Histological Studies on the Genus *Fucus*

I. Light Microscopy of the Mature Vegetative Plant

By

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With 9 Figures

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Introduction

The genus *Fucus* offers much of interest to both the plant and animal histologist. The developing embryos of these plants are widely recognized as providing an almost ideal system for the study of the establishment of polarity and anisometry in plant cells. In the course of a study of the histology and fine structure of *Fucus* embryos it was found necessary to examine also mature plants since many of the findings with the embryos were uninterpretable in the absence of histological and fine structural details of the mature tissue.

Quite unexpectedly the cells and tissues of the mature *Fucus* plant have proven of interest in their own right and it is with this work that the present paper is concerned.

A section of mature *Fucus* thallus is remarkable in its resemblance to a section of animal cartilage. This resemblance is more than superficial. In both cases the cells are embedded in a massive, biphasic matrix composed of rigid, oriented fibres and amorphous polysaccharide. The fibrous components of cartilage and of tissues of the *Fucus* thallus are of course quite different, being the protein collagen in cartilage and the polysaccharides cellulose and alginic acid in *Fucus*. However, the amorphous polysaccharides of the matrices are remarkably similar; both are sulphated polysaccharides, chondroitin sulphate in cartilage and fucoidin in *Fucus*.

Recent work shows that some polysaccharides both of animals (Godman and Lane 1964, Berendes 1965) and of plants (Northcote

and Picket-Heaps 1966) are polymerized into units of large molecular weight which are contained within membrane-bounded vesicles in the cells. These polysaccharides are probably secreted from the cells by a reverse pinocytosis process in which the vesicle membrane is incorporated into the plasma membrane. It is of interest to know if the extracellular polysaccharides of *Fucus* are also polymerized within the cells and similarly secreted as macromolecules.

There is as yet no histological evidence in either plant or animal tissue for the secretion of different polysaccharides of high molecular weight from the same cell. Mature *Fucus* tissue is ideal material in which to look for such a multiple secretion since the two polysaccharides alginic acid and fucoidin can be distinguished histochemically (McCully 1965). As a system for the study of the production of extracellular material the *Fucus* thallus has the advantage of having continuous apical growth. Such a growth pattern is useful since it gives both a temporal and a spacial sequence of matrix formation within an individual plant.

Algal polysaccharides such as alginic acid, fucoidin, carrageenin and agar make up a large proportion of the mass of the algae in which they are produced as extracellular material. Because these algal polysaccharides are of considerable economic importance there has been much investigation of their chemistry (see reviews by Kreger 1962, O'Colla 1962) and studies have also been made on the influence of the environment on their production (Moss 1948, MacPherson and Young 1952, Black et al. 1953). No studies have been made on their origin or mode of secretion. The absence of such work is especially surprising in view of the light which such a study would throw on the production of extracellular materials in general.

In addition to the obvious advantages of *Fucus* tissues for the study of matrix formation, the cellular components of this material are also of interest. Microscopical examination of cells teased out of a piece of fresh *Fucus* thallus reveals a confusion of intracellular components. Of these only the plastids are easily identified by their pigment. In some cases the large nuclei can be seen, but in general the cells are so crammed with vacuoles and inclusions that even the nuclei cannot be distinguished. Although the exact nature of the various cellular inclusions has not been described, it is known from the earlier work that many of these contain polyphenolic materials (see Fritsch 1945). Recently there has been considerable interest in these substances. It has been shown that intact *Fucus* plants secrete large amounts of polyphenolic material (Craigie and McLachlan 1964a) and there are strong suggestions that these secreted materials are of considerable ecological and physiological significance. In this regard Craigie and McLachlan (1964b) have demonstrated differential antibiotic effects of these substances on pure cultures of various algae, and Provasoli (1965) has shown that the secreted polyphenols from *Fucus* have marked morphogenetic effects on cultures of *Monostroma*. The origin and mode of secretion of these polyphenolic materials of *Fucus* are therefore of considerable interest as is also the nature of the various

other cell components. This information, however, is not available in the earlier literature.

The morphology and histology of the mature *Fucus* plant was studied by a number of early workers (see review in Fritsch 1945) and more recently by Moss (1948 and 1950). Unfortunately the inadequate methods then available limit the usefulness of these observations.

There is good reason for the absence of histological information on *Fucus*. These plants have long had the reputation of being "unfixable". The extensive extracellular matrix and the large amount of intracellular polyphenols have made these tissues quite resistant to the efforts of the plant histologist. Not only are their non-cytoplasmic components not fixed by conventional fixatives, but they greatly impede the penetration of almost all fixing solutions so that it has been impossible to preserve the cell components faithfully. In addition, earlier workers have been hampered by the use of embedding matrices such as paraffin and celloidin (Wetmore 1932) which could not be cut thin enough to allow the resolution of intracellular detail.

More detailed histological work is now possible with *Fucus* because new methods of fixation and embedding have recently been developed (Feder 1960, Ashley and Feder 1966, and Feder, personal communication).

The present observations which were made on *Fucus* prepared by these new methods are concerned with determining the origin and development of the extracellular matrix, the histogenesis of the different cell types and the identification of the various cellular components.

Materials and Methods

Specimens of *Fucus vesiculosus* L. and *Fucus edentatus* De la Pyl. were collected at Bass Rocks, Gloucester, Massachusetts, from November 1964 to April 1965 and in September 1965. Thallus and holdfast tissues were immediately cut up in the fixative solutions into pieces of 2 mm^3 or less. All collections and fixations were done at low tide.

Fixation: Two fixatives were used—10% acrolein (Feder 1960) or a mixture of 6% acrolein and 6% glutaraldehyde, both in 0.025 M phosphate buffer at pH 6.8. Tissues were fixed for 24 or 48 hours at 0° C. It was found that the longer fixation was necessary for uniform preservation throughout the tissue pieces. In most cases the material was post-fixed for 24 hours, in 1% $HgCl_2$ at 0° C then thoroughly washed in 5 changes of buffer over 24 hours. Although not necessary for the preservation of cytoplasmic components, the mercuric chloride stabilizes the polyphenols, some of which tend to migrate through the specimen after aldehyde fixation alone.

Dehydration and Embedding: A sequence of 4 dehydration steps was used—methoxyethanol, absolute ethanol, n-propanol, and n-butanol, all at 0° C. Tissues were transferred directly from buffer into cold methoxyethanol and given 2 changes over 24 hours in each dehydrating agent. After

the final butanol change, material was either stored for up to 5 months in n-butanol at -25° C or brought to room temperature in n-butanol and transferred directly into 100% glycol methacrylate "monomer mix." Specimens were changed to fresh "monomer mix" after 2 days and left to infiltrate for at least 2 weeks in the dark at room temperature[1].

