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.

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Relation between Effects of Auxin on Cell Wall Synthesis and Cell Elongation [1, 2]

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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.

[1] Received March 12, 1964.

[2] Supported by National Science Foundation grants G-8705 and 23769, and by a Newcombe Fellowship to D. B. Baker. Some of the experiments were performed at the Botanisches Institut, Tübingen, Germany, during tenure of a NSF postdoctoral fellowship by D. B. Baker. We thank Professor Erwin Bünning for the facilities and for his interest in this work.

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Methods

Oat seedlings of variety Victory were raised and 8 mm long coleoptile segments were cut, deleafed, 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

Table I. *Effect of IAA Concentration*

Figures give means and average deviations (in percent of mean) for 4 replicate samples of 5 segments each, incubated 7.5 hours in 0.05 M glucose (0.8 μc/ml) containing 0.02 M $CaCl_2$ and IAA at the concentrations indicated. The last column gives mean elongation for 10 segments (initially 8.3 mm long) incubated for the same period in 0.05 M glucose at different concentrations of IAA without $CaCl_2$.

IAA mg/liter	Cell wall incorporation cpm	Uptake cpm	Elongation in Ca^{++} mm	Elongation without Ca^{++} mm
0	2770 ± 6 %	15800 ± 7 %	0.1	1.1
0.03	3390 ± 8	15700 ± 7	0.3	2.8
0.30	3680 ± 14	15800 ± 14	0.3	3.6
3.0	4350 ± 18	17000 ± 15	0.5	3.1
30	2740 ± 9	15400 ± 8	0.4	2.8
300	375 ± 9	6110 ± 10	0.1	0.2

of the filter. The filter was allowed to dry on a planchet coated with Haupt's adhesive and was then counted, the side bearing the cell wall material being exposed to the counting element. Plating out of the material on the filter was found not to influence the efficiency of counting as compared with plating on a planchet.

In all instances uptake denotes the sum of cell wall activity and soluble activity extracted from the segments, and does not include any activity that was lost as CO_2.

Counting efficiency was approximately 20 %.

Results

Effect of Auxins. The effect of IAA concentration on wall synthesis and elongation of segments is shown in table I. In this experiment synthesis was measured in Ca^{++}-inhibited segments so as to avoid indirect effects associated with elongation, and elongation was also measured in controls lacking Ca^{++}. At 0.03 mg/liter, the lowest concentration of IAA used, a definite promotion of synthesis was observed, as well as a promotion of elongation. The largest promotions of synthesis occurred at 0.3 and 3.0 mg/liter IAA. At 30 mg/liter and above, inhibition of elongation and a strong inhibition of cell wall synthesis occurred.

The effect of several auxin analogues on elongation (without Ca^{++}) and cell wall incorporation (when elongation was inhibited with Ca^{++}) is shown in table II. Only those compounds that caused a significant promotion of elongation (IAA, indolepropionic acid, 2,4-D, 4-chloro-2-methylphenoxyacetic acid) induced an increase in cell wall incorporation above the control.

Effects of Inhibitors. Table III gives results of an experiment in which the effect of IAA on cell wall incorporation was tested in the presence of divalent cations, inhibitory to elongation, other than Ca^{++}. A comparable promotion by IAA was obtained when growth was inhibited by Sr^{++}, but only a weak one in the presence of Mg^{++} and none in the presence of Ba^{++}.

We previously reported (15) that promotion of wall synthesis by auxin could not be observed when elongation was inhibited by mannitol. Table IV gives results of an experiment in which the effect of IAA was tested in the presence of mannitol and several other presumably osmotic inhibitors of elongation, and also in the presence of Ca^{++} for comparison.

Table II. *Effect of Different Auxins and Auxin Analogs*

For each treatment 45 segments 8 mm long were incubated 5 hours in 1.5 ml 0.05 M labeled glucose (0.66 μc/ml) containing 0.02 M $CaCl_2$ and one of the indicated compounds (25 mg/liter). Means and average deviations (in percent of means) are for 3 replicate subsamples of 15 segments each. Elongation was measured for samples of 10 segments incubated 5 hours in the same media without $CaCl_2$.

Growth substance	Elongation mm*	Cell wall incorporation cpm	Uptake cpm
None	0.6	7040 ± 5.5 %	35600 ± 3.0 %
Indole-3-acetic acid	1.8	8630 ± 5.0	36800 ± 5.3
Indole-3-propionic acid	0.8	7720 ± 1.2	38200 ± 2.6
2,4-Dichlorophenoxyacetic acid	1.2	7890 ± 9.3	39200 ± 5.5
2,6-Dichlorophenoxyacetic acid	0.4	6700 ± 1.9	37900 ± 2.0
2-Chlorophenoxyacetic acid	0.4	6510 ± 5.1	35200 ± 3.9
4-Chloro-2-methylphenoxyacetic acid	1.8	7940 ± 1.4	36600 ± 4.0
Phenylacetic acid	0.6	6830 ± 4.5	37800 ± 3.5

* Without Ca^{++}.

