The Effect of Auxin on Synthesis of Oat Coleoptile Cell Wall Constituents 1, 2

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In the previous paper (2) we reported that a substantial increase in gross cell wall synthesis occurs when oat coleoptile cylinders whose elongation is inhibited by Ca++ are treated with IAA; this effect of auxin is direct in the sense that it is due to hormone action rather than to the occurrence of elongation. This paper presents information on the components of the cell wall whose synthesis is affected directly by auxin under the conditions of these experiments.

Methods

Oats (Avena sativa L. var. Victory) were grown and coleoptile segments 8.0 mm long were cut, the leaf was removed from within (except in the experiment in table III), and the coleoptile segments were incubated in uniformly C14-labelled glucose solutions, as described previously (2).

Preparation of Cell Wall Material. Different methods were used in the several experiments described here.

I. In the experiments of tables I and II the whole cell wall material was prepared and counted as described in reference 2.

II. In the experiment of table III, at the end of the incubation in labelled glucose, the coleoptile segments were quickly washed 4 times with ice water, and frozen on solid CO2. They were subsequently thoroughly ground, beginning while still frozen, in a small ice-cold mortar, 2.5 ml of ice water was then added and the grinding continued for about 5 minutes. The slurry, together with about 10 ml of ice water that was used in several small portions to rinse the mortar,

Table I. Incorporation of Activity into Wall Components of Ca++-Inhibited Segments

Means and average deviations for 4 replicate samples of 16 coleoptile segments, each incubated 7.5 hours in 1 ml 0.05 M glucose containing 11.9 μC of U-C14 glucose and 0.02 M CaCl2, with or without 3 μg/ml IAA.

<table>
<thead>
<tr>
<th>Component</th>
<th>Mean amount isolated* μg</th>
<th>Specific activity, cpm/μg, ± % of mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05 × H2SO4 extract, sugars</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>61</td>
<td>96,200 ± 2.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>196</td>
<td>113 ± 8</td>
</tr>
<tr>
<td>Arabinose</td>
<td>180</td>
<td>103 ± 2</td>
</tr>
<tr>
<td>Xylose</td>
<td>158</td>
<td>118 ± 3</td>
</tr>
<tr>
<td>0.05 × H2SO4 extract, acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucuronosylxlose</td>
<td>46</td>
<td>17,400 ± 7.5</td>
</tr>
<tr>
<td>4-O-methylglucuronosylxlose</td>
<td>36</td>
<td>101 ± 4</td>
</tr>
<tr>
<td>4-O-methylglucuronic acid</td>
<td>8</td>
<td>66 ± 9</td>
</tr>
<tr>
<td>Galacturonic acid</td>
<td>32</td>
<td>56 ± 6</td>
</tr>
<tr>
<td>Galacturonosylrhamnose</td>
<td>14</td>
<td>41 ± 6</td>
</tr>
<tr>
<td>4 × KOH extract, sugars</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>50</td>
<td>13,300 ± 3.8</td>
</tr>
<tr>
<td>Xylose</td>
<td>19</td>
<td>78 ± 4</td>
</tr>
<tr>
<td>72% H2SO4 extract, sugars</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>505</td>
<td>35,500 ± 8.7</td>
</tr>
<tr>
<td>Mannose</td>
<td>21</td>
<td>68 ± 3</td>
</tr>
<tr>
<td>Xylose</td>
<td>14</td>
<td>22 ± 12</td>
</tr>
</tbody>
</table>

Specific activity, cpm/μg, ± % of mean

No IAA 119,000 ± 6.3 1.23
3 μg/ml IAA 20,900 ± 3.8 1.20

Ratio 1.23

* Mean of all samples, with and without IAA. The amounts recovered did not differ systematically between the series with and without IAA. Amounts shown for aldobiouronic acids were calculated, from hexuronic acid determination, for the molecular weight of the aldobiouronic acid in question.

** Specific activity in Roman figures, total activity in italics, ± average deviation as percent of mean.
Table II. **Effect of IAA on Cell Wall Incorporation in Uninhibited and Ca**++-Inhibited Segments

