Plant Physiol. (1977) 59, 653-657

myo-Inositol Metabolism in *Lilium longiflorum* Pollen

UPTAKE AND INCORPORATION OF *MYO*-INOSITOL-2-^{3}H[1]

Received for publication August 17, 1976 and in revised form November 15, 1976

MINSHEN CHEN[2] AND FRANK A. LOEWUS[3]
Department of Agricultural Chemistry, Washington State University, Pullman, Washington 99164

ABSTRACT

Germinating *Lilium longiflorum* pollen absorbs and metabolizes *myo*-inositol-2-^{3}H (MI-2-^{3}H) with a pronounced lag when label is supplied from the beginning of germination. If MI-2-^{3}H is given after 3 hours of germination, incorporation of labeled metabolic products into pollen tube polysaccharides is constant over a range of 0.56 mM to 2.78 mM MI. When MI-2-^{3}H is supplied as a 0.5-hour pulse 3 hours after germination, the proper precursor-product relationship to tube wall polysaccharides is observed. Replacing 10% of the germination media with sigmatic exudate from a compatible cultivar hastens germination and tube elongation. Enhanced MI metabolism accompanies tube growth in this exudate-enriched media.

The process of tube wall formation during pollen germination and pollen tube growth is dependent on carbon sources that are stored in the pollen grain or that are provided by the environment through which the tube must grow. During initial stages, reserves within the grain supply this need. Later, as the pollen tube emerges, nutrients in the pistil or artificial growth medium supplement and eventually supplant depleted reserves. In artificial media, sucrose, raffinose, and stachyose function as superior carbon sources for tube elongation (5). D-Glucose and D-fructose are also utilized though less effectively than sucrose.

Pectin, a major constituent of pollen tubes, is highly localized in the tube wall region, particularly at the growing tip, a site of intense metabolic activity. Stanley and Loewus (13) reported that labeled MI,[4] a precursor of D-galacturonosyl and L-arabinosyl units in pectin, was readily taken up and utilized for tube wall biosynthesis by germinating pear pollen, *Pyrus communis*, in media containing sucrose. Later, cytochemical evidence of localization of label in tube wall substance was provided (16).

Dickinson (2) opened the way for experiments on MI metabolism in pollen that had been germinated in artificial media. By substituting nonmetabolizable pentaerythritol (2,2-bis[hydroxymethyl]-1,3-propanediol) for sucrose as osmoregulator, he showed that a growth medium devoid of utilizable carbohydrate could be used to study lily pollen germination. With his system, Kroh and Loewus (7) examined MI metabolism in germinating pollen of Easter lily, *Lilium longiflorum*. When 1 mM MI was supplied to lily pollen 2 hr after germination, uptake remained linear for the next 4 hr. When MI-2-^{14}C was present in the germination medium, label rapidly appeared in galacturonate and arabinose residues of pollen tube wall polysaccharides. Label also appeared in xylose and hexose residues, but only after 6 hr of exposure to MI-2-^{14}C.

The current study was initiated to examine in greater detail the conditions of MI uptake and utilization in lily pollen. Subsequent studies deal with inhibition of MI utilization by 2-O,C-methylene MI (1), the role of free D-xylose as an intermediate in glucogenesis, and the utilization of MI and related sugars for glucogenesis via this xylose-mediated pathway. A preliminary report on the latter two studies has appeared (12).

MATERIALS AND METHODS

Pollen. On the day of dehiscence, anthers of *L. longiflorum*, cv. Ace and 44, were harvested and air-dried in open Petri dishes. After 2 to 3 days, anthridial thecae hardened, and the loose pollen grains were readily separated from dried anthers. This pollen was loosely packed in 20-ml glass vials with perforated plastic closures and stored at 4 C. Upon storage, the rate of germination declined for several days and then remained unchanged at 40 to 70% germination over several months. The pollen used in this study was gathered in March, 1971, from lilies grown for commercial purposes. All experiments were run during 1971. Except as noted, cv. 44 was used throughout this study.

Chemicals. Sugars were purchased from Fisher Scientific Co., pentaerythritol from Eastman Organic Chem., and Pectinol R-10, a fungal pectinase, from Rohm and Haas, Inc. *myo*-Inositol-2-^{3}H, 4.9 Ci/mol, was prepared chemically in this laboratory. It was recrystallized from water and ethyl alcohol before use.