Specimens were placed in size 00 gelatin capsules filled with fresh "monomer mix." Capsules were capped and polymerized for 48 hours at 60° C.

The "monomer mix" consisted of glycol methacrylate (Rohm and Haas Co., Philadelphia) plus 0.15% α, α-azodiisobutyronitrile (Eastman Chemical, Rochester, N.Y.) and 5% polyethylene glycol 200 (Ruger Chemical Co., Irvington-on-Hudson, N.Y.). The glycol methacrylate was purified initially by column chromatography using Amberlyst A-21 resin (Rohm and Haas Co.). This procedure removes methacrylic acid, the presence of which results in staining of the plastic by basic dyes.

The above methods of dehydration and embedding are essentially those first developed by Feder (personal communication 1965, and Ashley and Feder 1966), the chief modification being increased time in each step, especially the final methacrylate infiltration.

Sectioning and Staining: Sections 1 to 2 μ thick were cut with a glass knife on a Huxley ultramicrotome. These were placed in drops of Seitz-filtered aqueous 2×10^{-3} M $CaH_4(PO_4)_2 \cdot H_2O$ on new glass slides and allowed to dry overnight at room temperature. The use of calcium phosphate increases the clarity of staining by stabilizing a metachromatic component which tends to smear the sections if they are dried down on water alone. Dried sections were washed for 1 minute under running tap water to remove the excess of calcium phosphate, then stained with toluidine blue (O'Brien, Feder and McCully 1964), or acidified acid fuchsin (Robinow 1966), or by the periodic acid-Schiff's (PAS) reaction (see Jensen 1962). Before using the latter method it is necessary to block the free aldehyde groups introduced by acrolein fixation. Two blocking agents were used; a saturated solution of 2,4-dinitrophenylhydrazine (DNP) in 15% aqueous acetic acid for 10 minutes at room temperature (modified from Danielli 1949), or chlorous acid (Rappay and Van Duijn 1965). The former method, while completely blocking Schiff positivity, leaves many cell components stained yellow while the latter blocks aldehydes equally well and does not stain the tissue.

Photomicrographs in colour were taken on Ektachrome type B sheet film using a didymium filter and appropriate colour correction filters. A didymium filter was also used for the black and white photomicrographs of sections stained with toluidine blue. The PAS and acid fuchsin stained material was photographed using a Corning #4010 green filter.

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[1] Even when small pieces of tissue were used, the infiltration of the extracellular material was incomplete if tissues were left in the methacrylate "monomer mix" for less than 2 weeks. The "monomer mix" should be kept in the dark to prevent light-induced polymerization.

General Morphology

The mature vegetative *Fucus* plant consists of a leathery, strap-like thallus and a flattened conical holdfast which secures the plant to the rocky substratum. The irregularly dichotomously branched thallus grows apically, and has a thickened mid-rib region and much thinner lateral wings.

The thallus consists of a single layer of columnar epidermal cells, several underlying layers of cortical parenchyma and a central network of branched primary filaments and elongated secondary fibres embedded in a highly metachromatic matrix (Fig. 1 *A*). There are a few randomly oriented filaments and fibres in the wing areas (Fig. 1 *A*) but in the much thicker midrib both the filaments and the numerous fibres run axially (Fig. 6).

All 4 cell types are differentiated within 0.5 cm of the apex. The epidermal cells and primary filaments of the midrib originate from a group of 4 to 8 initials in the peripheral layer at the base of a small furrow, elongated in the plane of the thallus, at the tip of each branch. A single, large apical cell as described by Woodworth (1888) for the same species was never observed although numerous apices of both *F. edentatus* and *F. vesiculosus* were sectioned serially. The apical cells are shorter and wider than mature epidermal cells and are further distinguished by large, spherical nuclei and a low level of intracellular staining with toluidine blue. The lateral walls of these cells are thin and sometimes appear only as an interrupted metachromatic line.

Files of small cells are cut off by unequal periclinal divisions of the apical cells (Figs. 1 *C* and 4) and these in turn give rise to the network of orientated primary filaments of the midrib area. Figs. 1 *C* and 6 show clearly this development of mycelium-like tissue from the parenchymatic tissue. As the young derivative cells round up, the extracellular space greatly increases so that cells a few layers beneath the apex are in contact over only a relatively small area. The wall in this contact region is thin and intensely PAS-positive.

The filamentous network of the midrib appears to form as the result of predominately transverse divisions and axial cell elongation accompanied by stretching caused by more rapid growth of the epidermal layer. If cross sections of fresh, young thallus are cut by means of two razor blades held tightly together, the tension existing between the inner and outer layers of the thallus is readily demonstrated by the strong inward curling of the ends of the tissue slices. During the formation of the filamentous tissue, the lateral connections between the original parenchyma cells remain intact and become the pit connections between adjacent filaments (Fig. 8). Remnants of the original primary walls (Elliot 1951) can be seen where filament cells have not extended their full length (Fig. 9). Subsequent elongation of these cells must rupture the original walls and the small wisps of PAS positive material in the intercellular spaces of older filament tissue (Fig. 5) are the remains of these torn walls. The formation of the filaments is accompanied by the laying down of a thick, moderately PAS-positive inner wall. None of this material is deposited over the lateral pits or on

the end walls so that the latter are in effect very large pits. The pits are strongly PAS-positive and metachromatic. No plasmodesmata can be seen with the light microscope using the present methods, although they were described by Hick (1885) in sections of *F. vesiculosus* and *F. serratus* which had been swollen by alkali treatment, and their presence in *F. vesiculosus* has been confirmed with the electron microscope (McCully, unpublished).

The epidermis consists of cells formed by anticlinial divisions firstly of the apical initials and subsequently the epidermal cells themselves, especially in the upper 1 cm of the thallus. Unequal, periclinal divisions at the base of these cells cut off the cortical parenchyma.

The network of filaments of the thallus wings originates from the inner layers of the cortex. The mode of production of this reticulum from a parenchyma tissue resembles that in the midrib except for a more random orientation of division and elongation of the inner cortical cells.

There are large pits in the lower lateral and basal walls of the epidermal cells and similar pits also occur between cortical cells and their filamentous derivatives.

