Plant Physiol. (1982) 69, 220–225
0032-0889/82/69/0220/06/$00.50/0

Redistribution of Tritium during Germination of Grain Harvested from *myo*-[2-^{3}H]Inositol- and *scyllo*-[*R*-^{3}H]Inositol-Labeled Wheat[1]

Received for publication June 2, 1981 and in revised form August 25, 1981

KEN SASAKI AND FRANK A. LOEWUS[2]
Institute of Biological Chemistry, Washington State University, Pullman, Washington 99164

ABSTRACT

Wheat kernels from *myo*-[2-^{3}H]inositol- or *scyllo*-[*R*-^{3}H]inositol-labeled plants (Sasaki and Loewus 1980 Plant Physiol 66: 740–745) were used to study redistribution of ^{3}H into growing regions during germination. Most of the labeled 1-α-galactinol (or the analogous *scyllo*-inositol galactoside) was hydrolyzed within 1 day. Water-soluble phytate was dephosphorylated within 3 days. A large reserve of bound phytate continued to release *myo*-inositol over several days. Translocation of free *myo*-inositol to growing regions provided substrate for the *myo*-inositol oxidation pathway and incorporation of ^{3}H into new cell wall polysaccharides.

Cell wall polysaccharides in the kernel were degraded during germination. The labeled residues were translocated to growing regions and reutilized for new cell wall formation. Pentosyl residues accounted for most of this label.

Free *scyllo*-inositol followed a path of translocation from kernel to seedling similar to that of *myo*-inositol. Unlike *myo*-inositol, it did not furnish substrate for the *myo*-inositol oxidation pathway but accumulated as free *scyllo*-inositol in the seedling.

The fate of phytate-derived *myo*-inositol during germination of wheat is discussed in relation to a recent scheme of phytate metabolism proposed by De and Biswas (1979 J Biol Chem 254: 8717–8719) for germinating mung bean seedlings.

The role of MI[3] in developing seed and in subsequent seed germination is masked by the multiple involvement of MI in separate processes of sugar transport, storage of phosphate, and participation in polysaccharide biosynthesis (3, 9, 11). An attempt to identify and to assign to each process its relative place in the sequence of events that accompany germination led to this study. If wheat is given [2-^{3}H]MI through the peduncle during the post-anthesis stage of kernel development, the bulk of the ^{3}H in the mature kernel, about 50% injected label, is found in two products, phytate and "bran" polysaccharides, together with lesser amounts of MI-Gal and free MI.

If [*R*-^{3}H]SI[4] replaces [2-^{3}H]MI as the source of label in such experiments, an even more efficient translocation of ^{3}H is effected with about 80% label appearing in the bran fraction. Unlike MI-labeled kernels, neither phytate nor bran polysaccharides are labeled and the accumulated ^{3}H appears in SI-Gal and free SI (18).

This paper reports the results of experiments in which labeled grain from [2-^{3}H]MI- or [*R*-^{3}H]SI-labeled wheat was germinated and the distribution of labeled products from seedling and residual kernel was examined to determine such changes as occur during germination. The study provides new information on the metabolic fate of the MI portion of phytate as well as other labeled products that are deposited in the kernel during grain development.

MATERIALS AND METHODS

Labeled wheat kernels used in this study were obtained from plants used in the preceding study (18). The ^{3}H content per kernel ranged from 3 to 5 nCi (13-day experiment) to 50 nCi (1- and 3-day experiments) for MI-labeled grain and was 10 nCi for SI-labeled grain. Determinations were made from pooled tissue of 10 or more kernels. Surface-sterilized grain (1 g in 5 ml 2% NaOCl, 10 min, 20°C) was rinsed in sterile H_2O, spread on moist filter paper in covered containers, and germinated in the dark at 25°C. Seedlings 3 days or older were separated into roots, shoot, and (residual) kernel. Younger seedlings were separated into embryo (scutellum attached) and residual kernel. Seedling parts were lyophilized and stored in a desiccator.

Dried kernel tissues were ground to a fine powder and extracted successively with 80% ethyl alcohol (60°C, 3 h), water (25°C, five extractions), and 0.2% Na_2EDTA (50°C, 1 h). Following each extraction, the residues were rinsed with fresh extracting medium to remove remaining traces of soluble radioactivity. EDTA-extracted residues from kernel tissues were lyophilized and resuspended in 50 mM acetate buffer (3 ml, pH 4.6, 90°C, 1 h) to solubilize suspended starch particles. To the cooled suspension was added 5% amyloglucosidase (Sigma A-7255) in 50 mM acetate buffer (3 ml, pH 4.6, 55°C, 5 h). Following this step of starch hydrolysis, the suspension was spun at 10,000*g* and the insoluble pellet was rinsed (three times) with distilled H_2O. Rinses were combined with the supernatant. In some experiments, the amyloglucosidase-resistant residue was resuspended in 2 N trifluoroacetic acid (100°C, 3 h) to hydrolyze pectic and hemicellulosic constitutents.

