The Chemical Composition and Structure of the Cell Wall of *Chlorella pyrenoidosa*

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The distribution of chemical substances in a complex organized structure such as a plant-cell wall can be studied by correlating a chemical analysis with a microscopical examination of the material. With green plants it is necessary to obtain a homogeneous tissue and to separate the cells before isolating the walls, and these are difficult experimental procedures. In the present work they have been avoided by using a unicellular green alga, *Chlorella*. It has been possible to isolate from this plant a cell-wall preparation which appears to be free from cell contents, and by using the electron microscope much of the fine detail of its structure has been shown. However, no histochemical stains, even of the limited specificity of those used in optical microscopy, are available for use with the electron microscope and it was difficult to assess the chemical nature of the structures observed. We have attempted to isolate the structural components from the wall and analyse them directly. To do this the wall has been degraded by chemical and enzymic methods which have destroyed one or more components but have left a discrete part of the wall which could be seen with the aid of the electron microscope.

The wall is composed of two distinct phases: an organized microfibrillar structure embedded in a continuous matrix, and it has been possible to separate these more or less intact one from the other. Treatment of the wall with dilute sodium or potassium hydroxide solutions removed the matrix and bundles of microfibrils were isolated. Incubation with an enzyme preparation made from the intestinal tract of the snail removed the microfibrillar elements and left a large part of the matrix material still in the shape of the original cell wall. In this way not only the chemical composition of the various parts of the wall were determined but also a more detailed knowledge of the distribution of the components was obtained.

The chemical composition of the cell walls of algae by direct isolation and chemical analysis has not previously been studied, although some indirect staining reactions and X-ray studies have been correlated with the cell-wall structure in certain species (Nicolai & Preston, 1952–53; Kreger, 1957).

EXPERIMENTAL AND RESULTS

Material used and general analytical methods

Alga. Chlorella pyrenoidosa was grown under sterile conditions on a completely defined salt medium (Emerson & Lewis, 1939) in large flasks (1–3 l.). A current of air + CO_2 (95:5) was passed through the medium (600 ml./min.) and growth was maintained for 10–14 days in daylight in a window facing west. The cells were harvested by centrifuging (1500 *g* for 20 min.), and washed with water and recentrifuged. Yield was approx. 0·7 g. dry wt./l.

Isolation of the cell-wall material by mechanical breakage of the cell. Chlorella cells (50 mg. dry wt.) were suspended in 10 ml. of water with 4 g. of fine glass beads (Ballotini no. 12, 0·15 mm. diam., Chance Bros. Ltd., Birmingham) and the mixture was placed in a vertical cup (internal measurements 5 cm. × 2·2 cm.) of a Mickle cell disintegrator (Mickle, 1948). Vibrations were continued for 90 min., after which the resultant suspension was treated as shown in Fig. 1, where the yields of the various fractions are also given. The temperature of the mixture during the breakage rose 15° from the initial room temperature.

Washed whole *Chlorella* cells centrifuged at 800 *g* for 10 min. and at 190 *g* for 30 min. came down completely into the sediment, leaving a clear colourless supernatant.

All the analyses were carried out on material dried at 0·01 mm. Hg over P_2O_5 at room temperature. Total N was determined by micro-Kjeldahl digestion (Chibnall, Rees & Williams, 1943) followed by distillation and titration. Total P was determined according to Fiske & Subbarow (1925).

Chromatography and electrophoresis of sugars. Descending chromatograms were run on Whatman no. 1 papers with pyridine–ethyl acetate–water (1:2:2, by vol.) for 14 hr. (Jermyn & Isherwood, 1949). Electrophoreses were run at 210 v in an apparatus similar to that described by Consden & Stanier (1952), with borate buffer, pH 9·2. The sugar spots were coloured in both procedures with aniline hydrogen phthalate (Partridge, 1949).

Chromatography of amino acids. Two-dimensional chromatograms were run in butanol–acetic acid–water (4:1:5, by vol.) and phenol buffered at pH 9·3 with borate according to Levy & Chung (1953). The spots were coloured with 0·3% triketohydrindene hydrate in butanol.

Chromatography of amino sugars. These were investigated by the methods described above for both sugars and amino acids. The spots were coloured by the spray reagents used for sugars and amino acids and also by the Elson–Morgan reagent described by Partridge (1948).

Enzyme preparation from Helix pomatia. The snails were dissected according to Keilin (1956) and the digestive tracts removed and cooled in ice. They were mixed with an equal volume of ice-cold water and crushed in a homogenizer with a Perspex pestle (Potter & Elvehjem, 1936). The resultant extract was dialysed against distilled water at 4° for 24 hr. and any precipitate which formed was removed by centrifuging at 15 000 *g* at 4° for 20 min.

This preparation has been quantitatively analysed and shown to contain only weak proteolytic activity but active lipases and carbohydrases. Some twenty different carbohydrases were examined, including cellulase, xylanase and mannanase (Myers & Northcote, 1958).

Chemical investigation of the cell wall

Elementary analysis and mineral content. The total N was 4·6%, and the total P 0·67% of the dry weight. When maintained at red heat (approx. 800°) in a platinum boat in a stream of clean dry air for 1 hr., 2·11 mg. of walls yielded a light yellow ash; 0·11 mg., 5·2%. This ash was dissolved in dil. HNO_3 and investigated chromatographically on Whatman no. 4 paper run with butanol saturated with N-HCl at 35° for 48 hr. The spots were detected by spraying with kojic acid and 8-hydroxyquinoline (Lederer & Lederer, 1955) and examination of the paper, before and after exposure to NH_3 vapour, under ultraviolet light. Iron, calcium and possibly aluminium were detected; no other spots were visible.

Lipid analysis. The lipid was determined by boiling the cell walls (55·1 mg.) with aq. 95% (v/v) methanol for 2·5 hr., and centrifuging and siphoning off the alcoholic supernatant from the walls, which were then redried at 0·01 mm. Hg over P_2O_5. The material was re-extracted with boiling dry ether for 2·5 hr. Yield of extracted cell walls was 50·1 mg. The alcohol and ether solutions were evaporated and the fat was weighed directly. Yield was 4·9 mg. (8·9%) and the wt. loss of the walls was 5·0 mg. (9·2%).

