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Studies on the Secretion of Maize Root Cap Slime

II. LOCALIZATION OF SLIME PRODUCTION[1]

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ABSTRACT

The distribution of fucose-containing polysaccharides in apical 1-cm sections of corn (*Zea mays* cv. SX-17) root tips was analyzed. Fucose-containing polysaccharides were localized predominantly in the apical 1 mm of the root, *i.e.*, in the apical initials and root cap. An analysis of the distribution of incorporated radioactive label from L-fucose[^{3}H] gave similar results. After a 2-hr incubation with fucose[^{3}H], label was found principally in two components, namely a water-soluble slime fraction and hemicellulose. The incorporation of fucose into the water-soluble, ethanol-insoluble fraction was primarily in the apical 1 mm of the root, whereas incorporation into a water-insoluble, potassium hydroxide-soluble fraction was in the region 2 to 5 mm behind the root cap. Addition of sucrose to the incubation medium during fucose[^{3}H] incorporation reduces label uptake but increases the amount of label in the fucose-rich secreted polysaccharide. The utility of fucose as a marker for the secreted polysaccharide was confirmed by demonstrating that no appreciable metabolism of this sugar occurs.

Roots of many terrestrial plants produce a slimy secretion which is mainly polysaccharide (2–6, 8). The polysaccharide produced by roots of corn contains a high proportion of fucose and galactose, together comprising 69% of the neutral sugars of the polymer (9). Using glucose[^{14}C], Harris and Northcote (3) showed that when the distribution of label appearing in fucose was expressed as a ratio of label in xylose, the ratio was higher in the apical 0.5-mm section of the root. An autoradiographic study by Kirby and Roberts (7) found that fucose[^{3}H] was incorporated into polysaccharides mostly in the root cap and epidermis. They also presented chemical fractionation data which showed that fucose[^{3}H] was incorporated into two principle fractions: a 2 M KOH-soluble fraction and a cold water-soluble fraction, but they did not attempt to localize these fractions in different regions of the root tip.

We have studied the production and incorporation of L-fucose into maize root tips to localize fucose definitively in the root tip, and to show spatial separation of the 2 M KOH-soluble and water-soluble fractions.

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MATERIALS AND METHODS

Plant Material. Seeds of a single cross maize hybrid (*Zea mays*, cv. SX-17), coated with Captan and Malathion, were soaked in continuously aerated tap water for 36 hr with three changes of water. Seedlings were then germinated on damp vermiculite or coarse sand covered with 4 layers of damp paper towelling for 36 hr in the dark at 23 C, after which time the roots were 1.5 to 2 cm long.

Incubation. Excised root tips, 2 or 5 mm in length, were incubated in a medium containing 20 to 40 mM sucrose or fucose, 0.5 Hoagland's solution, 0.025 mM boric acid, 20 μg/ml of chloramphenicol, and 5 μg/ml of streptomycin (9). For incorporation of labeled fucose, 25 root tips were incubated in duplicate for periods of up to 5 hr in 1 ml of medium at 28 C in 25-ml Erlenmeyer flasks with shaking at 150 cycles/min.

Tissue Homogenization and Fractionation. Tissue was homogenized and fractionated as outlined in Figure 1. Twenty-five root tips and 1 ml of medium were removed from the incubation flask and washed four times with 1 ml of chilled distilled H_2O. The medium and washings were combined and filtered through a glass fiber disc. The filtrate was made to 80% (v/v) alcohol and allowed to stand at 4 C overnight. The precipitate (secreted material) was then collected on a glass fiber disc and counted. The final soluble filtrate was reduced to a standard volume and is referred to as the supernatant medium. The washed roots were homogenized in 1 ml of chilled glass-distilled H_2O in a glass-Teflon homogenizer at 4 C. The brei was either filtered through a glass fiber disc, and the filter was washed with water or centrifuged at 20,000g for 30 min, then resuspended and recentrifuged. The centrifuged pellet or filter disc is referred to as the insoluble cell wall material. The remaining filtrate was combined with the washings, made to 80% (v/v) alcohol, and allowed to stand at 4 C overnight; it was then filtered through a glass fiber disc. The resulting precipitate is designated the homogenate precipitate, and the filtrate, which was reduced in volume, is referred to as unincorporated uptake (Fig. 1).

