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An Extracellular Macromolecular Complex from the Surface of Soybean Suspension Cultures[1]

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ABSTRACT

A cell-free, insoluble cell wall fraction is described which floats on the surface of suspension cultures of *Glycine max* L. Merrill var. Acme cells. Its accumulation is governed by both the shaking speed and the medium volume, a shaking speed of 110 to 120 revolutions per minute with a medium volume of about 100 to 120 milliliters in a 250-milliliter flask being optimal. Various factors which could control the accumulation of the complex were tested and are discussed, and scanning electron micrographs of the complex being released from the cell surface are presented.

The composition of the complex by weight is 46% galacturonic acid, 36% protein, 11% lignin (apparent), 4.4% arabinose, 2% ash, and 0.5% methyl ester. Evidence for an intimate relationship between the uronic acid and protein fractions is presented. The protein contains hydroxyproline, and the bulk of it is tightly bound to the complex, although a portion can be removed with high salt treatments.

The release of cell wall components into the medium of plant cells in suspension culture has been described by several workers, and extensive analyses of certain of these fractions have been performed. Most studies to date have dealt with soluble macromolecules which are thought to be either noncellulosic wall matrix polysaccharides or intercellular material (1, 2, 9, 21). More recently, Leppard *et al.* (16) have described the loss of small fibers from the surface of cells of three kinds of plants in suspension culture. These fibrils were isolated by centrifugation and are believed to derive from the middle lamella. In this report the formation and composition of a cell wall fraction from soybean cells which forms a mat on the surface of the medium under certain conditions are described. A substance perhaps related to this was described briefly by Hayashi (10) in tobacco cultures.

MATERIALS AND METHODS

Culture Techniques. *Glycine max* L. Merrill var. Acme callus tissue was obtained from Dr. Carlos O. Miller. The tissue was routinely grown in 100 ml of liquid medium (19) in 250-ml Erlenmeyer flasks. A 14-day culture cycle was maintained by inoculating fresh medium with 2.5-ml samples from 14-day-old cultures. Stock cultures were shaken in continuous white light (52,800 erg/cm²·sec) at 150 rpm on gyrotary shakers with a 0.75-inch stroke. Exceptions in shaking speed, medium volume, and medium composition are indicated in the text.

For fresh and dry weight determinations cells were filtered onto a piece of Miracloth (Calbiochem) and then transferred to a tared dish. Dry weights were determined following drying for 48 hr at 80 C. The cell numbers were obtained by counting an aliquot of cells on a hemocytometer following separation of the cells with EDTA by the method of Letham (17).

Sizing of cell clumps was accomplished by passing the tissue and medium through a series of five filters and washing with 1 to 1.5 liters of water to insure that all the clumps washed through to the appropriate filter. The first four filters were of nylon mesh with pore diameters of 2.3, 1.1, 0.6, and 0.25 mm, and the final filter was Miracloth. The cells remaining on each filter were collected and weighed.

Isolation of the Complex. In order to isolate the material, the mat was lifted from the surface of the medium and rinsed briefly in a shallow dish of water. The dry weight was then determined as described above.

For the analytical studies adhering cells were removed from the complex as follows. The mat was placed into a test tube two-thirds filled with water and shaken vigorously for 10 sec. The complex was allowed to float to the surface while the cells and cell clumps settled. This procedure was repeated a total of five times, after which microscopic examination showed only very minor contamination of the material with intact cells.

The complex was then washed five times each with 80% ethanol, absolute ethanol, and acetone, and finally once with ether. The material was dried *in vacuo* overnight over calcium chloride and stored *in vacuo* over phosphorus pentoxide. Drying the complex in an oven following this procedure resulted in no further weight loss.

Analytical Methods. Dry weights and the weights of hydrolysis residues were measured following drying for 48 hr at 80 C. For all treatments of the material, weighed portions were dispersed in water using a ground glass tissue homogenizer.

Hydrolysis of the carbohydrate moiety was performed in 2 N trifluoroacetic acid for 30 min at 120 C in tubes with Teflon-lined screwtops. For more exhaustive hydrolysis the material was placed in a small amount of 72% H_2SO_4 at 4 C for 2 hr followed by dilution to 3% H_2SO_4 and heating at 100 C for 4 hr. Following hydrolysis the H_2SO_4 solution was neutralized with $Ba(OH)_2$, and the precipitate was removed by centrifugation. After hydrolysis the products were dried under an airflow at 55 C and the sample was redissolved as required. For gas chromatography, the trifluoroacetic acid hydrolysates were dried for 5 days *in vacuo* over NaOH.

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Protein hydrolysis was performed in evacuated, sealed tubes with redistilled 6 N HCl for 24 hr. These samples were dried *in vacuo* over NaOH.

Chromatography of carbohydrates was performed on cellulose thin layer plates with *n*-butanol-pyridine-water (2:2:1, v/v), ascending for 3 hr, or with ethyl acetate-pyridine-water (8:2:1, v/v), descending for 14 hr.

