A CHEMICAL AND PHYSICAL INVESTIGATION OF THE CELL WALLS OF SOME MARINE ALGAE

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INTRODUCTION

The modern physical techniques which are available for the determination of the fine structure of biological materials have been fully applied to the cell walls of the algae[1]. The results obtained from this study however have never been correlated with a determination of the chemical nature of cell wall constituents. It seems likely that a combination of the biophysical methods with processes for the complete chemical analysis of the cell wall such as devised by JERMYN AND ISHERWOOD[2] for higher plant material would repay some attention. These methods have not hitherto been adapted to the cell walls of the algae, although many individual components have been isolated and analysed.

The object of the work to be described has been to conduct a paper chromatographic analysis of the sugars and sugar derivatives in the hydrolysis products of various cell wall fractions, as defined by physical methods. In this preliminary survey a wide range of marine algal types was chosen from the three major groups — green, brown and red algae. The wall material was separated into arbitrary fractions comparable to the pectin, hemicellulose and cellulose of the higher plants; before and after each fractionation, the wall was examined by electron microscopy and the method of X-ray diffraction analysis.

MATERIALS

The seaweeds used in this investigation were collected at Cullercoates on the north-east coast of England during the first week of January 1957; at this time of the year the storage products are at a minimum (FOGG[3]). The algae selected from species common in the littoral flora included as large a morphological range as was possible at this time of the year, and are listed below.

Green algae

Cladophora rupestris
Chaetomorpha melagonium
Enteromorpha sp.
Ulva lactuca

Red algae

Ptilota plumosa
Griffithsia flosculosa
Rhodymenia palmata
Porphyra sp.

Brown algae

Halidrys siliquosa
Fucus serratus
Himanthalia lorea
Ascophyllum nodosum
Pelvetia canaliculata
Laminaria saccharina
Laminaria digitata

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METHODS

(a) *Preparation of starting material*

The algae were frozen in liquid air, ground to a flour and transferred to ethanol which was quickly brought to the boil. After extraction for ½ hour the residue was washed several times in boiling ethanol, twice in acetone, and dried *in vacuo*. This ethanol-insoluble product constituted the starting material.

(b) *Fractionation of cell wall material*

The methods used were modified from those described by JERMYN AND ISHERWOOD[2] who used pear cell walls as experimental material. The ethanol-insoluble algal material was first fractionated by extracting with boiling water, and then with 4 *N* potassium hydroxide. The polysaccharide material remaining (α-cellulose) was divided into two parts, one of which was subjected to a mild chlorination. The major modification from the methods described by JERMYN AND ISHERWOOD, necessitated by circumstances which will be mentioned later, was thus a deferment of the chlorination until after the alkali extraction.

The four fractions into which the wall has been separated were calculated as a percentage of the dry weight of the starting material, and were then hydrolysed and analysed for their constituent sugars. Before and after each fractionation of the material, samples were taken for a physical examination.

The details of the various extractions and hydrolysis procedures are as follows.

Materials soluble in hot water

The cell wall preparation (1.5–2 g) were extracted with water (150 ml), for 12 hours on a boiling water bath. The mixture was filtered through a sintered-glass funnel and the residue washed with boiling water. The residue was thoroughly mixed with acetone and washed on the filter with more acetone. It was then dried in a current of air. The filtrate and washings, from the extract described above, were concentrated under reduced pressure at a maximum temperature of 40° C to a final volume of 75 ml. The whole concentrate was used for the subsequent analysis.

Materials soluble in 4 N *alkali*

The material remaining after the water extraction (approximately 1 g), was placed in a 100 ml Erlenmeyer flask and 60 ml of potassium hydroxide were added. The air above the liquid was replaced by nitrogen. The flask was closed with a rubber-bung and placed in an incubator at a constant temperature of 25° C. The contents were gently swirled at 10 minute intervals for 4 hours. The mixture was filtered through a sintered-glass funnel, washed with 15 ml of 4 *N* potassium hydroxide, 150 ml of water and 15 ml of 2 *N* acetic acid. The filtrate and the washings were run into a suction-flask containing excess of glacial-acetic acid. The solution was transferred to a litre flask diluted to 1 litre with ethanol, and allowed to stand overnight. The flocculent precipitate was centrifuged off, and washed on the centrifuge successively with ethanol, acetone and ether, and then dried *in vacuo*.

The chlorite treatment

The physical separation of the cell wall from the cytoplasm proved impossible. A chemical removal of the protein material was carried out by a chlorination such as is usually used for the delignification of woody tissues. It was found experimentally that the chlorite treatment, when carried out immediately after the water extraction, removed some carbohydrate material, revealed by the sensitive anthrone test of DREYWOOD[4]. As it is difficult to recover carbohydrate extracted in this way it was decided to postpone the chlorite treatment until after the extraction with alkali had been carried out. The α-cellulose was therefore split into two parts and one subjected to the chlorite treatment. Hydrolysis of the treated and untreated material gave, by subtraction, information about the carbohydrates which were being removed by the chlorination.

The material (*ca.* 0.2 g) was suspended in 16 ml of water, one drop of glacial acetic acid and 0.15 g of sodium chlorite were added. The treatment was carried out at a temperature of 75°–80° C. At hourly intervals one drop of glacial acetic acid and 0.15 g of sodium chlorite were added. After treatment for four hours, the material was filtered through a sintered glass funnel, washed repeatedly with water and then acetone. The powder was dried in a current of air and then *in vacuo*.

