ions in much the same manner as are the physical properties of the cell wall itself. This suggests that pectic materials of the coleoptile cell wall may be of importance in determining the mechanical deformability of the structure.

Literature Cited

1. Bonner, J. and Foster, R. J. The growth-time relationships of the auxin-induced growth in Avena coleoptile sections. Jour. Exptl. Bot. 6: 293–302. 1955.
2. Cooil, B. and Bonner, J. Effects of calcium and potassium ions on the auxin-induced growth of Avena coleoptile sections. Planta (In press).
3. Heyn, A. N. J. Der Mechanisms der Zellstreckung. Rec. trav. bot. néerl. 28: 113–244. 1931.
4. Heyn, A. N. J. Weitere Untersuchungen über den Mechanismus der Zellstreckung und die Eigenschaften der Zellmembran. Jahrb. wiss. Bot. 79: 753–789. 1934.
5. Ordin, L., Applewhite, T. H. and Bonner, J. Auxin-induced water uptake by Avena coleoptile sections. Plant Physiol. 31: 44–53. 1956.
6. Söding, H. Wachstum und Wanddehnbarkeit bei der Haferkoleoptile. Jahrb. wiss. Bot. 74: 127–151. 1931.
7. Söding, H. Über das Streckungswachstum der Zellwand. Ber. deut. bot. Ges. 50: 117–123. 1933.
8. Thimann, K. V. and Schneider, C. L. The role of salts, hydrogen-ion concentration and agar in the response of the Avena coleoptile to auxin. Amer. Jour. Bot. 25: 270–280. 1938.

EFFECT OF GALACTOSE ON GROWTH AND METABOLISM OF AVENA COLEOPTILE SECTIONS[1,2]

LAWRENCE ORDIN and JAMES BONNER

Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, California

During the course of investigation of the effect of various osmotically active solutes on Avena coleoptile section growth, it was found that galactose is more inhibitory to such growth than are other solutes in general. Mannitol for example is essentially without effect on section growth in concentrations up to 0.02 M, but galactose is highly inhibitory at this concentration. The inhibition caused by galactose is thus not due to the osmotic properties of the substance. Earlier Burström (2) found galactose to inhibit the growth of wheat roots. He indicated indirectly that galactose is respired but not apparently used synthetically, i.e., not assimilated into structural components. Thimann (11) also found that galactose inhibits growth of pea stem sections but he supposed galactose to be metabolically inert.

The galactose-induced growth inhibition of Avena sections has now been further investigated. The question is posed; is the inhibition exerted on energy producing enzymatic steps of respiration or is it exerted through an interference with cell wall metabolism, such as the production of cell wall components. The latter possibility is of inherent interest in connection with studies of auxin action since it has been shown that the primary act of auxin in cell elongation is upon the cell wall (3). Auxin increases rate of methylation of a cell wall fraction which possesses the properties classically ascribed to pectin (7).

The data presented here suggest that galactose inhibits cell elongation primarily by interfering with the synthesis of a cell wall fraction having the solubility properties of cellulose rather than by inhibiting respiration. The cellulose fraction of the cell wall is, however, as shown earlier (7), not influenced directly by auxin.

Methods

The material used in this investigation consisted of 5-mm sections cut 3 mm below the apex of Avena (var. Siegeshafer) coleoptiles. The Avena seedlings were grown as described earlier (6). All solutions were made up with redistilled water. Primary leaves were removed except in cases where elongation was measured.

Respiration measurements were made in the standard Warburg apparatus by following oxygen uptake. These measurements were made over a three-hour period.

Cell wall metabolism was studied by incubating sections in galactose-1-C^{14} or uniformly labeled glucose-C^{14} and by analyzing the cell walls as described by Ordin, Cleland and Bonner (7).

Aliquots of the hot water (pectin), hot acid (protopectin) and hot oxalate (pectate) extracts were plated on copper planchets. The alkali soluble fractions (hemicellulose and non-cellulosic polysaccharides) were neutralized with 0.5 N and 9 N H_2SO_4 before plating. The planchets were counted with a micromil window counting tube using Q gas. The final residues (cellulose) were counted with a thin-window GM tube. All counts are corrected to infinite thinness. The counts incorporated are expressed on a 10-mg cell wall basis.