Pollen Germination. The germination medium, containing 0.29 M pentaerythritol, 1.27 mM $Ca(NO_3)_2$, 0.16 mM H_3BO_3, and 0.99 mM KNO_3, was adjusted to pH 5.2 (2). Other additions are indicated in the text.

In a typical experiment, 5 mg of pollen was suspended in 1 ml of medium in a 10-ml borosilicate flask. Pollenkitt, a gummy substance attached to the surface of lily pollen, was removed by gently stirring the suspension with a glass rod until all pollenkitt adhered to the rod. Pollen grains were kept in suspension by gentle agitation on a reciprocal shaker at 30 oscillations/min at 27 C.

Germination was measured by viewing pollen at 40× magnification. A pollen grain was scored as germinated if a tube was visible and protruded more than 20% of the long axis of the grain. The percentage of grains that germinated was calculated from duplicate counts involving 300 to 500 grains.

Tube length was measured as the mean of 20 to 30 pollen tubes using 40× magnification. Reported values do not include the diameter of the pollen grain.

Fractionation of Germinated Pollen. Germinated samples

[1] Scientific Paper No. 4689, Project 0266, College of Agriculture Research Center, Washington State University, Pullman, Wash. 99164. This work was supported in part by Grants GM-12422 and GM-22427 from the National Institute of General Medical Sciences, National Institutes of Health.

[2] Present address: Department of Biochemistry, Purdue University, West Lafayette, Ind. 47907.

[3] To whom inquiries and requests for reprints should be addressed.

[4] Abbreviations: MI: *myo*-inositol; TFAA: trifluoroacetic acid.

were transferred to 12-ml centrifuge tubes with the aid of additional rinses of fresh medium, adjusted to a final volume of 6 ml, and centrifuged at 1,000*g* for 2 min. The Medium was removed with a Pasteur pipette and pollen tissues were washed repeatedly with more medium until radioactivity external to the tubes had been removed, usually two to three washes. Washes and supernatant fluid were combined and analyzed for radioactivity to determine the uptake of labeled MI.

Each sample of washed pollen was resuspended in 2 ml of 70% (v/v) ethyl alcohol at 0 C and finely ground in a motor-driven glass homogenizer. This suspension was transferred to a 12-ml centrifuge tube with the aid of additional 70% ethyl alcohol, adjusted to 5 ml, and centrifuged at 1,000*g* for 2 min. Pollen residues were twice extracted with 2-ml portions of 70% ethyl alcohol and centrifuged. Original supernatant solution and washes were combined and further centrifuged at 20,000*g* for 15 min to yield a high speed pellet and a 70% ethyl alcohol-soluble fraction.

Enzymic Hydrolysis of Pollen Residues. Residue from the low speed centrifugation was resuspended in 1 ml of 0.5% (w/v) pectinase containing 0.1% (w/v) Na_2EDTA. The suspension was hydrolyzed for 24 hr at 27 C, diluted to 5 ml with H_2O, and centrifuged at 1,000*g* for 2 min. Enzyme was destroyed by heating the supernatant solution to 100 C. Denatured protein was removed by centrifugation. The final solution was passed through a column of Dowex 50-H^+ exchange resin (1 × 10 cm) and stored for later analysis of carbohydrate constituents. Pectinase-resistant residue was resuspended in H_2O and analyzed for tritium in thixotropic counting fluid.

Acid Hydrolysis of Pollen Residues. Residue from the low speed centrifugation was hydrolyzed in 1 ml of 2 M TFAA in a sealed ampule for 40 min at 121 C. Acid-resistant residue was removed by centrifugation and TFAA by drying the supernatant solution at reduced pressure. Acid-resistant residue was analyzed for tritium.

Analysis of Hydrolyzed Products. Hydrolyzed products were fractionated into neutral and acidic fractions on a column of Dowex 1-formate exchange resin (1 × 12.5 cm). Acids were eluted as a mixture with 3 N formic acid. The neutral fraction was chromatographed on Whatman No. 1 paper in one or more of the following solvent systems: (A) ethyl acetate-pyridine-H_2O, 8:2:1, v/v; (B) ethyl acetate-pyridine-H_2O, 10:6:5, v/v; and/or (C) ethyl acetate-H_2O-acetic acid-formic acid, 18:4:3:1, v/v. Radioactive regions were detected by a chromatographic scanner with 1% efficiency for tritium. Sugars and sugar acids were located with alkaline silver nitrate (14) and sugar phosphate esters by the method of Hanes and Isherwood (4). Other radioactive samples were counted by liquid scintillation in naphthalene-dioxane or toluene-Triton X-100 scintillation fluid containing 2,5-diphenyloxazole and *p*-bis(*o*-methylstyryl)-benzene.