(c) *Hydrolysis of cell wall fractions*

Material soluble in hot water

The concentrated extract (75 ml) to which had been added a crystal of urea was heated in a boiling water bath under reflux. When the temperature of the solution reached that of the bath, 3 ml

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of colourless nitric acid (1.42 sp. gr.) was then added slowly with shaking. The acidified extract was heated for twelve hours and then cooled. The solution of sugars after hydrolysis was neutralised (using bromo-thymol-blue as indicator) with 2 *N* sodium hydroxide and evaporated to dryness under reduced pressure at a temperature of less than 40° C. A mixture of ethanol and benzene was distilled through the residue to remove any traces of water remaining. The dried residue was extracted with acetic acid, filtered from suspended nitrate, and the sugars precipitated by the addition of anhydrous ether. The precipitate was washed with ether and dissolved in an appropriate volume of water to give a 2% (w/v) solution.

Material soluble in alkali

The alkali-soluble fraction (400 mg) was mixed with 10 ml of 0.5 *N* nitric acid containing a crystal of urea in a test tube which was closed with a glass bubble and the whole heated in a boiling water bath for 4 hours. The nitric acid was removed from the hydrolysate as described for the water soluble fraction, with the modification that the residue after evaporation under reduced pressure was taken up in ethanol instead of acetic acid and was filtered from insoluble salts. The sugars were recovered by evaporation under reduced pressure and dissolved in water as before.

α-Cellulose

Each of the two α-cellulose fractions (100 mg) were dissolved in 1 ml of 72% (w/w) sulphuric acid with stirring. The solution was diluted to 1% acid and the whole boiled under reflux for 12 hours. The solution was then neutralised whilst hot with barium carbonate until neutral to methyl red and quickly filtered through a sintered glass funnel into a flask containing a few drops of 0.1 *N* sulphuric acid. The filtrate was evaporated to dryness under reduced pressure at a temperature of less than 40° C and the sugars redissolved in water to give a 2% solution.

(*d*) *Analysis of the sugars in the hydrolysis products*

The sugars in the hydrolysis products were separated and identified by the method of descending paper chromatography, using Whatman's No. 1 filter paper.

The developing solvents used were:

(a) Pyridine: ethyl acetate: water 1:2:2;

(b) Water-saturated phenol.

The positions of the sugars, after separation, were revealed by spraying the dried chromatograms with one of the following reagents.

(a) 2% ammoniacal silver nitrate;

(b) 3% *p*-anisidine hydrochloride in moist butanol;

(c) 2.5% aniline hydrogen phthalate in water-saturated butanol.

The sugars and sugar derivatives were identified by comparison with the positions and colours of standard sugars run on the same chromatogram.

(*e*) *The physical examination of the cell wall*

X-ray diffraction studies

Powder diagrams were obtained in the usual way, using a flat film at a distance of approximately 3 cm from the specimen. The X-ray beam of Cuk_{α} radiation was collimated to 0.5 mm diameter. The intermolecular spacings were calculated from the diameters of the rings, the specimen film distance being accurately calibrated using a silver diagram.

Electron microscopy

Observations were made on Pd-Au shadowed material which had been blended in a Potter homogeniser, washed with water on the centrifuge, suspended in water, and dried onto a formvar film supported on a metal grid. Comparison of micrographs was facilitated by making all the observations at a screen magnification of × 20,000.

RESULTS

1. *Electron microscopic observations*

Preparations of wall material in a natural condition, after water extraction, after 4 *N* alkali extraction and after the chlorite treatment, were observed in the electron microscope. From the many observations recorded, representative series are illus-

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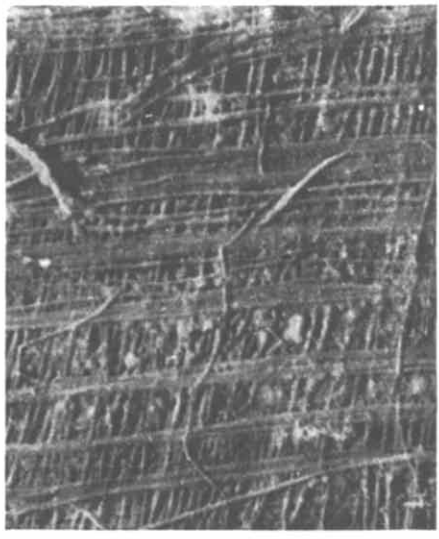

a

b

c

Fig. 1. *Cladophora rupestris*, electron micrographs of wall material in the conditions stated. Specimens shadowed with Pd-Au. a. Natural. Magnification × 30,000. b. After water extraction. Magnification × 31,500. c. After full treatment. Magnification × 30,000.

trated for *Cladophora rupestris* (Fig. 1), *Laminaria saccharina* (Fig. 2), *Fucus serratus* (Fig. 3), *Rhodymenia palmata* (Fig. 4), *Porphyra* sp. (Fig. 5).

Chaetomorpha has a structure identical with that of *Cladophora*; and *Laminaria digitata* with that of *Laminaria saccharina*. Apart from differences in the size of the microfibrils, *Fucus serratus* is representative of the structure we have observed for the other members of the Fucales, *Ulva lactuca* and *Enteromorpha* sp. Similarly *Rhodymenia* is representative of the structure of *Ptilota plumosa* and *Griffithsia*