The amount of radioactive substrate taken up by the tissue varied somewhat from experiment to experiment. Therefore a calibration experiment was done in which the extent of incorporation into each tissue component was determined as a function of

[1] Received December 8, 1956.

[2] Report of work supported in part by the Herman Frasch Foundation and in part by the National Science Foundation.

amount of labeled substrate supplied and taken up. The calibration curves so obtained have been used to convert all of the data to incorporation per unit absorption of substrate. The data are expressed as counts per minute incorporated per 80,000 cpm of substrate absorbed.

The variability of Avena coleoptile section growth rates under the present conditions has been considered by Cleland and Bonner (3). In general growth increment differences greater than 0.06 mm are significant at the 5 % level. Variability in the measured incorporation of metabolite into tissue fraction is greater and differences in such incorporation of less than 10 % are not considered in this paper as meaningful. Each experiment here reported has been repeated many times.

The galactose-1-C^{14} was a gift from the National Bureau of Standards and the glucose-C^{14} was purchased from the California Foundation for Biochemical Research. Incubation of homogenized tissue with the radioactive substrates, followed by extraction and analysis indicated very little contamination of cell wall fractions by adsorption or occlusion of substrate.

Results

Figure 1 gives the results of an experiment which examines the effect of galactose on section elongation. Sections were placed in either 0.01 M galactose or 0.01 M mannitol. Potassium free indoleacetic acid (IAA) was added at the point in time indicated by the arrow. It may be seen that there is a marked effect of galactose on auxin-induced growth. If the galactose concentration is increased to 0.09 M in the presence of IAA, sections elongate only 0.09 mm in 20 hours whereas in a control solution of 0.09 M mannitol, elongation is 0.94 mm.

The effect of these two concentrations of galactose on respiration was investigated in order to determine if galactose blocks the normal respiration of glucose.

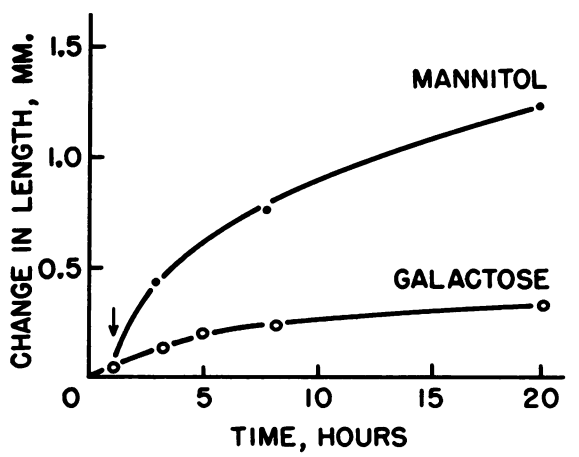

Fig. 1. Effect of 0.01 M galactose and of 0.01 M mannitol on the elongation of Avena coleoptile sections as a function of time in the presence of IAA. Five mg/l K-free IAA added at arrow, no buffer, final pH 6.1.

Table I

Effect of Galactose and of Mannitol Solutions on Oxygen Uptake of Avena Coleoptile Sections

Solutions	Conc	−IAA	+IAA
	M	*μl/20 sections × hr*	
Mannitol	0.09	13.0	21.0
Galactose	0.09	7.5	7.5
Mannitol	0.01	15.5	22.0
Galactose	0.01	24.0	27.0

Basal medium contains 5 mg/l K-free IAA, no buffer, initial pH 5.0 to 5.3.

Table I shows that 0.01 M galactose has no inhibiting effect on basal respiration but that 0.09 M galactose does inhibit respiration somewhat. The auxin induced increment in respiration is, however, almost eliminated by the lower concentration of galactose. This might be expected since the auxin induced respiratory increment accompanies elongation rather than the presence of auxin alone (cf Ordin, Applewhite and Bonner (6)).

The higher galactose concentration apparently interferes with respiration. That this might occur at the hexokinase stage is indicated by Hele (4) who has shown that phosphorylation of sugar mixtures by ATP in the presence of hexokinase yield lower rates than are obtained in the presence of single acceptors. Galactose in particular was found to be phosphorylated by intestinal mucosa at a slower rate than glucose. A mixture of the two sugars was phosphorylated more slowly than either alone.

The basis of this behavior is unknown but might account for the inhibition of respiration evident in table I at the higher galactose concentration. At the lower concentration however such inhibition does not appear to be a factor of importance.

