Plant Physiol. (1976) 57, 249-256

Studies on the Secretion of Maize Root Cap Slime

IV. EVIDENCE FOR THE INVOLVEMENT OF DICTYOSOMES[1]

Received for publication June 27, 1975 and in revised form October 15, 1975

ROBERT E. PAULL AND RUSSELL L. JONES
Department of Botany, University of California, Berkeley, California 94720

ABSTRACT

The involvement of dictyosomes and their vesicles in secretion of slime by maize root cap cells is demonstrated by kinetic and organelle fractionation experiments using L-fucose as a specific marker for the secreted slime. Pulse-chase experiments show that L-[1-^{3}H]fucose is incorporated into two distinct fractions of root cap cells. Incorporation into a water-soluble, ethyl alcohol-insoluble fraction of the homogenate has a peak at 20 minutes of chasing followed by rapid loss of label. Seventy per cent of the radioactivity in this fraction is secreted from the tissue during a 2-hour chase period. Incorporation of label from [^{3}H]fucose into a water-insoluble fraction is kinetically different suggesting that *in situ* incorporation of label is occurring into the cell wall. Labeling of the water-soluble, ethyl alcohol-insoluble fraction with an ^{14}C-amino acid mixture differs from that of [^{3}H]fucose. Thus, while release of the [^{3}H]fucose-containing polymer begins after 10 to 15 minutes of chasing, the release of the ^{14}C-amino acid polymer is delayed an additional 5 to 10 minutes and occurs at a lower rate. Cesium chloride density gradient centrifugation of secreted material labeled with radioactivity from [^{3}H]fucose indicates the presence of only one major component having a buoyant density similar to that of purified root cap slime (1.63 g cm^{-3}). Sucrose density gradient centrifugation of homogenates of [^{3}H]fucose-labeled root cap tissue shows that radioactivity in nondialyzable material occurs as a broad band between densities 1.12 and 1.18 g cm^{-3} with a peak at density 1.15 g cm^{-3}, the same density at which dictyosomes were localized by electron microscopy. Autoradiography of organelle fractions shows that radioactivity was associated almost exclusively with dictyosomes.

The outer root caps cells of corn (*Zea mays*) secrete a polysaccharide slime of high fucose content (4, 6, 8, 16) which adheres to the root tip for several hours. A number of studies using cytochemical (13, 15, 18, 21) and biochemical (2, 6) techniques have implicated dictyosomes in slime production. Bowles and Northcote (2) labeled corn root cells with [^{14}C]glucose and isolated various membrane fractions via differential centrifugation and sucrose step gradients. The presence of radioactive fucose (6%) in the hydrolysate of the dictyosome fraction from whole roots indicated dictyosome involvement. It is possible that another membrane fraction having a density similar to that of dictyosomes contains the labeled fucose in such homogenates. Further difficulties in the interpretation of this work (2) are the poor specificity of glucose as a marker for the secreted slime, and the absence of evidence that radioactivity was associated with a polysaccharide.

Fucose is an ideal marker for the secreted slime (9, 17), and this characteristic was exploited in our experiments designed to confirm the site of fucose incorporation in maize root cap cells. Our results show the involvement of dictyosomes in the compartmenting and transport of the fucose-rich polysaccharide secreted from these cells.

MATERIALS AND METHODS

Plant Material. Corn (*Zea mays* cv. SX-17A) was germinated and grown as described previously (17). After 72 hr of germination, the root tip was 1 to 2 cm long. Excised root tips (0.1 or 0.5 cm) were placed in glass distilled H_2O containing 100 μg/ml chloramphenicol and 10 μg/ml streptomycin at or near 4 C until needed.

Pulse-Chase Experiments. Four hundred root tips (0.1 cm long) were incubated for 10 min at 28 C with 150 rpm shaking in a 50 ml-Erlenmeyer flask with 0.4 ml of standard incubation medium (16) containing 70 μCi L-[1-^{3}H]fucose (Amersham Searle TRA366, 2.82 Ci/mmole, 1 mCi/ml), and 10 μCi D-[^{14}C]glucose (Amersham Searle; CFB 96, 268 μCi/mmole, 253 μCi/ml). At the end of the pulse, the root tips were washed five times at 4 C for 10 min with 2 ml of standard incubation medium containing 10 mM fucose and 10 mM sucrose. Following washing, the root tips were incubated at 28 C with 150 rpm shaking in 8.5 ml of the same medium. The start of this incubation was regarded as chase time zero. After 0, 10, 20, 30, 45, 60, 90, and 120 min chase, duplicate samples of 25 root tips and 0.5 ml of medium were removed and fractionated as described previously (17). The incubation medium was fractionated into unincorporated, ethyl alcohol-soluble material, water-insoluble material on filter disc, and water-soluble, ethyl alcohol-insoluble secreted slime. The homogenate was similarly fractionated into an ethyl alcohol-soluble fraction, a water-soluble, ethyl alcohol-insoluble fraction, and water-insoluble wall fraction.

In another experiment, 850 root tips (0.1 cm) were incubated for 10 min in standard incubation medium containing 100 μCi of [^{3}H]fucose and 10 μCi of uniformly labeled ^{14}C-amino acid mixture (Amersham Searle CFB 152, 10 μCi/mmole, 50 μCi/ml). The washing and chase media contained 10 mM sucrose, 10 mM L-fucose, and 1 mM of each amino acid contained in the ^{14}C-amino acid mixture. At the end of the chase period, 25 root tips and 0.5 ml of medium were removed and fractionated as described above.

