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Partial Chemical Characterization of Corn Root Cell Walls[1]

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Abstract. **The present study reports on chemical changes which occur in the cell wall of *Zea mays* during early phases of growth. Roots of seedling corn plants were divided into a meristematic zone, the zone of elongation, and the maturation zone, and the cell wall isolated from each of these zones. The wall preparations were then extracted sequentially to obtain pectin, hemicellulose, cellulose, and lignin fractions. Each of these, except for the lignin fraction, was hydrolyzed and the resultant sugars isolated, identified, and estimated quantitatively. Quantitative analysis of the products of hydrolysis of these fractions demonstrated that the classical scheme of fractionation is a valuable indicator of the changes in solubility properties which the various polysaccharide components for the wall undergo. It does not however yield definite chemical entities. For example, the "pectin" fraction contains only about 3 % galacturonic acid; the bulk of it being composed of glucose, xylose, and galactose. By summation of analysis of these various fractions, it was found that substances yielding glucose and xylose upon hydrolysis increase with advancing age of the tissue. Galactose- and arabinose-yielding compounds decrease and mannose appears during maturation. Anhydrouronic acids first decrease, then increase. Most interestingly, of the total dry weight of the cell wall, only 24, 45, and 50 % of the meristematic, elongation, and maturation zones respectively are accounted for as simple sugars in the acid hydrolysates. Oligosaccharides were not encountered in large amounts so that the 50 to 75 % of the wall weight unaccounted for would consist of polysaccharides or oligosaccharides not precipitated by ethanol from the extracting solutions employed and by polysaccharides in the hemicellulose fraction which are resistant to acid hydrolysis.**

Increased attention is being focused on the chemical composition of plant cell walls with the intent of better understanding the relationship between structure, development, and metabolic function. The necessity for more comprehensive studies is evident in light of the current view that cell wall rigidity is an important determinant of elongation growth.

Recent, thorough, studies have utilized coleoptile tissue (1, 10, 20, 21), tubers (9), and cambial tissues (22, 23, 24). Few studies have been made of the cell walls of roots and, in fact, in these cases the starting material was whole roots, either dried or fresh, and without isolation of the cell walls. In addition, prior studies were based primarily upon solubility properties of the various extractable fractions (4, 18, 19). Consequently, percent composition of pectin, hemicellulose and α-cellulose is expressed as total weight of material extracted with a particular solvent without qualitative identification and quantitative estimation of the constituent sugars. Bishop *et al.* (1) have indicated that fractionation of cell wall components into the classical pectin, hemicellulose, cellulose and lignin fractions may be misleading since many polysaccharides may occur in each fraction. This paper reports a partial chemical characterization of the cell wall of *Zea mays* roots with emphasis on the carbohydrate-containing fractions: pectin, hemicellulose and α-cellulose. The various growth zones were examined separately in order to detect changes in cell wall composition concomitant with root development.

Methods and Materials

Preparation of Plant Material. Kernels of *Zea mays*, L. Michigan 300 Hybrid, obtained from the Michigan Farm Bureau, were soaked in running tap water for 15 hours and then surfaced sterilized by treatment for 5 minutes in a 1 % Chlorazene solution (Frost Laboratories, Inc., Boston, Massachusetts). After thorough rinsing with glass distilled water the kernels were germinated between moist paper towels for 5 days at 25° in the dark. The 3 root zones utilized were cut with a razor blade and stored in the freezer until enough tissue was collected for examination. The 0 to 2 mm section, including the root cap, is designated as the meristematic zone; the 2 to 17 mm section as

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the elongation zone; and the 17 to 32 mm section as the maturation zone.

Isolation of the Cell Wall. Cell wall was isolated from the frozen sections of root by the method of Kivilaan *et al.* (10). This method, involving homogenation in glycerol, has previously been shown to yield a cell wall product which is substantially free from contamination by soluble enzymes or cell organelles. Following the final sedimentation from glycerol, the cell wall material was collected by centrifugation at 54,000 × *g* for 1 hour and the glycerol removed by 2 washings with absolute alcohol followed by 1 of acetone and finally 1 of ether. The wall material was dried *in vacuo* over anhydrous $CaSO_4$ for 3 hours, then stored *in vacuo* over P_2O_5. The present preparation contained no unbroken cells when examined with the light microscope and closely resembled the wall fragments obtained by Kivilaan *et al.* (10).