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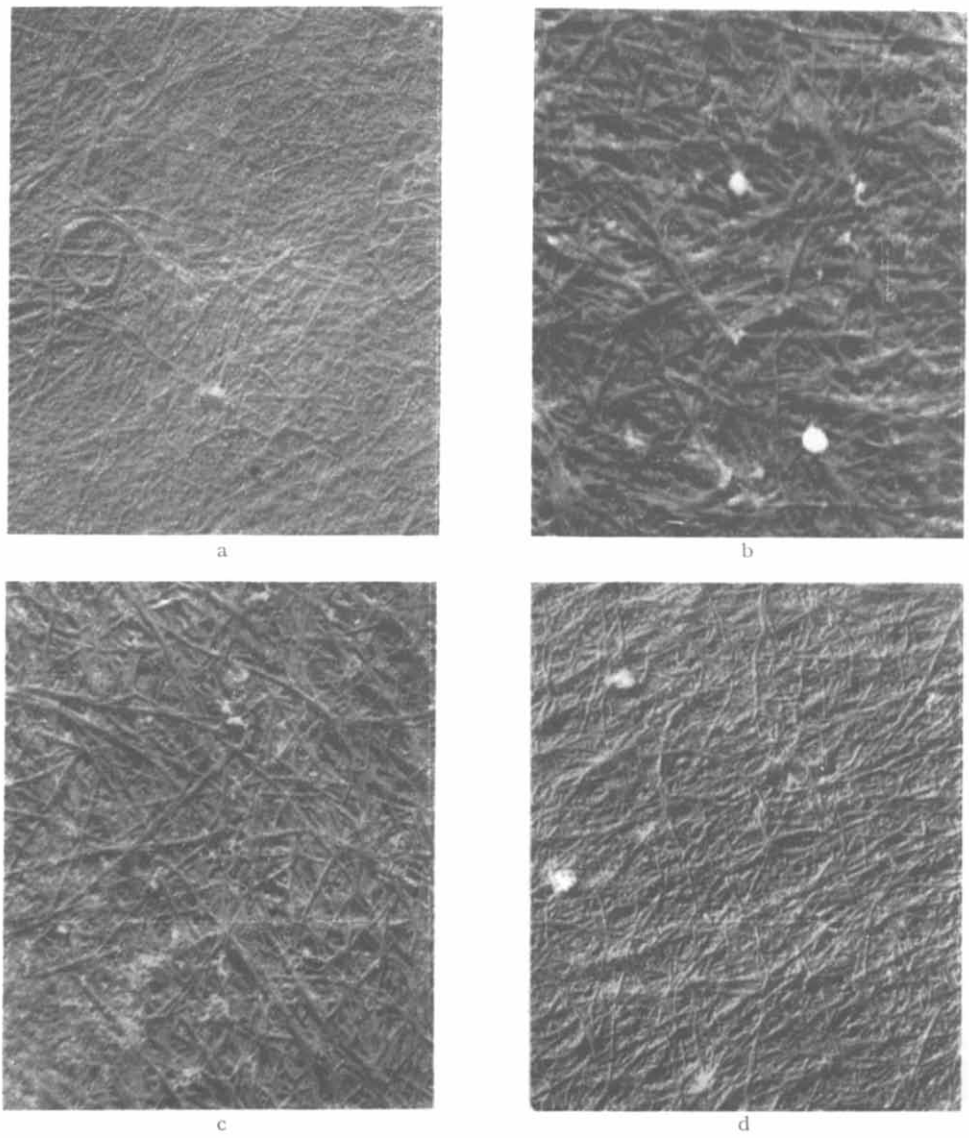

Fig. 2. *Laminaria saccharina*, electron micrographs of wall material in the conditions stated. Specimens shadowed with Pd-Au.a. Natural. Magnification × 30,000. b. After water extraction. Magnification × 30,000. c. After 4 *B* alkali extraction. Magnification × 30,000. After full treatment. Magnification × 30,000.

flosculosa. The dimensions of the individual microfibrils of each of the algae studied are given in Table I.

2. *The X-ray diagrams*

The spacings of the intermolecular planes calculated from the X-ray diagrams of all fifteen algae investigated, in a natural condition and after the fractionation processes, are set out in Tables II, III, IV, V.

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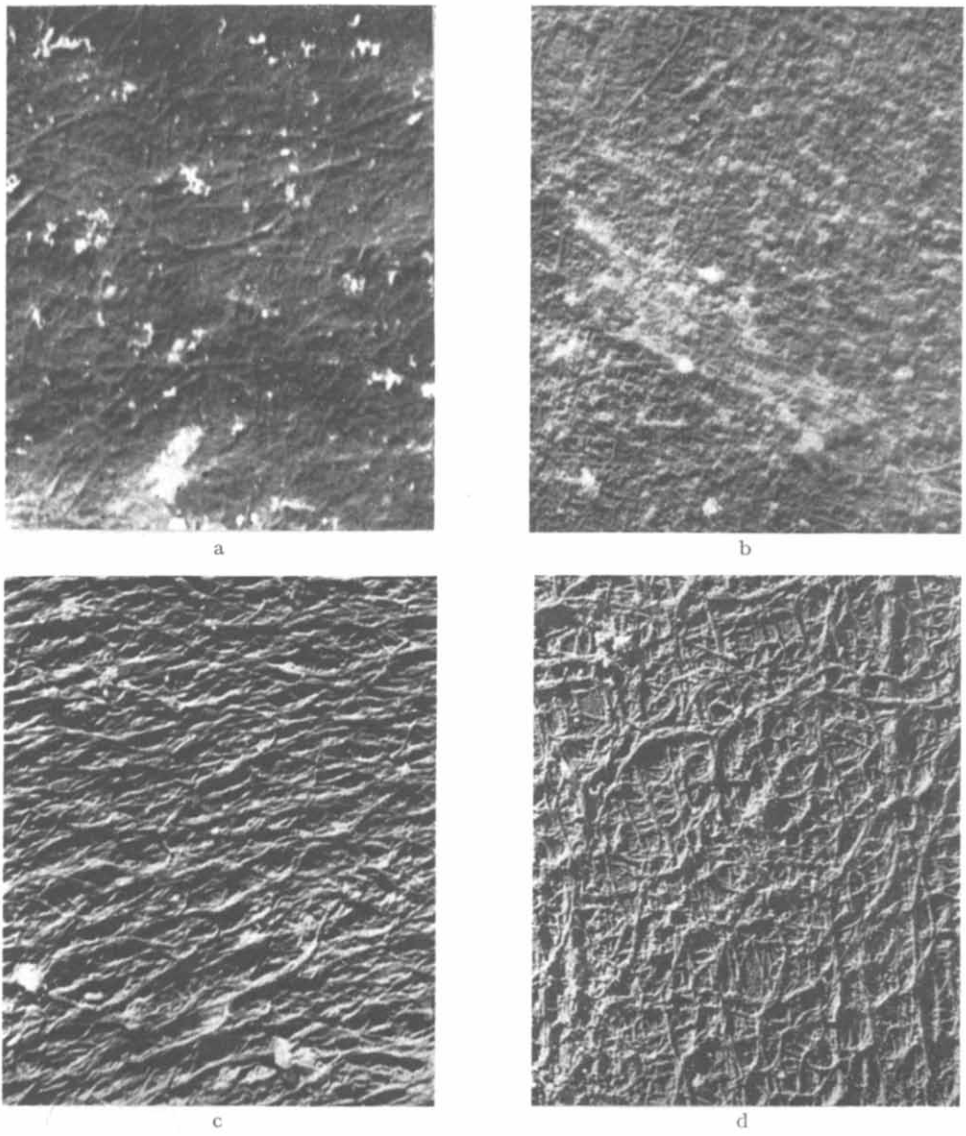

