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Patterns of Polysaccharide Biosynthesis in Differentiating Cells of Maize Root-Tips

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1. The patterns of incorporation of radioactivity from D-[6-^{3}H]-, D-[1-^{14}C]-, D-[U-^{14}C]- and D-[6-^{14}C]-glucose and [U-^{14}C]*myo*inositol into the neutral sugars and uronic acids of the polysaccharides synthesized in different regions of the root-tip of maize were determined. 2. The root-cap tissue synthesized a slime in which a polysaccharide that contained a high proportion of fucose (32%) and galactose (21%) was found. This polysaccharide is synthesized only by the root-cap cells, and very little polysaccharide containing fucose is synthesized in adjacent tissues. Part of the meristematic tissue of the root is surrounded by the cap cells. A section of the root that contains both these tissues can be analysed, and the polysaccharide synthesized by the meristematic region can be obtained since the contribution of the root-cap cells can be found by the amount of fucose formed. 3. It was shown that there is very little difference in the polysaccharide synthesis of the meristematic region from that of the cells immediately behind it. In the more mature cells, however, the amount of xylose synthesized relative to that of arabinose is increased, and the proportion of xylose and arabinose formed in the matrix polysaccharides is increased whereas that of galactose is decreased. 4. The effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on polysaccharide synthesis was to bring about a decrease in the relative amount of galactose synthesized in the matrix polysaccharides of cells immediately adjacent to the meristematic region and also in the more mature tissue. The growth factor also increased the amount of xylose synthesized relative to that of arabinose in the more mature tissue. These metabolic effects were related to a very obvious change in the morphological appearance of the root-tips. 5. Radioactivity from [U-^{14}C]*myo*inositol was incorporated mainly into xylose, arabinose and galacturonic acid rather than into the hexoses, although small amounts of these sugars were formed.

Different synthetic pathways involved in cell-wall synthesis are active in the cell at different stages in its history and these are recorded in the polysaccharide components of the fully differentiated cell (Northcote, 1969). Also various chemical substances can determine the direction of differentiation of a cell and therefore the nature of the cell-wall biosynthetic pathways.

To obtain information about the pattern of polysaccharide biosynthesis in a tissue at a definite time radioactive precursors of these polysaccharides have to be used. In the study reported here the patterns of incorporation of radioactivity from different labelled glucose precursors and *myo*-inositol into component neutral sugars and uronic acids of the polysaccharides synthesized in the different tissues present in the maize root tip were examined. The effect of the artificial plant growth hormone 2,4-dichlorophenoxyacetic acid (2,4-D) on polysaccharide synthesis was also determined.

MATERIALS AND METHODS

Radioactive chemicals. The radioactive precursors used were D-[6-^{3}H]glucose (specific radioactivity 1.12 Ci/mmol), D-[6-^{14}C]glucose (specific radioactivity 45 mCi/mmol), D-[1-^{14}C]glucose (specific radioactivity 54.2 mCi/mmol), D-[U-^{14}C]glucose (specific radioactivity 320 mCi/mmol) and [U-^{14}C]*myo*inositol (specific radioactivity 324 mCi/mmol), all of which were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Preincubation treatment of seedlings. Seeds of maize (*Zea mays*) var. Orla 266 coated with a copper-containing fungicide were soaked in tap water for 10 h and germinated in damp vermiculite in the dark at 25°C. Seedlings were taken when their roots were 2–3 cm long and grown in aerated tap water in the dark at 25°C for 24 h. The water was changed after 12 h. In one experiment in which the

effects of the artificial plant growth hormone 2,4-dichlorophenoxyacetic acid were examined the roots were transferred after 12h to a solution containing 6p.p.m. of this substance dissolved in tap water.

Growth of seedlings under sterile conditions. D-[1-^{14}C]-Glucose was used as a precursor with roots that were grown under both sterile and non-sterile conditions. In all other experiments non-sterile conditions of growth were employed.

Maize roots were grown under sterile conditions by treating the seeds with Milton (Milton Division, Richardson–Merrell Ltd., London W.1, U.K.) for 30min, washing them in sterile water and then presoaking the seeds in a sterile solution of chloramphenicol (50mg/l) (Parke, Davis and Co. Ltd., Hounslow, Middx., U.K.) for 8h. The seeds were then washed in sterile water and germinated in moist sterile vermiculite. All transfers of material were carried out in a sterile cabinet fitted with an ultraviolet light and an air filter. The root slime from these seedlings was checked for bacterial and fungal contamination by light microscopy and by plating out on a nutrient agar (28g/l) (Oxoid Ltd., London, EC.4, U.K.). The roots were used when they were approx. 3cm long. No contamination was visible on microscopic examination of the tissues and no micro-organisms grew on the plates after 72h incubation at 25°C.

Incubation of the seedlings with radioactive precursor. The radioactive solutions (200μl; glucose, 20μCi, *myo*-inositol, 10μCi) were contained in glass vials (height 0.7cm, diam. 1.2cm). In one experiment 2,4-dichlorophenoxyacetic acid (6p.p.m.) was also present. Ten root tips were suspended in the solutions for 2h. The seedlings were held in position by threading their roots through holes (2mm diam.) in a sheet of Perspex (6cm × 6cm) supported by four glass tubes fixed at the four corners. The incubations were carried out in the dark at 25°C in a sealed glass tank containing a little water to maintain a moist atmosphere. After 2h the roots were removed from the radioactive solutions, washed with distilled water (the washings were collected), excised with a razor blade and stored in 50% (v/v) ethanol for approx. 48h at 4°C, with several changes of ethanol to extract all the soluble sugars. The quantity of label taken up by the roots was measured by determining the amount of radioactivity in a sample (10μl) of the radioactive solution before incubation and the amount present in the solution remaining after incubation. The samples of the radioactive solutions before and after the incubation were made up to 15ml with glass-distilled water, and 0.1ml samples of each were dried down under an infrared lamp on glass-fibre discs (Whatman GF/C; 2.5cm diam.).

Sectioning of the roots. The extracted roots were placed on a clean microscope slide and sectioned with a scalpel blade under a dissecting microscope fitted with a calibrated eye-piece. The first section (Fig. 1) was cut 440μm from the tip and consisted of the root-cap. The second section was 750μm long and represented the meristematic region of the root. The third and fourth sections of the root were 1000μm long and mainly contained expanding cells. The sections were placed in small test tubes and dried *in vacuo* over P_2O_5.