Table III. *Effect of IAA in Presence of Divalent Cations Other than Ca^{++}*

Thirty segments initially 8.4 mm long were incubated 4 hours in 1.4 ml 0.05 M labeled glucose (0.75 μc/ml) containing the indicated additions. Means and average deviations are for triplicate subsamples of 10 segments each.

Salt	Conc M	IAA 3 mg/liter	Elongation mm	Cell wall incorporation cpm ± % of mean	Uptake cpm ± % of mean
None	—	—	0.8	4220 ± 11.7	30300 ± 8.0
		+	2.3	6360 ± 8.2	35800 ± 2.0
$SrCl_2$	0.02	—	0.1	3590 ± 2.0	24900 ± 3.4
		+	0.4	4670 ± 1.9	23800 ± 4.8
$MgCl_2$	0.06	—	0.1	1790 ± 7.6	21700 ± 5.4
		+	0.2	2040 ± 2.0	19900 ± 4.9
$BaCl_2$	0.02	—	0.04	3170 ± 5.4	22070 ± 9.6
		+	0.6	3200 ± 2.0	22900 ± 7.7

Table IV. *Effect of Osmotic Inhibitors as Compared with Ca^{++}*

Thirty segments 8.9 mm long were incubated 4 hours in 1.4 ml of 0.05 M labeled glucose (0.92 μc/ml) containing the indicated inhibitors with or without 3 mg/liter IAA. Mean elongation for all groups of segments was less than 2 % during the period. Means and average deviations are for triplicate subsamples of 10 segments each.

Inhibitor	Conc	Cell wall incorporation cpm ± % of mean		Uptake cpm ± % of mean	
		− IAA	+ IAA	− IAA	+ IAA
$CaCl_2$	0.021 M	3300 ± 5.8	3750 ± 2.5	21400 ± 7.6	21100 ± 0.5
Mannitol	0.24 M	1210 ± 5.4	1350 ± 10.3	22600 ± 5.9	20350 ± 0.4
Cellobiose	0.24 M	1270 ± 11.8	1070 ± 5.6	20200 ± 3.5	19800 ± 2.9
Raffinose	0.24 M	845 ± 6.5	705 ± 7.1	19400 ± 9.6	20700 ± 1.9
Carbowax 4000	14 % w/v*	1540 ± 6.5	1430 ± 6.3	22200 ± 5.6	20000 ± 9.6
KCl	0.126 M	1480 ± 5.7	1810 ± 4.1	25000 ± 1.7	26000 ± 6.0

* 0.24 Osmolar, by freezing point depression.

It may be noted that all the osmotic inhibitors depressed cell wall synthesis, but not glucose uptake, very substantially as compared with that which occurs when elongation is inhibited instead by Ca^{++}. With none of the carbohydrates (including the polyethyleneglycol Carbowax) was a substantial promotion of cell wall incorporation by IAA obtained. However, a promotion by IAA was found in the presence of KCl; this effect was obtained again in a repetition of the experiment.

Table V and figures 1 to 4 summarize a number of experiments in which we examined the effects of

Table V. *Effects of Metabolic Inhibitors*

Segments were incubated 7.5 hours in 0.05 M glucose (0.84 μc/ml) with or without 3 mg/liter IAA and other additions as indicated. Counts and average deviations are for 4 or more replicate samples of 5 segments each.

Expt.	Treatment		Elongation mm		Uptake cpm ± % of mean		Cell wall incorporation cpm ± % of mean	
			− IAA	+ IAA	− IAA	+ IAA	− IAA	+ IAA
1	Control		1.4	4.6	18700 ± 4	25100 ± 6	3080 ± 4	6310 ± 5
	DNP*	7×10^{-5} M	0.1	0.1	6180 ± 14	6570 ± 12	230 ± 27	250 ± 36
	NaN_3	3×10^{-4} M	0.8	0.6	5650 ± 17	6390 ± 1	265 ± 2	225 ± 7
2	Control		0.9	—	22900 ± 8	—	4310 ± 3	—
	$CaCl_2$ 0.01 M		0.1	0.6	20500 ± 8	22100 ± 11	3980 ± 11	5950 ± 5
	$HgCl_2$	5×10^{-5} M	0.2	1.5	12800 ± 4	14900 ± 5	3020 ± 7	4260 ± 5
		2×10^{-4} M	0	0.1	7630 ± 9	6460 ± 18	1150 ± 7	1210 ± 20
	$NaAsO_2$	3×10^{-5} M	0.5	2.5	21100 ± 7	22300 ± 4	3740 ± 8	6200 ± 8
		6×10^{-5} M	0.2	1.6	16600 ± 6	18300 ± 3	3600 ± 7	5000 ± 1
	NaF	5×10^{-3} M	0.7	0.8	18100 ± 3	21900 ± 9	3740 ± 10	3870 ± 10
3**	Control (22°)		2.4	7.5	44400 ± 3	60500 ± 14	7440 ± 4	12900 ± 16
	2°		1.2	1.4	8000 ± 6	7690 ± 8	1260 ± 6	12[illegible]0 ± 8

* 2,4-Dinitrophenol.

