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FINE-STRUCTURAL STUDIES ON THREE SPECIES OF RED ALGAE
WITH SPECIAL EMPHASIS ON ASPECTS OF SPOROGENESIS,
SPORES AND SPORELING DEVELOPMENT

By

ABDULLA SIRAG MANDURA

VOLUME I

A thesis submitted for the degree of
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List of Contents

<u>Volume I</u>	<u>Page no.</u>
<u>Acknowledgments</u>	i
<u>List of Contents</u>	ii
<u>Summary</u>	vi
<u>1. General Introduction</u>	1
<u>2. Material and Methods</u>	5
2.1. Location, collection, transportation and storage. ...	5
2.2. Culture studies. 	5
2.2.1. Media and culture vessels. 	5
2.2.2. Control of contamination. 	6
2.2.3. Illumination. 	7
2.2.4. Aeration and temperature. 	8
2.2.5. Culture of spores. 	8
2.2.6. Measurement of growth of sporelings. 	8
2.2.7. Transplantation of sporelings of <u>Palmaria palmata</u>	8
2.3. Processing specimens for light microscope and transmission electron microscope. 	9
2.3.1. Processing of vegetative tissues. 	9
2.3.2. Modified processing for spores and sporelings. ...	11
2.4. Sectioning and staining for light microscope and transmission electron microscope. 	11
2.5. Microscopes. 	15
2.6. Photomicrographs. 	15

<u>3. Culture Studies</u>	16
3.1. Introduction.	16
3.2. The effects of different light intensities on the growth of tetrasporelings of <u>Palmaria palmata</u>	16
3.3. The effects of different light intensities on the growth of parasporelings of <u>Plumaria elegans</u>	17
3.4. Growth in culture of tetrasporelings of <u>Palmaria palmata</u> .						17
3.5. Growth in culture of parasporelings of <u>Plumaria elegans</u> .						18
3.6. Discussion.	19
<u>4. Ultrastructure of Vegetative Cells</u>	23
4.1. Introduction.	23
4.2. Ultrastructure of vegetative cells of <u>Palmaria palmata</u> .						24
4.3. Ultrastructure of vegetative cells of the upright filaments of <u>Plumaria elegans</u>	26
4.4. Ultrastructure of vegetative cells of <u>Audouinella</u> <u>sagraeanum</u>	28
4.5. Discussion.	30
<u>5. Ultrastructure of Tetraspore Formation of Palmaria palmata.</u>						35
5.1. Introduction.	35
5.2. Cytoplasmic organelle differentiation.	36
5.3. The behaviour of the nucleus and the nucleolus during the tetrasporogenesis.	46
5.4. Discussion.	50
<u>6. Plasmalemmavilli and Endoplasmic Reticulum</u>	64
6.1. Introduction.	64
6.2. Plasmalemmavilli and endoplasmic reticulum in newly released tetraspores of <u>Palmaria palmata</u>	65

6.3.	Plasmalemmavilli and endoplasmic reticulum in a newly released spore of unknown red alga epiphyte on <u>Plumaria elegans</u> (possibly <u>Callithamnion</u>).	65
6.4.	Plasmalemmavilli and endoplasmic reticulum in newly released paraspores of <u>Plumaria elegans</u>	66
6.5.	Discussion.	68
7.	<u>Floridean Starch</u>	72
7.1.	Introduction.	72
7.2.	Floridean starch of <u>Plumaria elegans</u>	72
7.3.	Floridean starch of <u>Palmaria palmata</u>	73
7.4.	Floridean starch of <u>Audouinella sagraeanum</u>	74
7.5.	Discussion.	75
8.	<u>Chloroplast Development</u>	78
8.1.	Introduction.	78
8.2.	Chloroplast development in <u>Palmaria palmata</u>	79
8.3.	Chloroplast development in <u>Plumaria elegans</u>	81
8.4.	Discussion.	84
9.	<u>Annulate Lamellae and Nucleolus-like Bodies</u>	90
9.1.	Introduction.	90
9.2.	Intranuclear annulate lamellae of <u>Plumaria elegans</u>	90
9.3.	Annulate lamellae and nucleolus-like bodies of <u>Palmaria palmata</u>	91
9.4.	Discussion.	92

<u>10. Additional Features</u>	94
10.1. The origin and development of vacuoles during tetrasporeling development of <u>Palmaria palmata</u>	94
10.2. Osmiophilic bodies of <u>Palmaria palmata</u> tetraspores.	95
10.3. Adhesion of paraspores and parasporelings of <u>Plumaria elegans</u>	98
10.4. Pit connections of parasporelings of <u>Plumaria elegans</u> .						99
10.5. Mitochondria of paraspores and parasporelings of <u>Plumaria elegans</u>	103
10.6. The occurrence of crystalline bodies in parasporelings of <u>Plumaria elegans</u>	107
10.7. Contribution of Golgi bodies in wall formation during parasporeling development of <u>Plumaria elegans</u>	107
<u>11. General Remarks</u>	111
<u>12. References</u>	113

Summary

1. The introductory experiments revealed that the suitable light intensity for growth in culture of tetraspores of Palmaria palmata was 3000 lux and the paraspores of Plumaria elegans was 1000 lux. Palmaria palmata tetrasporelings attained the length of 6.7 cm at 3000 lux after twenty-one months in culture while Plumaria elegans parasporelings attained the length of 2 cm at 1000 lux after seventeen months in culture.

2. The ultrastructure of vegetative cells of the three species (Palmaria palmata, Plumaria elegans and Audouinella sagraeanum) shows

(a) Presence of unusual structures within the cell walls of Audouinella sagraeanum and the cell walls of the stipes of Palmaria palmata. The cell walls of the three species are composed of microfibrils of probably random arrangement embedded in an amorphous matrix and are covered by a cuticle though it is not always clear in Audouinella sagraeanum.

(b) Floridean starch lies free in the cytoplasm but small grains of similar electron density to floridean starch grains were observed within chloroplasts of Audouinella sagraeanum. (c) Each cell of the three species possesses several chloroplasts. The thylakoid arrangement within the chloroplasts of Plumaria elegans and the small chloroplasts of Audouinella sagraeanum is represented by a peripheral thylakoid enclosing several internal thylakoids, though juxtaposition of two to three thylakoids was observed in some chloroplasts within the parasporangia of Plumaria elegans and appressed thylakoids in groups of four and six were observed within a chloroplast of a newly released paraspore. The thylakoid arrangement in chloroplasts of Palmaria palmata is represented by a peripheral thylakoid enclosing one or more concentric thylakoids and several internal thylakoids. Each cell of Audouinella sagraeanum possesses a single large chloroplast in addition to several small chloroplasts. The large chloroplast has no peripheral thylakoid but a single pyrenoid (two pyrenoids on rare occasions) is present which may protrude towards the cytoplasm or be

embedded within the stroma. The pyrenoid is enclosed by thylakoids which may penetrate into the matrix. Tubular structures were also observed within the matrix of some pyrenoids. There is no spatial relationship between the starch grains and the pyrenoid. (d) Pit connections of the three species are composed of a plug core and plug caps. The plug cap of Plumaria elegans and Audouinella sagraeanum is a single layer while in Palmaria palmata it is multilayered. Pit connections are also observed between immature paraspores within the parasporangium and immature tetraspores within the tetrasporangium of Plumaria elegans. (e) Mitochondria accompanied by Golgi bodies are scattered at different locations within the cytoplasm of the three species. (f) Spherical osmiophilic and crystalline bodies are present in the cytoplasm of Plumaria elegans only, and are also found in parasporangial cells growing in culture. (g) The protoplasts of the ageing cells of Audouinella sagraeanum degenerate but a new cell replaces them. The new formed cell is surrounded by two cell walls.

3. The tetrasporogenesis of Palmaria palmata has here been interpreted as a three stage process. Stage 1 is characterized by synthesis and incorporation of cell wall material into the differentiating vegetative cell wall and a possible reorganization of the cell wall microfibrils as the cell enlarges and divides into a stalk cell and a tetrasporangium mother cell. The chloroplasts within the tetrasporangium mother cell start to divide, deposition of starch grains begins and Golgi bodies secrete mucilage vesicles 1. At stage 2, deposition of mucilage layer 1 at the region between the cell wall and the plasmalemma is completed, secretion of endoplasmic reticulum vesicles by the endoplasmic reticulum and mucilage vesicles 2 by Golgi bodies begins and initiation of the first cleavage furrow takes place. By late stage 3, deposition of mucilage layer 1 and mucilage layer 2 around each spore is completed, secretion of adhesive vesicles is taking place and secretion of small fibrous vesicles and their transformation into large fibrous vesicles has been completed. Degeneration of endoplasmic

reticulum vesicles is at a maximum and the tetrasporangium mother cell has divided into four spores. The tetraspores escape through an aperture leaving an empty cavity. The stalk cell may enlarge or may divide to form a second stalk cell and a second tetrasporangium mother cell which differentiates and enlarges to occupy the empty cavity and form the second generation of tetraspores.

The nucleus completes its first division by late stage 2 - early stage 3 when the first cleavage furrow is in progress. At mid-stage 3, the second nuclear division takes place and by this time the tetrasporangium mother cell possesses four nuclei occupying peripheral positions. Each nucleus represents the mature tetraspore nucleus. At stage 1, the nucleus enlarges in size, a dark electron dense element appears within the nucleoplasm on rare occasions, and there is an increase in the nuclear pore density. The nucleus is surrounded by a sheath of endoplasmic reticulum which disappears in nuclei of mature tetrasporangium. Microbodies were located on rare occasions between the sheath of the endoplasmic reticulum and the nuclear envelope. The nucleolus also enlarges in size, becomes highly granulated and nucleolar vacuoles appear. The nucleolus migrates towards the porous area of the nuclear envelope and discharges its products to the perinuclear cytoplasm.

4. The newly released tetraspore (up to four hours from release) is naked and possesses a centrally located nucleus, a small amount of starch grains, few chloroplasts, adhesive vesicles and large fibrous vesicles. The adhesive vesicles fuse with the plasmalemma and secrete their contents to form the adhesive layer which helps to stick the spore to the substratum while the large fibrous vesicles contribute in the formation of the spore wall.

5. Plasmalemmavilli were observed in newly released naked spores of three species (Palmaria palmata, Plumaria elegans and Callithamnion?). Plasmalemmavilli are tubular structures associated with the plasmalemma,

closed at the lower end and abut the endoplasmic reticulum. The endoplasmic reticulum underlies most of the plasmalemma and is in direct contact with it in some regions. Both the plasmalemmavilli and the endoplasmic reticulum underneath the plasmalemma disappear soon after the spore starts depositing cell wall material.

6. There is an increase in the amount of starch as the newly released spores of Palmaria palmata and Plumaria elegans grow but in Palmaria palmata, it decreases again and cells of the tetrasporelings (differentiated into a holdfast and an upright frond) possess a small number of grains. In rhizoidal cells of Plumaria elegans, the large grains aggregate at one side of the cell while the small grains occupy the other side. Some grains composed of two to several concentric layers were observed in Plumaria elegans but in Palmaria palmata, some grains are of two concentric layers in which the outer one is electron dense and some grains are surrounded by endoplasmic reticulum cisternae. Floridean starch grains may fuse together.

7. Chloroplasts of Palmaria palmata and Plumaria elegans are maintained from cell to cell and generation to generation by growth and differentiation of proplastids and by fission of chloroplasts. Proplastids may divide or bud from mature or immature chloroplasts and the internal thylakoids of the proplastids originate by invagination of the peripheral thylakoid. The fission process in Palmaria palmata is accomplished by the appearance of a constriction between elongated or increased in diameter chloroplasts which result in the formation of two daughter chloroplasts of either equal or unequal sizes. There can be a multiple division. In Plumaria elegans, a small group of parallel thylakoids appears within the stroma beside the group of large thylakoids. Differentiation and growth of the small thylakoids may result in their projection towards the cytoplasm and attain the size of the parent, or the parent may appear having two sets of parallel thylakoids. Appearance of a constriction results in the formation

of two daughter chloroplasts. Growth, invagination or fragmentation of the thylakoids is essential for chloroplast replication. Genophores may also elongate, divide and be transmitted into the daughter chloroplasts. Phycobilisomes were observed attached to thylakoids of some chloroplasts of Palmaria palmata and some chloroplasts have thylakoids with wavy appearances. Plastid ribosomes in chloroplasts of Plumaria elegans aggregate in groups and in parallel rows between the thylakoids and localized inflations of the thylakoids in some chloroplasts were also observed.

8. Intranuclear annulate lamellae (as cisternae interrupted by pore complexes) were observed within the nucleoplasm in differentiating cells of Plumaria elegans and Palmaria palmata. Nucleolus-like bodies were also observed within the nucleoplasm of Palmaria palmata. Cytoplasmic annulate lamellae in a form of circular configurations of several perforated cisternae were observed in the cytoplasm of Palmaria palmata.

10. Two types of osmiophilic bodies were observed within the cytoplasm of Palmaria palmata tetraspores before they divide.

11. Vacuole formation during the tetrasporeling development of Palmaria palmata is achieved by the activity of Golgi bodies.

12. Adhesion of paraspores of Plumaria elegans is the result of fusion of the cored vesicles with the plasmalemma and secretion of their contents. A layer is formed around the germinating parasporeling of probably mucilaginous nature and may possibly be secreted by endoplasmic reticulum.

13. Mitochondria of Plumaria elegans parasporelings have various sizes and shapes. Some have a pouch and some probably have an extra part or compartment. They may aggregate in groups which may appear to be surrounded by endoplasmic reticulum cisternae. Mitochondria were observed to undergo division.

14. Golgi bodies contribute in cell wall formation during parasporeling development of Plumaria elegans but not in Palmaria palmata tetrasporelings. Plasmalemmasomes were observed in both species.

1. GENERAL INTRODUCTION

Three species of three families of the class Florideophyceae were chosen for these studies. They belong to the orders Palmariales, Ceramiales and Nemaliales. These plants are:

1. PALMARIA PALMATA (L.) O. Kuntze
2. PLUMARIA ELEGANS (Bonnem.) Schm.
3. AUDOUINELLA SAGRAEANUM (Mont.) Born.

Palmaria palmata (Fig. 1) is a member of the family Palmariaceae. Palmaria palmata commonly grows in the low intertidal or upper subtidal zone on the stipes of Laminaria digitata, some on Fucus serratus and some on bare rocks. Plants are gregarious or solitary, flat, membranaceous to coriaceous, about 10-15 cm long and 4-6 cm wide. The older specimens are thick and deep pinkish red in colour while the young specimens are relatively thin and of pale red colour. The plants have more or less a dichotomously dividing thallus with the main division having sometimes many proliferations. The blade is gradually attenuated towards the base ending in a stipe and attached by a holdfast of about 5 mm in diameter. Fertile plants can be easily distinguished from sterile ones in field by holding the thallus against the light when the reproductive sori will appear as small dark patches.

Dried blades of Palmaria palmata are called "dulse" and are commonly eaten, or simply chewed like tobacco in certain regions, such as Maine and Maritime provinces of Canada (Bold and Wynne, 1978). For poor peasants on the west of Ireland dulse was the only addition to potatoes in many of their meals (Newton, 1931) and it was regarded as having medicinal properties.

The genus Palmaria palmata was first proposed by Stackhouse (1801) as a substitute name for Ceramium (Stackhouse, 1797). However, the genus Rhodomencia (Greville, 1830) was later corrected orthographically to

Rhodymenia by Agardh (1852). Following the suggestion of Montagne (1839), the name Rhodymenia was conserved against Palmaria and the full binomial Rhodymenia palmata was given. Recently, comparing Rhodymenia palmata with the type species Rhodymenia pseudopalmata (Lamouroux) Silva, Guiry (1974, 1975) revived the generic name for the former species as Palmaria palmata, separating it from the genus Rhodymenia and creating a new family Palmariaceae. Subsequently, Guiry (1978) separated the family Palmariaceae from the order Rhodymeniales into a new order Palmariales which includes three genera, Palmaria, Halosaccion and Leptosarca. According to Guiry (1974, 1975) the genus Palmaria is characterized by a solid, flat thallus, multiaxial, composed of cortical and medullary layers. The tetrasporangium is cruciately divided bearing a stalk cell and the carpogonium and the carposporophyte generation is lacking in the life history (but see section 3).

Plumaria elegans (Fig. 2) is a member of the family Ceramiaceae of the order Ceramiales. Plumaria elegans commonly grows in the mid-intertidal zone. It is a shade-loving plant, usually found in shaded crevices overhung by other seaweeds.

The thallus is filamentous, flaccid with a silky texture, branched 5-15 cm, and dark red. At a very short distance behind the apex cortication is initiated and develops extensively over the main axes, the lower part of the branchlets remain uncorticated. Norton (1975) reported that the plants exhibited marked and progressive morphological changes the deeper they penetrated into a cave, but in culture, spores of different samples from different distances from the cave mouth gave rise to morphologically similar sporelings. There has been a great deal of confusion by early workers between Plumaria elegans and Ptilota plumosa. Frequently specimens of the former were misidentified as the latter, but the reverse situation is extremely rare (Cullinane and Murphy, 1976).

Audouinella sagraeanum is a member of the family Acrochaetiaceae of the order Nemaliales. The thallus consists of uniseriate filaments of cylindrical cells measured 20 - 23 μm long and 6 - 8 μm wide, brownish red in colour.

The above plants were chosen for these studies for several reasons:

1. Palmaria palmata and Plumaria elegans are fertile the whole year around which makes them available at any time of the year and convenient for spore formation and germination studies. Audouinella sagraeanum is cultured growing material supplied by the Culture Collection of Algae and Protozoa.

2. They are members of different orders of the class Florideophyceae and offer scope for studying the different structural features and differentiation of cytoplasmic organelles.

3. They bear different types of reproductive organs, e.g. Palmaria palmata plants bear spermatia and cruciate tetrasporangia. Plumaria elegans plants in the south of England bear tetrahedral tetrasporangia, spermatia and carpogonia, while in Scotland, they bear parasporangia and a few of tetrasporangia on the same plant. Audouinella sagraeanum plants bear mainly monosporangia.

4. They have different patterns of sporeling development, e.g. sporelings of Plumaria elegans show a clear polarity in a form of vegetative filaments and rhizoids while Palmaria palmata sporelings are represented by a disc-like holdfast and upright multicellular fronds and Audouinella sp. sporelings consist of disc-like holdfasts and upright unicellular filaments.

5. They display different life histories, e.g. Plumaria elegans plants are characterized by the occurrence of two completely independent parts of the life history (Drew, 1939) in which the triploid parasporophyte plants reproduce themselves by parasporangia and the normal diplobiontic floridean life history (Polysiphonia type), where the haploid

sexual generations alternate with the diploid asexual generations. Many Audouinella sp. plants are reproduced by means of monosporangia, the simplest asexual reproductive organs described in Florideophyceae. In the case of Palmaria palmata knowledge of the life history was not completed until early 1980 (see sections 3 and 5 for more details). Tetrasporangia are formed in most plants and spermatia have been reported by numerous investigators from different localities (Delf and Grubb, 1924; Lee and Kurogi, 1972; Guiry, 1974, 1975; Lee, 1978). Carpospores and carposporophytes have not been found in nature. Several investigators have given different chromosome numbers for plants from different localities.

The above points show obvious differences between the plants and a comparative ultrastructural study of them would be of interest.

The original aim of this investigation was to compare for the three species the ultrastructure of vegetative cells, sporogenesis, spore and sporeling developments with particular emphasis on the mobilization of cell organelles prior to and immediately following spore release, spore adhesion and the early stages of sporeling development. During the course of the work, Poeschel (1979) published a paper on the ultrastructure of tetrasporogenesis in Palmaria palmata from the east coast of the United States. My preliminary results indicated clearly that there would be differences in interpretation of the process as observed in the material from Scotland. Hence a critical examination of the overall process in Palmaria palmata was thought to be essential and in consequence less time was available for comparative studies with Plumaria elegans and Audouinella sagraeanum. The processes of cleavage of the cell contents of the tetrasporangium are considered to be of taxonomic importance especially with Palmaria palmata (Poeschel, 1979; Guiry, 1974, 1978). Again my observations on these processes were not in accord with those described in the two papers and they are described in detail in the thesis.

Each section of the thesis will have a separate introduction and discussion

2. MATERIAL AND METHODS

2.1. Location, collection and storage.

Palmaria palmata (tetrasporophyte) and Plumaria elegans (parasporophyte) were collected from Isle of Cumbrae in the vicinity of the University Marine Biological Station (Fig. 1). The plants used for culture experiments were kept damp but not submerged in polythene bags and brought to the laboratory within a period of two hours at a temperature not exceeding 15°C, stored under refrigeration in darkness at 5°C and used within twenty-four hours. The plants used for light microscope and transmission electron microscope were fixed on shore and transported on ice. Audouinella sagraeanum was supplied by Professor A.D. Boney, Botany Department, University of Glasgow, from material already growing in culture, but originally supplied by the Culture Collection of Algae and Protozoa at Cambridge.

2.2. Culture studies.

2.2.1. Media and culture vessels.

All algae were cultured in an enriched seawater medium (Boney and Burrows, 1966). Seawater collected from the Firth of Clyde was aged for at least two weeks, filtered through a double layer filter paper No. 1 and then pasteurized by heating it twice at 75°C at room temperature. The formula of the medium was as follows:

To 1 litre of filtered and pasteurized seawater were added:

60 ml of solution A, which contained:

50 ml of 0.4% NaNO_3 in distilled water.

2 ml of each of the following:

1.47 g/l $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$

0.0023 g/l $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$

0.064 g/l $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$

0.005 g/l $\text{LiCl} \cdot \text{H}_2\text{O}$

0.23 g/l $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$.

15 ml of solution B, which contained:

2.6 g/l tetrasodium salt EDTA

0.2 g/l $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$.

15 ml of solution C, which contained:

1.5 g/l $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$.

2 ml of solution D, which contained:

4.98 g/l $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$.

Each of the above solutions was autoclaved separately and stored in a refrigerator (5°C) until used.

Small square petri dishes measuring 10 x 10 x 2 mm and rectangular glass tanks measuring 180 x 250 x 170 mm and having a capacity of about 5 litres, were used in culture studies.

2.2.2. Control of contamination.

Contamination was successfully reduced by:

1. Using carefully washed small pieces of fertile plant which carry large numbers of sporangia at the onset of each experiment.
2. Changing the medium once a week.
3. Using Germanium dioxide by adding 2 ml of 250 mg/l in distilled water to 1 litre of the culture medium at pH 7.5 which brought the concentration of GeO_2 to 0.5% (Lewin, 1966).

4. Removal of other algal sporelings.

5. Using a cleaning apparatus (Fig. 2) which consists of a cylinder 50 cm in length and 18 cm in diameter. At a height of 12 cm from the bottom, a circular gauze with 1 mm square holes was fitted. Underneath the gauze, there are three perforated plastic tubes, two in criss-crossing position and the third peripheral. All tubes were connected to two aeration pumps.

The cylinder was filled with filtered seawater. The slides with attached sporelings were fitted to Microid flask shaker and hung submerged at the top of the cylinder. By switching on the shaker and the aeration pumps, two water currents were created at the upper part of the cylinder, one coming from the movement of the shaker and the other one from the air bubbles induced by the aeration pumps. The seawater underneath the gauze remains calm. The two water currents help to detach the epiphytes growing on the slides and the sporelings, hence they sink to the calm part of the seawater at the bottom of the cylinder.

This method of controlling the contamination was only successful in Palmaria palmata cultures, where the sporelings strongly adhere to the slides.

The materials were treated by this method once every three weeks.

2.2.3. Illumination.

The cultures were illuminated from above with 6' Atlas Daylight 85W tubes at a distance of 23-35 cm. The glass shelves upon which the culture vessels were placed were covered with opaque construction paper to eliminate illumination from the tubes over the shelf below. The illumination was measured with an EEL portable electric photometer serial no. 1132 and controlled by muslin layers. The tubes were changed whenever necessary to keep the illumination as required. The day length was 12 hours.

2.2.4. Aeration and temperature.

In culture experiments using the rectangular 5 litre tanks, the tanks were aerated by bubbling compressed air continuously through the culture medium. The air was cleaned and humidified by passing the tubes through two flasks, one containing cottonwool and the other containing tap water. All experiments were run at $10^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in a thermostatically controlled culture cabinet.

2.2.5. Culture of spores.

Spores were obtained from small pieces cut from mature plants freshly collected from the shore, washed several times in sterile seawater and then laid down on glass slides ground on one side and incubated at the illumination required, at 10°C for 24 hours.

For light microscope and transmission electron microscope observations, the materials were laid direct on Falcon tissue culture dishes, 60 x 15 mm in measurement, to get the spores released and attached direct to the dishes and incubated as above for the age of spores required.

2.2.6. Measurement of growth of sporelings.

Growth of sporelings was measured by the increase in the diameter of the holdfast, the increase of the total length of the sporelings, or the increase in the total number of cells. The mean of 30-50 sporelings selected at random was obtained from different microscopic fields of ten slides.