Hydrolysis of the extracted root sections. The dried root sections were allowed to dissolve in 72% (w/w) H_2SO_4 (12.5μl) at room temperature for 4h. The solution was

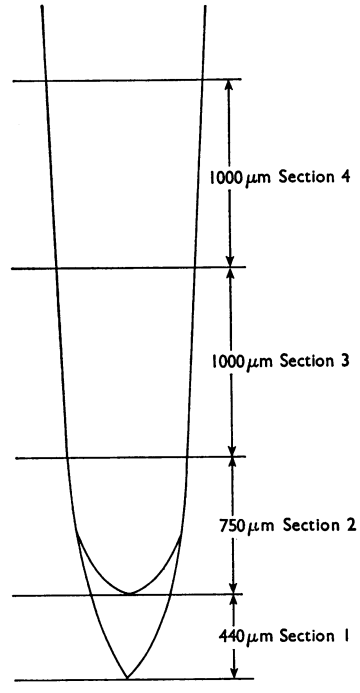


Fig. 1. Diagram of the maize root to illustrate the sections (1–4) that were analysed.

then diluted to 3% (w/w) H_2SO_4 and autoclaved at 120°C at 15lb/in^2 for 1h. The hydrolysates so obtained were filtered through sintered-glass micro-filters (porosity 3) under reduced pressure and neutralized by mixing with three changes of an equal volume of a 10% (v/v) solution of methyldi-*n*-octylamine (Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K.) in chloroform. Excess of amine was removed by extracting with four changes of chloroform. The neutralized hydrolysates were freeze-dried in a desiccator containing P_2O_5 and were taken up in either 50 or 100μl (section 1) or 100μl (sections 2–4) of water.

Radioactivity counting procedure. The radioactivities on glass-fibre paper discs were counted in 20ml Packard liquid-scintillation spectrometer bottles to which was added 2ml of toluene scintillation fluid [0.005% 1,4-bis-(5-phenyloxazol-2-yl)benzene and 0.35% 2,5-diphenyloxazole in sulphur-free toluene].

The paper chromatograms and electrophoretograms were dissected into 4cm × 1cm strips with the short side parallel to the direction of the run. These strips were placed in counting vials containing toluene scintillation fluid (0.5ml), and their radioactivities were counted inside 20ml Packard liquid-scintillation spectrometer bottles. Radioactivities of samples were counted in an ambient-temperature Nuclear–Chicago Unilux scintillation spectrophotometer for periods of 1, 4 and 10min, depending on the radioactivity of the sample.

Paper chromatography. Separation of the neutral sugars present in the hydrolysates was carried out by

descending paper chromatography on Whatman no. 1 paper with ethyl acetate–pyridine–water (8:2:1, by vol.) as the solvent system (solvent 1). Standard solutions of neutral sugars made up in saturated benzoic acid and stored at 4°C were run in parallel as markers. The fucose and xylose were separated with butan-1-ol–ethanol–water (5:1:4, by vol.) (solvent 2) and also with water-saturated phenol (100g of phenol, 39ml of water) (solvent 3) (Hough, 1954).

The sugars were detected by the aniline hydrogen phthalate method of Wilson (1959).

In all determinations of the radioactivity incorporated into the neutral sugars these were first separated on chromatograms with solvent 1. The paper strips containing the radioactive material that ran at the region of the xylose marker in this solvent were removed from the scintillation fluid, washed with clean toluene three times, dried and eluted with water. The eluent was freeze-dried and taken up in a small quantity of water. A sample of this solution was then rechromatographed with solvent 3 for 18.5h. The chromatogram was dried and the phenol was removed by dipping the chromatogram eight times in chloroform. It was then dissected and the radioactivities of the strips were measured in the scintillation spectrophotometer.

Electrophoresis. The uronic acids (D-glucuronic acid and D-galacturonic acid) present in the hydrolysates were separated from peptides and amino acids by electrophoresis on Whatman no. 1 paper strips (57cm × 23cm). The starting line was 17cm from the anode. Electrophoresis was carried out in acetic acid (8%, v/v)–formic acid (2%, v/v) buffer, pH2.0, at 4kV and approx. 50mA for between 30 and 45min. The positions of the uronic acids and neutral sugars were determined by running known markers in parallel. A strip of paper approx. 6cm wide containing the uronic acids was cut out and stitched on to another strip of Whatman no. 1 paper. The starting line was approx. 17cm from the cathode. The electrophoresis, which separated galacturonic acid and glucuronic acid, was carried out in pyridine–acetic acid–water (1:10:89, by vol.) buffer, pH3.5, at 4kV for 45min. All the electrophoreses were carried out in tanks filled with 'white spirit' (Esso Petroleum Co. Ltd., Cambridge, U.K.) cooled by water circulating in a glass coil. The electrodes were made of platinum foil. The positions of the sugars and uronic acids were determined with the aniline hydrogen phthalate reagent of Wilson (1959).

Collection of the maize root-cap slime. The roots from 500–800 seedlings were threaded through the holes of a sheet of perforated zinc supported on a shallow vessel containing aerated distilled water into which the roots were dipped. This treatment was continued for 5–10h at 25°C in the dark. The roots were then removed and the slime secreted by the root-caps was wiped on to weighed 2.5cm-diam. glass-fibre discs (Whatman GF/C) that had been previously heated to approx. 500°C to remove any carbohydrate present. The discs containing the absorbed slime material were dried over P_2O_5 in a desiccator and then cut up into small fragments and dissolved in 72% (w/w) H_2SO_4 (0.5 or 1.0ml) and hydrolysed. The hydrolysate was neutralized with either 10% (v/v) methyldi-*n*-octylamine or Amberlite IRA-400 resin (CO_3^{2-} form) and evaporated to dryness in a rotary evaporator.

Gas–liquid chromatography of the maize root-cap slime hydrolysate. (i) Preparation of the trimethylsilyl derivatives. Known sugar mixtures (5mg of each sugar) or hydrolysates were dried over P_2O_5 in a desiccator. Pyridine (1ml) (dried over KOH pellets for 12h), hexamethyldisilazane (0.2ml) and chlorotrimethylsilane (0.1ml) were added (Ludlow, Harris & Wolf, 1966). The substances were thoroughly mixed in a vortex mixer and kept at room temperature for 1h before use. The reactions produced a precipitate of NH_4Cl that was not removed. Known volumes (0.5–5.0μl) of the derivatives prepared in this way were injected on to the top of the column with a micro-syringe.

(ii) Preparation of the alditol acetates. Known sugar mixtures (5mg of each sugar) or hydrolysates of 5.5mg of slime were dissolved in water (1ml) containing $NaBH_4$ (5mg). After 1.5–2h at 20°C the excess of borohydride was removed by the addition of acetic acid drop by drop until effervescence ceased. The borate ions were removed by evaporating to dryness ten times with methanol (5ml). The alditols were acetylated by treatment with acetic anhydride (1ml) and 98% (w/w) H_2SO_4 (0.1ml) for 1h at 50–60°C. The reaction mixture was cooled and poured into ice-cold water (15ml). The alditol acetates that were precipitated were then extracted by shaking the mixture with methylene chloride (10–15ml) three times. The combined methylene chloride solutions were evaporated to dryness, water (2ml) was added and they were re-evaporated to dryness. The alditol acetates were dissolved in methylene chloride (0.4–1ml), and known volumes of the solution (1–5μl) were applied to the column (Borchardt & Piper, 1970).

The instrument used was a Pye series 104 model 24 chromatograph fitted with a flame ionization detector. Argon was used as the carrier gas at a flow rate of 40ml/min. The column used was 10% polyethylene glycol adipate on Celite (100–120 mesh). For the separation of the trimethylsilyl derivatives it was operated over the temperature range 90–190°C and the temperature was increased linearly at the rate of 3°C/min. For the detection of the alditol acetates it was operated isothermally at 210°C.