Roots and shoot tissues were successively extracted with 80% ethyl alcohol and 0.2% Na_2EDTA before hydrolyzing the residues in 2 N trifluoroacetic acid as described above. The step involving enzymic hydrolysis of starch was omitted.

Recovery and Analysis of Radioactive Components. Free MI and SI, and their corresponding galactosides, were extracted in the 80% ethyl alcohol step. The inositols were separated from their galactosides by passage through a Sephadex G-15 column (98 × 1.2 cm diameter). Phytate appeared in extracts from water, EDTA,

[1] Supported by Grant GM-22427 from the National Institute of General Medical Sciences, National Institutes of Health, United States Public Health Service. Scientific Paper No. 5934, Project 0266, College of Agriculture Research Center, Washington State University, Pullman, WA 99164.

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

[3] Abbreviations: MI, *myo*-inositol; MI-Gal, 1L-1-(*O*-α-D-galactopyranosyl)-*myo*-inositol (also galactinol); SI, *scyllo*-inositol; SI-Gal, *O*-α-D-galactopyranosyl-*scyllo*-inositol.

[4] The symbol *R* denotes the random position of 1 atom of ^{3}H per labeled molecule of *scyllo*-inositol.

and amyloglucosidase steps. It was separated from polysaccharides in these extracts by passage through a Sephadex G-50 column (45 × 0.9 cm diameter). The eluting solvent was 0.4 M acetic acid. Analysis of inositols, inositol galactosides, and phytate have been described (18). Phosphoric esters of MI were separated by ion exchange chromatography (20).

Mixed polysaccharides, eluted in the void volume of the Sephadex G-50 column step, were hydrolyzed in 1 N H_2SO_4 (100°C, 2.5 h). The hydrolysate was passed through a column (10 × 0.9 cm diameter) of Dowex 1 (acetate) exchange resin to remove acidic components. Neutral sugars in the effluent were converted to their alditol acetates (1) and identified by GLC on an isothermal column (1.8 m × 0.64 cm diameter) of 3% XE-60 on 100 to 120 mesh Gas-chrom Q (Applied Sci., State College, PA) at 180°C.

Position of ^3H in Arabinosyl Residues from Seedlings of [2-^3H]MI-Labeled Wheat Kernels. Water-insoluble polysaccharide (3 mg) from root tissue of 3-day-old seedlings was hydrolyzed in 1 N H_2SO_4 (100°C, 2 h), deionized by passage through short columns of Dowex 50 (H^+) and Dowex 1 (acetate) resins, and evaporated to 0.1 ml. The neutral sugars in this hydrolysate were separated by HPLC (Waters model 201 with refractive index detection) on Aminex HPX-87 ("organic acid" column, BioRad Labs, Richmond, CA) with 0.1 M formic acid (0.3 ml/min, 30°C). Fractions under the arabinose peak were combined, evaporated to dryness, redissolved in methyl alcohol (2 ml) with additional L-arabinose (20 mg) as carrier, and evaporated to a syrup. This syrup was redissolved in dry methyl alcohol (2.5 ml) to which was added I_2 (41 mg in 1 ml methyl alcohol) and 4% (w/v) KOH in methyl alcohol (0.8 ml), in that order. The solution was held at 40°C until the yellow color faded, then stored at 4°C overnight to permit the crystallization of K arabinate. The arabinate was oxidized with Na periodate to release C-5 as formaldehyde which was trapped as its dimedon derivative (2) and analyzed for ^3H by liquid scintillation spectrometry.

RESULTS

Mature kernels of wheat from plants that had been labeled with [2-^3H]MI or [*R*-^3H]SI (18) were used in this study. In these kernels, 70% or more of the ^3H was deposited in the outer (bran) layers. With [2-^3H]MI as source of label, ^3H accumulated primarily in phytate and polysaccharide (uronosyl and pentosyl units) accompanied by lesser amounts of MI-Gal and free MI. A comparison of ^3H distribution in whole, ungerminated grain with that in the bran fraction is given in Table I. Virtually none of the ^3H present in the kernels was lost during surface sterilization and the subsequent rinse with water preliminary to germination.

