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EFFECTS OF LIGNIFICATION, CELLULOSE CRYSTALLINITY AND ENZYME ACCESSIBLE SPACE ON THE DIGESTIBILITY OF PLANT CELL WALL CARBOHYDRATES BY THE RUMINANT

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Abstract

Intrinsic characteristics of plant cell walls limiting susceptibility of structural carbohydrates to microbial attack in the ruminant's gastrointestinal tract are lignification of the cell wall, covalent bonding of phenolic acids to cell wall polysaccharides, the crystalline structure of cellulose and limited fibrolytic enzyme accessible space. The exact mechanism by which or degree to which each of these characteristics affect rate and/or extent of cell wall polysaccharide hydrolysis by gastrointestinal tract microbes is not well understood. Lignification and limited enzyme accessible space probably affect the extent of cell wall degradation by preventing contact between microbial enzymes and cell wall polysaccharides. Phenolic acids may limit cell wall carbohydrate degradation by steric hindrance of the fibrolytic enzyme, which could affect both rate and extent of degradation, and by their potentially toxic effects on microbes. Crystalline cellulose, occurring in secondary cell walls, may be degraded at a slower rate than amorphous cellulose. Further research is needed to gain a better understanding of the mechanisms by which these characteristics limit structural polysaccharide degradation by gastrointestinal tract microbes and to determine to what degree each contributes to limiting digestibility of cell wall carbohydrates by ruminants.

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Introduction

Pond et al. (1980) identified the poor digestibility of lignocellulose as a major obstacle constraining animal protein production in the face of an expanding world population. Digestion of plant material by ruminants is dictated, in part, by the rate and extent with which gastrointestinal tract microorganisms can degrade cell wall polysaccharides. Increasing digestibility of lignocellulosic fiber by ruminants, therefore, is dependent upon a better understanding of the reasons that cell wall carbohydrates are limited in their susceptibility to microbial degradation.

Based on the model of the cell wall proposed by Albersheim (1978), there are three possible factors primarily responsible for limiting susceptibility of cell wall polysaccharides to microbial degradation in the ruminant's gastrointestinal tract: (1) the close physical and chemical association among cellulose, hemicelluloses and lignin; (2) the presence of crystalline regions within cellulose; and (3) a limited enzyme accessible space (Stone et al., 1969; Northcote, 1972; Bailey, 1973; Van Soest, 1973; Cowling, 1975; Rowland, 1975; Gordon et al., 1977; Fan et al., 1980 Chambat et al., 1981; Jung and Fahey, 1983). Before these limitations to cell wall degradation can be overcome by plant breeding or chemical treatments, one must understand how these factors exert their negative influence on microbial degradation of cell wall carbohydrates.

Factors limiting microbial degradation of cell wall polysaccharides

Phenolic acids / lignin

A comprehensive review of the nutritional implications of phenolic monomers and lignin was presented by Jung and Fahey (1983). Microbial degradation of lignocellulosics is negatively correlated with total phenolic acid content (Burns and Cope, 1974; Jung and Fahey, 1981) and cell wall lignification (Patton and Gieseker, 1942). While phenolic acids and polyphenolic polymers appear to be primary factors limiting susceptibility of cell wall polysaccharides to microbial digestion, their mechanism of protection is unclear (Chesson et al., 1983).

Cell wall polymeric lignin is covalently bound to hemicelluloses in the plant cell wall (Van Soest, 1981; Brice and Morrison, 1982). Smith and Hartley (1983) noted that ferulic and para-coumaric acids were esterified to cell wall polysaccharides, and appear to be the primary means of lignin attachment to

cell wall polysaccharides. The mechanism by which ester linkages between cell wall polysaccharides and ferulic and (or) para-coumaric acids limit enzymatic hydrolysis of cell wall polysaccharides is not well understood. These phenolic acids probably limit structural carbohydrate degradation by inhibiting microbial growth and (or) enzyme activity, by inactivating the enzyme or by sterically hindering its attachment to the structural carbohydrate.