RESULTS

Uptake of radioactive precursors by the seedlings

A high percentage of the total radioactivity supplied by the glucose precursors was taken up by the seedlings. (The growth factor 2,4-dichlorophenoxyacetic acid had no effect on the uptake of glucose.) The uptake of the [U-^{14}C]*myo*inositol was, however, much lower (Table 1). A low rate of uptake of *myo*inositol by maize root-tips has also been reported by Roberts, Deshusses & Loewus (1968).

Fucose-containing polysaccharide synthesized by the cells of the root-cap (section 1 and part of section 2; see Fig. 1)

D-[6-^{3}H]- *and* D-[6-^{14}C]-*Glucose used as the radioactive precursors.* When solvent system 1 was used for the chromatography of neutral sugars of the hydrolysates obtained from sections 1 and 2, a

Table 1. *Uptake of radioactive precursors by maize seedlings*

Ten seedlings were incubated in the solutions for 2h at 25°C in the dark in a moist atmosphere. 2,4-D, 2,4-Dichlorophenoxyacetic acid.

Radioactive precursor	10^{-6} × Amount of radioactivity in incubation medium (c.p.m.)	10^{-6} × Amount of radioactivity taken up by seedling (c.p.m.)	% of the radioactivity taken up by seedling
D-[U-^{14}C]Glucose	37.2	32.3	87.0
D-[U-^{14}C]Glucose+2,4-D	34.6	30.0	86.9
D-[1-^{14}C]Glucose	34.9	26.0	74.5
[U-^{14}C]*myo*Inositol	15.4	5.6	36.7
D-[6-^{3}H]Glucose	47.9	31.9	66.6
D-[6-^{14}C]Glucose	35.8	23.1	64.5

Table 2. *Relative amounts of radioactivity incorporated into xylose and fucose by the different sections of the root-tip of maize seedlings*

2,4-D, 2,4-Dichlorophenoxyacetic acid.

	Section 1		Section 2		Section 3		Section 4	
Radioactive precursor	Fucose (%)	Xylose (%)	Fucose (%)	Xylose (%)	Fucose (%)	Xylose (%)	Fucose (%)	Xylose (%)
D-[U-^{14}C]Glucose	66.5	33.5	48.6	51.4	3.6	96.4	0	100
D-[U-^{14}C]Glucose+2,4-D	74.2	25.8	43.1	56.9	3.3	96.7	1.0	99.0
D-[1-^{14}C]Glucose	68.9	31.1	40.4	59.6	2.4	97.6	1.5	98.5
[U-^{14}C]*myo*Inositol	Trace incorporation only		10.1	89.9	0	100	0	100
D-[6-^{3}H]Glucose	97.1	2.9	79.3	20.7	16.4	83.6	11.3	88.7
D-[6-^{14}C]Glucose	96.7	3.3	82.7	17.3	13.4	86.6	9.5	90.5

Table 3. R_{Glc} *values of sugars in different chromatographic solvents*

The compositions of the solvents are given in the Materials and Methods section. Values for solvents 1 and 2 were determined experimentally, those for solvent 3 were calculated from the results of Hirst & Jones (1949).

	R_{Glc} values		
Sugar	Solvent 1	Solvent 2	Solvent 3
Galactose	0.8	0.8	1.1
Mannose	1.4	1.2	1.2
Arabinose	1.8	1.3	1.4
Fucose	2.5	2.3	1.7
Xylose	2.5	1.7	1.2
Ribose	3.6	2.3	1.6
Rhamnose	4.1	3.3	1.6

sugar was found that had the mobility of xylose. This region contained a high proportion of the total radioactivity of the hydrolysate. Fucose runs only very slightly behind xylose in solvent 1. When the radioactive sugar was eluted and re-run on a chromatogram with solvent 2 or 3 clear separations of xylose and fucose were obtained, and by this means it was shown that 97% of the radioactivity of the region (section 1) was in fucose and only 3% was in xylose (Table 2). Table 3 shows the R_{Glc} values of the sugars in the different solvents used in these investigations.

D-[U-^{14}C]- *and* D-[1-^{14}C]-*Glucose used as the radioactive precursors.* Radioactive fucose accounted for a large proportion of the total radioactivity incorporated into sections 1 and 2 when D-[U-^{14}C]- and D-[1-^{14}C]-glucose were used as precursors (Fig. 2).

Distribution of the fucose-containing polysaccharide. Only a very small amount of radioactive fucose was

Table 4. *Incorporation of radioactive precursors into the neutral sugars and uronic acids of each section of the root-tip of maize seedlings*

2,4-D, 2,4-Dichlorophenoxyacetic acid.

Radioactive precursor	10^{-3} × Radioactivity incorporated (c.p.m.)			
	Section 1	Section 2	Section 3	Section 4
D-[U-^{14}C]Glucose	11.4	174.3	238.9	490.4
D-[U-^{14}C]Glucose+2,4-D	27.1	65.3	143.8	320.3
D-[1-^{14}C]Glucose	76.6	388.8	636.5	843.2
[U-^{14}C]*myo*Inositol	Trace incorporation only	2.5	4.9	8.7
D-[6-^{3}H]Glucose	14.4	47.5	96.4	163.2
D-[6-^{14}C]Glucose	75.3	302.4	301.0	395.6

Table 5. *Relative amounts of radioactivity incorporated from* D-[1-^{14}C]*glucose into section* 1 (*see Fig.* 1) *of the root-tip when the roots were grown under sterile and non-sterile conditions*

The values in parentheses represent the amounts of radioactivity calculated as percentages of the total amount of radioactivity in the neutral sugars and the uronic acids excluding glucose. The other values represent the amounts of radioactivity calculated as a percentage of the total amount of radioactivity in all the neutral sugars and the uronic acids.

Sugar	Radioactivity (%)	
	Roots grown in sterile medium	Roots grown in non-sterile medium
Galactose	25.7 (31.4)	28.1 (32.0)
Glucose	18.3 (–)	12.3 (–)
Mannose	4.7 (5.7)	5.2 (5.9)
Arabinose	9.6 (11.8)	10.9 (12.4)
Fucose	22.1 (27.0)	23.7 (27.1)
Xylose	8.8 (10.7)	10.7 (12.2)
Galacturonic acid	9.3 (11.4)	7.7 (8.8)
Glucuronic acid	1.6 (1.9)	1.5 (1.7)

synthesized from the radioactive glucose precursors and from [U-^{14}C]*myo*inositol in sections 3 and 4 of the root (Fig. 2). Hydrolysates of section 2 from roots incubated in the radioactive glucose precursors contained about 50–75% of the amount of the radioactive fucose of that present in section 1. Radioactive fucose occurs in section 2 because the section contains root-cap tissue at its periphery (Fig. 1). Unlike section 1, section 2 incorporated a small amount of [U-^{14}C]*myo*inositol that could be measured (Table 4), but this precursor did not form much radioactive fucose in section 2 and is presumably not a good precursor of the 6-deoxyhexose.

Fucose was synthesized in large amounts only by the root-cap tissue (Fig. 2). Table 5 shows the relative amounts of radioactivity incorporated into section 1 (Fig. 1) of the root-tip when the roots were grown under sterile and non-sterile conditions.

