Effect of Boron on the Incorporation of Glucose from UDP-Glucose into Cotton Fibers Grown in Vitro

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ABSTRACT

Boron is required for fiber growth and development in cotton ovules cultured in vitro. Incorporation of L-glucose by such fiber from supplied UDP-L-glucose into the hot alkali-insoluble fraction is rapid and linear for about 30 minutes. Incorporation of L-glucose from such substrate by fibers grown in boron-deficient ovule cultures is much less than in the case with fibers from ovules cultured with boron in the medium. Total products (alkali-soluble plus alkali-insoluble fractions) were also greater in fibers from ovules cultured with boron. The fraction insoluble in acetic-nitric reagent was a small part of the total glucans; however, in the boron-sufficient fibers, there was significantly more of this fraction than in fibers from boron-deficient ovule cultures. The hot water-soluble glucose polymers from the labeled fibers had a significant fraction of the total L-glucose incorporated from UDP-L-glucose. Both β-1,4- and β-1,3-water-soluble polymers were formed in the boron-sufficient fibers, whereas the same water-soluble fraction from the boron-deficient fibers was predominantly β-1,3-polymers. The incorporation of L-glucose from UDP-L-glucose by the fibers attached to the ovules was insignificant.

One of boron's possible roles in higher plant growth and development is that of regulating metabolic processes that result in the build-up of specific products, including UDP-glucose, glucose-1-P, or 6-P-glucanate (13-15, 22, 23, 34). If boron does play such a regulatory role, it would have some influence on cell wall metabolism including the biosynthesis of cellulose and pectin compounds. Torsell (44) proposed that the "complexes between boric acid and carbohydrates control the deposition of oriented cellulose micelles and the accompanying stiffening of the cell wall." Spurr (39) observed that boron deficiency in celery plants did alter plant cell walls, and concluded that boron apparently affects the rate and process of carbohydrate condensation into wall materials. Odhoff (26) also proposed that boron's influence on bean root cell elongation was probably related to the deposition of new cellulose microfibrils.

Whittington (50) suggested that cessation of cell division in boron-deficient field bean radicles was related to abnormalities in cell wall formation which, in turn, prevented the cell wall from becoming organized for mitosis. Later work in the same laboratory (36) revealed that L-glucose was incorporated into pectic substances of boron-deficient field bean radicles at a higher level than in boron-sufficient radicles. The authors proposed that boron's role in plant growth is as a bonding agent between cell wall polysaccharides. Wilson (51) also observed that boron deficiency affected cell walls of tobacco pith parenchyma grown in tissue culture by doubling the amount of cell wall fraction with no apparent change of the cellulose: pectic substance ratio as compared to control grown tissue. Boron enhanced the incorporation of myo-[3H]inositol into 1-arabinosyl units from pectin of pear pollen tube membranes. Stanley and Loewus (41) provided evidence suggesting that boron plays a definite role in pectin synthesis of germinating pollen. Oil palm seedlings grown in the absence of boron showed an increased level of hemicellulose and pectic substance in the root tissue as compared to the roots of boron-sufficient grown seedlings (29). Callose, β-1,3-glucan, accumulated in tissue of boron-deficient bean and cotton plants; however, the deposition of callose in such plants occurs after the observed effect of boron deficiency on translocation through the phloem tissue (47).

A previous study of boron's effect on cotton fiber growth and development showed the advantage of using an in vitro culture of unfertilized cotton ovules grown on defined medium (3). Our subsequent work (5) with the cotton ovule tissue culture technique showed that boron deficiency symptoms observed with cotton tissue are not related to a reduction in nucleic acid biosynthesis. That work (5) also suggested that boron has a role in regulating UDP-glucose synthesis. Earlier work (14) observed that the in vitro activity of UDP-glucose pyrophosphorylase was stimulated when boron was included in the reaction mixture. In addition, it has been reported that boron deficiency resulted in UTP accumulation and a decline in the level of bean root UDP-glucose (43).

Recently, Wainwright et al. (unpublished) showed that [6-14C]orotic acid incorporated into cotton fibers is inhibited when the ovules are cultured with 10 μM boron as compared to the control level (100 μM boron). Prior to the onset of growth reduction induced by lower boron levels (8th day of culture), incorporation into RNA was higher and that into UDP-glucose was lower at the 10 μM boron level as compared to the control level.