Distribution of ^3H in [2-^3H]MI-Labeled Wheat Kernels during Initial Stages of Germination. In the first 24 h following imbibition, there was a sharp decrease in labeled MI-Gal (Fig. 1). To examine this change more closely, a short term experiment was run with kernel samples taken 0, 5, 10, and 20 h after imbibition. The kernels were divided into embryo (with scutellum) and residual kernel. A few kernel parts from each sample were combusted in a biological oxidizer. The ratio of ^3H present in embryo and residual kernel of ungerminated grain was 1:20 and this ratio remained unchanged throughout the 20-h experiment. Samples were extracted with 80% ethyl alcohol to obtain the distribution of ^3H between extract and residue as given in Table II. Of the ^3H present in the embryo, 74% was soluble in 80% ethyl alcohol. It was exclusively MI-Gal and free MI in the ratio 5:1. There was no significant change in these results for 10 h following imbibition but at 20 h, only 61% ^3H was extracted, largely as free MI. The increase in insoluble labeled material probably corresponded to an increase in cell wall polysaccharide biosynthesis. During the same 20-h period, changes in distribution of ^3H within the residual kernel were less pronounced. Only 10% ^3H appeared as MI-Gal and MI. During the period 24 to 72 h after imbibition, the ^3H content of water-soluble constituents reached a maximum (Table I, sum of water-soluble phytate and polysaccharides) and began the decline that continued to the end of the 13-day study. At 72 h, only 3% water-soluble ^3H was phytate as compared with 15% in the same extract from ungerminated kernels (Table I), suggesting

Table I. *Distribution of ^3H in Ungerminated Kernels, Bran Fraction, and Germinated Kernels from [2-^3H]MI-Labeled Wheat (cv. Twin)*

Embryo and scutellum were removed from germinated kernels.

Fraction	Ungerminated Kernel	Bran Fraction	Germinated Kernel (h after imbibition)		
			24	48	72
	% total ^3H in grain or fraction				
myo-Inositol	8	5	8	8	13
Galactinol	13	7	2	2	1
Phytate					
Water extract	15	19	12	16	3
Bound[a]	20	36	29	20	17
Polysaccharide					
Water extract	18	7	17	21	34
Insoluble residue	18	16	21	23	23
Other	8	10	11	10	9

[a] Sum of radioactivity in phytate from EDTA extract and supernatant from amyloglucosidase-treated residue.

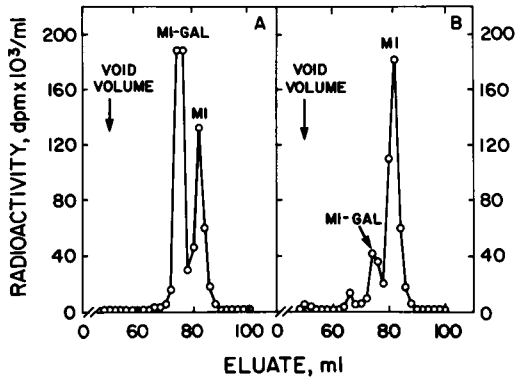

FIG. 1. Sephadex G-15 gel filtration of 80% ethanol-soluble fraction from wheat kernels. A, Ungerminated whole kernels; B, residual kernels 1 day after imbibition.

Table II. *Distribution of ^3H in Embryo and Residual Kernel of [2-^3H]MI-Labeled Wheat in Initial Stages of Germination*

Kernel Part	Germination	80% Ethyl Alcohol-Soluble Fraction			80% Ethyl Alcohol-Insoluble Fraction
		MI	Galactinol	Other	
	h	*% total ^3H in embryo or kernel*			
Embryo (scutellum attached)	0	12	58	4	26
	5	11	54	5	30
	10	13	57	5	25
	20	57	2	2	39
Residual grain	0	4	6	1	89
	5	5	5	1	89
	10	5	5	1	89
	20	7	3	1	89

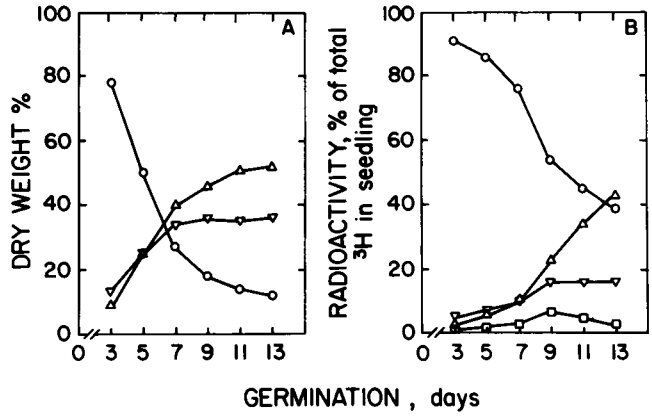

FIG. 2. Changes in the dry weight and the distribution of radioactivity in germinating wheat. A, Dry weight; B, radioactivity; (○——○), residual kernel; (▽——▽), roots; (△——△), shoot; (□——□), radioactivity recovered from the moist filter paper support.

