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CHANGES IN CELL WALL POLYSACCHARIDES OF GERMINATING BARLEY GRAINS

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Key Word Index—*Hordeum distochon*; barley; cell walls; polysaccharides; gums; aleurone layer; starchy endosperm; embryo.

Abstract—Decorticated barley grains were germinated at 25° for 6 days, until the endosperm reserves were nearly exhausted. The neutral monosaccharide components of the hydrolysates of the cell walls and gums from the embryo, aleurone layer and starchy endosperm and the endospermic starch were determined at daily intervals. The amount of embryo cell wall polysaccharide increased 40 times and glucose became the major component, followed in abundance by xylose and arabinose. The cell wall and gum polysaccharides of the aleurone layer (plus testa) and the starchy endosperm declined during germination and their compositions altered. The endospermic starch also decreased. In the early stages of germination the apparent composition of the cell walls of the aleurone layer and starchy endosperm depended upon how they had been prepared. After 6 days the cell walls and gums had provided a significant carbohydrate supply to the living tissues, equivalent to 18.5% of the endospermic polysaccharide degraded during growth, starch having provided the remaining 81.5%.

INTRODUCTION

During germination the scutella and the aleurone layers of barley grains release enzymes which, together with enzymes already present, hydrolyse the reserves of the starchy endosperm. The products of hydrolysis, together with those released by the aleurone layer, are used by the growing embryos [1–3]. The thick cell walls of the aleurone layer are slowly degraded whereas the starchy endosperm cell walls are rapidly dissolved by a 'wave' of hydrolysis passing from the base to the apex of the grain [4–7]. In contrast, during germination new cell walls are synthesized in the embryo.

The cell walls of the starchy endosperm are *ca* 2 μm thick and consist of an amorphous ground substance (*ca* 75% β-glucan, 25% arbinoxylan) associated with a microfibrillar component of 'holocellulose' containing glucose and mannose [8]. The starchy endosperm is rich in 'gums', soluble in water, which appear to have been associated with the cell walls. The β-glucans consist of chains of β(1 → 4) and β(1 → 3) linked D-glucopyranosyl units which are believed to be mainly cellotriosyl and cellotetraosyl units mainly linked by single β(1 → 3) linkages but with a few longer runs of glucose residues linked by consecutive β(1 → 3) linkages [9]. Initially the β-glucans in the walls have high MWs and are cross-linked through peptides. These peptides are cleaved early in germination, liberating more soluble β-glucans of lower MWs [10]. The arabinoxylans are chains of β(1 → 4) D-xylopyranosyl residues, variously substituted at 2′ and 3′ positions with L-arabinofuranosyl residues to give a highly branched polymer [11, 12]. Other minor polysaccharides containing glucose, pentoses, galactose and mannose are also found in the walls and gums [8]. The cell walls, during germination, separate into lamellae and lose their ability to stain with Congo Red. An outer non-staining lamella is more slowly degraded [13, 14].

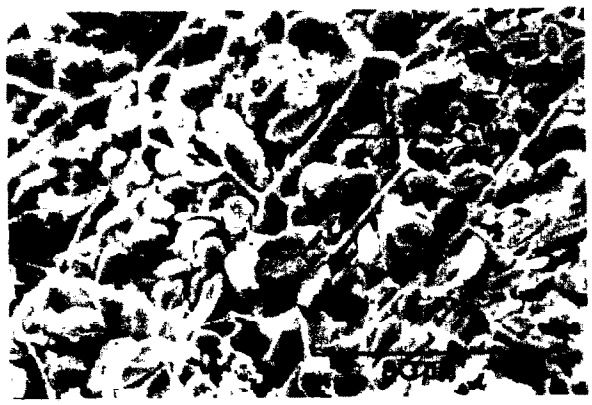
The cell walls of the aleurone layer are 3–5 μm thick and are composed of layers of differing susceptibilities to enzymic degradation [15–19]. During germination breakdown is most rapid in the walls parallel to the starchy endosperm-aleurone layer interface, particularly adjacent to the plasmodesmata [5, 6, 20–22]. Some preparations of barley aleurone layer cell walls contained 8% 'holocellulose' and 85% arabinoxylan [23].

Both aleurone layer and starchy endosperm cell walls fluoresce in UV light due to the presence of esterified phenolic substances, probably ferulic acid and diferulic acid [8, 24–26]. Pectins (uronic acids) and lignin do not occur in the walls of the starchy endosperm or aleurone layer and the associated protein contains little or no hydroxyproline [8, 23, 27, 28]. However, pectins do occur in the quiescent embryo and lignin is formed in the vascular tissues during germination [29].