RESULTS

In this study, uptake and utilization of MI were examined during metabolic events following pollen germination. Preliminary experiments in which MI-2-^{3}H was present in the medium at the time the pollen was introduced revealed a lag in uptake of MI related to the germination process. Such an experiment is described in Figure 1, where 5 mg of pollen was suspended in germination medium containing 0.17 mM MI-2-^{3}H, 5 × 10^5 cpm. This concentration is below the level of maximal MI uptake. During the first 2 hr, only 5% of the pollen grains produced visible tubes and uptake of label was less than 5%. During the 3rd hour, virtually all viable grains formed pollen tubes and uptake rose to 17%, accompanied by substantial incorporation of label into tube wall polysaccharides. As tube growth continued, the MI content of the medium was depleted and most of the label accumulated in 70% ethyl alcohol-insoluble residue as pectic substance. After 8 hr of growth, 82% of the MI-2-^{3}H was present in pollen tubes, primarily as pectin.

A series of experiments in which 5-mg samples of pollen were incubated for 4 hr in 1 ml of media containing MI-2-^{3}H at concentrations ranging from 0 to 50 mM revealed that above 0.33 mM, uptake of MI during the 4-hr period was proportional to MI concentration, averaging 25 ± 4% of the MI supplied. In these runs, germination ranged from 63 ± 2% in samples lacking MI to 73 ± 1% in samples containing 50 mM MI. Tube length at 4 hr ranged from 0.67 ± 0.1 mm to 0.74 ± 0.1 mm within the same MI concentrations.

When pollen grains were allowed to germinate in pentaerythritol media for 3 hr prior to introduction of MI-2-^{3}H, the low uptake encountered during the lag of pollen tube appearance was avoided. With pregerminated pollen, over 50% of the MI-2-^{3}H supplied at 0.22 mM was taken up by the pollen tubes in 2 hr (Table I). At 0.56 mM MI-2-^{3}H, incorporation of label into 70% ethyl alcohol-insoluble substance was 2 μg of MI/mg of pollen/hr, the maximum rate. Increasing the MI level to 2.78 mM had no further effect.

When labeled pollen tubes were ground in 70% ethyl alcohol, most of the labeled polymeric material contained by the tubes, as well as the tube wall substance, appeared in the ethyl alcohol-insoluble fraction. By grinding the tubes in water rather than 70% ethyl alcohol, the cytosol remained soluble or in suspension after low speed centrifugation. A substantial portion of the labeled polysaccharide associated with the tube wall was undoubtedly solubilized by this procedure, but valuable information comparing appearance of label in cytoplasm and tube wall was gained. Pollen samples, pregerminated for 3 hr, were labeled with 0.28, 1.11, and 2.77 mM MI-2-^{3}H and allowed to metabolize the MI for a given period of time. At the end of this period, samples were homogenized with water and separated by centrifugation into soluble and insoluble fractions (Fig. 2). Total

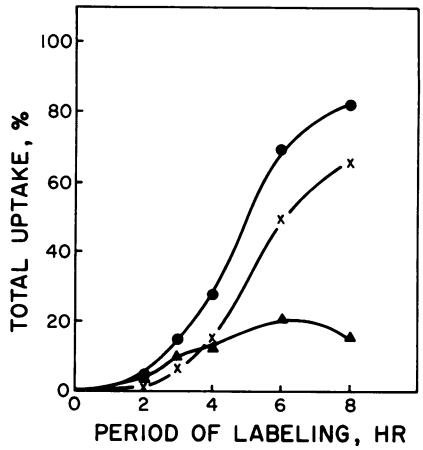

FIG. 1. Uptake of MI-2-^{3}H by *L. longiflorum* pollen. Five mg of pollen was incubated in 1 ml of Dickinson's pentaerythritol medium containing 0.17 mM MI-2-^{3}H (5 × 10^5 cpm). ▲: 70% ethyl alcohol-soluble fraction; X: 70% ethyl alcohol-insoluble fraction; ●: total uptake and incorporation of tritium.