Samples were obtained from 20 segments incubated 7 hours in 0.05 M glucose (0.84 μc/ml) with or without 3 mg/liter IAA and/or 0.01 M CaCl₂.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Fraction</th>
<th>No CaCl₂ - IAA cpm</th>
<th>+ IAA cpm</th>
<th>0.01 M CaCl₂ - IAA cpm</th>
<th>+ IAA cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05 N H₂SO₄</td>
<td>Sugars</td>
<td>6,250</td>
<td>13,500</td>
<td>6,090</td>
<td>8,690</td>
</tr>
<tr>
<td></td>
<td>Uronic acids</td>
<td>840</td>
<td>1,670</td>
<td>1,040</td>
<td>1,540</td>
</tr>
<tr>
<td>4 N KOH</td>
<td>Sugars</td>
<td>990</td>
<td>1,850</td>
<td>830</td>
<td>1,370</td>
</tr>
<tr>
<td>72 % H₂SO₄</td>
<td>Sugars</td>
<td>2,370</td>
<td>5,790</td>
<td>2,500</td>
<td>2,500</td>
</tr>
<tr>
<td>Recovery of total activity*</td>
<td>83 %</td>
<td>81 %</td>
<td>85 %</td>
<td>72 %</td>
<td></td>
</tr>
</tbody>
</table>

* Percent of the activity of the whole cell wall preparations that was recovered in the extracted fractions and the residue after extraction with 72 % H₂SO₄.

was centrifuged and the cell wall material was washed twice with cold water (the first washing being added to the original supernatant, this constituting the cold water-soluble fraction), and twice with 100 % ethanol, which was discarded.

The residue (cell wall material) was dried in vacuo at room temperature, then extracted twice with 2.5 ml water at 100°C for 1 hour, washed with 1.5 ml water at room temperature and with 100 % ethanol.

The cell wall material was transferred to a planchet, and by addition of about 1 ml of chloroform, which was used to rinse the centrifuge tube, the material was spread evenly over the surface of the planchet and allowed to dry.

III. The procedure used in the experiment of table IV was the same as procedure II above except that A) the segments were ground in 2.5 ml of ice-cold 0.15 M sodium acetate buffer, pH 4.4, rather than water, and B) because of its large amount (about 15 mg), the cell wall material was not spread on a planchet but, after being washed with ethanol and dried, it was extracted with successive solvents as described in the next section.

**Fractionations.** I. In the experiments of tables I and II the cell wall material was removed from the plantlets, extracted and hydrolyzed as described by Ray (9), except that extraction with hot water was omitted. The sugars were separated from the uronic acids of each hydrolysate by passing through Dowex-I (acetate form) and eluting with 6 N acetic acid (10). Each fraction or an aliquot thereof was evaporated on a planchet and counted.

II. In the experiment of table III the cold-water-soluble fraction was heated 10 minutes at 100°C, cooled to 4°C, evaporated over P₂O₅ to about 3 ml, and insoluble material (heat precipitable fraction) was removed by centrifugation. The precipitate was washed with 1 ml of water, which was added to the supernatant, and then with 95 % ethanol, then dried down on a planchet.

By addition of absolute alcohol the supernatant was made 75 % in ethanol and kept at 4°C for 4 days. The white precipitate was washed 3 times with 95 % ethanol, then suspended in water, centrifuged, and the water-soluble material (cold-water soluble, ethanol-insoluble fraction) dried down on a planchet (a small amount of water-insoluble material, probably protein, remaining at this stage possessed only slight activity).

The cell wall material was first extracted with hot water, and then dried on a planchet, as described in section II of Preparation of Cell Wall Material. The water extract was dried down on a planchet also.

After being counted, each of the above fractions was treated on the planchet 3 times for 1 hour with 1 M NH₄OH at room temperature, the reagent being evaporated under a stream of warm air after each treatment [this sufficed to reduce to a constant value the activity of extracts of cell wall material of coleoptile segments that had incorporated radioactivity from methyl-labelled methionine; see also (7)]. In the present experiment a decrease in activity was detected only with the hot-water-soluble fraction, and is shown as ester groups in table IV (the remaining activity given for this fraction being the residual activity after NH₄OH treatment).

The cell wall residue was then extracted on the planchet with 0.5 ml of dimethylsulfoxide (Eastman, practical grade) for 5 days, then washed with 2 ml water and 2 ml of ethanol. The solvent and washings were evaporated on a planchet.

The air dried residue was extracted twice on the planchet with 1 ml of 0.5 % ammonium oxalate for 1 hour on a steam bath in a water-saturated atmosphere, 0.5 ml of water being added halfway through each heating period, then washed with 1 ml of water. Extracts and washings were centrifuged, passed through 3 g of Amberlite IR-120 (H⁺ form), and an amount of calcium acetate equivalent to the oxalate in the extract was added. Calcium oxalate was removed by centrifugation, washed once with 0.01 N acetic acid, and the supernatants (ammonium oxalate-soluble fraction) were evaporated on a planchet.