Acid hydrolysis. (i) For monosaccharide constituents. The whole cell walls (4 mg.) were hydrolysed by 2 ml. of 2N-H_2SO_4 for 6 hr. at 100°, then neutralized with $BaCO_3$ and filtered and the filtrate evaporated to dryness. Glucose, galactose, mannose, arabinose, xylose and rhamnose were identified in the hydrolysate by chromatographic and electrophoretic investigations.

(ii) For amino acids. Whole cell walls (4 mg.) were hydrolysed by boiling in 5 ml. of a mixture (1:1, v/v) of conc. HCl and formic acid for 24 hr. The hydrolysate was evaporated at 25° under vacuum, and dissolved in water and re-evaporated several times. Serine, glycine, glutamic acid, threonine, arginine, lysine, histidine, alanine, proline, tyrosine, valine, methionine, phenylalanine and leucine were identified in the hydrolysate.

(iii) For amino sugars. Whole cell walls (4 mg.) were hydrolysed by 1 ml. of 3N-HCl at 100° for 6 hr. The hydrolysate was evaporated at 25° under vacuum, dissolved in water and re-evaporated several times. The hydrolysate was finally dissolved in 5 ml. of 0·5N-HCl and put through an ion-exchange column (Dowex 50), which was washed with 10 ml. of water, and then the amino sugars were eluted with 2N-HCl and estimated according to Boas (1953). Calculated as glucosamine the yield was 3·3%. A neutral solution of the amino sugar was investigated chromatographically and electrophoretically and was identified as glucosamine.

Polysaccharides of the cell wall. Walls (50·1 mg.) from which fat had been extracted were treated with 10 ml. of

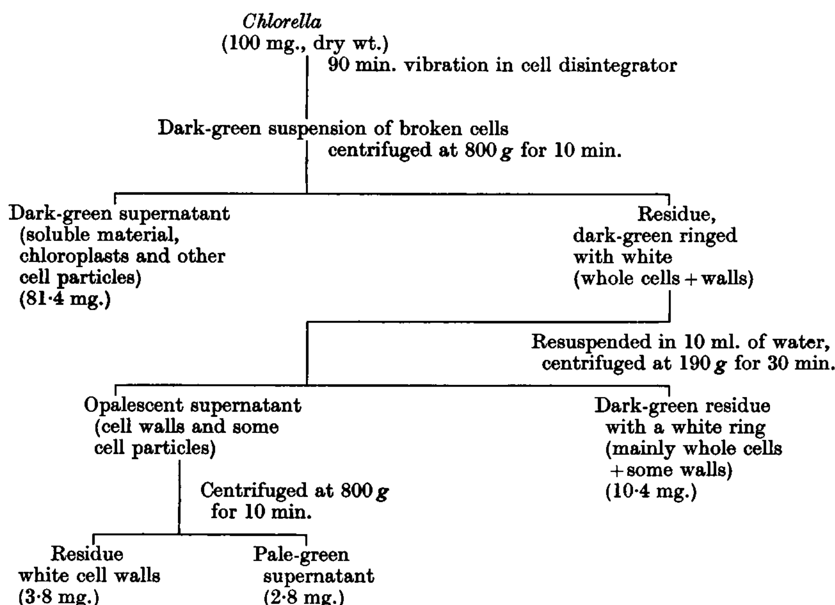

Fig. 1. Differential centrifuging of mechanically disintegrated *Chlorella* cells. The total recovery of material was 98·4 mg.

24% (w/v) KOH at room temperature under N_2 for 2 hr. The solution was siphoned off after centrifuging and the residue re-extracted with a further 10 ml. of KOH for 2 hr. The residue from this extraction was thoroughly washed with water by suspension and centrifuging. Normally six to eight washings were required to give a supernatant with the same pH value as the original wash water. The washings and alkali extracts were combined and the residual α-cellulose was dried over P_2O_5 at 0·01 mm. Hg. Yield of white preparation was 8·5 mg., 15·4% (N, 0·0%; ash, 0·05%).

The alkaline solution of hemicellulose was neutralized at 4° with cooled acetic acid and then evaporated under reduced pressure to half its volume. It was poured into a volume of ethanol so that the final concentration of alcohol was 85%, and the precipitate which formed was allowed to settle for 24 hr. This precipitate was centrifuged, washed with ethanol and dried. Yield, 34·1 mg. From a previous experiment it was known to contain mineral matter (approx. 27%) and N (approx. 3%) and a hydrolysate contained both amino acids and the amino sugar. It was therefore redissolved in water and reprecipitated with a mixture (1:4, v/v) of acetic acid and 85% ethanol. This precipitate was well washed with ethanol and dried. Yield of white powder was 17·1 mg., 31·0% (N, 0·2%; ash, 0·3%).

The α-cellulose was hydrolysed and the sugars were investigated by chromatograms and electrophoresis. Glucose, galactose, arabinose, mannose, xylose and rhamnose were identified. The colour of the spots of glucose and galactose was relatively much more intense than that of the other sugars. A hydrolysate of the hemicellulose contained galactose, mannose, arabinose, xylose and rhamnose. The colour of the galactose spot was relatively much more intense than that of the other sugars. A solution of the hemicellulose gave no colour with dilute iodine solution and contained no starch.

Separation of alkali-soluble and -insoluble polysaccharides from the whole cell

Freeze-dried *Chlorella* cells (50 g.) were extracted with 1·2 l. of methanol on a boiling-water bath for 6 hr. and filtered, and the pale-green residue was washed with methanol. The cells were then re-extracted with 600 ml. of boiling acetone for 6 hr. and filtered, and washed with acetone and dried. The cells, which were still coloured a light green, were treated with 500 ml. of 3% NaOH at 100° under N_2 for 6 hr. The residue was centrifuged and washed twice with water and then re-extracted at room temperature with 200 ml. of 24% KOH, centrifuged and washed six times with water. The residual material was dried. Yield, 1·05 g., 2·1% (N, 0·02%; ash, 0·8%).

The 3% NaOH extract and the washings were combined, cooled in ice and brought to pH 4·2 with acetic acid. A precipitate formed, which was centrifuged off. After evaporation to small bulk under reduced pressure, the supernatant liquid was poured into 2 l. of ethanol, whereupon a precipitate formed. This was separated by centrifuging, dried and dissolved in 130 ml. of water. The solution was cooled in ice and 100 ml. of trichloroacetic acid added in small portions until precipitation was complete. The mixture was centrifuged and the supernatant liquid was added to 2 l. of ethanol, whereupon a white flocculent precipitate of the soluble polysaccharides was obtained. This was collected, washed with ethanol and dried. Yield 4·34 g., 8·7% (N, 0·1%; ash, 0·05%).