Cesium Chloride Gradients. Four hundred root tips (0.5 cm) were incubated for 2 hr in 3 ml of standard incubation medium containing 20 μCi of [^{3}H]fucose. At the end of the incubation period, the root tips were divided into two lots and each washed three times with 2 ml of glass-distilled H_2O. The medium and washings were combined and filtered through a glass fiber filter to remove debris and whole cells. The filtered solution was dialyzed against distilled H_2O to remove low mol wt labeled material, frozen and lyophilized. The root tips were homogenized in 2 ml of glass-distilled H_2O in a motor-driven, Teflon and glass homogenizer. The brei was centrifuged at 21,000g for 30 min, the supernatant was poured off and retained, and the

[1] This work was supported by Grant GB-27468 from the National Science Foundation.

pellet was resuspended in 2 ml of glass-distilled H_2O and recentrifuged twice. The washings were combined with the original supernatant and dialyzed against distilled H_2O with frequent changes of H_2O. The nondialyzable material was frozen, lyophilized, and centrifuged on cesium chloride gradients (16). Radioactivity in cesium chloride gradient fractions was determined after addition of 0.2 ml of each fraction into a plastic scintillation vial with 0.8 ml of H_2O followed by 10 ml of water-miscible fluor (1).

Organelle Isolation. Root tips (6–8 g wet weight, 0.5 cm long) were incubated at 28 C for 2 hr in 0.5 ml of standard medium containing 80 to 90 μCi of [^{3}H]fucose, and less than 1 g (wet weight) 0.1 cm long root tips, consisting mostly of cap tissue, was incubated for 45 min in standard medium containing 100 μCi of [^{3}H]fucose. After incubation, root slime and excess nutrients were washed off with excess non-radioactive incubation medium or 50 mM sodium phosphate (pH 7), then twice with excess of homogenizing medium. To facilitate the isolation of organelles from 0.1-cm root tips, they were mixed with 4 g of unlabeled 0.5-cm root tips before homogenization. Root tips were homogenized with motorized razor blades for 4 min at 2 C in 3 ml of homogenizing medium containing 50 mM tris buffer (pH 7.2 at 25 C) or 100 mM PIPES[2] buffer (pH 6.65 at 25 C), 0.5 M sucrose, 10 mM KCl, 1 mM EDTA Na_2 (pH 7.4), 0.1 mM $MgCl_2$, 10 mM dithiothreitol, 0.1% BSA, 2.5% Ficoll, and 5% dextran (average mol wt 40,000). Alternatively the motorized razor blades were replaced by hand chopping with a single edge razor blade for 30 min in 2 ml of the above homogenizing medium at 2 C. The homogenate was squeezed through four layers of cheesecloth, and the residue was resuspended in 2 ml of homogenizing medium and squeezed through the same cheesecloth. The crude filtrate was centrifuged for 10 min at 500g at 2 C, and the pellet was discarded.

Density gradient centrifugation of the homogenate supernatant was on either nonlinear or linear sucrose gradients. Nonlinear gradients consisted of a 2-ml cushion of 60% (w/w) sucrose, 25 ml of sucrose increasing linearly from 30 to 50% (w/w), and an 8-ml layer of 20% (w/w) sucrose. Linear gradient consisted of 2 ml of 60% (w/w) sucrose, 30 ml of sucrose increasing linearly from 20 to 50% (w/w), and a 4-ml layer of 20% (w/w) sucrose. Sucrose solutions contained 50 mM tris (pH 7.2 at 25 C) or 50 mM PIPES (pH 6.5 at 25 C), 1 mM EDTA (pH 7.4), and 0.1% BSA. Gradients were centrifuged in a Beckman SW27 rotor at 22,500 rpm (average 68,000g) for 2 to 4 hr at 4 C. Fractions were collected by displacement with 65% (w/w) sucrose and monitored at 280 nm at a flow rate of 1.5 ml/min. Fractions of 1.5 ml were collected and maintained at 2 to 4 C. Density of the fractions was determined with a Zeiss Abbé refractometer.

Enzyme assays were performed on the fractions within 5 hr of collection. Phosphorylcholine-glyceride transferase was assayed according to the procedure of Lord *et al.* (10) using [methyl-C^{14}]CDP-choline (New England Nuclear NEC-572, lot 744-069, 40 mCi/mmole) as substrate. In some experiments, we used one-quarter of the volumes used by Lord *et al.* NADH oxidase activity was assayed using a 0.05% solution of NADH (Calbiochem Corp.) following the procedures of Mackler and Green (11) and Crane (3). The decrease in absorbance at 360 nm with time was measured in a Varian Model 635 spectrophotometer operated at room temperature.

For the determination of radioactivity, 0.5-ml aliquots of each fraction were dialyzed against distilled H_2O for 3 days at 4 C with 4 changes of water. The nondialyzable material was transferred to a standard scintillation vial, and the dialysis bags were washed 5 times with distilled H_2O into the vial (total 10 ml). The H_2O was evaporated under a stream of filtered air at 50 C, 0.3 ml of NCS solubilizer (Amersham/Searle) was added, and the vials were capped and incubated overnight at 40 C. Glacial acetic acid (0.4 ml) was then added to the vials, followed by 10 ml of toluene fluor (4 g PPO/l toluene). The vials were capped, shaken, cooled at 4 C for 4 hr, and counted in a Beckman Model 150 liquid scintillation counter.

Preparation of fractions for electron microscopy was similar to that of Lord *et al.* (10). Two 1.5-ml aliquots from each major density band (densities 1.12, 1.135, 1.15, 1.18, and 1.24 g cm^{-3}) were mixed with an equal volume of 5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) and the appropriate sucrose concentration. Fractions were allowed to stand overnight at 4 C, mixed with one-third of the volume of 0.5 M sodium phosphate buffer (pH 7.2), and centrifuged at 150,000g for 1 hr. The pellet was washed with 50 mM sodium phosphate buffer (pH 7.2) containing the appropriate sucrose concentration and postfixed in 2% osmium tetroxide in the same buffer and sucrose concentration for 4 hr, followed by 2% osmium tetroxide in 50 mM sodium phosphate buffer (pH 7.2) overnight at 4 C. The pellet was washed twice (15 min each) with 50 mM sodium phosphate buffer (pH 7.2), dehydrated in a graded ethyl alcohol series, and embedded in a low viscosity epoxy resin (22). The blocks were sectioned parallel to the direction of centrifugation and gray to light gold sections were collected on Parlodion-coated, 200-mesh, copper grids. Sections were stained for 15 min in uranyl acetate (5% w/v in 70% v/v alcohol), washed with distilled H_2O, and stained in Reynold's lead citrate (20) for 20 min.

