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Incorporation of UDPGlucose into Cell Wall Glucans and Lipids by Intact Cotton Fibers[1]

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ABSTRACT

The [^{14}C] moiety from [^{3}H]UDP[^{14}C]glucose was incorporated by intact cotton fibers into hot water soluble, acetic-nitric reagent soluble and insoluble components, and chloroform-methanol soluble lipids; the [^{3}H] UDP moiety was not incorporated. The ^{3}H-label can be exchanged rapidly with unlabeled substrate in a chase experiment. The cell wall apparent free space of cotton fibers was in the order of 30 picomoles per milligram of dry fibers; 25 picomoles per milligram easily exchanged and about 5 picomoles per milligram more tightly adsorbed. At 50 micromolar UDPglucose, 70% of the [^{14}C]glucose was found in the lipid fraction after both a short labeling period and chase. The percent of [^{14}C]glucose incorporated into total glucan increased slightly with chase, but the fraction of total glucans incorporated into insoluble acetic-nitric reagent (cellulose) did increase within a 30-minute chase period. The data supports the concept that glucan synthesis, including cellulose, as well as the synthesis of steryl glucosides, acetylated steryl glucosides, and glucosyl-phosphoryl-polyprenol from externally supplied UDPglucose occurs at the plasma membrane-cell wall interface. The synthase enzymes for such synthesis must be part of this interfacial membrane system.

There are a number of concepts about the process of cell wall glucan biosynthesis that are generally accepted. Recent reviews discuss the processes and supporting data leading to the *in vivo* cellular events of β-1,4- and β-1,3-linked glucan synthesis, cellulose microfibril production, formation of neutral and polar glycolipids, pectic and hemi-cellulosic matric polysaccharides (5–7, 13, 20, 22, 23, 27). Several of these general concepts about cell wall synthesis that are pertinent to this report are: (a) UDPglucose is considered the precursor for wall glucan biosynthesis or is a compound on the pathway of synthesis (4). (b) Cellulose synthesis in higher plants probably occurs at the plasma membrane or plasma membrane-cell wall interface with the substrate produced on the the cytoplasmic side and cellulose on the outside (28). Conceivably, cellulose synthase is located in the plasma membrane and/or adjacent cell wall. (c) Golgi apparatus and ER are involved in the synthesis of matrix polysaccharides and the transport of these materials and synthase enzymes to the plasma membrane (22, 23). (d) Preparations of membrane fractions through density gradient centrifugation and subsequent assay for glucan synthesis has led to the concept that there exist at least two glucan synthases in plants. Glucan synthase I, an enzyme associated with Golgi cisternae, has a high affinity for UDPglucose and a Mg^{2+} requirement; the product has been reported to be β-1,4-glucan. Glucan synthase II isolated with the plasma membrane vesicles has a low affinity for UDPglucose, no requirement for Mg^{2+}, and the glucan produced has predominately β-1,3 linkage (20, 26, 27). (e) Particulate fractions from plants will incorporate UDP glucose into steryl glucosides, acetylated glucosides, and glucosyl-phosphoryl-polyprenol (11, 12, 17–19, 24). However, there is no unequivocal evidence for the involvement of lipid-linked intermediates in glucan synthesis (7). (f) Intact tissue or unaltered cell membranes are impermeable to UDPglucose and will not incorporate this externally supplied substrate into β-1,4-linked glucans (3, 4, 6, 29). (g) Although a number of reports indicate that plant tissue slices, particulate fractions, or detached cotton fibers will synthesize hot-alkali insoluble products from UDP[^{14}C]glucose; a better criterion for estimating the incorporation of this substrate into cellulose is to hydrolyze the noncrystalline glucans with acetic-nitric reagent at 100°C for 30 to 60 min (3, 30). This reagent will, however, solubilize some β-1,4-glucans (7, 9). In all cases, the linkage of the synthesized glucans need to be confirmed by methylation analysis (3, 4, 7, 16).

In this report, we submit evidence supporting the concept that intact cotton fibers are not permeable to UDPglucose. However, they will incorporate the glucose moiety into acetic-nitric reagent soluble and insoluble products; the insoluble product has β-1,4 linkage. For the most part, the acetic-nitric soluble products are β-1,3-linked polymeric material. These intact fibers also rapidly synthesize neutral and polar glucolipids. This work indicates that the plasma membrane-cell wall interfacial region of *in vitro*-grown cotton fibers are fully capable of utilizing externally-supplied UDPglucose and to partially mimic the synthetic processes observed when labeled substrates, that will penetrate the plasma membrane, are used to investigate cell wall glucan synthesis by intact tissue (3, 10, 25).

