4. The results suggest that inactivation by difunctional agents results from inter-strand cross-linking of DNA. With monofunctional agents it seems likely that the principal mode of inactivation results from degradation of the DNA consequent upon its alkylation.

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Sugar Composition of Oat-Coleoptile Cell Walls

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Much interest centres on the primary cell wall as the probable locus of processes immediately responsible for the growth of the plant cell. However, little has been published about the chemical composition of polysaccharides of primary cell walls of higher plants, especially of growing tissues. Wilson (1961) reported the total monosaccharide composition of growing tobacco-pith cell walls without fractionation. Thornber & Northcote (1961, 1962) fractionated and analysed cell walls of cambium, and of xylem and phloem derived from it, of several species of trees.

Cell walls of the oat coleoptile, a material commonly used for studies on the growth of plant cells, were fractionated by Bishop, Bayley & Setterfield (1958), and analysed partly quantitatively and partly qualitatively. In the present work the monosaccharide composition of the oat-coleoptile cell wall was examined by a different extraction procedure, resembling those that have been employed in most of the radioisotope studies on cell-wall metabolism of this material (Boroughs &

Bonner, 1953; Ordin, 1960, 1962; Ordin, Cleland & Bonner, 1955, 1957).

Results are given on changes in monosaccharides during the growth of oat-coleoptile cylinders, with emphasis on the growth that occurs in the absence of sugar in the medium, when little net increase in cell-wall material takes place (Ray, 1962*a*). It was thought that under these circumstances it might be possible to detect metabolic alterations of cell-wall polysaccharides that could be involved in the process of cell enlargement, since such changes would not tend to be obscured by the simultaneous addition of new cell-wall material.

METHODS

Plant material. Oats of variety Victory, obtained from Svenska Allmänna Utsädes A.B., Svalöf, Sweden, and of variety Milford, obtained from the National Institute of Agricultural Botany, Cambridge, were grown on moist filter paper, for 1 day under red light followed by 2 days in the dark, at about 26°. Coleoptile cylinders 8 mm. long, begin-

ning 3 mm. below the tip, were cut under red light, the leaves removed, and the cylinders floated on water for about an hour. For growth experiments, lots of 15–20 cylinders were transferred to 4 ml. of growth solution at 26° in the dark, the initial samples being taken at the time of the transfer of the remaining cylinders to the growth solutions.

Cell-wall preparations were made by crushing the cylinders between glass plates, soaking the residues in 50% (w/v) urea overnight, and washing them with water and 95% (v/v) ethanol; they were weighed after being dried to a moisture content of about 4%. (For details of these procedures see Ray, 1962a.)

Extraction and hydrolysis of polysaccharides. Samples of 3–7 mg. of cell-wall material were extracted successively with hot water, hot 0.05N-H₂SO₄, 4N-KOH, and 72% (w/w) H₂SO₄. Extractions and hydrolyses were performed in Pyrex tubes (18 mm. × 185 mm.) closed with ground-glass stoppers. Because of the way in which the cell-wall preparations were made, the cell-wall material of each coleoptile cylinder remained as a coherent unit throughout the procedure, so that the extracts and washes were separated from the residues by decanting. After each extraction the residue was washed with ethanol, dried and weighed.

Samples were first extracted twice with 1.0 ml. of water at 100° for 1 hr. The residue was washed with two 0.5 ml. portions of water and the combined extracts and washes were heated at 100° for 6 hr. with 0.4 ml. of 10N-H₂SO₄.

Samples were next extracted for two 1 hr. periods at 100° with 1.0 ml. portions of 0.05N-H₂SO₄ and washed with two 0.5 ml. portions of water; the combined extracts and washings were made 1N with respect to H₂SO₄ and heated at 100° for 6 hr.

