Phytochemistry, 1978, Vol. 17, pp. 923–931 © Pergamon Press Ltd. Printed in England 0031-9422/78/0501-0923 $02.00/0

MATRIX POLYSACCHARIDES OF OAT COLEOPTILE CELL WALLS

SHUNJI WADA* and PETER M. RAY†

* Department of Biology, Faculty of Science, Tohoku University, Sendai 980, Japan; † Department of Biological Sciences, Stanford University, Stanford, CA 94305, U.S.A.

(*Received* 27 *July* 1977)

Key Word Index—*Avena* coleoptile; Gramineae; cell wall; polysaccharides; hemicellulose; arabinoxylan; glucuronoarabinoxylan; mixed-linked glucan.

Abstract—Separation of component polysaccharides in extractable fractions of the noncellulosic matrix of *Avena sativa* coleoptile cell walls shows that the principal classes of polymers present are glucuronoarabinoxylans (GAX) and iodine-negative hemicellulosic β-glucans. Rhamnogalacturonan is a minor component. GAX contains about 5–10% glucuronic acid and its 4-*O*-methyl ether, arabinose in amount almost equal to xylose, and a small amount of galactose; some subfractions contained appreciable amounts of glucose and galacturonic acid but these may derive from separate, contaminating polysaccharides. From the sedimentation and diffusion coefficients and intrinsic viscosities of one subfraction each of the GAX and of the hemicellulosic glucan that had been purified to apparent homogeneity by criteria of sedimentation and borate electrophoresis, MWs of about 200000 were calculated by two methods. The viscosity characteristics and gel-forming ability of the hemicellulosic glucan give evidence of appreciable molecular interactions which suggest that this polymer is an important structural component of the cell wall.

INTRODUCTION

Because of the importance of the coleoptile of *Avena sativa* in study of auxin-induced cell enlargement and the acknowledged significance of the primary cell wall in this process, considerable data on the monosaccharide composition of oat coleoptile cell walls and the changes in their composition during growth, have been published [1–8]. However, almost no information is yet available regarding the kinds of polysaccharide macromolecules that occur in this material. Here we present findings on this subject obtained by electrophoretic and chemical fractionation of polymers extracted from *Avena* coleoptile cell walls. The polysaccharides that were isolated have been characterized structurally as reported in a companion paper [9].

RESULTS

The extraction procedure and resultant yields of *Avena* coleoptile wall matrix polysaccharide fractions are summarized in Fig. 1. Although dimethylsulfoxide, which was reported to be a mild but effective solvent for hemicelluloses [10, 11], actually extracted very little material (fraction DS), this treatment doubled the yield (fraction OX) of the next extraction step (ammonium oxalate) probably by its swelling action on the cell wall. Much the largest amount of material was obtained in the conventional alkali extraction step; this material was separated into the traditional hemicullulose-A fraction (HA) which becomes insoluble upon neutralization and the hemicellulose-B fraction (HB) which remains in solution. Fig. 2 shows the monomer composition of each of these fractions, and of the water-soluble, alcohol-

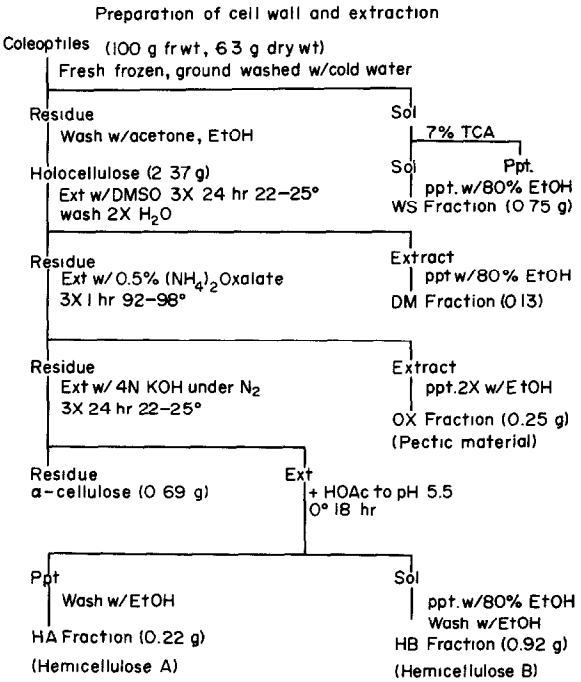

Fig. 1. Procedure for preparation of oat coleoptile cell wall material and polymer fractions. The washed wall material is here called holocellulose because it contains only 3.6% lignin [2], probably located mainly in secondary walls of the xylem elements in the two vascular bundles of the coleoptile. The material was not delignified, which should reduce extraction of matrix components from the small amount of secondary wall material that it contains [44]. Abbreviations: DMSO, dimethyl sulfoxide; EtOH, ethanol; ext, extract; HOAc, acetic acid; ppt., precipitate; sol, soluble; TCA, trichloroacetic acid. Additional details appear in the Experimental.