The dried residue was extracted on the planchet twice for 40 hours with 4 N KOH at room temperature, then washed 3 times with 1.5 ml of water. Extract and washings were centrifuged, passed through 2 g of IR-120 (H⁺), and dried on a planchet. The residue (α-cellulose) was spread evenly on a planchet and dried.

III. In the experiment of table IV the cold-water-
Table III. *Effect of IAA on Incorporation in Ca**++*-Inhibited and Uninhibited Coleoptile Segments

Incubation was 5 hours. The procedure is given in Methods section. Activities are shown as cpm/17 segments and are means and average deviations (in percent of mean) for 3 replicate samples.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Principal components*</th>
<th>Segments in 0.02 m CaCl₂</th>
<th>Segments without Ca**++</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IAA</td>
<td>cpm</td>
<td>IAA</td>
</tr>
<tr>
<td>Cold water soluble, + heat precipitate</td>
<td>Protein</td>
<td>—</td>
<td>670 ± 6.6 %</td>
</tr>
<tr>
<td>Cold water soluble, + EtOH insoluble</td>
<td>Glucan, Arabinogalactan</td>
<td>—</td>
<td>620 ± 7.4</td>
</tr>
<tr>
<td></td>
<td>Glucan, arabinan, Pectic acid</td>
<td>—</td>
<td>375 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>Ester groups (methyl, acetyl, etc.)</td>
<td>+</td>
<td>575 ± 5.6</td>
</tr>
<tr>
<td>Hot water soluble</td>
<td>—</td>
<td>30 ± 20</td>
<td>36 ± 31</td>
</tr>
<tr>
<td>Dimethylsulfoxide soluble</td>
<td>Glucans (I₃ + and I₂ —)</td>
<td>—</td>
<td>420 ± 5.7</td>
</tr>
<tr>
<td>Ammonium oxalate soluble</td>
<td>Glucans (I₃ + and I₂ —)</td>
<td>—</td>
<td>370 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>Pectic acid</td>
<td>+</td>
<td>700 ± 10.6</td>
</tr>
<tr>
<td>4 n KOH soluble</td>
<td>β-glucan (I₅ —)</td>
<td>—</td>
<td>2290 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>Glucuronoarabinoxylan</td>
<td>+</td>
<td>3260 ± 3.0</td>
</tr>
<tr>
<td>Residue</td>
<td>α-Cellulose</td>
<td>+</td>
<td>1820 ± 1.1</td>
</tr>
<tr>
<td>Sum of preceding 5</td>
<td>Total cell wall</td>
<td>+</td>
<td>5400 ± 1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>7220 ± 3.3</td>
</tr>
<tr>
<td>Recovery in cell wall fractionation**</td>
<td>—</td>
<td>103 % ± 2</td>
<td>108 % ± 1</td>
</tr>
<tr>
<td>Mean elongation, % of initial length</td>
<td>—</td>
<td>105 ± 0.5</td>
<td>108 ± 2</td>
</tr>
</tbody>
</table>

* I₃ + and I₂ — denote iodine-positive and iodine-negative polysaccharides, respectively. Note that in addition to the components listed, about 10% of glucuronoxarabinoloxylan is present in the hot water, dimethylsulfoxide and ammonium oxalate-soluble fractions. Some galacturonic acid is obtained from the 4 n KOH soluble fraction but appears to be associated with glucuronoxarabinoloxylans.

** Sum of last 4 fractions as percent of activity previously found in residue after hot-water extractions. Values above 100 % are due to self-absorption (about 10 %) by the unfraccionated preparations.

The cold-water-soluble polysaccharide fraction was made 80% in ethanol and kept 3 days at 4°. The precipitate (cold-water-soluble polysaccharides) was washed 4 times with 95% ethanol. The cell wall material was extracted successively with hot water (twice with 5 ml for 1 hour at 100°), dimethylsulfoxide (3 ml for 4 days at room temperature), 0.5% ammonium oxalate (twice with 3 ml for 2.5 hours at 100°), and 4 n KOH (twice under N₂ with 2 ml for a total of 30 hours at room temperature). Polysaccharides were recovered from the extracts by precipitation with 80% ethanol (in the case of the KOH extract, after neutralization with acetic acid), or, with the dimethylsulfoxide extract, by evaporation of the solvent in a vacuum desiccator under a heat lamp. Each polysaccharide fraction, or an aliquot thereof, and an aliquot of the alcohol-soluble material after precipitation of cold-water-soluble polysaccharides, was dried down on a planchet and counted.

