CYTOLOGICAL STUDIES ON PHYSODES IN THE VEGETATIVE CELLS OF CYSTOSEIRA STRICTA SAUVAGEAU (PHAEOPHYTA, FUCALES)

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SUMMARY

Physodes have been recognized in meristodermic and promeristematic cells by correlated light- and electron-microscope investigations using different fixation procedures. They are vesicles which contain an osmiophilic material of phenolic nature. Their content changes in appearance according to the fixative used. Osmiophilic deposits are often associated with coiled and disturbed lamellar formations.

It has been possible to distinguish several ultrastructural stages which occur during the secretion of the content of the physodes, namely: a chloroplast accumulation and exudation, and a reticular transport to accumulation vacuoles where materials undergo evolution or hydrolysis. Inside plastids, osmiophilic granules are found in close association with thylakoid stacks. They may contain the polyphenolic precursors of physodes, though this has not yet been proved by electron-microscope procedures. They are expelled from plastids to the chloroplast endoplasmic reticulum. The mechanism of transfer through the chloroplast envelope remains to be elucidated. Lytic activities have been reported inside physodes which might thus act in the same way as the secondary lysosomes of animals and higher plants. Occasionally, the physode content seems to be excreted from the cytoplasm to the cell walls by exocytosis after the probable fusion of plasmalemma and tonoplast.

These cytological changes, observed in the vegetative apex of a brown alga, recall some ultrastructural characteristics of the secretory processes described in various glandular tissues of higher plants and which consist of the synthesis, the transport and the elimination of an exudate of flavonic, terpenic or lipophenolic nature.

INTRODUCTION

One distinguishing feature of the brown algal cell is possession of several refractive and irregularly shaped bodies called ‘fucosan vesicles’ or, more usually, ‘physodes’.

Small light-refracting inclusions, now known to be physodes, were referred to as early as 1847 by Nägeli. Many phycologists have since studied them by light microscopy and light cytochemistry in an attempt to find out their nature and physiological role (see Ragan, 1976). Nowadays, it is generally agreed that vegetative and reproductive cells of Phaeophyceae contain phloroglucinol compounds and other tannins, most of which are included in the physodes. However, many biochemical and cytological problems remain unsolved. As pointed out by Ragan, physodes have not been isolated and characterized by modern biochemical techniques, so that one does not know whether all the physodes enclose phloroglucinol and whether phloroglucinol derivatives are (or are not) associated therein with other compounds. Moreover,
electron-microscope studies have not clarified the nature of brown algal physodes and their biogenesis is still problematical.

This account concerns correlated light- and electron-microscope observations made on the physodes present in the promeristematic and meristodermic cells of the vegetative apex of Cystoseira. Particular attention has been given to the origin of the physode content and to the ultrastructural characteristics of physode secretion.

MATERIAL AND METHODS

Plants of Cystoseira stricta Sauvageau were collected throughout the vegetative growth period from the rocky shores of Provence between Marseilles and Toulon. They were either directly prepared for examination by light and electron microscopy or placed in tanks containing seawater with added mineral salts and left at 18 °C in complete darkness for several days, as described previously (Pellegrini, 1976).

Light microscopy

Living tips of main axes were observed after vital staining in neutral red and cresyl blue diluted in seawater. Other apices were fixed by various procedures using (a) the fixative of Karpechenko modified by Papenfuss (1946) for 15 to 17 h, (b) the fixative of Johansen (1940) for 48 h, or (c) the fixative of Dalton (1955) for 5 h. Then they were washed, embedded in paraffin wax and sectioned for conventional light microscopy. Most of the sections were observed directly without staining.

The chemical nature of physodes was investigated using various specific reactions of phenolic compounds, especially (a) 1 % caffeine in distilled water: the appearance of white globules proves the presence of tannins (Laurent, 1966), (b) 1 % vanillin dissolved either in a freshly mixed 9:1 solution of 95° ethanol and concentrated hydrochloric acid (Ragan & Craigie, 1976), or in 70 % aqueous sulphuric acid (Forrest & Bendall, 1969), (c) diazotized benzidine: the reagent consists of a freshly mixed solution v/v of 0.5 % benzidine in 0.2 N hydrochloric acid and 10 % aqueous sodium nitrite (Jensen & Haug, 1952; Gaillard, 1962). The 2 last reagents are specific for condensed tannins. Fresh sections of apices were immersed for a few minutes in these various stains and observed directly. Sections of fixed apices were also treated with diazotized benzidine.