Root tips and secreted materials were also fractionated chemically as outlined by Ray and Baker (10). After the required incubation time in L-fucose-1-[^{3}H], 100 root tips (5 mm) were ground in a glass-Teflon motorized homogenizer at 4 C with 4 ml of incubation medium. The brei was centrifuged at 20,000g for 30 min. The supernatant was retained and the pellet was resuspended with 1 ml of distilled H_2O and recentrifuged. The supernatants were combined and the pellet was retained. The supernatant was made 80% (v/v) alcohol and allowed to stand at 4 C for 24 hr. The precipitate was collected on a glass fiber filter disc and is referred to as the cold water-soluble material. The pellet was extracted with 5

ml of distilled H_2O at 100 C for 1 hr and filtered through a glass fiber filter disc. The residue was extracted twice with 2 ml of distilled H_2O at 100 C for 15 min and was filtered. The filtrate and washings were combined, frozen, and lyophilized. This fraction is referred to as the hot water-soluble fraction.

The residue remaining on the filter was extracted with 5 ml of 0.5% ammonium oxalate at 80 C for 1 hr and was washed with an additional 2 ml for 15 min at 80 C. Evaporation was prevented by lightly capping the tubes with an inverted cone of aluminum foil. The original extract and washings were cooled and desalted on a 30-ml Dowex 50W-X8 (H^+ form) column, and the eluant was lyophilized. This fraction is referred to as the ammonium oxalate-soluble fraction.

The residue after ammonium oxalate extraction was collected on a glass fiber disc and further extracted for 48 hr with two changes of 4 M potassium hydroxide (5 ml, 2 × 2 ml) under nitrogen. Extract and washings were filtered and passed through a 30-ml column of Dowex 50W-X8 (H^+ form). The eluant was made 80% (v/v) with ethanol and the precipitate collected on a glass fiber disc. The filtrate was reduced by lyophilization. These two fractions made up the 4 M potassium hydroxide-soluble material, also referred to as hemicellulose. The remaining insoluble material is referred to as the residue or cellulose fraction.

Radiochemical Methods. Samples to be counted were transferred to standard plastic scintillation vials and dried at room temperature. Materials were not eluted from the glass fiber discs since these discs have a refractive index similar to the scintillation liquid. Liquid samples were frozen and lyophilized, or evaporated under a stream of filtered air at 50 to 60 C. After drying, 0.3 ml (or enough to wet the sample) of NCS tissue solubilizer (Amersham/Searle, Inc., Arlington Heights, Ill.) with 40 μl of water was added to each vial which was then tightly capped. The vials were allowed to stand at 40 C overnight. The vials were uncapped and 0.4 or 0.5 ml of glacial acetic acid was added, followed by 10 ml of toluene fluor (4 g of PPO/l). The vials were capped, shaken to mix the contents, and placed in a refrigerator at 4 C. After cooling, the vials were counted in a Beckman LS 150 liquid scintillation counter (Beckman Instruments, Palo Alto, Calif).

Segmentation of Excised Roots. Excised roots were cut with a razor blade on a clean microscope slide, against a graduated background, into an apical 1-mm section and a subapical 19-mm section, (Table I) or were sectioned at varying distances from the root tip as indicated (Table II; Fig. 2).

With nonincubated roots, the sections were cut and frozen. Then 2 mg of myoinositol were added as a standard and the sample was lyophilized. The sections were hydrolyzed and the alditol acetates were prepared (9).