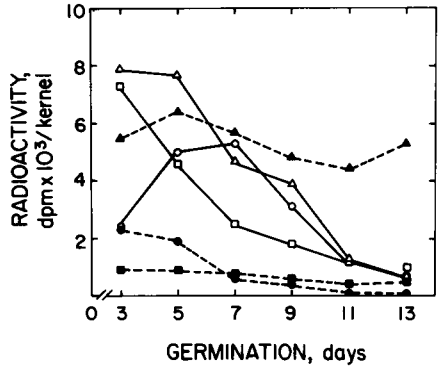

FIG. 3. Changes in the distribution of radioactivity in the residual kernel. (○——○), 80% ethyl alcohol-soluble fraction; (●- - -●), EDTA-soluble fraction; (△——△), water-soluble fraction; (▲- - -▲), trifluoroacetic acid-soluble fraction; (□——□), amyloglucosidase-soluble fraction; (■- - -■), trifluoroacetic acid-insoluble fraction.

that the greatest portion of this ^3H was present as water-soluble labeled polysaccharide.

Changes in Distribution of ^3H among Constituents of [2-^3H]MI-Labeled Kernels (Residual) during Period 3 to 13 Days after Germination. Three days after imbibition, root and shoot portions of the seedling were sufficiently developed to provide material for dry weight comparisons with the residual kernel (Fig. 2A). At this stage of germination, mobilization of reserves within the kernel and subsequent transfer of this material to the seedling were well underway. The dry weight of the residual kernel continued to decrease almost linearly to the 7th day while the dry weights of roots and shoot increased. Beyond that period, increase in dry weight was limited to shoot tissue. Unlike the dry weight changes, movement of ^3H from kernel to seedling developed slowly in the 7 days following imbibition (Fig. 2B). Thereafter, rapid movement of ^3H, especially to shoot tissue, resulted in accumulation of 60% label in the seedling at 13 days.

Changes in appearance of ^3H in extracted fractions of the residual kernel in the 3- to 13-day period following germination are plotted in Figure 3. There was a notable increase in 80% ethyl alcohol-soluble label during the first 7 days, primarily free MI. Only traces of MI-Gal were present in this fraction beyond the 3rd day. Glucose, sucrose, and raffinose, all unlabeled, were also present in this extract.

At least 43% labeled phytate in ungerminated kernels was extractable with water (Table I). This was rapidly hydrolyzed during the first 3 days. Small amounts of MI mono-, bis-, tris-, tetrakis-, and pentakisphosphate were also found in ungerminated and in 1- and 2-day germinated kernels (data not given). Water-extractable ^3H in residual kernels on the 3rd day gave two radioactive peaks when eluted from a Sephadex G-50 column (Fig. 4A). Further resolution of the retarded peak on a Sephadex G-15 column produced three radioactive components with mobilities similar to MI, MI monophosphate, and tentatively, MI bisphosphate (Fig. 4B). Only a trace of ^3H remained in the void volume region where phytate normally appears. The water-soluble polysaccharides eluted in the void volume of the G-50 column contained xylose (43%), arabinose (42%), glucose (10%), and galactose (5%). Only xylose and arabinose contained ^3H.

Only a portion of the phytate in the water-insoluble residue was extracted with 0.2% Na_2EDTA (Fig. 3), but heating followed by treatment with amyloglucosidase solubilized all remaining phytate. Most of the ^3H present in these two extracts was phytate as identified by gel filtration and paper electrophoresis.

Acid hydrolysis of amyloglucosidase-resistant residue released 80 to 90% of the remaining ^3H (Fig. 3). The labeled products were not analyzed but previous studies (8, 13, 18) have shown that uronic acids and pentoses are the principal labeled products.

Appearance of ^3H in Developing Seedlings of Labeled Kernels from [2-^3H]MI-Labeled Wheat. As noted earlier, about 5% ^3H in ungerminated kernels was found in the embryo and scutellum, primarily as MI-Gal and MI. After imbibition, most of the MI-Gal was hydrolyzed leaving labeled MI as the major source of ^3H

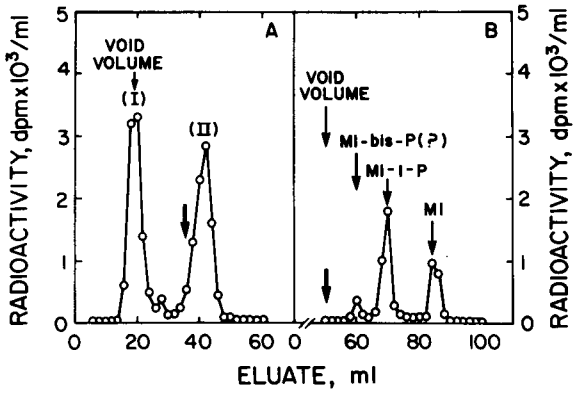

FIG. 4. Gel filtration of the water-soluble fraction from 3-day-old residual kernel. A, Sephadex G-50 gel filtration of water-soluble fraction; B, Sephadex G-15 gel filtration of peak II in Figure 4A; ↓, retention volume in which phytic acid would be detected.