The cold-water-soluble polysaccharide fraction and the hot-water and ammonium oxalate extracts of the cell wall material were then treated with 0.5% EDTA (sodium salt, pH 11.5); after 1 hour the pH was brought to about 5 with acetic acid, and freshly dialyzed commercial pectinase (Nutritional Biochemicals Corporation) was added (0.2% final concentration). After 5 hours at room temperature each sample was passed through Dowex-1-acetate, and adsorbed acids were eluted with 6 n acetic acid. The material not adsorbed on Dowex-1 in the foregoing step, all remaining cell wall extracts, and a sample of the residue (α-cellulose) that had been dissolved in 72% H₂SO₄, were then hydrolyzed with 1 n H₂SO₄ for 6 hours, and separated into neutral and acidic fractions with Dowex-1-acetate (10).

An aliquot of the alcohol-soluble fraction (after precipitation of cold-water-soluble polysaccharides) was passed through Dowex 50 (H⁺) and Dowex 1 (acetate form) to remove amino acids and organic acids. These latter were eluted with formic acid and counted. The sugar fraction, which contained almost all of the alcohol-soluble radioactivity, was chromatographed (see below).

Separation and Determination of Sugars and Uronic Acids. Paper chromatography was used to separate individual sugars (9) or uronic acids [(10), solvent a] of each fraction. The labelled compounds were located by autoradiography and were identified by comparison with guide strips bearing known sugars.
Table IV. Incorporation into Cell Components during 2 Hour Incubation

Six samples of 100 coleoptile segments were each incubated 1 hour in 3 ml 0.05 M labelled (16 μC) glucose, followed by 1 hour in the same medium with or without 3 μg/ml IAA (3 samples each; mean final lengths, minus IAA 8.5 mm, plus IAA 8.8 mm). Figures in italics show total incorporation (cpm × 10⁻³), those in Roman show specific activity in cpm/μg, ± average deviation as percent of mean. Specific activity found for glucose isolated from the incubation medium was 420 cpm/μg.

<table>
<thead>
<tr>
<th>Fraction and constituent</th>
<th>Mean amount isolated μg*</th>
<th>Total activity in cpm × 10⁻³</th>
<th>− IAA</th>
<th>+ IAA ± % of mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol soluble</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>7800</td>
<td>750 ± 3.9</td>
<td>855 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>3000</td>
<td>40.5 ± 4.9</td>
<td>41.2 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Cold water soluble, alcohol insoluble</td>
<td></td>
<td>16.9 ± 4.7</td>
<td>19.1 ± 7.8</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>45</td>
<td>60.7 ± 1.7</td>
<td>62.4 ± 5.1</td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>25</td>
<td>24.6 ± 11.8</td>
<td>28.3 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>22</td>
<td>22.5 ± 2.7**</td>
<td>26.8 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Galacturonic acid**</td>
<td>&lt; 3</td>
<td>0.20 ± 46</td>
<td>0.39 ± 29</td>
<td></td>
</tr>
<tr>
<td>Hot water soluble</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>273</td>
<td>35.5 ± 4.4</td>
<td>38.8 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>32</td>
<td>21.0 ± 10.9</td>
<td>22.3 ± 6.3</td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>135</td>
<td>11.7 ± 4.3</td>
<td>12.8 ± 4.9</td>
<td></td>
</tr>
<tr>
<td>Galacturonic acid</td>
<td>104</td>
<td>7.82 ± 5.9</td>
<td>7.82 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>Gal U(1 → 2) rhamnose</td>
<td>141</td>
<td>5.00 ± 5.0</td>
<td>5.34 ± 5.4</td>
<td></td>
</tr>
<tr>
<td>Dimethylsulfoxide soluble</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>369</td>
<td>16.7 ± 2.2</td>
<td>15.4 ± 7.0</td>
<td></td>
</tr>
<tr>
<td>Arabinose**</td>
<td>52</td>
<td>12.9 ± 5.4</td>
<td>13.3 ± 8.3</td>
<td></td>
</tr>
<tr>
<td>Xylose**</td>
<td>55</td>
<td>18.9 ± 2.1</td>
<td>18.5 ± 7.5</td>
<td></td>
</tr>
<tr>
<td>Ammonium oxalate soluble</td>
<td></td>
<td>7.72 ± 5.6</td>
<td>7.82 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>30</td>
<td>40.6 ± 21**</td>
<td>41.5 ± 14</td>
<td></td>
</tr>
<tr>
<td>Galacturonic acid</td>
<td>41</td>
<td>11.5 ± 3.7</td>
<td>12.5 ± 7.2</td>
<td></td>
</tr>
<tr>
<td>4 N KOH soluble</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>640</td>
<td>71.3 ± 1.1</td>
<td>79.2 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>650</td>
<td>22.0 ± 3.2</td>
<td>23.7 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Xylose</td>
<td>860</td>
<td>12.7 ± 7.6</td>
<td>14.4 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Glu U(1 → 2) xylene</td>
<td>170</td>
<td>14.3 ± 6.8</td>
<td>15.2 ± 5.1</td>
<td></td>
</tr>
<tr>
<td>Residue (α-cellulose)</td>
<td></td>
<td>17.1 ± 2.2</td>
<td>17.5 ± 4.1</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>695</td>
<td>53.8 ± 2.4</td>
<td>55.1 ± 2.5</td>
<td></td>
</tr>
</tbody>
</table>