In radioactive labeling experiments, root sections were cut and incubated for 2 hr in the radioactive incubation medium at 28 C. Twenty-five root tips or sections were used per milliliter of medium.

Metabolism of Fucose-1-[^{3}H]. One hundred root tips (5 mm) were incubated for 60 min in 4 ml of standard incubation medium containing 20 mM sucrose at 28 C. The roots were either fractionated chemically using the procedures of Ray and Baker (10) as outlined above or as described in Figure 1.

The alditol acetates were prepared after hydrolysis, as described by Paull *et al.* (9). The neutral sugar alditol acetates were separated on a 183 × 0.318 cm column of 3% ECNSS-M on (100/120 mesh) Gas Chrom Q. This was placed on one channel of a Varian Aerograph chromatograph Model 204B, Varian Associates, Walnut Creek, Calif.) fitted with a 10:1 splitter. Nitrogen gas flow to the flame ionization detector was 28 ml/min and at the splitter it was 240 ml/min. Hydrogen flow was 29 ml/min. The injector was maintained at 275 C, the detector at 245 C, and the column operated isothermally between 205 C and 210 C.

Efficiency of collection from the splitter, using known fucose

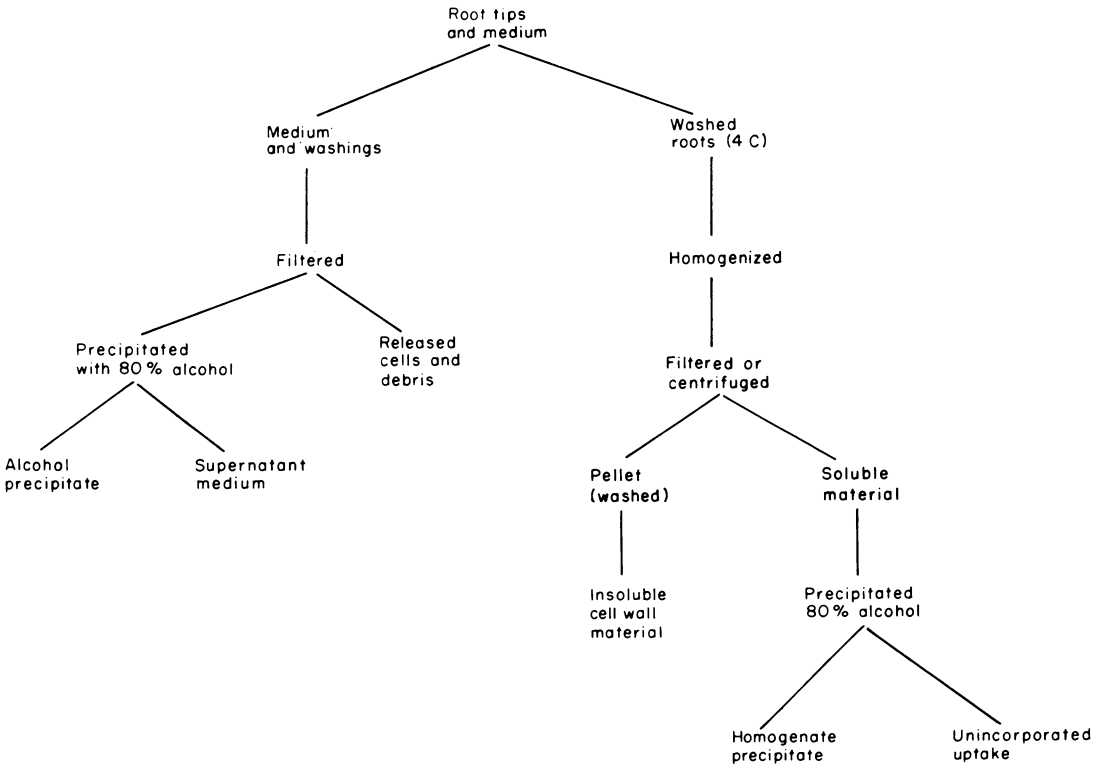

FIG. 1. Fractionation procedure.

hexaacetate[^{3}H] injections, was 41.7% (±1.2%). The collector consisted of a small detachable piece of glass tubing which allowed the gas to pass into a vial containing 2 ml of methyl alcohol. In order to determine the start and end of a neutral sugar peak, it was necessary to add a standard mixture of sugar acetates so that sufficient material reached the detector to give adequate recorder deflection.