The dried residue was extracted with 0.2 ml. of 4N-KOH for 6 hr. at room temperature (about 25°). Water (1 ml.) was added and the diluted extract passed through about 3 g. of Amberlite IR-120 (H⁺ form). The residue was washed with four 1 ml. portions of water, the last washing having pH 7 or below. Each wash was passed in turn through the resin. To the combined effluent 0.5 ml. of 10N-H₂SO₄ was added and the sample heated 6 hr. at 100°. During the hydrolysis a small flocculent white precipitate formed. This could be separated by centrifuging, but ordinarily it was not removed. In larger-scale experiments this precipitate, presumably of the hemicellulose A type, yielded glucose and xylose on hydrolysis, but complete hydrolysis could not be achieved owing to its insolubility.

The dried residue from the alkali-extraction step (α -cellulose) was treated with 0.10 ml. of 72% (w/w) H₂SO₄ for 15 hr. at room temperature; 2.2 ml. of water was then added. The brownish insoluble residue weighed less than 1% of the initial cell wall and ordinarily was not removed. The sample was heated for 6 hr. at 100°.

In trial experiments with larger amounts of cell-wall material the hydrolysis of each fraction was followed with time by determinations of reducing sugar as described below. Under the conditions given above, hydrolysis was largely completed within 2 hr. and the maximum reducing value was reached at or before 6 hr.

Separation of sugars. Each hydrolysate was passed through a column containing about 10 ml. of Dowex 1 (acetate form), which replaced H₂SO₄ by acetic acid and absorbed uronic acids. The column was washed with 40 ml. of water, and the effluent was evaporated under reduced pressure at about 40° with a rotary evaporator to about

5 ml., transferred to a test tube and evaporated to dryness over P₂O₅ in a vacuum desiccator. The entire sample was chromatographed, except for the hot-0.05N-H₂SO₄ and 72% (w/w) H₂SO₄ extracts from cell-wall preparations weighing 7 mg. or more, where half was used to avoid the possibility of overloading the chromatogram with certain sugars.

Whatman no. 1 filter paper in sheets (9 in. × 22 in.) was prepared by washing it with 5N-acetic acid followed by water, in packets of about 50 sheets in a trough similar to that described by Isherwood & Hanes (1953). The papers were then washed chromatographically twice with water. These washings were found necessary to decrease the paper blank value to a satisfactorily low level.

Sugar samples dissolved in 0.1 ml. of 6N-acetic acid were applied as spots to the starting line followed by two washings of the sample tube with 0.1 ml. of water. Samples and guide mixtures (60 μ g. each of galactose, glucose, mannose, arabinose and xylose) were applied as illustrated in Fig. 1 (a). The chromatogram was developed, without prior equilibration, for about 40 hr. at 25° by the descending method in the solvent butan-2-one-acetone-water-pyridine (150:30:20:1, by vol.).

A part of each guide strip was removed as shown by the solid lines in Fig. 1 (a) and sprayed with aniline phthalate

Fig. 1. Diagrams illustrating the method of chromatographic separation used in analyses. In (a), O indicates the starting line, the solid lines show where the paper was cut to remove guide strips for determining the positions of mannose (M), arabinose (A) and xylose (X), and the broken lines show how the paper was cut to remove the piece containing glucose (Gc) and galactose (Ga). This piece was sewn on a new piece of paper at the broken line in (b), and was developed further until the sugars were moved to positions shown by Gc and Ga, which were found by cutting off guide strips as shown by solid lines.

(Partridge, 1949) to reveal xylose, arabinose and mannose. The paper was then cut as shown by the broken lines in Fig. 1 (a).

To the portion of the paper containing glucose and galactose (which were not yet sufficiently separated for analytical purposes) a new piece of Whatman no. 1 paper was sewn on with a sewing machine, as shown in Fig. 1 (b), with an overlap of about 4 mm. Development was continued for about 80 hr. with the same solvent; the sugars migrated across the sewn boundary and became completely separated as illustrated in the lower part of Fig. 1 (b).

By reference to the guide strips, rectangles containing the sugars of the unknown samples were cut out.