Each of the experiments described above was carried out at least three times with similar results.

Autoradiography was performed on organelle fractions prepared for electron microscopy as described above. Organelles were isolated from 2 g root tips (0.1 cm) incubated in 100 μCi of L-[^{3}H]fucose for 40 min, and mixed with 4 g of unlabeled 0.5-cm long root tips before homogenizing. Grids with unstained sections of the organelle fractions were mounted with double-sided adhesive tape on a microscope slide and Ilford L4 emulsion applied by the loop method. After exposure, grids were developed as described previously (18) and stained for microscopy as described above.

RESULTS

The incorporation of label from [^{3}H]fucose and [^{14}C]glucose into the water-soluble, ethyl alcohol-insoluble material of the homogenate and incubation medium during a 10-min pulse and a 2-hr chase is shown in Figure 1A. Secretion of labeled material begins after a 10-min lag period for both isotopes, and the slopes remain linear during the subsequent chase period. Incorporation into the material precipitated from the homogenate continues to increase during the first 20 min of the chase and then shows a marked decline (Fig. 1A). Of the total radioactivity incorporated into the water-soluble, ethyl alcohol-insoluble fraction of the homogenate from [^{3}H]fucose and [^{14}C]glucose, more than 70% is eventually secreted into the incubation medium after 2 hr of chasing with carrier monosaccharide (Fig. 1A). Incorporation into the water-insoluble fraction of the homogenate differs markedly from that of the water-soluble fraction (Fig. 1B). Incorporation of both [^{3}H]fucose and [^{14}C]glucose into this water-soluble fraction continued with a declining rate throughout the chase period (Figs. 1B and 2). There is a marked change in the rate of incorporation into this fraction between 30 and 45 min (Fig. 2).

Pulse labeling of root cap tissue with [^{3}H]fucose and an ^{14}C-amino acid mixture is shown in Figure 3. Incorporation of these isotopes into the water-soluble, ethyl alcohol-insoluble fraction of the homogenate is kinetically similar showing accumulation of label up to 15 min of chase followed by a rapid decline. The kinetics of release of these isotopes into the medium, however, is different. Release of the [^{3}H]fucose-containing material be-

[2] Abbreviation: PIPES: piperazine-N,N'-bis(2-ethanesulfonic acid).

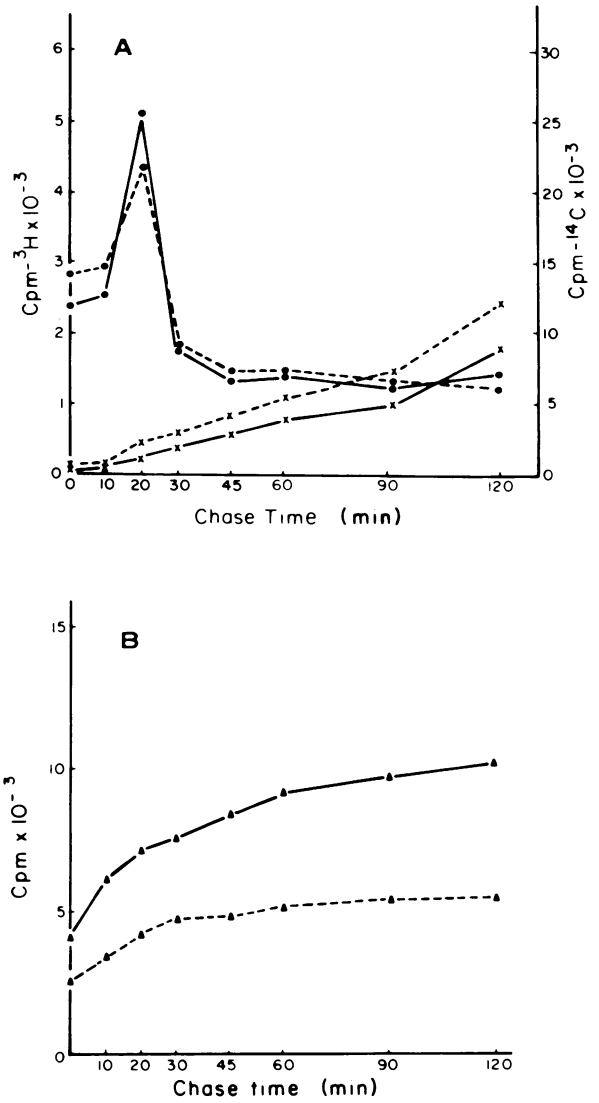

FIG. 1. Incorporation of L-[^{3}H]fucose and [^{14}C]glucose into 0.1-cm root tips. Material pulsed for 10 min and chased for specified times up to 2 hr with non-radioactive 10 mM L-fucose and 10 mM sucrose. A: Label incorporated into water-soluble, ethyl alcohol-insoluble fractions of homogenate (○) and incubation medium (×); B: label incorporated into water-insoluble material of homogenate (▲); ^{3}H (——); ^{14}C (– – –).

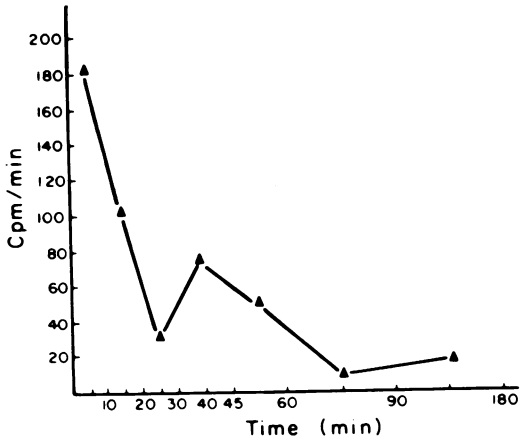

FIG. 2. Rate of [^{3}H]fucose incorporation into the water-insoluble material of 0.1-cm root tips pulsed and chased as in Fig. 1.