This report is concerned with the effect of boron on short term incorporation of exogenously supplied UDP-[14C]glucose into intact cotton fibers grown in vitro as described previously (4).

MATERIALS AND METHODS

Growth of Plants and Preparation of Cotton Fibers for Assaying. Unfertilized cotton ovules (Gossypium hirsutum L., Acala S1) were cultured as described previously (4, 5). Fourteen days after the start of culture, the ovules from several flasks were removed from the medium and washed by stirring in several hundred ml of 50 mM Tes buffer (pH 7.5), containing 5 mM MgCl2 and 0.01% Tween 20 (polyoxyethylene sorbitan monolaurate). Twenty ovules were transferred to a 30-ml screw cap vial containing 19 ml of this buffer and then incubated in a water bath at 34 C for 15 min. The shaker oscillated the vials in the vertical plane at approximately a 25° arc. After preincubation, 1 ml of a UDP-glucose solution in the same buffer (containing UDP-[14C]glucose at approximately 100 dpm/nmol of UDP-glucose) was added to the vials. The final concentration of UDP-glucose was 1 mM. After rapid mixing, 0.1 ml of the solution was removed for radioactive assay, the vials were recapped and returned to the shaker and incubated for the
appropriate times. Duplicate tubes containing 20 ovules each were prepared for each treatment of all experiments.

At various points in the time studies, and at 20 min in the assays of other variables, ovules were removed from the incubation solution, transferred to several layers of paper towels until excess liquid was absorbed, and then washed with 400-500 ml of water on a Büchner filter funnel. After being washed, ovules were blotted dry with filter paper and their fibers were removed. Fibers from five ovules were pooled, placed in aluminum pans, frozen on solid CO₂, and lyophilized. After drying the fibers from the five ovules (which constituted one replication), they were transferred to plastic microfuge tubes for storage and later extraction.

Assay of Fibers. The procedure for extracting the fibers is given in Figure 1. The precise components in plant cell walls extracted with hot water, dilute acid, or aqueous base are not known; it is generally considered that pectic polysaccharides are extracted by hot water and that hemicellulose components are solubilized by subsequent extraction with hot alkali (42).

Delmer et al. (11) have shown that chloroform-methanol (1:2) extraction of cotton fibers labeled with UDP-[¹⁴C]glucose solubilized labeled sterlyglycosides and acetylated sterlyglycosides. Only 5-10% of the [¹⁴C]glucose incorporated into cotton fibers from UDP-[¹⁴C]glucose in the present study was solubilized by the chloroform-methanol extraction. The extract was not analyzed further.

Heiniger and Delmer (19) recommended that hot alkali extraction to solubilize β-1,3-linked glucans should be "abandoned." Their work and that of others (7, 20, 21, 28, 31) has shown that not all β-1,3-glucans in cotton fibers are solubilized with the hot alkali extraction. Acetic-nitric reagent (46) has been used to free cotton fibers of noncellulosic components (19). In our study, extracting the UDP-[¹⁴C]glucose-labeled cotton fibers with hot alkali followed by an acetic-nitric reagent treatment made it possible to report the results as: (a) the hot alkali-insoluble glucans; and (b) acetic-nitric insoluble products (cellulose).

An alternative procedure was also used to hydrolyze alkali-insoluble glucans. After the alkali treatment, the insoluble products were incubated 24 h at 50 C in 50 mm Na-acetate buffer (pH 4.8) containing 3 mm NaNO₃ and 80 μg *Rhizopus* β-1 → 3)-glucanase (S 178K) (1, 19, 27, 31, 33, 48). Hydrolysis was terminated by boiling, followed by centrifugation to separate the hydrolyzed products from the remaining insoluble cell wall material. After being washed, the soluble components were lyophilized, redissolved in a minimum volume of water, and chromatographed. The insoluble material was subjected to *Streptomyces* cellulase (S 199g) hydrolysis at the same pH, buffer, and temperature. Solubilized products were separated from the insoluble material by centrifugation and the hydrolyzed product was lyophilized for subsequent chromatography. The two hydrolytic enzymes were the gift of E. T. Reese, U.S. Army Laboratory, Natick, Mass.