Determination of sugars. Rectangles of chromatogram paper, blank or containing individual sugars, were eluted with water, dried and weighed. The paper blank value depended mainly on the particular sheet of paper and on the weight of the rectangle employed, and was little influenced by position on the chromatogram sheet, so that one paper blank served for all samples on a given chromatogram. The eluted sugars were evaporated to dryness over P_2O_5 in a vacuum desiccator and redissolved in a known volume of water.

Sugars were determined by the copper reduction-arsenomolybdate method as described by Wager (1954), with one-fifth the volumes he recommended, so that the sensitivity was five times as great. The assays were performed in specially-constructed Pyrex determination tubes utilizing a ground joint instead of the stopper shown in Wager's (1954) apparatus. The heating time (at 100°) was 30 min. The blue reaction product was measured at 660 m μ with a spectrophotometer, or with a Klett colorimeter and a red filter. With paper blanks or minor sugar components the whole eluate was taken for the determination, but with major sugar components repeated measurements could be made on portions of eluate containing 10–20 μ g. Paper blanks were generally equivalent to less than 6 μ g. of sugar; the major sugar samples usually contained more than 100 μ g.

In the Tables the amounts of sugars found are expressed as anhydrosugar. No attempt has been made to correct for possible losses. The sugars were stable to the hydrolysis procedure employed.

Other determinations. Protein and uronic acid were determined on samples removed from the extracts used in the determinations of Table 1, before hydrolysis. Protein is calculated as $6.2 \times$ Kjeldahl nitrogen (Ray, 1962a). Uronic acid was determined by the carbazole method (Dische, 1947), and calculated as galacturonic acid, uncorrected for interference from sugars and other materials in the extracts.

The remaining components listed in Table 2 were determined on separate samples of wall material. Lipid was determined gravimetrically as material soluble in three successive 24 hr. extractions with boiling benzene-ethanol (2:1, v/v). Lignin was determined as material insoluble in 72% (w/w) H_2SO_4 (without the preliminary extraction steps used in polysaccharide extractions). Methoxyl was determined by the method of Clark (1932). Xylose in aldobiouronic acids was calculated from the aldobiouronic acids recovered after a 6 hr. hydrolysis of the fraction soluble in hot 0.05N- H_2SO_4 (cf. Ray, 1962b).

RESULTS

Qualitative composition. The predominant sugars in the fraction soluble in hot water were arabinose and glucose, with smaller amounts of galactose and xylose; in the fraction soluble in hot dilute acid, arabinose and xylose were predominant, with decreasing amounts of glucose and galactose; in the alkali-soluble fraction, glucose and xylose were predominant, with small amounts of mannose and galactose, not always detectable in qualitative chromatograms; in the α -cellulose fraction, glucose was predominant, with a consistently observed minor proportion of mannose and xylose.

Rhamnose was detected, isolated and determined in hydrolysates of the fraction soluble in hot dilute acid, and in a hydrolysate of oat-coleoptile hemicellulose B, by chromatographing the entire hydrolysate derived from 50–100 μ g. of cell-wall material. The yield was about 0.05% from whole cell-wall material and 0.1% from hemicellulose B.

Table 1. *Composition of extracts of cell walls of oat coleoptiles, variety Victory*

The results show the mean amounts of anhydrosugar $\pm$ average deviation in μ g./40 coleoptile cylinders 8 mm. long, for the number of replicate cell-wall samples indicated in parentheses. A dash indicates that a significant amount was not found. The mean weight of cell-wall material was 6.97 mg./40 cylinders.