We are unaware of detailed analyses of the cell walls from individual parts of barley grains sampled during germination, although there are several studies on the gums extracted from quiescent and germinating grains [30]. The work reported here was undertaken to make good this deficiency.

RESULTS AND DISCUSSION

During the 6 day germination period most of the starchy endosperm of the decorticated grain liquefied, except for material around the ventral furrow (Figs. 1 and 2) [7]. Embryos were easily separated from all samples of grain and the aleurone layers plus testae were peeled from the starchy endosperms of grains that had been germinated



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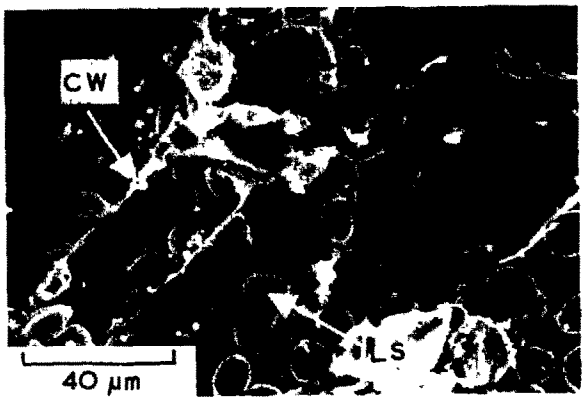
(e)

(b)



(f)

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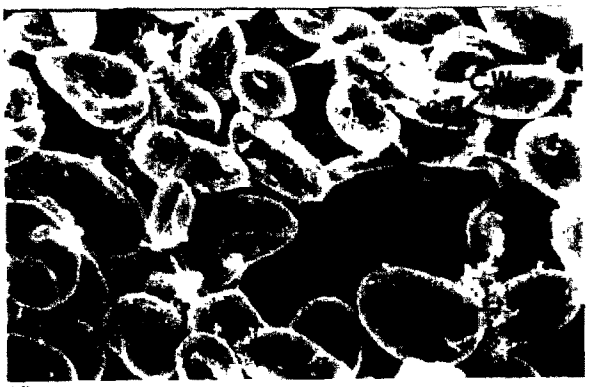

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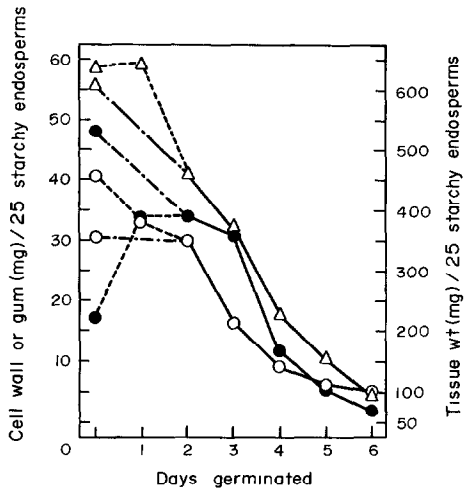

Fig. 2. The freeze-dried weights of the starchy endosperm △——△; ○——○ the starchy endosperm cell wall fractions; ●——● the starchy endosperm gum fractions. - - - - Samples for days 0 and 1 prepared by the 'pectinase' method. —·—·— Samples for day 0 prepared by the 'peeling' method.

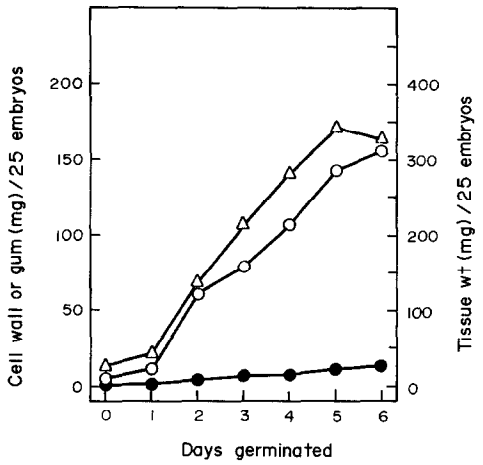

Fig. 3. Changes during germination in the freeze-dried weights of △——△ the embryos, ○——○ the cell wall fractions and ●——● the gum fractions.