Cell Wall Fractionation. A sample of the cell wall material from each of the 3 growth zones was fractionated by a modification of the method of Bishop *et al.* (1).

I. *Pectin Fraction.* Wall preparations were first extracted twice with 0.5 % ammonium oxalate-oxalic acid at 90° for 24 hours. After each extraction the residue was separated from the supernatant fluid by centrifugation. To each extract 8 volumes of absolute alcohol were added, slowly with stirring, and the mixtures were allowed to stand at −10° overnight. The resulting precipitates were collected by centrifugation, dried *in vacuo* over anhydrous $CaSO_4$ for 3 hours, stored over P_2O_5 and are here designated as pectin fractions 1 and 2 respectively.

II. *Hemicellulose Fraction.* The residues from the oxalate extractions were next extracted sequentially with 4 % NaOH, twice with 10 % NaOH, and finally once with 17.5 % NaOH. Each extraction was at room temperature for 18 hours. After removal of the insoluble residues by centrifugation, the alkaline extracts were neutralized with HCl, diluted with 10 volumes of absolute alcohol, and stored overnight at −10°. The resultant precipitates were washed with ethanol, acetone and ether, and dried *in vacuo* over P_2O_5. The 2 10 % extracts were combined and the fractions designated as hemicellulose 1, 2, and 3 for the 4, 10, and 17.5 % alkaline extractions respectively.

III. *Cellulose Fraction.* The residues from the above alkaline extractions were washed with ethanol, acetone and ether, and dried. They were suspended in 72 % H_2SO_4 and kept at 0 to 4° for 48 hours with occasional stirring. The suspensions were then diluted with distilled water to a final concentration of 1 % H_2SO_4, autoclaved for 2 hours, and, after cooling, neutralized with NaOH. Aliquots of these fractions were desalted with a Warner-Chilcott Laboratories electric desalter, Model 1930C, and used for paper chromatographic analysis. They are here designated as the α-cellulose fractions.

IV. *Lignin-like Fraction.* The non-hydrolyzable residue remaining after acid hydrolysis of the α-cellulose was filtered off, weighed and designated as lignin-like material. No attempt was made to characterize this fraction chemically.

In addition, samples of the unfractionated cell wall were analyzed for nitrogen by the Dumas method at a combustion temperature of 900°. These analyses were done by the Micro-Tech Laboratories, Skokie, Illinois.

Characterization of the Pectin Fraction. Samples from each of the 3 root zones were hydrolyzed by the method of Jermyn (8). Each pectin fraction was refluxed with concentrated HNO_3 and a few crystals of urea at 100° for 12 hours. The resultant solutions were cooled and, after dilution to a standard volume, were analyzed by paper chromatography. Qualitative identification of the sugars was accomplished utilizing 5 solvent systems and 3 color reagents. The solvent systems and duration of chromatography times were: (I) pyridine:ethyl acetate:water 2:8:1 (v/v), 30 hours; (II) pyridine:ethyl acetate:acetic acid:water 5:5:1:3 (v/v), 48 hours; (III) butanol:ethanol:water 3:1:1 (v/v), 48 hours; (IV) 80 % phenol, 30 hours; and (V) butanol:acetic acid:water 4:1:5 (v/v), 66 hours (3, 6). Whatman No. 1 paper and descending chromatography were used in all cases. Aniline hydrogen phthalate, diphenylamine and aniline hydrogen phthalate-diphenyl amine reagents were used to visualize the resolved sugars (7). Quantitative determinations of sugars were by the method of Wilson (25), and uronic acid determinations by the carbazole method of McCready and McComb (15, 16), modified by the addition of sodium tetraborate (2). The uronic acids remain at the origin in the pyridine solvent (I). Thus, following chromatography a square, sufficiently large enough to include the uronic acid spot, was removed from the origin prior to application of a color reagent to the chromatogram. The sugar acid was eluted from the paper with distilled water and the eluant used directly for the carbazole assay.

Characterization of the Hemicellulose Fraction. A sample of each of the hemicellulose fractions was hydrolyzed with 3 % HNO_3 in a boiling water bath for 4 hours following the method of Jermyn (8). Qualitative identification of the hydrolysis products was made using solvents I, III, and V, utilizing the 3 color reagents described. Quantitative measurements were made by the methods used in the analyses of the pectin fractions.