MATERIALS AND METHODS

Cotton Ovule Culture. Unfertilized cotton ovules (*Gossypium hirsutum* L., Acala SJ1) were cultured as previously described (1). Fourteen d after the start of culture, the ovules from several flasks were washed with the culture medium, less glucose, adjusted to pH 7.0; 20 ovules were transferred to vials containing 19 ml of medium, and preincubated as previously described (9). After preincubation, 1 ml of a UDPglucose solution in medium containing 1 μCi of UDP[^{14}C]glucose or [^{3}H]UDP[^{14}C]glucose was added; for the dual-labeled compound, the ratio of ^{3}H to ^{14}C was 7.5. With the concentration of UDP[^{14}C]glucose *versus* rate of incorporation experiment, sufficient UDPglucose was added to obtain the desired level. In the dual labeling experiments, the final concentration of UDPglucose was 50 μM. After adding the UDPglucose, the procedures for radioassay of medium and incubation were those previously reported (9, 10). To compare the incorporation rates of UDP[^{14}C]glucose and [^{14}C]glucose into fibers for short periods, 1 mM levels of substrates were used.

At various times after adding the labeled substrate, the ovules from duplicate tubes were removed, washed, and blotted dry (9).

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The fibers from five ovules were removed to a small test tube, 2 ml water added, and boiled for 10 min. The fibers were removed, frozen, and lyophilized; the hot water extract was frozen and stored for combining with additional hot water extracts obtained at the time of assay. By using the fibers from five ovules per assayed sample, there were eight replications for each incubation period.

When ovules were labeled with [^{3}H]UDP[^{14}C]glucose and chased with unlabeled UDPglucose, the following procedures were followed: (a) the ovules were washed two times with medium (pH 7.0), 34°C; (b) 20 ml of the same medium containing 50 μM unlabled UDP-glucose added; (c) the tubes were returned to the water-bath shaker. After the chase period, the ovules were handled as described above.

Assay of Fibers. At the time of assay, the fibers were extracted: (a) two more times with water at 100°C and the three hot water soluble extracts combined; (b) with two, 2 ml of chloroform-methanol, once with 2:1 mix then with 1:2 mix and combined. The chloroform-methanol extracts were added to counting vials, dried, and Betaphase liquid scintillation solution (Westchem, San Diego, CA) was added; (c) with acetic-nitric reagent (9); (d) the residue was combusted, as previously reported (9). Radioactivity was determined by an LS 3801 Beckman system.

UDP[^{14}C]glucose and [^{3}H]UDP[^{14}C]glucose were purchased from ICN, Anaheim, CA.

Methylation Analyses. Cotton fibers extracted with hot water and chloroform-methanol, and the fiber residue after extracting with acetic-nitric reagent were subjected to permethylation as described by Harris *et al.* (16) and the permethylated alditol acetates separated on a WCOT glass-capillary column (0.5 mm × 25 m, Silar 10C, 0.8 μm film, Altech Associates). The oven temperature was programmed at 2°/min from 160 to 255°C. Helium carrier gas flow was 9.5 ml/min, with makeup gas to 35 ml/min at the detector. A Varian model 2740 GC, equipped with a flame ionization detector and 1:10 stream splitter was used for all analyses. Injector and detector were at 270°C. Peak areas were integrated by an IBM 9000 computer. An HP5790A GC, coupled to a VG ZAB high-resolution mass spectrometer was used for all GC-MS analyses, with partially per-*O*-methylated alditol acetates and per-*O*-acetyled alditol sugars separated on a CPS 2 capillary column (0.25 mm × 15 m, Quadrex).

Separation and Analyses of Labeled Lipid Components. The chloroform-methanol soluble components from labeling cotton ovules were evaporated under N_2, made to 0.5 ml with chloroform, and stored at −20°C in sealed vials until analyzed. The sample was loaded onto a flurosil Sep-Pak previously washed with 5 ml of $CHCl_3$. Five ml of $CHCl_3$ followed by 5 ml of $CHCl_3$:$CH_3COOC_2H_5$ (6:4) were passed through the Sep-Pak, this was followed by 10 ml of methanol which removed between 90 to 97% of the radioactivity. The eluant was evaporated to a small volume and transferred to a silica G TLC plate and subjected to two-dimensional chromatography (24). Radioactive areas were identified by audioradiography and lipids by iodine. The radioactive areas were removed for radioactivity determination.

One-dimensional TLC with solvent IV from Forsee *et al.* (12) was also conducted on samples not subjected to prior separation on the flurosil Sep-Pak. Lipids were identified with iodine, and radioactivity was determined on 1-cm sections scraped from the TLC plates.

A modified column method was used to separate steryl glucoside, acetylate steryl glucosides, and glucosyl-phosphoryl-polyprenols from cotton fibers labeled with UDP[^{14}C]glucose (11, 24). Lipid extracts in $CHCl_3$ were placed on $CHCl_3$ washed flurosil Sep-Pak, and the column eluted sequentially with aliquots of increasing concentration of CH_3OH in $CHCl_3$; after the 10% CH_3OH in $CHCl_3$, the column was eluted with 1% CH_3COONH_4 in CH_3OH. Peaks of radioactivity were collected and subjected to one-dimensional TLC; with peaks 1 and 2 of Figure 7, solvent IV of Forsee *et al.* (12) used; solvent A of Forsee and Elbein (11) was used to rechromatogram peak 3. Steryl glucoside and acetylate steryl glucoside were identified by the methods reported by Ongun and Mudd (24) and glucosyl-phosphoryl-polyprenol by the methods of Forsee and Elbein (11).