Electron microscopy

The main branches about 1–2 cm long were cut from young plants. They were quickly immersed in the chosen fixative and, after 15 min, 1–2 mm apical regions were excised and prepared for observation by electron microscopy. Different procedures of fixation, made between 5 and 10 °C, were used: (a) osmium tetroxide fixations for 60–90 min, with either 2 or 4 % osmium tetroxide in acetate veronal buffer (pH 7.4), sometimes with 0.01 % calcium chloride added, or with the chrome-osmium mixture of Dalton (1955); and (b) glutaraldehyde fixations followed by postfixations. Material was fixed for 8 h in 5–6 % glutaraldehyde in phosphate buffer (0.05 or 0.1 M, pH 7.3). It was rinsed in six 30-min changes of the same buffer. Then, it was postfixed either in 2 % osmium tetroxide (same buffer) for 7 h or in 2 % potassium permanganate in veronal buffer for 1 h. Sometimes, 1.5 % potassium ferrocyanide was added to the osmium tetroxide postfixation solution according to Endress & Thomson (1976). Then, fixed apices were rapidly washed in several changes of the same fixation buffer, dehydrated for 15 min each through an acetone or ethanol series, embedded either in Araldite M or in a mixture of Epon and Araldite (Mollenhauer & Totten, 1971) and sectioned on a Porter-Blum MT2 ultramicrotome. Some thin sections were viewed directly using a Philips EM-300 electron microscope at 80 kV. Others were prestained with 2 % aqueous potassium permanganate. The periodic acid-thiocarbohydrazide-silver proteinate method of Thiéry (1967) was sometimes used to obtain a better contrast of cytomembranes: 25 min oxidation in 1 % aqueous periodic acid, 2.5 h in thiocarbohydrazide, 30 min in silver proteinate in darkness. Staining in phospho-
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Tungstic acid–chromic acid (PTAc) was also employed according to Roland, Lembi & Morre (1972): 25 min oxidation in 1 % aqueous periodic acid, 30–50 min in 1 % phosphotungstic acid in 10 % chromic acid.

Acid phosphatase localization

Small apical regions from main branches were prefixed for 3–4 h at 4 °C in the normal buffered glutaraldehyde solution plus 3 or 4 drops of hydrogen peroxide according to Perracchia & Mittler (1972). They were rinsed in a series of citrate buffers of decreasing pH (7–6.5–6–5.5–5) to remove calcium salts. They were then incubated at 4 °C for 2 h and 37 °C for 1 h in the standard medium with sodium β-glycerophosphate as substrate and lead nitrate as coupler. After the incubation wash, specimens were osmicated, dehydrated and embedded normally. Controls were (a) medium with substrate omitted, or (b) complete medium containing 0.01 M sodium fluoride as inhibitor. Thin sections were examined without post-staining.

RESULTS

The different regions of a vegetative apex of Cystoseira stricta can be seen in a longitudinal section (Fig. 1). As in other Fucales, the apical cell (a) is located at the tip of the axis at the bottom of a groove which is filled with mucilage. Its segmentation gives rise to a parenchymatous construction, the 'promeristem' (pm) of Moss (1967). Medullar and cortical cells of the adult thallus result from the differentiation of promeristematic cells. The most external cell layer of promeristem and cortex is the 'meristoderm' (m) (Fritsch, 1945). Meristodermic cells divide to accommodate thickening of the thallus.

Observations on physodes in light microscopy

The physodes appear as inclusions of various sizes. In fresh sections, they stain red with neutral red and turquoise with cresyl blue, differing from the neighbouring iridescent bodies which are refractive formations unstained by these dyes (Fig. 2).