** Incubation was for 24 hours in this experiment.

metabolic inhibitors, as compared with Ca^{++}, on the action of IAA on elongation and wall metabolism. Inhibition, by metabolic inhibitors, of elongation and the effect of auxin thereon was in all cases associated with inhibition of the promotive effect of auxin on wall synthesis. Dinitrophenol, azide, and galactose depressed growth and wall synthesis more or less in parallel, both without and in the presence of auxin. Fluoride and arsenite, on the other hand, had (like Ca^{++}) relatively little effect on wall synthesis in the absence of auxin, but (unlike Ca^{++}) at concentrations inhibitory to growth they inhibited the promotion of wall synthesis by auxin.

Figures 5 to 8 show effects of some inhibitors of protein and nucleic acid metabolism. Ethionine, chloramphenicol, puromycin and actinomycin all reduced elongation, wall synthesis and the effect of auxin on both of these more or less in parallel. They also depressed glucose uptake; this recalls the effect of chloramphenicol on ion transport by plant tissue (7, 20).

At higher concentrations of several of the inhibitors (fig 2, 3, 5–8) some promotion of elongation was observed with IAA even though its promotive effect on wall incorporation had been eliminated. A similar observation was made when growth and wall synthesis were tested at 2° (table V, expt. 3). In these instances the promotion by auxin of elongation was, however, relatively small compared with the increase in elongation that auxin causes in the absence of inhibitors.