Insoluble polysaccharides. These were hydrolysed with acid and then investigated by chromatography and electrophoresis, which showed the presence of glucose and galactose. This fraction probably corresponded to the α-cellulose isolated from the cell walls.

Soluble polysaccharides. A solution of this fraction gave a blue colour with dilute iodine solution. Acid hydrolysis followed by chromatography and electrophoresis showed the presence of galactose, glucose, arabinose, mannose, xylose and rhamnose. A solution of the polysaccharides (100 mg. in 10 ml. of borate buffer, pH 9·2) was investigated in a Tiselius electrophoresis apparatus (Perkin-Elmer and Co., U.S.A.; Model 38) by the method described by Northcote (1954). Three peaks were observed with mobilities of $2·5 \times 10^{-5}$ cm.^{2}v^{-1} sec.$^{-1}$, $7·48 \times 10^{-5}$ cm.^{2}v^{-1} sec.$^{-1}$ and $12·5 \times 10^{-5}$ cm.^{2}v^{-1} sec.$^{-1}$. Calculated from the areas under the curves the relative amounts of the components present were approximately 2:4:1 respectively.

The solution of the polysaccharides was investigated by zone electrophoresis on glass paper with borate buffer, pH 9·2 (Fuller & Northcote, 1956). Three spots were obtained. The distances of the spots from the starting line towards the cathode were 0·0, 3·0 and 5·0 cm.; 2:3:6-tri-*O*-methyl-D-glucose moved 13 cm. Starch used as a marker gave a spot at 5·0 cm. from the origin. The spot from the mixture which moved to 5·0 cm. was shown to be starch by its blue-staining reaction with iodine vapour. A solution of the hemicellulose isolated from the cell wall gave a spot at 3·0 cm. from the origin.

Purification of a soluble polysaccharide from the whole cell. The alkali-soluble polysaccharide (1 g.) was dissolved in 50 ml. of water to give a clear solution. To this was added a solution (2·5 ml.) containing amylase (prepared from human saliva by adding 10 vol. of water and centrifuging) and the mixture was kept at 25° for 30 hr. under toluene. A control solution containing 0·5 g. of soluble starch in 50 ml. was also treated in the same way and this, after incubation, gave a light-red colour with iodine solution. The solution of the polysaccharides from *Chlorella* was dialysed against running tap water for 12 hr. and then against 10 l. of distilled water for 6 hr. A light precipitate formed in the polysaccharide solution and this was removed by centrifuging. The clear supernatant was precipitated with 350 ml. of 80% (v/v) ethanol. The precipitate was collected and dried; yield, 0·4 g. A solution of this material gave no colour with iodine. In the Tiselius apparatus it gave two peaks with mobilities of $8·7 \times 10^{-5}$ cm.^{2}v^{-1} sec.$^{-1}$ and $12·5 \times 10^{-5}$ cm.^{2}v^{-1} sec.$^{-1}$; the relative amounts of the components present were approximately 3·5:1. Thus the peak with the mobility of $2·5 \times 10^{-5}$ cm.^{2}v^{-1} sec.$^{-1}$ in the original alkali-soluble polysaccharide fraction was identified as starch. Electrophoresis on glass paper showed one spot corresponding to the substance moving at 3·0 cm. in the original material.

A solution of 0·1 g. of the alkali-soluble polysaccharides in 2 ml. of borate buffer (pH 9·2) was put on a glass-powder electrophoresis column (Hocevar & Northcote, 1957) and a separation of the components obtained. A polysaccharide ($[\alpha]_D^{17}$ + 17·1 in water *c*, 1·0; *l*, 2·0) was obtained which gave one peak in the Tiselius electrophoresis apparatus with a mobility of $9·9 \times 10^{-5}$ cm.^{2}v^{-1} sec.$^{-1}$. This corresponded

with the component with a mobility of $7 \cdot 48 \times 10^{-5}$ cm.^{2}v^{-1} sec.$^{-1}$ in the original mixture or that with a mobility of $8 \cdot 7 \times 10^{-5}$ cm.^{2}v^{-1} sec.$^{-1}$ in the amylase-treated material. On glass-paper electrophoresis it gave one spot corresponding to the substance moving at 3·0 cm. in the original mixture and to the hemicellulose isolated from the cell wall. An examination of the acid hydrolysate of this polysaccharide showed the presence of galactose, arabinose, mannose, xylose and rhamnose. The colour of the galactose spot on the chromatogram was relatively much more intense than that of the other sugars.

Analyses of the cell walls treated with snail digestive enzyme

Cells walls (32·7 mg.) were incubated at 25° at pH 6·0 with snail digestive-enzyme preparation and control experiments were carried out concurrently with enzyme in the absence of cell walls and with cell walls in the absence of enzyme. After 14 hr., the cell walls were centrifuged, and washed with water and dried; yield, 25·8 mg. (loss of wt. 21·1%). No precipitate was observed in the incubation without cell walls and no loss in weight was apparent in the incubation without enzyme.

The digested walls were then analysed by the procedure already described. Found: lipid, 1·7 mg., 5·2%; hemicellulose, 8·8 mg., 27% (N, 0·5%; ash, 0·2%); α-cellulose 1·5 mg., 4·6% (N, 0·0%; ash, 0·2%). Compared with the original walls the material lost was made up of lipid, 4·0%, hemicellulose, 4·0%, and α-cellulose, 10·8%, which accounts for a total loss of material of 18·8%. Very little protein was removed, probably because of the weak proteolytic activity of the enzyme preparation. In terms of the individual constituents of the wall the digestion had removed 70% of the total α-cellulose, 43% of the lipid but only 13% of the hemicellulose.

Microscopical examination of the cell wall

The cell walls were examined by the optical and electron microscope. Whole cells can easily be distinguished under the optical microscope. Many fields of numerous preparations were examined and few whole cells were detected. The electron-microscope examinations showed that in nearly all cases few small cell particles contaminated the preparations.

A comprehensive examination of the cell wall was made under the electron microscope with a Sieman's Elminskop and with a high-tension voltage of 80 kv. Both unsectioned and sectioned material was examined. The preparations were supported on nitrocellulose films stabilized with carbon, which were mounted on Athene copper grids (New 200, diam. 2·3 mm.; Smethurst, High-Light Ltd., Bolton, Lancs.).