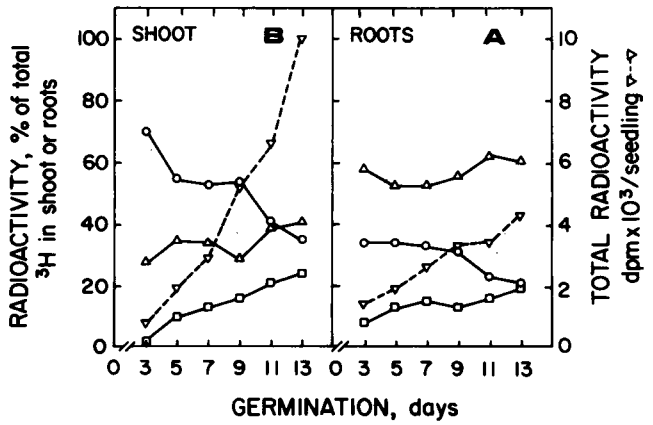

FIG. 5. Changes in the distribution of radioactivity in root (A) and shoot (B) fractions during germination. (○——○), 80% ethyl alcohol-soluble fraction; (△——△), trifluoroacetic acid-soluble fraction; (□——□), trifluoroacetic acid-insoluble fraction; (▽- - -▽), total radioactivity per seedling.

during early stages of radicle emergence. Additional MI from MI-Gal and free MI pools in the residual kernel supplemented this flow into rapidly growing roots, later augmented by MI from breakdown of water-soluble phytate. After 3 days, labeled polysaccharidic material in the kernel was available as a carbon source for seedling development. Over 60% ^3H in root tissue was ethyl alcohol-insoluble, principally cell wall polysaccharide (Fig. 5A). While the dry weight of root tissue did not increase after 7 days (Fig. 2A), accumulation of ^3H in cell wall polysaccharides continue to the end of the study, ultimately accounting for 80% radiolabel in that tissue.

Shoot tissue, in contrast to root tissue, accumulated more free MI during seedling development (80% ethyl alcohol-soluble fraction, Fig. 5B). Incorporation of ^3H into cell wall polysaccharides, as judged by presence of label in ethyl alcohol-insoluble material (acid-soluble and -insoluble fractions, Fig. 5B), proceeded slowly, reaching a level comparable to that of free MI only after 11 days.

Position of ^3H in Cell Wall-Derived Arabinose. A portion of root tissue from 3-day-old seedlings was hydrolyzed in 1 N H_2SO_4 and the sugar residues were separated by HPLC. Most of the ^3H was present in xylose and arabinose. Oxidation of the latter to arabinate followed by oxidative degradation with periodate released C-5 as formaldehyde which was recovered as its dimedon derivative. Of the ^3H present in arabinose, none was lost during oxidation to arabinate and 93% ^3H in arabinate was recovered in the dimedon derivative corresponding to C-5.

Distribution of ^3H in Germinated Kernels from [*R*-^3H]SI-Labeled Wheat. Wheat labeled with [*R*-^3H]SI in the post-anthesis stages of grain development sequestered 80% of the injected ^3H in outer (bran) layers (18). Free SI and SI-Gal were the only labeled constituents. Neither phytate nor cell wall polysaccharides were labeled. Upon germination (Fig. 6), ^3H moved from the kernel to the developing seedling, both roots and shoot, notably the latter after the 3rd day. Virtually all ^3H in 7-day-old seedlings (shoot, roots, and residual kernel) was soluble in 80% ethyl alcohol. Free SI was the only labeled constituent of this extract.

To trace the fate of SI-Gal during initial stages of germination, an experiment similar to that described earlier (Table II) was performed with kernels from [*R*-^3H]SI-labeled wheat. Results are given in Table III. In ungerminated kernels, all ^3H in the embryo was soluble in 80% ethyl alcohol, equally distributed between free SI and SI-Gal. This distribution of label remained unchanged for 10 h following imbibition but at 20 h, two-thirds of the ^3H previously present in SI-Gal was recovered as free SI. The residual kernel had a distribution similar to that in the embryo but extraction with 80% ethyl alcohol released only 83% total label. Within 5 h of imbibition, the amount of ^3H present as SI-Gal began to decrease but the rate of that decrease was less than that noted in the embryo at 10 to 20 h. The nature of the labeled material remaining in alcohol-insoluble residues was not investigated.

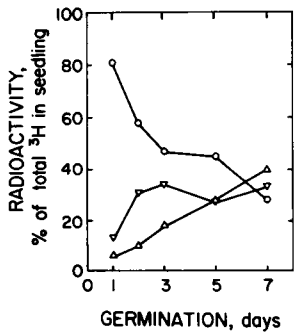

FIG. 6. Changes in the distribution of radioactivity in germinating wheat labeled with [*R*-^3H]SI. (○——○), residual kernel; (△——△), shoot; (▽——▽), root.