The acetic-nitric insoluble residue and the insoluble products after hydrolysis with β-1 → 3)-glucanase and cellulase were subjected to combustion in a biological material oxidizer (Beckman Instrument Co.) and the [¹⁴C]CO₂ was absorbed in Oxfluor-Co₂ scintillation fluid (New England Nuclear). The efficiency of combustion, collecting, and counting the [¹⁴C]CO₂ was determined by oxidizing calibrated methyl-[¹⁴C]methacrylate chips (New England Nuclear).

Determination of Radioactivity. Aliquots of aqueous extracts were counted in a scintillation fluid containing: 333 ml Triton X-100, 625 ml toluene, and 42 ml Tolu Scint/l (ICN Pharmaceuticals, Inc.). Chloroform-methanol-extracted samples were evaporated under N₂ prior to adding scintillation fluid. All samples were counted in an LS 100 or LS 9000 Beckman scintillation counter. Counts per minute were converted to dpm by correcting for counting efficiency.

Chromatography. The [¹⁴C]-labeled products extracted from fiber with hot water consisted of a number of products other than glucose and sucrose. This is in contrast to the products from UDP-[¹⁴C]glucose labeling of detached cotton fibers reported previously (12). To identify these hot water-soluble labeled products, we chromatographed samples on a Bio-Gel P₂ (200-400 mesh) column (2.5 × 10 cm) and collected the eluate containing glucose larger than pentaose. This heterogeneous glucan sample was lyophilized and redissolved in a small amount of water incubated with *Rhizopus* β-(1 → 3)-glucanase and/or *Streptomyces* cellulase. The rate of glucan hydrolysis was followed by spotting small aliquots of TLC cellulose plates at different times after the start of incubation with the enzyme. The solvent system used for separating components on TLC plates was formic acid-methylethyl ketone-tetr butanol-water (3:5:7:5) as used by Damonte et al. (9). The TLC plates were first placed in contact with x-ray film to develop latent images of separated radioactive components, and then treated with alkaline AgNO₃ to identify the separated carbohydrates colorimetrically.

Material solubilized from alkali-insoluble glucan by β-(1 → 3)-glucanase or cellulase was chromatographed on cellulose TLC

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**Figure 1.** Protocol for extracting and assaying cotton fibers after labeling with UDP-[¹⁴C]glucose.
plates (9) or on Whatman 3MM paper with a propanol-ethyl acetate-water (7:1:2) solvent. Laminaribiase and laminaritiose were separated initially from the partial hydrolysis of pure laminarian by chromatography on a Bio-Gel P2 column; subsequently they were separated by paper chromatography. Cellotriose and cellulbiose were prepared identically after partial hydrolysis of pure cellulose (25). These di- and tripolysaccharides, along with known monosaccharides, were used for standards in chromatographic separation of the soluble cotton fiber cell wall components.

Periodate Oxidation. Periodate oxidation of hot water- and chloroform-methanol-insoluble fiber components was performed by the method described by Heiniger and Delmer (19). The procedure was further modified for water-soluble glucan components. Hot water-soluble polysaccharides larger than the pentaose from the Bio-Gel P2 column were lyophilized and subjected to periodation for 5 days. Ethylene glycol (100 ml) was added and the sample was desalted through Dowex 1 and Dowex 50 columns. One hundred mg of NaB₄H₄ was added and the reduction was continued at room temperature for 1 h. The excess NaB₄H₄ was destroyed with glacial acetic acid and borate was removed by repeated additions of methanol and evaporation under N₂. Total hydrolysis was performed with 2 N trifluoroacetic acid at 121 C for 90 min. The solution was concentrated and chromatographed on TLC plates. The chromatographed oxidation products were identified by use of audiordiography and alkaline AgNO₃ solution. Cellulose from the TLC plates, with separated radiooxidative oxidation products, were scraped from the plates in 1-cm increments, and transferred to scintillation fluid where the radioactivity level was determined.

RESULTS

Figure 2 shows the incorporation of UDP-[¹⁴C]glucose within a 20-min period by intact cotton fibers grown in vitro on a complete medium. This log plot shows that at the 1 mM UDP-glucose concentration, the incorporation sites have not been saturated. In turn, this indicates that the amount of UDP-[¹⁴C]glucose incorporated may be limited by the rate of diffusion to the incorporation site or that Kₘ for the glucan synthetase enzyme is large (12, 31).