Fig. 3. *Fucus serratus*, electron micrographs of wall material in the conditions stated. Specimens shadowed with Pd-Au. a. Natural. Magnification × 30,000. b. After water extraction. Magnification × 30,000. c. After 4 *N* alkali extraction. Magnification × 30,000. d. After full treatment. Magnification × 30,000.

3. *Fractions expressed as a % of the dry weight of the starting material, and the sugars in the hydrolysis products*

These data are presented in Tables VI and VII for the green algae, VI and VIII for the brown algae, and VI and IX for the red algae. Identical results were obtained concerning the sugars in the hydrolysis products of all the members of the Fucales investigated, and are denoted as such. Similarly only one member of the Laminariales

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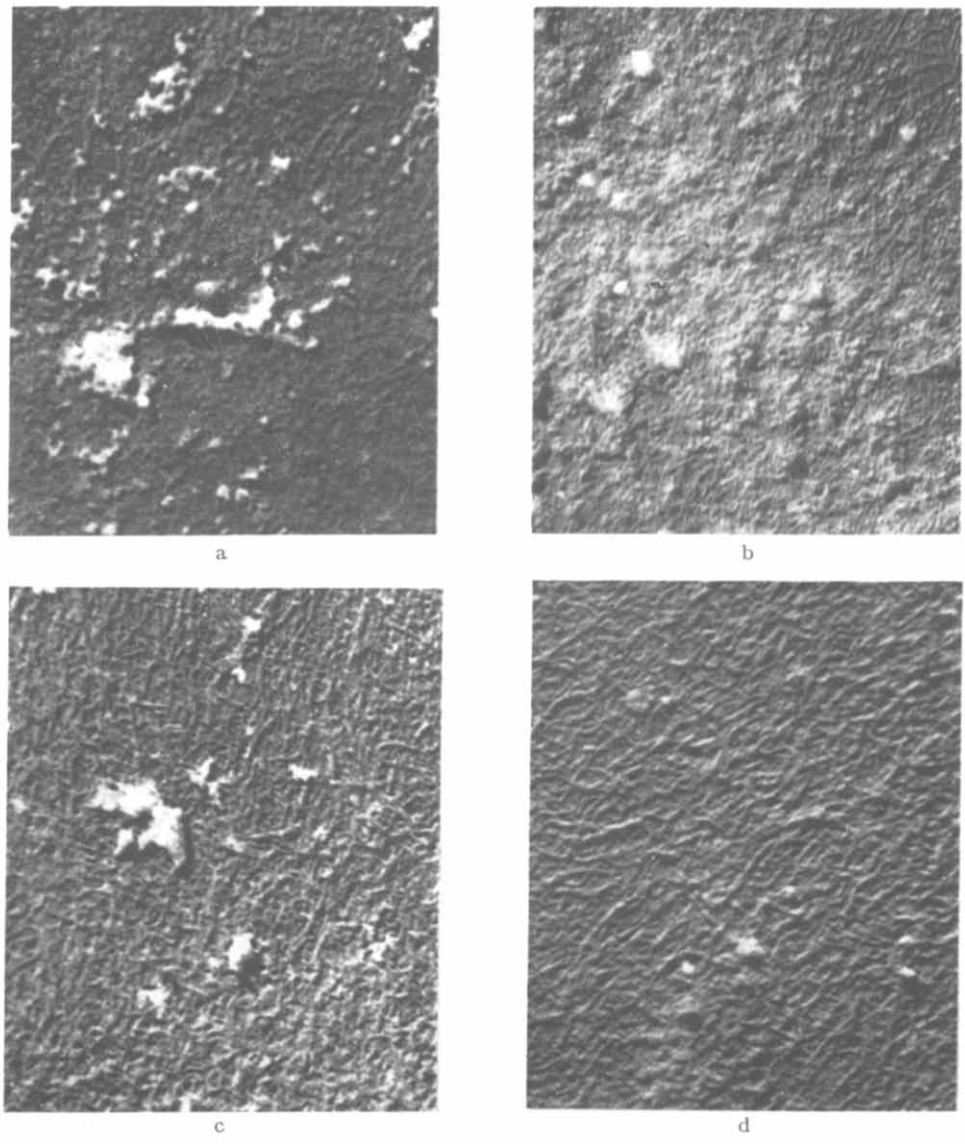

Fig. 4. *Rhodymenia palmata*, electron micrographs of wall material in the conditions stated. Specimens shadowed with Pd-Au. a. Natural. Magnification × 30,000. b. After water extraction. Magnification × 30,000. c. After 4 *N* alkali extraction. Magnification × 30,000. d. After full treatment. Magnification × 30,000.

is represented, the other giving the same results. In the tables the gradings strong, moderate and weak (S, M and W) refer to the intensity of the corresponding spots.