for two or more days. However, for ungerminated grains it was necessary to remove the embryos and resort to 'damp incubations' before the aleurone tissue (plus testae) could be separated by peeling. Alternatively combined starchy endosperms with attached aleurone layers and testae from ungerminated and one day germinated grains were analysed, aleurone layers plus testae were loosened by a pectinase treatment and were separated and analysed. The composition of the starchy endosperm was calculated by difference. Analyses of both types of preparation are given. The yields of the freeze dried tissue fractions, prepared in triplicate, agreed within 2–10%. The chosen hydrolytic procedure, using a mixture of carbohydrases followed by hot dilute acid, was preferred to dissolution in cold concentrated acid followed by dilution and heating because sugar recoveries were higher and the reproducibility of replicate analyses was superior for several polysaccharide preparations. Replicated analyses of the cell wall and gum preparations gave results within s.d. ±10% of the mean. Inevitably losses occurred in preparing the gums. The polysaccharide content of each freeze dried fraction was checked by 'total carbohydrate' analyses and these usually confirmed the yields obtained by GLC. Nitrogen determinations showed that protein (N × 6.25: 7.5–19.6%) polysaccharides (40–108%, xylose equivalents) and water (4–15%) accounted for most of each fraction. *meso*-Inositol, used as an internal standard, was initially absent from all the preparations.

During germination the embryo cell wall polysaccharide increased in amount *ca* in proportion to the embryonic weight. The check in growth which occurred between day 5 and 6 coincided with the near exhaustion of the endosperm reserves (Figs. 2 and 3). The yields of gums from ungerminated embryos were too small for

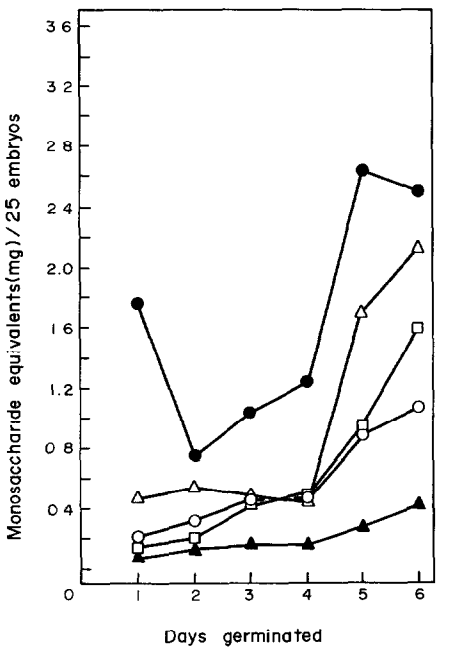

Fig. 4. The monosaccharide components of the embryo gum polysaccharides during germination. ○——○ Arabinose; ●——● xylose; △——△ glucose; □——□ galactose; ▲——▲ mannose.

Fig. 1. Scanning electron micrographs of germinating barley grains. Transverse sections were cut half way down the grain parallel with the scutellum. (a) and (b): Ungerminated grain, starchy endosperm, showing intact cells, large (LS) and small (SS) starch grains, undegraded cell walls (CW) and material adhering to the walls. (c) and (d): Two day germinated grain. (c) Modification (endosperm breakdown EB) has occurred all round the grain beneath the aleurone layer (AL). (d) Detail of the dorsal edge of the modified starchy endosperm showing only traces of degraded cell walls (CW), slightly pitted large starch grains (LS) and an absence of small grains. (e) and (f): Four day germinated grain in which endosperm degradation has progressed further. (f) The dorsal edge of the partly degraded region region contains partially degraded cell walls (CW) coexisting with pitted starch granules (LS). (g) and (d): Six day germinated grain, showing the resistant residues of cell walls (CW) radiating from the nucellar structures (NS) situated over the ventral furrow and extensively pitted starch grains.

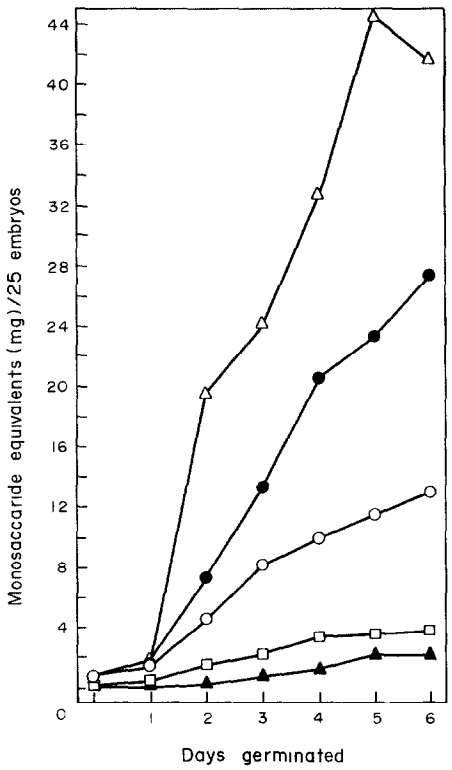

Fig. 5. The monosaccharide components of the embryo cell walls during germination. ○——○ Arabinose; ●——● xylose; △——△ glucose; □——□ galactose, ▲—▲ mannose.