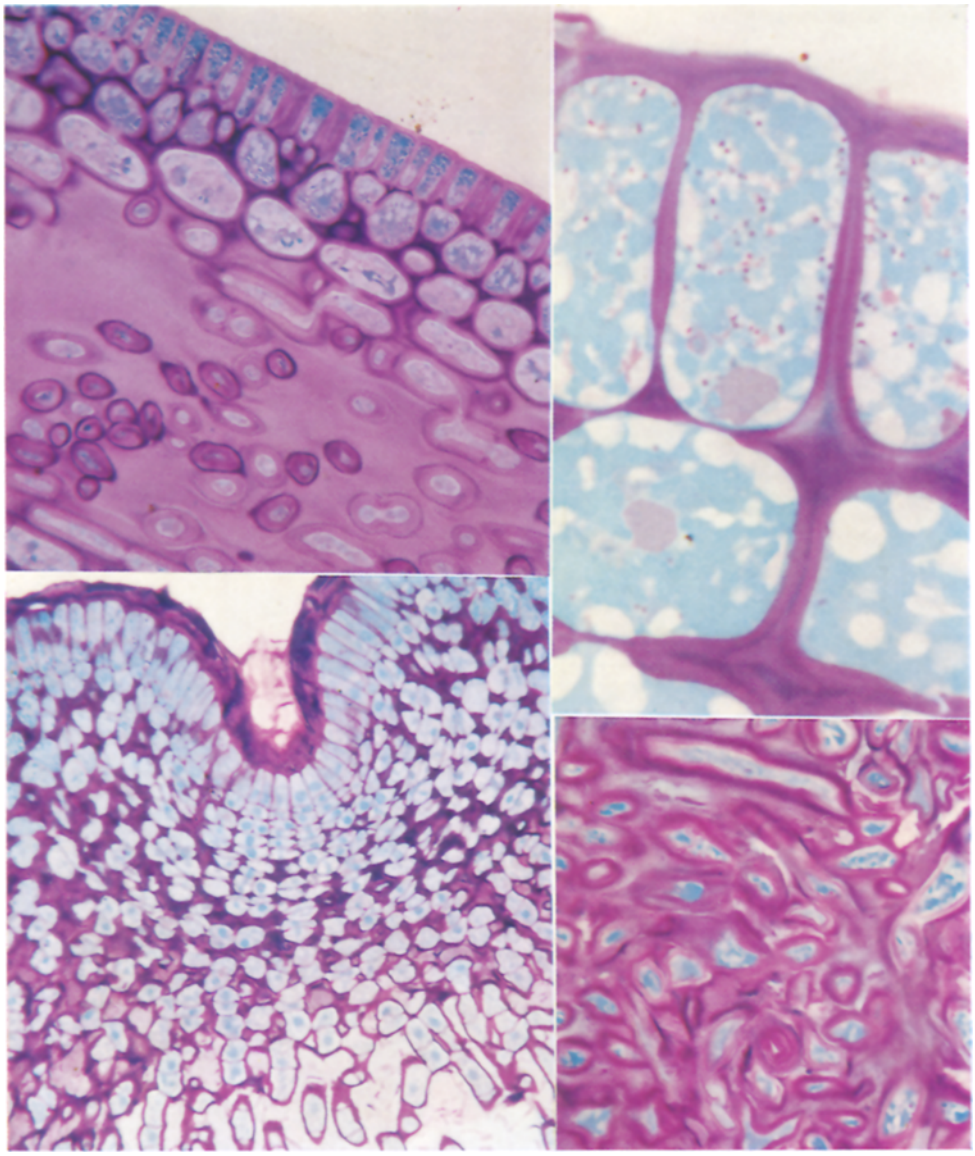
Secondary fibres first appear ca. 0.5 cm behind the apex, as thick-walled, narrow-lumened lateral projections from primary filaments (Fig. 7). These fibres grow intrusively down through the thallus toward the holdfast. Their growth is apical and they have few cross walls. Most of the fibres grow through the midrib but a few are found in the wing medulla.

The holdfast consists of a narrow, callus-like cortex surrounding a main body of closely packed fibrous elements (Fig. 1 *D*). The organ is formed entirely by the continuous downward growth of fibres from the thallus, which on reaching the substratum turn outward and differentiate parenchyma toward the periphery of the disc. This peculiar growth habit, which has been described by Moss (1950b), continues throughout the life of the plant (several years in the case of *F. vesiculosus*) and results in a continuous increase in the diameter of the holdfast. The diameter of the lumen of a holdfast fibre is about twice that of a thallus fibre, and the cells of the former are shorter. The fibre crosswalls are thin and pitlike but there are no lateral connections between fibres. Pits, however, do occur between the cortical parenchyma cells.

Histological Detail

Extracellular Material: Previous light and electron microscopy of thallus tissue has distinguished 2 components in the extracellular matrix—(a) thick fibrillar walls which surround all the cells and (b) amorphous material which fills the intercellular spaces (McCully 1965). All the extracellular material of the thallus stains metachromatically (pinkish) with toluidine blue (Fig. 1 *A*). The PAS reaction, however, gives differential staining of the walls and the matrix—the walls being PAS-positive and the intercellular material either PAS-negative or only weakly stained (McCully 1966).

Metachromatic staining by thiazin dyes such as toluidine blue occurs with high molecular weight compounds having free carboxyl, sulphate or



