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CORRELATED LIGHT AND ELECTRON MICROSCOPE STUDIES ON BROWN ALGAE

I. LOCALIZATION OF ALGINIC ACID AND SULPHATED POLYSACCHARIDES IN *DICTYOTA*

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Summary

By electron microscopy and light microscope histochemical techniques, alginic acid and sulphated polysaccharides (probably largely fucoidan) have been located in the cell walls of *Dictyota dichotoma*.

The cell walls of the vegetative thallus and those of mature reproductive cells (tetrasporangia, tetraspores, antheridia and oogonia) are composed of three to four well-defined layers, mainly of alginic acid, sulphated polysaccharide or a mixture of these substances. Prior to their secretion the two types of polysaccharide can generally be detected in conspicuous, peripheral, intracellular cytoplasmic vesicles. Alginic acid is liberated from vesicles through the plasma membrane by reversed pinocytosis.

The results are discussed in relation to other work on the polysaccharides and histochemistry of brown algae.

Introduction

Perusal of the considerable literature published over the last eighty years or so on aspects of the cytology of brown algae (for a review of some of these see Fritsch (1945)) will reveal many inconsistencies, conflicting opinions and large gaps in our knowledge with respect to the origin, development, chemical nature and physiological significance of the various intracellular inclusions and extracellular products. This is largely due to the inadequacy of the techniques hitherto available for detailed histochemical work on plants. Whilst modern methods of electron microscopy can help to clarify this situation, the use of these methods alone imposes obvious limitations in this sort of investigation and leaves many uncertainties, in particular with respect to the identification of the many substances revealed. However, there is now considerable information, gained from chemical analyses, on the range of polysaccharides and other materials produced by brown algae (for a summary see Black *et al.*, 1953, and Percival and McDowell, 1967). Using this information combined with electron microscopy and procedures allowing resolution of fine intracellular details with the light microscope (Feder, 1960; Ashley and Feder, 1966; summarized by Feder and O'Brien, 1968) many long-standing histological anomalies may now be resolved.

The first investigations on brown algae using these newly developed methods, in conjunction with electron microscopy, were carried out on the genus *Fucus* by McCully (1965, 1966a, b, 1968b, 1969) and clearly the techniques are of considerable value for brown algal histology. We (Evans and Holligan, 1969) applied the techniques first to

some of the unsolved histochemical problems revealed during an electron microscope study of the distribution of pyrenoids amongst certain brown algal orders (Evans, 1966, 1968). The results presented in the present paper mainly concern the application of these methods to determine whether alginic acid and sulphated polysaccharide (probably fucoidan) are present in the thallus and reproductive cell walls of *Dictyota dichotoma* (Huds.) Lamour. The formation of extra-cellular material by peripheral vesicles, abundant in epidermal and apical cells but present also in medullary cells, oogonia and tetrasporangia, is also reported.

MATERIALS AND METHODS

Plants of *Dictyota* were collected from the shore at Trearddur Bay, Anglesey, North Wales, during low water of spring tides. Whenever possible fixations were carried out on the sea shore.

Electron microscopy

Material was generally fixed for 5 hours in 4 or 5% glutaraldehyde in 0.1 M cacodylate or phosphate buffer, pH 7, containing 0.25 M sucrose. The fixative was then washed out by three half-hourly washes in 0.1 M cacodylate or phosphate buffer of decreasing sucrose content. This was followed by post-osmication in 1 or 2% osmium tetroxide in 0.1 M cacodylate or phosphate buffer for periods ranging from 5 hours to overnight. Dehydration in an ethanol series was followed by transfer to propylene oxide and the material was embedded in Epon 812. Fixation, buffer washes, post-osmication and dehydration as far as 95% ethanol were carried out on ice. Sections were cut with a diamond knife on an L.K.B. Ultratome, stained with lead citrate (Reynolds, 1963) and examined with a Siemens Elmiskop 1 electron microscope.

Light microscopy

Material fixed in 5% glutaraldehyde and embedded in Epon, though primarily for electron microscopy, could be examined with the light microscope. For this, 1-μm sections (often of a block being cut for electron microscopy) were floated off in 10% acetone (Hoefert, 1968) and put in drops of the same liquid on a slide which had previously been cleaned and covered with a minimum amount of albumen. The slides were heated to expand the sections and to evaporate the acetone. They were then exposed to bromine vapour for 30 seconds, washed in acetone for 10 minutes to remove the Epon (Yensen, 1968), mounted in dammar xylene and examined with phase or interference contrast microscopy. Occasionally such sections were stained and examined by ordinary transmitted light microscopy; the material was then stained with toluidine blue (see later) before mounting in dammar xylene.

Generally, material for light microscope histochemical studies was fixed for 24–48 hours at 0° C in either 10% acrolein (Feder, 1960) in tap or sea water, or in 10% formalin in tap water. McCully (1966a), Ashley and Feder (1966) and Feder and O'Brien (1968) give detailed schedules for fixation in acrolein and for the subsequent dehydration, embedding, sectioning and staining procedures so that only an outline will be given here. Following fixation, dehydration of *Dictyota* material was carried out on ice, the material being immersed in turn in the following solvents: methoxyethanol, absolute ethanol, *n*-propanol and *n*-butanol. Transfer of the material from one solvent to the next was usually broken into a series of graded steps, each step consisting

of mixtures of the two solvents in the ratios 2 : 1, 1 : 1, 1 : 2, 3 : 0. The material was left in each mixture for 2–3 hours and in the pure solvents for 15 hours the latter being changed after 6 hours.