Chesson et al. (1982) showed that phenolic acids inhibited growth and cellulolytic activity of ruminal bacteria. Akin (1982) found that para-coumaric, ferulic and sinapic acids depressed in vitro cellulose digestion by ruminal bacteria. Akin (1982) found that para-coumaric acid was more inhibitory to cellulose degradation than ferulic acid. Jung (1985), however, found ferulic acid more inhibitory to cellulose degradation than para-coumaric acid. As shown by Chesson et al. (1982; Table 1), different bacterial species respond differently to the various phenolic acids present in plant cell walls. The contrasting results of the previously mentioned experiments could be explained by differences in the primary species of bacteria degrading cellulose in these experiments. Whether phenolic acids actually affected total microbial numbers, lowering the total amount of bacterial cellulase present, or decreased the cellulase enzyme activity, is unclear. In either case, in vitro cellulose degradation would be reduced. Further research is needed to identify the mechanisms by which free phenolic acids and (or) complexes of phenolic acids covalently bound to cell wall monosaccharides depress microbial degradation of plant cell wall polysaccharides.

Jung and Sahlu (1986) found that filter paper cellulose degradation by ruminal bacteria was depressed when phenolic acids were esterified to cellulose fibers (Table 2). The negative effect of phenolic acids on structural carbohydrate degradation was apparently greater when phenolic acids were esterified to the cellulose than when free in solution. Jung and Sahlu (1986) also found that different sources of cellulose, presumed to vary in their structure, differed in terms of which phenolic acids were most inhibitory to microbial degradation of the cellulose. If the different celluloses used in these experiments were degraded by different species of cellulolytic bacteria, variation in the negative effects of the various phenolic acids tested on cellulose degradation could have been due to differences in the predominant cellulolytic organism present (as previously discussed). Further research needs to be conducted to determine (1) the primary bacterial species degrading various hemicelluloses and cellulose in the plant cell wall and (2) the effects of various phenolic acids esterified to cell wall structural carbohydrates on rate and extent of microbial degradation.

Smith and Hartley (1983) isolated a lignin-carbohydrate complex from wheat bran cell wall after fungal cellulase treatment. The complex was composed primarily of xylose, arabinose and ferulic acid. They identified the complex as 2-O-[5-O(feruloyl)-β-L- arabino-furanosyl]-D xylopyranose. Because this compound could be isolated, it appears that steric hindrance inhibited hydrolyses of monosaccharides bound to phenolic acids. Chesson et al. (1983) noted that xylans substituted with arabinose residues were preserved during ruminal digestion, and the extent of substitution at the O-5 position of arabinose was closely related to the amount to phenolic material present further indicating that ruminal microbes are limited in their ability to degrade cell wall polysaccharides bound to phenolic acids.

Core lignin (Gordon and Neudoerffer, 1973) is a complex three-dimensional structure formed by free radical-induced polymerization of phenolic monomers synthesized by the shikimic acid pathway (Harkin, 1973). The mechanism by which core lignin limits cell wall polysaccharide digestion is also unknown, but it is possible that this limitation is due to lignin's physical protection of cell wall carbohydrates and its hydrophobic character (Van Soest, 1982). The physical protection and hydrophobic nature of core lignin would exclude microbes from reaching and attaching to the polysaccharides, thus reducing their ability to hydrolyze the cell wall carbohydrates. Disrupting the structure of core lignin which encrusts the cell wall polysaccharides should result in increased attachment and penetration by microbes and, subsequently, in an increased digestibility of the cell wall polysaccharides. Completely removing core lignin from the cell wall with permanganate oxidation was shown to increase microbial degradation of cell wall polysaccharides (Barton and Akin, 1977; Table 3). Kerley et al. (1985) demonstrated that partial (approximately 50%) delignification of plant cell walls by alkaline hydrogen peroxide treatment (Gould, 1984) allowed extensive attachment of ruminal microbes, accompanied by rapid degradation of cell wall carbohydrates (Figure 1).

It is not known to what extent core lignin's negative effect on digestion is dependent on its binding with cell wall polysaccharides. It is known that non-core (alkali-labile) phenolic acids form diaryl (Hartley and Jones, 1976) and alkyl-alkyl (Stafford and Brown, 1976) bonds with proteins. Scalbert et al. (1985) provided evidence of ferulic acid (non-core lignin) attachment to core lignin by a similar bonding mechanism, indicating that core lignin may be bound to cell wall polysaccharides via non-core lignin. This could limit digestion, in that core lignin could physically exclude and non-core lignin could sterically hinder enzymatic attachment to and hydrolysis of cell wall carbohydrates.

Cellulose crystallinity.