In sections of material fixed in the solutions of Karpechenko (Fig. 1), Dalton (Fig. 3) and Johansen (Fig. 4), phenolic-containing vesicles are more easily distinguished since these fixatives contain various substances – chromic acid, potassium bichromate, ferrous salts or osmium tetroxide – which give black or dark-brown precipitates with tannoids. Such coloured inclusions are located preferentially at the external pole of meristodermic cells and around the nucleus in promeristematic cells (Figs. 3, 4), and are larger in lateral branches (Fig. 1, l).

These inclusions are clearly equivalent to physodes. Moreover, their contents darken with the chrome–osmium fixative (Fig. 3) and, in living material, precipitate as large white globules with caffeine solution (Fig. 5), stain red with vanillin-acid (Fig. 6) and diazotized benzidine (Fig. 7). Therefore, they contain condensed tannins.

Observations on physodes in electron microscopy

General aspect. Physodes have been located, unambiguously, at the ultrastructural level after correlated studies of sections prepared for light microscopy and for electron microscopy. They lie at the external pole of meristodermic cells and scattered throughout the cytoplasm of promeristematic cells (Figs. 8, 9). They are vesicles
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bounded by a thin unit membrane. The fine structure of their contents seems to change according to the fixative used. Comparative observations of promeristematic cells have therefore been made with different fixation procedures.

In cells fixed with buffered osmium tetroxide (Fig. 8), physodes contain an electron-transparent or finely granular matrix and an osmiophilic material. The latter may be a number of small particles or may fill the whole vesicle. It may be alveolate in material kept in darkness for 10 days (Fig. 10). The various aspects of physode content may be observed in one and the same cell, where the physodes can be grouped or confluent (Fig. 8).

The fine structure of physode content fixed according to Dalton reveals striking analogies with the preceding description (Fig. 9). Compounds have reacted with osmium tetroxide and chrome salts in such a way that the resulting micrographs suggest fluid or semifluid contents which have taken various forms in response to the fixative.

Inside cells fixed with glutaraldehyde–osmium tetroxide (Fig. 11), the cytoplasm still contains large or small vesicles with an electron-transparent or finely granular matrix and osmiophilic deposits. However, the appearance of the latter dense material has changed to globular compact masses associated with membrane configurations. With modified postfixation, either by replacing osmium tetroxide with potassium permanganate (Figs. 12, 13) or by adding potassium ferrocyanide to osmium tetroxide solution (Figs. 14, 15), the aspect of the physodes is similar.

Origin and evolution of the physode content. Plastids of meristodermic and promeristematic cells sometimes contain electron-dense inclusions reaching about 0.5 μm long (Fig. 16). These bodies seem not to be surrounded by a membrane. They are frequently in close association with thylakoid stacks. Their central area appears electron transparent, in some cells, probably due to loss of material during fixation and inclusion. The largest and most numerous of them are found in material fixed during spring. They are also present inside the plastids of algae which have been in darkness for several days (Fig. 10).

These dense formations become protruded from the plastids and move either into the chloroplast endoplasmic reticulum (Figs. 17, 18) or directly into the physodes intimately associated with the plastids (Fig. 19). The chloroplast endoplasmic

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Fig. 1. Apical region in longitudinal section showing the general distribution of physodes in a young lateral branch (l) and in the apical (a), promeristematic (pm) and meristodermic (m) cells of the main axis. Karpechenko fixation, benzidine reaction. × 125.

Fig. 2. Physodes (ph) vital stained by cresyl blue are clearly distinct from unstained iridescent bodies (ii) in promeristematic cells. × 800.

Figs. 3, 4. Longitudinal sections of apices fixed according to Dalton (Fig. 3) and Johansen (Fig. 4). Localization of physodes (ph). m, meristoderm. No staining. Fig. 3, × 680; Fig. 4, × 640.

Figs. 5–7. Cytochemical reactions on fresh sections with solutions of caffeine (Fig. 5), vanillin–sulphuric acid (Fig. 6) and diazotized benzidine (Fig. 7) proving the phenolic nature of physodes. × 650.
Fig. 8. Portion of a longitudinal section of material fixed in buffered osmium tetroxide. Physodes (ph) are located beneath the cuticle (c) of the meristodermic cells (m). Different aspects are illustrated in the promeristematic cells (pm). ci, iridescent body; d, dictyosome; mi, mitochondrion, n, nucleus; p, plastid. No staining. × 3500.
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Fig. 9. Portion of a longitudinal section of material fixed in chrome-osmium mixture. The content of physodes (ph), in varying quantities, shows different aspects. c, cuticle; ci, iridescent body; d, dictyosome; mi, mitochondrion; n, nucleus; p, plastid. No staining. x 3350.
Fig. 10. Meristodermic cell after 10 days in total darkness showing iridescent bodies (ci), a physode (ph) and a plastid (p) with osmiophilic granules. Fixation: osmium tetroxide. Thiery test. ×31 400.