Figure 3 shows that at the level of 1 mM substrate, the incorporation rate in alkali-insoluble product is linear for 20-30 min. This linear rate was also observed for total incorporation and for the formation of hot water-soluble products. All subsequent labeling experiments were done for 20 min. Figure 3 also shows that the incorporation rate into fibers grown on boron-sufficient culture medium is significantly larger than that in fibers grown in medium without added boron.

In contrast with other reports (2, 11, 16, 35), there was not appreciable incorporation of radioactivity from GDP-[¹⁴C]glucose. Less than 1 nmol of GDP-glucose was incorporated per mg dry weight of fiber into the water-soluble fraction. Also, less than 0.1 nmol per mg dry weight of fiber was incorporated into each of the other fractions within the 20-min period. Similar low incorporation levels within a 20-min labeling period were observed when [¹⁴C]glucose was used as substrate.

Table I gives the results from an experiment conducted to compare the effectiveness of acetic-nitric extraction with enzymic hydrolysis of cotton fiber cell wall glucans. The acetic-nitric extraction removed almost all of the [¹⁴C]-labeled products after a 20-min labeling period with UDP-[¹⁴C]glucose. Only about 1% of the glucan is insoluble to this extraction. On the other hand, the β-(1→3)-glucanase- and cellulase-insoluble products were about 13% of the total glucan in the samples. These results substantiate the fact that in a short incubation period with exogenously supplied UDP-glucose, the alkali-insoluble product formed is not cellulose even though it may constitute more than 50% of the newly formed wall glucans.

Table II shows the result obtained when cotton ovules were cultured in vitro with [¹⁴C]glucose in the medium for the 14 days. After the fibers were extracted with hot water and chloroform-methanol, one-half were extracted with hot aqueous alkali and the other half with acetic-nitric reagent. Between these two methods, there is no significant quantitative difference in the solubilized wall glucans. The insoluble wall glucans constitute 39% of the total in the hot alkali-extracted fibers and 36% when extracted by acetic-nitric reagent (46); that is not a significant difference.

Without added boron in the growing medium, there is only about one-fourth to one-third the quantity of fiber per ovule as compared to growth in medium where 100 µM boron is added. There are two possible ways to express the difference in the glucan synthesis from added UDP-glucose: (a) as the quantity of [¹⁴C]-glucose from UDP-[¹⁴C]glucose incorporated into products per mg

![Fig. 2. Plot of log UDP-glucose concentration versus log of glucose incorporated by cotton fibers grown in vitro for 14 days. Total is incorporated into all fractions; "glucan" is incorporation into alkali-soluble and insoluble fractions; alkali-insoluble is incorporation into fractions not solubilized by three extractions with 1 N NaOH at 100 C for 5 min each. The labeling period was for 20 min.](image-url)

![Fig. 3. Time course for the incorporation of [¹⁴C]glucose into alkali-insoluble glucan after supplying 14-day-old in vitro grown cotton ovule culture UDP-[¹⁴C]glucose at 1 mM. +B is incorporation by fibers grown in culture with 100 µM boron added. −B is incorporation by fibers grown in culture without added boron.](image-url)
Boron and Glucan Synthesis

In vitro grown cotton ovules were cultured for 14 days at 34°C. The ovules with attached fibers were incubated and assayed. One-half of the samples were subjected to acetic-nitric extraction and one-half to enzymic hydrolysis.

<table>
<thead>
<tr>
<th>Hot Water-soluble</th>
<th>Chloroform-Methanol-soluble</th>
<th>Hot Alkali-soluble</th>
<th>Acetic-Nitric-soluble</th>
<th>Insoluble</th>
<th>Glucans</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>3-5</td>
</tr>
<tr>
<td>9.7 ± 0.5</td>
<td>1.5 ± 0.1</td>
<td>11.2 ± 2.3</td>
<td>0.1 ± 0.2</td>
<td>17.3 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>1.3-Glu-osoluble</td>
<td>Cellulase-soluble</td>
<td>5.3 ± 0.7</td>
<td>4.0 ± 0.8</td>
<td>2.4 ± 0.4</td>
<td>18.6 ± 2.8</td>
</tr>
</tbody>
</table>

*Expressed as mean ± two standard errors.