RESULTS

Figure 1 shows that intact cotton fibers cultured *in vitro* for 14 d incorporated the [^{14}C]glucose moiety from UDP[^{14}C]glucose into glucans over a range of substrate concentrations. The results appear to indicate incorporation by two mechanisms dependent on UDPglucose concentration. At a lower level, the substrate incorporation had a V_{max} of 2.83 nmol/mg·h and a K_m of 132 μM UDPglucose. At the higher level, the UDPglucose had a V_{max} of 18.76 nmol/mg·h with a K_m of 1.08 mM. The data points more appropriately fit a two-sigmoid lines computer model than the single-sigmoid line model; this is consistent with reports that there exist two glucan synthases in plants that will utilize UDP-glucose as substrate.

For fibers removed from ovules after labeling with 50 μM UDP[^{14}C]glucose for 30 min and subjected to the hot water and chloroform-methanol extractions, the majority of the insoluble labeled glucans were not cellulosic (Fig. 2, A and B) with 65% of the label in the permethylated alditol acetate product from 3-linked glucose and 23% as 4-linked glucose; 6% of the label was in terminal glucose and the several cross-linked glucose fraction made up 3.5% of the label. After extracting with the acetic-nitric reagent, 72% of the insoluble ^{14}C-labeled glucans was in the 4-linked glucose, 12% in 3-linked glucose, 5% in terminal glucose, and 12% in cross-linked glucose (Fig. 2, C and D). The acetic-nitric reagent did extract the majority of labeled noncellulosic glucans. The GC trace from 3-linked glucose (Fig. 2C) shows no detectable level in the acetic-nitric insoluble fraction as compared to the trace for total glucans (Fig. 2A); however, there were a few radioactive counts eluting at the β-1,3-linked region of the GC trace (Fig. 2D). This could be caused by incomplete solubilization of the noncellulosic glucans or incomplete washing of the insoluble residue. The counts and detecting of cross-linked permethylated alditol acetate, may, in part, be due to incomplete per-

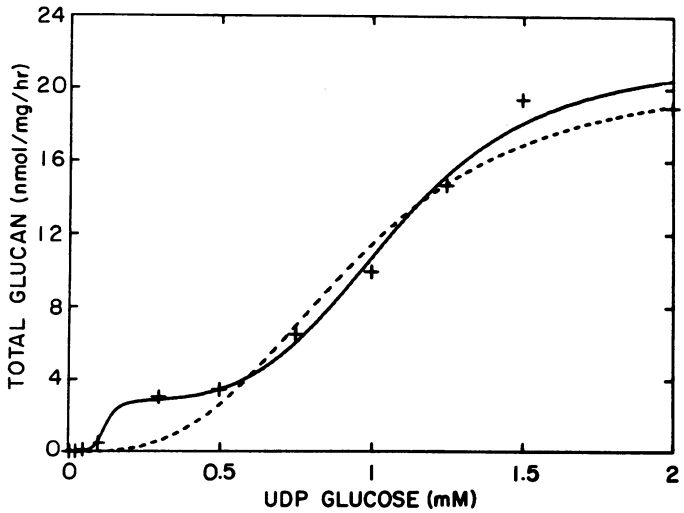

FIG. 1. Incorporation of UDPglucose into glucans by intact cotton fibers as a function of substrate concentration. Results are the fit of 1 and 2 sigmoid lines with values obtained at 11 concentrations of UDP-glucose. Number of replicatios of UDPglucose concentrations = 8 at 0.005, 0.025 μM, 1.25, 1.5 mM; 16 at 50, 100, 500, 750 μM; 32 at 1 and 2 mM. Values are average ± 2 × SE.