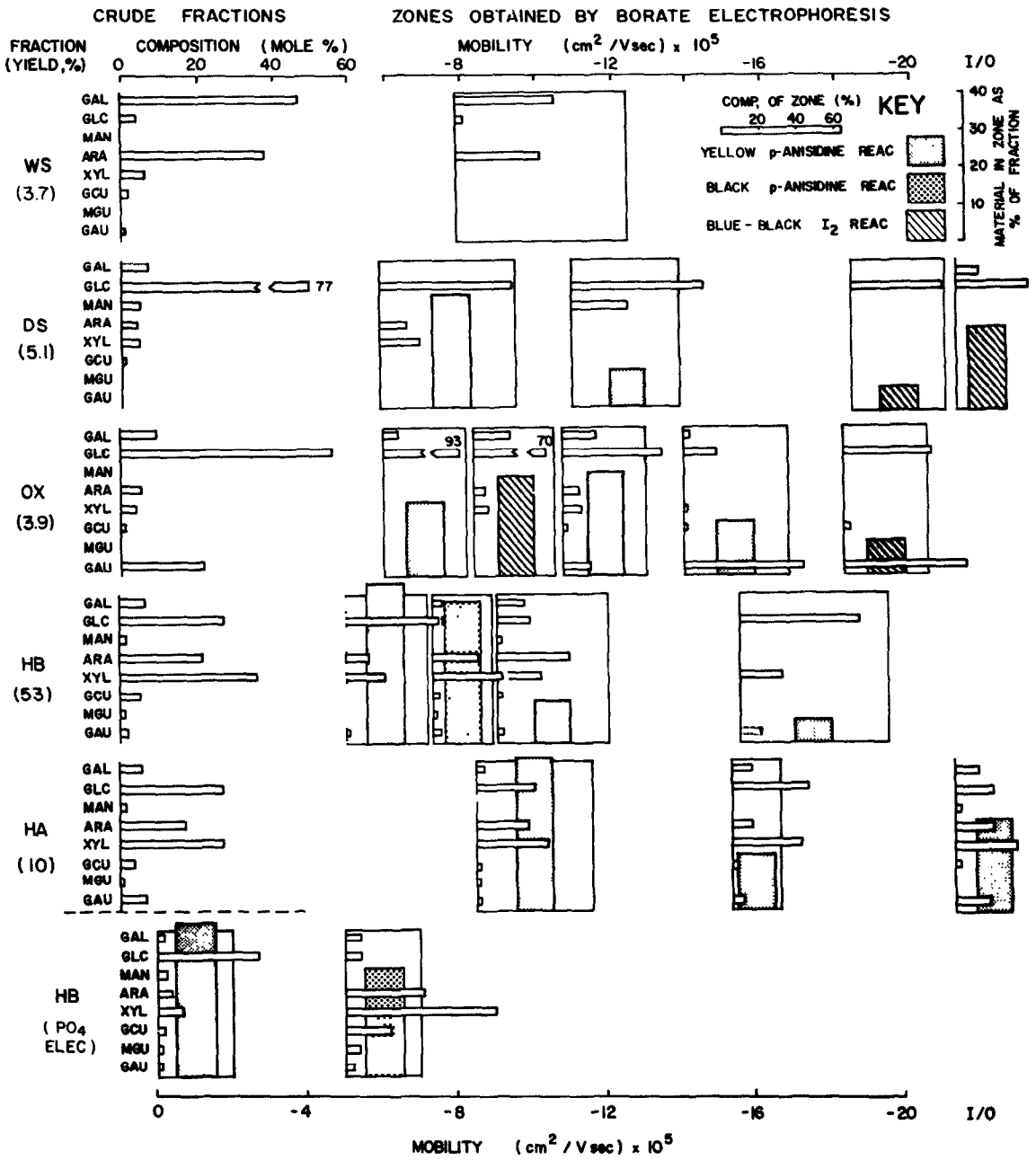

Fig. 2. Yields and composition (left side) of fractions extracted according to the protocol of Fig. 1, and (center and right side) of polysaccharide components separated from these fractions by electrophoresis. The large rectangles represent, on the mobility scale given at top and bottom, the pattern of polysaccharide zones observed on electropherograms by spraying with *p*-anisidine. The horizontal bars within each rectangle show the monomer composition of that zone, and the vertical bars within the rectangles give the relative amounts of material in the different zones as percent of total polysaccharide recovered after electrophoresis (scales given at upper right). Special shading of these bars is used to indicate zones that give a blackish color reaction with *p*-anisidine or that give a positive color reaction to iodine (see key at upper right). The column labeled I/O at right indicates material that did not move from the electropherogram origin despite electroosmotic flow and which is presumably insoluble in the electrophoresis buffer or becomes adsorbed to the glass fiber paper support. Electrophoresis was in borate at pH 9.2, excepting HA (next to last line) which was in borate at pH 11, and the last line which shows electrophoresis of HB in phosphate, pH 7. Conventional abbreviations are used for sugars, and: GCU, glucuronic acid; MGU, 4-*O*-methylglucuronic acid; GAU, galacturonic acid

insoluble polysaccharide material (WS fraction) obtained from the coleoptiles.

Fractionation by electrophoresis

The right-hand part of Fig. 2 shows that each of the fractions except WS could be separated by electrophoresis in the presence of borate into two or more zones and was evidently a mixture of different polysaccharides. The electrophoretic patterns of fractions DS, OX and HB were relatively similar. However, zones that give a blue–black color with I_2, presumably amylose from starch grains that contaminated the cell wall preparation, were found only in the DS and OX fractions. Each of the fractions yielded at least 2 other zones in which glucose was the major or a prominent constituent, but other sugars were often present in these zones. Xylose and arabinose were especially prominent in two of the zones of the HB fraction, accompanied by glucuronic and 4-*O*-methylglucuronic acids, but these components also occurred to some extent in zones of similar mobility from the OX and DS fractions. Fast-migrating zones that contain galacturonic acid as a prominent constituent, and can be called pectic polysaccharides, were found as expected primarily in fraction OX, but HA and HB, which contained minor amounts of galacturonic acid, also yielded such zones.

Electrophoresis of HB in neutral phosphate buffer yielded two major polysaccharide zones which could be separated completely from one another (lowest line in Fig. 2). The zone closest to the neutral marker is composed mainly of glucose with only minor amounts of pentoses and is apparently neutral, but its displacement during electroosmotic flow appears to be retarded, relative to the displacement of the neutral marker, by adsorption to the glass fiber substrate. The second zone clearly migrates toward the anode from the position of the neutral marker, and is composed primarily of pentoses with prominent secondary amounts of glucuronic acid most of which is obtained, after hydrolysis, as glucuronosyl- (and 4-*O*-methylglucuronosyl-) xylose.

Because of insolubility, the hemicellulose-A fraction could be successfully electrophoresed only in a more alkaline borate buffer (pH 11) than afforded the best electrophoretic separations of the other fractions. The slowest-migrating of the resulting zones (see HA, Fig. 2) corresponded roughly in mobility and composition to the single zone that was obtained from hemicellulose-B under these conditions, and may consist of polymers similar to those found in hemicellulose-B.

The complex monomer composition of most of the electrophoretically separable zones obtained from cell wall fractions suggested that many of these might still be mixtures of more than one polymer. This was confirmed by chemical fractionation of the hemicellulose-B fraction as described in the next section.

The water-soluble, ethanol-insoluble polysaccharide obtained from coleoptiles (WS fraction, Fig. 2) differed markedly from any of the wall fractions in consisting largely of galactose and arabinose. It yielded a single electrophoretic zone of essentially the same composition (Fig. 2). This material is probably an arabinogalactan. Because of the prominence of galactose and presence along with it of arabinose in zones of similar electrophoretic mobility from the OX and HB fractions, it seems that a similar arabinogalactan may occur as a minor wall-bound component.

