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The Sites of Synthesis and Transport of Extracellular Polysaccharides in the Root Tissues of Maize

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1. Subcellular fractionation of maize roots resulted in the isolation of the following enriched fractions: cell wall, dictyosome, smooth-membrane and rough-microsomal fractions. In addition, extracellular polysaccharide of the root slime was isolated. 2. Maize-seedling roots were incubated *in vivo* with D-[U-^{14}C]glucose, and the pattern of incorporation of radioactivity into the polysaccharides of each fraction was investigated. 3. The differentiation of maize-root cells with respect to the synthesis of specific extracellular polysaccharide directly relates to the polysaccharide synthesized and transported within the membrane system of the cell. A fucose-containing polysaccharide, characteristic only of root slime, was present only in the membrane system of the root-tip region of the root. Regions of typical secondary wall development within the root were characterized by an increased incorporation of radioactivity into xylose of polysaccharide within the membrane system. 4. The incorporation of radioactivity into glucan polymers in the membrane fractions was very low in all regions of the root. Since in regions of secondary wall development greater than 60% of all radioactive incorporation was into a glucan polymer, it can be inferred that this polymer, most probably cellulose, is not synthesized or transported within the compartments of the membrane system. It is suggested that synthesis of cellulose occurs at the surface of the plasmalemma. 5. Maize-root cells contained 40 times more rough endoplasmic reticulum than dictyosome membrane. The relative specific radioactivities of each fraction indicated that polysaccharide was concentrated in the region of the Golgi apparatus, which showed a 100% increase in specific radioactivity compared with the rough endoplasmic reticulum. The Golgi apparatus can thus be regarded as a localized focal point on the synthetic and transport system of polysaccharide by the intracellular membrane compartments.

The polysaccharide components of the matrix of the cell wall vary according to the stage of differentiation of the cell. During growth there is a change in the relative extent of the formation and incorporation into the wall of pectin, hemicellulose and cellulose, so that if a comparison is made between regions involved in primary wall formation and those involved in secondary wall formation, the components of the cell wall of the two regions should be found to be different (Thornber & Northcote, 1961; Northcote, 1969*a*; Stoddart & Northcote, 1967). The maize root is an ideal system for such an investigation into the changes that occur during development (Roberts & Butt, 1967, 1969). The root-cap zone of the root has been shown to produce a slime polysaccharide containing a high proportion of fucose (Harris & Northcote, 1970; Kirby & Roberts, 1971). Since fucose is present only in slime, and the root cap is the only part of the root system that produces the slime, the presence of fucose can be used as a marker to investigate the change in polysaccharide synthesis between the differentiated root-cap cells and the cells immediately adjacent to them but further back in the root.

The Golgi apparatus has been shown to be involved in the production of cell-wall polysaccharides both by radioautographic methods and observations of fine structure (Northcote & Pickett-Heaps, 1966; Whaley *et al.*, 1966; Wooding, 1968; Roberts & Northcote, 1970) and by the presence of polysaccharide in isolated Golgi cisternae and vesicles after cell fractionation (Van der Woude *et al.*, 1971; Harris & Northcote, 1971). It has been suggested that the Golgi apparatus may also be concerned with slime production (Northcote & Pickett-Heaps, 1966; Morré *et al.*, 1967; Kirby & Roberts, 1971).

In the present study we investigate the involvement of the membrane system comprising rough endoplasmic reticulum, dictyosomes and smooth-membrane vesicles, in the formation of exportable polysaccharide. The results indicate that the type of polysaccharide synthesized within the membrane system

is directly correlated with the state of differentiation of the root zones.

Methods

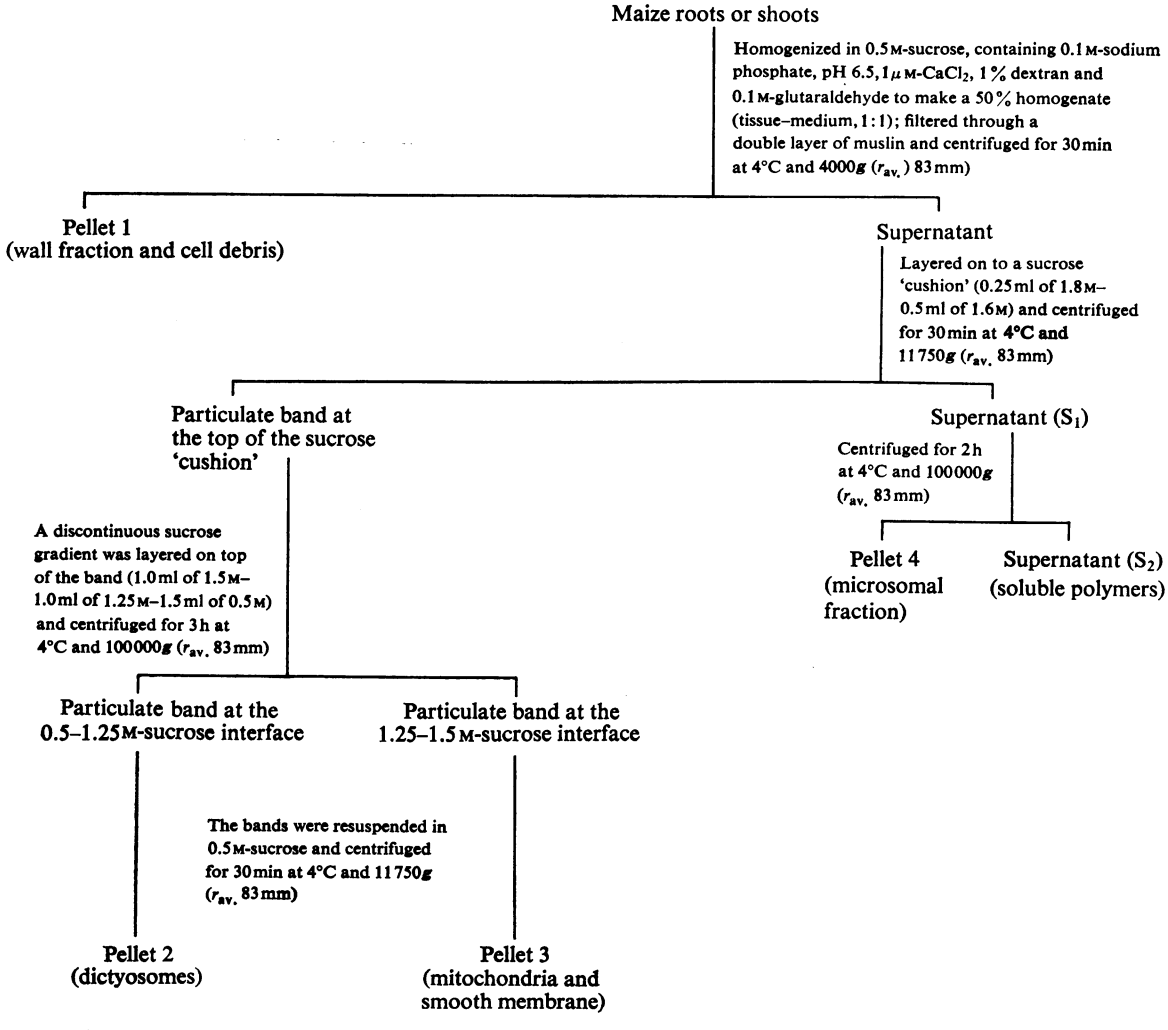
Growth of tissue and general method of incubation

Seedlings of *Zea mays* variety Caldera were grown under sterile conditions on filter paper (Harris & Northcote, 1970) and 48h after sowing 80–120 seedlings were selected. They were washed several times in sterile water, surface-dried and then incubated for 2h in daylight at room temperature in D-[U-^{14}C]-glucose (specific radioactivity 320mCi/mmol) from The Radiochemical Centre, Amersham, Bucks., U.K., diluted with sterile water. The radioactive glucose solutions (150μl, 5μCi) were contained in glass Durham tubes (height 25mm, diam. 5mm). Five seedlings were incubated in each tube with the main root suspended in the nutrient.