Polysaccharide biosynthesis in the root tissues

Radioactive glucose used as the precursor. The amount of radioactive glucose relative to the total amount of radioactivity incorporated into the neutral sugars and uronic acids of the polysaccharides of the root cells when the roots were incubated with the radioactive glucose precursors showed a very great increase from section 1 to section 4 (Fig. 3). The percentage contribution of radioactivity in galactose, mannose, galacturonic acid and glucuronic acid decreased progressively in sections 1–4 (Figs. 4–7), whereas the percentage radioactivity in xylose (Fig. 8) increased and that in arabinose (Fig. 9) remained approximately constant. There is a rapid change in the contribution of glucose (starch and cellulose) to the total radioactivity in the neutral sugars and uronic acids from the root-cap, where the amount is small, to the fourth section, where it is very high. The amount of radioactivity in the various sugars was therefore recalculated to show the percentage of the total radioactivity in the uronic acids and neutral sugars excluding glucose. These values represent the contribution of the various sugars to the matrix polysaccharides of the cell wall, the hemicelluloses

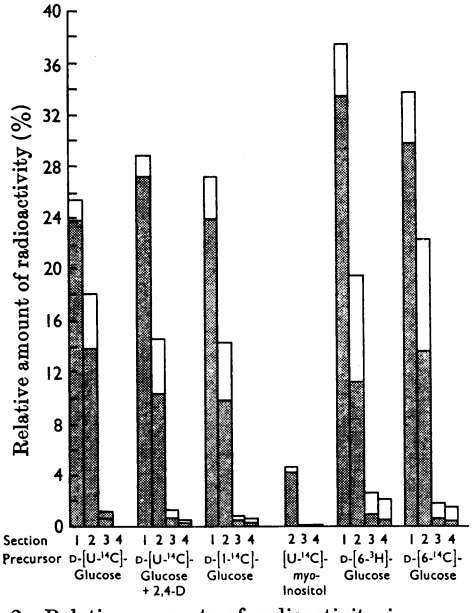


Fig. 2. Relative amounts of radioactivity incorporated into the fucose of the polysaccharides synthesized by the four sections of the root-tip when radioactive glucose and *myo*inositol were used as precursors. The open columns represent the amounts of radioactivity calculated as percentages of the total amount of radioactivity in the neutral sugars and the uronic acids excluding glucose. The stippled portions of the columns represent the amounts of radioactivity calculated as a percentage of the total amount of radioactivity in all the neutral sugars and the uronic acids. 2,4-D, 2,4-Dichlorophenoxyacetic acid.

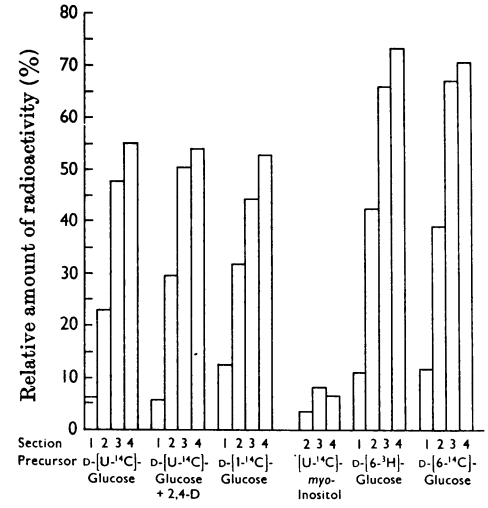


Fig. 3. Relative amounts of radioactivity incorporated into the glucose of the polysaccharides synthesized by the four sections of the root-tip when radioactive glucose and *myo*inositol were used as precursors.

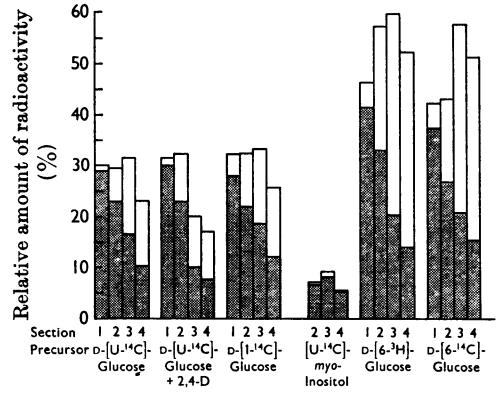


Fig. 4. Relative amounts of radioactivity incorporated into the galactose of the polysaccharides synthesized by the four sections of the root-tip when radioactive glucose and *myo*inositol were used as precursors. For explanation see Fig. 2.

and pectins. The amounts of mannose synthesized were very low (Fig. 5). Mannose is a component of the glucomannans of the hemicellulose. The percentage radioactivity in xylose and arabinose increased sharply from section 1 to section 4 (Figs. 8 and 9). The amount of xylose synthesized relative to arabinose increased from section 1 to section 3 and then remained approximately constant (Table 6). In most cases the relative amounts of radioactive galacturonic acid in the matrix polysaccharides decreased from the root-cap region to section 4 (Fig. 6). The changes in percentage of the amount of radioactivity in glucuronic acid in the four sections were low, although there was a distinct increase from section1 to section 4 in tissues incubated with D-[6-^{14}C]glucose (Fig. 7).

The relative amounts of radioactive label in the neutral sugars and uronic acids when the tissue was incubated with D-[U-^{14}C]-, D-[1-^{14}C]-, D-[6-^{14}C]- and D-[6-^{3}H]-glucose were consistent with the known pathways involved in their biosynthesis (Scheme 1). Thus the contribution of D-[6-^{14}C]- and D-[6-^{3}H]-glucose to the radioactivity of the xylose and arabinose was low relative to that made by D-[U-^{14}C]glucose. Also, far more radioactivity was transferred from D-[6-^{14}C]glucose to galacturonic acid and glucuronic acid than from D-[6-^{3}H]glucose. The formation of radioactive pentoses and uronic acids from D-[6-^{3}H]glucose and the formation of radioactive pentoses from D-[6-^{14}C]glucose were probably due to equilibration of the C-1 and C-6

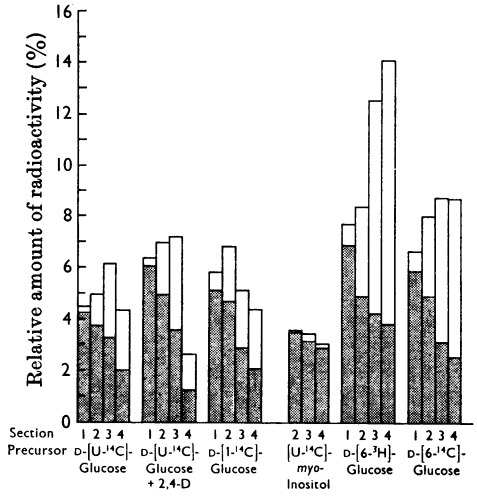


Fig. 5. Relative amounts of radioactivity incorporated into the mannose of the polysaccharides synthesized by four sections of the root-tip when radioactive glucose and *myo*inositol were used as precursors. For explanation see Fig. 2.