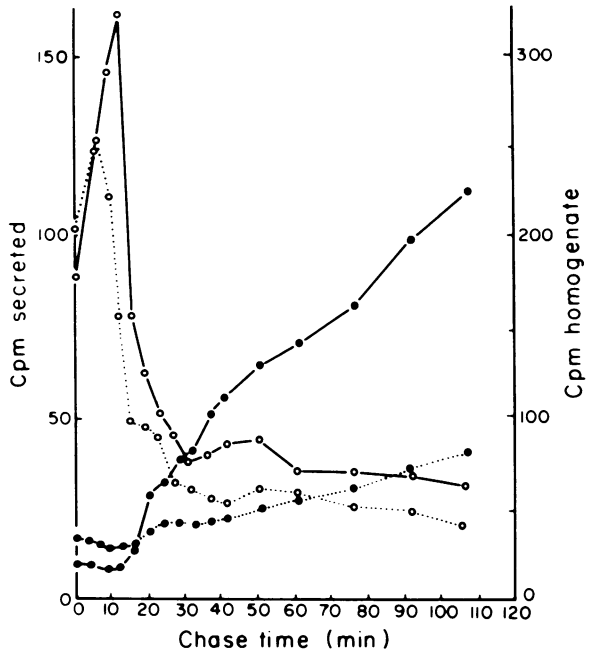

FIG. 3. Incorporation of [^{3}H]fucose and ^{14}C-amino acid mixture into 0.1-cm root tips. Material pulsed for 10 min and chased for specificed times up to 105 min with non-radioactive fucose and amino acid mixture. Label incorporated into water-soluble, ethyl alcohol-insoluble fractions of homogenate (○) and incubation medium (●); ^{3}H (——); ^{14}C (···).

gins after 10 min of chasing, whereas the release of the ^{14}C material does not begin until 20 min of chasing time has elapsed. The rate of release of the fucose-containing material is four times that of the amino acid-containing component.

We have shown (16) that the fucose-rich, secreted slime of corn root cap cells is primarily carbohydrate having a buoyant density of 1.63 g cm^{-3}. A comparison was therefore made by equilibrium centrifugation in CsCl of the densities of water-soluble, nondialyzable materials from the homogenate and medium after a 2-hr pulse of [^{3}H]fucose (Fig. 4). Labeled material from the homogenate has three distinct components having densities of 1.63, 1.55, and less than 1.4 g cm^{-3} (Fig. 4A). The secreted material on the other hand has only one component with a buoyant density of 1.63 g cm^{-3}, which is characteristic of secreted slime (Fig. 4B)

The subcellular location of [^{3}H]fucose incorporation was investigated using sucrose density gradient centrifugation of root tip homogenates. The analysis of corn root cap cell organelles was first accomplished on nonlinear or step gradients similar to those used by Lord *et al.* (10). Root tips (0.5 cm) were pulsed with [^{3}H]fucose for 2 hr, homogenized, centrifuged at 500*g* to remove cell debris, and the supernatant was centrifuged on a nonlinear gradient (Fig. 5D). Radioactivity from [^{3}H]fucose in nondialyzable material (Fig. 5C) bands at a density of 1.13 g cm^{-3} coinciding with CDP-choline transferase activity (Fig. 5B). Mitochondria were located at the expected density of 1.18 g cm^{-3} by NADH-oxidase activity (Fig. 5A). The sharp banding of CDP-choline transferase activity and of the fucose-containing, nondialyzable material at the 20 to 30% sucrose gradient step (1.13 g cm^{-3}) suggests that boundary effects cause accumulation of light membrane fractions at this point. This suspicion was confirmed when linear sucrose gradients were used to separate organelles. A homogenate was prepared from 0.5-cm root tips pulsed with [^{3}H]fucose for 2 hr and layered on a linear 20 to 50% (w/w) sucrose gradient. Radioactivity associated with nondialyzable material appears as a broad band having peaks at 1.12 and 1.15 g cm^{-3}, while absorbance at 280 nm shows two

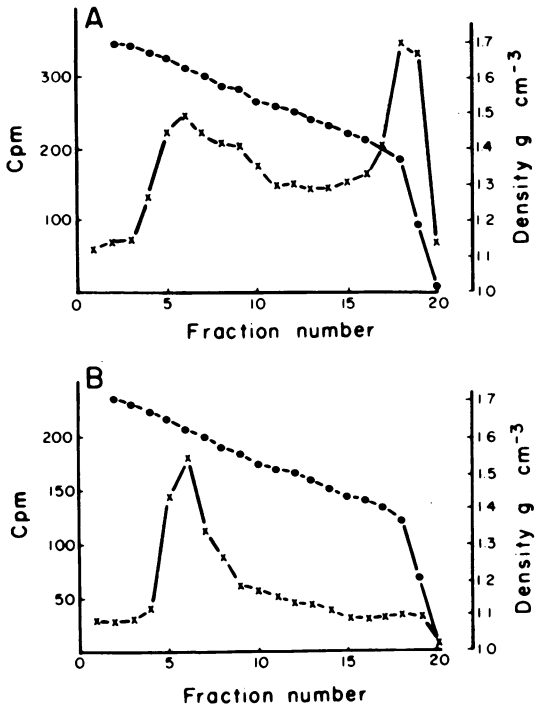

FIG. 4. CsCl gradient fractionation of water-soluble, alcohol-insoluble material from 0.5-cm root tips incubated in [^{3}H]fucose for 120 min. A: homogenate; B: incubation mledium; ×: ^{3}H; ●: CsCl density, g cm^{-3}.

major peaks corresponding to densities 1.18 and 1.25 g cm^{-3} (Fig. 6). CDP-choline transferase exhibits two peaks of activity, one at densities between 1.25 and 1.14 g cm^{-3} and the other at 1.18 g cm^{-3} (Fig. 6).