Characterization of the α-cellulose Fraction. Qualitative analysis of the desalted α-cellulose fractions was accomplished by paper chromatography using ethyl acetate:acetic acid:water 9:2:2 (v/v), descending 28 hours, in addition to solvents I and III. The products were visualized with silver nitrate or aniline hydrogen phthalate. Total

reducing sugar was determined by the arsenomolybdate method of Nelson (5, 17) and glucose was determined by the glucose oxidase reaction (Glucostat, Worthington Biochemical Corporation, Freehold, New Jersey).

Results

Composition of the Cell Wall. The recovered wall material represents 1.00, 0.74, and 1.03 % of the original fresh weights of the meristematic, elongation and maturation zones respectively. Recovery is probably not complete but the losses should be comparable for the 3 zones. Table I presents an analysis of the 3 root growth zones based on the weight of the extracted fractions and expressed as percent of the cell wall. As expected, α-cellulose is present in very small amounts in the meristem tissue, increases in the elongation zone and finally, in the maturation zone, is approximately 5 times greater than in the root tip. By contrast, pectin constitutes about one-fourth of the cell wall in the tip and elongation zones and decreases significantly in the maturing tissue. Hemicellulose is in lowest concentration in the root tip and increases through the older growth zones. Protein, calculated as 6.25 times the percent N, is greatest in the tip and gradually decreases with tissue age.

Table I. *Composition of Corn Root Cell Walls*

	Meristem	Elongation	Mature
	% *of dry wt*		
Pectin	27	26	15
Hemicellulose	25	46	40
α-Cellulose	4.6	13	24
Protein	9.0	5.4	4.8
Lignin-like residue	0.7	3.7	1.3
Total recovery	66	94	85

About 34 % of the weight of the wall from the meristematic zone is not accounted for in the 5 fractions isolated. Thus, appreciable amounts of material were not precipitated from the oxalate and alkaline extracts. Satisfactory recovery was obtained for the other 2 zones. Absolute recoveries cannot be calculated since protein contents were determined using unfractionated walls. Thus, at least a portion of the protein could be included in the weights of the pectin, hemicellulose and α-cellulose fractions. The maximum error, assuming all protein is extracted by the procedures used, would be 9.0, 5.4, and 4.8 % of the wall dry weight for the meristem, elongation and maturation zones respectively.

Characterization of the Pectin Fraction. No detectable residue remained after hydrolysis under reflux. Paper chromatography indicated a similar composition for each of the 3 root zones. Glucose, xylose, galactose, arabinose, and galacturonic acid were found in each. Table II illustrates the quantitative composition of the 2 ammonium oxalate-oxalic acid extracts of each of the root zones. The values for sugars are the average of 4 replicates and those for galacturonic acid, 8 replicates. Identification of the uronide as galacturonic acid was accomplished using chromatography in solvents II and III. While the other solvents, especially I, gave satisfactory separation of the sugars, they failed to separate the uronic acids. Neither glucuronic nor mannuronic acids were detected.

Glucose was the most abundant sugar in the pectin fractions from all 3 zones. Xylose appeared as the second most abundant sugar, except in the meristematic zone, where its concentration was approximately that of galactose. The tip of the root contains approximately 3 times as much galactose as the other zones. Arabinose and galacturonic acid were minor constituents in all sections.

Characterization of the Hemicellulose Fraction. Appreciable residues remained after hydrolysis with HNO_3. Subsequent hydrolysis of these residues with 72 % H_2SO_4 in sealed tubes at 100° for 6 hours failed to produce perceptible solubilization as detected by recovery and reweighing of the original residues. No further attempts were made to determine the composition of these residues. Qualitatively, the soluble portions of the hemicellulose fractions of the 3 root zones yielded the same sugars and galacturonic acid. Nevertheless, there are marked changes in the solubility properties of the hemicellulosic components as evidenced by comparing the 4, 10, and 17.5 %-soluble glucose and xylose polymers in, for example, the meristematic and maturation zones. Table III presents the quantitative composition of the hemicellulose

Table II. *Sugar Analysis of Pectin Fractions from Corn Root Cell Wall*
The sugars are expressed as percent of total fraction.