In a separate experiment we looked at wall syn-

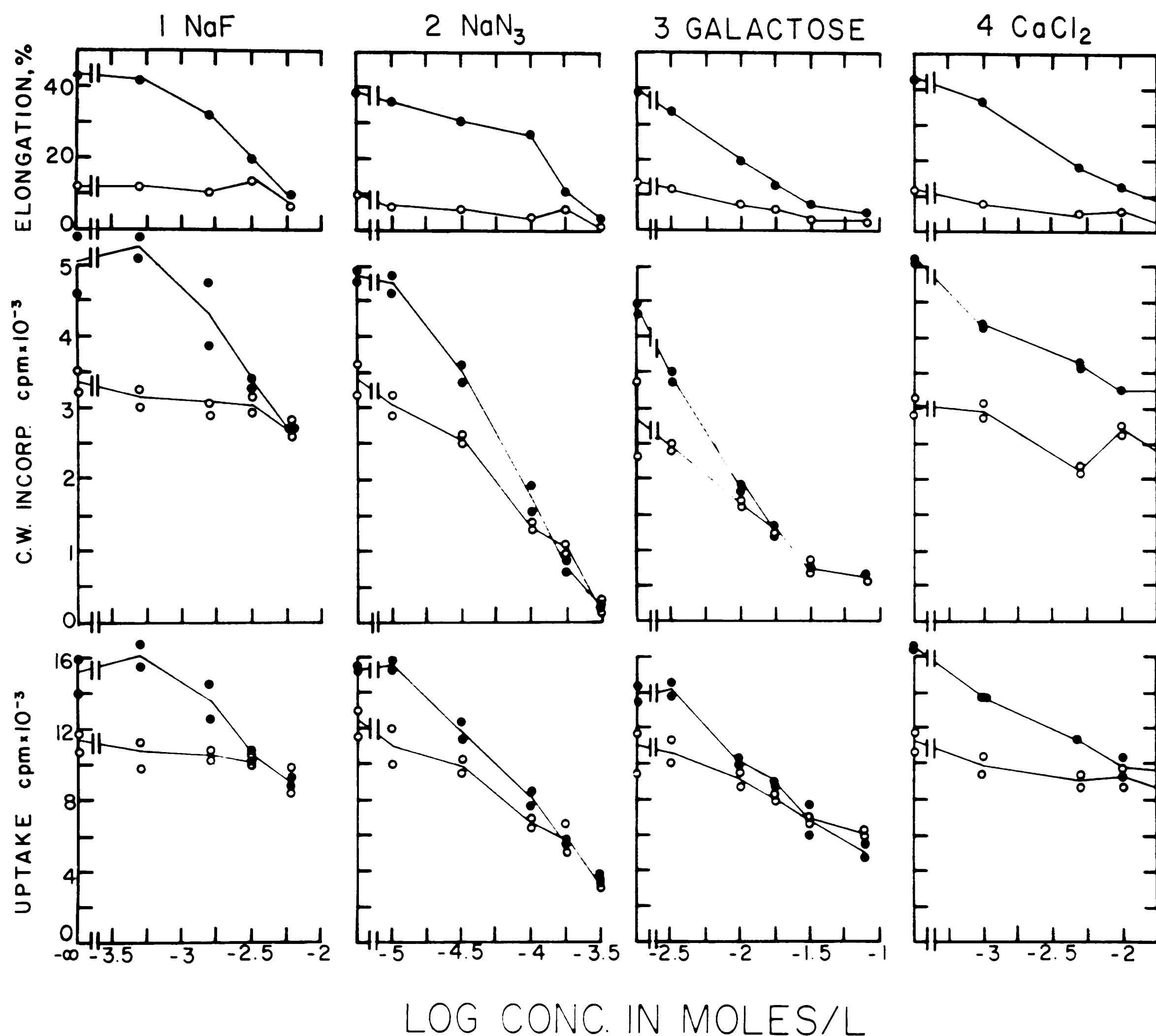


FIG. 1–4. Effects of inhibitors on elongation (*top row*), cell wall incorporation (*middle row*) and glucose uptake (*lower row*). Open circles, segments without IAA; solid circles, segments treated with IAA. In each treatment 20 segments were incubated 6 hours in 2 ml 0.05 M labeled glucose (0.31 μc/ml) containing the indicated concentrations of inhibitors, with or without 3 mg/liter IAA. For each treatment results are given for duplicate samples of 10 segments each. Figure 1, NaF; figure 2, NaN_3; figure 3, D-galactose; figure 4, $CaCl_2$.

thesis in coleoptile segments cut from 4-day-old oat seedlings, whose coleoptiles had ceased to grow as indicated by emergence of the primary leaf. The average cell wall incorporation found was 3010 cpm without auxin and 2590 cpm with auxin (incubation and sampling as given in heading of table V); elongation was nil for both groups of segments. Evidently mature segments can synthesize wall material at a rate comparable with growing segments (table V), but this synthesis is not promoted by auxin.

Timing of the Response to Auxin. We reported earlier (3) that the promotive effect of IAA on wall synthesis in the presence of Ca^{++} could not be detected until more than 1 hour after the start of treatment with IAA; whereas the rate of elongation of uninhibited oat coleoptile segments is known to increase within 15 minutes after treatment with auxin is begun (17).

During the first hour of exposure to labeled glucose the possibility exists that because of utilization of and isotope dilution by endogenous substrates, isotope incorporation fails to reflect differences in rates of wall synthesis between untreated and auxin-treated segments (16). In several experiments we tried to circumvent initial isotope dilution effects by pretreating with labeled glucose in the hope of attaining isotope equilibrium on the pathway to cell wall material prior to addition of IAA. In no case was a substantial promotive effect observed 1 hour after addition of IAA. For example, in 1 experiment the cell wall activity found at the end of a 1.5 hour pretreatment was 590 ± 50 cpm (uptake 2560 ± 180