RESULTS AND DISCUSSION

Localization of Fucose Polysaccharide along the Root. Because the secreted slime of the SX-17 cultivar of corn is fucose-rich (9), a comparison of the ratios of fucose to arabinose, glucose, and xylose provides an excellent indication of the distribution of the polymer along the primary root (Table I; Fig. 2). The highest proportion of fucose is in a polysaccharide which occurs in the apical 1 mm of roots, with the proportion of fucose rapidly decreasing behind the tip. Extraction of root sections with hot and cold water indicates that the bulk of the cold water-soluble material from the apical 1-mm section of the root contains the highest proportion of fucose which, when expressed as a ratio of fucose-xylose, is 13-fold greater than in the 2- to 19-mm section, and as a ratio of fucose-glucose, is 800-fold greater in the apical 1 mm of the root. The hot water-soluble fucose fractions is located almost entirely in the first 1 mm of the root and is probably secreted slime located in the interstices of the wall and not readily extractable with water at 20 C (Table I). The residue from such extractions, which contains pectins, hemicelluloses, and cellulose, does not show such a marked drop in the ratio of fucose to glucose or xylose from the apical 1 mm to the subapical 19-mm sections. The fucose-xylose ratio is only 5-fold greater than in the 1 mm section, whereas the fucose-glucose ratios change only slightly between the sections (Table I).

The neutral sugar composition of all ethanol-insoluble material including cell wall was determined in sections taken at intervals along the length of 3.4-cm root tips (Fig. 2). When the distribution of fucose is expressed as a ratio of arabinose, glucose, or xylose, it is clear that this monosaccharide predominates in the apical 1 mm of the root.

These results were confirmed by examining the incorporation of L-fucose-1-[^{3}H] into the different regions of the root. The results are presented as cpm/μg of protein (Table II) in the fractions prepared as indicated in Figure 1. Incorporation into the water-soluble, ethanol-insoluble fraction occurs predominantly in the first 1 mm with a rapid decline from the tip. Label in the homogenate precipitate fraction does not change with distance from the root tip to the same extent, but is higher in the apical 2 mm (Table II). Incorporation into the wall fraction has a peak at 2 to 10 mm behind the root tip, being low in the apical 1 mm.

These results suggest two different sites of fucose[^{3}H] incorporation: the water-soluble, ethanol-insoluble material in the apical 1-mm region, and the insoluble wall fraction in the 2- to 10-mm region (Table II). The ratio of secreted material incorporation to wall fraction incorporation emphasizes the predominance of secretion of a fucose-containing, water-soluble polysaccharide by the apical 1 mm of the maize root (Table II).

Metabolism of L-fucose-1-[^{3}H]. To establish the usefulness of fucose[^{3}H] as a marker for root cap slime, the metabolism of this sugar was followed in various chemical fractions of corn

Table I. *Fucose to Xylose and Fucose to Glucose Ratios in Two Apical Segments of Maize Roots*

Fraction	Apical Section (0.0-0.1 cm)			Subapical Section (0.1-2.0 cm)		
	Fucose	Fucose/ xylose	Fucose/ glucose	Fucose	Fucose/ xylose	Fucose/ glucose
	μM	ratio		μM	ratio	
Cold water-soluble, ethanol-insoluble	1.219	3.22	0.58	0.018	0.024	0.00003
Hot water-soluble	0.142	1.78	0.36	0	0	0
Total water-soluble	1.561	2.50	0.42	0.018	0.012	0.000015
Residue	0.372	0.102	0.049	0.331	0.019	0.046