After incubation, root slime and excess of nutrient were washed off with sterile water, the seedlings were surface-dried and the main root was used for the investigation.

Whole-root investigation

After removal close to their origin the main roots were mixed with 16g of washed, surface-dried unlabelled roots. Homogenization and fractionation techniques were based on previous methods (Morré,



Scheme 1. *General procedure for the preparation of the cell fractions from the maize root or shoot*

1970; Harris & Northcote, 1971). The cell-fractionation procedure used is summarized in Scheme 1. Centrifugations were carried out at 4°C by using a Beckman Spinco L2 centrifuge with a SW 50L rotor; the radius of the rotor was 83mm. The sucrose solutions used to make the discontinuous gradients contained all the constituents of the homogenization medium. The four pellets obtained were washed first with several changes of aq. 75% (v/v) ethanol containing D-glucose (50g/l), and then with aq. 75% (v/v) ethanol and dried *in vacuo*.

Dictyosomes were also prepared from non-radioactive shoots of the seedlings by a method identical with that described for the roots.

Slime and extracellular polysaccharides

The post-incubation radioactive solutions were collected and added to the excess of nutrient that had been washed off the roots. The solutions were then evaporated to small volume at 50°C *in vacuo*, mixed with toluene and dialysed against three changes of 3 litres of glass-distilled water for 3 days at 4°C. The non-diffusible material was evaporated to dryness and stored at −20°C.

Preparation of Golgi bodies and other membranes from the root cap and older root zones

The number of dictyosomes in the root cap is too small for the usual procedures of preparation to be used. However, if organelles of the root cap are labelled by incubation in a radioactive nutrient, they remain distinct from unlabelled organelles from another source, provided that possible mixing of the radioactivity between the two is eliminated. Thus unlabelled dictyosomes from an abundant source can be used to 'carry' labelled dictyosomes, thereby providing a useful tool in the investigation of the metabolism of organelles from initial low-bulk sources.

The tip (approx. 1–2mm) of each root was removed to include the root cap, the next 10mm was discarded and the remainder, constituting the older tissue, was cut off close to the origin of the root. Each sample was ground in a small volume of homogenization medium containing 0.2M-glutaraldehyde. The cap-segment homogenate was not filtered through muslin but was mixed with an unlabelled filtered whole-root homogenate (0.2M-glutaraldehyde in the medium) to a final volume of 5.0ml. The homogenate of the older root tissue was filtered through two layers of muslin and then mixed with unlabelled filtered homogenate, to a final volume of 10.0ml. The centrifugation procedure for both samples was the same as that shown in Scheme 1.

Electron microscopy

The particulate fraction sedimenting at the 0.5–1.25M-sucrose interface was examined by negative staining with 2% phosphotungstic acid, neutralized by NaOH to pH6.8. In addition, the pellets (1–4, Scheme 1) were fixed for 30min in phosphate (0.02M, pH7.2)-buffered glutaraldehyde (6%) containing 0.5M-sucrose, post-fixed for 15min in osmic acid (1%) buffered with veronal, dehydrated with ethoxyethanol and embedded in Araldite. Thin sections of the embedded material were cut and mounted on grids (400 mesh), stained with uranyl acetate and alkaline lead hydroxide and then examined in a GEC EM6B at 60kV (Burgess & Northcote, 1967).

Determination of lipid content of pellets

The subcellular fractions were prepared from a known fresh weight of whole maize roots, by using the scheme of cell fractionation summarized in Scheme 1. The pellets 2, 3 and 4 were washed once with water.

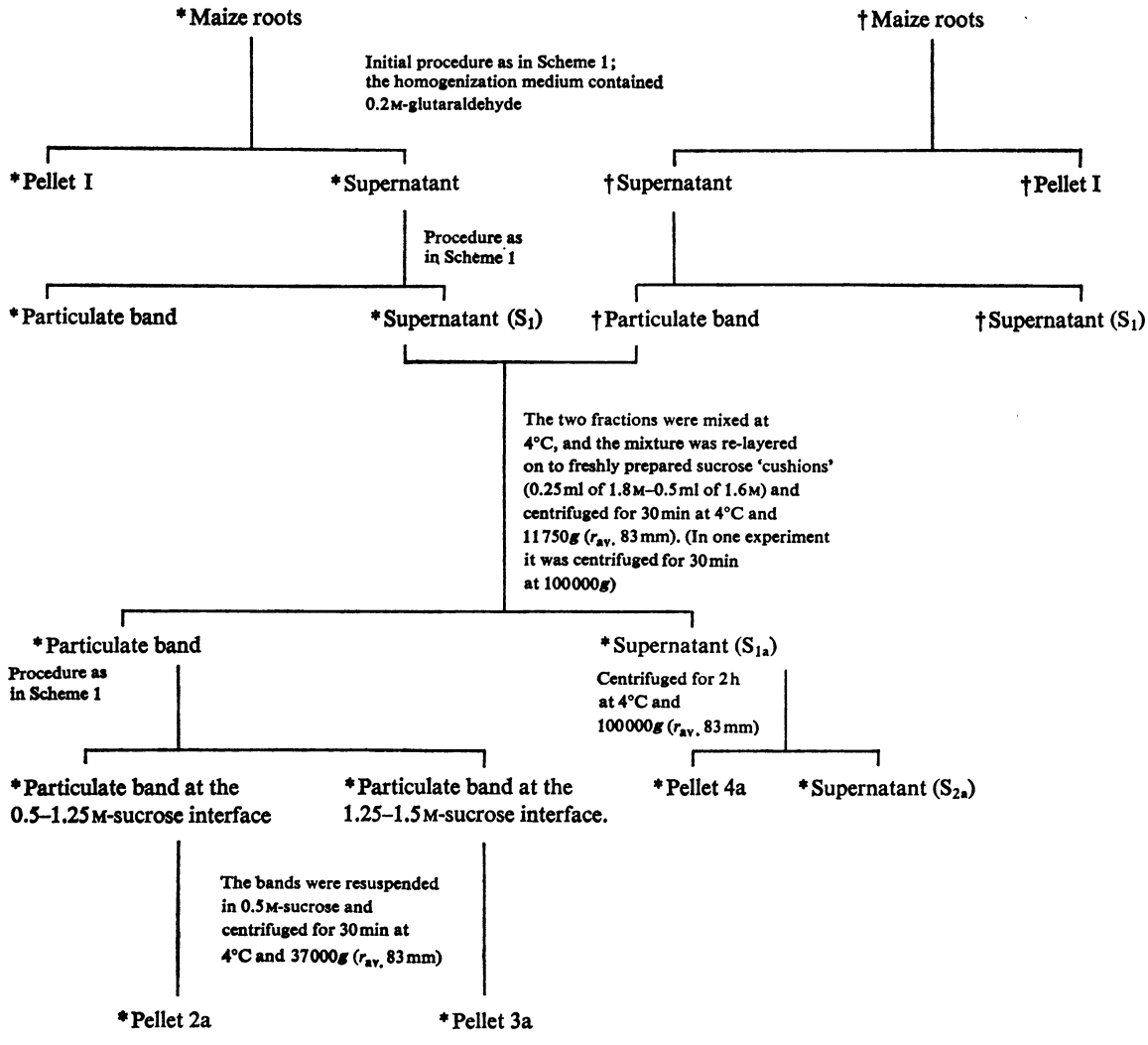
The apparatus used in the extraction was washed with chromic acid and stored before use in dust-free containers. The organic solvents were redistilled before use. Lipid extraction from the samples was based on the method of Bligh & Dyer (1959). The apparatus used was subsequently washed with chloroform–methanol (2:1, v/v; Folch *et al.*, 1957) and the washings, after decrease in volume, were added to the main extracts.

Vacuum distillation of the solvents in a grease-free glass apparatus was used to decrease the volume of the washings, and to evaporate the final main extracts to dryness.

The lipid was finally dissolved in a small volume of diethyl ether, and was transferred to aluminium boats that had been previously washed in chloroform and weighed on a Sartorius microbalance model 4125. The boats were re-weighed and the weight of the lipid from each fraction was calculated.

