning its relationship to the zoosporangial sori. Since sorus inception is a phenomenon of the meristoderm in part (Guignard 1892, Skottsberg 1907), the relationship of the mucilage duct system to the lamina surface would be of particular interest. Even though the process of mucilage duct system inception has been in part assigned by most authorities to the meristoderm, there is disagreement as to the first events of its inception (Fritsch 1945). Guignard (1892) has designated duct lumen differentiation as the first event of inception and he assigned secretory cell differentiation to the cortex. However,
Skottsberg (1907) claims that secretory cells differentiate first in the meristoderm and that the duct lumen differentiates subsequently in the cortex. There is, to the authors, knowledge, only one report that the ducts of *Laminaria saccharina* do indeed open to the surface on the lamina (Evans, Simpson, and Callow 1973), but these opening points illustrated in the sorus are not discussed at any length. MacMillan (1899) published an extensive anatomical description of *Nereocystis* which essentially corroborates the views of Guignard (1892) concerning the mucilage duct system. He reported however, that the mucilage ducts of the lamina are derived from a periclinal division and schizogeny rather than anticlinal schizogeny. In an attempt to explain the field and laboratory observations indicated above light microscope findings of the mode of mucilage duct inception and differentiation in the lamina of *Nereocystis* are reported here and the mucilage duct system's ultimate relationship to the mature zoosporangial sori is illustrated. Finally the functional relationship between the sorus and the mucilage duct system and conflicts in the literature concerning inception are discussed.

2. Observations
2.1. Mucilage Duct Inception

Through light microscope investigations it is observed that the meristoderm cells in the transition zone of *Nereocystis* generate new cortical cells internally by periclinal divisions. In juvenile plants the mucilage duct system differentiates on either side of the transition zone between pneumatocyst and lamina. Transverse sections through the transition zone meristoderm on the lamina side showed a gradation of mucilage duct differentiation from apparent stages of initiation at the margin of the developing lamina to more mature stages at an approximate midline of the lamina (Fig. 1). In these tissues the perinuclear cytoplasm of the meristoderm cells stains densely and metachromatically with TBO indicating the presence of acid and/or sulfated polysaccharides (Fig. 2). The first recognizable morphological evidence of mucilage duct system differentiation is the appearance of enlarged inner progeny cells and a partial schizogeny of an adjacent anticlinal wall to produce the new duct lumen (Fig. 3), both of which are observed to correlate with a periclinal division of a meristoderm cell. In contrast to neighboring new cortical cells, these cells show a persistent metachromatic staining of the perinuclear cytoplasm with TBO (Fig. 3 and 4) as well as a dense staining of the cytoplasm for proteins with ABB comparable to the meristoderm cells (Fig. 7). One also sees profiles of small duct lumen with no associated enlarged cells.
Moving closer to the midline of the developing lamina apparently both the meristoderm progeny and the newly produced secretory progeny cell have divided again (Fig. 4), the former to produce a new cortical cell which borders the enlarged duct lumen and the latter to produce more secretory cells. Both the meristoderm and secretory progeny cells of the inception division continue to divide. The meristoderm progeny cell proceeds to produce cortical cells which border the expanding duct lumen as it keeps up with neighboring meristoderm cells (Fig. 5). The schizogeny of the anticlinal wall is also apparently continued as the proximity of the most superficial extent of the duct lumen adjacent to this meristoderm progeny cell remains quite constant (Fig. 3-5). The secretory progeny cell of the inception division continues to divide producing a cup-shaped group of secretory cells on the inner surface of the duct lumen (Fig. 4, 5 and 10). From the first appearances of duct lumen, traces of mucilage can be detected within (Fig. 4 and 5) or occasionally the lamina ducts are seen to be full (Fig. 6).

2.2. Mature Mucilage Ducts and Secretory Cells

Observations from sections taken in mature portions of the lamina and in the plane of the lamina revealed an anastomosing network of mucilage ducts (Fig. 8). This system is morphologically identical in both vegetative and fertile
portions of the lamina. In slightly oblique sections, the system is observed to extend to within one layer of the meristoderm (Fig. 9). From transverse and longitudinal sections it is apparent that the mucilage duct system is confined to the cortex of the lamina (Fig. 10-14 and 16). The ducts reach their greatest diameter in cross section at their deepest extensions in the cortex just above the cups of secretory cells (Fig. 11). In one instance the deepest secretory cells were seen to be located on the border of the medulla. Their diameter in sections appeared to be the smallest at their most superficial extensions, where they are unlined by secretory cells (Fig. 10). In essence three different types of morphology of the system were observed. The most common was that of the small unlined portions which approach the inner border of the meristoderm, either epidermal cells in vegetative regions or cells subtending paraphyses and zoosporangia in soral regions (Fig. 10, 13 and 14). Although these portions of the system are unlined they are easily distinguished by their metachromatically staining mucilaginous contents and often also by their shape and size. The types less frequently observed are the completely lined ducts (Fig. 12) or those with a cup-shaped profile of secretory cells lining the deepest inward extensions of the ducts (Fig. 11). The clusters of secretory cells may often become irregular in shape.
2.3. Secretory Cells

Ultrastructurally the secretory cells are not unlike those reported by previous authors (Schnepf 1963, Evans, Simpson and Callow 1973). Nuclei are polymorphic, presumably in accord with the presence of numerous perinuclear, prominent dictyosomes (Fig. 20). The NE, ER and CER clearly constitute a single luminal continuum (Fig. 20). Besides the small degree of vacuolation a few other features are of interest. Chloroplasts were small with few thylakoids as those of the transition and expansion zone meristoderm cells. The cell walls of the secretory cells facing duct lumen characteristically are structurally looser than those of the same cell facing adjacent cortical cells. The plasma membranes of these cells possess osmiophilic deposits far in excess of neighboring cortical cells. It is also curious that dictyosomes, although numerous and prominent, certainly do not match the cisternal hypertrophy seen in transition zone meristoderm cells.

2.4. Possible Duct Venting Points

Careful inspection of many sections of mature lamina in both vegetative and sorus regions revealed the presence of characteristic points where the lumen of the duct system is in
contact with the basal end of the anticlinal walls of either adjacent epidermal cells or of subtending cells of paraphyses and zoosporangia (Fig. 13 and 14). In the case of the sorus, which differentiates after the lamina has all but finished lateral expansion, only the lateral junctions or middle lamellae between adjacent subtending cells appeared to separate the lumen of the mucilage duct system from the extracellular lumen of the sorus (arrow, Fig. 14). With the preparation procedures used in this study these cell junctions are not disturbed (Fig. 14). In vegetative regions of the lamina both the middle lamellae and the outer walls of adjacent epidermal cells separate the lumen of the mucilage duct system from the external environment. These points are not necessarily in apparent association with clusters of secretory cells in any particular plane of section. Scanning EM examinations of large areas of lamina surface as well as LM examinations of large numbers of sections failed to demonstrate any direct opening of the mucilage duct system to the surface in any vegetative or in any immature or mature intact sorus regions of undisturbed lamina.

2.5. Physical Disruptions

When infiltration with methacrylate was carried out on a tissue shaker, structural disruptions were observed. Young sorus material at the uninucleate zoosporangial mother cell
stage forms blisters on the surface (Fig. 15). Small blisters formed in vegetative regions of the same material (Fig. 17). Sorus blisters contained paraphyses which were torn loose basipetally from the subtending cells but were still firmly attached acropetally to the outer wall of the sorus (Fig. 15). The zoosporangia, which are unattached to the outer wall, remained firmly attached to the subtending cells (Figs. 15 and 16). The volume of the blisters appeared to be filled with material histochemically identical to the mucilage duct system contents (Fig. 15). This material was metachromatically red and purple with TBO and the granular component showed a positive reaction for sulfated polysaccharides with AB/SO while the fibrillar component showed positive alcianophilia for acid polysaccharides. Where the blisters had ruptured the mucilage like material, paraphyses and outer wall were no longer present (Fig. 16). In mature sori the tissue was found to be in a much greater state of disorganization. The paraphyses may remain attached basipetally while the outer wall has been removed (Fig. 18). The zoosporangia are often also torn loose at the base in a mass of mucilage like material (Fig. 18). It is of importance to note here that there is an apparent disruption of the middle lamellae in cortical tissues (Fig. 18). In contrast to young and mature disrupted tissues, mature and undisrupted tissues which have released all zoospores naturally are characterized by the walls of empty zoosporangia and old paraphyses, both still attached basally to the subtending cells (Fig. 19). However, careful inspection of this material
reveals the occasional possible mucilage duct venting point which has blown out (Fig. 19).

3. Discussion

3.1. Mucilage Duct Differentiation

The assignment of mucilage duct inception to the regions on either side of the transition zone between lamina and pneumatocyst by previous authors (Guignard 1892, MacMillan 1899, and Fritsch 1945) is confirmed here. From materials such as that in Fig. 1 it is apparent that as the lamina expands laterally at the margins mucilage duct inception and subsequent events of differentiation follow along. This results in the apparent gradient of development seen in transverse sections of lamina distal to the transition zone (Fig. 1). Examinations of this tissue have led to the conclusion that mucilage duct system inception is solely a function of the meristoderm and is not shared with the cortex as previously supposed (Guignard 1892, MacMillan 1899, Skottsberg 1907). In Guignard's review of 1892, secretory cell differentiation is reported to follow duct lumen differentiation. Although he described duct lumen differentiation as occurring among the meristoderm cells, secretory cell differentiation was thought to have occurred
after repeated periclinal divisions of meristoderm had buried the ducts (Guignard 1892). The secretory cell initials were considered to be of cortical origin (Guignard 1892, MacMillan 1899). Despite the fact that MacMillan (1899) corroborated Guignard's (1892) observations in the stipe, he proposed that the schizogeny which marks duct lumen inception occurs in a periclinal rather than an anticlinal wall in the lamina of *Nereocystis*. Furthermore, he proposed that the cells on either side of this schizogeny differentiate to produce the duct lumen completely lined with secretory cells, which he illustrated (MacMillan 1899). Skottsberg (1907) arrived at almost the opposite interpretation in identifying the first event of inception as secretory cell differentiation. He diagramatically figured in surface view groups of secretory cells with denser cytoplasm within the meristoderm with no associated duct lumen (Skottsberg 1907, Fig.166). He proposed that these groups of secretory cells become buried by further meristoderm activity, followed by the differentiation of a spherical duct lumen. I confirmed Guignard's (1892) location of duct lumen inception in the meristoderm by means of an anticlinal schizogeny. However, it is highly probable that Guignard overlooked the concurrently formed secretory initial cells of the inception division described here (Fig.3). I also confirm Skottsberg's (1907) identification of secretory cell inception in the meristoderm layer, although I suspect that he missed the actual inception division and from his figures identified a stage closer to my Fig.5 in surface view.
Furthermore, as pointed out above I designate duct lumen inception as a meristoderm phenomenon in contradiction to Skottsberg (1907). Observations made in this work (Figs. 1-5, summarized in Fig. 21) show that secretory cell and duct lumen differentiation are simultaneous events embodied in the periclinal division of a single meristoderm cell. It has not been possible to corroborate MacMillan’s (1899) suggestion that the duct lumen of the lamina results from schizogeny of a periclinal cell wall. Based on evidence presented here it is proposed rather that the inception of the lumen occurs in the radial anticlinal cell wall of the former meristoderm mother cell whose periclinal division produced a shorter meristoderm progeny cell on the outside and a secretory progeny cell, overlooked by Guignard (1892), on the inside (Fig. 3). This single secretory progeny cell is easily distinguished from similarly produced and adjacent cortex cells by its proximity to the new lumen, shape, and histochemical properties. It follows then that each single secretory progeny cell of the inception division undergoes subsequent divisions to form an isolated cup-shaped group of secretory cells. The fact that mucilage is detectable in the duct lumen from inception is evidence for early differentiation of the secretory cells. Although brown algal meristoderm and epidermal cells are capable of producing mucilage for secretion, they are reported to secrete this material to the exterior of the thallus (McCully 1966, 1968, Davies, Ferrier, and Johnston 1973, Evans and Holligan 1972, Evans, Simpson, and Callow 1973).
assumed to be the case here as well, and the presence of perinuclear red metachromasia in secretory progeny cells with TBO would support their role in mucilage secretion to the duct lumen as do Evans, Simpson, and Callow (1973) for mature secretory cells of *Laminaria saccharina* mucilage ducts. However, this does not eliminate the possibility that the meristoderm progeny cells of the inception division may contribute some mucilage to the new lumen. The presence of small lumen in section which are not associated with secretory cell initials can be interpreted as a continuing anastomosis of the new duct lumen begun schizogenously at inception. This continuous anastomosis leads to the reticulum of the mature lamina. This anastomosis of the lumen was proposed by Guignard (1892), Oliver (1887) and Skottsberg (1907) and is still ascribed to in Fritsch (1945). The occasional full duct in section implied that the duct lumen does in fact originate at isolated points, which would allow pockets of lumen to be undisturbed by the initial cutting of the tissues at the time of fixation. In mature laminae bearing sori, where blade expansion has ceased, the duct system is continuous from its deepest points possessing the greatest sectional diameter to those located within one cell layer of the former meristoderm and having the smallest diameter. I believe that this gradient in duct volume is influenced by the activities of the secretory cells, responsible for their filling and the process of lamina maturation in which this duct system occurs. The observation of apparent completely lined ducts (MacMillan 1899)
is corroborated, however, their development is not attributed to a periclinal schizogeny, but rather to the effects of surrounding tissue morphogenesis associated with lamina expansion. This hypothesis finds support in the works of Skottsberg (1907) and Oliver (1887).

3.2. Potential Duct Venting Points and Physical Disruptions

Guignard (1892) reported many points where the lumen of the duct system approaches the surface of the lamina but does not directly vent. MacMillan (1899) failed to report these for Nereocystis. From the observations made here it is apparent that these points do occur in Nereocystis. Although Guignard (1892) only considers these points in vegetative tissues, Evans, Simpson, and Callow (1973) report them in soral tissues of L. saccharina and further state that they are open to the lamina surface. With careful preparation of soral tissues as seen here these points are not found to open into the extracellular lumen of the sorus of Nereocystis as long as exuviation or the removal of the outer cell wall of the sorus has not yet occurred (Fig. 13, 14, and 21f). Furthermore, these points may be disrupted and open to the surface if tissues are physically agitated (Fig. 15-18) or they may apparently open during the natural course of sorus exuviation and abscission from the lamina (Fig. 19).
3.3. Mucilage Duct System Structure and Function

The observation of large quantities of mucilage associated with mature sori led to this study. One certain source of some of this mucilage is the zoosporagia in which this substance functions in zoospore release (Toth 1976b). The mucilage duct system found in a number of Laminariales and considered in this study of *Nereocystis* also may contribute some mucilage during sorus exuviation. Certain aspects of the relationship between the mucilage duct system and the meristoderm from inception (Guignard 1892, MacMillan 1899, Skottsberg 1907) to maturity (Guignard 1892, Evans, Simpson, and Callow 1973) has been imperfectly defined. From observations made here it is seen that mucilage duct inception is solely a phenomenon of the meristoderm. Also duct secretory cells are distinct from adjacent cells and remain similar to meristoderm cells both histochemically and meristematically. It is proposed that the mucilage duct system maintains an association with the meristoderm all through development by means of the points of lumen inception (Figs. 3 and 21a) to maturity where these points have become the points described here as potential venting points (Fig. 14 and 21d and f). Guignard (1892) hinted at this but the loss of the secretory cell/inception point association as well as his incomplete understanding of the events of inception discouraged this. Both Guignard’s misunderstanding and MacMillan's
completely lined mucilage ducts can be explained by physical distortions which occur during lamina growth combined with the interpretation of the plane of section. Close examination of the relationship between mature mucilage duct and meristoderm systems (diagrammed in Figs. 21d and f) revealed that the mucilage duct system develops as a closed system separated from the external environment. In vegetative tissues the outer cell wall and middle lamella of adjacent epidermal cells stand between the lamina surface and the mucilage duct system (Fig. 21d). In the sorus prior to exuviation the former outer wall of the meristoderm which remains attached to the paraphyses tips; the extracellular lumen of the sorus; and the middle lamella of adjacent subtending cells stand between the lamina surface and the mucilage duct system. Apparently these potential venting points may be prematurely disrupted (Figs. 15-18) resulting in the opening of the mucilage duct system to the lamina surface. It is suggested with some confidence that these potential venting points may naturally be opened to the lamina surface as needed under certain circumstances. The most obvious circumstance imaginable from this study would be natural sorus exuviation where the balance between the sorus lumen and the cut lumen is lost, leaving only the middle lamella of adjacent subtending cells standing between duct lumen and the outside. I would propose that this is the condition of the material described by Evans, Simpson, and Callow (1973) and that the middle lamella has in fact given way resulting in the opening of the duct system. In this work
Fig. 19 would represent a later stage at which the zoospores have been released from their sporangia and washed from the surface. Other factors which could possibly cause an opening of the duct system might be wounding by grazers or any other abrasive agents. Consequences of this type of event are under investigation. Finally, exposure of lamina tissues, especially in intertidal Laminariales possessing mucilage duct systems, to the stress of desiccation may also lead to the opening of the venting points of the duct system. It is believed that through opening of the mucilage duct system by the mechanisms suggested above the reserve of mucilage contained in the ducts may participate significantly in zoospore dispersal, response to wounding and play a role in the resistance to desiccation. It is supposed that wherever this type of mucilage duct system occurs in the Laminariales it functions in the roles suggested but, it is emphasized that in all of these cases participation of the mucilage duct system is possible but not an obligatory role.
CHAPTER 2 FIGURES, MUCILAGE DUCT SYSTEM
Plate I

Figure 1. One quadrant of a lamina cross section. Note the gradient of mucilage duct system differentiation. Inception is at the lamina margin to the right in the figure. More mature stages are at the midline at the left of figure. Staining is with Alcian Blue/Safranin O to differentiate acid and sulfated polysaccharides. Dense staining of outer wall and mucilage ducts (arrowheads) is due to safranin for sulfated polysaccharides and heavier alcianophilia for acid polysaccharides. The cortex cell walls show alcianophilia, although less intense and no safranin staining. Scale equals 100 um.

Figure 2. Meristoderm cells from inception region. Staining is with TBO. There is purple metachromasia in the cell walls for acid and/or sulfated polysaccharides. The cytoplasm is characterised by a prominent nucleus and chloroplasts. Reddish metachromatic material, acid or sulfated polysaccharides, forms a basal cap on the nucleus (arrowhead) and is present as granules in apical cytoplasm (arrowhead). Scale equals 10 um.
Figure 3. Earliest recognized mucilage duct stage of development. Note the meristoderm (a) and secretory (b) progeny of the periclinal inception division. The adjacent newly formed duct lumen is already present (*). Staining is with TBO. Scale equals 10 um.

Figure 4. Subsequent progeny cell divisions. Meristoderm progeny cell (a) of inception produces new cortical cells (a) lining the duct lumen. The secretory progeny cell also divides producing more secretory cells (b). With TBO staining the perinuclear region of these new secretory cells shows red metachromasia (arrowheads). Scale equals 10 um.

Figure 5. Continued progeny cell divisions. Secretory cells begin to form the characteristic cup (b's). The meristoderm progeny cell continues producing cortex cells (bottom a's) to keep up with the meristoderm and buries the secretory cell initials. The continuity of these progeny cells is still clear (follow the a's). Staining is with TBO. Scale equals 10 um.

Figure 6. A full mucilage duct. Note much more homogeneous contents, implying a preservation of duct lumen integrity. Staining with TBO. Scale equals 10 um.

Figure 7. Mucilage duct stained with ABB for proteins. Secretroy cells show staining comparable to that of meristoderm cells and dense in contrast to cortex cells. There is little if any staining of mucilage duct contents. Scale equals 10 um.
Plate II

Figure 8. Surface section of lamina cortex. Mucilage duct system is a reticulum in the cortical layer of the lamina. TBO stained. Scale equals 50 μm.

Figure 9. Oblique surface section of lamina. Note portions of epidermal cell bases (arrowed circle). The duct lumen extends to within one cortex cell thickness of the meristoderm layer. TBO stained. Scale equals 50 μm.

Figure 10. Most superficial extensions of mucilage duct lumen. Note that their shape and contents make them easy to distinguish from their cortical neighbors. TBO staining. Scale equals 50 μm.

Figure 11. Cup of secretory cells. These cups give ducts partially lined appearance when cut in section. TBO stained, nomarski optics. Scale equals 50 μm.

Figure 12. Cross section of an apparently completely lined mucilage duct. Secretory cells are numbered and the figure is positioned so that the meristoderm runs along the right margin of the figure. TBO stained. Scale equals 50 μm.
Plate III

Figure 13. Young undisturbed sorus material. Paraphyses are still attached to the outer cell wall and zoosporangial mother cells are present. The potential duct venting point is still intact (arrowhead). PAS staining. Scale equals 10 μm.

Figure 14. Mature undisturbed sorus material. The potential mucilage duct venting point is still closed maintaining the integrity of the duct lumen (arrowhead). Only the middle lamella of the two adjacent subtending cells to the paraphyses and zoosporangia stands between the lumen of the sorus and the mucilage duct system. Stained with TBO, cut from Spurr's. Scale equals 10 μm.

Figure 15. Cross section of physically agitated young sorus. Mucilage filled blister is clear. The paraphyses have torn loose at their attenuated bases and left zoosporangial mother cells below a layer of mucilage. The paraphyses are still attached to the outer wall. Nomarski optics. Scale equals 10 μm.

Figure 16. Blistered young agitated soral material. Most paraphyses, outer wall and mucilage are absent, blister has lysed. A possibly disrupted potential venting point is indicated (arrowhead). Nomarski optics. Scale equals 10 μm.
Plate IV

Figure 17. Cross section of physically disturbed young lamina. Meristoderm shows small blisters after physical agitation. PAS stained. Scale equals 10 um.

Figure 18. Agitated mature zoosporangial material. The outer wall is gone, paraphyses and zoosporangia are torn loose in a mass of mucilage. There seems to be a breakdown of middle lamellae of cortical cells. TBO stained. Scale equals 10 um.

Figure 19. Old abscissed sorus tissues. Zoospores have been released leaving paraphyses and empty zoosporangia. A blown-out mucilage duct venting point is clearly shown (arrowhead). TBO stained. Scale equals 10 um.
Figure 20. Secretory cell of a mature mucilage duct system. Polymorphic nucleus is deformed by numerous prominent dictyosomes. NE/ER (arrow) and CER/ER (Ch) continuities are clear. Note many mitochondria and osmiophilic deposits on plasma membrane. Mucilage duct is poorly infiltrated. Compare cell walls adjacent to cortical cells with that bordering the mucilage duct. Scale equals 0.5 um.
Plate VI

Figure 21. A diagrammatic summary. These are the events of mucilage duct inception and critical stages in development leading to the mature undisrupted structural relationship between mucilage duct and zoosporangial sorus systems.

Figure 21a. The meristoderm cell source. The meristoderm cells are characterised by an approximately central nucleus with a basal cap of red metachromatic material (arrowhead) and apical chloroplasts. Mucilage duct inception entails a periclinal division of a single meristoderm cell producing a meristoderm progeny cell (1) and a secretory progeny cell (2). Concurrently a duct lumen is produced schizogenously in the adjacent anticlinal cell wall (stippled area 3).

Figure 21b. Immediate progeny cell divisions. The meristoderm progeny cell (1) of the inception division divides to produce a cortical cell (1), while the secretory progeny cell of the inception division divides to produce more secretory cells (2). The lumen of the duct enlarges with the products of secretory cell activity and the addition of cortical lining cells.
Figure 21c. Subsequent divisions. The meristoderm progeny cell continues to produce cortical cells (those numbered 1 in their vacuoles) which line the enlarging duct lumen (stippled area). The direct relationship of secretory cells to the meristoderm is still obvious (1's and 2's). The secretory cells are beginning to form a cup shaped cluster on the inner face of the duct lumen or a lining in section.

Figure 21d. Mature relationship of secretory cells, duct lumen and the meristoderm. The secretory cells form a cup shaped cluster in the bottom of the duct lumen. The direct lineage between secretory cells and meristoderm cells has been obscured and the former point of inception is now a potential duct venting point consisting of outer cell wall and middle lamella (*) of two adjacent meristoderm cells.

Figure 21e. Mucilage duct system and sorus inception. The extracellular lumen of the zoosporangial sorus develops between outer cell wall and the basal half of the former meristoderm cells (2) by a schizogeny of the anticlinal cell walls (*) of the paraphyses initials (1).

Figure 21f. Mucilage duct system, mature sorus undisturbed relationship. The potential venting point is now reduced to the middle lamella (*) of two adjacent subtending cells.
1. Introduction

The process of zoosporangial sorus development is generally assumed to be well defined. Most morphological components, zoosporangia, paraphyses and outer cell wall, were observed as early as 1850 by Thuret. Amongst similar observations for many other members of the Laminariales, *Nereocystis* was included (MacMillan 1899). In Sauvageau (1915) and Kylin's (1916) redefinition of the basic life history of the order, again *Nereocystis* was included (Hartge 1928). Fritsch (1945) has summarized these early works. Morphological work with sorus development in some members has continued and this has been summarized by Ohmori (1967). With *Nereocystis* the location of meiosis and syngamy has been clearly established in its life history (Kemp and Cole 1961). With the advent of electron microscopy attention was refocused on brown algal reproductive cells (Manton 1957, Scagel 1966 for review), however, not until later did work begin to appear dealing with zoosporogenesis in Laminariales (Chi and Neushul 1972, Kugrens and Reeves 1972, Toth and Wilce 1972, Toth 1972, Collins and Kugrens 1975). Yet little more of the process of sorus development has been explored with electron microscopy.

This chapter is devoted to the process of sorus
development in *Nereocystis luetkeana* at both ultrastructural and light microscope levels. Ultrastructurally paraphyses are central to this chapter. Attention has also been given to the sorus extracellular lumen, outer cell wall or slime cuticle, subtending cells and zoosporangia. The observations made here shed new light on older observations of sorus development and require new interpretations of structure and function before the events of sorus abscission may be understood.

2. Observations

2.1. Paraphysis and Sorus Inception

It has not been possible to observe paraphysis inception in youngest sori since all sori visible both to the naked eye and with infrared photography already contain zoosporangial initials, partially elongate paraphyses and extracellular spaces (Fig. 5). It has only been possible to reconstruct the events involved in the process of sorus inception through an examination of the margins of young sori. Here one finds a linear sequence of stages from vegetative meristoderm cells to whatever stage of development the bulk of the sorus is at (Fig. 1, 2 and 3). Ultrastructurally it will be necessary to anticipate sorus inception in order to observe it since the aspects of the sorus abscission process may have already begun
in cells at the margin by the time the sorus has become visible. For this reason ultrastructural observations of this tissue are included in that chapter dealing with abscission.

It is possible to reconstruct the events of sorus inception from young sorus margins with light microscopy at the cellular level. Here a gradual gradient of development is present and presumably not completely arrested. The first indication of sorus inception is probably the resumption of the elongation of meristoderm cells followed by periclinal cell divisions (Fig. 1), resulting in the often referred to "palisade" appearance (MacMillan 1899, Fritsch 1945). This first step is apparently followed then by the appearance of the extracellular spaces and zoosporangial initials (Fig. 2). MacMillan (1899) did not clarify whether his observations of inception are from the central or marginal regions of sori. Since he states that preserved material was used (MacMillan 1899); it is most probable that the sorus margins were also his source of observations.

The extracellular spaces appear schizogonously in the anticlinal cell walls of paraphysis initials which were elongate meristoderm cells. This schizogeny does not extend the full length of the walls (Fig. 2, 3 and 5) for adjacent paraphysis initials and the subtending cells share some portions of their anticlinal walls (Fig. 1, 2 and 3). With the appearance of extracellular space and zoosporangial initials, the basal portions of the paraphyses become narrower than their
apical ends and also the basal cells subtending them (Fig. 2 and 3). The zoosporangial initials are produced by continued mitotic activity of the subtending cells (Fig. 3 and 4, MacMillan 1899, Hartge 1928, Kemp and Cole 1961). The resultant sporangial initials protrude into the extracellular space of the sorus (Fig. 2, 3 and 5). These events produce all of the morphological components of the zoosporangial sorus of Nereocystis. These observations confirm the descriptions of MacMillan (1899) with Nereocystis and generally agree with those of other members of the Laminariales (Fritsch 1945, Papenfuss 1951, Chapman and Chapman 1973, Ohmori 1967).

2.2. Paraphyses of the Post-inception/Pre-meiotic Sorus

In the youngest visible sori all of the structural components of the mature sorus, the outer cell wall, extracellular space, paraphysis initials, subtending cells and the zoosporangial initials are present (Fig. 5). In the larger pieces of tissue used for light microscopy the outer wall remains firmly attached to the paraphysis tips. On the other hand as with the transition and expansion zone meristoderm cells, the outer cell wall and walls of the paraphysis and zoosporangial initials do not preserve well in material processed for electron microscopy (Fig. 8, 10, 12, 15 and 19). Using observations with the light microscope the extracellular space of the sorus is seen to contain a reticulum of material
in both the sorus proper (Fig. 5) as well as at its margin (Fig. 2 and 3). Strands of this reticulum attach to the anticlinal walls of the paraphyses and the walls of zoosporangial initials as well (Fig. 2, 3, 5 and 6). The paraphyses are already basally attenuated and are approximately twice the height of the zoosporangial initials (Fig. 4-7). The smallest basal dimensions of paraphyses are seen when both the paraphysis and zoosporangial initial of a single subtending cell are included in the plane of section, while the greatest basal dimensions of paraphyses, although still attenuated, are seen when the associated zoosporangial initial is not included in the plane of section (compare paraphyses of Fig. 5). In contrast, the zoosporangial initials are ovoid to oblong in shape with little, if any, basal attenuation (Fig. 5 and 18). An individual subtending cell may produce more than one zoosporangial initial (Fig. 4). The only apparent changes in the subtending cells are in their outer periclinal cell walls which now delimit them from one paraphysis and at least one zoosporangial initial (Fig. 5). Their anticlinal walls and inner periclinal cell wall appear unchanged (Fig. 1, 2 and 5).

2.2.1. Histology

2.2.1.1. The Outer Cell Wall of the Pre-meiotic Sorus

The cell wall covering the sorus stains metachromatically purple with TEO for acid and/or sulfated polysaccharides and
shows a positive response for acid polysaccharides with AB/SO staining (Fig. 6). In both cases there is an increase in staining intensity in the outermost layer (seen best in Fig. 6). With AB/SO the innermost layer of the outer cell wall adjacent to the plasma membrane of the paraphysis apex may stain more densely for acid polysaccharides (Fig. 6).

2.2.1.2. The Extracellular Lumen of the Sorus

This space contains a reticulum of material which stains metachromatically purple with TBO for acid and/or sulfated polysaccharides and is positive for acid polysaccharides with AB/SO as well. There is no staining of the contents of this space with the ABB/PAS technique for proteins.