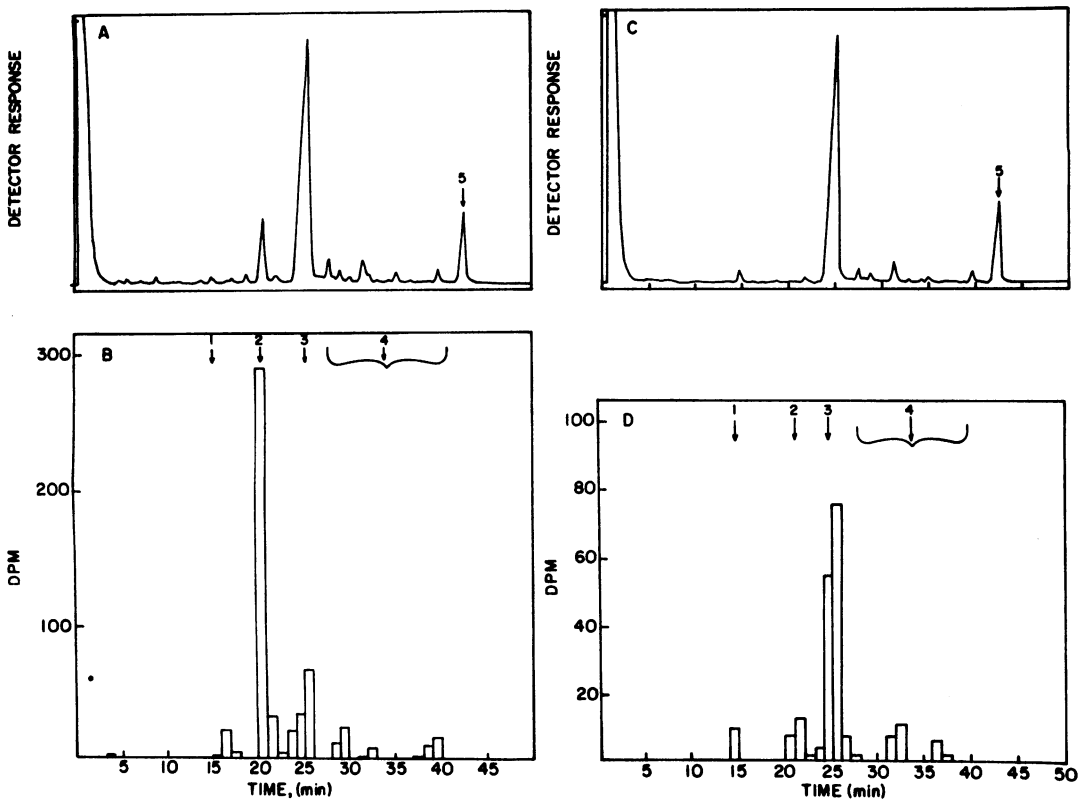

FIG. 2. Permethylated alditol acetates prepared from labeled cotton fibers were separated by GC with a flame ionization detector and a 1:10 stream splitter to collect the labeled samples. Linkage analyses were confirmed by a GC-mass spectrometer. A, B, total glucans; C, D, acetic-nitric reagent insoluble fraction. 1, terminal-linked glucose; 2, 3-linked glucose; 3, 4-linked glucose; 4, cross-linked glucose; 5, myo-inositol hexa acetate standard.

methylation of the β-1,4-linked glucan. We were satisfied that the acetic-nitric reagent insoluble fraction does approximate the cellulosic fraction from cotton fibers.

In vitro-grown cotton fibers were incubated with 50 μM [^{3}H] UDP[^{14}C]glucose for periods up to 60 min and extracted as described in the previous section; Figure 3 presents the ^{3}H- and ^{14}C-labeled products extracted with hot water. After a 10-min labeling period, there was no significant increase in the hot water extracted ^{3}H-label; there was a continued increase in the ^{14}C-labeled products for the 60 min. As the incorporation of ^{14}C into the cellulosic fraction (Fig. 4B) was nearly linear for 30 min, 20- and 30-min pulse labeling periods were used prior to chase periods of 10, 20, and 30 min. There was no significant level of ^{3}H-label present in fractions other than the hot water soluble ones after the various labeling periods and with chase there was no significant change in the distribution of the ^{3}H-label (Table I); the incorporation of the [^{14}C]glucose moiety into glucans and lipids as well as the hot water soluble fraction was substantial (Figs. 3 and 4; Table I). Although there was no increase in the labeled total glucans fraction with chase, the percent of labeled glucans that was insoluble in acetic-nitric reagent increased from 14% after a 30-min labeling to 21% after a subsequent 30-min chase (Table I).

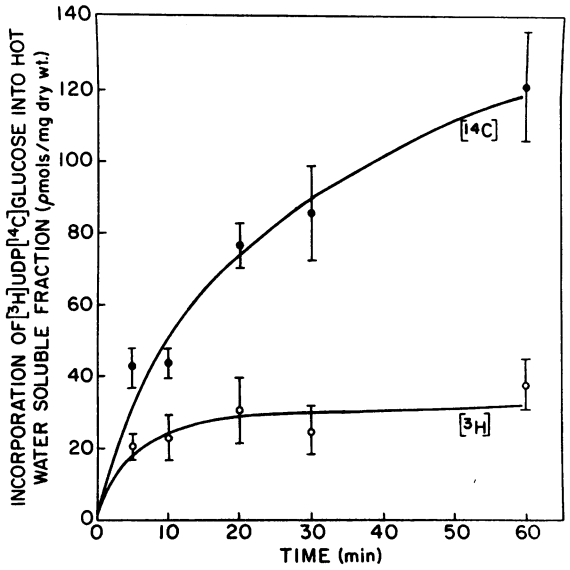

FIG. 3. Incorporation of ^{3}H and ^{14}C from 50 μM [^{3}H]UDP-[^{14}C] glucose into the hot water soluble fraction of *in vitro*-grown cotton fibers *versus* time of labeling.