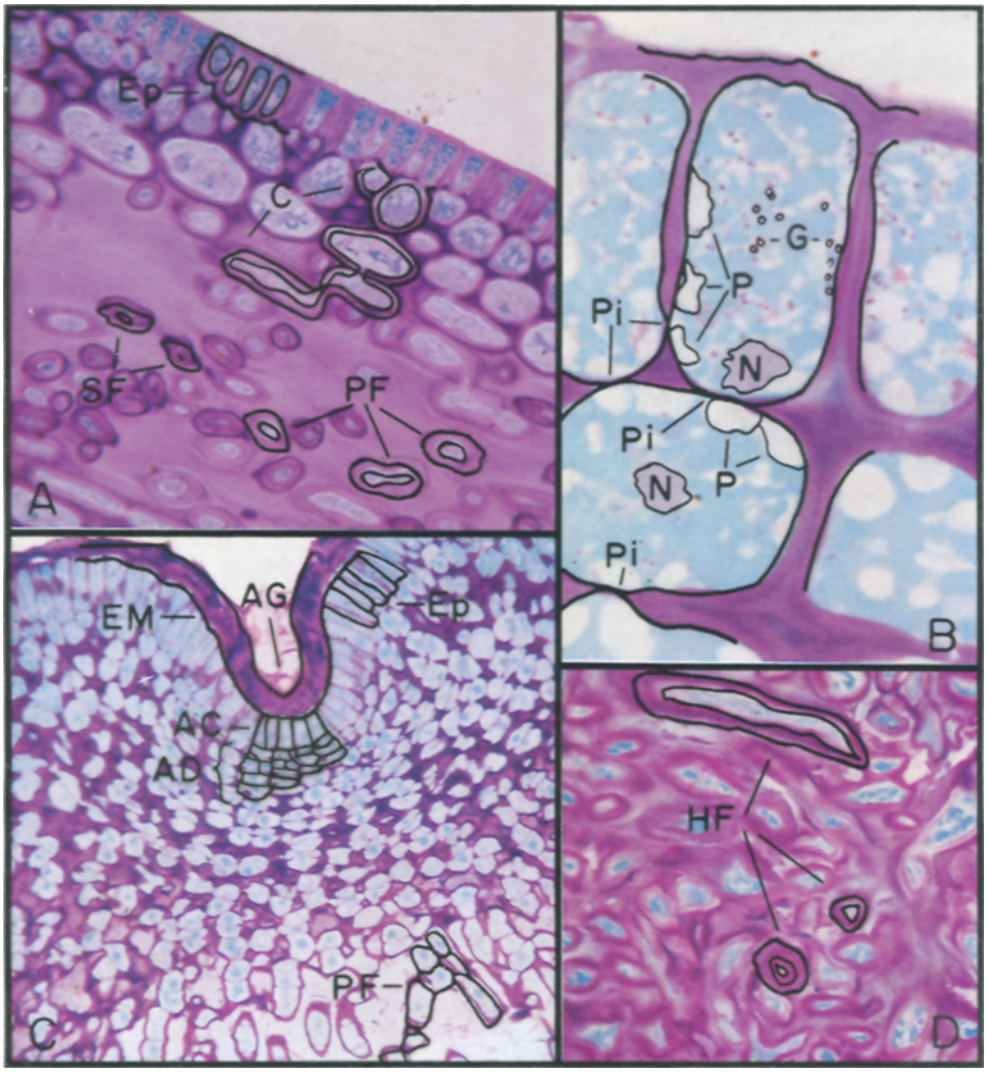


Fig. 1.

Fig. 1 *A*. Cross section thallus 1 cm from apex, showing epidermal cells (E_p), cortical parenchyma (*C*), primary filaments (*PF*), and secondary fibres (*SF*). Acrolein-$HgCl_2$ fixation. Toluidine blue staining. ×190.

Fig. 1 *B*. Detail of young epidermal and cortical cells which are just outside the apical groove, showing pits (*Pi*), nuclei (*N*), plastids (*P*), and metachromatic granules (G). Acrolein-$HgCl_2$ fixation. Toluidine blue staining. ×1,100.

Fig. 1 *C*. Longitudinal mid-section of the thallus cut across the long axis of the apical groove (*AG*) showing epidermal cells (E_p), apical cells (*AC*), and the young primary filaments (*PF*), and the external mucilage (*EM*) in the apical groove. Acrolein-$HgCl_2$ fixation. Toluidine blue staining. ×130.

Fig. 1 *D*. Section through medulla of holdfast showing fibres (*HF*). Acrolein-$HgCl_2$ fixation. Toluidine blue staining. ×100.

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phosphate groups (Barka and Anderson 1963). In particular, this colour shift is characteristic of sulphated polysaccharides, polyuronic acids and polyphosphates. On the other hand, the PAS reaction is considered quite specific for polysaccharides having free hydroxyl groups on 2 vicinal carbon atoms (Hotchkiss 1948). The principal *Fucus* polysaccharides of high molecular weight are the polyuronic acid, alginic acid and the sulphated polysaccharide, fucoidin. Both these compounds should stain metachromatically because of the free carboxyl groups in alginic acid and the sulphate esters in fucoidin. Moreover, alginic acid although carboxylated, still has free adjacent vicinal hydroxyl groups (see Percival 1964) and therefore must also be PAS-positive (Hotchkiss 1948). However, fucoidin lacks free vicinal hydroxyl groups (Conchie and Percival 1950) and is therefore PAS-negative. Analysis of *Fucus* tissue indicates only two other polysaccharides, cellulose and laminarin, present in addition to the alginic acid and fucoidin (Black et al. 1953). Of these, cellulose is present in very small quantity and, although PAS-positive, would not stain metachromatically. Laminarin is a short chain compound (see Meeuse 1962) and therefore would probably not be retained by the fixation procedures. Laminarin has neither adjacent hydroxyl groups nor free sulphate or carboxyl groups and even if present it would not stain with either toluidine blue or the PAS reaction.

On the basis of toluidine blue metachromasy and reaction to the PAS procedure, it appears that in the thallus the alginic acid is mainly confined to the fibrillar wall areas whereas the fucoidin is the main component of the amorphous matrix. This distribution of the polysaccharides has been confirmed by additional histochemistry and differential extraction procedures (McCully, in preparation).

The staining reactions of the holdfast are similar to those of the thallus. The fibrillar walls, especially of the holdfast fibres are intensely PAS-positive. Both these walls and the matrix material are metachromatic but this metachromatic colour is more red (Fig. 1 *D*) than that developed by the thallus matrix and possibly the polysaccharides present may be somewhat different.

The origin of the intercellular matrix can be seen by comparing sections proximal to the apex (Fig. 1 *C*) with more distal sections (Fig. 1 *A*). There is no intercellular material between the thin lateral walls of the apical cells but it appears between the earliest periclinical derivatives of these cells and continues to fill the increasing intercellular space up to the time when filament formation begins. From this point up to about 0.5 cm from the apex the intercellular space greatly increases but this is not matched by matrix formation. However, by 1 to 2 cm from the apex all the spaces are filled (Fig. 1 *A*).

The outer epidermal walls of cells close to the apical groove are thicker than those 0.5 cm or more behind the apex. All areas of these walls are metachromatic, but PAS staining shows 3 distinct layers present in cells near the thallus apex—a thick inner layer which is weakly PAS-positive, a strongly PAS-positive middle layer and a thin weakly PAS-positive

outer layer (Figs. 1 *B*, 1 *C*, 5). These 3 layers are especially well developed in the apical groove, most of which is filled by a proliferation of the outer layer. The outer layer becomes quite discontinuous in the older parts of the thallus and in some cases much of the second layer disappears as well.

Cytoplasma: There are no non-vacuolated cells in the vegetative tissues and even the apical cells contain many small vacuoles. At maturity, the cytoplasm in cells of all types is distributed in a thin, perinuclear sheath a delicate reticulum ramifying between the vacuoles and inclusions, and in a thin peripheral layer. Toluidine blue staining reveals some cytoplasmic basophilia in the apical cells, their immediately underlying derivatives, and the cells of the primary filaments. In some material the inner parenchyma cells also show some basophilic strands. No cytoplasmic basophilia can, however, be seen among the densely packed vacuoles of the epidermal and outer cortical cells (Fig. 1 *B*) or in the fibres and parenchyma of the thallus and the holdfast. On the other hand, acid fuchsin staining shows a tenuous network of cytoplasm in all the cell types. This network is most prominent in the young cells close to the apical groove and in the cells of the holdfast.