* Constituents were isolated from one-tenth of the sample in the case of 4 N KOH extracts, one-twelfth in the case of the alcohol soluble, about 15% in the case of the α-cellulose, and the entire sample in the remaining instances. All yields shown are calculated for the entire sample. Galacturonic acid and galacturonosyl-(1 → 2) rhamnose were isolated from pectinase hydrolysates, the remaining constituents were obtained from H₂SO₄ hydrolysates, except for the alcohol-soluble sugars.

** Due to accident, means are based on duplicate rather than triplicate samples.

*** As explained in Methods, the identity of the compound is doubtful, so the figures for total activity (which varied greatly among the replicates) are given.

† Amounts recovered for different samples were variable, so total incorporation is reported as cpm/0.611 mg, which was the mean amount recovered.

or uronic acids [for details on identification of the uronic acids, see (10)]. Representative portions of autoradiographs, illustrating the separations attained between different components, are shown in figure 1. 

The labelled compounds were eluted on to planchets with water, dried and counted. They were then dissolved and made up to a known volume with water, and aliquots (or the whole sample in the case of some minor components) were taken for determination, sugars according to (9) and uronic acids by the borax-carbazole method (3) according to (10). Replicate determinations of each sample were run insofar as the amount permitted, and the mean of the determinations, minus a paper blank similarly eluted and determined (9), was used to find the specific activity of the sample.

In the experiment of table IV the acetic acid eluates of acids adsorbable on Dowex-1 after pectinase treatment proved to contain both galacturonic acid (data quoted in table IV) and material that did not move with the solvent but remained at the origin, the latter comprising about half the activity recovered in this fraction of the hot-water and ammonium oxalate extracts and 70 to 90% in the case of the cold-water-soluble, alcohol-insoluble fraction. Hydrolysis of this material with H₂SO₄ gave labelled xylose, arabinoce,
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Fig. 1. Portions of autoradiographs of chromatograms of hydrolysates, illustrating separation attained between components. Origins are below bottom of illustration except in strip 4. Strips 1 to 3 are sugar chromatographed in butanone, acetone, water, pyridine (150: 30: 20: 1). Strip 1, hot 0.05 N H₂SO₄ soluble fraction after first development. Strip 2, glucose and galactose of same after second development (19). Strip 3, a-cellulose, after 1 development. Strip 4, uronic acids of hot 0.05 N H₂SO₄ soluble fraction, chromatographed in ethyl acetate, acetic acid, water, formic acid (18: 3: 4: 1): 0, origin; Z, 1, mixture of uronic acids not further separated in this experiment; GX, glucuronosyl(1→2)xylose; GR, galacturonosyl(1→2) rhamnose; GU, galacturonic acid (with small amount of glucuronic acid probably also present); MGX, 4-O-methylglucuronosyl(1→2) xylose; MG, 4-O-methylglucuronic acid.

Hexose and glucuronosylxylose, so it was evidently mainly glucuronoxarabinoxylan that had probably been partly degraded by pectinase (10). In the case of the pectinase hydrolysate of the cold-water-soluble, alcohol-insoluble fraction, the labelled compound that was detected in the position where galacturonic acid was expected ran slightly slower than known galacturonic acid on the guide strips and gave a negligible reaction (equivalent to less than 3 μg) in the carbazole determination. The apparent specific activity of this compound, if it were galacturonic acid, would have been at least 100 cpm per μg, which is far out of line with the specific activities of other constituents and causes us to suspect that the compound was not galacturonic acid. The amount obtained did not permit further study. Because the determination and therefore the specific activity was unreliable, the total activity found in this compound is quoted for completeness in Table IV as galacturonic acid, due to the interest that has attached to galacturonic acid of the cold-water-soluble fraction in previous work (1).

Counting. Radioactive samples were counted with a Nuclear-Chicago D-47 gas flow counter with a micromil window using Q-gas (efficiency 25%) and an automatic sample changer.