2.2.1.3. The Paraphyses, Subtending Cells and Zoosporangia

The paraphyses are distinctive in that the nucleus and cytoplasm stain a turquoise to greenish blue with TBO, as opposed to the zoosporangia and subtending cell's cytoplasm and nuclei which stain a blue-gray. In all three cell types the chloroplasts stain very lightly with TBO. There are points in the apical cytoplasm of the paraphyses which stain a more intense green indicative of phenolics in contrast to the rest of the cytoplasm and there is an occasional clear vesicle with a metachromatically stained core suggesting acid and/or sulfated polysaccharides. The zoosporangial initial nuclei of
this stage frequently have a reddish-purple metachromatic mass at their basal hemisphere (Fig. 18) suggesting acid and/or sulfated polysaccharides. The cell wall of the zoosporangial initial and the anticlinal cell walls of the paraphyses stained lightly for acid polysaccharides with AB/SO. The basal portion of the zoosporangial initial and the apical cytoplasm of the paraphyses (Fig. 6) may stain positively for acid polysaccharides at points. The chloroplasts, nuclei and cytoplasm of both cell types stain positively for proteins with ABB/PAS in that order of decreasing intensity (Fig. 7). The apical cytoplasm shows some clear or vesiculate regions and a few similar regions also exist in the basal cytoplasm (Fig. 7). The cytoplasm, nucleus and chloroplasts of the zoosporangial initials stain for proteins with quite a uniform density with ABB/PAS with the exception of occasional clear areas in the basal cytoplasm (Fig. 7). The intensity of staining with ABB in the subtending cells varies a great deal depending upon whether or not zoosporangial initial inception has occurred. The staining for proteins is intense prior to inception and less intense with some clear areas afterwards (Fig. 7). The middle lamellae of the anticlinal and basal periclinal walls of the subtending cells show a very light positive PAS staining for neutral polysaccharides.

2.2.2. Ultrastructure of the Paraphyses

In paraphysis cells the nucleus, golgi apparatus with its
derivatives, and the chloroplasts dominate the cytoplasm (Fig. 8). Also in evidence are mitochondria, ER and a number of presumptive lipid droplets of light electron density which appear to have been partially extracted during processing (Fig. 11 and 12). The nuclei are typically located in the apical portion of the cytoplasm. They contain a nucleolus and often a homogeneous region of uniform fibrillar material (Fig. 9 and 10), as reported in the meristoderm cells (Chapter 1). The nucleoplasm is fairly homogeneous showing no pronounced heterochromatic regions and the nuclear envelope is periodically interrupted by nuclear pores (Fig. 10). The golgi apparatus consists of a few dictyosomes, all with their formative faces adjacent to the basal hemisphere of the nucleus. Usually the cisternae of the maturing faces show some hypertrophy and the derived golgi vesicles contain apparent cell wall material (Fig. 10 and 11). The chloroplasts of these paraphyses are typical (Fig. 13). Occasional plastoglobuli are observed (Fig. 13 and 14), and in contrast to adjacent epidermal cells (Chapter 1) no membrane configurations reminiscent of the concentric bodies of Brown and Wiper (1970) have been observed. The apical cytoplasm contains most of the chloroplasts, a number of hypertrophied golgi vesicles, a few mitochondria, lightly electron dense droplets (Fig. 9 and 14, identified by a lack of a delimiting membrane) and some profiles of ER. At the cell apex the plasma membrane often shows caveolae suggestive of exocytosis (Fig. 15) in contrast to the plasma membrane along the anticlinal cell walls (Fig. 13).
Coated vesicles are observed (Fig. 14 and 16), and these may be associated with golgi vesicles (Fig. 16). The plasma membrane of the basal cytoplasm is characterised by deep convolutions and the cytoplasm by numerous mitochondria, fewer chloroplasts and small golgi vesicles.

2.2.3. The Subtending Cells

These cells are in continuity with the paraphysis and zoosporangial initials by means of plasmodesmata. Their nuclei and chloroplasts appear much the same as those of the paraphyses they subtend. Although there is little evidence of golgi activity there is usually some degree of vacuolation and some lipid droplets are present. Adjacent subtending cells are invariably connected with one another and to the underlying cortical cells by plasmodesmata. The lower portions of their anticlinal cell walls are thick and intact in comparison to those of both paraphysis and zoosporangial initials. However, the extracellular space of the sorus may extend down the middle lamella of the distal portions of these walls. Centrioles are present adjacent to the nuclear envelope.

2.2.4. The Extra-cellular Space and Zoosporangial Initials

Ultrastructurally there is little material in the extracellular spaces of the sorus to correlate with the reticulum observed in light microscope material. However,
there is some fibrillar material extending into the extracellular space from the very thin walls of the sporangial initials. Adjacent to the nuclei there are regions which are often metachromatic with TBO staining indicating acid and/or sulfated polysaccharides (Fig. 18) and equally AB positive with the AB/SO method indicating acid polysaccharides (Fig. 29). Ultrastructurally these regions are occupied by a group of dictyosomes adjacent to the basal hemisphere of the nucleus. Their cisternae are highly hypertrophied (Fig. 20) and contain fibrillar material morphologically identical with the material of the cell walls (Fig. 20 and 21).

2.3. Paraphyses of the Meiotic Sorus

The paraphyses of meiotic sori have continued to elongate and reached their mature length by the end of meiosis (Fig. 21, 24 and 26). From field and in vitro observations of sori (Chapter 2) it is clear that this outer cell wall/paraphysis tip association is strongest at this point. From in vitro disruptions of soral tissue it was determined that the attenuated bases of the paraphyses may fail first when stressed (Chapter 2) resulting in mucilage blisters. These blisters consist of an intact outer wall, with basally detached paraphyses suspended in an expanded extracellular space above the developing sporangia and are filled with mucilage (Fig. 23). This additional mucilage apparently originates from the
mucilage duct system below the sorus (Chapter 2). Even in undisturbed tissues there may be a large extracellular space in the sorus at this stage (Fig. 21 and 24). The contents of the lumen may show variation in densities (compare fig. 21 and 22). Control tissues (Fig. 22) which were held during high light intensities for 24 hr under a carbon fourteen autoradiography experiment showed the greatest density of reticulate metachromatic material observed in this study of Nereocystis sori.

2.3.1. Histology of the Meiotic Sorus

2.3.1.1. The Outer Cell Wall

The outer cell wall covering the sorus stains essentially as in the pre-meiotic sorus with all three staining procedures, TBO, AB/SO and ABB/PAS. However, the most apical portion of the cell wall of the paraphysis has become more distinct from the remainder of the outer cell wall in its intensity of staining for acid polysaccharides with AB/SO.

2.3.1.2. The Extracellular Sorus Lumen

The extracellular lumen of the sorus now appears to be at its greatest volume relative to the other constituents of the sorus. Its contents exhibit the same staining patterns with TBO and ABE/PAS as in the pre-meiotic sorus. However, the
reddish background staining for sulfated polysaccharides with AB/SO reaches a maximum at this stage of development.

2.3.1.3. The Paraphyses, Subtending Cells and Zoosporangia

The nuclei and cytoplasm retain their turquoise to greenish-blue staining properties with TBO, although the cytoplasm may be lighter in intensity. The anticlinal cell walls of the paraphyses stain metachromatically purple with TBO indicating acid and/or sulfated polysaccharides and positively for acid polysaccharides with AB/SO, although, the chloroplasts maintain their protein staining intensity with ABB of the ABE/PAS method, the nuclei and cytoplasm may stain lighter (Fig. 26), and the basal cytoplasm may become heterogeneously stained (Fig. 34). The enlarged zoosporangia show a marked increase in the heterogeneity of staining with TBO and ABB of the ABB/PAS method but no change in the color. The subtending cells are apparently much more vacuolate as is evident from their staining patterns with both TBO and ABB/PAS (Fig. 24, 26 and 34).

2.3.2. Ultrastructure of the Meiotic Sorus

2.3.2.1. The Paraphyses

The apical cytoplasm of the pre- and post-meiotic sori paraphyses is characterized by vesiculation and large
chloroplasts (Fig. 27-31). The somewhat apically located nucleus separates the basal and apical cytoplasm. In the nucleus little chromatic material is evident and nucleoli are small (Fig. 28, 29, 32 and 33). Despite paraphysis attenuation and elongation during meiosis and sporangium enlargement the anticlinal cell walls of the pre- and post-meiotic paraphyses are of approximately the same thickness (Fig. 27 and 28). While the zoosporangia of pre-meiotic sori are small enough to fall down amongst the paraphyses during processing (Fig. 27, see cross section of reclining zoosporangium). Zoosporangia within post meiotic sori have enlarged enough so that they may not fall down amongst the paraphyses (Fig. 28). In both cases the cuter cell wall shows three distinct fibrillar layers and a fourth flocculent cuter layer. Fibrils of the first and innermost layer, layer 1, are oriented tangentially to the plasma membrane of the paraphysis apex and are continuous with the anticlinal cell walls (Fig. 27 and 28). Fibrils of the next layer are oriented both tangentially and perpendicular to the plasma membrane (Fig. 29 and 31). This layer would be comparable to layer 2/3 of the meristoderm cells adjacent to the youngest sorus (Chapter 1), and as in the meristoderm this layer is continuous over the sorus showing extensions into what remains of the middle lamellae of anticlinal walls of paraphyses (now sorus lumen, Fig. 27 and 28). Fibrils of the next and last layer are oriented tangentially to the paraphyses also forming a continuous layer over the sorus (Fig. 29 and 31) and are identical to layer 4 of the meristoderm (Chapter 1).
There is an outermost layer of flocculent material which is perpendicular to and covers the outermost fibrillar layer (Fig. 29 and 31), and is identical to the layer of flocculent material at the outer limit of the meristoderm cell wall adjacent mature sori (Chapter 1). It is of interest to note that if a paraphysis is damaged or dying this flocculent material is eroded away (Fig. 30). Even though the paraphysis tip/outer cell wall relationship is still firm in both cases, there is already a perceptible differentiation between layer 1 of the outer cell wall and layers 2/3 and 4 of the sorus (Fig. 28 and 31).

The apical cytoplasm is dominated by the large chloroplasts and numerous vesicles (Fig. 27 to 31). Some vesicles are always found to contain irregular flocculent material (Fig. 29 and 31). A number of these apical vesicles are apparently without any electron opaque contents (Fig. 29 and 31). There are cisternae of peripheral ER (Fig. 29) as in the other meristoderm cells (Chapter 1), as well as large vesicles containing material similar in density to the adjacent cytoplasm. Another inclusion of the apical cytoplasm is the often irregular shaped, extremely homogeneous structures which associate with the chloroplasts (Fig. 29,L). These are most similar to presumed lipids which are more prevalent in associated sporangia (Appendix 1). The large chloroplasts which dominate the apical cytoplasm are characterized by their numerous stacks of thylakoids and lack of pyrenoids (Fig. 27 and
Plastoglobuli are not very common at this stage (Fig. 35 arrowhead). The Golgi apparatus consists of a few dictyosomes adjacent to the nucleus (Fig. 32 and 33) and they appear to be more active in post-meiotic sorus paraphyses (Fig. 33) than in pre-meiotic sorus paraphyses (Fig. 32).

It is below the nucleus that the most severe attenuation of the paraphyses is evident (Fig. 39), however the cell walls are no thinner here than around the apical cytoplasm (Fig. 40 and 41). With ABB protein staining the basal cytoplasm appears vesiculate alternating between stained and unstained portions of cytoplasm (Fig. 39). Ultrastructurally the unstained regions are found to vary from simply vesiculate (Fig. 38) to containing a variety of membranous configurations (Fig. 35 and 36). Tangential sections through the basal cytoplasm often reveal cisternae of the peripheral ER system (Fig. 37). The densely stained portions of cytoplasm often consist of a core of material which is very homogeneous and surrounded by an electron translucent region (Fig. 35, 36 and 39). In some areas this electron-translucent border is clearly delimited by two membranes suggesting derivation from ER (Fig. 40 arrows), while in others there appears to be only one membrane (Fig. 39 arrow). It is noteworthy that paraphyses are still joined to subtending cells by plasmodesmata (Fig. 39) as are neighboring subtending cells.
2.3.2.2. Subtending Cells

Subtending cells of the pre-meiotic sorus have begun vacuolation. Centrioles are still in evidence. Chloroplasts are large and contain numerous stacks of thylakoids similar to those of the paraphyses above (Fig. 48). In contrast to the paraphyses, nuclei show distinct regions of electron dense chromatic material. By the end of meiosis, vacuolation and nuclear condensation are advanced. Subtending cells remain in contact with the underlying cortical cells as well as with the paraphyses above by means of pit fields of plasmodesmata.

2.4. The Mature Sorus

This stage is indicated by completion of cytokinesis of most zoosporangia in the sorus. The zoosporangia have enlarged and become swollen so that the only remaining extracellular space extends between their thickened tips and the outer wall of the sorus which is still attached (Fig. 41) to the paraphysis tips. The basal portions of the paraphyses are most attenuated at this stage of development. Their nuclei and most chloroplasts reside in that portion of cytoplasm above the sporangia (Fig. 41 to 43). The reticulum of metachromatic material is essentially confined now to the small remaining extracellular space between outer cell wall and the zoosporangial apices (Fig. 41).
2.4.1. Histology

2.4.1.1. The Outer Cell Wall and the Extracellular Lumen

The staining characteristics of the outer cell wall with TBO and AB/SA are still as in the previous two stages. However, the paraphysis cell wall is very distinct from the remainder of the outer cell wall and stains lightly PAS positive at its tip (Fig. 42). The compressed volume of the extracellular lumen of the mature covered sorus still contains its reticulum of TBO metachromatic material (Fig. 41).

2.4.1.2. The Paraphyses, Subtending Cells, and Zoosporangia

The cytoplasm and nuclei of the paraphyses still stain a greenish-blue with TBO. The apical cytoplasm, nucleus included, are densely and heterogeneously stained with ABB for proteins of the AEE/PAS method (Fig. 42, large arrow). The subtending cells are now highly vacuolate (Fig. 41) and some vacuoles may contain a TBO metachromatic reticulate material presumably acid and/or sulfated polysaccharides. The constituents of the zoosporangia are the most altered within the sorus. They now have a metachromatically pink thickened tip which may also be slightly PAS positive suggesting sulfated polysaccharides (Fig. 42). At completion of cytokinesis the extracellular space which forms around the spores was filled with a metachromatically pink material as seen with TBO. This
material also stains positively for sulfated polysaccharides with AB/SA. The flagella are visible with ABB at this stage as well (Fig. 42, small arrows). The nuclei and chloroplasts stain blue and blue-gray respectively with TBO and blue-black with ABB.

2.4.2. Ultrastructural Aspects of the Mature Sorus

The junction between paraphysis tips and the outer cell wall is now very distinct and in many instances signs of separation are evident (Fig. 44 and 45). Although some of these areas are aggravated by tissue preparation, there are often signs of localized erosion of the outer cell wall of the sorus (Fig. 44). In this latest stage of development when paraphyses appear most crowded by the zoosporangia the first signs of senescence become apparent.

2.4.2.1. The Outer Wall and the Paraphyses

At this stage of development the continuous portion of the outer cell wall covering the sorus is now very distinct from that confined to individual paraphyses. The outer two fibrillar layers and outermost flocculent layer are often partially separated from the paraphyses (Fig. 43-45), which are now surrounded only by the innermost layer, layer 1 (Fig. 45). Some of layer 2/3 of the outer cell wall which extends down into the former region of middle lamella between paraphysis
initials may remain attached to the adjacent paraphyses (Fig. 43, arrows). However, the plane of separation is clearly between the innermost layer, layer 1, and the next fibrillar layer out, layer 2/3 (Fig. 45). Thus separation occurs between the continuous and discontinuous constituents of the outer cell wall. In areas of sorus where separation of the outer cell wall is evident there is localized erosion of the outermost flocculent layer as well as some of the outermost fibrillar layer, layer 4, (Fig. 44).

2.4.2.2. The Paraphyses

From appropriate sections it is apparent that the paraphysis apical cytoplasm has become even more vesiculate (Fig. 43) and that the remaining ground substance has become more electron dense (Fig. 43 and 45). Vesicles may show a considerable range in both size and density of electron opaque material (Fig. 43), or granular material (Fig. 46) while some vesicles may be devoid of electron opaque material (Fig. 45), and others may contain distinctly granular material (Fig. 46, bottom). Some vesicles can be described as multi-vesiculate. There are regions of dense cytoplasm surrounded by what would appear to be ER cisternae (Fig. 47). As in the previous section the inner membrane may be absent in some of these (Fig. 49). Furthermore, there may be evidence of ER cisternal hypertrophy (Fig. 46, clear region at top) similar to that in the provacuolar regions of meristoderm cells of older tissues.
The outstanding feature of the chloroplasts is the abundance of pyrenoids (Fig. 46 and 49). Furthermore, it appears as though the chloroplast ribosomes are aggregated in the stroma between adjacent stacks of thylakoids and numbers of plastoglobuli are now observed as well (Fig. 45, 46 and 49).

The relationship of the golgi apparatus to the nuclear envelope becomes obscured by the irregular hypertrophy of dictyosome cisternae. Occasionally mitochondria may be found in apparent cytosegrosomes with the internal membranes or cristae already somewhat obscure (Fig. 48). Finally invaginations of the plasma membrane are becoming more common in this region of the apical cytoplasm.

2.5. Sori After Exuviation and Zoospore Release

After exuviation or removal of the outer cell wall which has covered the sorus and segregated the extracellular space of the sorus from the external environment, the zoosporangia and paraphyses appear to be of equal size. Paraphysis morphology is essentially identical to that of the pre-exuviation sori (Fig. 50), however, these paraphyses certainly are more vesiculate with the granular cytoplasm now confined in dense cytoplasmic masses (Fig. 50). It is not until most of the zoosporangia have released their zoospores that there is any
change in paraphysis morphology. In sori which still contain some indehiscent sporangia, these sporangia will continue to deform the adjacent paraphyses (Fig. 55, *). In these paraphyses their central volume is occupied now by large vacuoles and only a small quantity of peripheral cytoplasm remains (Fig. 51 and 55). The nucleus and chloroplasts are clustered loosely in the apical end (Fig. 51-53, 55 and 56). After exuviation and before zoospore release there is no extracellular space between zoosporangia and paraphyses and the reticulum of metachromatic material formerly occupying the remaining extracellular space has disappeared. Once the sporangia have released their spores there is space amongst the walls of the empty sporangia and remaining paraphyses. This space is now a site of bacterial growth (Fig. 52).

2.5.1. Histology of the Released Sorus

The outer cell wall and sorus lumen contents are, of course, lost and the zoosporangia are with rare exceptions empty (Fig. 52). The paraphysis cell walls are metachromatically purple, tending towards blue with TBO as are the cell wall remains of the dehisced zoosporangia indicating decreased amounts of acid and/or sulfated polysaccharides. The only recognizable organelles are the nuclei and the large vacuole of the paraphyses (Fig. 52 and 53). The apical cytoplasm is the only portion of the peripheral cytoplasm surrounding the large vacuole stained with TBO (Fig. 52). The
same is essentially true for the subtending cells (Fig. 52). With the AB/SO method only the paraphyses cell walls and subtending cell anticlinal cell walls show a positive staining for acid polysaccharides (Fig. 54). There is no staining for sulfated polysaccharides in any of the other cell types of the lamina. The zosporangial cell walls are extremely difficult to see (Fig. 52, small arrowheads). With ABB/PAS staining the only structures stained and visible are the proteins of nuclei and chloroplasts of the paraphyses and subtending cells in addition to the peripheral cytoplasm around the large vacuole (Fig. 53).

2.5.2. Ultrastructure

Ultrastructurally the most obvious feature of both the post-exuviation/pre-spore release sorus and the post-spore release sorus is the extent of cytoplasmic vacuolation. The only recognizable organelles are the chloroplasts, the nucleus and a few mitochondria.

2.5.2.1. Paraphyses of the Post-exuviation/Pre-spore Release Sorus

The cytoplasm of these paraphyses is almost completely vacuolate (Fig. 50), and with the exception of a few isolated masses of homogeneously electron opaque dense cytoplasm there is no ground cytoplasmic matrix or free ribosomes left
The nucleus and chloroplasts of the apical cytoplasm along with a few mitochondria are the only recognizable organelles (Fig. 50). The dictyosomes are extremely difficult to identify amongst the profusion of vesicles and vacuoles. The nuclei of these paraphyses now contain distinct patches of chromatic material. The chloroplasts show little change from the previous stage of development (Fig. 50). The cell walls of the paraphyses are still intact and the region of former association with the outer cell wall of the scrus remains distinguishable (Fig. 50, arc between the arrowheads). At this stage of development there is a notable difference between the thickness and densities of the cell walls of the paraphyses and adjacent zoosporangia. In this material the cell walls of the paraphyses when cut perpendicularly are approximately 0.3 μm in thickness and of moderate fibrillar density while the lateral walls of the indehiscent zoosporangia are only 0.1 μm in thickness and electron dense. Only in zoosporangial tip apical thickenings does the fibrillar density of the sporangial cell wall diminish (Fig. 55).

2.5.2.2. Paraphyses of the Post-exuviation and Post-spore Release Scrus

The outstanding feature of these paraphyses is a further increase in vacuolation (Fig. 51 and 55). There is little if any change in the nucleus or chloroplast morphology although
they are less electron dense. Occasionally, however, a chloroplast may show signs of disorganization (Fig. 56, arrow). The masses of homogeneous electron opaque material are absent and the occasional mitochondrion is observed (Fig. 56). The matrix material of the mitochondria has become very translucent (Fig. 56). Except for a few electron opaque inner fibrillar layers the cell walls of the paraphyses may show signs of erosion, especially at their apical ends (Fig. 55 and 56). Furthermore, it is of great interest to note the differential in cell wall thickness between those sporangia which still contain zoospores as opposed to the cell walls of empty sporangia (Fig. 55).

2.5.2.3. The Subtending Cells

The subtending cells are completely vacuolate with little peripheral cytoplasm (Fig. 52 and 56). There remain a few ribosomes associated with the nuclear envelope and the nucleoplasm is extremely condensed.

2.6. Morphological Observations of Sori and Paraphyses with SEM

In the course of this work attention has often been directed to shapes of paraphyses and zoosporangia in particular, as well as morphology of the extracellular space in
sori. From scanning electron micrographs of young and intermediate soral tissues which have been freeze-dried it is apparent that both the extracellular spaces and reticular material contained therein are not artifactual (Fig. 57 and 62). Furthermore, mature sori which have just undergone exuviation or removal of the outer cell wall have also been examined with the SEM after freeze drying. The sporangia and paraphyses are clearly visible at points where they have separated from each other (Fig. 64). Their tips are partially obscured by a layer of amorphous material, mucilage (Fig. 64). The attenuate shape of the paraphyses is confirmed in the freeze-dried material as well.

Soral material destined for TEM has also been examined with the SEM by two other techniques. The first is by critical point drying after normal dehydration and the second is by breaking the plastic embedded blocks of tissue and then mounting them directly on stubs for viewing after gold coating. The observations afforded by both of these techniques confirm results from sectioned and freeze-dried material. The paraphyses are attenuated throughout development of the sorus (Fig. 58-60 and 61), while the zoosporangia remain swollen and turgid during their elongation in the extracellular space of the sorus (Fig. 58-61 and 63). The nature of the extracellular space and its reticulate contents emanating from the tips of the sporangia are even more clearly seen in the critical point dried material than in the freeze-dried (Fig. 58 and 61). It
should be noted here that the tips of the paraphyses and sporangia of mature sori prepared by means of critical point drying do not have an amorphous material clinging to their tips (Fig. 63). The zoosporangial tips are easily identified in this material by their puckered tips while the paraphyses are equally distinct with their fuzzy apical tips (Fig. 63).

The outer cell wall can be easily removed from mature sori and it can be retrieved from the surface of mature sori which are releasing zoospores. This outer cell wall can be manipulated with forceps and air dried directly on an SEM stub for subsequent coating and viewing. Its inner surface, where paraphyses formerly were attached (Fig. 65), is easily distinguished from the outer surface which was often found to be coated with epiphytic bacteria (Fig. 66).

2.7. Morphological Observations of sori on Laminarian Hybrids and Manipulated laminae of Nereocystis

The author has had the opportunity to examine material of young and old sori from a hybrid of *Pelagophycus* and *Macrocystis* which was grown in culture by M. Neushul (Sanbonsuga and Neushul 1978). Although the parental types are of different genera they are members of the same order and family as *Nereocystis* and likewise produce zoosporangial sori. It is believed that some observations can be made on these
somewhat irregular sori which may contribute to our understanding of normal zoosporogenesis. Two observations may be made in this light. First in this hybrid the inception of zoosporangial initial cells is retarded (Fig. 67) and with this retardation there is also a lack of extracellular space. Even when they do finally appear they are diminutive but they are still associated with the appearance of small extracellular spaces (Fig. 68). Secondly the paraphyses of these sori are not metabolically quiescent as the outer cell wall is quite thick (Fig. 68), and there is a very thick layer of material which has apparently been deposited since inception (Fig. 68).

During the course of this study one opportunity to hold a thallus in culture presented itself. During this time five fertile laminae of *Nereocystis* were suspended so that one side was always up. The sori of *Nereocystis* develop on both sides of the lamina. At the end of one week these lamina were collected and selected portions fixed and sectioned. It was discovered that although sori did develop on both sides of the lamina there was an obvious differential rate of development between the two sides (compare Figures 69 and 70), which may be presumably attributed to differences in illumination.

3. Discussion
3.1. Sorus Inception

The process of sorus inception has been described by many authors. The first very brief description (Thuret 1850) established the presence of paraphyses, considered by that author to be epidermal cells of a particular structure. It was also observed that the paraphyses contained several pigmented granules, presumably chloroplasts. Their cell walls were described as being thin basally while thickened and mucilaginous apically. It was further noted that their apices were laterally joined by their mucilaginous summits, while their bases were separate and constricted, and the sporangia were present in these spaces. The outer wall or slime cuticle according to Fritsch (1945) was faceted where the paraphyses tips insert (Thuret 1850). Finally it was observed that the spores ultimately fill all but an apical portion of the sporangium and that the paraphyses and sporangia are superficial and protrude perpendicular to the lamina (Thuret 1850), in other words they are superficial.

Subsequently, MacMillan (1899) described morphological stages in the inception and development of the sorus of *Nereocystis*. Inception was described as beginning with the periclinal division of epidermal cells (the term meristoderm was applied by Sauvageau, 1918). The superficial progeny of this division were described as larger and more densely
staining than their subepidermal counterparts (MacMillan 1899) and their subsequent elongation was said to produce the typical 30-40 um long club shaped paraphyses. The concurrent attenuation has been suggested to produce the thinner, 2 um basal points of attachment to the subepidermal cells and afford the extracellular space in which the zoosporangia would develop (MacMillan 1899). The sporangia were described as originating by hemispherical budding of the basal cell beside the base of the paraphysis. This sequence of events, in other words, the periclinal division; the outer cell elongation and attenuation; and the budding of the subepidermal cell to protrude the zoosporangial mother cell into the extracellular space afforded by paraphyses basal attenuation is essentially reiterated in the review of Fritsch (1945). Papenfuss (1951) has suggested that it is lateral enlargement of the basal or subtending cells which produces the basal attenuation of paraphyses and the extracellular space of the sorus. In light of the numerous descriptions of this phenomenon it was disturbing not to be able to find any youngest sori on the laminae of Nereocystis in which all of the morphological constituents of the mature sori subtending cells, zoosporangia, paraphyses, extracellular lumen and outer cell wall, were not present. It is thus concluded that the various events of sorus inception must either occur in rapid sequence or perhaps simultaneously. In fact morphological stages figured by MacMillan (1899) were only obtainable for this study from sections taken across the sorus margin (see Materials and Methods). It is of interest to note
that although MacMillan (1899) had numerous thalli at his disposal, information to clarify this problem was not indicated. Furthermore, it is probably no coincidence that Sauvageau (1918) makes it clear that he located the early stages of sorus development at the margins of sori. This approach is perhaps not uncommon (Kaneko, personal communication, Chi 1973, Toth 1974), although rarely stated in publications. The only possible exceptions to this author's knowledge are for Chorda (Toth 1976a) and Macrocystis (Chi 1973) where observations of regions where early stages of sorus development could in fact be located with some degree of certainty. However, observations at the EM level of the early stages are notably lacking.

Thus for Nereocystis at least it seems that the events of inception requisite to the establishment of paraphysis initials, zoosporangial initials, extracellular sorus lumen and subtending cells occur quickly if not simultaneously, and that observations of early stages of inception are rather observations from the margins of the already extant sori possessing all constituents.

From the observations made in this study it is clear that the morphological events which must occur in the process of inception are periclinal division of the meristoderm cells, continued elongation of the outer progeny cell, the schizogenous production of an extracellular lumen between adjacent paraphysis initials and further enlargement and
division of the internal progeny or subtending cell. If these events are considered to occur simultaneously or very quickly as indicated here then they are strikingly similar to the sequence of events now believed to occur during mucilage duct inception (Chapter 2). There are several lines of evidence to support the similarities of these two processes as discussed below.