Amino acid analysis was performed on a Beckman model 120C amino acid analyzer. The hydroxyproline value was calculated from the 440 nm channel peak under the normal aspartate peak with the proper corrections. The validity of this method was verified both by direct hydroxyproline analysis (20) on hydrolysates and with the Beckman method for physiological amino acid analysis.

The uronic acid content of the complex was measured routinely by the 100 C boron assay described by Knutson and Jeanes (13). The identity of the uronic acid was established by testing under the four conditions they described. Estimates of the total carbohydrate content were made using the test of Dubois *et al.* (8) and the orcinol assay of Dische (7) with galacturonic acid and arabinose as standards. Total nitrogen was estimated by the method of Jensen (12). The methyl ester content was measured by the method of Wood and Siddiqui (25) following treatment in 0.1 N NaOH at room temperature for 30 min. The lignin content was estimated as Klason lignin following the H_2SO_4 hydrolysis described above and by reaction with *N*-2,6-trichloro-*p*-benzoquinoneimine after extraction with 0.5 N NaOH overnight at 70 C as described by Carceller *et al.* (6).

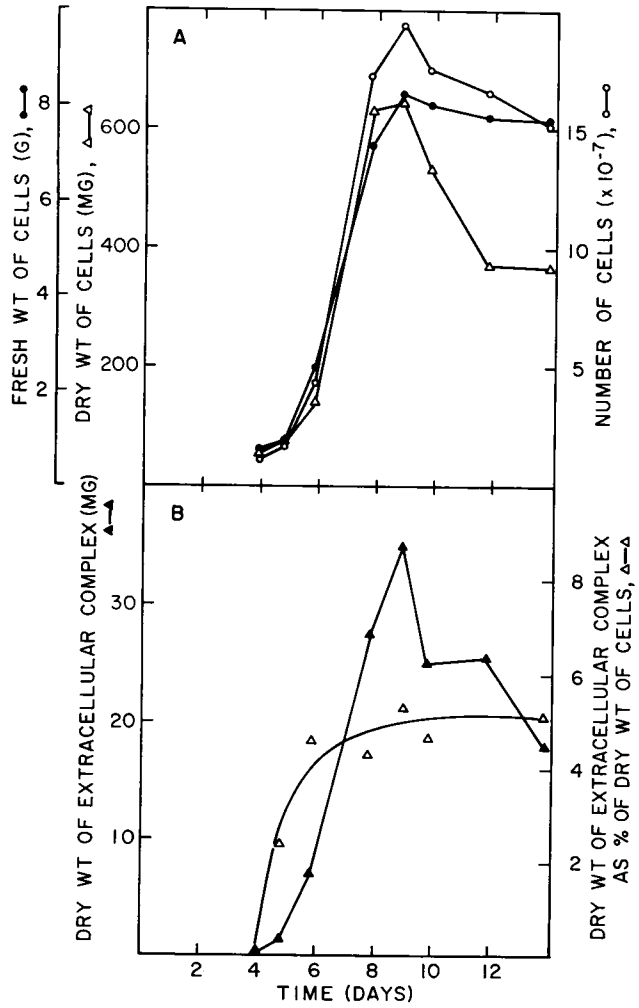

FIG. 1. Growth of soybean cells and accumulation of the complex over the culture period. A: Fresh weight (●), dry weight (△), and number (○) of the cells. B: Dry weight of the complex (▲) and dry weight of the complex as the percentage of the cell dry weight (△).

Gas chromatography was performed on derivatives obtained with Tri-Sil-Z (Pierce Chemical Co.). A Hewlett-Packard model 5750 research chromatograph equipped with a flame detector was used. The samples were injected into a 6-ft × 0.5-inch stainless steel column containing 1.5% OV-17 supported on 100/120 mesh Chromatosorb G. The temperature program consisted of 20 min at 120 C followed by a 2 C/min rise to 200 C.

Enzyme Assays. The assay mixture for peroxidase contained 10 mM potassium phosphate buffer, pH 6.0; 0.003% hydrogen peroxide; and 0.009% *o*-dianisidine in a total of 3 ml. The change in absorbance was followed at 460 nm; the extinction coefficient used was 6.4 $cm^2/\mu mole$ (11, 26).

For acid phosphatase, the assay included 0.61 mM *o*-carboxyphenylphosphate and 0.10 M Na acetate, pH 5.0, in a total of 3.0 ml. The extinction coefficient was 3500 cm^2/mole at 300 nm (3). Invertase was assayed by incubating the enzyme in 0.1 M sucrose at pH 4.5 (10 mM sodium acetate) for 3 hr at 37 C followed by assaying for free glucose by the Glucostat method (Worthington Biochemical Corp.).

Enzyme Digestion. Portions of the complex were ground in either 0.1 M sodium acetate buffer, pH 5.0, containing 0.3% pectinase (Sigma) or in 10 mM potassium phosphate, pH 7.5, containing 0.1 mg/ml protease (Worthington Biochemical Corp.). The samples were incubated at 37 C for 3 hr, and aliquots were periodically removed and chilled. The aliquots were centrifuged to recover the insoluble complex; total nitrogen and uronic acid assays were made on those insoluble fractions. After 3 hr the total reaction mixtures were centrifuged, the precipitates were washed, and pectinase was added to the sample which previously had protease and *vice versa*. In the protease incubations, the protease was renewed every 30 min.