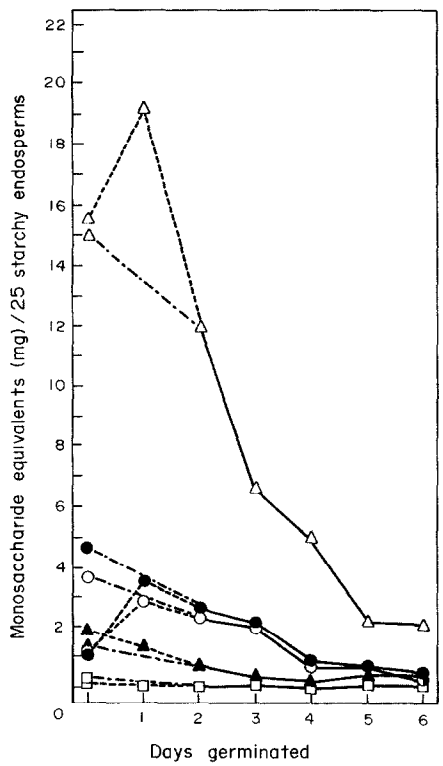

Fig. 7. The monosaccharide components of the starchy endosperm cell wall polysaccharides during germination. ○——○ Arabinose; ●——● xylose; △——△ glucose; ▲——▲ mannose; □——□ galactose. – – – – – Samples for days 0 and 1 prepared by the 'pectinase' method. –·–·–·– Samples for day 0 prepared by the 'peeling' method.

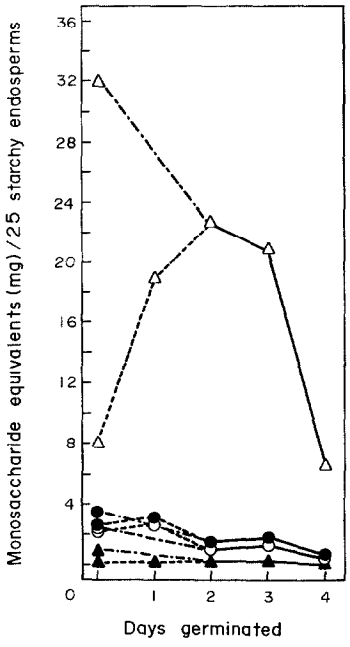

Fig. 6. The monosaccharide components of the starchy endosperm gum polysaccharides during germination. ○——○ Arabinose; ●——● xylose; △——△ glucose; ▲——▲ mannose. – – – Day 0 and 1 samples prepared by the 'pectinase' method. –·–·– Day 0 samples prepared by the 'peeling' method.

reliable analyses. The composition of both the embryo cell wall and gums changed markedly during germination (Figs. 4 and 5). In the cell wall, glucose increased rapidly from 25% of the polysaccharide, to become the major component (45%) followed in abundance by xylose and arabinose (Fig. 5). The arabinose/xylose ratio fell from 1.1 (day 0) to 0.48 (day 6). Galactose and mannose were present and traces of fucose and rhamnose were detected. The composition of the walls of ungerminated barley embryos resembled those of brome grass and rye embryos grown in suspension tissue culture [31]. These cell walls contained *ca* 10% uronic acids. During germination the barley seedling cell wall composition resembled that of oat coleoptile polysaccharide [32].

In the initial stages of germination the yields and compositions of the starchy endosperm fractions depended upon their method of preparation (Figs. 2, 6 and 7). The yields of gum and wall glucan and arabinoxylan on day 0, calculated by difference, appeared to be low. Consequently other samples were prepared using damp incubations and peeling. The composition of the combined gum and cell wall fractions of ungerminated Proctor barley was similar to those reported for other varieties [8]. During germination the starchy endosperm gum and cell wall polysaccharides declined in amount and changed in composition (Figs. 6 and 7). The major decline was in the level of glucose, presumably occurring as β-glucans. The mannose containing gums, which altered relatively little in amount, have never been

