CHEMICAL COMPOSITION OF THE WALLS OF CERTAIN ALGAE.*

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INTRODUCTION.

In spite of the fact that numerous investigations of the nature of cell walls of algæ have been made, the recorded data are still far too incomplete to be of the greatest service to science. It is usually impossible to obtain from the literature a complete record of the composition of the walls of the most commonly occurring fresh water algæ. The physiological nature of the cell walls of algæ appears to play an important role in such phenomena as the resistance of certain algæ to desiccation during dry seasons, the rate of entrance of certain mineral elements, the attachment or nonattachment of epiphytic algæ, and the utilization of algæ as food by fishes and other aquatic animals. It seems important that a thorough knowledge of the composition of the cell walls of algæ should be obtained. The object of the investigation reported below was to determine the composition of the cell walls of some of our common algæ. Representatives of the following genera were studied: *Vaucheria*, *Cladophora*, *Oedogonium*, *Spirogyra*, *Zygnema*, and *Draparnaldia*.

LITERATURE.

Only those papers dealing with investigation of the genera listed above will be reviewed in this paper. In 1913, Mirande (4) reported that the cell walls of *Vaucheria* were composed entirely of cellulose-pectic compounds. He did not state whether these compounds were intermingled or in separate layers, or in what form the pectic compounds occurred.

Brand (1) states that stratification of the cell walls of *Cladophora glomerata* begins in a very early stage, young filaments of two cells being composed of two layers. The outermost layer surrounds the entire filament and is loosened from

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it by dilute acetic acid or other weak acids. Potassium hydroxide, alcohol, and acid free glycerine have no effect on it. This membrane appears to be independent of either of the other two membranes constituting the second layer of the cell wall. It gave no positive reactions for cellulose. The cellulose reaction was obtained with difficulty, so that it seemed that the pectic compounds predominated in the membrane. Chlorzinc iodide gave no blue color even after boiling with hydrochloric acid.

Hirn (3) found the cell walls of Oedogonium to be composed of two layers. The inner one was a moderately thick layer of cellulose and the other a "cuticle" which enclosed the entire filament. This "cuticle" could not be identified with that of the higher plants by means of either stains or other reagents.

Hirn also reports (3) that the beginning of the new partition wall in Oedogonium was at first a thin cellulose ring. This ring enlarges and appears stratified, and separates into two entirely different regions. The outer one of these regions gives a positive cellulose reaction and the inner one a negative reaction for cellulose. Klebahn says [according to West (7) and Oltmanns (6)] that the ring is of a gelatinous nature and stains with hæmatoxylin. The young membrane which comes from the ring stains in the same manner while the older membranes do not.

Czapek (2), in his discussion of the cell walls of the green algæ, states that Klebs found the wall inside the gelatinous sheath of *Zygnema sp.* to have no homogeneous composition. Part of the membrane, which in the normal cell membrane stained with the aniline dyes, was dissolved by heating in dilute hydrochloric acid. The remainder of the cell wall was readily dissolved in copperoxide ammonia, stained with congo red and gave the usual cellulose reactions.

West (7) reports all the members of the group Conjugatæ to have a firm cellulose wall and some to have a great development of the mucilaginous pectose constituents of the cell wall. This development may be through the conversion of the outer cellulose layers into mucilage or by mucilage being continually exuded through the cell wall.

METHODS OF INVESTIGATION.

All the filaments were mounted in distilled water and examined in polarized light. The results of these data were then checked by color reactions, hydrolysis, and solubility of the membranes.

The following tests were used in the identification of the substances found:

1. Cellulose. Cellulose is doubly refractive in polarized light, stains blue with iodine potassium iodide and 70% sulphuric acid, and is soluble in copperoxide ammonia. The filaments are left intact when the cellulose has been removed and will then give a negative hydrocellulose reaction and show no double refraction in polarized light.

2. Pectic Compounds. All pectic compounds are singly refractive in polarized light. The different compounds are distinguished by their different solubilities. Pectic acid is soluble in 2% potassium hydroxide or in 5% sodium carbonate, and is dissolved from the filament by treating it with either of these reagents. If the filament still shows the presence of a pectic compound it is then treated for the removal of calcium pectate.

Calcium pectate is broken down by 2% oxalic acid, giving calcium oxalate crystals and pectic acid. The calcium oxalate crystals may be recognized by their tetragonal shape and their double refraction of polarized light. The pectic acid may be removed by dissolving it in 2% potassium hydroxide or 5% sodium carbonate as stated above.

