A Function of the Golgi Apparatus in Polysaccharide Synthesis and Transport in the Root-Cap Cells of Wheat

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1. A radioautographic study of the cells of the root tips of wheat incubated with D-[1- or 6-^{3}H]glucose has shown that labelled material is formed in the golgi apparatus of the root-cap cells. This material passed to the vesicles associated with the golgi bodies and then moved through the cytoplasm across the plasmalemma and was incorporated into the cell wall and slime layer of the tissue. 2. Analysis of the labelled material extracted from the root tips showed that the bulk of the radioactive material was polysaccharide; there were relatively small amounts of labelled lipids and protein in the tissue. 3. Starch was formed from the exogenous labelled glucose and it was located in the plastids of the cell. The synthesis of starch depended on the metabolic activity of the cells, which varied with the position of the cell in the various tissues of the root tip and with the amount of the exogenous glucose. 4. Isolation of the radioactive polysaccharides from the root tip incubated in the radioactive glucose has shown that the glucose was very rapidly incorporated into the galactosyl residues of the polymers. 5. Analysis of the radioactive polysaccharides has indicated that the material transported in the golgi vesicles is probably pectic substance. 6. A scheme for the synthesis of the storage and wall polysaccharides by separate routes and their location within the cell has been put forward.

During the last 5 years there have been many indications that part of the synthesis of cell-wall material in plants takes place away from the site of wall formation and that the material is subsequently transported in vesicles to the wall where it is incorporated across the plasmalemma (cell membrane) by a process of pinocytosis (Northcote, 1962). During pinocytosis the membrane of the vesicle fuses and becomes continuous with the plasmalemma, and the content of the vesicle is passed out of the cell without any breakage of the cell membrane. This evidence is based on observations with the electron microscope, when it is seen that the golgi apparatus in particular seems to produce both the vesicles and the wall material. However, no direct evidence has yet been presented for this process. The golgi apparatus of plant cells is an organelle consisting of a stack of about six cisternae each bounded by a unit membrane and each approx. 0·6–1μ across. These cisternae are arranged on top of one another like a pile of plates.

The work described below has attempted two correlated lines of investigation: (*a*) a radioautographic study, in which 'pulses' of radioactive labelled glucose were applied to growing root tips, and the sequence of the movement of the label within the cell after various periods was followed by means of electron microscopy; (*b*) a direct chemical isolation and analysis of the resultant labelled material that had been incorporated into the wall and that was detected by the radioautographic investigation.

MATERIAL AND METHODS

Seedlings. Wheat seeds (*Triticum vulgare*) were soaked in water overnight, washed and germinated on damp filter paper. When the roots were about 1cm. long they were washed gently, blotted on filter paper and used for the experiments.

Chemical fractionation of root tips. The tissue was heated with 3% (w/v) NaOH (1ml.) for 1hr. in a boiling-water bath and the solution was separated from the insoluble material by a fine pipette. The extraction was repeated and the tissue was washed twice with boiling water (1ml.). The alkali solution and washings were combined. The insoluble residue represented the α-cellulose fraction.

The solution was dialysed against two changes of water (each 10l.) overnight at 4°, and then 1ml. of an α-amylase preparation (Olaitan & Northcote, 1962) was added to the non-diffusible material in solution and it was left for 6hr. at room temperature. The activity of the α-amylase solution was checked against a 1% (w/v) solution of soluble starch that reached the achromic point with iodine in 2hr. at room temperature.

The solution was redialysed against two changes of water

(each 10l.) at 4° for 24hr. and the solution inside the dialysis sac was freeze-dried. The residue represented the hemicellulose fraction.

Hydrolysis of polysaccharides. The polysaccharides were dissolved in 1 or 2 drops of H_2SO_4 (72%, w/v) at room temperature and the solution was diluted to 3% H_2SO_4 and autoclaved at 120° for 1hr. The solution was neutralized with $BaCO_3$ and filtered and the residue was well washed. The filtrate and washings were combined, deionized with Amberlite IR-120 (H^+ form) (British Drug Houses Ltd., Poole, Dorset) and freeze-dried.

Hydrolysis of whole root tips for amino acids. Dried lipid-extracted root tips (six) (see below) were hydrolysed in 6N-HCl for 24hr. in a sealed tube at 100°. The solution was evaporated to dryness under reduced pressure at 100° and re-evaporated four times with 5ml. of water. The dried residue was dissolved in 0·2ml. of water and applied to an electrophoretogram.

Lipid extraction. Dried root tips (six) were extracted with boiling dry methanol (4ml.) for 6hr. The roots were washed in methanol, and the extract and washings were combined and evaporated to dryness. The root tips were redried in a vacuum desiccator and then further extracted with boiling dry diethyl ether (5ml.) for 6hr. The roots were washed in ether, and the extract and washings were combined and evaporated to dryness.

Chromatography. Neutral monosaccharides present in polysaccharide hydrolysates were separated by descending paper chromatography on Whatman no. 1 paper in ethyl acetate–pyridine–water (8:2:1, by vol.) (solvent *A*).

The sugars were detected and estimated by the aniline hydrogen phthalate method of Wilson (1959) and results were calculated as percentage of each glycosyl radical present. Standard solutions of the monosaccharides were made up in saturated benzoic acid solution and stored at 4°. Definite volumes of standard and unknown solutions were applied to chromatograms by means of E-mil pipettes (H. J. Elliott Ltd., Treforest, Glamorgan).

Uronic acids were separated by chromatography with ethyl acetate–acetic acid–pyridine–water (5:1:5:3, by vol.) (solvent *B*) in a tank equilibrated with ethyl acetate–pyridine–water (40:11:6, by vol.) (Fischer & Dörfel, 1955).

Electrophoresis. Sugars were separated from amino acids on strip supports of Whatman no. 1 paper (57cm. × 25cm.; effective length 45cm.). The starting line was 19cm. from the anode. Electrodes were of platinum foil.

Electrophoresis was carried out with acetic acid (8%, v/v)–formic acid (2%, v/v) buffer, pH2·1, at 2000v and 50mA for 1hr. in a tank filled with white spirit (Esso Petroleum Co. Ltd., Cambridge), which was cooled by water in a glass coil.

Sugars were detected by the aniline hydrogen phthalate dip and the amino acids by a 0·2% solution of ninhydrin in acetone, which was used as a dip.

Radioactive chemicals. The glucose used was D-[6-^{3}H]-glucose of specific activity 1·3c/m-mole, D-[1-^{14}C]glucose of specific activity 4·0mc/m-mole (The Radiochemical Centre, Amersham, Bucks.) and D-[1-^{3}H]glucose of specific activity 350mc/m-mole (Volk Radiochemical Co., Skokie, Ill., U.S.A.).

Counting procedure. The chromatograms and electrophoretic supports were cut down their length into a series of consecutively numbered 1cm.-wide strips; each series corresponded to an initial spot on the starting line. The strips were placed in the counting vials together with 0·5ml. of toluene scintillator fluid [1,4 bis-(5-phenyloxazol-2-yl)benzene (0·005%) and 2,5-diphenyloxazole (0·35%) dissolved in toluene] and counted for 10min. in a scintillation counter (Tri-Carb Liquid Scintillator Spectrometer, series 314A; Packard Instrument Co. Inc., La Grange, Ill., U.S.A.). The position of the radioactive material found in the strips was compared directly with marker spots that were shown up by the aniline hydrogen phthalate dip on the same chromatogram or electrophoretogram.