DISCUSSION OF RESULTS

A consideration of the results presented in the above survey enables us to make a division of the algal types studied into three groups. These are: (1) *Cladophora*

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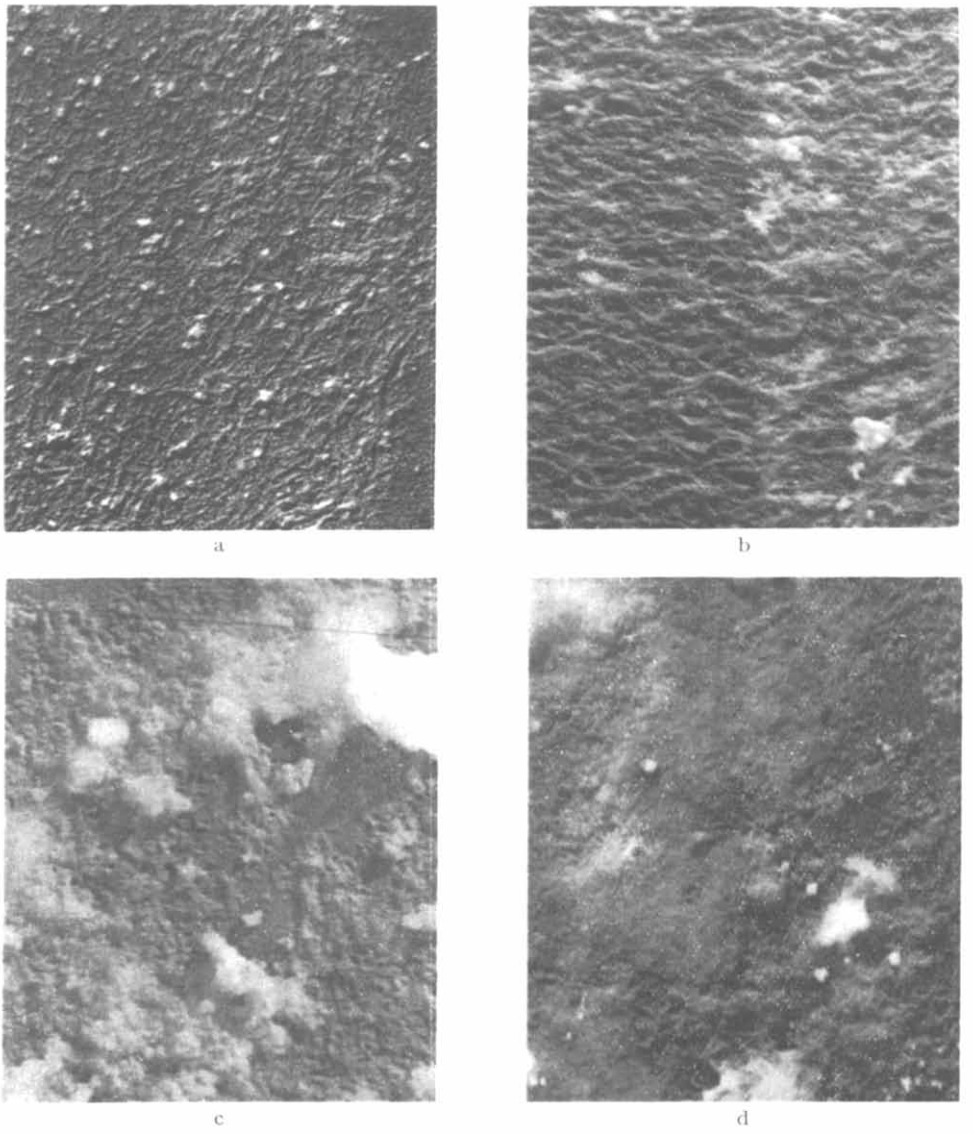

Fig. 5. *Porphyra* sp., electron micrographs of wall material in the conditions stated. Specimens shadowed with Pd-Au. a. Natural. Magnification × 22,500. b. After water extraction. Magnification × 30,000. c. After 4 *N* alkali extraction. Magnification × 30,000. d. After full treatment. Magnification × 30,000.

rupestris and *Chaetomorpha melagonium*; (2) *Ulva lactuca, Enteromorpha* sp., the brown algae, together with *Ptilota plumosa, Griffithsia flosculosa* and *Rhodymenia palmata*; (3) *Porphyra* sp.

A detailed discussion of the characteristics of these three groups is now given.

(1) *Cladophora rupestris and Chaetomorpha melagonium*

The cell walls of these two algae have been previously thoroughly investigated

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TABLE I

THE RANGE OF SIZE OF THE MICROFIBRILS

Species	*Width in A*	*Thickness in A*
Cladophora rupestris	300–250	100–70
Chaetomorpha melagonium	300–250	100–70
Enteromorpha sp.	190–140	80–45
Ulva lactuca	180–90	60–30
Halidrys siliquosa	250–150	130–40
Fucus serratus	250–150	130–40
Himanthalia lorea	250–150	130–40
Ascophyllum nodosum	180–160	75–70
Pelvetia canaliculata	190–100	55–40
Laminaria saccharina	230–220	90–60
Laminaria digitata	230–220	90–60
Ptilota plumosa	260–190	105–70
Griffithsia flosculosa	260–190	105–70
Rhodymenia palmata	260–190	105–70
Porphyra sp.	190–125	65–30

TABLE II

X-RAY DIFFRACTION RESULTS (A) NATURAL MATERIAL

Species	*Major intermolecular spacings A*				
Cladophora	6.07	5.3	4.4	3.89	2.61
Chaetomorpha	6.21	5.38	4.42	3.93	2.64
Enteromorpha	6.2	4.4	3.02	2.82	
Ulva	7.35	4.46	2.56		
Halidrys	5.2	3.94	2.6		
Fucus	4.78	3.85	2.6		
Himanthalia	6.8	4.4	3.85	2.7	
Ascophyllum	3.95				
Pelvetia	8.85	6.45	4.55	3.85	
L. saccharina	5.4	4.0			
L. digitata	5.25	3.85	2.6		
Ptilota	4.4	2.6			
Griffithsia	10.8	7.18	4.2	3.76	2.62
Rhodymenia	4.4	2.6			
Porphyra	4.45				