A further test of the hypothesis that respiratory inhibition by galactose is not of primary importance

Table II

Effect of Various Treatments on Elongation of Galactose-Pretreated Avena Coleoptile Sections

Treatment	Elongation, mm *
0.08 M Glycol	0.26
0.08 M Mannitol	0.14
0.08 M Sucrose	0.54
0.08 M Glucose	0.52
0.045 M Tartrate	0.12
0.045 M H_2PO_4	0.27
0.045 M Malate	0.12
0.045 M Fumarate	0.26
0.045 M Citrate	0.17
0.045 M Succinate	0.28

Pretreatment: Incubation for 5 hrs in 0.01 M galactose, 0.0025 M potassium maleate, 3.5 mg/l IAA, pH 4.5.
Treatment: Incubation for 15 hrs in 0.01 M galactose and potassium salts or carbohydrate with maleate buffer, 3.5 mg/l IAA, pH 4.5.
* Elongation after 15 hrs.

TABLE III

$C^{14}O_2$ RESPIRED BY AVENA COLEOPTILE SECTIONS INCUBATED IN GALACTOSE-1-C^{14} OR GLUCOSE-C^{14}

SUBSTRATE	CPM/1000 CPM INITIAL SUBSTRATE
Glucose-C^{14}, 0.004 M	54.1
Galactose-1-C^{14}, 0.004 M	9.3

Initial specific activity: Glucose—182×10^6 cpm/mM, galactose—246×10^6 cpm/mM.

in the growth inhibition consisted in investigation of the effect of acids of the tricarboxylic acid cycle and of certain sugars on growth of galactose inhibited sections. Sections were pre-treated for 5 hours with 0.01 M galactose, 5 mg/l IAA and 0.0025 M potassium maleate pH 4.5. This was followed by 15-hour treatment in IAA, galactose and 0.045 M solutions of the acids or 0.08 M solutions of the sugars. The results are presented in Table II. Only glucose and sucrose had any effect in reducing the inhibition and even their effects were relatively small.

That galactose is indeed respired can be shown conclusively by incubating sections in a Warburg vessel with galactose-1-C^{14}. The CO_2 absorbed by the center well KOH was precipitated with $BaCl_2$ and counted as $BaC^{14}O_3$. Table III compares the amount of radioactive CO_2 respired with galactose-1-C^{14} or glucose-C^{14} as the substrates. The results are presented as cpm per 200 sections per 1000 cpm of initial culture solution. Each solution was 0.004 M with respect to the given sugar.

If it is assumed that both the galactose and the glucose are respired completely, then glucose is respired 6 times as rapidly as galactose. Under these conditions section growth is inhibited about 30 %.

Seegmiller, Axelrod and McCready (9) and Ordin, Cleland and Bonner (7) have shown that glucose gives rise to pectic substances in plant tissue. Because of the structural relationship between galactose and the pectic substances, experiments were performed to study the comparative incorporation of the two radioactive substrates into the various cell wall fractions.

Table IV gives the results of an experiment in which sections were incubated in 0.001 M radioactive galactose or glucose. For purposes of comparison it is assumed that the hexoses enter the cell wall fractions intact, i.e., without being dissimilated and reorganized. This concentration of galactose is non-inhibiting to growth. It may be seen that there is relatively more activity in the hot water soluble fraction (pectin) with galactose as substrate than with glucose, but that relatively less activity goes into the residue (cellulose) and hot acid soluble fractions (protopectin) with galactose as substrate. In fact, the differences between the two substrates are such that all fractions except cellulose and protopectin are more radioactive with galactose as substrate than with glucose as substrate. This is particularly marked for cellulose. It may be noted that auxin treatment causes an increase in the amount of galactose carbon incorporated into cellulose. Although in this particular experiment auxin does not cause a significant increase in cellulose activity with glucose as substrate, other experiments indicate that when cell wall expansion occurs, incorporation of glucose-C^{14} into cellulose is increased significantly by auxin treatment. This has also been shown by Boroughs and Bonner (1). The effect of IAA upon the pectin fraction is small both for the galactose and glucose cases. The small effects have been shown, in the glucose case, to be due entirely to the effects on a saponifiable substituent, presumably methoxyl (8).