Radioautography. (*a*) Light microscopy. The tissue was fixed with glutaraldehyde (6%, v/v) in phosphate buffer, pH7, for 2hr. It was then washed, dehydrated in ethanol and embedded in paraffin wax (m.p. 49°). Sections (6–8μ) were collected on gelatine-coated glass slides. After removal of the wax in xylene the sections were hydrated in an ethanol series and placed finally in water or in EDTA (50mM in phosphate buffer, pH7). The EDTA extraction was carried out at 70° for 2–5hr. and the extraction with water was carried out at room temperature.

After extraction the slides were coated with photographic emulsion (Ilford L–4 diluted 1:1 with water) by pipetting the solution on to the slides and allowing them to drain. This latter operation was done in the dark room with a safety light (Wratten series 1; 25w bulb). The coated sections were stored for 3 days in light-tight boxes containing silica gel and then developed and fixed (Ilford ID–19 for 6min., Kodafix diluted 1:5 for 10min.). After washing the slides were dried and examined with phase optics.

(*b*) Electron microscopy. The tissue was fixed in $KMnO_4$ (4%, w/v) containing NaCl (90mM), KCl (3·5mM) and $CaCl_2$ (1·2mM) (Pickett-Heaps & Northcote, 1965) for 2hr. After washing in water it was dehydrated in ethanol series and finally in propylene oxide and then embedded in Araldite resin.

Sections were cut on a mechanical advance microtome with glass knives. 'Gold' and 'silver' sections were collected on carbon-coated copper grids and these were stained with lead (Millonig, 1961) for 5–15min. After staining the sections were coated with carbon (Koehler, Mühlethaler & Frey-Wyssling, 1963) and then photographic emulsion (Ilford L–4) according to the method of Caro & van Tubergen (1962). The grids after being coated with the photographic film were kept for various periods (2–4 weeks) at 4° in small light-tight boxes containing silica gel.

The film was developed in filtered Ilford ID–19 for 1min., washed in water and fixed in Kodafix (diluted 1:4) for 1min. The grids were then washed and soaked in water for 10min., dried and examined with an electron microscope (Phillips EM 100) at 80kv.

RESULTS

Radioautographic observations

The sections of the tissue used for the radioautographic work were very similar to those taken from the fresh tissue without the incubation in the radioactive glucose (Mollenhauer, Whaley & Leech, 1961) and thus there seems to be little damage or alteration of the metabolism of the root tip either from the incubation procedure up to a period of 3hr. or because of the incorporated radioactivity.

Continuous incorporation. Four root tips were used for each experiment. The tips (1·5mm. long) were excised with a clean razor blade on a glass slide and immediately placed into a solution of D-[6-^{3}H]glucose (0·2ml., 0·2mc) in a small test tube lightly plugged with cotton wool. After incubation for 0·5–6·0hr. at room temperature the tips were removed, washed in water and prepared for electron microscopy. Similar experiments were performed with D-[1-^{3}H]glucose (0·1ml., 0·1mc) with incubation times of 0·5–4·0hr.

After exposure of the root tips to the radioactive glucose for 30min. labelled material was found in the golgi apparatus, its associated vesicles, the wall and in the new material of the wall under the plasmalemma (*m* and *n* on Plate 4). Similar results were also observed after exposure for 15min. to the D-[6-^{3}H]glucose and during the next few hours the label accumulated steadily in the external layer of material surrounding this tissue. This layer is presumably the protective slime of the root-cap cells (*k* and *l* on Plate 3).

The golgi bodies of the internal root-cap cells are not hypertrophied (Mollenhauer, 1959) and the vesicles incorporated into the wall of these cells are smaller than those of the outer root cap (Pickett-Heaps & Northcote, 1965). The radioactive label is found in the small golgi vesicles and in positions at the wall where it is known that vesicular incorporation occurs (*p* on Plate 4), but it is also found in various other parts of these cells. A continuous incorporation of labelled material into the wall was evident when the period of incubation of the tissue in the labelled glucose was extended for several hours.

An important difference in the labelling pattern of the organelles of the outer and more internal cells of the root cap can be seen by comparison of the plastids in these cells (*s* and *t* on Plate 5). This difference indicates variations in the metabolism of the cells. The outer root-cap cells of excised tissue that was incubated in media containing only the very small amounts of the D-[6-^{3}H]glucose (47μg.) contained plastids in which only very little of the label had been incorporated into their starch grains (*t* on Plate 5). In the more internally situated cells of the same tissue the plastids contained starch that had become appreciably labelled (*s* on Plate 5). Plastids in these two types of cell in a single radioautographic section are shown in (*s*) and (*t*) on Plate 5.

Long-pulse incorporation. The previous experiments showed that the labelled glucose was incorporated very quickly into the material of the cell, and an examination of the sections of the excised roots indicated that these underwent cytological changes after about 3hr. Pulse experiments with intact tissue were therefore designed to investigate the metabolism of the supplied radioactive glucose in more detail.

Intact seedlings were gently washed and blotted dry with filter paper. These were then placed with one of their root tips dipping into a drop (0·05ml.) of D-[6-^{3}H]glucose solution (2mc/ml.) placed on a clean glass slide covered by a Petri dish. The atmosphere within the Petri dish was kept moist by means of damp filter paper that was not in contact with the seedlings. Seedlings were removed and prepared for electron microscopy after 15 and 30min. incubation. The remainder of the plants were incubated for 45min. in the radioactive solution and were then washed with water, and the particular root tip was placed in a solution of unlabelled D-glucose (1·0ml., 500μg.) (i.e. an unlabelled glucose 'chase') in a Petri dish for 0·25–3·25hr. and then prepared for electron microscopy.

A radioautograph of root-cap cells after incubation in a solution of unlabelled glucose for 2·25hr. is shown in (*q*) on Plate 5. The label is no longer present to any extent in the golgi apparatus nor in the associated vesicles. The label is much more randomly distributed in the cell but there is a considerable concentration of labelled material in the starch grains of the plastid and in the slime and walls (*q* and *r* on Plate 5).

The internal root-cap cells also show a similar random distribution of the label after the chase with unlabelled glucose although here again the accumulation of labelled material in the starch grains and wall is very apparent (*o* on Plate 4).

Short-pulse incorporation. The incubation procedure was similar to that described for the long-pulse incorporation experiments. The intact seedlings were treated with D-[6-^{3}H]glucose (0·05ml., 5mc/ml.) and were removed and prepared for electron microscopy after 5 and 10min. incubation. The remainder of the plants were placed in unlabelled glucose (1·0ml., 1·0mg.) for 10, 30 and 60min. before being prepared for electron microscopy.

The results of these experiments are shown in (*a*)–(*j*) on Plates 1–3. These radioautographs were prepared from sections all of which were exposed to the photographic emulsion for the same period (3 weeks).

After 5min. exposure to the radioactive labelled glucose, derivatives appear within the cell but these are confined mainly to those areas of the section representing the golgi apparatus of the cell (*b* on Plate 1). The labelling of the cytoplasm is generally sparse and no labelled material appears in the wall although new wall substance can be detected in the section under the plasmalemma (*a* and *b* on Plate 1). Within 10min. the golgi apparatus and its associated vesicles are markedly labelled (*c* on Plate 1) but even at this stage there is

little labelled material deposited in the wall (*d* on Plate 1). After 10min. incubation in the radioactive labelled glucose followed by incubation in unlabelled glucose for periods of 10, 30 and 60min., there is a progressive loss of radioactivity from the golgi apparatus and vesicles and a progressive increase in the radioactivity of the material of the wall and slime (*e–j* on Plates 2 and 3).

Radioautography with the light microscope. Excised root tips were placed in D-[6-^{3}H]glucose (0·1ml., 2mc/ml.) in a test tube for 2hr.