Fractionation of hemicellulose-B

Only the HB fraction afforded enough soluble material to warrant attempting a chemical fractionation. After exploration of several possible fractionation methods, the material was fractionated by precipitation of glucans as their copper complexes, and fractional precipitation of acidic polysaccharides as their cetyl trimethylammonium (CTA) salts, as summarized in Fig. 3 which gives the yield of different components. The composition of these fractions is given in Table 1. The CTA-precipitated products (A components) correspond in composition to the acidic pentosan zone obtained by electrophoresis in phosphate buffer; the products precipitated by Fehling's solution (G components) correspond to the neutral (glucan) zone in phosphate electrophoresis (lowest line in Fig. 2). With other materials Fehling's solution has been used to precipitate xylans (e.g. [12, 13]), which probably fail to form Cu complexes in the present case because of the high degree of substitution by arabinose [9]. Material not precipitated by either Fehling's solution or CTA, designated fraction R, has an intermediate composition probably reflecting a mixture of smaller molecular weight or partially degraded A and G components, but is noteworthy in containing also all of the mannose found in HB; therefore it contains mannan(s) or perhaps glucomannan(s).

After many of the steps in HB fractionation that involved precipitation, significant amounts of previously dissolved material became insoluble in water (see Fig. 3). The largest loss of this kind occurred upon precipitation of crude glucan from the bulk of the nonglucose poly-

Table 1. Composition of hemicellulose-B subfractions

Component*	A1	A2	A3	A4	R	G1	G2	G3
N	—†	1.0	0.6	—†	0.5	0.25	0.4	1.2
Glc	2	2	1	27	26	94	96	52
Man	—	—	—	—	10	tr	tr	1
Gal	6	6	6	3	8	tr	tr	6
Ara	35	31	34	24	25	2	1	10
Xyl	49	52	53	40	29	4	3	28
GlU	6	5	5	5	1	—	—	}
MGU	2	2	2	1	1	—	—	} 4‡
GaU	1	1	tr	tr	—	—	—	}

Sugars and uronic acids are given in percent of total residues, rounded to the nearest integer; N is in weight %; tr = trace detected by qualitative chromatography. * Abbreviations as listed for Fig. 3. † Not determined. ‡ Titrable acidic groups, as uronic acid.

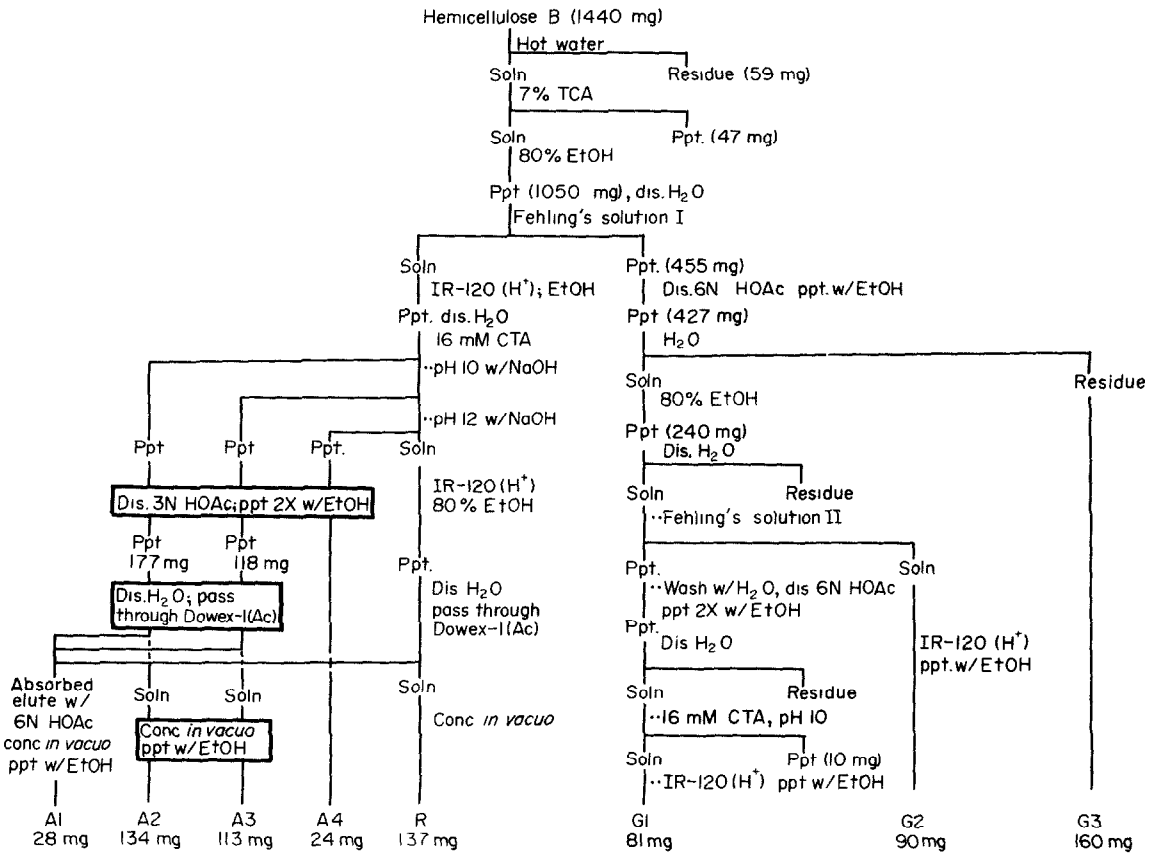

Fig. 3. Fractionation of hemicellulose-B (HB fraction of Fig. 1). Abbeviations as in Fig. 1, and: CTA, cetyl trimethylammonium bromide; conc *in vacuo*, concentrate by vacuum evaporation; dis, dissolve in; IR-120, Amberlite cation exchange resin. Details, including composition of the 2 Fehling's solns, are given in the Experimental.

saccharides (fraction G3, Fig. 3). The insoluble material consisted mainly of glucose, but also contained pentoses and uronic acids (Table 1). The appearance of G3 and similarly insoluble materials at other steps in the fractionation indicates a tendency, illustrated also by the polymers of the HA fraction, for hemicellulosic glucans and pentosans to associate rather strongly to yield water-insoluble complexes.