Unsectioned material was examined as follows. The walls were suspended in water and allowed to dry at room temperature in air on to the specimen grids. These were shadowed with uranium or chromium in an experimental evaporator (Coslett & Horne, 1955). The shadowing angle was 60°.

Sectioned material was prepared by fixing the walls in buffered (pH 7·3) osmium tetroxide soln. (1%, w/v) for 2 hr. (Palade, 1952); they were then washed with water and dehydrated with ethanol in the normal manner. The specimens were embedded in a mixture of 85% (v/v) of butyl methacrylate and 15% (v/v) of methyl methacrylate which was polymerized with 1·5% (w/v) of benzoyl peroxide at 50°. The sections were cut with glass knives in a microtome described by Porter & Blum (1953).

Unsectioned material. The microfibrils of the cell wall could be seen in the untreated preparations (Plate 1, Fig. 2), but were more apparent when the walls were treated with dilute NaOH soln. at room temperature (Plate 2, Fig. 3*a–e*). The NaOH soln. used was either 0·5 or 3% (w/v), and the time of exposure to the alkali varied from 5 min. to 24 hr. With the stronger solution and times of over 30 min. the wall lost its shape and eventually disintegrated into bundles of isolated fibres (Plate 1, Fig. 4). The milder treatment revealed the microfibrils as structures having a diameter of approx. 30–50 Å existing as a complete network over the wall and lying in two principal directions at right angles to one another (Plates 1 and 2, Figs. 5 and 3). In some of the specimens (Plate 1, Fig. 2) the inner surface of the wall could be seen at the characteristic cleavage which occurred when the cells were broken in the cell disintegrator. The orientation of the microfibrils in this region was the same as that on the outer surface. Generally the microfibrils were irregularly interwoven throughout the entire wall and covered the wall completely (Plates 1 and 2, Figs. 2 and 3). The microfibrils could also be exposed by treating the walls with ethanolamine (Wise, Peterson & Harlow, 1939) for 4 hr. at 25° (Plate 1, Fig. 5). The characteristics of these preparations were the same as those described above.

The intermicellar material was revealed as a granular substance packed around the microfibrils and could be seen *in situ* in the preparations which were treated with NaOH soln. for short times (Plate 2, Fig. 3). When the walls were treated with snail digestive enzyme (see above) they retained their shape but no microfibrils could be seen. Instead the surface of the wall appeared rough and made up of a granular substance (Plate 3, Fig. 6). The walls under these conditions were quite mechanically sound and could be centrifuged, placed on the supporting grids and embedded in the acrylate without disintegration. However, if these preparations were treated with 0·5% NaOH soln. for 5 min. or longer they completely fell apart and very little insoluble material could be recovered. Material previously treated with 0·5% NaOH soln. for 25 min. and then with the enzyme preparation still retained the shape of the cell wall and although very thin and fragile could be recovered. When examined microscopically it had a granular appearance (Plate 1, Fig. 7).

Sectioned material. The difficulty in the interpretation of the sectioned material was that of distinguishing definite layers in the wall from a layer-like appearance due to the representation by a two-dimensional photograph of a three-dimensional section in which the top and bottom edges of the section could appear as layers in the photograph. In some cases (Plate 4, Fig. 8) sections of the wall could easily be recognized as ribbon-like structures having a top and bottom edge. But generally our interpretation of the sectioned material is of necessity cautious and depends upon a close study of a very large number of such preparations.

The section of the whole cell (Plate 3, Fig. 9) showed that the preparation as isolated (Plate 4, Fig. 8) did represent the outer membranes of the cell. The thickness of the wall measured from these sections (Plates 3 and 4, Figs. 9 and 8)

was approx. 210 Å. The overall diameter of the cells was about 3–4 μ. In the section shown in Plate 3, Fig. 9, the outer wall appeared to consist of at least two membranes and after treatment with the digestive enzyme this division into membranes became much more apparent (Plate 4, Fig. 10). Each membrane indicated by the dark line, i.e. electron-dense or osmium-stained material, was approx. 50 Å thick and the space between them was approx. 100 Å. Thus the section at this region was in fact approx. 200 Å deep, which represents the thickness of the intact wall.

An interesting characteristic of these sections is the pronounced curling which takes place at the breakage cleft (Plate 4, Figs. 8 and 10). It was not ascertained at what stage after breakage this occurred.

The preparations treated with dilute NaOH soln. showed no definite lamellae but the walls seemed to have swollen (Plate 3, Fig. 11). The reticulate nature of the microfibrils was continuous throughout the section with no obvious lamellae due to local concentrations or variations in orientation. The microfibrils when stained with osmic acid appeared as a rather fluffy haze.

Sections of walls treated first with dilute NaOH soln. and then with the enzyme preparation were difficult to stain with osmic acid and showed no appearance of membranes.

DISCUSSION

The cell wall of *Chlorella pyrenoidosa* has been obtained by mechanical breakage and differential centrifuging. It is not contaminated by whole cells or cell debris. The material isolated has a fairly constant composition and when examined microscopically it resembles the wall of the intact cell. The cell wall has a thickness of approximately 210 Å whereas the diameter of the whole cell is 3–4 μ.

The general analysis of the cell wall shows an approximate composition of protein, 27 %; lipid, 9·2 %; α-cellulose, 15·4 %; hemicellulose, 31·0 %; glucosamine, 3·3 %; ash, 5·2 % (containing iron and calcium), which accounts for over 90 % of the material. The protein content of the wall is high compared with the few analyses that have been made on other plant-cell walls from soft tissues (Thimann & Bonner, 1933; Tripp & Rollins, 1952; Wirth, 1946). It has been calculated in this analysis from the nitrogen content of the walls after correcting for the amount of amino sugar present. This protein may be structural in function or metabolically active as part of the synthetic system of the cell-wall constituents, or both. The presence of the amino sugar is of interest in relation to the high protein content of the wall and might indicate the presence of a glycoprotein. It seems unlikely that an insoluble polysaccharide such as chitin is present since the glucosamine component is soluble in dilute sodium hydroxide.

No uronic acids were detected in the chemical analyses and if these are present they are presumably in very low concentrations. Normally the uronic acid constituents of the plant-cell wall are associated with the middle lamella or intercellular material which is absent in this unicellular alga.

The α-cellulose of the wall as isolated by the analytical procedure is composed of polysaccharide(s) made up of galactose, arabinose, mannose, xylose and rhamnose in addition to glucose.