2.2.7. Transplantation of sporelings of Palmaria palmata.

Some sporelings of Palmaria palmata, while they were still attached to the slides, were transferred to the shore after they had been left growing

in culture for about 18 months and attained the length of 3.5 - 6 cm. The slides were fixed on the slide holders (Fig. 3). Some of the slide holders were fixed on rocks (Fig. 4) and some were fixed on Keppel Pier (Marine Station pier) using a quick setting epoxy resin (Araldite rapid) and Braid dacron line for further support (Fig. 4).

2.3. Processing specimens for light microscope and transmission electron microscope.

2.3.1. Processing the vegetative tissue.

1. Fixation.

Fertile tissues were cut into 5 mm lengths and fixed on shore in 5% glutaraldehyde in 0.1 cacodylate buffer containing 0.25M sucrose at pH 7 on ice for 24 hours (Evans and Christie, 1970).

2. Washing.

The specimens were then given three 30-minute washes of 0.1M cacodylate buffer with decreasing sucrose concentrations (0.25, 0.125 and zero) on ice.

3. Post fixation.

The specimens were post-fixed in 1% OsO_4 (buffered as above) for 5 hours on ice.

4. Dehydration.

The specimens were dehydrated in a graded series of ethyl alcohol on ice.

30% ethyl alcohol for 30 minutes

50% " " " " "

70% " " " " "

85% " " " " "

95% " " " " "

100% " " " " "

100% " " " " " at room temperature

100% propylene oxide for 30 minutes (2 changes) at room temperature.

5. Infiltration.

Samples for infiltration in resin were placed in a 1:1 mixture of resin : propylene oxide at room temperature. The amount of propylene oxide in the mixture was gradually decreased through evaporation by leaving the vials open overnight in the fume cupboard.

6. Embedding.

When infiltration of the tissues was completed, the samples were dried by paper tissue and embedded in a pure resin in small aluminium dishes.

The formula of the resin was as follows:

Epon (Epicote) 812	40 cm ³] mixed well] mixed well
DDSA	40 cm ³		
MNA	20 cm ³		
BDMA	2 cm ³		

The resin was prepared during the infiltration and stored in a deep freeze to be used the following day.

7. Polymerization.

Polymerization was carried out in ovens of three different temperatures:

35°C for 12 hours

45°C " " "

60°C " " "

2.3.2. Modified processing for spores and sporelings.

The spores were released in Falcon tissue culture and allowed to grow using the dish as a substratum to the age required. They were then fixed, washed, post-fixed and dehydrated according to the procedure mentioned in (2.3.1.) with the following exceptions:

1. All solutions were removed and added carefully by pipette direct to the culture dish to avoid any damage to the spores and the sporelings and a large number of them were processed with relatively little effort.
2. Propylene oxide was omitted from the procedure because it dissolves the culture dish.
3. Infiltration was done in 1:1 mixture of resin : absolute ethyl alcohol over a period of 48 hours, since ethyl alcohol does not evaporate as quickly as propylene oxide.
4. The material was embedded and the resin was polymerized in the same culture dish.

When polymerization was completed the culture dishes were broken and the resin removed quickly before it had cooled down and hardened.

2.4. Sectioning and staining for light microscope and transmission electron microscope.

Semi-thin sections (2 μ m) for light microscope were cut with either LKB 11800 pyramitome, the LKB ultratome 1 type 4802A or the LKB 8800 ultratome III and dried down in a drop of water on gelatine coated slide.

Serial sections of specimens were obtained by mounting each section in order on the slides. Permanent preparations were made (following staining) by using a drop of resin as mounting medium and sealing with a cover slip. The following stains were used for staining the sections of the light microscope:

1. Toluidine blue: Most sections were routinely stained for about 10 seconds on a hot plate with 1% solution of toluidine blue in 1% borax.
2. Alcian stains: These were used to localize acid and sulphated polysaccharide (Park^{er} and Diboll, 1966). It is necessary to remove the resin from the sections before staining with alcian stains (Hoff and Rayburn, 1974). The resin was removed by treating the sections with sodium ethoxide (saturated absolute ethanol with NaOH and left for several days before use) for 15 minutes, followed by three 1-minute washes in absolute ethanol, three 1-minute washes in distilled water, and one 5-minute wash in running tap water. Then the sections were stained as follows:

1. Alcian blue (0.5% pH 0.5 adjusted with N HCl) for 60 minutes.
2. Wash in distilled water adjusted to pH 0.5 for 10 minutes.
3. Wash well in distilled water.
4. Alcian yellow (0.5% pH 2.5 adjusted with N HCl) for 60 minutes.
5. Wash in water.
6. Dehydrate, clear and mount.

3. Periodic acid - Schiff's Reagent: Sections were oxidised in an 0.5% periodic acid (H IO_4) solution in distilled water for 2 hours (Jensen, 1962), washed in running tap water for 10 minutes, stained in Schiff's reagent for 6 hours and finally washed in running tap water for 10 minutes. Polysaccharides will stain purplish-red. For control, the oxidation with periodic acid was omitted (Grimstone and Skaer, 1972).

Schiff's reagent was prepared freshly and stored in refrigerator.

according to Jensen's (1962) procedure which is as follows:

1. Dissolve 0.5g of basic fuchsin and 0.5g of sodium metabisulphite in 100 ml of 0.15 N HCl.
2. Shake the mixture until the dye is converted to fuchsin-sulphuric acid.
3. Add 300 mg of fresh decolorizing charcoal and shake for at least 5 minutes.
4. Filter through hard filter paper.
5. Repeat steps 3 and 4 once or twice until the filtrate becomes clear and colourless.

4. Iodine: (0.2% iodine in 2% potassium iodide). Iodine solution was used to locate starch (Jensen, 1962).

5. Chloramine-T-Schiff's reagent: This was applied to trace protein (Jensen, 1962). Sections were treated as follows:

1. Stain in 1% solution of chloramine-T in 0.1M phosphate buffer at pH 7.5 for 6 hours at 40°C.
2. Wash in water.
3. Place in dilute solution of sodium thiosulphate for 1 minute.
4. Place in Schiff's reagent for 8 hours.
5. Wash in running tap water.

Protein will stain pink.

For control the following blocking procedures were run on alternate sections before staining with chloramine-T:

1. Deamination: The tissue was placed in a mixture made up of 20 ml of 60% sodium nitrite and 60 ml of 1% acetic acid at room temperature for 24 hours.
2. Acetylation: The tissue was placed in a 10% solution of acetic anhydride in pyridine at room temperature for 20 hours.

6. Sudan black B: This was used to detect lipid according to the procedure given by Bronner (1975). The sections were placed in 70% ethanol for 2 minutes, stained with sudan black at 60°C in an oven for 1 hour (Sudan black was left in the oven for 30 minutes before use), rinsed in 70% ethanol for 1 minute and washed in water. Lipid will stain black.

Ribbons of ultra-thin sections for the transmission electron microscope (silver or gold) were cut with either the LKB ultratome 1 type 4802A or the LKB 8800 ultratome III using 6 mm thick glass knives made with the LKB 7800 knife maker. The ribbons were floated on to the surface of water in a bath made from silver self-adhesive tapes attached to the knife. The ribbons were then flattened with chloroform and collected on copper grids (size either G75 or G100) coated with a thin collodion support film (2% cellulose nitrate in amylacetate).

A double staining procedure with uranyl acetate and lead citrate was used for transmission electron microscope staining:

1. The grids were floated (sections side downwards) on the meniscus of solution of saturated uranyl acetate in water (7.5%) at room temperature for 30 minutes. The stain was contained in an overfilled polythene cup (6 mm in diameter). The grids were then washed with distilled water and dried on velin tissue.
2. The grids were floated on drops (sections side downwards) of lead citrate (Reynolds, 1963) in a petri dish. The lid of this dish was lined with dental wax to make an air-tight seal and pellets of KOH surrounded the drops of the stain on the wax seal, so as to absorb CO₂ and hence prevent contamination of the specimens with lead carbonate crystals which are insoluble and extremely hydrophobic (Hayat, 1970).

2.5. Microscopes.

For light microscopic observations a Zeiss photomicroscope and a Nikon microscope were used.

The initial transmission electron microscopic studies were made on an AEI 6B in the Botany Department and a Philips 300 instrument in the Department of Microbiology. Later a Philips 301 electron microscope was installed in the Botany Department and the majority of the studies were carried out on this instrument.

2.6. Photomicrographs.

The photomicrographs illustrated in this thesis were selected out of 3179, including 698 of light microscopy. All negatives (except the colours) were developed and subsequently printed in the electron microscopy laboratory of the Botany Department. The colour film which was used is a Kodak Ektachrome, 50 tungsten, EPY 135-36.

During this investigation, three different electron microscopes were used and these were each calibrated (in the magnification range used) with a 216011 mm standard specimen. However, variations up to $\pm 10\%$ may be present from the print magnifications listed due to inevitable errors occurring owing to inaccuracy of the enlargers and rounding off of the print magnifications.

3. CULTURE STUDIES

3.1. Introduction

Some introductory experiments were carried out, both to determine the suitable light intensity (optimum) for sporeling growth to be used for the cultures for electron microscopic studies, and as control cultures for comparison with those of the electron microscopic cultures, and also to gain some information regarding the life history of Palmaria palmata in particular. This was not successful since the sporelings did not show any fertility even after they reached the age of twenty-one months and attained sizes of 6.7 cm in culture. Although tetrasporangial and spermatangial plants of Palmaria palmata are found in nature, plants bearing female reproductive structures had never been identified (see section 1) and many investigators have favoured the interpretation of no sexual reproduction for this species (e.g. Magne, 1959, 1964; Bold and Wynne, 1978).

3.2. The effects of light intensity^{ies} on the growth of the tetrasporelings of Palmaria palmata.

Tetraspores of Palmaria palmata collected from the Isle of Cumbrae were cultured under various levels of illumination (800 lux, 1000 lux, 2000 lux, 2200 lux, 2400 lux and 3000 lux). Details of the culture conditions were described in section 2. Although good growth and healthy sporelings were obtained at all illumination levels there was a gradual increase in growth rates from illumination 800 lux to 3000 lux (Fig. 1). At 800 lux the mean diameter of the sporelings was about 88 μm and at 3000 lux it was about 127 μm after four weeks. An illumination of 3000 lux was considered to be the optimum for growth of tetrasporelings of Palmaria palmata and was used to grow them for transmission electron microscopic studies. Illumination above this level was not tested.

3.3, The effect of different light intensities on the growth of parasporelings of Plumaria elegans.

Paraspores of Plumaria elegans were obtained and were cultured at various levels of illumination (40 lux, 280 lux, 320 lux, 680 lux, 1000 lux and 1400 lux) under daylength regime and temperature previously described (see section 2). At an illumination of 40 lux, although the parasporelings remain healthy, the mean increase in total number of cells per sporeling was hardly noticeable compared with the other levels of illumination (Fig. 2). In addition, some of the paraspores did not either divide or adhere to the slide over a period of four weeks. At an illumination of 1400 lux the parasporelings were dying after three weeks and some cells showed bleaching (Fig. 3C). However, at 1000 lux the maximum growth rate (Fig. 2) and the typical parasporeling configurations (Fig. 3B) were obtained compared with growth at 680 lux (Fig. 3A). Therefore, the illumination 1000 lux was taken as the optimum level for growth of parasporelings for transmission electron microscopic studies.

3.4. Growth in culture of tetrasporelings of Palmaria palmata.

The illumination level 3000 lux and the other laboratory-induced environmental factors reported in section 2 were used for growth in culture of tetrasporelings of Palmaria palmata. The tetraspores differentiated into disc-like holdfasts and erect branches within the first three weeks (Fig. 4A). By the time a year has passed the tetrasporelings may form two populations in which some have broad erect branches (Fig. 4C) while others have thin erect branches and are smaller in length (Fig. 4B). After another three months both types of plants were branched (Figs. 4D, 4E) but the second type was still stunted (Fig. 4D) when compared with the first type (Fig. 4E), and both types had not shown any fertility at this stage. Some of these tetrasporelings, whilst still attached to the slides, were

transferred to the shore and fixed according to the procedure reported at section 2.2.7. Unfortunately they were grazed by the marine animals before any information regarding their fertility could be obtained. At that age (15 months from release) the stunted tetrasporelings were removed and discarded from the cultures. The remaining tetrasporelings reached lengths of about 6.7 cm at the age of twenty-one months from release (Fig. 4F) with extensive branching, but the blades themselves never attained the same breadth as the nature-growing plants. Fig. 5 shows a slide upon which sixteen sporelings were growing. At this age (21 months) the sporelings died and the experiment was terminated before any information regarding their fertility was obtained.

3.5. Growth in culture of parasporelings of Plumaria elegans.

The illumination level of 1000 lux and the other laboratory-induced environmental factors used in culturing the tetrasporelings of Palmaria palmata were used in culturing the parasporelings of Plumaria elegans. The paraspores had differentiated into upright filaments and rhizoids within a period of five weeks (Fig. 3B) in which the upright filaments had branched as well as the rhizoids, giving secondary rhizoids which spread horizontally on the substratum. At the age of fifteen weeks from release, the erect branches of the parasporelings had branched extensively and the rhizoids also branched and gave rise to upright filaments as well as secondary rhizoids (Fig. 3D). By the time seventeen months had passed the parasporelings attained the maximum length of about 2 cm in which most of the parasporeling growth was concentrated in anastomosis and spreading of the rhizoids which gave rise to upright filaments and secondary rhizoids (Fig. 6). At this stage of development the experiment was terminated and no information regarding the fertility was obtained.

Contamination of cultures with bacteria and diatoms was a common experience in all cultures examined in the present work. However, they were

controlled by using Germanium dioxide, manual removal of epiphytes and using the cleaning apparatus (see section 2.2.2).

3.6. Discussion.

Neither the tetrasporelings of Palmaria palmata nor the parasporelings of Plumaria elegans showed any fertility over a period of twenty-one months for the former and seventeen months for the latter. However, monosporelings of Audouinella secundata (Lyngbye) Dixon were brought to fertility in culture within a period of five weeks in which the resulting plants produced monosporangia (Fig. 7) which gave monospores (Fig. 8), under the same laboratory-induced environmental factors of temperature, daylength and culture medium. The only difference between the three cultures was that illumination 1000 lux was used in Plumaria elegans cultures while in Audouinella secundata and Palmaria palmata cultures, the illumination was 3000 lux. Rueness (1968) cultured the paraspores of Plumaria elegans from Norway in Erd-Shreiber medium at a temperature of 12°C and illumination of 2000 lux. The parasporelings attained the length of 1.5 - 2 cm after six months which is the same length obtained after seventeen months in the present work. However, the parasporelings cultured by Rueness (1968) were fertile at that age (six months) in which about 600 sporangia per plant were produced from the apical cells of the upright filaments and the apical cells of the rhizoids. Whittick (1977) cultured paraspores of Plumaria elegans from Newfoundland in Provasoli ES medium and found that the rate of axial cell production is temperature dependent and the light intensity does not have a significant effect on growth rate at temperatures below 15°C, but at 15°C higher light intensities promote faster growth rate. The parasporelings cultured by Whittick (1977) became fertile after sixty-eight days at a temperature of 15°C and photoperiod 16 light : 8 dark, and after 101 days at a temperature of 10°C and photoperiod 16 light : 8 dark. The plants were sterile over a period of 151 days at a temperature

of 10°C and short day (8 light : 16 dark), as also were sporelings growing at a temperature under 10°C with long day (16 light : 8 dark). In all the above cultures the illuminations 150, 500 and 1200 lux were used (Whittick, 1977).

In the case of Palmaria palmata, the attempt by Sparling (1961) to bring the tetrasporelings to fertility was also unsuccessful over a period of twenty months in culture using Erd-Shreiber medium and light intensity of about 108-540 lux at a temperature of 11-15°C and a daily photoperiod of 12-14 hours.

The search for a chromosome number as another indicator for the life history of Palmaria palmata has been carried out by several investigators from different localities and the results are conflicting. For instance, Westbrook's (1928) estimation for the chromosome number was $2n = 20$ at least with all meiosis stages observed in the tetrasporangium, but the chromosome number was not counted in the resulting tetraspore. Austin (1956, 1959) reported $n = 21$, Magne (1959, 1964) counted $2n = 14$ with all meiosis stages observed in the tetrasporangium but the number of the chromosomes in the resulting tetraspore was the same as the diploid tetrasporophyte and the male plant, and hence meiosis was considered to be non-functional (apomeiosis). Sparling (1961) counted the chromosome number of the tetraspores and reported $n = 14$ which could also represent $2n$ since the diploid stages were not examined. Yabu (1971, 1976) reported the chromosome number as $2n = 40-50$, $n = 21$ or 26 and concluded that the material had at least two races. Yabu (1976) reported the presence of some peculiar structures such as two long chromosomes and a small rod-shaped body found on rare occasions in the first meiotic division; an extra large chromosome appeared among the bivalents, which takes an O shape and which may play an important role in the life history. Finally, Van der Meer (1976) gave $n = 22-23$, $2n = 44-46$, corrected later to $n = 21$ (Van der Meer and Chen, 1979), who also reported that Palmaria palmata would become fertile if incubated at a low temperature

(5-7°C) with short light period (8h light : 16h dark). Van der Meer and Chen (1979) obtained from individual tetrads a 2:2 segregation of large haploid plants (phene I) versus small haploid plants (phene II) in culture. Plants of phene I gave rise to spermatia and phene II gave rise to aborted (sterile) sporangia. Their conclusion was that phene I plants represent the male forms while phene II plants are believed to be the female forms, and because of the culture conditions fertilization did not take place and aborted sporangia were formed on the female plants. Similar segregation of large and small plants (the ratio is not known) was observed in the cultures in the present work, though it was not realized at that time that they were sexually different types of plants and hence they were removed from the culture since variations in growth rates between sporelings are a common experience within a population in a culture. However, Van der Meer and Todd (1980) reported that the female gametophytes of Palmaria palmata attain sexual maturity when only days old and a fraction of a millimetre in size, while the male gametophytes require several months to develop spermatangia and to grow as large as the tetrasporophytes. Because the female plants mature so quickly they are virtually unable to mate with male plants of the same generation and must be fertilized by older male plants in the population. Tetrasporophytes establish independent holdfasts by overgrowing the parental females, and carposporophytes are lacking from the life history (Van der Meer and Todd, 1980). It is interesting to note that Van der Meer and Chen (1979) found fertile tetrasporic fronds in culture (without mating the females with older males) on one occasion in which segments of juvenile tetrasporophytes collected from the field were brought to fertility and a large population of sporelings was established in culture. The sporelings grew well for several months then suddenly sickened and died back severely leaving only spheroid galls on which new fronds regenerated and became fertile after several months. Many of these fronds formed spermatangia, others developed large sori containing tetra-

sporangia and few produced both. Spores released from these tetra-sporangia gave rise to another generation of sporelings which segregate into small and large plants as well, but both the sporelings and the parent died before further data on their reproductive morphology or chromosome number were obtained (Van der Meer and Chen, 1979). Van der Meer (1981) reported that Halosaccion ramentaceum (L.) J. Agardh, the other member of the order Palmariaceae, has the same life history as Palmaria palmata.

Whilst my own attempts at growing new generations of the plants to a fertile condition proved unsuccessful, a comparison of the morphological forms obtained in the cultures with those described from elsewhere and in nature confirmed that the culture conditions for early sporeling growth were not likely to give rise to abnormal germination stages.

4. ULTRASTRUCTURE OF VEGETATIVE CELLS

4.1. Introduction.

The fine-structure of red algal vegetative cells generally shows some characteristic features, e.g. (1) Floridean starch grains lying freely in the cytoplasm (see section 7). (2) The presence of phycobilisome granules on the outer surface of the chloroplast thylakoids (see section 8), though phycobilisomes are also present in the chromatoplasts of blue green algae (Hara and Chihara, 1974). (3) The absence of plasmodesmata (Wetherbee, 1979); nevertheless, translocation has been demonstrated using autoradiography (Goff, 1979a), but the exact avenue has not been identified (Wetherbee and Kraft, 1981). (4) The presence of pit connections between neighbouring cells which has been suggested to function for translocation (for details see section 10.4). (5) The double membrane comprising the chloroplast envelope not associated with endoplasmic reticulum (Duckett and Peel, 1978). (6) The chloroplast thylakoids lie more or less parallel across the stroma or in concentric circles and there is often a single thylakoid lying parallel to the chloroplast envelope (peripheral thylakoid) and enclosing the others (Dodge, 1973, 1974). Hara and Chihara (1974) classified the chloroplasts of the Rhodophyta into seven types based on presence or absence of the pyrenoids, presence or absence of the peripheral thylakoids, presence or absence of the bundle of tubular thylakoids, the shape of the chloroplasts, number of chloroplasts in each cell, sites of the chloroplasts within a cell, presence or absence of floridean starch grains covering the pyrenoid matrix, presence or absence of the thylakoids surrounding the pyrenoid matrix and, finally, the pattern of the thylakoid system within a pyrenoid matrix. These types are (1) "Nemalion type"; (2) "Modified-Nemalion type"; (3) "Helminthocladia type"; (4) "Liagora type"; (5) "Rhodella type"; (6) "Polysiphonia type"; (7) "Batrachospermum type".

In this section features of the ultrastructure of vegetative cells of three species of red algae are described.

4.2. Ultrastructure of vegetative cells of Palmaria palmata.

A transverse section through the blade of Palmaria palmata shows that it is composed of cortical cells on both sides sandwiching the internal medullary cells (Figs. 1,2,3). Guiry (1974) has divided the cortical cells into an outer cortex and an inner cortex, and this will also be quoted in the present work. The tetrasporangia may be present as a single row (Fig. 1) or several rows (Figs. 2,3) between the cortical cells on both sides of the blade (Figs. 1,2), or on one side only in a given piece cut from the blade (Fig. 3). The outer cortical cells are of one to several layers (Figs. 3,4,6). Each cell is more or less elongated perpendicular to the surface, measuring about 6.5 μm in width and 10.5 μm in length (Fig. 7), and possesses a centrally located nucleus of about 4 μm in diameter, a vacuole (Fig. 5), a few mitochondria (Figs. 6,7) often accompanied by Golgi bodies (Fig. 9), proplastids and chloroplasts occupying a peripheral position (Fig. 7). A few cells were observed to possess two nuclei (Fig. 13) and in a single sample an elongated cell with four nuclei was observed (Fig. 14). Cells with more than one nucleus were also reported in Chondrus crispus (Harvey and McLachlan, 1973). The inner cortical cells of Palmaria palmata are of one to several layers (Figs. 1,3) and their fine-structure is similar to those of the outer cortical cells with the exception that they are slightly larger and their long axes are more or less parallel to the blade surface. The medullary cells are also of one to several layers (Figs. 1,2,3). They are often elongated (Figs. 2,4) but sometimes they may appear rounded (Fig. 3). The cells are highly vacuolated (Fig. 4) and the cytoplasm is represented by a narrow strand occupying the periphery of the cell (Figs. 4,8). The nucleus is in a peripheral position (Fig. 10). The chloroplasts of the medullary cells are smaller and narrower (Fig. 8)

compared to the chloroplasts of the cortical cells but the mitochondria and Golgi bodies are similar.

The cross section through the stipe and the holdfast of Palmaria palmata shows that the stipe can also be divided into cortical, subcortical and medullary cells (Fig. 15) but not the holdfast which is composed of highly vacuolated cells (Fig. 16). The fine-structure of the cells of both the stipe and the holdfast are similar to those of the blade with the exception that most nuclei of the stipe and holdfast cells show electron dense patches which may possibly represent heterochromatin (Figs. 16,17).

On the surface of the thallus there is a single layer covering (Figs. 6,7), referred to here as cuticle. The cuticle is about 0.7 μm thick and appears more electron dense than the cell walls. According to Myers and Preston (1959) microfibrils of the vegetative cell wall of Palmaria palmata (as Rhodymenia palmata) are randomly orientated (as shown by X-ray diffraction and shadowing techniques) and the middle lamella is considerably increased in thickness at the corner where neighbouring cells meet. This statement agrees with the observations in the present work. The middle lamella often can hardly be resolved in the cortical region (Figs. 5,7) but is clearly distinguishable from the immediate cell walls in the subcortical and medullary regions of both the blade (Figs. 6,8) and the stipe (Figs. 17,18) as well as the holdfast (Fig. 16) where it is much wider.

Unusual cell wall structures were observed within the immediate cell walls of the stipe cells (Figs. 17,18). They are not bounded by membrane and are of various shapes and sizes (Figs. 19 to 29). They are composed of dark electron dense bands (varying from 0.04 - 0.06 μm) alternating with less electron dense bands (varying from 0.008 - 0.02 μm), though a few of them were observed to be of uniform electron density (Fig. 28) or partly banded (Fig. 29). Both the dense and the less dense bands within a structure have a more or less uniform width for each type of band but these dimensions may vary from one to another structure. The bands are orientated

at different angles to the plasmalemma or the cytoplasm (Figs. 19 to 29, arrows pointed towards the direction of the plasmalemma and the nearest cytoplasm). Two to three sets of parallel bands were sometimes observed in a single structure (Figs. 26,27). The cell wall structures are often separated by a distance from the plasmalemma (Figs. 19,21,24,26,29) but in a few cases they are in direct contact with it (Fig. 20). Large dark electron dense indentations were sometimes observed (Fig. 24).