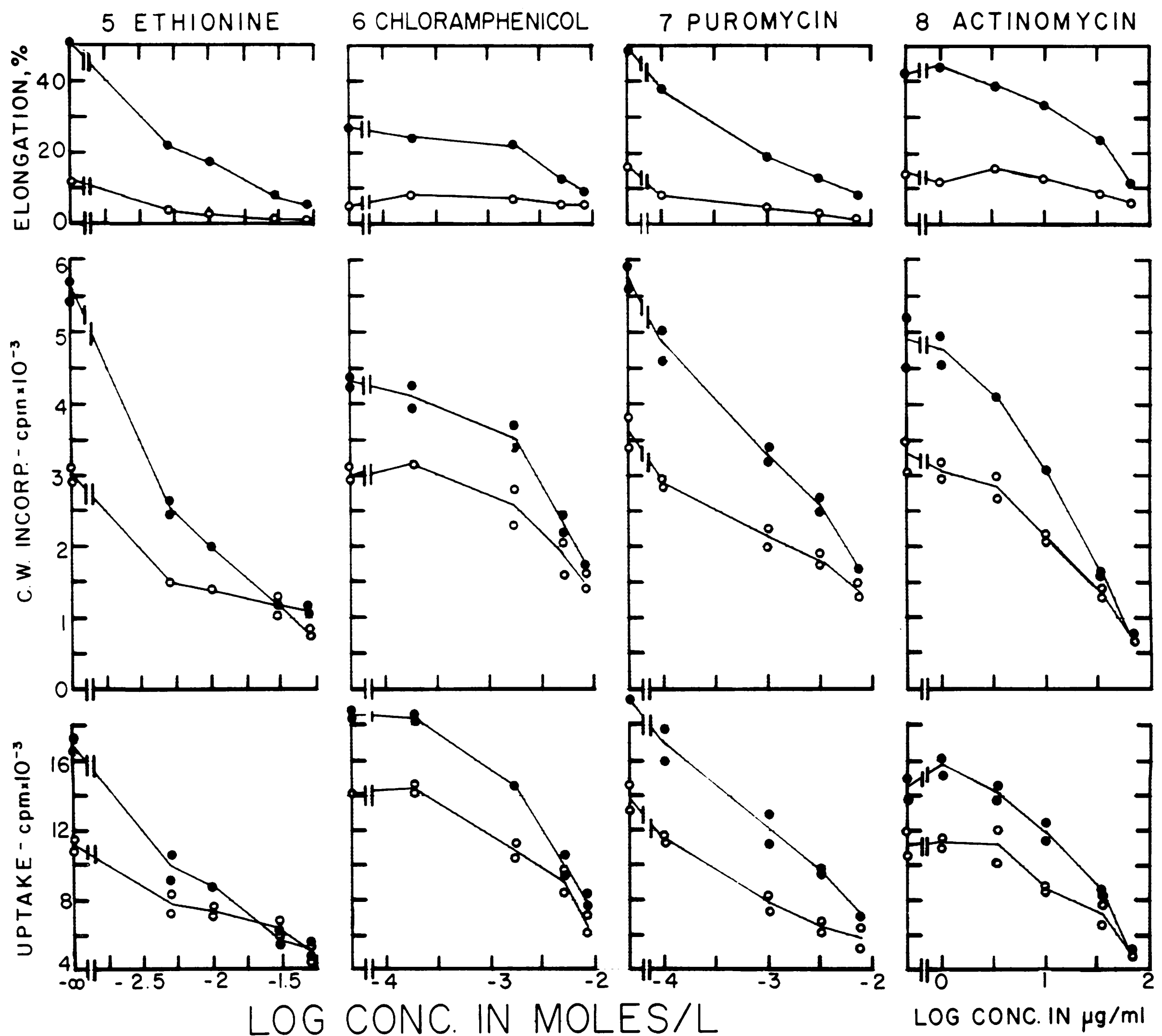


FIG. 5–8. Effects of protein synthesis inhibitors on elongation (*top row*), cell wall incorporation (*middle row*) and glucose uptake (*lower row*). Open circles, segments without IAA; solid circles, segments treated with IAA. Conditions the same as for figures 1 to 4, except that in the chloramphenicol experiment incubation was 5 hours. Note that the ordinate for plots of uptake data begins at 4000 cpm. Figure 5, DL-ethionine; figure 6, chloramphenicol; figure 7, puromycin; figure 8, actinomycin C.

cpm); to half the segments auxin was then added and 1 hour later the wall activity was 1020 ± 80 cpm for segments in auxin and 1070 ± 60 cpm for those that received no auxin (uptake 3920 ± 190 and 4190 ± 380 cpm, respectively); after 6 hours more the wall activity was 2310 ± 320 cpm without auxin and 2810 ± 140 cpm with auxin (uptake 11600 ± 1280 and 11100 ± 670 cpm, respectively; 4 replicate samples of 5 segments for each sampling; incubated in 0.02 M $CaCl_2$ containing 0.1 M labeled glucose, 8.4 μc/mmole, and 3 mg/liter IAA where added).

We found, however, that substantial promotion of isotope incorporation during a 1-hour exposure to labeled glucose could be detected following a pretreatment of several hours with auxin (in $CaCl_2$ to inhibit elongation), as compared with pretreatment in $CaCl_2$ alone. Data demonstrating this are given in table VI, and similar results have been obtained in several other experiments. This appears to show that if a substantial promotion of wall synthesis occurred during the first hour after exposure to auxin, we should be able to detect it despite the existence of isotope dilution effects. Since, as illustrated further by data in table VI, an effect of auxin is not seen within 1 hour, the conclusion seems warranted that the promotion of wall synthesis by auxin in the presence of $CaCl_2$ does not appear until after 1 hour.

It is of course impossible to determine whether the elongation response to auxin takes place within 1 hour in concentrations of $CaCl_2$ that are strongly inhibitory to elongation. Cooil and Bonner (6) concluded that Ca^{++} prevents the growth-promoting action of auxin in treatments of about 1 hour duration. Because of the possibility that the biochemical effect of auxin that is critical to cell enlargement is delayed by Ca^{++}, we examined the effect of a 1-hour treatment with auxin on wall synthesis in uninhibited coleoptile segments. This experiment involved a 1-hour pretreatment with labeled glucose alone in an effort again to minimize isotope dilution effects at the time IAA was added. The cell wall material from these segments was fractionated extensively, in case a significant effect of auxin on some components occurred which might be missed by determination simply of total incorporation.