Table I. *Uptake and Utilization of MI-2-^{3}H by L. longiflorum Pollen*

Five mg of pollen was germinated in 1 ml of Dickinson's pentaerythritol medium at 27 C for 3 hr. The indicated amount of MI-2-^{3}H (10^4 cpm/μg) was added and pollen growth continued for 2 hr. Five replicates were run at each concentration of MI.

MI Concn.		Uptake of Label	Incorporation into Tube Wall	MI Utilized for Tube Wall
μg/ml	mM	%	%	μg
40	0.22	53.2	38.5	15.4 ± 0.7
100	0.56	31.2	20.1	20.1 ± 1.4
200	1.11	17.8	10.6	21.2 ± 0.5
500	2.78	7.9	4.2	21.0 ± 1.6

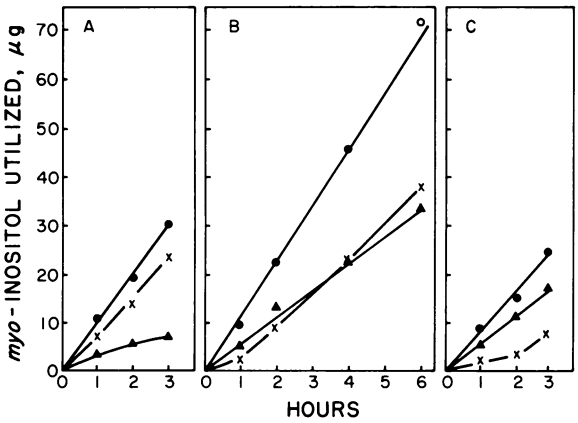

FIG. 2. Uptake of MI-2-^{3}H by *L. longiflorum* pollen after prelabel germination of pollen for 3 hr. ▲: water-soluble fraction; X: water-insoluble fraction; ●: total uptake and incorporation of tritium. MI concentrations: A, 0.28 mM; B, 1.11 mM; C, 2.78 mM.

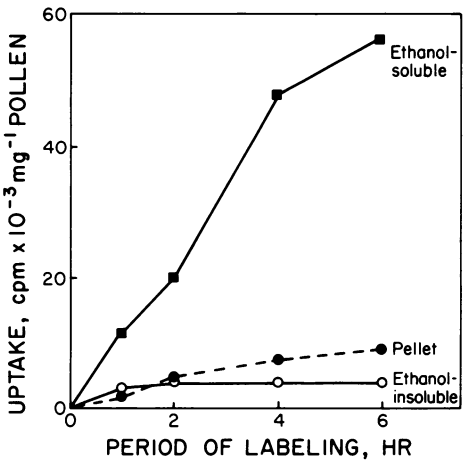

FIG. 3. Distribution of tritium from MI-2-^{3}H in water-extracted cytoplasmic portion of labeled *L. longiflorum* pollen after further fractionation at 20,000*g* followed by alcohol precipitation.

uptake was linear over a period of 6 hr in 1.11 mM MI. At 0.28 mM, 62% of the MI-2-^{3}H was taken up in 3 hr. At this low concentration, conversion of MI-2-^{3}H to wall polysaccharides was so rapid that even 1 hr after labeling, more tritium appeared in the insoluble than in the soluble fraction. Beyond 1 hr, soluble label decreased due to depletion of MI-2-^{3}H in the medium and to continued rapid incorporation of label into the wall. Incorporation of tritium into water-insoluble polysaccharides remained linear over the 3-hr period. At 1.11 mM as well as 2.77 mM MI, label from MI-2-^{3}H first accumulated in the soluble fraction. Although the rate of uptake of MI was not affected by MI concentration, appearance of label from MI into wall substance as measured by the water-insoluble fraction was greatly repressed, especially at the higher concentration. These data, as well as earlier studies in which incorporation was measured on 70% ethyl alcohol-insoluble residue, suggest that incorporation of MI-2-^{3}H-derived label saturates at 20 to 40 μg of MI/mg of pollen. Beyond this range of concentrations, MI represses incorporation of the products of MI metabolism into water-insoluble tube substance, but does not repress uptake and incorporation into the water-soluble fraction.