The results of the chase out with UDPglucose at 10, 20, and 30 min after 20- and 30-min labeling with [^{3}H]UDP[^{14}C]glucose appears in Figure 5. The average loss of both ^{3}H- and ^{14}C-labels for the 10, 20, and 30 min chase was 26.4 ± 4 ρmol/mg dry weight of fiber with an average retention of ^{3}H after a 20 to 30 min chase of 6.5 pmol/mg dry weight. If preboiled ovules with fibers were labeled with [^{3}H]UDP[^{14}C]glucose for 30 min, then chased with UDP-glucose for 30 min the results presented in Table II were obtained. These results approximate the ^{3}H-label in the hot water soluble fraction, about 30 pmol/mg dry weight, and the more strongly adsorbed component, 5 pmol/mg, after a 20- and 30-min chase (Fig. 5). It appears that cotton fibers have the capacity to adsorb about 30 pmol of [^{3}H]UDP[^{14}C]glucose/mg dry weight with about 25 pmol/mg easily chased out by UDPglucose within 20 to 30 min and about 5 pmol/mg adsorbed more strongly and only removed by several extractions with hot

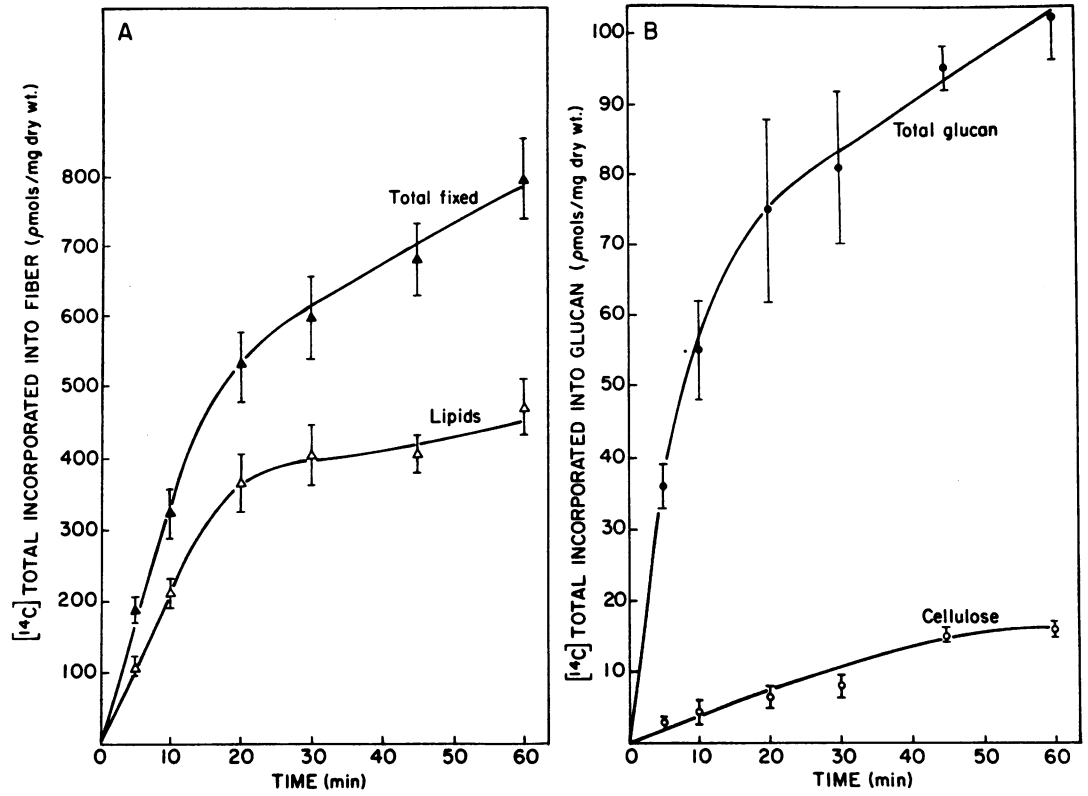

FIG. 4. Incorporation of [^{14}C]glucose from [^{3}H]UDP[^{14}C]glucose into cotton fibers grown *in vitro versus* time of labeling. A, total incorporated and into chloroform methanol soluble fraction; B, incorporation into total glucans (soluble and insoluble in acetic-nitric reagent) and cellulose (insoluble in acetic-nitric reagent).

Table I. *Incorporation of [^{3}H] and [^{14}C] into Glucan and Lipid Fractions Extracted from Cotton Fibers Labeled with [^{3}H]UDP[^{14}C] Glucose for 30 Minutes and Chased with Unlabeled Medium*

Values are average of four replications ± 2 × SE.

Time of Chase	Lipids		Total Glucans		Percent of Glucans as Cellulose
min	*pmol/mg fiber*		*pmol/mg fiber*		*%*
	[^{3}H]	[^{14}C]	[^{3}H]	[^{14}C]	[^{14}C]
0	1 ± 1	332 ± 54	0	48 ± 5	14 ± 1
10	1 ± 1	389 ± 54	0	50 ± 6	17 ± 1
20	2 ± 1	365 ± 33	0	52 ± 2	17 ± 2
30	2 ± 1	341 ± 9	0	53 ± 3	21 ± 2

water. The easily chased label represents the exchangeable cell wall apparent free space.