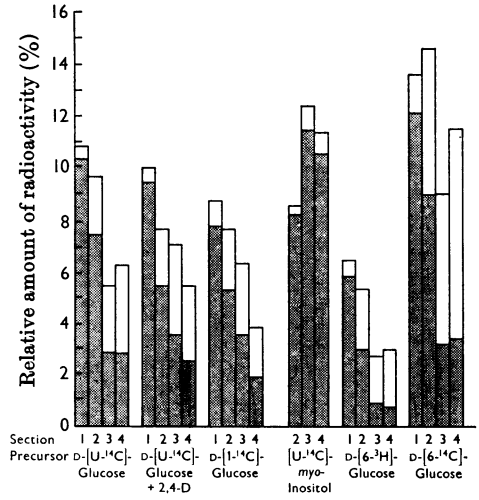


Fig. 6. Relative amounts of radioactivity incorporated into the galacturonic acid of the polysaccharides synthesized by the four sections of the root-tip when radioactive glucose and *myo*inositol were used as precursors. For explanation see Fig. 2.

positions by triose phosphate isomerase followed by the resynthesis of glucose. Glycolysis has been found to be more important than the pentose phosphate pathway in the oxidation of glucose in root-

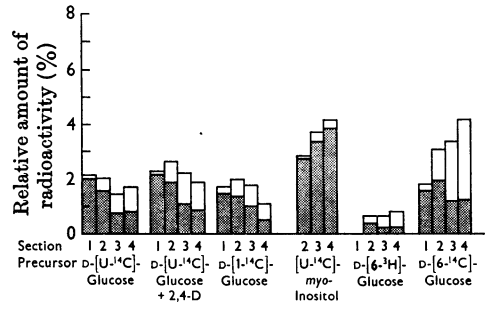


Fig. 7. Relative amounts of radioactivity incorporated into the glucuronic acid of the polysaccharides synthesized by the four sections of the root-tip when radioactive glucose and *myo*inositol were used as precursors. For explanation see Fig. 2.

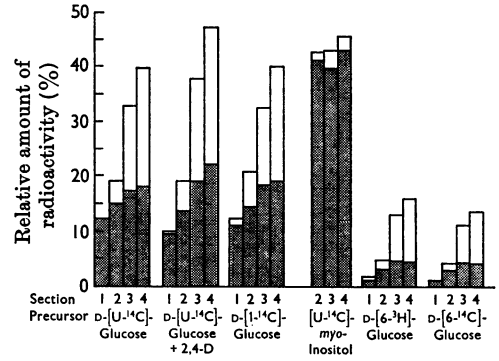
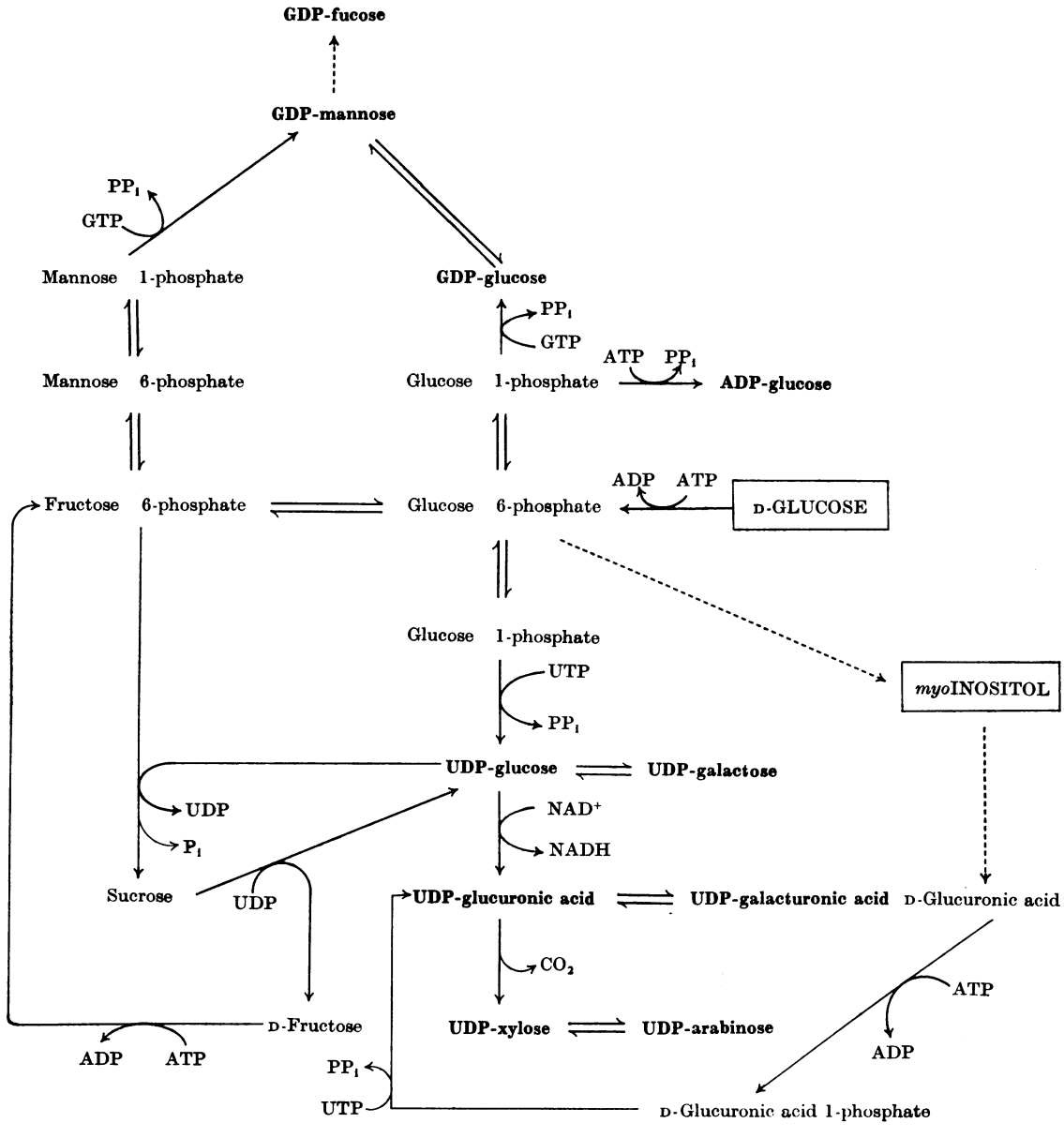


Fig. 8. Relative amounts of radioactivity incorporated into the xylose of the polysaccharides synthesized by the four sections of the root-tip when radioactive glucose and *myo*inositol were used as precursors. For explanation see Fig. 2.

tips (Fowler & ap Rees, 1970). Tissues incubated with D-[1-^{14}C]- and D-[U-^{14}C]-glucose gave very similar distributions of the amount of radioactivity incorporated into neutral sugars and uronic acids (Figs. 2–9). The relative amounts of radioactive galactose in the sugars and uronic acids of the matrix polysaccharides were different when D-[1-^{14}C]- and D-[U-^{14}C]-glucose were supplied from those when D-[6-^{3}H]- and D-[6-^{14}C]-glucose were used (Fig. 4). The radioactive glucose precursors that were labelled at the primary alcohol group gave a greater percentage contribution to the radioactivity of all hexoses in all four sections (Figs. 2–5), as would be expected since the pentoses formed directly from these glucose precursors would not carry the radioactive label. Similarly the uronic acids derived directly from D-[6-^{3}H]glucose would not be radio-



Scheme 1. Diagram to illustrate the biosynthetic pathways from glucose and *myo*inositol of the sugars present in plant polysaccharides. The nucleoside diphosphate sugars in **bold** type are the donor compounds for the synthesis of the polysaccharides of the cell. The broken lines represent pathways about which the details are unknown.

active. Tissues incubated with D-[U-^{14}C]- and D-[1-^{14}C]-glucose gave the same relative amounts of radioactive galactose in the first three sections and markedly less in the fourth (Fig. 4). When D-[6-^{3}H]- and D-[6-^{14}C]-glucose were used, however, there was a lower incorporation of radioactivity into galactose in sections 1 and 2 relative to section 3, probably because of the considerable formation of fucose, followed by a smaller incorporation in section 4 (Figs. 2 and 4).