Fig. 11. A glutaraldehyde-osmium tetroxide-fixed promeristematic cell with various physodes (ph). Stain: potassium permanganate. × 21 500.
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Figs. 12–15. Aspects of physodes (ph) in material fixed in glutaraldehyde-potassium permanganate (Figs. 12, 13) and in glutaraldehyde-osmium tetroxide-potassium ferrocyanide (Figs. 14, 15). The arrows point to lamellar configurations ci, iridescent body; n, nucleus; p, plastid. No staining.

Fig. 12. Meristematic cell, x 22,600. Fig. 13, promeristematic cell, x 37,000. Fig. 14, promeristematic cell, x 22,600. Fig. 15, promeristematic cell, x 36,900.
Figs. 16–18. Sections of plastids (p) in promeristematic cells showing osmiophilic bodies (ob) closely associated with thylakoid stacks. The thin arrow on Fig. 16 points to a protrusion of dense material. The thick ones on Figs. 17 and 18 show dense material lodged between the 2 chloroplast endoplasmic reticulum membranes (cer). mp, plastidial membrane. Fixation: glutaraldehyde–osmium tetroxide. Fig. 16. potassium permanganate staining, × 51 300. Fig. 17, Thiéry test, × 64 000. Fig. 18, Thiéry test, × 80 000.
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Figs. 19-22. Cytochemical localization of acid phosphatases in promeristematic cells. The arrows on Fig. 20 indicate 2 deposits of lead phosphate inside the dense material of a physode. ci, iridescent body; er, endoplasmic reticulum; p, plastid; ph, physode; vl, lysosome-like vesicle. No staining. Fig. 19, × 39900; Fig. 20, × 28000; Figs. 21, 22, × 31400.
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reticulum connects with the smooth endoplasmic reticulum and the physode envelope (Fig. 23).

The cytochemical test for localization of acid phosphatases allows better understanding of the evolution of the physode content (Figs. 19–22). Lysosome-like vesicles empty into the physodes (Fig. 21) and the enclosed material is slowly lysed. Micrograph 20 shows 2 aggregates of lead phosphate precipitates inside a physode dense deposit, indicating the localized presence of lytic enzymes. The intraplastidial osmiophilic formations do not give a positive reaction to the cytochemical test (Fig. 21). Compounds ejected from the plastids become positive only when they are included inside the vesicles (Figs. 19, 21). There, the products of lysis appear as compact masses and/or unfurled membrane configurations (Fig. 22).

However, the physode content may also be excreted from the cell towards the cell walls (Figs. 24, 25). The physode envelope probably fuses with the plasmalemma which implies a similar configuration of the 2 cytomembranes. These latter clearly show the same positive reaction with the PTAc test (Fig. 26). After the membrane fusion, the physode content may then be discharged against the walls where it lies on both sides of the middle lamella (Fig. 25).

DISCUSSION
Notion of physodes

In the light microscope, brown algal physodes are distinguished by their reactivity with neutral red, cresyl blue, caffeine, vanillin–hydrochloric acid and diazotized benzidine. The first 2 compounds are vacuolar vital stains; the last 3, precipitate phenolics. This is evidence that physodes are phenolic-storing vacuoles.

In the electron microscope, the identification of physodes is more difficult. Indeed, one cannot adapt light-microscopic methods for they profoundly alter cell contents. However, a few workers have tried to locate phenolic compounds in higher plants at the ultrastructural level either by fluorometry (Charrière-Ladreix, 1976) or by adding to fixatives potassium bichromate (Dumas, 1975), ferric sulphate (Endress & Thomson, 1976), caffeine (Mueller & Greenwood, 1978) or else ferric chloride (Robb, Brisson, Busch & Lu, 1978). Tannins have also been located by their strong affinity for osmium (Baur & Walkinshaw, 1974; Chafe & Durzan, 1973). In Cystoseira, the localization of physodes has been realized on the basis of comparative fixation.
techniques in light and electron microscopy by adding to fixatives chemicals which react with phenolics.