Table III. *Distribution of ^3H in Embryo and Residual Kernel of [R-^3H]-SI-Labeled Wheat in Initial Stages of Germination*

Kernel Part	Germination	SI	SI-galactoside
	h	*% total ^3H in embryo or kernel*	
Embryo[a] (scutellum attached)	0	50	50
	5	49	51
	10	50	50
	20	82	18
Residual kernel[b]	0	40	43
	5	45	38
	10	47	36
	20	55	28

[a] One hundred % ^3H in embryo was extracted with 80% ethyl alcohol.
[b] Eighty-three% ^3H in this fraction was extracted with 80% ethyl alcohol.

DISCUSSION

An earlier paper (13) examined the utilization of [2-^3H]MI by young wheat seedlings in two ways, uptake by imbibition, and injection into partially digested endosperm of 72-h seedlings. In 96-h seedlings, labeled by either method, 70% ^3H was recovered in cell wall polysaccharides. Clearly, the MI oxidation pathway was operative in this tissue. 2-*O*,*C*-Methylene-MI, a known antagonist of MI oxidation, inhibited utilization of MI. Uptake of [2-^3H]MI by imbibition was used to trace the utilization of free MI during early stages of germination preceding hydrolysis of phytate reserves. Injection of 72-h seedlings with [2-^3H]MI was used to trace MI utilization during subsequent stages of germination in which hydrolysis of phytate was a major source of MI for the new seedling. Results suggested that reserves of MI within the dormant kernel function as a carbon source for uronosyl and pentosyl units of cell wall polysaccharides during germination.

More recently, we turned to the method of Sakri and Shannon (17) in an attempt to obtain mature grain containing [2-^3H]MI-labeled phytate (18). This method was largely successful since translocation of label through the vascular system was selectively channeled into the aleurone (bran) layer in the kernels. [2-^3H]MI was phosphorylated and deposited as phytate. It also accumulated as MI-Gal and free MI in these outer layers. One limitation was encountered. During injection of [2-^3H]MI into the peduncle of the head of wheat, considerable MI was oxidized to D-glucuronate and then utilized for cell wall biosynthesis, not only in the region of the puncture but in the grain itself. Whether this oxidation occurred only in the region of injection or also in the developing grain was not determined. In any case, it resulted in labeling of cell wall material within the grain, especially the outer layers (bran fraction). Since polysaccharide-bound uronic acids and pentoses are major metabolic products of D-glucuronate, arabinoxylan, the principal cell wall polysaccharide of endosperm and the aleurone layer, was heavily labeled.

When [*R*-^3H]SI rather than [2-^3H]MI was injected, none of the labeled SI was utilized by developing kernels for phytate or polysaccharide biosynthesis although 50% SI was glycosylated to form SI-Gal. Both SI-Gal and free SI accumulated in the outer layers of the kernel and jointly accounted for 80% injected dose. Thus, [*R*-^3H]SI offered a convenient method in which to mimic MI transport yet circumvent oxidation of the cyclitol.

Changes in Labeling Pattern during Germination. The first notable change was hydrolysis of MI-Gal (or SI-Gal). The labeled inositol from this hydrolysis augmented reserves of free inositol. This change was most apparent in the embryo and it occurred within 1 day after imbibition.

Phytate was found in at least two physical states, one readily

extracted by water and a second solubilized only after treatment with EDTA and a heat step followed by treatment with amyloglucosidase. The former was rapidly hydrolyzed, almost completely within 3 days, but the latter followed a much slower course that continued to release MI over an extended period. The combined effect of the hydrolysis of MI-Gal and phytate, particularly the water-soluble form of phytate, was an accumulation of free MI in the residual kernel, and to a lesser degree in the shoot, during the first few days of germination. This was followed by a decrease in free MI as reserves diminished and as MI in the shoot was oxidized.

About one-third of the ^3H in the kernels was found in pentosyl units of cell wall polysaccharides, 50% of which was water-soluble. During germination, most of this carbohydrate was broken down and translocated to growing parts. The trend toward mobilization of labeled pentose-containing polysaccharides and translocation of breakdown products was well underway on the 3rd day. Aleurone and endosperm cell walls of cereal grains are rich sources of arabinoxylan (14, 15), and the breakdown of this polysaccharide is a gibberellic acid-induced process triggered early in germination (4, 19).

Additional information regarding the pattern of release of inositol to growing parts was obtained from grain of plants labeled with [*R*-^3H]SI. In this study, the only labeled constituents in the kernel were SI-Gal and free SI. SI-Gal was rapidly hydrolyzed in an early stage of germination leaving free SI as the sole ^3H-containing constituent for translocation to the seedling. Within 5 days, 60% SI moved into the seedling, particularly the shoot. In this respect, free SI and MI followed a common path, differing only in the failure of SI to act as substrate for the MI oxidation pathway once it reached the growing parts. Since neither phytate nor polysaccharides were labeled by [*R*-^3H]SI, prolonged translocation of ^3H-labeled products associated with hydrolysis of these reserves was absent.