Hydrolysis and analysis of the fractions

All of the wall-fraction pellets (pellet 1) were dissolved in 0.5ml of 72% (w/w) H_2SO_4; the dialysed post-incubation medium and supernatants S_2 (Scheme 2) were dissolved in 0.25ml of 72% (w/w) H_2SO_4. Each of the membrane pellets (pellets 2–4) was dissolved in 50μl of 72% (w/w) H_2SO_4. After 2–4 h the acid was diluted to 3% (w/w) H_2SO_4 and the preparations were hydrolysed by autoclaving at 103.4kN/m^2 (15lb/in^2) at 120°C for 1h. The hydrolysates were centrifuged to remove any insoluble particles, and neutralized by mixing with sufficient changes of equal volumes of a 10% (v/v) solution of *NN*-dioctylmethylamine (Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K.) in chloroform until the pH value was greater than 6.0. Some excess of amine was removed by washing with several changes of chloroform. The neutralized hydrolysates were



* Radioactive; † non-radioactive or very little radioactivity.

Scheme 2. *Experimental scheme used to investigate the amount of contamination in the membrane-fraction pellets 2 and 3 by particulate and soluble material produced during the homogenization*

evaporated at 40°C, under reduced pressure, to dryness, the residues dissolved in a small quantity of water and then run electrophoretically on Whatman no. 1 paper (pH2.0, 30min, 5kV) to separate any remaining amine and contaminant peptides and amino acids from neutral sugars and uronic acids (Northcote & Pickett-Heaps, 1966). The electrophoretograms were cut into 4cm×1cm strips and their radioactivities were counted (Harris & Northcote, 1970). The paper strips containing the radioactive material that ran at the region of the neutral sugar/uronic acid marker were removed from the scintillation fluid, washed three times with toluene, twice with benzene, and then dried and eluted with water. The eluate was evaporated at 40°C *in vacuo*, the residue dissolved in a small quantity of water, and either part or all of the sample, dependent on the amount of radioactivity present, was run chromatographically in solvent I [ethyl acetate–pyridine–water (8:2:1, by vol.)] for 24h. The radioactive incorporation was counted, and the paper strips containing radioactive material that ran respectively at the region of uronic acids, galactose/glucose/mannose and xylose markers in solvent I were removed from the scintillation fluid,

washed as before, dried and eluted. The eluates were evaporated under reduced pressure and the residues dissolved in small quantities of water. The solution of uronic acids was run electrophoretically (pH3.5, 45min, 4.0kV) (Harris & Northcote, 1970) and the radioactivity in the regions corresponding to galacturonic acid and glucuronic acid was counted. The solution of galactose/glucose/mannose was run chromatographically in solvent I for 56h and the radioactivity in the regions corresponding to galactose, glucose and mannose was counted. The solution in the region of the xylose marker was run chromatographically in water-saturated phenol (phenol–water, 100:39, w/v) for 18h, and the radioactivity in regions corresponding to xylose and fucose was counted.

Results

Electron-microscopic examination of the fractions (Scheme 1)

Pellet 1. Thin sections of the pellet showed that it was heterogeneous but that it was mainly composed of wall fragments; nuclei and mitochondria were also present.

Pellet 2. Negative staining showed that the particulate band at the 0.5–1.25M-sucrose interface contained high proportions of Golgi cisternae, either completely separate from one another (Plate 1,*a*), or in groups of three to six (Plate 1,*b*). In the central region of the cisternal elements very densely packed tubules and membrane sheets were present (Plate 1,*c*), whereas in others the innermost zone appeared hollow and it was surrounded by loosely packed tubules and fenestrated membrane (Plate 1,*d*). Each cisterna of a group had a different and distinct central region. The peripheries of all cisternae were composed of vesiculating tubules and fenestrated membrane. The extent of the tubular region depended on the type of central morphology. Vesicles of two types were observed: rough-surfaced vesicles that formed at the end of tubules by constriction and smooth-surfaced vesicles (Plate 2,*a*), the majority of which seemed to be formed by vesiculation of a mid-region of a tubule (Plate 2,*a*). There was a possible tubular interconnexion between the cisternae in any one group of the negatively stained material (Plate 1,*b* and Plate 2,*b*).

Thin sections of the Golgi pellet indicated that the fraction isolated had little contamination from other organelles or from other parts of the membrane system. Sections through the depth of the pellet showed striations, with smooth- and rough-membrane vesicles that were less dense than the complete stacks of the Golgi cisternae (Plate 3,*a*). In some cases intercisternal material was visible in the stacks. This can also be seen in some negatively stained preparations (Plate 1,*a*).

Pellet 3. Thin sections of the pellet showed striations through its depth and a great variation in components. The most-dense layer consisted of mitochondria but they were virtually absent from less-dense layers that were made up of smooth-membrane vesicles, proplastids and, to a lesser extent, rough-membrane vesicles (Plate 3,*c*,*d*).

Pellet 4. Thin sections of the pellet showed that although smooth and rough membrane were present the pellet consisted mainly of rough membrane and free ribosomes (Plate 3,*b*).

Lipid content of pellets 2, 3 *and* 4

Lipid content was determined by a direct gravimetric method. The method used for the lipid extraction (Bligh & Dyer, 1959) gives 96% recovery of lipid from the original material. The results shown in Table 1 indicate that the lipid content of pellet 4 (microsomal fraction) is much greater than that of either pellet 2 (dictyosome fraction) or pellet 3 (smooth-membrane fraction). Since the amount of lipid is related to the amount of membrane present, these

Table 1. *Amounts of the three membrane fractions isolated from maize roots, and the relative specific radioactivities of the radioactivity incorporated from* D-[*U*-^{14}*C*]*glucose into the polysaccharide components of the membrane fractions*

Lipid was extracted from pellets 2, 3 and 4 (Scheme 1), chloroform–methanol–water being used to give an optimum extraction of total lipids from the membrane fractions.

	Wt. of lipid extracted from membrane fractions of maize roots		Average relative amounts of the different membrane fractions	Relative specific radioactivities of the radioactivity in the polysaccharide components
	(μg/11g fresh wt. of roots)	(μg/15g fresh wt. of roots)		
Pellet 2 (dictyosomes)	80	264	1	6.0
Pellet 3 (smooth membrane)	159	502	2	1.0
Pellet 4 (rough membrane)	4001	6950	40	2.9

results show that in a known fresh weight of maize-root tissue, the rough endoplasmic reticulum constitutes the bulk of the membrane system.

Distribution and location of radioactivity in the various fractions

The results of five separate experiments showed that the incorporation of radioactivity into the fractions obtained from whole roots was constant and ranged from 4500 to 5250c.p.m. for pellet 2, from 1500 to 2000c.p.m.for pellet 3 and from 88000 to 90000c p.m. for pellet 4. The results given in Tables 1–5 refer to a single experiment.

Pellet 1. The percentage distribution of radioactivity in the polysaccharide components of the wall-fraction pellets from the whole-root, root-cap and older tissue as defined in the Methods section is shown in Table 2. The total amount of radioactivity that was incorporated into the older walls was very much less than that incorporated into the root cap, although the actual bulk of tissue of the former was greater (20–30mm) than that of the root cap (1–2mm). When the results are calculated to show the radioactivity ratios galacturonic acid/glucuronic acid, galactose/glucose and arabinose/xylose it can be shown that in the young tissue before secondary thickening of the walls has occurred there is a greater relative incorporation of the glucose into galacturonic acid, galactose and arabinose than in the older walls (Table 6).

Table 2. *Relative amounts of radioactivity incorporated from* D-[*U*-^{14}*C*]*glucose into the polysaccharide components of pellet* 1 (*wall fraction*) *from maize-root tissue*

Roots were incubated with radioactive glucose for 2h and then homogenized. Wall fractions were obtained by centrifugation of the initial homogenate for 30min at 4°C and 4000*g* ($r_{av.}$ 83mm).