	Meristem zone Extract		Elongation zone Extract		Mature zone Extract	
	1	2	1	2	1	2
Glucose	22.0	22.8	46.4	60.8	56.0	51.2
Xylose	11.2	9.2	35.6	21.6	12.5	10.8
Galactose	12.8	10.8	4.1	2.6	4.8	3.6
Arabinose	5.6	4.2	3.3	2.0	4.2	3.6
Galacturonic acid	4.6	3.8	2.4	3.2	2.8	2.6

Table III. *Sugar Analysis of the Hemicellulose Fractions from Corn Root Cell Walls*
Each component is expressed as percent of weight of total hemicellulose fraction.

Hemicellulose fraction sugar	Meristem NaOH Extraction			Elongation NaOH Extraction			Mature NaOH Extraction		
	4 %	10 %	17.5 %	4 %	10 %	17.5 %	4 %	10 %	17.5 %
Glucose	8.6	15.8	3.1	15.7	6.8	4.3	27.4	17.6	22.4
Galactose	2.2	3.5	0.8	1.4	1.8	2.4	3.9	2.8	3.1
Mannose	2.2	1.8	1.4	1.2	0.6	2.3	0.4	0.9	2.4
Xylose	7.6	7.4	7.1	9.4	5.2	4.1	22.9	16.1	12.7
Arabinose	1.9	1.2	1.7	1.0	1.6	2.3	4.3	1.6	2.6
Galacturonic acid	0.9	0.4	0.1	1.0	0.6	0.1	4.1	2.1	2.6

Table IV. *Analysis of the α-Cellulose Fractions from Corn Root Cell Wall*

Root zone	α-Cellulose[1]	Total reducing sugar	Glucose	Reducing sugar	Glucose
	mg	*mg*	*mg*	*% of α-cellulose*	*% of reducing sugar*
Meristem	9.3	7.3	6.6	79	90
Elongation	64.8	55.4	54.7	86	99
Mature	122.7	88.2	73.9	72	84

[1] Calculated as the difference in weight between the residue after extraction of hemicellulose and the residue after digestion with 72 % H_2SO_4.

fractions by root zone. As in the pectin fractions, glucose, and xylose are the predominant sugars with galactose, arabinose, and galacturonic acid occurring in lesser proportions. In addition, mannose is present in small amounts throughout the root. In all fractions significant and sometimes large amounts of the hemicellulose could not be accounted for either as simple sugars or the insoluble residues previously referred to. The presence of unidentified spots (presumably aldobiuronic acids, although no positive identification was attempted) prevent a complete and quantitative analysis of the hemicellulose fractions. In addition, since only a single time of hydrolysis was used, it is probable that some loss occurred through breakdown of the sugars.

Characterization of the α-cellulose Fraction. Paper chromatography of these fractions with solvent I indicated only glucose in the meristematic and elongation zones, and glucose plus a small amount of mannose in the maturation zone. Subsequent chromatography in solvent III resolved the streaking at the origin into 3 discrete spots with (migration compared to glucose) r_g values of 0.12 to 0.13, 0.42 to 0.43 and 0.60 to 0.65. One spot was found to cochromatograph with mannobiose (r_g = 0.61) and a second with gentiobiose (r_g = 0.47) while the third unknown was not identified. Total reducing sugar and glucose oxidase analyses are presented in table IV. As can be seen, from 72 to 86 % of the weight of the α-cellulose can be accounted for as reducing sugar and this is almost entirely glucose. Thus the glucose oxidase assay supports the chromatographic evidence that the α-cellulose fraction is composed primarily of glucose except in the maturation zone where mannose is detectable. The 14 to 28 % of the weight unaccounted for would be the oligo- and polysaccharides referred to above.

When the constituent sugars, which occur in the various fractions, are summated a distribution as shown in table V is observed. Glucose remains the most abundant sugar. Except for galactose in the meristem, the only other sugar occurring in a relatively large amount is xylose. Galactose, arabinose and mannose constitute a very small proportion of the wall. The small amount of arabinose is interesting in view of the fact that it is reportedly present in significant quantities in coleoptile (20) and tuber tissue (9). It is significant to note that if pectin is defined on the basis of galacturonic acid content, it constitutes only 1 to 2 % of the corn

Table V. *Summary of the Sugar Composition of Corn Root Cell Walls*

Component	Meristem	Elongation	Maturation
	% of total wall wt		
Glucose	12.8	29.2	31.9
Xylose	4.6	11.2	9.5
Galactose	3.3	1.6	2.0
Arabinose	1.7	1.4	1.8
Mannose	0.8	0.7	3.1
Galacturonic acid	1.3	1.0	1.7
% Of dry wt of wall accounted for by the above sugars	24.5	45.1	50.0

root cell wall, while if based on total dry weight of the oxalate-extractable material, it would represent 15 to 27 % of the wall.