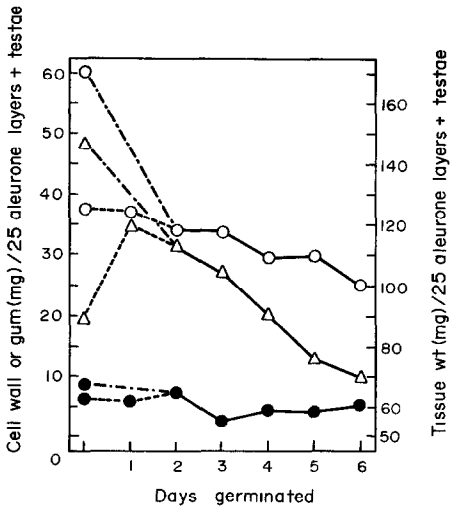

Fig. 8. The freeze-dried weights of aleurone layer and testa preparations. △——△ Whole tissue; ○——○ cell wall fractions; ●——● gum fractions. - - - - - Samples for days 0 and 1 prepared by the 'pectinase' method, –·–·– Day 0 samples prepared by the 'peeling' method.

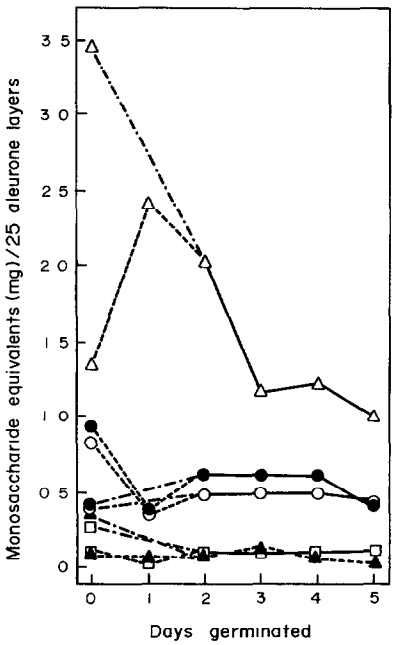

Fig. 9. The monosaccharide components of the gums from the aleurone layer and testa. ○——○ Arabinose; ●——● xylose; △——△ glucose; ▲——▲ mannose; □——□ galactose. - - - - Days 0 and 1 samples prepared by the 'pectinase' method. –·–·– Day 0 samples prepared by the 'peeling' method.

characterized. The final glucose and mannose level in the cell wall (day 6) may represent the holocellulosic component of the fibrillar wall fraction and/or residual nucellar tissues situated above the ventral furrow (Figs. 1 and 7) [8]. The arabinose and xylose containing polysaccharides, presumably mainly arabinoxylans, were also considerably degraded. In the wall, the arabinose/xylose ratio (0.8) does not alter appreciably, suggesting that rapid dissolution occurred and not selective degradation. The lower ratio in the gums (0.69) suggests an initial removal of arabinose from the arabinoxylans or the preferential degradation of an arabinose-rich polysaccharide [33]. Galactose was essentially absent from the gums. On days 5 and 6 the gum yields were too low for reliable analysis.

The yields of aleurone layer and testa fractions from ungerminated grain varied according to the method of preparation (Fig. 8). Predictably the crude 'pectinase' treated tissues (day 0 and 1) were low in glucose, because the enzyme preparation contained β-glucanase. Yields of gums were low and analyses could not be obtained for the very small day 6 samples (Figs 8 and 9). The testae and pigment strands, which apparently remained unchanged during germination, were estimated to contribute *ca* 14.7 mg/25 grains to the tissue and cell wall preparations. This exceeded the yield of polysaccharide remaining in the aleurone layer and testa and pigment strand cell wall preparation after 6 days germination [14.1 mg/25 grains; Table 1]. In agreement with analyses of aleurone layers of another variety, Himalaya, the major wall components were xylose and arabinose (85% in total) [22]. In our pectinase treated preparation the pentoses comprised 80% of the wall; in the damp-incubated preparations, 59%. The higher levels of glucose occurring in the 'damp incubated' and peeled aleurone

Table 1. Changes in total gums, cell wall polysaccharides and starch in different tissues of germinating decorticated grains (mg monosaccharide equivalents/25 grains)*

Days germinated	Embryo	Aleurone layer and testa			De-germed grain Starchy endosperm			Combined total		
	Cell wall + gum	Cell wall	Gum	Total (A)	Cell wall	Gum	Total (B)	A + B	Starch	A + B + starch
0	2.0	35.7	4.9	40.6†	24.8	39.2	64.0†	104.6†	445	550
1	7.8	25.0	3.2	28.2‡	27.0	25.1	52.1§	80.3‡,§	265	345
2	34.8	24.1	3.2	27.3	17.8	25.4	43.2	70.5	226	297
3	50.1	23.3	2.5	25.8	11.2	24.3	35.5	61.3	153	214
4	70.2	17.6	2.5	20.1	6.9	7.7	14.6	34.7	140	175
5	91.4	17.9	1.9	19.8	4.3	[2.5]	[6.8]	[26.6]	126	[153]
6	95.9	14.1	[1.5]	[15.6]	3.7	[1.5]	[5.2]	[20.8]	73	[93.8]

* Cell wall and gum polysaccharides calculated from GLC data.
† 'Damp incubated' samples.
‡ Pectinase treated samples.
§ Calculated by subtraction of aleurone layer values from whole endosperm values.
[] Approximate calculated values.