Floridean starch grains are hardly present in the vegetative cells, but on very rare occasions cells possessing moderate amounts of them were observed (Fig. 9).

Pit connections of Palmaria palmata vary in shape and size. They may be spherical, oval or more or less rectangular (Fig. 6,11,12). The plug core is apparently finely granulated and moderately electron dense and is covered by plug caps at the cytoplasmic sides. Each cap is composed of three layers in which two dark electron dense layers sandwich a less electron dense layer (Fig. 12). The rim of the aperture fits into a shallow groove around the short axis of the pit connection, thus firmly locking the plug into the aperture (Figs. 11,12).

The chloroplasts consist of a double membraned envelope surrounding the chloroplast matrix within which DNA areas and plastoglobuli were observed (Figs. 6,7, for more detailed micrographs see section 8). The peripheral thylakoid is parallel to the chloroplast envelope and encloses one or more concentric thylakoids and several internal thylakoids (Figs. 7,9).

4.3. Ultrastructure of vegetative cells of the upright filaments of Plumaria elegans.

The ultimate branches or ramuli of Plumaria elegans are ecorticate (Fig. 30). Cortication is initiated and develops extensively at a very short distance behind the apex (Fig. 31). Chloroplasts are the main constituent of these cortical cells (Fig. 32), though a small amount of

floridean starch and other cytoplasmic organelles are also observed. The axial cells of the ecorticate branches are surrounded by a cell wall measuring about 2.5 μm in width in which the outer part is more electron dense and often referred to as cuticle (Fig. 33). This layer (cuticle) is also observed in the outer region of the cell walls of the branchlet cells (Figs. 34,35), apical cells (Fig. 36), parasporangia (Fig. 31 section 10) and tetrasporangia (Fig. 32 section 10) of the parasporophytes. Microfibrils of the cell walls are probably distributed randomly and the inner ones are apparently loosely arranged (Figs. 33 to 36). The nucleus is often centrally located (Fig. . . ,36) with a large nucleolus (Fig. 36), but in highly vacuolated cells the nucleus may be peripheral (Fig. 35). Some nuclei have a pouch within which cytoplasm and some floridean starch granules were observed (Fig. 41). Chloroplasts are generally composed of a chloroplast envelope surrounding a stroma in which the thylakoids lie more or less parallel and enclosed by a single peripheral thylakoid (Fig. 38, for more detailed micrographs see section 8). The chloroplasts often occupy peripheral positions within the cell (Figs. 33,35). Proplastids and immature chloroplasts represent the major proportion of the apical cells (for more details see section 8). Floridean starch grains, in which some show concentric layers, are present freely in the cytoplasm (Figs. 33,34,37, for more details see section 7). Multilamellar bodies (myelin figures) and several Golgi bodies, often accompanied by mitochondria, are found in various locations in the ribosome-rich ground cytoplasm (Figs. 33,37,40). Osmiophilic bodies of different electron density were observed in most cells (Figs. 33,35). They are spherical or often angular in crystalline forms or other shapes and are of various sizes (Figs. 39,40). These bodies do not appear to have a limiting membrane and consist of amorphous or finely granular substances (Figs. 39,40). Pit connections are ultrastructurally similar to those reported between the cells of parasporangia (see section 10.4).

4.4. Ultrastructure of vegetative cells of Audouinella sagraeanum.

The vegetative cells of Audouinella sagraeanum are cylindrical and of more or less equal size, measuring about 8 μm in diameter and about 23 μm in length (Fig. 42). The cell wall is approximately 1 μm wide and the microfibrils are probably in a random distribution (Figs. 43,46,49). The outermost layer of the cell wall is thin (0.06 μm) and electron dense and probably represents the cuticle (Figs. 46,49), though it is not always clear. At the outermost side of the cell wall (facing the seawater) patches of teeth-like structures were observed (Figs. 42,43, indicated by arrows, 54) in addition to other peculiar structures which were also located at this region (Figs. 55,59 to 67). These structures are of various shapes, sizes and substructures. The nucleus of the vegetative cell of Audouinella sagraeanum is centrally located and is more or less spherical, measuring about 2.6 μm in diameter with a nucleolus of about 1 μm in diameter (Figs. 42,48). Floridean starch grains lie freely in the cytoplasm and may represent up to about 60% of the cell volume (Figs. 42,44). Small grains of similar electron density to floridean starch grains were observed within the chloroplasts (Figs. 44,49,50, see section 7 for more details). Each cell of Audouinella sagraeanum possesses a single large chloroplast and several small chloroplasts (Figs. 43,44) and sometimes a few proplastids (Fig. 47). The difference between the large and the small chloroplasts is that the thylakoid arrangements within the small chloroplasts are represented by a peripheral thylakoid parallel to the chloroplast envelope and enclosing the internal thylakoids (Figs. 43,51), or with no internal thylakoids in the case of young proplastids (Fig. 47), whilst the large chloroplasts lack a peripheral thylakoid but possess a single pyrenoid (Figs. 44,46), or on rare occasions two pyrenoids per chloroplast were observed (Fig. 45). All chloroplasts occupy a more or less peripheral position within a cell. The large chloroplast may occupy one side of the cell (Figs. 42 and Fig. 18 section 7) or the two sides. In the latter, the chloroplast appears as U-shaped (Fig. 45) or as an A-

shape in which the pyrenoid is located at the bridge connecting the two arms (Fig. 44 and Fig. 24 section 7). The pyrenoid may be embedded in the chloroplasts (Figs. 45,46) or protrude from it, but even then the pyrenoid is bounded by a continuation of the chloroplast envelope and few thylakoids (Fig. 42, and 23 section 7). The pyrenoid is more or less spherical, measuring about 2 - 3.8 μm in diameter (Figs. 42,44,46) and is composed of a matrix of finely granular appearance (Figs. 49,50) of proteinaceous material (Dodge, 1973; Bisalputra, 1974). The matrix is penetrated by a number of chloroplast thylakoids (Figs. 46 arrows, 56). These thylakoids may branch and rejoin (Fig. 46, arrow with tail). Tubular structures were seen within the matrix of some pyrenoids as shown in Fig. 45 in a cross section and in Figs. 49,50 in a longitudinal section. These tubular structures were sometimes observed attached to the thylakoids (Fig. 50, arrows), which may indicate that they possibly originate from the pyrenoid-penetrated thylakoids. There seems no spatial relationship between the pyrenoids and floridean starch grains. Pit connections of Audouinella sagraeanum are composed of a plug core of finely granular substance and moderate electron density (Fig. 52). The plug caps at both sides of the plug core are composed of a single electron dense layer upon which electron dense granular material (at the sides facing the cytoplasm) is aggregated (Fig. 52). The plasmalemma is apparently continuous from cell to cell passing around the sides of the plug (Fig. 52, arrows). Mitochondria are often accompanied by Golgi bodies and some were observed to possess three DNA areas in a single section (Fig. 53). Senescence or ageing of cells is followed by autolysis of the cytoplasm and its organelles (Fig. 55). Floridean starch grains are the last to degenerate (Figs. 55,58). A degenerated pit connection has a peculiar appearance (Fig. 57) when compared to a normal one (Fig. 52). Senescence and cytoplasmic autolysis of a cell may be followed by division and growth of the neighbouring one to replace the dying one (Fig. 56) in which the newly

formed cell is surrounded by two cell walls (Fig. 56). This newly formed cell may or may not occupy the whole area of the dead one (Figs. 56,58).

4.5. Discussion.

Palmaria palmata is the only species reported in the present work in which what is termed "middle lamellae" by Myers and Preston (1959) is present. The middle lamella is similar to those described in Chondrus crispus by Harvey and McLachlan (1973) who termed it as an intracellular substance composed of fibrils and is distinct from the immediate cell walls in that the fibrils are not arranged in definite rows. The microfibrils of the cell walls of the three species (Palmaria palmata, Plumaria elegans and Audouinella sagraeanum) are probably randomly arranged and the single electron dense layer which is in contact with the seawater probably represents the cuticle (see Dodge, 1973), though this layer in Audouinella sagraeanum does not always appear clearly. In contrast, the cuticle of Chondrus crispus is multilayered (Harvey and McLachlan, 1973). The cell walls of Audouinella sagraeanum cells and the stipe cells of Palmaria palmata possess unusual structures. They are in direct contact with the seawater in Audouinella sagraeanum and embedded within the immediate cell walls in Palmaria palmata. Their function, origin and chemical composition is not known but the presence of large dark electron dense indentations (Fig. 24) in the case of Palmaria palmata may suggest a support function for these structures. These structures are hitherto undescribed, at least in algae. The cell walls of ageing cells of the brown alga Ectocarpus sp. show local thickening (ingrowth) of electron dense material trapped between the primary cell wall and the newly deposited wall material (Oliveira and Bisalputra, 1977c). In Antithamnion defectum the cell wall possesses osmiophilic bands (Young, 1980).

The thylakoid arrangements within chloroplasts of Plumaria elegans and the small chloroplasts of Audouinella sagraeanum are represented by a

peripheral thylakoid parallel to the chloroplast envelope and enclosing the internal thylakoids. Chloroplasts possessing this pattern of thylakoid arrangements were classified as "Polysiphonia type" chloroplasts by Hara and Chihara (1974). The pattern of thylakoid arrangements within chloroplasts of Palmaria palmata is that a peripheral thylakoid parallels the chloroplast envelope and encloses one or more concentric thylakoids and several internal thylakoids similar to those described in Batrachospermum moniliforme (Brown and Weier, 1970) with the exception that the tubular thylakoids which were observed in chloroplasts of only the cortical cells of Batrachospermum moniliforme were not observed in the present work. Chloroplasts possessing bundles of tubular thylakoids were classified as "Batrachospermum type" chloroplasts by Hara and Chihara (1974). In the large chloroplast of Audouinella sagraeanum, the peripheral thylakoid is absent but a pyrenoid is present. This observation agrees with Hara and Chihara's (1974) observation in which they reported that the peripheral thylakoid cannot be detected in chloroplasts possessing pyrenoids (see also Cole and Sheath, 1980). The large chloroplast of Audouinella sagraeanum has more or less the character of "Modified-Nemalion type" chloroplasts in Hara and Chihara's (1974) classification. The pyrenoids of Audouinella sagraeanum are probably in the category of "compound internal pyrenoid with random arrangement of thylakoids" according to Dodge's (1973) classification, due to the following features: (a) the pyrenoid is penetrated by a number of thylakoids which may branch and rejoin; (b) no spatial relationship between the pyrenoid and floridean starch grains exists; (c) each large chloroplast possesses mainly one or sometimes two pyrenoids.

The tubular structures (see Figs. 45,49,50) found within the matrix of the pyrenoid are similar to those reported in Glenodinium foliaceum (Dodge and Crawford, 1971) with the exception that tubules in Glenodinium foliaceum do not have any connection with the thylakoids and were reported to be invaginations which bring chloroplast stroma into the pyrenoid (Dodge and Crawford, 1971). The pyrenoids might serve as a store of enzymes

particularly for the use of newly formed cells (Dodge, 1973) or as a site for the storage of protein (Bisalputra, 1974).

The pit connections of Palmaria palmata consist of a plug core which is covered at both sides by plug caps. Each cap consists of three layers in which two layers are electron dense, sandwiching a less electron dense layer (Fig. 12). The number of the layers of the cap disagrees with the observation reported by Pueschel (1977a) who stated that the plug cap appears to be composed of two moderately electron dense layers sandwiched among three more heavily electron dense layers in ultrathin section, but with the freeze-etch technique it appears to consist of two layers separated by a membrane which is continuous with the plasmalemma. The plug cap of Audouinella sagraeanum consists of a single layer upon which electron dense material, at the cytoplasmic side, aggregates. Such aggregation of electron dense material was also noticed adjacent to pit caps of Nemalion helminthoides (Duckett et al., 1974). However, according to Aghajanian and Hommersand (1978), there is some confusion as to the interpretation of the pit cap in which the outer amorphous layer of material was considered by some authors as being part of the pit cap. It is generally agreed that the plasmalemma is continuous from cell to cell passing around the sides of the plug (Dodge, 1973; Bold and Wynne, 1978; Lee, 1980). This is true in Audouinella sagraeanum (see Fig. 52, arrows point at the plasmalemma) and during the tetrasporogenesis of Palmaria palmata in which the two mucilage layers were formed between the plasmalemma and the tetrasporangium cell wall did not form upon pit connections (see section 5). In Audouinella sagraeanum, pit connections change their appearance and degenerate by ageing and death of the cells (Fig. 57) which indicates that pit connections in Audouinella sagraeanum do not break down and establish cytoplasmic continuity between living cells as in Batrachospermum sirodotti in which they undergo breakdown and the cytoplasmic continuity is established between axial cells and between pleuridial cells (Aghajanian and Hommersand, 1978). The origin and function

of pit connections is discussed in section 10.4.

The aspects of floridean starch granules of the three species (Palmaria palmata, Plumaria elegans and Audouinella sagraeanum) is discussed in section 7 and the fine-structure of mitochondria of the vegetative cells of the three species is similar to those of the parasporangia of Plumaria elegans with the exception that cup-shaped mitochondria and mitochondria possessing extra parts or compartments were not observed in the vegetative cells (see section 10.5).

The osmiophilic bodies of different electron density were observed in only vegetative cells of Plumaria elegans among the three species examined in the present work. The osmiophilic bodies are spherical, crystalline shapes or other shapes, and they are not limited by membranes. These bodies are similar to those reported in the vegetative cells of Ptilota hypnoides (Scott and Dixon, 1973a) in which they are also not bounded by membranes and have different electron density. Spherical and crystalline bodies were also reported in many species of Ceramiales (Fritsch, 1945) and were thought to be composed of protein. Crystalline bodies with crystal lattice were observed to be a regular feature of the rhizoidal cell cytoplasm of Harveyella mirabilis (Goff, 1979b) but their chemical composition was not determined. The cytoplasmic crystalloids, without a binding membrane, of Antithamnion defectum (Young, 1979a) were reported to be protein and may serve as protein reserves, as well as the spherical vacuolar bodies. The densely staining bodies appear in the cytoplasm of gonimoblast cells of Polysiphonia novae-angliae (Wetherbee, 1980) and some were observed to crystallize, these were also reported to be of proteinaceous nature. In Plumaria elegans, the crystalline bodies were also observed in the cells of parasporangia growing in culture (see section 10.6) and spherical osmiophilic bodies were also observed during tetraspore germination in Palmaria palmata (see section 10.2). The chemical composition of these osmiophilic bodies (spherical and crystalline) of

Plumaria elegans is not known but they are probably of proteinaceous nature similar to those of Antithamnion defectum (Young, 1979a) and Polysiphonia novae-angliae (Wetherbee, 1980). However, the histochemical tests carried out in the present study did not prove either positive or negative for protein.

5. ULTRASTRUCTURE OF TETRASPORE FORMATION OF PALMARIA PALMATA

5.1. Introduction.

The amount of information accumulated in the last ten years on the differentiation of the reproductive organs of the class Florideophyceae is extensive, as will be shown in the ensuing discussion. The majority of the work so far reported deals with members of the order Ceramiales. An ultrastructural study of tetrasporogenesis of Palmaria palmata, the sole British member of a newly created order Palmariales (Guiry, 1978) would be of interest, since transmission electron microscopy should increasingly play a role in red algal taxonomy (Duckett and Peel, 1978). While this work was in progress a paper was published by Pueschel (1979) describing tetrasporogenesis in Palmaria palmata from the east coast of the U.S.A. My results, however, offer different interpretations of several aspects of the process to those given by Pueschel (1979) and these different interpretations will be fully discussed in the following text.

The literature contains conflicting reports on the chromosome numbers found in Palmaria palmata (see section 3). This is probably due to the presence of large numbers of tiny chromosomes in relatively small nuclei, a fundamental difficulty involved in counting chromosomes in red algae (Dixon, 1966). At ultrastructural level, a major breakthrough was achieved when Kugrens and West (1972b) announced the discovery of synaptonemal complexes in tetraspore mother cells of four genera of Florideophyceae, Janczewskia, Levringiella, Gonimophyllum and Polycoryne. To date, however, ultrastructural information on karyokinesis of red algae is limited to a report (as far as is known) describing some stages of meiosis (Scott and Thomas, 1975) and a few reports on the stages of mitosis (McDonald, 1972; Bronchart and Demoulin, 1977; Schornstein and Scott, 1980; Scott et al., 1980).

In the present work, it was difficult to correlate the cytoplasmic

organelles differentiation directly in relation to nuclear activities as attempted by Pueschel (1979) since the nucleus did not appear in all sections examined and the stages of the nuclear division were not observed. The development of tetraspores, however, can be conveniently separated into three stages based on the presence or absence of certain vesicles, and the activity of the cytoplasmic organelles in relation to the number of layers outside the plasmalemma of the differentiating cell. At stage 1 the tetrasporangium mother cell is surrounded by the cell wall only; at stage 2 a mucilage layer is added, and at stage 3 a second mucilage layer is incorporated.

5.2. Cytoplasmic organelle differentiation.

One of the cortical cells enlarges anisotropically and becomes highly vacuolated with a large nucleus, a few mitochondria and with the chloroplasts more or less occupying the peripheral region (Fig. 1). The cell wall is broad but of irregular thickness, approximately 0.5 - 2.4 μm wide (Fig. 2). The cell wall is of two layers, an outer electron dense layer and a less electron dense inner layer. The inner layer is several times thicker than the outer layer (Fig. 2). This cell is at early stage 1 of tetrasporogenesis. At mid-stage 1 the vacuolation is gradually replaced by an increase in the volume of the cytoplasm (Fig. 3); the chloroplasts divide (Fig. 4) (chloroplast division may have started earlier); the cell wall becomes less broad and less irregular (Fig. 3) and the cell divides unequally into a small somewhat cup-shaped stalk cell and a tetrasporangium mother cell (Fig. 3). They are separated by a regular cell wall 0.4 - 0.5 μm wide. This cell wall in most cases consists of three layers in which the middle layer is a thin dark electron dense layer, continuous with the outer dark electron dense layer of the tetrasporangium mother cell and the stalk cell (Figs. 6,7). At late stage 1, the tetrasporangium mother cell wall becomes much thinner (about 0.4 - 0.5 μm wide)

and is of uniform thickness (Fig. 5). This wall will stay constant in thickness until tetraspore maturity (see Figs. 30,40A,40C) and represents the tetrasporangium cell wall. The vacuolation is much reduced at this stage and the reduction probably proceeds from cell apex to base (Figs. 5,7,9). The chloroplast number increases and deposition of floridean starch begins (Fig. 4).

The stalk cell occupies the lower end of the tetrasporangium mother cell and is highly vacuolated with the nucleus in a lateral position and about 3.5 μm in diameter (Fig. 7). The chloroplasts occupy peripheral positions (Fig. 7). The stalk cell is connected to the tetrasporangium mother cell by a pit connection (Fig. 3) which was also observed between a vegetative cell and the stalk cell (Fig. 9). Floridean starch was observed in random distribution within the stalk cell (Fig. 6) and Golgi bodies are represented by small stacked cisternae frequently accompanied by mitochondria (Fig. 8).

As the cortical vegetative cell elongates to form the tetrasporangium mother cell at early stage 1, the surrounding cortical cells undergo considerable modification and develop into a structure of 3-4 cells termed a paraphysis (Guiry, 1974). The paraphysis may have a bulbous cell at the apex (Fig. 10) and occasionally two nuclei per cell were observed (Fig. 11). Small amounts of floridean starch were observed within some of the inner cortical cells which lie underneath the developing tetrasporangium (Fig. 12, arrow) but not all (Fig. 12, arrow with tail) and PAS staining did not reveal them (Fig. 39B). These observations are in disagreement with Pueschel's (1979) observations, who stated that deposition of starch within the tetrasporangium is preceded by massive accumulation of starch in the inner cortical cells which subtend the fertile filaments.

Fig. 14 shows an apparently inactive Golgi body at early stage 1 accompanied by a mitochondrion, a feature which has been observed repeatedly in the higher forms of red algae (Kugrens and West, 1973;

Alley and Scott, 1977; Wetherbee and West, 1977). Fig. 15 shows a Golgi body at the beginning of activation at early stage 2 in which the distal ends of the cisternae are swelling. Small vesicles near the forming face of Golgi body, possibly originated from the endoplasmic reticulum, might contribute to the formation of new Golgi cisternae (Fig. 14 arrows) (Konrad Hawkins, 1974b; Alley and Scott, 1977; Wetherbee and West, 1977). Such vesicles were also reported to have originated from the chloroplast membranes (Konrad Hawkins, 1974b) and the nuclear envelope (Dodge, 1973; Rascio et al., 1980). The clear zone observed by Pueschel (1979) between the surface of the mitochondrion and the forming face of the associated Golgi body in which ribosomes and other organelles were excluded, was not observed in the present work.

At mid stage 2, Golgi bodies increase in number and actively produce small vesicles of about 80 - 200 nm in diameter (Fig. 16), and each Golgi cisterna produces more than one vesicle. These vesicles (mucilage vesicles 1) migrate towards the peripheral cytoplasm (Fig. 17), fuse with the plasmalemma and discharge their contents by reverse pinocytosis (Fig. 17, arrow) to form a layer termed mucilage layer 1. The smooth crenulation of the plasmalemma seen during stage 1 (Figs. 2,5,14) is replaced during this stage by a massive convolution as a result of non-uniform deposition of the mucilaginous material from mucilage vesicle 1 between the tetrasporangium cell wall and the plasmalemma (Figs. 17,18). More deposition of the mucilaginous material results in the mucilage layer 1 becoming thicker (about 1 - 2.8 μm wide). The mucilaginous material then becomes evenly distributed around the tetrasporangium mother cell and the plasmalemma regains its normal smooth crenulate appearance (Figs. 19,20). By this time the first cleavage furrow has initiated as an annular ingrowth of the plasmalemma (Figs. 19,20). The mucilaginous material participates in formation of the cleavage furrow but not the cell wall. The vacuolation in the cell is reduced considerably and replaced at the same time by the increased number of the chloroplasts and the other

cytoplasmic organelles (Fig. 19).

At late stage 2 - early stage 3, large mucilage vesicles are produced in large numbers by the Golgi bodies. Each vesicle is produced from the transformation of a single cisterna (Figs. 21,22); such vesiculation of entire Golgi cisterna has been described for other red algae (Kugrens and West, 1972a,1973; Konrad Hawkins, 1974a,1974b; Wetherbee and West, 1977). These vesicles are termed in the present work mucilage vesicles 2. They migrate to the peripheral cytoplasm (Fig. 23 arrow with tail), fuse with the plasmalemma and discharge their contents by reverse pinocytosis (Fig. 23, arrow) to form the second mucilage layer (mucilage layer 2). The plasmalemma convolutes again as a result of such deposition (Figs. 23,25). Mucilage layer 2 consists of fine fibrillar material compared with mucilage layer 1 (Figs. 24,25). The material in mucilage vesicles 2 (but not mucilage layer 2 when deposited) seems to be delicate and is destroyed if exposed to the electron microscope beam for relatively long periods, as is shown in Fig. 22 in which some vesicles appear to have lost their contents even before they have separated from the Golgi bodies (Fig. 22, arrow). This distortion is possibly induced by the heat coming from the beam. Production of mucilage vesicles 1 did not cease with the initiation of mucilage vesicles 2. Fig. 25 shows a Golgi body producing mucilage vesicles 1 whilst deposition of mucilage layer 2 is in progress. These type 1 vesicles are involved in deposition and advancing the cleavage furrows (Fig. 26); at the same time mucilage vesicles 2 are involved in depositing mucilage layer 2 in the cleavage furrows (Fig. 27). Fig. 28 shows a cleavage furrow in which a short distance at the end consists of mucilage layer 1. This indicates that mucilage vesicles 1 material mainly spearheads the progress of the cleavage furrows (Fig. 28, 27). Deposition of the mucilaginous material results in a broad lenticular indentation at the apex of the tetrasporangium (= apical plug) and mainly consists of mucilage layer 1 (Figs. 29, 40A).