The sections from these root tips were extracted with EDTA or cold water before being prepared for microscopy. In the sections treated with water, radioactive labelled material was detected in the slime layer around the root cap and in the walls of the cells (*u* on Plate 6), whereas in the sections treated with EDTA the labelled material of the slime layer was nearly all removed (*v* on Plate 6).

Chemical investigation

Root tips (5·8mg.) were divided into cap (2·4mg.) and meristematic portions (3·4mg.) and each portion was separated into α-cellulose and hemicellulose fractions. These were analysed for their component sugars and the results are shown in Table 1.

The course of the incorporation of the radioactive glucose into the root tips was followed by the analysis of material obtained from the tips after incubation in the glucose at room temperature for 0·25, 0·5, 1, 2, 3 and 4hr. For each incubation, six root tips cut from two seedlings were placed in a solution of D-[6-^{3}H]glucose (0·2ml., 1mc/ml.). After incubation, the tips were washed with water and placed in ethanol (50%, v/v). The root tips were subdivided into a cap (the apical 0·7–1·0mm. segment) and a meristematic portion (the remaining 0·5–0·7mm. segment) and these were analysed separately into α-cellulose and hemicellulose fractions, which were hydrolysed and the monosaccharides were separated chromatographically (solvent *A*). The results are shown in Tables 2 and 3.

The strips of filter paper carrying the origin spots of hemicellulose hydrolysates (Table 2) after the 1, 2, 3 and 4hr. incubations were collected, washed with toluene to remove the scintillator, dried in a current of cold air and eluted with water, and the aqueous solution was divided into two halves. One half was rehydrolysed with sulphuric acid (3%, w/v). The neutralized hydrolysate and the unhydrolysed solutions were freeze-dried. The residues were run on a chromatogram (solvent *B*) with galacturonic acid and glucuronic acid markers. No radioactive material coinciding with the uronic acid markers was detected. Two components (A and B) with positions relative to galacturonic acid (R_{GalA}) 0·85 and 1·4 respectively were found in the unhydrolysed material, and relatively small amounts of component A together with mannose and galactose were detected in the hydrolysed solution.

Separate experiments to determine the total amount of radioactivity taken up by the excised roots and the percentage recovered in the sugars of the hemicellulose, starch and α-cellulose fractions, the protein and lipid were carried out by incubating six root tips in the D-[6-^{3}H]glucose (0·2ml., 1mc/ml.) for 2hr. and washing the roots five times in unlabelled glucose (1ml., 2%, w/v) which was added to the remaining radioactive incubation

Table 1. *Composition of polysaccharide fractions of root tissue*

	% dry wt. of whole tissue	% dry wt. of segment	Sugar components of each polysaccharide (% of total sugars estimated)				
			Gal	Glc	Ara	Xyl	Man
Tip segment	41						
Hemicellulose fraction		10·5	15·1	42·5	25·5	17·5	Trace
α-Cellulose fraction		9·5	Trace	71·0	10·0	19·0	Trace
Meristem segment	59						
Hemicellulose fraction		14·0	13·0	32·0	27·0	28·0	Trace
α-Cellulose fraction		10·0	Trace	73·0	15·0	12·0	Trace

EXPLANATION OF PLATES 1–6

Abbreviations: W, Wall of cell; S, slime layer; G, golgi apparatus; GV, vesicles derived from the golgi apparatus; ER, endoplasmic reticulum; P, plastid; M, mitochondria; EC, epidermal cell of the root meristem; V, vacuole; N, nucleus; XC, external root-cap cell; IC, internal root-cap cell. (*a*)–(*j*) show the passage of labelled material formed from a short pulse of radioactive labelled glucose over a period of 70min. in the outer root-cap cells. All the radioautographs were treated in an identical manner.

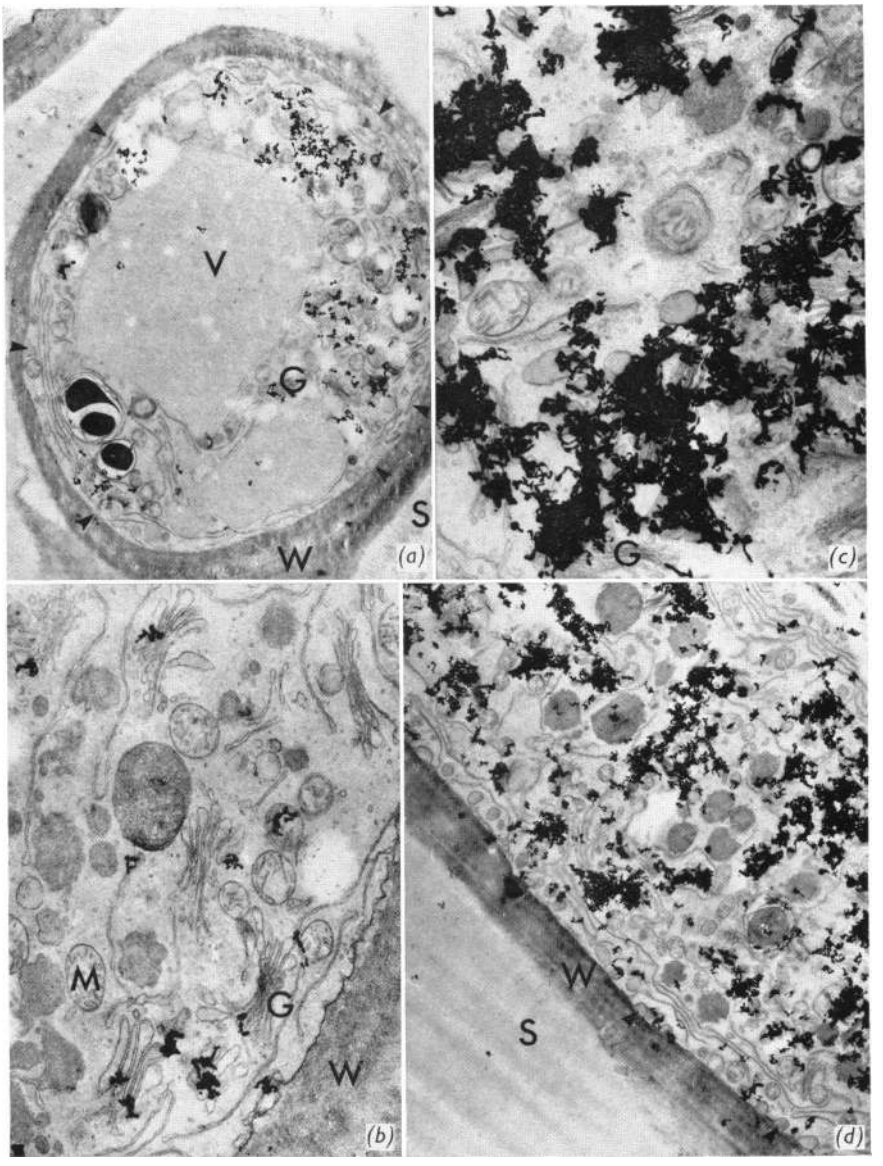

Plate 1. (*a*) Tissue exposed to D-[6-^{3}H]glucose (5mc/ml.) for 5min. before fixation; note the generally sparse labelling of the cell. New material being added to the wall is arrowed. Magnification × 3750. (*b*) A higher magnification of a cell from the same section as that shown in (*a*). Most of the labelled derivatives are located in the golgi apparatus. Magnification × 11 625. (*c*) Tissue had been exposed to D-[6-^{3}H]glucose (5mc/ml.) for 10min. before fixation. A massive accumulation of the labelled derivatives in the golgi apparatus is evident. Magnification × 14 435. (*d*) A longitudinal section of a root-cap cell, equivalent to that shown in (*c*). The labelled material is concentrated in the golgi apparatus. New material (arrowed) is being added to the wall but this and the wall are almost free of the label. Magnification × 3750.