The turbid, water-soluble fractions from pollen tubes labeled at 1.11 mM MI-2-^{3}H were recentrifuged at 20,000*g* to yield a clear supernatant fraction and a pellet. The former was evaporated to a small volume at reduced pressure, adjusted with ethyl alcohol to 70% (v/v) to precipitate ethyl alcohol-insoluble polysaccharide, and centrifuged (Fig. 3). Most of the label was soluble in 70% ethyl alcohol. Chromatography of this fraction in solvents B and C revealed that most of the tritium was MI or a compound that chromatographically resembled MI. A small amount of labeled glucuronic and/or galacturonic acid was found, as well as an unknown labeled component with a mobility in solvent system B slightly slower than MI. Composition of neither pellet nor ethyl alcohol-insoluble residue was examined chromatographically, although it should be noted that hydrolysis of the pellet with 2 M TFAA for 40 min at 121 C in a sealed vial completely solubilized bound tritium.

Pollen tube residues recovered from the low speed centrifugation of pollen grown in 1.11 mM MI-2-^{3}H were hydrolyzed in 2 M TFAA, a treatment that released 99% of the bound label. Aliquots of hydrolysate were chromatographed in solvent A and scanned for tritium (Fig. 4). Radioactivity was found at the origin and in regions characterized by co-chromatographed standards as galactose-glucose, arabinose, and xylose. The ratio of radioactivities among these four regions remained unchanged over the 6-hr labeling period as 3:1:12:0.8. In solvent A, uronic acids remained at the origin with oligosaccharides and other partially hydrolyzed tube wall fragments. In an earlier study (7), labeled hexose and xylose did not appear in hydrolysates until 6 hr after labeling, as compared to 2 hr in this study. This difference in appearance of labeled hexose and xylose was due to the 3-hr prelabel germination procedure introduced in the present study.

To trace the course of MI utilization for tube wall formation, 1.11 mM MI-2-^{3}H (2×10^6 cpm) was supplied as a 0.5-hr pulse to pregerminated pollen. At the end of this period, labeled medium was replaced with unlabeled medium and pollen tube growth continued for 1 to 6 hr. Microscopic examination of pollen tubes and tips following the change in media revealed no evidence of tube damage or ruptured tips. During the exposure to MI-2-^{3}H, about 5% of the label was taken up. At the moment pollen tubes were transferred from labeled to label-free media, about 87% of the tritium was in water-extractable cytosol, nearly all of it remaining in the supernatant fraction after centrifugation at 20,000*g* and 75 to 80% of that still soluble in 70% ethyl alcohol. Within 1 hr (Fig. 5), 50% of this soluble label had moved into tube wall residue. Longer intervals of tube growth resulted in a further decrease in water-soluble label, but this was not accompanied by a corresponding increase in wall-bound label. Apparently, a portion of the MI-2-^{3}H taken up during the pulse was lost, presumably as tritiated water to unlabeled medium (12). At the time that these experiments were run, this loss was overlooked and no attempt was made to analyze final media for radioactivity.

Centrifugation of the water-extracted, turbid supernatant from 0.5-hr pulsed experiments into a clear soluble fraction and an insoluble pellet, followed by precipitation of 70% ethyl alcohol-insoluble polysaccharide from the clear supernatant fraction, produced the distribution of radioactivity plotted in Figure 6. During the 1st hr after removal of labeled media, a rapid drop occurred in 70% ethyl alcohol-soluble label, and most of this was recovered in the 70% ethyl alcohol-precipitable fraction and the pellet. Label continued to accumulate in the pellet up to 2 hr after removal from labeled media. Beyond 2 hr, there were no further changes despite the fact that 60 to 70% of the tritiated material in the 70% ethyl alcohol-soluble fraction had the same chromatographic properties as MI.

All of the tritium present in tube wall residues from these pulsed experiments was solubilized by acid hydrolysis. In samples hydrolyzed immediately after removal of tubes from labeled media, chromatographic analysis in solvent A produced the same pattern observed in Figure 4. Further incubation in unlabeled media led to the disappearance of the galactose-glucose peak, but there were no changes in relative amounts of tritium found at the origin or in the arabinose and xylose regions.

