Direct and Indirect Effects of Auxin on Cell Wall Synthesis in Oat Coleoptile Tissue [1, 2]

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It has been observed that promotion of cell enlargement by auxin is accompanied by an increase in synthesis of cell wall material (2, 3, 5, 6, 18, 25, 26, 27). Because auxin does not cause an increase in wall synthesis in oat coleoptile segments whose elongation is inhibited with mannitol, the increase in synthesis caused by auxin in uninhibited segments has been regarded as induced by elongation rather than directly by auxin (2, 23). This kind of effect, in which a promotion of synthesis results from a promotion of elongation, we term an indirect effect of auxin.

We reported previously (20) that when elongation is inhibited by Ca^{++}, a substantial promotion by auxin of incorporation of labelled glucose into the cell wall can still be detected. This shows that auxin actually exerts a direct promotive effect on wall synthesis, direct in the sense that it is not a consequence of the promotion of elongation. Of course this effect is not necessarily a primary action of auxin.

This paper presents a more detailed comparison of the effect of IAA on wall synthesis in oat coleoptile segments in the absence and presence of Ca^{++}, which makes it possible to distinguish the occurrence of both kinds of effects on wall synthesis during auxin-promoted elongation, namely direct (due to the auxin) and indirect (due to the elongation).

Methods

Plant Material. Oats (*Avena sativa* L., var. Victory) obtained from Svenska Allmänna Utsädes A. B., Svalöf, Sweden, were husked, soaked in distilled water for 2 hours, then planted on moist filter paper supported by inclined glass slats, according to the method of Wiegand and Schrank (24). The seeds were irradiated for the first 36 to 44 hours by a 25-w ruby red bulb placed about 45 cm over the chamber, after which they remained in complete darkness (about 26°) until they were harvested. Coleoptiles 2.5 to 3.0 cm long were harvested 72 to 84 hours after planting, under dim red light, and from each a segment 8 mm long (2 mm in 1 experiment) was cut with a double-bladed cutter beginning 3 mm below the tip; the primary leaf was removed from the segment. Segments were kept in a petri dish of distilled water from the time they were cut until they were placed in the growth solutions. This period varied from 1 to 3 hours depending on the number of segments handled in the experiment. In the tables, initial length refers to length at time of transfer to incubation media.

Experimental Treatments. Unless otherwise indicated, all incubation solutions contained 0.05 M unlabelled glucose and, in those containing auxin, 3 mg per liter IAA. Uniformly C^{14}-labelled D-glucose (from Calbiochem), 30 μc per μmole, was added in amounts indicated in each experiment.

The incubation solutions were contained in stender dishes (37 mm diameter by 25 mm high) with a ground rim and cover. The ground surfaces were greased with white vaseline to prevent evaporation during the course of the experiment. Each dish contained 1 ml of growth solution and from 8 to 15 coleoptile segments. The dishes were held on a reciprocating shaker (120 oscillations/minute) in the dark at room temperature (about 23°). Usually less than 15 % of the glucose in the medium was absorbed by the tissue during the experiment.

Preparation and Counting of Samples. After the incubation period the segments were removed from the stender dishes and the adhering medium was washed off by transfering them through 2 successive petri dishes of distilled water and finally passing a stream of distilled water followed by air through the central cavity of each segment. During the washing procedure the segments were in distilled water for about 8 minutes. The segments from a single growth solution were then divided into 2 or 3 groups for subsequent extraction and counting. The segments were measured to the nearest 0.1 mm by placing them on a thin glass coverslip over 1 mm ruled graph paper and observing them with a binocular dissecting microscope. The segments were then slit down 1 side and placed in 1.5 ml of 80 % ethanol contained in a screw cap vial at room temperature, to extract alcohol-soluble compounds. After 24 hours the alcohol extracts were transferred to 1.25 inch planchets. The segments were then washed in the vial with a further 1 ml of 80 % alcohol for 1 hour, the washings being added to the initial extracts on the planchets. The segments were washed with distilled water 4 times, and were then individually unrolled on a piece of plate glass and crushed by pressing a second piece of glass on top of them.

The segments were next extracted for 24 hours in 2 ml of 2 mg per ml pepsin in 0.03 M phosphate buffer (pH 2) at 23°. This reduced their protein content from about 35 % to about 8 % of their dry weight.