Distribution of radioactivity from homogenates of 0.1-cm long root tips incubated with [^{3}H]fucose for 2 hr is shown in Figure 7. Label in nondialyzable material is also distributed over a density range from 1.12 to 1.165 g cm^{-3}. The distribution of CDP-choline transferase activity is also broad; however, two peaks of activity at densities 1.13 and 1.15 g cm^{-3} are apparent.

Organelles separated by sucrose gradient centrifugation were examined by electron microscopy. Material from density 1.135 g cm^{-3} was heterogenous with respect to membrane components. Smooth and rough microsomes were commonly observed at this density as were dictyosomes (Fig. 8). The organelle fraction from density 1.15 g cm^{-3} was rich in dictyosomes which were identifiable as both stacked and separate cisternae but mitochondria were also frequently observed (Fig. 8). Mitochondria were the preponderent organelles at density 1.18 g cm^{-3} (Fig. 8).

Autoradiography was performed on organelle fractions obtained by sucrose gradient centrifugation of tissue incubated in [^{3}H]fucose for 40 min. Silver grain distribution on sections exposed for 12 weeks was exclusively associated with dictyosomes and single membrane-bound vesicles at densities between 1.135 and 1.15 g cm^{-3} (Fig. 8). Background silver grain counts in these autoradiographs were nearly nil; no silver grains were associated with mitochondria, the most abundant organelles in the 1.18 cm^{-3} zone of the gradient.

DISCUSSION

Secretion of material by a cell involves either direct secretion through the plasmalemma after a recognition process or the packaging into secretory vesicles, movement of these through

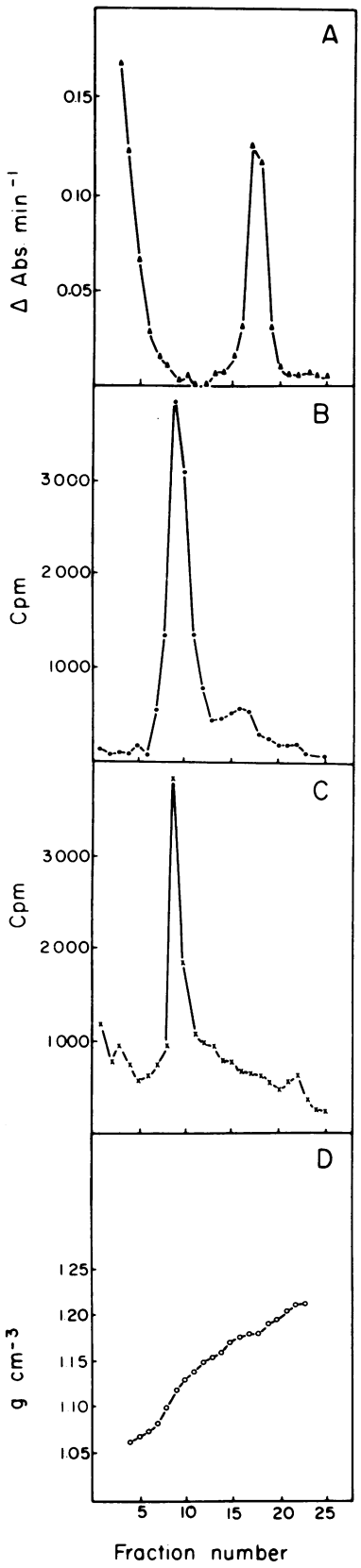

FIG. 5. Distribution of NADH oxidase (A), CDP-choline transferase (1/10 actual value) (B), and [^{3}H]fucose incorporated into nondialyzable material (C) on a nonlinear sucrose gradient (D) of homogenate of 0.5-cm root tips incubated with 90 μCi of [^{3}H]fucose for 2 hr.

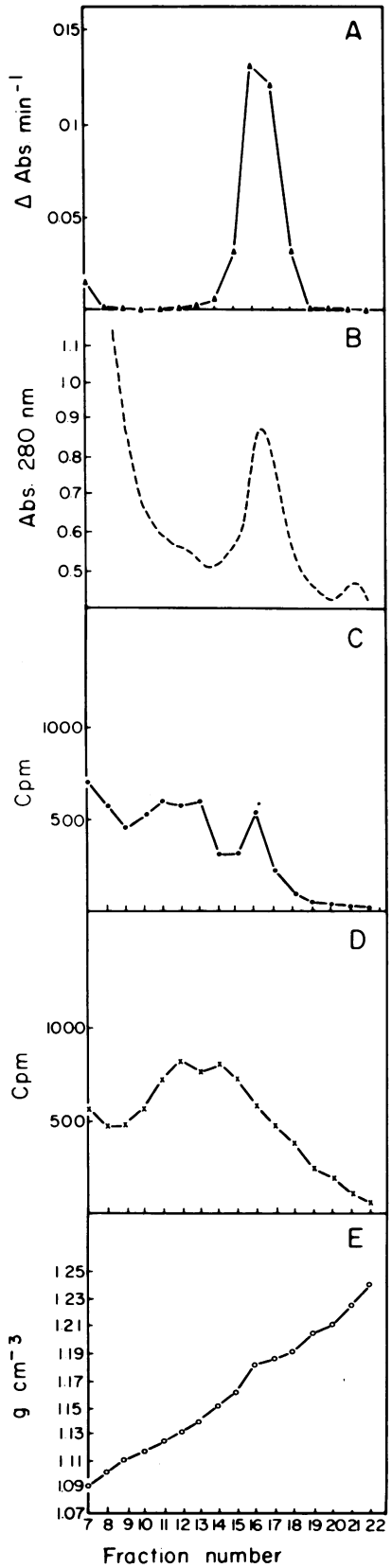

FIG. 6. Distribution of NADH oxidase (A), 280 nm absorbance (B), CDP-choline transferase (1/10 actual value) (C), and [^{3}H]fucose incorporated into nondialyzable material (D) on a shallow, linear sucrose gradient (E) of homogenate of 0.5-cm root tips incubated in 80 μCi of [^{3}H]fucose for 2 hr.

the cytoplasm to the plasmalemma, fusion with the plasmalemma, and expulsion of vesicular contents (5). Secretion of materials via dictyosome vesicles has been well characterized in animal systems (7) and, to a lesser extent, in plants (12). Our results support the involvement of dictyosomes in the secretion of corn root cap slime.