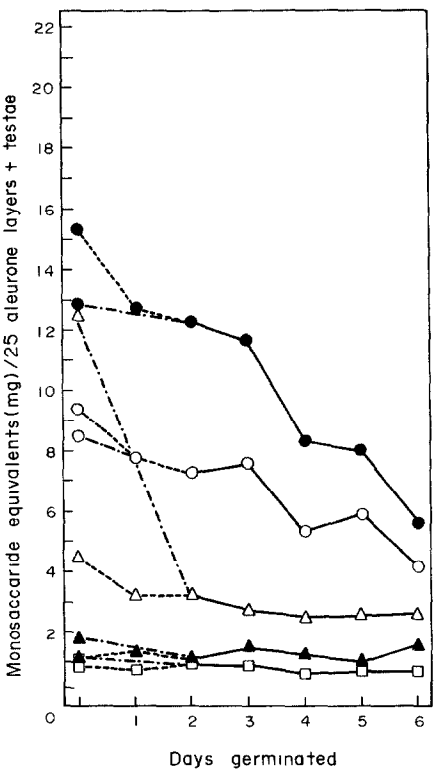

Fig. 10. Changes in the monosaccharide components of the aleurone layer and testa cell walls. O——O Arabinose; ●——● xylose; △——△ glucose; ▲——▲ mannose, □——□ galactose. - - - - Samples for days 0 and 1 prepared by the 'pectinase' method. - -·- Samples for day 0 prepared by the 'peeling' method

layers were not caused by adhering starch or cell walls from the sub-aleurone layer. We hypothesize that they may have been due to a glucan-rich layer between the aleurone layer and the starchy endosperm. During germination the levels of glucose in both the cell walls and gums initially fell (Figs. 9 and 10). Isolated aleurone layers are reported to release β-glucans during early germination perhaps due to an endo-$\beta(1 \rightarrow 3)$ glucanase, detectable in imbibed grain [21, 34]. The glucose which remained in the cell wall after day 3 may be combined in a more resistant 'holocellulosic' polysaccharide [23]. The latter may also contain the wall mannose and galactose, which did not decline either, forming an enzyme-resistant wall layer (Fig. 10) [18, 19]. The pentose content of the aleurone cell walls fell steadily by 60% during germination. The arabinoxylans of the gums had a higher arabinose/xylose ratio (0.98) than those of the cell walls (0.65), perhaps giving them an inherently higher solubility and lower affinity for the insoluble cell wall components [23, 35]. Attempts to correlate the structural changes in the aleurone layer cell walls with the chemical analyses were confounded because the cell walls underwent slow progressive changes rather than a rapid simultaneous dissolution and the stage of degradation depended upon the position of the cell in the grain.

Scanning electron microscopy showed that initially starchy endosperm cell wall dissolution preceded starch grain hydrolysis, in agreement with earlier reports (Figs. 1c and d) [36, 37]. However, by day 4 very thin partially degraded cell walls were seen to co-exist with pitted starch grains (Figs. 1e and f). By day 5 only the cell walls and nucellar material radiating from the ventral furrow remained. This residual tissue contained only a few, heavily pitted starch grains whereas the cell walls were only partly degraded (Figs. 1g and h). These observations support previous reports that these walls are comparatively resistant to enzyme attack [7]. The change in the balance between cell wall and starch grain hydrolysis during germination may be due to a gradient of chemical and structural differences in the cell walls along the path of modification. The chemical composition of the residual cell walls on day 6 is in agreement with this idea (Fig. 7).

Table 2. Fall in endospermic and rise in embryonic polysaccharide components during six days germination (mg monosaccharide equivalents/25 grains)

	Glucose*	Galactose	Mannose	Xylose	Arabinose
Fall in endosperm	429	1.2	2.0	14	10
Rise in embryo	43	5	2.5	29	13

* Including starch.