The first cleavage furrow is periclinal and the second and the third are anticlinal. One may be produced before the other. However, Guiry (1974) stated that the first cleavage furrow is completed (Fig. 31) whilst the second and the third cleavage furrows are being initiated. But Pueschel (1979) reported a different process in which the first cleavage furrow is arrested shortly before completion (Fig. 30) and remains in this condition until the anticlinal cleavage furrows are also nearly completed (Fig. 32) to ensure synchrony of spore maturation. Ten tetrasporangia were observed in the present work behaving in this way. Figs. 34A to Q are light microscope serial sections of a single tetrasporangium in which a clear cytoplasmic continuity exists between spore A, spore B, spore C and spore D, through the incompleted cleavage furrows (Fig. 34H, arrows). By contrast when random sporangia were examined by light microscopy in order to construct a serial sequence, it appears that the first periclinal cleavage furrow seems to be completed (apart from some areas of doubtful interpretation as in Fig. 35E,G,H,^K arrows) even when the second and the third cleavage furrows are just being initiated (Figs. 35A to Q). However, within the former ten selected sporangia, the cytoplasmic continuity between the four spores seems not always to be clear. For instance, Figs. 36A to R show light microscopic serial sections of a single sporangium in which there is a clear cytoplasmic continuity between spore A, spore B and spore C but spore D seems to be completely isolated (Fig. 36K) apart from one doubtful area (Fig. 36I, arrow). In Figs. 37A to R, it appears that the cytoplasmic continuity exists between spore A, spore B, spore C and spore D (Fig. 37J, arrows), but there seems to be another part of the second cleavage furrow (Fig. 37K, arrow) not completed which suggests that the cytoplasmic continuity between spore C and spore D exists through two incompleted parts of the second cleavage furrow, which is unlikely.

The cleavage furrow growth and plane are summarized in Fig. 85 in which Pueschel's conclusion was taken initially as the correct one. On

sheet 1, A represents a sporangium at stage 2 (see Fig. 19). The first periclinal cleavage furrows have been initiated and growth of this cleavage furrow is accompanied by incorporation of more material of mucilage vesicles 1 (arrows indicate the growth direction). By adding sheet 2, sporangium A1 at mid stage 3 is shown (see Fig. 30) which is also represented by sporangium B on sheet 1. When sheet 3 is placed in position, the initiations of the second and the third cleavage furrows are shown on sporangium A1+2 which is also represented at sporangium C on sheet 1 (see Figs. 72A to Q). Addition of sheet 4 shows a sporangium A1+2+3 at late stage 3 and the summary diagram is shown as sporangium D of sheet 1 (see Fig. 40A). Fig. 85 sporangium B1+2, C1+2 and D1+2 (seen by combining sheets 1, 2 and 3) are three sporangia in three different stages of development. The sporangium B1+2 is the earliest developmental stage in which B, B1, B1+2 of the three sheets are at different level of sectioning of a single sporangium. At levels B and B1+2, the first cleavage furrow takes the appearance of being fully completed at the cut face when the sheets are examined separately. At level B1 of the same sporangium on sheet 2, the first cleavage furrow appears incomplete (see serial sections Figs. 72A to Q). The same applies for sporangia C1+2 and D1+2. Sporangium D1+2 at section level D on sheet 1 only and section level D1+2 on sheet 3 alone gives the appearance of a completely mature sporangium but at section level D1 on sheet 2 appears as an immature sporangium in which the three cleavage furrows are not completed (see serial sections Figs. 34A to Q). Guiry's (1974) mistake was probably due to misinterpretation of the random sections of a single sporangium in which it appears mature in some sections and immature in others. On the other hand, the failure of the light microscope in giving an accurate resolution, as discussed before, weakens this probability. In addition to the presence of a small region at the end of each cleavage furrow shown by electron microscopy (random sections) to consist of mucilage layer 1 only (Figs. 27, 28, 32) gives the impression of verifying both Guiry's and Pueschel's conclusions in which the first

cleavage furrow is not arrested (as Pueschel describes it) but slowed down for some reasons in some sporangia while the second and the third cleavage furrows are fast growing.

The first cleavage furrow is always along the short axis of the sporangium (Figs. 19,30,35A to Q, 71A to R, 72A to Q) and the second and third cleavage furrows are along the long axis in the majority of the sporangia. The orientation of the second and third cleavage furrows varies through 180°. They may be in a single plane, giving the sporangium the appearance of a typical cruciate form (Figs. 34A to Q) or they may be in a single plane, but are not in the plane of the section, thus the tetrasporangium appears as bisporangium (Figs. 37C,D,E and Figs. 36C,D) or one of the longitudinal cleavage furrow may be at an angle of 90° to the other which gives the appearance of a sporangium with a single large spore and two smaller spores. This is referred to as decussate cruciate (Fig. 40C). Further, one of the longitudinal cleavage furrows does not meet the first cleavage furrow. This gives the tetrasporangium the intermediate appearance between cruciate and zonate (Figs. 40A,D) and in some cases the other one may not be on the plane of the view (Fig. 40B). These variations in the orientation of the cleavage furrows in cruciately dividing tetrasporangium were discussed in detail by Guiry (1978).

Fig. 41 represents part of a tetrasporangium mother cell at early stage 2 in which the rough endoplasmic reticulum cisternae are extensive among the cytoplasmic organelles, around the vacuoles (Fig. 42), in a close juxtaposition with the plasmalemma (Fig. 44) and around the nucleus (Figs. 73,74,76,78,81). The lumens of the endoplasmic reticulum cisternae expand at the rims of the cisternae (Fig. 43, arrow) and bud off as vesicles (Figs. 45,46,15). These vesicles show amorphous or finely granular electron dense cores of about 0.2 - 0.4 μm in diameter surrounded by an electron transparent zone of about 30-62 \AA wide and a membrane of irregular outline (Figs. 45 to 50). Ribosomes may be attached to their outer surface (Figs.

47,48). These vesicles are termed endoplasmic reticulum vesicles. They were seen to fuse together through short tubules of about 40 A° wide with different lengths (Figs. 47,48,49). Fusion of up to three vesicles was observed (Figs. 48, 49, arrows). The endoplasmic reticulum vesicles may exchange their material through the fusion tubules (Fig. 49, arrow with tail) but they do not show a remarkable increase in size. These vesicles degenerate eventually (Figs. 48,49,50, double arrows with tail pointing at the degenerated vesicles). Their fusion and degeneration were maximum at late stage 3. They disappear by sporangium maturity and they were not present in newly released naked tetraspores (Figs. 54 and Fig. 1 section 6).

At mid stage three (Fig. 30) of the tetrasporangium mother cell, some Golgi bodies are actively releasing dark electron dense vesicles in large numbers (Fig. 51,52). Five vesicles were observed attached to a single Golgi body at one time (Fig. 51). These vesicles are interpreted as the adhesive vesicles. Fig. 52 shows a Golgi body and the associated adhesive vesicles in which the vesicles before their detachment from the Golgi bodies, and some detached vesicles near to Golgi bodies (Fig. 52, arrows), are less electron dense than those vesicles which lie free in the cytoplasm (Fig. 53). This phenomenon suggests that the adhesive vesicles either complete their reaction after release from the Golgi body or more material is added shortly after. The former is more likely to be the case than the latter.

These vesicles are present in newly released tetraspores (Fig. 54 and Fig. 1 section 6). They were observed to fuse with the plasmalemma (Fig. 55) and discharge their contents by reverse pinocytosis to form the adhesive layer (Fig. 60) which helps to stick the tetraspores firmly to the substratum.

At mid stage 3 of the tetrasporangium mother cell, another group of Golgi bodies are actively secreting another type of vesicle (Figs. 56,57) in which the whole cisternae are involved in producing a single vesicle

with a dark electron dense core (Figs. 56 to 59). These vesicles are termed small fibrous vesicles. They fuse together and enlarge in size (Fig. 53, arrow indicates the site of fusion). As more small fibrous vesicles fuse, the resulting large vesicles are termed large fibrous vesicles. The large fibrous vesicles lose their dark electron density as they become larger (Figs. 58, 59, arrow indicates the site of fusion). The small fibrous vesicles are short-lived. Their production and their transformation into large fibrous vesicles is completed before tetraspore maturity; hence the reason for the small fibrous vesicles not being present in the majority of the tetrasporangia shortly before and at maturity. However, the large fibrous vesicles are present in the spore after release (Fig. 60). They were seen to fuse with the plasmalemma and discharge their contents by reverse pinocytosis to form the spore cell wall (Fig. 61, arrow points at the site of fusion). The vesicles which are not located directly underneath the plasmalemma may discharge their contents into the vesicles near the plasmalemma which may in turn fuse with the plasmalemma and discharge their contents (Fig. 62).

During these three stages of tetrasporogenesis the number of mitochondria increase tremendously. Such increase was noted during the tetrasporogenesis in Levringiella gardneri by Kugrens and West (1972A). They may branch (Fig. 13) and enlarge in size, in which some may gain a slightly larger size than small chloroplasts (Fig. 33). Enlargement of mitochondria was also reported during mitosporogenesis of Ectocarpus parvus (Lofthouse and Capon, 1975).

The pit connection which is formed between the stalk cell and the tetrasporangium mother cell at stage 1 (Fig. 3) remains intact until stage 3 and the two mucilage layers appear not to be deposited upon it (Fig. 23) which may indicate that the plasmalemma is continuous between the two cells. It is not known, however, when the pit connection is ruptured or degenerates.

The cytoplasmic organelle activities during the tetrasporogenesis of

Palmaria palmata are summarized in Fig. 86 in which stage 1 is characterized by synthesis and incorporation of cell wall material into the differentiating vegetative cell wall and a possible reorganization of the cell wall fibrillar material as the cell enlarges and the chloroplasts start dividing. There is initiation of floridean starch deposition, and secretion of mucilage vesicles 1. At stage 2 deposition of mucilage layer 1 at the region between the cell wall and the plasmalemma is completed, secretion of the endoplasmic reticulum vesicles and mucilage vesicles 2 begins, and initiation of the first cleavage furrow takes place. By late stage 3, deposition of mucilage layer 1 and mucilage layer 2 around each spore is completed, secretion of adhesive vesicles is taking place, secretion of small fibrous vesicles and their transformation into large fibrous vesicles has taken place, degeneration of endoplasmic reticulum vesicles is at a maximum and the tetrasporangium mother cell has divided into four spores. Each spore, after release, possesses the adhesive vesicles and the large fibrous vesicles. Products of the adhesive vesicles function for spore adhesion and the large fibrous vesicles form the spore cell wall.

The tetraspores were released by rupturing the tetrasporangial cell wall at the upper part and the "cuticle". They escape through an opening of about 4 μm dimension (Fig. 64). The newly released tetraspores are naked. They lose their angular shape and become spherical. They are surrounded by an irregular thin layer (Figs. 54 and Fig. 1 section 6) possibly representing remnants of the mucilage sheath (Boney, 1975a, 1981). The newly released tetraspore contains adhesive vesicles, large fibrous vesicles, a few chloroplasts, floridean starch grains and a centrally located nucleus (Fig. 1 section 6). After tetraspore release, a distinct cavity remains within the vegetative blade (Fig. 63). This cavity is lined by the tetrasporangium cell wall in which the major part of the inner layer has apparently dissolved (Figs. 63,66,67). The cavity contains some strands of mucilaginous material which reacts positively with PAS stain

(Fig. 39C). The stalk cell may enlarge as a result of a central vacuolation (Fig. 65) and function as a second tetrasporangium mother cell, or it may divide before enlarging into another stalk cell and a second tetrasporangium mother cell (Fig. 66). The cell wall of this second tetrasporangium mother cell differentiates again into two layers, an outer electron dense layer and an inner less electron dense layer (Fig. 64,65,66,67) and more cell wall material will be synthesized and incorporated. As the second tetrasporangium mother cell enlarges, it occupies the whole cavity (Fig. 64,67) and the second generation of mature tetraspores use the former aperture, which remains open, to emerge (Fig. 64). It is not known how often this process can be repeated but up to three tetrasporangium cell wall remnants were observed (Fig. 67). The tetrasporangial cavity is liable for invasion by marine organisms (Fig. 68) and phytoplankton (Fig. 67 arrows).

5.3. The behaviour of the nucleus and nucleolus during the tetrasporogenesis.

Light microscopic serial sections of tetrasporangia were followed in order to correlate the behaviour of the nucleus and the time of karyokinesis with the three developmental stages already described. It was found that the tetrasporangium mother cell at stage 1 possesses a single nucleus (serial section, Fig. 69A to K). Fig. 70A to P are serial sections of a tetrasporangium mother cell at early stage 2 in which the nucleus appears not to have divided. At late stage 2 - early stage 3, when the first cleavage furrow is in progress (Fig. 71I, arrows indicate the far end of the cleavage furrow), the nucleus has completed its first division and now the tetrasporangium mother cell possesses two nuclei occupying the central part of each half at opposite poles (Fig. 71A to R). At midstage 3, the second nuclear division takes place and by this time the tetrasporangium mother cell has four nuclei. These nuclei occupy the peripheral positions of the tetrasporangium mother cell (Fig. 72A to Q) in which the second and the

third cleavages isolate them from each other and each one represents the nucleus of the mature tetraspore (Fig. 34A to Q). At stage 1 of the tetrasporogenesis, the nucleus enlarges in size to a maximum of about 6.6 μm in diameter (compare with a vegetative cell nucleus) with a nucleolus approximately 2.6 μm in diameter, and may frequently show an irregular outline (Figs. 78,79,81). The nucleolus is more or less centrally located (Fig. 73) and composed of granular and non-granular zones (Fig. 83). The nucleoplasm is amorphous or finely granular and is surrounded by a nuclear envelope composed of an inner and outer membrane showing some increase of nuclear pore density (Fig. 73) compared to the vegetative nucleus. The nucleus is surrounded by a sheath of endoplasmic reticulum. Microbodies between this sheath of endoplasmic reticulum and the nuclear envelope were observed on rare occasions (Fig. 73 arrows). They are spherical or elongated, of about 0.42 - 0.6 μm in diameter. Their granular contents are membrane bound. These nucleus associated microbodies did not react with DAB (Pueschel, 1980a) and were presumed to contain catalase inactivated by fixation and were reported to have originated from endoplasmic reticulum (Pueschel, 1980a). As the tetrasporogenesis progress the nuclear pores increase in density and size (Fig. 81 arrow indicates the largest size of a nuclear pore). It is not clear whether increasing numbers of nuclear pores are distributed in random clusters at first (Fig. 73) and eventually cover the whole surface area of the nuclear envelope in a three-dimensional nucleus or the concentration of nuclear pores begins in a localized area and then spreads around the nucleus in different directions. The latter is more likely to be the case since Fig. 81 shows a nucleus in a single random section with large nuclear pores spread across the whole surface of the nuclear envelope, whilst Figs.78,79 show two nuclei with large nuclear pores more or less localized to half (Fig. 78) or two-thirds (Fig. 79) of the sectioned outline of the nuclear envelope. During the period in which the nuclear pore density is increasing the nucleolus enlarges and undergoes

changes in substructure in which the proportion of the granular zone increases (Figs. 76,77,81) and nucleolar vacuoles appear (Figs. 3,5,74,76) as a large single vacuole (Fig. 76) or several small vacuoles of variable size and number (Figs. 3,5). However, the ability of small vacuoles to coalesce to form one large vacuole within the same nucleolus was reported elsewhere (Burns and Soloff, 1972). The nucleolus migrates towards the peripheral nucleoplasm, pressing against the nuclear envelope, and is flattened in a cup-shaped form (Figs. 78,79,80), which is also interpreted as showing nucleolar products being discharged directly to the perinuclear cytoplasm. It is not clear whether that part of the nuclear envelope against which the nucleolus presses breaks down and is removed or remains intact and the nucleolar products merely flux through its enlarged nuclear pores. The latter situation is more likely to be the case if the arrowed parts of Fig. 80 represent the continuous part of the nuclear envelope and do not represent parts of the perinuclear endoplasmic reticulum cisternae. This interpretation is supported by Fig. 77 which shows part of a highly granulated nucleolus adjacent to the nuclear envelope with patches of the nucleolus (presumably nuclear products) apparently detached and probably making their way out to the cytoplasm through the enlarged nuclear pores (Fig. 77 arrows) shortly before the nucleolus reached the nuclear envelope. The other interpretation which may exist is that the nucleolar products flux through the enlarged nuclear pores in a quantity filling the whole area of a nuclear pore with dark electron dense materials which make such a pore and envelope invisible in a cross section. There is a notable similarity in size to the naked eye between the nucleolar granules and the cytoplasmic ribosomes. The similarity in size between the nucleolar granules and the cytoplasmic ribosomes was observed by Gunning and Steer (1975). The movement of the nucleolus is always towards the porous area of the nuclear envelope indicated by the presence of the nuclear pores at both sides of the nucleolus (Figs. 78,79,80). This movement takes place

in any direction within the nucleus of the tetrasporangium mother cell taking the stalk cell as a longitudinal axis. A centrally located nucleolus is shown in Fig. 84A and the migrations of the nucleoli within the nuclei towards the other directions are shown in Figs. 84 B to F. Figs. 1, 3 and 5 represent tetrasporangium mother cells at three different developmental phases of stage 1 in which the tetrasporangium mother cell in Fig. 1 is at the earliest phase of differentiation and the nucleolus lies in peripheral position in the nucleus, while in Fig. 5 the nucleolus is in a central position in a tetrasporangium mother cell at early stage 1 similar to those of Figs. 78 to 81, which suggests that the nucleolus migrates back to the central position after releasing its products and possibly repeats this process. This conclusion is supported by the presence of the nucleoli in nuclei of all tetrasporangia examined either by transmission electron microscopy or by light microscopy, which in turn indicates that the nucleolus here is in the category of persistent nucleolus type according to the classification of Pickett-Heaps (1970). During these nuclear activities a dark electron dense element appears in the nucleoplasm on rare occasions. These elements are more or less straight (Figs. 19, arrow with tail, 74,75) or curved (Figs. 81, arrow with tail, 82). They were sometimes observed to be attached to the inner membrane of the nuclear envelope (Figs. 74,75). Their function is not known, but their presence in a highly activated nucleus suggests that a functional significance cannot be eliminated. They probably correspond to the peculiar small rod-shaped bodies observed by Yabu (1976) and they resemble what have been considered the synaptonemal complexes by Pueschel (1979). He described them as being cryptic in structure and composed of a single lateral element in longitudinal section whilst in transverse section the pairing of some lateral elements is apparent but the central element cannot be distinguished. This description is quite different from the synaptonemal complexes described in other algae (Kugrens and West, 1972b;

Toth and Markey, 1973). The pairing of some lateral elements that appear in Pueschel's micrograph (Fig. 13 in Pueschel 1979) probably represent two individual juxtaposed dark electron dense elements in a transverse section. However, a nucleus possessing more than a single dark electron dense element was not observed in the present work (serial sections were not followed). This opposes the possibility that they are synaptonemal complexes since Van der Meer ^{and Chen} (1979) and Yabu (1976) reported the diploid chromosomes number as over forty, but not Magne (1964), and therefore at least twenty synaptonemal complexes would be expected to appear in the nucleus if meiosis really takes place in Palmaria palmata during tetrasporogenesis, and more than one would sometimes be expected to appear in a single random section.

The resulting four nuclei of the nuclear division are small in size, about 5 μm in diameter, with a centrally located nucleolus of about 1.9 μm . The perinuclear endoplasmic reticulum apparently moves away and the nuclear pore density returns to normal (Fig. 83). The ultrastructure of the two nuclei of the first nuclear divisions has not been followed in the present work.

5.4. Discussion.

At early stage 1 the differentiation of a vegetative cell into a tetrasporangium mother cell in Palmaria palmata involves an increase in size, mainly caused by a high level ^{of} vacuolation and partly due to an increase of the cytoplasmic organelles, e.g. chloroplasts, mitochondria, endoplasmic reticulum, Golgi bodies etc. (Fig. 1). The high vacuolation is attributed to an intake of water into vacuoles (Preston, 1974). As the tetrasporangium mother cells enlarge, the cell wall expands to exactly the extent necessary in such way as to continue as a smooth unbroken envelope. This process possibly involves synthesis and differentiation of cell wall material into an outer dark electron dense layer and an inner

less electron dense layer, though there is no indication of involvement of any of the cytoplasmic organelles, such as Golgi derived vesicles, in cell wall deposition during the tetrasporogenesis in Palmaria palmata.

Myers and Preston (1959) reported that the thallus of Palmaria palmata is very elastic whilst wet, so that a fresh or rewet piece cut from a frond could be stretched up to twice its original length, and when dried remain stretched until rewet when it rapidly reassumed its original shape. This phenomenon is attributed to the low cellulose content of the cell wall (Cronshaw et al., 1958). The amorphous cell wall components, of which xylan is the most important, are in a semi-fluid state, so that the microfibrils are free to move under stress. The microfibrils which are completely unorientated in the natural state, become aligned in the direction of the stretching force until they lie almost parallel to it in stretched material (Myers and Preston, 1959). It is not fully clear from the transmission electron micrography shown in the present work (Figs. 2,14,17, 20) whether such alteration of cell wall microfibrillar organization also exists when the cell wall expands, at early stage 1 of the tetrasporogenesis, as a response to the turgor pressure resulting in the cell wall microfibrillar material changing their random distribution to a longitudinal one parallel to the longitudinal axis of the cell. However Fig. 44, which represents the maximum expansion of the tetrasporangium mother cell wall, shows some apparent longitudinal arrangement of the cell wall microfibrillar compared to their apparent random distribution in vegetative cells. These observations indicate a possible existence of multinet growth (see Preston, 1974 for review). Multinet growth has been reported in some algae such as Nitella opaca (Probine and Preston, 1961), Nitella axillaris (Green, 1960), two species of Cladophora and two species of Chaetomorpha (Frie and Preston, 1961). Studies by X-ray diffraction and shadowing techniques need to be conducted in order to have a definite establishment of the existence of this phenomenon during tetraspore development in Palmaria palmata.

Pueschel (1979) reported a different mechanism of cell wall formation during tetrasporogenesis in Palmaria palmata in which a completely new cell wall is deposited upon the vegetative cell wall of a differentiating tetrasporangium. If this situation does exist, several questions will be raised; for instance, what is the fate of the original vegetative cell wall?; would it be stretched as the tetrasporangium mother cell expands?; would the microfibrils break up or reorientate as a response to the stretching force?; would a new cell wall material be incorporated into it since the tetrasporangium mother cell expands to roughly 5-8 times the original vegetative cell size? However, in no case in the present study was a tetrasporangium observed surrounded by two cell walls (the vegetative cell wall + the tetrasporangium cell wall). According to Pueschel (1979) the dark electron dense layer of the tetrasporangium cell wall is first laid down, followed by deposition of the less electron dense layer. This observation is weakened by the fact that after the spores are discharged, the stalk cells expand to form the second generation of tetraspores. The cell wall of this stalk cell consists of two layers (the dark electron dense and less electron dense layers) before and after the cell enlargement (Figs. 64,65,66) which indicates that the cell wall material has differentiated into the two layers as new material is incorporated during the cell expansion. However, careful inspection of Pueschel's micrographs (Figs. 9 and 10 Pueschel 1979) does not indicate clearly that the electron dense layer is deposited first. In Fig. 10 at the upper left corner and lower right corner, shows vegetative cells with an electron dense layer on the side near to the plasmalemma of the wall as well as the tetrasporangium mother cell which could be either artifacts induced by fixation or by staining these particular cells. Therefore, it is more likely for the tetrasporangium cell wall of Palmaria palmata to have transformed from the vegetative cell wall similar to that of Levriengiella (Kugrens and West, 1972A) rather than deposition of completely new cell wall.

Between late stage 1 and late stage 3, Golgi bodies were observed to be involved in producing four types of vesicle. These vesicles are mucilage vesicles 1, mucilage vesicles 2, adhesive vesicles and small fibrous vesicles. Their terminology was based on their origin, fate and their similarity in morphology to those of other red algae cited in the literature. The four types of Golgi derived vesicles may be observed in a single developing tetrasporangium concomitant with tremendous increase of the total number of Golgi bodies, which suggests that each type of vesicle is secreted by independent groups of Golgi bodies rather than transformation of Golgi bodies from one type to another as is the case in Polysiphonia novae-angliae (Wetherbee and West, 1977). Here, Golgi bodies at early stage of carposporogenesis are involved in cell wall formation as carpospore formation progresses. When deposition of cell wall material ceases, they secrete what is termed as striated vesicles type b and later on they produce striated vesicles type C.