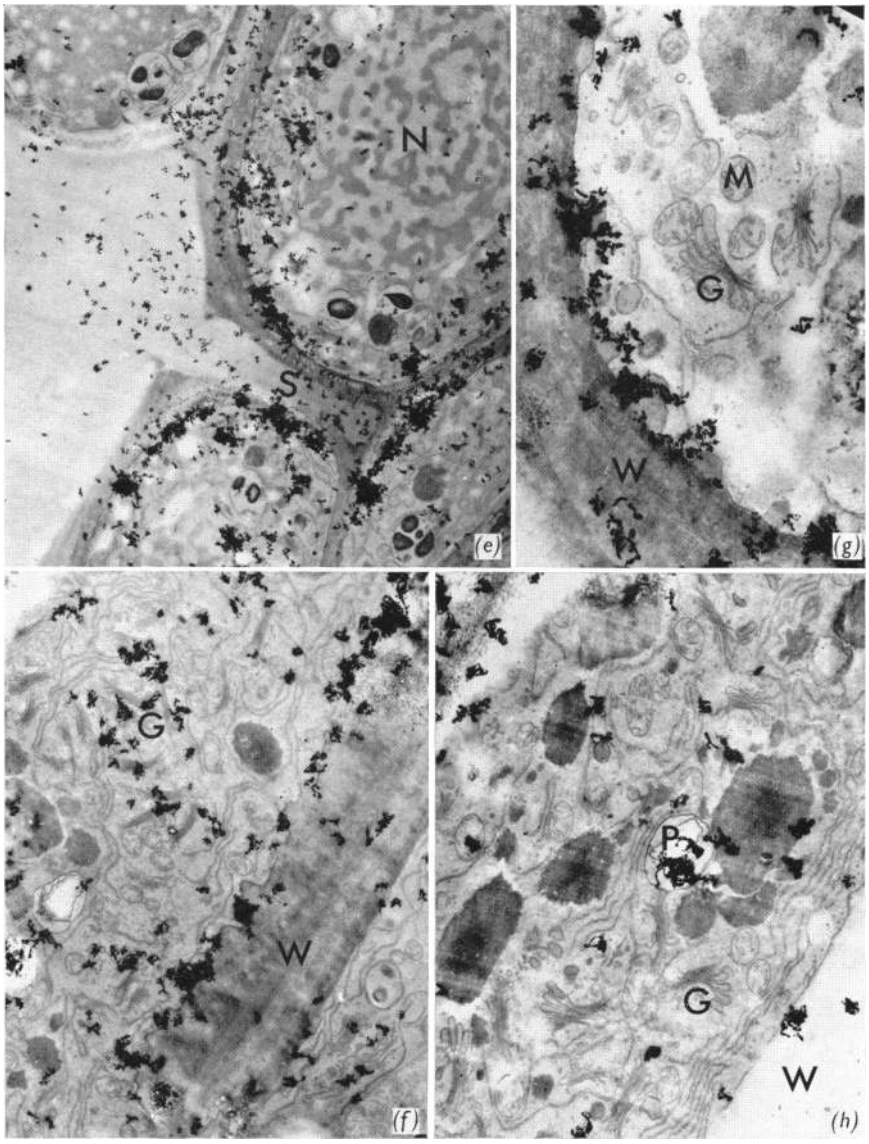

Plate 2. (*e*) After exposure to the labelled glucose (10 min.) the cells had been placed in a chase of unlabelled glucose (10 min.). Labelled material is apparent in many areas of the internal wall surfaces; traces of activity are also apparent in the slime material. The plastids show traces of radioactive incorporation. Magnification × 3000. (*f*) A higher magnification of cells equivalent to those shown in (*e*). The golgi bodies are still labelled, but now the new wall material also contains some activity. Magnification × 4875. (*g*) After exposure to the labelled glucose (10 min.) the cells had been placed in a chase of unlabelled glucose (30 min.). The golgi have lost almost all of their activity and the new wall material and the wall itself contain appreciable amounts of the labelled derivatives. Magnification × 10 685. (*h*) Another section equivalent to that shown in (*g*). The absence of labelled material in the golgi bodies is shown more clearly. The starch grain of the plastid has accumulated some radioactivity. Magnification × 7500.