The most obvious difference between the two systems is of course the end products, mucilaginous material in one case and zoospores in the other. Closer inspection, however, revealed that the distinction between the two processes is not so clear. From observations at sorus margins of a schizogenous origin of new sorus lumen with a concomitant deposition of TBO metachromatic material in the new lumen, the evidence for a similarity is strengthened. Surprisingly, the hyperactive morphology of the golgi apparatus in zoosporangial initials described here and in Appendix 1 before the onset of meiosis implies a primarily secretory function. This function is here interpreted as bipartite in that new fibrillar cell wall material as well as a nonfibrillar mucilaginous transient component must also be in the process of synthesis and secretion. Presumably the clearly fibrillar contents of these secretory vesicles is destined to be new cell wall of the sporangium while the remaining material is to be the mucilaginous contents of the new sorus lumen. In fact if the zoosporangial initials are now seen as growing secretory cells
in their earliest stage of development, in other words meristematic, then numerous other observations here and in Chapter 2 make more sense. It is the author's belief that earliest stages of sporangium development are absent from ultrastructural literature in the Laminariales because of the physical infirmity of the cell walls of this tissue, (only in Toth and Markey 1973 is a premeiotic sporangium shown at the ultrastructural level). The golgi apparati of these cells are almost identical in appearance to those of the meristoderm cells of the transition and expansion zones and are presumably secreting new fibrillar and transient material (Chapter 1). Furthermore, if the current auxin, hydrogen ion model of cell wall loosening in elongation (Cleland and Rayle 1978) is assumed to be operating here then the instability of this tissue as well as that of the meristoderm of transition zone and expansion zone might easily be explained. It might be asked why, then, is the mucilage duct system so easily demonstrable in the mature lamina with the EM (Schnepf 1963)? In the current interpretation of mucilage duct differentiation and development (Chapter 2) all of the morphological processes occurring during sorus inception and meristoderm cell activity which tend to loosen walls will have ceased in the mature lamina. It is suggested that the high degree of golgi activity observed in Laminaria secretory cells by Evans, Simpson and Callow (1973) are a response to wounding which was not controlled for in their experiments. Further evidence for the artifactual nature of the secretory cell structure in
earlier observations (Evans, Simpson and Callow 1973) comes from the obvious difference in the compactness of the secretory cell walls crossed by secreted mucilage as opposed to those adjacent to surrounding cortical or secretory cells (Chapter 2 Fig. 20). It is proposed that the secretory cells of the mucilage duct system will prove to be more similar morphologically and synthetically to the meristoderm cells of transition and expansion zones and the zoosporangial initials when examined with the TEM. Secretory duct system inception was seen to entail a special periclinal division which produced an internal progeny cell maintaining meristematic activity while both division and secretion continued (Chapter 2). Schizogenous production of the mucilage duct system lumen was observed. This lumen received the secretory products of the secretory initial cell. This secretion causes the enlargement and ramification of the system (Chapter 2.). The outer, still meristematic, progeny cell was seen to continue elongation, division and secretion to the lamina surface (Chapter 2). Morphological differences in mucilage duct system lumen and sorus lumen are easily explained by two points. First it should be remembered that mucilage duct system inception occurred in the meristematically active tissues and at point loci which were separated from each other during development by continued anticlinal divisions of the active meristoderm cells. Furthermore, the secretory initials continued to form cups of cells, hence, the volume of mucilage is greater (Chapter 2). In contrast the scri differentiate in essentially static
tissues where lamina expansion and thickening have ceased. Therefore, the sorus lumen immediately becomes one localized continuous lumen with no lateral separation in the plane of the lamina and the external progeny cells of sorus inception are paraphysis initials. Although it has not been considered before, the paraphyses are seen here as essentially comparable to the external meristematic progeny cells in that they continue to elongate and secrete to the outside laminar surface and not to the sorus lumen. The only differences are the short duration of paraphysis elongation and the lack of continued periclinal and anticlinal divisions. However, it has been noted in the review of Ohmori (1967), that a species of *Chorda* normally produces septate paraphyses. Even more interesting, in the regions of sori where zoosporangia fail to develop, (Angst 1929, Kaneko 1972) paraphyses are also septate. In other words they are meristematic and continue to both elongate and divide periclinaly. Another feature of these abnormal regions illustrates the secretory nature of the zoosporangial initials. If the paraphysis initials produced the extracellular lumen by their own secretory activity one might expect these lumen to be present amongst the abnormal septate paraphyses of Angst (1928) and Kaneko (1972), but this is not the case. Furthermore, it is very interesting to note that in the young sori of the hybrids (Neushul 1978, and Fig.68 here) where the appearance of zoosporangia is delayed the extracellular sorus lumen is also absent. Still more favorable evidence supporting zoosporangial initial secretory
function is cell shape. It is the zoosporangial initials which exhibit a turgid appearance while the inverted flask-shaped paraphysis initials of the sorus have a basally compressed morphology (Fig. 6). Also the schizogeny has begun in sorus margin tissues before the subtending cells have budded as evidenced by the small wedge shaped extracellular lumen adjacent to the end of the inception periclinal cell walls (Fig. 2). A final and very interesting case in favor of the similarities between sorus inception and mucilage duct system inception as functions of the meristoderm is the cases of sori in which subtending cells of the inception division produce secondary paraphyses rather than zoosporangia (Nishibayashi and Inoh 1958a, b and 1963, Ohmori 1967) in their first division as well as a sorus lumen. Although this may at first seem to deny the secretory role of the zoosporangial initial, such is not the case. Indeed this is easily explained. Paraphyses like the meristoderm cells continue to secrete both fixed and transient material to the surface of the lamina as will be seen in the subsequent discussion. The often reported thickened apical paraphyses appendages (Fritsch 1945) are due to paraphyses secretory activity which is evidenced here by maintenance of the outer layers of finely flocculent material (Fig. 30). The thickened tips of Pelagophyclus-Macrocytis paraphyses hybrids shows that the paraphyses have been active and the differential in soral features suggest that this outer deposition is dependent on available energy (Fig. 69 and 73). Thus, the secondary paraphyses of the sorus of Alaria
(Nishibayashi and Inoh 1958a,b and 1963) probably behave as the zoosporangial initials of other genera and secrete the mucilage to produce the sorus lumen into which the sporangia will later develop. Finally, it has long been established that sorus development was exclusively a meristoderm phenomenon. Such was not the case for the mucilage duct system (Fritsch 1945), however, from Chapter 2 it is apparent that mucilage duct system inception is also a phenomenon of the meristoderm.

3.2. Paraphysis Cytomorphogeneses

From the previous section of discussion it would seem that sorus inception to some degree involves a return to meristematic activity in localized regions, the sori. This is by virtue of the periclinal division and elongation of the paraphyses initials. The latter is the most pronounced feature of paraphysis morphogenesis. From Chapter 1 it is apparent that these young sori exist in photosynthetically mature tissues where mitotic activity and perhaps cell enlargement have ceased. Furthermore, cell walls of adjacent meristoderm cells are stable in contrast to those of their predecessors in the transition and expansion zones of the lamina. Thus the fragility of the cell walls of both the paraphyses and zoosporangial initials of these youngest sori is in accord with the similar observations in the transition and expansion zones of the laminae. This strengthens the argument
for the meristematic nature of sorus inception and development. This view is furthermore supported by the apparent golgi activity of these youngest paraphyses. Although it is not as prominent as in the active cells of meristematic transition and expansion zones it must be remembered that these cells are already post-inception. Furthermore, the presence of coated vesicles in these paraphyses cells and the presence within the apical cytoplasm of numerous vesicles and uneven apical plasma membrane are reminiscent of the transition and expansion zone cells. Yet in spite of the meristematic nature of these early paraphyses their well developed chloroplasts reflect their origin from presumably photosynthetically mature tissue. Another point of interest is their supply of lipid material. This perhaps reflects a retention and storage of photosynthates in contrast to neighboring vegetative meristoderm cells which are presumably actively exporting photosynthates to meristematic tissues of the transition and expansion zones.

The nuclei of these paraphyses are quite homogeneous excepting small nucleoli and homogeneously fibrillar regions as seen in adjacent meristoderm cells, which may attest to the determinant nature of their growth. Thus it would seem ultrastructurally that the young paraphyses are active in elongation and cell wall growth. However, their basally attenuate morphology might seem to deny this in light of the role turgidity is assumed to play in cell enlargement (Cleland and Rayle 1978, Kristen 1976, Prat, Vian, Preis and Roland
1977). Such a morphology is not an artifact as the attenuation is present in both freeze-dried fresh material as well as fixed and critical point dried material. This attenuation becomes more pronounced with age and its persistence after spores have been released from the neighboring sporangia further suggests that this morphology is acquired during development. The variations in cell wall thickness between paraphyses, loaded zoosporangia and dehisced zoosporangia further supports this point of view. The initial mode of acquisition of this basal attenuation has been linked with zoosporangial initial formation and secretory activity in the previous discussion and presumably its continuation follows from the pressures exerted by zoosporangial enlargement. Although it has been suggested by some authors (Sauvageau 1918, Kemp and Cole 1961, Ohmori 1967) that paraphyses squeeze spores from sporangia, evidence presented by Toth (1976b) for the mechanisms of zoospore release from unilocular sporangia and in this work would seem counter to such an interpretation.

By the intermediate stages of soral development when meiosis occurs, paraphyses have caught up with the adjacent meristoderm cells, their cell walls have become stabilized and upon the outer surface of their outer cell wall a layer of fine flocculent material has developed. The activity of the golgi apparatus has increased and apical vesiculation kept pace. However, coated vesicles are now absent. The correlation of golgi activity with the presence of the outermost layer of
Flocculent material is evidenced by the decline of flocculent material thickness with the death of underlying paraphyses and by its increase in thickness after meiosis. These cells have also arrived at a state where endomembrane system luminal continuities are difficult to locate as is the case in the adjacent meristoderm cells of these and youngest sori. This is not believed to coincide with export of photosynthates to internal medullary elements, although the decreased quantities of lipids implies some export or utilization of the material. It is essential to recall that zoosporangial initials are meiotic at this point in development and just entering a phase of rapid nuclear, mitochondrial, and chloroplast replication and enlargement of other membrane systems. Furthermore, these paraphyses still maintain protoplasmic continuity with zoosporangial cells by way of the pitfields between themselves, subtending cells and zoosporangia. It seems likely then that export from paraphyses to developing zoosporangia rather than internal cortical cells may have begun. This is supported indirectly by observations of Lobban (personal communication) that carbon fourteen assimilated distal to the sori is not diverted by developing sori but passes on to the intercalary meristem tissues. As was observed by Sauvageau (1918) the paraphysis/outer wall junction is firmer than the basal attachment of paraphyses at this stage. In sharp contrast to both younger and older sori (Chapter 2), indeed the dumping of mucilage from the mucilage duct system into the sorus lumen will tear the paraphyses loose at their bases rather than
lifting the outer wall from their apices. Although there is very little vesiculation, regions of basal cytoplasm become segregated by double membrane bound cisternae which subsequently lose the internal single membrane. This process apparently continues into the paraphyses of the oldest sori. It seems most likely to represent the basal provacuolar system of the adjacent meristoderm cells (Chapter 1) which, however, now shows more similarities to that described by Khera and Tilney-Bassett (1976) in higher plant meristem cells as well as some red algal cells (Goff 1979) and also more closely resembles cytosegrosome formation characteristic of the process of autophagy (Matile 1975, review). This would then be perhaps an acceleration of the process seen in adjacent meristoderm cells. This is further supported by figures of autophagy of some mitochondria and a generalized increased vacuolation of the cytoplasm. It is noteworthy that this vacuolation continues to the extreme in abscised sori and that vacuolation and senescence go hand in hand has been reported in both Ectocarpus (Oliviera and Bisalputra 1977a,c,d) and Ascophyllum (Rawlence 1973). This is of particular interest in the brown algae as the role of pyrenoids in brown algae remains obscure (Russel 1973). From these two tissues at least it would appear that they correlate with the culmination of the differentiation process. Two more lines of evidence support the onset of senescence in the paraphyses of the oldest sori and those which have released spores. The first is that not only is the outermost layer of flocculent material
dissipated but also there is a break down of cell wall cohesion between layer 1 of the outer cell wall and 2/3 which results in exuviation of the sorus. The second and most conclusive line of evidence is that once the spores have been released from the sorus (in vitro as well as in the field) the tissues remaining lack pigmentation. Thus even though, as described here, the paraphyses still contain chloroplasts, nuclei, a few mitochondria and the plasma membrane is still intact, they have lost photosynthetic capacity.

3.3. Summary, Sorus Development and the Role of Paraphyses

In light of the observations reported here and the preceding discussions it is now possible to propose a model for sorus development with particular emphasis on the paraphyses and their mechanical interactions with other components of the sorus such as the sporangia, subtending cells, sorus lumen and the outer cell wall. A knowledge of these interrelationships is critical for any understanding of the significance of each soral component in the development of the sorus and the events occurring at maturity which expose the sporangia. It must be recalled that the actual sequence of events in the process of inception is not known and can only be hypothesized from observations at the margins of youngest sori. Regardless of the actual sequence of events meristoderm cell enlargement and periclinal division are certainly the first events of sorus
inception. The precise timing of anticlinal wall schizogeny as well as the budding and mitosis of the internal subtending cells remains undetermined. It is certain that these events occur quickly since no intermediate stages are found in youngest sori, only those with all components present. Therefore, it is proposed that sorus inception is very similar to mucilage duct system inception and that these events are essentially simultaneous for the bulk of the sorus.

It is the combination of the middle lamellar dissolution of schizogeny along with subtending cell budding and zосsporangial initial secretion which compresses the basal portions of the paraphyses initials. For the bulk of paraphyses elongation, the outer cell wall is firmly attached to the apices of paraphyses. This cell wall, not cuticle, is simply the outer cell wall of the former meristoderm cells. From the higher plant model of cell elongation (Cleland and Rayle 1978) turgor pressure or mechanical force is required to continue the elongation process. Paraphysis morphology does not appear turgid. Although these cells may maintain turgidity, their attenuate morphology is interpreted as resulting from the continued secretory activity of zосsporangial initials into the sorus lumen which is a closed volume while paraphyses remain attached to the outer cell wall. This sorus volume can only be increased by continued paraphyses attenuation and elongation. Indeed the ovate to elliptical sporangial morphology is in agreement with turgid cell
morphology. Thus, although paraphyses may be turgid, the hydration of secretory products in the sorus lumen and the continued enlargement of zoosporangial initials which maintain a turgid morphology in effect squeeze and stretch the elongating paraphyses as long as they are firmly attached to the outer cell wall. Secretory activity of the developing zoosporangia ceases by the onset of meiosis. The morphology of young paraphysis cytoplasm and the characteristics of the outer cell wall flocculent layer imply that paraphysis secretory activity is directed to the surface and not the adjacent sorus lumen as is the case in the mucilage duct system. It is presumed that the continuing growth of the zoosporangia against the hydrated mucilaginous contents of the sorus lumen which is delimited by the outer cell wall of the paraphyses and sorus that continues to cause the elongation and attenuation of these paraphyses. It must be emphasized that this does not mean that the paraphyses are not turgid but rather that the differential between that of the paraphyses and zoosporangia and hydrated sorus lumen mucilage squeezes and attenuates the paraphyses rather than the zoosporangia. This would mean that arguments to the effect that paraphyses help squeeze spores from the sporangia at maturity is probably not tenable. Toth (1976b) has satisfactorily explained the mechanisms of sporangium dehiscence without the reliance on paraphyses. The failure of detached sporangia to release spores noted by Kemp and Cole (1961) is dealt with later (Appendix 1).
Ultrastructural observations of the cytoplasm of paraphyses revealed that they revert in some respects to a meristematic condition and then rapidly catch up with the adjacent meristoderm cells. By the time the sporangia have matured, the cytoplasmic continuity between paraphyses and zoosporangia through pitfields of subtending cells, has been terminated by zoosporangial protoplast cytokinesis. By this point in development the cytoplasm of the paraphyses is showing signs of senescence beyond anything seen in the adjacent meristoderm cells. It is tempting to suggest that the loss of cytoplasmic continuity between paraphyses and zoosporangia may somehow contribute to the accelerated senescence of the paraphyses. As in the case of damaged younger paraphyses or meristoderm cells adjacent to oldest sori (Chapter 1), when golgi function ceases, the outermost layer of fine flocculent material on the outer cell wall will dissipate and Layer 4 may also begin to degenerate. A further aspect is the presence of the deposition of dense black material on the inner surface of the apical portion of the paraphyses cell walls, Layer 1 of the outer wall. This is accompanied by the deterioration of the interface between layers 2/3 and Layer 1 of the outer wall/paraphyses junction. This process may explain the lamellar nature of the outer wall described for sori of *Nereocystis* by MacMillan (1899) which were near exuviation or removal of the outer wall. Thus even without the processes of abscission (Chapter 4) there exists a cytological process which
brings about a disjunction of outer cell wall from paraphyses apices which appears to correlate with the final stages of zoosporogenesis. This break-down of the outer wall/paraphysis tip junction coupled with the mechanical forces exerted by mucilaginous contents of the sorus lumen and turgid zoosporangia constitutes the mechanism of sorus exuviation or the removal of the outer cell wall which separated the zoosporangia from the external environment. Any mechanical disturbance which would move the laminae of *Nereocystis* will abrade the loosened, mucilage backed and deteriorating outer cell wall covering the sorus, perhaps tearing it. Thus exuviation may commence.

In conclusion the sorus is derived morphologically in a manner essentially identical to that which produces the mucilage duct system. The paraphyses in association with the developing sporangia hold on to the outer cell wall of the sorus until zoosporangia have adequately matured and signal their senescence. A corollary of paraphyses senescence is the release of the outer cell wall from their apices. It was the secure attachment to this outer wall and their return to a somewhat meristematic state which allowed for the development of a sorus lumen into which the sporangia could develop. The size of this lumen the zoosporangia regulate by their own secretory activity and enlargement. From this work it can also be concluded that the mucilaginous contents of the sorus lumen might account for some of the mucilage characteristic of
spore releasing exuviated sori as well as ruptured mucilage ducts (Chapter 2). Mucilaginous appendages have not been observed on the apices of these paraphyses. They are common on some members of the Laminariales (Fritsch 1945 and Fig. 68), and may also be a source of mucilage at the time of exuviation.
CHAPTER 3 FIGURES, SORUS AND PARAPHYES DEVELOPMENT
Plate I

Figure 1.a. Diagram of the whole thallus. The remaining chapters are based on observations made from the youngest, intermediate and the oldest or most distal sorus of the fertile laminae.

Figure 1.b. Cross section of the youngest sorus of a lamina. All morphological constituents of the sorus are present yet there are no gross morphological signs of sorus release.

Figure 1.c. Cross section of an intermediate aged sorus. Signs of sorus abscission commencement are at its margins.

Figure 1.d. The mature sorus. The outer cell wall may be loose and tissue deterioration at the margin has begun.
Plate II

Figure 2. The margin of a youngest sorus. A few paraphysis initials have begun to elongate (arrowhead) and the first evidence of the inception of a zoosporangial initial and the extracellular lumen of the sorus may be found (arrows). Note the lack of extracellular lumen where zoosporangial initial inception is not visible. Scale equals 10 um.

Figure 3. Youngest sorus margin. Closer to sorus margin budding of subtending cells to produce zoosporangial initials is further along (arrows) and the extracellular space has enlarged. Scale equals 10 um.

Figure 4. Youngest sorus margin still closer to the sorus. Paraphysis initials have begun to elongate and attenuate at their bases. Where zoosporangial initials are present, they are larger and paraphyses are separated from each other through schizogeny of their anticlinal walls. Away from budding zoosporangia adjacent paraphyses may be adherent (*). Scale equals 10 um.
Figure 5. The subtending cell products. Subtending cells (*) may produce two zoosporangial initials (1 and 2). Scale equals 10 μm.

Figure 6. The youngest visible sorus. Here all morphological constituents are present. Note the zoosporangia, paraphyses, subtending cells, extracellular lumen (*) and outer cell wall. Remnants of its extension into the former anticlinal walls are visible (arrowhead). Staining with TBC. Dense staining of outer wall is metachromatic purple as is the reticulum of material in the sorus lumen indicating acid and/or sulfated polysaccharides. Scale equals 10 μm.

Figure 7. AB/SO staining of youngest visible sorus. Predominant stain here is alcian blue for acid polysaccharides. Note more dense staining of outer layer of outer cell wall. There may be some safranin 0 positive material sulfated polysaccharides extending from zoosporangia (arrowheads), and within the apical cytoplasm of the paraphyses (arrows). Scale equals 10 μm.

Figure 8. ABB/PAS staining of youngest visible sorus. Note ABB protein staining of the cytoplasmic constituents with the exception of vesiculate areas in paraphyses and vacuoles. The outer layer of the outer cell wall and middle lamellae (arrowheads) are PAS positive for neutral
polysaccharides while the reticulum of the extracellular lumen (*) does not stain. Scale equals 10 μm.
Figure 9. Paraphyses of a youngest sorus. Note centralized nucleus and mix of chloroplasts and vesicles in the apical cytoplasm. There is some basal vacuolation. Scale equals 1 um.

Figure 10. Nucleus of paraphyses of youngest sori. Nuclear envelope occasionally interrupted by nuclear pores (arrowhead). The nucleolus and homogeneous regions (*) are the most prominent features. There is little chromatic material. Scale equals 1 um.

Figure 11. A dictyosome adjacent to the nucleus. See associated hypertrophied golgi vesicles. Scale equals 0.5 um.

Figure 12. Two dictyosomes located basally on the nuclear envelope. Few hypertrophied vesicles present although the nuclear envelope displays morphological features associated with vesiculation (arrow). Large electron translucent regions may contain presumptive lipid droplets. Scale equals 0.5 um.
Plate V

Figure 13. A chloroplast from youngest paraphyses. They are characterized by a modest number of stacks of thylakoids, occasional plastoglobuli, ring genophore and chloroplast envelope and ER. Scale equals 0.5 μm.

Figure 14. Apical cytoplasm of a paraphysis from a youngest sorus. Electron translucent regions without a delimiting membrane (L), membrane bound golgi vesicles (*) and chloroplasts dominate the apical cytoplasm. Coated vesicles may be observed in the golgi region (arrowhead). The apical region of the plasma membrane (top of figure) is cavitated in contrast to adjacent anticlinal regions. Scale equals 1 μm.

Figure 15. Exocytotic vesicle on apical region of the paraphysis plasma membrane. Scale equals 0.5 μm.

Figure 16. Secretory paraphysis apical cytoplasm. Presumptive secretory vesicles at apical paraphysis plasma membrane. Note associated coated vesicle (arrowhead). Scale equals 0.5 μm.
Plate VI

Figure 17. Zoosporangia from a youngest sorus.
Metachromatically reddish-purple nuclear caps presumably acid and/or sulfated polysaccharides are seen in TBO stained material (arrowheads). Scale equals 10 um.

Figure 18. AB/SO stained youngest sorus zoosporangia.
Nucleus is basally capped with alcian blue positive material indicating acid polysaccharides (arrowheads). Scale equals 10 um.

Figure 19. Basal hemisphere of a zoosporangial initial nucleus. Note prominent golgi apparatus associated with numerous hypertrophied golgi vesicles and distal cisternae. The extracellular lumen of the sorus (*) contains fibrillar material similar to the thin cell wall of the zoosporangial initial. Scale equals 1 um.

Figure 20. Golgi vesicles of these zoosporangial initials. These vesicles contain fibrillar material morphologically identical to that of the sporangial cell wall (arrows). Golgi vesicles are associated with coated vesicles (arrowheads). Scale equals 0.5 um.
Plate VII

Figure 21. Intermediate aged sorus at meiosis. Paraphyses have elongated and are greatly attenuated in their basal portions. The amount of metachromatic reticulate material with TBO staining in the sorus lumen (small arrowhead) is typical. The extracellular lumen is at its greatest volume relative to that of the paraphyses and zoosporangia. The occasional metaphase figure may be observed (large arrowhead). Scale equals 10 um.

Figure 22. Intermediate aged sorus. Fixation was preceded by 24 hrs. under high light intensity. Note the density of reticulate material in the extracellular lumen of the sorus (*). Scale equals 10 um.

Figure 23. Intermediate aged sorus disrupted. Tissue has been physically disturbed after fixation, during infiltration with methacrylate. Dumping of mucilage from the duct system has torn most paraphyses loose from the subtending cells at their attenuated bases to form a blister. Scale equals 10 um.

Figure 24. Intermediate aged sorus TBO stained. Note extremely attenuated paraphyses and enlarging zoosporangia. The sorus lumen contains reticulate metachromatic material presumably acid and/or sulfated polysaccharides. The cell walls of the paraphyses, zoosporangia and the outer wall all show purple
metachromasia. Scale equals 10 μm.

Figure 25. AB/SO stained intermediate aged sorus. Density of staining here is due primarily to alcianophilia (acid polysaccharides) although there may be faint staining with SO in the sorus lumen and the outer-most layer of the outer cell wall of the sorus for sulfated polysaccharides. Scale equals 10 μm.

Figure 26. ABB/PAS staining of intermediate aged sorus. Cytoplasmic constituents stain positively with ABB for proteins while staining of the outer cell wall outer layer is PAS positive for neutral polysaccharides. Note chloroplasts (small arrowhead) and nuclei (larger arrowhead) of the paraphyses. Scale equals 10 μm.
Figure 27. Intermediate aged sorus paraphysis prior to meiosis. Zoosporangia may fall down amongst the paraphyses in the sorus lumen (*) during processing. Chloroplasts contain numerous stacks of thylakoids and are usually located distally to the nucleus. Apical cytoplasm contains numerous vesicles and there is little distinction between the paraphyses cell walls and the outer cell wall to which they attach. The dictyosomes usually are adjacent to some portion of the basal hemisphere of the nucleus. Hypertrophied golgi vesicles are not clustered around the golgi apparatus (arrowhead). Scale equals 1 μm.

Figure 28. Intermediate aged post-meiotic sorus paraphysis. The zoosporangia are too large to fall amongst the paraphyses in the sorus lumen (*). Chloroplasts are essentially distal to the nucleus which has the golgi apparatus adjacent to its basal hemisphere (arrowhead). Hypertrophied golgi vesicles are observed in the golgi region and the apical cytoplasm is vesiculate. The outer wall is distinct from the paraphysis cell wall. Scale equals 1 μm.
Figure 29. Cell wall and cytoplasm of a pre-meiotic sorus parapysis note the outermost layer of perpendicular flocculent material. Cell wall layers 1, 2/3, and 4 are present and clearly seen. Scale equals 1 μm.

Figure 30. Outer cell wall over dead paraphysis. The outer cell wall layers are as in Figure 29. Note the irregular and dissipated appearance of the outermost layer of finely flocculent material. Scale equals 1 μm.

Figure 31. Paraphysis outer cell wall and apical cytoplasm from a post-meiotic sorus. Layer 1 shows a distinguishable difference in electron opacity from layers 2/3 and 4. The outer layer of flocculent material beyond layer 4 is well developed. Golgi vesicles are crowding the apical cytoplasm. Scale equals 1 μm.
Plate X

Figure 32. Golgi apparatus and nucleus of a premeiotic sorus paraphysis. Golgi vesicles are not particularly abundant in the golgi region. The nucleoplasm is fairly homogeneous with occasional slightly electron denser patches of heterochromatin. Scale equals 1 um.

Figure 33. Dictyosomes of a paraphysis from a post-meiotic sorus. Peripheral extremities of some dictyosomes cisternae show pronounced hypertrophi. Scale equals 1 um.

Figure 34. Paraphyses of meiotic sori. See uneven staining of the basal cytoplasm with ABB. Scale equals 10 um.

Figure 35. Paraphysis basal cytoplasm from a meiotic sorus. The occasional chloroplast contains a few plastoglobuli (arrowhead) and the chloroplast endoplasmic reticulum may show hypertrophy with flocculent contents (large arrow). Prominent features of the basal cytoplasm are electron translucent regions containing membrane configurations and regions of homogeneous electron dense cytoplasm. There are peripheral cisternae of endoplasmic reticulum as well (small arrows). Scale equals 1 um.
Figure 36. Paraphysis basal cytoplasm, intermediate sorus. Electron translucent regions containing membrane configurations may be open to the intramural space (arrows). Scale equals 1 um.

Figure 37. Paraphysis basal cytoplasm, intermediate sorus. Peripheral cisternae of endoplasmic reticulum in basal cytoplasm of a paraphysis from a pre-meiotic sorus. Scale equals 1 um.
Plate XI

Figure 38. Paraphysis from a meiotic sorus. See electron translucent vesicles (arrowheads) possibly associated with a dictyosome of the golgi apparatus (arrowhead to left). Scale equals 1 um.

Figure 39. Basal periclinal wall separating paraphysis from subtending cell. Large arrowhead indicates extent of remainder of the original anticlinal cell wall of meristoderm cell which produced the paraphysis and subtending cell. Anticlinal walls of the paraphysis and the distinct inner layer of the subtending cell have been deposited since inception. Several pit fields penetrate the basal periclinal end wall of the paraphysis between opposing small arrowheads. Regions of homogeneous dense cytoplasm (*) may be enclosed by an electron translucent region with one defining membrane in some cases (arrow). Scale equals 1 um.

Figure 40. Paraphysis cytoplasm, intermediate sorus. Note electron translucent region partially delimiting a dense cytoplasmic region, defined by two membranes (arrows). Scale equals 1 um.
Plate XII

Figure 41. Section from a sorus with zoosporangia in cytokinesis. The outer cell wall may still be attached although often it may come loose during processing for EM. The extracellular sorus lumen is relatively small and crowded now by zoosporangia. Basal portions of the paraphyses are difficult to locate between the zoosporangia. Scale equals 10 um.

Figure 42. ABB stained sorus. Zoosporangial cytokinesis is visible (small arrows). Some have completed cytokinesis (to left) while some have not (center). Paraphysis apical cytoplasm reaches its densest staining and still occupies the same location (larger arrow). Scale equals 10 um.

Figure 43. Paraphyses and outer cell wall of a sorus in cytokinesis. Cell walls of the paraphyses are distinct from the outer cell wall and partially separated. A skirt of outer wall material is still associated with the paraphyses anticlinal walls (arrows) capping the paraphysis tips. The paraphyses apical cytoplasm is crowded with a varied assortment of vesicles and chloroplasts. The nuclei may be quite polymorphic and the nucleoplasm is increasingly chromatic. The dictyosomes are difficult to identify in the numerous vesicles and condensed cytoplasm. Zoospore is seen in
sporangium at lower right. Scale equals 1 um.

Figure 44. A region of advanced paraphysis, outer wall disjunction. Note outer wall deterioration and lack of flocculent material. Separation indicated by double ended arrows. The skirt of outer wall material sheathing the paraphyses tips has disjoined. Paraphyses tips are rounded up. Scale equals 1 um.
Plate XIII

Figure 45. Paraphysis tip at separation from the outer wall. Note separation at the interface between layers 1 and 2/3 of the outer cell wall/paraphysis junction (small arrowheads). Some of layer 1 appears to remain with the outer wall (arrow). Layer 2/3 shows lesions (*). At this early stage of disjunction flocculent material persists outside layer 4 (between parallel bars). Scale equals 1 um.
Figure 46. **Paraphysis apical cytoplasm during sorus cytokinesis and exuviation.** Chloroplasts usually possess a pyrenoid containing chloroplast matrix and numerous electron dense granules about 20 nm in diameter. These granules are also aggregated between the stacks of thylakoids within the chloroplast. There is a variety of vesicles present. Note vesicle containing granules adjacent to the dictyosome. Scale equals 0.5 um.

Figure 47. **Paraphysis cytoplasm, mature sorus.** Mass of dense homogeneous cytoplasm contained within double membrane cisternae and assortment of various vesicles. Scale equals 0.5 um.

Figure 48. **Paraphysis cytoplasm, mature sorus.** Presumptive cytosegresome containing a mitochondria. Note the ER outside of the double membrane of mitochondria (large arrowhead), and the light profiles of cristae (small arrowheads). Scale equals 0.1 um.

Figure 49. **Paraphysis cytoplasm, mature sorus.** Pyrenoid and adjacent mass of dense cytoplasm contained within a single membrane. Note the narrow neck of the pyrenoid. Scale equals 0.5 um.
Figure 50. Paraphysis tip after exuviation, before zoospore release. With the exception of residual masses of dense cytoplasm (*) and organelles such as chloroplasts, nuclei and occasional mitochondrion, most of the cytoplasm is vesiculate. The region of former attachment to the outer wall is still distinguishable between the large arrowheads. Scale equals 1 μm.
Plate XVI

Figure 51. Sorus after exuviation and dehiscence of most zoosporangia. Note empty zoosporangia (arrows). Sporangia withholding zoospores have intact thickened tips (*) and zoospores exhibit organized eye spots (arrowheads). Paraphyses are extremely vacuolate leaving chloroplasts, nuclei and occasional mitochondria identifiable at the light microscope level. Material is thick-thin sectioned from Spurr's, osmium fixed and TBO stained. Scale equals 10 μm.