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(The observed decrease in weight following pepsin extraction exceeded that which could be accounted for by the decrease in protein by about 10 %; the buffer was found to extract a small amount of radioactivity from the cell walls by itself. The activity found in the pepsin extract was about 10 % of the activity of the extracted cell wall residue.)

The segments were then washed 4 times with water, and unrolled onto a planchet previously moistened with a 10-fold dilution of Haupt's adhesive (10), and dried under a heat lamp. The adhesive prevented the segments from curling on drying, thus insuring uniform geometry and self-absorption characteristics. At this dilution the adhesive did not appreciably alter the self-absorption of the samples or the backscattering of the planchets. Four to 6 segments were placed on a single planchet. The pepsin extract was also dried on a planchet.

Planchets were counted with a Nuclear Chicago Model D 47 Gas Flow Counter with a micromil window and Q-gas; counting efficiency was about 25 % (typical sample thickness: alcohol extract, 0.21 mg/cm^2; pepsin extract, 1.57 mg/cm^2; cell wall samples, 0.60 mg/cm^2). Calculations based on sample thickness, and direct estimates obtained from placing unlabelled segments on top of labelled segments, indicated that the self-absorption of the cell wall samples was approximately 10 %. Activity figures have not been corrected for self-absorption except where indicated.

In the tables, each number shows the mean for the indicated number of replicate samples consisting of 4 to 6 segments each. Activities are given on a per sample basis. Variation among replicate samples is indicated as plus or minus the average deviation from the mean expressed as percentage of the mean. Absorbed activity is the sum of the activity found in cell walls, alcohol extracts and pepsin extracts, and does not include any activity that was lost as CO_2.

Results

Table I gives data from an experiment designed to compare gravimetrically detectable changes in amount of cell wall material with incorporation of C^{14} into the cell wall from 0.05 M labelled glucose. The changes in amount of cell wall material calculated from the incorporation and the specific activity of the glucose supplied, corresponded closely with the gravimetrically determined increases. Therefore, wall synthesis was taking place essentially at the specific activity of the glucose supplied externally, without significant isotope dilution by endogenous substrates, and the incorporation represents largely net synthesis, not turnover (see also below). Under these conditions isotope incorporation provides a direct measurement of wall synthesis independent, for example, of how much radioactivity has been absorbed by the tissue as a whole.

In a number of experiments we found that radioactivity that had previously been absorbed by coleoptile segments from labelled glucose solutions is utilized very sluggishly for cell wall synthesis. An example is given in table II. This experiment was designed to test both for utilization of internal alcohol-soluble radioactivity and for gross turnover of cell wall material.

During the 2-hour pretreatment in labelled glucose about 3500 cpm were incorporated into the cell wall and 3800 cpm taken into the alcohol-soluble fraction, whereas during the following 2 hours in unlabelled medium only about 500 cpm were added to the cell wall from the alcohol-soluble fraction; thereafter no more activity was added to the wall even though considerable activity remained in the alcohol-soluble fraction.

Comparison of the results with and without an excess of unlabelled glucose in the medium indicates that turnover of cell wall material as a whole is slight even over a 24-hour period. The figures for total cell wall activity do not, of course, preclude conversion of 1 wall polysaccharide into another. To investigate this replicate samples for each treatment and time of sampling were combined, extracted successively with hot 0.05 N H_2SO_4, 4 N KOH at room temperature, and 72 % H_2SO_4, as described by Ray (19), then hydrolyzed with 1 N H_2SO_4 for 6 hours at 100° and separated into a neutral (sugar) and acidic (uronic acid) fraction using Dowex-1 (acetate form) (21). Each fraction was evaporated on a planchet and counted.

A significant decline in activity was observed only in the sugar fraction of the material soluble in hot 0.05 N H_2SO_4, and the data for this fraction are therefore included in table II. This is the fraction in which a substantial decrease in the amounts of glucose and galactose has been observed when coleop-

Table I. *C^{14} Glucose Incorporation and Increase in Mass of Cell Wall Material*

Each treatment involved 32 8-mm segments incubated 24 hours in 0.05 M glucose containing 1.67 μc/ml; extracted and counted as 8 replicate samples of 4 segments each; cell wall samples combined by pairs, weighed as 4 replicate samples of 8 segments each, and compared with 4 similar initial samples (mean weight 999 μg ± 4 %) to determine increase in weight of cell wall material. Data are expressed on an 8-segment basis.