Constituent	Extract			
	Hot water	Hot 0.05N- H_2SO_4	4N-KOH	72% (w/w) H_2SO_4
Xylose	11.3 $\pm$ 5.4 (3)	512 $\pm$ 55 (4)	96 $\pm$ 3.1 (3)	58 $\pm$ 16 (3)
Arabinose	41.3 $\pm$ 5.9 (3)	488 $\pm$ 18 (4)	12 $\pm$ 1.7 (3)	—
Glucose	18.5 $\pm$ 3.2 (4)	336 $\pm$ 10 (2)	202 $\pm$ 21 (4)	1460 $\pm$ 139 (4)
Galactose	11.3 $\pm$ 3.3 (4)	151 $\pm$ 19 (3)	20 $\pm$ 8 (3)	17*
Mannose	—	12 $\pm$ 3.3 (3)	27 $\pm$ 3.3 (4)	122 $\pm$ 23 (3)
Total sugars	82	1499	357	1640
Uronic acid	49	342 $\pm$ 21	98 $\pm$ 7	—
Total carbohydrate	131	1841	455	1640
Loss in weight on extraction	380 $\pm$ 40	3410 $\pm$ 110	1440 $\pm$ 150	1740 $\pm$ 80

* Separate determination of galactose was made with only one sample. The mean value for glucose for this extract includes the small amount of galactose that was present.

Rhamnose was also obtained from an aldobiouronic acid that is released in the hydrolysis of the fraction soluble in hot dilute acid or of hemicellulose B. Because of the different chromatographic procedure needed to isolate rhamnose and the low content of this sugar, it was not determined in the remaining experiments described below.

Quantitative composition. Table 1 gives the results of analysis of cell-wall monosaccharides of oat-coleoptile cylinders of the variety Victory. These results were obtained from four replicate samples of cell-wall material carried through the entire procedure. (The number of replicate samples reported is sometimes less than four, owing to accidental loss of samples, or rejection of results in a few instances in which there were gross discrepancies between a given sample and its replicates in the same series.)

Variation between results with replicate samples was larger than might be desired, but the variation observed was of the same magnitude as that reported in the extensive analyses of pear cell walls by Jermyn & Isherwood (1956). (In other analyses of primary cell-wall materials that have been published, replicate samples have not been reported.)

The variation between replicate determinations probably represents primarily variations in recovery rather than in composition. Variation may also be due to variable extraction of components by solvents in which they are only slightly soluble, or variable carry-over of minor amounts of the more soluble constituents to later extraction steps.

Variation was especially large in the sugar components of the fraction soluble in hot water. Because of the small amount and variability of this fraction, extraction with hot water was not performed in the other experiments; its constituents

are then presumably included in the fraction soluble in hot dilute acid.

Sugar constituents of the α -cellulose [soluble in 72% (w/w) sulphuric acid] fraction accounted almost completely for the loss in weight of the cell-wall material on extraction (Table 1). With the other fractions, however, the sugars found were much less than the loss in weight of the material. This is presumed to be due both to incomplete recovery of sugars, and to the fact that major non-carbohydrate constituents, especially protein, were removed during these extraction steps.

The total sugars, and other constituents that were determined, are expressed as percentages of the cell-wall material in Table 2. The protein probably represents mainly contamination of the cell-wall preparations with protoplasm, since considerably lower protein contents have been obtained by other methods of preparation (Bishop *et al.* 1958; Kivilaan, Beaman & Bandurski, 1959). About 6% of the coleoptile cell-wall preparation remained unaccounted for, presumably owing to incomplete recovery of sugars, and to lack of determination of some possible constituents (e.g. acetyl).

Cell-wall monosaccharide composition of coleoptile cylinders prepared from oat seedlings of the variety Milford are given in Table 3 (first column). The findings were generally similar to those with the variety Victory, except that pentoses appeared somewhat more predominant in the fraction soluble in hot dilute acid, and the ratio of xylose to glucose in the fraction soluble in 4N-potassium hydroxide was greater.

Changes during growth. Analyses of samples of cylinders after a period of auxin-induced elongation in the presence and absence of glucose in the medium are shown in Table 3. With glucose an increase was observed in almost all monosaccharides of all fractions. The increase in the major components, for which the results are more reliable, was 60–87% in all fractions except the glucose in the fraction soluble in 4N-potassium hydroxide (46%), showing that during growth in the presence of glucose there is a general synthesis of cell-wall polysaccharides.