Figure 52. TBO stained released sorus, retrieved from a holding tank. Zoosporangial cell walls have lost metachromasia (small arrowheads) while paraphyses retain it. Although vacuolate paraphyses show no marked swelling. Broad paraphysis morphology (*) is partly due to the plane of section. Bacteria are amongst the paraphyses and zoosporangial cell walls (larger arrowheads). Scale equals 10 μm.

Figure 53. ABB/PAS stained, released sorus retrieved as above. Except for the apical cluster of organelles (arrowheads) and the occasional mitochondria (arrow) the paraphyses are vacuolate. The basal cluster of ABB positive organelles are in the subtending cells. Scale equals 10 μm.
Figure 54. AB/SO stained sorus as above. Staining here is for AB. Paraphyses and subtending cells are stained lightly compared to cortical cells. Zoosporangial cell walls do not stain with either the AB or the SO. Scale equals 10 um.
Figure 55. Mature, post-exuviation sorus subsequent to release of most zoospores. Vacuolation of the paraphyses cytoplasm is complete. Chloroplasts and nuclei are recognized in the apical regions where the cell wall appears to have begun thinning. Note empty zoosporangia (arrow). Some zoosporangia still contain zoospores and their thickened tips (*) are intact. Zoosporangia may still deform neighboring paraphyses. Zoospore maturity is attested to by their organized eye spots (arrowhead). Scale equals 1 um.
Figure 56. Paraphysis apical cytoplasm from a released sorus. The nucleus, chloroplasts and mitochondria are the most persistent and recognizable organelles. Chloroplast deterioration commences with thylakoid hypertrophy (arrow). Note the layer of electron opaque material on the inner surface of the apical portions of the paraphysis cell wall (large arrowhead) which is further disorganized. Scale equals 1 μm.
Plate XIX

Figure 57. Live young sorus tissue cut and freeze-dried. Zoosporangia and reticulate material of the sorus fall out from between the already somewhat attenuate paraphyses which are firmly attached to the outer wall. Scale equals 10 μm.

Figure 58. Critical point dried intermediate aged sorus. Note attenuate paraphysis and club-shaped zoosporangia with associated dense reticulum of material within the sorus lumen (arrowhead). Scale equals 5 μm.

Figure 59. Fracture face through Spurr's embedded intermediate aged sorus. Note the attenuate paraphyses and shorter turgid club-shaped zoosporangia. Cortex is also visible. Scale equals 10 μm.

Figure 60. Detail of paraphyses prepared as in figure 59. Letter P is on the plasma membrane and the small arrows indicate where the fracture plane dropped through the paraphysis cell wall to the plasma membrane. Paraphyses tips (top) are still fairly embedded in the outer cell wall. Scale equals 10 μm.
Plate XX

Figure 61. Critical point dried almost mature sorus. Note extremely attenuate paraphyses and reticulum of material associated with tips of the turgid zoosporangia. Scale equals 10 μm.

Figure 62. Freeze dried almost mature sorus. Note leaning out zoosporangia and associated reticulum of material. Paraphyses are still attached to the outer cell wall. Scale equals 10 μm.

Figure 63. Critical point dried mature zoosporangial sorus. Paraphysis tip/outer wall junction was weakened such that the outer wall was lost during processing. Puckered tips are of zoosporangia while the fuzzy tips are of paraphyses. Scale equals 1 μm.

Figure 64. Mature sorus, freeze dried zoosporangia and paraphyses tips coated with mucilage. Material is post-exuviation/pre-release. Scale equals 50 μm.

Figure 65. The inside surface of the outer wall of a mature sorus. Retrieved after natural exuviation in the field, fixed in glutaraldehyde, and air dried. The surfaces where paraphyses formerly attached (*) are bordered by a continuous skirt (arrowhead). Some of the outer surface has folded back on the inner (arrow). Scale equals 1 μm.
Figure 66. The outer surface of the outer wall of a mature sorus. Prepared as in figure 65. The depressions (*) coincide with paraphyses attachment surfaces underneath as do the ridges with the skirts. Rods and spheres are epiphytic bacteria. Scale equals 1 um.
Figure 67. Young sorus of a hybrid thallus from *Pelagophycus* and *Macrocystis*. Zoosporogenesis is late. Note the lack of extracellular space between paraphyses. One subtending cell has begun to bud (arrowhead). The outer wall is made of two distinct layers. The outer wall is continuous and has interposed between it and the paraphyses tips disjunct pads of intensely metachromatic material (arrowhead large). Scale equals 10 um.

Figure 68. More mature sorus from same thallus as figure 67. Where zoosporangia (small arrowheads) have been initiated extracellular spaces (*) have formed. The old outer wall (layer between arrows) has been lifted from the tips of the paraphyses by pads of material associated with their tips. Scale equals 10 um.

Figures 69 and 70. Sorus material taken from directly opposite surfaces of a lamina excised from the pneumatocyst and fixed with one side always up in a floating dock holding tank for one week.

Figure 69. Sorus material on one side of excised lamina. Note the sizes of paraphyses and zoosporangia which appear to be post-meiotic but pre-cytokinetic. Scale equals 10 um.
Figure 70. Sorus material on other side of excised lamina. Note the apparent lack of zoosporangia and shorter paraphyses. Scale equals 10 um.
VI CHAPTER 4. ABSCISSION

1. Introduction

According to Rigg (1912) it was a graduate student, S. M. Zeller who in 1911, working at the Puget Sound Marine Station, observed that the soral patches of *Nereocystis luetkeana* (Mert.) P. and R., "disappear from the frond with the maturing of the spores in them." These early observations have been subsequently confirmed and somewhat extended (Rigg 1917, Hartge 1928, and Scagel 1947). However, the mechanism of disappearance or release has remained undetermined. The only other genus for which sorus release has been indicated is *Lessoniopsis* (MacMillan 1900), and the account is both brief and inconclusive, "In this species the sorus itself falls away from the frond and the old leaves must present a very different appearance from the younger ones." In a subsequent examination of the sporophylls of *Lessoniopsis* (Griggs 1909) sporophyll differentiation is clearly defined but no comment is made concerning sorus release. The closely related genus *Pelagophycus* would seem to be a likely place for sorus release to occur. Frye (1930) reports that *Pelagophycus* like *Nereocystis* also bears its sori on the laminae. However, no information suggesting sorus release is available either from histological (Herbst and Johnstone 1937, Parker and Fu 1965) or gross morphological observations (Areschoug 1884, Farlow 1889,
Setchel 1896, Dawson 1962, Parker and Dawson 1964, and Parker and Bleck 1965). Another genus which is of interest is *Macrocystis* which usually bears sori on sporophylls at the base of the thallus (Hoffman 1911, Delf and Levyn 1926) and occasionally on other laminae (Lobban, personal communication). However, the sori and the sporophylls are not released but become necrotic and disintegrate (Neushul 1963).

In the early account of sorus release in *Nereocystis* (Rigg 1912) it was also observed that fertile fronds exhibit young sori at the basal ends (nearest the pneumatocyst), maturing sori in the middle and open spaces near their distal tips where older sori have "disappeared". This process of sorus differentiation is suggested to be the cause of the "ragged appearance" of older fertile fronds (Rigg 1912). These observations are later confirmed during field observations at low tide when empty holes from which soral patches had disappeared were observed (Rigg 1917). In the course of a description of the gametophytic portion of the life history of *Nereocystis* (Hartge 1928) the sequential development and subsequent release of mature sori from the distal end of fertile laminae is reconfirmed. This author (Hartge 1928) adds that "They may be found in a series on the same frond, from those which are just forming and scarcely discernible to those mature, dark olivaceous ones which are ready to shed.", thus establishing a pigment change which accompanies morphogenesis. Scagel (1947) confirmed the previous
observations of the developmental sequences of sori, the increasing pigmentation, their release from the lamina at maturity, and the subsequent damage to the lamina. In addition Scagel (1947) has noted that not all sori separated from the lamina prior to the completion of zoospore release. This was evidenced at the gross morphological level by a whitening or loss of pigmentation in the sorus (Scagel 1947), and thus it would appear that zoospore release, beginning at the surface may either be completed there or after separation from the lamina.

From these reports it is evident that sorus release in *Nereocystis* (Rigg 1912, Rigg 1917, Hartge 1928, and Scagel 1947) is important in the distribution of this genus. However, there is a lack of morphological data concerning the process of sorus release which is prerequisite to any further understanding of the entire releasing process and its regulation.

The process of lamina splitting, a more ubiquitous morphological phenomenon in the Laminariales (Fritsch 1945), provides superficially at least some context with which to approach the process of sorus release in *Nereocystis*. The most obvious similarity is the localized process of tissue separation. The histological survey of Wells (1910) stands out as the broadest and most thorough consideration of this process. From this work (Wells 1910) and others (see Fritsch 1945 for review) it is apparent that morphogenetic events
following the actual splitting are important.

In light of the foregoing remarks an examination of the margins of the sori of *Nereocystis* at varied stages of development leading up to and after release has been made using light and electron microscope techniques. In addition, a brief examination has been made of the initial events of the wound response in *Nereocystis* laminae. Observations from these examinations are presented here and then compared with further observations in adjacent vegetative and soral tissues. Finally these findings are discussed in relation to other relevant morphogenetic phenomena in the Laminariales and ultimately the process of abscission in higher plants.

2. Observations

2.1. Sori and Soral Margins

In the course of field collections it was observed that often there are at least three sori per fertile lamina. Those most proximal to the transition zones of the lamina (Fig. 4M&M) are lightest in pigmentation when viewed with transmitted light and least distinct. From adjacent vegetative tissue more distal sori are progressively denser in pigmentation and therefore become more distinct. The distinctiveness of
progressively distal sori is accentuated by the establishment of a narrow band at the soral margins by means of a progressive loss of pigmentation which culminates in a distinct white perimeter about the most distal mature sori (Fig. 4M&SM). In truly mature sori the pigmentation changes from a dark olive brown to dark chocolate brown. Beyond the most distal sorus may exist holes from which previously matured sori have been released. Often the most distal sorus may fall from the lamina during the collection of tissue samples. The physical process of separation of the sorus from the lamina is confined to the above mentioned cleared tissue band at the sorus perimeter. The distal holes from which earlier sori have been released may be entire or disrupted by tears in the lamina. Occasionally (once in a holding tank and once in a quiet tidal channel) the most distal sorus may be essentially cleared of pigmentation as are mature sori which have shed all their spores in vitro (Ch. 3 and Appendix 1).

None of the most proximal sori examined were found at developmental stages prior to zoosporangial initial inception. It was only at the soral margins that earlier developmental stages were found (Ch. 3). From observations with SEM (Fig. 1 and 2) and in sections traversing the sorus margin it was observed that a sequence of the developmental stages antecedent to zoosporangial initial inception is present. Earliest stages are located at the vegetative end and progressively advanced stages are observed as the sorus proper is approached.
At the margins of older, more distal sori this sequential array of early developmental stages is interrupted by an area of outer cell wall disjunction and surface cell erosion (Fig. 2 and 5). However, from observations of young sorus margins (Ch. 3) and using the cellular events during sorus inception it is possible to both delimit the sorus margin from the adjacent vegetative and soral tissues and to subdivide the margin into three regions.

The vegetative limit of the sorus margin and the beginning of region I is the point at which the epidermal cells commence periclinal divisions (Fig. 2 and 3). In the other direction is the soral limit of the margin and region three. Region I is designated as the area between the vegetative limit of the margin to the point where paraphyses initials produced by the periclinal division begin to elongate (Fig. 3). Region II extends from the point where paraphysis initial elongation begins to that where zoosporangial initials commence (Fig. 3). Region III extends between the points of zoosporangial initial inception and the point at which the zoosporangia are at a developmental stage identical to that of the bulk of the sorus proper (Fig. 3). Since the emphasis of this work has been on the surface cells of the thallus, the process of release is divided, for convenience, into two phases. The first is the process of erosion of the surface cells in the marginal tissues (Fig. 2, 4 and 5) and the second is the process of separation through the cortical and medullary tissues.
2.2. Young Sorus Margin

In the most proximal sori where pigmentation is not readily distinguishable from that of adjacent vegetative tissues the sorus margin shows no loss of pigmentation and is difficult to delimit. It is only through microscopic observations that the limits of the sorus margin can be distinguished and the three regions identified (Fig. 1 and 3). It is important to note here that these three regions across the sorus margin are determined by cells of the epidermis and their immediate derivatives only. With light microscopy the cells of the medulla and cortex are morphologically indistinguishable from adjacent regions at this stage. The processes of sorus inception have raised the surface of the lamina in the region of the sorus. Therefore, the region of the sorus margin is seen here as a somewhat uneven incline plane as one proceeds from the vegetative limit across regions I, II, and III to the sorus proper (Fig. 3). The outer cell wall covering the paraphysis initials of region I and elongating paraphyses of regions II and III is continuous and indistinguishable from any adjacent regions morphologically and histochemically (Fig. 3). The anticlinal walls between paraphysis initials and subtending cells of regions I and II are apparently intact, while in region III zoosporangial initials are protruding into an extracellular space between the now distinct anticlinal walls of adjacent paraphyses (Fig. 3).
This space does not extend between the deeper subtending cells which produced the zosporangial initials. Cytoplasmic density is quite uniform in all of the cell types across the three regions, especially in the paraphysis initials and paraphyses. Whether cuboidal or elongate they show little vacuolation (Fig. 3 and 6) and possess numerous cytoplasmic ribosomes. There may be some vesiculation at the paraphyses apices in regions II and III (Fig. 7) but the moderately hypertrophied dictysosomes lie adjacent to the nuclei in all three regions. Microbody-like structures are frequently observed as are the scattered mitochondria (Fig. 6). The nuclei are not remarkable and occupy a central position in region I paraphysis initials and are more distally located in those of regions II and III. Chloroplasts are distributed about the nucleus (Fig. 6), and tend to be located more distally to it in the elongating paraphyses of regions II and III. The paraphysis chloroplasts (Fig. 6) appear similar to those of photosynthetically mature adjacent meristoderm cells (Ch. 1) with a moderate number of thylakoid stacks and occasional plastoglobuli.

2.3. Margin of the Intermediate Sorus

With laminae bearing three or more visible sori it is the intermediate sori which become clearly defined from the adjacent vegetative tissues by their denser and more olivaceous
pigmentation. They may be further accentuated by the beginning of pigmentation loss at their perimeters (Fig. 4 sorus C in M and M).

With continued elongation of the zoosporangia (Appendix 1) and paraphyses (Ch. 3) in the sorus the slope across the margin from sorus to vegetative tissue becomes somewhat steeper (Fig. 1). The regions across the margin are still easily determined since individual cell wall disorganization has not occurred (Fig. 4). However, the outer cell wall covering the paraphyses of region II is frequently found dislodged (Fig. 4) or disorganized having partially elongate paraphyses still delimited by a fine cell wall but exposed at their apices to the external environment (Fig. 4). Regions I and III may still be covered with the outer cell wall, although loosely in region III and the sorus (Fig. 4). The anticlinal walls of the paraphyses in region II are not physically separated to produce extracellular spaces or sorus lumen in region II, however, the lack in uniformity of cell shape and orientation relative to adjacent paraphyses and subtending cells implies reduced lateral adhesion (Fig. 4). It is important to note that the paraphyses initials, paraphyses and subtending cells of regions I and II are somewhat vacuolated in contrast to those of the younger sori (Fig. 3 and 4). In region III where zoosporangial initials and sorus lumen are present, the paraphyses are somewhat compressed basally and do not appear so extensively vacuolated (Fig. 4).
Ultrastructurally the majority of the organelles including nuclei, chloroplast, mitochondria and golgi apparatus are located apically in the paraphysis initials, and paraphyses of all three regions of the sorus margin. The golgi apparatus is elaborate, consisting of several dictyosomes and associated vesicles (Fig. 8). The apical cytoplasm is typically crowded with vesicles, chloroplasts and profiles of ER (Fig. 8, 9 and 10), while the basal portions are dominated by a vacuole defined by a tonoplast and peripheral cytoplasm (Fig. 8). For paraphyses bordering and in region III the sorus lumen is present (Fig. 8). The ER system of these paraphyses is hypertrophied with a finely flocculent material as is the chloroplast ER with which it is continuous (Fig. 10 arrow). The chloroplasts are as in the previous stages with numerous stacks of thylakoids and numbers of plastoglobuli. Neither pyrenoids nor membrane whorls are found associated with these chloroplasts. The outer cell wall at the paraphyses consists of the three fibrillar layers numbered 2, 2/3, and 4 with an external layer of perpendicular flocculent material (Fig. 9 and 10). In accord with light microscopic observations of regions II and III, frequently there are signs of disorganization and disjunction in the outer cell wall, usually coming up from the former middle lamellae of anticlinal walls (Fig. 9 arrow) and extending between layers 1 and 2/3 of the outer cell wall (Fig. 8 and 9, arrowheads). Finally it has been observed that in the paraphyses of regions II and III at
this stage there may be a deposition of particularly electron-opaque material on the inner fibers of the anticlinal cell walls which continues up over the apical dome along the inner surface of layer 1 of the outer wall (Fig. 11 and 8 arrows).

2.4. The Mature Sorus Margin

Where three sori are simultaneously present on a single lamina the most distal, and most mature sorus is delimited by a region devoid of pigmentation (Fig. 4 in M and M). The sorus itself, if mature, is a dark chocolate brown and clearly distinguished from the adjacent vegetative tissues. Frequently the sorus separated from the lamina during handling.

At this late stage of development the sorus margin may show great regional differences, and the exact boundary between region II and III may be difficult or impossible to locate. By this time the difference in elevation between the sorus and vegetative tissue is at its greatest. This is accentuated by the disjunction and disintegration of the outer wall over regions II and III along with the disintegration of surface cells resulting in the exposure of subtending and cortical cells (Fig. 5). On the vegetative side of the lytic zone there are typically some partially elongate paraphyses remaining. These show decreased metachromasia for acid and/or sulfated polysaccharides in their remaining cell wall and the outer wall
is in varying stages of deterioration or is absent (Fig. 5). On the soral side there are incompletely developed zoosporangia which grade into the mature sorus proper (Fig. 5 and 17).

In region I both paraphysis initials and subtending cells are extremely vacuolate with a thin layer of peripheral cytoplasm (Fig. 12 and 15). Although there is vesiculation of the apical cytoplasm (Fig. 12 and 15), the golgi apparatus is not a prominent feature in these cells. While the nuclei are small, their envelopes remain intact, and only occasionally interrupted by pores. The nucleoplasm is dominated by prominent masses of heterochromatin (Fig. 18). Osmiophilia of the chloroplast thylakoids approximates that of the chloroplast matrix and there may be slight thylakoid hypertrophy (Fig. 18). Plastoglobuli are numerous in the chloroplast matrix as are ribosomes (Fig. 18) and the genophore is still present. There is no marked difference between the numbers of stacks of thylakoids here and in the intermediate margin paraphysis initials. Cytoplasmic ribosomes and intact mitochondria are still present (Fig. 18). The cell walls show weak osmiophilia and the flocculent material outside layer 4 is difficult to visualize if present (Fig. 15). Finally, it is noteworthy that there is no coating of the inner surface of the paraphysis initials with the electron dense material seen in those of region II.

In region II when partially elongate paraphyses are intact they appear to be the most disorganized of any region (Fig. 13).
General cytoplasmic protein staining with ABB and osmiophilia are weakest in most cells of this region relative to regions I and III (Fig. 13, 16 and 19). When present, the outer cell wall or fragments thereof show varied patterns of osmiophilia and organization. In some, the outer wall layers show a decreased osmiophilia with the exception of that portion of region 2/3 lying to the sides of the apical dome of the paraphyses (Fig. 16 and 19). Although lightly osmiophilic the remainder of layers 2/3 and 4 are essentially undisturbed. However, the perpendicular flocculent material is definitely absent from the outer surface of layer 4 which may show signs of erosion (Fig. 19). Layer 1 of the outer wall and the anticlinal walls of the paraphyses show a marked reduction in osmiophilia due to an apparent loss of fibrillar material and only the innermost material which was coated with electron dense material in the paraphyses of the intermediate margin persists (Fig. 9). This dense layer may be multilamellar and there are often very electron translucent regions between these layers (Fig. 16). There may also be schizogenously produced spaces adjacent to these paraphyses where anticlinal walls are disrupted (Fig. 16 and 19*). The cytoplasmic constituents of these partially elongated paraphyses may be completely disrupted. Frequently only chloroplasts are recognizable by their persistent thylakoids and plastoglobuli and the plasma membrane may be completely disrupted (Fig. 19).

In region III the outer cell wall has usually come loose
or is dislodged during fixation. There are zoosporangia in this region which are in various stages of development and showing signs of deterioration similar to the surrounding paraphyses (Fig. 17). Most of the paraphyses in this region show condensed apical cytoplasm and basal vacuolation with ABB protein staining (Fig. 14 and 20). However, there are paraphyses where disruption of the cytoplasm and plasma membrane breakdown is apparent (Fig. 21). In the denser paraphyses the apical portion of their cell walls, formerly layer 1 of the cuter wall, and their anticlinal walls show signs of dissolution (Fig. 20) and the electron dense inner material is multilamellar and intact (Fig. 20). In the intramural space and in plasma membrane invaginations there may be membrane bound masses of electron dense material similar to the cytoplasm (Fig. 20). The nucleus is seen as a polymorphic mass of granular/fibrillar appearing electron dense material with no matrix (Fig. 20 and 21). It is only in the lighter paraphyses that the fragments of nuclear envelope are clearly seen (Fig. 21, large arrowhead). Chloroplasts are visible in both types of paraphyses, (Fig. 20 and 21) although, in the lighter ones only membranes and plastoglobuli persist. In the lighter paraphyses shown here (Fig. 21) the apical dissolution of the cell wall (between small arrowheads) is evident. The inner layer of electron dense material persists (Fig. 21).

In some of these oldest sorus margins examined there were signs of cell wall dissolution in the anticlinal cell walls of
subtending cells in region II near the border between it and region III. This dissolution was also apparent in the anticlinal walls and layer 1 and 2/3 of the outer cell wall of the paraphysis initials of region I. This resulted in a bilamellar appearance of the outer wall and was preceded by a split between layers 2/3 and 4 which had lost its outer coating of perpendicular flocculent material. The dissolution of cortical cell walls begins centrally away from corners, with a loss of osmiophilia and thickening of the wall by a spreading of the fibrillar elements. As this spreads to the corners schizogenous lumen begin to appear separating adjacent cortical cell walls.

2.5. Separation

It has been very difficult to find any sorus margins in which the process of separation has proceeded only part of the way through the cortex and medulla in the few cases where separation has been found to be in progress there is an apparent loss of both metachromasia and a decrease in the overall staining intensity with TBO. Concomitant with this there is an apparent loss of middle lamella and subsequent cellular dissociation.

At the ultrastructural level the process is seen to entail a swelling of the cell wall, decreased osmiophilia of fibrils
and perhaps decreasing numbers of fibrils. Eventually a schizogencusly derived lumen appears between walls of adjacent cells. This process, as seen with light microscopy, begins in the middle of cell walls between corners and extends out to the corners. This type of process is seen to occur also laterally into region I paraphysis initials, however, it is primarily cell wall layers 2/3 and to some extent 1 which are affected. This study has been based primarily on the meristoderm (Fig. 25 large arrows) and sufficient data has not been gathered to correlate cell wall changes with the protoplasts of the cortical and medullary cells.

This process of dissociation appears first at the outer limits of the cortex in the sorus margin just where meristoderm erosion is most acute (Fig. 22). This is more clearly seen in sections of the cortex taken across the margin in the plane of the lamina (Fig. 23). This process is apparently initiated simultaneously at both surface of the lamina (Fig. 22, top and bottom). The margins of both the hole in the vegetative lamina and the released sorus show this loss of metachromasia and middle lamella (Fig. 24 and 25). The loss of metachromasia and middle lamella appears to proceed laterally into the cortex and medulla and apparently advances fastest in the medulla (Fig. 24). In both the cortex and medulla metachromasia persists longest in the corners of cell associations and in the cross walls of medulla cells (Fig. 25 and 26). The matrix of the medulla shows a loss of all staining with the loss of
metachromasia except immediately adjacent to the plasma membranes of these cells (Fig. 24). It is of particular interest that at this time there is bacterial invasion in the medulla and cortex which has now become exposed (Fig. 27). There is no evidence of mitosis in either the cortex of medulla cells at these margins.

2.6. Wound Response in the Lamina

The cortex of unwounded lamina consists of several layers of roughly isodiametric cells which increase in diameter towards the medulla (Fig. 22, 28 and 29). The interface between cortex and medulla is indicated by the apparent separation and stretching of cortical cells into the elongate elements of the medulla (Fig. 28). The space between cells is not a void but metachromatic with TBO and birefringent in polarized light.

Sections of freshly wounded lamina reveal some apparent folding of cortical cell walls, a curling in of the meristoderm and complete disruption of some cortex and medulla cells (Fig. 29). There is an apparent thinning of the extracellular matrix surrounding the cells of the medulla immediately adjacent to the cut (Fig. 29).

After the wounded edge has had a week of repair activity the most remarkable response is the apparent clotting of the
medulla, presumably through expansion and division of medulla cells which remained intact at the site of wounding. In contrast to the margins of sori and the holes from which they drop there is no bacterial invasion (Fig. 30). The disrupted cells of the medulla and cortex have lost their metachromasia with TBO but stain faintly blue (Fig. 30). These are often metachromatically pink masses in the vacuoles of the "clotted" medulla cells and green granules may be observed around some nuclei. Their cell walls are typically metachromatically purple.

3. Discussion

3.1. Gross Morphology of Sorus Release

From the observations made in the course of this study it is possible to confirm and significantly extend the previous accounts of the gross morphology of sorus release in Nereocystis. As in previous reports and in the results of this work it has been observed that in Nereocystis the sori develop sequentially with the youngest located most proximally and the oldest most distally to the transition zone (Riggs 1912 and 1917, Hartge 1928, and Scagel 1947); the mature sori are released or dropped from and leave holes in the lamina (Riggs 1912, and 1917, Hartge 1928 and Scagel 1947); the release of
sori is often followed by lamina deterioration (Riggs 1912 and 1917, Hartge 1928 and Scagel 1947); the sori increase in pigmentation with age to a dark olivaceous color (Hartge 1928 and Scagel 1947); and the release of zoospores from the sorus in some cases may occur prior to the release of the sorus from the lamina as evidenced by a clearing of pigmentation (Scagel 1947). To extend the above observations it has been observed here that not only do the sori become more olivaceous as they mature but at maturity there is a shift in pigmentation from the dark olivaceous coloration to a dark chocolate brown. Furthermore, the increase in distinction of the sorus from the rest of the lamina is accentuated by a progressive clearing of pigmentation at the perimeter of the sorus and that the physical locus of separation is through this perimeter of cleared tissue. Finally from observations of sorus pigment clearing during in vitro release of zoospores from mature sori in the lab (Ch. 3 and Appendix 1) it is possible to add support to the previous reports (Scagel 1947) that white sori have shed all of their spores prior to being released from the lamina.

3.2. Meristoderm Morphogenesis at the Sorus Margin

There was no previous information concerning the cellular and subcellular events associated with the process of sorus release from laminae in Nereocystis. From the observations made here it is now possible to histologically describe and
characterise the sequence of events at the margins of the sori leading to their release from laminae. From the gross morphological observations reported and discussed above (sections 2.1., 2.2. and 3.1.), two are of particular interest. First there is a clearing of tissue which precedes the actual event of separation, and secondly the process of separation is consistently limited to this perimeter zone of pigment-cleared tissue surrounding the sorus. In light of these observations and the fact that the greatest density of chloroplasts is in the epidermal and/or meristoderm cells (Fritsch 1945, Smith 1939 and Ch.1) it might be expected that the process of sorus release begins first in the meristoderm layer and later proceeds into the internal tissues. It is apparent that changes which are peculiar to the sorus margin make the margin distinct from both the vegetative and soral tissues which it separates. Thus changes at the cellular and subcellular levels in the sorus margin must be considered from the point of initiation and compared with conditions in adjacent tissues.

From previous observations made on soral development in *Nereocystis* (MacMillan 1899 and Hartge 1928) and other Laminariales (Fritsch 1945, review, Papenfuss 1951, Ohomori 1967, Chapman and Chapman 1962 and Bold and Wynne 1978) sorus inception can be broken down into several distinct events. The first is the periclinal division of the meristoderm cell to produce a superficial paraphysis initial and an internal subtending cell (see also Ch.3). Next, while the paraphysis
initial elongates the subtending cells bud a zoosporangial initial into the developing extracellular sorus lumen. In figure 3 and the first sections of observations 2.1 and 2.2, it was pointed out that the margins of the youngest and most proximal sori reveal a sequential array of the early stages of soral development. Thus scanning a section taken across a sorus margin one first sees a number of meristoderm cells which have divided periclinaly to produce outer paraphysis initials and internal subtending cells of equal size.

In region I of youngest soral margins the meristoderm has divided periclinally to produce an external paraphysis initial and an internal subtending cell. The periclinal and anticlinal walls of all of these cells are intact and stain metachromatically as adjacent epidermal cells indicative of acid and/or sulfated polysaccharides. Region II consists still of just paraphysis initials and subtending cells. However, paraphysis initials have begun to elongate, as is apparent from their longer anticlinal walls. In region III not only have paraphyses continued to elongate, but zoosporangial initials have been budded into the concomitantly formed new sorus lumen.

A comparison of this young margin (Fig. 3) and its cellular constituents with mature sorus margins (Fig. 5) immediately reveals two important points. First, in spite of the zone of eroded meristoderm, the gradient of cellular stages seen in the
youngest margin has persisted. This is demonstrated on the one hand by the persistence of paraphysis initials which have not elongated, indicating region I (Fig. 5, 2 and 15) as well as a few of those which have just begun, indicating the vegetative end of region II (Fig. 5), and on the other hand by the persistence of partially developed zoosporangia on the sorus side of the eroded region (Fig. 5 and 17). The second point is that the internal tissues, the cortex and medulla, may remain fairly intact even until sorus maturity (Fig. 5). It is therefore apparent that phase one of sorus release is an erosion of the meristoderm at the sorus margin, which involves mostly region II meristoderm derived cells nearest to the boundary between regions II and III, and which is antecedent to the second phase or actual separation of the sorus from the lamina. Phase two will be discussed further after the mechanism of erosion has been considered.

From a comparison of paraphysis initials, paraphyses and their cell walls from regions I, II and III at the margins of young, intermediate and mature sori it is possible to outline the mechanism of meristoderm cell erosion reported here.