TABLE III

X-RAY DIFFRACTION RESULTS
(B) MATERIAL AFTER WATER EXTRACTION

Species	*Major intermolecular spacings A*				
Cladophora	6.04	5.25	4.3	3.9	2.6
Chaetomorpha	5.95	5.35	4.32	3.81	2.55
Enteromorpha	6.15	4.4	2.77		
Ulva	9.7	7.1	4.45	2.46	
Halidrys	5.2	3.94	2.6		
Fucus	8.95	5.2	4.32	3.86	2.56
Himanthalia	6.25	3.85	2.6		
Ascophyllum	8.5	5.1	3.95	2.54	
Pelvetia	4.46	3.68			
L. saccharina	5.08	3.85			
L. digitata	6.04	5.25	4.64	3.85	2.6
Ptilota	9.25	7.1	4.35	2.52	
Griffithsia	10.8	7.18	4.32	2.6	2.5
Rhodymenia	5.35	4.48	3.94	2.55	
Porphyra	5.4	4.28	3.7	3.42	2.63

TABLE IV

X-RAY DIFFRACTION RESULTS
(C) MATERIAL AFTER ALKALI EXTRACTION

Species	*Major intermolecular spacings A*						
Cladophora	6.04	5.25	4.3	3.9	2.6		
Chaetomorpha	5.94	5.2	4.24	3.84	2.6		
Enteromorpha	7.1	4.35	3.42	2.52			
Ulva	4.32	2.52					
Halidrys	4.45	3.85	2.6				
Fucus	4.4	4.0	2.62				
Himanthalia	7.1	4.35	3.85	2.46			
Ascophyllum	4.23	3.94					
Pelvetia	4.4						
L. saccharina	4.4	3.95	2.62				
L. digitata	6.15	4.4	3.94	2.6			
Ptilota	7.0	4.4	2.52				
Griffithsia	0.8	6.94	4.32	2.5			
Rhodymenia	4.4						
Porphyra	8.1	4.4	3.8	3.5	2.66	2.6	2.24

(NICOLAI AND FREY-WYSSELING[5]; NICOLAI AND PRESTON[6]). It has been shown that the wall is built up of many lamellae. Each lamella consists of straight microfibrils of Cellulose I arranged parallel to each other and surrounded by amorphous material. The directions of orientation of the microfibrils in adjacent lamellae are approximately at right angles. This structure has been confirmed in the present investigation (Fig. 1a). As judged by the X-ray results, the Cellulose I remains unaffected by our chemical treatments. The electron microscope, however, reveals a considerable change in the visible organisation of the wall. The treatments successively remove the amorphous binding materials, so that the microfibrils become easily separated, and show random arrangement after blending.

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TABLE V

X-RAY DIFFRACTION RESULTS
(D) MATERIAL AFTER THE CHLORITE TREATMENT

Species	*Major intermolecular spacings A*				
Cladophora	5.94	5.1	4.24	3.78	3.46
	2.99	2.54	2.37		
Chaetomorpha	6.04	5.25	4.35	3.85	2.55
Enteromorpha	4.35				
Ulva	7.0	4.4	2.54		
Halidrys	4.45	3.85	2.6		
Fucus	4.45	3.98	2.6		
Himanthalia	7.15	4.35	3.85	2.46	
Ascophyllum	4.47	3.88			
Pelvetia	4.4	3.86	2.52		
L. saccharina	4.45	3.85	2.6		
L. digitata	4.45	3.85	2.6		
Ptilota	7.0	4.4	2.52		
Griffithsia	7.1	4.35	4.0	2.46	
Rhodymenia	4.4				
Porphyra	7.2	4.47	3.94		

TABLE VI

	Water-soluble fraction %	*Alkali-soluble fraction %*	*Chlorite-soluble fraction %*	*α-cellulose %*
Cladophora rupestris	31.5	2	38	28.5
Chaetomorpha melagonium	41.5	8	9.5	41
Enteromorpha sp.	30	39	9	21
Ulva lactuca	52	25	4	19
Halidrys siliquosa	62	14	10	14
Fucus serratus	44.5	29	13.5	13.5
Himanthalia lorea	67	14	11	8
Ascophyllum nodosum	68.5	16	8.5	7
Pelvetia canaliculata	70	16	12.5	1.5
Laminaria saccharina	59	17.5	5.5	18
L. digitata	49	25	6	20
Ptilota plumosa	36	17.5	23	24
Griffithsia flosculosa	41.5	14	22.5	22
Rodymenia palmata	50	36.5	6.5	7
Porphyra sp.	49	47.5	0	3.5

These physical methods enable us to see that there are two fractions into which the wall is divisible; the microfibrillar fraction which is Cellulose I, and the amorphous matrix. The microfibrils hydrolyse giving glucose only; a result which would be expected from physical considerations. The sugars in the hydrolysis products of the amorphous materials are shown in Table VII.