Mucilage vesicles 1 and mucilage vesicles 2 are two independent groups of Golgi-derived vesicles which differ from each other in the manner they were released from Golgi bodies and in their size. They are involved in depositing mucilage layer 1 and mucilage layer 2 around the developing tetraspores (Fig. 30). These two layers are of different chemical composition in which mucilage layer 2 appears to have a fine fibrillar material compared to mucilage layer 1 and they react differently to the histochemical stains. Alcian stains (Parker and Diboie, 1966) react when the resin (epoxy embedded tissue) ^{is} removed from the sections (Hoff and Rayburn, 1974). By treating them with saturated absolute ethanol with NaOH for 15 minutes, mucilage layer 2 was destroyed and removed from the sections (Fig. 38A,B,C) even when the sections were treated for 4 minutes, but mucilage layer 1 was stained greenish, which indicates that it has acid mucopolysaccharide nature (Fig. 38A). The cytoplasm was harmed (Fig. 38C) and mucilage layer 1 in some sections has moved away slightly from its normal position (Fig. 38B) when the resin was removed,

which indicates that this layer has an elastic nature. PAS stained mucilage layer 1 pinkish, but not mucilage layer 2 (Fig. 39A). The pale pinkish colour that appears in the micrograph probably represents a reflection of the pink colour of mucilage layer 1, judging from the disappearance of this pink image and the material becoming colourless if the same specimen was out of focus. However, Scott and Dixon (1973a) reported a negative reaction of the mucilage layer around the differentiating tetraspores of Ptilota hypnoides when the sections were treated with PAS. A completely different pathway for mucilage deposition during the tetrasporogenesis of Palmaria palmata was reported by Pueschel (1979) in which the Golgi bodies at the early stage of development are involved in producing vesicles with microfibrillar contents. Soon after this another type of vesicle appears in the cytoplasm with dense contents and these are believed to have originated by fusion of several of the former Golgi-derived vesicles. Their derivation was based only on the timing of their appearance in the cytoplasm and they were termed as mucilage vesicles (the term endoplasmic reticulum vesicles is applied to them in the present work). These vesicles were reported by Pueschel (1979) either to fuse directly with the plasmalemma or to fuse with what are termed mucilage sacs, which in turn fuse with the plasmalemma and form a single mucilage layer around the developing tetraspores. In no case, however, were these observations confirmed by micrographs except a single micrograph representing a fusion between what is believed to be a mucilage vesicle and a mucilage sac (Pueschel, 1979; Fig. 23). This micrograph does not show a clear fusion between the two organelles but the misinterpretation could be partly due to the two organelles being pressed against each other, and partly due to poor preservation of membranes - a problem often encountered. The mucilage sacs were shown by Pueschel (1979) to be formed by dilation of endoplasmic reticulum cisternae and lateral fusion of cisternae to give them the appearance of several parts of the endoplasmic reticulum cisternae being

involved in forming a single mucilage sac (Pueschel, 1979; Figs. 24,25). The interpretation of these two micrographs could be mistaken for actual vacuoles of non-mucilaginous material (the vacuole that caused cell enlargement). This criticism is supported by Fig. 41 in the present work, which represents an early stage 2 of a tetrasporangium mother cell in which the vacuolation is being gradually replaced by increasing numbers of cytoplasmic organelles and of the volume of the cytoplasm which presses against the tonoplast of the vacuole. In some cases the two sides of a tonoplast of a single vacuole lie close and parallel to each other for a considerable distance, giving the vacuole the appearance of a vesicle or a sac secreted by endoplasmic reticulum (Fig. 41 double arrows). The vesicles which are termed mucilage vesicles by Pueschel (1979) and supposed to have originated by fusion of several Golgi-derived vesicles (but with no evidence given) were observed in the present work to be budding off the endoplasmic reticulum (Figs. 15, arrow with tail, 45, arrow, 46). They fuse together (Figs. 47,48,49,50 arrows) and assume a degenerative appearance as the spore development progresses (Figs. 48,49,50 double arrows with tails). Therefore, the term endoplasmic reticulum vesicles was given to them. These vesicles probably contain proteinaceous material and function as storage products utilized during the developmental stages. However, morphologically similar vesicles, believed to be originated from the endoplasmic reticulum, have been repeatedly reported from different algae, viz. Bryopsis hypnoides (Burr and West, 1970), Griffithsia flosculosa (Peyriere, 1970), Ceramium rubrum (Chamberlain and Evans, 1973) and Callithamnion roseum (Konrad Hawkins, 1974b). These vesicles were also observed to disappear as the sporogenesis progresses (Chamberlain and Evans, 1973; Konrad Hawkins, 1974b). Konrad Hawkins (1974b) also reported a fusion between the vesicles and Golgi-derived vesicles and a possibly relationship with cell wall deposition was suggested.

The endoplasmic reticulum vesicles are probably equivalent, but not

morphologically identical with the striated vesicles of Levringiella gardneri (Kugrens and West, 1972a, 1973); Polysiphonia sertularioides (Tripodi, 1971a) and Polysiphonia novae-anglia (Wetherbee and Wynne, 1973; Wetherbee and West, 1976, 1977). These striated vesicles were reported to have originated from endoplasmic reticulum and disappear or degenerate later (Kugrens and West, 1972a, 1973; Wetherbee and West, 1976, 1977). They are of a proteinaceous nature and are probably deposited as reserve materials utilized during growth (Kugrens and West, 1973; Tripodi, 1971a; Wetherbee and Wynne, 1973; Wetherbee and West, 1976, 1977). The endoplasmic reticulum vesicles of Palmaria palmata show some resemblance to the striped vesicles reported in Erythrocytis saccata (Kugrens and West, 1974) in being secreted by endoplasmic reticulum and disappearing during the late stages of carpospore maturation. These were again thought to be reserve products, possibly lipids utilized during the differentiation.

The manner in which the four spores in a tetrasporangium were arranged was stated by Guiry (1978) to have some taxonomic significance. Guiry described three types of arrangements - cruciate, tetrahedral and zonate. The timing of the cleavages which precede the formation of three types follows two pathways:

- 1) Successive cleavages in which the median cleavage takes place before the longitudinal cleavages have been initiated.
- 2) Simultaneous cleavages in which the three cleavages take place at the same time.

According to Pueschel (1979), Palmaria palmata's mode of cleavage in tetrasporogenesis is a new type, intermediate between successive and simultaneous, in which successive initiations and simultaneous completion of the median and the longitudinal cleavages take place. This new type disagrees with results of successive types of cleavage reported for Palmaria palmata by Guiry (1974, 1975). The two conclusions were drawn from evidence from the limited resolution of the light microscope which does not resolve the

aperture clearly in the median septum as Pueschel (1979) mentioned. However, the light microscope serial sections of several tetrasporangia reported in the present work do not disagree with either Pueschel or Guiry, and the electron microscopic random sections support both of them. Figs. 30 & 32 support Pueschel's conclusion but this is weakened by the presence of a short distance at the far end of the median cleavage being composed of mucilage layer 1 (Figs. 28,32). Fig. 31 supports Guiry's conclusion, but is weakened by the possibility that the other two longitudinal cleavages and the aperture in the median cleavage may not be seen in this particular section. Therefore, it will be appropriate to leave this point open for further investigation, using serial sections at ultrastructural level for sufficient number of tetrasporangia to get a definite answer. Palmaria palmata tetrasporogenesis is unique among the red algae for which tetraspores development have been studied so far, in having two mucilage layers around each spore and a lenticular indentation at the apex of the tetrasporangium. It is generally assumed that liberation of spores is accomplished by rupturing the cell wall, which is thought to be under the control of the pressure induced by enlargement of the sporangium (Kugrens and West, 1972a; Scott and Dixon, 1973a), possibly enzymatic digestion (McBride and Cole, 1971; Hawkes, 1980) or internal osmotic pressure existing in the sporangia (Scott and Dixon, 1973a; Chamberlain and Evans, 1973; McBride and Cole, 1971). The ability of the mucilage around the mature spore to absorb water and expand in a confined space would create the pressure responsible for rupturing the cell wall and acting as a propellant for the spores (Chamberlain and Evans, 1973; Boney, 1981). The presence of a lenticular indentation at the apex of the sporangium in Palmaria palmata mainly consists of mucilage layer 1 generating the pressure necessary to rupture the overlying wall and expanding of mucilage layer 1 around the tetraspores, then propelling them from the sporangia, leaving loose strands of mucilage which give a positive reaction with PAS in the chambers of discharged tetrasporangia (Fig. 39c). These positive PAS

strands indicate that they are remnants of mucilage layer 1 and that mucilage layer 1 is not firmly attached to mucilage layer 2. The escaping tetraspores are surrounded by a thin irregular layer (Fig. 54 and 1 section 6). This layer probably represents the remnants of mucilage layer 2 in which a major part is probably lost during the preparation of spores for electron microscopy. Such problems were encountered by Chamberlain and Evans (1973). However, this layer was reported in several algal spores and termed mucilage sheath (Boney, 1975a, 1981; Ngan and Price, 1979). The mucilage sheath volume decreases with time (Ngan and Price, 1979), which probably gives another explanation for the presence of only a thin mucilage layer around spores of Palmaria palmata fixed four hours after release. The mucilage sheath has functional significance (Boney, 1981) viz. slowing down spore movements on sloping surfaces, influencing spore buoyancy and so assisting in dispersal, possibly assisting in the early stages of spore adhesion, acting as protection against desiccation, maintaining ionic equilibrium within the spore, forming an antibiotic layer or a physical buffer in a robust habitat and related to metabolic activity (see Boney, 1981 for review).

Pueschel (1979) reported the presence of only a single mucilage layer around the developing tetraspores of Palmaria palmata. Although at later stages of development this layer appears to consist of two parts, his explanation was that the second layer is an artifact, due to shrinkage of the mucilage layer induced by fixation or dehydration, judging from its disability to react with histochemical stains and the absence of substructure in the electron micrographs (Pueschel, 1979; Figs. 28,29). Indeed, Fig. 29 of Pueschel's micrographs does not show microfibrils in this second layer even though the whole mucilage layer cannot be seen in some regions, but careful inspection of Fig. 28 shows the presence of some microfibrillar substructure on some region of the second layer. This second layer appears in Fig. 26 of Pueschel's micrograph as well, although it seems to be devoid of substructure, it is quite similar to Fig. 22 which has been represented as

a mucilage layer at its early stage of deposition. This second layer could be affected and poorly preserved by the use of a glutaraldehyde-acrolein mixture used by Pueschel or could be merely a staining or printing problem. However, the failure of glutaraldehyde-acrolein mixture in preserving some structures (microtubules) has been reported elsewhere (Steer and Newcomb, 1969). The two mucilage layers have been represented clearly in the present work (Figs. 24,25,30,31, etc.).

The third type of Golgi-derived vesicles (the adhesive vesicles) are present in the newly released tetraspores. Their fusion with the plasmalemma, the formation of the adhesive layer around the tetraspores and their morphological similarity to the vesicles containing adhesive substances in other algae (Evans and Christie, 1970; Callow and Evans, 1974; Braten, 1975) make it safe to describe such vesicles as adhesive vesicles containing the adhesive polysaccharide material (Pueschel, 1979) that is responsible for firmly sticking the tetraspores to the substratum.

The formation of large fibrous vesicles by fusion of several small fibrous vesicles produced during the tetrasporogenesis has been reported several times, (Brown, 1969; Kugrens and West, 1972a, 1974; Konrad Hawkins, 1974b; Chamberlain and Evans, 1973; Scott and Dixon, 1973a; Alley and Scott, 1977; Hawkes, 1978, 1980; Teveter-Gallagher et al., 1980). These were present in the released tetraspore, and were observed to fuse with the plasmalemma to form the tetraspore's cell wall. A similar process was suggested for Batrachospermum carpospores (Brown, 1969). The need for such vesicles to be formed during tetraspore formation rather than after spore discharge is probably due to the rapid demand for a suitable source of protection against the robust habitat conditions. The large fibrous vesicles were observed by Pueschel (1979) in the tetrasporangia and released tetraspores and were termed fibrous bodies, but their origin and function were not established.

An empty cavity remains after spore discharge. This cavity is lined

by the outer layer and small dissolved parts of the inner layer of the tetrasporangium cell wall. This outer layer proved to consist of protein-rich substances and may provide a jacket within which hydrostatic pressure could be developed as part of the process of tetraspore expulsion in addition to a function equivalent to the cuticle after spores are discharged (Pueschel, 1979). A second generation of tetraspores is repeated by the stalk cell within the empty cavity (Guiry, 1974; Pueschel, 1979).

The observations reported in the present work on the nuclear behaviour at ultrastructural level did not contribute towards elucidating the type of nuclear division which takes place in the tetrasporangium. Earlier reports using other methods have described meiosis (Van der Meer, ^{and Todd} 1980) or apomeiosis (Magne, 1964) (see section 3). Further experiments were carried out in which three-hourly interval fixed specimens of twenty four hours were processed for electron microscope but could not be examined mainly due to shortage of time and partly due to scarcity of reports on this aspect among the numerous reports dealing with the reproductive organs' development, which may indicate that a peculiar mode of nucleus behaviour might exist among red algae. However, the nucleus was observed to have completed its first division by late stage 2 - early stage 3 of the tetrasporogenesis of Palmaria palmata in which the median cleavage furrow has already been initiated and the second nuclear division was completed by midstage 3 in which the other two cleavage furrows have initiated, which indicates that there is a pause between telophase 1 and prophase II if meiosis is considered to take place, similar to those reported in Levringiella gardneri (Kugrens and West, 1972a) and dissimilar to Polysiphonia denudata in which uninuclear meiosis was reported (Scott and Thomas, 1975). This observation does not correspond to Pueschel's observation, who stated that both meiotic divisions occur before cytokinesis begins and both meiotic nuclei were distinguished by their peripheral position in the uncleaved cell.

Concentration of endoplasmic reticulum around the active nucleus similar to those of Palmaria palmata has been described in other red algae

(Peel et al., 1973; Scott and Dixon, 1973a; McDonald, 1972; McBride and Cole, 1971). The perinuclear endoplasmic reticulum was reported to have functional significance. This includes isolating the dividing nucleus, the centrioles and a thin layer of the cytoplasm around the nucleus from the rest of the cell in Hydrodictyon reticulatum (Marchant and Pickett-Heaps, 1970). It might serve to contain the area at the poles, which is then possibly a reservoir of microtubule protein in a restricted space during their breakdown and assembly in Membranoptera platyphylla (McDonald, 1972). It probably receives messenger RNA for sufficient translation into manufactured products in Smithora naiadum (McBride and Cole, 1971). The production of swollen ER cisternae containing an electron dense substance and vesicular transport from these cisternae to the forming face of Golgi bodies has been reported (Peel et al., 1973). Heath and Greenwood (1971) have suggested that stacks of endoplasmic reticulum at the base of the nuclei in zoospores of the fungus Saprolegnia ferax may be the result of cytoplasmic dehydration caused by vacuole development. In Palmaria palmata, Pueschel (1979) reported that abundant transcriptional activity and rapid egress of RNA from the nucleus might be expedited by an increased number of nuclear pores and by concomitant relaxation of perinuclear sheath of endoplasmic reticulum in addition to that which the perinuclear ER involves in formation of nucleus associated microbodies, preventing them from migrating elsewhere (Pueschel, 1980a).

The increase in nuclear pores density as an indication of nucleocytoplasmic interaction has been suggested elsewhere (Peel et al., 1973), and a positive correlation between the number of nuclear pores and the metabolic state of the cell were reported by La Cour and Wells (1972). McPham and Lane (1969) have suggested that nuclear pores are sites at which polyribosomes are assembled before leaving the nucleus. Another feature indicative of nuclear activity is the presence of vacuoles within the nucleolus (Peel et al., 1973; Gimenez-Martin^{etal}, 1977). Johnson (1969)

found that vacuolated nucleoli of tobacco callus cells incorporated tritiated-uridine more readily than did nucleoli lacking vacuoles and his conclusion was that nucleolar vacuoles are closely related to the RNA synthetic activity of the nucleolus.

Several authors have indicated that the fibrillar zones of the nucleolus are the sites of synthesis of preribosomal RNA which then matures and moves to accumulate at the granular zone (Gunning and Steer, 1975; Busch and Sementana, 1970; Daskal et al., 1974; Sementana and Busch, 1974; Gimenez-Martin et al., 1977). Gunning and Steer (1975) stated that the fibrils are difficult to resolve and that they may lie in a matrix of amorphous protein. Sementana and Busch (1974) reported that in some nucleoli the fibrillar and granular zones cannot be distinguished because these components are mixed together; such zones are referred to as fibrillo-granular zones. In Palmaria palmata the nucleoli appear to consist of hardly distinguishable granular and non-granular zones similar to those reported in Corallina officinalis (Peel et al., 1973).

The increased density of nuclear pores, the increased volume of the nucleus and the nucleolus, the presence of vacuoles within the nucleolus, the increase in the proportion of granular zones with the nucleolus, and the migration of the nucleolus to the peripheral position in Palmaria palmata, make it reasonable to hypothesize that these changes are of functional significance. The discharge of the nucleolar products directly into the perinuclear cytoplasm (Figs. 78,79,80) provides strong support for the concept that the contact area between the nucleolus and the nuclear envelope, either directly or through canals, seems to be favourable morphological basis for rapid nucleolocytoplasmic transfer of material (Bourgeois et al., 1979), who also report that the preribosomal RNA first synthesized in the dense fibrillar component and pass here and/or in the granular component, then migrate into the cytoplasm. The morphological substrate of this transfer is unknown, since the granules cannot be followed

after they have left the nucleolar body. As for autoradiographic data, it is not known whether or not the RNAs labelled by tritiated-uridine enter the nuclear pole before they leave the nucleus. If so, a large part of the autoradiographic labelling observed over the nucleoplasm would be of nucleolar origin. If not, the nucleoplasmic labelling would essentially reflect premessenger RNA synthesis and the preribosomal RNA could be transferred directly from the nucleolus into the cytoplasm (Bourgeois, 1979). The latter situation is more likely to take place during the nucleus activity of Palmaria palmata.

6. PLASMALEMMAVILLI AND ENDOPLASMIC RETICULUM

6.1. Introduction.

Tubular structures associated with the plasmalemma and protruding into the cell wall have been described in a few species of red algae and are considered to be of a possible taxonomic significance (Duckett and Peel, 1978). Cottler (1971) first described regular villus-like outgrowths in the cortical, outer medullary and inner medullary cells of Chondrus crispus and named them plasmalemmavilli. Subsequently, plasmalemmavilli have been reported in a few species of red algae, including Nemalion helminthoides (Duckett and Peel, 1978), Botryocladia pseudodichotoma (Young, 1978) and Harveyella mirabilis (Goff, 1979b). More recently, they were described in the medullary cells of Palmaria palmata by Pueschel (1980b) who also claimed that these villus-like outgrowths are fixation artifacts in red algae. Tubular structures also associated with the plasmalemma have been described in Polysiphonia novae-angliae (Wetherbee, 1978b) but they were located in the peripheral cytoplasm. They were reported by Wetherbee to have originated from endoplasmic reticulum since they abut on it. He considered them to be different structures from plasmalemmavilli.

In higher plants, tubular invaginations of the plasmalemma similar to those described by Wetherbee (1978b) have been found in leaf cells of Zea mays (Evert et al., 1977) and there is no continuity between the tubules and other cellular membranes.

In this section tubular structures associated with the plasmalemma, similar to those described by Wetherbee (1978b) and Evert et al. (1977), are reported for the three species of the red algae. Their authenticities were discussed and the term plasmalemmavilli applied to avoid any confusion in naming.

6.2. Plasmalemmavilli and endoplasmic reticulum in newly released tetraspores of Palmaria palmata.

Plasmalemmavilli of a uniform diameter (Fig. 2) appear in the peripheral cytoplasm of the naked tetraspores fixed up to four hours after release (Fig. 1). The plasmalemmavilli are numerous and consist of hollow convoluted tubules, which sometimes show continuities with the plasmalemma and an orientation at right angles to it (Figs. 3,4). Their maximum length is about 1.6 μm (Fig. 2, assuming that the outer and the inner tubules are in fact continuous) with a uniform external diameter of about 38 nm and a wall thickness (which is difficult to resolve) of about 8 nm (Fig. 4, arrow). They are closed at the lower end (Fig. 2, arrow with tail) and may apparently bifurcate (Fig. 3, arrow). A secondary bifurcation was observed in a single tubule (Fig. 3, arrow with tail). The plasmalemma in some regions evaginates to form irregular vesiculate projections about 200 nm long and 45-131 nm in diameter (Fig. 4).

The endoplasmic reticulum in the peripheral cytoplasm of the naked tetraspores is abundant (Figs. 2,5,6), running parallel to the plasmalemma and distant from it by 50-150 nm (Figs. 4,6). It underlines most of the plasmalemma of the spore (Figs. 1,5), abutting the plasmalemmavilli and in direct contact with the plasmalemma in some regions (Figs. 3,6, arrow).

The plasmalemmavilli disappear soon after the spores start depositing cell wall material at 10-16h after spore discharge. At the same time the endoplasmic reticulum cisternae become less conspicuous in the peripheral region.

6.3. Plasmalemmavilli and endoplasmic reticulum in a newly released spore of an unknown red alga epiphyte on Plumaria elegans (possibly Callithamnion).

This spore was found in a Plumaria elegans spore culture. The identification will be discussed later. The plasmalemmavilli appear again

in the peripheral cytoplasm of the naked newly released spore fixed up to 10 hours after release (Fig. 7). They are of two slightly different types, since in one (Figs. 8,9) there is a bulbous, electron dense structure (of unknown chemical nature) at the junction with the plasmalemma whereas in the other type this swollen junction at the plasmalemma is absent (Fig. 10). Otherwise, all plasmalemmavilli have a uniform external diameter of approximately 31 nm with a wall thickness of about 8 nm (Fig. 9, arrow with tail) and in a single section are up to 200 nm in length (Figs. 9,10). The plasmalemmavilli are orientated at right angles to the plasmalemma and are straight or slightly convolute (Figs. 9,11). They are closed at the inner end and lack bifurcations. The two types of plasmalemmavilli are of mixed distribution and not preferentially restricted to any one part of the spore (Fig. 8).

In this alga also the plasmalemma evaginates in some regions to form irregular projections about 140 nm long and 100 nm in diameter (Fig. 12).

The endoplasmic reticulum in the peripheral cytoplasm is abundant, running parallel to and about 60-260 nm from the plasmalemma. It almost underlines the whole surface of the plasmalemma of the spore (Fig. 7), abutting the plasmalemmavilli (Figs. 11,13) and is in direct contact with the plasmalemma in some regions (Fig. 13 arrows).

6.4. The plasmalemmavilli and endoplasmic reticulum in newly released paraspores of Plumaria elegans.

Plasmalemmavilli of a uniform diameter are met again in the peripheral cytoplasm of the naked paraspores fixed up to 10 hours after release (Fig. 15). Plasmalemmavilli in this species are less abundant than in the former two species and consist of tubules which show continuity with the plasmalemma. These tubules have a uniform external diameter of approximately 25 nm and are up to 480 nm in length (Figs. 16,20). The plasmalemmavilli are orientated at right angles to the plasmalemma, are straight

(Fig. 17), slightly convolute (Figs. 16,18) or sharply convolute (Figs. 19,20) towards the central cytoplasm. They are closed at the lower end (Figs. 17,20, arrows) and no bifurcations were observed.

The plasmalemma in this species shows a more irregular outline (Fig. 18) than in the two previous species. No evaginations in the form of tubular projections were observed.

The endoplasmic reticulum in the peripheral cytoplasm is less abundant than in the two other species, running parallel to the plasmalemma and usually separated from the latter by about 55-188 nm (Figs. 21,22, arrows).

The plasmalemmavilli again disappear soon after the spore starts depositing cell wall material at 10-16 hours after spore release and the extensive endoplasmic reticulum cisternae become less conspicuous in the peripheral region.

Several characteristics distinguish the spore of the unknown red alga (possibly Callithamnion) and those of Plumaria elegans, viz.,

1. The spore of Callithamnion (?) is elongated, approximately 34 μm long and 17 μm wide (Fig. 7), while Plumaria elegans paraspores are more or less spherical with a diameter of about 25 μm (Fig. 15).

2. The chloroplasts of the spores of Plumaria elegans show a radiating arrangement around the nucleus (Fig. 23). The chloroplasts of the Callithamnion (?) spore do not show this arrangement (Fig. 14). The chloroplasts are randomly distributed in the cytoplasm similar to those of Palmaria palmata (Fig. 1).

3. The cristae of the mitochondria of the Callithamnion (?) spore are fewer (Figs. 10,13) than those of Plumaria elegans and the peculiar mitochondria observed in the latter (Fig. 21, see section 10.5 for more details) were not observed in the former.

4. Finally, the plasmalemmavilli of Callithamnion (?) spore are of two types, while the plasmalemmavilli of Plumaria elegans paraspores are

of one type, similar to those of Palmaria palmata, and the endoplasmic reticulum in the peripheral cytoplasm of Plumaria paraspores is relatively less abundant than those of Callithamnion (?).

6.5. Discussion.

Tubular plasmalemmal structures of a uniform diameter were reported by Evert et al. (1977) in leaf cells of Zea mays. These tubules are not in association with any of the cytoplasmic membranes and were believed to have originated by invaginations of the plasmalemma. However, Wetherbee (1978b) observed similar tubular structures continuous with the plasmalemma and with a uniform diameter, during the final phase of cell wall formation of carposporogenesis in Polysiphonia nova-angliae. These tubules were thought by Wetherbee to be derived from endoplasmic reticulum since they abut to it but no evidence of transformation of endoplasmic reticulum cisternae into tubules was presented. Wetherbee's conclusion on their origin was based on the observations made by Steer and Newcomb (1969) in which cytoplasmic tubules showed continuities with the endoplasmic reticulum at their early stage of formation. Subsequently large tubules appeared among the smaller tubules and gradually replaced them. The endoplasmic reticulum was therefore thought to be involved in the production of the small tubules which in turn developed into large tubules (Steer and Newcomb, 1969). The large tubules, however, were neither connected with the endoplasmic reticulum nor with the plasmalemma. They were of uniform diameter with electron dense material occupying their lumens.