Discussion

The present results indicate that the cell wall constitutes 1 % of the fresh weight of the meristem tissue in corn roots. The percentage decreases to 0.74 % in the elongation zone and increases again to 1.03 % in mature tissue, reflecting the metabolic changes accompanying root development. Kivilaan (personal communication) reports that cell walls of 4 day old corn coleoptiles comprise 1.22 % of the fresh weight, and that this amount decreases to 0.77 % in 8 day old plants.

Correlated with these developmental differences are significant changes in the major carbohydrate fractions of the isolated cell wall, and in the chemical composition of each fraction. The pectin fraction accounts for 27 % of the meristem wall and decreases to 15 % of the mature wall, while the α-cellulose constitutes only a small portion (4.6 %) of the undifferentiated root tip, but increases 5 fold in the mature tissue. Hemicelluloses account for one-quarter of the meristem wall and approximately one-half of older walls.

Since there are but few reports of root cell wall analyses, an absolute comparison with other plants is difficult. Burstrom (4) reported 11 % pectin, 10 % hemicellulose, and 26 % cellulose in wheat root cell walls. Odhnoff (18) calculated 7 % pectin, 15 % hemicellulose, and 21 % cellulose in bean root cell walls, and Phillips (19) found that the oat root cell wall contained 25 % hemicellulose and 30 to 35 % cellulose. The difficulties in drawing comparisons between prior reports and the present data arise primarily from differences in germination periods and methods of extracting the various fractions. Older seedlings were used by the other workers and the wall components were extracted from whole roots without first isolating the cell wall. Only total root weights were reported in those papers with no reference to the portion of the roots used. It seems probable that these workers exercised more than the 23 mm of root used in the present analyses. Therefore, beginning with older and presumably longer roots it is possible that their analyses would involve a greater proportion of mature tissue, and as a result, would yield a higher cellulose to pectin ratio.

When the pectin, hemicellulose and cellulose contents reported in the present study are expressed on the basis of the entire 23 mm root, instead of on the individual growth zones, values of 21, 40, and 16 % are obtained respectively. The pectin and cellulose fractions compare favorably with the published data. Only the hemicellulose differs significantly; the current data being higher than those previously reported. Burstrom stated that his values seemed low in this respect but offered no explanation. Phillips reported a considerable amount of xylan in his hemicellulose fraction. In view of this and the fact that these earlier results were based mainly on total reducing sugars, without benefit of qualitative analysis, it appears quite reasonable to assume that the low hemicellulose content of roots, previously reported in the literature, arises from (1) less rigorous techniques leading to incomplete extraction and cross-contamination with other fractions and (2) incomplete hydrolysis of the fractions leading to deceivingly low total reducing sugar values.

It has already been noted that root cell wall characterization in all prior reports was based primarily on the solubility properties of the various fractions. The ability of the extracting solvent to selectively extract wall components is certainly not good. The oxalate-soluble, so called, pectin fraction, serves here as an example. By weight, ammonium oxalate-oxalic acid extractable material represents 27, 26, and 15 % of the meristem, elongation and maturation cell walls respectively. However, the total galacturonic acid content for these same growth regions accounts for only 1.3, 1.0, and 1.7 %. A similar occurrence was reported in corn coleoptiles (10) where Kivilaan *et al.* found that the ammonium oxalate fraction constituted 28 % of the dry weight of the wall, but the galacturonic acid content was only 8 %. It seems then, that anhydrouronic acid represents only a small fraction of the cell wall, and thus it is not likely that uronic acid polymers are a major determinant of wall rigidity.