The hemicellulose fraction of the cell wall on hydrolysis gives rise to galactose in relatively large amounts and also to mannose, arabinose, xylose and rhamnose. The alkali-soluble polysaccharides from the whole cells have been investigated electrophoretically and shown to contain at least three components. One of these has similar characteristics to that obtained from the cell wall and another is starch. An electrophoretically pure polysaccharide has been obtained from this mixture which resembles the material isolated from the wall. The purification was achieved and followed analytically by using electrophoretic procedures. The hemicellulose has a mobility of $9{\cdot}9 \times 10^{-5}$ cm.^{2} v^{-1} sec.$^{-1}$ in 0·05 M-borate buffer, pH 9·2, 0° and $[\alpha]_D^{17}$ of +17·1°.

The yield of hemicellulose and α-cellulose from the whole cell together with the analytical amounts of these constituents in the isolated wall enables the percentage (w/w) of wall in the whole cells to be calculated. If the α-cellulose yield from the whole cell is taken to have been 2·1 % then the cell wall represents 13·6 % (w/w) of the dry weight of the cell. Thus in the isolation procedure employed in this work about 28 % (w/w) of the total available wall material is isolated. The yield of walls was undoubtedly lowered by the differential centrifuging, as indicated in Fig. 1, but this loss is necessary if the preparation obtained is to be free from cell debris and whole cells.

The electron-microscope studies have shown the wall to consist of the usual two-phase system which is known to occur in the cell walls of most higher plants (Northcote, 1958). This consists of a microfibrillar structure embedded in a continuous matrix. The microfibrils have a diameter of 30–50 Å and are irregularly interwoven in a continuous network over the wall. Two main directions of the microfibrils are apparent, lying at right angles to one another. There seems to be no difference in the general arrangement and orientation of the microfibrils on the inner and outer surfaces of the wall and sections of wall treated with dilute sodium hydroxide solution showed no local concentrations of microfibrils. In these respects the wall resembles the primary cell wall of higher plants rather than the secondary wall. In some primary walls, however, differences in orientation of the microfibrils from the outside to the inside of the cell have been observed (Scott, Hamner, Baker & Bowler, 1956). Chemically the microfibrils correspond to the

isolated α-cellulose fraction and thus contain polysaccharides composed of monosaccharides other than glucose. It is possible that some of the material containing these other sugars may be adsorbed on to the microfibrils from the continuous matrix during their preparation. The matrix does contain a polysaccharide composed of these non-glucosidic sugars. However, treatment of the wall with 3 % solutions of sodium hydroxide seems to give microfibrils which are free from the matrix substance when these are examined microscopically.

The matrix has a granular appearance and is continuous over the cell surface. Chemically it is related to the substances soluble in dilute sodium hydroxide. These are the hemicellulose, protein, amino sugar and possibly the lipid. The impure hemicellulose contains a high proportion of the nitrogen present in the original cell wall. The walls digested with the snail enzyme lose 70 % of their α-cellulose, 43 % of their lipid and only 13 % of their hemicellulose. This accounts for nearly all the material digested by the enzyme, so that very little of the protein of the wall is removed. The material left shows no microfibrillar structure but resembles the granular matrix, and when sectioned these digested walls have a laminated appearance. Two distinct layers approximately 50 Å thick can be seen, one near the outer edge and one near the inner edge, separated by a space of 100 Å. This would indicate local concentrations of some of the materials of the matrix in these outer and inner lamellae although it is not implied that these matrix materials are completely localized in these regions. The sections of the wall show an interesting characteristic curling at the breakage cleft, and although several explanations of this are possible it might well indicate differences in molecular composition of the outside and inside regions of the cell wall.

SUMMARY

1. A cell-wall fraction of *Chlorella pyrenoidosa* has been isolated after disintegration of the whole cells in a Mickle cell disintegrator followed by differential centrifuging.

2. The isolated wall has been shown to be free of whole cells and debris. It is approximately 210 Å thick and represents 13·6 % of the dry weight of the whole cell.

3. A quantitative chemical analysis of the wall has been made with respect to the following fractions: α-cellulose, hemicellulose, protein, lipid and ash. Glucosamine has been estimated.

4. The structure of the wall has been studied by the electron microscope and shown to be a two-phase system in which microfibrils approximately 30–50 Å in diameter are enclosed in a continuous matrix.

5. The protein, some of which may exist as a glycoprotein, is associated with the hemicellulose and with these polysaccharides makes up the greater part of the continuous matrix.

6. The microfibrillar structure, which is nearly all polysaccharide, corresponds to the α-cellulose of the chemical analysis. It is composed of polymers of glucose, galactose, mannose, arabinose and rhamnose.

7. The hemicellulose can be isolated as an electrophoretically pure polysaccharide from the whole cell and some of its characteristics have been studied quantitatively. An acid hydrolysate contains galactose, mannose, arabinose, xylose and rhamnose.

8. The microfibrils are present as a continuous irregular network over the cell wall and throughout its thickness. They lie approximately in two directions at right angles to one another.

9. The continuous matrix is granular in appearance. Microscopical examination and chemical analysis of cell walls treated with snail digestive enzyme show that some of the substances of which it is composed are partially localized in two regions. One of these is near the outer surface and the other near the inner surface of the wall.