The tubules observed in the three species described in the present work are within an individual species of uniform diameters. They show continuities with the plasmalemma and abut on to the endoplasmic reticulum in spores of Palmaria palmata and the spore of Callithamnion (?). In spores of Plumaria elegans, there seems neither to be any continuity nor abutting between these tubules and endoplasmic reticulum. These

observations suggest that these tubules are more likely to have originated from invaginations of the plasmalemma rather than by involvement of endoplasmic reticulum. There is no evidence of transformation of endoplasmic reticulum into tubules in any of the three species examined, and the plasmalemma in two species only evaginates to form irregular projections. Evaginations and invaginations of the plasmalemma to form tubular projections have been reported several times in the plant kingdom.

In algae, the term *plasmalemmavilli* was applied to these evaginations (Cottler, 1971; Duckett and Peel, 1978; Young, 1978; Goff, 1979b) and a possible involvement in cell wall synthesis or nutrient uptake attributed to them. Wetherbee (1978b), however, considered the tubular plasmalemmal structures to be distinct from *plasmalemmavilli* in morphology, origin and location; their function was an extension of the plasmalemma. In higher plants, the term tubular extensions of the plasmalemma was applied to them (Evert et al., 1977) and transport and secretory functions were proposed. Verbelen (1977) applied the term tubular plasmalemmasomes to tubules (mainly present against the cell wall surrounding a degenerative protoplast) possibly involved in secondary wall synthesis in the xylem of Phaseolus. The wall papillae were reported by Frey-Wyssling (1962) to have possibly originated by invagination of the plasmalemma and their possible involvement in cell wall formation was discussed. In lichens, Brown and Wilson (1968) observed the plasmalemma of the fungal cells, unlike that of the algal cells, to be highly invaginated, forming projections termed microvilli and involved in nutrient uptake by the mycobiont of the lichen Physcia aipolia.

The above cited literature clearly indicates that the elaboration of the plasmalemma in a form of evaginations or invaginations to form tubular structures (except as described by Wetherbee, 1978b) is a widespread phenomenon in the plant kingdom. They possibly share a common physiological function as extensions for either cell wall synthesis or material exchange. Hence, in this section the term *plasmalemmavilli* has been given

to both invaginations and evaginations of the plasmalemma of newly released spores of Palmaria palmata, Callithamnion(?) and Plumaria elegans to avoid similar structures being given different names. Plasmalemmavilli are similar to the microvilli described in the animal toad oocyte (Dick et al., 1970) as being extensions of the plasmalemma. They differ, however, in being smaller in diameter and do not contain filamentous substructures as in the microvilli. The hypothesis that the greater the expanse of the membrane, the greater the diffusion flux, was confirmed statistically in these microvilli.

The extensive cisternae of endoplasmic reticulum concentrated in the region just under the plasmalemma in the internodal cells of the alga Nitella translucens (Costerton and Macrobbie, 1970) are considered barriers to the inward diffusion of ions as it is unlikely that the membranes of endoplasmic reticulum are very permeable to ions. A similar phenomenon has been observed in transfer cells of higher plants. The close juxtaposition of the plasmalemma and endoplasmic reticulum might allow for local modifications of the molecular constitution and permeability properties of the membrane (Gunning and Steer, 1975).

The presence of the plasmalemmavilli and the extensive arrangement of endoplasmic reticulum under the plasmalemma in the three red algal spores described in the present work, suggest that they might function in regulating ion diffusion in and out of the spores (e.g. nutrient uptake), whilst these functions might be regulated by the newly-formed cell wall after disappearance of the plasmalemmavilli.

In a recent publication Pueschel (1980b) observed plasmalemmavilli and what are called paramural bodies in the medullary cells of Palmaria palmata fixed in 5% glutaraldehyde for three hours. The cytoplasm was very dense and amorphous and individual ribosomes could not be distinguished whilst even the endoplasmic reticulum, mitochondria and dictyosomes were difficult to discern. However, in material fixed in a mixture of 5%

glutaraldehyde and 3% acrolein for 24-72 hours, cytoplasmic organelles were generally clearly discernible, but the plasmalemmavilli and paramural bodies were lacking and the freeze-etching technique failed to reveal them as well. Pueschel's conclusion is that plasmalemmavilli are artifacts since they were neither observed in material fixed in glutaraldehyde plus acrolein for a prolonged period nor in freeze-etched specimens. According to him, most published micrographs of plasmalemmavilli (e.g. Duckett and Peel, 1978; Goff, 1979b) show poor cytoplasmic fixation. But the authenticity of paramural bodies in Palmaria palmata was nevertheless defended by Pueschel since they were observed in Batrachospermum moniliforme (Brown and Weier, 1970) in both thin-sectioned and freeze-etched specimens. Pueschel did not give an explanation as to why neither prolonged fixation (glutaraldehyde + acrolein) nor freeze-etching revealed this organelle. Indeed, paramural bodies were interpreted as a real constituent of the living cells in several species of fungi using freeze-etching (Marchant and Moore, 1973) and by means of autoradiography in the higher plant (Cox and Juniper, 1973).

Although I did not observe the plasmalemmavilli in the medullary cells of Palmaria palmata, their presence or absence might be related to the environmental factors or may be merely an uncommon feature of these cells. Their occurrence in newly released spores appears to be a genuine cell feature.

7. FLORIDEAN STARCH

7.1. Introduction.

Floridean starch is the reserve polysaccharide of the red algae. It occurs in grains, composed of an α 1:4 glucan, identical or almost so with the branched or amylopectin fraction of higher plant starch (Dixon, 1973). A further difference between floridean starch and higher plant amylopectins is that floridean starch gelatinizes in water only after prolonged boiling. Floridean starch grains of red algae lie freely in the cytoplasm and are of variable shape and size (Dodge, 1973). They show inrolling, warping and bending after contact with iodine solution in numerous species of red algae (Boney, 1975b). They may be composed of a number of concentric layers (Sheath et al. (in press). A Golgi body origin of floridean starch granules was suggested by Tripodi (1971 a,b) in which the starch granules are surrounded by a membrane which disappears later on.

Several aspects of the floridean starch granules of the three species are discussed in this section.

7.2. Floridean starch of Plumaria elegans.

Newly released naked paraspores of Plumaria elegans possess relatively small amounts of floridean starch grains (Fig. 15 section 6). As the spore grows and differentiates into upright filaments and rhizoids (see section 3.5), floridean starch grains increase considerably in number and size. Fig. 1 shows a rhizoidal cell of a nine months old sporeling of Plumaria elegans growing in culture under the condition reported in section 2. This cell accumulated vast amounts of floridean starch grains, up to 80% of its volume. These starch grains lie freely in the cytoplasm and vary in shape and size ranging from 0.7 - 6.9 μ m in length. The most common shapes of the grains are spherical and ovoid (Figs. 1 to 4).

Fusion between some of these granules was observed (Fig. 2). In a few cases the granules appear to be composed of two to several concentric layers (Figs. 3,4). Some granules show cracks along their longitudinal axes (Fig. 9) which probably spread transversely and give the granules a degenerative appearance (Fig. 10). These could represent an enzymatic attack or could be artifacts induced during the preparation for the electron microscope. One noticeable feature was observed in the rhizoidal cells of Plumaria elegans, which is that the large granules are always aggregated at one side of the cell while the small granules occupy the other side (Figs. 1,5,6,7). This particular distribution is uniform along the cells of the filaments (Fig. 5), and it is not related to the rhizoidal branches of either secondary rhizoids or upright filaments; nor is it seen in the early formation of such branches. Fig. 6 shows the zone of the large granules near to the upright filament while at Fig. 7, the zone of the small granules is near to the upright filament. The early stages of these branches are located near the small granules-zone (Fig. 5), the large granules-zone (Fig. 7, arrow) or in the middle between the two zones (Fig. 7 arrow with tail). Such distribution of starch grains was not observed in the cells of the upright filaments (Fig. 8).

7.3. Floridean starch of Palmaria palmata.

Newly released naked tetraspores of Palmaria palmata possess relatively small amounts of floridean starch grains (Fig. 1 section 6). As the spore grows, floridean starch grains increase in number (Fig. 7 section 10) but decrease again by the time the sporeling differentiates into a holdfast and an upright frond (Figs. 4,5 section 10) in which each cell possesses small amounts of starch. Floridean starch grains of Palmaria palmata lie free in the cytoplasm and vary in shape and size ranging from 0.2 - 2.5 μm in length. The most common shapes are spherical and ovoid, although other shapes have been observed (cylindrical,

ellipsoidal, asymmetrical (Figs. 11 to 17). Some starch grains are surrounded more or less completely or partially by endoplasmic reticulum cisternae, leaving a distance between the granules and the cisternae which is about 0.05 - 0.2 wide (Figs. 11,12,13). The endoplasmic reticulum cisternae were observed to be continuous with the outer membrane of the nuclear envelope (Fig. 13 arrow) and passing between the chloroplasts in a close juxtaposition (Figs. 11,13). Fig. 14 shows a starch granule completely surrounded by a double membrane with a tiny gap appearing between the granule and the double membrane in some regions; such examples of starch granules closely invested by a double membrane are frequent. It is not known whether this double membrane represents an integral part of the granule (such an observation seems not to have been recorded in the literature) or merely endoplasmic reticulum cisternae pressing against the starch granule; or possibly artifacts induced during the preparation for the electron microscope. Some granules appeared to be fusing together (Fig. 15). In a few cases the granules appear to consist of two concentric layers with the outer layer composed of dark electron dense material measured about 0.03 - 0.15 μm wide (Figs. 16,17).

7.4. Floridean starch of Audouinella sagraeanum.

Vegetative cells of Audouinella sagraeanum accumulate vast amounts of floridean starch grains (Fig. 18) such that up to 60% of the cell volume is occupied by starch. Floridean starch grains lie free in the cytoplasm with sizes ranging from 0.3 - 3.6 μm and showing various shapes. They may assume spherical, ovoid, cylindrical (Figs. 18,19,24), geometrical shapes (Fig. 20) and may be of unusual shape (Fig. 21). Small grains similar in appearance to the floridean starch grains in the cytoplasm were observed within the chloroplasts (Figs. 18,22,23,24). Their chemical composition is not known since, because of their small sizes, they cannot be detected by Iodine solution at light microscopic level. Their occurrence within

the chloroplasts where the carbohydrates are formed in photosynthesis, and their similarity to the other starch grains described (Fig. 23), suggest that they are possibly of the same chemical composition as floridean starch. These small grains are more or less of uniform size of about 0.14 - 0.2 μm in length; they never attain the size of the cytoplasmic floridean starch grains. They aggregate in groups in random distribution within the chloroplasts (Fig. 22) and they may aggregate at the base of the pyrenoid (Fig. 23) or are distributed around it (Fig. 24).

7.5. Discussion.

Floridean starch grains of the three species reported in the present work vary in size. According to Sheath et al. (in press) this variation is attributed to (a) diversity in the maturity of the starch grains, (b) the fact that they are not subject to a well regulated environment in the plastid stroma as are amyloplast starch granules of higher plants, and (c) they may be subject to a variety of chemical and physical conditions due to their scattered distribution in the cytoplasm. The separation of starch granules into two groups depending on their size in Plumaria elegans rhizoidal cells only, seems not to be related to formation of branches and it is unlikely to be caused by gravitational force (Gunning and Steer, 1975) since the granules occupy the major volume of the cell contents (Fig. 1). However, its occurrence only in Plumaria elegans rhizoidal cells among the three species examined in the present work suggests a characteristic significance, and perhaps it is that the activity of the enzymes necessary for the synthesis of starch on one side of the cell is different from that of the other side.

It is generally agreed that floridean starch granules of red algae lie free in the cytoplasm (Dodge, 1973; Gunning and Steer, 1975; Trainer, 1978; Bold and Wynne, 1978; Lee, 1980) but the presence of small granules - possibly of the same chemical composition of floridean starch -

within the chloroplasts of Audouinella sagraeanum clearly invites further investigations. Due to their small size, which never attains that of the cytoplasmic floridean starch granules size, may explain why previous workers using other methods did not report them. However, Konrad Hawkins (1974b) has reported the presence of floridean starch granules within some chloroplasts of Callithamnion roseum. She concludes that these chloroplasts possess the enzymatic machinery necessary for synthesis and breakdown of starch.

Floridean starch granules composed of layers have been described in other red algae. They were observed either after treating the granules with IKI in Bonnemaisonia nootkana (Boney, 1975b) and Rhodomenia pertusa (Boney, 1978) or with NaOH in Ceramium sp., or even without any chemical treatments (Sheath et al., in press). These layers were reported in higher plant starch granules (Gunning and Steer, 1975). Buttrose (1962) reported that starch granules of potatoes, formed in a constant environment of light and temperature, differentiate into layers indistinguishable from that of granules under field conditions, while the layers did not develop in wheat starch granules growing in a constant environment, but they could be produced at will by imposing a dark period. His conclusion was that the formation of layers in potato starch granules must be controlled by endogenous rhythm, whereas in wheat starch granules it must be controlled by external environment. The layers observed in starch granules of Plumaria elegans are similar to those observed in other red algae (Boney, 1975b, 1978; Sheath et al. (in press) and the number of layers is few compared to those of higher plants (Buttrose, 1962; Sterling, 1968). Their formation is more likely to be controlled by endogenous rhythm like potato starch granules (Buttrose, 1962) rather than external environmental control since few of them per cell were observed. However, the question concerning the nature of the outer layers of some floridean starch grains of Palmaria palmata remains unanswered. This layer develops an electron

dense appearance (Figs. 16,17); the possibility of it having the same chemical composition as floridean starch is unlikely. The reason for deposition of such layers around some of the starch granules of Palmaria palmata is not known.

In some species of higher plants, the periplastid endoplasmic reticulum was suggested to help in transporting sugar moving to and from the starch in the plastids (Gunning and Steer, 1975). The development of starch grains during carposporogenesis of Lithothrix aspergillum is intimately associated with endoplasmic reticulum cisternae. In addition to this, starch grains are first recognized between these cisternae, suggesting that the endoplasmic reticulum is the site and/or source of the enzymes necessary to polymerize hexone molecules into floridean starch (Borowitzka, 1978). In Palmaria palmata some of the floridean starch grains are enclosed by endoplasmic reticulum (Figs. 11,12,13) confirming the above hypothesis. Particles associated with starch granules of Palmaria palmata, and presumed to be ribosomes which may play a part in the deposition of starch granules (Pueschel, 1979), were not observed in the present work.

Fusions between starch granules are evident in the two species Palmaria palmata and Plumaria elegans. Such fusions were reported in algae (Meeuse et al., 1960) and higher plants (Gunning and Steer, 1975).

Palmaria palmata seems to be the only species reported in the present work in which an increase of the density of starch granules takes place for a short period, then the population of granules declines as the sporelings grow and small amounts of starch granules were observed in the vegetative cells of plants collected from the field (see section 4). Such depletion of reserves is difficult to explain. However, the absence of starch from other species such as Porphyra naiadum, Porphyra perforata and Bangia fuscopurpurea has been reported (Meeuse et al., 1960).

8. CHLOROPLAST DEVELOPMENT

8.1. Introduction.

It is now well known that chloroplasts are derived from pre-existing chloroplasts. Two ways in which chloroplasts are formed have been described in several red algae. The chloroplast may divide by fission into either two daughter chloroplasts (Burton, 1971; Honsell et al., 1978; Borowitzka, 1978; Nonomura and West, 1980; Gori, 1980) or there can be a multiple division resulting in the formation of more than two chloroplasts (Nichols et al., 1966; Burton, 1971; Honsell et al., 1978; Gori, 1980). Each daughter chloroplast contains membranaceous and other stromal components (Bisalputra, 1974). Growth of the daughter chloroplasts usually follows their replication (Honsell et al., 1978). The other way in which chloroplasts may be formed is through growth and differentiation from a population of structurally much more simple proplastids (Brown and Weier, 1968; Burton, 1971; Honsell et al., 1978; Borowitzka, 1978). By these methods chloroplast number, size and continuity from cell to cell and generation to generation are maintained.

In section 4, the majority of the chloroplasts of the vegetative cells of Palmaria palmata were shown to consist of a double membraned envelope with an outer thylakoid parallel to it and enclosing one or more concentric thylakoids and several internal thylakoids, similar to the thylakoid arrangement in Batrachospermum moniliforme (Brown and Weier, 1970), with the exception that thylakoids consisting of parallel tubules were not observed in the present work. The thylakoid arrangement within the chloroplasts of the vegetative cells of Plumaria elegans was reported to be represented by a single peripheral thylakoid lying parallel to the plastid envelope and enclosing the other internal thylakoids. These arrangements are reported in the present work and will be shown to be the same as the vegetative cells, in immature

and mature spores, as well as sporelings growing in culture for both Palmaria palmata and Plumaria elegans.

8.2. Chloroplast development in Palmaria palmata.

Fig. 1 shows the apical region of a twenty-four days old tetrasporeling of Palmaria palmata growing in culture. The majority of the plastid population is represented by proplastids and a few immature chloroplasts. The proplastids are more or less spherical or ovoid measuring about 0.76 - 1.2 μm in diameter and limited by a double membraned envelope. Lying parallel to this envelope, and separated from it by a distance of about 0.03 μm , is the peripheral thylakoid. The stroma is homogeneous, rich in ribosomes and contains a single genophore or DNA region (Fig. 2) of about 0.14 - 0.25 μm wide (single section). The immature chloroplast has a double membraned envelope and a peripheral thylakoid encloses randomly arranged internal thylakoids, genophores and plastoglobuli (Fig. 2). The youngest proplastids have no internal thylakoids (Fig. 2, arrowed proplastid). However, they soon form the internal thylakoids by invagination of the inner membrane of the peripheral thylakoids (Fig. 2, arrow with tail indicates the site of the invagination). The proplastids with no internal thylakoids are formed by budding from immature chloroplasts of the apical actively growing cells of the tetrasporelings (Fig. 2). In the subapical cells of tetrasporelings and in mature and immature tetraspores, the proplastids were seen to bud from mature chloroplasts and the first internal thylakoid may be formed by invagination of the peripheral thylakoid even before their detachment from the parent chloroplast (Fig. 3). Fig. 4 shows a mature chloroplast in the process of budding off a proplastid in which the peripheral thylakoid of the resulting proplastid is fragmented (at the point indicated by arrow) and grows to encircle the other internal thylakoids and may form a second peripheral thylakoid or may fragment (at the point indicated by arrow with tail) to

add more internal thylakoids. Proplastids consisting of two peripheral thylakoids, as well as others with a single peripheral thylakoid, were observed in both immature and mature tetraspores as well as the subapical cells of the tetrasporelings (Fig. 5). In the subapical cells and mature and immature tetraspores, chloroplasts were observed to replicate by fission. This fission process takes several pathways and is more conspicuous in the tetraspores than in the tetrasporelings. The chloroplasts may slightly elongate and some of the internal thylakoids grow and curve at one end of the chloroplast, running parallel to the peripheral thylakoids, whilst invaginating at some points to form more thylakoids. As a result of this process a distinct part of the chloroplast appears (Fig. 6, C1 arrows indicate the thylakoids which grow and curve at one end of the chloroplast). With differentiation of this part of the chloroplast more thylakoids are formed, a constriction appears (Fig. 7, arrows indicate the site of a constriction) and finally fission occurs resulting in the formation of two unequal daughter chloroplasts (Fig. 8). The second pathway of chloroplast fission is where the chloroplasts may increase in diameter and the internal thylakoids segregate into two groups (Fig. 6, C2). A constriction occurs between the two groups separating them into two parts (Fig. 9 and Fig. 4 section 5), then the two parts pull apart from each other resulting in stretching, rupturing the chloroplast envelope (Fig. 10) and dividing the parent chloroplast into two daughter chloroplasts as in Fig. 11 (but only one daughter chloroplast is showing). This figure also shows the ruptured part of the chloroplast envelope surrounding a homogeneous matrix continuous with the chloroplast stroma but similar to the cytoplasm in density (Fig. 11 arrow). This part may degenerate later (Fig. 12 arrow and Fig. 12 section 7) and the envelopes of the two daughter chloroplasts presumably retain their normal appearance. The third pathway of chloroplast fission is accomplished through elongation of the parent chloroplast with elongation of the peripheral and the

internal thylakoids; a constriction appears perpendicular to its longitudinal axis (Fig. 13). This constriction penetrates deeper through the chloroplast resulting in fragmentation of the thylakoids (Fig. 14) and dividing the parent chloroplast into two daughter chloroplasts. This constriction may proceed from both or only one side of the chloroplast (Figs. 15 and 17, C1). Fig. 15 also shows two daughter chloroplasts, each containing a single genophore (indicated by arrows) which possibly indicates that the genophores also divide and are transmitted into the daughter chloroplasts (this interpretation is based on a single section). The last pathway of chloroplast fission observed in the present work is that the internal thylakoids grow in different directions causing an irregular expansion of the chloroplast and resulting in the disorganized appearance of the total thylakoids but distinguished into groups of parallel thylakoids (Figs. 16 and 17, C2). Constrictions appear between the groups resulting in a multiple division and production of several chloroplasts from a single one (Fig. 18).

The budding process of proplastid formation and the four pathways of chloroplast fission of Palmaria palmata are summarized in Fig. 43. Some chloroplasts during tetrasporogenesis have thylakoids with a wavy appearance (Fig. 19). Phycobilisomes were seen attached to the thylakoids in some chloroplasts (Fig. 20) including some in a process of division (Fig. 13). Chloroplasts possessing phycobilisomes were not observed in either the vegetative cells (see section 4.2) or tetrasporelings growing in culture.

8.3. Chloroplast development in Plumaria elegans.

Chloroplasts of Plumaria elegans within the parasporangium as well as mature paraspores and parasporelings growing in culture were observed to have the arrangement of thylakoids similar to those of vegetative cells collected from nature (see section 4.3). This arrangement is

represented by a single peripheral thylakoid enclosing several internal thylakoids (Fig. 21). However, a juxtaposition between two to three thylakoids was observed in some chloroplasts of immature paraspores (Fig. 22). Appressed thylakoids in groups of four and six were seen in a single example (of many chloroplasts examined) in a newly released paraspore fixed 10 hours after release (Fig. 23). In the majority of the chloroplasts of Plumaria elegans the ribosomes are seen to aggregate in groups and in rows parallel to the thylakoids; this feature was observed in vegetative cells collected from nature (see section 4.3), immature paraspores (Figs. 21,22), mature paraspores (Figs. 24,29,30) and parasporelings (Fig. 37). These aggregations are quite conspicuous in mature paraspores and (in some chloroplasts) they may occupy a large area and cause local displacement of the internal thylakoids from their normal position (Fig. 25, arrow). Chloroplast ribosomes appear to be slightly smaller than the cytoplasmic ribosomes (Fig. 24). Phycobilisomes were not observed in this species.

Fig. 26 is a portion of an apical vegetative cell collected from nature in which the majority of the plastids are represented by proplastids and immature chloroplasts. The proplastid consists of a double membraned envelope and a single peripheral thylakoid which in some cases encloses a few internal thylakoids. However, some proplastids seem to show a lack of these photosynthetic lamellae, including the peripheral one, but this is possibly due to fixation problems. Genophores in these species are quite conspicuous and have different size and shape ranging from spherical to broad cylindrical and some may appear in a process of division (Fig. 26, arrow). The proplastids may have one or two of these areas (Fig. 27) and up to eleven of these areas were observed in a probably mature chloroplast in a single section (Fig. 28). Some proplastids appear to be dividing (Fig. 27). In mature paraspores and in parasporelings, proplastids were seen to originate through a localized blebbing of mature chloroplasts (Figs.

29, arrow and Fig. 30, arrow). This process involves extension of the chloroplast envelope, the peripheral thylakoid and some of the internal thylakoids which grow and curve around the smaller thylakoids which may be formed by fragmentation and invagination of these thylakoids (Fig. 29, arrows with tails point at the growing thylakoids). As a result of this process, proplastids consisting of an envelope, peripheral thylakoid and a few internal thylakoids are pinched off free into the cytoplasm (Fig. 30, arrow with tail).