After dehydration, the material was brought to room temperature and immersed for up to 1 week in a mixture of equal parts of *n*-butanol and monomer mixture. The tissues were then placed in monomer mixture alone and left in the dark at room temperature for a week. The monomer mixture consisted of a monomer glycol methacrylate (96% hydroxyethyl methacrylate) to which was added 0.15% of a catalyst, 2,2′-Azobis (2-methylpropionitrile) and 5% of a plasticizer, polyethyleneglycol 200. Before use the monomer was purified (to remove methacrylic acid) by passage through a column of Amberlyst A-21 resin. Subsequent polymerization was most successful if the monomer was then passed through activated charcoal. Following infiltration the material was covered with fresh monomer in an aluminium weighing dish (Catalogue No. 8-732-5, size B, made by Fisher Scientific) as described by Feder and O'Brien (1968). A second similar dish was then placed in the first to exclude air. Polymerization was carried out at 60° C for 16 hours.

One-μm sections were cut with a glass knife and mounted in drops of membrane-filtered distilled water on slides; evaporation of the water caused the sections to stick to the slides. The sections were stained with either 0.05% toluidine blue O in sodium benzoate buffer at pH 4.4 (Sidman, Mottla and Feder, 1961) or in 0.1 M phosphate buffer at pH 6.8 (O'Brien, Feder and McCully, 1964); in 1% acid fuchsin in water (Feder and O'Brien, 1968) or in acidified acid fuchsin (Robinow and Marak, 1966; McCully, 1966a); or by the periodic acid-Schiff (PAS) reaction (Jensen, 1962; McCully, 1966a, Feder and O'Brien, 1968). Before carrying out the PAS reaction aldehyde groups introduced by acrolein fixation were blocked by immersing slides in chlorous acid (Rappay and Van Duijn, 1965) for 30–60 minutes. The slides were then put into 1% periodic acid for 10 minutes, washed, and immersed in Schiff reagent for 1–2 hours (Feder and O'Brien, 1968). All stained material was mounted in dammar xylene.

Light microscopy was also carried out on material stained with the dyes alcian blue and alcian yellow. Such material was either fixed in acrolein and embedded and sectioned in glycol methacrylate as already described, or fixed in 10% formaldehyde, dehydrated through a tertiary butyl alcohol series and embedded in paraffin wax as described by Parker and Diboll (1966). The tissue was then stained for 30–60 minutes in 0.5% aqueous alcian blue at pH 0.5, washed in distilled water and stained for a similar time in 0.5% aqueous alcian yellow at pH 2.5.

Cellulose was detected by the potassium iodide solution/sulphuric-acid method (Johansen, 1940). The schedule followed was basically that of Jensen (1962). Sections of living thallus were mounted on a slide in 0.2% iodine in 2% aqueous potassium iodide solution and the slide irrigated with a few drops of 65% sulphuric acid.

Alginic acid was extracted from fresh pieces of thallus by treatment for 3 hours in 3% sodium carbonate at 30° C. Extraction of fucoidan (O'Colla, 1962) was carried out by boiling fresh thallus pieces for 10–15 minutes in distilled water.

Light microscope observations were carried out with a Reichert Zetopan microscope using apochromatic or planachromatic objectives, and, for unstained material Reichert phase contrast and transmitted-light interference contrast equipment was used. Black and white photographs were taken on Ilford FP4 35-mm film or plates, and colour photographs on Kodachrome II 35-mm film or Ektachrome type B sheet film. The Reichert Photoautomatic system was used for both black and white and colour 35-mm

film and the Reichert plate camera, with an additional base-shutter to reduce vibration, for the black and white plates and the colour sheet film.

RESULTS

General histology

It is now well known (Barka and Anderson, 1965; McCully, 1966a) that thiazin dyes, such as toluidine blue, stain compounds with free carboxyl, sulphate or phosphate groups metachromatically, whilst a positive reaction with PAS is generally accepted as indicating the presence of polysaccharides with adjacent free hydroxyl groups (Hotchkiss, 1948; Jensen, 1962). The four principal brown algal polysaccharides are cellulose, laminaran, the polyuronic acid alginic acid and the sulphated polysaccharide fucoidan (Percival and McDowell, 1967). A number of fucose-containing sulphated polysaccharides occur in brown algae in addition to fucoidan. Percival and McDowell (1967) consider it desirable to name these glucuronoxylofucan sulphates, and since it is not possible to differentiate histochemically between these and fucoidan, the general term sulphated polysaccharides will be used in this paper to refer to the whole complex. Although cellulose would be PAS positive, it would not stain metachromatically with toluidine blue. Polyphosphates, known to occur in algae (Kuhl, 1962) are not present in brown algae as far as is known and so are not relevant to the present work. From the work of McCully (1965, 1966a, 1968a, b) on the brown alga *Fucus*, it can be seen that the intercellular PAS-negative polysaccharide fucoidan stains pink metachromatically with toluidine blue, whilst the PAS-positive carbohydrate alginic acid which occurs mainly in the cell walls stains a metachromatic reddish purple colour (Plate 1, No. 1).

Staining living material with a solution of iodine in potassium iodide followed by sulphuric acid treatment, results in a characteristic heavy blue reaction in the cuticle. This test is regarded as a fairly reliable indicator of cellulose, though hemicelluloses may also stain blue (Whaley, Mericle and Heimisch, 1952). These, however, are generally restricted to land plants (Percival and McDowell, 1967).

Thallus cells

Careful light microscope examination of sectioned *Dictyota* thallus shows that the thick outer epidermal cell wall is composed of three distinct layers (Plate 2, No. 9). There is a wide outer 'cuticle' which stains deep purple with toluidine blue and which is highly PAS positive. On the basis of the staining methods described it would appear that the 'cuticle' is composed of alginic acid together with cellulose and perhaps other materials. Beneath the 'cuticle' is a narrower middle layer which stains deep pink with toluidine blue and this layer generally varies from being PAS negative to moderately PAS positive. It is continuous with the narrow 'middle lamella' which lies between adjacent lateral epidermal cell walls, between basal epidermal walls and medullary cells, and between the medullary cells themselves. The middle layer is also in continuity with the material occupying the small intercellular spaces which occur, e.g. where anticlinal epidermal cell walls abut on to medullary cells. This layer is likely to be composed of a mixture of sulphated polysaccharides and alginic acid. The innermost layer of the outer epidermal cell wall is also narrow and it continues around the anticlinal and basal walls of the cells. It stains reddish purple metachromatically with toluidine blue and is PAS positive. Alginic acid is almost certainly the main if not the sole component of this inner layer.