	Radioactivity (%)		
Sugar	Whole roots	Root-cap tissue	Older tissue
Galacturonic acid	2.6	7.8	2.7
Glucuronic acid	1.2	1.3	0.8
Galactose	7.0	17.4	6.9
Glucose	63.8	38.3	62.5
Mannose	1.2	2.5	1.8
Arabinose	7.2	12.5	8.8
Xylose	15.7	13.5	15.6
Fucose	0.9	4.9	0.4
Ribose+rhamnose	2.7	1.2	0.7
Total c.p.m.	720300	130500	18200

Pellet 2. The percentage distribution of radioactivity in the polysaccharide components of the dictyosome fraction (pellet 2) is shown in Table 3. Irrespective of the origin of the Golgi apparatus,

Table 3. *Relative amounts of radioactivity incorporated from* D-[*U*-^{14}*C*]*glucose into the polysaccharide components of pellet* 2 (*dictyosome fraction*) *from maize-root tissue*

Roots were incubated with radioactive glucose for 2h and then homogenized. Dictyosome fractions were obtained by discontinuous sucrose-density-gradient centrifugation. The particulate band present at the 0.5–1.25M-sucrose interface was centrifuged to give a pellet.

	Radioactivity (%)		
Sugar	Whole roots	Root-cap tissue	Older tissue
Galacturonic acid	14.0	11.6	10.3
Glucuronic acid	1.9	4.7	1.6
Galactose	26.6	34.1	21.5
Glucose	6.6	6.8	3.95
Mannose	3.5	1.8	1.0
Arabinose	22.3	14.7	19.8
Xylose	20.1	18.5	41.4
Fucose	2.8	6.2	0.2
Ribose+rhamnose	1.7	1.7	—
Total c.p.m.	4500	1100	790

Table 4. *Relative amounts of radioactivity incorporated from* D-[*U*-^{14}*C*]*glucose into the polysaccharide components of pellet* 3 *from maize-root tissue*

Roots were incubated with radioactive glucose for 2h and then homogenized. The fractions were obtained by discontinuous sucrose-density-gradient centrifugation, and the particulate band present at the 1.25–1.5M-sucrose interface was centrifuged to give a pellet.

	Radioactivity (%)		
Sugar	Whole roots	Root-cap tissue	Older tissue
Galacturonic acid	10.2	8.6	10.2
Glucuronic acid	4.9	4.6	5.6
Galactose	24.4	37.4	26.0
Glucose	12.9	6.4	7.3
Mannose	4.0	5.3	—
Arabinose	22.4	16.3	14.7
Xylose	15.3	13.4	32.8
Fucose	1.6	8.1	2.8
Ribose+rhamnose	3.7	—	—
Total c.p.m.	1500	950	350

EXPLANATION OF PLATE 1

Electron micrographs of negatively stained preparations of a dictyosome-rich fraction isolated from maize-root (b, c) and shoot (a, d) tissue

The fraction was isolated by centrifugation of a plant homogenate in the presence of 0.1 M-glutaraldehyde. (*a*) A single cisterna showing the characteristic fenestrated membrane and outward-radiating tubules. Rough-surfaced and smooth-surfaced vesicles are formed towards the periphery. Fibrous material, which could be intercisternal elements, can be seen (arrow) (compare with Plate 3,*a*) (×33000). (*b*) A group of cisternae (×24000). (*c*) A cisterna depicting one extreme of form of the central zone, where the region is composed of solid membrane sheets and very densely packed tubules. Few vesicles are observed (×45000). (*d*) A cisterna showing the other extreme in type of central zone, which is hollow with fenestrated membrane and loosely packed tubules radiating outwards from it (×48000). The horizontal bars in (*a*)–(*d*) represent 1 μm.

EXPLANATION OF PLATE 2

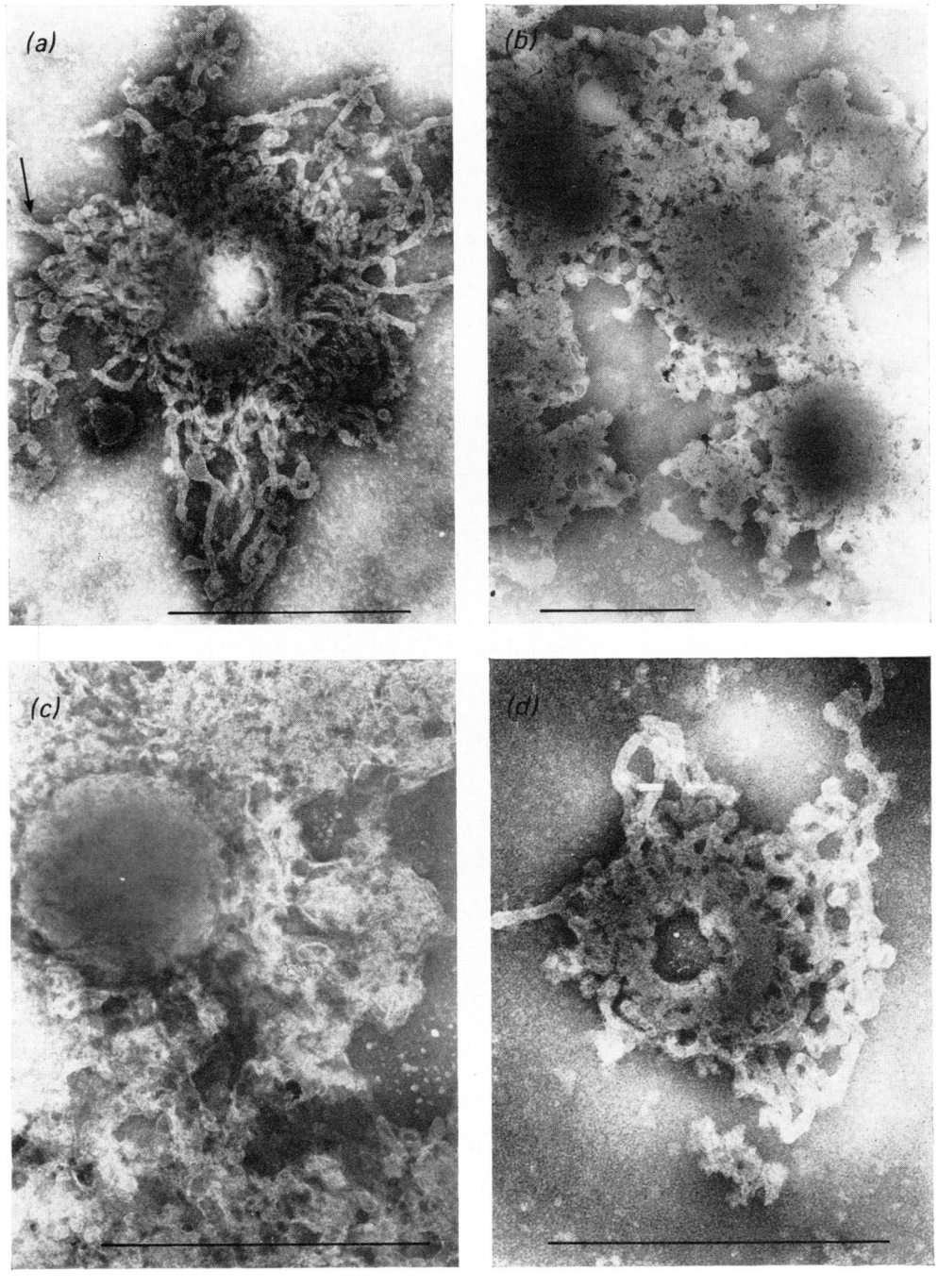
Electron micrographs of negatively stained preparations of a dictyosome-rich fraction isolated from maize-root (b) and shoot (a) tissue

The fraction was isolated by centrifugation of a plant homogenate in the presence of 0.1 M-glutaraldehyde. (*a*) Detail of part of a cisterna, showing the formation and structure of the two types of vesicles. The rough-surfaced vesicles (r) are formed by constriction of the ends of tubules, whereas the smooth-surfaced vesicles (s) are formed more often from a mid-region of a tubule (×65000). (*b*) Detail of part of two cisternae, showing possible tubular links (t) between the cisternae (×55000). The vertical bars on the micrographs represent 1 μm.