Pulse-chase experiments show that [^{3}H]fucose is incorpo-

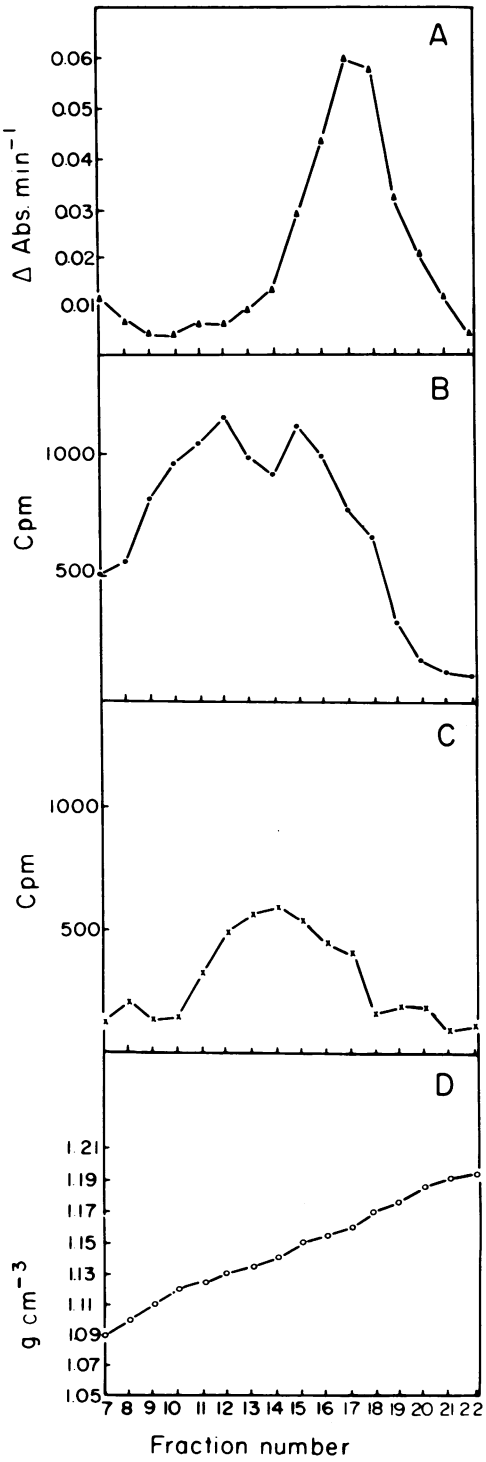

FIG. 7. Distribution of NADH oxidase (A), CDP-choline transferase (B), [^{3}H]fucose incorporated into nondialyzable material (C) on a shallow, linear sucrose gradient (D) of homogenate from 0.1-cm root tips incubated in 100 μCi of [^{3}H]fucose for 45 min.