Seen with the electron microscope (Plate 2, No. 10) the 'cuticle' often appears two-layered. This is usually most apparent in young cells. There is an outer, more amorphous layer where alginic acid may predominate and an inner finely fibrillar layer perhaps of cellulose or other polysaccharides. The fibrillar and characteristically more electron-dense middle layer is clearly visible and is continuous with the narrow band of material of similar appearance which forms the 'middle lamella' between the lateral epidermal cells walls. The innermost homogeneous layer of the outer epidermal cell walls, which is less granular and more finely fibrillar when mature than when first laid down and which is believed to be composed mainly of alginic acid, is also distinguishable in Plate 2, No. 10. Thicker areas of the anticlinal epidermal walls in between thinner areas (which often contain pores), appear to be the result of increase in the inner cell wall layer on either side of a less thickened 'middle lamella' (Plate 4, No. 15). Medullary cells are circumscribed by a narrow peripheral band of material similar to the innermost layer of epidermal cell walls, on the outside of which is the 'middle lamella' between adjoining cells.

Alginic acid is confirmed as a major constituent of the walls by differential extraction with sodium carbonate solution or the sodium salt of EDTA. Whilst the 'cuticle' on the outside of the outer epidermal cell walls is not visibly affected by this treatment, the middle layer is partially removed and the innermost layer very markedly reduced. Similarly with the other epidermal cell walls and medullary walls, the 'middle lamella' is partially removed, leaving a space and the inner layer very extensively reduced. The small amount of material remaining in the 'middle lamella' after treatment probably consists of sulphated polysaccharides. Although these results indicate a low alginate content in the cuticle, it is likely that the composition of this alginate in mature outer walls renders it less soluble. It has been shown that where glucuronic acid units predominate, alginate is more resistant to attack (Percival and McDowell, 1967). Application of a technique known to remove fucoidan caused no significant change in the staining or other visible properties of the walls, so the amount of fucoidan present is not likely to be great. However, glucuronoxylofucan sulphates which are unlikely to be affected by this treatment may still be present.

The use of alcian dyes (Ravetto, 1964; Lev and Spicer, 1964; Parker and Diboll, 1966) also supports the conclusion that *Dictyota* cell walls consist largely of alginic acid, in marked contrast with the observations of Parker and Diboll (1966), who found that in *Taonia automaria* (Woodw.) J. Ag. fucoidan predominated.

Tetrasporangia

The constitution of tetrasporangial walls changes with their development. Young tetrasporangia (Plate 1, No. 4) are enclosed in an outer layer which stains deep purple with toluidine blue and is very strongly PAS positive. This layer appears to be continuous with the similarly staining 'cuticle' over the epidermal cells, and like it, is considered to be mainly composed of alginic acid, though possibly some cellulose and perhaps other materials may be present as well. Beneath the outer layer of young sporangia is a thin second layer which stains a pale purple-pink with toluidine blue and is moderately PAS positive. It is likely to consist of a mixture of sulphated polysaccharides and alginic acid. In older tetrasporangia (Plate 1, No. 5) a thicker third layer which stains reddish purple with toluidine blue and is PAS positive develops beneath the second layer. This third layer is considered to be predominantly composed of alginic acid. At about the time the nucleus undergoes division into four, a fourth inner wall layer starts

to appear and this rapidly increases in thickness. It stains a deep pink with toluidine blue, is PAS negative and its chief constituent is undoubtedly sulphated material. During cytoplasmic cleavage further amounts of this are secreted to fill the region between the four separating protoplasts within the sporangium (Plate 1, No. 5). As cleavage proceeds, small gaps appear in those regions of the intersporangial wall that lie opposite the junctions between the four spores. These extend into the material between spores so that each individual spore within the sporangium becomes invested by its own complete sheath of sulphated material. Finally, beneath this sheath, a wall of material showing the characteristic staining reactions of alginic acid appears. Inside this is the plasma membrane of the spore. Plate 2, No. 11 shows the wall layers as seen with the electron microscope.

Antheridia

Like tetrasporangia, antheridial sori are covered by a 'cuticle' whose main component is considered to be alginic acid. Beneath the 'cuticle' and extending downwards between individual antheridia, is a region whose staining reactions are consistent with the presence of a mixture of alginic acid and sulphated polysaccharide. Occasionally discontinuous areas of material with the staining properties of alginic acid alone occur in this mixture. Each antheridium within the sorus is further enclosed completely by a wall of alginic acid and extensions of this continue into the antheridium to form the 'middle lamellae' of the male gamete-containing compartments (Plate 1, No. 6). On each side of the 'middle lamellae' and forming a wall inner lining to each compartment is a thick layer of sulphated material. Inside this is the plasma membrane enclosing the protoplast of the male gamete. Plate 3, No. 12 shows the antheridial wall layers with the electron microscope.

Oogonia

Oogonial sori also are invested by an outer layer whose staining properties suggest alginic acid to be its main constituent. This layer is continuous with the 'cuticle' on the epidermal cells. It occurs as a horizontal layer over the oogonial surfaces (though it is of course present on the sides of oogonia at the edges of a sorus) and extends vertically for short distances where oogonia are more loosely packed. Beneath the outer layer is a middle layer whose staining characters suggest a mixture of alginic acid and sulphated polysaccharide (Plate 1, No. 7). As the oogonium approaches maturity (Plate 1, No. 8) a thick inner sulphated layer is laid down round the inside of the oogonial wall. Plate 3, No. 13 shows the three wall layers with the electron microscope. The single enclosed ovum lacks the wall of alginic acid present round mature tetraspores and is bounded only by a plasma membrane. The use of alcian blue and yellow supports these results.