Physical characterization of hemicellulose-B components

The electrophoretic behaviour of HB components in borate buffer is shown in Fig. 4. Comparison with the pattern obtained from crude HB indicates that components G2 and R contribute to the zone of lowest mobility; A2, A3 and A4 comprise the zone of intermediate mobility that gives a blackish color with the *p*-anisidine reagent; G1 and A1 comprise the zone of still higher electrophoretic mobility (about 6×10^{-5} cm^2/V sec) that usually overlaps the blackish zone. Fractions A1, A4 and G2 each exhibit a secondary component of much higher electrophoretic mobility (about 15×10^{-5} cm^2/V sec) resembling that of a minor zone obtained from crude hemicellulose-B.

Fig. 5 shows the sedimentation behavior of several of these fractions in the ultracentrifuge. A2 showed two components: A1 and G2 each gave a broad, slowly sedimenting boundary indicative of polydisperse, relatively low molecular weight material. A3 and G1, however, each gave a single sharp sedimentation boundary, with a hint of the presence of a minor amount of faster sedimenting material in A3.

Therefore, although most of the chemically separated

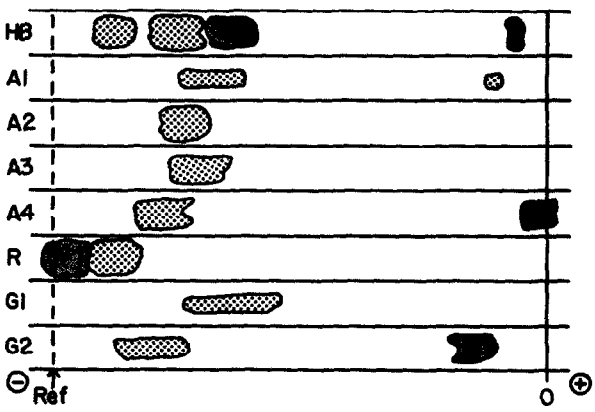

Fig. 4. Borate electropherograms of hemicellulose-B (top line) and of component fractions isolated from it by the procedure of Fig. 3. Cathode to left, anode to right; line at right is point of application of samples. Arrow shows position of the marker for electroosmotic flow, α-methylglucoside. Major component(s) marked by coarse stippling, minor components by fine stippling.

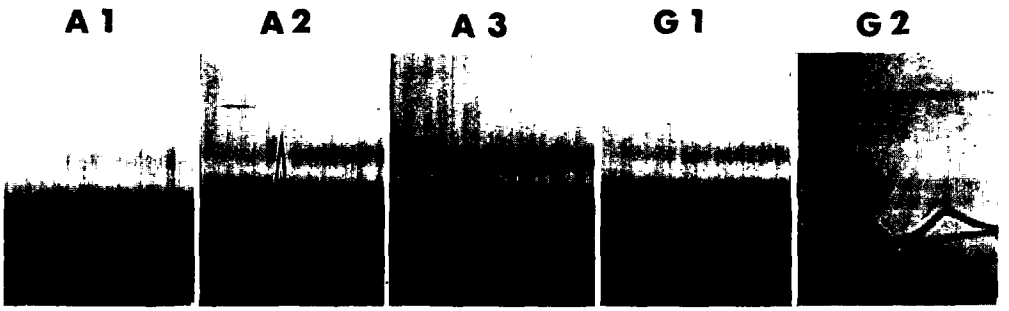

Fig. 5. Sedimentation of hemicellulose B components isolated by the procedure of Fig. 3. The solutions were 1% except G1 and G2 which were 0.5%. Photographs represent the following times of sedimentation at 59780 rpm and 20°, A1, 160 min; A2, 244 min; A3, 256 min; G1, 128 min; G2, 180 min. Bar angle 65°. Baseline, where present, is solvent only (phosphate buffer) in second sector of a double sector cell.

subfractions of HB are heterogeneous by either or both the criteria of electrophoresis and/or sedimentation, glucan G1 and glucuronoarabinoxylan A3 appeared to be nearly homogeneous by these criteria and so were selected for further characterization.

Fig. 6 (left side) shows that the sedimentation coefficients of G1 and A3 depended relatively weakly and linearly upon concentration, permitting values for S^0 to be obtained by extrapolation to zero concentration. These values, combined with the diffusion coefficients that were measured for these polysaccharides, lead to estimated MWs of about 200000 for both products (Table 2).

Viscosity of solutions of G1 and A3 as a function of concentration is shown in Fig. 6 (right side). For A3 the relation was simple but in the case of G1 viscosity increased more and more steeply with concentration at higher concentrations, indicating increasingly strong interaction between molecules. This property is manifest also in the fact that solutions of G1 at concentrations of 0.5% or above will set to a gel when cooled to 4°.

The intrinsic viscosities of these polymers (Table 2) in combination with S^0 from sedimentation measurements again gave molecular weights in the 200000 range (Table 2).

The MWs calculated for glucan G1 and GAX fraction A3 correspond to DPs of about 1500 and 1700, respectively. These values are high compared with DPs previously estimated for hemicellulosic polysaccharides of monocots [13–16]. The previous determinations were on polysaccharides from older tissues, and evidence has been presented that the molecular weight of hemicellulosic glucan decreases with age of the tissue [15, 16]. The relatively low DP values in the literature quoted were obtained by end group determination on hemicelluloses rather than from their physical characteristics as in our estimates, which could err because of molecular aggregation especially in view of the evidence for interaction between molecules of the glucan in dilute solution. Although the calculation of molecular weights from S^0 and $[\eta]$ (Table 2) employs parameters estimated for infinite dilution (Fig. 6), which should minimize the effects of any tendencies toward association, we cannot exclude the possible occurrence of irreversible aggregation. But, considering the lack of extensive polydispersity indicated by sedimentation of G1 and A3 (Fig. 5) it appears that if these polymers were aggregated the aggregates would have been remarkably uniform in size. Data on other monocot hemicelluloses have indicated a comparatively high degree of polydispersity [14, 17]. In