EXPLANATION OF PLATE 3

Electron micrographs of ultra-thin sections of cell fractions isolated from maize roots

The cell fractions were isolated by differential centrifugation after stabilization of the homogenate by the presence of 0.1 M-glutaraldehyde. (*a*) Section through pellet 2, showing stacks of Golgi cisternae. Intercisternal material is visible in transverse sections through the stacks (arrow) (compare with Plate 1,*a*) (×24000). (*b*) Section through pellet 4, showing rough endoplasmic reticulum membrane fragments and vesicles (×45000). (*c*) Section through the densest region of pellet 3, showing mitochondria and rough-membrane vesicles (×24000). (*d*) Section through a less-dense region of pellet 3, showing smooth-membrane vesicles and proplastids in addition to mitochondria (×22000). The horizontal bars on the micrographs represent 1 μm.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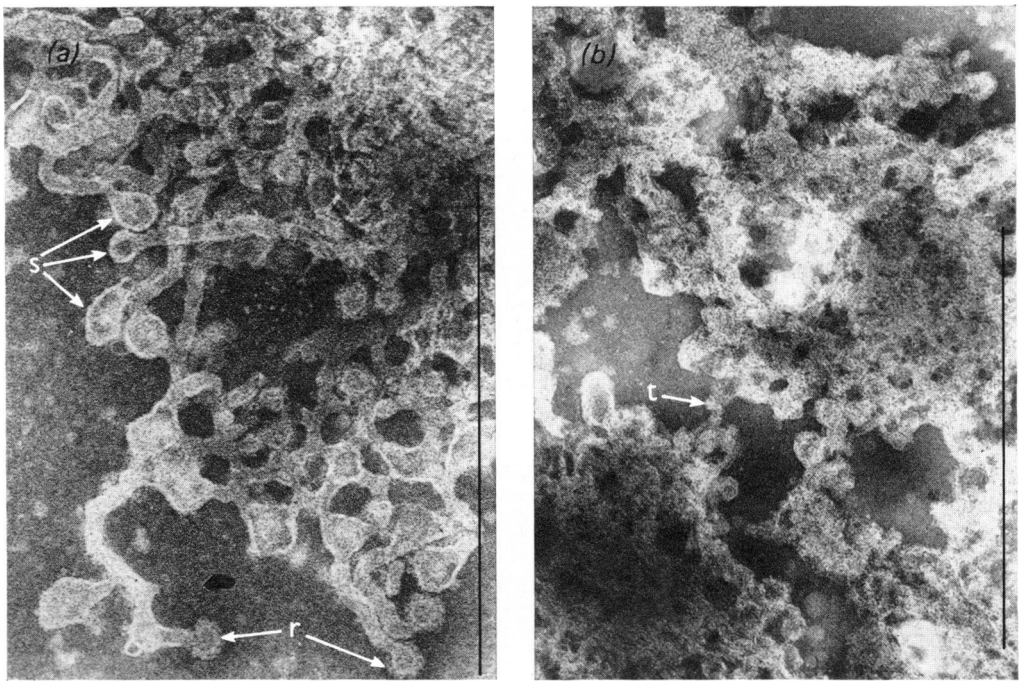
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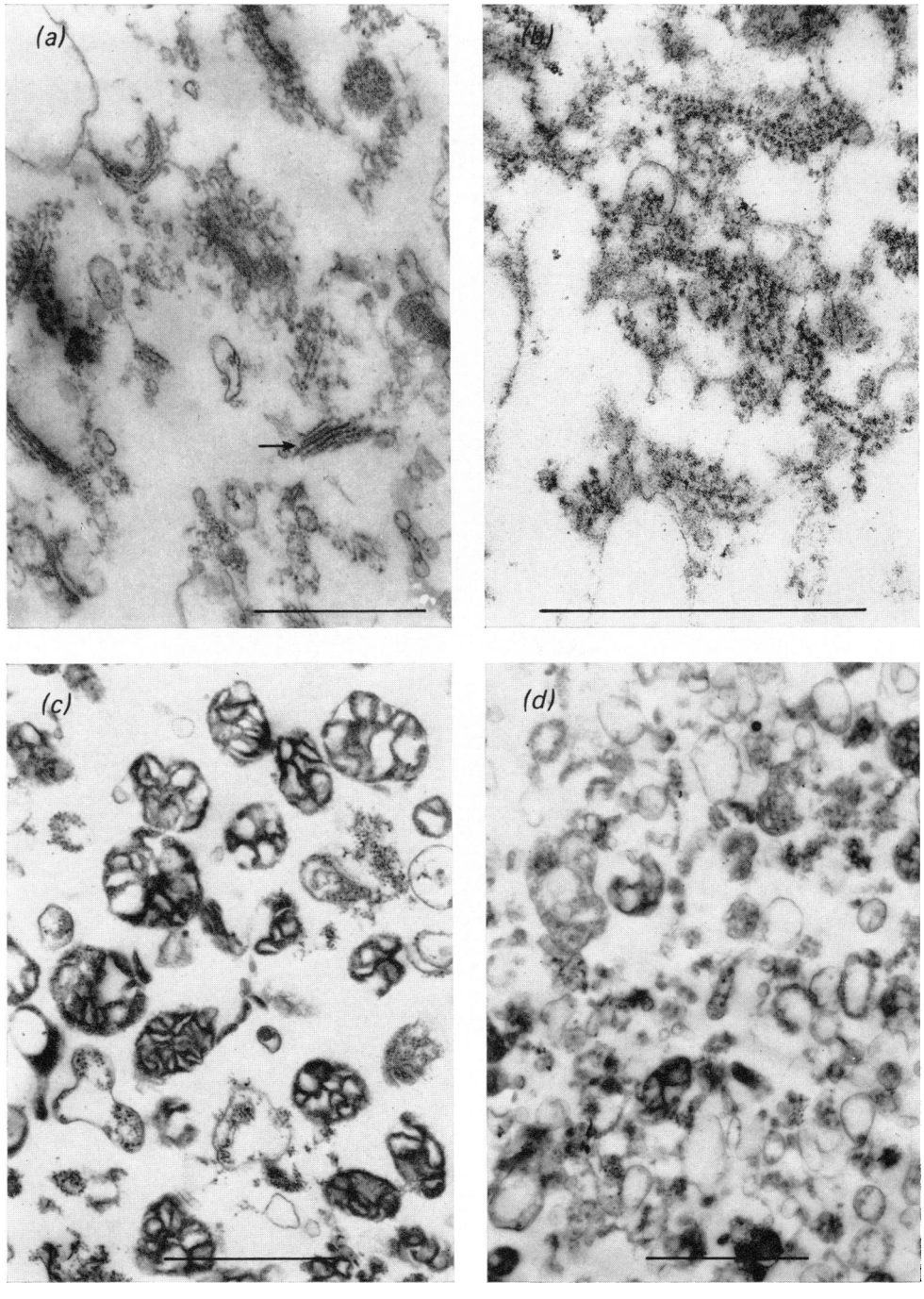
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(d)

incorporation of radioactivity into glucose was very low compared with the high incorporation into galacturonic acid, galactose and arabinose. The main distinction between the dictyosomes of the root cap and those of the older tissue was the presence of radioactivity in fucose in the organelles isolated from the root cap. In the Golgi bodies isolated from the older tissue there was a comparatively high amount of incorporation into xylose. The ratios of radioactive incorporation into the monosaccharides of the pectic polysaccharides (galactose, galacturonic acid and arabinose) to the incorporation into the monosaccharides of hemicellulose (glucose, glucuronic acid and xylose) have been calculated in a similar manner to that described for the wall fraction and are shown in Table 6.

Carrier dictyosomes of a partially disrupted membrane system might have been able to utilize radioactive nutrient present in cytoplasmic pools, and adsorbed to the surface of organelles. To test this

Table 5. *Relative amounts of radioactivity incorporated from* D-[*U*-^{14}*C*]*glucose into the polysaccharide components of pellet* 4 *from maize-root tissue*

Roots were incubated with radioactive glucose for 2h and then homogenized. The fractions were obtained by centrifugation for 2h at 4°C and 100000*g* ($r_{av.}$ 83mm) of a supernatant (S_1) free from heavier membrane components.