Table II. Comparison of Extracting in Vitro Grown Cotton Fibers with Hot Aqueous Alkali and Acetic-Nitric Reagent

In vitro grown cotton ovules were cultured for 14 days at 34°C with [14C]glucose. At 14 days the tissue was washed and blotted, and fibers were removed and lyophilized.

<table>
<thead>
<tr>
<th>Hot Water-soluble</th>
<th>Chloroform-Methanol-soluble</th>
<th>Hot Alkali-soluble</th>
<th>Acetic-Nitric-soluble</th>
<th>Insoluble</th>
<th>Total Incorporated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>1-5</td>
</tr>
<tr>
<td>1.43 ± 0.10</td>
<td>0.29 ± 0.08</td>
<td>0.81 ± 0.16</td>
<td>1.61 ± 0.22</td>
<td>4.13 ± 0.38</td>
<td></td>
</tr>
<tr>
<td>1.53 ± 0.24</td>
<td>0.24 ± 0.06</td>
<td>0.94 ± 0.10</td>
<td>1.54 ± 0.41</td>
<td>4.23 ± 0.54</td>
<td></td>
</tr>
</tbody>
</table>

*Expressed as mean ± two standard errors.

Table III. Effect of Boron on Incorporation of UDP-[14C]Glucose into Fiber Cells of in Vitro Grown Cotton Ovules

In vitro grown cotton ovules cultured for 14 days at 34°C. The ovules with attached fibers were incubated and assayed.

<table>
<thead>
<tr>
<th>Growth Media</th>
<th>Hot Water-soluble</th>
<th>Chloroform-Methanol-soluble</th>
<th>Hot Alkali-soluble</th>
<th>Acetic-Nitric-soluble</th>
<th>Insoluble</th>
<th>Glucans</th>
<th>Total Incorporated</th>
</tr>
</thead>
<tbody>
<tr>
<td>+B</td>
<td>6.9 ± 2.2</td>
<td>2.7 ± 0.2</td>
<td>6.0 ± 1.5</td>
<td>20.8 ± 6.7</td>
<td>0.4 ± 0.20</td>
<td>28.9 ± 11.2</td>
<td>36.2 ± 7.0</td>
</tr>
<tr>
<td>−B</td>
<td>4.3 ± 0.4</td>
<td>1.5 ± 0.3</td>
<td>2.3 ± 0.2</td>
<td>10.8 ± 1.3</td>
<td>0.1 ± 0.02</td>
<td>13.1 ± 1.4</td>
<td>18.8 ± 1.3</td>
</tr>
<tr>
<td>+B</td>
<td>13.1 ± 6.2</td>
<td>5.0 ± 6.0</td>
<td>10.8 ± 1.9</td>
<td>37.4 ± 8.6</td>
<td>0.7 ± 0.20</td>
<td>48.8 ± 1.0</td>
<td>66.9 ± 9.4</td>
</tr>
<tr>
<td>−B</td>
<td>2.2 ± 0.5</td>
<td>0.8 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>5.5 ± 1.0</td>
<td>0.2 ± 0.01</td>
<td>6.7 ± 1.2</td>
<td>9.6 ± 1.7</td>
</tr>
<tr>
<td>−B as % of +B</td>
<td>17</td>
<td>16</td>
<td>11</td>
<td>15</td>
<td>3</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

*Expressed as mean ± two standard errors.

Dry weight of fiber per ovule from −B-grown ovules as per cent of fiber per ovule from +B-grown ovules ± two standard errors = 25% − 35%; determined from 80 ovules per treatment.

The dry weight of fibers from ovules grown without added boron averaged between 25 and 35% of the +B control. In each of the extracted fractions the per cent of 14C-labeled products in the −B treatment was below this range, indicating boron’s apparent role in the process of glucan synthesis in addition to its effect on overall fiber growth during the 14 days of culture. Although all extracted fractions show this effect, the very low value for the fraction insoluble in all solvents points out the difference in the synthesis of insoluble β-1,4-polymers during the 20-min incubation period.