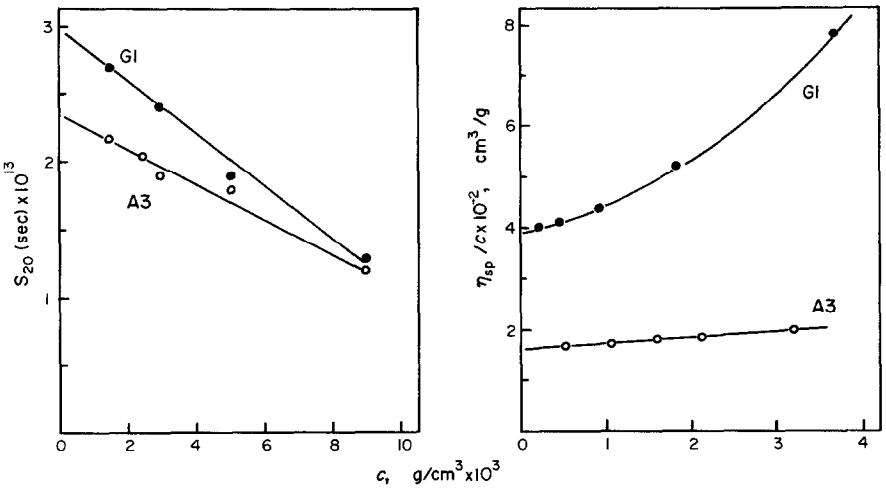

Fig. 6. (left) Sedimentation coefficient, and (right), specific viscosity, of hemicellulose-B fractions A3 and G1 as a function of concentration (c). Ordinate of right hand side is specific viscosity (increase in viscosity caused by solute as a fraction of the viscosity of the solvent) divided by the concentration (c) at which viscosity was measured.

Table 2. Physical constants of *Avena* coleoptile polysaccharides

Parameter	Units	Glucurono-arabinoxylan† A3	β-glucan† G1
Specific rotation	$[\alpha]_D$ deg/cm × 10	−85.2	−7.0
Sedimentation coefficient	$S^0_{20,w}$ sec × 10^{13}	2.31	2.97
Diffusion coefficient	D cm²/sec × 10^7	1.89	0.93
Intrinsic viscosity*	$[\eta]$ cm³/g × 10^2	1.60	3.88
Partial specific volume	$\bar{V}$ cm³/g	0.827	0.673
Apparent MW	from S^0 and D	180000	240000
	from S^0 and $[\eta]$	220000	260000

The parameters were all measured at 20°. * Viscosity of the solvent (phosphate buffer) was 0.950 centipoises. † See Figs 3–5.

view of the results of electrophoretic and chemical fractionation given above, the relative homogeneity indicated for the polysaccharides A3 and G1 is probably due at least in part to selective isolation, in the fractionation procedure, of particular components from a polydiverse and polydisperse mixture of matrix polysaccharides, as in previous work on older tissues of oat plants [18, 19].

The specific optical rotations found for G1 and A3 (Table 2) are similar to those reported for analogous hemicellulosic polysaccharides in the literature. The slightly negative specific rotation of G1 and the strongly negative rotation of A3 indicate that the backbones of both polymers contain mainly β linkages [20, 21].

DISCUSSION

The results show that the matrix of *Avena* coleoptile cell walls consists primarily of two kinds of polysaccharides, neutral iodine-negative β-glucan and acidic glucuronoarabinoxylan (GAX). The amounts of these that were actually isolated from the hemicellulose-B fraction plus the amounts that can be estimated from data given here and in [9] to occur in other fractions add up to at least 65% of the wall matrix (noncellulosic material). Both the glucan and the GAX are to some degree heterogeneous and are separable into components having somewhat different properties. Structural studies [9] show that the hemicellulosic β-glucans of the G series from wall hemicellulose B are mixed-linked (1,3- and 1,4-) glucans.

Several kinds of evidence show that the glucuronic (and 4-*O*-methylglucuronic) acid, xylose and arabinose of the GAX, and probably also the galactose that is associated with it, are joined into a heteropolysaccharide molecule: (a) sedimentation and electrophoretic homogeneity of the A3 components, which contains all these constituents; (b) migration of xylose, arabinose and some galactose along with glucuronic acid in the GAX zone during electrophoresis in phosphate buffer, in which neutral polysaccharides should not migrate; (c) release, by hydrolysis, of most of the glucuronic and 4-*O*-methylglucuronic acid as glucuronosyl-xylose and 4-*O*-methylglucuronosyl-xylose; and (d) occurrence, as non-reducing terminal groups, of most of the arabinose and all of the galactose that are associated with the GAX [9].

While GAXs similar to those reported here for *Avena* coleoptiles have been obtained from wall material of many monocots including mature *Avena* stem [13, 14, 22, and references there cited], the best-known mixed-linked β-glucans, those of barley and oat kernels [20], are water-soluble materials that are not necessarily structural wall components. Although mixed-linked glucan has been found in vegetative tissues of various monocots [12, 15, 23, and references there cited], it has been postulated that this material is a storage product, not a structural wall component [22, 24, 25]. Smith and Stone [26], however, have extracted a mixed-linked glucan from wall material of endosperm cell cultures of the grass *Lolium*, and evidence for a similar product in purified cell walls of maize coleoptiles has been given [27].

We consider that the hemicellulosic β-glucan of *Avena* coleoptiles must be a structural wall component. It remains insoluble after all iodine-positive glucan (starch) has been removed by extractions with DMSO and ammonium oxalate, and the starch grains thereby have presumably been disrupted. Its physical properties described above, and its quantitative conspicuousness in this material, also suggest a structural role.

It might be suggested that some of the 'hemicellulosic' glucan, particularly that which becomes insoluble (G3 component) during fraction of HB, could actually represent cellulose which, unlike typical α-cellulose, happens to be solubilizable by alkali. This is not tenable because the material remains soluble in neutral aqueous media through several steps of the procedure, and because evidence shows that it contains 1,3 as well as 1,4 linkages [9]. Similar conclusions were reached regarding other monocot alkali-soluble β-glucans, including that of mature oat plants [15, 28].

Only one polysaccharide component, a minor zone of high mobility (17×10^{-5} cm²/V sec) in borate electrophoresis of hemicellulose-B (Fig. 2), was found that consisted of glucose and xylose without arabinose, and which might be analogous to the xyloglucans which are important components of dicot primary walls [24]. The ratio of xylose to glucose in this material, however, was much lower than in dicot xyloglucans that have been characterized [24]. The zone also contained a small amount of galacturonic acid (Fig. 2), which could have comprised a separate, admixed polyuronide. Although there is no proof that the glucose and xylose in this zone are linked to one another, structural information [9] shows that at least some of the xylose present in the G3 subfraction obtained by chemical fractionation of hemicellulose-B is probably linked to glucose in the same manner as in dicot xyloglucans. Therefore a minor number of xyloglucan-like polysaccharide chains seem

to be present in the *Avena* coleoptile wall, but the results agree with evidence on other monocot walls [22, 23, 29] indicating that xyloglucan is not a prominent component.