Cytological. Ruthenium red staining for pectin was used as described by Jensen (12). Material for the scanning electron microscope was lyophilized onto the surface of the sample holder, coated with gold, and observed directly. A Japan Electron Optics Co. JSM-2 scanning electron microscope was used.

RESULTS AND DISCUSSION

RELATIONSHIP BETWEEN GROWTH AND COMPLEX FORMATION

Figure 1A shows some quantitative aspects of the growth of the soybean cells in suspension culture when shaken continuously at 125 rpm over a period of 14 days. A 4-day lag phase is followed by exponential growth for 2 to 3 days, linear growth to day 9, and then a period during which fresh weight declines slowly and cell number and dry weight decrease more rapidly.

Figure 1B shows that the accumulation of the material described here on the surface of the cultures begins when growth starts and ceases when growth stops at day 9. When the dry weight of the material is expressed as the per cent of the dry weight of the cells, it becomes obvious that the period of its most rapid accumulation is when the cells are most rapidly dividing (Fig. 1B). This rate falls off as the rate of division slows and finally decreases in parallel with the decrease in total dry weight. Thus, from day 9 to day 14 the weight of the complex remains at some 5% of the weight of the cells.

ELECTRON MICROSCOPY

Electron micrographs of the complex and of cells removed from cultures producing the complex are presented in Figures 2A through 2D. From these pictures it is evident that the material is derived from the surface of the cells in strands or sheets. Figures 2A and 2B are of cells either embedded in or surrounded by material pulling loose while Figures 2C and 2D are more highly magnified pictures of the attachment of the material to the surface of the cells.

COMPLEX ACCUMULATION UNDER DIFFERENT CULTURE CONDITIONS

Figure 3, which shows the response to different shaking speeds, demonstrates that there is no direct relationship between accumulation of the complex and growth of the cells. The most extensive accumulation occurred in the range 110 to 120 rpm where growth was not at its maximal rate. At shaking speeds less than 100 rpm, where the growth rate was considerably reduced, and at speeds 140 rpm and above when