Ray (1962*a*) noted that, when sugar is not added to the growth medium, relatively little increase in total cell-wall material takes place, but a substantial rise in α -cellulose occurs. This rise in the amount of α -cellulose is borne out by the increase in glucose in the fraction soluble in 72% (w/w) sulphuric acid. This was accompanied by a large decrease in glucose and galactose in the fraction soluble in hot dilute sulphuric acid.

Changes in cell-wall constituents during elongation without sugar were examined also in coleoptile cylinders of the variety Victory (Table 4). In this

Table 2. *Summary of the composition of cell-wall preparations of oat-coleoptiles, variety Victory*

The yields of the free sugars are summed from Table 1.

Constituent	Percentage of cell-wall material
Glucose	28.9
Xylose { obtained as free sugar	9.7
{ in aldobiouronic acids	1.2
Arabinose	8.0
Galactose	2.9
Mannose	2.3
Uronic acids (calc. as galacturonic acid)	7.0
Methoxyl (calc. as CH ₃)	0.6
Lignin	3.6
Lipid	2.8
Ash	2.9
Protein	19.5
Water	4.0
Total	93.4

Table 3. *Cell walls of coleoptile cylinders, variety Milford*

The initial value is the mean for two samples of 31 cylinders each. The growth samples were taken after 20 hr. in 3 mg. of indolylacetic acid/l. (31 cylinders) and 18 hr. in 3 mg. of indolylacetic acid/l. containing glucose (0.1 M) (25 cylinders). Cell-wall constituents are given in $\mu\text{g.}/30$ cylinders.

Coleoptile-cylinder sample	Initial	After growth in indolylacetic acid only	After growth in indolylacetic acid + glucose
Mean length (mm.)	8.9	15.2	18.8
Total cell wall ($\mu\text{g.}$)	5360	5770	8380
Hot-0.05N- H_2SO_4 extract			
Xylose	630	745	1035
Arabinose	570	750	1055
Glucose	250	68	405
Galactose	175	97	205
Mannose	27	10	38
4N-KOH extract			
Xylose	135	155	235
Arabinose	5	9	8
Glucose	120	110	175
Mannose	13	14	12
72% H_2SO_4 extract			
Xylose	25	34	50
Glucose	1215	1600	2270
Mannose	140	223	156
Total sugars recovered	3305	3815	5640

Table 4. *Elongation of coleoptile cylinders, variety Victory*

The mean and average deviation for four replicate samples of 15–16 cylinders at each time of sampling is given in $\mu\text{g.}/15$ cylinders.

Time in 3 mg. of indolylacetic acid/l. (hr.)	0	3.5	16
Mean length (mm.)	8.9	11.1	14.5
Total non-protein cell wall ($\mu\text{g.}$)	1870 $\pm$ 30	2010 $\pm$ 45	2020 $\pm$ 45
Hot-0.05N- H_2SO_4 extract			
Glucose	116 $\pm$ 10	78 $\pm$ 6	28 $\pm$ 5
Galactose	30.7 $\pm$ 2.8	14.7 $\pm$ 3.7	15.0 $\pm$ 3.7
Arabinose	133 $\pm$ 8	113 $\pm$ 6	123 $\pm$ 9
Xylose	156 $\pm$ 25	*	134 $\pm$ 25
4N-KOH extract†			
Glucose	42.5 $\pm$ 2.7	43.5 $\pm$ 2.5	38.6 $\pm$ 0.4
Xylose	18.5 $\pm$ 0.7	25.7 $\pm$ 2.1	40.2†
72% H_2SO_4 extract			
Glucose	538 $\pm$ 33	589 $\pm$ 27	672 $\pm$ 34
Mannose	27.3 $\pm$ 4.0	23.9 $\pm$ 0.5	30.0 $\pm$ 6.5

* Owing to accident no satisfactory values were obtained for these samples.

† Replicate samples were combined by pairs for the determinations; the deviation given is that for the duplicate determinations.