Crystalline cellulose, in contrast to amorphous cellulose, refers to aggregates of cellulose polymers held tightly together by extensive hydrogen bonding. Cellulose is a polymer of β-1,4-linked D-glucose units (Frey-Wyssling, 1969). This type of linkage results in the relative inversion of alternate glucose units. This places the C-3 hydroxyl of one glucose unit in close proximity to the ring oxygen of the next glucose unit in the chain. Hydrogen bonding between the hydroxyl and ring oxygens stabilize the cellulose polymer, giving it a straight, flat structure (Frey-Wyssling and Muhlethaler, 1963). This linear structure of cellulose allows adjacent polymers to fit closely together, favoring hydrogen bond development between the C-6 hydroxyl glucose in one chain with C-2 or C-6 hydroxyls of glucose in an adjacent chain. Since cellulose chains consist of 8,000 to 15,000 glucose residues, extensive hydrogen bonding can occur, conferring considerable strength to the microfibrils (Frey-Wyssling and Muhlethaler, 1963).

Cellulose polymers are held so tightly together in the microfibril structure by hydrogen bonding that water molecules may be excluded from the crystal

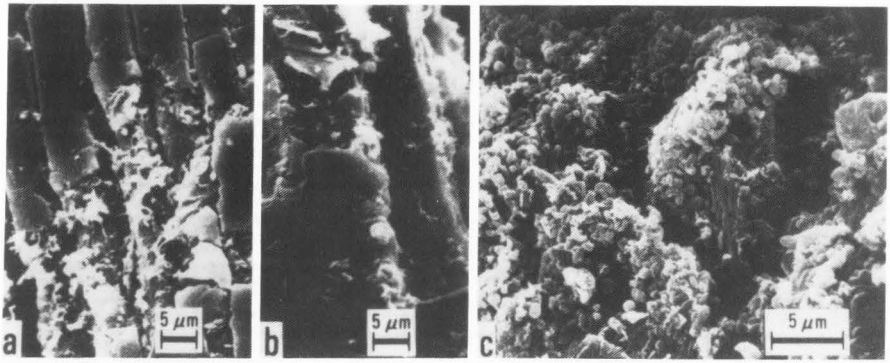

Figure 1. Scanning electron micrographs of straw particles isolated from the rumen of fistulated mature sheep fed diets containing 72 percent untreated wheat straw (a and b) or 72 percent wheat straw treated with alkaline hydrogen peroxide (c; Gould, 1984).

Table 1. Effect of phenolic acids on the cellulolytic activity of ruminal bacteria[a]

Phenolic acid	Concentration (mM)	% of cellulolytic activity retained[b]		
		Bacteroides succinogenes BL2	Ruminococcus flavefaciens 007	Ruminococcus albus SY3
p-Coumaric acid	1	89	96	100
	5	87	71	83
	10	37	39	55
Ferulic acid	1	93	96	97
	5	58	47	84
	10	29	27	81
Vanillic acid	1	100	100	100
	5	90	100	100
	10	89	100	100

[a](Chesson et al., 1982).
[b]The amount of cellulose digested after 7 to 10 days of incubation at 39°C is expressed as a percent of that digested by control cultures without added acid under the same conditions.

Table 2. Effects of ester-linked cinnamic acids on in vitro filter paper cellulose digestion by ruminal microorganisms[a]

Compound	Cellulose digestion (%)
Control	23.9
Cinnamic acid	24.0
p-Coumaric acid	22.7
Caffeic acid	11.7[b]
Ferulic acid	19.1[b]
Sinapic acid	22.4[b]

a(Jung and Sahlu, 1986).
[b]Significantly different from the control ($P \leq 0.05$)

Table 3. Percent in vitro dry matter disappearance of untreated and permanganate-treated neutral detergent fiber fraction of Tall Fescue and Coastal Bermudagrass[a]

Grass	Neutral detergent fiber residue	Permanganate-treated neutral detergent fiber residue
Tall Fescue	63.1	77.0
Coastal Bermudagrass	63.5	79.8

[a](Barton and Akin, 1977).

inner structure. The inability of water to penetrate the microfibril prevents hydration of the internal cellulose polymers of the microfibril, which in turn prevents cellulose hydrolysis by cellulolytic enzymes or microorganisms. As a result, microbes are limited to attacking cellulose polymers on the outer surface of the unhydrated microfibril unit. Haworth et al. (1969) noted that 44% of the cellulose polymers were on the surface of the microfibril unit, which is rectangular in cross-section with eight and ten cellulose polymers along each of two sides. Assuming that this is the correct structure of the cellulose microfibril, 56% of the cellulose polymers in the microfibril would be protected from microbial hydrolysis until the outer layer of cellulose polymers was removed. This could greatly affect rate of cellulose degradation by ruminal microbes. Fan et al. (1980) demonstrated that the degree of crystallinity affected the rate of cotton cellulose hydrolysis by Trichoderma reesei cellulase.