	IAA	
	None	3 μg/ml
Final length of segments, mm	10.6	15.7
Observed increase in cell wall μg ± % of mean	199 ± 7	331 ± 6
Cell wall activity cpm ± % of mean	23,800 ± 4	41,200 ± 16
Calc. increase in cell wall* μg ± % of mean	188 ± 1	330 ± 8
Total activity absorbed cpm ± % of mean	142,000 ± 3	193,400 ± 14

* Calculated from specific activity of glucose in medium (as anhydroglucose), and activity of cell wall material corrected for 10 % self-absorption.

Table II. *Utilization of Previously Absorbed Activity*

Segments (210) were kept 2 hours in 15 ml of 0.002 M labelled glucose (4.0 μc), then washed and transferred (zero time) to 3 mg/liter IAA with or without 0.05 M unlabelled glucose, and harvested after the incubation periods shown. Each figure is for 6 replicate samples of 5 segments each; the cell wall samples were combined for extraction with hot 0.05 N H_2SO_4 but the figures are given on a 5 segment basis to be comparable with the rest.

Incubation period hr	0.05 M unlabelled glucose	Length mm	Cell walls cpm ± % of mean	Sugars of 0.05 N H_2SO_4 soluble wall material cpm	Alcohol soluble cpm ± % of mean	Total activity* cpm ± % of mean
0	...	8.8	3,450 ± 17	2,180	3,800 ± 10	7,530 ± 13
2	—	9.7	3,910 ± 9	2,050	2,960 ± 5	7,340 ± 7
	+	9.7	3,940 ± 12	2,430	3,370 ± 6	7,690 ± 9
8	—	10.7	3,900 ± 14	1,950	2,660 ± 12	7,180 ± 13
	+	11.2	4,170 ± 15	2,200	2,960 ± 11	(7,500) ± 11**
24	—	11.2	3,570 ± 8	1,450	2,610 ± 8	6,790 ± 8
	+	13.0	3,330 ± 19	1,680	2,450 ± 10	6,420 ± 15

* Includes some activity lost to medium (<7 % of total activity even after 24 hr).

** Total activity found was 8,300 cpm, indicating excess absorption or sample size. All figures for this sample have been multiplied by 7500/8300 to put them on a basis comparable with the other samples.

tile segments are maintained in a medium that does not contain sugar (19). The decline in activity in this fraction is probably due to the same phenomenon, although in the present experiment the individual sugar components were not studied. The decrease in activity in this fraction accounts at least approximately for the decline in activity of the cell wall as a whole; it is evident from the data that the rate of this turnover effect is very small compared to the rate of incorporation of new polysaccharide material when 0.05 M glucose is present in the external medium.

Promotion by Auxin. The effect of IAA on uptake of activity and incorporation into the cell wall at several $CaCl_2$ concentrations is shown in table III. Promotion of elongation and wall synthesis by IAA in uninhibited segments (no Ca^{++}) was always accompanied by an increase in uptake of C^{14}. As elongation was inhibited by increasing concentrations of $CaCl_2$, the effect of IAA on uptake disappeared, but promotion of cell wall incorporation by IAA persisted. This indicates that the increase in glucose uptake caused by IAA in uninhibited segments is an indirect effect of IAA, i.e., a consequence of its effect on elongation, whereas the action of IAA on cell wall incorporation in the presence of sufficient Ca^{++} to inhibit elongation is evidently a direct effect. To what extent this direct effect contributes to the larger effect of IAA on wall incorporation in uninhibited segments remains to be determined.

Table III. *Effect of $CaCl_2$ on Uptake and Wall Incorporation*

Four replicate samples of 5 segments were incubated 7 hours in 0.05 M glucose (0.84 μc/ml) with or without IAA and $CaCl_2$. Initial length was 8.4 mm.

$CaCl_2$ M	IAA 3 μg/ml	Final length mm	Cell wall activity cpm ± % of mean	Absorbed activity cpm ± % of mean
0	—	9.7	2,860 ± 7	14,300 ± 5
	+	12.6	6,400 ± 2	26,200 ± 9
0.01	—	8.8	2,730 ± 10	13,700 ± 6
	+	9.2	4,460 ± 5	15,100 ± 3
0.02	—	8.7	2,720 ± 17	12,700 ± 1
	+	8.9	3,850 ± 10	13,200 ± 9
0.03	—	8.6	2,300 ± 14	12,300 ± 7
	+	8.7	3,660 ± 8	12,900 ± 5

The effect of IAA on cell wall synthesis was also examined in a medium containing 0.003 M maleate buffer, pH 4.7, without or with 0.01 M Ca^{++}. The results are noted as experiment 12 in table VII; a substantial promotion by IAA of cell wall incorporation, similar to that in the experiment already cited, was observed.