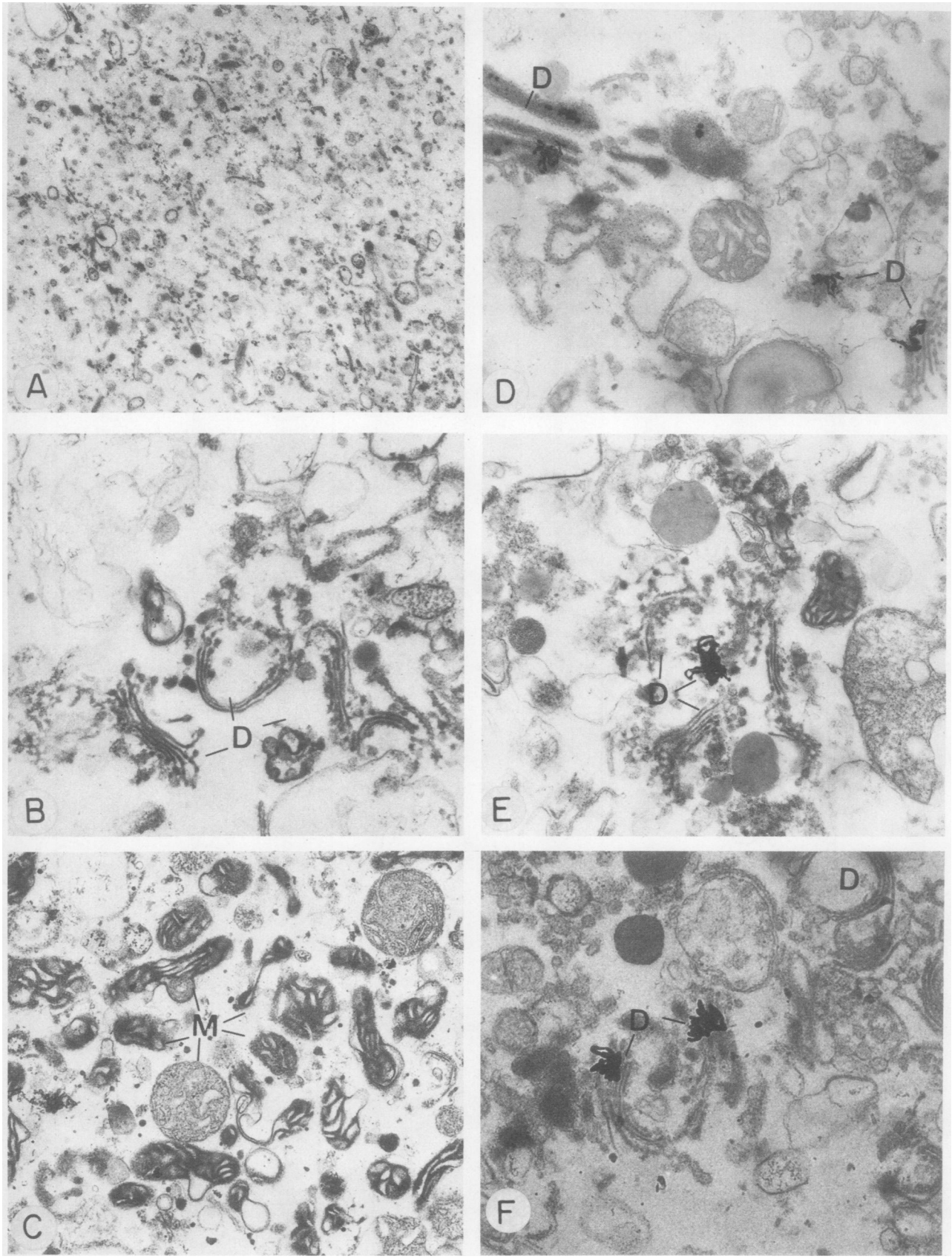

FIG. 8. A–C: Electron micrographs of organelle fractions from linear sucrose density gradients. A: Fraction from density 1.135 g cm^{-3}, × 28,500; B: density 1.15 g cm^{-3}, × 26,000; C: density 1.18 g cm^{-3}, × 26,000. D–F: Electron microscope autoradiographs of organelle fractions from density 1.15 g cm^{-3} showing silver grains associated with dictyosomes. D: × 26,250, E: × 26,250, F; 2 44,000. Dictyosomes (D) and mitochondria (M) are abundant.

rated into two distinct fractions in root cap cells. Incorporation of label into a water-soluble ethyl alcohol-insoluble fraction of root homogenates had a peak at 20 min of chasing followed by rapid loss of label. Seventy per cent of the label found in the water-soluble, ethyl alcohol-insoluble component of the homogenate is secreted to the outside medium after 2 hr of chasing (Fig. 1A). Although the peak of label accumulation in the homogenate at 20 min of chasing is characteristic for cells which sequester polymers in secretory organelles, we observed no accumulation of label in the medium during 20 to 30 min of chasing corresponding to the decline in the ethyl alcohol-insoluble radioactivity of the homogenate. Rather, the rate of secretion of the fucose-rich polymer remained constant during the 20- to 90-min chase period (Figs. 1A and 3). The kinetics of label accumulation in the water-insoluble cell wall fraction suggest that this secreted water-soluble component becomes trapped in the interstices of the wall (Fig. 2), and its appearance in the medium is thus delayed. We have shown by electron microscopy that material released from dictyosome vesicles of peripheral cells of the corn root cap accumulates between the plasmalemma and the cell wall (18).

The sequential accumulation and release of the labeled, water-soluble polymer suggests that cellular or extracellular barriers prevent its secretion from the cell. Cellular barriers to secretion would involve the participation of a transport system in export of the polymer while the cell wall could function as the extracellular barrier. Labeling of the water-insoluble fraction of the cell homogenate is markedly different from the water-soluble component (Fig. 1B). There is no evidence of the involvement of a secretory organelle in the transport of this material. Rather, the evidence suggests the *in situ* addition of label occurs. Chemical analysis of the water-insoluble fraction has shown that it is primarily hemicellulose while differential extraction of labeled sections shows that it is located in the cell wall (17, 18). It is clear from the kinetic data presented in Fig. 1B that the addition of fucose label to a cell wall component does not occur by a mechanism analogous to the secretion of the water-soluble polymer.

The incorporation of [^{14}C]glucose into both water-soluble and insoluble components is qualitatively similar to that of [^{3}H]fucose (Fig. 1). The incorporation of ^{14}C-amino acid mixture, however, is different from that of fucose or glucose (Figs. 1 and 3). Thus, while release of label into the medium from [^{3}H]fucose and [^{14}C]glucose begins after 10 to 15 min of the chase period, the release of the amino acid polymer is delayed an additional 5 to 10 min and occurs at a lower rate (Fig. 3). This suggests that amino acids are not added to the [^{3}H]fucose or [^{14}C]glucose containing polysaccharide and agrees with our analysis showing that the secreted polymer has no detectable protein (16).

The water-soluble, nondialyzable component from root cap homogenates was compared with the secreted material by CsCl equilibrium density gradient centrifugation (Fig. 4). While only one major component with a density of 1.63 g cm^{-3} is released from [^{3}H]fucose-labeled root tip sections, a broader spectrum of water-soluble materials is present in the homogenate (Fig. 4). Whether the materials at densities of 1.55 and 1.35 g cm^{-3} are precursors of the polysaccharide which is eventually secreted can only be conjectured. It is significant that a major fraction (32%) of the [^{3}H]fucose label located in the homogenate is physically similar to that secreted to the incubation medium.

Sucrose density gradient centrifugation of root cap homogenates provides evidence for the involvement of smooth membranes in the intracellular transport of secreted, fucose-rich polysaccharides (Figs. 6 and 7). With the exception of nonlinear gradient fractionation, label from fucose is distributed over a wide range of densities with a peak between 1.13 to 1.17 g cm^{-3} (Figs. 6 and 7). Although superior resolution of fucose label is obtained on a nonlinear gradient, our evidence suggests that for light membrane fractions, these gradients are unsatisfactory since boundary effects cause accumulation and nonspecific banding of material (Fig. 5).

In shallow linear gradients (Figs. 6 and 7), the broad peak of nondialyzable [^{3}H]fucose corresponds with the location of CDP-choline transferase activity. Although CDP-choline transferase is a suitable marker for endoplasmic reticulum in some plant tissues (14), we were unable to resolve the activity of this enzyme to the band having the characteristic density at 1.12 g cm^{-3}. Rather, activity of the enzyme was broadly distributed over a range of densities from 1.12 to 1.18 g cm^{-3}. In nonlinear gradients (Fig. 5) both CDP-choline transferase activity and [^{3}H]fucose incorporation resolve into a sharp band at a density of 1.12 g cm^{-3} but, as stated above, such sharp banding is undoubtedly a result of the boundary effects established in such gradients.