It has been estimated that with in vivo grown cotton there are about 384,000 lint fibers per boll (24) and, on the average, 30 ovules per boll (C. A. Beasley, personal communication). There appear to be fewer fibers per ovule with in vitro cultured tissue but no definite count has been made. Assuming that the in vivo values are correct, the 29 nmol glucan per mg dry weight of fiber per 20 min calculates out to 48.5 ng glucose incorporated into glucan per fiber per day. The average length of 14-day-old control grown fibers is 16 mm. The calculated incorporation rate of ng glucose incorporated into glucan per day per mm fiber is 3.0.

When 10 mM cellulose was included in the incubation medium for the 20-min incorporation period, there was no significant difference between the glucan fraction extracted with hot aqueous alkali and acetic-nitric reagent than that when cellulose was not included. However, the results were quite different (Table IV) when 10 mM laminaribiose was included in the incubation solution containing UDP-[14C]glucose. The dry weight of fiber per ovule for the −B treatment varies between 25 and 37% of the +B treatment; however, the nmol per ovule value for the −B treatment (expressed as per cent of +B treatment) indicates that a disproportionate amount of UDP-[14C]glucose was incorporated into all of the extracted fractions except the insoluble one. With laminaribiose in the reaction mixture and with all fractions except the insoluble, there was significantly more UDP-[14C]glucose incorporated into the fiber of −B-grown fibers. In the insoluble fraction, the proportion was within the fiber per ovule dry weight ratio for...
The data indicate a significant difference between the +B and -B-grown fibers when either no disaccharide or cellobiose was used. With laminaribiose, there was no significant difference in the pmole of UDP-[14C]glucose incorporated into cellulose between the +B and -B-grown fibers. There was also no significant difference observed between incorporation into the insoluble fractions with or without cellobiose in the incubation mixture. Either with or without cellobiose included during the 20-min labeling period, the average quantity of labeled insoluble product per ovule in the -B-grown fibers amounts to 9.3 ± 4.4% of that observed in the +B-grown fibers. That is considerably less than the fiber per ovule dry weight ratio for +B and -B-grown tissue.

A specturm of labeled hot water-soluble products was also observed, including glucose, sucrose, laminaribiose, cellobiose, laminaritriose, laminaritetrose, cellobiose; also included were radioactive products that do not migrate at all from the origin of TLC cellulose plates and those that move slightly (between Rglucosae of 0.2 for cellobiose and the origin). Table VI lists the Rglucosae for radioactive compounds observed in the hot water-soluble fraction and known compounds. There were no latent images formed on x-ray film from compounds with an Rglucose larger than 1.0.

If the hot water-soluble products were subjected to hydrolysis with R. solani β-(1 → 3)-glucanase for 24 h, desalted, lyophilized, redissolved in a small volume of water and chromatographed on cellulose TLC plates, and if the plates were then autoradiographed, the following 14C-containing compounds were observed: glucose, laminaribiose, laminaritriose, laminaritetrose, and a continuous image of Rglucose of 0.35 to the origin. There appeared to be radioactive hot water-soluble polymers that were not hydrolyzed by β-(1 → 3)-glucanase. The labeled product(s) that did not migrate on the TLC plate was subjected to Streptomyces cellulase hydrolysis and rechromatographed at 1, 2, 4, and 24 h after adding the hydrolytic enzyme. Latent images developed on x-ray film from the water-soluble fraction of the +B treatment revealed only a 14C-compound having the mobility of glucose after 24 h of incubation with the cellulase enzyme. There was no residual radioactive product remaining at the origin. With the hot water-soluble fraction from the -B treatment, the latent images developed at Rglucose of 0.75 and 0.50 after 24 h appear to be laminaribiose and laminaritriose. After periodate oxidation of the +B-grown fibers, radioactive as revealed by autoradiography was sufficiently intense in the chromatographed products to cause a strong latent image on x-ray film at the known Rglucose of glucose, erythritol, and glycerol. With the -B-grown fibers, this method of identifying radioactive compounds indicated that the only radioactive product was [14C]glucose. Figure 4 is a plot of total radioactivity recovered for the +B and -B-grown fibers plotted against the distance from the origin of the chromatogram. Considerably different total radioactivity was recovered from the two chromatograms; in fact, the +B-treated water-soluble fraction which had been oxidized by periodate had several times more total radioactivity. The periodate oxidation products of the hot water-soluble fraction from -B-grown fiber are predominantly glucose (65%), with about 12% migrating to an Rglucose region of (8.5–11 cm) (erythritol). With the oxidized and hydrolyzed water-soluble products from the +B-grown fibers, 52% of the radioactive product had an Rglucose of 1.4 (the value of erythritol) and 28% had an Rglucose of 1.6 (the value for known glycerol). About 16% was present in the glucose area (6.5–8.5 cm).
by an *in vivo* or *in vitro* plant system have complicated the comparison of various investigators’ results.