Any pectic compound remaining is pectose and is dissolved by heating the filament on the water bath 20—30 minutes in 2% hydrochloric acid and then treating with 2% potassium hydroxide. The hydrochloric acid breaks the pectose down to pectin or pectic acid. If to pectin, the compound is removed by washing the filament in water. If to pectic acid the potassium hydroxide dissolves it.

If fresh untreated tissue is used in making these tests, the cells are left intact after all the pectic compounds have been removed. These cell walls will then react negatively for pectic compounds in polarized light, but positive by for any other substance composing the walls.

3. Chitin. Chitin, when present, seems to be in a thin layer on the outside. It is singly refractive and can not be distin-

guished from the pectic compounds in polarized light. Tissues containing chitin give a positive chitosan reaction. Filaments of the alga are placed in boiling concentrated (50%) potassium hydroxide, and allowed to boil in a covered dish for 30 minutes. The filaments are then hardened in 90% alcohol, mounted in iodine potassium iodide, and dilute sulphuric acid added. A red violet color, characteristic of chitosan, is obtained.

REPORT OF INVESTIGATION.

VAUCHERIA GEMINATA. In polarized light the cell walls of *Vaucheria geminata* are composed of two layers, the outer one being singly refractive and the inner one doubly refractive. This outer layer gives positive reaction to all tests for pectose. Other pectic compounds, if present, occur only in small amounts. Before treating with the pectose solvents the inner layer reacts negatively to the hydrocellulose reaction, although it is dissolved in copper oxide ammonia if left in the solution two days, the solution being changed three times. After the removal of the cellulose the filament, now composed only of pectose walls, is left intact although very fragile. After removing the outer layer of pectic compounds, the inner layer of the cell wall reacts readily to the cellulose reagents, giving a positive test to the hydrocellulose reaction and dissolving rapidly on the slide in copperoxide ammonia. The entire cell wall is dissolved when treated with the solvents for both pectic compounds and cellulose.

The mature oospore seems to have a layer in addition to the two present in the cells of the filament. A thin membrane is left intact after both the pectic compounds and the cellulose have been removed. This membrane is broken by the pressure of the cell contents while the swelling cellulose is being dissolved. The cell contents move out of the oospore wall leaving it intact as an empty shell. This shell is singly refractive. It may be chitin but the exact composition has not been determined.

To summarize the work on *Vaucheria geminata:* the cell walls are composed of pectose and cellulose, the former being on the outside. This outer pectic layer is very difficultly permeable to cellulose reagents, particularly those used in the hydrocellulose reaction, presumably iodine, though this point was not

definitely determined. The mature oospore has an additional outer layer which may be chitin.

CLADOPHORA GLOMERATA. The cell walls of *Cladophora glomerata* are composed of three layers. The outermost layer is of chitin and can be separated from the rest of the filament by dilute acetic acid. This layer is singly refractive in polarized light, insoluble in either pectic or cellulose solvents, and gives a positive chitosan reaction.

The second layer is also singly refractive in polarized light but is soluble in the pectose solvents. The inner layer is doubly refractive in polarized light but gives the characteristic cellulose reactions only after the filament has been treated with pectose solvents. Then the cellulose is readily dissolved leaving only the outermost layer of chitin.

Therefore, the cell walls of *Cladophora glomerata* are composed of three layers: chitin, pectose, and cellulose. The layer of chitin is readily separated from the rest of the cell wall and is the only membrane left intact after the filaments have been treated with pectose and cellulose solvents. This layer is also evidently very difficultly permeable to cellulose reagents until partially hydrolyzed. It appears to be more impermeable to copper than pectose is. It is interesting to note in this connection that Moore and Kellerman (5) find that the per cent of copper sulphate required to destroy Cladophora in reservoirs is twenty-five times that required to kill Spirogyra which has no chitinous membrane.

OEDOGONIUM IRREGULARE. The young filaments, of from one to two cells in length, of *Oedogonium irregulare* give no reaction for the presence of cellulose. They are composed of two layers, the outer one of which may be separated from the other and be made to bulge out by dilute acetic acid. This layer gives the chitosan reaction and is singly refractive in polarized light. It surrounds the entire filament but does not form a part of the cross cell walls. The inner layer of the wall is of pectose and may be dissolved by heating in 2% hydrochloric acid for one-half hour and then leaving in 2% potassium hydroxide for 15 minutes or longer. The outer layer is left intact after this treatment.