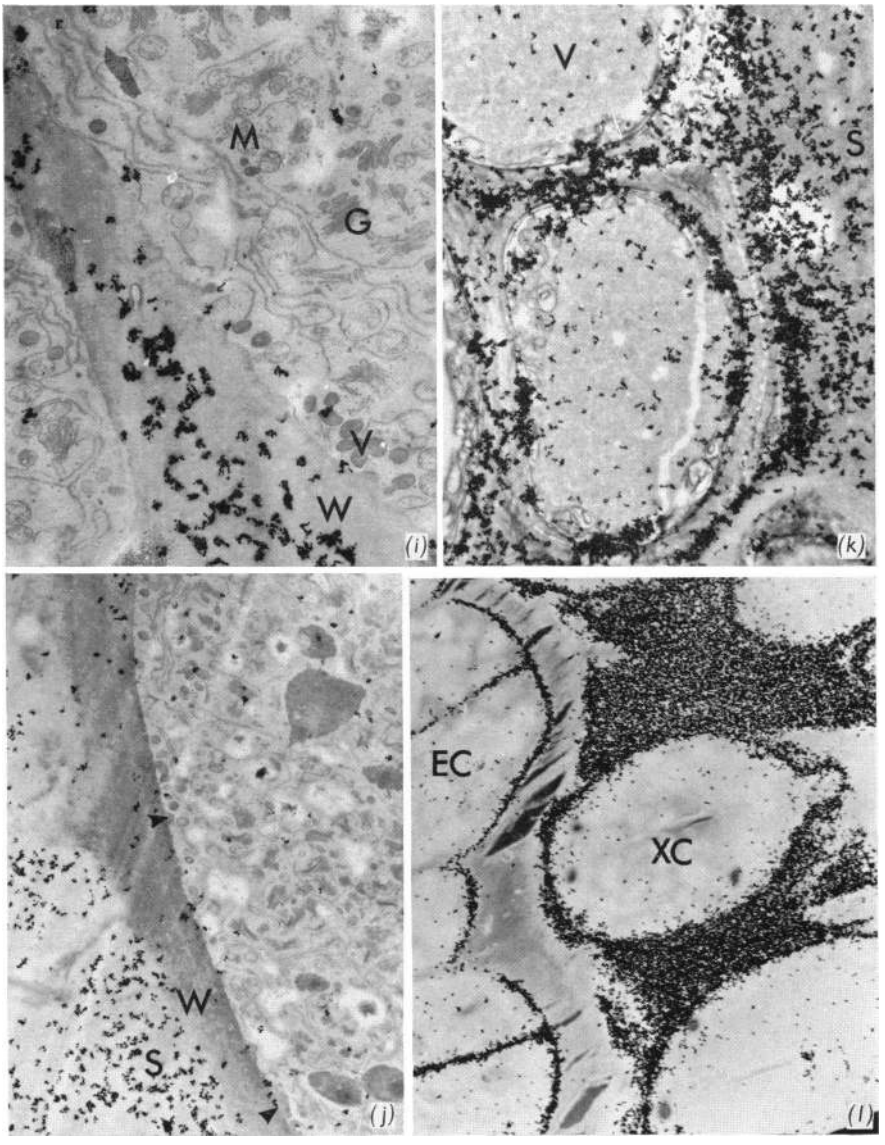

Plate 3. (*i*) The chase of unlabelled glucose in this experiment has been extended to 60 min. The cell contains little activity. The golgi vesicles contain very small amounts of label. There is a band of activity in the wall between the cells. Magnification × 5625. (*j*) A longitudinal section of a root-cap cell equivalent to that shown in (*h*). The small amounts of labelled material still present in the cytoplasm and the various cell organelles are clearly shown. An accumulation of radioactive material in the outer slime layers of the root cap is evident. Magnification × 3375. (*k*) Outer cap cell. Excised root tips were exposed to D-[1-^{3}H]glucose (1mc/ml.) for 2 hr. The specific activity of this glucose was much lower than that of the D-[6-^{3}H]glucose. Large amounts of labelled derivatives can be seen on the internal surface of the wall, in between the cells, and particularly in the outer slime layer. Magnification × 3185. (*l*) Excised root tips were exposed to D-[6-^{3}H]glucose (1·0 mc/ml.) for 4 hr. An unstained transverse section of cap cells over the epidermal cells (EC) of the root meristem is shown. The large accumulation of labelled material in the slime layer of the cap cells is typical of that seen in all radioautographs taken from tips that were incubated for various periods in the labelled glucose without subsequent incubation in unlabelled glucose. Incorporation has also occurred in the walls of the epidermal cells. Magnification × 1910.

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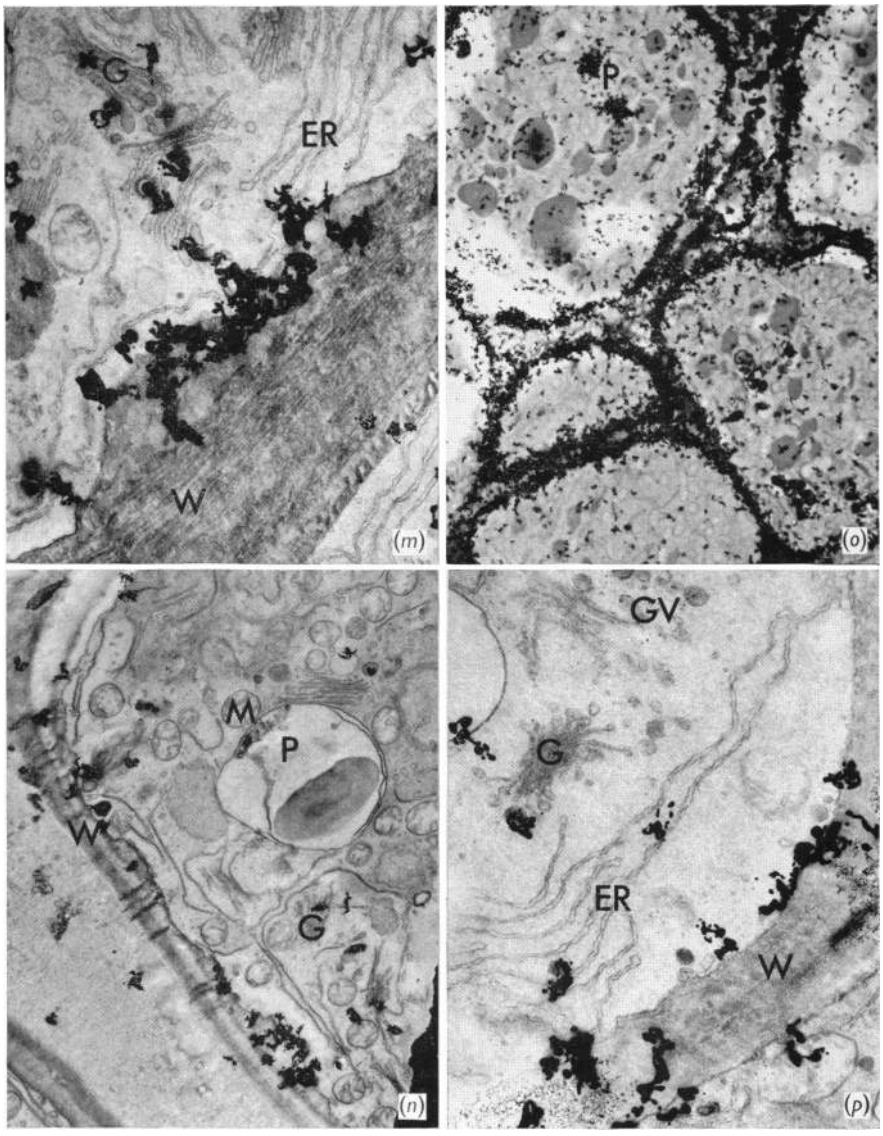

Plate 4. (*m*) Outer cap cell. The excised tissue was exposed to D-[6-^{3}H]glucose for 30 min. The radioautograph clearly shows the labelling of the new wall material and the golgi apparatus. Magnification × 13685. (*n*) Outer cap cell. The excised tissue was exposed to D-[1-^{3}H]glucose for 30 min. This labelled precursor was incorporated into the new wall material and golgi apparatus of the cell in a similar manner to that shown with D-[6-^{3}H]glucose (*m*). Magnification × 13685. (*o*) Long-pulse experiment. The intact root tips were exposed to D-[6-^{3}H]glucose (2 mc/ml.) for 45 min. followed by a chase of unlabelled glucose for 3·25 hr. The label is randomly distributed in these internal root-cap cells. The wall and starch grains show some accumulation of the label. Magnification × 1875. (*p*) Short-pulse experiment in which the experimental conditions were similar to those stated in the legend to (*g*). The golgi vesicles and the wall between these internal root-cap cells contain labelled material. Magnification × 16875.