Histological details

Epidermal cells

Transverse (Plate 4, No. 15), and longitudinal sections both parallel to or at right angles (Plate 4, No. 14) to the flat plane of the thallus, show that these cells are typically brick-shaped with thick outer walls and relatively thin inner walls. Outer walls of epidermal cells near the apex are thicker than those of more mature epidermal cells lower down the thallus. The inner walls are frequently pitted and adjacent cells are in contact by protoplasmic connections which run through pores in these pit areas (Plate 3, No. 9, Evans and Holligan, 1972). Examination of Plate 4, Nos. 14 and 15

shows that epidermal cells are highly polarized, the peripheral chloroplasts being arranged next to the three inner walls so as to form a cup in the centre of which lies the nucleus and other cytoplasmic organelles. Plate 1, No. 2, Plate 2, No. 10 and Plate 4, No. 15 show that chloroplasts do not occur beneath the outer walls of mature epidermal cells. This region of the cell is occupied by an aggregation of very well defined vesicles averaging 1.8 μm and 1.7 μm respectively when measured with the light and electron microscope. Although mainly confined to the region beneath the thick outer epidermal walls some vesicles may occasionally be seen also beneath the other walls of epidermal cells. Toluidine blue staining shows the content of these vesicles to be a delicate metachromatic reddish purple (Plate 1, No. 2). Treatment of sections with cold trichloroacetic acid, a method known to remove the metachromatic part of polyphosphates (Keck and Stich, 1957; McCully, 1966a), did not affect the staining reactions of the vesicles in any way. Plate 4, No. 16 shows the outside edge of an epidermal cell containing vesicles with granular content. Further, the vesicle membranes may be seen in continuity with the cell plasma membrane, so that the vesicle content appears to be liberated to the outside of the cell by a reverse pinocytosis. As stated earlier a layer of material, identical in appearance and staining properties with that in the vesicles may be seen lining the inside of the outer cell wall, and all the evidence points to this being mainly alginic acid. After its deposition, this layer becomes finely fibrillar in appearance.

Apical cells

Apical cells also contain great numbers of small vesicles whose contents stain reddish purple metachromatically with toluidine blue. These vesicles occur mainly beneath the thick convex outer wall. There are very few adjacent to the thin concave basal wall except at the edges of the cell (as seen in longitudinal section) where, because of its lens-shape, the outer and basal walls run close together. The contents of the vesicles are difficult to retain for electron microscopy and are generally lost during fixation and subsequent processing. Similar membrane-bounded vesicles were seen in the apical cell of *Zonaria farlowii* by Liddle and Neushul (1969).

Medullary cells

These are relatively unpolarized and all their walls are more or less of equal thickness. The nucleus, chloroplasts and other cytoplasmic inclusions occupy a relatively small area in the centre of the cell, the remainder being occupied by very large peripheral vesicles (Plate 1, No. 3) whose membranes are continuous with the cell plasma membrane and whose contents form part of the cell wall. Examination of Plate 1, No. 3 further shows that the contents of these vesicles stain purple pink with toluidine blue, as do the cell walls themselves. The presence of these large vesicles of alginic acid around the whole periphery is a constant feature of medullary cells and such extensions of cell wall material projecting deeply into the cell cytoplasm may be a feature contributing to the structural rigidity of the thallus.

Tetrasporangia

Examination with the light microscope of stained sections of tetrasporangia at different developmental stages shows large vesicles containing material of at least two distinct sorts. At any one stage vesicles containing material of one sort generally predominate. In young tetrasporangia and in tetrasporangia where the four spores have cleaved, vesicles whose contents stain reddish purple with toluidine blue and are PAS

positive are the more abundant. In older tetrasporangia, in particular those in which the inner layer of sulphated material is being laid down around the inside of the sporangial walls and between the four spores (Plate 1, No. 5), the sporangial protoplasts are completely dominated by vesicles containing toluidine blue, pink staining, PAS negative material. These may also be seen with the electron microscope, as in Plate 2, No. 11.

Oogonia and eggs

Light microscope examination of developing oogonia and eggs show vesicles with two sorts of staining reactions, as encountered in tetrasporangia. Those believed to contain alginic acid predominate in young oogonia but at the time of the laying down of the thick inner oogonial wall layer, vesicles containing sulphated polysaccharide become the more numerous (Plate 1, No. 7). The electron microscope also shows an abundance of large peripheral vesicles at this stage.

Newly released eggs are devoid of all walls, being enclosed by a plasma membrane only (see Plate 1, No. 3, Evans and Holligan, 1972) until fertilization occurs.

Discussion

Many early investigations on alginic acid are concerned with its isolation and structure (for a summary of these see Black, Cornhill and Dewar (1952)). More recent work has centred around (a) variation in alginic acid content of some of the common larger brown seaweeds according to season and depth, etc. (e.g. Black, 1950; Macpherson and Young, 1952), (b) evaluations of some of these algae as sources of alginates (e.g. Rose, 1951; Black *et al.*, 1952), and (c) the different ratios of the two main constituents of alginic acid (D-mannuronic and L-guluronic acids) which occur in different brown algae (Fischer and Dörfel, 1955) and in different regions of single plants (Frei and Preston, 1962; Haug, 1964; Haug, Larsen and Baardseth, 1969).