Figure 1 shows the time course of uptake and incorporation in Ca^{++}-inhibited segments. Promotion of wall synthesis by IAA became apparent within 2 hours. Rate of incorporation during the first hour was less than during subsequent hours, indicating that during much of the first hour isotope equilibrium had not been reached on the pathway from glucose to cell wall material.

Effect of Glucose Concentration. Table IV shows the effect of varying the concentration of externally supplied glucose on the uptake and wall incorporation with and without IAA in Ca^{++}-inhibited segments. Since, in this experiment, the specific activity of glucose was different at different glucose concentrations, the activities of all samples have been expressed as weights of glucose absorbed or incorporated, based on the specific activity of the glucose supplied.

As the external glucose concentration was decreased, the amount of glucose that was incorporated into the cell wall from the medium decreased greatly over the whole range of concentrations tested. The rate of incorporation varied less than in proportion to external concentration, since a 10,000-fold change in glucose concentration caused only a 300-fold change in incorporation. The amount of incorporation cor-

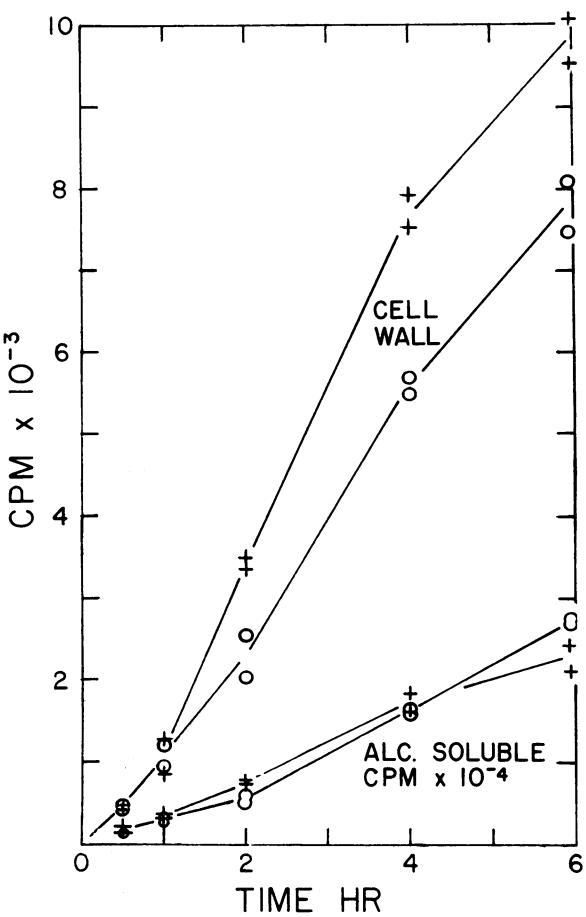


FIG. 1. Time course of uptake into alcohol-soluble fraction and incorporation into cell wall, in oat coleoptile segments in 0.05 M C^{14}-glucose (1.67 μc/ml) containing 0.01 M $CaCl_2$, without IAA (circles) and with 3 mg/liter IAA (crosses). Duplicate lots of 5 segments at each time of sampling. Note that alcohol-soluble activity is shown on one-tenth the scale used for cell wall activity.

related more closely with the amount of glucose absorbed. The relation was not exactly proportional, since incorporation, as percent of absorbed activity, rose from about 15 % at the highest glucose concentration to about 60 % at the lowest concentration.

The maximum promotion of cell wall incorporation by IAA was found at glucose concentrations of 0.01 to 0.10 M. At 0.25 M there was a substantial decrease in the promotion by IAA while at 2.67×10^{-5} M glucose the promotion appeared to be greatly reduced or absent.

Effect of Segment Length. In order to assess what factors determine the rate of cell wall synthesis in uninhibited segments it is important to have information on how uptake and wall incorporation in coleoptile segments might vary with the length of the segment. Coleoptile segments of different lengths were obtained either by differential elongation between initially identical groups of segments, brought about by pretreatment with or without IAA, or by cutting segments of different initial lengths.