Table II. *Incorporation of Fucose[^{3}H] by Various Lengths of Maize Root tip*

Distance from Tip	Secreted Alcohol Precipitate	Homogenate Precipitate	Insoluble Cell Wall Material	Ratio of Secreted/ Wall	Proteins
mm	cpm/μg protein				μg
1	895.6	57.1	64.0	13.99	2.5
2	148.6	71.9	231.2	0.64	14.4
5	39.9	17.2	225.1	0.18	64.0
7.5	34.9	23.2	169.4	0.21	84.8
10	40.2	23.7	100.5	0.40	102.4
2-10	47.2	32.5	188.5	0.25	66.4
5-10	75.1	72.8	188.9	0.40	40.0

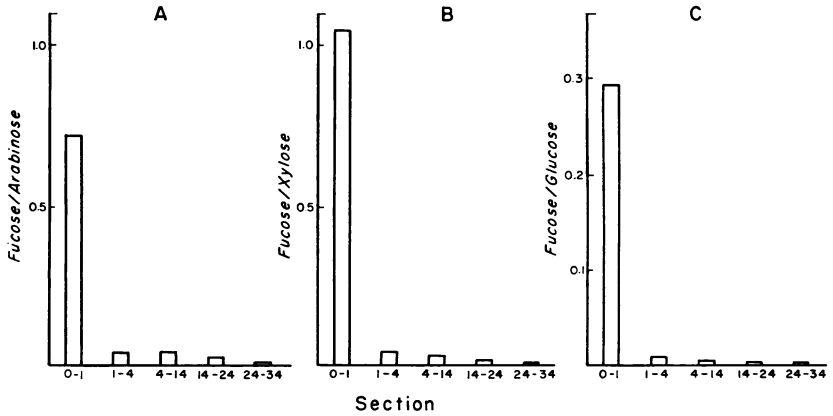

FIG. 2. Fucose-containing polysaccharides in maize root tip sections expressed as (A) fucose to arabinose ratio; (B) fucose to xylose ratio; and (C) fucose to glucose ratio in the neutral sugars.

root tips. Hydrolyzed products of the fractions obtained as outlined in Figure 1 were reduced, acetylated, and separated by GLC. The neutral sugar fractions from the column were collected and their radioactivity was counted. The number of cpm minus background in the neutral sugars is given in Tables III and IV. In root fractions, the majority of counts (66%) were at the position of fucose in the chromatogram. There is a small contribution of label from the fucose peak to the sugars which elute to either side of fucose, namely erythrose and arabinose (Tables III and IV). There is only a small percentage of label eluting with other sugars with no sugar showing preferential labeling.

In those fractions which became most heavily labeled (insoluble homogenate of Table III and secreted ethanol-insoluble fraction of Table IV), there is no evidence of interconversion of the added fucose to other sugars. It is significant that when a high number of counts were collected, the percentage of label eluting as fucose increased to a maximum of 84%. These results are in agreement with the findings of Bekesi and Winzler (1) who showed that there was little metabolism of fucose in serum and tissues of rats. The results demonstrate the utility of fucose as a marker for corn root slime.

Table III. *Distribution of Tritium Label in Various Neutral Sugars Obtained from Corn Root Tips*

The sugars were isolated from the tips after incorporation of fucose[^{3}H] for 2 hr. The data are the means of two determinations minus background.

Neutral Sugars	Secreted Ethanol-insoluble	Homogenate		Insoluble Wall Fraction
		Ethanol-soluble	Ethanol-insoluble	
	%			
Erythrose	2	2.9	4.5	7.1
Fuc	82	84.4	35.2	79.3
Ara + Rib	7	5.4	17.8	6.6
Xyl	3	4.2	19.6	2.4
Man	2	1.8	10.1	1.3
Gal	2	0.8	7.4	1.8
Glc	2	0.4	5.5	1.5
Total cpm	108	5652	78	421

Table IV. *Distribution of Tritium Label in Neutral Sugars Obtained from Hydrolysis of Various Chemical Fractions Prepared From Maize Root Tips*

Root tips had been incubated for 2 hr in fucose[^{3}H]. The data are the means of two determinations minus background.