	Radioactivity (%)		
Sugar	Whole root	Root-cap tissue	Older tissue
Galacturonic acid	7.5	11.2	5.2
Glucuronic acid	1.7	1.6	1.2
Galactose	24.1	31.4	21.2
Glucose	4.0	7.3	8.5
Mannose	3.1	5.0	3.4
Arabinose	23.0	22.4	20.4
Xylose	31.0	16.4	38.8
Fucose	1.9	4.5	1.1
Total c.p.m.	89000	68000	26000

D-[U-^{14}C]glucose (50μCi; specific radioactivity 320mCi/mmol) was added to a homogenate of unlabelled maize roots. At a glutaraldehyde concentration of 0.2M, no radioactivity was observed in the neutral sugar components of polysaccharide in either the dictyosome fraction or in the cell-wall fraction.

Supernatant 2 (S_2, *Scheme* 1). Table 7 shows the distribution pattern of radioactivity incorporation into the polysaccharide component of the soluble polymers present in the supernatant S_2.

Analysis of soluble polymers from both regions of the root showed that approx. 70% of the radioactivity was present in polysaccharide and the remaining 30% occurred in protein that was removed in the electrophoresis at pH2.

Soluble polysaccharides in the incubation medium. Soluble polysaccharides were present in the incubation medium containing radioactive glucose in which the seedlings were placed for 2h initially. These solutions were collected and analysed and the results are shown in Table 8. The high amount of radioactivity in the fucose is characteristic of the slime produced by the outer root-cap cells (Harris & Northcote, 1970; Kirby & Roberts, 1971).

Pellet 3. Table 4 shows that the distribution pattern of radioactivity incorporation into pellet 3 was similar to that exhibited by the dictyosome fraction.

Radioactive polysaccharides may be adsorbed on to the membranes present in the pellets (2 and 3), or they may be present because they are of sufficient molecular weight to sediment at the same level in the density gradient as the components of the pellets. In addition some contamination by small fragments of broken membranes may occur. A series of experiments was designed to investigate these possibilities.

Contamination by soluble polysaccharides and membrane fragments

The fractionation used is shown in Scheme 2. Unlabelled particulate fractions were removed from the 1.6–1.8M-sucrose 'cushion' and were added to an equivalent supernatant S_1, which was obtained from whole roots that had been previously incubated with

Table 6. *Ratios of amounts of radioactivity incorporated into galacturonic acid and glucuronic acid, galactose and glucose, and arabinose and xylose, by the different fractions obtained from maize root-cap* (1) *and older root tissue* (2) *by differential centrifugation*

Sugar radioactivity ratio	Pellet 1		Pellet 2		Pellet 3		Pellet 4		S_2	
	1	2	1	2	1	2	1	2	1	2
Galacturonic acid/glucuronic acid	6.1	3.4	2.5	6.4	1.9	1.8	7.0	4.3	2.7	3.6
Galactose/glucose	0.45	0.11	5.0	5.4	5.8	3.6	4.3	2.5	7.97	5.5
Arabinose/xylose	0.92	0.56	0.79	0.47	1.2	0.4	1.4	0.5	2.6	2.2

Table 7. *Relative amounts of radioactivity incorporated from* D-[*U*-^{14}*C*]*glucose into soluble polysaccharides of maize-root tissue*

Roots were incubated with radioactive glucose for 2h and then homogenized. Soluble polysaccharides were isolated as a supernatant from which all membrane fragments had been removed by differential centrifugation (30min at 4000*g*; 30min at 11750*g*; 2h at 100000*g*, all at 4°C and $r_{av.}$ 83mm). The supernatant was dialysed against 9 litres of glass-distilled water (unbuffered) for 3 days. The non-diffusible material was ultrafiltered, acid-hydrolysed and analysed.

	Radioactivity (%)		
Sugar	Whole root	Root-cap tissue	Older tissue
Galacturonic acid	7.1	5.7	18.2
Glucuronic acid	1.5	2.1	5.1
Galactose	29.4	35.9	31.2
Glucose	3.2	4.5	5.7
Mannose	2.7	0.8	1.1
Arabinose	24.4	33.3	23.0
Xylose	23.5	12.9	10.6
Fucose	7.9	4.9	5.1
Total c.p.m.	366300	237600	57600

Table 8. *Relative amounts of radioactivity incorporated from* D-[*U*-^{14}*C*]*glucose into polysaccharide components of root slime (polysaccharide present in the incubation medium) from maize roots*

Roots were incubated with radioactive glucose for 2h. The post-incubation medium and washings were collected and dialysed against 9 litres of glass-distilled water (unbuffered) for 3 days. The non-diffusible material was acid-hydrolysed and analysed.

Sugar	Radioactivity (%)
Galacturonic acid	10.1
Glucuronic acid	1.8
Galactose	23.9
Glucose	4.0
Mannose	2.1
Arabinose	13.8
Xylose	15.0
Fucose	28.3
Total c.p.m.	150800

radioactive glucose. The mixture was layered on to freshly prepared sucrose 'cushions', and the particulate fractions were re-sedimented on to the 'cushion'. In a preliminary investigation the centrifuge speed used for this re-sedimentation was 36000rev./min (100000*g*); on all subsequent occasions 12000rev./min (11750*g*) was used. The supernatant S_{1a} was removed and the discontinuous sucrose gradient was layered on to the 'cushion' and centrifuged as shown in Scheme 2. Particulate bands from the gradient were centrifuged at 20000rev./min (37000*g*) to give pellets 2a and 3a. The supernatant S_{1a} was centrifuged at 100000*g* for 2h to form pellet 4. All pellets were washed and analysed.

The membrane bands were labelled and the extent of labelling was dependent on the centrifuge speed of the re-sedimentation step on to the sucrose 'cushion'; if the particulate layer was sedimented at 100000*g*, the radioactivity present subsequently in the 0.5–1.25M-sucrose interface band was 2000c.p.m., and 1500c.p.m. in the 1.25–1.5M-sucrose interface band. However, when the speed of re-sedimentation on to the sucrose 'cushion' was decreased to 11750*g*, the subsequent labelling of the membrane bands was also decreased (1800c.p.m. and 660c.p.m. respectively) (Table 9). Thus contamination of the heavier 1.25–1.5M-sucrose interface band was most affected by the centrifuge speed of the re-sedimentation step.