‡ One determination only.

case the determinations were made on four replicate samples, each of 15–16 cylinders or about 2 mg. of cell-wall material. The principal sugar constituents of each fraction are reported in Table 4. This experiment was disappointing in that the recovered sugars accounted for a smaller percentage of the cell wall than in the previous experiments. However, the results confirm an increase in glucose in the α -cellulose fraction and an approximately compensating decrease in glucose in the fraction soluble in hot dilute acid. In this fraction decrease in galactose also occurred, but only during the first time-interval. Unlike the similar experiment in Table 3, small decreases in pentoses in the fraction soluble in hot dilute acid were recorded, although the variability with the xylose samples was large.

An increase in xylose in the alkali-soluble fraction was noted, but, as in Table 3, glucose in this fraction neither increased nor declined substantially.

DISCUSSION

The present results on the monosaccharide composition of oat-coleoptile cell-wall material may be compared with values given by Bishop *et al.* (1958). They reported the relative amounts of different sugars in hydrolysates as determined by a densitometric measurement of spots developed directly on the paper by reaction with silver nitrate. Their alkali-soluble polysaccharide fraction, comprising 89% of the non-cellulosic polysaccharides recovered in their fractionation, yielded 29.8% of xylose,

29.1 % of arabinose, 34.3 % of glucose and 6.7 % of galactose. If the amounts of these four sugars obtained by the present method from the fractions soluble in hot dilute acid and alkali (Table 1), which include about 95 % of the non-cellulosic material, are added together, we find 33.6 % of xylose, 27.7 % of arabinose, 29.7 % of glucose and 9.4 % of galactose. The agreement is close, considering the differences in the methods employed in the two studies, including different extraction procedures and methods of determination, and the use of different varieties of oats.

The nearly 1:1 ratio of arabinose to xylose in the coleoptile cell wall is noteworthy. Compared with secondary cell-wall material, in which the content of arabinose is typically low (see, for example, Thornber & Northcote, 1961; Gustafsson, Pettersson & Lindh, 1951), a high ratio of arabinose to xylose appears to be characteristic of such primary cell-wall material as has been examined thus far (cf. Andrews, Hough & Stacey, 1960). Compared with water-soluble pectin-rich and arabinan-rich materials, e.g. pear (Jermyn & Isherwood, 1956), the coleoptile cell wall, according to the present results and those of Bishop *et al.* (1958), is of interest in that most of the arabinose is represented not in water-soluble polysaccharides but in hemicellulose fractions (soluble in hot dilute mineral acid or in alkali).

The composition of the non-cellulosic polysaccharides of the oat-coleoptile cell wall rather resembles that found for plum-leaf cell-wall material by Andrews & Hough (1958).

Probably most of the arabinose of the oat-coleoptile hemicellulose fraction is derived from polysaccharides of the arabinoxylan (or arabinoglucuronoxylan) type, which are much less readily solubilized than are straight arabinans. That the xylose of hemicellulose fractions is derived from glucuronoxylans is indicated by the isolation of xylose-containing aldobiouronic acids from this material (Ray, 1962*b*).

Although in quantitative composition the fraction of the coleoptile cell wall soluble in hot dilute acid differs markedly from the alkali-soluble fraction, these fractions do not necessarily represent separate classes of polysaccharides. On completion of extraction with hot 0.05*N*-sulphuric acid the extract possesses about half the maximum yield of reducing sugar that can be produced by further hydrolysis. By chromatography, arabinose was found to be the principal free sugar present after extraction (but before further hydrolysis), as might be expected from the well-known lability of the arabinofuranosyl linkage to acid hydrolysis. The major proportion of this arabinose is probably derived by splitting off arabinose side groups from arabinoxylan chains. Thus there is no assurance that the xylan found in the alkali-soluble fraction

did not possess, in the native state, arabinose side groups that became hydrolysed off and contributed to the fraction soluble in hot dilute acid. The present extraction procedure, including the use of hot dilute acid, was adopted primarily because this type of procedure has been employed in most of the radioisotope studies on cell-wall metabolism of oat-coleoptile tissue (see references cited in the introduction), but the fractions evidently may to some extent be artifacts.