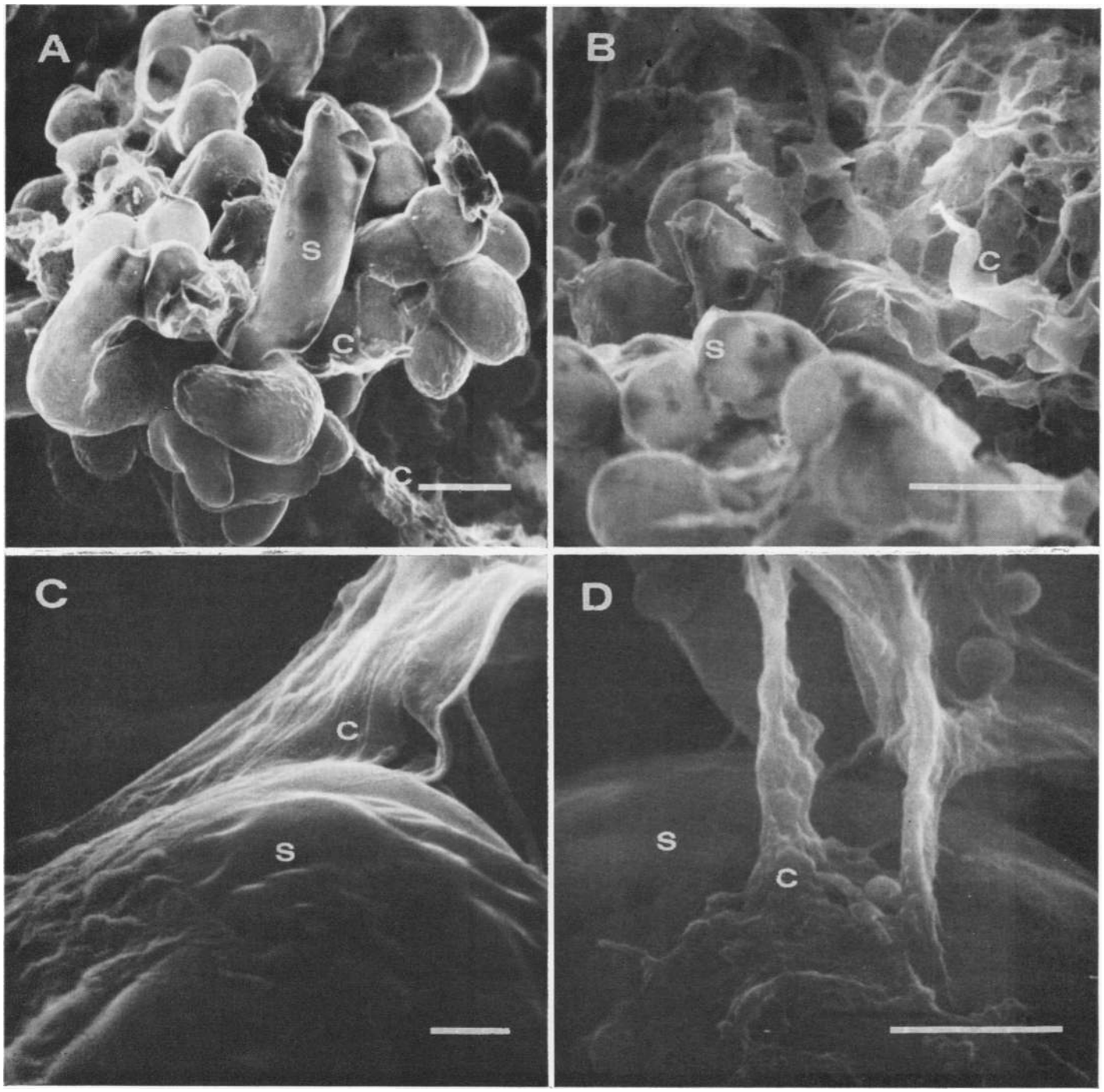

FIG. 2. Scanning electron micrographs of cultured cells enmeshed in the complex (A, B) and the complex being released from the surface (C, D). In A and B the bar is 50 μ, in C, 10 μ, and in D, 5 μ. Abbreviations: cell surface, S; complex, C.

growth was at its maximum, no material accumulated on the surface of the cultures.

Results obtained after 14 days of growth at 125 rpm in different volumes of medium are shown in Figure 4. Here the amount of tissue finally obtained was fairly directly affected by increasing the volume of the suspending medium, but the accumulation was again limited to a narrower range of conditions than those allowing rapid growth.

In seeking an explanation of these results, production of material comprising the complex must be distinguished from its accumulation on the surface, and the possibility must be considered that a soluble counterpart of the complex accumulates under some conditions. The high content of uronic acid in the complex (see analytical data below) allows a test of these possibilities. The complex itself stains intensely with ruthenium red, and microscopic examination of cultures grown at higher

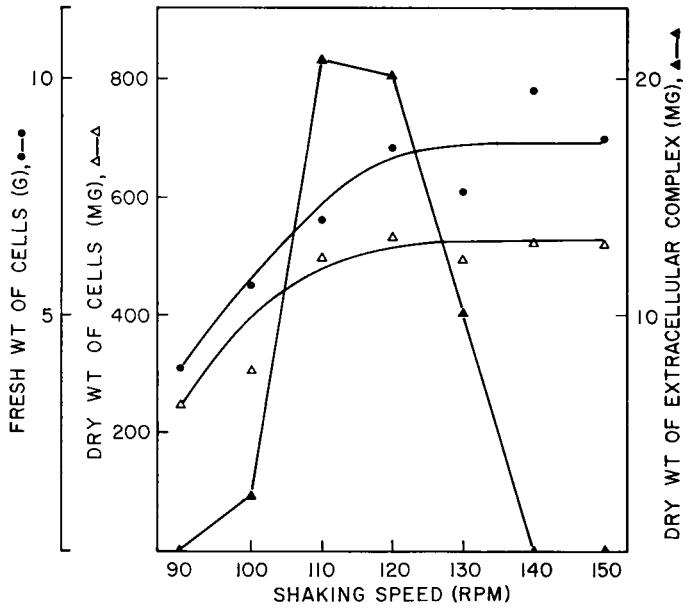

FIG. 3. Accumulation of the complex in 100 ml of medium at different shaking speeds: fresh weight (●), dry weight (△), and complex dry weight (▲).

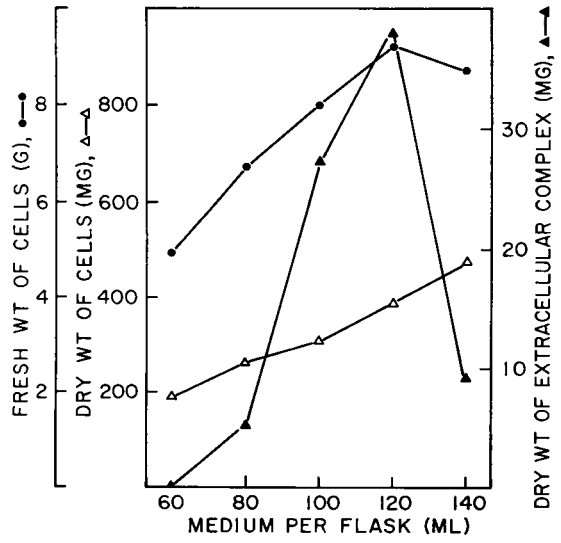

FIG. 4. Accumulation of the complex at 120 rpm in different volumes of medium: fresh weight (●), dry weight (△), and complex dry weight (▲).