Enzymatic hydrolysis of glucosidic bonds in crystalline cellulose may also be hindered by the restricting influence of hydrogen bonding (Rowland, 1975). Hydrolysis of the glucosidic bonds in solution is reversible. For hydrolysis to occur, the glucosidic linkage must be available for protonation and the chain ends must move apart to implement hydrolysis. Separation of chain ends may be prevented or delayed by the restricting influence of interchain hydrogen bonding. Therefore, the microbes must first disrupt the hydrogen bonds. Decreased hydrogen bonding could be controlling the rate at which fragmentation, swelling, loss in tensile strength, transverse cracking and lowering of the degree of polymerization occurring in cellulose prior to release of glucose and cellobiose by cellulase enzymes (Lee and Fan, 1980). All of these occurrences would be expected if the extent of hydrogen bonding was reduced.

The process of bacterial attachment and hydrolysis of cellulose is further complicated by the manner in which crystalline microfibrils are interconnected with one another. In the past, microfibrils were thought to be interconnected by cell wall matrix components (hemicelluloses and lignin). Therefore, it was hypothesized that the major factor limiting the cellulose microfibril from bacterial attack was encrustation and attachment of lignin and hemicelluloses to the cellulose polymers. While this undoubtedly occurs, Colvin and Sowden (1985) reported that microfibril units in cotton cellulose were interconnected with one another by cellulose polymers, which themselves were arranged in a crystalline structure. If the crystalline arrangement prevents microbial access to cellulose, separation of the microfibrils, which is necessary for extensive microbial attachment, would be limited, slowing the rate of microfibril hydrolysis.

Limited data exist regarding the degree of cellulose crystallinity of lignocellulosics commonly fed to ruminants or the effect of their crystalline arrangement on cellulose degradation by ruminal microbes. Since microfibrils in plant cell walls are known to become more tightly packed and lie more parallel to one another upon maturation and secondary cell wall formation (Northcote, 1972), it would be expected that most crop residues, which are harvested at advanced stages of maturity, are comprised primarily of crystalline cellulose. Therefore, determining the extent of cellulose crystallinity in lignocellulosics and understanding the effect of cellulose crystallinity on bacterial hydrolysis of cellulose might be important in predicting the degree of susceptibility of lignocellulosic materials to microbial attack, providing crystallinity is an important component affecting cell wall breakdown.

Gould (1984) reported that treating wheat straw with dilute, alkaline solutions of hydrogen peroxide greatly increased its water absorption capability. Alkaline hydrogen peroxide treatment also increased susceptibility of wheat straw structural carbohydrates to ruminal microbial degradation (Kerley et al., 1985). These findings were attributed to a decrease in the crystallinity of the cellulose in wheat straw. However, based upon X-ray and neutron diffraction studies, it was concluded that no change in the degree of cellulose crystallinity occurred due to alkaline hydrogen peroxide treatment (Martel and Gould, 1987), indicating that other factors are involved. Therefore, cellulose crystallinity does not appear to greatly deter microbial hydrolysis of cellulose in forages.

Cellulase Enzyme Accessible Space

The surface area of cell wall carbohydrates accessible to ruminal cellulase enzymes could also limit their degradation. The accessible surface area is defined by size, shape and surface properties of microscopic and sub-microscopic capillaries within the fiber in relation to size, shape and diffusibility of microbial cellulase enzyme molecules themselves. Microscopic capillaries include the cell lumina, pit apertures and pit-membrane pores that are visible under the light microscope and range between 20 nm and 10 or more microns in diameter (Cowling, 1975). Sub-microscopic capillaries include spaces between microfibrils and cellulose polymers in the amorphous regions of cellulose. Some sub-microscopic capillaries expand to 20 nm in diameter when fully hydrated, but most are considerably smaller. Total surface area exposed in microscopic capillaries is approximately 2×10^3 cm^2 per g of wood or cotton, whereas total surface ares exposed in sub-microscopic capillaries is approximately 3×10^6 cm^2 per g of wood or cotton (Cowling, 1975). If cellulolytic bacteria could penetrate into the sub-microscopic capillary area, substantially greater rates of cellulose degradation would be expected than if they were prevented from entering this area of the cell wall.