The amount of radioactivity incorporated into mannose was also different when the tissue was

incubated with glucose precursors labelled at the primary alcohol group from tissue incubated with D-[1-^{14}C]- and D-[U-^{14}C]-glucose (Fig. 5). The percentage of the total radioactivity in mannose rose in sections 1 and 2 and sometimes in section 3 and then fell in section 4 in those roots incubated with D-[1-^{14}C]- and D-[U-^{14}C]-glucose; but when D-[6-^{3}H]- and D-[6-^{14}C]-glucose were used the percentage radioactivity in mannose increased progressively from section 1 to section 4 (Fig. 5).

Radioactive myo*inositol used as a precursor.* Incorporation of radioactivity obtained with tissues incubated with [U-^{14}C]*myo*inositol was very similar whether the amounts of radioactivity in each sugar were expressed as a percentage of the total or of the matrix polysaccharides. This is because only very small amounts of glucose were synthesized from *myo*inositol. A large proportion of the radioactivity was incorporated into xylose, arabinose and galacturonic acid (Figs. 6, 8 and 9). The changes in the relative amounts of radioactivity in the various sugars between the four sections were only small. This is to be expected since there was only a little incorporation of radioactivity into the hexoses (Figs. 2–5). The proportion of xylose relative to arabinose that was synthesized in the four sections was similar to that obtained with the glucose precursors except that more xylose relative to arabinose was synthesized in the second section (Table 6).

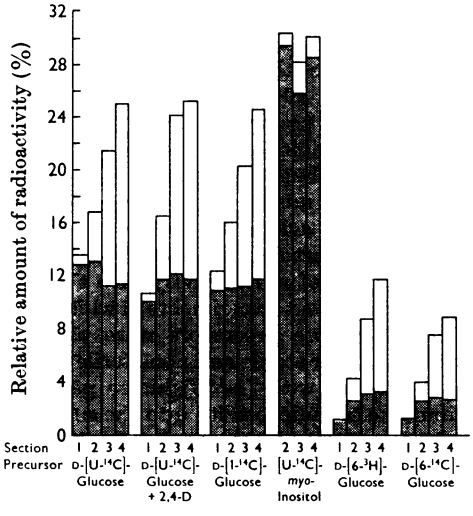


Fig. 9. Relative amounts of radioactivity incorporated into the arabinose of the polysaccharides synthesized by the four sections of the root-tip when radioactive glucose and *myo*inositol were used as precursors. For explanation see Fig. 2.

Effect of 2,4-dichlorophenoxyacetic acid on the polysaccharide synthesis with D-[U-^{14}C]*glucose as a precursor.* There was no change in the incorporation of the radioactivity into the sugars of the polysaccharides formed by the root-cap in the presence and absence of 2,4-dichlorophenoxyacetic acid (Figs. 2–9). However, in sections 2, 3 and 4 of the root the amount of radioactivity incorporated into the galactose decreased in section 3 rather than in section 4, and it decreased to a lower value than in roots incubated without the growth hormone (Fig. 4). The amount of xylose synthesized relative to arabinose increased to a greater extent in the presence of 2,4-dichlorophenoxyacetic acid (Table 6).

Analysis of the slime of the root-cap

Determination of the neutral sugars by g.l.c. showed that the slime contained: galactose, 21%; glucose, 22%; mannose, 6%; xylose, 4%; arabinose, 15%; fucose, 32%. Galacturonic acid and gluc-

Table 6. *Ratios of amounts of radioactivity incorporated into xylose and arabinose by the different sections of the root-tip of the maize seedlings*

2,4-D, 2,4-Dichlorophenoxyacetic acid.

	Xylose/arabinose ratio				
Radioactive precursor	Section 1	Section 2	Section 2 (analytical data corrected for contribution from slime of the cap cells)	Section 3	Section 4
D-[U-^{14}C]Glucose	0.94	1.13	1.4	1.52	1.58
D-[U-^{14}C]Glucose+2,4-D	0.94	1.15	1.3	1.56	1.88
D-[1-^{14}C]Glucose	0.99	1.31	1.6	1.65	1.63
[U-^{14}C]*myo*Inositol	—	1.40	—	1.53	1.52
D-[6-^{3}H]Glucose	0.86	1.13	1.2	1.50	1.51
D-[6-^{14}C]Glucose	0.93	1.15	1.2	1.50	1.38

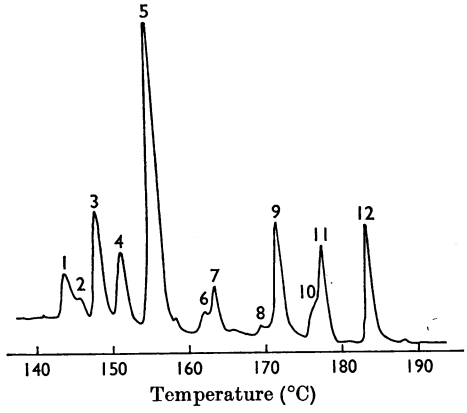


Fig. 10. Trace of a g.l.c. separation (column: 10% polyethylene glycol adipate on Celite) of 5 μl of a pyridine solution of the trimethylsilyl derivatives prepared from a hydrolysate of the slime. The column was operated over the temperature range 90–190°C and the temperature was increased linearly at the rate of 3°C/min. Peaks: 1, arabinose; 2, fucose; 3, fucose; 4, arabinose; 5, fucose, arabinose and xylose; 6, xylose; 7, mannose; 8, galactose; 9, galactose and glucose; 10, mannose; 11, galactose; 12, glucose.

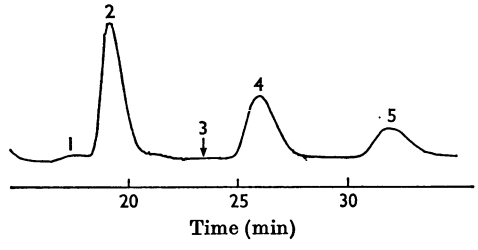


Fig. 11. Part of a trace of a g.l.c. separation (column: 10% polyethylene glycol adipate on Celite) of 5 μl of a methylene chloride solution of alditol acetates prepared from a hydrolysate of the slime. The column was operated isothermally at 210°C. Peaks: 1, rhamnose; 2, fucose; 3, position of ribose not present in hydrolysate; 4, arabinose; 5, xylose.

uronic acid were also present in the hydrolysate. The traces of the chromatographic separations of the trimethylsilyl derivative and the alditol acetates prepared from hydrolysates of the slime are shown in Figs. 10 and 11. A further chromatographic separation of the alditol acetates mixed with fucitol acetate (prepared from authentic fucose) gave a quantitative reinforcement of the suspected fucitol acetate peak in the hydrolysate.