The paraphysis initials of region I are consistently intact for all three aged soral margins. Comparing paraphysis initials from region I of the margins of youngest sori (Fig. 3, 6 and section 2.2. here) with the same from margins of mature sori (Fig. 12, 15, 18 and section 2.4. here) it is apparent that the cytoplasm has usually become basally
vacuolated and more vesiculate apically (Fig. 12 and 15). Furthermore, the nucleoplasm is dominated now by electron dense granular/fibrillar heterochromatin regions and few regions of lighter nucleoplasm. In contrast to chloroplasts from young region I paraphysis initials their thylakoids have begun to hypertrophy slightly (Fig. 18). Vacuolation has been noted to correlate with differentiation in other brown algal cells (Oliveira and Bisalputra 1977ac, Fagerberg and Dawes 1977, Pellegrini 1976, Rawlence 1973, and Neushul and Dahl 1972) as well as in other algae (Brown and Weir 1970, Goff 1979) and higher plants (Prat Vian, Reis and Roland 1977, Khera and Tilney-Bassett 1976, Matile 1974 and 1975, reviews, Matile and Moor 1968, and Ragetli, Weintraub and Lo 1970, Gomez, Harris and Walne 1974). The increased heterochromatin density seen here is similar to that seen in other plant cells differentiated or beginning to senesce (Ragetli, Weintraub and Lo 1970, Eratcn 1966 and Shaw and Manocha 1965) as well as some animal systems (Nevalaina and Anttiner 1977). The hypertrophy of thylakoids has been associated with the onset of senescence in Ectocarpus (Oliveira and Bisalputra 1977cd) another brown alga and some higher plants (de Vecchi 1971 and Dodge 1970). In this vain it is interesting to note that the increase in size and numbers of plastoglobuli also follows aging (Lichtenthaller and Peveling 1967, Freeman, Pratt-Aloia, Mudd and Thomson 1978). Although the prominent golgi apparatus of region I paraphysis initials does not show much hypertrophy (Fig. 6) it stands in marked contrast to that of paraphysis
initials in mature margin region I paraphysis initials. Decline in golgi apparatus has been related to aging in *Ectocarpus* vegetative cells (Oliveira and Bisalputra 1977cd). Hence, these morphological changes from paraphysis initials of region I in youngest sorus margins to paraphysis initials of mature sorus margins are interpreted here as a cytomorphogenesis from a photosynthetically mature condition to one verging on senescence.

For region II the morphogenetic changes are even more dramatic than those of region I and III. Partially elongate paraphyses of region II at younger sorus margins differ from the initials only in apparent golgi activity indicated by hypertrophy and apical cytoplasm vesiculation (Fig.7). This stands in sharp contrast to the apparent autolysed mature sorus region II paraphyses where the chloroplast membranes and plastoglobuli may be the only recognizable organelles remaining from the protoplast (Fig.19). It is clear in this material that the plasma membrane and other organelles have been completely disrupted, a consequence of senescent plant cell autolyses (brown algae, Oliveira and Bisalputra 1977d, higher plants, Matile 1975, review). It is of particular interest that layer 1 of the outer wall, continuous with the anticlinal walls has undergone dissolution and dissolution was preceded by deposition of an inner lining of electron dense material in the intermediate sorus margin region II paraphyses which is all that persists after dissolution (Fig.19 and 16). This
phenomenon occurs later in the paraphyses of the sorus (Ch. 3 mature and released sorus). The only parallel this author is aware of in plants is found in barley aleurone cell walls, where an electron dense material is deposited prior to cell wall dissolution (Taiz and Jones 1973, see their Fig. 3-15) and persists. The flocculent material outside layer 4 of the outer wall is gone (Fig. 19) as has been previously observed in the case of prematurely necrotic paraphyses (Ch. 3), and aging meristoderm cells (Chapter 1). Region II paraphyses of intermediate sori are highly vacuolated basally and the apical cytoplasm is crowded with vesicles from the elaborate hypertrophied dictyosomes always found adjacent to the nuclei. The CER, NE, ER system is hypertrophied with flocculent material similar to that of golgi vesicles and the outer layer of flocculent material of the outer wall. This morphology is interpreted as representing high metabolic activity related to secretion for maintaining an outer layer of transient cell wall material, probably a sulphated polysaccharide (see Bouck 1965, Gibbs 1962, Evans 1974 and Leppard 1974 for endomembrane system in brown algae and Chapter 1 Schnepf 1963, Leppard 1974, McCully 1968, Evans and Holligan 1972, Evans, Simpson and Callow 1974, Evans 1974, Pellagrini 1976, and Davies, Ferrier and Johnston 1973 for brown algal secretory morphology). These region II paraphyses of the intermediate aged sori are very important for a number of reasons. Their maintenance of an intermediate or partially elongate morphology up to and including the time of autolysis implies that they have been
arrested during a stage of cell wall expansion associated with cell elongation and those near the boundary between regions II and III have been arrested at a time when anticlinal wall schizogeny has begun. This most probably explains the loosened and often disjointed outer wall and mild state of paraphyses disarray already mentioned (section 2.3.) in light microscopic material as well as the minor lesions between layers 1 and 2/3 of the outer wall. It is also important to note that the deposition of electron dense material on the inner face of the apical portions of the paraphyses of the sorus and that in both cases it precedes localized dissolution of the cell walls in the mature sorus margin and later in the bulk of the sorus (Ch.3). Therefore, it is possible to assume that the onset of meristoderm erosion in region II paraphyses begins while they are in the process of anticlinal wall elongation, as evident by their intermediate size and secretion of a transient cell wall component, which continually dissipates from the outer surface of the outer wall (section 2.3. here and Ch.1 and 3) as proposed by Evans and Callow (1978) in Fucus zygotes. It should be noted that although signs of autolysis are not evident in paraphyses of this stage it will be essentially complete by maturity, well in advance of any other adjacent meristoderm components. Furthermore, the electron dense layer deposited at this stage on the inner face of the cell wall, similar to that in barley aleurone cell walls also destined to undergo hydrolysis (Taiz and Jones 1973) at least implies a correlation if not a causal relationship with paraphyses cell
wall dissolution and outer wall disjunction. This is further supported by the latter occurrence of the same phenomenon in the paraphyses of released sori (Ch.3.p).

Region III paraphyses span developmental stages from a point similar to those of region II to a stage approximating conditions in the sorus proper. Beginning in the youngest sorus margin at the developmental stage somewhere between that region II they may be comparable as well to paraphyses of the youngest sorus (Ch.3). They will be close to half of their final length with fairly stable cell walls in equilibrium with the already existant sorus lumen and zoosporangial initials (Fig.3 and 4). They are, based on morphological features already discussed, metabolically active and involved in mild secretion by the golgi apparatus. In turning to the paraphyses of region II in mature sori which were found in an apparent state of senescence and occasionally autolysis somewhere between paraphysis initials of region I and paraphyses of region II (Fig.20 and 21). The heterochromatin appears relaxed into a fibrillar reticulum the granular nucleoplasm is absent and the nuclear envelope may be beginning to break down (Fig.21) as has been seen in aging and autolytic Ectocarpus cells (Oliveira and Bisalputra 1977cd). A stage of heterochromatin condensation seen here (Fig.18 and 20) and in other organisms discussed above may have been either overlooked or not present in Ectocarpus. Chloroplasts seem to pass from a condensed stage where thylakoids stain little denser than the
matrix to hypertrophy and matrix dissolution (compare Fig. 20 to 21). Prior to plasmalemma breakdown, there is also an apparent exclusion of membrane bound electron dense material morphologically similar to that observed in Ectocarpus (Oliveira and Bisalputra 1977cd). Electron dense material has also been deposited on the apical portions of the inner faces of the paraphysis cell walls which show progressive dissolution and even separation into a multi-lamellar morphology of the electron dense material (Fig. 20). The outer wall is almost always absent after usual processing. In light of the above discussion and the observations made in regions I, II and III of youngest and intermediate sori it is possible to suggest an outline of the principle events in the erosion of the meristoderm at the sorus margin which is designated here as phase one of sorus release. The gross morphological, histological and ultrastructural observations made and reported here support the following interpretation of phase one of sorus release.

Immediately after sorus inception, which would seem to occur simultaneously for the bulk of the sorus, the margin is indistinct because of the lateral spread of continued inception at a slowed pace. This is supported by the long gentle gradient of early stages as observed for regions I, II and III of youngest sori and by ultrastructural observations on paraphysis initials and paraphyses of these three regions. From morphological information about the process of sorus
inception in general it is possible to assume that at least the paraphyses of all of regions II and III are in the process of cell wall extension associated with their elongation and at least at the border line between regions II and III schizogeny of paraphyses anticlinal walls is occurring or has just completed, most probably by middle lamella dissolution. Based on similar morphological processes in higher plants (Yager 1960, Morre 1968, Valdovenos and Jensen 1968, Jensen and Valdovinos 1968, Taiz and Jones 1973, Sexton and Hall 1974, Sexton 1976, Wilson, Nessler and Mahlberg 1976) it would seem reasonable to assume that some sort of enzymatic activity is operating on one hand to loosen anticlinal paraphyses walls for extension and on the other to dissolve middle lamellar material allowing the budding of zoosporangial initials (Ch.3) and the concomitant formation of the new sorus lumen. This then effectively characterized the situation in a youngest sorus margin. The indistinctness of the margin may thus be attributed to the juvenile condition of the sorus proper and the undisturbed but slowed lateral spread of the sorus primarily on the observation that regions I, II and III are essentially present although not undisturbed at maturity it is proposed that after inception and at the time of or slightly before the intermediate stage as characterized here, the process of lateral spread of inception is halted. Further evidence for this is found in the ultrastructural observations that paraphyses of region II although morphologically little different in shape and size from those of region II in the
youngest sorus, are very similar to those of the adjacent sorus (Ch. 3 post meiotic/pre-cytokinetic sori). A final piece of evidence is the presence of the apical cap of electron dense material lining the inner surface of the paraphysis walls in this and some of region II but absent from region I and the paraphyses of the scrus proper. This may morphologically signal the onset of meristoderm erosion as some disruption may be evident between the paraphyses and the outer wall. No evidence is presently available as to the means of or mechanism whereby this arrest is achieved. The arrest of lateral spread and the continued development of the zoosporagia and paraphyses of the sorus proper are most probably the cause of the increased distinction of the sorus margin visible even prior to any loss of pigmentation. The last event of phase one of meristoderm erosion at the sorus margin involved the dissolution of cell walls and a differential senescence of paraphyses protoplasts resulting in autolysis. Evidence for this comes from a comparison of ultrastructural observations in the three regions of the sorus margin and those in adjacent sorus and vegetative tissues. There is little doubt of the senescence process here (compare sections 2.2., 2.3. and 2.4. with Oliveira and Bisalputra 1977cd, in Ectocarpus and Gomez, Harris and Walne 1974 in Euglena and in higher plants (Buttler 1967, Buttler and Simon 1971, Matile and Winkenbach 1971, Hernandez-Gil and Schaedle 1973). Most relevant here is that both senescence and cell wall dissolution are most acute in region II near its border with region III. It is of
interest to point out that paraphysis initials of region I in the mature sorus are most similar to paraphyses of the mature sorus (see Ch.3) and that autolysis is most acute or complete in region II paraphyses at this time. Paraphyses of the mature sorus are more aged than are adjacent vegetative cells (compare Ch.1 meristoderm adjacent oldest sorus and Ch.3). The clearing of pigmentation from the sorus margin during the morphogenesis from intermediate to mature sorus is caused, therefore, by the process of senescence and autolysis of paraphyses in region II and subsequently III of the sorus margin. Loss of chlorophyll is well established (Woolhouse 1967, Spencer and Titus 1973, Freeman, Platt-Aloia, Mudd and Thomas 1978) as a part of plant senescence. As for the observation that in the second phase of release, separation is confined to the pigment cleared region it is relevant to recall that cell wall dissolution is most acute here at the border of region II and III among the paraphyses. This may be related to the earlier observation that this is a region also where paraphysis elongation, paraphysis anticlinal wall schizogeny and subtending cell budding of zoosporagia has occurred prior to arrest (Ch.3). At this point it is necessary to consider the second phase of sorus release, separation.

3.3. The Internal Tissues and Sorus Separation

Although it has not been difficult to interpret the
morphological events involved in the process of meristoderm erosion at the sorus margin, things are not as clear for the second phase of sorus release for two reasons. The most obvious one is that the mechanical aspects of release may confuse any interpretations of morphological observations of the internal tissues made either during or after the completion of physical separation. As was pointed out previously, it has been difficult to obtain mature sorus margins where separation was partially completed because many mature sori fell from the lamina during collection or vegetative and soral ends of tissue blocks become separated during processing. Hence, the bulk of observations discussed here are from tissues prior to or after separation. The second reason contributing to the confusion is that because of the lack of cytological data concerning the condition of the protoplasts of cortical and medullary cells in this region it is difficult if not impossible to determine to what extent cell wall dissolution occurring here is due to enzymatic activity as opposed to exposure. These problems are not uncommon for this sort of phenomenon is evident from Fritsch's (1945) review of the early literature dealing with lamina splitting in the Laminariales. Evidence for both intrinsic and extrinsic factors involved in separation will be discussed and evaluated as much as is possible. There is good evidence for the role of intrinsic mechanisms in the process of separation. The most obvious is the faithfulness with which the process of separation is preceded by and is confined to the pigment cleared tissues at the sorus margin. From the
previous discussions (sections 3.1 and 3.2) not only has there been autolysis (a process often associated with the release of hydrolytic enzymes, Matile 1975) or meristoderm cells antecedent to separation but also cell wall dissolution as well. From older material it is apparent that cell wall dissolution may continue to spread laterally in the meristoderm of region I before (Fig. 22) and after (Fig. 25, large arrows) sorus release. Furthermore, ultrastructural observations of cell wall swelling, and schizogeny in subtending cells and cortical cells agrees with morphological observations at the light microscope level in cortex of both soral and vegetative sides after release. From the biochemical work with laminae of Nereocystis by Whyte and Englar (1975) the presence of three carbohydrate groups, a cellulose consisting of B 1-4 linked glucose molecules, an acid polysaccharide consisting of polyguluronic and polymannuronic acids and a sulfated polysaccharide was established. Sulfated and acid polysaccharides such as determined by Whyte for Nereocystis are presumed to stain metachromatically with TBO (McCulley 1965, 1968ab and 1971 and Evans and Holligan 1972a). In light of this it is possible to interpret the dissolution of cell walls as at least consisting of a removal of acid and sulfated polysaccharides from the cell walls, one or both of which may function as a middle lamella leaving some or all of the cellulosic contents of the wall behind as blue dissociated ghosts of cortical cells. It would appear that the same process is occurring or continues to occur in the medulla as
well as is evidenced by the disappearance of all the extracellular matrix excepting a blue ghost of wall material around these cells as well (Fig. 24 and 26).

It could be argued that these swellings of cell wall are due to mechanical disturbances of the tissue since Nereocystis usually inhabits regions of considerable water motion. However, when the splitting of cortical walls has been produced by deliberate mechanical disturbance (Fig. 29) there is no sign of wall swelling just discussed. Further, evidence against mechanical production of this schizogeny is the centripetal movement of the process (Fig. 22) for if mechanical disturbance was the source of this it seems unlikely that the medulla and inner cortex should escape stresses which should be evidenced across the whole lamina. However, mechanical factors may not be entirely eliminated from the physical event of separation since in some instances (Scagel 1947) sori are still present in laminae after the spores have been shed. The two instances where this author observed this retention of sori were in exceptionally still water situations where mechanical disturbance was minimal. The second question is whether or not the cell wall dissolution which results in schizogeny is caused by enzymatic activity. Evidence in favor of enzymatic mechanisms as opposed to exposure to the elements comes from the observation that in some way the outer cell wall had to be dislodged before there could be exposure. Furthermore, as is well established, autolysis involves enzymatic activities.
Once the outer wall is discontinuous then effects of exposure must be considered. Where erosion has occurred to this point bacterial invasion is already evident (Fig. 27) and apparently continues into the internal tissues (Fig. 27). Another aspect of this exposure is the effect of exposing internal tissue cell walls to ambient sea water. It seems likely that there could be concomitant disturbances in the calcium content of the acid polysaccharide component of these walls which may influence the physical characteristics of this component in the wall. In other words calcium alginates behave like jells while sodium and potassium alginates behave more as mucilage (Mackie and Preston 1974). This in conjunction with the observation of bacterial invasion puts considerable importance on the nature of the outer cell wall covering the sorus in *Nereocystis*. In light of the above discussion it seems reasonable to assume that at least the process of cell wall dissolution and schizogeny may be initiated enzymatically and that this process in essence may continue down into the cortical and medullary tissues of the lamina. This process may well be accelerated or augmented by water action and various microbes. Cytological and enzymological experimentation will greatly contribute to the resolution of this problem. Mechanical disturbance may apparently play a temporal role in sorus release. Given pre-weakening of the marginal tissues by the events of phase one and then their continuation into the internal tissues during phase two, mechanical disturbances may produce separation within a broader time period rather than
when meristoderm, cortex and medulla have completed cell wall
dissolution. Phase two then is seen as a process of cell wall
dissolution between cortical and medullary elements which may
be accelerated or delayed in accordance with various extrinsic
factors.

3.4. Sorus Release and Lamina Splitting

Having characterized phases one and two of sorus release
in *Nereocystis* it is now possible to compare this process with
related phenomena in other members of the Laminariales,
especially other members that abscise their sori. To this
author's knowledge there is only the one suggestion by
MacMillan (1900) that *Lessoniopsis* may release its sori.
However, this was not confirmed in subsequent considerations
(Reinke 1903, Griggs 1909, and Setchell 1925). The next
possibility to consider is those members of the Laminariales
which bear sporophylls which might be abscised, particularly
amongst the Alariaceae. There is one reference to sporophyll
abscission in *Macrocystis* (Neushul 1963), however, in this
paper the term meant is "excission" or the surgical removal of
sporophylls. In fact the process of spore release was matched
and the concomitant loss of pigment was reported. This begins
distally and progresses up the sporophyll towards its point of
attachment and is followed by necrosis and sloughing off of all
distal tissues. The beginnings of this necrosis have been
observed in released and captured sori (Ch3). It would appear that a similar phenomenon is occurring in *Alaria*. If the whole sporophyll becomes soral but erodes away from the distal end up to the point of attachment at the stipe, then the process if not abscission. If, on the other hand, sorus development goes up to the attachment point of sporophyll to stipe and disjunction occurs prior to the end of sporangial dehiscence then perhaps a phenomenon similar to that in *Nereocystis* is occurring. In either case it would be interesting to histologically investigate the events leading to sporophyll loss at the stipe in genera such as *Alaria*.

Another possible related morphological phenomenon is the production of holes in the lamina of *Agarum cribrosum*. This process was carefully described by Humphery (1886) and although there is reported loss of pigmentation in the small piece of tissue ultimately released it is accomplished by a peculiar annular meristematic activity which essentially proceeds from one or the other side of the lamina cutting out an approximately 0.5 mm piece of tissue. The implication of localized cell death is interesting but the meristematic activity sets this in contrast to the events described here for *Nereocystis* sorus release. A somewhat similar problem arises when considering the process of lamina splitting in the Laminariales. From the early literature dealing with the process of lamina splitting one finds that there is usually some meristoderm activity (Will 1897, Grabendorfer 1885, Reinke 1903, Skottsberg 1907, Killian 1911 and Wells 1910) and in
those where the meristoderm does not participate in the process then cells of the cortex become meristematic to allow splitting and then become epidermal (Wells 1910, see *Macrocystis* and *Dictyoneurum* split advance). Where the meristem is active there is usually some internal gelatinization producing a cavity into which the anticlinally dividing meristoderm furrows. However, from these works, as has been pointed out by Fritsch (1945) it is not clear what the relationship is between furrowing and internal tissue gelatinization. It would be interesting to examine this phenomenon ultrastructurally. It is noteworthy that lamina splitting in *Nereocystis* is reported to begin by a deliquescence of internal tissues followed by a furrowing in of meristoderm from both sides (MacMillan, 1899). This was refuted by Wells (1910) who states that it is the rapid periclinal divisions of meristoderm cells in the shallow furrows which produce juvenile tissue assumed to be weak and thus eventually torn to enlarge the split. This tearing is then followed by a curving in to meet over the exposed medulla and cortex by the two meristoderm layers (Wells 1910). Although preliminary observations have been made it is not possible to confirm one or the other theory. However, it is apparent that in the process of lamina splitting the activity of the meristoderm clearly differentiates lamina splitting from what has been described here for sorus release in *Nereocystis*. Rather it seems that the process of sorus release is most directly related to the process of sorus inception which has been combined with
mucilage duct system inception into a single morphological phenomenon (Ch.3). Both mucilage duct system and sorus inception are seen as related to special periclinal divisions of meristematic tissues which produce internally meristematic or dividing cells which secrete carbohydrates into newly produced schizogenous extracellular lumen (Ch.3). Sorus release is interpreted as commencing by an apparent arrest of this process, lateral extension, resulting in premature senescence and autolysis of young paraphyses as well as dissolution of cell walls of internal tissues ultimately consumated in sorus separation from the lamina.

3.5. Sorus Release and Vascular Plant Abscission

The term abscission has been applied to the process of sorus release in *Nereocystis* (Bold and Wynne 1978) and now it is possible to explore the applicability of this term. The process of abscission in higher plants has received a great deal of attention for a long period of time (Lee 1911, review covering 19th and 18th centuries). This is of course due to the direct economic importance in the context of crop plants. Information about the process of abscission is available at many levels. For instance there is a considerable literature dealing with hormonal aspects of abscission in higher plants (Rubenstein and Leopold 1964, Cracker and Ables 1969, Addicott 1970, Jacobs 1968, Addicott and Lynch 1955, Addicott 1965 and
Osborne 1973) and other substance of endogenous origin such as ethylene (Burg 1968, Jackson and Osborne 1970, Wright and Osborne 1974, Osborne 1973, Fowler and Morgan 1972). Numerous other factors may play a role in the regulation of, or process of abscission as well (Addicott 1968, 1976, Cooper, Rasmussen, Rogers, Reece and Henry 1968). This information may become valuable in the search for factors controlling sorus release in Nereocystis. However, literature concerning hormones in the algae (Augier 1978, review), the lack of information concerning algal growth regulators in general and in Nereocystis specifically, and finally the sole morphological approach used here limit the basis for comparison of sorus release and higher plant abscission to primarily morphological aspects. However, it is extremely interesting to note that Overbeek (1940ab), using coleoptile bending as a bioassay, proposes an auxin distribution for the fertile laminae of Nereocystis. Having assumed that low concentrations are indicative of regions of auxin use he observed that the intercalary meristem and youngest sori are areas of lowest concentration while the concentration is increasing distally in the lamina. This data integrates beautifully with the observations here from the transition zone (Ch. 1 and 2), and the youngest sori (Ch. 3) and with Cleland and Rayle (1978) and their model for the role of auxin in cell wall loosening.

Although there is variation in morphological features of abscission zones as well as mechanisms of separation (Esau
1977) there are apparently some general aspects of the process which are consistent (Cutter 1971, Addicott 1976 and Esau 1977). It is generally accepted that there is usually an identifiable abscission zone which can be characterized as a region where typical cellular differentiation is reduced and as a region of weakness relative to adjacent tissues (Esau 1977). Cells of the abscission zone may be smaller, with thinner cell walls and a richer cytoplasm (Addicott 1976). There may be no intercellular spaces, little starch in the cytoplasm and fibers small or absent (Cutter 1971). In many cases the abscission zone is subdivided into a protection layer and a separation layer. The protection layer may often be identified by mitotic activity which produces cells which become suberized during the process of abscission (Cutter 1971, Esau 1977). However, it should be noted that cell divisions and layer differentiation need not precede septation (Gawadi and Avery 1950), but some form of protection, often periderm activity (Addicott 1976, Esau 1977) will eventually cover sites of abscission. In the vascular tissues there are modifications which may parallel abscission as well. Callose deposition on the sieve plates of phloem elements and numbers of tyloses may increase (Poovaiah 1974). Other than references to discoloration (Cutter 1971, Esau 1977) it would appear that the higher plant epidermis may not play much of a role in abscission. Before comparisons can be made with Nereocystis it should be pointed out that the differences in the degree of morphological specialization between thalli of the Laminariales
and higher plant bodies must be accounted for in the selection of comparable morphological features. To begin with one can eliminate, for the most part, tissues and cell types related to support and water conduction. That is not to say that there are no structurally significant adaptations in the thalli of the Laminariales. There are elements in the Laminariales apparently involved in the conduction of photosynthates and other substances however, such adaptations are not on the level of that seen in vascular plants (Ziegler 1963, Ziegler and Ruck 1967, Schmitz and Schrivastiva 1974, 1976, Nicholson 1975).

From the observations presented here the site of sorus release can be definitely anticipated at about the intermediate stage of sorus development (about the time of meiosis Ch.3 and Appendix 1). It is noteworthy perhaps that the tissues where phase one begins are cells of the meristoderm which have just reverted to an essentially meristematic condition involving cell enlargement, schizogeny of cell walls through middle lamellar dissolution and mitosis as well. Meristematic activity has been associated with weakness in other members of the Laminariales in the context of lamina splitting and this is true of *Nereocystis* (Wells 1910) and consistent with observations made in the transition zone of young thalli as well (Ch.1 transition zone).

It might be argued that there is no differentiation of the internal tissues of the lamina in anticipation of abscission but in light of the aforementioned caution about support tissue
it is not necessary for *Nereocystis* to prepare the cortex for
it is little different either morphologically perhaps than that
of the undifferentiated pith and cortical cells in the
separation layers of higher plants. Although the term
secondary walls has been used for innermost layers of cell
walls in the medulla of *Nereocystis* (Schmitz and Schrivastva
1976) and other brown algae (Evans 1972a, McCully 1966, 1968)
it does not entail the permanence associated with secondary
wall formation in higher plants. Dissolution of middle
lamellae is accepted as a crucial aspect of abscission in many
higher plants (Morre 1968, Sexton 1976, Esau 1977) as is
primary wall dissolution and even rupture in a number of cases
(Webster 1968, Addicott 1976). Webster working mostly with
*Phaseolus* attributes great importance to cell disruption in the
pith (Webster and Leopold 1972) and differential swelling of
tissues which generates internal tissue tensions to tear
weakened cells in the separation layer. Although there is no
evidence of differential swelling to either side of or in the
sorus margin, there definitely is evidence of middle lamellar
dissolution by the loss of acid and sulfated polysaccharide
components of the cell walls resulting in the separation of
cells. Separation in the medulla and cortex of the lamina
agrees beautifully with observations by Sexton (1976) of the
abscession zone of *Impatiens* and the cell ghosts left from the
cortex and medulla are identical with the secondary walls of
Schmitz and Srivastava (1976) and probably represent higher
proportions of cellulose deposition. The autolysis seen at
the surface bears some similarity to observations by Webster (1968) and cell wall swelling, thinning and schizogeny strongly resembles similar events in higher plant abscission zones.

Strength would be added to this argument if cytochemical and biochemical information concerning enzymes such as cellulase (Ables 1969, Lewis and Varner 1970), pectinases (Mcrre 1968, LaMott, Gochnauer, LaMott, Mather and Davies 1969, Moline, LaMott, Gochnauer and McNamer 1972), acid phosphatases (Gillian, Bornman and Addicott 1976) and peroxidases (Henry and Jensen 1973, Hall and Sexton 1974, Webster, Dunlap and Craig 1976) were available also for *Nereocystis*.

Work in this direction is currently under way. In this author's opinion there is but one aspect which is validly comparable to the condition in higher plants which must be dealt with. This is the observation that there is apparently no wound response to cover the exposed medulla and cortex of the vegetative tissues left after sorus release and in fact there is an obvious invasion of microbes which involves cell wall deterioration and cell lysis (Section 2.5.). *Nereocystis* is capable of responding to wounding (Fig. 30) and does so in a fashion indistinguishable from other thallose brown algae (Moss 1961, Fulcher and McCully 1969, 1970, Fagerberg and Dawes 1977). However, this cutting experiment was not done in the tissues adjacent to a mature sorus so it is not known whether there is some distance from an active transition zone meristem beyond which wound response would not
occur and would only create an unnecessary sink for photosynthates which might better be used to build new lamina and push the deteriorating fruited distal ends further away. It is definitely confirmed here, however, that the terminal deterioration of the lamina is in fact augmented by the changes wrought by the process of sorus release in *Nereocystis*. Finally it is the opinion of the author that the process of sorus release seen in *Nereocystis* (see summary diagram Fig.31) is in fact a laminarian equivalent to higher plant abscission in the morphological and most probably biochemical sense as well. It will be extremely interesting to see what are the mechanisms controlling sorus abscission.
CHAPTER 4 FIGURES, SORUS ABSCISSION
Plate I

Figure 1. SEM of an intermediate sorus margin. Note the cut edge where the sorus tissue is the elevated region to the right and vegetative tissues are to the left. The central sloped region is the margin. Paraphyses and sporangia hang out of the cut sorus (arrowheads). Scale equals 50 um.

Figure 2. The margin of an abscissing mature sorus with SEM. Sorus is the plateau to the right. Note disjunction and apparent erosion of outer cell wall in margin region. Scale equals 50 um.

Figure 3. Methacrylate section of a young sorus margin TBO stained. The three regions of the margin are indicated by roman numerals. It is important to note the continuous outer cell wall firmly attached to the paraphysis tips, the lack of vacuolation of the paraphysis initials and the points where the extracellular space of the sorus originates (arrow). Scale equals 50 um.

Figure 4. The margin of an intermediate aged sorus. The paraphyses and paraphysis initials to the right are vacuolate and the outer cell wall is both looser and discontinuous across the sorus margin. Scale equals 50 um.
Figure 5. The margin of a mature sorus. The deterioration of the outer cell wall has spread. Paraphyses of region II have lysed leaving subtending and cortical cells exposed in some areas. Scale equals 50 um.
Plate II

Figure 6. Paraphysis initial from a youngest sorus margin. Note the lack of vacuolation, the presence of perinuclear dictyosomes, the microbody-like structure (arrowhead), and the essentially apical chloroplasts. The system of peripheral ER is visible and in continuity with the chloroplast ER (arrow). The subtending cell is to the left. Scale equals 1 um.

Figure 7. Elongating paraphysis apical cytoplasm from a youngest margin. Note the accumulation of golgi vesicles and loosely defined wall. Scale equals 1 um.

Figure 8. Intermediate sorus margin paraphyses. The basal cytoplasm is vacuolate while the apical cytoplasm contains the nucleus and other organelles. The outer wall is still attached and the sorus lumen is intact. Plasmodesmata connect paraphyses and subtending cells (arrowheads). Note the electron dense inner layer of the anticlinal cell walls (arrows) of the paraphyses. Scale equals 1 um.

Figure 9. Intermediate sorus margin paraphysis apical cytoplasm and cell wall. The apical extremity of the cytoplasm is crowded with dictyosome vesicles. The outer wall consists of four distinct layers 1, 2/3, 4 and a layer of perpendicular flocculent material (between parallel bars). Separation of layer 1 from 2/3 is
indicated by the arrow and arrowheads. Scale equals 1 um.