Was the [^{14}C]glucose moiety from [^{3}H]UDP[^{14}C]glucose incorporated into glucans at the cell wall-plasmalemma interface or was the substrate hydrolyzed at the cell surface, with the glucose moiety entering the cytoplasm, processed by the endomembrane system, and retransported to the interface? A comparison of the incorporation of UDP[^{14}C]glucose and [^{14}C]glucose under the same conditions of labeling *versus* time of incorporation into total glucans and lipids was made (Fig. 6). The incorporation of the [^{14}C]glucose moiety from UDP[^{14}C]glucose into glucans and lipids was several times more rapid than the incorporation of [^{14}C]glucose; incorporation of [^{14}C]glucose by cotton fibers was linear over a longer time than for UDP[^{14}C] glucose (10).

The majority of the [^{14}C]glucose moiety incorporated by cotton fibers from UDP[^{14}C]glucose at 50 μM appears in the chloroform-methanol soluble fraction (60–70%). The data points

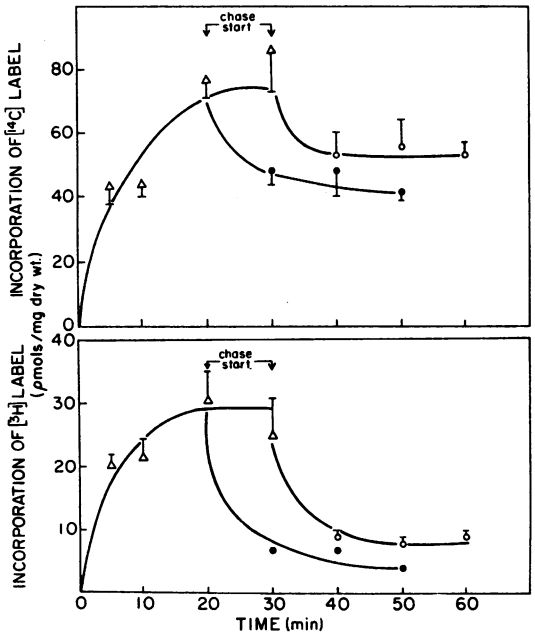

FIG. 5. Incorporation of ^{3}H and ^{14}C from [^{3}H]UDP[^{14}C]glucose into the hot water soluble fraction from *in vitro*-grown cotton fibers and the subsequent chase with unlabeled UDPglucose.

from incorporation rates into this fraction *versus* concentration of UDPglucose also fit a two-sigmoid lines model (data not reported). Figure 7 shows the separation of the major components in this fraction. The three fractions constitute 84% of the labeled lipids applied to the flurosil Sep-Pak. The techniques, as

Table II. *Incorporation of ^3H and ^{14}C Labels from 50 μM [^3H]-UDP[^{14}C]Glucose by Boiled Cotton Fibers and Subsequent Chase with 50 μM UDPglucose*

Values are average of four replications ± 2 × SE.

Treatment	[^3H]	[^{14}C]
	pmol/mg fiber	
After 30-min labeling	29 ± 5	28 ± 4
After 30-min chase	5 ± 1	5 ± 1

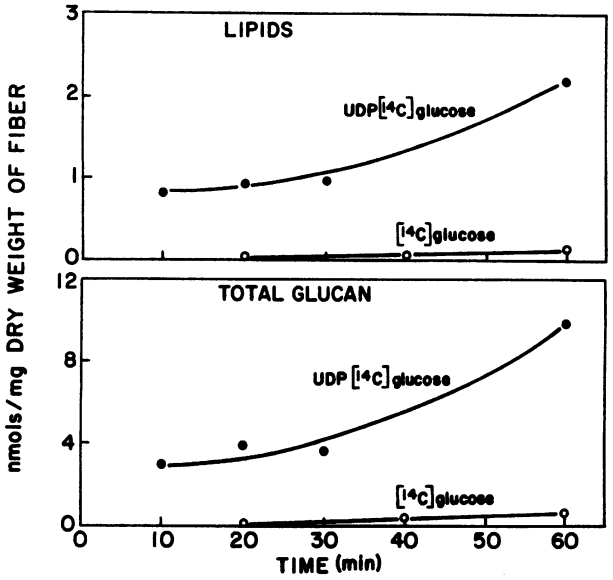

FIG. 6. Incorporation of 1 mM [^{14}C]glucose and 1 mM UDP[^{14}C]-glucose into glucans and lipids by *in vitro*-grown cotton fibers *versus* time of labeling.