DISCUSSION

It is clear from the experiments with radioactive precursors that the maize root-cap (section 1) synthesizes an acidic polysaccharide material containing a high proportion of galactose and fucose. Northcote & Pickett-Heaps (1966) showed that the outer root-cap cells of wheat roots contained hypertrophied Golgi bodies that were involved in the secretion and probably the synthesis of a slime material that accumulated around the caps. Thus it is extremely likely that the labelled material being synthesized by the maize root-cap is slime, and this is confirmed by the chemical analysis of this slime, which shows a high proportion of galactose and fucose. From its composition it is probable that the slime contains a polysaccharide related to tragacanthic acid and pectin (Aspinall, 1967; Northcote, 1969), although the high fucose content is very unusual. The large amount of galactose could indicate the presence of a separate neutral galactan, but large neutral blocks composed of galactose and arabinose are attached to the main polygalacturonic acid chain of some pectin preparations (Stoddart & Northcote, 1967).

Incorporation of radioactivity into the slime is constant in different experiments in which [U-^{14}C]- and [1-^{14}C]-glucose precursors were used. Also, roots grown under sterile and non-sterile conditions when incubated with [1-^{14}C]glucose gave similar distributions of radioactivity into the sugars of the root-tip tissue (Table 5). These results indicate that the polysaccharides synthesized during the experiments were those of the root tissue and were not produced by bacterial or fungal contamination.

Northcote & Pickett-Heaps (1966) found by high-resolution radioautography that exogenous glucose contributed to the cell walls and starch of the inner root-cap tissues of wheat. The starch was contained in the plastids of the cell. Thus some of the labelled glucose found in hydrolysates of the maize root-cap sections must be derived from these polysaccharides. In addition, the proportion of the total radioactivity in the uronic acids in the slime is probably underestimated because of the difficulty with which uronosidic bonds are hydrolysed.

Fucose is synthesized in large amounts only by the cap tissues in maize roots. It is synthesized to only a very small extent in the rest of the root, probably as a minor component of the galacturonorhamnans of pectin. This represents an excellent example of metabolic differentiation, in which the biosynthetic pathway from glucose to fucose is operating to a very different extent in adjacent tissues. Nevins, English & Albersheim (1967) studied the constituent sugars of the cell walls in maize roots and leaves by g.l.c. and found that fucose was only a minor component. It is possible that fucose is synthesized

in large amounts in other very localized regions of the maize plant and would thus not be detected by gross analytical procedures.

Root-cap mucilage is secreted by a large proportion of land plants, and it would be interesting to know whether fucose was a general constituent of the slime. In the root-cap slime of wheat fucose was not a major component (Northcote & Pickett-Heaps, 1966).

In higher plants fucose is usually reported as a trace constituent of the galacturonorhamnans of the pectic substances. Aspinall, Molloy & Craig (1969) have, however, reported the isolation of a fucoxyloglucan from the culture medium of suspension-cultured sycamore cells. The polymer consisted of a main glucan chain with xylose and fucose side chains.

Fucose almost certainly occurs more widely in higher plants than is realized since it is probably very widely confused with either xylose or ribose. The hydrolysate of the slime secreted by the maize root-cap has been analysed by Juniper & Roberts (1966). It was said to contain glucose, galactose, xylose, arabinose and small amounts of uronic acids. However, fucose and xylose have similar R_{Glc} values on chromatograms run with the solvent system used by these workers and were probably confused. Jones & Morré (1967) carried out a similar analysis and concluded that the slime contained a highly hydrated acidic polysaccharide that yielded on hydrolysis 37% glucose, 35% galactose, 12% galacturonic acid, 11% ribose and 5% xylose. Jones & Morré (1967) considered that the arabinose found by Juniper & Roberts (1966) was the result of cell-wall contamination. The high proportion of ribose reported by Jones & Morré (1967) is extremely unusual. There have been no reports of a riban occurring in any polysaccharide preparation. Ribose was not found in the chemical analysis of our material, and in the radioactivity experiments it was found to be labelled only to a very small extent. A possible explanation is that Jones & Morré (1967) confused ribose with fucose; alternatively, the ribose could have been derived from the RNA of a massive bacterial contamination. It is also possible that there may be varietal differences in slime composition.

Ribose and fucose were confused by Kawamura & Narasaki (1956), who first stated that ribose was a constituent of the B_1 hemicellulose fraction of *Vicia faba* seeds. Later it was reported (Kawamura & Narasaki, 1958) that the sugar was fucose.

Surface scales of the alga *Chrysochromulina chiton* are produced by the Golgi bodies of the cells (Manton, 1967*a*,*b*) and are composed of polysaccharide. Radioactive polysaccharide formed by cells incubated with $NaH^{14}CO_3$ was said to contain radioactive galactose and a radioactive sugar tentatively identified as ribose. The ribose accounted for 25% of the radioactivity on the chromatogram (Green & Jennings, 1967). In at least two of the chromatographic solvent systems used fucose and ribose have similar R_{Glc} values. It may well be that the scales contain a high proportion of fucose as well as galactose. Ribose has also been reported as a constituent of the scales of the alga *Pleurochrysis scherffelii* (Brown, Franke, Falk & Sitte, 1969).

The biosynthesis of fucose has not been studied in plants. Kornfeld & Ginsburg (1965) found that in *Acetobacter aerogenes* and *Salmonella urbana*, which contain fucose but not mannose polymers, the pyrophosphorylase involved in the formation of GDP-mannose from mannose 1-phosphate and the oxidoreductase enzyme (involved in the last step in GDP-fucose synthesis) are inhibited by GDP-fucose, whereas the pyrophosphorylase is not inhibited by GDP-mannose. Only the mannose-containing polysaccharide is found in *Salmonella paratyphi*, and the pyrophosphorylase is inhibited by GDP-mannose but not by GDP-fucose. In *Salmonella hvittingfoss* and *Salmonella champaign* both fucose- and mannose-containing polysaccharides are present; here only GDP-mannose is capable of inhibiting the pyrophosphorylase, and GDP-fucose specifically inhibits the oxidoreductase reaction. It would be interesting to discover whether such feedback inhibitions operate in plants where changes in amounts of mannose- and fucose-containing polysaccharides occur during growth and differentiation (Scheme 1).

Although the second segment of the maize root-tip immediately behind the cap contains a high proportion of meristematic tissue it also contains some cells of the root-cap. If fucose was only synthesized by the root-cap cells in the slime, the amount of this 6-deoxyhexose allows a correction of the analytical data of the meristematic section of the root to be made so that any contribution from the slime of the cap cells can be accounted for (Table 6 and 7). Corrected in this way the pattern of radioactivity incorporated into the polysaccharides of the meristematic region (section 2) was very similar to that obtained in the third section of the root, which also contained some meristematic cells but which consisted mainly of cells that were beginning to enlarge. The cell elongation was continued in the tissue isolated in the fourth section.