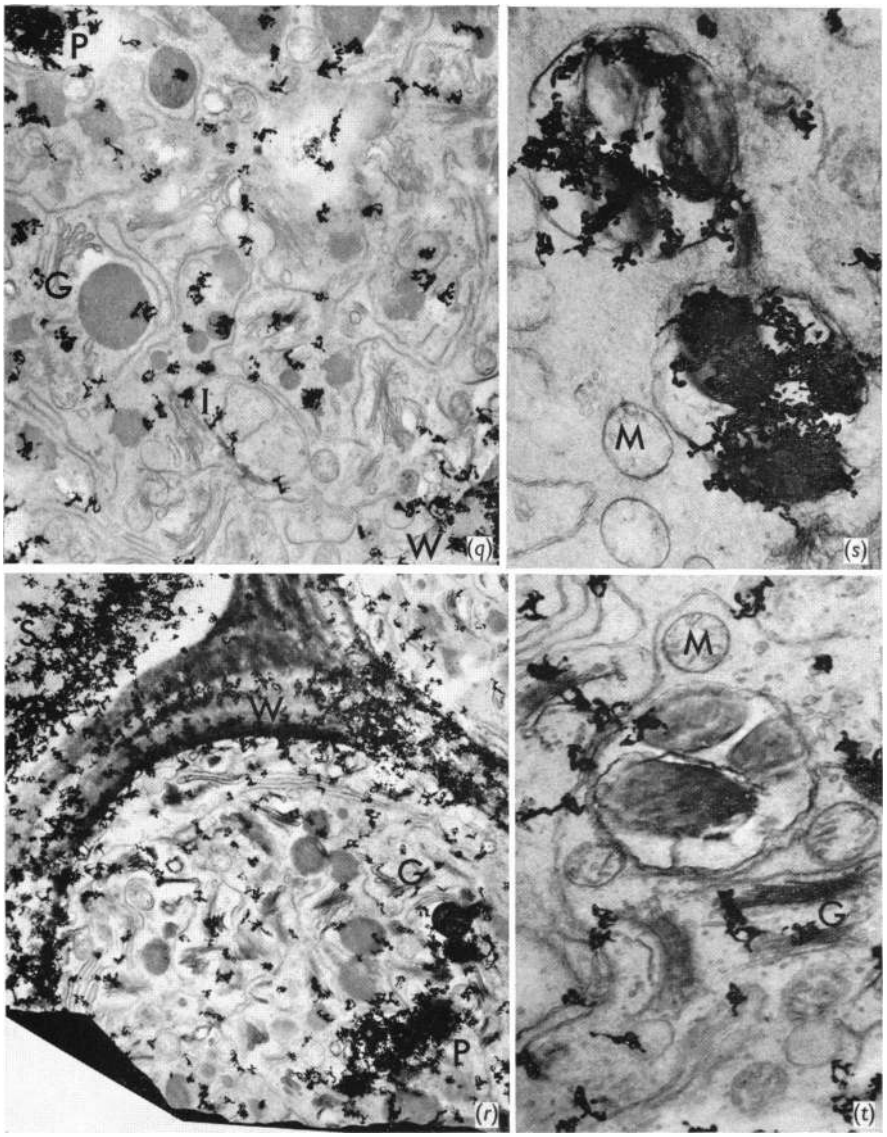

Plate 5. (*q*) Long-pulse experiment: outer cap cells. Intact root tips were exposed to D-[6-^{3}H]glucose (2 mc/ml.) for 45 min. followed by an incubation in unlabelled glucose for 2·25 hr. The label is randomly distributed in the cells. The golgi apparatus is free of activity. The starch in the plastid and the wall material is labelled. Magnification × 8810. (*r*) Long-pulse experiment: outer cap cell. Intact root tips were exposed to D-[6-^{3}H]-glucose (2 mc/ml.) for 45 min., followed by an incubation in unlabelled glucose for 3·25 hr. The starch in the plastids of the external cap cells is clearly labelled (compare with *t*). The golgi bodies are almost free of the label, but an incorporation of radioactivity into the wall is evident. The outer slime layer of the cells show an accumulation of labelled material. Magnification × 6975. (*s*) Excised root tips were exposed to D-[6-^{3}H]glucose (1 mc/ml.) for 1 hr. The starch in the plastids found in the internal root-cap cells is heavily labelled. Magnification × 15 750. (*t*) This photograph shows a typical plastid found in external root-cap cells after exposure of root tips to D-[6-^{3}H]glucose (1 mc/ml.) for 1 hr. It was taken from the same grid square as that containing the cell shown in (*s*). Magnification × 15 750.

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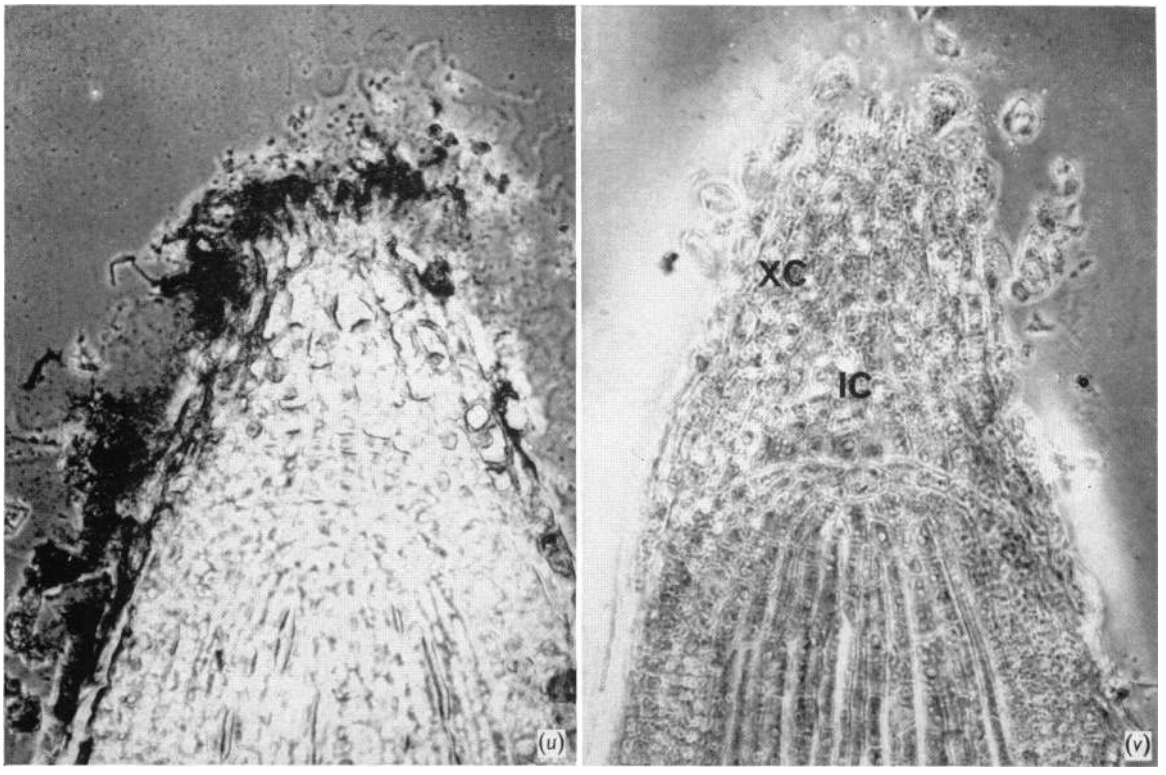


Plate 6. (*u*) Radioautograph of a longitudinal section of an excised root tip incubated in D-[6-^{3}H]glucose (0·1ml., 2mc/ml.) for 2hr. The section was extracted with water before the photographic film was put over the section. The labelled material of the slime layer of the cap is clearly visible. Magnification ×180. (*v*) Radioautograph of the same root tip used in the preparation shown in (*u*) but the section was treated with hot EDTA before the application of the photographic film. Magnification ×180.

Table 2. *Radioactivity of separated sugars isolated on chromatograms from the hydrolysate of polysaccharide fractions obtained from the cap segments of the root tips*

The root tips were incubated in D-[6-^{3}H]glucose (0·2ml., 1mc/ml.).

	Time of incubation (hr.)	Radioactivity				
	0·25	0·5	1	2	3	4
Hemicellulose fraction						
Total counts/min.	1805	2435	8330	11020	14810	14310
Origin material (% of total counts/min.)	9·8	10	9·1	9·8	13	11
Galactose (% of total counts/min.)	70	52	53	54	52	52
Glucose (% of total counts/min.)	11	21	22	17	14	15
Mannose (% of total counts/min.)	3·5	2·7	2·7	2·9	2·7	2·4
Arabinose (% of total counts/min.)	4·4	8·4	7·4	10	11	12
Xylose (% of total counts/min.)	1·4	5·4	5·5	7·5	8·1	8·2
Cellulose fraction						
Total counts/min.	958	6312	2930	10970	14800	12000
Origin material (% of total counts/min.)	5·5	9·5	5·5	3·2		
Galactose (% of total counts/min.)	85	65	61	61		
Glucose (% of total counts/min.)	8	18·5	29	34		
Mannose (% of total counts/min.)	1·6	3·8	2·0	1·3		
Arabinose (% of total counts/min.)	1	2·7	1·7	0·8		
Xylose (% of total counts/min.)	0·5	0·6	1·0	0·2		

Table 3. *Radioactivity of separated sugars isolated on chromatograms from the hydrolysate of polysaccharide fractions obtained from the meristematic segments of the root tips*

The root tips were incubated in D-[6-^{3}H]glucose (0·2ml., 1mc/ml.).