The changes in carbohydrates recorded here, together with those for the endospermic starch, take no account of the soluble sugars and fructosans which occur and at times accumulate in the germinating grain [30, 38]. Ignoring the small amounts of soluble xylose and arabinose present in ungerminated grain, the balance of individual monosaccharide incorporation into embryo polysaccharide and the corresponding disappearance from the endosperm polysaccharide shows that sugar interconversion occurs before incorporation (Table 2). Starch was the major carbohydrate reserve, declining by 372 mg glucose equivalents/25 grains in the 6 days studied (Table 1). The cell wall and gum polysaccharides contributed 84 mg monosaccharide equivalents/25 grains or *ca* 18.5% of the total polysaccharide that was degraded. Of the contribution made by the cell walls and gums, 25.0 mg/25 grains was pentose and 59 mg/25 grains was hexose, predominantly glucose. The thick cell walls of the seeds of other species are well recognized carbohydrate reserves [39–41]. It appears that the cell walls (and gums) of the starchy endosperm and aleurone layer serve a similar function in the barley grain.

EXPERIMENTAL

Grains of *Hordeum distochon*, cv. Proctor, passed by a 2.8 mm slotted screen but retained by a 2.5 mm slotted screen, were decorticated. Mealy, undamaged grains were selected and surface sterilized [42]. Grains (25) were germinated in Petri dishes on filter paper discs (2 × 9 cm, Whatman No. 1) wetted with 6 ml H_2O, in a moist atmosphere in the dark, at 25° [38]. Samples were stored frozen. Embryos, with scutella, were easily separated from all grain samples. With samples taken after 2 days germination, aleurone layers plus attached testae were peeled from starchy endosperms and cleaned by scraping. For ungerminated and one day germinated grains different separation methods had to be used. (a) 'Pectinase treatment'. Starchy endosperm and aleurone layers and testae, (degermed, decorticated grains) were prepared and analysed. Separated aleurone layers and testae were prepared by the 'pectinase' method and analysed [43] Starchy endosperm values were calculated by difference. (b) 'Peeling method'. Aleurone layers and testae were peeled from the starchy endosperms of ungerminated, de-germed grains which had been incubated on moist sand [14]. All separated tissues were freeze-dried and weighed before analysis.

Cell wall and gum preparation. Freeze-dried tissues (from 25 grains) were homogenised in 4 ml of EtOH–H_2O (4:1) with a Willems Polytron (type P.T 10, 22000 rpm, 1 min). The solid residues were re-extracted with aq. EtOH (2 × 10 ml, 80°, 1 hr) cooled (4°) and centrifuged. The solids were washed (EtOH–H_2O 24:1, 4°), extracted × 2 with 10 ml $CHCl_3$–MeOH (2:1) at 30° for 1 hr and dried (N_2, 40°). Starch was gelatinized by heating in buffer (5–10 ml, veronal, 25 mM; NaN_3, 5 mM; $CaCl_2$, 25 mM; β-mercaptoethanol, 0.1 mM; pH 6.9 80°, 15 min). After cooling, dialysed pancreatin (4 mg/ml, same buffer) was added. The mixtures were incubated at 37° with stirring. When the starch had been dissolved (I_2/KI staining) the residual cell wall was collected by centrifugation, rinsed with H_2O (2 × 5 ml) and freeze-dried. The combined supernatants were heated to coagulate proteins (90°, 20 min), cooled and centrifuged. The supernatant was dialysed against H_2O then freeze-dried. The solid was redissolved in H_2O and gums were precipitated by adding 15 vols satd $(NH_4)_2SO_4$ (24 hr, 4°). The precipitated gum fraction was dissolved, dialysed, then freeze-dried.

Analyses. Carbohydrase mixture for cell wall hydrolysis. 'Cellulases' from *Aspergillus niger* (Sigma Ltd., Prac. Grade II), *Trichoderma viride* (B.D.H. Ltd.) and Onozuka-S.S. enzyme (Japan Biochemicals Ltd.) and a 'pectinase' from *T. viride* (Koch Light Ltd.) (5 g of each) were dissolved in buffer (350 ml., Pi, 10 mM; NaN_3, 5 mM; $CaCl_2$, 1 mM; β-mercaptoethanol, 1 mM; pH 6) and centrifuged (15000 *g*, 1 hr, 4°). The enzymes were precipitated with satd $(NH_4)_2SO_4$ (1.2 l. 4°, 18 hr) and collected by centrifugation (15000 *g*, 1 hr, 4°). The ppt., re-dissolved in Pi buffer, was separated from NH_4^+ ions by passage through a Biogel P4 column (45 × 2 cm) and freeze-dried.