The findings on changes in monosaccharides during coleoptile-cylinder elongation indicate that when sugar is not supplied in the medium there occurs a striking decrease of glucose in the fraction soluble in hot dilute mineral acid, and a more or less compensating increase in α -cellulose glucose. We must consider whether the decrease in glucose soluble in hot dilute acid represents simply disappearance of starch, rather than a decrease in a true cell-wall glucan.

The decrease observed in glucose in Tables 3 and 4 amounts to about 4.4 % of the cell wall or to slightly more than 50 % of the total glucose present in non-cellulosic polysaccharides. Bishop *et al.* (1958) obtained only 1.2 % of iodine-positive glucan from oat-coleoptile holocellulose, comprising apparently about 7 % of the non-cellulosic glucose. Unpublished fractionation studies by Dr Shunji Wada in this Laboratory indicate the presence of three distinct iodine-positive glucans in oat-coleoptile cell-wall preparations, making up approx. 22 % of the non-cellulosic glucan. Although these two findings are not in close agreement, they both suggest that the decrease in non-cellulosic glucose observed in the present experiments was considerably greater than the content of iodine-positive glucan. Hence the observed fall in the amount of glucose probably involves hemicellulose components.

Questions about starch do not arise with regard to the decrease observed in galactose, although the decreases in Tables 3 and 4 were not so conspicuous as with glucose. The finding agrees with the report of Matchett & Nance (1962) indicating metabolic turnover of galactan in pea-stem cell walls, and with the decreases in cell-wall galactan with time found by Jermyn & Isherwood (1956) and Thornber & Northcote (1961).

The decrease in hemicellulose glucose and galactose, or the increase in α -cellulose glucose, or both, might be involved in the mechanism of cell enlargement. The present results do not establish a causal relationship, but indicate that some cell-wall polysaccharides are not inert during cell enlargement. Metabolic dissolution of cell-wall polysaccharides was suggested as the basis for cell enlargement in pea-stem segments by Matchett & Nance (1962) and Maclachlan & Young (1962).

SUMMARY

1. The yields of monosaccharides obtained by the hydrolysis of extracts of oat-coleoptile cell-wall material are reported. The material has a high content of hot water-insoluble arabinose, probably mainly derived from arabinoglucuronoxylans.

2. Changes in amounts of monosaccharides that occur during elongation of isolated oat-coleoptile cylinders were determined. When sugar was supplied in the medium, a general increase in cell-wall monosaccharides occurred. When elongation occurred without sugar, a decrease in non-cellulosic glucose and galactose and an approximately compensating increase in α -cellulose glucose was observed.

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Particle Uptake by Polymorphonuclear Leucocytes and Ehrlich Ascites-Carcinoma Cells

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It is now well known that the rate of oxygen uptake by guinea-pig polymorphonuclear leucocytes is accelerated by the presence of particles that are engulfed by these cells; for example, insoluble starch particles, polystyrene-latex spherules, or particles of carbon and sulphur (Sbarra & Karnovsky, 1959; Iyer, Islam & Quastel, 1961), or bacteria such as *Mycobacterium tuberculosis*, *Bacillus subtilis*, *Escherichia coli* (Stähelin, Suter & Karnovsky, 1956a, b; Iyer *et al.* 1961). It is also known that the phagocytic stimulation of respiration by the particles taken up is proportional to the amount of particles present [e.g. with starch (Sbarra & Karnovsky, 1959) or with killed *E. coli* (Iyer *et al.*

1961)] until a maximum value is reached. According to Iyer *et al.* (1961), phagocytic stimulation of metabolism is proportional, within certain limits, to the amount of dead bacteria engulfed by the leucocytes and to the number of leucocytes used. It was suggested that the process of phagocytic stimulation might be used for an assay method for phagocytosis.

However, since phagocytosis takes place under anaerobic conditions as well as aerobically, and the presence of substances that suppress respiration may be without effect on the phagocytic process, it is unlikely that a satisfactory assay of the extent of phagocytosis can be based solely on measure-