	Time of incubation (hr.)	Radioactivity				
	0·25	0·5	1	2	3	4
Hemicellulose fraction						
Total counts/min.	598	428	3410	18880	10840	12740
Origin material (% of total counts/min.)	11	21	19	13	22	13
Galactose (% of total counts/min.)	35	30	34	33	30	41
Glucose (% of total counts/min.)	30	34	22	27	20	14
Mannose (% of total counts/min.)	1·6	—	2·8	2·4	2·4	2·1
Arabinose (% of total counts/min.)	8	6·8	14	13	15	16
Xylose (% of total counts/min.)	14	8·4	7·5	10	9·3	14
Cellulose fraction						
Total counts/min.	1720	4146	15350	26100	30400	20130
Origin material (% of total counts/min.)	1	1	2	2·4	2·7	3·6
Galactose (% of total counts/min.)	24	29	46	55	58	49
Glucose (% of total counts/min.)	72	68	50	39	34	41
Mannose (% of total counts/min.)	1	1	0·6	1	0·5	1
Arabinose (% of total counts/min.)	1	0·6	0·2	1	0·7	2·3
Xylose (% of total counts/min.)	0·6	0·3	0·7	1·3	1·2	3·1

solution. The root tips were then washed in 10ml. of cold ethanol (50%, v/v). The radioactivity of the solution before and after incubation and that of the ethanolic solution was measured so that the amount of radioactivity retained by the tissue could be calculated. The polysaccharide fractions were isolated from the roots in the usual manner, and these were hydrolysed and the sugars separated from the other material in the neutralized hydrolysates by electrophoresis at pH 2·1, and their radioactivity was measured. The radioactivity of the sugars in the polysaccharides represented 2·2% of the total radioactivity removed from the incubation solution by the tissue during the 2hr. period, and of this recovered radioactivity 73% was present in the α-cellulose fraction, 23% in the hemicellulose fraction and 4% in the starch fraction.

The lipid of six root tips incubated with the D-[6-^{3}H]glucose (0·2ml., 1mc/ml.) for 2hr. was extracted with methanol and diethyl ether. The

methanol extract contained 0·3% of the total radioactivity removed from the incubation mixture and the ether extract was completely unlabelled. The total radioactivity of the amino acids recovered from an electrophoretogram of a hydrolysate of the lipid-extracted root tips represented 0·4% of the total radioactivity removed from the incubation mixture.

Six excised root tips were incubated for 2hr. in a solution of D-[1-^{14}C]glucose (0·25ml., 0·1mc/ml.) at room temperature and the results for the analysis of the cap portions of the root tips are shown in Table 4. The material representing the origin spot of the hemicellulose hydrolysate (Table 4) was recovered from the filter-paper strip in the manner described above and it was re-run on a chromatogram with solvent *B*. The only radioactive material that was detected on this latter chromatogram ran at the same position as that of the marker galacturonic acid.

Radioactive galactose and glucose were eluted with water from the chromatograms of the hydrolysates of polysaccharides that had been obtained from root tips incubated in D-[6-^{3}H]glucose. The aqueous solutions were freeze-dried and the residues were run electrophoretically at pH 2·1 with glucose, galactose and amino acid markers. A clear separation of neutral sugars and amino acid markers was obtained. The sugars were found at 2cm. and the amino acids were detected 8–25cm. from the starting line towards the cathode. Of the total radioactivity of the applied radioactive sugars eluted from the chromatograms 90% was recovered in the neutral sugar position. The bulk of the remaining 10% formed a streak lying between the sugar and the beginning of the amino acid zones. An electrophoretogram of a fresh solution of the original D-[6-^{3}H]glucose used in the incubation experiments gave a similar quantitative pattern of the distribution of radioactivity. The sugars of the hydrolysates of the α-cellulose and hemicellulose fractions prepared from root tips incubated for 2hr. with D-[6-^{3}H]glucose were isolated from an electrophoretogram by elution with water of the zone found at 2cm. from the starting line. The total radioactivity of this zone was measured for each polysaccharide fraction and the eluates were freeze-dried and run on a chromatogram with solvent *A*. The distribution of radioactivity in the separated sugars was similar to that shown for the 2hr. incubation experiment in Table 2. The total amount of radioactivity in the various sugars accounted for 90% of the activity of the sugar zone of the electrophoretogram.

Table 4. *Radioactivity of separate sugars isolated on chromatograms from the hydrolysate of polysaccharide fractions obtained from the cap segment of the root tips*

The root tips were incubated in D-[1-^{14}C]glucose (0·25ml., 0·1mc/ml.) for 2hr.

	Radioactivity	
	Hemicellulose fraction	Cellulose fraction
Total counts/min.	1290	3260
Origin material (% of total counts/min.)	14·9	16·3
Galactose (% of total counts/min.)	16·9	36·4
Glucose (% of total counts/min.)	8·9	22·2
Mannose (% of total counts/min.)	9·6	4·1
Arabinose (% of total counts/min.)	27·4	12·0
Xylose (% of total counts/min.)	22·2	8·9

DISCUSSION

Previous electron-microscopic studies of the root tip of maize and wheat have shown that the outer root-cap cells possess characteristic hypertrophied golgi bodies (Branton & Moor, 1964; Mollenhauer & Whaley, 1963; Mollenhauer *et al.* 1961; Pickett-Heaps & Northcote, 1965). These organelles produce vesicles that seem to give rise to the massive amount of wall material and protective coating of the root tip. The cells of the tissue are thus particularly suited to a radioautographic study to characterize the material that the golgi produces and to provide direct evidence for the sequence of events leading to incorporation of material into the wall from these organelles.

The radioautography experiments have shown that within 5min. the label could be detected in the root-cap cells and was almost entirely confined to the golgi apparatus. Because of the treatment of the section before its examination by the electron microscope it seems likely that the radioactive material was insoluble in water and was therefore of high molecular weight. Within 15min. high-molecular-weight non-diffusible polysaccharides carrying the label could be isolated from the cells both in the hemicellulose and α-cellulose fractions. Analysis of these polysaccharides after incorporation of D-[6-^{3}H]glucose showed that over 70% of their radioactivity could be accounted for by the activity of the galactose content. Behind the root cap, in the portion of the root tip that contained the meristematic tissue, radioactive material derived from the administered D-[6-^{3}H]glucose could be detected on the radioautograph within 15min. of its application. In this tissue there was relatively no localization of material in the golgi bodies although some incorporation of the label into the wall had occurred. The polysaccharides extracted from this tissue contained 30–40% of their total radioactivity as galactose and the remainder was mainly that of glucose.

During the 3hr. incubation of the root tip in D-[6-^{3}H]glucose there was in both polysaccharide fractions of the root-cap tissue an increase in the proportion of the radioactivity of the glucose to the total radioactivity of the polysaccharides for the first hour of the incubation. The percentage of the total radioactivity of the galactose fell over this period since the radioactivity of the other sugars had slowly increased. It seems therefore that the pool of precursors of the glucans became saturated with the introduced radioactive glucose more slowly than the pools of the precursors of the slime and wall polysaccharides containing galactose. This would be expected in the outer root-cap cells where the passage of glucose through the precursor pools giving rise to these latter polysaccharides appeared to be very rapid indeed. The galactose, even in the α-cellulose, contributed a high proportion to the total radioactivity of this fraction, although the actual amount of galactose present is exceedingly small (Table 1). This again demonstrates very clearly the quick transformation of labelled glucose into the galactose polysaccharides of the various fractions of the wall.

The course of the labelling in the meristematic tissue (Table 3) was different from the root-cap tissues in that the radioactivity of the galactose slowly increased or remained fairly constant over a 3hr. period and that of the glucose decreased. It is likely that this difference resulted from the special processes of the cap cells that form material for their walls and the slime in addition to the polysaccharides required in the normal growth of the wall.