Alkali-soluble materials have generally been considered as noncellulosic glucan polymers, and the insoluble components as cellulosic; \(\beta-1,4\)-glucosyl linkage is the predominant type of bonding between sugar monomers. More recently, four groups (19, 21, 28, 31) have reported as invalid the assumption that hot alkali insolubility denotes \(\beta-1,4\)-linked glucan polymers. Herth et al. (20) have also reported that in pollen tube walls of *Lilium longiflorum*, alkali-resistant fibrils of crystalline \(\beta-1,3\) along with cellulose constitute a structural polysaccharide component. In addition, this \(\beta-1,3\)-glucan fibrillar glucan is different from cellulose, which is an amorphous \(\beta-1,3\)-glucan solubilized by alkali (20). The authors propose that such noncellulosic fibrillar glucans may be contained in variable proportions in cell walls of other plant species. Recently, more specific analytical methods for cellulose synthesis have been used (1, 16, 19, 21, 24, 31, 37, 40, 42, 46).

If hot alkali insolubility is used as a criterion for rapid cellulose glucan synthesis (within 20 min), then more than 50% of the incorporation of \([\text{\textsuperscript{14}}\text{C}]\)glucose from UDP-[\(\text{\textsuperscript{14}}\text{C}\)]glucose in the present study end up in this form of glucan. Others (19, 21) have observed that the glucan product formed by short time labeling of cotton fibers with UDP-[\(\text{\textsuperscript{14}}\text{C}\)]glucose is primarily a \(\beta-1,3\)-linked glucan that may or may not be extracted from fibers with hot aqueous alkali. It has been reported (21) that strong alkali solution solubilizes \(\beta-1,4\)- as well as \(\beta-1,3\)-glucans from the cell walls of cotton fibers.

In both the present and other studies of glucan synthesis by *in vitro* grown cotton fibers, it seems that the \(\beta-1,3\)-glucan synthetase system is much favored over the \(\beta-1,4\)-glucan system (12, 19, 21). Meinert and Delmer (24) reported a large increase in noncellulosic glucan glucose in cotton fiber development just before the onset of secondary wall deposition. Delmer (12) discussed the possibility that noncellulosic glucans may serve as reserve polymers for later incorporation into cellulose. Huwyler et al. (21) suggested that \(\beta-1,4\)-glucan solubilized by hot aqueous alkali may be cellulose in *Statu nascendi*. Satoh et al. (33) reported cytoplasmic \(\beta-1,4\)-glucans in the homogenate prepared from *Phaseolus aureus* seedlings and suggested that these glucans might be involved in cell wall cellulose synthesis. Incorporation of \([\text{\textsuperscript{14}}\text{C}]\)glucose into cytoplasmic \(\beta-1,4\)-glucan could be chased out of the cytoplasm into the cell wall fraction. Coumarin inhibited the incorporation of \([\text{\textsuperscript{14}}\text{C}]\)glucose into the cytoplasm \(\beta-1,4\)-glucan product as well as into cell wall cellulose. Others (7, 35, 37, 38) have reported both \(\beta-1,4\)- and \(\beta-1,3\)-glucans to be present in hot aqueous extract of glucan synthesizing *in vitro* or *in vivo* systems.

Table I shows that the glucan fraction which is insoluble in hot aqueous alkali is larger than the soluble fraction, and is almost completely solubilized by the acetic-nitric reagent (Table I, col. 4). A combination of glucan hydrolytic enzymes that many use to indicate the kind of glucan product synthesized by a glucan synthetase system is much less complete in solubilizing the alkali-insoluble fraction than is the acetic-nitric reagent. The glucan products synthesized by cotton fibers supplied \([\text{\textsuperscript{14}}\text{C}]\)glucose over the 14 days of growth in culture (rather than UDP-[\(\text{\textsuperscript{14}}\text{C}\)]glucose for 20 min after 14 days of culture) showed about the same quantity of glucan solubilized by the hot alkali treatment as by the acetic-nitric reagent (Table II). There was no significant difference in the glucan fraction insoluble to the two extraction procedures.