Table V shows results of an experiment where coleoptile segments initially 8.0 mm long were kept for 8 hours in water or IAA so as to produce segments of different lengths which were then allowed to absorb and incorporate labelled glucose. During the incorporation period Ca^{++} was present to inhibit further elongation and IAA was added to ensure the same auxin effect in all the segments. The amounts of activity absorbed and incorporated into the cell walls were larger in the longer segments even though all the segments had equal numbers of cells. The uptake and incorporation per mm of length were, however, similar in the longer and shorter segments. This indicates that the length of the segment affects both wall synthesis and uptake of sugar proportionally. Note that the absorption of unlabelled glucose during the pretreatment period had essentially no effect on subsequent incorporation of labelled glucose into the cell wall.

A comparison of uptake and incorporation between

Table IV. *Effect of Glucose Concentration*

Each figure is for 3 samples of 5 segments each, incubated 7 hours in 1 ml 0.02 M $CaCl_2$ containing 0.80 μc of C^{14}-glucose, and unlabelled glucose to give the indicated chemical concentration, with or without 3 mg/liter IAA. Activity in cpm converted to equivalent weights of anhydroglucose in μg.

Glucose M	IAA 3 μg/ml	Cell wall incorporation μg ± % of mean	ratio	Absorbed glucose μg ± % of mean
0.25	—	151 ± 10	1.18	1,030 ± 10
	+	177 ± 4		918 ± 4
0.10	—	95.4 ± 2	1.39	615 ± 6
	+	133 ± 10		652 ± 8
0.05	—	66.5 ± 8	1.42	364 ± 10
	+	94.9 ± 7		371 ± 7
0.01	—	27.8 ± 8	1.39	105 ± 8
	+	38.7 ± 13		111 ± 12
0.001	—	8.27 ± 10	1.30	18.4 ± 4
	+	10.7 ± 10		19.2 ± 6
2.67×10^{-5} M	—	0.51 ± 5	0.98	0.96 ± 4
	+	0.50 ± 4		0.81 ± 2

Table V. *Effect of Segment Length on Uptake and Incorporation*

Segments were pretreated 8 hours in solutions with or without 0.05 M unlabelled glucose and/or 3 μg/ml IAA, then incubated in 0.05 M glucose (1.67 μc/ml) containing 0.01 M $CaCl_2$ and 3 mg/liter IAA for 24 hours. Each treatment contained 6 replicate samples of 5 segments each. Mean initial length 8.2 mm.

Pretreatment		Length after pretreatment	Final length	Cell wall activity cpm		Absorbed activity cpm		Percentage incorporation*
Glucose	IAA	mm	mm	± % of mean	cpm/mm	± % of mean	cpm/mm	± % of mean
+	+	12.4	12.5	17,100 ± 9	1,370	98,600 ± 6	7,920	17.4 ± 5
—	+	12.2	12.2	16,200 ± 24	1,330	102,000 ± 17	8,360	15.7 ± 8
+	—	9.1	9.8	13,000 ± 22	1,380	68,000 ± 17	7,190	19.0 ± 6
—	—	9.1	9.7	12,000 ± 11	1,280	68,200 ± 11	7,250	17.7 ± 5

* Cell wall activity as percent of absorbed activity.

segments which were cut initially to lengths of 2 mm and 8 mm is shown in table VI. In this case uptake and incorporation per mm of length was about twice as great in the short segments as in the long ones. This is probably because glucose penetrates more rapidly through the cut ends of the segment than through the epidermis, as suggested by Ochs and Pohl (14).

Discussion

Three ways by which elongation might cause an increase in wall synthesis may be considered: A) The rate of wall synthesis might depend directly on the surface area of the cell walls, as suggested by Busse (5). B) Elongation might open up new sites for wall synthesis, as discussed by Heyn (9). C) Elongation might cause an increase in uptake of sugar, and thereby increase wall synthesis, as proposed by Ochs and Pohl (14).

Evidence for A might be seen in the proportional relation between segment length and wall synthesis in table V. On the other hand these data are contrary to B since according to it, wall synthesis should occur in proportion to elongation: the elongation of the IAA-pretreated segments in table V was more than 4 times that of the segments not pretreated with IAA, whereas the cell wall synthesis was only about 30 % greater in the former group. Moreover, the fact that pretreatment with unlabelled glucose did not inhibit wall synthesis in the experiment of table V is against B, because much more rapid wall synthesis is known to take place when glucose is added than when it is not (18). This synthesis should have filled up, at least in part, the hypothetical new sites created by elongation. Therefore, we regard B as disproved.