Electron-opaque vesicles are confined to the external pole of meristodermic cells and around the nucleus of promeristematic cells. They occupy the same location as the phenolic vacuoles which stain vitally with neutral red and cresyl blue and whose contents precipitate with caffeine, vanillin–hydrochloric acid and diazotized benzidine. It is clear that these vesicles are the physodes. The present account shows therefore that physodes of *Cystoseira* cells are really equivalent to higher plant tannin vacuoles.

It agrees with the ideas of Mangenot (1930) who implied the same from light-cytological investigations on several Phaeophyceae.

**Genesis of physodes**

The development of higher plant vacuoles involves several successive stages: (a) formation of provacuoles, (b) fusion of provacuoles into small vacuoles, and (c) extension of the vacuoles. Sometimes, other processes may occur during the last 2 stages: (a) association with reticular profiles, (b) intervention of lysosomal activities, and (c) accumulation of various substances, for example, tannins.

The genesis of physodes in *Cystoseira* must involve these processes, some of them related to the vacuole proper and the others more especially to the secretion product which is enclosed therein. The origin and development of vacuoles in *Cystoseira* have been described in another paper (Pellegrini, 1978). They involve the sequestration of cytoplasmic materials by formations originating probably from GERL as in some higher plants (Buvat, 1977; Buvat & Robert, 1979; Marty, 1974, 1978). Some of these vacuoles may evolve into physodes after accumulation of phenolics. The secretion of the physode content consists of several successive stages: plastidial accumulation and exudation, reticular transport to accumulation vacuoles, and excretion into cell walls. A few of these processes were suggested as early as 1974 (Pellegrini, 1974a, b).

**Plastidial accumulation.** The first phase of physode secretion is seen inside the plastids. These osmiophilic inclusions are rarely observed in osmium tetroxide-fixed material, except when the algae live in darkness for a long time. They may often be seen inside cells fixed by glutaraldehyde and postfixed by osmium tetroxide or potassium permanganate. However, they do not always seem to be present in all materials collected during 1 year’s growth. This suggests that physode activity may change according to the season. In several *Cystoseira* species of the Adriatic, the highest amounts of reducing compounds (e.g. polyphenols) are measured during winter and during spring, with a maximum in March (Munda, 1962). Large intra-plastidial inclusions are most numerous in *Cystoseira stricta* apices collected in March. They are irregularly shaped formations, not surrounded by a membrane.

Plastidial bodies, more or less identical in appearance, have been reported (a) in other Phaeophyceae (McCully, 1968; Evans, 1968; Magne, 1971; Bisalputra, Shields & Markham, 1971; Feldmann & Guglielmi, 1972; Evans & Holligan, 1972; Bisalputra, 1974; Oliveira & Bisalputra, 1977a, c; Forbes & Hallam, 1978) and (b) in different higher plant cells which elaborate terpene-like resins (Wooding & Northcote, 1965; Carde, 1976), essential oils (Amelunxen & Arbeiter, 1967; Amelunxen &
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The chemical nature of the dense inclusions in brown algal plastids has been interpreted in various ways (Magne, 1971; Feldmann & Guglielmi, 1972; Evans & Holligan, 1972; Bisalputra, 1974; Oliveira & Bisalputra, 1977a). As in Evans & Holligan’s study, the present investigation suggests, though does not likewise prove by electron-cytochemical procedures, that they may contain the polyphenolic precursors of physodes. Several papers would seem to confirm this suggestion, although most of them refer only to higher plants. Indeed, phenolics are considered as usual components of angiosperm plastids and their amounts may fluctuate according to the season (Monties, 1969). Flavonoids have been detected by fluorometry inside the plastids of *Populus nigra* (Charrière-Ladreix, 1976). Concerning Phaeophyceae, Davies, Ferrier & Johnston (1973) wrote that biochemical studies made in their laboratory ‘indicate that polyphenol precursors appear to be derived from plastids, and fractionation studies provide evidence for the presence of enzymes involved in the metabolism of phloroglucinol-containing phenolics’. Our attempts to locate polyphenoloxidases inside *Cystoseira* plastids by electron cytochemistry are still unsuccessful. However, Davies et al. added: ‘the degree of polymerization achieved in or on leaving the plastid may vary, and similarly any interaction with other metabolites and the species involved’. This might explain difficulties in obtaining positive cytochemical results in some cases.