Figure 10. Intermediate sorus margin paraphysis apical cytoplasm. Apical cytoplasm of another paraphysis showing the slightly hypertrophied peripheral ER system is in continuity with the chloroplast ER (arrow). The hypertrophied ER contains a fine flocculent material. Scale equals 1 um.

Figure 11. Electron dense lining of the inner surface of the anticlinal walls. The innermost reticulum of cell wall fibrils (arrowheads). Scale equals 0.1 um.
Plate III

Figure 12. Mature sorus margin region I in methacrylate, ABB/PAS stained. Note extreme vacuolation of both paraphysis initials and subtending cells. The cytoplasm stains densely for proteins with ABB. Scale equals 10 um.

Figure 13. Mature sorus margin Region II in methacrylate, ABB/PAS stained. Paraphyses (*) show some variety in staining and collectively stain less densely than cells of region I with ABB, even with lateral compression of paraphyses. Scale equals 10 um.

Figure 14. Mature sorus margin Region III in methacrylate. ABB/PAS staining here is variable as above, however, the overall density is comparable to cells of region I. Scale equals 10 um.

Figure 15. Mature sorus margin Region I meristoderm cells. Cytoplasm of Region I cells show dense osmiophilia due to the crowding of the cytoplasm and organelles by the extensive vacuole. Cell walls show a general decrease in osmiophilia and arrowheads indicate a periclinal wall separating paraphysis initial from the subtending cell. Scale equals 1 um.
Figure 16. Mature scrus margin Region II paraphyses.
Disorganization of cytoplasm is evident. Multi-layered electron dense material appears on the inner surface of the paraphysis cell walls (small arrowheads). Electron translucent regions also appear in the cell walls of the paraphyses (arrow). The loose outer cell wall retains some electron density around perimeters of junction regions with paraphysis tips. Scale equals 1 um.

Figure 17. Region III paraphyses and aborted zoosporangial initials. Note the zoospore in a mature zoosporangium. Extra cellular lumen of the sorus indicated by *, s. Scale equals 1 um.

Figure 18. A paraphysis initial from Region I. Much of nuclear volume is occupied by heterochromatin. See electron dense particles clustered between stacks of thylakoids in the chloroplasts. Little osmiophilia relative to chloroplast matrix and some hypertrophy (arrowhead). They contain a number of plastoglobuli. Mitochondria show electron dense matrix. Scale equals 0.5 um.

Figure 19. Paraphyses of Region I. Plasmalemma has degenerated and the cytoplasm is disorganized. Chloroplast thylakoids and plastoglobuli (arrow) may be last organelle recognizable. The electron dense inner wall lining is still visible. The outer cell wall consists of only three layers 1, 2/3 and 4. The outer flocculent material is gone. The next layer in, layer 4,
may have begun to deteriorate as well (see right). The anticlinal cell walls are dissipating where the middle lamella is gone and lumen are present (*). Scale equals 1 um.
Plate IV

Figure 20. Mature scrus margin paraphysis in region III.
Only the nucleus and chloroplast are recognizable in the condensed cytoplasm. The inner electron dense layers of the paraphysis cell wall are loosened into a multi-layered state in its apical extremity. The majority of layer 1 is deteriorated in these parts. Scale equals 1 um.

Figure 21. Mature scrus margin paraphysis in Region III.
The nucleus is recognizable by the persistent envelope (arrowhead) and chloroplast by its persistent thylakoid lamellae and plastoglobuli. The outer wall is gone and the inner layer, layer 1, which was continuous with the anticlinal cell wall (between opposing small arrowheads) has deteriorated in its apical portions. Scale equals 1 um.
Plate V

Figure 22. Separation of internal tissues at the mature sorus margin. In Region II of both sides of the lamina separation is characterized by a loss of metachromasia, acid and/or sulfated polysaccharides, and swelling of the cell walls of the underlying cortical cells. This is seen as a decrease in staining density in these figures and increased sectional thickness (arrowheads). Scale equals 1000 um.

Figure 23. Mature sorus margin separation in the cortical layer. Here separation passes mucilage ducts and a cup of secretory cells is seen in surface section (*). The first step is a loss of metachromasia (large arrows) followed by a progressive separation along the middle lamella (small arrow) to finally culminate in separation of the walls of adjacent cells (opposing arrowheads). Scale equals 100 um.

Figure 24. Margin of a mature abscissed sorus. At the site of separation loss of metachromasia and middle lamella produces loose ghosts of cortical (large *) and medulla (small *) cells. The extracellular matrix of the medulla has vanished with the loss of metachromasia. This degradation apparently continues after separation (extent marked by large arrowheads). This dissolution appears to begin in the midsections of cell walls (small arrowhead)
and spread to corners where metachromasia persists (small arrows). Scale equals 100 um.
Figure 25. The margin of a hole left in a lamina after sorus abscission. Morphological consequences of separation are identical to those in Figure 24. Note loss of metachromasia and separation of adjacent cell walls proceeding to corners of cortical cells. Dissolution of medulla has not proceeded as far. Region of the meristoderm remains. Note the peeling back of the outer wall (upper large arrow) from cuboidal paraphysis initials and apparent dissolution of anticlinal walls of paraphysis initials to release outer wall below (large arrow). Anticlinal walls of subtending cells persist (small arrows). Scale equals 50 μm.

Figure 26. Section in lamina plane at the inner cortex level in vegetative tissues of post abscission margin. Note the production of cell ghosts by the loss of metachromasia and extracellular matrix of the medulla. The cross walls of "sieve elements" persists as do corners (arrow). Scale equals 50 μm.

Figure 27. Abscission margins. Note invasion of cortical cells by rod-shaped bacteria which bore holes through the walls (arrows). Scale equals 25 μm.
Plate VII

Figure 28. Unwounded laminar cortical and medullary tissues. The cortex consists of large isodiametric cells with discrete cell walls of uniform thickness. Three developmental classes of cells are discernable in the medulla. The most prevalent are cells of the cortex which have become separated laterally and stretched longitudinally (small arrowheads). The second class is a group of cells arising from the lateral separations which appear "hyphal" but did not result from intrusive growth (arrow). The third class is the trumpet cells seen only in cross section here (*). Scale equals 50 um.

Figure 29. A just cut edge of a vegetative lamina. Note disrupted cortical cells (*) and buckled cell walls (arrowhead). There is also a little extracellular matrix around the cells of the medulla. Scale equals 50 um.

Figure 30. A one week old cut edge of lamina. Note the apparent clotting of the medulla. Remnants of walls of disrupted cells are still visible (arrowheads) although they have lost their metachromasia. Vacuoles contain masses of red metachromatic material (arrow) as well as clusters of blue-green granules. Scale equals 50 um.
Figure 31a. Diagram of the sorus margin prior to abscission. Three of the five tissue layers of the lamina are illustrated, meristoderm (Me), cortex (C) and medulla (Md). To either side of the sorus margin are characteristic vegetative (V) and soral (S) regions of the meristoderm. The sorus margin is divisible into three regions. Region I begins with meristoderm cell division and ends with paraphysis initial elongation. Region II starts here and extends to the site of zoosporangial cell initial inception. Region III starts here and continues to whatever stage of development the sorus is at. Middle lamella dissolution begins between elongating paraphysis cell walls (arrow). Zoosporangial initials acting as secretory cells produce and fill the sorus lumen with mucilage (stippling in sorus). Internal tissues are unaffected morphologically.

Figure 31b. Meristoderm erosion. Outer cell wall loosening and disjunction and paraphysis initial senescence occur approximately at the border between regions II and III where middle lamellae are present as is some extracellular lumen (between arrows). Other than vacuolation and condensation of cytoplasm neighboring cells are intact. Some paraphysis initials may lyse (*).
Figure 31c. Sorus exuviation and separation. Disjunction of the outer wall may lead to sorus exuviation as paraphyses of the sorus proper release it. At the border between regions II and III meristoderm erosion gives way to dissolution of middle lamellae of cortical cell walls (arrows) followed by the matrix of the medulla culminating in sorus separation with mechanical disturbance.
VII REFERENCES CITED


Matile, Ph. And H. Moor. 1968. Vacuolation: origin and development of the lysosomal apparatus in root tip cells. Planta 80:159-175.


Postels, A. And F. Ruprecht. 1840. Illustrationes algarum, oceani pacifici, imprimis septemtrionalis. Petropoli.


1. Introduction

The essential constituents of the laminarian zoosporangial sorus were described by Thuret in 1850 and the precise role of zoospores in the laminarian life history was determined by Sauvageau (1915) and Kylin (1916).

MacMillan (1899) briefly described the zoosporangia and spores from the sori of *Nereocystis*. Zoospores were described as being hyaline, slightly longer than a micrometer, numbering a hundred or more per sporangium and dehiscence was proposed to occur at the apically thickened tip (MacMillan 1899).

After clarification of the debate concerning the laminarian life history by Sauvageau (1916) and Kylin (1916), subsequently MacMillan's (1899) observations on *Nereocystis* were extended to include sexual reproduction by Hartge (1928). Although this study emphasized the gametophytic portion of the life history of *Nereocystis*, it included a brief description of the zoospores. Free spores were described as ovoid (3.8 to 4.2 μm long by 1.5 to 3.0 μm wide) with unequal flagella emergent from a lateral depression, one the length of the spore and the other as twice that and with a parietal chloroplast located in the smaller end of the spore.
The work of Kemp and Cole (1961) with Nereocystis established that meiosis occurs during the first two karyokineses of zoosporogenesis which results finally in the thirty two haploid spores. Similar data had been reported for other genera in the Laminariales (see table I in Kemp and Cole 1961). Further evidence for meiosis at this stage of development in the Laminariales comes from the recent observation of synaptonemal complexes in the young zoosporangial initials of Chorda by Toth and Markey (1973). The process of zoosporogenesis in the unilocular sporangia of a few members of the Laminariales and other orders of the Phaeophyta have been described ultrastructurally (Chi and Neushul 1972, Kugrens and Reeves 1972, Chi 1973, Loiseaux 1973, Toth 1974, Collins and Kugrens 1976, Baker and Evans 1973b, Markey and Wilce 1976a). Similar ultrastructural observations have been made for plurilocular sporangial development as well (Baker and Evans 1973a, Markey and Wilce 1975, 1976b, Lofthouse and Capon 1975). Finally there are several transmission electron microscopy studies which deal with the ultrastructure of the free brown algal zoospore (Manton 1957 and Toth 1976a, Gherardini and North 1972, Baker and Evans 1973a and b).

In light of the lack of an ultrastructural examination of the process of zoosporogenesis in Nereocystis and this author's concern with the whole process of sorus development and release it is essential to obtain data concerning the subcellular events of zoospore morphogenesis. These observations are
reported here and compared with similar reports in the Laminariales and other Phaeophycean orders. Furthermore, with the data presented here and other relevant cytological information a generalized structural and functional model is presented and discussed for the brown algal zoospore.

2. Observations

2.1. Pre-meiotic zoosporangial initials

It was noted previously that youngest sori contain all the constituents of mature sori including paraphyses, subtending cells, extracellular sorus lumen, outer cell wall and the zoosporangial initials (Fig. 2, 8 and Ch. 3). In any youngest sorus all zoosporangia are approximately the same size (Fig. 2) and at the same stage of development.

In zoosporangial initials shortly after inception the most prominent fine structural feature is the nucleus. It is positioned centrally and contains small distinct masses of chromatin material which are accentuated by the relatively electron translucent nucleoplasm (Fig. 1). The nuclear envelope is occasionally interrupted by nuclear pores and may also show luminal continuities with the endoplasmic reticulum (ER) and chloroplast ER (Fig. 1). Typically the nucleus
contains a prominent nucleolus showing distinct granular and fibrillar regions (Fig. 1) as well as a region of homogeneously fibrillar material (Fig. 1*) which is identical to that noted in meristoderm cells (Ch. 1). In favorable sections a pair of centrioles surrounded by radiating microtubules are found adjacent to the nuclear envelope (Fig. 4). At this stage no evidence of synaptonemal complexes is discernable.

The golgi apparatus consists of a number of prominent dictyosomes which are usually located in the basal perinuclear cytoplasm (Fig. 1). These dictyosomes show outstanding hypertrophy of maturing face cisternae and small vesicles at the formative face between the nuclear envelope and the closest cisternae (Fig. 3). The nuclear envelope morphology suggests that a process of vesiculation must be occurring (Fig. 3 arrowhead). Hypertrophied dictyosome cisternae and free vesicles contain a fibrillar material morphologically similar to the fibrous material of the very thin cell walls (Fig. 3 and Ch. 3). Invariably there are 100 nm coated vesicles in proximity to or associated with the golgi vesicles and hypertrophied regions of dictyosomal cisternae. On occasion these coated vesicles may be observed in the vicinity of the plasma membrane.

In addition to the chloroplast ER observed and ER in continuity with the nuclear envelope there is a system of ER cisternae in the peripheral cytoplasm parallel to the plasma membrane (Fig. 4 and 5). This system of ER is present in both
apical and basal portions of the cytoplasm. The ER may also be in luminal continuity with the peripheral system. The typical double membrane chloroplast envelope encloses the triple stacks of thylakoids (Fig. 1) and there may be central stacks of three thylakoids per chloroplast. Plastoglobuli are scarce and pyrenoids have not been observed.

Elongate and spherical profiles of mitochondria in section illustrate as well as their cristae the tubular morphology of both. Bilobed mitochondrial profiles suggestive of division are also observed (Fig. 5). Small microbody-like structures are observed infrequently in section, implying that they are not present in particularly great numbers at this stage of development (Ch. 3). These initials maintain cytoplasmic continuity with the subtending cells by means of plasmodesmata.

2.2. Pre-meiotic sporangial initials close to meiosis

By the time of meiosis the sporangia and paraphyses have both enlarged and the cytoplasm may show more heterogeneity in staining with ABB for proteins (Fig. 8).

In these initials the nuclei remain the dominant organelle. The nucleoplasm has become quite dense and heterochromatric regions are not as easily identified (Fig. 7). However, linear electron dense masses suggestive of leptotene chromosomes have been observed in longitudinal and cross
section (Fig. 7). These linear elements often terminate at the nuclear envelope (Fig. 7). In favorable sections a mass of electron dense material distinct from both the nucleoplasm and nucleolus may be observed adjacent to the nuclear envelope (Fig. 7 and 12). From favorable sections it was determined that there is at least one pair of centrioles per nucleus (Fig. 12) and in contrast sporangia of the previous section, often two (Fig. 10). In the cases of nuclei with more than one pair of centrioles, ribosome-free cytoplasm surrounds each centriole (Fig. 10) and extends between separated pairs of centrioles (Fig. 11). Few profiles of microtubules were observed.

There are some distinct differences in the organization of the golgi apparatus compared to that of the previous stage. The dictyosomes are no longer confined to a specific area of the perinuclear cytoplasm (Fig. 7) nor are they strictly confined to that cytoplasm (Fig. 9). Furthermore, the formative face may be associated with endoplasmic reticulum rather than nuclear envelope (Fig. 9). Although not as prominent and devoid of fibrillar material some hypertrophied golgi vesicles are observed about the dictyosomes (Fig. 9 and 7), coated vesicles are absent and the cell walls are substantial (Fig. 7).

In spite of the fewer luminal continuities between the nuclear envelope and either the ER or the chloroplast ER an extensive system of peripheral endoplasmic reticulum is evident
The chloroplasts are delimited as in the previous section by ER and an envelope. Stacks of thylakoids and plastoglobuli are more numerous and there is the typical cluster of vesicles between chloroplast ER and chloroplast envelope (Fig. 9). Although not illustrated here the chloroplast envelope frequently shows convolutions and pocketings within the limits of the chloroplast ER as observed by Evans (1968) in the fucoids. Pyrenoids typical of the Phaeophyceae have not been observed in this stage of development. Continuities between chloroplast ER and the peripheral ER system are common and small microbody-like structures with dense matrices are often associated with these points of continuity (Fig. 9 *'s). Mitochondrial profiles are more numerous and distributed throughout the cytoplasm.

The cytoplasm is rich in free ribosomes, contains structures presumed to be lipid droplets and is still in continuity with that of the subtending cell through plasmodesmata.

2.3. Post-meiosis, pre-cytokinesis zoosporangia

Although the actual processes of meiotic nuclear divisions have not been observed here, due to the lack of suitable culture facilities for entire thalli, some zoosporangia have been observed at stages prior to completion of post-meiotic
mitotic divisions. These observations coupled with those from sporangia which have completed nuclear divisions make it possible to discuss the cytological events which occur during the karyckinesis of zoosporogenesis.

Post-meiotic sporangia have nearly attained their mature length, just short of the length of the adjacent paraphyses (Fig. 13). The cytoplasmic heterogeneity visualized by ABB protein staining is more pronounced at this stage and the protoplast for the most part is still associated with the zoosporangial cell wall (Fig. 13).

The increase in zoosporangial size and nuclear divisions have been accompanied by a proliferation and re-organization of organelles. From longitudinal sections of sporangia which are tangential it is apparent that chloroplasts are arranged regularly in the peripheral cytoplasm. The distribution of most other organelles, does not appear to be as regular in section. However, mitochondria are frequently found in clusters (Fig. 14, bracket).

At the end of karyokinesis the nuclei are smaller (1.5-2.0 μm), contain a relatively homogeneous nucleoplasm and a small nucleolus (0.5 μm). Nuclei in the midst of the karyokinesis are of intermediate sizes (see previous section) and in contrast to both, possess outstanding masses of chromatic material and nucleoli are either absent or obscured (Fig. 18). The nuclear envelope is periodically interrupted by pores and
regularly shows continuities with adjacent ER cisternae (Fig. 24). A pair of centrioles is present at each pole of the nuclei even between the mitotic divisions which follow meiosis (Fig. 19). At least one pair of centrioles is associated with each nucleus by the end of karyokinesis (Fig. 20). In the former case cytoplasmic microtubules are preserved about the nucleus and centrioles (Fig. 19) while they are not in evidence at the conclusion of karyokineses.

By the end of karyokineses there is at least one dictyosome associated with each nucleus and the previous association with the nuclear envelope is restored (Fig. 20). As with the centricles, the polar arrangement of dictyosomes adjacent to the nuclear envelope between post-meiotic, mitotic divisions (Fig. 18) strongly suggests a nuclear membrane involvement in replication of golgi apparatus elements. These dictyosomes by virtue of their small size, lack of hypertrophied cisternae and associated vesicles differ markedly from those of the pre-meiotic zoosporangia.

The endoplasmic reticulum proliferation has kept pace with other membrane components during this phase of karyokinesis. By the termination of nuclear divisions each nucleus is surrounded by at least one layer of cisternal ER. There are luminal continuities between the nuclear envelope, this cisternal ER, and the peripheral system of ER which parallels the plasmalemma (Fig. 24, upper right arrow) and interconnects the chlorocplasts. This creates a unified luminal continuum of
ER throughout the sporangium (Fig. 16 and 17).

The chloroplasts are discoid (compare planar, Fig. 15, with transverse sections Fig. 14). Characteristically the chloroplast ER adjacent to the plasma membrane and the continuous peripheral ER system may contain an electron opaque material (Fig. 15). This opaque material is also seen in masses on the plasma membrane (Fig. 15, 14 and 20). Within the chloroplast envelope a moderate number of stacks of thylakoids are observed (Fig. 14). Neither pyrenoids nor out-pocketings of the chloroplast envelope have been observed during this phase (2.2). Plastoglobuli are not characteristic of these chloroplasts. Finally, from sequential sections it is apparent that by termination of karyokineses there is one chloroplast associated with each nucleus and its two persistent centrioles and dictyosome (Fig. 20). This close association of nucleus, chloroplast, centrioles and dictyosome is maximized when the centrioles have arrived at a position adjacent to the plasma membrane (Fig. 20). Furthermore, at this time the nucleus may often be deformed from its usual spherical shape (Fig. 20).

The mitochondria show no morphological changes except for an increase in numbers and an apparent clustering (Fig. 14 and 16). Small microbody-like structures are no longer associated with the peripheral ER/CER junctions. Instead they are located adjacent to nuclei and their sheathing ER cisternae (Fig. 21).
There has been a clear increase in the amount of non-membrane bound material which shows light homogeneous electron density and is presumed to represent lipoidal storage material (Fig. 14). This material is often associated with chloroplasts (Fig. 14).

In addition to the presumed storage material there are also some electron translucent vacuoles present at the end of karyokineses which had not been present earlier. Their origin remains obscure.

A final interesting cytoplasmic inclusion is the clusters of membrane bound bodies containing electron opaque material which may eventually obscure their own delimiting membranes (Fig. 14, arrows). Morphologically they can be found close to the presumed lipid masses (Fig. 14, 17, 22 and 23), and infrequently connected to the chloroplast ER (Fig. 17). They may show some variation in electron opacity but they remain distinct from the co-existent microbody-like structures (compare Fig. 21 and 23). These structures were never associated with the dictyosomes which morphologically possess neither hypertrophied cisternae nor associated vesicles during this phase of development.

2.4. Sporangia and Cytokineses
Mature sori may often be found with zoosporangia in various stages of cytokinesis. At the light microscope level zoosporangia destined to undergo cytokinesis change from a heterogeneously staining protoplast which is in contact with its cell wall to a homogeneously dense multiflagellate protoplast separated from its cell wall by a space (Fig. 25). The contents of the intramural lumen represented by this space stain metachromatically with TBO indicating acid and/or sulfated polysaccharides. With AB/SO staining there is both blue and red staining material within the lumen indicating the presence of both acid and sulfated polysaccharides. In mature sporangia where release has begun the extracellular lumen is difficult to see (Fig. 26).

The onset of cytokinesis is preceded by the production of flagella. It appears that flagellar production occurs by the protrusion of the axoneme and flagellar membrane into the intramural lumen. Indeed by the time the first signs of cytokinesis are present naked flagella consisting of axonemes and delimiting membrane are already present in the intramural space of the sporangium (Fig. 27). These flagella are typical with a basal plate separating the basal bodies from the 9 plus 2 microtubular axoneme. No mastigonemes are present at this time. However, in sori where some sporangia have begun or just completed cytokinesis, some pre-cytokinetic sporangia have been found to contain membrane bound bodies with fibrillar elements suggestive of mastigonemes. Some other stages of
mastigoneme maturation similar to those reported by Loiseaux (1973) and Markey and Wilce (1976a) in unilocular sporangia have been observed as well.

Except for modifications in the organization of the ER there are no dramatic changes in organelle associations characteristic of the previous stage.

By the onset of cytokinesis, there are distinct granulofibrillar masses of heterochromatin lining the nuclear envelope within the nucleoplasm (Fig. 29). These are easily distinguished from the small dense nucleoli (Fig. 29, lower left corner). There is also a dramatic increase in vesiculation of the outer nuclear membrane adjacent to the dictyosomes formative face (Fig. 27, top), which is accompanied by an increase in hypertrophy of the maturing face cisternae (Fig. 27) and increased numbers of golgi vesicles in the cytoplasm which are easily identified by their reticulate contents (Fig. 27, 28 and 29). Golgi vesicles often are found apparently poised for exocytosis (Fig. 28). In instances where cytokinesis is imminent the maturing face or most distal dictyosome cisterna was observed to be in various degrees of hypertrophy. The former fenestra, still visible in the adjacent more proximal cisternae (Fig. 30, arrowheads), have become stretched into membrane bound tunnels through the hypertrophied regions of these outer cisternae (Fig. 30, arrows). This is of great interest since the cleavage of the cytoplasm is accomplished by hypertrophied membrane bound cisternae containing apparent
membranous tubular invaginations and vesicles (Fig. 29, arrows). These cleavage vesicles are distinct from the individual golgi vesicles which are presumed to be secreting reticulate, non-membranous materials into the enlarging intramural lumen (Fig. 29) and certainly not elements of ER.

Although the nuclei of these sporangia are still at least partially ensheathed by cisternae of ER (Fig. 29), it is important to emphasize that the peripheral portion of the ER system which was so clear in the previous section is now obscured (Fig. 27).

Chloroplast and mitochondrial ultrastructural details show little variation from their morphologies in the previous stage except that chloroplasts may evidence some outpocketings of their envelope and ER, but there are no clearly defined phaeophycean pyrenoids (Fig. 29).

Other cytoplasmic inclusions discussed previously show no marked morphological changes. The csmiophilic vesicles, nevertheless are now uniformly electron opaque which makes their bounding membranes completely indistinguishable from their contents.

2.5. Mature Zoospores Awaiting Dehiscence

Once cytokinesis is finished individual zoospores are
easily observed at the light microscope level (Fig. 26). The segregation of organelles and other cytoplasmic inclusions has been completed. From this time until that of sporangial dehiscence not only do new cytoplasmic inclusions appear but also the organelle distribution and inter-relationships become very regular.

The zoospores are essentially spherical within the sporangium (Fig. 26 and 31) except when deformations are induced by crowding and lipid inclusions. The nucleus occupies a central location with the other organelles and inclusions distributed around it. Masses of chromatic material are still prominent in the nuclei which remain small (3 um in diameter), and the nuclear envelope is infrequently interrupted by pores (Fig. 31 and 32). The nuclear association with chloroplast, dictyosome and the centrioles, now appropriately considered basal bodies persists and may yet deform the nucleus (Fig. 38). Luminal continuity with the ER (Fig. 43) and the chloroplast ER (Fig. 31 and 32) is clear. Indeed the nuclear envelope and chloroplast ER are one and the same for the surface area where the two organelles adjoin one another (Fig. 32).

The opposite surface of the nucleus is usually occupied by the single dictyosome (Fig. 32). The outer nuclear membrane adjacent to its formative face remains vesiculate as in the cytokinesis phase (Fig. 40). Associated with the maturing face of this dictyosome are vesicles of varying sizes. These vesicles when small and/or still associated with the dictyosome
cisternae contain electron dense material (Fig. 39). These are clearly distinguishable from the homogeneous osmiophilic vesicles which appeared prior to cytokinesis (Fig. 39, compare with ES in Fig. 37). They all show a distinct variation in density, becoming more condensed with time (Fig. 31 and 32, large arrows vs. Fig. 39 and 44). They attain a slightly cupped discoid shape with a diameter of about 0.5 um. Those numbered 1 in Figure 39 are cut in surface section and the light centers represent cytoplasm in the depressed surface. Those numbered 2 in Figure 39 and indicated with arrowheads in figure 44 are cut in perpendicular cross section. Golgi vesicles containing incomplete structures such as these have been infrequently observed associated with and separated from the dictyosome just after cytokinesis.

Areas of perinuclear cytoplasm opposite the chloroplast not occupied by the golgi apparatus may contain restricted interconnected stacks of rough ER (Fig. 31) showing clear luminal continuities with the nuclear envelope (Fig. 43).

There is one chloroplast per zoospore which is bilobed and draped over the nucleus (Fig. 32). Small vesicles are located in the central constriction adjacent to the nucleus between the chloroplast envelope and the chloroplast ER which is now one and the same with the nuclear envelope. It is of interest to note that in these segregated zoospores proper pyrenoids may now be observed (Fig. 42) and plastoglobuli are infrequent but present (Fig. 35 and 38).
Microbody-like structures are small, and although present they are few in number (Fig. 31 and 32) and usually lie adjacent to the nucleus. Mitochondria consistently present a spherical to elliptical profile in section (Fig. 31 and 41). Although they possess the typical tubular cristae they have acquired electron opaque filamentous cores (Fig. 41 arrowheads).

Another prominent feature of most zoospores is the presence of storage material. This lightly osmiophilic material is believed to be most probably triglycerides. Indeed from biochemical assays of sori it has been determined that there is an increase in lipids during development with over half of this lipid being in the form of triglycerides (Lee and Walker, unpublished observations). Furthermore, none of the other cell types within the sorus possess any material comparable in morphology or quantity (Ch. 3), and internal tissues do not differ morphologically between soral and vegetative regions. In spores of this post cytokinetic stage this material is found to be continuous with apparent membrane profiles (Fig. 41). Finally with the freeze-etch technique they fracture in a manner similar to lipids of other organisms.

Zoospores awaiting sporangial dehiscence are biflagellate. Both flagella consist of an axoneme of the typical 9 plus 2 arrangement of microtubules which is surrounded by a membrane (Fig. 33), one of these has acquired mastigonemes which are apparently attached to one side of the flagellum (Fig. 34).
Each axoneme terminates at the spore with a basal plate and the flagellar membrane is continuous with the plasma membrane of the spore (Fig. 35). On the cytoplasmic side of each basal plate is a basal body and one of these (Fig. 25, basal body 1) is connected to the base of the other (Fig. 35 basal body 2) by an electron dense bridge (Fig. 35 large arrow) that seems to correlate with the angular emergence of the axonemes from the spore. Also the second basal body is associated with an electron dense mass of material (Fig. 35 arrowhead) which is interpreted as the rhizoplast. This appears in perpendicular section as a crescent of electron dense material (Fig. 36, arrowhead). Often portions of the rhizoplast are seen in the cytoplasm of the zoospore away from the basal bodies, usually near the plasma membrane (Fig. 31, arrowhead). Also apparent in the cytoplasm adjacent to the basal bodies are a few cytoplasmic microtubules (Fig. 35, small arrows). In appropriate sections these microtubules can be seen to project from the basal bodies along the plasma membrane into the cytoplasmic region on the dictyosome side of the nucleus where they lie as a sheet of microtubules behind homogeneously electron opaque vesicles. A plate of this complex of csmichilic vesicles and microtubules constitutes the eyespot of the _Nereocystis_ zoospores (Fig. 37).

Finally it should be mentioned that the sporangial wall has accumulated a thick layer of material at its most distal extent. It is interesting to note that the sporangial wall
tends to be altered in its electron opacity, perhaps in preparation for dehiscence (Fig. 44).

2.6. Zoospores

2.6.1. Release of Live Zoospores

In mature live material the tightly packed nature of the zoosporangia is obvious (Fig. 26). The thickened apical tips of these sporangia are clearly visible as the apical portion of the sporangium from which the tightly packed spores are excluded (Fig. 26, *). The process of zoosporangial dehiscence is best described as a sudden extrusion which removes the spores from the sporangium as a single mass. At the time of sporangial dehiscence the spores are essentially spherical and embedded in a mass of mucilaginous material (Fig. 45, cluster to right). As the mucilage dissipates, the spores separate, begin to assume a pyriform to conical shape (Fig. 45, cluster at left), and commence motility. The motile zoospore is of this pyriform to conical shape (Fig. 46).

With Nomarski optics it is possible to identify dehisced sporangia and differentiate them from paraphyses. These observations revealed that the dehisced sporangia are completely emptied of zoospores and opened apically where the thickened tips are absent (Fig. 26, arrowhead). Frequently
during in vitro release of zoospores intact but dislodged, indehiscent zoosporangia and numerous released spherical spores showing little or no motility were observed. These latter types often dominated zoospore suspensions collected in vitro at room temperature or in the confines of slide and coverslip.