As pointed out already, tables IV and VI show a strong positive correlation between uptake of activity and incorporation into the cell wall. The data in table VI show that cell wall synthesis will vary when the rate of uptake varies even without differences in length of the cells or in external concentration of glucose. The nearly proportional relation between uptake and incorporation, established by the foregoing evidence, is also followed closely in table V. Thus increase in uptake accounts completely for the increased incorporation in the segments that had increased in length by growth (table V). The data indicate, therefore, that there is no independent effect (A) of wall area on rate of wall synthesis.

As noted previously, promotion by auxin of elongation in uninhibited segments is always accompanied by an increase in glucose uptake. In such segments a substantial indirect effect of auxin or cell wall incorporation, due to the increase in uptake, must therefore occur. The data given in table I show that this is a real increase in wall synthesis, not merely an incorporation artifact resulting from an increased specific activity of internal pools.

We can conclude from the results that the correlation observed between uptake of labelled glucose and incorporation into the cell wall is not a correlation between incorporation rate and amount of isotope already taken up, but between incorporation rate and rate of uptake. Figure 1 shows that the rate of incorporation remains nearly constant while the total absorbed activity rises by a large factor, during incu-

Table VI. *Uptake and Incorporation in Segments Cut 2 mm and 8 mm Long*

Duplicate samples of 5 segments each were incubated 6 hours in 0.05 M glucose (2.5 μc/ml) with or without 3 mg/liter IAA.

Initial length mm	IAA	Final length mm	Cell wall activity cpm ± % of mean	cpm/mm	Absorbed activity cpm ± % of mean	cpm/mm	Percentage incorporation* ± % of mean
2.0	—	2.7	2,530 ± 1	1,050	15,600 ± 8	6,500	16.3 ± 8
	+	3.1	3,250 ± 2	1,250	16,300 ± 4	6,270	20.1 ± 6
8.0	—	9.9	3,830 ± 2	417	28,700 ± 8	3,120	13.4 ± 6
	+	12.8	6,000 ± 13	555	30,700 ± 13	2,870	19.5 ± 1

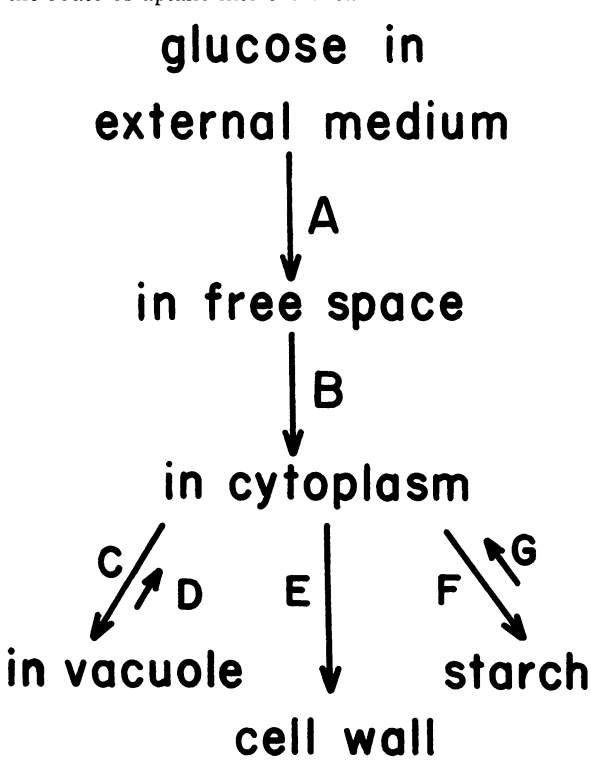
* Cell wall activity as percent of absorbed activity.

bation in a medium containing C^{14}-glucose. Table II shows that previously absorbed activity is incorporated sluggishly compared with the incorporation that took place while the aforementioned activity was being absorbed.

After incubation of coleoptile tissue in labelled glucose, most of the alcohol-soluble radioactivity of the tissue is contained in glucose [see ref. (21)]. Moreover, as shown by data in table IV of reference 21, glucose that enters the alcohol-soluble fraction undergoes a large isotope dilution, due to unlabelled glucose already within the tissue. However, this dilution evidently does not affect substantially either the rate of cell wall incorporation or the specific activity of the material incorporated (table I), when substrate levels of glucose are supplied in the external medium over several hours.

These facts indicate the existence of substrate compartmentation within coleoptile tissue, that permits utilization of externally supplied glucose without passing through and dilution by the bulk of the alcohol-soluble pool. Further evidence for such compartmentation, with respect to polysaccharide formation, is given by data in table IV of reference 21 and is discussed there.