In some of the mature chloroplasts a group of small parallel thylakoids become evident in a localized region (Figs. 28,31,32). These small thylakoids arise by growth, invagination and fragmenting of the large thylakoids (Fig. 31, arrow points at the growing large thylakoid and a possible fragmentation takes place at that region, and arrow with tail indicates the site of thylakoid invagination). These groups of small parallel thylakoids grow independently and the enclosing plastid envelope is bulged out into the cytoplasm (Fig. 33). More differentiation of such parts results in the formation of more thylakoids which enlarge in size to almost the same size as the parent chloroplast, whilst still attached to it (Fig. 34). The formation and growth of a constriction results in the formation of two daughter chloroplasts (Fig. 35). The group of small parallel thylakoids may become evident along the long axis of the parent chloroplast at one end. These small thylakoids probably originate by growth of the thylakoids of the parent chloroplast (Fig. 36). This group grows independently and elongates giving the chloroplast the appearance of a very long one with two sets of parallel thylakoids of more or less equal size (Fig. 37). Constriction between the two sets probably gives the two daughter chloroplasts the appearance of being connected by a narrow tubule (Fig. 38). On the other hand, these two chloroplasts of Fig. 38 may represent two mature chloroplasts in a process of fusion rather than division. This view is based on the fact that the outer membranes of the two chloro-

plast envelopes are joined together but not the inner membranes, and a small island continuous with the stroma of one chloroplast is located in the chloroplast envelope space of the other (Figs. 39,40). Localized inflation of the thylakoids with some contents appears sometimes in some chloroplasts (Fig. 41, arrows). Fig. 42 shows a chloroplast with two sets of parallel thylakoids. This chloroplast may represent the second stage of the development of the chloroplast of Fig. 32 in which the group of the small parallel thylakoids has grown within the space of the parent chloroplast without projecting outside, and the dividing chloroplast of Fig. 35 may represent the following stage of the chloroplast of Fig. 42 in which the constriction is separating them into two daughter chloroplasts. Chloroplast development of Plumaria elegans including proplastid formation is summarized in Fig. 44.

8.4. Discussion.

Thylakoid arrangements in chloroplasts of Palmaria palmata differ from those of Plumaria elegans (see section 4). The juxtaposition of two to three thylakoids in some chloroplasts of immature paraspores within the parasporangia of Plumaria elegans, and the appressed thylakoids in a group of four and six observed in a newly released paraspore, are similar to the granum-like structures reported in chloroplasts of Smithora naiadum monospores (McBride and Cole, 1971,1972). Such a phenomenon was not observed in either Palmaria palmata chloroplasts or Plumaria elegans parasporelings or vegetative cells collected from nature, suggesting that thylakoids appression is either a rare event or a very short-lived one in Plumaria elegans paraspores (Mandura et al., in press). The latter corresponds with McBride and Cole's (1972) observation in which smaller numbers of appressed thylakoids were observed in chloroplasts of germinating Smithora naiadum monospores compared with those of newly released monospores. McBride and Cole (1971) have suggested that formation of granum-like structures in

Smithora monospores is perhaps due to physical effects of pressure arising from the accumulation of metabolic products and possibly linked with the altered metabolism of the spore.

Phycobilisomes which have been reported in red algal chloroplasts (e.g. Dodge, 1973; Wanner and Köst, 1980; Gantt and Lipschultz, 1980) were seen attached to thylakoids of some chloroplasts of Palmaria palmata (Figs. 13,20) among the three species examined in the present work. They were not observed in Ceramium rubrum (Chamberlain and Evans, 1973) and in Batrachospermum moniliforme in both thin section and after freeze-etching (Brown and Weier, 1970). However, they were reported to be fixation labile (Gantt and Conti, 1966) and easily lose integrity, especially in Osmium post-fixed material (Borowitzka, 1978). Phycobilisomes presumably dissolve out if the material is suspended in glycerol prior to freezing (Dodge, 1973).

The significance of aggregation of plastid ribosomes in groups and in parallel rows between the thylakoids (e.g. Figs. 22,24,25) and the localized inflations of the thylakoids (Fig. 41) in some of the chloroplasts of Plumaria elegans is not known, though the possibility of the latter being artifacts induced during the preparation for electron microscopy is not precluded. In Palmaria palmata, although the significance of the wavy appearance of the thylakoids in some chloroplasts is not known, similar observations were reported in Porphyridium cruentum (Wanner and Köst, 1980).

Chloroplast development and replication in both Palmaria palmata and Plumaria elegans are summarized in Figs. 43 and 44. The apical cells of both plants are characterized by the presence of a high population of proplastids, though proplastids were observed in low proportion in other cells (e.g. subapical cells, mature and immature spores). The high proportion of proplastids in these cells may be attributed to their high division rates which prevent them from developing further (Honsell et al., 1978). Proplastids of both Plumaria elegans and Palmaria palmata consist of plastid envelopes and peripheral thylakoids similar to those described in Nitophyllum

punctatum (Honsell et al., 1978), Batrachospermum moniliforme (Brown and Weier, 1968, 1970) and Antithamnion subulatum (Burton, 1971), though in Plumaria elegans some proplastids appear to lack photosynthetic lamellae (Figs. 26,27) but these are probably due to processing problems. However, proplastids consisting of only the plastid envelope were observed in Lomentaria baileyana (Bouck, 1962); Erythrocytis montagnei (Tripodi and De Masi, 1977) and Lithothrix aspergillum (Borowitzka, 1978). Proplastid differentiation begins with the formation of one or a few internal thylakoids by invagination of the inner peripheral thylakoid membrane (Fig. 2) in Palmaria palmata as in Batrachospermum moniliforme (Brown and Weier, 1968) and Lomentaria baileyana (Bouck, 1962). By contrast the internal thylakoids in Lithothrix aspergillum are formed from the pro-lamellar body in the DNA region of the proplastids (Borowitzka, 1978). In Euglena gracilis the thylakoids are derived from the inner membrane of the plastid envelope (Ben-Shaul et al., 1964). Vesicles present in the stroma of the proplastids which probably have originated from the peripheral thylakoid and might possibly contribute in forming some of the internal thylakoids (Honsell et al., 1978) were not observed in the present work. Proplastids in the apical cells were observed to undergo division in Plumaria elegans (Fig. 27) and to pinch off immature chloroplasts in Palmaria palmata (Fig. 2). Division of proplastids was reported in algae (Borowitzka, 1978; Honsell et al., 1978) and higher plants (Leech, 1977). In other cells (e.g. subapical cells, immature and mature spores) of both Palmaria palmata and Plumaria elegans, proplastids may also originate by budding from mature chloroplasts and the internal thylakoids may be formed before their complete detachment, by invagination of the peripheral thylakoid (Fig. 3) or by extension and fragmentation of either the internal thylakoids of the parent chloroplast (Fig. 29) or the peripheral thylakoid itself (Fig. 4). Proplastid formation by budding off chloroplasts was reported in other algae such as Antithamnion subulatum (Burton, 1971) and Erythrocytis montagnei

(Tripodi and De Masi, 1977).

Genophores in Plumaria elegans and Palmaria palmata are similar to those reported in various algae (e.g. Dodge, 1973; Bisalputra, 1974; Honsell et al., 1978). Genophores in Plumaria elegans proplastids are very conspicuous and of different size and shape. Some may appear in a process of division and some of the proplastids may have two of these regions (Figs. 26,27). However, genophore divisions were reported by Bisalputra (1974) and their transmission into the daughter chloroplasts is essential (Bisalputra, 1974; Bisalputra and Bisalputra, 1970).

The noticeable increase in number of chloroplasts during sporogenesis (see section 5), spores and sporeling development of both Palmaria palmata and Plumaria elegans, is not only a result of proplastid division and differentiation but also due to replication of young and also probably mature chloroplasts, if the definition of mature chloroplasts adopted here is accepted as having several photosynthetic lamellae bearing phycobilisomes (Fig. 13), since there are no criteria by which it is possible to judge whether a particular chloroplast has reached the state of a maximum development. However, in higher plants mature chloroplasts were reported to divide (Leech, 1977). In Palmaria palmata, chloroplast replication is completed by fission. This fission process is accomplished by the appearance of a constriction which may be in the plane perpendicular to the long axis, resulting in the separation of the parent chloroplast into two daughter chloroplasts either of unequal size (Figs. 6,7,8 and Fig. 43b) or of equal size (Figs. 13,14,15 and Fig. 43d). In both cases growth, invagination and fragmentation of the thylakoids of the parent chloroplasts are essential. The plane of the constriction may be parallel to the long axis of the chloroplast separating the thylakoids into two groups which pull apart after increase in size into two daughter chloroplasts (Figs. 9, 10,11,12 and Fig. 43c). Multiple division also takes place in this species (Figs. 16,17,18 and Fig. 43e). Chloroplast fission into two daughter

chloroplasts has been reported in other red algae (Honsell et al., 1978; Borowitzka, 1978; Burton, 1971; Nonomura and West, 1980; Bouck, 1962; Brown and Weier, 1968; Nichols et al., 1966; Gori, 1980) and higher plants (Gunning and Steer, 1975; Leech, 1977). Multiple division has been reported in other red algae (Burton, 1971; Honsell et al., Gori, 1980). Completely different mechanisms of chloroplast replication take place in Plumaria elegans in which a group of small parallel thylakoids are formed by growth, invagination and fragmentation of the parent chloroplast thylakoids. They are located either at the corner of the chloroplast (Fig. 31) or along the short axis (Fig. 32) or along the long axis (Fig. 36). Growth and differentiation of this part either results in the projection of this part into the cytoplasm, or takes place within the space of the parent chloroplast (Figs. 32 to 35 and Figs. 42, 44b) with final separation into two equal chloroplasts by fission. In the case of aggregation of this group of small parallel thylakoids along the long axis of the chloroplast, its growth and elongation gives the chloroplast the appearance of being very long with two sets of parallel thylakoids of more or less equal size. Eventually fission occurs, dividing them into two daughter chloroplasts (Figs. 36,37 and Fig. 44C). A somewhat comparable way of division was described in Nitophyllum punctatum (Honsell et al., 1978) in which the first internal thylakoids are formed and orientated on different planes within the stroma of the proplastid. Differentiation of this proplastid results in a complex chloroplast with different sets of parallel thylakoids and finally fission occurs, separating each set into a single chloroplast. Chloroplast division by central baffle formation reported in higher plants (Cran and Passingham, 1972; Leech, 1977) was not observed in the present work. The two chloroplasts of Fig. 38 in the present work may represent a chloroplast in a state of fission or equally well they may represent fusion between two mature chloroplasts (perhaps temporary). The latter is more likely to be the case based on the fact that part of the stroma of

one chloroplast appears to have moved to the plastid envelope space of the other through a tubule (Figs. 39,40). Fusion between chloroplasts has been reported in the green alga Clamydomonas reinhardii (Cavalier-Smith, 1970), the higher plant Mimosa pudica (Esau, 1972) and the fern Osmunda regalis (Allen et al., 1973).

9. ANNULATE LAMELLAE AND NUCLEOLUS-LIKE BODIES

9.1. Introduction.

An annulate lamella consists of two parallel membranes separated by a cisternal space and periodically interrupted by pore complexes similar to those of the nuclear envelope (Kessel, 1968). The pore density in annulate lamellae can be much higher than in the nuclear envelope (Franke and Scheer, 1974). Annulate lamellae are found either in the cytoplasm or within the nucleus. They are infrequent and transitory structures (Kessel, 1968; Steinkamp and Hoefert, 1977) and have been reported from a wide variety of animal cell types (Kessel, 1968; Franke and Scheer, 1974) but there seem to be only a few reports of their occurrence in algae (Peel et al., 1973; Duckett and Peel, 1978; Tripodi, 1974; Wetherbee et al., 1974; Wetherbee, 1980; Brawley et al., 1977) and higher plants (Scheer and Franke, 1972; Franke et al., 1972; Steinkamp and Hoefert, 1977; Bowes, 1979). Annulate lamellae were described from normal cells or virus infected cells (Steinkamp and Hoefert, 1977) and have been induced to form in cells under stimulatory factors (De Br^{ab}ander and Borger, 1975; Gulys, 1975).

In this section annulate lamellae are reported in the two species Plumaria elegans and Palmaria palmata during sporeling development in addition to the presence of nucleolus-like bodies in some nuclei of Palmaria palmata tetrasporelings.

9.2. Intranuclear annulate lamellae of Plumaria elegans.

Intranuclear nuclear annulate lamellae were observed in differentiating cells of Plumaria elegans during parasporeling development. They occurred singly or in pairs in straight, curved or circular configurations (Figs. 1 to 4). They consist of a pair of closely associated, approximately parallel membranes (without associated ribosomes) frequently interrupted by

pores. Annulate lamellae commonly occur in random orientation in the nucleoplasm adjacent to the nuclear envelope (Fig. 1) though they may be present elsewhere in the nucleoplasm (Fig. 3) or close to the nucleolus (Fig. 4). They may show continuity with the inner membrane of the nuclear envelope (Fig. 2).

9.3. Annulate lamellae and nucleolus-like bodies of Palmaria palmata.

Intranuclear annulate lamellae observed in differentiating cells of Palmaria palmata (Fig. 5) are morphologically similar to those of Plumaria elegans but they occur much less frequently in the former than in the latter. However, in Palmaria circular configurations of several cisternae arranged in concentric stacks (Fig. 6) were observed in the cytoplasm on rare occasions during tetrasporeling development. Each cisterna consists of double membranes separated by an irregular space and interrupted by pores, indicated in Fig. 6 by arrows, and arrows with tails which show the pore rims through which the level of the section has passed. These concentric stacks are interpreted as cytoplasmic annulate lamellae as distinct from whorls of unperforated rough endoplasmic reticulum.

It is worthwhile to note that nucleolus-like bodies with apparently fibrillar contents appear free in the nucleoplasm as well as the intranuclear annulate lamellae in Palmaria palmata during tetrasporeling development (Fig. 7). They are spherical and measure about 0.3 - 0.76 μm in diameter (Fig. 7 arrows). Fig. 8 arrow possibly represents the degenerative appearance of these nucleolus-like bodies. There does not seem to be any relationship between these bodies and the intranuclear annulate lamellae. The nucleolus-like bodies are similar to the fibrillar bodies observed in the nuclei of some siphonous green algae (Roth and Friedmann, 1980). However, nucleolus-like bodies (as distinct from nucleoli) are of common occurrence in plants and animals. They are generally dense, spherical and may have a tightly packed fibril component

(Gimenz-Martin et al., 1977). Their origin and function is not known, but they could be products of nucleolar material or they could be structures newly synthesized during the prophase periods (Gimenz-Martin et al., 1977).

9.4. Discussion.

The basic criterion for annulate lamellae is the occurrence of pore complexes in the cisternae. Annulate lamellae appear most frequently in rapidly growing and differentiating cells under both normal and abnormal circumstances (Kessel, 1968; Steinkamp and Hoefert, 1977). Hence these organelles may have a multiple function depending upon the cell type (Sun and White, 1979). There has been much speculation on the function of these structures. Their function was expressed as nucleocytoplasmic exchange (Kessel, 1968; Peel et al., 1973); a site for tubulin synthesis or microtubule polymerization in animal cells which have completely lost their microtubule apparatus (De Brabander and Borgers, 1975); protein synthesis (Wischnizer, 1970) and storage site or dispersal mechanism for materials such as ribonucleoprotein (Steinkamp and Hoefert, 1977).

Their origin was attributed to a transformation of the endoplasmic reticulum (Steinkamp and Hoefert, 1977; Sun and White, 1979); coalescence of vesicles pinched off the nuclear envelope (Kessel, 1968; Gulyas, 1971); local proliferation of the nuclear envelope (Ollerich and Carleson, 1970) and fusion of smooth endoplasmic reticulum vesicles (De Br^{ab}ander and Borgers, 1975).

The intranuclear annulate lamellae of Plumaria elegans and Palmaria palmata are similar to those reported in Polysiphonia novae-angliae (Wetherbee et al., 1974), Corallina officinales (Peel et al., 1973; Duckett and Peel, 1978), higher plants (Scheer and Franke, 1972; Franke et al., 1972) and animals (Kessel, 1968). Their direct continuity (in some sections) with the inner membrane of the nuclear envelope in Plumaria elegans (Figs. 1,2) and the apparent lack of the intranuclear vesicles or

membranes in the nuclei that may be involved in intranuclear annulate lamellae formation (Kessel, 1968; Franke et al., 1972) suggest that annulate lamellae are derived from the inner membrane of the nuclear envelope. A similar conclusion was reported for giant cells of the rat placenta by Ollerich and Carlson (1970), who also found an annulate lamella in direct contact with the nucleolus, and reported that the intramembrane space in intranuclear annulate lamellae was continuous with the perinuclear space of the nuclear envelope. The latter is known to be continuous with the space between membranes of intercisternal elements of endoplasmic reticulum. Consequently, a continuous system of spaces, extending from the nucleolus to the cytoplasm, is conceivable and exchange of material could possibly occur in either direction, from the nucleolus to the cytoplasm or vice versa. Peel et al. (1973) suggested that during the tetrasporogenesis of Corallina officinalis, the intranuclear annulate lamellae may be indirectly involved in cytoplasmic differentiation or they may represent stored or incomplete information released at mitosis on germination of the tetraspore.

The concentric stacked cisternae of the cytoplasmic annulate lamellae observed in differentiating cells of Palmaria palmata tetrasporelings has been previously encountered in Polysiphonia nova-angliae by Wetherbee et al. (1974). They offered evidence in support of its derivation from the vesiculate smooth endoplasmic reticulum, which was seen to be attached to the end of the cisternae. Cytoplasmic annulate lamellae in Palmaria palmata are of rare occurrence but when found they appear to be mature. Their apparent absence in Plumaria elegans make conclusions difficult regarding their origin. Their absence from the specimens examined in Plumaria elegans does not exclude their presence in this species since the annulate lamellae are reported to be short lived structures (Kessel, 1968). The occurrence of ribosomes on the outer surface of cytoplasmic annulate lamellae seems to be a variable situation (Kessel, 1968).

10. ADDITIONAL FEATURES

10.1 The origin and development of vacuoles during tetrasporeling development of Palmaria palmata.

According to Gunning and Steer (1975), there may be no one answer to how and where vacuoles originate, depending upon how narrowly or widely vacuoles are defined. Some of the ideas put forward regarding their origin (Gunning and Steer, 1975) are that they develop (a) from membrane-bounded vesicles originating in the Golgi apparatus; (b) as swelling of endoplasmic reticulum cisternae; (c) from pre-existing vacuoles which may enlarge and divide by fission, so that there are always enough to provide every newly formed cell with its quota; (d) by a de novo process of molecular association, starting when water-attracting compounds become surrounded by a tiny aqueous shell, and this in turn develops a membranous skin of lipid and protein molecules to give rise to the tonoplast.

In newly released tetraspores of Palmaria palmata, the cytoplasmic organelles (chloroplasts, mitochondria etc.) occupy the major volume of the cytoplasm (Fig. 1 section 6). Vacuoles or provacuoles at this stage of tetraspore development seem to be very rare, if not absent (excluding the large fibrous vesicles which contribute to tetraspore cell wall formation, see section 5). Soon after that, vesicles are released to the cytoplasm by dilation of the whole cisternae of Golgi bodies which appear not to be accompanied by mitochondria (Fig. 1). These vesicles (provacuoles) contain granular or fibrillar material. They fuse together (Fig. 2) and increase in size and number (Fig. 3). Continuous increase in their size and number results in the formation of vacuoles of the tetrasporeling which occupy a major proportion of each cell (Figs. 4,5). Such origin of vacuoles from Golgi bodies was reported during monospore germination of Smithora naiadum (McBride and Cole, 1972) and in the green alga Chlorogonium elongatum (Ueda, 1966). In some algae vacuoles were reported to have been derived either

from fusion of Golgi body-vesicles to vacuoles believed to be originated by inflation of endoplasmic reticulum cisternae (Brown and Weier, 1970); Young, 1979a) or coalescence of endoplasmic reticulum vesicles only (provacuoles) (Young and West, 1979; Olivera and Bisalputra, 1977a; Young, 1979b). In Cystoseira stricta, specialized vacuoles (irridescent bodies) are derived from Golgi bodies, whereas the vacuolar system at its early stage of development appears as anastomosed cavities, probably originating from G.E.R.L. (smooth reticular region located against the distal pole of Golgi bodies). These cavities partition off areas of the cytoplasm by lying in a circle, then fusing with each other, and finally the sequestered portions or areas undergo degradation (Pellegrini, 1979). Such sequestered portions or areas are achieved by progressive invagination of the tonoplast or by endoplasmic reticulum in Ectocarpus sp. (Oliveira and Bisalputra, 1977a).

10.2. Osmiophilic bodies of Palmaria palmata tetraspores.

Storage products (including crystalline bodies) of different shape with no limiting membrane were observed in the vegetative cells of Plumaria elegans plants collected from nature (see section 4). Their chemical constitution is not known but a possible proteinaceous nature was discussed. Crystalline bodies were observed during parasporing development of Plumaria elegans in culture (see section 10.6).

Osmiophilic bodies, of the same electron density as the crystalline bodies of Plumaria elegans parasporings, appear frequently in the cytoplasm of tetraspores of Palmaria palmata after five hours from release (Figs. 6,7). They were not observed in newly released tetraspores up to four hours from release (Fig. 1 section 6) which indicate that they were not formed during tetrasporogenesis. They disappear after tetraspore division or germination (Figs. 3,4,5) suggesting that these bodies may represent storage products consumed during the division or the germination. Two types of these

osmiophilic bodies of apparently different morphological composition were observed in Palmaria palmata tetraspores (Figs. 6,7). The first type are more or less spherical in shape (Fig. 6) and measure about 1 - 2.5 μm in diameter. They consist of electron dense material which at first glance appears not to be limited by a membrane (Fig. 6,8), but careful inspection reveals what appears to be a discontinuous membrane around each body (Fig. 8, arrows). It is possible that this membrane may completely surround the electron dense material and the apparent discontinuity may be partly due to the similarity in electron density between the membrane and the electron dense material, and partly due to the tight appression between this membrane and the dense material at some regions. Endoplasmic reticulum and polyribosomes were observed around the osmiophilic bodies (Fig. 8). The second type of osmiophilic body is much less frequent than the former. Each body of the second type is more or less spherical, about 4 μm in diameter, and consists of two matrices plus the electron dense material. Both matrix B and the electron dense material are enclosed by matrix A, which in turn is bounded by a double membrane (Fig. 9). Matrix A is a granular material in which the granules appear to be similar in size to the cytoplasmic ribosomes to the naked eye (Fig. 10). The outer membrane is thicker than the inner and ribosomes seem to be attached to its outer surface (Fig. 10, arrows). The inner membranes invaginate in a form of narrow tubules or projections (Fig. 10, arrow with tail). Matrix B consists of homogeneous fibrillar material which is at least partly surrounded by a double membrane (Fig. 11, arrow). Matrix A and B intermingle with each other. This conclusion is based on Fig. 12 which is from a serial section of Fig. 9, and the proportion of matrix A and B are different at different levels of sectioning. Fig. 9 at the region indicated by arrow appears not to be surrounded by membranes, but at Fig. 12 the same region (indicated by arrow) at a different level of sectioning appears to be surrounded by membranes. This indicates that because of the similarity between the densely staining membrane and the

electron dense material, the membranes at that region are indistinguishable (Fig. 9, arrow) and this supports the above possibility that the first type of osmiophilic bodies are membrane-limited structures.

Electron dense spherical bodies limited by membrane similar to the first type of osmiophilic bodies of Palmaria palmata tetraspores were observed in Janczweskia morimotoi (Nonomura and West, 1980) and Batryocladia pseudodichotoma (Young, 1978). In Janczweskia morimotoi they were termed "black bodies" and were suggested to have a proteinaceous composition, at least at their early stages of their formation, since they were observed in association with endoplasmic reticulum and numerous ribosomes (Nonomura and West, 1980). The same association of osmiophilic bodies, endoplasmic reticulum and polyribosomes, were observed in Palmaria palmata (Fig. 8). In Batryocladia pseudodichotoma the osmiophilic bodies were reported to be of endoplasmic reticulum origin and a possible lipid or protein nature was discussed (Young, 1978). The osmiophilic bodies of Palmaria palmata tetraspores resemble in electron density the unusual lipid bodies observed in the cortical vegetative cells of Palmaria palmata by Pueschel (1977b). They differ, however, in some aspects. For instance, most lipid bodies are reported to be homogeneously dense with few or no membranous associations. Some have a lone unit membrane or several concentric ones attached to the surface of the body, and some are composed of a homogeneous dense core surrounded by a sheath of reticulum membranes appearing as a honeycomb in which the chambers are filled with lipid (extracted by chemical fixation). The entire lipid body together with its reticulate sheath are surrounded by a unit membrane (Pueschel, 1977b). It is worthwhile to note that these lipid bodies observed by Pueschel (1977b) were not observed in the present work, and therefore their formation may be controlled by environmental factors.

10.3. Adhesion of paraspores and parasporelings of Plumaria elegans.

Two morphologically different types of vesicles were reported to be responsible for spore adhesion in red algae. The first type are commonly termed cored vesicles and are of widespread occurrence (Chamberlain and Evans, 1973; Kugrens and West, 1972a, 1974; Wetherbee, 1978a; Scott and Dixon, 1973a; Duckett and Peel, 1978). Each vesicle consists of a dark electron dense core surrounded by a less electron dense region. The second type of adhesive vesicles are dark and electron dense (see section 5). Both types of vesicles are derived from Golgi bodies and are formed during sporogenesis.