**Plastidial exudation.** The dense globules of *Cystoseira* plastids are ejected into the chloroplast endoplasmic reticulum (Figs. 17, 18). Transfer through the chloroplast envelope has not still been elucidated.

Similar protrusions of portions of plastids have been mentioned in *Dictyota* (Evans & Holligan, 1972) and in *Ectocarpus* (Oliveira & Bisalputra, 1977a). Plastidial exudations of osmiophilic material into the endoplasmic reticulum surrounding plastids also occur during secretory processes in various higher plants, for example in mature resin canal cells of *Pinus picea* (Wooding & Northcote, 1965), in oil cells of *Acorus calamus* (Amelunxen & Gronau, 1969), in transfer cells of *Pinus pineaster* leaves (Carde, 1973), in glandular cells of *Forsythia intermedia* stigma (Dumas, 1975, 1977) and in glandular tissue of *Populus nigra* buds (Charrière-Ladreix, 1973, 1978).

**Reticular transport.** Electron-opaque material has been shown inside the chloroplast endoplasmic reticulum and then inside vacuoles. Two types of association must therefore assist the transport, one between plastids and the chloroplast reticulum, the other between this reticulum and physodes.

Brown algal plastids are surrounded by a reticular profile: the ‘chloroplast endoplasmic reticulum’ (see Gibbs, 1962, 1970; Dodge, 1973; Bisalputra, 1974).

Associations between plastids and the endoplasmic reticulum have also been found among higher plants, in various secretory cells or tissues but always in materials with effective metabolic functions (see Dumas, 1974b for a review) where they would be involved in the synthesis and transport of several compounds (Galatis, Apostolakos & Hatzopoulou, 1974; Dumas, 1974b, 1975; Whatley, 1977).
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In Phaeophyceae, Bouck hypothesized as early as 1965 the participation of chloroplast endoplasmic reticulum during the transport of elaborated products. This intervention might concern (a) carbohydrate transport where the chloroplast endoplasmic reticulum would play an important part during the transport of alginic acid precursors from plastids to dictyosomes (Leppard, 1973, 1974), and (b) transport of polyphenolic precursors to the physodes. Dumas (1974b) discussed the role of plastid-ER associations in phenolic synthesis during stigmatic secretion in Forsythia and Verbascum. He suggested 2 pathways. In one, precursors would be synthesized inside plastids and then collected by the periplastidial reticulum. In the other, this reticulum might bring essential precursors of polyphenolic biogenesis towards the plastids. It seems likely that the first Dumas' possibility may occur in Cystoseira. We cannot, of course, rule out the second, but Fig. 16 suggests more an exudation than an intrusion of dense material into the plastid. This material is clearly lodged between the 2 endoplasmic reticulum membranes (Figs. 17, 18). In Dictyota, a process of extrusion of bodies from the chloroplast matrix had also been seen but Evans & Holligan (1972) thought that these bodies were 'probably surrounded by a membrane' and that the chloroplast endoplasmic reticulum was not involved in the transport of these 'physodes'. However, it is difficult to imagine the mode of transfer of a vesicle through membranes whereas the crossing of material only assumes a particular permeability of these membranes to certain molecules.

Electron-dense bodies have also been reported closely associated with either the periplastidial reticulum or with the plastids in Laminaria (Davies et al. 1973), Fucus (Brawley, Quatrano & Wetherbee, 1977) and Cutleria (La Claire & West, 1978). Membrane continuities between the periplastidial reticulum and the chloroplast envelope have been described in Ectocarpus by Oliveira & Bisalputra (1973, 1977b). Similar continuities have been observed in Cystoseira but only with a low frequency, so that the possibility of fixation artifacts must also be considered (Pellegrini, 1978). It is evident that these membrane contacts might facilitate substance exchanges.