Nuclei: All the cells are uninucleate. The nuclei of apical cells and immediately underlying derivatives are spherical and ca. 10 μ in diameter. Those of the cortex, filaments and fibres are also spherical but somewhat smaller. Mature epidermal cell nuclei are quite different; the plastids are arranged to form a cup at the base of each cell and the nucleus is flattened into this cup. The nuclei of the apical cells also tend to be basal, but there is no preferred position of the nucleus in cells of the other types.

All the nuclei reveal a uniform slight acidophilia with acid fuchsin. With toluidine blue, however, different nuclei give quite different reactions. When the tissue is post-fixed with mercuric chloride and stained with toluidine blue, the nuclei of the apical cells and their nearby derivatives are either unstained or faintly green, while those of the older cortex and medulla region and of the holdfast are basophilic. The colour of the latter, however, is blue rather than the purplish blue basophilia of higher plant nuclei. Moreover, the colour is uniform over both nucleus and nucleolus and there is no clumping of chromatin. On the other hand, epidermal cell nuclei are never basophilic but stain distinctly pink with toluidine blue. Rarely, nuclei of young parenchyma cells which have recently been cut off from the epidermis also stain pink (Fig. 10).

The peripheral clumping of chromatin characteristic of higher plant nuclei is not seen in any *Fucus* nuclei although it is maintained in higher plant tissue fixed and stained by the same methods. Roy (1938) observed a prominent chromatin network in all nuclei of *F. vesiculosus* following a different fixation procedure. One or two large nucleoli are present in all the nuclei except those of the epidermal cells in which they are not revealed by these staining methods.

The epidermal cell nuclei of tissues not post-fixed with mercuric chloride stain pink as before with toluidine blue but many of those of the parenchyma and filaments stain green. The only basophilic nuclei are those

in some of the older filaments and secondary fibres of the thallus. This anomalous staining results apparently from the mordanting of nuclear components by unfixed polyphenols which are subsequently stained green by toluidine blue. The absence of green staining in the pink nuclei perhaps reflects a peculiar arrangement of nuclear components which does not allow binding with polyphenols.

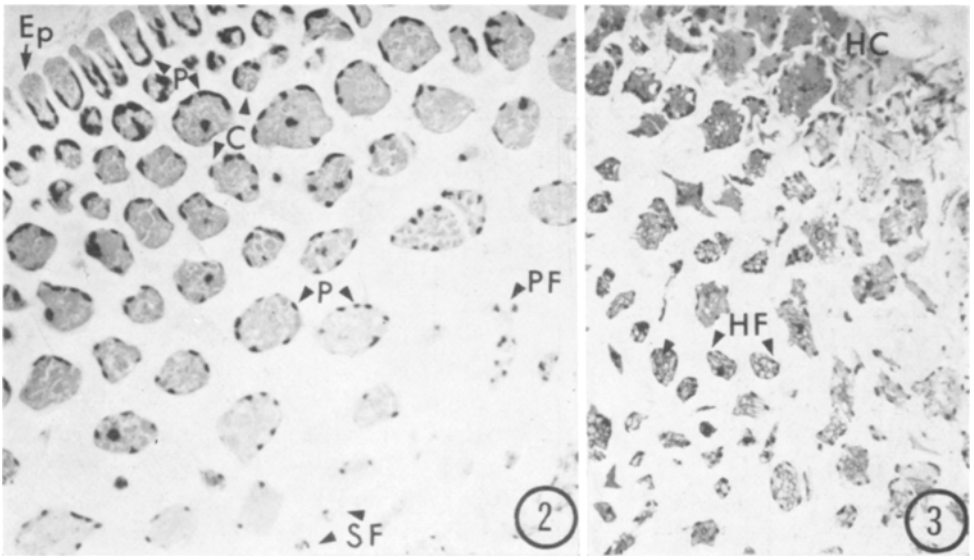


Fig. 2. Cross section through thallus 1 cm behind the apex showing distribution of plastids (*P*) in the epidermal cells (E_p), cortical parenchyma (*C*), primary filaments (*PF*), and secondary fibres (*SF*). Acrolein fixation, acid fuchsin staining. ×170.

Fig. 3. Section through cortex and medulla region of holdfast showing plastids in the holdfast parenchyma (*HC*) and fibres (*HF*). Acrolein fixation, acid fuchsin staining. ×170.

Only 2 division figures were seen in all the tissue examined and these were in cortical cells. This is interesting in the light of observations by Farmer and Williams (1898), Yamanouchi (1909), and Roy (1938) who found appreciable numbers of division figures only in material which had been collected and fixed from plants covered by the incoming tide. Since all the plants used in the present study were collected and fixed at low tide, it seems likely that mitosis is infrequent in exposed plants.

Plastids: Figs. 2 and 3 show the distribution of plastids in cells of the vegetative tissue. They occur in cells of all types but are most numerous in the mature epidermis and cortex of the thallus. Epidermal cell plastids are discoid and lie at the base of the cell in a cup-shaped shell around the nucleus (Figs. 1 *B* and 2). Those of the cortical cells are slightly larger and are preferentially distributed against the outer periclinal walls, especially in the outer cell layers (Fig. 2). The immature plastids of the apical cells and nearby derivatives are small spheres, ca. 1 μ in diameter and

are scattered throughout the cells. There are only a few plastids in the primary filaments but although they are small in diameter they are elongated up to ca. 4 μ. There are a few small, spherical plastids in the fibres of the holdfast and thallus and they resemble the immature ones of the apex region. In addition, there are small, discoid plastids in the holdfast parenchyma.

Table 1. *Vacuoles and Inclusions Distinguished by Toluidine Blue Staining.*

Colour	Shape	Approx. Diam.	Distribution
Colourless	1-irregular	1 μ	All cells. Most prominent in meristematic cells around apex and in epidermal cells.
	2-roughly spherical	4 μ	Inner cortical parenchyma and primary filaments.
Pale green	Roughly spherical	4 μ	Many in young epidermal and cortical cells of thallus and in cortical cells of holdfast. Few in older epidermal cells, filaments and fibres.
Turquoise	Amoeboid	1 μ to 4 μ	Many in mature epidermal cells and outer cortical parenchyma. Few in primary filaments and fibres.
Grass-green	Amoeboid	4 μ	Fibres of holdfast.
Pale pink	Roughly spherical	4 μ	Inner cortical parenchyma. Few in mature epidermal cells.
Deep purple	Granules	0.5 μ	All cells except apical group. Most prominent in epidermal cells.
Bright pink	Roughly spherical	3 μ	All cells except apical group-most prominent in cells of epidermis, cortex and filaments.