(2) *Ulva lactuca, Enteromorpha* sp., *the brown algae, together with Ptilota plumosa, Griffithsia flosculosa and Rhodymenia palmata*

By contrast with *Cladophora* and *Chaetomorpha* the fine structure of the cell walls of the algae of this group has not previously been fully elucidated. The walls of

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TABLE VII

	Cladophora rupestris	*Chaetomorpha melagonium*	*Enteromorpha sp.*	*Ulva lactuca*
Water-soluble fraction	S Uronic acid S Galactose S Glucose M Arabinose W Xylose	S Arabinose M Galactose	S Glucose M Uronic acid M Galactose W Xylose	S Uronic acid S Glucose S Xylose W Rhamnose
Hemicellulose		S Arabinose	S Xylose S Rhamnose M Glucose	S Galactose S Arabinose S Rhamnose W Xylose
α-Cellulose before chlorite	S. Glucose M Galactose M Arabinose W Xylose	S Glucose M Arabinose	S Glucose M Xylose M Rhamnose	S Glucose S Xylose
α-Cellulose after chlorite	S Glucose	S Glucose W Arabinose	S Glucose M Xylose M Rhamnose	S Glucose S Xylose

TABLE VIII

	Halidrys siliquosa and all Fucales	*L. digitata and L. saccharina*
Water-soluble fraction	S Uronic acid M Galactose M Glucose M Xylose M Fucose	S Uronic acid M Galactose W Xylose W Fucose
Hemicellulose	S Xylose S Fucose	S Xylose S Fucose
α-Cellulose before chlorite	S Glucose M Xylose M Fucose	M Uronic acid S Glucose W Xylose
α-Cellulose after chlorite	S Glucose W Xylose W Fucose	S Glucose M Uronic acid

Ulva and *Enteromorpha* have been shown (NICOLAI AND PRESTON[6]) to give X-ray diagrams basically different from that of Cellulose I. KYLIN[7] has described two unidentified pectin-like polysaccharides obtainable from the walls, which he calls Ulvacin and Ulvin. The brown algae have been described by PERCIVAL AND ROSS[8] as having Cellulose I as the main structural component of the walls, which also contain up to 25% of a salt of polymannuronic acid (BLACK[9]).

On the evidence from staining reactions and solubility tests, cellulose has been described as a wall component of many species of the red algae (NAYLOR AND RUSSEL-WELLS[10]), though the cell walls of *Griffithsia flosculosa* have been shown to contain no Cellulose I (MYERS, PRESTON AND RIPLEY[11]). The commercially important agar-

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TABLE IX

	Ptilota plumosa	*Griffithsia flosculosa*	*Rhodymenia palmata*	*Porphyra sp.*
Water-soluble fraction	S Galactose M Uronic acid M Glucose M Xylose M Ribose W Arabinose	S Galactose M Uronic acid M Xylose	S Xylose M Galactose W Uronic acid W Glucose W Ribose	S Galactose M Uronic acid M Fucose W Ribose W Mannose
Hemicellulose	S Xylose	S Xylose	S Xylose	S Xylose M Galactose M Mannose
α-Cellulose before chlorite	S Glucose S Galactose M Xylose	S Glucose S Galactose M Xylose	S Glucose S Xylose	S Mannose W Xylose
α-Cellulose after chlorite	S Glucose W Galactose W Xylose	S Glucose W Galactose W Xylose	S Glucose S Xylose	S Mannose

agar and carragheen are water-soluble polysaccharides present in large amounts in the walls of some red algae. From *Rhodymenia palmata* a pure sample of xylan has been isolated by BARRY, DILLON, HAWKINS AND O'COLLA[12].

The walls of all the algae in this group, when observed in the electron microscope show the same basic structure of randomly arranged microfibrils embedded in an amorphous matrix. When treated by the methods described, they all show the same series of changes. The material presenting an amorphous appearance is gradually removed leaving after the final treatment a pure preparation of microfibrils. With the two species of *Laminaria*, however, a small amount of inter-microfibrillar material remains even after the final treatment.

As in the first group, the electron microscope observations enable us to visualise the wall as a two phase structure. The continuous amorphous phase, in which are embedded the microfibrils, is chemically further divisible on the basis of the solubility of the various components.

In contrast with the results obtained from the group I algae, the microfibrillar fraction hydrolysate is a mixture of sugars. Reference to the tables shows that glucose is still the major structural unit in these microfibrils, but it is invariably associated with other sugars, frequently xylose. The percentage of the microfibrillar fraction is amazingly low, reaching the extremely small value of 1.5% for *Pelvetia*. Each alga has its own characteristic mixture of sugars in the hydrolysis products of the water-soluble and alkali-soluble fractions, these being the same for the members of the Fucales, and similarly for the two members of the Laminariales, but differing for all the others.

A diagrammatic representation of the values of the major intermolecular spacings calculated from the 48 diagrams obtained from this group is displayed in Fig. 6. The X-ray diffraction diagrams from natural wall materials of the 12 algae of this group differ widely from each other. This is even more clearly illustrated in the results after the water extraction, when the diagrams obtained show a higher degree of

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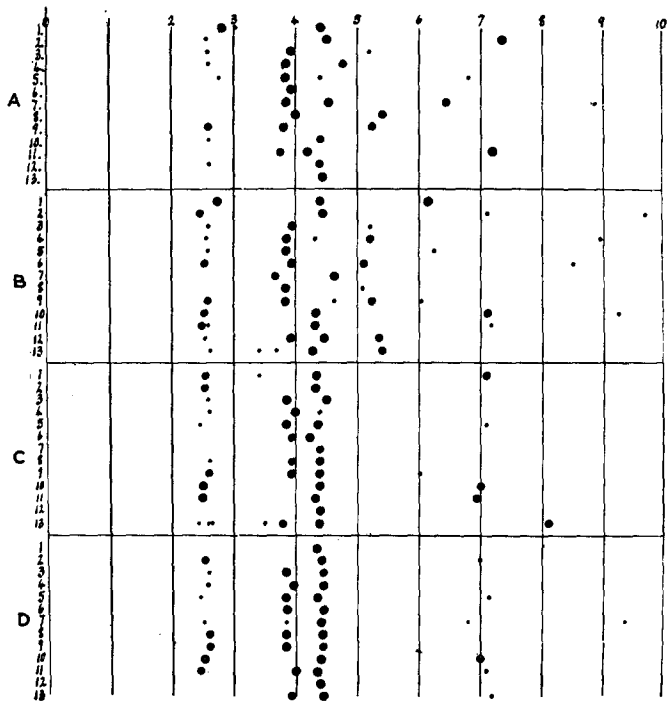