The results of this experiment, which are of interest also in other respects, were given in the preceding paper (ref. 16, table IV). As explained there, IAA caused an increase of not more than 20 % in incorporation into cell wall constituents during the 1 hour of auxin treatment. Moreover, uptake of glucose was increased by IAA to a similar extent, so that the entire promotive effect of IAA seen within 1 hour was apparently an indirect effect (due to increase in glucose uptake caused by elongation), according to the interpretation advanced in the first paper of this series (3).

Discussion

The results examined in the last section indicate that the promotion of cell wall synthesis that is induced directly by auxin (as opposed to by elongation) is a delayed effect compared with the effect of auxin on rate of cell enlargement, and thus the latter cannot be a consequence of the former.

However, the other data presented here show a rather general parallelism between effects on elongation and on wall metabolism under treatment with different auxins and inhibitors. No treatment has been found that inhibits wall synthesis without inhibiting growth. All inhibitors of cell enlargement that have been tested inhibit the promotive effect of auxin on wall synthesis, except Ca^{++} and Sr^{++} (and perhaps Mg^{++} and osmotic concentrations of KCl) which presumably inhibit growth mechanically rather than by interfering with its metabolic basis (22). These observations imply that wall synthesis plays a causal role in growth and that the direct effect of

Table VI. *Effect of Pretreatment with IAA on Glucose Uptake and Cell Wall Incorporation*

In each treatment coleoptile segments were incubated 1 hour in labeled glucose, either beginning 0.5 hours after cutting (expt. 1, first 2 lines), or following a 4-hour pretreatment in 0.02 M $CaCl_2$, containing IAA and/or unlabeled glucose where shown in the left hand column. The labeled glucose (0.05 M, 0.65 μc/ml) incubation medium contained 0.02 M $CaCl_2$, and 3 mg/liter IAA where indicated in the second column. Each mean and average deviation (in percent of mean) is for 4 replicate samples of 14 segments.

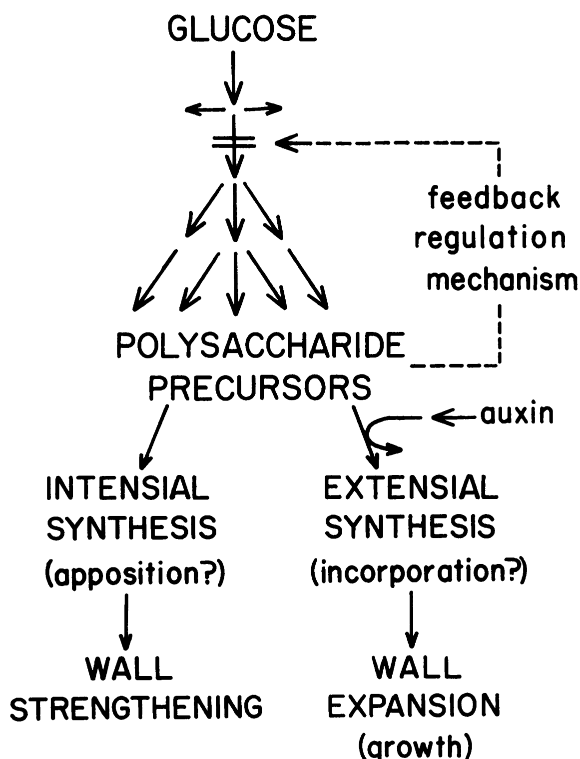
Pretreatment medium 0.02 M $CaCl_2$ plus	Incubation medium IAA	Cell wall incorporation cpm	Uptake cpm
Expt. 1			
No pretreatment	—	1380 ± 4.3 %	11000 ± 8.7 %
No pretreatment	+	1490 ± 7.4	13700 ± 12.2
$CaCl_2$ only	—	900 ± 10	18500 ± 8.1
IAA 3 mg/liter	+	1350 ± 3.0	17850 ± 4.9
Expt. 2			
$CaCl_2$ only	—	605 ± 8.3	12470 ± 4.9
$CaCl_2$ only	+	620 ± 8.1	12700 ± 4.3
IAA 3 mg/liter	+	1010 ± 4.7	13510 ± 6.7
Glucose 0.05 M	—	665 ± 7.2	9290 ± 9.8
Glucose 0.05 M	+	675 ± 3.0	9700 ± 7.5
Glucose + IAA	+	820 ± 2.2	10800 ± 5.5

auxin on wall synthesis is in some manner connected with its effect on growth. In what follows we suggest an explanation of this connection that appears to account for the evidence that is available at present.