Results

Table I shows the effect of IAA on the incorporation of radioactivity into extractable fractions of the cell wall material of oat coleoptile segments whose elongation was inhibited by Ca++. The 0.025 N H₂SO₄-soluble and the 4 N KOH-soluble fractions, which consist mainly of hemicelluloses, comprise the matrix portion of the cell wall; the 72% H₂SO₄-soluble fraction is the a-cellulose (the microfibrillar portion).

Auxin caused a definite promotion of incorporation into the matrix fractions. The promotion was about the same in the sugar fraction and uronic acid fraction of the dilute-acid-soluble material, and in the sugar fraction of the alkali-soluble material, viz., 23%, 20% and 24% respectively. In contrast to the matrix fractions, auxin caused an insignificant promotion of incorporation into the a-cellulose fraction.

The specific activity of the individual sugars and uronic acids that were isolated from the different fractions in this experiment are also shown in Table I. The specific activities of all components were increased by IAA except for the glucose of the a-cellulose fraction.

Comparison Between Elongating and Ca++-Inhibited Segments. Table II gives data on cell wall incorporation by uninhibited as compared with Ca++-inhibited segments with and without IAA. As noted in the previously discussed experiment, IAA did not cause a significant increase of incorporation into α-cellulose in the Ca++-inhibited segments. However, IAA caused a large promotion of incorporation in all cell wall fractions of uninhibited segments, including α-cellulose. This latter result agrees with the finding that during wall synthesis by uninhibited coleoptile segments given glucose with or without IAA, the percentage α-cellulose in the cell wall remains approximately constant (8).

Table III gives the results of a more extensive experiment in which a different fractionation procedure was employed. This was performed in order to examine incorporation into some fractions that have been emphasized in previous work on the effect of auxin on cell wall metabolism, viz., cold- and hot-water-soluble fractions. Further fractionation of the cell wall was by methods that, unlike the extraction with hot dilute mineral acid employed in the foregoing experiments,
do not extensively degrade the hemicellulose polysaccharides and afford a better (but by no means perfect) separation of different types of polysaccharides. The major constituents of each fraction ([11], data of table IV, and unpublished experiments), are listed in table III. We also sought to determine what proportion of the effect of IAA on incorporation into the water-soluble and water-insoluble fractions might be due to incorporation of methyl and other ester groups, by ascertaining loss of activity after saponifying samples with NH₄OH.

A detectable decrease in activity upon saponification was found only in the hot-water-soluble fraction; this is shown as ester groups in table III. It amounted to less than 10% of the total activity of this fraction in all series and was therefore determined with poor precision, as reflected by the large deviations. The results show that a negligible part of the total effect of IAA on cell wall metabolism, detected in this type of experiment, is due to incorporation of ester groups.

In uninhibited segments a substantial promotion by IAA was detected in incorporation into all the fractions examined, including a-cellulose, and cold-water-soluble protein and polysaccharides. With segments inhibited by Ca⁺⁺ a substantial effect of IAA was found in all fractions except the 3 just mentioned. This suggests that the direct effect of auxin is specifically on synthesis of cell wall matrix polysaccharides, as opposed to water-soluble polysaccharides, protein, or a-cellulose.

After treatment with Ca⁺⁺, the amount of activity found in the cold-water, hot-water and ammonium oxalate-soluble fractions of segments not given IAA was substantially less than in control segments (glucose only). This does not necessarily mean that Ca⁺⁺ inhibited incorporation into the polysaccharides normally extracted by these solvents, because it is quite possible that treatment with Ca⁺⁺ altered the solubility of some polysaccharides or polyuronides.

**Fractionation of Tissue after 2-Hour Incubation in Labelled Glucose.** The experiment whose results are given in table IV was performed to obtain detailed information about the relation between cell wall incorporation and uptake into the alcohol-soluble fraction during incubation in labelled glucose, and about the effect on cell wall metabolism of a short treatment with auxin. The data also afford evidence for the composition of the various fractions examined in table III, although the figures should not be considered a quantitative analysis since recoveries were rather poor in some cases and data on minor constituents are not reported (more complete analyses of oat coleoptile polysaccharides will be published elsewhere).

In this experiment, 6 samples of 100 coleoptile segments each were incubated 1 hour in labelled glucose only, IAA was then added to 3 of the samples and incubation in labelled glucose was continued (with or without IAA) for a second hour. Table IV shows A) the total activity found in the alcohol-soluble fraction, in cold-water-soluble polysaccharides and in the extracted cell-wall fractions, and B) the amounts and specific activities of the principal constituents isolated from each fraction, after hydrolysis in the case of all but the alcohol-soluble fraction. Glucose was also isolated from the incubation medium and its specific activity was determined by the same method.