The present observations closely resemble the plastidial origin and reticular intervention during the processes of synthesis and transport of the terpene-like resin precursors in Pinus secretory cells (Wooding & Northcote, 1965; Carde, 1976). They also agree with the suggestions of Mueller & Beckman (1974) about polyphenolic synthesis in specialized cells of banana. It will be of interest to find if a similar pathway of physode formation takes place during cell differentiation of other Phaeophyceae.

In Cystoseira, the chloroplast endoplasmic reticulum connects the smooth endoplasmic reticulum and the physode tonoplast. These latter associations are probably temporary for they are seldom observed. They may facilitate exchanges between cellular compartments.

Some connexions between the endoplasmic reticulum and vesicles have been reported (a) in 3 other brown algae: Dictyota (Feldmann & Guglielmi, 1972), Ectocarpus (Oliveira & Bisalputra, 1973) and Laminaria (Davies et al. 1973), and (b) in various higher plants (Berjak, 1972; Fineran, 1973; Dumas, 1974a, 1975; Baur & Walkinshaw, 1974; Endress & Thomson, 1976; Carde, 1976; Ramsey & Berlin, 1976; Kristen, 1977; Coulomb, 1978). In particular, these ER-vacuole contacts might
function during the tanniferous synthesis in banana (Mueller & Beckman, 1974) or Pinus elliottii cells (Baur & Walkinshaw, 1974) and during the lipo-polyphenolic secretion of Forsythia stigma (Dumas, 1975, 1977). They would also allow a regulation of the synthesis of terpene hydrocarbons in Pinus pinaster (Carde, 1976).

**Vacuolar accumulation.** Dense material is carried to physodes where it is accumulated sparsely or in plentiful quantities. Sometimes, amorphous electron masses are associated there with membrane configurations.

Most ultrastructural studies on higher plants confirm that phenolics are stored inside vacuoles where they appear either as dense globules or as lamellar bodies which may or may not be enclosed inside osmiophilic and amorphous deposits. The presence of lamellar configurations raises some problems. They have been reported in animal cells and in cells of higher and lower plants. Their significance is not clear and various interpretations are possible. Firstly, lamellar formations might result from modifications of membranar systems, especially the ER and the tonoplast. That view has sometimes been taken into consideration to explain the origin of myelin-like material included inside brown algal vesicles or physodes (Feldmann & Guglielmi, 1972; Oliveira & Bisalputra, 1973; Loiseaux, 1973; Brawley, Wetherbee & Quatrano, 1976; Forbes & Hallam, 1978). Secondly, lamellar formations might represent some partially lipid aggregates which would be more or less hydrated (Curgy, 1968; Marty, 1974). Recently, Mollenhauer, Morrè & Jelsema (1978) proposed lamellar bodies as intermediates in endoplasmic reticulum biogenesis from seed reserves. They reported that these myelin-like figures would be produced by the transformation of some protein bodies. These different suggestions do not seem to justify the origin of the lamellar content in brown algal physodes because no lipid and no protein have been detected there by modern cytochemical or biochemical procedures (see Ragan, 1976). Thirdly, lamellar formations might occur in relation to cellular lysis as Coulomb & Buvat (1968) and Coulomb (1972) considered to be the case for vacuoles of higher plants. It would seem that the last interpretation would be reasonable for *Cystoseira* physodes where myelin-like arrangements are often disturbed, which would involve the intervention of a lytic process.

**Destiny of the secretion product.** Lytic enzymes have been located inside physodes which could thus act in the same way as the secondary lysosomes of animals and higher plants. The appearance of their contents would change according to the intensity of lytic processes which occur therein. These variations of aspect have been reported in several works (Chadeau, 1932, 1934, 1935; Ando, 1951, 1958; McCully, 1966, 1968; Feldmann & Guglielmi, 1972; Rawlence, 1973). However, the present account also suggests that the various appearances of physode content observed in *Cystoseira* cells may relate to several stages of an evolutionary process: the phenolic secretion consisting of a synthesis and a transformation of phenolics. On the other hand, these observations do not exclude the possibility of the presence of several biochemical classes of physodes both within brown algal orders and within distinctive species.