Neutral Sugars	Secreted Ethanol-insoluble	Hot Water-soluble	0.5% Ammonium Oxalate-soluble	4 M KOH-soluble		Residue
				Ethanol-insoluble	Ethanol-soluble	
	%					
Erythrose	2.4	1.6	11.7	2.1	3.3	9.4
Fuc	84.0	66.0	65.6	81.3	72.1	74.1
Ara + Rib	4.9	11.9	5.8	5.2	11.2	5.0
Xyl	4.2	9.7	3.8	2.8	7.0	4.1
Man	1.9	9.2	7.1	2.7	5.5	3.1
Gal	1.4	0	5.8	0.2	0	2.6
Glc	1.1	1.4	0	0.8	0.9	1.9
Total cpm	575	36	15	309	68	144

Uptake and Incorporation of Fucose[^{3}H] into Tissue and Chemical Fractions. The incorporation of fucose into tissue fractions obtained from root tips as outlined in Figure 1 was followed with time (Fig. 3). Incorporation of fucose into the insoluble cell wall material and the homogenate precipitate fraction of root tips (2 mm) incubated in fucose[^{3}H] for 5 hr is linear with time. Label from fucose in the alcohol-insoluble secreted material shows a low rate of incorporation for the first 2 hr of the labeling period, followed by a more rapid labeling from 2 to 5 hr (Fig. 3). Some of the label incorporated into both the cell wall material and homogenate precipitate was later shown to be in material similar to the secreted slime that was probably not removed by distilled H_2O washing of the root sections because of its location inside intact cell walls and the interstices of the cell wall. After a 5-hr incorporation period, 4.5% of the added fucose[^{3}H] was taken up by root sections. Of the radioactivity taken up, 57% was incorporated into the wall material, 29% into the homogenate precipitate, and 14% into the alcohol precipitate fraction from the medium (Fig. 3).

Addition of sucrose up to 40 mM to the incubation medium during incorporation of fucose[^{3}H] reduces uptake of label but increases the proportion of label appearing in the fucose-rich polysaccharide (Fig. 4). Sucrose also causes a marked reduction in incorporation of label into the pellet fraction (Fig. 4).

The incorporation of fucose into chemical fractions defined by their solubility in various solvents was followed during a 2-hr period of incubation of 5-mm long root tips with fucose[^{3}H]. Label was found principally in two fractions, namely, the characteristic secreted material and the hemicellulose fraction (Fig. 5). There was only a small amount of label in the residue (cellulose) and in the ammonium oxalate-soluble (pectin) fraction. Hot water extracted some label along with the glucan, araban, and pectic acids, but this was only one quarter of the label in the cold water-soluble fraction. After a 2-hr incubation, 5% of the radioactivity was incorporated into macromolecules. When expressed as a percentage of the total incorporated label, 42% appeared in the cold water-soluble, ethanol-insoluble fraction and 36% in the 4 M KOH-soluble fraction. Lesser amounts occurred in the other fractions: hot water-soluble fraction 13%, ammonium oxalate-soluble fraction 3%, and residue 6%.

Kirby and Roberts (7) have also followed the incorporation of fucose[^{3}H] into various chemical fractions of corn root tips. These workers found that 67% of the applied fucose was incorporated into a 2 M KOH-soluble fraction, whereas in this study, only 36% of the label from fucose[^{3}H] appeared in a 4 M KOH-soluble fraction. This discrepancy can be accounted for by differences in experimental conditions. Kirby and Roberts (7) incubated 1-cm long root tips in the absence of a carbon source, whereas we incubated 2- or 5-mm sections in the presence of 40 mM sucrose. We have shown that the labeling of homogenate fractions with fucose[^{3}H] can be varied by manipulation of the sucrose concentration of the medium (Fig. 4). The proportion of label appearing in the water-soluble secreted material increases with an increase in the sucrose concentration of the medium.