In 2 ways the data of table IV demonstrate the existence of internal compartmentation of coleoptile tissue, with respect to substrates for polysaccharide formation. First, the specific activity of glucose that was isolated from cold-water-soluble glucan was higher than the specific activity of glucose in the alcohol-soluble fraction. Second, the specific activities of glucose isolated from the major cell wall polysaccharide fractions were about 40% or more of the specific activity of alcohol-soluble glucose, even though the major polysaccharides could not have increased in amount by more than 5% during 2 hours (8, 9).

Since the mean specific activity of alcohol-soluble glucose during the period was no more than half the final value (the initial value being zero), it is certain that the glucose of major cell wall polysaccharides was not drawn from the glucose in the bulk of the alcohol-soluble fraction.

Specific activity varied markedly among different constituent monosaccharides and uronic acids. This may have been because either A) during much of the 2-hour period, isotope equilibrium had not yet been established on the pathway to cell wall constituents, or B) the composition of the new cell wall material was different from that of the wall material already present. The latter seems unlikely because it appears that the composition of the cell wall remains approximately constant during growth of oat coleoptile segments (8, 9). However, the specific activity of a given monosaccharide also differed widely in different extractable fractions of the wall, which might suggest explanation B. But alternate explanations are C) there is compartmentation of the pathways leading to different polysaccharides and the time required to attain isotope equilibrium differs for different polysaccharides even when they contain the same monomer unit, and D) the solubility characteristics of the original cell wall polysaccharides are changing with time. In the case of arabinose, xylose and glucuronosylxylose of the 4 × KOH-soluble fraction, these units are known to be constituents of a single polysaccharide (11), and it seems necessary to invoke questions of isotope equilibrium (A) to explain the data.

While the precision of the data is certainly sufficient to establish the differences in specific activity discussed above, the situation is otherwise with respect to the effect of treatment with IAA. Compared to the differences between treatments with and without auxin, the variation among replicate samples is such as to preclude laying stress on results for any single component, except to state definitely that no large increases (e.g. 2-fold) were caused by IAA. (Considering the large number of coleoptile segments in each sample this variation is probably due almost entirely to difficulties inherent in such fractionation

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of all the major matrix polysaccharides, runs between 20 and 50% of the synthesis of the cell wall as a whole.

In elongating segments (not inhibited with Ca\(^{++}\)) the effect of auxin on rate of cell wall synthesis is considerably greater, commonly up to 100% promotion, and involves a large increase in formation of \(\alpha\)-cellulose as well as of matrix polysaccharides. According to the interpretation advanced in the foregoing paper (2) the effect on \(\alpha\)-cellulose would be an indirect effect induced by elongation; the results in Table III indicate, however, that matrix polysaccharides are also involved in the indirect effect.

The results of the experiment shown in Table IV suggest that a much smaller increase in rate of cell wall metabolism, no more than 20%, takes place within the first hour of treatment of uninhibited segments with auxin. However, the experiment also demonstrates features of internal compartmentation that will lead to isotope dilution effects whenever endogenous substrates are being utilized: This seriously restricts quantitative interpretation of radioisotope experiments on cell wall synthesis, particularly experiments of short duration. For example, because auxin has been found to promote wall synthesis from endogenous substrates [(8) and references there cited], it must be anticipated that the increased transfer of sugar from a metabolically inactive pool to the site of utilization for wall synthesis may there create an increased isotope dilution of incoming labelled substrate that will tend to reduce or prevent an increase in isotope incorporation from accompanying the increased rate of synthesis.

In the experiment of Table IV, before addition of auxin the tissue was pretreated with labelled glucose for 1 hour because it appeared, on the basis of time course data [e.g. fig 1 of ref. (2)], that isotope equilibrium would be attained within 1 hour on the pathway from glucose to cell wall material. However, the various possible explanations listed above for features of the specific activity data in Table IV indicate that an influence of isotope dilution effects on the results cannot be rigidly excluded even after 1-hour pretreatment. Moreover it is easily possible that the intracellular route through which auxin promotes wall synthesis involves compartments other than those utilized in the cell wall synthesis that takes place without auxin, in which case isotopic equilibrium might be reached more slowly with respect to the IAA-promoted fraction of wall synthesis than in the remainder.