All the plastids are strongly acidophilic (Fig. 2) but unlike those of higher plants they have little basophilia and appear unstained with toluidine blue (Fig. 1 *B*).

Mitochondria: Rod-shaped acidophilic bodies resembling mitochondria are present in cells of all the types and are especially numerous in the holdfast fibres and parenchyma. There is a notable accumulation of these organelles at the apical poles of mature epidermal cells and here their identity as mitochondria has been confirmed with the electron microscope (McCully, unpublished).

Vacuoles and Inclusions: Toluidine blue staining distinguishes a variety of non-cytoplasmic components in the vegetative tissue. These are listed in Table 1.

Greenish-staining vacuoles, which are the most conspicuous of these inclusions are present in all cells except the apical group. Those of the holdfast fibres stain a distinct grass-green colour and are peculiar to this

tissue (Fig. 1 *D*). All the remaining cells have both pale-green and turquoise-staining vacuoles, the former being more numerous in young epidermal and outer cortical cells (Fig. 1 *B*), while there are more of the latter in mature cells of the same types (Fig. 1 *A*). Outer cortical cells, especially those lying over the midrib are crammed with green-staining vacuoles and only close examination reveals the tenuous network of cytoplasm ramifying throughout the non-cytoplasmic cell contents.

Observations of toluidine blue staining of both sections of lignified cell walls and solutions of pure compounds have shown that a green-staining reaction in aqueous solution is commonly associated with polyphenols (O'Brien, Feder and McCully 1964, and unpublished results). It seems clear, therefore, that the green-staining bodies in *Fucus* cells are the polyphenolic physodes first described by Crato (1893). The 3 different green colours of these vacuoles may simply reflect different concentrations of a single tannin-like compound or they may indicate the presence of different polyphenols. Indeed, the results of Chadefaud (1932) and Ando (1958 a and b) suggest strongly that several different tannin-like compounds are present in the Phaeophyta. None of the polyphenolic bodies give a positive PAS reaction but they are lightly stained by acid fuchsin.

There are 2 sorts of vacuoles which are not stained by toluidine blue—(a) small, irregularly-shaped ones present in all cells but most prominent as the only non-cytoplasmic component in the apical cells, and (b) the larger, spherical ones which fill most of the volume of the primary filaments and inner cortical parenchyma cells but which are uncommon elsewhere. It is also to be noted that neither of these types of vacuoles is stained by either acid fuchsin or the PAS reaction and the vacuolar contents have apparently not been preserved.

There are 3 types of metachromatic (red or pink-stained) inclusions and these have quite different distributions. Pale pink vacuoles are present mainly in inner parenchyma cells which are ca. 0.5 cm or more from the apex, and there are also a few of these vacuoles in mature primary filaments. The most widespread metachromatic component, the irregular, bright pink-staining bodies are in all cells except those of the apical group. These inclusions are found mainly in a perinuclear position although they occur elsewhere, especially just inside the plasma membrane. These bodies are most prominent in the early periclinal derivatives of the apical cells and in the primary filaments. The third type of pink-stained inclusion is quite different from the others, being dense and granular in appearance and very deeply metachromatic. There are a few of these granules in cortical and medullary cells but they are most prominent in the epidermis. Here they are at first scattered throughout the young cells which are close to the apex (Fig. 1 *B*) but increase in number and accumulate at the apical pole in older epidermal cells, where they tend to aggregate into larger particles (Fig. 5). They also accumulate against newly-forming anticlinal walls. These granules are PAS-positive but all other metachromatic inclusions are PAS-negative.

Although it is most likely that the various PAS-positive and/or metachromatic inclusions contain polysaccharides, other possibilities should be considered. Polyphosphates are also metachromatic (Barka and Anderson 1963), but although these substances are widespread in the algae (see review by Kuhl 1962), they have not been reported in the Phaeophyceae. However, because of the possibility that some of the metachromatic material in *Fucus* cells could be polyphosphate, sections were extracted with cold trichloracetic acid. This extraction is considered to remove the metachromatic component of polyphosphates (Keck and Stich 1957). None of the metachromatic components of the *Fucus* cells were extracted by this procedure although the same method removed the metachromacy from known polyphosphate granules in sections of higher plants which had been similarly fixed and embedded.

There is also reason to question the polysaccharide nature of the PAS-positive granules in this polyphenol-rich material. It is known firstly, that lignin is stained by Schiff's reagent (see Jensen 1962) and secondly, that some tannin-like components of higher plant cells are PAS-positive (Hršel 1960). The following observations, however, seem to confirm that the PAS-positive granules in *Fucus* cells are polysaccharide.

1. Unhydrolysed tissue in which the free aldehyde groups have been properly blocked by DNP or chlorous acid treatment do not stain with Schiff's reagent. After blocking and subsequent periodic acid hydrolysis the only PAS-positive intracellular materials are the small granules—the various physodes do not stain.

2. When sections are treated for 24 hours with chlorous acid, the toluidine blue green-staining components disappear. Although not conclusive, it is likely that the polyphenolic materials are extracted by this treatment; however, the granules remain PAS-positive.

Fig. 4. Longitudinal mid-section of thallus cut across the long axis of the apical groove (*AG*) showing apical cells (*AC*) and their periclinal derivatives (*AD*). The cell nuclei (*N*) and the intensely metachromatic intercellular material (*IM*) can be seen. Acrolein-$HgCl_2$ fixation. Toluidine blue stain. ×630.

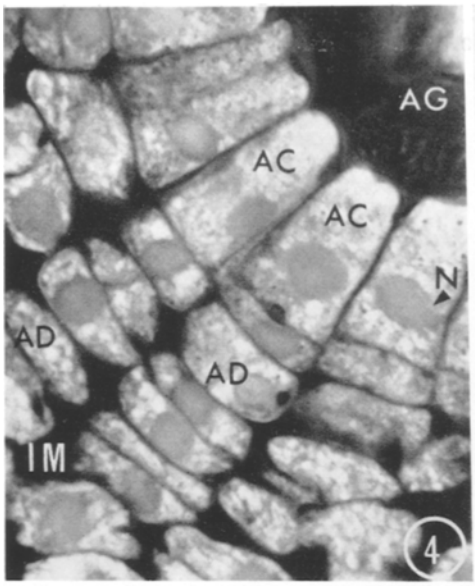
Fig. 5. Cross section of thallus just outside the apical groove showing outer wall (*OW*) of the epidermal cells (E_p) and the PAS-positive granules (*G*) within these cells. Acrolein-$HgCl_2$ fixation. DNP block. PAS reaction. ×850.