2.6.2. Shadowed and Negatively Stained Zoospores

From shadowed material (Fig. 47, 48 and 49) it is apparent that the anterior flagellum is approximately twice the length of the posterior one which is about the length of the spore (Fig. 47). This confirms previous light microscope observations of Nereocystis zoospores (Hartge 1928, Kemp and Cole 1961). It is clear that the anterior flagellum, in contrast to the posterior one, bears mastigonemes (Fig. 48). These mastigonemes consist of a basal shaft of approximately 1 μm length with an apical extension which is about as long again but of a lesser diameter (Fig. 48). At the junction of the mastigoneme's basal shaft with its lesser extension are two short spurs also of a lesser diameter (Fig. 49, arrowheads). From negatively stained material it is clear that the chloroplast/nucleus and flagellar apparatus are still associated (Fig. 50, arrowheads indicated emergent flagella) and that the eyespot consisting of a plate of spherical granules separated from the chloroplast is still intact (Fig. 50, between arrows and Fig. 51). Finally from partially disrupted zoospores the flagellar basal body and rhizoplast apparatus are
apparently intact in the motile spore as well (Fig. 52).

2.6.3. Non-motile, Spherical Zoospores

Samples of non-motile, spherical zoospores revealed three different types of zoospore morphology. The first type is characterized by its electron translucent cytoplasm, the presence of un-released plaques, apparently naked plasma membrane and most significantly the mound created by the withdrawn axoneme which appears as a matching pair of typical 9 plus 2 profiles of microtubules (Fig. 53). The second type of zoospore is very much like the first one with two exceptions. On the one hand the previously tightly coiled axonemes have relaxed to an equatorial diameter of the spore and on the other, plaques have been released to the outside of the cell by means of exocytosis (Fig. 54). The third type of zoospore is characterized by a significantly more electron opaque cytoplasm, osmiophilic deposits on the plasma membrane, and perhaps most significantly by the presence of a fibrillar circumferential cell wall between the plasma membrane and the secreted plaque material (Fig. 55) It is important to note that the eyespot has been disorganized in all three spherical zoospore types as well.

2.6.4. Cases of Delayed Zoosporangial Dehiscence

Electron microscope observations come from sori which have
undergone in vitro release of spores for approximately 4 to 6 hours while light microscope observations are from soral material recovered several days after release from the lamina.

The subcellular organization of these retained zoospores is essentially the same as that of mature spores awaiting sorus exuviation and sporangial dehiscence. The basal body/nucleus/chloroplast association is still intact and the full organelle complement is present (Fig. 56 and 58). However, there are some differences which must be noted here. First it is interesting that the intramural space surrounding the zoospores no longer contains the reticulate material presumed to be acid and sulfated polysaccharides (compare Fig. 37 and 56). Also it is of interest to note that the zoospores contain numerous plaques (Fig. 56) which in favorable surface sections show their annular sculpturing (Fig. 59). Although they are still within the protoplast fused vesicles have been observed.

Flagellar withdrawal of the naked posterior flagellum has occurred (Fig. 56 and 58) and evidence of longitudinal flagellum/flagellum fusions has also been observed. These latter are presumed to occur by tight coiling of a single flagellum upon itself as zoospores apparently rarely fuse (Fig. 57). Another curious aspect of these zoospores is the masses of osmiophilic material associated with their plasma membranes as well as the finely fibrillar material emanating from them (Fig. 58).
Finally from the abscised soral material it is very interesting to discover that zoospores for which dehiscence is delayed even for a few days may remain alive and secrete a cell wall while still within the zoosporangial cell walls. Although this was rare (Fig. 57) it is not unreasonable to assume that the numerous dislodged sporangia observed by Kemp and Cole (1961) which had failed to dehisce were viable and suggest an heretofore unconsidered form of zoospore dispersal.

3. Discussion

3.1. Pre-meiotic Zoosporangial Initials

It was previously pointed out that a number of authors have already ultrastructurally examined various stages of zoosporogenesis and gametogenesis in brown algae. Unilocular sporogenesis has been studied in several orders of the brown algae. However, a number of these reports do not include any of the pre-meiotic or meiotic stages of development (Manton 1957, Bouck 1965, Gherardini and North 1972, Baker and Evans 1973b, Toth 1974 and 1976). Those that do include pre-meiotic or meiotic stages do so either incompletely (Chi and Neushul 1972, Chi 1973, Toth and Markey 1973) or do not deal with the Laminariales (Markey and Wilce 1976a, Loiseau 1973). Clearly
the observations made here (sections 2.1 and 2.2) and in Chapter 3 contribute to a better understanding of these earlier stages of unilocular zoosporogenesis in the Laminariales and the brown algae in general.

The nucleus and golgi apparatus are the most outstanding participants in the morphogenetic processes prior to meiosis (Ch.3 and Kemp and Cole 1961). The golgi apparatus morphology and staining characteristics together with the circumstances of zoosporangial inception and sorus differentiation are indicative of a very active secretory role (Ch.3). The coated vesicles observed in association with the maturing face of the dictyosomes may perhaps be derived from the hypertrophied regions (Fig.3 and 6). The precise role of these coated vesicles remains obscure. It is noteworthy that as meiosis approaches the hypertrophy of the golgi apparatus dictyosomes decreases and the dictyosomes become both more loosely associated with and dispersed around the perimeter of the nucleus (Fig.7 and 9). This has been interpreted as a stage of dictyosome replication by other authors in other unilocular initials (Loiseaux 1973, Marke and Wilce 1976a) and in the mother cell of Macrocystis (Chi and Neushul 1972). The secretory activities apparently required of the zoosporangial initials in Nereocystis sori during the sporangial initiation phase prior to meiosis (Ch.3) explain the contrast between the golgi apparatus here and in unilocular sporangia of Pilayella which do not occur in a sorus (Markey and Wilce 1976a). The
apparent replication of the golgi apparatus at the end of the pre-meiotic phase agrees with its continued close relationship with the nuclei here and in other members of the Laminariales (Chi and Neushul 1972, Chi 1973, Toth 1974, Kugrens and Reeves 1972, Kugrens and Collins 1976) and in other brown algal sporangia as well (Markey and Wilce 1975, 1976a and b, Loiseaux 1973). Thus it would seem that the golgi apparatus begins in a meristematic or secretory capacity (Ch.3) and then with the onset of meiosis shifts to a replicative phase during which the nuclear association is less distinct.

Changes in the nuclear organization observed during and up to meiosis would appear to correlate with the proposed shift in function from secretion (Ch.3) to the mechanics of meiosis (Kemp and Cole 1961) and subsequent mitoses. In accord with the dominant nucleolus of this phase, still present at the onset of meiosis (Fig.7) there is an apparent increase in the density of cytoplasmic ribosomes and granular matrix of the nucleoplasm (compare Fig.1 and 7). It would seem logical to assume that this prominent nucleolus (not common in many brown algal cell types (Russell 1973)) would correlate with preparation for internally directed processes such as the imminent rapid organelle replication which follows with meiosis. A free ribosome rich cytoplasm is generally associated with this type of cellular activity. In light of the clear demonstration by Kemp and Cole (1961) of meiosis in this first sporangial initial division, and replacement of the
masses of electron dense material in the just produced zoosporangial initials by electron dense linear elements is assumed here to represent the shift into leptotene of meiotic prophase I. Centriole replication has begun at the end of this pre-meiotic phase and presumably therefore correlates with prophase I of meiosis as was the case in *Pilayella* (Markey and Wilce 1976a).

It is interesting to note that the homogeneous fibrillar region is absent and there is a mass of electron dense fibrillar material associated with the nuclear envelope (Fig. 7 and 12). The fate of this material would be worth investigating in light of Kemp and Cole's (1961) description of a supposed synizetic knot at the nuclear envelope where chromatic material concentrates.

This is reinforced by the observation that the relationship between the nuclear envelope, chloroplast endoplasmic reticulum and endoplasmic reticulum is clear during this pre-meiotic phase (sec. 2.1) and becomes less so at the onset of meiosis as nuclear envelope continuities with the rest of the endomembrane system are difficult to locate although these systems are still present if not prominent.

A similar obscuring of direct continuities between the nuclear envelope and elements of the ER and CER has been associated with the morphogenesis in meristoderm cells from the younger expansion zone to the photosynthetically mature tissues
adjacent youngest scri. Perhaps more specifically it correlates with a slowdown in the secretory activity of the meristoderm cells' golgi apparatus (Ch. 1) as these cells presumably become exporters of photosynthates. Although it is doubtful that these zoosporangia are becoming exporters of photosynthates it would seem that they undergo a change from external to internally directed synthetic activities. In fact they would most probably either continue to or begin to import metabolites, the paraphyses being the nearest source through plasmodesmatal continuity.

Although the chloroplasts of the zoosporangial initials are not identical to those of meristematic cells (Ch. 1, transition zone), they do contain fewer stacks of thylakoids than at the end of the pre-meiotic phase (compare sections 2.1. and 2.2.) and certainly fewer than chloroplasts of adjacent meristoderm cells (Ch. 1). The proliferation of chloroplast envelope identical to those of Cystoseira (Evans 1968), are interpreted here as preparatory to the chloroplast divisions which must occur by the end of the impending karyokineses.

Homogeneous lightly electron dense masses, believed by this author to be primarily neutral lipids are small but are present in zoosporangial initials (Fig. 2) and increase during this phase to become a prominent feature of the post-karyokinetiC protoplast (Sec. 2.3.). These masses of material have been called variously "food vacuoles" (Chi and Neushul
The small spherical microbody-like structures (Ch.3 Fig.20) either are formed de novo or are carried over from the subtending cells. They are present in small numbers in the newly differentiated zoosporangial initials and appear to increase in numbers and become associated with the chloroplast ER during the pre-meiotic phase of *Nereocystis*. Although, present in micrographs of pre-cytokinetic zoosporangia of *Macrocystis* (Chi 1973) they are not considered there or in other relevant publications (Markey and Wilce 1975, 1976a,b).

Finally it should be noted that the cell walls of the sporangium have thickened and stabilized by the beginning of meiosis. It is interesting that the cell walls of the meristoderm behave in a similar manner when their golgi apparatus shifts from the hyperactive morphology as well (Chapter 1, Sec.2.3.). This would again re-enforce the argument for the similarities between sorus inception, mucilage duct system inception and meristematic activity.

3.2. Post-meiotic Cytomorphogenesis and Cytokinesis

Although all stages of these post-meiotic cytomorphogeneses which accompany the mitotic divisions have not been observed here, comparisons with earlier observations in *Macrocystis* (Chi and Neushul 1972, Chi 1973), *Chorda* (Toth
1974) and *Pilayella* (Markey and Wilce 1976a) make it possible to comment on and add to the understanding of some aspects of this process.

The historically central cytomorphogenetic feature of this phase of zoosporogenesis is the several post-meiotic nuclear divisions which culminate in the regular distribution of the nuclei throughout the zoosporangial protoplast prior to cytokinesis (Fritsch 1945, Ohmori 1967). This is, furthermore, characteristically accompanied by a diminution of nuclear size (Fritsch 1945, Ohmori 1976). From these light microscope observations, chloroplast replication has also been reported to follow nuclear divisions (Fritsch 1945). All three of these phenomena, identified by light microscope studies, have been subsequently confirmed ultrastructurally in various brown algal unilocular sporangia (Baker and Evans 1973b, Chi and Neushul 1972, Chi 1973, Loiseaux 1973, Toth 1974, Markey and Wilce 1976a). These observations have also been confirmed in *Nereocystis* with both light microscopy (Kemp and Cole 1961) and ultrastructurally here.

The ultrastructural observations just cited have demonstrated that other organelles, like the chloroplasts, may replicate almost synchronously with karyokineses. Although Toth (1974) and Markey and Wilce (1975, 1976a and b) have noted centriole pairs in nuclear envelope pockets at various stages during this phase of Toth uni- and plurilocular zoosporogenesis they suggest that centrioles and/or basal bodies may arise de
novo. Their chief argument being that whole sporangia may sometimes reveal no centrioles (Toth 1974). However, they illustrate several stages where pairs of centrioles are in nuclear envelope pockets even at both poles in some dividing nuclei (Markey and Wilce 1975). In Chi (1973) similar centriole behaviour is demonstrated for *Macrocystis* and no evidence for a disjunction of extant centrioles was presented. This would also be true in the few other studies where brown algal cell divisions have been considered (Neushul and Dahl 1972, Walker, Chi and Neushul 1975, Penney personal comm.). If disassembly and then de novo synthesis of these structures were to have occurred it would most likely be between the last karyokinesis and the production of basal bodies for flagella. However, any of the previous works dealing with this phase of zoosporogenesis in brown algae (Markey and Wilce 1976a, Chi 1973, Loiseaux 1973) have failed to produce either evidence of a centriolar organizer as reported for some epithelial cells (Sorokin 1968) or even evidence of simple centriole nucleated centriole replication during this stage. If anything, the observations made here would agree with those of Chi and Neushul (1972) and Chi (1973) and even go further in that separating pairs of centrioles have been reported here suggesting they certainly are not synthesized de novo at the poles of dividing nuclei. Also as will become clear the cytoplasm of the zoosporangia is very regularly organized and thus even a median section of a sporangium could easily miss all centrioles. Therefore, it would seem safe for the present
to assume that the centrioles of *Nereocystis* are replicated from extant ones in synchrony with the karyokineses and at the end of this period the pair associated with each nucleus directly becomes a basal body when axonemes are produced.

From observations of the mitoses in unilocular (Markey and Wilce 1976a) and plurilocular (Markey and Wilce 1975) sporangia of *Pilayella* it has also been suggested that the dictyosomes of the golgi apparatus (Fig. 18) replicate and are segregated with the nuclei by virtue of their polar positioning during these karyokineses. This is apparently the case in *Macrocystis* as well (Chi 1973, Chapter 1, Fig. 4). The observations from both the sporangia described here and the meristoderm cells of the transition and expansion zones of young thalli (Ch. 1) would also support this suggested nuclear directed golgi replication and segregation. The close relationship between nuclei and golgi apparatus is supported by a diversity of observations but classically in the experiments of Flickenger (1968) in which dictyosomes of enucleate amoebae run down. From the final one to one relationship of dictyosome to nucleus observed here at the end of karyokineses and in zoospores it seems that the golgi apparatus is also replicated and segregated in something close to if not synchrony with nuclear divisions in *Nereocystis*.

Various elements of the endoplasmic reticulum, the microbody-like structures and the mitochondria are the three obvious remaining organelles to consider. Only in the studies
of Chi and Neushul (1972) and Chi (1973) have either the ER or the microbody-like structures present during this stage received any attention. Although the mitochondria are briefly considered by all no evidence for a synchronous replication and segregation has been presented by any of the previous authors, only suggestions of preferential distribution in peripheral or central cytoplasmic regions. The subsequent consideration of the endomembrane system which follows will clearly establish its close adherence to nuclear and organellar replication and segregation as has been considered for chloroplasts, golgi apparatus, and centrioles.

It is clear that the mitochondria do not follow nuclear divisions in obvious synchrony. However, each post-cytokinesis zoospore invariably contained its complement of mitochondria. Although they do not show as restricted a distribution throughout the protoplast it is extremely interesting to note the apparent clumping of some as cytokineses approaches during this phase of zoosporogenesis. Although the area of mitochondrial behaviour is a little explored topic, studies like those of Heath and Heath (1978), Franke and Kartenbeck (1971) and Bracker and Grove (1971) may prove fruitful in considerations such as this. It certainly would be premature to dismiss the possibility that mitochondria may follow the replicative process of the nuclei which is consumated in their segregation amongst zoospores.

The microbody-like structures also present a confusing
picture in terms of their distribution. Only in the work of Chi (1973) are they even considered and then they are only pointed out in their association with nuclei and ensheathing ER. From the present study it can be added that they are apparently present from the beginning (Fig. 20 Ch. 3) and associated with ER subsequently they are more numerous and principally occur at ER/CER junctions. They then shift during this phase to a primarily perinuclear locus amongst the ensheathing ER and later at least one invariably is present in the golgi/mitochondrial region of each mature zoospore. In addition one is reminded that the current theory for the origin of microbbody-like structures points to the ER which as will be seen, closely follows nuclear replication and segregation. Thus it is not unreasonable to suggest that microbody-like structures are as closely tied to nuclear divisions as mitochondria, if not more so.

The presumed lipid masses have also kept up with organelle replication. As is seen from favorable sections each chloroplast is associated with some of this material (Fig. 14). Thus through their chloroplast association, and most probably origin, they are within this tight organelle association. Likewise the appearance of the osmiophilic membrane bound masses may be associated with the chloroplasts (Fig. 17) and cannot be separated from the lipid masses. It is proposed that these masses may originate from out-pocketings of the chloroplast ER and that their subsequent maturation is
accomplished to some extent through their association with the presumed lipid masses. Furthermore, it is clear from observations made here that these masses are the future granules of the *Nereocystis* eyespot.

During the pre-meiotic phase of development the endomembrane system began as an interconnected entity consisting of some peripheral ER, cytoplasmic ER, chloroplast ER and the nuclear envelope. Direct luminal continuities of nuclear envelope with cytoplasmic ER and chloroplast ER were obscured by the onset of meiosis. With this the peripheral system of ER became more prominent. There is little or no data concerning the dynamics of this endomembrane system in other considerations of laminarian unilocular sporangia prior to meiosis. Only in Chi (1973) and Chi and Neushul (1972) is there any serious consideration of this system outside of the phenomenon of mastigoneme production (Markey and Wilce 1976ab). Although NE/EE continuities are pointed out for similar stages in unilocular sporangia of other brown algae (Loiseaux 1973, Baker and Evans 1973b Toth 1974, Markey and Wilce 1976a) some portions of the endomembrane system were either not present or omitted from discussions all together. From the post-meiotic observations of *Nereocystis* reported here a more complete model is possible. This more complete morphological picture allows for some consideration of functional aspects as well.

This phase of zoosporogenesis sees numerous changes in this endomembrane system. The peripheral system of ER
continues its expansion from the last stage as the sporangia elongates. A new component the nuclear ensheathing ER is very prominent until the production of flagella. It presumably arose from an elaboration and organization of cytoplasmic ER it is with its appearance that NE/ER continuities are also clearly re-established. In fact at this point the NE, ensheathing ER, peripheral ER and CER constitute one completely confluent endomembrane system. This ensheathing ER system has been noted by Chi and Neushul (1972) and Chi (1973) in *Macrocystis* and it was likened to the model of Bouck (1965) for the generalized brown algal cell. This marks the point at which both the amount of continuity and extent of continuity in the endomembrane system is greatest. This period is ended at the time of the appearance of flagella. The occurrence of an almost identical ER system in the basidia of *Pholiota terestris* (Wells 1978) during meiosis suggests some general significance for this nuclear ensheathing ER system which should be pursued. This turnabout is marked by a disjunction and reduction of the peripheral ER portion of the endomembrane system, a reduction of and confinement of the ensheathing ER to a localized portion of the nuclei, and a juxtaposition of chloroplasts and nuclei such that the NE and CER may be one in the same for some portion of both. Thus it would appear that the endomembrane system consisting of NE, ensheathing ER, CER and peripheral ER go through a series of morphogenesises closely related to nuclear divisions as was the case for previously considered organelles. It certainly represents an attractive candidate
for a mechanism of distributing or coordinating the
distribution of nuclei about the sporangium in an orderly
fashion. There is little doubt from the observations of Chi
(1973) and here that each zoospore receives its complement of
the endomembrane system during cytokinesis. From Chi (1973)
the ensheathing system of ER is even implicated in the process
of cytokinesis. Others have suggested the golgi apparatus.
Thus, there is still some question as to how the internal
vesicles of cytokinesis are produced. Toth (1974) and Markey
and Wilce (1976a) suggest that there is an accumulation of
small vesicles probably of golgi origin, while Chi and Neushul
(1972) suggest ER elements as a source. The sheathing ER of
the just post-karyokinetic sporangia are a possibility,
however, this ER is apparently not present in Pilayella (Markey
and Wilce 1976a) or Chorda (Toth 1974) and no intermediate
stages have been shown in Macrocystis (Chi and Neushul 1972,
Chi 1973). Furthermore, in both Macrocystis and Nereocystis
there develops a restricted mass of rough ER with each zoospore
about the time of cytokinesis. It is suggested here that this
mass of ER so well illustrated in Chi's account of Macrocystis
represents retracted former nuclear sheathing ER. It
suggested, furthermore, that another source may be present for
the origin of the internal portion of the developing cell plate
in cytokinesis. Portions of this internal member have been
clearly illustrated in Macrocystis and Nereocystis here.
Their difference from golgi vesicles contents (the tubular
inclusions) (Fig. 29), strongly suggests a different mode of
origin than for those typical spherical golgi vesicles seen
secreting to the intramural lumen of the sporangium (Fig. 28 and
29, Chi 1973, Toth 1974) at this time.

There is evidence that dictyosomes of the golgi apparatus
may show intra-cisternal functional differentiation (Coulomb
and Coulon 1971, Sage and Jersild 1971, Mignot, Brugerolle and
Metenier 1972, Ovtracht and Thiery 1972, Dawson 1973, Locke and
Sykes 1975). It is proposed here that the internal cell plate
member originates from a whole dictyosome cisterna which has
hypertrophied and dissociated from the dictyosome stack and
continues to enlarge to meet others and ultimately the
infurrowing plasma membrane as well. This departure of
distal-most cisternae has also been suggested by Mollenhauer
(1971) in the context of golgi apparatus replication.

In concluding this discussion it should be clear that
throughout the karyokineses of zoosporogenesis the contents of
the sporangium are regularly organized in a nucleocentric
manner and that by the onset of cytokinesis organelle
segregation into zoospore units is an established fact. It is
suggested that the proposal of Collins and Kugrens (1976) is
inadequate and that rather the model of Bouck (1965) (also
suggested by Chi and Neushul 1972) is more adequate but
requires for Nereocystis the addition of microbody-like
structures, centricles and neutral lipid storage products as
part of the organization. It is believed that closer
examination of this phase of zoosporogenesis may shed light on
the mechanisms of replication of the elements of the typical brown algal cytoplasmic unit of Bouck (1965) as well as contribute much to our understanding of the interrelationships of the constituent organelles.

3.3. Zoospore Structure and Function

3.3.1. Ultrastructural Constituents of the Zoospore

As noted by Chi and Neushul 1972 and Chi 1973 in *Macrocystis* mature spores awaiting sporangial dehiscence possess both a consistently uniform complement of ultrastructural constituents and an essentially identical internal organization. The observations made here from zoospores of *Nereocystis* agree with those from *Macrocystis* (Chi 1973). The *Nereocystis* zoospores are delimited by a plasma membrane which is continuous over the flagella. One of these bears mastigonemes and is longer than its naked posteriorly directed counterpart. Although not described for swimming zoospores of *Macrocystis* (Chi 1973), in *Nereocystis* there is a single long terminal filament and then two very short ones projecting from the distal end of each mastigoneme. Baker and Evans (1973b) have noted single terminal filaments on mastigonemes of *Ectocarpus* unilocular zoospores but not the two short adjacent members. The basal bodies of these flagella are apparently anchored to each other at 90 degrees in both
sporangia and free zoospores as has been noted in *Macrocystis* (Chi 1973). This differs from zoospores of other brown algae (Toth 1974), as well as a number of brown algal spermatozoa where basal bodies are at an obtuse angles to each other (Manton 1957). The single sheet of microtubules penetrating into the cytoplasm away from the basal bodies observed in *Macrocystis* (Chi 1973) are also present in *Nereocystis*. These sheets of cytoplasmic microtubules or roots differ from those of fucoid sperm cells. They do not clearly associate with the chloroplasts which contain the eyespot in fucoid sperm (Manton 1964). *Macrocystis* (Chi and Neushul 1972, Chi 1973) and *Nereocystis* stand apart from the other orders of the Phaeophyta and *Chorda* as well (Toth 1974 and 1976) in their possession of a cytoplasmic eyespot. It is of particular interest that these eyespot granules, although cytoplasmic, apparently originate from and are presumably still bounded by membranes of the chloroplast ER (Sect. 2.3, Fig.17). As more brown algal reproductive cells are examined it will be of interest to see if this difference is of any phylogenetic significance. From light microscopy of rounded up free zoospores, thin sections of still contained zoospores and negatively stained free zoospores it appears that the chloroplast associates with the basal bodies at one end coincident with the nuclear association to the basal bodies and that it is wrapped around the nucleus such that it occupies a parietal position in the zoospore (see Fig. 50). The cytoplasmic eyespot remains in the posterior, broadest hemisphere of the zoospore (Fig. 50 and 51), a position
usually occupied by the eyespot bearing chloroplast in other brown algal zoospores (Baker and Evans 1973b, Toth 1976a, Markey and Wilce 1975, 1976a and b). Opposite the chloroplast, adjacent to the nuclear envelope and closest to the posterior basal body is the single dictyosome of the Golgi apparatus. This same configuration is seen in *Macrocystis* (Chi and Neushul 1972, Chi 1973) and the "plaques" apparently produced by this Golgi apparatus were first described in *Macrocystis* (Chi 1973) and their role in adhesion which was suggested there has been further supported (Oliveira, Walker and Bisalputra, manuscript in preparation). It is believed that these plaques are homologous to the vesicles observed in *Ectocarpus* unilocular and plurilocular zoospores (Baker and Evans 1973a, b), *Pylaiella* zoospores (Markey and Wilce 1976a) and *Chorda* (Toth 1974 and 1976a). In accord with these observations of plaques, and the chloroplast/nucleus/Golgi apparatus complex in both *Macrocystis* (Chi and Neushul 1972, Chi 1973) and *Nereocystis* it is essential to point out a further similarity. Both also contain a mass of stacked, fenestrate luminally interconnected rough ER adjacent to remaining portions of the nuclear envelope where luminal continuities with it are also evident. Thus *Macrocystis* and *Nereocystis* are distinct from *Chorda* in the possession of this particular tight endomembrane system of chloroplast ER/rough ER and Golgi apparatus. It is suggested that this endomembrane unit containing chloroplast, RER and Golgi reflects the apparent production of the presumed (Oliveira, Walker and
Eisalputra unpublished observations) glycoprotein adhesive plaques. These will be distributed throughout the zoospore eventually (Fig. 44). The masses of lipids are still as prevalent as they were observed to be in both *Macrocystis* and *Chorda* zoospores (Chi and Neushul 1972, Chi 1973, Toth 1974 and 1976a). The suspicion that these are lipid is well supported here and has been held previously (MacMillan 1899 and Sauveageau 1918). The posterior cytoplasm is seen to contain a number of apparently spherical mitochondria and invariably at least one microbody-like structure is present. The occurrence of filaments in mitochondrial cristae of zoospores during and just after cytokinesis has been previously reported (Chi 1973, Markey and Wilce 1976a). This is apparently true of the *Fucus* sperm as well (Pollock and Cassell 1976). As yet there is no evidence as to their functional significance.

It becomes apparent that although the zoospores of *Nereocystis* are very similar to those of other orders of brown algae there are some differences and many of these are shared in common with *Macrocystis* but not *Chorda*. Furthermore, it should be clear that other than the appearance of plaques all of the constituents of these zoospores were present and roughly organized prior to cytokinesis. The appearance of the plaques conceivably represents further cause for the modifications in the endomembrane system noted to occur at the time of cytokinesis.
3.3.2. Zoospore Release and Behavior

The process of zoospore release from dehiscent sporangia has been observed and described for *Nereocystis* in the course of this study. The observations made here are in agreement with those made previously (Kemp and Cole 1961). The flagellate, essentially spherical spores are suddenly ejected from the distal end of mature sporangia in a mass of mucilage. The same observations have been made ultrastructurally for *Chorda* (Toth 1976a). As the mucilage dissipates the spores become motile and anteriorly pointed (Kemp and Cole 1961, here Fig. 46). This acute anteriorly pointed morphology is typical of most swimming brown algal zoospores (Fritsch 1945). Subsequently zoospores either become attached by their anterior flagellum, round up and move about their point of attachment, or round up without attachment and gradually lose motility. Kemp and Cole (1961) have also observed the anterior flagellar attachment and propose flagellar withdrawal as the mechanism whereby the zoospore is drawn to the point of its anterior flagellar attachment to the substrate. This view has been supported by Toth (1976a) who also has demonstrated the axoneme within the protoplast of settled *Chorda* zoospore protoplasts. Similar observations of withdrawn axonemes have been made here as well. The observation of rounded up non-motile zoospores from *Eisenia* led Clare and Herbst (1938) to erroneously (Hollenberg 1939) suggest that these zoospores were not motile and not flagellate. It was noted during this work that
rounded up essentially non-motile zoospores may often be obtained during in vitro collection of zoospores from mature sori of *Nereocystis*. Furthermore, as was previously observed by Clare and Herbst (1938), these spores may produce gametophytes. However, it has been observed here that these spores contain withdrawn flagellar axonemes (Fig. 53). Thus it seems that *Nereocystis* zoospores may successfully adhere to a substrate either by primary anterior flagellar attachment followed by secondary spore adhesion or primary attachment may be accomplished by the spore after its flagellum has been withdrawn. Kemp and Cole (1961) noted large numbers of dislodged sporangia where dehiscence had failed. Similar observations were made during this study as well as additional observations of sporangia still attached to sori which had failed to dehisce. The observations here of retained spores which have withdrawn flagella and even deposited individual cell walls within the sporangium suggest the interesting possibility of another mode of gametophyte dispersal. The numbers of dislodged indehiscent sporangia observed by Kemp and Cole (1961) and here may represent a dispersal unit which contains pre-packaged male and female gametophyte progenitors. In Kemp and Cole (1961) it was proposed as did Sauvageau (1918) that in the absence of paraphyses to squeeze the sporangia laterally they could not dehisce, hence dislodged sporangia do not dehisce. It is proposed here that rather the dislodging of the sporangia may partially disrupt the sporangial wall to a degree which allows the mucilage and its concomitant hydration
pressure (Toth 1976b) to be bled off and escape before the apical thickening can dehisce in the usual fashion and release the spores. Furthermore, from observations made concerning paraphysis function (Ch.3) it is believed that the paraphyses are compressed by the sporangia rather than the reverse as others have suggested (Sauvageau 1918, Fritsch 1945, Kemp and Cole 1961, Ohmori 1967).

It is clear from observations made here that zoospores will continue with flagellar withdrawal and cell wall deposition within the sporangium. However, it is not known whether plaque excytosis is a pre-requisite to cell wall deposition. It is the case in zoospores which either attach first by their flagella or those which round up and subsequently attach (Toth 1976a, here Figs. 53, 54 and 55). These plaques first described for *Macrocystis* zoospores (Chi 1973) are presumed to be analogous to the presumptive adhesion vesicles of *Chorda* (Toth 1976a) and other brown algal zoospores (Baker and Evans 1973b). The goal of adhesion is common to all of the brown algal zoospores and the contents of these vesicles are presumed to play a role in zoospore adhesion. Evidence of their believed role in adhesion is accumulating (Baker and Evans 1973b). Similarly derived golgi vesicles presumed to play a role in zoospore adhesion have been demonstrated and studied in other algae (Evans and Christie 1970, Christie, Evans and Shaw 1970, Callow and Evans 1974) and even fungi (Sing and Bartniki-Garcia 1975) where they have been
associated with the process of spore encystment. With the abundance of literature dealing with the process of secretion and excytosis and comparative data in other groups it is both possible and desirable to construct a model to both explain zoospore structure and function as well as to suggest future approaches in order to further our understanding of these spores and how they work.