The maximum dimensions of various cellulolytic enzymes studied (Ishikawa et al., 1963) appear to be smaller than microscopic capillaries of both wood and cotton. Only a small fraction of the sub-microscopic capillaries in hydrated wood or cotton, however, are sufficiently large enough to allow penetration of the microbial cellulase enzymes. Stone et al. (1969) showed that the initial rate of cellulose hydrolysis by Trichoderma cellulase was proportional to the surface area accessible to a solute molecule of 4 nm. Ruminal bacteria, ranging from 0.3 - 2.0 m in diameter and 1.0 - 6.0 m in length (Church, 1976), would be greatly limited in their ability to enter the sub-microscopic capillary space in the plant cell wall. Since the cellulase enzyme complex is probably bound to the bacterial cell wall or subcellular membrane vesicles of ruminal microorganisms (Groleau and Forsberg, 1981; Forsberg et al., 1981), the surface area of the sub-microscopic capillaries would be

inaccessible to the cellulase enzyme complex.

Dehority (1961) and Dehority and Johnson (1961) found that physical reduction of forage particle size by ball milling increased the amount of cellulose degraded by ruminal microbes (Table 4). The increase in cellulose digestion may have been due to an increase in the cellulase accessible surface area of the forage due to ball milling. Lin et al. (1985) found that increasing the surface area of cornstalk residue was necessary for effective increases in cellulose digestion. Further research is needed to determine the effects of cellulase accessible surface area on plant cell wall degradation by ruminal microbes.

Table 4. Effect of surface area on in vitro cellulose digestibility by ruminal microorganisms[a]

	Cellulose digested (%)	
Grass	Ground (40 mesh screen)	Ball milled (72 h)
Bromegrass	42.7	75.8
Orchardgrass	53.7	82.3

[a](Dehority and Johnson,1961).

Conclusion

The plant cell wall is a complete entity rather than merely a complex of isolated fractions. Use of techniques such as those involved in the determination of cellulase enzyme accessible space allows the cell wall to be treated as a holistic unit. Chesson (1982) noted that the rate of plant cell wall degradation by microbes was determined more by the nature of the cell walls themselves than by the physicochemical properties of their individual component polymers. Therefore, to identify factors constraining degradation of cell wall structural polysaccharides by ruminal microorganisms, researchers must view the cell wall as a single entity and not as a complex of individual fractions which are studied independently of each other. This is exemplified by the findings of Chesson et al. (1982) which showed that residual fractions of barley straw cell wall remaining after extensive ruminal degradation had a similar cell wall composition as the undigested, original cell wall material. Even though cell wall composition was similar between undigested and digested residues, the residual material could not be further degraded by ruminal microorganisms, indicating that analyses of individual components of the cell wall, aimed at identifying factors limiting structural carbohydrate degradation by ruminal microbes, may not totally encompass the major factors constraining cell wall degradability.

As indicated by Harbers (1985), further research is needed to separate components or fraction of the plant cell wall based on their susceptibility to microbial degradation in the ruminant's gastrointestinal tract, without destroying the infrastructure of the cell wall. These fractions need to be characterized according to their structure and composition, using microscopic and chemical techniques, so that differences in structure and composition among the various fractions might be used to explain differences in their susceptibility to microbial attack in the ruminant's gastrointestinal tract. Once the limitations to microbial degradation of cell wall carbohydrates has been elucidated, plant breeding methods and chemical treatments can be developed to increase utilization of plant carbohydrates by the ruminant.

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Discussion with Reviewers

H.G. Jung: Published data suggest virtually no free phenolic acids exist in plant tissue. How can the study of inhibition of microbial fermentation by free phenolic acids aid our understanding of plant cell wall biodegradability?

Authors: Because of syntrophic relationships in the rumen, it is not illogical to think that free phenolic compounds do exist in the rumen liquor. Also, it is possible that microenvironments around the microbial cells do contain phenolic monomers generated upon plant cell wall hydrolysis. Effects of phenolic monomers in solution around the microorganism on the cells microenvironment is unknown. Another important area of consideration is the effect of phenolic-carbohydrate complexes on microbial activity, as both a toxin and a sterical hindrance to structural polysaccharide hydrolysis.

H.G. Jung: Could the differential results seen for phenolics esterified to different cellulose preparations be an example of crystallinity effects on microbial fermentability?

Authors: It is possible that crystallinity has some affect on selection of bacterial species by a particular microorganism having a competitive advantage in hydrolyzing crystalline cellulose. Our research, as

does others, indicates that cellulose crystallinity of forages is not sufficient to limit cellulose hydrolysis. Therefore, in forage research, while crystallinity may play a minor role in limiting cellulose digestion, the limited susceptibility of structural carbohydrates appears to be a composite of several factors negatively affecting digestion.