When the filament is older and is composed of two or more cells, a third layer appears inside the layer of pectose. This

layer is doubly refractive, but gives the cellulose reactions only after the filament has been treated for the removal of pectose. The layer of chitin is evidently partially hydrolyzed by this treatment since a slight chitosan reaction is also obtained when applying the hydrocellulose test. This layer of chitin and the layer of pectose are undoubtedly the membranes which prevent the entrance of copper and iodine into the cell when tests are made for cellulose and may explain why it has been generally considered that the cell walls of water plants were composed of difficultly soluble cellulose. In these investigations it was found that when these impermeable layers were removed or hydrolyzed the cellulose in the walls of algæ is no more difficultly soluble than that in higher plants.

OEDOGONIUM CRASSUM AMPLUM. The walls of the vegetative cells of *Oedogonium crassum amplum* are stratified and composed of three substances. The outer layer surrounds the filament as a whole and can be readily separated from it by treatment with copperoxide ammonia. When this solution is run under the coverglass the outer membrane swells and bulges out from the other membranes, but does not dissolve. It is not caused to separate from the filament when treated with acetic acid as was the case in *Oedogonium irregulare*. It is singly refractive and, although it is soluble in neither pectose nor cellulose solvents alone, it is finally dissolved with the rest of the cell wall when both solvents are used. It apparently is not a mixture of cellulose and pectic compounds since it does not give a positive reaction for celluolse when the pectic compounds are removed. It gives a negative reaction for pentosans.

The second layer of the cell wall is of pectose and is dissolved in hydrochloric acid and potassium hydroxide. This membrane is singly refractive in polarized light.

The inner layer is doubly refractive and gives the characteristic hydrocellulose reaction for the presence of cellulose. When the sulphuric acid is added in the hydrocellulose reaction, the cellulose membrane seems to loosen at one end of the cell and expand, remaining attached at the other end. In the same reaction the filament undergoes a series of color changes before the final blue color is attained. The filament becomes red and then goes through violet to the characteristic deep blue color of cellulose. After being treated with the pectose solvents, the

filament is stained red directly with iodine potassium iodide, then changes to blue when 60% sulphuric acid is added. These peculiar color changes indicate the presence of some unidentified substance in the walls of this alga.

When the sulphuric acid or copperoxide ammonia is added the cells separate from each other, the cellulose layer swells rapidly becoming many times its original thickness, then it divides into two or more parts forming distinct cylinders which rapidly slip out of each other before going into solution. The entire filament is soluble in copperoxide ammonia after the pectic compounds have been removed. The pectose solvents evidently partially hydrolyse the outermost layer which may be composed partly of some form of chitin or a hemicellulose.

The ring, which is the first indication of the new partition wall in cell division, is singly refractive in polarized light, indicating that it is not pure cellulose. It is insoluble in either the pectic solvents or the cellulose solvent when they are used alone, but when the filament is treated with both groups of solvents the ring is dissolved as well as the rest of the cell wall. It gives a faint blue when treated with the hydrocellulose reagents, but gives a negative test for cellulose under polarized light both before and after treatment with pectose solvents.

To sum up, the cell walls of *Oedogonium crassum amplum* are striated and are composed of three substances: an unidentified substance on the outside, which can be separated from the rest of the filament; a middle layer of pectose; and an inner layer of cellulose. The ring formed in the dividing of the cells may be in part a mixture of pectose and cellulose, though it resembles rather closely the unidentified outer layer of the filament.

SPIROGYRA FLUVIATILIS. The cell walls of this Spirogyra are composed of two layers. The outer layer is of pectose, being singly refractive in polarized light and dissolved from the wall by 2% hydrochloric acid followed by 2% potassium hydroxide. The inner layer is of cellulose, but unlike that of Vaucheria, Cladophora, or Oedogonium, it reacts readily to both the hydrocellulose and solubility reactions for cellulose before any previous hydrolysis of the outer membranes of the cell walls. This indicates that the pectic compound present in *Spirogyra fluviatilis* is not as impermeable to copper and iodine as in the

other cases, and may explain why Spirogyra is so readily killed by copper sulphate as found by Moore and Kellerman (5).

In the formation of the conjugation tubes there is a gradual change in the composition of the walls as they increase in age. During the slight bulging of the vegetative cell wall, which is the first indication of the formation of the conjugation tube, there is no obvious alteration in the original cell wall. When the tube is completely formed, the cellulose extends only a short distance into the tube, the rest of the wall being composed of a single layer of pectose. In a more mature conjugation tube of another species it was noted that the cellulose lined the entire tube, making the cell wall of two layers, the outer one of pectose and the inner one of cellulose as in the wall of a vegetative cell.