Concerning Pentose Requirements of Cell Wall Formation in Seedling. Several potential sources for filling the pentose requirements of cell wall formation in the seedling can be identified in ungerminated grain. These include free MI, MI-Gal, phytate (and lower MI polyphosphates), breakdown products of arabinoxylan and other pentose-containing polysaccharides, and sugar phosphate metabolism (starch and sugar reserves).

Although direct evidence is still lacking, it appears that free MI is the form in which MI diffuses to the scutellum and ultimately enters the seedling. MI-containing compounds such as MI-Gal and phytate are hydrolyzed to free MI prior to translocation. Subsequent conversion of MI to UDP-pentose and incorporation of pentosyl residues into cell wall polysaccharides completes the process. The biosynthetic processes leading to MI-Gal and to phytate do not affect the position or attachment of ^3H in [2-^3H]MI when this form of radiolabel is used. Similarly, hydrolysis of MI-Gal and phytate leaves this radiolabel intact (6, 13, 18). Since the position of labeling in [2-^3H]MI is unaffected prior to its entry into the MI oxidation pathway, the location of ^3H in pentose will be determined by the stereochemistry of oxidative cleavage of MI and subsequent decarboxylation of UDP-glucuronate (7). In the absence of MI-linked gluconeogenesis, pentose will be labeled at C-5 (7, 10).

That portion of injected [2-^3H]MI that is oxidized to D-[5-^3H]-glucuronate in developing grain will ultimately appear as labeled uronosyl and pentosyl units of arabinoxylan and other acidic and pentose-containing polysaccharides (18). The pentosyl residues will be labeled with ^3H on C-5 as described earlier. Hydrolytic release during germination followed by translocation to growing parts will provide the young plant with L-[5-^3H]arabinose for new cell wall biogenesis. Carbon-5-bound ^3H remains undisturbed during incorporation of arabinose into polysaccharide (16). D-[5-^3H]Xylose, which undoubtedly accompanies labeled arabinose, lacks a direct route of incorporation into cell wall polysaccharides and is recycled through hexose phosphate, losing in the process most of its ^3H by exchange and by dilution with unlabeled carbohydrate (7, 16).

Finally, consideration must be given to the contribution made by hexose phosphate metabolism when reserves are mobilized for biosynthesis of pentose-containing polysaccharides in the seedling. Kernels from plants injected with [2-^3H]MI during the post-anthesis stage accumulate 5% or less of the total label in the endosperm (flour fraction) (18). Although no attempt was made to analyze this fraction, one may assume from the studies of Mares and Stone (14) that arabinoxylan in the endospermic cell walls, not starch, is the site of labeling. Major hexose-rich polysaccharides such as starch were unlabeled, an indication that MI-linked gluconeogenesis played an insignificant role in MI metabolism during kernel development. Therefore, a contribution of label from hexose phosphate to pentose biosynthesis will be minimal whether it involves the nucleotide sugar oxidation pathway, or MI-1-phosphate synthase and the MI oxidation pathway.

To summarize, sources of labeled pentose for the synthesis of pentose-labeled polysaccharides in the seedling of germinated kernels from [2-^3H]MI-labeled wheat are limited to free MI, MI-Gal, phytate (and lower MI polyphosphates), and the arabinose that is released during cell wall breakdown in the residual kernel. Each source will supply pentose precursor ^3H-labeled at C-5 exclusively. Results support this. Arabinose from root polysaccharides of 3-day seedlings had 93% its ^3H attached to C-5. The choice of 3-day seedlings has added significance. At this stage of development, most of the label coming from the kernel originates in free MI, MI-Gal, or water-soluble phytate. The contribution from pentose-rich polysaccharides only becomes significant about 5 to 7 days after germination. Since phytate is a major source of ^3H at the 3-day stage, results indicate that the MI oxidation pathway is the principal route of conversion for phytate-derived MI to pentose in the seedling cell walls.

De and Biswas (5) have proposed an alternate pathway for breakdown of phytate in germinating mung bean. Their scheme selects MI-1-phosphate as the sole product of phytate breakdown and they assign to a novel enzyme, MI 1-phosphate dehydrogenase, the role of oxidizing MI 1-phosphate to 3-keto-D-gluconate-6-P, which in turn generates hexose via the pentose phosphate pathway. According to their scheme, phytate containing [2-^3H]MI would be converted to 3-keto-D-[5-^3H]gluconate-6-P. De and Biswas (5) suggest an enzymic or spontaneous decarboxylation of this product to ribulose-5-P (^3H would be attached to C-4). Normally, one regards ribulose-5-P as an intermediate in hexose phosphate formation, not a direct precursor of pentosyl units for cell wall formation. If one assumes that the principal pathway from ribulose-5-P to pentosyl units of the cell wall proceeds through hexose phosphate, one expects to find the entire pool of hexose-related products labeled by this process. Further, redistribution of ^3H between opposite halves of hexose due to triose-P metabolism would be considerable. That portion of the hexose phosphate converted to cell wall via MI 1-phosphate synthase and the MI oxidation pathway encounters a synthase-linked hydrogen isotope effect (12) that further diminishes the amount of ^3H reaching pentose.