Polygalacturonic acid is also a very minor component of *Avena* coleoptile cell walls as compared with primary walls of dicots. But the small amount of polygalacturonic material that is present in *Avena* coleoptile wall resembles at least to some extent that of dicot walls since it contains covalently bound rhamnose as shown by the isolation of galacturonosyl-rhamnose from it [3].

The electrophoretically separated zones that contained galacturonic acid as a prominent constituent did not contain substantial amounts of the other sugars that often occur attached to dicot galacturonans [30]. Although galacturonic acid was detected in certain GAX subfractions (A1, A2) of hemicellulose-B, these subfractions contained more than one polymer component and it is possible that the galacturonic acid represents a contaminating pectic polymer (probable, in the case of A1, which contained a fast-migrating component electrophoretically similar to the pectic components of the OX fraction).

The overall impression that has emerged from recent studies [12–29], as well as the present one, is that the matrix material of monocot and dicot primary walls is rather different. Dicots lack the GAX and mixed-linked glucan that are major components in monocots, possessing instead xyloglucan, rhamnogalacturonan, arabinogalactan and often hydroxyproline-rich polypeptides as important components, none of which figures prominently in the monocot primary wall. Arabinogalactan occurs mainly as a soluble polysaccharide in *Avena* coleoptiles. This material may well occur within the cell wall but in unbound form, so that it becomes leached into the soluble fraction during tissue homogenization and washing of the cell walls.

These preceding generalizations regarding monocot versus dicot cell walls may be qualified by certain facts and observations. Glucuronoxylans, structurally similar to the GAX of monocot walls except for the virtual absence of arabinose, are important components of dicot secondary walls [21]. A water-soluble mixed-linked β-glucan has been reported to occur transiently in a dicot tissue [31]. Very young dicot walls were found to give the staining reaction for callose [32, 33] a test usually regarded as indicative of β-1,3-glucan but which conceivably might be given also by mixed-linked glucans. On the other hand the walls of certain cultured monocot tissues have been reported to lack mixed-linked glucans [22, 29]. This may have been a procedural artefact, for reasons given in the companion paper [9].

The question arises whether the glucan, the GAX and the minor rhamnogalacturonan components of monocot primary walls are covalently attached to one another as has been proposed for the matrix polysaccharides of dicot primary walls [24]. The present evidence shows that if any such interconnections are present they must be labile to relatively gentle extraction treatments that do not degrade the major polysaccharides into small fragments, and to which most ordinary glycosidic bonds are stable. This suggests that the different kinds of monocot matrix polysaccharides are not attached one to another as side chains by glycosidic bonds. Alkali-labile bonds such as ester linkages could, however, conceivably cross link some of the polysaccharides; such linkages could involve either uronic acids, or other organic acids [17].

Since by pectinase treatment it is possible to remove all of the rhamnogalacturonan from *Avena* coleoptile walls while leaving much of the GAX and matrix glucan still in place [3], the latter evidently do not depend upon linkages to galacturonan for their structural attachment to the wall. The tendency of hemicellulose-A and -B components to associate with one another to form gels and water-insoluble complexes, which became apparent during manipulation of the matrix polysaccharides as described above, suggests that the noncovalent bonding capabilities of these matrix polysaccharides may suffice to create a stable structural material without need of covalent cross-linking. This view of polymer organization has been held for cell walls [34] including those of a dicot [35].

Regarding the functional roles of GAX and hemicellulosic glucan in the wall it may be significant that much of the hemicellulosic glucan became insoluble after it was separated from the glucurono-arabinoxylan, and as noted in Results even the glucan that remained soluble, but not the GAX, showed evidence of strong tendencies toward intermolecular association. Similarly, purification of a mixed-linked glucan from soluble hemicelluloses of oat leaves [23] and bamboo [12] led to an insoluble product. This suggests that the matrix glucan may represent the principal structural component that binds the microfibrillar phase of the wall together, while the GAX may serve to weaken the forces of interaction sufficiently that the wall can act as a plastic structure during growth. This is consistent with the finding that breakdown [2, 5–7] and decrease in molecular weight [15, 16] of hemicellulosic glucan can occur during development, a process which could serve to further weaken the wall to the point of actual yield. In growing coleoptile tissue this breakdown has been observed, however, only under sugar starvation* and it could be that during normal, sugar-sufficient growth a different process such as insertion of new GAX actually controls the occurrence of wall yielding. McNeil *et al.* [29], however, suggest the opposite view that in barley aleurone cells arabinoxylan serves as the principal bonding material between cellulose microfibrils.

EXPERIMENTAL

Plant material. Oat seeds, *Avena sativa* L. cv Victory, were germinated for 3–3.5 days at 25° on moist filter paper, in the dark except for exposure to dim red light during the first day. Whole coleoptiles, after removal of the primary leaf, were frozen, ground in ice-cold H_2O in a mortar, centrifuged for 15 min at 1500 *g*, and the pptd cell wall material was washed twice with H_2O, 95% EtOH and Me_2CO, and vacuum dried. The supernatant and H_2O washings of the cell wall material were concd by vacuum evapn and suspended in EtOH (80% final concn). Pptd material was resuspended in H_2O, centrifuged and the supernatant was made 7% in TCA. Pptd protein was removed by centrifugation and the supernatant was made 80%

* An apparent exception is the report of Reid and Wilkie [18] who found a decrease in mole percent of glucose with age in hemicellulose of various organs of field and laboratory grown oat plants, including coleoptiles. This does not demonstrate an actual decrease in the amount of hemicellulosic glucan, as has been suggested [28], because the total amount of hemicellulosic material was probably increasing with age.

in EtOH. Pptd material comprised the H_2O soluble polysaccharide (WS) fraction.

Extraction of cell wall material. The procedure is summarized in Fig. 1. Dimethylsulfoxide (3 extractions for 24 hr each at room temp.) itself extracted but little material (DS fraction), but this treatment doubled the yield of material in the subsequent ammonium oxalate extraction (OX fraction). Of the material recovered from the dimethylsulfoxide extract by EtOH pptn, 28% was insoluble in hot H_2O and consisted of iodine-positive glucan; the water-soluble remainder was used for electrophoretic characterization of the DS fraction. From the alkali extract, material that pptd at 0° for *ca* 18 hr after neutralization at 0° with HOAc to pH 5.5, comprised the HA fraction (hemicellulose-A); material that remained soluble constitutes HB (hemicellulose-B). The residue from alkali extraction (α-cellulose) consisted as expected almost entirely of glucose.