Samples (5–15 mg) of cell wall or gum were incubated with enzyme mixture (1 mg in 5 ml Pi buffer and 1 mg *meso*inositol internal standard, 38°, 72 hr) with magnetic stirring. The samples were freeze-dried and further hydrolysed with H_2SO_4 (0.5 M, 5 ml, 100°, 4 hr). The acid was neutralized with $PbCO_3$, the $PbSO_4$ centrifuged (1500 *g*, 30 min) and the supernatant concentrated. The monosaccharides were reduced with $NaBH_4$ (25 mg/2 ml, 0.1 M NH_4OH, 16 hr). Excess $NaBH_4$ was decomposed with HOAc. Borate was removed by repeated evaporation (6 × 2 ml) with MeOH–HOAc (9:1) at 45°. The alditols were acetylated (5 ml Ac_2O–Py 1:1, 80°, 4 hr). The reaction mixture was concd (vacuum, 90°) then dissolved in DMF. Alditol acetates were analysed by GLC 305 cm × 6 mm, 3% ECNSS-M on Gas Chrom Q, 100–120 mesh, dual FID chromatograph, isothermal 195°; N_2, 40 ml/min). Known sugar mixtures were subjected to the standard procedure to obtain calibration factors. The sugars in each hydrolysate were quantified and 'blank' values from the sugars derived from the hydrolytic enzyme mixture were subtracted. Each cell wall fraction was prepared in triplicate, each fraction was hydrolysed and chromatographed × 4. The total carbohydrate content of each fraction was estimated using the PhOH–H_2SO_4 method, using xylose as standard [45].

Nitrogen determinations. Sub-samples (*ca* 0.25 mg) of the wall and gum fractions were analysed by the Jaenicke micro-method [46].

Testa content. Aleurone layers and testae were peeled from grains germinated for 72 hr, freeze-dried and weighed. The cell walls were hydrolysed with the hydrolytic enzyme mixture (5 mg/10 Pi buffer, 5 days), washed (H_2O, 2 × 4 ml), freeze-dried and further hydrolysed with 72% w/w H_2SO_4 (0.6 ml, 0°, 1 hr) followed by 0.5 M H_2SO_4 (100°, 4 hr). The residue, the testae, was washed (H_2O, 3 × 3 ml), freeze-dried and weighed.

Starch estimations. Grains (25) were de-germed, homogenized (Polytron, 22000 rpm, 150 sec, 7.5 ml H_2O) freeze-dried and weighed. Sub-samples were extracted with EtOH–H_2O 4:1 (× 2, 4 ml, 80°, 1 hr). The extracts were discarded. Starch was gelatinized with 90% DMSO (0.5 ml, 100°, 1 hr) then hydrolysed with α-amylase (20 mg *B. subtilis* α-amylase, Koch Light. Ltd. in 4 ml $CaCl_2$, 1 mM; NaN_3, 1 mM; Tris, 1 mM; pH 6.9, 55° magnetic stirring). After heating (100°, 15 min) and centrifugation (10000 *g*, 35 min, 5°) the supernatants were incubated with amyloglucosidase (4 ml of 15 mg/ml Glaxo Agidex, acetate buffer 15 mM; NaN_3 1 mM; pH 4.5, 25°, magnetic stirring, 40 hr). The vols were adjusted to 50 ml, aliquots (1 ml; 0–100 μg glucose) were added to 3.5 ml of a glucose oxidase reagent (glucose oxidase, Sigma type V, 10 mg; *o*-dianisidine HCl, 10 mg; peroxidase, Boehringer type I, 1 mg; glycerol, 400 ml; in 1 l. of Tris 0.3 M, adjusted with NaH_2PO_4 to pH 7) and incubated (37°, 1 hr). Acid (5M HCl, 4 ml) was added and the A at 540 nm measured. The *iso*-maltose contents of aliquots were measured by GLC of the TMSi ethers [47].

Scanning electron microscopy. Grains were sectioned at −13° and critical point dried (Me_2CO–CO_2). Samples were attached to Al stubs with double sided Sellotape and coated with Au (500 Å, splutter coater). Stubs were examined at an angle at 45° to the electron beam (25 kV).

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ADDENDUM

Recently it has been reported (Fulcher, R. G., Setterfield, G., McCully, M. E. and Wood, P. J. (1977) *Aust. J. Plant Physiol.* **4**, 917) that the cell walls of the aleurone layer–sub-aleurone layer interface are rich in β(1–3) glucan. This would appear to be the readily degraded layer of glucan whose existence we have deduced in this paper.

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