It appears that the \([\text{\textsuperscript{14}}\text{C}]\)glucose-labeled products incorporated by cotton fibers after a short labeling period with UDP-[\(\text{\textsuperscript{14}}\text{C}\)]glucose are quite different, with respect to their solubility in hot aqueous alkali and acetic-nitric reagent from the labeled products formed when \([\text{\textsuperscript{14}}\text{C}]\)glucose was supplied over the normal 14 days of growth. Perhaps the short labeling period with UDP-[\(\text{\textsuperscript{14}}\text{C}\)]glucose results in glucans that are less polymerized and, therefore, more soluble to acetic nitric reagent (19, 21). It could also indicate
that the glucan product formed with UDP-[14C]glucose is a different polymer than that formed with [14C]glucose (1).

Although an earlier report (12) identified sucrose as the major water-soluble product formed when UDP-glucose was supplied to cotton fibers, we consistently observed that the hot water extracts contained both β-1,3- and β-1,4-linked glucans. Periodate oxidation of the aqueous extract with subsequent TLC showed that +B-grown tissue had three oxidation products: erythritol (indicating β-1,4-linkage), glycerol, and glucose (Fig. 4). In fibers grown without added boron in the growth medium, the primary periodate oxidation product was glucose, with only a trace of erythritol and glycerol. Perhaps the β-1,4-linked water-soluble glucans synthesized are polymers that subsequently will be incorporated into cell wall cellulose (21, 33). There was no increase observed in the acetic-nitric insoluble fraction or total glucan component after a 4-h chase with nonradioactive UDP-glucose (data not given).

Periodate oxidation of the labeled cotton fibers after aqueous and chloroform-methanol extraction indicated that the majority of the alkali-soluble and -insoluble glucans synthesized in the 20 min with UDP-[14C]glucose have β-1,3-linkage. There was insufficient [14C]erythritol on the TLC plates after chromatography to cause the formation of a latent image on x-ray film.

The observed effect of having boron in the growing medium on all fractions extracted from the [14C]-labeled fibers is significant (Table III). Torsell (44), Spurr (39), and Odhnoff (26) report that this may indicate that boron does affect the rate and process of carbohydrate condensation into cell wall materials. Chao and Maclachlan (8) reported a heat-stable, dialyzable component present in extracts from pea plants that enhances the synthesis of alkali-soluble- and -insoluble glucans from mm levels of UDP-glucose. They also reported a heat-labile, nondialyzable component which suppresses glucan synthesis at mm levels of UDP-glucose. Both factors primarily affect the proportion of β-1,4- rather than β-1,3-linkage synthesis with the enhancing factor increasing Vmax and Km. The authors suggested that the two components may moderate glucan synthetase activity in vivo. Perhaps the heat-stable, dialyzable enhancer is an inorganic boron compound, something which should be easily determined.

It is interesting to note the stimulation of [14C]glucose incorporation by fibers from UDP-[14C]glucose grown with added boron when laminariae is added to the incubation mixture. Perhaps the cellular conditions (without added boron) resulted in the stimulation of cellular β-1,3-glucan synthetase enzyme that can be expressed more completely if a β-1,3-disaccharide is present in the incubation mixture. Rajaratnam and Lowry (29) reported that boron deficiency leads to an increased level of pectic compounds and hemicellulose in root tissue of oil palm seedlings. Also, callose is known to accumulate in boron-deficient tissues (47).

The observation that periodate oxidation of the hot water-soluble fraction showed considerably more β-1,4-product when boron was added to the ovule tissue growing medium is consistent with the possibility that there is greater β-1,4-synthetase activity in the fibers (Fig. 4). It does appear that boron influences the glucan synthetase system of in vitro grown cotton fibers. The predominant glucan synthetase system of 14-day-old cultures appears to be the β-1,3-glucan synthetase rather than the cellulose synthetase system, thought to be more predominant in older fibers. It may be fruitful to subculture the in vitro grown ovules with fibers to extend the fiber growth period into the secondary cell wall synthesis phase (24). Such attempts are now under way in our laboratory.

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