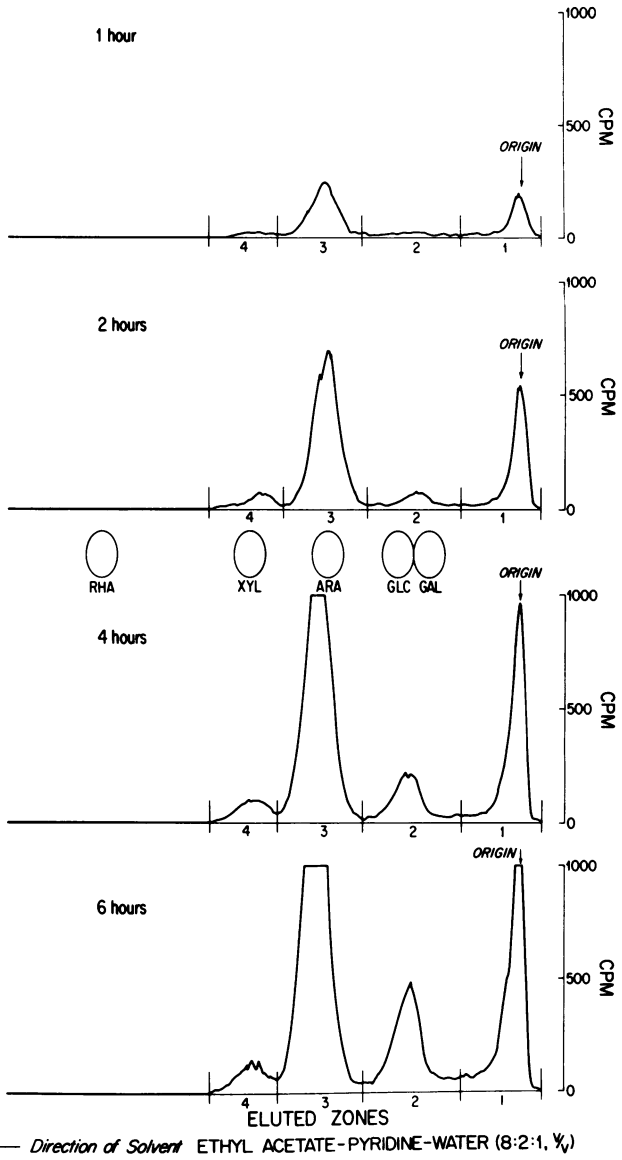

FIG. 4. Radiochromatographic scans of acid-hydrolyzed 70% ethyl alcohol-insoluble residue from *L. longiflorum* pollen grown in MI-2-^{3}H. Intervals of labeling following a 3-hr prelabel germination period are given in the figure. Positions of authentic sugar standards are indicated for RHA, rhamose; XYL, xylose; ARA, arabinose; GLC, glucose; and GAL, galactose.

Effect of Stigmatic Exudate on Uptake of MI. Replacement of 10% of the artificial media with fresh stigmatic exudate from cv. Croft pistils accelerated germination and tube elongation of freshly harvested cv. Ace pollen. In the absence of added exudate, 50% of the grains germinated in 2 hr and 8 hr was required to reach 95% germination. In the presence of exudate, 50% germination was reached in less than 0.5 hr and all grains had germinated in 4 hr. The effect of exudate on tube elongation was similar. When 0.03 mM MI-2-^{3}H was included in the media, labeling of tube wall polysaccharides was also accelerated by the presence of exudate. Virtually all of this labeled MI was taken up within 4 hr in exudate-enriched media and 60% was incorporated into tube wall material. Tubes grown in the absence of exudate required twice as much time to reach the same level of incorporation.

DISCUSSION

Once the process of pollen germination is triggered, metabolic events within the grain initiate tube growth and the emerging

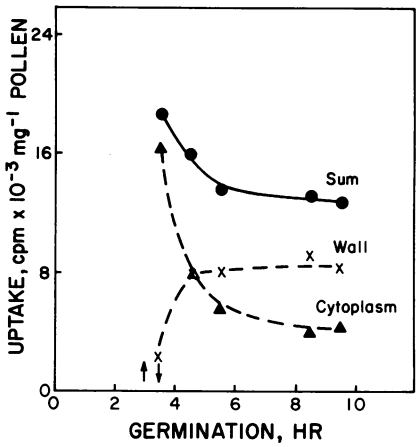

FIG. 5. Distribution of label in *L. longiflorum* pollen following 0.5-hr pulse-labeling with MI-2-^{3}H. Symbols as in Figure 1. ↑: addition of MI-2-^{3}H to the medium; ↓: replacement of labeled medium with unlabeled medium.