At the outset one must accept the premise that a substantial proportion of the new wall material that is being made cannot contribute to the growth process. This conclusion, which has been documented previously (13), is shown for example by the substantial wall synthesis that continues in mature nongrowing coleoptile cells, or when elongation of growing segments is inhibited by fluoride. If formation of cell wall substances does cause cell wall expansion and thereby growth, the kind of cell wall synthesis that leads to wall expansion (called hereafter extensial synthesis) must be occurring in addition to or in competition with a kind of wall synthesis that does not cause wall expansion (to be called intensial synthesis, because it is not connected with wall stretching).

The finding that direct promotion of wall synthesis by auxin involves all cell wall components except α-cellulose (16) suggests that extensial synthesis cannot be identified with any specific chemical fraction other than the wall matrix as a whole. A reasonable model is that intensial synthesis is appositional, while extensial synthesis occurs when new matrix polysaccharides are introduced within the existing wall structure in such a way as to cause stress relaxation and thus expansion.

The simplest supposition, as shown in the following scheme, is that auxin (probably as an indirect consequence of its primary action) promotes the extensial component of wall synthesis.



Kinetic analysis shows that the flux through any biosynthetic pathway must in general be controlled by one or at most only a few key or sensitive steps (8), typically located at or near the beginning of the pathway. If auxin acts, at the end of the pathway from glucose to cell wall polysaccharides, to promote utilization of precursors for the extensial type of wall synthesis, the immediate result would have to be that concentrations of precursors fall, the intensial type of synthesis suffers a compensating inhibition, and the total flux remains the same as before. Such an action would lead to promotion of cell enlargement without an immediately detectable promotion of the rate of polysaccharide synthesis.

That an overall promotion of wall synthesis by auxin does gradually appear may be attributed to a regulatory mechanism that controls flux through the pathway in response to utilization of end products. Such negative feedback controls of 2 general types (end product inhibition and repression) are well known in bacteria, and although they have been relatively little studied as yet in other plants (23, 24), the necessity for feedback control in growing systems is generally admitted. The lowering of polysaccharide precursor levels that may be expected to result, as explained above, from a promotive action of the hormone on the extensial component of wall synthesis, should tend to release the pathway from feedback control, until the overall rate of synthesis has been raised by the same amount as the extensial component had initially been promoted by auxin. The promotive effect of auxin on wall synthesis is, in this view, a consequence of the developmentally crucial action of auxin and under appropriate conditions may reflect the latter quantitatively and permit its investigation.

This hypothesis enables a number of otherwise dissident observations to be reconciled with the evidence that the effect of auxin in promoting cell wall metabolism is connected with its effect on growth. For example, if conditions do not permit an increased flux through the polysaccharide synthesis pathway, such as when wall synthesis is held back by inhibitors or by insufficient substrate, it is still possible for auxin to exert an influence on the extensial component and thus to promote growth without bringing on an increase in rate of wall synthesis as a whole. The same would be true under circumstances that prevent response of the feedback control, for example in the presence of inhibitors of RNA or protein synthesis if repression is involved (21).

The data in table VI afford some suggestion that feedback controls are operating in the carbohydrate metabolism of oat coleoptile tissue. It appears from experiment 1 that the capacity for glucose uptake rises during incubation of segments in a medium lacking sugar, and experiment 2 indicates that glucose tends to prevent this rise. On the other hand experiment 1 shows that the capacity for wall synthesis falls during incubation without auxin and that the promotive effect of auxin is actually a prevention of this decline. Now at the start of the experiment the tissue had recently been removed from the plant, where the cells

were growing rapidly under the influence of the native auxin. Treatment of segments with auxin tended to maintain the previous level of activity in the wall synthesizing system, while in the absence of auxin an inhibition set in that might be attributed to a reduced demand for precursors of wall polysaccharides.

It seems possible that the inhibitory effects of galactose (fig 3) and mannose (13) on growth and cell wall synthesis are instances of feedback regulation by end products, since these sugars are normal, but minor, constituents of the cell wall (14).

Of interest relative to these ideas is the evidence reported by Sacher, Hatch and Glasziou (18) that in sugar cane stem slices glucose represses invertase, and auxin can induce derepression of this enzyme.

The existence of a dynamic state and a repression phenomenon involving enzyme activities on the cell wall synthesis pathway might explain the dependence of cell enlargement upon protein synthesis that is indicated by inhibitor experiments (9, 11: fig 5–8) and the promotive effect of auxin that has been reported on nucleic acid metabolism (9, 10).

The presently studied general promotion of matrix polysaccharide synthesis by auxin may reflect the same promotive effect as has been investigated previously with respect to methyl ester groups (12, 19) and galacturonic acid (1, 2). The kind of scheme given above can account both for the evidence that suggests a connection between these latter effects and the action of auxin on growth (12), as well as for results that have appeared to show that the biochemical effects are independent of the effect on growth (4, 5).