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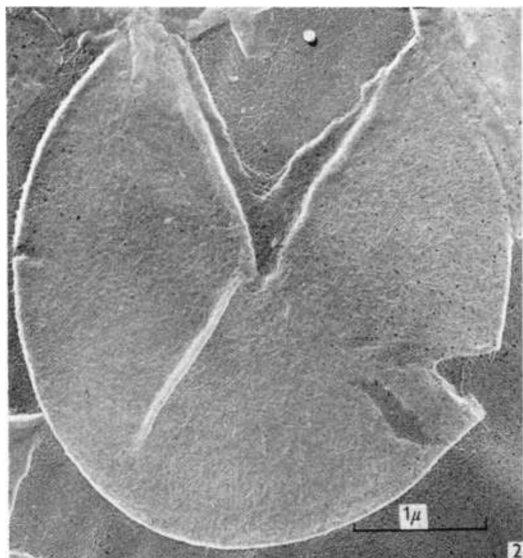
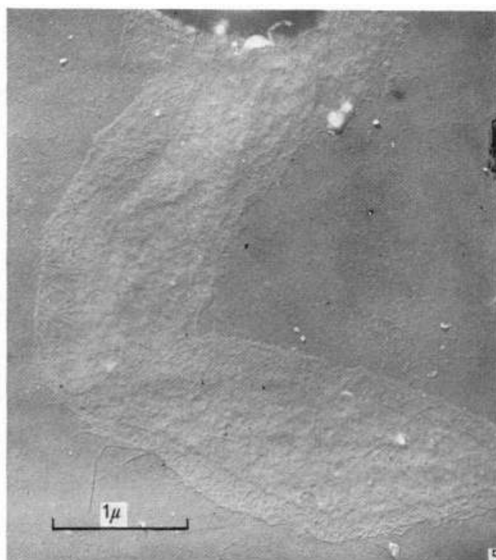
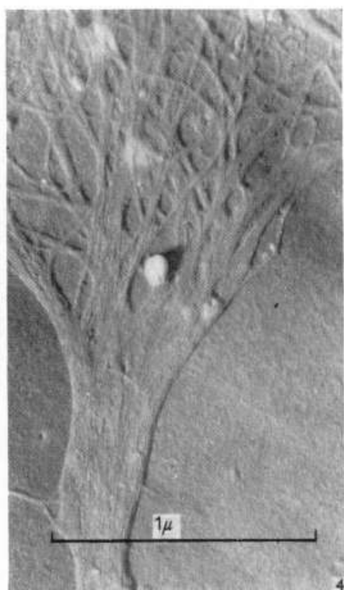
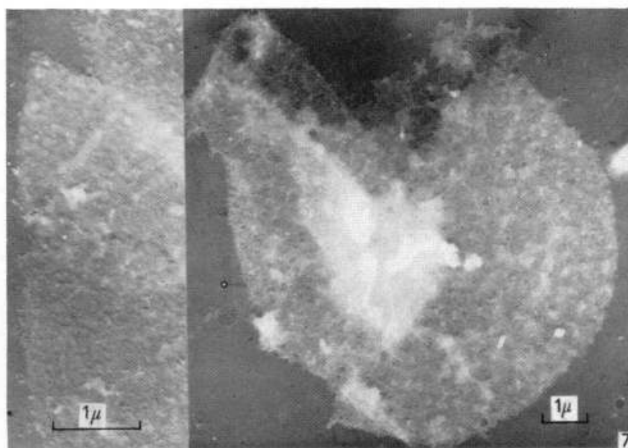
REFERENCES

Boas, N. F. (1953). *J. biol. Chem.* **204**, 553.
Chibnall, A. C., Rees, M. W. & Williams, E. F. (1943). *Biochem. J.* **37**, 354.
Consden, R. & Stanier, W. M. (1952). *Nature, Lond.*, **170**, 1069.
Coslett, V. E. & Horne, R. W. (1955). *Vacuum*, **5**, 109.
Emerson, R. & Lewis, C. M. (1939). *Amer. J. Bot.* **26**, 808.
Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
Fuller, K. W. & Northcote, D. H. (1956). *Biochem. J.* **64**, 657.
Hocevar, B. J. & Northcote, D. H. (1957). *Nature, Lond.*, **179**, 488.
Jermyn, M. A. & Isherwood, F. A. (1949). *Biochem. J.* **44**, 402.
Keilin, J. (1956). *Biochem. J.* **64**, 663.
Kreger, D. D. R. (1957). *Nature, Lond.*, **180**, 914.
Lederer, E. & Lederer, M. (1955). *Chromatography*, 1st ed., p. 318. London: Elsevier Publishing Co.
Levy, A. L. & Chung, D. (1953). *Analyt. Chem.* **25**, 396.
Mickle, H. (1948). *J. R. micr. Soc.* **68**, 10.
Myers, F. L. & Northcote, D. H. (1958). *J. exp. Biol.* **35**, 639.
Nicolai, E. & Preston, R. D. (1952–53). *Proc. Roy. Soc.* B, **140**, 244.
Northcote, D. H. (1954). *Biochem. J.* **58**, 353.









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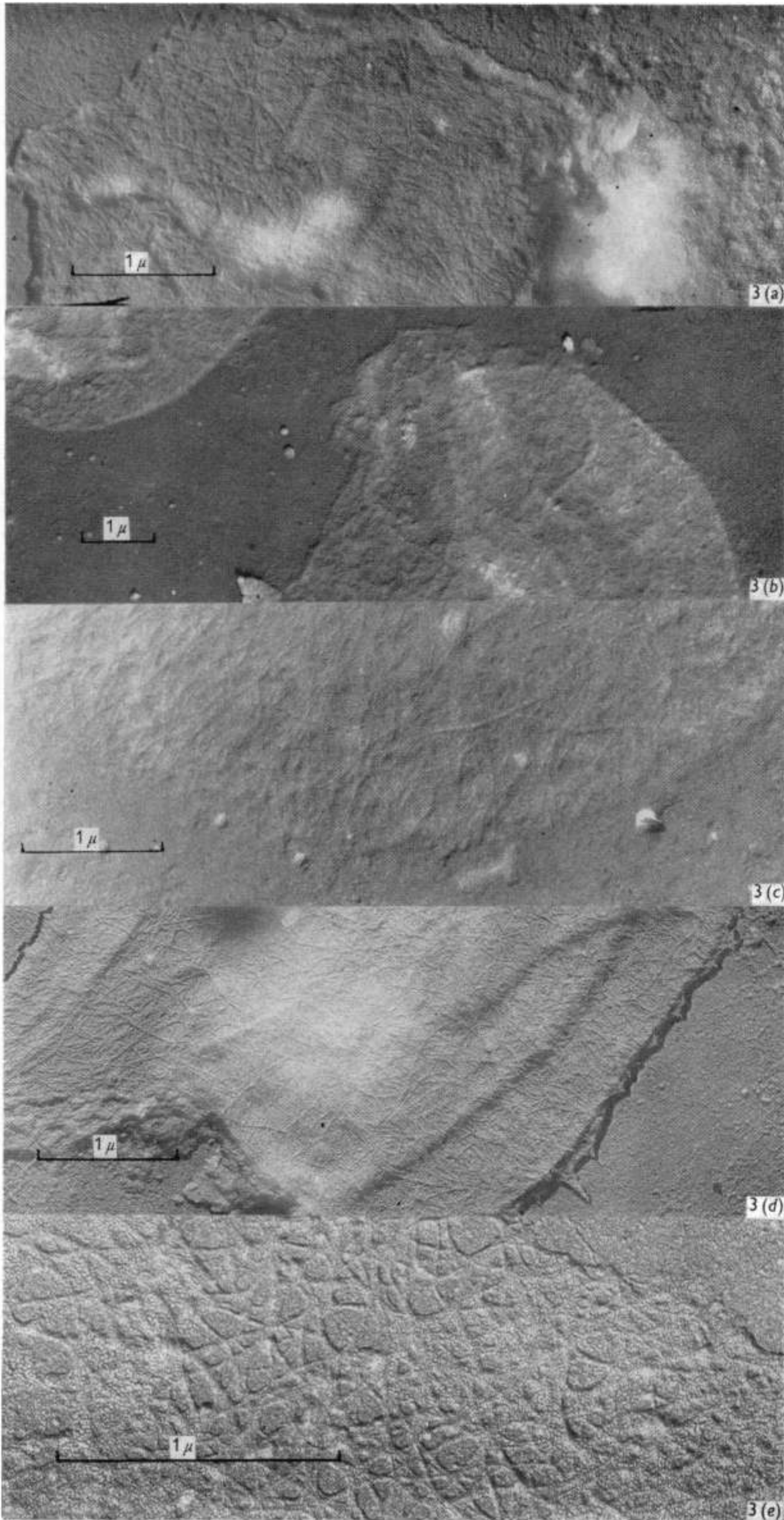