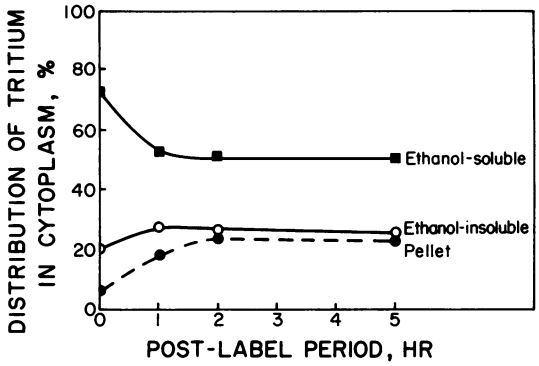

FIG. 6. Distribution of label in cytoplasmic fraction of experiment described in Figure 5 after further fractionation at 20,000*g* followed by alcohol precipitation.

pollen tube begins to take up nutrients from sources external to the grain itself. MI, a precursor of pectic residues (9), is readily incorporated into pollen tube tissues from the moment of tube emergence (16). As growth proceeds, newly formed tube wall substance deposits behind the growing tip and utilization of MI for pectin biosynthesis at the tip increases (8). When labeled MI is present in the medium at the onset of germination, uptake lags until a population of viable grains has germinated and formed tubes. As the tubes emerge and grow, there is a steep rise in MI uptake and utilization, as seen in Figure 1 and earlier experiments (7). Delaying introduction of label for 3 hr after initiation of germination eliminates the lag in MI uptake due to a growing population of new tubes and permits evaluation of MI uptake and utilization in a uniform population of elongating tubes.

Fractionation of germinated pollen in 70% ethyl alcohol provides a quick, convenient procedure for evaluating the incorporation of label from MI-2-^{3}H into pectic substance, but it fails to distinguish between pectin deposited in the tube wall, and new formed polysaccharide still within the pollen tube. For this reason, experiments were run in which germinated pollen labeled with MI-2-^{3}H were fragmented in water and separated by low speed centrifugation into a crude cytoplasmic fraction and a sedimented tube wall fraction. Higher speed centrifugation sedimented the suspended particles from the cytosol, including contents of vesicles responsible for transport to tube walls of polysaccharide newly formed from cytoplasmic organelles (15). About 25% of the labeled material that remained in solution after sedimentation was precipitated with ethyl alcohol. Although the amount of label accumulated by germinated pollen continued to increase with time over the 6-hr period of MI-2-^{3}H

uptake (Figs. 2 and 3), this increase was seen only in the tube wall fraction, the high speed pellet, and the final 70% ethyl alcohol-soluble fraction. Label in water-extracted, 70% ethyl alcohol-precipitable material increased only during the first 2 hr. Beyond that time, no increase was noted. Presumably, this fraction, corresponding to newly formed pectic substance, is labeled as readily as other fractions, but turnover due to deposition of this material at the tube wall causes continuous depletion. Evidence of this is seen in a 0.5-hr pulse experiment described in Figure 5 in which disappearance of tritium from cytoplasm is largely accounted for by its appearance in the tube wall fraction.

When Kroh and Loewus (7) supplied MI-2-^{14}C to lily pollen at the onset of germination, the first incorporated label detected in 70% ethyl alcohol-insoluble residues appeared in galacturonic acid and arabinose. Labeled xylose and hexose were not detected until 6 hr after labeling. In the present study, appearance of labeled xylose and hexose was seen at 2 hr after labeling due to the prelabel germination procedure. The disappearance of labeled hexose from hydrolysates of tube wall residues after short term labeling provided the first clue that this form of labeling was associated with a polysaccharide fraction other than pectic substance. This hexose, identified as glucose, came from starch co-precipitating with tube wall fragments (12).

L. longiflorum pollen contains 3 to 5 μg of free MI/mg of freshly harvested grains (M-A. Mitchell, unpublished observation), a MI reserve less than 25% of that needed to saturate the germinating pollen's capacity for MI uptake under conditions used in this study. Given an adequate supply of MI, uptake reaches 1 μg/mg of pollen·hr. About 20% of this MI is converted to tube wall pectic substance in this same period. It should be pointed out that growth of *L. longiflorum* pollen in artificial media seldom reaches even 1% of the tube length required for fertilization under normal conditions of pollination. To achieve that growth potential, this lily pollen relies on nutritional sources supplied by pistil exudate, as well as carbohydrate reserves stored within the grain. Evidence of the nutritional dependence of growing pollen tubes on pistil exudate was obtained by Loewus and Labarca (10). Similar observations apply to pollen grown in artificial media as shown in present experiments and earlier studies (6). In results reported here, the rate of germination and tube elongation doubled when 10% pistil exudate from a compatible pistil source was added to the pentaerythritol medium. Incorporation of label from MI-2-^{3}H into tube wall pectin was also stimulated by pistil exudate, possibly a reflection of accelerated tube elongation. *L. longiflorum* pollen contains the enzyme MI-1-P synthetase, needed for conversion of D-glucose-6-P to MI-1-P (11). It also contains UDP-D-glucose dehydrogenase (1, 3). Given an adequate supply of D-glucose-6-P, biosynthesis of UDP-D-glucuronic acid can occur either by the MI oxidation pathway or by the sugar nucleotide oxidation pathway. Since it is well established from results obtained in this and earlier studies that MI is converted to galacturonosyl and arabinosyl residues of tube wall pectic substance, one must ask if this process of MI metabolism is a major route to UDP-D-glucuronate and subsequent pectic polysaccharides. The following paper examines this aspect.