D. E. Akin: Is there any direct evidence that cellulose crystallinity varies in forages or that crystallinity affects forage breakdown?
Authors: The hypothesis that crystallinity affected mature forage cellulose hydrolysis was derived from research examining the degradation of wood cellulose, which does appear to be affected by crystallinity. It is our opinion, based on X-ray crystallinity research, that cellulose in forages examined to date does not appear to affect digestion.

D. E. Akin: Do plants vary in size of submicroscopic capillaries?
Authors: It is expected that plants, like wood, have a wide range of submicroscopic capillary pore sizes. Extensive research into the various range of sizes which exist and the effect of pore size on microbial degradation has not been done to our knowledge.

D. E. Akin: Are a, b and c of the same plant region? Figure a and b seem to show cuticle (usually with little attachment) while c is of internal plant tissue. This should be addressed as it will influence attachment.
Authors: Panel a and b of Figure 1 show a broken portion of the external face of a wheat straw particle. The upper-left half of panel a probably is the actual outer surface of the straw, while the lower-right half reveals the inner surfaces of the underlying tissue. Attached cells are apparent in the lower-right area, but are sparsely distributed. Panel b shows the inner surface of an epithelial cell, again with relatively sparse cell attachment. As Dr. Akin has so elegantly shown in many publications, these attachment patterns are typical for relatively indigestible lignocellulosic materials such as straw. Panels a and b merely reiterate the many pictures documenting this fact that are already in the literature. Panel c, on the other hand, shows a typical view of rumen-incubated alkaline peroxide-treated wheat straw. Because the treatment process so completely disrupts the organization of the straw tissue, it is impossible to tell what portion of the original tissue is present in this view (for additional SEM data detailing the effects of alkaline peroxide treatment on wheat straw tissue morphology, see Gould, J. M., Biotechnol. Bioeng. 27, 225-231 (1985). In any event, the panel shown is representative of all samples of rumen-incubated treated straw samples examined. In other words, untreated straw samples were characterized by having regions where the density of attached cells was very low as well as by regions where the density was moderate. In contrast, treated straw was characterized by a uniform, dense coat of attached cells on all surfaces.

L. H. Harbers: How well do the currently accepted methods of fiber analyses (crude fiber, neutral detergent fiber, acid detergent fiber, etc.) aid in explaining the limitations of cell wall degradation?
Authors: It is our belief that neutral detergent fiber and acid detergent fiber offer superior alternatives to fiber analyses of foods and feeds compared to crude fiber. Neutral detergent fiber and acid detergent fiber have obvious merit in non-specific fractionation of food and feed, and for use as adequate predictors of digestibility and intake. However, detailed analyses of components in food and feeds requires more extensive study of the composition and structure of the cell wall than can be achieved by detergent analyses.

L. H. Harbers: Assuming all three factors discussed do limit cell wall degradation, what types of tests should we concern ourselves with in reference to feed formulation in the future?
Authors: Cellulose crystallinity will probably have little influence on ration formulation. Enzyme accessible space could be important in determining digestibility of forage material. The most potential in predicting digestibility and use of forages in ruminant diets lies in understanding intrinsic factors in the cell wall structure which limit structural polysaccharide hydrolysis.

L. H. Harbers: Do each of the limitations to digestion affect monocotyleden (C3 versus C4) and dicotyledon species equally?
Authors: There are obvious differences between species of C3, C4 and legume forages. The differences within a class (i.e. legumes), or even within a variety, can be as great as are expected to occur among classes. It may well be that factors(s) limiting hydrolysis is similar among all forages (particularily C3 and C4), but the repitition or number of these negative factors varies from one class to another. It is also possible that each class and possibly species within a class differ in factor(s) which control digestion, which would make the search for a specific factor controlling digestion of forages by the ruminant virtually impossible.

S. H. Cohen: Figure 1 is an electron micrograph of wheat straw, untreated or treated with alkaline hydrogen peroxide, fed to sheep, and then isolated from the rumens. According to the text the alkaline hydrogen peroxide removed lignin and allowed extensive attachment of rumen organisms and rapid digestion of cell wall carbohydrate. When I examined the figure these effects were not apparent to me. The figures need some indicators pointing them out.
Authors: The effects of alkaline peroxide treatment on the disruption of tissue integrity as a result of cell wall delignification are pretty well obscured in these pictures by the attached bacteria. A better view of the effects of treatment on tissue morphology is given in Gould, J. M., biotechnol. Bioeng. 27, 225-231 (1985).