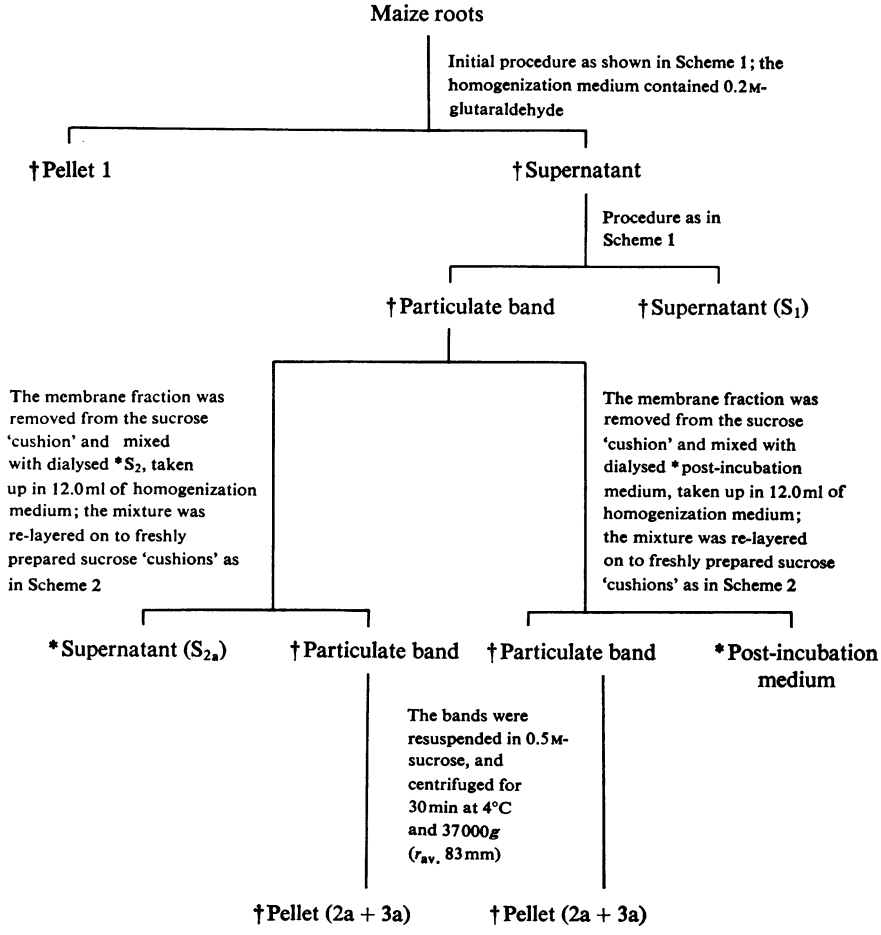
Contamination by soluble polysaccharide

Clarified radioactive supernatant S_2 was mixed with toluene and dialysed against three changes of 3 litres of glass-distilled water for 3 days at 4°C, and the non-diffusible material was filtered through a glass-fibre disc and Millipore filter to remove material that precipitated during the dialysis. Approximately 50% of the radioactive polymers were retained during ultrafiltration. The filtrate, containing approx. 150000c.p.m., was evaporated to dryness *in vacuo* and the residue was dissolved in 12.0ml of homogenization medium. The high-molecular-weight dextran used in the medium (mol.wt. 250000–750000) was probably still present after the dialysis, and consequently dextran was omitted from the medium used to dissolve the soluble polymers of the supernatant. This radioactive solution was mixed with a freshly prepared unlabelled particulate fraction (Scheme 3). After mixing, the particulate fraction, which was re-sedimented at 11750*g*, was taken from the sucrose 'cushion' with a Pasteur pipette and centrifuged at 37000*g* for 30min to give pellets of the membrane fractions (pellets 2a and 3a), which were washed and analysed. The radioactivity in the previously unlabelled membrane fraction was 240c.p.m. Thus centrifugation of the radioactive supernatant before addition to the unlabelled particulate fraction decreased contamination of that fraction by one order of magnitude (Table 9). Therefore the radioactivity obtained in the pellets 2 and 3, that is shown in Tables 3 and 4, could not be due to contamination by the soluble polysaccharides pre-

Table 9. *Incorporation of radioactivity (c.p.m.) into the components of polysaccharides of non-radioactive unlabelled membrane fractions, after mixture with radioactive polysaccharide (S_1, S_2 or the post-incubation medium)*

Non-radioactive particulate membrane fractions were removed from a sucrose 'cushion' (1.6M/1.8M), added to solutions of radioactive polysaccharides, and the mixture was re-layered on to a sucrose 'cushion' and centrifuged for 30min at 4°C (11750*g*, $r_{av.}$ 83mm) to re-sediment the particulate fraction (Schemes 2 and 3).

Membrane fraction mixed with radioactive S_1 (450000c.p.m.) and centrifuged to separate pellets 2a and 3a (Scheme 2)		Membrane fraction mixed with radioactive S_2 (150000c.p.m.) and centrifuged to give combined pellet 2a and 3a (Scheme 3)	Membrane fraction mixed with post-incubation medium (75000c.p.m.) and centrifuged to give a combined pellet 2a and 3a (Scheme 3)
Pellet 2a	Pellet 3a		
1800	660	240	150



* Radioactive; † non-radioactive or very little radioactivity.

Scheme 3. *Experimental scheme used to investigate the amount of contamination in the membrane fractions pellets 2 and 3 by soluble material produced during the homogenization*

sent in the initial supernatant from the homogenized tissue.

A radioactive post-incubation medium, and washings from an incubation of the roots in radioactive glucose, were evaporated under reduced pressure to dryness, dissolved in 5.0ml of water, centrifuged at 100000*g* for 2h and the supernatant was then dialysed under the same conditions as supernatant S_2, to remove the unused radioactive glucose. After ultrafiltration and evaporation to dryness the residue was dissolved in 12.0ml of homogenization medium and added to sufficient unlabelled particulate material to provide two pellets after re-sedimentation and centrifugation at 37000*g* for 30min. One pellet was washed in the usual way, and the other pellet was washed with a solution of glucose and unlabelled root-slime polysaccharide in aq. 75% ethanol and then in aq. 75% ethanol. After drying *in vacuo* and analysis of these pellets only a small amount of radioactivity was found, and its presence was unaffected by washing with unlabelled slime polysaccharide (Table 9).

Radioactivity in pellet 4

Total incorporation into pellet 4 was much higher than into the other membrane fractions. The labelling of polysaccharide components again showed a distribution pattern similar to that of pellets 2 and 3 (Table 5).

The radioactivity of the polysaccharides in pellet 4 may have been due either to vesicles derived from dictyosomes or to such membrane fragments as broken cisternae, or vesicles, or to smooth endoplasmic reticulum or plasmalemma that are actively involved in polysaccharide synthesis. Membranes broken during homogenization may form vesicles in an environment of radioactive polymers to give a radioactive pellet.

To investigate these possibilities, D-[U-^{14}C]glucose was given to the seedlings, and samples of root-tip and older tissue were obtained in the same way as before. To avoid mixing small amounts of root cap with unlabelled root homogenates, which would result in dilution of radioactive polymers of the root cap, 5–10mm of root tip was used instead of the 1–2mm used previously. Radioactive supernatants S_2 were obtained from both root samples, and were dialysed, filtered and evaporated to dryness under reduced pressure. A sample (20%) of the root-tip supernatant was retained for analysis, and 80% was dissolved in 15.0ml of homogenization medium that contained no dextran. Older root tissue (15.0g) was prepared from unlabelled roots, and was homogenized in a mixture of the homogenization medium and radioactive root-tip polysaccharide. Pellet 4 was obtained by fractionation and analysed. The radioactivity recovered in the pellet was only 50c.p.m., although the radioactivity of the root-tip polymers in the homogenization medium was approx. 100000c.p.m.

Discussion

Negatively stained preparations of dictyosomes indicated that the structure of the cisternae and vesicles of the Golgi apparatus in maize-root and shoot tissue is similar to that previously described (Cunningham *et al.*, 1966; Beams & Kessel, 1968; Harris & Northcote, 1971).

The cisternae were often arranged in groups, with possible tubular connexions between the individual cisternae; the morphology of the central region of the cisternae within a group was observed to vary considerably. It is suggested that a group may represent the cisternal elements of one Golgi apparatus. The process of 'destacking' is most probably the result of the negative staining and drying procedure, and indicates that in such conditions the presence of the intercisternal elements is insufficient to maintain an intact stack. It is probable that the cisternae are kept in close proximity to one another by the presence of connecting tubules, which, in the organized functional stack, represent vertical connexions between cisternae.

Much work has been done in the characterization of the polysaccharide components of maize roots (Roberts & Butt, 1967, 1969; Kirby & Roberts, 1971; Harris & Northcote, 1970). In the present work we have investigated the change in those components during differentiation, and the involvement of different membrane fractions in the production of polysaccharide polymers in particular zones of differentiation within the root.

D-[U-^{14}C]Glucose was given to maize seedling roots *in vivo*, so that all the metabolic reactions had already occurred before glutaraldehyde was added. The patterns of distribution of radioactivity in the various membrane and wall fractions are therefore representative of the polymers that have been formed and are present at the time of homogenization, i.e. they do not necessarily correspond to the composition of the completed polysaccharide structure but rather indicate the rate of conversion of D-glucose into other sugar components, and of the incorporation of these into the polysaccharides that are being synthesized.