However, in spite of the number of investigations carried out on alginic acid and the large quantities present in brown algae (Rose (1951) records figures varying between 13 and 23% of the dry weight of air-dry weed), relatively little is known about the origin, subsequent development and transport, or of the eventual location of this polysaccharide at the cellular level.

Stanford (1883) regarded alginic acid as a main component of brown algal cell walls and Kylin (1915), Bird and Haas (1931), Miwa (1932) and Naylor and Russell-Wells (1934) appear to agree with this. More recently, Andersen (1956) reported that the alginic acid in *Laminaria* spp. occurs in 'the middle lamella and the primary membrane', outside the secondary (cellulose) membrane. Dawes, Scott and Bowler (1960) in a preliminary report stated that the cell wall of *Dictyota flabellata* consists of cellulose and pectic materials, and in 1961, Dawes, Scott and Bowler reported that in the twelve brown algal genera they investigated, the mature wall consists of 'a thin, outer, reticulate layer of loosely arranged microfibrils and a thick, inner, layer composed of parallel cellulose microfibrils'. The observation of Ziegler (1963) on *Macrocystis pyrifera* (L.) Ag., Cole (1964) on the gametophytic generation of three members of the Laminariales, Bourne and Cole (1968) on *Phaeostrophion irregulare* Setchell and Gardner, and Bailey and Bisalputra (1969) on *Ectocarpus acutus* Setchell and Gardner and *Elachista fucicola* (Velley) Areschoug, in general, concur with this.

On the other hand, the work of Moss (1948) suggests that in *Fucus vesiculosus* L. alginic acid does not form a major part of the (cortical) cell walls. Baardseth (1966)

also found no evidence that there is a primary wall of alginate though 80–85% of the intercellular material in *Ascophyllum nodosum* (L.) Le Jol. was found to consist of alginate. (This author does state, however, and this is reiterated by Haug *et al.* (1969), that since alginate has been extracted from *Ectocarpus* sp. (by Haug, 1964), a plant which has no intercellular spaces, it is probable that the wall may be a site of alginate.) Thiele and Andersen (1955) also report that alginic acid is not present in the cell walls of *Fucus* and *Laminaria* spp., this substance being confined to the intercellular mucilage (which they regard as greatly swollen primary walls).

There are very few studies on the polysaccharides of *Dictyota* and little quantitative information. The presence of alginic acid has been shown by chemical analyses, e.g. Fischer and Dörfel (1955), Haug (1964), Kappanna, Rao and Mody (1962), and by X-ray diffraction analysis by Frei and Preston (1962). From the results of the present work quantities of alginic acid recorded by these workers are lower than might be expected. However, this may be due to variation in the alginic acid content of *Dictyota* according to season or geographical location.

The present communication has confirmed the presence of alginic acid in *Dictyota* and for the first time has shown the precise location of this substance, and also of sulphated polysaccharide in the vegetative and reproductive cells of this alga. The thallus cell walls and the walls of mature tetrasporangia, tetraspores, antheridia and oogonia are composed of well-defined layers of alginic acid or sulphated material or a mixture of these substances, a situation similar to that described for *Fucus* (McCully, 1965, 1966a, 1968a) and the oogonial walls of *Zonaria farlowii* Setch. and Gardn. and *Dictyota binghamiae* J. Ag. (Neushul and Liddle, 1968). On the other hand, the alginic acid and sulphated polysaccharide destined for secretion from these cells may generally be seen within conspicuous peripheral vesicles in their cytoplasm. Where alginic acid is being added to the thick outer epidermal cell walls, continuity between the vesicle membrane and the cell plasma membrane may frequently be observed and material in the 'open' vesicles can be seen to be continuous with identical material forming a thin inner cell wall layer. It is interesting that in *Dictyota* both the alginic acid and sulphated materials are produced by the ordinary vegetative and reproductive cells, although some of the vegetative cells are specialized for secretion. In *Laminaria*, another brown alga currently being investigated, the sulphated material is the product of much more highly specialized secretory cells, associated with canals in the sporophytic thallus.

The results indicate that there is almost certainly some cellulose present also, although since it is not yet possible to distinguish between alginic acid and cellulose, it is not clear exactly where the cellulose is situated. It is now generally recognized that at least some cellulose does occur in brown algal cell walls (see Frei and Preston, 1961; Northcote, 1963) and Percival and Ross (1948) considered cellulose to be the main structural component of the cell walls of *Fucus* and *Laminaria* species.

Vesicles have been implicated in transfer of materials out of or into the cell in many plants, e.g. *Phaeostrophion irregulare* (Bourne and Cole, 1968), *Fucus* (McCully, 1968b), *Ectocarpus acutus* and *Elachista fucicola* (Bailey and Bisalputra, 1969), *Pinus* (Wooding and Northcote, 1965), *Diplotaxis erucoides* D.C., *Helleborus niger* L. and *H. foetidus* L. (Eymé, 1966) and in *Citrus* (Thomson, 1967).

The origin of the alginic acid and sulphated polysaccharides has not been established although autoradiographic studies have begun. It is probable that one or more of these substances will turn out to originate from the Golgi complexes in the nuclear area where (highly distended Golgi cisternae are commonly encountered.

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EXPLANATION OF PLATES

PLATE 1

No. 1. Surface section *Dictyota* thallus showing epidermal cells (e) with nuclei (n), chloro-

plasts (c), etc. and medulla cells (m), all with metachromatic reddish purple-staining walls. Formaldehyde fixation, toluidine blue staining. × 350.

No. 2. Epidermal cell in T. S. showing reddish purple walls, nucleus (n), chloroplasts (c) some with small peripheral bodies (p), and numerous vesicles (v) beneath the outer wall (left-hand side) with delicate purple red content. Also visible are parts of two medulla cells (right-hand side). Acrolein/toluidine blue. × 1250.