These conclusions agree with those reached, on other grounds, about internal compartmentation in a variety of plant tissues, including coleoptiles (8, 11, 12, 13). Since the bulk of the alcohol-soluble fraction of coleoptiles is doubtless the vacuolar contents, the present observations concerning wall incorporation can be explained simply by the fact that externally supplied glucose is utilized for cell wall synthesis along the route of uptake into the vacuole:



This also affords an explanation of the nearly proportional relation between rates of glucose uptake and incorporation that is involved in the indirect effect. Any factor that increases transport at A—such as increase in external concentration, a change in tissue geometry (e.g. segment length), or the simple influx of external solution that takes place during elongation —will raise the internal concentrations governing transport at B and C and thereby promote uptake. Evidently the kinetic characteristics of the system are such that even when the tissue is in a rather concentrated external medium, the level of glucose in the cytoplasm is low enough that not only transport at C, but also rate of utilization for wall metabolism (E), are strongly concentration-dependent (this could be because of vigorous active transport at C in company with rather sluggish diffusion at A). Thus, any circumstance that increases influx into the free space raises the level of glucose in the cytoplasm and increases the rates of both transport into the vacuole (C) and wall synthesis (E) in proportion.

That oat coleoptile segments, like other plant tissues (7), carry on active transport of glucose is indicated A) by inhibition of uptake by dinitrophenol and respiratory inhibitors (1), and B) by the occurrence of accumulation. For example from the data of table IV it is easily computed, using the average fresh weight of about 6 mg per 8-mm coleoptile segment (22), that substantial internal accumulation of glucose occurred in the segments treated with 10^{-2} to 10^{-3} M glucose; we have made similar observations repeatedly.

Although processes D and G, providing endogenous substrates for cell wall formation, appear to be relatively unimportant when substrate levels of glucose (> 0.01 M) are supplied in the external medium, it is clear that endogenous substrates are utilized for wall synthesis to some extent when external sugar is not supplied (3, 18). This must be true also when low concentrations of sugar ($\leq 10^{-3}$ M) are furnished in the external medium in tracer experiments, for the rate of sugar incorporation from such media is small compared with the rate at which endogenous substrates can be utilized. Under these circumstances a substantial isotope dilution of the tracer must be occurring. And in this event incorporation of radioactivity into the cell wall cannot correctly be taken as a measure of cell wall synthesis unless the specific activity of the material being deposited can be determined for each separate experimental condition or treatment.

For this reason we feel that a number of experiments in the literature, in which auxin did not promote incorporation into the cell wall of labelled sugar supplied in low concentrations (4, 15, 16, 17), do not show that auxin did not affect the rate of wall synthesis.

Evaluation of Direct and Indirect Effects in Elongating Segments. We would like to estimate the magnitudes of the direct and indirect effects of auxin on wall synthesis in elongating segments on the basis of the foregoing evidence that the indirect

effect is due to changes in the rate of sugar uptake during elongation.

Let c^+ and c^- represent incorporation into the cell wall, and a^+ and a^- represent glucose absorption, with (+) or without (−) auxin. Since the rate of incorporation is nearly proportional to rate of glucose uptake, the increase in incorporation Δc^i (over the control level c^-) that should take place in auxin as a result of the indirect effect is $\Delta c^i = c^- (a^+/a^- - 1)$. As a fraction of the control incorporation the indirect effect should thus be $\Delta c^i/c^- = (a^+/a^- - 1)$. The direct effect Δc^d of auxin, i.e. that part of the total auxin-induced increase in incorporation $c^+ - c^-$ that is not due to the indirect effect, should be $c^+ - c^- - \Delta c^i$ or, as a fraction of the control incorporation, $\Delta c^d/c^- = (c^+/c^-) - (a^+/a^-)$.

In table VII such data are collected from the experiments presented here, in other papers of this series, and in several other similar experiments as noted. Included also is the effect of IAA on cell wall incorporation in segments whose elongation was inhibited by Ca^{++}, where determined in the same experiments.

It appears from these figures that in most experiments the direct and indirect effects of auxin are about equal in uninhibited coleoptile segments. The actual values vary considerably among the experiments, but incubation time and conditions were not the same in all cases and of course the growth rate and auxin sensitivity of coleoptile segments varies among different lots of seedlings even with the same incubation conditions. However, in most instances the direct effect seen in segments whose elongation was inhibited by Ca^{++} was similar to the direct effect estimated for uninhibited segments in the foregoing manner. This supports the interpretation of the indirect and direct effects explained above.