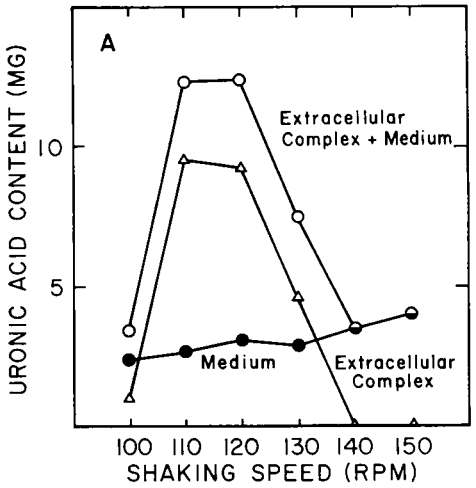

FIG. 5. Effect of different shaking speeds on the amount of nondialyzable, soluble uronic acid (●), complex uronic acid (△), and soluble plus complex uronic acid (○).

shaking speeds shows that densely stained sheets of material are present. Furthermore, when cultures in which the complex has accumulated during growth at 120 rpm are then shaken at higher speeds, the complex clumps and sinks, and if the complex itself is dispersed by treatment in a glass homogenizer it also sinks spontaneously.

Examination of the dialyzed medium and the complex by the carbazole test (Fig. 5) shows that there is a relatively small accumulation of polymers rich in uronic acid in the medium and no evidence that the complex is solubilized when it fails to accumulate at the higher shaking speeds. The resistance to solubilization observed in the chemical analysis (see below) also speaks against this possibility. Thus, it appears that the complex is produced under conditions of active cell growth but its accumulation on the surface is prevented when a certain degree of turbulence is exceeded.

An interesting correlation between complex accumulation and cell clumping in the experiments on shaking speed and volume of suspending medium is shown in Figures 6 and 7. It is clear from the graphs that under the different conditions the fraction of the total cells in clumps of the various sizes changed in a systematic way. Of particular interest is the percentage of cells in the largest clumps, labeled 5 in Figures 6A and 6B, since the form of these curves is very similar to those for the accumulation of complex in the corresponding experiments (Figs. 3 and 4).

EFFECT OF CHANGES IN THE MEDIUM ON ACCUMULATION OF THE COMPLEX

Removing inositol, a uronic acid precursor (18), from the medium resulted in a 14% decrease in fresh weight and a 19% decrease in the dry weight of the cells (Table I). The dry weight of the accumulated complex was reduced 47%. Similar results were obtained even after three passages in medium without inositol. Thus, it appears that inositol does promote synthesis of the complex somewhat, but the tissue itself must synthesize enough for most of its purposes.

Introducing 2,4-D in place of α-naphthaleneacetic acid slowed down the growth of the cells but drastically reduced the amount of complex accumulating in the cultures (Table I). When kinetin and NAA[2] were omitted and 2,4-D was added,

[2] Abbreviation: NAA: α-naphthalene acetic acid.

growth was still further reduced (Table I), and there was no accumulation of complex.

CHEMICAL ANALYSIS OF THE COMPLEX

Table II summarizes the effects of a variety of extraction and hydrolysis conditions on the material isolated from the surface of the medium and washed and dried as described for analytical preparations under "Materials and Methods." Extractions with chloroform and petroleum ether showed that lipids and waxes were absent. Pectin extraction procedures using EDTA (23) also resulted in no reduction in weight. Sodium sulfite extraction, used to extract lignins (22), dissolved 26% of the material, but its specificity is not complete since 61% of the protein was also extracted; the Russian method of lignin determination with 86% H_2SO_4 (22) left 41% of the material as residue, but 64% of the protein was also undissolved. Prolonged extraction with 0.1 N NaOH did dissolve, or hydrolyze, 45% of the material, and extraction with 17.5% NaOH resulted in solubilizing 91%. The residue of the 17.5% NaOH extraction was about 80% protein. Sulfuric acid hydrolysis of that residue did not yield any glucose upon chromatography, and the IKI-H_2SO_4 test for cellulose (12) was negative, although cell wall material stained strongly under the conditions used. These results and others in the table which will be discussed later emphasize the relative insolubility of the material and show that, even under the rather harsh conditions described in Table II, it cannot all be dissolved.

Carbohydrate Constituents. Thin layer chromatography of trifluoroacetic acid and sulfuric acid hydrolysates showed that the number of carbohydrate constituents in the material was limited; arabinose and a uronic acid were positively identified.

The presence of galacturonic acid in the complex, suggested

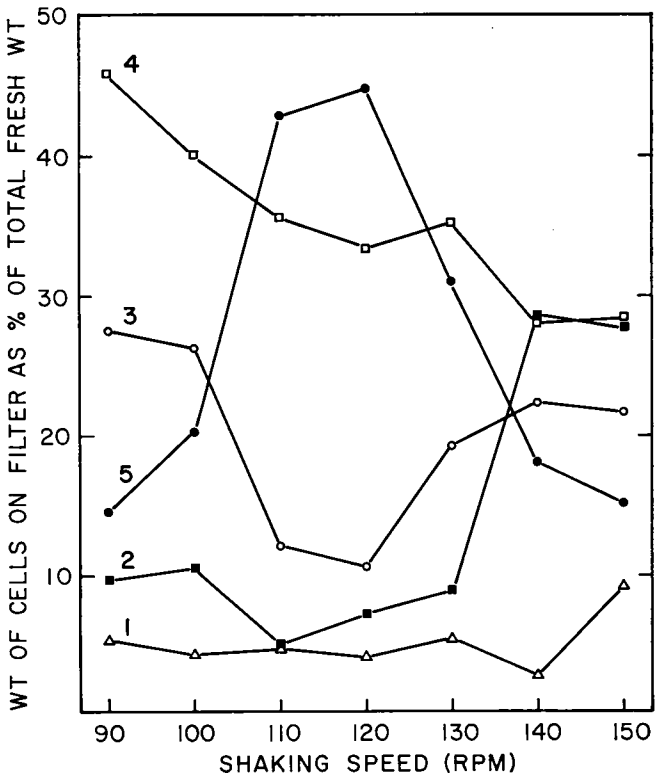

FIG. 6. Effect of different shaking speeds on cell clumping in the soybean cultures. The smallest through the largest clumps are represented by 1 through 5 in that order. See "Materials and Methods" for details.