Fig. 6. A diagrammatic representation of the values of the major intermolecular spacings calculated from the X-ray diagrams obtained from the 13 algae of groups 2 and 3. 1. *Enteromorpha*. 2. *Ulva*. 3. *Halidrys*. 4. *Fucus*. 5. *Himanthalia*. 6. *Ascophyllum*. 7. *Pelvetia*. 8. *Laminaria saccharina*. 9. *Laminaria digitata*. 10. *Ptilota*. 11. *Griffithsia*. 12. *Rhodymenia*. 13. *Porphyra*. A. Natural. B. After extraction with hot water. C. After extraction with 4 *N* alkali. D. After treatment with chlorite.

crystallinity. After treatment of the wall materials with 4*N* alkali, on the other hand, the diagrams show a striking similarity, which is even closer after the chlorination. The final diagrams show at least one, but usually all three of the rings corresponding to planes of intermolecular spacings 4.4, 3.9, and 2.6 A. In comparing this result with that obtained from *Cladophora* and *Chaetomorpha*, it is clear that in this group the wall does not contain Cellulose I. The diagrams of *Laminaria* interpreted by PERCIVAL AND ROSS[8] as indicating the presence of Cellulose I cannot be due to this substance, since the diagram is changed by our mild treatment. The diagram of *Enteromorpha* which was previously uninterpretable (NICOLAI AND PRESTON[6]) is after treatment comparable to those of the other algae of this group.

The crystalline component of the wall, responsible for the X-ray diffraction diagrams, is probably to be identified with the microfibrils, as in other plant cell walls. There are two possibilities for the change in the X-ray diagrams; either a crystalline component is being extracted or the crystalline form of the remaining material is changed. A careful consideration of the results make the latter alternative the more probable.

(3) *Porphyra* sp.

The fundamental difference of *Porphyra* from all the other algae investigated has

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necessitated its separation into a third group. A polymannose has previously been described from *Porphyra umbilicalis* (JONES[13]) from an acid hydrolysis product, together with galactose and fucose. The special relationship of these substances in the plant however has not been previously described.

Porphyra in a natural condition when observed in the electron microscope presents no major differences from the algae of Group 2. Again the picture is one of randomly arranged microfibrils embedded in apparently structureless material. Water extraction removes much of this amorphous matrix (Fig. 5) and a chlorination at this stage removes the remainder. After treatment with alkali, however, microfibrils are no longer visible. The remaining material presents the appearance of an accumulation of particles, the dimensions of which are comparable with the diameter of the original microfibrils. We must conclude, therefore, that the alkali extract contains polysaccharides from the microfibrils. These polysaccharides hydrolyse to give a mixture of xylose, galactose and mannose, whilst the remaining particulate fraction after the final treatment hydrolyses to give only mannose. Glucose was not identified from any of the four fractions.

The X-ray diagram, whilst changing with every treatment, finally shows rings corresponding to molecular planes of spacing identical with those of Group 2.

CONCLUSIONS

The first conclusion to be drawn from the results that have been presented is that, of the algae considered, only the cell walls of *Cladophora* and *Chaetomorpha* show any resemblance to the condition in higher plants, supporting the view expressed by NICOLAI AND PRESTON[6]. In the future any attempts to derive the higher plants from an algal ancestry must be made bearing this in mind.

The species which have been collectively placed in Group 2 are characterised by a uniform wall structure. The major crystalline component is certainly not Cellulose I. It hydrolyses to give a mixture of sugars always containing glucose and usually xylose. Since the xylose has been shown to be an integral structural unit of the microfibrils, then these contain fewer hydrogen bonds than corresponding microfibrils of Cellulose I. This may be connected with the invariably observed phenomenon that the microfibrils are extremely intertwined, never running straight over more than a very short distance.

Porphyra illustrates a case in which the basic building unit of the microfibrils is not glucose, although the structural features of the wall characteristic of Group 2 may be observed in the electron microscope. Mannose which appears to replace glucose in the wall of this plant is, like glucose, an aldohexose sugar, and this difference in the end product of carbohydrate metabolism probably reflects a fundamentally different physiological system.

The method of X-ray analysis which has been used in the past for the identification of crystalline cell wall components can give confusing results; the present investigation has revealed instances of chemically differing carbohydrates giving the same diagram. X-ray diffraction, which in modern laboratories had come largely to replace staining reactions for the identification of cell wall constituents, must now be used with caution.

Finally it may be said that this study of algal cell walls has shown that the

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physical and chemical methods used are compatible and that their combined application puts a tool of considerable power in the hands of biologists.

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SUMMARY

A study has been made of the cell walls of 15 species of marine algae. The cell walls were separated chemically into four fractions which were hydrolysed and analysed by paper chromatography for their constituent sugars. Before and after each chemical extraction samples were examined by X-ray diffraction analysis and electron microscopy.

The results indicate that the algae investigated may be divided into three groups on a basis of their cell wall structure. Only Group 1 (*Cladophora rupestris* and *Chaetomorpha melagonium*) shows any resemblance to the condition in higher plants by the presence of Cellulose I. The algae of Group 2 (comprising the remaining sp with the exception of *Porphyra*) are characterised by a crystalline microfibrillar fraction which is certainly not Cellulose I and hydrolyses to give a mixture of sugars always containing glucose and often xylose. *Porphyra* is quite unique and forms a third group in which mannose replaces glucose as the basic structural unit of the microfibrils.

The importance of the material presenting an amorphous appearance in the electron micrographs is stressed.

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URIDINE PYROPHOSPHOGLYCOSYL COMPOUNDS AND THE FORMATION OF GLUCURONIDES BY ISOLATED ENZYME SYSTEMS

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The isolation of uridine pyrophosphoglucuronic acid (UPPGA) from liver tissue by SMITH AND MILLS[1] and the demonstration that this compound acted as a glucuronic acid donor in the formation of glucuronides by liver homogenates opened the way to

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