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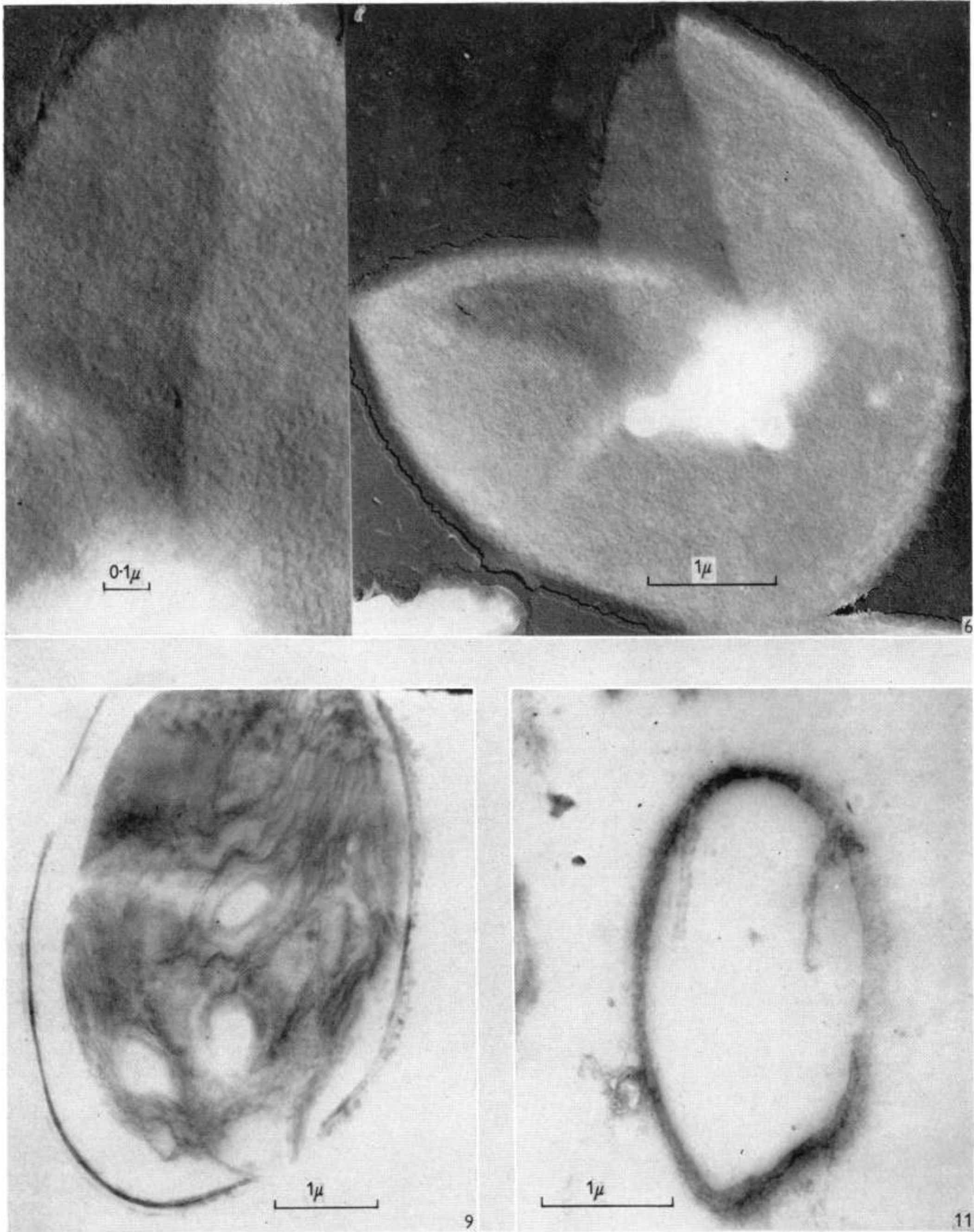