The possibility that a separate small indirect effect, unrelated to rate of uptake, occurs in oat coleoptile tissue is suggested by the following facts. In the second paper of this series (21) it is shown that the direct effect of auxin, seen in the presence of Ca^{++}, is on synthesis of matrix polysaccharides and not on α-cellulose. Ray (18) found that formation of α-cellulose (from endogenous substrates) by segments that were not supplied with sugar, and whose elongation was not inhibited by Ca^{++}, was promoted slightly by auxin. It is possible, though not proved, that this is an indirect effect, and of course it could not be due to an increase in uptake from the external medium. In any case the effect was small compared to the indirect effect that occurs in auxin-treated segments elongating in the presence of substrate amounts of external sugar, and so the former effect would probably have little influence on the interpretation of the present experiments.

Table VII. *Auxin Effects on Cell Wall Synthesis*

Figures show the increases caused by IAA, as a fraction of the control value, in total incorporation into the cell wall ($c^+/c^- - 1$), uptake of activity ($a^+/a^- - 1$), and the difference between these 2 ($c^+/c^- - a^+/a^-$) which is considered a measure of the direct effect of auxin on wall synthesis. Experiments which are not followed by a reference to Literature Cited are from tables in this paper except as noted.

Expt. no.	Segments without Ca^{++}			With Ca^{++}	Ref.
	Total effect $\frac{c^+}{c^-} - 1$	Indirect $\frac{a^+}{a^-} - 1$	Direct $\frac{c^+}{c^-} - \frac{a^+}{a^-}$	$\frac{c^+}{c^-} - 1$	
1	0.73	0.36	0.37	...	Table I
2	1.24	0.83	0.41	0.38	Table III (0.02 M Ca)
3	0.56	0.07	0.49	...	Table VI (8 mm)
4	0.46	0.16	0.30	0.23	(1) Table II
5	0.51	0.18	0.33	0.30	(1) Table III (Sr^{++})
6	1.05	0.34	0.71	...	(1) Table IV expt. 1
7	0.57	0.31	0.26	...	(1) Fig. 3
8	0.67	0.49	0.18	0.23	(1) Fig. 4
9	0.71	0.23	0.48	0.43	(20) Table 1
10	0.92	0.36	0.56	0.37	See note below*
11	0.80	0.22	0.58	0.37	(21) Table III
12	0.85	0.44	0.41	0.50	See note below**
13	0.61	0.36	0.25	0.27	See note below***
Mean	0.75	0.34	0.41	0.34	

* Treatments and conditions the same as expt. 9.

** Six replicate samples of 5 segments for each treatment incubated 9 hours in a total of 3 ml of 0.05 M glucose (1.67 μc/ml) containing either K maleate or Ca maleate, 0.003 M, pH 4.7, with and without 3 μg/ml IAA. In the case of Ca maleate the Ca^{++} concentration was made 20 meq/liter by addition of $CaCl_2$. Figure given for IAA promotion in Ca^{++} is $c^+/c^- - a^+/a^-$, since absorbed activity was 17 % greater in IAA owing to incomplete (80 %) inhibition of elongation by Ca maleate.

*** Duplicate samples of 20 segments incubated 8 hours in 2 ml 0.05 M glucose (0.625 μc). Cell wall material was prepared and counted by method III of ref. (1).

Summary

When substrate amounts of glucose are supplied in the medium, indoleacetic acid causes an increase of 20 to 50 % in the rate of gross cell wall synthesis in oat coleoptile segments whose elongation is inhibited by Ca^{++}, showing that the promotive effect on synthesis is due directly to hormone action and not simply the result of elongation. Evidence is presented to show that, in addition, elongation of coleoptile segments promotes their cell wall synthesis. This indirect effect appears to occur because the rate of absorption of glucose from the medium is increased by elongation, and the rate of wall synthesis is strongly dependent on glucose concentration at internal sites of utilization even when high glucose concentrations are supplied in the external medium. In elongating segments treated with auxin the indirect effect on wall synthesis appears to be of about the same magnitude as the direct effect of auxin.

The data indicate that utilization of sugar for wall synthesis takes place along the route of uptake rather than from sugar already in the bulk of the alcohol-soluble fraction of the tissue.

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