No. 3. Medulla cells in section parallel to the thallus surface, showing peripheral vesicles (v), with light reddish purple content, in continuity with similarly staining cell walls. Glutaraldehyde/osmic fixation, Epon embedding, toluidine blue staining. × 675.

No. 4. L.S. young tetrasporangia on thallus surface, showing walls consisting of an outer purple layer (o) and a thin underlayer (u). Also nuclei with nucleoli and peripheral turquoise-staining bodies. Formaldehyde fixation, toluidine blue staining. × 150.

No. 5. T.S. older tetrasporangia showing four wall layers: purple outer layer (o), thin pale purple-pink second layer (s), wider reddish purple third layer (t), and a broad inner pink staining layer (i). The last is continuous with the cleavage furrows between the four developing spores. Note also vesicles (v) with pink contents in the spores, and nuclei (n) with nucleoli. Formaldehyde fixation, toluidine blue staining. × 300.

No. 6. L.S. antheridia on thallus surface. Note outer reddish purple layer (o) and wide pale purple-pink middle layer (m). Each antheridium is enveloped by a deep reddish purple inner layer (i); this extends inwards to form the 'middle lamellae' of the male gamete containing compartments. The latter have a lining of pale pink staining material (arrows) on all walls. Formaldehyde fixation, toluidine blue staining. × 600.

No. 7. Oogonia in L.S. showing outer (o) and middle (m) wall layers. The third inner wall layer is not yet present but note abundant vesicles (v) with pink-staining contents in egg cytoplasm. Acrolein fixation, toluidine blue staining. × 500.

No. 8. Oogonia in T.S. showing outer deep purple red (o), middle pale purple pink (m) and inner deep pink (i) wall layers. Also visible are nuclei (n) with nucleoli and many empty vesicles (v). Acrolein fixation, toluidine blue staining. × 250.

PLATE 2

No. 9. Epidermal cells in T.S. to show with the light microscope the three layers of the outer wall (large arrow). The middle (lighter) layer continues as the 'middle lamella' (small arrows) of the lateral wall and into the intercellular space (small triangle) between the epidermal and medulla cells. Acrolein/toluidine blue. × 1000.

No. 10. Parts of two epidermal cells in L.S. perpendicular to flat plane of thallus to show the wall layers. The exterior 'cuticle' (left-hand side) consists of an amorphous outer layer (o) (here itself resolvable into an outer more amorphous (o_i) and an inner finely fibrillar (o_{ii}) zone) a darker middle layer (m) and a narrow inner layer (i). Layer (m) is continuous (arrowed) with the thin 'middle lamella' in the centre of lateral and basal epidermal cell walls and layer (i) forms a very thin lining band closely pressed to the 'middle lamella' of these walls. Part of a medulla cell (md), chloroplasts (c), nuclei (n), etc. are also visible. × 7000.

No. 11. Part of a tetrasporangium showing its four wall layers: outer (o), second (s), third (t) and inner (i); the inner layer continues as the cleavage furrow (arrowed) between the spores. The protoplasts of the spores contain vesicles of sulphated material (v) and are not yet enclosed by their own (alginic acid) walls. × 7000.

PLATE 3

No. 12. Parts of two antheridia in L.S. showing outer layer (o) and middle layer (m) the last extending between the antheridia. Each antheridium is enclosed by a thin third layer (arrowed) which continues inwards (arrowheads) to form the 'middle lamellae' of the male gamete-containing compartments. On each side of this is a fourth lining inner layer (i). × 7000.

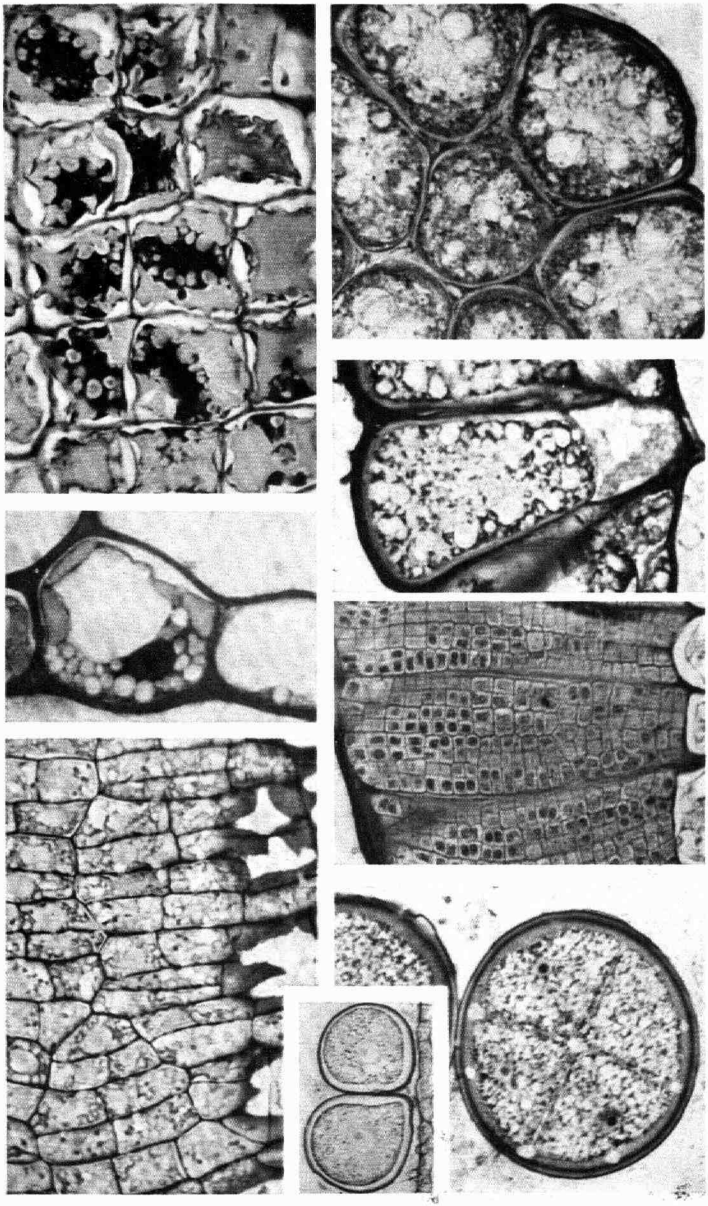
No. 13. Part of an oogonium in T.S. showing the outer (o), middle (m) and inner (i) wall layers. (Some loss of the material in the latter is probably due to fixation.) Some of the more or less empty vesicles (v) thought to have contained the sulphated material may be seen in the egg cytoplasm. × 7000.