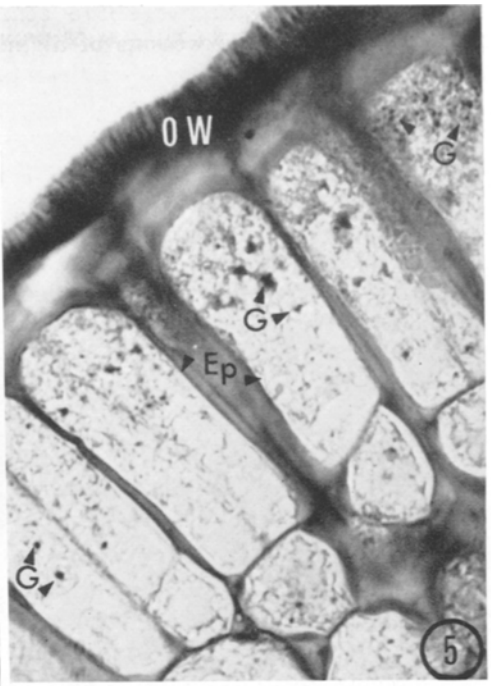
Fig. 6. Longitudinal mid-section of thallus just below apical cells, showing the pulling apart of the young apical derivatives (*AD*) and the formation of the primary filaments (*PF*). Acrolein-$HgCl_2$ fixation. DNP block. PAS reaction. ×100.

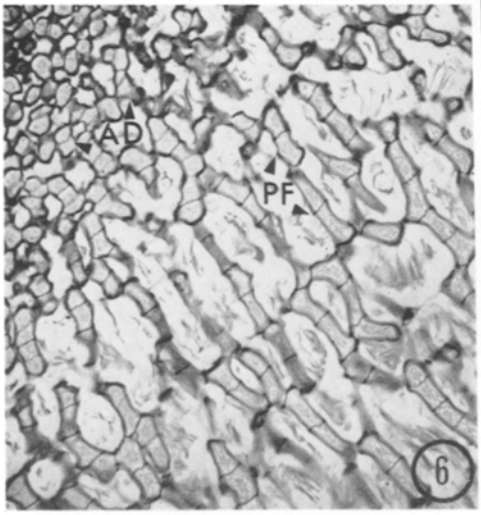
Fig. 7. Section through medulla, 0.5 cm from thallus apex showing origin of secondary fibre (*SF*) from primary filament (*PF*) cell. Acrolein-$HgCl_2$ fixation. DNP block, PAS reaction. ×400.

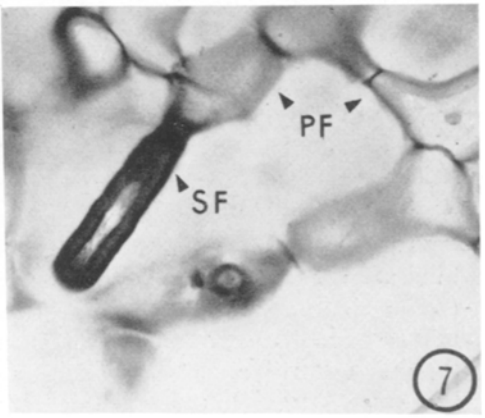
Fig. 8. Longitudinal section 1 cm from thallus through cells of primary filaments. A lateral pit (*Pi*) and pit-like cross walls (*CW*) can be seen. Acrolein-$HgCl_2$ fixation. DNP block. PAS reaction. ×450.

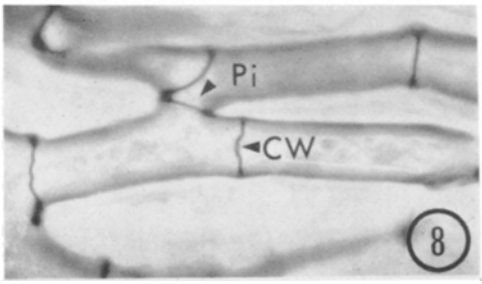
Fig. 9. Section through elongating primary filaments showing remnants of original primary walls (*O*), inner thickening of walls (*T*) and pit-like cross walls (*CW*). Acrolein-$HgCl_2$ fixation. DNP block. PAS reaction. ×520.

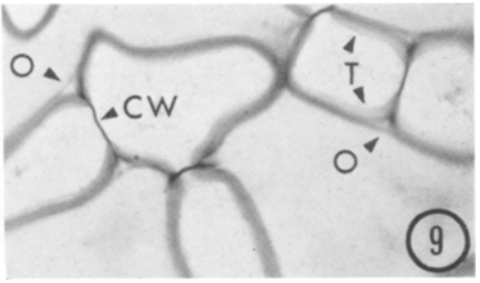












Figs. 4—9.

On the basis of the above considerations and the PAS and metachromatic staining reactions of alginic acid and fucoidin discussed in the section dealing with extracellular materials, it is now possible to identify with considerable certainly the various PAS-positive and metachromatic intracellular components of *Fucus* cells. The vacuoles which show pink metachromasy but are PAS-negative and which are often observed in a perinuclear or peripheral position in the cells probably contain the sulphated polysaccharide fucoidin and this polysaccharide is also present in the intercellular matrix. The metachromatic but PAS-positive granules which are scattered throughout almost all the cells probably contain alginic acid and this polysaccharide is the major component of the fibrillar walls which surround all of the cells.

Discussion

The massive production of extracellular polysaccharide is the most outstanding feature of the vegetative tissue. The origin of this material is of interest. The thick, fibrillar walls are probably best considered as primary, since even in the case of the medullary filaments where there is considerable thickening of the walls during the transition from parenchyma to filament, this thickening of the walls is laid down while the cells are still growing (Fig. 6). On the other hand, the intercellular matrix is secondary in the sense that it is secreted through an existing primary wall into an extracellular space. This secondary formation is apparent from comparison of Figs. 1 *A*, 1 *C* and 6 where it can be seen that the secretion of matrix does not keep pace with the expansion of the intercellular space and that it is not until some distance from the apex that secretion catches up and completely fills the space.

Electron microscopy has not revealed any pores in the inner walls, hence the secreted polysaccharide must escape by permeation through the fibrillar region of the wall.

The idea of a secondary origin of the matrix material does not occur in the earlier *Fucus* literature. It was considered to result from a swelling (Strasburger 1887) or gelatinization (Reinke 1876) of an existing middle lamella. Although the possibility of polysaccharide movement through a fibrillar wall has not been given much consideration in botanical literature, it has been postulated by Schnepf (1963) for the mucilage secreting cells of *Drosophyllum*, and Buer (1964) has presented a good case for the "Ausschwitzen" of the mucilage through the fibrillar walls of several species of the Zygnemataceae.