Electrophoresis. Polysaccharides in the cell wall fractions were separated by borate electrophoresis [36, 37]. The dry test samples were first dissolved in 0.4 N NaOH at a concn of 1–2%, an equal vol. of 0.8 M boric acid was added to bring the pH to 9.2, and the sample was applied to a line near the anodal end of a 7 × 53 cm strip of glass fiber paper soaked in 0.1 M Na tetraborate, usually of pH 9.2. α-Methyl-D-glucoside, applied at the starting line to one side of the test samples, usually served as a reference spot for detection of electroosmotic flow [37]. Electrophoresis was performed normally at 800 V (16 V/cm) for 3–5 hr in a sandwich-type H_2O-cooled electrophoresis apparatus [38] (E-C apparatus Co., Swarthmore, PA). After electrophoresis the paper was dried and sprayed (or a strip cut from the edge of it was sprayed) with 1% *p*-anisidine/2% H_2SO_4 in H_2O-satd *n*-BuOH, then heated at 100–120° for 30–60 min to detect polysaccharides [39]. To determine composition, the unsprayed portion of each zone was eluted from the paper with H_2O, passed through Amberlite IR-120 (H^+), vacuum evapd 3 times from MeOH to remove boric acid, and hydrolyzed for analysis as described below. The mobility of α-methylglucoside in borate relative to that of 2,3,4,6-tetramethylgucose, which has zero mobility [37], was determined separately to be -4.36×10^{-5} cm^2/V sec; the mobilities of polysaccharides as reported were corrected by this value when measured in borate relative to α-methylglucoside as reference compound. Negative values represent displacement toward the anode relative to the position to which nonmigrating substances would be carried by electroosmotic flow, which occurred toward the cathode at about 8 cm/hr. Electrophoresis was performed similarly using 0.1 M K phosphate buffer, pH 7, as medium. To determine electroosmotic flow α-methylglucoside was assumed to have zero electrophoretic mobility in this medium.

Fractionation of hemicellulose-B. Prior to the fractionation (Fig. 3) the aq. sample of crude HB was treated with 7% TCA and after removal of a small amount of insoluble material was repptd with EtOH and redissolved in H_2O for treatment with a modified Fehling's soln (Fehling's soln I: 0.2 M $CuSO_4$ 0.33 M Na K tartrate, 0.9 M NaOH; 7 ml added to 100 ml of 1% soln of HB) to ppt. copper complexes of glucans. The gelatinous blue ppt. was twice dissolved in 6 N HOAc and repptd with EtOH, then suspended in H_2O and centrifuged to remove material that had become insoluble (G3 fraction), the supernatant then being fractionated further using Fehling's soln II (0.14 M $CuSO_4$, 0.61 M Na K tartrate, 1.75 M NaOH; 3.4 ml added to 40 ml of 0.5% soln of polysaccharides). The redissolved ppt. from this step was treated with 16 mM cetyl trimethylammonium bromide at pH 10 and this supernatant, as well as that from the second pptn with Fehling's soln was deionized with Amberlite IR-120 (H^+) before repptn with EtOH (yielding fractions G1 and G2, respectively). After every pptn step in this procedure some additional hemicellulosic material became water-insoluble like the G3 fraction and was removed by centrifugation. The supernatant from the first Fehling's soln pptn, which contained predominantly glucuronoarabinoxylans, was deionized with IR-120 (H^+), concd by vacuum evapn, repptd twice with EtOH, then dissolved in H_2O and made 16 mM in cetyl trimethylammonium bromide, yielding a ppt and a supernatant which was fractionated further by raising the pH successively to 10 and 12 with NaOH and removing material that pptd. The first two ppts (dissolved in 3 N HOAc) and the final supernatant were passed slowly through Dowex-1 (acetate); the adsorbed material was eluted with dil HOAc, combined, concd by vacuum evapn and pptd with EtOH (A1 fraction). The unadsorbed materials were pptd with EtOH to yield fractions A2, A3, A4 and R (see Fig. 3).

Determination of monosaccharide composition. Samples were dissolved in 0.2 ml of cold 24 N H_2SO_4, diluted to 1.14 N H_2SO_4 with H_2O, heated at 100° for 6–10 hr, and passed through sufficient Dowex-1 (acetate) to remove all the H_2SO_4, yielding the neutral sugar fraction. Sugar acids were eluted from the Dowex-1 with 6 N HOAc. Aliquots of the neutral and acidic fractions were chromatographed on Whatman No. 1 paper as described [40], and examined for qualitative composition by spraying with aniline phthalate. For quantitative composition the zones corresponding to known carbohydrates (except rhamnose, a minor component which was not determined) were eluted with H_2O. Sugars were determined using phenol–H_2SO_4 [41], and uronic acids, which were obtained both as free uronic acids and as aldobiouronic acids [3], were determined by the carbazole method [42]. The molar amount of xylose contained in aldobiouronic acids, taken to equal the amount of uronic acid found in these components, was added to the amount of free xylose to give the total yield of xylose. Yields of free and combined uronic acid were added to determine the amount of each uronic acid.