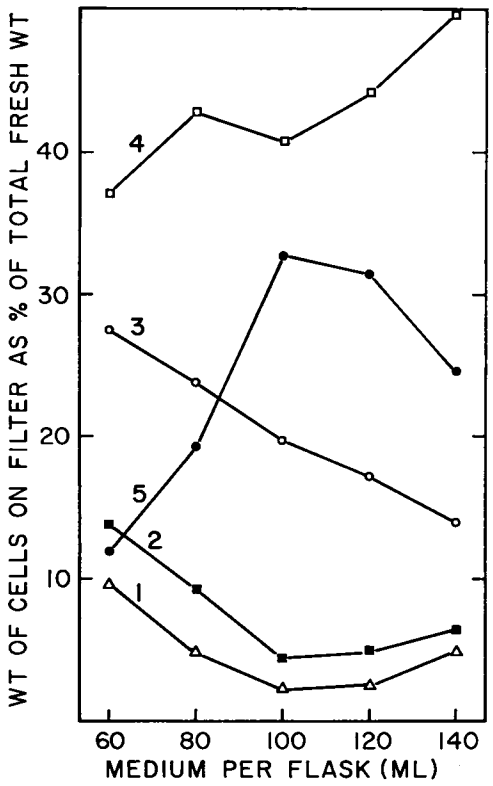

FIG. 7. Effect of different medium volumes on cell clumping in the soybean cultures. See Figure 6 for details.

Table I. *The Effect of Omitting Inositol from the Medium and of Substituting 2,4-D for NAA and NAA + Kinetin on Tissue Growth and Complex Accumulation*

Treatment	Culture Period	Fresh Wt of Tissue	Dry Wt of Tissue	Dry Wt of Complex	Complex Wt per Dry Wt
	days	*g*	*g*	*mg*	%
1. Normal	14	8.99	0.6828	33.3	4.9
-Myoinositol	14	7.68	0.5513	18.0	3.3
2. Normal	19	5.31	0.2255	10.4	4.6
-NAA, +2,4-D (1.4 μM)	26	4.92	0.2716	0.2	0.1
-Kinetin, -NAA, +2,4-D (45 μM)	56	5.07	0.3370	0.0	0.0

by the chromatography results, was confirmed by the carbazole assay as described by Knutsen and Jeanes (13). In tests using the four conditions of their assay the uronic acid component behaved as though it was entirely galacturonic acid. Further assays showed that 45 to 48% of the complex is galacturonic acid (Table III). Some galacturonic acid can be liberated from the complex by pectinase treatment, indicating that it occurs as polygalacturonic acid or pectin, in spite of the inability of the EDTA treatment to dissolve any of the complex.

Measurements of the methyl ester content of the complex suggest that 10 to 11% of the uronic acids occur as that ester (Table III). In addition, the ash content was 1.5 to 2% (Table III). Thus, it appears that the bulk of the uronic acids exist either as the free acid or are countered by some other component of the complex; evidence is presented below that at least some of the acids are countered by the protein fraction.

Several chemical assays were found to be impractical for

Table II. *Solubility of the Complex under a Variety of Conditions*

Treatment	Insoluble (Dry Wt)	Protein Insoluble[1]
	%	%
a. Diethyl ether, 7 days	96	...
b. Chloroform, 7 days	100	...
c. 0.1% EDTA, 30 C, 8 hr	99	...
d. 0.1 N NaOH, 23 C, 24 hr	55	43
e. 17.5% NaOH, 23 C, 48 hr	9	25
f. 1% acid sodium sulfite, 75 C, 1.5 hr	74	39
g. 86% H_2SO_4, 23 C, 4 hr; 8.6% H_2SO_4, 100 C, 10 min	41	64
h. 6 N HCl, 121 C, 24 hr	24	0
i. 72% H_2SO_4, 4 C, 2 hr; 3% H_2SO_4, 100 C, 4 hr	13	...
j. 0.02 N H_2SO_4, 100 C, 1 hr	...	60
k. 2 N trifluoroacetic acid, 120 C, 30 min	62	...
l. 0.25 M KCl, 23 C, 2 hr	...	83
m. 0.22 M $Ba(OH)_2$, reflux 6 hr;		
After KCl extract	...	85
$Ba(OH)_2$ + KCl	...	20

[1] Calculated from total nitrogen.