Acknowledgments – The authors wish to thank M. E. Walz-Loewus for valuable advice and suggestions regarding this paper, and M. Kroh, University of Nijmegen, for guidance in the initial stages of this research.

LITERATURE CITED

1. CHEN M-S, MW LOEWUS, FA LOEWUS 1977 Effect of a *myo*-inositol antagonist, 2-O,C-methylene-*myo*-inositol, on the metabolism on *myo*-inositol-2-^{3}H and D-glucose-1-^{14}C in *Lilium longiflorum* pollen. Plant Physiol. 59: 658–663
2. DICKINSON DB 1968 Rapid starch synthesis associated with increased respiration in germinating lily pollen. Plant Physiol 43: 1–8
3. DICKINSON DB, JE HOPPER, MD DAVIES 1973 A study of pollen enzymes involved in sugar nucleotide formation. *In* F. Loewus, ed, Biogenesis of Plant Cell Wall Polysaccharides. Academic Press, New York pp 29–48
4. HANES CS, FA ISHERWOOD 1949 Separation of the phosphate esters on the filter paper chromatogram. Nature 164: 1107–1112
5. HRABĚTOVÁ E, J TUPÝ 1964 The growth effect of some sugars and their metabolism in pollen tubes. *In* HF Linskens, ed, Pollen Physiology and Fertilization. North Holland Publ Co, Amsterdam pp 95–104
6. KROH M, C LABARCA, F LOEWUS 1971 Use of pistil exudate for pollen tube wall biosynthesis in *Lilium longiflorum*. *In* J Heslop-Harrison, ed, Pollen: Development and Physiology. Butterworths, London pp 273–278
7. KROH M, FA LOEWUS 1968 Biosynthesis of pectic substance in germinating pollen: labeling with *myo*-inositol-2-^{14}C. Science 160: 1352–1354
8. KROH M., H MIKI-HIROSIGE, W ROSEN, F LOEWUS 1970 Incorporation of label into pollen tube walls from *myo*-inositol-labeled *Lilium longiflorum* pistils. Plant Physiol 45: 92–94
9. LOEWUS F 1969 Metabolism of inositol in higher plants. Ann NY Acad Sci 165: 577–598
10. LOEWUS F, C LABARCA 1973 Pistil secretion product and pollen tube wall formation. *In* F Loewus, ed, Biogenesis of Plant Cell Wall Polysaccharides. Academic Press, New York pp 175–193
11. LOEWUS MW, FA LOEWUS 1971 The isolation and characterization of D-glucose-6-phosphate cycloaldolase (NAD-dependent) from *Acer pseudoplatanus* L. cell cultures. Plant Physiol 48: 255–260
12. ROSENFIELD C-L, FA LOEWUS 1975 Carbohydrate interconversions in pollen-pistil interactions of the lily. *In* DL Mulcahy, ed, Gamete Competition in Plants and Animals. American Elsevier, New York pp 151–160
13. STANLEY RG, FA LOEWUS 1964 Boron and *myo*-inositol in pollen pectin biosynthesis. *In* HF Linskens, ed, Pollen Physiology and Fertilization. North Holland Publ Co, Amsterdam pp 128–136
14. TREVELYAN WE, DP PROCTER, JS HARRISON 1950 Detection of sugars on paper chromatograms. Nature 166: 444–445
15. VAN DER WOUDE WJ, DJ MORRÉ, CE BRACKER 1971 Isolation and characterization of secretory vesicles in germinating pollen of *Lilium longiflorum*. J Cell Sci 8: 331–351
16. YOUNG LCT, RG STANLEY, FA LOEWUS 1966 *myo*-Inositol-2-t incorporation by germinating pollen. Nature 209: 530–531