In relation to the above, it is important to consider whether the phenomenon of hydrolysis is compatible with the usually accepted ideas on tannoid metabolism in plant cells. None of the cytochemical and biochemical investigations on Phaeophyceae
provide evidence on this but several studies of higher plant cells may do so. In particular, Vaudois & Laurent (1976) have reported, during cell differentiation of the fern prothallus, the existence of a morphological evolution of vacuoles. This evolution was associated with concomitant variations of the appearance and amounts of tannoids included in the vacuoles. The phenolic content underwent successively an impoverishment, which came from the preparation of materials, and a synthesis which included biogenesis of monomers, their condensation and finally a hydrolysis of tannoids. Hence, the tannins of higher plants are currently considered to be both waste products and substances which might provide some metabolic requirements. Similarly, it is likely that turnover or chemical evolution of phenolics may occur inside brown algal physodes. The presence of oligomers and polymers of phloroglucinol has been reported in extracts of Halidrys (Glombitza & Sattler, 1973), Bifurcaria (Glombitza & Rößner, 1974) and Fucus (Ragan & Craigie, 1976). As pointed out by Ragan (1976), the polymerization of phloroglucinol units might explain the ‘aging process’ of the physodes mentioned by a few early cytologists. Biochemical and development investigations of several Phaeophyceae should help to clarify the situation.

The physode content in Cystoseira cells may be excreted by a typical process of exocytosis which takes place after the probable fusion of plasma membrane and physode envelope. There may exist a mechanism of membrane flow similar to that described by Vian & Roland (1972) and Prat, Vian, Reis & Roland (1977) which would contribute to plasmalemma enlargement and produce the synthesis of wall components. An excretion of physodes has been reported by Chadefaud as early as 1935 in light-microscopic studies. At the ultrastructural level, Loiseaux (1973) observed, in the developing zoospores of Elachista and Pylaiella, physodes which were expelled from the cytoplasm, then located between the plasmalemma and the cell wall and finally integrated into the cell walls. Caram (1977) reported diffusion of physodes through the walls in the female gametangia of Cutleria adspersa. La Claire & West (1978) also noted during the gametogenesis of Cutleria hancockii a secretion of osmiophilic material into the extracytoplasmic spaces and finally into the medium. The presence of phenolic substances in the sea, excreted from various brown algae, has also been mentioned in several biochemical works (see Hellebust, 1974).

CONCLUSION

It is becoming evident that physodes are equivalent to the specialized vacuoles storing phenolics in higher plants but there has been only one previous ultrastructural study on the origin of their contents, Evans & Holligan (1972) having described a plastidial origin of physodes in Dictyota. The present account confirms the participation of plastids in the secretion of physode contents in Cystoseira but also allows the elucidation of some further cytological stages of the secretion which have not been reported before. This secretory process must consist of several successive stages. Firstly, there is a phase of plastidial secretion in which osmiophilic material is found in close association with the thylakoids. This material is secreted more actively during the spring than during other seasons of the annual growth cycle. Next, it is expelled
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from the plastids by an unknown mechanism which must concern the chloroplast envelope and the chloroplast endoplasmic reticulum and which implies a particular permeability of these membranes. Thirdly, there is a phase of intracellular secretion. The material from the plastids is carried by the chloroplast endoplasmic reticulum and then by the endoplasmic reticulum to vacuoles where it is accumulated and may undergo an evolution. Lytic processes occur inside physodes which thus function in the same way as the secondary lysosomes of animals and higher plants. Fourthly, a phase of extracellular secretion is occasionally observed. The release of physode content is facilitated by a typical exocytosis.

The infrastructural characters of the physode secretion in Cystoseira recall some peculiarities which have been mentioned during glandular processes in higher plants, especially the role of plastids in the elaboration of secretory products which will then be exuded and the role of the endoplasmic reticulum in the transport of these products to vacuoles. These comparisons strongly suggest that the physodes of Cystoseira are not special formations. It also suggests that their biogenesis fits into the general pattern of secretory phenolic activities in plants.

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