Table III. *Composition of the Complex Isolated from 7-day-old Cultures*

The results from isolates of two different batches are given.

Component	Percentage of Dry Weight	
	Isolation 1	Isolation 2
Galacturonic acid	45	46
Protein	38	35
Lignin	11 (10)[1]	15 (11)
Arabinose	4.4	4.4
CH_3 (as ester)	0.5	0.5
Ash	1.5	2.0
Total	100.3 (99.3)	102.9 (99.8)

[1] Values outside parentheses are for Klason lignin; those in parentheses are by the *N*-2,6-trichloro-*p*-benzoquinoneimine method.

estimating the arabinose content of the complex because of the high uronic acid content. They did, however, suggest that the amount was around 5% of the weight. Hence, gas chromatography was used to measure the arabinose content after trifluoroacetic acid hydrolysis. The predominance of arabinose over other neutral sugars in the complex was confirmed, and a value of 4.4% of the weight of the complex was obtained (Table III).

Estimates of the total carbohydrate content of the material using the orcinol assay (7) and the method of Dubois *et al.* (8) indicated that the galacturonic acid and arabinose probably account for all the carbohydrate present.

Protein. Hydrolysis in 6 N HCl followed by thin layer chromatography (5) showed that protein was present in the complex; a complete range of amino acids was found. Since complete dissolution of the complex was difficult, the total protein content was estimated from total nitrogen determinations. These results indicate that 35 to 38% of the weight of the complex is protein. No evidence was found for any other nitrogen-containing material. Upon further analysis of the protein fraction, the amino acid composition shown in Table IV was obtained. Hydroxyproline, a distinctive component of cell wall protein (14), is the most abundant amino acid followed by lysine, proline, serine, and valine, in that order.

Some evidence suggesting noncovalent bonding of a portion of the protein can be given. Table V shows the results of assays for three enzymes commonly reported to be present in cell walls. Invertase, peroxidase, and acid phosphatase were all found. Incubating the complex in solutions containing 0.05 M $CaCl_2$ or 0.25 M KCl released a high proportion of the enzyme activities—as much as 82% of the acid phosphatase and 70% of the peroxidase in 0.25 M KCl. Invertase was more tightly bound although it was more easily removed than total protein, which was only 19% solubilized. It should also be pointed out that the specific activity of these enzymes in the complex is low when compared with the purified enzymes.

All these enzymes are also found in the medium. In Figure 8, which shows the peroxidase activity in the medium and that calculated for the complex at different shaking speeds, the amount of enzyme present in the complex is a small portion of that in the medium. It does not show a distinct profile as was true for the uronic acid (Fig. 5).

Thus, it appears that a portion of the protein is nonspecifically bound to the complex; however, the bulk of the protein is held more strongly, and the total protein can be released only by vigorous hydrolysis methods. Treatment with 0.1 N NaOH

Table IV. *Amino Acid Composition of the Complex*

Amino Acid	Composition
	moles/100 moles total
Hydroxyproline	12.58
Lysine	8.89
Proline	8.75
Serine	8.53
Valine	8.32
Aspartic acid	8.24
Glycine	8.17
Leucine	7.23
Alanine	5.93
Glutamic acid	5.78
Threonine	3.90
Isoleucine	2.96
Histidine	2.46
Tyrosine	2.46
½-Cystine	2.31
Phenylalanine	2.17
Arginine	1.30
Methionine	Trace

Table V. *Enzyme Content of the Complex and Solubilization of Those Enzymes by Treatment with $CaCl_2$ or KCl*

Enzyme	Units in Complex[1]	Removal by Salt			
		0.05 M $CaCl_2$		0.25 M KCl	
		Soluble	Insoluble	Soluble	Insoluble
		%	%	%	%
Invertase	5.19	0	100	38	62
Peroxidase	2.28	64	36	70	30
Acid phosphatase	0.035	36	64	82	18
Protein		6	94	19	81

[1] Units for acid phosphatase and peroxidase are μmoles/min/mg protein; for invertase μg glucose/hr·mg protein.

for 24 hr released 57% of the protein, and weak acid removed 40% (Table II). Complete removal could be effected only by treatments such as refluxing with 0.22 M barium hydroxide for 6 hr, a treatment known to split specific covalent bonds (15). Hence, it seems that most of the protein is bound tightly into the structure of the complex, very likely by covalent bonds.

Pectin-Protein Relationship. One further indication of the intimate structure relationship between the major constituents of the complex is shown in Figure 9. From this figure it is evident that only about 44% of the total protein can be released by protease treatment alone, and only 48% of the galacturonic acid is released by pectinase alone. Sequential addition of the two enzymes can release essentially all of either fraction, however. Protease alone does not affect the uronic acid content of the material when applied initially, but, when applied subsequently to pectinase treatment, it can release all but 3% of the remaining pectin. Pectinase, on the other hand, releases 33% of the protein when applied initially and releases most of what is left after initial protease treatment. These results can best be explained by assuming a complex, intimate relationship between the pectin and the protein components of the complex.