We feel that at least until such time as some other substantial effect of auxin on the metabolism of a structurally significant component of the cell wall is demonstrated, its effect on matrix polysaccharide synthesis should be regarded as reflecting a probable means by which auxin promotes elongation of oat coleoptile cells. In our view the most attractive interpretation of existing evidence is that biosynthesis of matrix polysaccharides and their incorporation into the wall structure in a suitable manner is probably the immediate metabolic basis for cell enlargement in this tissue, or at least a process that contributes importantly thereto. The possibility of an interaction between the effect of auxin and a feedback mechanism regulating carbohydrate metabolism suggests a number of exciting avenues for further investigation.

Summary

The promotion by indoleacetic acid (IAA) and other auxins of cell wall synthesis in oat coleoptile tissue parallels the effect of auxins on elongation. At concentrations supraoptimal for elongation, cell wall synthesis is inhibited by auxin.

Among a variety of inhibitors that were tested, inhibition of elongation and of the effect of IAA thereon was invariably accompanied by inhibition of the promotive effect of auxin on wall synthesis, except when elongation was inhibited by Ca^{++}, Sr^{++}, Mg^{++}, or osmotic concentrations of KCl. Cell wall synthesis in mature coleoptiles that have ceased elongating is not promoted by IAA. At 2° or in the presence of certain inhibitors, IAA can cause a small promotion of elongation with little or no effect on wall synthesis.

In the presence of Ca^{++}, a net promotion of wall synthesis by IAA is not observed within 1 hour even if the segments are pretreated with labeled glucose. In uninhibited segments an increase in wall synthesis is detected in tissue treated 1 hour with IAA after pretreatment with labeled glucose for 1 hour, but the increase appears to be attributable to the promotion of sugar uptake by elongation, rather than to IAA directly. Treatment of coleoptile segments with auxin for 4 hours results in a strong promotive effect on wall synthesis detectable during a subsequent 1-hour incubation in labeled glucose. These observations show that the direct effect (not caused by elongation) of auxin on rate of wall synthesis is a delayed effect compared with the effect of auxin on elongation.

The data are satisfactorily explained by the hypothesis that auxin acts (possibly indirectly) to promote utilization of polysaccharide precursors for a particular kind of formation or incorporation of polysaccharides that is effective in inducing cell wall expansion, while net promotion of the rate of wall synthesis follows as a response of a feedback control mechanism that regulates the pathway of wall polysaccharide synthesis.

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The Photooxidation of Uric Acid by Anacystis nidulans [1, 2]

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It has previously been reported that several species of blue-green algae showed atypical growth with uric acid as the sole nitrogen source. The growth rate was much reduced and the photosynthetic pigment system was highly bleached (9). Further consideration of the growth data and the finding that allantoin accumulated in the medium during growth of *Agmenellum quadriplaticum,* strain PR-6 (9) led to the supposition that the initial oxidative attack on uric acid was nonenzymic and was perhaps a property of certain blue-green algae independent of their ability to utilize uric acid as the sole nitrogen source. Accordingly whole cells of several blue-green algae were tested for their ability to degrade uric acid. The best organism found was *Anacystis nidulans.* As shown by Birdsey and Lynch (1) *Anacystis* did not grow on uric acid or its oxidation products but did degrade it in the light. This report deals with the characteristics of the photooxidation of uric acid by *Anacystis,* with respect to rate, products, and effect of light quality. The data presented indicate that this photooxidation is not mediated by the photosynthetic pigments chlorophyll a and phycocyanin found in *Anacystis* (7). The sensitizing pigment is shown to be similar in properties to the unknown pigment absorbing at 750 mμ described by Gassner (3).

[1] Received June 8, 1964.
[2] Supported by Grant GB2006 from the National Science Foundation.

Materials and Methods

Cells of *Anacystis nidulans* were grown batchwise on Medium C (5) using the test tube culture apparatus as described by Myers (6). The temperature was 39° and 1 % CO_2 in air was continuously bubbled through the cultures. Illumination was provided by 2 high output cool white fluorescent tubes 12.5 cm from the tubes.

Uric acid was determined by decrease in OD at 293 mμ using a Beckman DU spectrophotometer. Numerous checks using a uricase preparation from Worthington Biochemicals consistently confirmed that $\triangle$ OD at 293 mμ was a valid measure for uric acid disappearance. The uric acid used was obtained from Nutritional Biochemicals Corporation.

Allantoin was determined using the method of Young and Conway (11). Allantoin was also identified as was urea by paper chromatography.

The pigment seen in a whole cell spectrum at 750 mμ was extracted with the solvent systems suggested