Our evidence on the distribution of neutral sugars along the primary root of corn and on the incorporation of fucose[^{3}H] into the various chemical fractions of corn root tips strongly indicates a spatial separation of the synthesis of two fucose-containing polysaccharides: a water-soluble, ethanol-insoluble

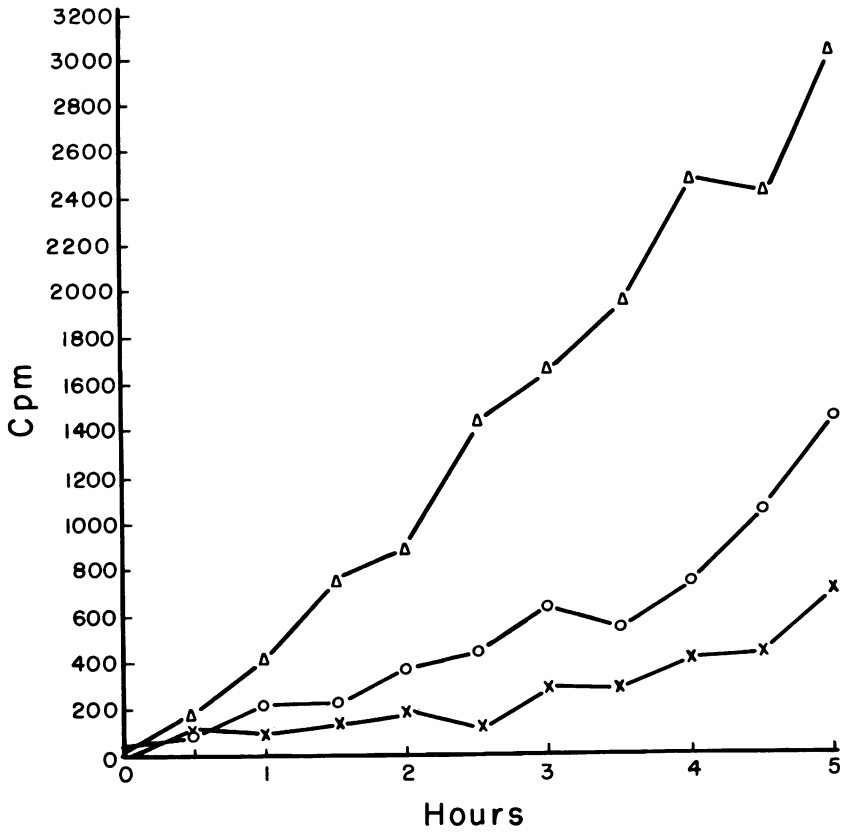

FIG. 3. Continuous incorporation (5 hr) of fucose[^{3}H] into insoluble cell wall material (△), homogenate precipitate (○), and secreted alcohol precipitate (×) fractions of 2-mm long maize root tips.