To summarize, the cell walls of *Spirogyra fluviatilis* are composed of two layers; the outer one is of pectose and is readily permeable to reagents containing either copper or iodine; the inner layer is of cellulose and gives all the characteristic cellulose reactions. In the conjugation tubes the cell wall is at first composed of a single layer of pectose, a layer of cellulose being formed later. Four other species of Spirogyra examined showed the same cell wall structure as described above.

ZYGNEMA CRUCIATUM. The cell walls of *Zygnema cruciatum* are composed of three layers. Around the entire filament is a thick mucilaginous sheath of pectic acid. This sheath is singly refractive in polarized light and is dissolved in 2% potassium hydroxide. Just inside the mucilaginous sheath is a more compact layer of pectose which may be removed from the filament by treatment with the pectose solvents. The innermost layer of the cell wall is left intact after the outer layers have been dissolved. This layer gives the characteristic cellulose reactions, being doubly refractive to polarized light, positive for hydrocellulose, and soluble in copperoxide ammonia. These reactions are positive both before and after the pectic compounds have been removed, indicating that the pectic compounds in this species are also readily permeable to copper and iodine.

DRAPARNALDIA PLUMOSA. The filaments of *Draparnaldia plumosa* are surrounded by a thick slimy sheath. This slime is singly refractive in polarized light and is partially soluble in 2% potassium hydroxide, more soluble in 5% sodium carbonate,

and entirely soluble in the pectose solvents, indicating that it is composed of both pectic acid and pectose.

The cell walls of the main filaments are composed of two layers. The outer layer is of pectose and the inner one of cellulose. Before the slimy sheath has been removed, the cellulose membrane gives a negative reaction to the hydrocellulose test. After the treatment with pectose solvents, the cell walls of the main filament give a positive reaction to the hydrocellulose reagents. The cell walls of the branch filaments are singly refractive in polarized light and give no hydrocellulose reaction, but when the filaments have been left in the potassium hydroxide of pectose solvent for several hours, they are dissolved, with the cell walls of the main filament, in copperoxide ammonia.

The above data seem to indicate that the main filaments of *Draparnaldia plumosa* are composed of cellulose and pectose in layers, while the branch filaments are of a combination of cellulose and a pectic compound. Of these two substances the pectic compound seems to predominate, masking the polarized light reaction of the cellulose present. The entire filament is surrounded by a slimy sheath of pectic acid and pectose. This sheath prevents the cellulose reagents from coming in contact with the cellulose, thereby preventing a positive test for cellulose until the slime has been dissolved.

TETRASPORA. The mucilaginous sheath surrounding the colonies of Tetraspora is singly refractive in polarized light and is soluble in dilute alkalies and alkalie carbonates, indicating pectic acid.

SUMMARY.

1. *The composition of the cell walls of algæ reported may be summarized as follows:*

Species	Outer Layer	Middle Layer	Inner Layer	Remarks
Vaucheria geminata	Pectose		Cellulose	Pectic layer difficultly permeable to copper and iodine reagents
Cladophora glomerata	Chitin	Pectose	Cellulose	Chitin very difficultly permeable to copper and iodine reagents
Oedogonium irregulare young filaments of 1-2 cells	Chitin		Pectose	Chitin very difficultly permeable to copper and iodine reagents
Oedogonium irregulare (older cells)	Chitin	Pectose	Cellulose	Chitin very difficultly permeable to copper and iodine reagents
Oedogonium crassum amplum	Unidentified	Pectose	Cellulose	Unidentified substance difficultly permeable to copper and iodine reagents
Spirogyra sp.	Pectose		Cellulose	
Zygnema cruciatum	Pectic acid (sheath) mucilaginous	Pectose	Cellulose	
Draparnaldia (main filament)	Pectic acid and pectose mucilaginous sheath	Pectose	Cellulose	

2. In all the forms studied the cell walls are composed of at least two layers, an inner one of cellulose and an outer one of pectose or of chitin. When chitin is present, pectose forms a middle layer of the cell wall.

3. In some forms, as in Vaucheria and Draparnaldia, the pectose is difficultly permeable to a solution of copper-oxide ammonia and iodine-potassium-iodide.

4. In other forms, *Cladophora glomerate* and *Oedogonsum irregulare*, a third layer composed of chitin is present; in *Oedogonium crassum amplum* a layer of an unidentified substance. These outermost layers surround the filament and may

be separated from it by appropriate reagents. These membranes are difficultly permeable to copper and iodine reagents until partially hydrolyzed.

5. The cellulose present in the algæ is readily soluble in copperoxide ammonia when the reagent is free to come in contact with the cellulose.

6. Mucilaginous sheaths of algæ studied are of pectic compounds, of which pectic acid predominates, with pectose in some cases.

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