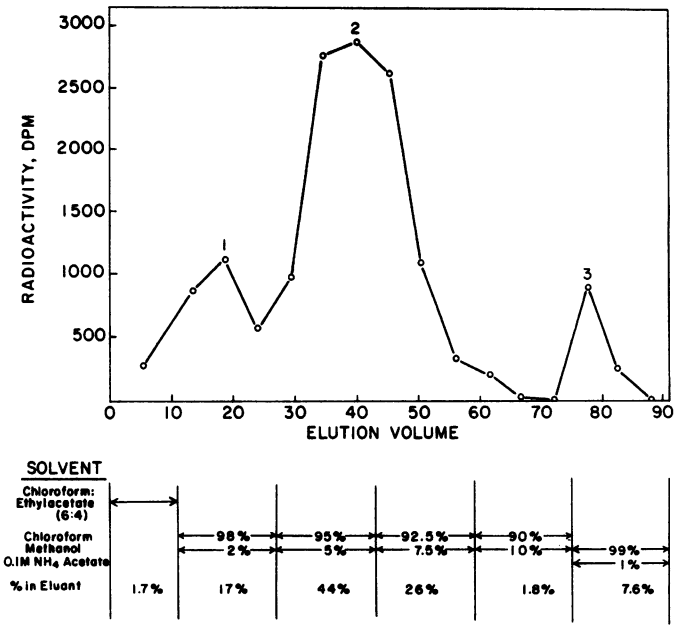

FIG. 7. Chromatographic separation of the neutral and acidic lipids from *in vitro*-grown cotton fibers. The chloroform-methanol soluble fraction was evaporated to dryness, redissolved in 0.5 ml chloroform, placed on a Sep-Pak Silica cartridge, and eluted with the solvents shown. The three peaks were collected and subjected to TLC (silica-gel G, 250 μ with $CHCl_3$:CH_3OH:NH_4OH, 70:20:2 for peaks 1 and 2; $CHCl_3$:CH_3OH:H_2O, 65:25:4 for peak 3); 1, acetylated steryl glucoside; 2, steryl glucoside; 3, glucosyl-phosphoryl-polyprenol.

reported in the previous section, indicate that these components are steryl glucoside, acetylated steryl glucoside, and glucosyl-phosphoryl-polyprenols.

DISCUSSION

A number of laboratories have shown that specific membrane fragments can be concentrated to give fractions rich in a particular kind; assay of these fractions for cell-wall glucan synthase activity has led to the concept that the cisternae vesicles from the Golgi apparatus will incorporate the glucose moiety from UDPglucose into a glucan different from that formed by the plasma membrane vesicles (20, 22, 23, 26). If the membrane flow of cisternae vesicles to the plasma membrane and incorporation by reverse pinocytosis was a rapid and continuous process (22), then the plasma membrane fraction should synthesize the same product as the Golgi cisternae fraction. Under the proper conditions, there should be no *a priori* reason why an externally supplied substrate, such as UDPglucose, cannot be used in the full spectrum of glucan synthesis by intact plant cells as with the glucan synthases from plasma membrane or from other endomembrane system of cells. With cotton fibers grown *in vitro*, where there was no restricting cutin or suberin layer, UDPglucose can be incorporated by these intact cells as shown in Figure 1; this incorporation was biphasic, showing V_{max} and K_m values somewhat similar to those reported for glucan synthase I and II. β-1,4-Linked acetic-nitric reagent soluble glucans make up a small fraction of the total soluble glucans, as previously shown (9); however, the majority of the soluble [^{14}C]glucans were β-1,3-linked glucans (Fig. 2, A and B) (3, 6–9).

As unaltered plasma membranes are impermeable to UDP-glucose (3, 7, 8, 21, 29), observations that [^{14}C]-labeled hot water soluble products, lipids, and glucans made by intact cotton fibers imply that products were synthesized by enzymes in the plasma membrane-cell wall interface (20, 28). On the other hand, the UDP[^{14}C]-glucose may be hydrolyzed, the glucose enters the fiber cells, processed and transferred by the endomembrane vesicles to the plasma membrane where it may be used in glucan synthesis. If this occurs, the acetic-nitric soluble products would contain glucans synthesized by the enzymes in the plasma membranes and the Golgi cisternae. The [^{14}C]glucose moiety of [^3H]-UDP[^{14}C]glucose was rapidly incorporated into components soluble in hot water, chloroform-methanol, and into labeled glucans (acetic-nitric reagent solule and insoluble products) (Figs. 3, 4). During the course of the labeling period, the acetic-nitric reagent insoluble glucan (cellulose) increases from 4 pmol/mg of fiber at 10 min of labeling to 16 pmol/mg of fiber at 1 h; during a subsequent chase after a 30-min labeling period, the cellulose fraction also increased (Table I). The hot water soluble ^{14}C-labeled component decreased from 75 to 50 pmol/mg of fiber during the 30-min chase. The [^3H]uridine moiety of [^3H] UDP[^{14}C]glucose was not incorporated and was present only in the hot water extract (Table I) and rapidly lost with chase (Fig. 5). The 25 to 30 pmol/mg fiber dry weight of ^3H-label in the hot water extract after 20- and 30-min labeling may be a good estimate of the labeled substrate in the cell wall apparent free space prior to the incorporation of the [^{14}C]glucose moiety. As the ^3H-label, assumed to be [^3H]UDP[^{14}C]glucose, was not incorporated into cell wall constituents, it would be appropriate to reduce the value for ^{14}C-label incorporation into hot water soluble components by the values for the ^3H-label at each of the labeling times (31 ± 9 pmol/mg fiber at 20 min and 25 ± 6 pmol/mg fiber at 30 min). If this was done, then for these two labeling periods, the corrected value for [^{14}C]glucose incorporated into hot water would be 46 and 61 pmol/mg fiber. The measurements of ^{14}C-label in the hot water extract after 20-min