Physical measurements. Sedimentation and diffusion of polysaccharides dissolved in 0.1 M K phosphate buffer, pH 7, were measured at 20° in a Beckman Model E ultracentrifuge, using the AN-D rotor and a sector cell centrifuged at 59780 rpm for sedimentation measurements (AN-E rotor, 50740 rpm and 30 mm deep cells for product concns below 0.5%), a synthetic boundary cell at 10000 rpm for diffusion measurements, and schlieren optics. Sedimentation coefficient (S) was determined by plotting $\log x_m$ vs t [43]. Diffusion coefficient was determined from the ratio of peak height to area [44] at 4 or more times from 20 to 120 min after formation of the boundary. The resulting D' values fell on a straight line when plotted against $1/t$; the D' value from extrapolation of this line to $1/t = 0$ was taken as the diffusion coefficient, D [44]. Partial specific volumes ($\bar{V}$) of polysaccharides as 0.6–0.8% solns in the above-mentioned buffer, and its density (ρ), were determined using a 5 ml pycnometer. Viscosities of polysaccharide solutions (η_c) and solvent (η_0) were measured with an Ostwald-type viscosimeter at 20°, using the phosphate buffer mentioned above as solvent. Intrinsic viscosity or limiting viscosity number, $[\eta]$, was obtained by extrapolating plots of $(\eta_c/\eta_0 - 1)/c$ vs polymer concn (c) to $c = 0$ (see text and [44]). MWs (Table 2) were calculated from S^0 and D using the Svedberg equation [43, 44], $M = (RTS_0)/(D[1 - \bar{V}\rho])$, and from S^0 and $[\eta]$ using the equation of Mandelkern *et al.* [45] for random-chain polymers, $M = [\eta]^{1/2} \cdot (4 \times 10^{-7} S^0 \eta_0 N/[1 - \bar{V}\rho])^{3/2}$. Specific optical rotation was determined for 0.6–0.7% solns of polysaccharides in the phosphate buffer mentioned above, using a standard polarimeter with 10 cm path length and sodium D light.

Acknowledgements—This work was supported by grants to PMR from the National Science Foundation and from the Institute of Science and Technology of the University of Michigan, where the isolation of polysaccharides described here was carried out.

REFERENCES

1. Bishop, C. T., Bayley, S. T. and Setterfield, G. (1958) *Plant Physiol.* **33**, 283.
2. Ray, P. M. (1963) *Biochem. J.* **89**, 144.
3. Ray, P. M. and Rottenberg, D. A. (1964) *Biochem. J.* **90**, 646.
4. Baker, D. B. and Ray, P. M. (1965) *Plant Physiol.* **40**, 353.

5. Katz, M. and Ordin, L. (1967) *Biochim. Biophys. Acta* **141**, 118.
6. Wada, S., Tanimoto, E. and Masuda, Y. (1968) *Plant Cell Physiol.* **9**, 369.
7. Loescher, W. and Nevins, D. J. (1972) *Plant Physiol.* **50**, 556.
8. Loescher, W. H. and Nevins, D. J. (1973) *Plant Physiol.* **52**, 248.
9. Labavitch, J. M. and Ray, P. M. (1968) *Phytochemistry* **17**, MS 409.
10. Hägglund, E., Lindberg, B. and McPherson, J. (1956) *Acta Chem. Scand.* **10**, 1160.
11. Maekawa, E. (1974) *J. Agric. Chem. Soc. Japan* **48**, 75.
12. Wilkie, K. C. B. and Woo, S.-L. (1976) *Carbohyd. Res.* **49**, 399.
13. Joseleau, J.-P. and Barnoud, F. (1974) *Phytochemistry* **13**, 1155.
14. Buchala, A. J., Fraser, C. G. and Wilkie, K. C. B. (1972) *Phytochemistry* **11**, 2803.
15. Buchala, A. J. and Wilkie, K. C. B. (1973) *Phytochemistry* **12**, 499.
16. Buchala, A. J. and Wilkie, K. C. B. (1974) *Phytochemistry* **13**, 1347.
17. Mares, D. J. and Stone, B. A. (1973) *Australian J. Biol. Sci.* **26**, 813.
18. Reid, J. S. G. and Wilkie, K. C. B. (1969) *Phytochemistry* **8**, 2059.
19. Fraser, C. G. and Wilkie, K. C. B. (1971) *Phytochemistry* **10**, 1539.
20. Clarke, A. E. and Stone, B. A. (1963) *Rev. Pure Appl. Chem.* **13**, 134.
21. Timell, T. E. (1964) *Adv. Carbohyd. Chem.* **19**, 247.
22. Burke, D., Kaufman, P., McNeil, M. and Albersheim, P. (1974) *Plant Physiol.* **54**, 109.
23. Fraser, C. G. and Wilkie, K. C. (1971) *Phytochemistry* **10**, 199.
24. Albersheim, P. (1976) in *Plant Biochemistry* (Bonner, J. and Varner, J. E. eds) 2nd edn, p. 225.
25. Buchala, A. J. and Wilkie, K. C. B. (1970) *Naturwissenschaften* **10**, 496.
26. Smith, M. M. and Stone, B. A. (1973) *Phytochemistry* **12**, 1361.
27. Kivilaan, A., Bandurski, R. S. and Schultze, A. (1971) *Plant Physiol.* **48**, 389.
28. Buchala, A. J. and Wilkie, K. C. B. (1971) *Phytochemistry* **10**, 2287.
29. McNeil, M., Albersheim, P., Taiz, L. and Jones, R. L. (1975) *Plant Physiol.* **55**, 64.
30. Aspinall, G. O. (1973) in *Biogenesis of Plant Cell Wall Polysaccharides* (Loewus, F. ed.) p. 95. Academic Press, New York.
31. Buchala, A. J. and Franz, G. (1974) *Phytochemistry* **13**, 1887.
32. Fulcher, R. G., McCully, M. E., Setterfield, G. and Sutherland, J. (1976) *Can. J. Botany* **54**, 539.
33. Waterkeyn, L. (1967) *Compt. Rend. Acad. Sci. (Paris)* **265**, 1792.
34. Rees, D. A. (1972) *Biochem. J.* **126**, 257.
35. Monro, J. A., Penny, D. and Bailey, R. W. (1976) *Phytochemistry* **15**, 1193.
36. Briggs, D. R., Garner, E. F. and Smith, F. (1956) *Nature* **178**, 154.
37. Foster, A. B., Newton-Hearn, P. A. and Stacey, M. (1956) *J. Chem. Soc.* 30.
38. Foster, A. B. (1952) *Chem. Ind. (London)* 1050.
39. Northcote, D. H. (1954) *Biochem. J.* **58**, 353.
40. Ray, P. M. and Baker, D. B. (1965) *Plant Physiol.* **40**, 353.
41. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956) *Anal. Chem.* **28**, 350.
42. Bitter, T. and Ewins, R. (1961) *Biochem. J.* **81**, 43P.
43. Schachman, H. K. (1959) *Ultracentrifugation in Biochemistry*. Academic Press, New York.
44. O'Donnel, I. J. and Woods, E. F. (1962) *Modern Meth. Plant Anal.* **5**, 250.
45. Mandelkern, L., Krigbaum, W. R., Scheraga, H. A. and Flory, P. J. (1952) *J. Chem. Phys.* **20**, 212.