PLATE 4

No. 14. Young epidermal cell in L.S. at right angles to plane of flattening of thallus to show the aggregation of cytoplasmic vesicles (arrowed) immediately beneath the outer cell wall (w). Normal cell organelles are also visible. × 8000.

No. 15. Older highly polarized epidermal cell in T.S.: the central nucleus (n) is enclosed by peripheral chloroplasts (c) adjacent to the lateral and basal walls whilst the area of cytoplasm beneath the outer wall (left-hand side) is occupied by vesicles (arrowed). Lateral walls show thickening (arrowheads) of the inner layer around the 'middle lamella'. × 2000.

No. 16. Part of an epidermal cell in L.S. at right angles to the flat thallus plane to show the vesicles (v) beneath the outer wall (w); their membrane is continuous with the cell plasma membrane and their content with identical material forming the inner wall layer (i). This has been shown to be alginic acid. × 15,000.



L. V. EVANS AND M. SUSAN HOLLIGAN—*CELL WALLS OF* DICTYOTA

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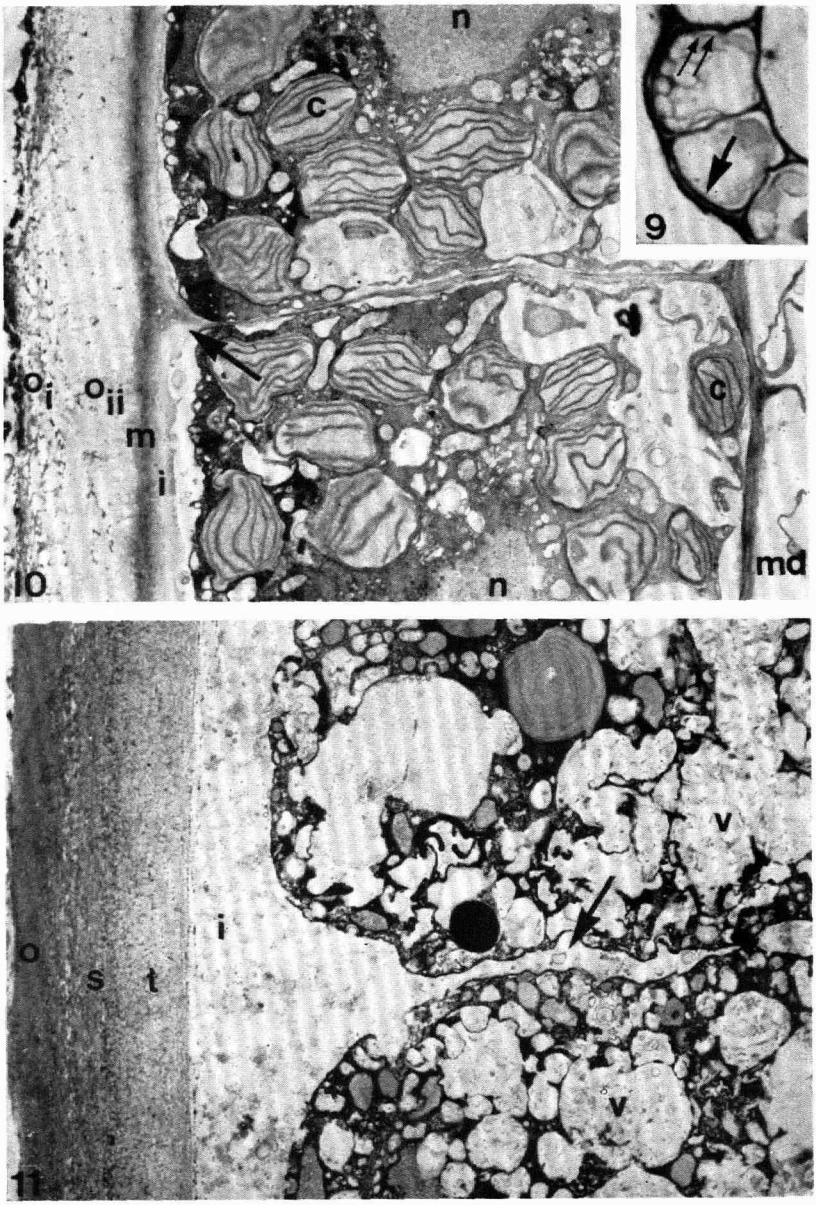