It is of interest that not all of the galacturonic acid is extracted in the pectin fraction: a considerable portion is removed during alkaline extraction of the hemicelluloses (table III). The differential solubilities thus observed may be interpreted as an indication of different pectin species, possibly related to the degree of association with accompanying sugars or simply the result of differences in molecular size. Of the total galacturonic acid in the meristem wall, 88 % is found in the pectin fraction. This proportion decreases significantly with age; only 67 and 24 % of the sugar acid is obtained in the oxalate extracts of the elongation and maturation zones respectively. This may be taken as evidence of metabolic turnover during growth and development, and could conceivably reflect a change in the role of this component with age.

Major changes in 2 other carbohydrate components of the wall are observed during root development. Glucose is the most abundant sugar in all of the growth zones, but a transition in its distribution between the cellulosic and non-cellulosic fractions is evident. While only 29 % of the total glucose in the meristem wall occurs as cellulose, this proportion increases to 37 % and finally 47 % in the elongation and maturation zones. The latter

value is probably an underestimation due to the significant amount of glucose present in the disaccharides resulting from incomplete hydrolysis of the cellulose fraction. In view of the fact that the total glucose content in the elongation and maturation zones are approximately equal (29 and 32 %), the net increase in the cellulosic fraction tends to support MacLachlan and Duda's report (13) that, in the apical section of pea epicotyls, grown without added substrate for cell wall synthesis, glucose disappears from their dilute-acid-soluble wall fraction while cellulose increases. Similar results have been reported by Ray (20) for oat coleoptile cell wall.

The present analysis also indicates a decrease in the galactose content of the pectin fraction concomitant with secondary growth (table II). Galactose constitutes 12 % of the meristem pectin and decreases dramatically to 3 and 4 % in the elongation and maturation zones. Similar loses have been reported in pea epicotyl (14), oat coleoptile (20) and various angiosperm cambiums (23). Although it is suggested in these reports that the plasticity of the wall and the mechanism of enlargement are associated with the breakdown of certain polysaccharides including galactans and starch-like glucans, the underlying relationships remain obscure.

With regard to chemical composition, the corn root hemicelluloses are similar in carbohydrate composition to that of other plants with the exceptions that rhamnose is not present and arabinose is found in lesser amounts than usual.

The hemicellulose fraction offers, perhaps, the most intriguing yet most difficult feature of the wall to evaluate. Marked quantitative changes in carbohydrate composition are accompanied by more subtle, but equally important, changes in the solubilities of the various polysaccharides within the fraction. The most striking example is the distribution of glucose-containing polymers. In the meristematic region, 57 % of hemicellulosic glucose is recovered in the 10 % NaOH extractions (table III) and in the elongation zone 59 % is extracted with 4 % NaOH. This indicates a shift in the molecular properties of the glucan-like component during cell expansion. In the zone of maturation, the greatest net increase is observed in the 17.5 % NaOH extractable material. The difficulty in evaluating this phenomenon arises from the fact that it is not known what portion, if any, represents metabolic turnover of existing wall material and what, in fact, is newly synthesized material. It is also apparent, by applying arbitrary extraction schemes, that the fractions obtained are complex mixtures of various polysaccharides. Obviously, the traditional fractionation does not constitute a reasonable chemical fractionation. It is, however, of interest in that it reveals that major changes are occurring in the solubility properties of the polysaccharide wall components.

The chemical analysis of the corn root cell wall presents, with a few minor exceptions, a profile quite similar to those of bean, oat and wheat roots, and the cambial, coleoptile and stem tissues of numerous plants. There is evidence of changes in the concentration of glucose, galactose, and galacturonic acid during growth and development. The fact that such changes do occur leads to the important question of what components of the wall determine its structural rigidity, and thus, its capacity for extension growth. The extremely small amount of galacturonic acid present in the roots prompts doubt as to its postulated role as a major determinant of the physical properties of the wall. However, if most of the uronic acid residues were arranged throughout the wall interspersed with and, in some unknown manner, affiliated with the non-cellulosic polysaccharides, it still seems possible that the reactive uronide carboxyl groups could control the plastic qualities contributing to cell expansion. Burstrom (4) and Ray (20) suggest that the hemicelluloses may be involved in expansion of coleoptile cells because of the large proportion of the wall that they occupy. This speculation has recently received experimental support (12) with the demonstration that enzymes that hydrolyze hemicelluloses are present in the wall. An attractive possibility has been advanced by Lamport (11) suggesting that a wall protein, which he calls "extensin", could be a determinant of cell wall rigidity.

The determination of which wall component determines the structural properties of the wall remains for further study.

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