3.3.3. The Motile Zoospore Structure and Function

It is possible to formulate a structural and functional model for the zoospore of *Nereocystis*, however, one assumption must first be discussed. It is assumed that the structural and organizational details of the mature zoospores awaiting release are essentially identical to those of motile zoospores. Chi and Neushul (1972) have made observations to this effect in zoospores of *Macrocystis*. The central location of the nucleus and the chloroplast and their association with the point of flagellar insertion is illustrated here with negatively stained and shadowed preparations of motile zoospores. This nucleus/chloroplast association in motile unilocular zoospores of other members of the Laminariales is supported by the observations of Chi and Neushul (1972), Chi (1973) in *Macrocystis* and by Toth (1974 and 1976a) in *Chorda*. Observations of the same type of association have been reported in other brown algal unilocular zoospores as well (Manton 1965, Loiseaux 1973, Markey and Wilce 1976a). Although Baker and
Evans (1973a) showed this tight nuclear/chloroplast relationship to exist in *Ectocarpus* plurilocular zoospores, the relationship was found to be more distant in the larger unilocular zoospores of *Ectocarpus* (Baker and Evans 1973b), however, a direct luminal continuity between chloroplast ER and the nuclear envelope was maintained by an ER bridge.

The lateral insertion of flagella and basal body orientation relative to nucleus and chloroplast are substantiated by observations in *Macrocystis* (Chi and Neushul 1972, Chi 1973). It is interesting to note that the angle between basal bodies never exceeds ninety degrees. This may be due to a lack of the posterior flagella and chloroplast contained eyespots association reported for other brown algal zoospores (Toth 1976a) and motile gametes (Bouck 1970). It is of interest that *Chorda* differs from *Macrocystis* and *Nereocystis* (Chi and Neushul 1972 and Chi 1973, Toth 1974) in this respect. Indeed, from this study there is clear negative staining evidence for the location of the eyespot both separate from the chloroplast and in the posterior portion of *Nereocystis* zoospores free of the sporangium. This would seem to be the case for *Macrocystis* as well (Chi and Neushul 1972). It is notable that in all other observations of brown algal zoospores (Manton 1957, Baker and Evans 1973ab, Loiseaux 1973, Toth 1974, 1976, Icfthouse and Capon 1975, Markey and Wilce 1975, 1976ab) the eyespots consist of a cluster of osmiophilic structures within the chloroplast envelope. This is also true
of the observations from brown algal sperm as well (Manton 1956, Bouck 1970, Cassell and Pollock 1977). The only exception to this is the sperm of Dictyota (Chi 1973) and Laminaria (Eisalputra, unpublished). However, as in all the previous observations of brown algal zoospores the maintenance of the eyespot appears to be dependent upon the rootlet of the posterior basal body which is intact here. Similar rootlets have been noted in brown algal unilocular and plurilocular zoospores (Baker and Evans 1972a, b, Manton 1957) as well as in fucoid sperm cells (Manton 1956, Bouck 1970) where they may produce the typical beak and in all cases where considered are suggested to position the eye spot bearing chloroplast by the posterior flagellum (Manton 1956, 1964, Baker and Evans 1973ab, Markey and Wilce 1976ab). Finally it is very interesting to recall that these eyespot granules in Nereocystis although separate from the chloroplast are most probably delimited by membranes of former chloroplast ER (Sect. 2.3, Fig. 17).

The presence of the golgi apparatus is not as clearly established. It certainly is not prominent within rounded up unattached Nereocystis zoospores. Even though the dictyosome is severely reduced in withheld zoospores (Sect. 2.6.4) the nuclear envelope continued to vesiculate in the appropriate locus relative to the chloroplast and basal body locations in the earlier stages of differentiation (compare Fig. 58, arrowheads with Fig. 32). The presence of the sculptured plaques which the dictyosome of the golgi apparatus produced
are persistent until the zoospores round up. Vesicles of similar nature and origin have been consistently reported in most studies of brown algal zoospores (Baker and Evans 1973ab, Loiseaux 1973, Toth 1974, 1976, Lofthouse and Capon 1975, Markey and Wilce 1976ab) but they are apparently absent from brown algal sperm cells (Manton 1956, Bouck 1970). The carbohydrate nature of the contents of these vesicles has been demonstrated in Ectccarpus zoospores (Baker and Evans 1973a) and in Macrocystis and Laminaria zoospores (Chi 1973, Oliveira, Walker and Bisalputra, unpublished observations).

The presence of mitochondria, lipids and the occasional microbody-like structures in favorable sections of withheld and unattached rounded up zoospores and previous stages strongly suggests their persistence through the motile stage. It is curious that microbody-like structures have only been reported in zoospores of Macrocystis and here. Finally it is certain that the anterior flagellum of Nereocystis bears mastigonemes as do those of other brown algae (Toth 1976, Scagel 1966, review). It is concluded then that the motile Nereocystis zoospore contains the same components as that of the mature zoospores awaiting sporangial dehiscence. With the establishment of this assumption a diagramatic representation of the ultrastructural architecture of the motile Nereocystis zoospore is provided (Fig.60) for reference during the remaining discussion.

From the previous discussions (Sec.3.3.2.) and studies
(Kemp and Cole 1961, Toth 1976a, Loiseaux 1973) the process of zoospore settling can be outlined by the events beginning with flagellar/substrate contact, then continuing with flagellar adhesion, spore thrashing, anterior flagellar coiling and resorption, morphological shift from pyriform to spherical, spore cell body adhesion and terminating with the commencement of cell wall synthesis.

At this time the following mechanistic model is put forward to explain these typical events of brown algal zoospore settlement. Then evidence from observations made here and elsewhere in support of each aspect of the model is discussed.

This first proposition is that the anterior flagellum functions in the three capacities of motility, tactile sensitivity, and adhesion. The second proposition is that in concert with the anterior flagellar tactile capacity, contact with a physical substrate induces zoospore thrashing and a disruption of calcium ion balance such that the calcium ion concentration in the cytosol is increased. The third proposition is that this increased calcium ion concentration causes anterior flagellar resorption, depolymerization of cytoplasmic microtubules changing the zoospores from pyriform to spherical and exocytosis of the plaques which simultaneously produces sticky zoospores and introduces new plasma membrane elements capable of synthesizing the fibrillar cell wall of the settled zoospore.
Flagellar function in unicell motility is an accepted proposition in cell biology. However, the role of mastigonemes has not been clearly indicated. The possibility that they may enhance motility in flagellate unicells has been considered (Bouck 1971). Although flagellar adhesion between *Chlamydomonas* is not perhaps exactly analogous it is noteworthy that mastigonemes may not be involved in flagellar adhesion (Snell 1976 ab) and thus Bouck's (1971) proposition seems more probable here as well. The lack of support for an adhesive function for mastigonemes coupled with the observations that the tip of the anterior flagella of *Nereocystis* (Kemp and Cole 1961) and *Chorda* (Toth 1976) zoospores may be the primary point of adhesion substantiates the suggestion of Toth (1976) that the most anterior portion of the anterior flagellum consisting of the two central microtubules and plasma membrane may be the locus of the adhesion mechanism. In this context it is of interest that this portion of the flagellum was also reported to be coiled in most preparations (Toth 1976). It has also been brought to the author's attention that while loose mastigonemes may often be found associated with but not properly attached to the anteriormost portion of the flagellum, they are absent from the axoneme portion unless properly anchored (Henry, personal comm.), suggesting they may be stuck.

There are two schools of thought as to the actual mechanism of cell to substrate adhesion. One would argue that the ionic charges of cell and substrate surfaces are the
critical factors (McKeenan and Ham 1976, Grinnell 1976) and thus any number of factors affecting the ionic charges of one or both surfaces might have a bearing on adhesion. The other school of thought argues that there are specific differentiated patches of plasma membrane possessing specific glycoproteins which act as foci for adhesion and that the usual treatments of tryptic digestions or chelating agents such as EDTA are affecting the relationship between cytoskeleton and plasma membrane rather than the actual points of adhesion (Vogel 1978). If the latter school of thought is applicable to these zoospores then the process of flagellar development becomes very interesting with regards to the origin of the anterior most portion of the flagellar membranes. In a couple of accounts of flagellar origin (Markey and Wilce 1976a) in some brown algae it appears that the golgi apparatus may contribute the original and possibly apical most portion of the flagellar membrane about the time of cytokinesis.

The third aspect of the first proposition is the capacity for tactile sensitivity of the anterior flagellum which in effect is inseparable from the second proposition that substrate contact triggers the violent zoospore thrashing and produces the fluctuations in internal calcium. The violent thrashing observed in yet unattached spores (Toth 1976a, and here) can be interpreted in two ways. Either the process of adhesion associated with the most anterior segment is one in the same in the process of tactile sensitivity and the
induction of thrashing and calcium fluxes or the two processes are separate phenomena. If the former is the case then unattached thrashing zoospores or rounded up non-motile zoospores might be considered zoospores which had made contact sufficient to trigger the next stages of settling but not adequate contact to adhere to the substrate. Another possibility to explore is that the tactile sensitivity is a separate phenomenon from adhesion and perhaps the mastigomemes ought to be reconsidered in this respect. At any rate what the actual mechanism is for the induction of the spore thrashing and induction of the calcium ion fluxes is not central to the validity of the third proposition.

That calcium is involved here, there is little doubt. The significance of calcium in a varied spectrum of cellular phenomena is well established (Perris 1971, Carafoli 1974, Carafoli and Crompton 1978, Douglas 1974, Rasmussen 1975). The quantities of calcium in sea water (Lyman and Fleming 1940) might exceed that presumed to exist in the cytosol where such data are available (Nilsson and Coleman 1977). Thus the surrounding sea water could act as a source for an influx of calcium given the proper tactile stimulus of the zoospore. Internal pools must also be considered. Not only is there evidence that the plasma membrane may store calcium (Sobota, Herbenda and Prezelecka 1977, Rossignol 1977) but also internal membrane bound organelles have been implicated (Nilsson and Colemen 1977, Kashiva and Park 1976, Perrelet and Bader 1978,
Sugaya and Onozuka 1978, Wooding and Morgan 1978). In preliminary studies using lanthanum chloride, a tri-valent ion which can replace calcium from labile binding sites (Haksar, Mandsley, Peron, Bedigian 1975), it has been observed that Nereocystis zoospores can be agglutinated. This would support the hypothesis that the zoospore plasma membrane may also store or accumulate calcium ions. Mitochondria have also been recognized as being involved in calcium accumulation and regulation for some time (Lehninger 1970). The mitochondria of Nereocystis zoospores are suitably located between the nucleus and eyespot along the rootlet microtubules and to the inside of or amongst the peripherally located plaque vesicles. In this vein it is tempting to speculate that the eye spot granules of Nereocystis and Macrocystis zoospores may play a role in calcium accumulation and regulation. Indeed they may be derived from the ER and special segments of ER such as the sarcoplasmic reticulum of muscle cells which stores calcium. Also all other brown algal zoospores at least possess densely osmiophilic granules (Baker and Evans 1973ab, Toth 1976, Lofthouse and Capon 1975, Gherardini and North 1972, Chi 1973) whose origins have not been closely enough examined to eliminate their possible homology with the eyespot granules of Nereocystis.

For proposition three, that of flagellar withdrawal, there is some evidence that calcium may participate in this phenomenon in Chlamydomonas (Quader, Cherniack and Filner 1978,
Faure-Fremiet and Ganchery 1957), and in addition to this calcium has been implicated in protoplasmic contractility (Ashley and Caldwell 1974, Disculescu and Popescu 1973, Martonosi, Pucell and Halpin 1971). There is ample evidence to support the second aspect of proposition three which states that the rootlets are calcium labile and their disappearance results in the change of the zoospore shape and in the dispersal of the eyespot. It should be recalled that after their emergence from the dehiscent sporangium the zoospores assume a conical or pyriform morphology as the ensheathing mucilage dissipates. It is proposed here that the anterior rootlet is responsible for this and that the spherical morphology was due primarily to the conditions of confinement in the zoosporangium. One likely prospect is the presumed pressure these spores are under as long as the sporangial wall remains intact (Toth 1976b). Another possibility to consider is the mucilage itself. It is reported that at mariculture facilities in Japan when zoospores are being collected from Laminaria species great care is taken not to damage the lamina and release any mucilage as subsequent zoospore harvesting is poorer (Mumford, and Kaneko personal communication). The anterior rootlet of some brown algal zoospores (Manton 1957, Baker and Evans 1973ab) has been described both as microtubular and as accounting for the non-spherical morphology of these cells. This is particularly pronounced in the fucoid sperm with their beaks (Manton and Clarke 1951, 1956, 1964). There is experimental evidence from other algae to support this role
of cytoplasmic microtubules and microtubular rootlets in the maintenance of cellular asymmetry. This work used both high pressures and antimicrotubular chemical agents which resulted in both cell shape changes and the disappearance of cytoplasmic microtubules (Bouck and Brown 1973, Brown and Bouck 1973). The possibility of microtubular growth at the time of release must also be considered but seems unlikely as cytoplasmic microtubules are present in the zoospores prior to release. In light of this role for cytoplasmic microtubules in the maintenance of cell asymmetry it is interesting to note that there is ample evidence in the literature not only for the calcium lability of cytoplasmic microtubules but also reports of changes in cell shapes related to disruptive increases in calcium ions in cytoplasm (Psote and Nicolson 1976, Vogel 1978, Voisky and Lcyter 1978, Goniakowska-Witalinska and Witalinski 1976, Weisenberg 1972, Shelanski, Gaskin and Cantor 1973, Olmsted, Marcum, Johnson, Allen and Borisy 1974, Kuriyama and Sakai 1974, Kirschner and Williams 1974)

Finally the last section of the third proposition is that this calcium influx induces the exocytosis of the plaques to produce sticky zoospores which may adhere and begin to secrete a fibrous cell wall. Golgi vesicles derived in a manner similar to that of the plaque containing vesicles of Nereocystis, Macrocystis (Chi 1973) and Laminaria (Oliveira, Walker and Bisalputra unpub. Obs.) have been studied in zoospores of the green algae Enteromorpha (Evans and Christie
1970, Christie Evans and Shaw 1970, Callow and Evans 1974) and Ulva (Braten 1975, Micalef and Gayral 1972). From these studies it was also concluded that these presumed glycoprotein secretory granules function in cellular adhesion to substrates. Similar conclusions have been reached in parallel studies of encysting zoospores of *Phytophthora palmivora* (Sing and Bartnicki-Garcia 1975abc, Nogueira, Pinto da Silva and Bartnicki-Garcia 1977) an aquatic fungus. An interesting additional feature of this work was the apparent role of "peripheral vesicles" (Sing and Bartnicki-Garcia 1975a) play in the initiation of cell wall deposition as was evidenced by their content of microfibrils. Essentially all of these works either present evidence for or speculate that these golgi derived vesicles participate in cellular adhesion. Thus there is evidence that these plaques may function as adhesive agents and their golgi origin is established. In addition this is apparently true for other algal zoospores as well. Of all the propositions put forth this by far finds the broadest support. The literature linking calcium with the process of exocytosis is extensive ranging from echinoderm eggs (Zucker Steinhardt and Winkler 1978) through mammals (Wooding and Morgan 1978, Schackmann, Eddy and Shapiro 1978) and others (Douglas and Kagayama 1977, Papahadjopoulas, Vail, Newton, Mir, Jacobson, Poste and Lazo 1977, Dean 1975, Sieghart, Theoharides, Alper, Douglas and Greengard 1978).

Finally with this model it is possible to offer
explanations for the different modes of zoospore dispersal described both in Kemp and Cole (1961) and here. The delayed dehiscence as observed by Kemp and Cole (1961) and here may be explained by leaky sporangia where the hydrostatic pressures of accumulated mucilage bleed off before the sporangium is dehisced. The retained spores, although contact is delayed, ultimately may encyst and go through cell wall deposition while remaining within the sporangium. Rounded up nonmotile zoospores represent spores for whom the calcium influx or release has occurred but successful anterior flagellar adhesion did not succeed. These cells are also still viable and their lipid content may explain their continued bouyancy and delayed adhesion.
APPENDIX 1 FIGURES, ZOOSPOROGENESIS
Figure 1. Post-inception zoosporangial initial. Note thin cell wall (lower left). Nucleus is large. The basal hemisphere is occupied by a large golgi complex of several dictyosomes showing hypertrophy of cisternae. A few chloroplasts are present in any section. Note the nuclear envelope ER and CER continuities (arrowheads). There is a distinct nucleolus and homogeneous fibrillar region (*) in the nucleoplasm. See also the electron opaque mass to the right of the nucleolus. Scale equals 1 um.

Figure 2. ABE/PAS stained youngest sorus tissue. Note the ovoid zoosporangial initials amongst the young paraphyses. Scale equals 10 um.

Figure 3. A dictyosome of the golgi apparatus. Dictyosomes lie adjacent to the nuclear envelope which vesiculates (larger arrowheads) to contribute to the formative face. Hypertrophied regions of cisternae contain fibrillar material similar to cell walls (small arrowhead). Coated vesicles are observed at the maturing face associated with hypertrophied portions of cisternae (arrows). Scale equals 0.5 um.
Figure 4. A pair of centrioles. A few cytoplasmic microtubules (arrowhead) are associated with each nucleus. Note also the ubiquitous peripheral system of endoplasmic reticulum just within the plasma membrane. Scale equals 1 um.

Figure 5. Mitochondrial morphology suggestive of division. Scale equals 1 um.

Figure 6. A coated vesicle. Note its association with the membrane of a hypertrophied region of a dictyosome cisterna. The diameter of the vesicle is approximately 90 nm and a 33 nm long neck connects it to the cisternal membrane (*). Scale equals 0.1 um.
Plate II

Figure 7. Zoosporangium approaching meiosis. The nucleus is the dominant organelle. The nucleolus is still present and electron dense masses of heterochromatin suggestive of leptotene chromosomes are found in cross section (arrow head) and longitudinal section associated with the nuclear envelope (arrow). Note the homogeneous mass of material associated with the nuclear envelope (*). The dictyosomes of the golgi apparatus are more loosely associated with the nucleus (top and bottom). The peripheral ER system is evident. The cytoplasm contains numerous free ribosomes. Scale equals 1 um.

Figure 8. ABE/PAS stained meiotic sorus. Note the still turgid shaped zoosporangia and the larger size. Scale equals 10 um.

Figure 9. A dictyosome not adjacent to the nucleus. Note the vesiculation of ER (small arrow) indicating the formative face, and golgi vesicles at the maturing face. Small microbody-like structures (*) are associated with the chloroplasts. Note the CER (arrowhead) and the chloroplast envelope (large arrow). Scale equals 0.5 um.
Figure 10. The perinuclear cytoplasm. Centrioles (*'s) have replicated and are somewhat separated. Note the ribosome free halo of cytoplasm around each. Scale equals 0.5 um.

Figure 11. The perinuclear cytoplasm. Cytoplasmic free ribosomes are excluded from more of the region adjacent to the nuclear envelope. Scale equals 0.5 um.

Figure 12. A pair of centrioles in a nuclear pocket. Note the more electron opaque homogeneous mass associated with the nuclear envelope. Note the lack of granular zone characteristic of a nucleolus. Scale equals 0.5 um.
Plate III

Figure 13. Spurr's embedded post meiotic zoosporangial sorus. Sporangia are large and cytoplasm is more heterogeneous in staining with TBO and OsO4. Scale equals 10 um.

Figure 14. Small nuclei distributed throughout the sporangium. Chloroplasts are peripheral and sometimes seen in tangential planes (*). They are associated with extremely homogeneous masses of non-membrane bound lightly electron dense material (presumptive lipids). These in turn are associated with clusters of electron opaque granules (arrows). Mitochondria occur individually in the peripheral cytoplasm and in clusters (brackets). Note also the osmiophilic material in the outer lumen of the CER and on the plasma membrane. Scale equals 1 um.

Figure 15. Favorable section showing the regular chloroplast distribution. Scale equals 1 um.

Figure 16. The peripheral cytoplasm. Note the peripheral ER continuities with the CER (arrows). The one continuity (to left) turns into the cytoplasm (arrowhead) while the other (right arrow) is enlarged peripheral ER system running the length of the figure. Scale equals 0.5 um.
Figure 17. The peripheral ER. The peripheral ER system may interconnect chloroplasts (opposing arrows). Note connections between osmiophilic vesicles and both presumptive lipid droplets as well as the chloroplast ER (arrowhead). Scale equals 0.1 μm.
Plate IV

Figure 18. Nucleus during the karyokineses or meiosis. There is a dictyosome associated with each pole of the elongate nucleus. Note the condensed chromatin. Scale equals 1 um.

Figure 19. Detail of nuclear pole during karyokinesis. Note the two centrioles, nuclear pocket and microtubules. Scale equals 0.5 um.

Figure 20. A post karyokinetic zoosporangium. Note the relationship between dictyosome, chloroplast, centrioles and nucleus. Scale equals 0.1 um.

Figure 21. Microbody-like structures. They are now found amongst the layers of nuclear ensheathing ER. Scale equals 0.1 um.

Figure 22. An osmiophilic vesicle. Note the association with a presumptive lipid droplet (above). Note distinct delimiting membrane in some places at its perimeter. Scale equals 0.1 um.

Figure 23. A cluster of osmiophilic vesicles. They range from relatively electron translucent with easily seen delimiting membranes (*) to electron dense ones with the delimiting membrane occasionally visible (arrowhead). Scale equals 0.1 um.
Figure 24. A post-karyokinetie nucleus. Nucleoli are small and pores are infrequent (Arrowhead). Layers of ensheathing ER surround each nucleus and show luminal continuities with the peripheral ER (Arrow to right) and the nuclear envelope (both arrows). Scale equals 0.5 μm.
Plate V

Figure 25. AEB/PAS stained sorus material at cytokinesis. Some zoosporangia have cleaved into spores while others have not. Scale equals 10 μm.

Figure 26. Nomarski optics of live mature, releasing zoosporangia. The thickened tips of waiting sporangia are intact (*) while they are not for dehisced zoosporangia (arrowhead) which are distinguishable from intact paraphyses. Scale equals 10 μm.

Figure 27. Post-meiotic-pre-cytokinetic zoosporangium. Flagella (arrowheads) already extended from former centrioles into the intramural space. Basal plates are now present at the base of each flagellum (arrow). Dictyosomes are hypertrophied and golgi vesicles contain a fibrillar material. Scale equals 0.5 μm.

Figure 28. An early stage of golgi vesicle exocytosis. Note plasma membranes prior to fusion (arrowhead). Scale equals 0.5 μm.

Figure 29. Zoosporangial protoplast during cytokinesis. Cytokinesis is begun by cisternae with tubular internal extensions of the delimiting membrane (arrows). These cisternae are distinct from golgi secretory vesicles. Scale equals 0.5 μm.
Figure 30. A dictyosome during zoosporangial protoplast cytokinesis. Note the fenestrae (arrowheads) of the un-hypertrophied cisternae. Localized hypertrophy of the cisternae stretches the fenestrae into membranous tunnels or tubular extensions at the maturing face of the dictyosome (small arrows). Scale equals 0.5 μm.
Plate VI

Figure 31 through figure 43 illustrate details of the post cytokinetic/pre-dehiscent zoospore, still contained within zoosporangia.

Figure 31. Typical organization of a zoospore. In cross section the central nucleus is about 2.5 um in diameter and contains chromatic material consisting of 50 nm granules. Regions of rough ER lie adjacent to the nucleus, as does the occasional microbody (small arrow). Note the chloroplast with shared CER and nuclear envelope. Other constituents are presumptive lipids, mitochondria, plaque containing vesicles of varying morphology (large arrow) and a portion of rhizoplast (arrowhead). Scale equals 1 um.

Figure 32. A zoospore prior to release. The chloroplast is in long section and the dictyosome is included. Note also the hypertrophied dictyosome cisternae and golgi vesicles contain plaques at varying stages of maturation (arrow). Microbody-like structures are present (arrowhead). Rough ER is not included in this section. Scale equals 1 um.
Figure 33. Cross section of a naked flagellum. Note the plasma membrane and the axoneme made of the 9+2 set of microtubules. Scale equals 0.1 um.

Figure 34. Oblique section of a flagellum. See mastigonemes (to the right). Scale equals 0.1 um.

Figure 35. Flagellar insertion. Note the electron dense material bridging the bottom of basal body 1 to an approximate midpoint on basal body 2 (large arrow). In both, the axoneme/basal body transition is marked by a basal plate. Note also the electron dense mass above basal body 1 (arrowhead) and the presumed cytoplasmic microtubules below basal body 2 (small arrows). Scale equals 0.5 um.

Figure 36. Transverse cross section of both basal bodies. Note lack of central tubules. From basal body 1 a crescent of electron dense material curves away (arrowhead). The distance between basal bodies and dense material in 2 implies that the plane of section is near the plasma membrane and basal plates. Scale equals 0.1 um.
Figure 37. Zoospore eyespot structure. The eyespot consists of osmiophilic vesicles arranged in a layer between the plasma membrane and a sheet of microtubules which originates from the region of flagellar insertion (arrow). Scale equals 1 μm.

Figure 38. The zoospore unit. The nucleus, chloroplast, dictyosome and centriole/basal body association. Dictyosome not included in this plane of section. Scale equals 0.5 μm.

Figure 39. Golgi apparatus of the post-cytokinetic contained zoospore. The formative face lies adjacent to the nucleus. At the maturing face hypertrophied extremities of cisternae contain small masses of electron dense material, nascent plaques (arrowhead). Note adjacent golgi vesicles with disc-shaped plaques of electron dense material sectioned in their plane (1) and transversely (2). Scale equals 0.5 μm.

Figure 40. The dictyosome formative face. Note the adjacent vesiculating nuclear envelope (large arrowhead). Scale equals 0.1 μm.
Figure 41. A pre-release zoospore. Presumptive lipid droplets may show continuity with membrane configurations (arrows). Mitochondria cristae contain a fibrillar element seen here in longitudinal and cross section (arrowheads). Genophore is to the left. Scale equals 0.1 μm.

Figure 42. Zoospore chloroplast during cytokinesis. Note the prominent pyrenoid surrounded by chloroplast ER the ER of the zoospore is slightly hypertrophied with a fibrillar material and covered with ribosomes. Scale equals 0.1 μm.

Figure 43. Nuclear envelope/ER association. Note continuity (arrowhead). Scale equals 0.1 μm.

Figure 44. Apical tip of a mature zoosporangium. Prior to dehiscence the cell wall is continuous around the tip but less electron dense. The apical thickening is added to the original cell wall. Zoospores contain numerous vesicles with electron dense, discoid plaques and large quantities of deforming lipids. Scale equals 1 μm.
Plate VIII

Figures 45 through 55 deal with free zoospores at various times after dehiscence of the zoosporangium.

Figure 45. Recently released live zoospores. At lower right the mostly spherical zoospores have just been released. The more dispersed mass to left was released earlier. Spores of this mass are becoming pyriform (arrowhead). Scale equals 10 um.

Figure 46. The free swimming zoospore is pyriform. Scale equals 10 um.

Figure 47. Platinum shadowed zoospore. Note the differences between anterior and posterior flagellar length. Scale equals 1 um.

Figure 48. The tip of the anterior flagellum. From Fig. 47. Note mastigonemes lying around the flagellum. Scale equals 0.5 um.

Figure 49. A platinum shadowed mastigoneme. From the distal end of the main shaft extends a long central filament and two short ones (arrowheads). Scale equals 0.1 um.

Figure 50. Whole free swimming zoospore. Negatively stained with PTA. The two flagella (arrowheads) emerge from below the chlorocplast which wraps over the nucleus and is anchored at one end with the basal bodies and nucleus. Note the posterior plate of dense spheres between the arrows, the eyespot. Scale equals 1 um.
Figure 51. The eyespot granules. Asterisks lie on two granules. Scale equals 0.1 um.

Figure 52. Negative stained flagellar apparatus. From a mechanically lysed zoospore. The two basal bodies lie between the basal plates and white bars. The lower one attaches to the lower position of the top one (compare with Fig. 35). See also the rhizoplast (arrowheads). Scale equals 0.1 um.

Figure 53. A free zoospore. It has rounded up and withdrawn its flagellum. Note the axonemes in cross section (arrowheads). Scale equals 1 um.

Figure 54. A free zoospore. It has rounded up and withdrawn its flagella. Presumptive remains of a plaque located in the depression marking its recent excytosis. Scale equals 0.1 um.

Figure 55. A free zoospore. It has rounded up, excreted the dense plaques and begun to deposit the first fibrillar cell wall components (arrow). Scale equals 0.1 um.
Plate IX

Figures 56 through 59 are all from material in which zoosporangial dehiscence has been delayed.

Figure 56. Zoospore detained in the zoosporangium. Axoneme of one flagellum has sunk into the cytoplasm. Note basal body (arrow). Basal plate and axoneme. One flagellum is still cut, note the mastigonemes. The vesicle contained plaques are still within the cytoplasm, note tangential (1) and cross sectiona views (2). In section pegs of the dense plaque material appears to extend to the vesicle membrane. Scale equals 0.5 um.

Figure 57. A zoosporangium which failed to dehisce. Each spore has a cell wall showing typical purple metachromasia. Nuclei stain in their centers. Scale equals 10 um.

Figure 58. Portion of a still contained zoospore. Note axoneme in cross section within the cytoplasm. The plasma membrane has electron opaque masses on it and close inspection reveals small fibrillar extensions. The golgi apparatus is reduced to a few small cisternae but the nucleus still shows vesiculation of its outer membrane (arrowheads). Note also the ubiquitous microbody-like structures (*). Scale equals 0.5 um.
Figure 59. A vesicle contained plaque. Cut tangentially the central mass of the disc reveals concentric rings of electron dense material. Scale equals 0.1 um.
Figure 60. Diagrammed zoospore model. Nucleus (N) with occasional pores is draped by the chloroplast (Ch). Both are associated with laterally positioned basal bodies (BB). The dictyosome (D) is positioned on posterior face of nucleus and surrounded by mitochondria (M). The eyespot (E) caps the posterior surface of the spore and is backed by microtubules (Mts) originating from the basal bodies. A few microtubules also extend anteriorly from basal bodies. Plague containing vesicles (Pg) are positioned below plasma membrane about the cell. These are discoid with annular ridges evident from cross section (Pg 1) and surface section (Pg 2). The anterior flagellum (AF) bears mastigonemes (Mst) along at least one edge. The posterior flagellum is naked.