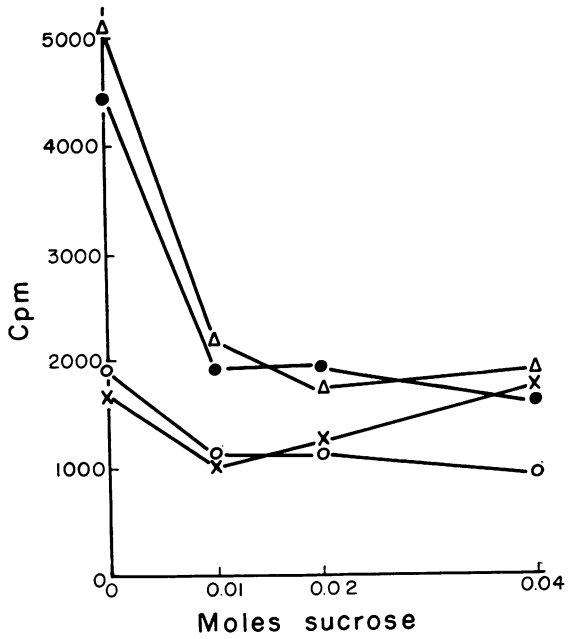

FIG. 4. Effect of sucrose on fucose[^{3}H] incorporation into different fractions of maize root tips: secreted alcohol precipitate (×); insoluble cell wall material (●); unincorporated uptake, 1/20 actual cpm (○); and homogenate precipitate (△).

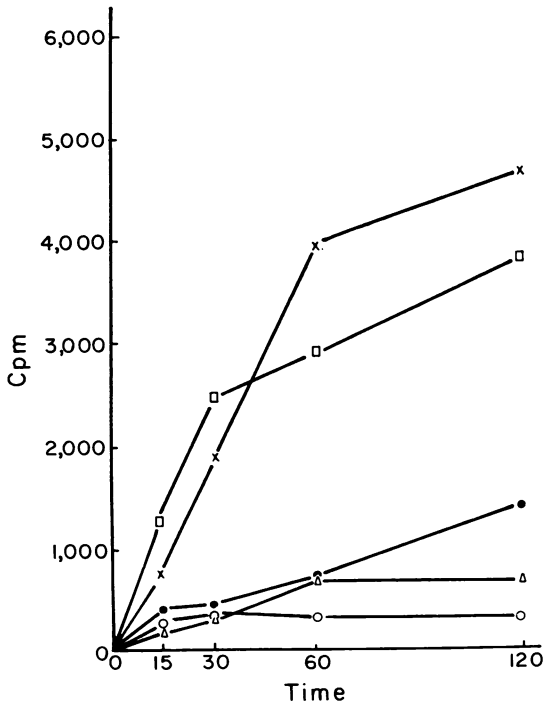

FIG. 5. Incorporation of fucose[^{3}H] into the following chemical fractions of 5-mm long root tips during the specified incubation times: cold water-soluble, ethanol-insoluble (×); hot water-soluble (●); ammonium oxalate-soluble (○); 4 M potassium hydroxide-soluble (□); and residue (△).

material being produced in the apical 1-mm region of the root and a 4 M KOH or hemicellulose material produced in the region distal to this section.

CONCLUSIONS

Extraction of regions of corn root tips shows that the bulk of the water-soluble, ethanol-insoluble material occurs in the apical 1-mm section. An analysis of the neutral sugar composition of water-soluble, ethanol-insoluble and of total ethanol-insoluble materials from various regions of the root indicates that fucose predominates in the apical 1-mm section (Table I; Fig. 2). The predominance of fucose in the apical 1 mm of the root was confirmed by experiments showing the localized incorporation of L-fucose[^{3}H] into alcohol-insoluble polysaccharide from this region. Fucose[^{3}H] was shown to be an ideal marker for studying the synthesis of fucose-rich polysaccharides in corn roots because metabolism of this sugar, particularly in the water-soluble, ethanol-insoluble fraction, was low. Fucose[^{3}H] is readily incorporated into cell wall, homogenate precipitate, and medium precipitable fractions of root cap tissue. It was shown that the distribution of label in various fractions of root homogenates could be changed by addition of sucrose to the incubation medium. Analysis of fucose[^{3}H] incorporation into chemically defined fractions of root homogenates showed that label appeared primarily in the cold water-soluble, ethanol-insoluble fraction and in the 4 M KOH-soluble fraction. Both localization and chemical fractionation experiments provide strong evidence for the incorporation of fucose into two chemically distinct and spatially separated components in the corn root tip.

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