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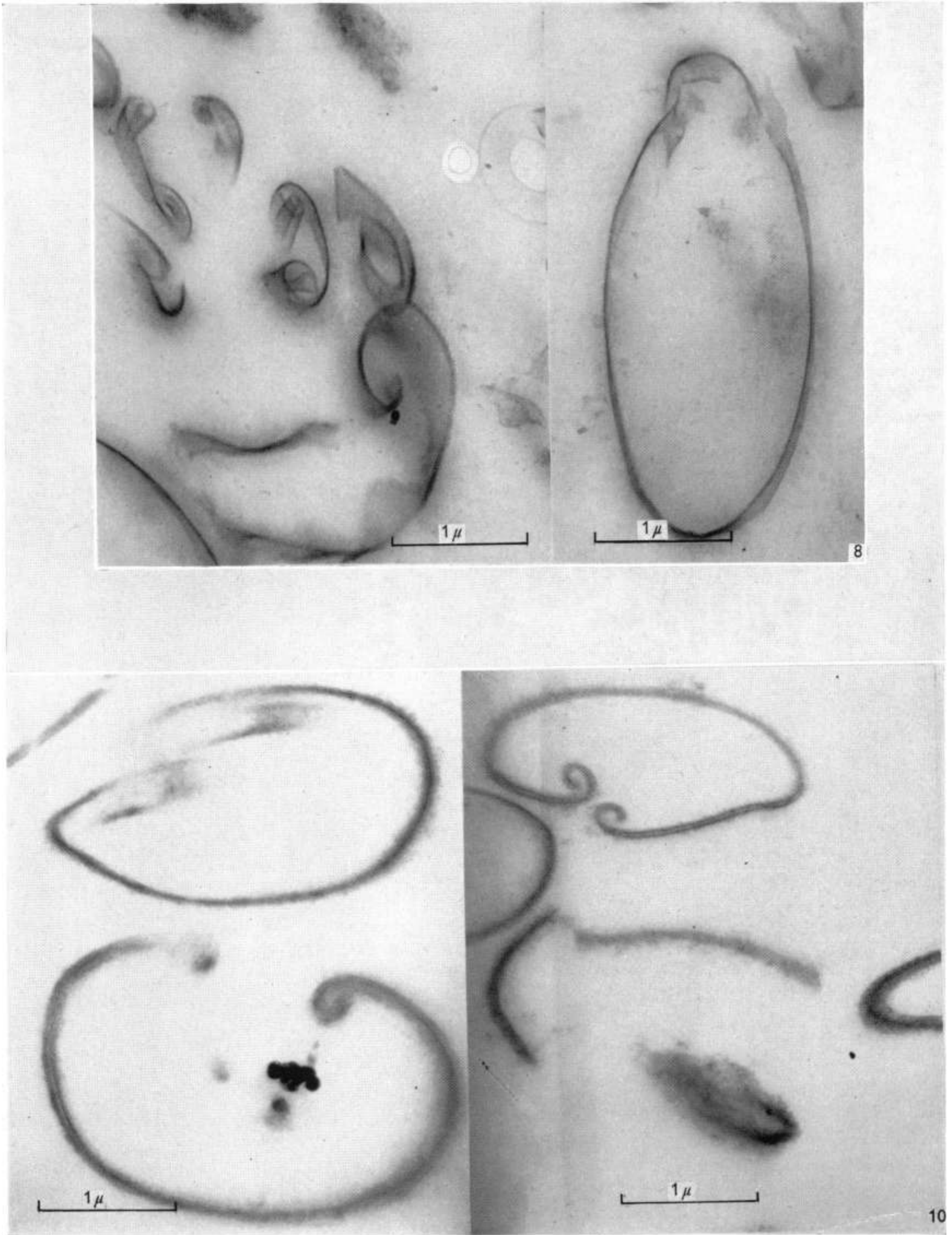

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Northcote, D. H. (1958). *Biol. Rev.* **33**, 53.
Palade, G. E. (1952). *J. exp. Med.* **95**, 285.
Partridge, S. M. (1948). *Biochem. J.* **42**, 238.
Partridge, S. M. (1949). *Nature, Lond.*, **164**, 443.
Porter, K. R. & Blum, J. (1953). *Anat. Rec.* **117**, 685.
Potter, V. R. & Elvehjem, C. A. (1936). *J. biol. Chem.* **114**, 495.
Scott, F. M., Hamner, K. C., Baker, E. & Bowler, E. (1956). *Amer. J. Bot.* **43**, 313.
Thimann, K. V. & Bonner, J. (1933). *Proc. Roy. Soc.* B, **113**, 126.
Tripp, V. W. & Rollins, M. L. (1952). *Analyt. Chem.* **24**, 1721.
Wirth, P. (1946). *Ber. schweiz. bot. Ges.* **56**, 175.
Wise, L. E., Peterson, F. C. & Harlow, W. M. (1939). *Industr. Engng Chem.* (*Anal.*), **11**, 18.

EXPLANATION OF PLATES 1–4

PLATE 1

Fig. 2. Untreated cell walls of *Chlorella*. Microfibrils can be seen and the undersurface of the wall is visible at the breakage cleft.

Fig. 4. Cell walls treated with 3% NaOH for 14 hr. at room temperature. The cell wall has completely disintegrated, giving bundles of microfibrils with very little matrix material visible.

Fig. 5. Cell walls treated with ethanolamine for 4 hr. at 25°, showing microfibrils.

Fig. 7. Cell walls treated with 0·5% NaOH for 25 min. at room temperature and then snail digestive enzyme at 25° for 14 hr. No microfibrils are visible and although the wall is very thin it still retains the cell shape.

PLATE 2

Fig. 3. Cell walls treated with NaOH at room temperature: (*a*) 0·5% NaOH for 5 min.; (*b*) 0·5% NaOH for 25 min.; (*c*) 0·5% NaOH for 25 min.; (*d*) 0·5% NaOH for 30 min.; (*e*) 3% NaOH for 30 min. Microfibrils lying in two principal directions at right angles to one another can be clearly seen. The granular material of the matrix is visible. In (*e*) the wall has lost its form and is disintegrating into microfibrils.

PLATE 3

Fig. 6. Cell walls treated with snail digestive enzyme for 14 hr. at 25°, showing rough granular surface and absence of microfibrils.

Fig. 9. Section of whole cell. The appearance of layers in the cell wall is visible. Compare with Plate 4, Fig. 8.

Fig. 11. Section of cell walls treated similarly to those shown in Plate 2, Fig. 3*c*. The walls have swollen and the microfibrils are visible as a diffuse material throughout the thickness of the sections.

PLATE 4

Fig. 8. Sections of untreated cell walls showing ribbon-like appearance and characteristic curling at the breakage cleft. Compare with Plate 1, Fig. 2 and Plate 3, Fig. 9.

Fig. 10. Section of cell walls treated similarly to those shown in Plate 3, Fig. 6. The appearance of two layers in the wall is very apparent.

Phosphorolysis of Citrulline by Mammalian Liver: the Effect of a Bacterial Activator

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In a previous paper (Krebs, Eggleston & Knivett, 1955) it was shown that washed suspensions of *Escherichia coli* N.C.I.B. 8571 accelerate the phosphorolysis of citrulline by mammalian liver extracts. The bacterial cells contained ornithine decarboxylase, and most of the acceleration could be ascribed to the removal of ornithine, which acted as an inhibitor of the phosphorolysis. However, an accelerating effect remained even when the activity of ornithine decarboxylase was abolished by hydroxylamine. From this it was concluded that this strain of *E. coli* contained an additional substance (or 'factor') which promotes the phosphorolysis of citrulline.

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