In Plumaria elegans the cored vesicles, measuring about 0.3 - 0.6 μm in diameter, are formed during parasporogenesis (Fig. 13) and were observed in newly released paraspores up to ten hours from release (Fig. 15 section 6). Some of these vesicles are formed by Golgi bodies in the newly released paraspores (Fig. 14). These cored vesicles fuse with the plasmalemma either directly (Fig. 15, arrows) or through tubules (Fig. 16, arrow) and discharge their contents by reverse pinocytosis to form the adhesive layer of the newly released paraspores. The vesicles may fuse to each other and probably discharge their contents from one to another, which may in turn be discharged to the exterior (Fig. 16, arrows indicate the site of vesicle fusion and the arrow with tail points at the fusion with the plasmalemma through a tubule). The cored vesicles which are not located near the plasmalemma may discharge their contents to vesicles near to it through long tubules (Fig. 18, arrow pointing at the fusion tubule). After the spore divides and germinates, a layer appears around the sporeling, which is probably of mucilaginous nature (Fig. 19, arrows). Fig. 20 shows part of a rhizoidal cell of a two week old parasporeling surrounded by this layer which also surrounds the cells of the upright filaments (Fig. 21). This layer probably contains adhesive substances which may help to stick the rhizoidal cells to the substratum as the rhizoid grows and extends above it (see section 3),

and/or it may act as a protecting layer for the cells of the upright filaments. The origin of this layer is not known but the endoplasmic reticulum is abundant in the growing cells of the rhizoids (Fig. 20) and the apical cells of the upright filaments (Fig. 21), and this may possibly suggest a role of the endoplasmic reticulum in the secretion of the adhesive layer. The endoplasmic reticulum is represented by rough endoplasmic reticulum (Fig. 22) and smooth endoplasmic reticulum (Fig. 23) but the proportion of the latter is much more than the former (Fig. 20,21). The smooth endoplasmic reticulum may be produced by a temporary loss of ribosomes from the rough endoplasmic reticulum (Gunning and Steer, 1975). The secretion process of these layers around the parasporangia of Plumaria elegans perhaps is that the endoplasmic reticulum mobilizes sugar and delivers the products to the exterior through fusion with the plasmalemma in which a rosette of particles (see Gunning and Steer, 1975) possibly appear in the plasmalemma at the site of the fusion as a result of local specialization at molecular level to permit such fusion, since the molecular architecture of the plasmalemma and the endoplasmic reticulum are dissimilar (Gunning and Steer, 1975). However, in nectar-secreting cells of higher plants, endoplasmic reticulum was reported to secrete sugar and proliferate and reach the maximum degree of proliferation at the stage of nectar secretion (Fahn, ^{and Rachmilevitz} 1970; Rachmilevitz and Fahn, 1973).

10.4. Pit connections of parasporangia of Plumaria elegans.

Pit connections are formed in the cross wall between daughter cells after incomplete cytokinesis. They vary considerably in size and shape, not only between species but also in different parts of the thallus (Dixon, 1973). The plasmalemma is continuous from cell to cell passing around the sides of the plug (Dodge, 1973; Duckett and Peel, 1978). This phenomenon has been demonstrated earlier, during the tetrasporogenesis of Palmaria palmata (see section 5) and in the vegetative cells of Audouinella

sagraeanum (see section 4). A pit connection consists of a plug fitted into the aperture. On each side of the plug is a membrane-bounded plug cap (Bold and Wynne, 1978). The plug caps may be of a single or multiple layers (Duckett et al., 1974). Pit domes may ^aoverly pit caps in some species such as Batrachospermum sirodotti (Aghajanian and Hommersand, 1978). In Batrachospermum sirodotti, some of the pit connections are composed of doughnut-shaped pit rings with central pores. The pit ring is bounded in all surfaces by the pit cap. The pit plug is a rivet-shaped structure that continues through and occludes the pore within the pit ring. The pit plug overlies the pit cap, isolating the pit ring and the pit cap from the cytoplasm. This type of pit connection may be characteristic of the fresh water red algae (Aghajanian and Hommersand, 1978), who also reported breakdown of pit connections and reopening of the septal pores to allow intracellular connection between the vegetative axial cells. Pit plugs are composed of protein and pit caps are reported to be at least partly composed of polysaccharides (Pueschel, 1980C).

In Plumaria elegans parasporangia, pit connections vary in size and shape. They may be lenticular (Figs. 24,25) or a horseshoe shape (Fig. 26). The latter is more common between the rhizoidal cells (Fig. 26). Pit connections of Plumaria elegans consist of pit plugs in which the marginal regions are more electron dense than the central region (Figs. 24,25,28,30) though some are uniformly electron dense (Figs. 26,27). The plug caps are represented by a very thin layer which is bounded by a membrane on both sides of the plug (Fig. 28, arrows). This layer may be indistinguishable in some cases, probably due to the high electron density of the plug core and the thinness of the plug caps (Fig. 27). The plug cap may be present on one side of the plug only (Fig. 24, arrow). The plug caps of Plumaria elegans are similar to those of Spermothamnion turneri and the Trailliella-stage of Bonnemaisonia hamifera (Lee, 1971) and unlike the clear multiple layer plug caps of Palmaria palmata and the clear single layer plug cap of

Audouinella sagraeanum (see section 4). The rim of the aperture fits into a shallow groove around the short axis, thus firmly locking the plug into the aperture (Fig. 24). Fig. 29 shows a pit connection between two cells of an upright filament of a Plumaria elegans parasporeling in which the middle part appears to have the same electron density as the cytoplasm. If the electron dense lines (pointed at by arrows) are merely artifacts and the middle part of the pit plug is part of the cytoplasm, this phenomenon would suggest that a narrow part at the rim of the aperture (indicated by Δ) protruded inwards upon which electron dense material from both daughter cells had been laid down (Fig. 29, arrow with tail), trapping part of the cytoplasm which occupies the aperture. Then electron dense material probably passes across the part indicated by arrow with tail at Fig. 29 and replaces the trapped cytoplasm, which may in turn degenerate or transform as part of the plug itself. The electron dense material may be transported from the cytoplasm by electron dense vesicles which were seen near to the pit plug continuous with tubules or cisternae, with electron dense material occupying their lumens, and some appear to have secreted a major part of their contents (Fig. 30, arrows point at the electron dense vesicles, and arrows with tail point at the vesicles with few contents). This way of pit connection formation is not what has been recorded in the literature. Pit connections were reported to be formed by centriptal polymerization of the content of elongated vesicles, derived from endoplasmic reticulum and aligned parallel to one another within the septal pore (Aghanjanian and Hommersand, 1978; Ramus, 1969), followed by deposition of pit caps, and finally deposition of pit domes which at least partly synthesized by Golgi bodies and transported by the endoplasmic reticulum, or directly by fusion of Golgi vesicles (Aghanjanian and Hommersand, 1978). However, another interpretation which may exist is that the middle part of pit connection of Fig. 29 in the present work may not represent trapped cytoplasm at all, but merely part of the plug that has the same electron density as the cytoplasm, and the vesicles

indicated by arrows at Fig. 30 may represent special vesicles which might play a role in transporting specific material across pit connections, and what appear as tubules or cisternae possibly represent the flux of such material, as a result of bursting or opening of the electron dense vesicles, across the cytoplasm adjacent to the pit connection and across the pit connection itself to the other cell, or vice versa. The role of pit connections in intracellular transport, however, is currently a much debated and controversial subject. This suggestion was put forward much earlier (see Fritsch, 1945). Other speculations regarding their functions include such as a role in spermatia liberation (Duckett et al., 1974; Duckett and Peel, 1978) or sites of mechanical strength (Kugrens, 1980) but there is no direct evidence. Wetherbee (1979, 1980) has reported that specific pit connections (transfer connections) occur between all cells of the carposporophyte of Polysiphonia nova-angliae and differ ultrastructurally from those of most vegetative cells in that the plasmalemma does not bifurcate to enclose the structure and the plug core is less dense fibrous material. In addition these transfer connections of the carposporophyte flare out acropetally. They appear to be intracellular and flaring increases the plug surface area to expedite translocation. This conclusion has been criticized by Pueschel (1980d) and Kugrens (1980). Wetherbee and Kraft (1981) have raised the point again and report that the modification of pit connections in the stalks of Cryptonemia sp., in which they become progressively more convoluted and fluted with increasing distance of the cells from the stalk surface, may serve to aid transport of solutes towards the more deeply-buried layers of living cells.

Plumaria elegans, however, offers interesting scope for gaining more information about pit connections. They were observed between immature paraspores within the parasporangium (Fig. 31) and immature tetraspores within the tetrasporangium (Fig. 32) of the triploid parasporophyte. More studies are needed.

10.5. Mitochondria of paraspores and parasporelings of Plumaria elegans.

One of the most significant features of eukaryotes is the possession of mitochondria, though small numbers of eukaryote cells (e.g. mammalian red corpuscles) contain no mitochondria at all, and were probably lost by degenerative evolution (Whittaker and Danks, 1978). Algal mitochondria have the typical construction found in other plants and animals (Dodge, 1973). A mitochondrion consists of an outer smooth membrane surrounding a highly infolding inner membrane which encloses a central matrix. The infoldings of the inner membrane (cristae) lie in the central matrix (Evans, 1974). Evenly-spaced bristle-like structures have been found projecting from the surface of some mitochondrial cristae in Oedogonium cells undergoing zoosporogenesis (Pickett-Heaps, 1971). These bristle-like structures are possibly related to the elementary particles or oxysomes which have been observed on the cristae of higher organisms (Dodge, 1973). The mitochondrial matrix normally consists of four components. Fine granules constitute a ground substance in which DNA areas, ribosomes of smaller size than the cytoplasmic ribosomes, and calcium-containing granules lie (Gunning and Steer, 1975). Mitochondria vary in shape and size (Evans, 1974; Herman and Sweeney, 1979; Blank and Arnold, 1980). There may be only one mitochondrion per cell but generally there are more (Evans, 1974).

In Plumaria elegans, the mitochondria consist of a double membrane in which the inner one infolds to form cristae (Fig. 33, arrow). The matrix contains an electron transparent area (single section) possessing DNA fibrils (Fig. 33, arrow with tail, Fig. 34 arrow) similar to those reported in other algae (Evans, 1974; Dodge, 1973; Tripodi et al., 1972; Tripodi and De Masi, 1977). In Polysiphonia sertularioides, in addition to the DNA areas, twisted structures interpreted as stages in replication of mitochondrial DNA were observed, and both the DNA areas and the twisted structures were removed after DNase treatment (Tripodi et al., 1972).

Two types of particles were observed in the mitochondrial matrix of Plumaria elegans. The first type of particles may represent the mitochondrial ribosomes (Fig. 33, arrowheads; Fig. 34, arrowheads) and the second type of particles are electron dense, spherical, measure about 0.04 μm in diameter (Fig. 35, arrow with tail, Fig. 35 arrows) and resemble the calcium-containing granules reported in higher plants (Gunning and Steer, 1975). Plumaria elegans mitochondria vary in shape and size. They may assume spherical (Fig. 33), cylindrical (Fig. 35), long cylindrical (Fig. 36) and dumb-bell shapes (Fig. 37), though the spherical configurations possibly represent transverse sections through the other shapes. Occasionally, mitochondria with U-shape configurations were observed (Fig. 38 M1) in which the lateral arms are club-shaped, connected together through a narrow bridge which appears to have cristae. This shape of a mitochondrion (Fig. 38 M1) may represent a longitudinal section through a cup-shaped mitochondrion similar to those reported by Bagshaw et al. (1969). Fig. 39 may represent a cup-shaped mitochondrion, in which the basal region branches towards the mitochondrial pouch as illustrated in Fig. 40A, or the mitochondrial pouch may have been invaded by another mitochondrion (Fig. 40B). In both cases (Fig. 40 A,B) at section level a1,a2 and b1,b2 result in the configuration of the mitochondrion C of Fig. 40 which is a diagram representing the mitochondrion of Fig. 39. Figs. 38 M2, 41 M1, 42 M1 and 43 show mitochondria appearances at first glance to be cup-shaped but some features - within the part which looks like mitochondrial pouches occupied by the cytoplasm - do not support this interpretation (e.g. cup-shaped) and offer another interpretation which may be that what appears as a pouch may in fact represent an additional mitochondrial part or compartment, and therefore each mitochondrion consists of two morphologically different parts or compartments. These features are:

1. Presence of a single partly or completely circular membrane of variable distance from the mitochondrial envelope within the extra part or compartment in some mitochondria (Figs. 41 M1, 42 M1 and Fig. 21 section 6).

2. The extra part or compartment may appear less electron dense than the cytoplasm in some mitochondria (Fig. 42 M1) and ribosomes may be absent.

However, one could argue on the grounds that the above two points are not sufficient to discriminate between a pouch in a cup-shaped mitochondrion and the extra part or compartment of these peculiar mitochondria. In addition to that, this extra part or compartment may in fact represent a pouch in a cup-shaped mitochondrion as illustrated in Fig. 40D, in which the basal region is very narrow and has no internal cristae. The cross section at level d1,d2 (Fig. 40D) gives the configuration of the mitochondrion E of Fig. 40, in which the arrowed part (Fig. 40E) corresponds to the arrowed part of Fig. 41 M1 and the pouch (marked ?, Fig. 40E) corresponds to the extra part or compartment (marked ?, Fig. 41 M1); and the membrane within the extra part or compartment is cytoplasmic membrane (Figs. 41 M1, 42, M1) and the particles are cytoplasmic ribosomes (Figs. 38 M2, 43). However, the arrowed part of Fig. 40E consists of two double membranes sandwiching part of the mitochondrial matrix. The outer double membrane represents the mitochondrial envelope facing the outside cytoplasm while the inner double membrane is the mitochondrial envelope facing the cytoplasm within the mitochondrial pouch. In no case were the extra parts or compartments of the peculiar mitochondria (Figs. 38 M2, 41 M1, 42 M1, 43 and 21 section 6) observed to be partly surrounded by two double membranes sandwiching part of the mitochondrial matrix, which confirms that each of these peculiar mitochondria consists of two morphologically different compartments or parts. Each is completely surrounded by a double membrane only. The function of these extra parts or compartments is not known and any attributed function is speculative. The possibility that part of the glycolate cycle may take place within this extra compartment may possibly exist, supported by the presence of microbodies (possibly peroxisomes) observed on rare occasions in the cytoplasm adjacent to the chloroplasts (Fig. 47). In Ectocarpus, degenerating

mitochondria show concentric double membrane structures in senescent cells (Oliveira and Bisalputra, 1977b).

Some mitochondria in paraspores and parasporangia of Plumaria elegans aggregate in groups (Fig. 35). Such aggregations were reported in monospores of Smithora naiadum (McBride and Cole, 1971) in the fusion cell of Erythrocytis montagnei (Tripodi and De Masi, 1977), in the carposporogenesis of Polysiphonia sertularioides (Tripodi, 1974), and aggregation of giant mitochondria in cells of Gonyaulax polyedra (Herman and Sweeney, 1979). Aggregation of mitochondria were also observed during parasporogenesis of Plumaria elegans, in which the majority of these aggregations were observed to be surrounded (partially or completely) by a sheath of endoplasmic reticulum (Fig. 44). The endoplasmic reticulum may serve in collecting raw material and/or the energy rich compounds produced by the mitochondria (Gunning and Steer, 1975). Tripodi and De Masi (1977) have suggested a functional relationship for the endoplasmic reticulum, which was seen in association with mitochondria, and the genesis of the mitochondria. An increase in the number of mitochondria accompanying growth and differentiation of spores and sporelings of Plumaria elegans was noticeable. However, constrictions were observed along the longitudinal axes of a few mitochondria (Fig. 45 arrow), and this may suggest a binary fission process. A mitochondrion with the inner membrane of the mitochondrial envelope partitioning the matrix compartment into two compartments enclosed by the outer membrane of the mitochondrial envelope, was observed (Fig. 46, arrow pointing at the partition). A constriction between these two compartments may result in separating them into two daughter mitochondria. According to Gunning and Steer (1975), mitochondrial population and continuity from cell to cell is maintained through division of the mitochondria, and they state that the possibility of the mitochondria of a new cell arising de novo is not supported by evidence. Septation of mitochondrial matrix into two compartments, and final separation into two daughter mitochondria, were reported elsewhere

(Whittaker and Danks, 1978; Gunning and Steer, 1975; Hanzely and Schjeide, 1971).

10.6. The occurrence of crystalline bodies in parasporangia of Plumaria elegans.

The crystalline bodies which have been observed in the vegetative cells of Plumaria elegans collected from nature (see section 4) were formed during parasporangial development in culture under the conditions reported in section 2. These crystalline bodies of the parasporangia (Figs. 26, 48) are more or less of the same size as those of the vegetative cells collected from nature. The possibility that they were proteinaceous was discussed earlier, though the histochemical attempts did not completely confirm it (see section 4). However, polyribosomes are abundant during parasporangial development. They were observed either free in the cytoplasm or attached to the endoplasmic reticulum (Figs. 22, 49, 50, 51) but the proportion of the former is more than the latter. According to Gunning and Steer (1975), the visual signal indicative of protein synthesis is the presence of polyribosomes either free in the cytoplasm or attached to the endoplasmic reticulum cisternae, and protein synthesis occurs at both locations, though the presence of numerous rough endoplasmic reticulum within a cell is not necessarily an indication of bulk protein synthesis.

10.7. Contribution of Golgi bodies in wall formation during parasporangial development in Plumaria elegans.

The cell wall of red algal cells typically have a fibrillar framework generally interpreted as cellulose (Duckett and Peel, 1978). The cellulose content of the walls is low (Preston, 1974). Cellulose is absent in the Bangiophyceae and the microfibrils were reported to be xylan (Bold and Wynne, 1978). The amorphous matrix of the cell wall in red algae is usually a sulphated galactan of which agar, porphyran, fucellaran and carageenan are

examples (Bold and Wynne, 1978). The outer layer or cuticle consists mainly of β 1-4 linked mannan which does not appear as microfibrils but is granular (Dodge, 1973). According to Mackie and Preston (1974), cellulose microfibrils may be synthesized by granules lying on the surface of the plasmalemma, but in the Chrysophycean alga Pleurochrysis scherfelli a claim has been made that cellulose (forming scales latterly found external to the plasmalemma) is also synthesized in Golgi bodies (Brown et al., 1970). Involvement of the cytoplasmic microtubules in cellulose deposition is no longer an acceptable theory (Mackie and Preston, 1974). According to Preston (1974), the concensus of opinion concerning biogenesis of the amorphous matrix is that the matrix substances are carried to the wall in Golgi vesicles within which at least part of the synthesis occurs, though O'Brien (1973) quotes plants in which deposition of the non-cellulosic wall components occurs without the intervention of these or other similar membrane bounded bodies.

During parasporeling development in Plumaria elegans, Golgi bodies were observed to be actively releasing small vesicles of about 0.12 - 0.37 μ m in diameter in large quantities (Figs. 52, 20). These vesicles migrate towards the periphery of the cell, fuse with the plasmalemma, and discharge their contents by reverse pinocytosis to form the cell wall (Fig. 53, arrow indicates the site of the fusion). Such involvement of Golgi bodies in the production of wall material has been reported in various algae (e.g. Dodge, 1973; Konrad Hawkins, 1974a; Kugrens, 1980; Aghajanian and Hommersand, 1980). Large elongated vesicles were also observed at different locations within the cytoplasm of the parasporeling cells of Plumaria elegans (Fig. 54). Multilamellar bodies were often observed attached to these vesicles and some of the reticulate contents were located within the concentric membranes of these multilamellar bodies, which suggests that the multilamellar bodies may probably have a role in their secretion (Figs. 55, 56). Multilamellar bodies were also observed free in the cytoplasm of the parasporeling cells (Fig. 59) as well as in the vegetative cells of Plumaria elegans collected from nature (Fig. 33 section 4). The multilamellar bodies of Plumaria

elegans closely resemble the concentric bodies of Batrachospermum moniliforme (Brown and Weier, 1970), Harveyella mirabilis (Goff, 1979b) and the multilamellar bodies of Bangia atropurpurea (Cole and Sheath, 1980). They were also observed using the freeze-etching technique (Brown and Weier, 1970). They were reported to have originated from the secondary vacuoles in Harveyella mirabilis and to have fused with the plasmalemma, or are invaginated into vacuoles (Goff, 1979b). In Bangia atropurpurea, the multilamellar bodies were observed to form single membrane-bound vesicles (Cole and Sheath, 1980).

An interesting feature was observed during the cell wall formation process in Plumaria elegans parasporangia; this is that the microfibrillar framework of the cell wall, in which they are connected by electron dense granules (Figs. 53,54,58), is similar in appearance to the microfibrillar framework of the small vesicles (Fig. 52) and the large vesicles (Figs. 54,55,56). Konrad Hawkins (1974a) also reported a similarity in appearance between the cell wall fibrils and the fibrils of Golgi-derived vesicles. She concluded that Golgi vesicles participate directly in the formation of the matrix material as well as the fibrillar network of the spore wall of Polysiphonia sp.

A very small region at the outermost tip of the wall of the apical rhizoidal cells (only appropriate level of sectioning reveals them) appears to be composed of a loose microfibrillar framework, whereas in upright filaments no such zone occurs in the apical cells. The parasporangia of Plumaria elegans are surrounded by a cuticle of electron dense material of about 0.28 μm thick (Figs. 19,57,58).

It is worthwhile to note that such involvement of Golgi bodies or other cytoplasmic organelles (except the large fibrous vesicles which were formed during the tetrasporogenesis and which were observed in newly released naked tetraspores, see section 5) in cell wall deposition during tetrasporangial development in Palmaria palmata was not observed but plasmalemmasomes were

observed in Palmaria palmata tetrasporelings (Fig. 60) as well as in Plumaria elegans parasporelings (Fig. 61). Plasmalemmasomes, however, are common in lower plants, particularly in fungi, but they also occur in higher plants and algae (Preston, 1974). They were reported using both thin section and freeze-etching techniques (Brown and Weier, 1970; Marchant and Moore, 1973). Their function in wall deposition is a topic of discussion (for references see Verbelen, 1977; Khan, 1976). Cox and Juniper (1973), using autoradiography, believed that plasmalemmasomes in the higher plant Apium graveolens participate in matrix polysaccharide deposition. Others, however, feel that they have nothing to do with cell wall deposition (Preston, 1974; Khan, 1976).

11. General Remarks

1. The cell walls of the three species (Palmaria palmata, Plumaria elegans and Audouinella sagraeanum) reported in the present work are generally similar to those of the other members of the class Florideophyceae in which they are composed of microfibrils embedded in amorphous substances and are covered by a cuticle, though the cuticle is not always clear in Audouinella sagraeanum but this is possibly due to the environmental conditions since they are culture grown plants. Cell walls of the stipe cells of Palmaria palmata and Audouinella sagraeanum show unusual structures which appear not to have been reported before in red algae.

2. The fine-structure of spore formation in Palmaria palmata offers different interpretations to those reported by Pueschel (1979) from specimens from the east coast of the U.S.A. and the possible existence of two races for the plants in different geographical regions which show different pathways of cell organelle differentiation is probably unlikely since some features of the different cell activities are not supported by evidence in Pueschel's paper.

3. Migration of the nucleolus towards a peripheral position within the nucleus and the discharge of its products directly into the perinuclear cytoplasm - as seen in the tetrasporogenesis of Palmaria palmata - may provide new evidence in support of the hypothesis that a direct relationship between the nucleolus and the cytoplasm exists (Bourgeois et al., 1979).

4. Floridean starch grains of the three species reported in the present work generally agree with the reports for the other species of the class Florideophyceae in which they lie freely in the cytoplasm and are of different sizes and shapes. But the presence of small grains of similar electron density to floridean starch grains and probably of the same chemical composition in chloroplasts of Audouinella sagraeanum should be given some further attention. They could be of important taxonomical significance if

they were proved to exist in chloroplasts of the other species of Audouinella by means of electron microscopy since they cannot be detected by the light microscope. These small grains seem not to have been reported, however, in the chloroplasts of Nemalion helminthoides, another member of the class Nemaliales (Duckett and Peel, 1978) or any other red algae (Dodge, 1973; Bold and Wynne, 1978; Lee, 1980). The separation of starch grains into two groups in Plumaria elegans rhizoidal cells appears to be unique (as far as I am aware).

5. Thylakoid arrangement within the chloroplasts (as well as the chloroplasts possessing a pyrenoid) of the three species reported in the present work is generally in correspondence with the thylakoid arrangement within chloroplasts of the other members of the class Florideophyceae. The fission process of chloroplasts of Palmaria palmata is more or less similar to those reported for the other members of the red algae. In Plumaria elegans, it is somewhat comparable (but not exactly the same) to those reported in Nitophyllum punctatum (Honsell et al., 1978). Proplastid formation is similar to those reported in the other red algae.

6. Plasmodesmata were not observed in any of the three species reported in the present work and this is in agreement with the report of their absence in red algae (Wetherbee, 1979; Wetherbee and Kraft, 1981) - at least until it is proved to be present in the other so far unstudied members.

7. The plastid envelope was not observed in association with endoplasmic reticulum in any of the three species and this agrees with the report of their ~~non~~-association in other red algae by Duckett and Peel (1978).

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