Lignin. The lignin content of the material was tested by a variety of methods. Estimation of the Klason lignin (Table III) indicated that the content was 11 to 15%. This value may be high, however, because of possible contaminating protein (14), and so a second assay with *N*-2,6-trichloro-*p*-benzoquinoneimine was used (16). This assay gave slightly lower values in the two samples, 10 and 11%, respectively. Further confirmation of the presence of lignin in the complex was obtained by assays with phloroglucinol after ethanolysis and by oxidation with nitrobenzene. The products of nitrobenzene oxidation separated into several UV-absorbing and fluorescing components upon chromatography (24), but none coincided with known samples of vanillin, *p*-hydroxybenzaldehyde, or syringealdehyde. Base extraction, which frequently gives ferulic acid from lignin (4), did not yield that compound from the complex. Although these results indicate the presence of lignins in the complex, the unusual results obtained with the nitrobenzene oxidation and base extraction necessitate further studies to confirm this conclusion and define the precise type of lignin present.

In summary, the material found floating on the surface of the soybean suspension cultures is composed of about 46% galacturonic acid, 4.4% arabinose, 36% protein, 11% lignin (apparent), 2% ash, and 0.5% methyl ester by weight. These results account for the total dry weight of the complex. Although a portion of the protein is nonspecifically bound, most is an integral part of the complex. This complex is distinctly different from any of the soluble extracellular polysaccharides from suspension cultures described in detail thus far (1, 2, 9, 21). It also appears different from the fibrils described by Leppard *et al.* (16); although high amounts of uronic acid were present in their material, it had a low protein content and neutral sugars were absent. A report by Hayashi (10) described a material which may be related to the one reported here since it floated on the surface of his tobacco culture medium and probably had a high pectin content. Carceller *et al.* (6) have also noted the occurrence of sheets of "hemicellulose" in their *Acer* cultures.

The role of the complex described here cannot be ascertained at this time. Clearly, more work must be done to relate it to the total cell wall, to learn more about factors controlling its synthesis, and to determine details about its structure.

Acknowledgments—I would like to thank Professor H. Beevers for his interest and advice during the course of this work. I am also grateful to Dr. Richard Tetley and Javier Sanz for their assistance and discussions, Gene Wolery for assistance with the electron microscope, Dr. Virginia Moore for assistance with the amino acid analyses, and Sarah Harman for technical assistance.

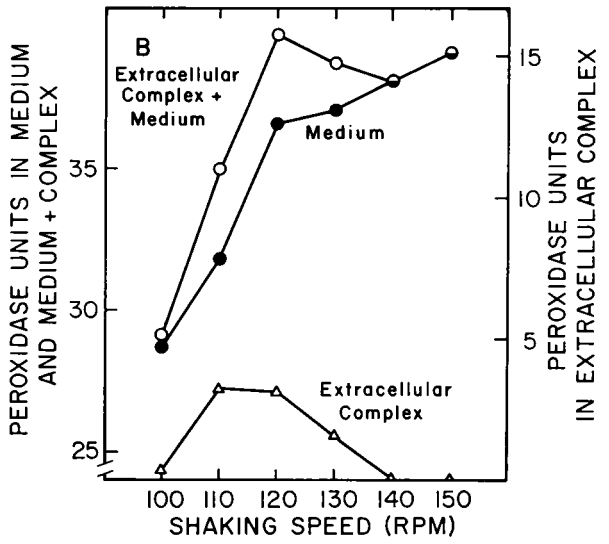

FIG. 8. Effect of different shaking speeds on the amount of peroxidase found in the medium (●), the complex (△), and the medium plus the complex (○).

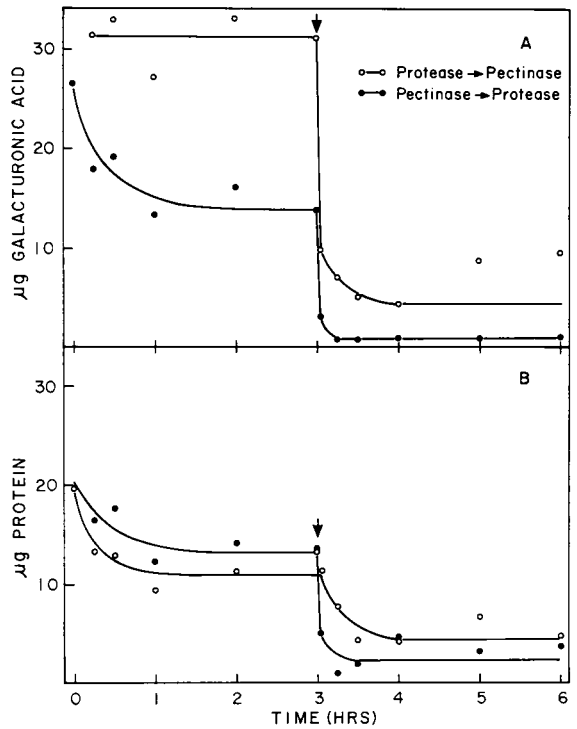

FIG. 9. Effect of sequential additions of proteolytic and pectin-degrading enzymes on the protein and uronic acid composition of the complex. See "Materials and Methods" for details. Arrows indicate the time of addition of the enzymes.

LITERATURE CITED

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