The results found in our study, which reveal a very specific labeling of pentose and a selective appearance of ^3H in pentose residues as opposed to the general labeling of all sugar residues including hexosyl residues, are best interpreted by the simpler scheme that assigns a direct link between phytate-derived MI and the MI oxidation pathway rather than the proposed cycle of De and Biswas (5). Possible differences in phytate metabolism in wheat and mung bean are not excluded.

LITERATURE CITED

1. ALBERSHEIM, P, DJ NEVINS, PD ENGLISH, A KARR 1967 A method for the analysis of sugars in plant cell wall polysaccharides by gas-liquid chromatography. Carbohydr Res 5:340–345
2. BLOOM, B 1962 The simultaneous determination of ^{14}C and ^{3}H in the terminal groups of glucose. Anal Biochem 3: 85–87
3. COSGROVE, DJ 1980 Inositol Phosphates. Their Chemistry, Biochemistry and Physiology. Elsevier, Amsterdam
4. DASHEK, WV, MJ CHRISPEELS 1977 Gibberellic acid-induced synthesis and release of cell-wall-degrading exoxylanase by isolated aleurone layers of barley. Planta 134: 251–356
5. DE, BP, BB BISWAS 1979 Evidence for the existence of a novel enzyme system. *Myo*-inositol-1-phosphate dehydrogenase in *Phaesolus aureus*. J Biol Chem 254: 8717–8719
6. FRYDMAN, RB, EF NEUFELD 1963 Synthesis of galactosylinositol by extracts from peas. Biochem Biophys Res Commun 12: 121–125
7. LOEWUS, FA 1964 Inositol metabolism in plants. II. The absolute configuration of D-xylose-5-t, derived metabolically from *myo*-inositol-2-t in the ripening strawberry. Arch Biochem Biophys 105: 590–598
8. LOEWUS, FA 1965 Inositol formation and cell wall formation in plants. Fed Proc Fed Am Soc Exp Biol 24: 855–862
9. LOEWUS, FA, DB DICKINSON 1982 Cyclitols. *In* FA Loewus, W Tanner, eds, Encyclopedia of Plant Physiology, New Series, Vol 13A. Springer Verlag, Berlin. In press
10. LOEWUS, FA, S KELLY 1963 Inositol metabolism in plants. I. Labeling patterns in cell wall polysaccharides from detached plants given *myo*-inositol-2-t or -2-^{14}C. Arch Biochem Biophys 102: 96–105
11. LOEWUS, FA, MW LOEWUS 1980 *myo*-Inositol: biosynthesis and metabolism. *In* PK Stumpf, EE Conn, eds, The Biochemistry of Plants, Vol 3. Academic Press, New York, pp 43–76
12. LOEWUS, MW, FA LOEWUS 1980 The C-5 hydrogen isotope-effect in *myo*-inositol 1-phosphate synthase as evidence for the *myo*-inositol oxidation pathway. Carbohydr Res 82: 333–342
13. MAITI, IB, FA LOEWUS 1978 *Myo*-inositol metabolism in germinating wheat. Planta 142: 55–60
14. MARES, DJ, BA STONE 1973 Studies on wheat endosperm. I. Chemical composition and ultrastructure of cell walls. Aust J Biol Sci 26: 793–812
15. MCNEIL M, P ALBERSHEIM, L TAIZ, RL JONES 1975 The structure of cell walls. VII. Barley aleurone cells. Plant Physiol 55: 64–68
16. ROSENFIELD, C-L, FA LOEWUS 1978 Metabolic studies on intermediates in the *myo*-inositol oxidation pathway in *Lilium longiflorum* pollen. II. Evidence for the participation of uridine diphosphoxylose and free xylose as intermediates. Plant Physiol 61: 96–100
17. SAKRI, FAK, JC SHANNON 1975 Movement of ^{14}C-labeled sugars into kernels of wheat (*Triticum aestivum* L.). Plant Physiol 55: 881–889
18. SASAKI, K, FA LOEWUS 1980 Metabolism of *myo*-[2-^{3}H]inositol and *scyllo*-[*R*-^{3}H]inositol in ripening wheat kernels. Plant Physiol 66: 740–745
19. TAIZ, L, WL HONIGMAN 1976 Production of cell wall hydrolyzing enzymes by barley aleurone layers in response to gibberellic acid. Plant Physiol 58: 380–386
20. WILSON, AM, GA HARRIS 1966 Hexose-, inositol-, and nucleoside phosphate esters in germinating seeds of crested wheat grass. Plant Physiol 41: 1416–1419