followed by chase periods of 20 and 30 min were 48 ± 8 and 41 ± 3 pmol/mg and after 30-min labeling and chases 56 ± 7 and 53 ± 4 pmol/mg fiber, in reasonable good agreement with the values obtained by using the hot water extracted ^{3}H-label as a measure of the exchangeable free space in the fiber cell wall. Fibers boiled before labeling also had an exchangeable cell wall apparent free space of about the same value (Table II). Most of the ^{3}H-label in the apparent free space was assumed to be the substrate, [^{3}H]UDP[^{14}C]glucose, as the [^{3}H]UDP residue after the incorporation of the [^{14}C]glucose moiety into products would be rapidly lost to the medium as the diffusion gradient would be steep from the fiber to the medium.

It does not appear that UDP[^{14}C]glucose was hydrolyzed and utilized after internal altering and retransported to the site of synthesis in the plasma membrane-cell wall interface (Fig. 6). Franz and Meier (14) obtained similar results with detached cotton fibers; their assay was for alkali-insoluble product and without a prior extraction to remove lipids. Delmer *et al.* (6) reported that the incorporation of UDP[^{14}C]glucose into alkali-insoluble product by older, detached fibers, from ovules cultured *in vitro*, was much greater than in young fibers and the product was soluble in chloroform-methanol. These same workers (8) reported that the utilization of UDP[^{14}C]glucose by intact cotton fibers at low and high levels was about 10% of that obtained with detached fibers; under the conditions used most of the [^{14}C] glucose moiety was recovered in [^{14}C]sucrose. Later (3) it was observed that the capacity of detached cotton fibers to synthesize [^{14}C]cellulose from [^{14}C]glucose was 50% restored by including PEG 4000 in the incubation medium. UDP[^{14}C]glucose was not utilized as a substrate for cellulose synthesis by such fibers, but could incorporate the substrate into β-1,3-glucan. It has also been reported that anucleated subprotoplasts from cotton fibers will incorporate the [^{14}C]glucose moiety from UDP[^{14}C]glucose into glucans; the inclusion of glucose at 0.2 to 1.0 mM did not change the amount of label incorporated into these glucans (15).

Particulate fractions prepared from cotton fibers have been reported to incorporate UDP[^{14}C]glucose into acidic lipids (11) and to transfer the [^{14}C]glucose from the sugar nucleotide to endogenous sterol acceptors as well as catalyze the acetylation of exogenous steryl glucoside (12). Mitochondrial preparations from a number of plants and chloroplasts from spinach leaves will incorporate [^{14}C]-glucose or [^{14}C]galactose from respective sugar nucleotides into steryl glucoside and to acetylate these products (24). Bowles *et al.* (2) reported that a particulate fraction from bean hypocotyls, correlated with Golgi apparatus, was active in incorporating UDP-[^{14}C]glucose into dolichylmonophosphate-glucoside, steryl and acetylated steryl glucosides. However, others have identified the particulate fraction from maize coleoptiles that have these capacities with the plasma membrane (17). Detached cotton fibers will also synthesize these lipids from UDP[^{14}C]glucose, but not from GDP[^{14}C]glucose (6).

That intact fibers from *in vitro*-grown cotton fibers rapidly incorporate the [^{14}C]glucose moiety from UDP[^{14}C]glucose into chloroform-methanol soluble components indicates that the enzymes for the synthesis and acetylation of steryl glucosides and the transfer of the moiety to dolichyl-phosphate were located in the plasma membrane-cell wall interface as were the glucan synthases. At 50 μM UDP[^{14}C]glucose, the majority of the incorporated label was into these lipid fractions; at 1 mM, incorporation into lipids drops to 12% and into total glucans increases from 17% at 50 μM UDPglucose to 52% (data not included). At the lower level of UDPglucose, steryl glucoside constitutes the major lipid (about 70%) with acetylated steryl glucoside about 15% and glucosyl-phosphoryl-polyprenol about 8% (Fig. 7). There was no evidence from the chase experiment that the [^{14}C] glucolipids were involved in glucan synthesis as there was no decrease in the lipid level with the 30-min chase period (Table I). Hopp *et al.* (19) has reported that such was the case for *Prototheca zopfii*; however, Helsper (18) did not show this to be the case for membrane fraction isolated from petunia pollen. Delmer (7) stated that there was no evidence to support a role of lipid intermediates in cellulose biosynthesis by higher plants.

It appears that under the right conditions, intact cotton fibers will incorporate UDP[^{14}C]glucose into the spectrum of cell wall components, including cellulose, observed when [^{14}C]glucose is used, but at a more rapid rate for 30- to 60-min labeling periods. These synthetic activities also appear to occur within the plasma membrane-cell wall interfacial region which has a substantial apparent free space capacity.

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