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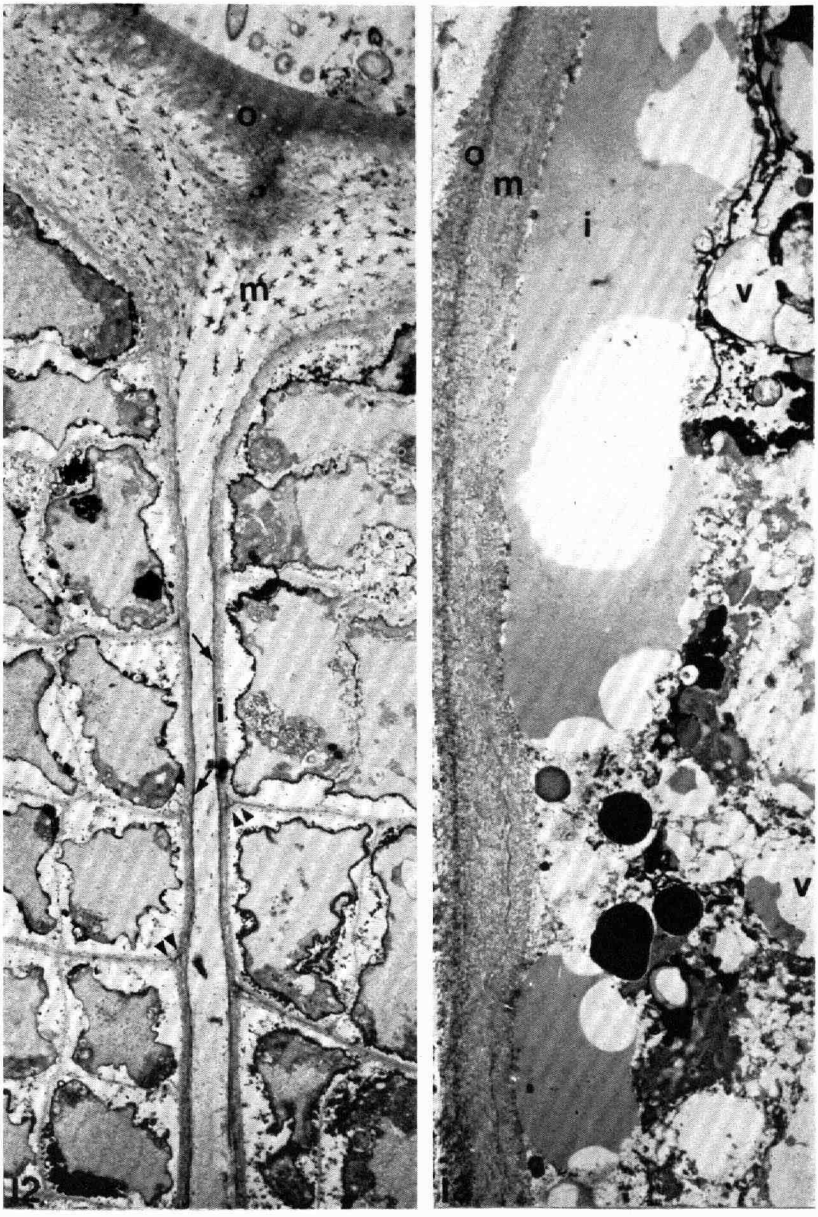
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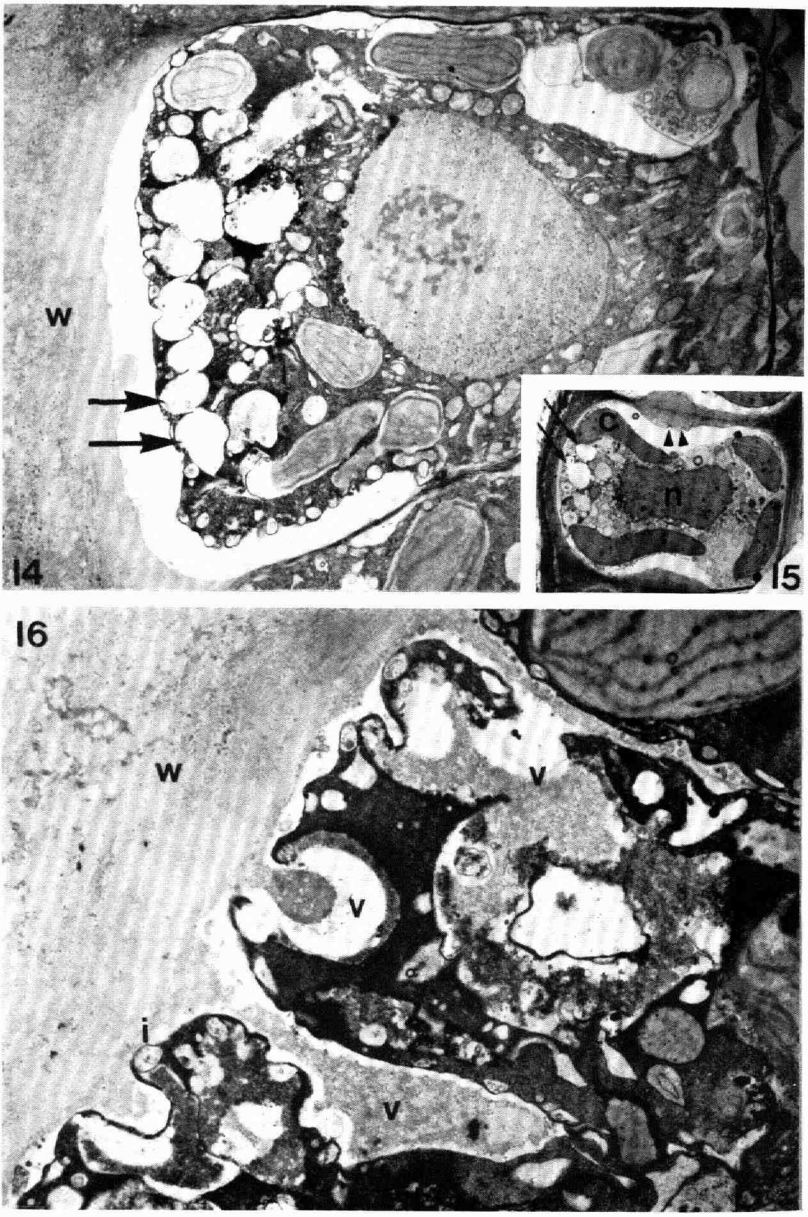

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