Electron microscopy showed that material from density 1.12 g cm^{-3} was composed principally of smooth membrane components, that from densities 1.13 to 1.15 g cm^{-3} was principally dictyosomes and dictyosome vesicles, and material at 1.18 g cm^{-3} principally mitochondria (Fig. 8). It is significant that the region of the gradient corresponding with the peak of [^{3}H]fucose incorporation is rich in dictyosomes and dictyosome vesicles.

Further evidence that dictyosomes contain label from [^{3}H]fucose is provided by electron microscope autoradiography of organelle fractions. Label was almost exclusively associated with dictyosomes (Fig. 8), and there was no significant labeling of material from densities 1.12 or 1.18 g cm^{-3}. Since negligible metabolism of [^{3}H]fucose occurs in cells of the corn root cap (17), it can be inferred that label associated with the dictyosomes is indeed from [^{3}H]fucose and not from a product of its metabolism. The exclusive association of label from L-[^{3}H]fucose with dictyosomes confirms their involvement in the transport of the fucose-rich polysaccharide and suggests that this organelle is also the site of polymer synthesis. Ray *et al.* (19) have demonstrated β-glucan synthetase activity in dictyosome-rich fractions from pea epicotyls isolated by methods similar to those used in this study. Our attempts to demonstrate L-fucose transferase activity (unpublished) in gradient fractions of corn root cap cells have indicated enzyme activity over the density range of 1.13 to 1.17 g cm^{-3}, the same region where the dictyosomes and their vesicles are found.

The data presented in this paper together with the *in situ* autoradiographic localization of label from [^{3}H]fucose (18) support the hypothesis that secretion of root cap slime of corn is via the dictyosome and dictyosome-derived vesicles. The site of synthesis and mechanism of assembly of this macromolecule are now being examined.

Acknowledgment—The assistance of E. Crump in the preparation of this manuscript is gratefully acknowledged.

LITERATURE CITED

1. Anderson, L. E. and W. O. McClure. 1973. An improved scintillation cocktail of high solubilizing power. Anal. Biochem. 51: 173–179.
2. Bowles, D. J. and D. H. Northcote. 1972. The sites of synthesis and transport of extracellular polysaccharides in the root tissue of maize. Biochem. J. 130: 1133–1145.
3. Crane, F. L. 1957. Electron transport and cytochromes of sub-cellular particles from cauliflower buds. Plant Physiol. 32: 619–625.
4. Floyd, R. A. and A. J. Ohlrogge. 1971. Gel formation on nodal root surface of *Zea mays*. I. Investigation of the gel's composition. Plant Soil 33: 331–343.
5. Grove, S. N., C. E. Bracken, and D. J. Morré. 1968. Cytomembrane differentiation in the endoplasmic reticulum golgi apparatus-vesicle complex. Science 161: 171–173.
6. Harris, P. J. and D. H. Northcote. 1970. Patterns of polysaccharide biosynthesis in differentiating cells of maize root tips. Biochem. J. 120: 479–491.
7. Jamieson, J. D. and G. E. Palade. 1967. Intracellular transport of secretory proteins in the pancreatic exocrine cell; role of the peripheral elements of the golgi complex. J. Cell Biol. 34: 577–596.
8. Jones, D. D. and D. J. Morré. 1967. Golgi apparatus mediated polysaccharide secretion by outer cap cells of *Zea mays*. II. Isolation and characterization of the secretory

product. Z. Pflanzenphysiol. 56: 166–169.
9. KIRBY, E. G. AND R. M. ROBERTS. 1971. The localized incorporation of ^{3}H-l-fucose into cell wall polysaccharide of the cap and epidermis of corn roots: Autoradiographic and biosynthetic studies. Planta 99: 211–221.
10. LORD, J. M., T. KAGAWA, T. S. MOORE, AND H. BEEVERS. 1973. Endoplasmic reticulum as the site of lecithin formation in castor bean endosperm. J. Cell Biol. 57: 659–667.
11. MACKLER, B. AND D. E. GREEN. 1956. Studies on the electron transfer system. II. On the opening phenomenon. Biochim. Biophys. Acta 21: 1–6.
12. MOLLENHAUER, H. H. AND D. J. MORRÉ. 1966. Golgi apparatus and plant secretions. Annu. Rev. Plant Physiol. 17: 27–46.
13. MORRÉ, D. J., D. D. JONES, AND H. H. MOLLENHAUER. 1967. Golgi apparatus mediated polysaccharide secretion by outer root cap cells of *Zea mays*. I. Kinetic and secretory pathway. Planta 74: 286–301.
14. MORRÉ, D. J., S. NYQUIST, AND E. RIVERA. 1970. Lecithin biosynthetic enzymes of onion stem and the distribution of phosphorylcholinecytidyl transferase among cell fractions. Plant Physiol. 45: 800–804.
15. NORTHCOTE, D. H. AND J. D. PICKETT-HEAPS. 1966. A function of the golgi apparatus in polysaccharide synthesis and transport in the root cap cells of wheat. Biochem. J. 98: 159–167.
16. PAULL, R. E., C. M. JOHNSON, AND R. L. JONES. 1975. Studies on the secretion of maize root cap slime. I. Some properties of the secreted polymer. Plant Physiol. 56: 300–306.
17. PAULL, R. E. AND R. L. JONES. 1975. Studies on the secretion of maize root cap slime. II. Localization of slime production. Plant Physiol. 56: 307–312.
18. PAULL, R. E. AND R. L. JONES. 1975. Studies on the secretion of maize root cap slime. III. Histochemical and autoradiographic localization of incorporated fucose. Planta. In press.
19. RAY, P. M., T. L. SHININGER, AND M. M. RAY. 1969. Isolation of β-glucan synthetase particles from plant cells and identification with golgi membrane. Proc. Nat. Acad. Sci. U.S.A. 64: 605–612.
20. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17: 208–212.
21. SCHNEPF, E. 1965. Physiologie und Morphologie, sekretorische Pflanzenzellen. *In:* Sekretion und Exkretion. pp. 72–88. Springer, New York.
22. SPURR, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26: 31–43.