Glucan synthesis represented a high proportion of the polysaccharides synthesized in the other three sections of the root behind the root-cap. This was greater in section 4 than in section 3. The glucans included both starch and cellulose, which were not distinguished by the analytical methods used, and the results could indicate high cellulose or high starch synthesis or both. It is possible that the increase in glucan synthesis of section 4 over that

Table 7. *Amounts of radioactivity incorporated into the neutral sugars and uronic acids from the various radioactive precursors by sections 2 and 3 of the root-tip of the maize seedlings*

The amounts of radioactivity incorporated into the sugars of the polysaccharides of section 2 have been corrected for the amounts of radioactivity incorporated into the slime of the cap cells.

Sugar into which radioactivity incorporated	Radioactive precursor ... Section	Radioactivity incorporated (%) D-[U-^{14}C]-Glucose	D-[U-^{14}C]-Glucose +2,4-D	D-[1-^{14}C]-Glucose	D-[6-^{3}H]-Glucose	D-[6-^{14}C]-Glucose
Galactose	2	14.0	18.6	17.7	28.8	17.6
	3	16.5	9.9	18.6	20.2	20.7
Glucose	2	46.6	41.3	45.6	58.7	61.7
	3	47.8	50.4	44.2	65.9	64.1
Mannose	2	3.2	4.3	4.4	2.8	4.1
	3	3.3	3.6	2.9	4.3	3.1
Arabinose	2	13.3	12.9	10.7	3.3	3.6
	3	11.3	12.1	11.1	3.0	2.8
Xylose	2	18.6	16.2	16.9	3.9	4.4
	3	17.1	18.9	18.3	4.5	4.2
Galacturonic acid	2	3.4	3.0	3.5	1.7	6.2
	3	2.8	3.5	3.5	1.0	3.2
Glucuronic acid	2	0.9	1.7	1.3	—	2.2
	3	0.8	1.1	1.0	0.2	1.2

of section 3 is associated with the laying down of cellulose during wall expansion. Roberts & Butt (1967) in a radioautographic study reported that the contribution of cellulose to the cell wall rose to a maximum 3mm behind the cap junction.

The amount of radioactive arabinose and xylose in the sugars of the matrix polysaccharides increased from section 3 to section 4, but their relative proportions remained the same. This probably represents a synthesis of an arabinoxylan, which is known to occur in maize tissues (Gramera & Whistler, 1963).

There was also a marked decrease in the contribution of galactose to the sugars of the matrix polysaccharides from section 3 to section 4; this was also paralleled to some extent by a fall in the relative amounts of galacturonic acid and glucuronic acid in these sections. Although this could be interpreted in terms of a decrease in the synthesis of the pectic substances, the situation is complicated by the fact that maize tissues contain a galactoarabinoxylan as a hemicellulosic constituent (although the galactose occurs in only relatively small amounts) (Gramera & Whistler, 1963). Roberts & Butt (1969) found that galactose was laid down in pectin in the first 4mm of the root and in hemicellulose 4–6mm behind the cap junction.

The distribution of radioactivity from administered [U-^{14}C]*myo*inositol was qualitatively very similar to that obtained by Roberts *et al.* (1968) in work with excised maize roots. However, our results have shown that, in addition to pentoses and uronic acids, small amounts of hexose sugars are also formed from the precursor.

No very great changes in the synthesis of polysaccharides were obtained by incubating the tissue with 2,4-dichlorophenoxyacetic acid. However, two effects were significant. In contrast with the untreated roots, the proportion of galactose in the matrix polysaccharides decreased to a lower value in the more proximal regions of the root. The galactose value corrected to remove the slime contribution in section 2 of roots treated with 2,4-dichlorophenoxyacetic acid was similar to those for sections 2 and 3 of the untreated tissue. Also, the proportion of xylose to arabinose was higher than in the control in section 4.

These changes in polysaccharide synthesis were associated with the morphological changes in the roots after 2,4-dichlorophenoxyacetic acid treatment. The roots were shorter and a swelling was seen behind the tip (part of which corresponds to section 4).

The anatomical changes associated with this swelling have not been investigated, but similar swellings caused by other plant growth substances have been shown to contain giant cortical cells (Levan, 1939). Torrey (1953) has shown that the application of indol-3-ylacetic acid caused primary zylem elements to differentiate nearer to the root-tip than in untreated roots.

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REFERENCES

Aspinall, G. O. (1967). *Pure appl. Chem.* **14**, 43.

Aspinall, G. O., Molloy, J. A. & Craig, J. W. T. (1969). *Can. J. Biochem.* **47**, 1063.

Borchardt, L. G. & Piper, C. V. (1970). *TAPPI*, **53**, 257.

Brown, R. M., Franke, W. W., Falk, H. K. H. & Sitte, P. (1969). *Science, N.Y.*, **166**, 894.

Fowler, M. W. & ap Rees, T. (1970). *Biochim. biophys. Acta*, **201**, 33.

Gramera, R. E. & Whistler, R. L. (1963). *Archs Biochem. Biophys.* **101**, 75.

Green, J. C. & Jennings, D. H. (1967). *J. exp. Bot.* **18**, 359.

Hirst, E. L. & Jones, J. K. N. (1949). *Disc. Faraday Soc.* **7**, 268.

Hough, L. (1954). In *Methods of Biochemical Analysis*, vol. 1, p. 231. Ed. by Glick, D. New York: Interscience Publishers Inc.

Jones, D. D. & Morré, D. J.(1967). *Z. PflPhysiol.* **56**, 166.

Juniper, B. E. & Roberts, R. M. (1966). *Jl R. microsc. Soc.* **85**, 63.

Kawamura, S. & Narasaki, T. (1956). *Bull. agric. chem. Soc. Japan*, **20**, 289.

Kawamura, S. & Narasaki, T. (1958). *Bull. agric. chem. Soc. Japan*, **22**, 436.

Kornfeld, R. & Ginsburg, V. (1965). *Fedn Proc. Fedn Am. Socs exp. Biol.* **24**, 534.

Levan, A. (1939). *Hereditas*, **25**, 87.

Ludlow, C. J., Harris, T. M. & Wolf, F. T. (1966). *Phytochemistry*, **5**, 251.

Manton, I. (1967*a*). *J. Cell Sci.* **2**, 265.

Manton, I. (1967*b*). *J. Cell Sci.* **2**, 411.

Nevins, D. J., English, P. D. & Albersheim, P. (1967). *Pl. Physiol., Lancaster*, **42**, 900.

Northcote, D. H. (1969). In *Essays in Biochemistry*, vol. 5, p. 89. Ed. by Campbell, P. N. & Greville, G. D. London: Academic Press.

Northcote, D. H. & Pickett-Heaps, J. D. (1966). *Biochem. J.* **98**, 159.

Roberts, R. M. & Butt, V. S. (1967). *Expl Cell Res.* **46**, 495.

Roberts, R. M. & Butt, V. S. (1969). *Planta, Berl.*, **84**, 250.

Roberts, R. M., Deshusses, J. & Loewus, F. (1968). *Pl. Physiol., Lancaster*, **43**, 979.

Stoddart, R. W. & Northcote, D. H. (1967). *Biochem. J.* **105**, 45.

Torrey, J. G. (1953). *Am. J. Bot.* **40**, 525.

Wilson, C. M. (1959). *Analyt. Chem.* **31**, 1199.