The radioautographs show that over a 3hr. period a very large amount of the radioactive label accumulated in the walls of the root-cap cells, and in the slime external to them, and most of the radioactivity of the tissue at this stage was confined to these regions. The chemical investigation has shown that the bulk of the radioactivity of the cells after 2hr. incubation in the radioactive glucose is present as polysaccharides and only relatively small amounts of labelled lipid and protein are present in the tissue. The radioactive material localized in the radioautographs therefore represented the position in the root tips from which the labelled polysaccharides were isolated.

The radioautographs of the pulse experiments show very clearly the dynamic sequence of the formation of the slime and wall material of the outer root-cap cells. The radioactive label was rapidly passed to the golgi apparatus through the vesicles and to the wall (*a–j* on Plates 1–3). The chemical analysis of the radioactive labelled hemicellulose derived from incubation with D-[6-^{3}H]glucose (Table 2) and D-[1-^{14}C]glucose (Table 4) showed that a large proportion of this radioactivity was due to the arabinose and galactose components. This result would be obtained if the radioactive label was rapidly incorporated into the polysaccharides of the pectic substances.

The radioactive material formed in the slime layer of the root cap was soluble in solutions of EDTA, as are the pectic substances.

The arabinose can be formed from the glucose by two pathways. One of these would involve the pentose phosphate pathway, which would form pentose sugars in which the radioactive label was randomized. The other route would involve a decarboxylation of UDP-glucuronic acid formed from UDP-glucose and an epimerization of the resultant UDP-xylose (Hassid, Neufeld & Feingold, 1959). This latter series of reactions would remove the primary alcoholic group of the glucose but leave the hemiacetal group intact. The occurrence of these two metabolic pathways for the formation of pentoses from hexoses would account for the difference in the relative amount of radioactivity recovered in the arabinose monomers when the seedlings were incubated with either D-[6-^{3}H]-glucose or D-[1-^{14}C]glucose (Tables 2 and 4).

The results of the pulse experiment show that variations in the metabolism of glucose occur in the cells of the root tip. These can be seen by the different appearance of the radioautographs of the internal and external cap cells and of these latter cells in the presence and absence of a large amount of exogenous glucose, supplied for the chase of unlabelled glucose. The outer root-cap cells synthesized very little starch because most of the soluble labelled glucose pool within the cell was channelled into the active golgi bodies of these cells and passed through them to accumulate in the slime and wall material. In the internal cells both starch and small amounts of wall material were finally formed from the soluble radioactively labelled pool. In these cells the golgi bodies and vesicles were not hypertrophied, and although they contain small amounts of labelled material they were not competing for the pool of the labelled glucose to the same extent as those of the outer root-cap cells. However, when unlabelled glucose was supplied starch synthesis did become apparent from the pool of labelled glucose within the cells of the outer root cap, and in the presence of the excess amount of glucose the label accumulated in the storage polysaccharides (*r* on Plate 5).

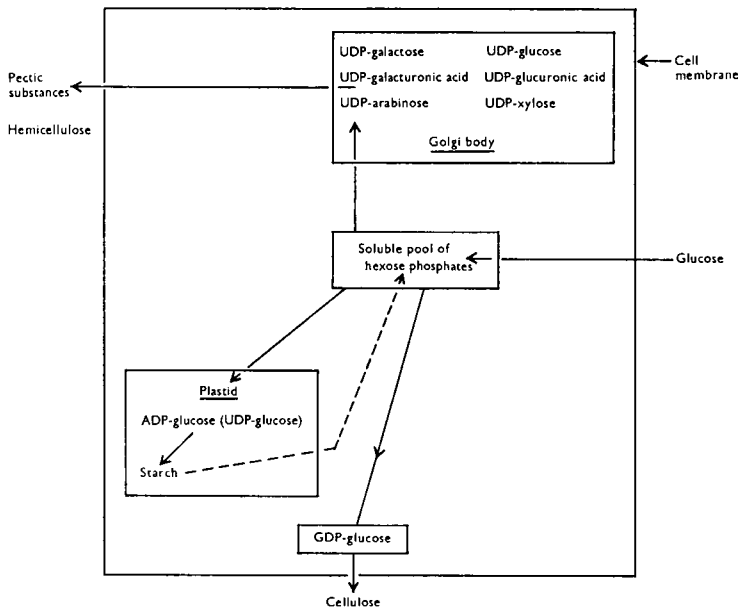
Our results are consistent with the idea that there occurs within the golgi apparatus a pool of precursors for the synthesis of polysaccharides that contain galactose, galacturonic acid and arabinose. These polysaccharides are probably identical with the pectic substances (Barrett & Northcote, 1965). The labelled glucose derivatives that enter the pool are not immediately available for cellulose

synthesis, and this is better regarded as arising from a separate pool of precursors that are derived from the same common pool of soluble glucose as those of the precursors of the galactose and arabinose (Andrews, Hough & Picken, 1965).

Galactose and arabinose monomers for polysaccharide synthesis are probably formed from UDP-galactose and UDP-arabinose that arise from the corresponding UDP-glucose compounds by epimerase activity; however, cellulose is probably formed from GDP-glucose (Barber, Elbein & Hassid, 1964) and starch from ADP-glucose and UDP-glucose (Leloir, Rongine de Fekete & Cardini, 1961; Recondo & Leloir, 1961). If this is correct then both the precursors and their locations within the cell are different for the different polysaccharides. These ideas are summarized in Scheme 1, which represents a working hypothesis for the routes of polysaccharide synthesis within the cell.

It has been indicated in animal (Caro, 1961; Caro & Palade, 1964; Porter, 1964; Wellings & Deome, 1961) and possibly in some plant tissues (Bonneville & Voeller, 1963) that the golgi apparatus is used to transport protein from the cell. The organelle may also take part in the formation and transport of mucopolysaccharides (Caro & van Tubergen, 1962; Godman & Lane, 1964; Fewer, Threadgold & Sheldon, 1964; Lane, Caro, Otero-Vilardebó & Godman, 1964; Peterson & Leblond, 1964). Manton and her colleagues (Manton, Oates & Parke, 1963; Manton & Parke, 1962; Manton, Rayns & Ettl, 1965) have shown that the golgi apparatus in certain flagellates can form inclusions of spicules and scales that are transported to the outer surface of the cell by means of vesicles formed from the cisternae of the golgi bodies, and excretory functions of the organelle in other plant tissues have been suggested (Bouck, 1962). During the growth of various plant cells material transported in golgi vesicles is incorporated into the enlarging wall (Gantt & Arnott, 1965; Rosen, Gawlik, Dashek & Seigesmund, 1963; Seivers, 1963) and also into the secondary thickenings of xylem elements and sieve tubes of sycamore stem and wheat seedling (Pickett-Heaps & Northcote, 1965; Wooding & Northcote, 1964, 1965). The polysaccharides laid down in the wall while the secondary growth takes place are derived from glucose, mannose, xylose and glucuronic acid, and very little pectic substance is used (Thornber & Northcote, 1961). Thus if polysaccharides are transported to the wall by the golgi apparatus at this secondary stage of development they are presumably those of the hemicellulose fraction.

The diverse nature of the materials transported by the golgi-body vesicles suggests that the function of the organelles varies with the metabolic conditions and state of development of the cell and that



Scheme 1. Scheme to show the separation of precursors and possible sites of synthesis of the polysaccharides formed by a plant cell.

generally it is concerned with the transport of materials in vesicles to sites outside the plasmalemma (Whaley, Kephart & Mollenhauer, 1959).

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