An analysis of the wall fraction indicated that the polysaccharide components present are dependent on the state of differentiation of the tissue. The values of the ratios galacturonic acid/glucuronic acid, galactose/glucose and arabinose/xylose were high in wall regions of the root tip compared with values for wall regions 2–3cm from the tip. This suggests that the two regions of the root taken for preparation of the membrane fractions represented zones typical of primary and secondary wall development (Northcote, (1969*b*). In addition, the slime produced by maize roots

contains fucose, which is absent from any other polysaccharide that is typical of maize roots (Harris & Northcote, 1970; Kirby & Roberts, 1971). Since slime production is restricted to the root-cap zone, and fucose is a marker for the presence of slime, it follows that the maize root represents an ideal control system for the study of the involvement of different membrane fractions in production of slime from distinct root-cap and non-cap regions of the root. The wall and slime can be regarded as constituting polysaccharides that are exported to the outside of the cell but that have been synthesized within the cell-membrane system. The slime was found to occur as a component of the extracellular polysaccharide that is liberated into the radioactive nutrient medium during the incubation period of 2h, and it also occurs in the washings of the roots before homogenization. In addition, slime polysaccharide is retained in the root-cap region and adsorbed on to the surface of the whole root, and it is this polymer together with free soluble polysaccharide that has been released from the wall and membrane compartments during homogenization and that constitutes a third class of exportable polysaccharide.

In the present investigation the percentage radioactivity in glucose increased from root-cap to older root tissue, suggesting an increased involvement in the synthesis of a glucan polymer, probably cellulose, in the latter region. The total pattern of distribution of radioactivity in the two parts of the root differed considerably. Total incorporation of radioactivity decreased markedly from root-cap to older root tissue, although the fresh weight of material used for preparation of the latter zone was very much greater. This may have been because of the decreased amounts of polysaccharides synthesized by this region, since slime is formed only by the root-cap cells (Harris & Northcote, 1970), or an inability of the radioactive nutrient to reach the innermost tissue of the older regions of the root in the limited time of the incubation.

The percentage distribution of radioactivity in the monosaccharide components of the soluble polysaccharide in the incubation medium was similar to the percentage composition of the slime determined by Harris & Northcote (1970). The percentage incorporation of radioactivity into the monosaccharides of the polysaccharide cannot be directly equated with its percentage composition. However, it seems likely that the greater part of this soluble polysaccharide was slime produced by the outer root-cap cells, especially as it contains a high percentage of radioactivity in fucose and this monomer occurs only in the slime.

Three membrane fractions were investigated. Thin sections showed that these were a Golgi fraction composed mainly of cisternal regions, a smooth-membrane fraction containing a small quantity of mitochondria and a rough-membrane fraction that contained free ribosomes. Such fractions have been isolated from onion stem by using a similar centrifugation scheme (Morré, 1970). In an investigation of pea-root fractions Harris & Northcote (1971) named the particulate band at the 1.25–1.5M-sucrose interface as mitochondria. However, it would seem that in maize root the fraction is heterogeneous, with mitochondria constituting only a localized heavy region of the total membrane pellet.

All fractions contained an appreciable amount of radioactivity incorporated into neutral sugars and sugar acids of polysaccharides. Artifacts may be caused by adsorption of polysaccharide on to membrane surfaces stabilized by cross-linking, and by non-specific vesiculation of endoplasmic reticulum within an environment of radioactive polymers. These artifacts could result in high radioactivity being recorded in membrane fractions even if these were not actively involved in polysaccharide synthesis. The results of experiments used to investigate these possibilities show that in the system used these artifacts did not occur.

The percentage distribution of radioactivity in polysaccharide components of the three membrane fractions was fairly similar but the polysaccharide synthesized within the membrane compartments varied in different zones of differentiation.

The lower radioactivity ratio (arabinose/xylose) that occurred in the membrane fraction from the older region relative to the root tip, was attributable mainly to the very high incorporation of glucose into the xylose component of polysaccharide in older root tissue membrane fractions. This may be indicative of a greatly increased synthesis of a neutral hemicellulose that is composed largely of xylose residues (Gramera & Whistler, 1963). The arabinose/xylose ratio present in the wall has been used as a measure of the relative amount of primary to secondary wall development (Thornber & Northcote, 1961) and in the present investigation we show that this change is brought about by a corresponding change in the synthetic and transport function of the membrane system of the cell.

The percentage of radioactivity in galactose, arabinose and galacturonic acid was comparatively high in all the membrane fractions, isolated from all the regions of the root. This suggested that although polysaccharide synthesis by the membrane system does reflect major wall-differentiation changes, the production of polysaccharides containing these monomers is still maintained.

It has been suggested that the Golgi apparatus is involved in the production of slime polysaccharide (Morré *et al.*, 1967; Harris & Northcote, 1970; Kirby & Roberts, 1971). The presence of radioactive fucose in the hydrolysate of the dictyosome fraction from the whole root provides the first direct experimental evidence for this suggestion. Fucose was also

present in both the smooth-membrane fraction and the microsomal fraction from whole roots. When fractions from the root tip were examined, the incorporation of glucose into fucose was greater than in the whole root, irrespective of the fact that much less root was used to provide the fractions. Membrane fractions from regions not containing root-cap tissue did not contain radioactive fucose to any significant extent.

Although the wall fractions from both parts of the root contained a very high percentage of radioactivity incorporated into a glucan, most probably cellulose, the membrane systems did not contain radioactive glucans to any comparable extent. The radioactive polysaccharides found in each membrane fraction are contained within the membrane, and consequently indicate the function of those membranes in the synthesis and transport of polymers during the time of incubation with radioactive nutrient. Polysaccharide formed only at a membrane surface, and not contained within a compartment, would not be found in any of the fractions. The results presented strongly suggest that even in regions of the plant where the greatest incorporation of radioactivity into the wall is into a glucan, most probably cellulose, that glucan is not contained within a membrane system, either during polymerization or for export to the wall. This is in accordance with the idea that cellulose is formed at the plasmalemma surface, where the polymers are synthesized at specific loci, and are organized into the basic network of microfibrils already present (Staehelin, 1966; Preston & Goodman, 1967; Northcote & Lewis, 1968; Roberts & Northcote, 1970; Jones & Northcote, 1972). Since the plasmalemma is formed and maintained by the active addition into it of vesicle membrane, it is possible that the enzyme complement necessary for β-(1→4)-glucan production is present within the membrane system before the plasmalemma, but the activity remains potential until the environment at the cell surface is reached (Northcote, 1969*a*; Van der Woude *et al.*, 1971).

The membrane system is made up of endoplasmic reticulum, Golgi bodies, vesicles and the plasmalemma and it serves both for the synthesis and transport of the polysaccharides. Any part of the system at any one time can carry polysaccharide which would account for the incorporation of radioactivity from glucose into the polysaccharides which are isolated with the membranes. However, the amount of incorporation does not indicate the turnover of material in any part of the system or the relative importance of the various fractions in polysaccharide synthesis. This information can only be deduced if the specific radioactivities of the radioactive incorporation are determined.

The measurement of the lipid content of the subcellular fractions was considered to be the most valid method for determination of the amount of membrane present. The protein content determined as N (Morré, 1970) is not satisfactory in the present system, owing to the presence of N in rRNA, which is present with the membranes of the rough endoplasmic reticulum. The results indicate that in maize roots there is 40 times more membrane constituting the rough endoplasmic reticulum than that of the Golgi apparatus and the relative specific radioactivities (c.p.m. in polysaccharides/unit amount of lipid) shows the Golgi apparatus to have a greater activity than the rough endoplasmic reticulum.

It is suggested that the Golgi apparatus can be considered as an important localized focal point in the synthetic and transport system of polysaccharide. Concentration of polysaccharide at the Golgi apparatus is indicated by the higher specific radioactivity in relation to other parts of the membrane system, and also by radioautographic evidence showing an accumulation of silver grains over the dictyosomes compared with a more diffuse scattering of grains over other parts of the system (Northcote & Pickett-Heaps, 1966). This concentration at the Golgi apparatus could be caused by two factors. Radioactivity could be incorporated into forming polysaccharides as additional sugar residues within the Golgi apparatus, as previously shown in the formation of glycoproteins in animal tissues (Zagury *et al.*, 1970; Bennet & Leblond, 1970; Wagner & Cynkin, 1971). In addition, polysaccharide synthesized within the diffuse membrane of the endoplasmic reticulum may undergo a physical concentration, since only part of the membrane may be used to package the polysaccharide for transport and to form the Golgi apparatus, and subsequently after modification to form the plasmalemma (Jamieson & Palade, 1968; Keenan & Morré, 1970; Leskes *et al.*, 1971; Northcote, 1971). It is probable that a combination of the two factors may occur in the maize-root system.

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