The increased resolution obtainable with the present histological methods gives some information on the location of the polysaccharide production. It seems very likely that the various metachromatic inclusions in these cells are polysaccharides of high molecular weight—probably alginic acid and fucoidin. The relatively large concentration of these inclusions suggests strongly that they are synthesized within the cell and subsequently move out through the plasma membrane and the fibrillar wall.

Recently, isotope labelling of *Triticum* root polysaccharides (Northcote and Pickett-Heaps 1966) has confirmed the suggestion from

previous work (Mollenhauer, Whaley and Leech 1961, Drawert and Mix 1961, Sievers 1963, Schnepf 1963, and Rosen et al. 1964) that polysaccharides of high molecular weight can move through the plasma membrane of plant cells. Moore (1965) has presented histochemical evidence for the production of gelatinous polysaccharide material in the cytoplasm and its subsequent movement through pores in the walls of some fungi, but there is no conclusive histochemical evidence for the movement of polysaccharides through the plasmalemma in other plants. On the other hand, it has been firmly established that polysaccharides are secreted through the plasma membrane of animal cells. Berendes (1965) has shown by correlated histochemical and electron microscope studies that polysaccharide granules move out of some cells in *Drosophila* salivary glands. It has also been demonstrated conclusively by histochemistry, isotope labeling and differential extraction techniques that chondroitin sulphate is polymerized inside chondrocytes and subsequently moves through the plasmalemma to the surrounding cartilage matrix (Godman and Lane 1964). It seems reasonable to postulate a similar movement of polysaccharides out of *Fucus* cells especially considering the similarity of the two sulphated polysaccharides, fucoidin and chondroitin sulphate.

The morphology of the highly polarized epidermal cells with their basal nuclei and plastids, numerous polysaccharide granules and apical mitochondria suggests that they are specialized for the secretion of polysaccharides to the outside of the plant. Although the cells lining the apical groove contain many fewer metachromatic inclusions than do the mature ones outside the groove, there is, nonetheless, a considerable accumulation of mucilage within the sheltered cleft. Since, in growing plants, a given set of epidermal cells remains within the groove for a short time only, it seems quite likely that mature cells outside the groove continue to secrete polysaccharides which are constantly being sloughed off into the ocean. Sections through outer epidermal walls in the upper 0.5 cm of the thallus have features strongly suggestive of such a process (Fig. 5).

The outwardly directed secretion of polysaccharides by the polarized epidermal cells is in contrast to that of the unpolarized cells which obviously secrete polysaccharides in all directions. Parallels exist in animal tissue, where, for example, the highly polarized goblet cells of the small intestine release mucopolysaccharides only through their apical poles, while unpolarized chondrocytes secrete large amounts of chondroitin sulphate in all directions.

The specialization of *Fucus* vegetative cells for the production and secretion of large amounts of extracellular polysaccharides, mainly alginic acid and fucoidin, is of fundamental ecological significance. The formation of a cartilage-like tissue is an admirable solution to the mechanical problems imposed by the environment. The presence of large amounts of hydrophilic polysaccharides is of obvious importance in preventing desiccation during exposure. The embedment of all the cells in a matrix which is in effect an ion exchange resin must provide an excellent buffer against sudden changes in the osmotic environment. Besides discouraging the growth of

epiphytes, the secretion of polysaccharides from epidermal cells can conceivably be involved in some form of osmoregulation by the transport of ions.

The proportion of cell volume occupied by the tannin-like materials is quite remarkable, and there was much speculation in the early literature on the role of these substances. Nothing is known, however, of their origin or function within the cells. In the thallus their presence seems directly correlated with apparent photosynthetic activity, i.e., they are mainly in the epidermis and outer layers of the cortex. This correlation, however, does not apply for holdfast fibres where they are also plentiful and where photosynthesis is probably minimal. Recent work by Craigie and McLachlan (1964a and b) has shown that tannin-like compounds are secreted from healthy, intact *Fucus* plants and that these compounds may be of considerable ecological significance to the plants by their differential control of the growth of other algae. The escape route of this tannin is not apparent from the sectioned material. No polyphenolic compounds were located in the outer walls or intercellular spaces, although toluidine blue staining of sections which had been deliberately mordanted in *Fucus* extract showed that even low tannin concentrations result in a green stain and a masking of polysaccharide metachromasy. It may be that the tannin release is rhythmic and perhaps associated with the incoming tide so that it was missed by fixing at low tide.

It is clear that the apical growth of the *Fucus* thallus needs further investigation. The finding of a group of initials instead of a single, large apical cell is surprising considering the classic, textbook use of *Fucus* as an example of apical growth from a single large cell. However, the observation is not new. Reinke (1876) described a group of apical initials of which one may be somewhat larger, and Rostafiński (1876) described several similar "Bildungszellen" in the peripheral layer at the base of the apical groove, distinguished from the epidermal cells by their truncated pyramidal shape and denser staining cytoplasm. These early observations, however, were refuted by Oltmanns (1889) and Woodworth (1888) who always found a single, large apical cell in the various *Fucus* species which they examined. The explanation of these differences is obscure, but may possibly reflect the growth conditions of the specimens examined. All the apices used in the present study were collected from September to April—seasons when thallus growth is maximum. Further, only apices from apparently vigorous thalli were fixed. No summer material was examined. It may be that a group of apical cells present during rapid growth is replaced by a single large cell under conditions of slower growth.

Summary

1. Vegetative tissues of *Fucus* have been fixed and embedded using new methods which greatly improve the resolution of histological detail.

2. The mature thallus consists of an outer layer of columnar epidermal cells, several underlying layers of parenchyma, and a medullary region

of branched primary filaments and elongated secondary fibres embedded in a metachromatic matrix. The holdfast is made up of an outer callus-like cortex and a medulla of thick-walled fibres also embedded in metachromatic extracellular material.

3. The growing point of the thallus lies at the base of an apical groove. In the plants examined which were rapidly growing, there is a group of 4 to 8 apical initials.

4. The results of toluidine blue staining and the PAS reaction indicate that the alginic acid and fucoidin of the extracellular matrix are synthesized within the cells and secreted through the plasma membrane.

5. The polarized epidermal cells are apparently specialized for the secretion of polysaccharides to the outside of the plant.

6. The results of toluidine blue staining suggest that there are possibly 3 different types of polyphenol-containing vesicles present. Two of these types are found in the cells of the thallus and the third is confined to cells of the holdfast.

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