**Summary**

The auxin-induced promotion of cell wall synthesis in oat coleoptile segments whose elongation is inhibited by Ca\(^{++}\), is a general promotion of synthesis of matrix polysaccharides including glucan, glucuronorabinoxylan, and polysaccharides containing galacturonic acid. Synthesis of \(\alpha\)-cellulose glucan is not promoted. By contrast, promotion by auxin of elongation of uninhibited segments is accompanied by large increases in synthesis of all polysaccharide fractions.

**Discussion**

These data indicate that the direct promotion of wall synthesis by auxin, seen in the presence of Ca\(^{++}\), is of a rather general nature, not confined to a particular solubility fraction or to individual sugar or uronic acid components. The effect of auxin is detected throughout the components of the cell wall matrix and is lacking only in the glucose of the 72% H\(_2\)SO\(_4\)-soluble fraction (\(\alpha\)-cellulose), i.e., the microfibrillar part of the cell wall.

The auxin-induced increases in incorporation demonstrated here for glucose from matrix polysaccharides on the one hand and for galactose, arabinose, xylose and xylose-containing aldobionarionic acids on the other hand, show that auxin promotes the synthesis of both the matrix \(\beta\)-glucan and the glucuronorabinoxylan, which are the major species of polysaccharides that have been isolated from the hemicellulose fraction (11).

As a result of this work it is no longer necessary to regard the promotion of incorporation of radioactivity into methyl ester groups and galacturonic acid of cold-water-soluble and hot-water-soluble polysaccharide fractions as the sole chemically characterized effect of auxin on the cell wall (1). It is worth noting that although the direct effect of auxin on synthesis of matrix polysaccharides as a whole is, by percent, of the same magnitude as the previously demonstrated (1, 4, 5, 6, 7) effect on incorporation into hot- and cold-water-soluble pectin, in absolute magnitude the presently examined effect is enormously greater, simply because the components that are involved constitute a very much larger proportion of the cell wall. According to Albersheim and Bonner (1) and Clelland (4) the hot- and cold-water-soluble uronic anhydride totals 0.7% of the oat coleoptile cell wall; the promotion of incorporation by auxin found therein would be equivalent to an increase of about 0.35% in the rate of synthesis of the cell wall as a whole. By contrast the direct promotion by auxin of cell wall synthesis studied in the present investigation, which as demonstrated here is due to increases in synthesis procedures and to the analytical errors involved in specific activity determinations.)

However, the mean incorporation was greater in IAA by about 10% in the cold-water-soluble, hot-water-soluble, and alkali-soluble fractions, and the specific activities of major constituents isolated from these fractions were for the most part increased in about the same proportion. The general trend of increased incorporation in the IAA-treated samples is consistent enough, we feel, to warrant the conclusion that a positive effect of IAA was detected. Considering that this effect could not have existed for more than half the period of exposure to labelled glucose (the other half being the pretreatment period with glucose alone) it appears that IAA may have increased the rate of incorporation by as much as 20% during the period of treatment.
including α-cellulose. It is concluded that the direct effect of auxin is probably on metabolism of matrix polysaccharides and that the promotion of α-cellulose synthesis is induced by elongation.

Fractionation of coleoptile tissue after 1-hour treatment with auxin does not show differences in incorporation as large as are observed after several hours of treatment. However, the data also demonstrate features of internal compartmentation of the tissue that restrict the interpretation of radioisotope experiments of short duration.

Literature Cited

Relation between Effects of Auxin on Cell Wall Synthesis and Cell Elongation

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We have demonstrated (3, 16) that in the presence of adequate amounts of substrate, indoleacetic acid (IAA) causes a substantial general promotion of synthesis of the matrix polysaccharides of the cell walls of oat coleoptile tissue, which can be detected even when elongation is inhibited by Ca++ and is, therefore, due directly to the hormone and not to its effect on elongation. This paper examines the question of whether this effect of auxin on wall synthesis may play a causal role in the action of auxin on cell enlargement.

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Methods

Oat seedlings of variety Victory were raised and 8 mm long coleoptile segments were cut, dejacketed, and incubated in labeled glucose as previously described (3).

In the experiments of tables I and V the cell wall and alcohol-soluble material was prepared and counted by the method of ref. (3).

In the experiments of tables II, III, IV and VI segments were harvested and the cell wall and cold water soluble material was prepared and counted by method II described under "preparation of cell wall material" in ref. (16), except that extraction of the cell wall material with hot water was omitted.

In the experiments of figures 1 to 8 the segments after incubation were washed briefly with water, and extracted with ethanol as in ref. (3). The residue was thoroughly ground in a mortar in ethanol and then passed through a cellulose acetate filter, the cell wall material becoming spread evenly on the surface.