Although the data imply interconversion of galactose to glucose, at least for the formation of cellulose, they also imply that this conversion is slow. That cellulose synthesis is essential to continued growth is indicated by the work of Wardrop (12) and of O'Kelley and Carr (5). The possibility may be considered therefore that galactose inhibits growth by being not

TABLE IV

INCORPORATION OF C^{14} FROM GALACTOSE-1-C^{14} OR FROM GLUCOSE-C^{14} INTO CELL WALL FRACTIONS OF AVENA COLEOPTILE SECTIONS IN THE PRESENCE AND ABSENCE OF IAA *

CELL WALL FRACTION	GALACTOSE		GLUCOSE	
	−IAA	+IAA	−IAA	+IAA
	Cpm/10 mg dry cell wall × 80,000 cpm absorbed by 150 sections			
Hot water soluble	833	938	575	624
Hot 0.05 *N* HCl soluble	7,725	8,390	13,300	11,050
Hot 0.5 % ammonium oxalate soluble	198	271	252	169
NaOH soluble, 4 %	2,210	2,345	2,370	1,560
NaOH soluble, 17.5 %	688	1,384	295	321
Residue	578	701	1,360	1,375
Totals	12,232	14,029	18,152	15,099

* Conc IAA used = 3.5 mg/l.

Initial specific activity of culture solutions: Galactose—142×10^6 cpm/mM, glucose—117×10^6 cpm/mM.

TABLE V

EFFECT OF 0.004 M GALACTOSE OR 0.004 M GLUCOSE ON INCORPORATION OF C^{14} FROM GLUCOSE-C^{14} INTO CELL WALL FRACTIONS OF AVENA COLEOPTILE SECTIONS

CELL WALL FRACTION	GALACTOSE	GLUCOSE
	Cpm/10 mg dry cell wall × 80,000 cpm absorbed by 150 sections	
Hot water soluble	453	528
Hot 0.05 *N* HCl soluble	2,270	3,040
Hot 0.5 % ammonium oxalate soluble	84	111
NaOH soluble, 4 %	1,204	968
NaOH soluble, 17.5 %	352	187
Residue	194	681
Totals	4,557	5,515

The culture solutions contained 3.5×10^5 cpm per 3.5 ml of C^{14}-labeled glucose in both cases; incubation for 3 hrs.

only itself a relatively poor substrate for cellulose synthesis but also by inhibiting glucose utilization for this purpose. An experiment was carried out to determine the effect of galactose on incorporation of glucose-C^{14} into the cell wall. To unlabeled glucose or galactose solutions, each 0.004 M, equal amounts of labeled glucose were added. Table V shows that the presence of galactose slightly inhibits incorporation of glucose carbon into pectic substance and slightly increases incorporation into the 4 % NaOH soluble fraction (hemicelluloses) and the 17.5 % NaOH soluble fractions (non-cellulosic polysaccharides). Most remarkable, however, is the fact that incorporation of glucose carbon into cellulose is severely inhibited by the galactose. This experiment was carried out in the absence of IAA to reduce artifacts due to elongation. Thus galactose not only is poorly utilized in cellulose synthesis itself but also inhibits glucose utilization in formation of this fraction of the cell wall. The inhibition is even greater than the growth inhibition under these circumstances.

Discussion

In contrast to Burström's finding that galactose is not used synthetically, it has been shown above that in the Avena coleoptile, galactose carbon is incorporated into the cell wall. Galactose is respired, as found by Burström, but not as rapidly as is glucose. Most noticeable is the fact that galactose carbon is not incorporated into cellulose as rapidly as is glucose. In low concentrations of galactose, auxin treatment causes radioactive galactose carbon to be incorporated more rapidly into cellulose than in the absence of auxin. Galactose also inhibits incorporation of radioactive glucose into cellulose. These lowered rates of incorporation are noticeable even when growth inhibition is small or nil.

Respiration is unaffected by growth inhibiting concentrations of galactose and the Krebs cycle acids are completely ineffective in overcoming the growth inhibition. Respiratory effects are not therefore involved in the inhibition of growth.

The depression of cellulose synthesis caused by galactose could be a factor of importance in limiting growth, albeit of a secondary nature as far as the auxin mechanism of elongation is concerned. Wardrop (12) has shown that as Avena coleoptiles grow the cellulose microfibrils tend to disperse. New microfibrils are, however, interpolated over the entire surface. This means that new synthesis does occur, a conclusion supported by the isotope incorporation data presented here as well as that of Boroughs and Bonner (1). O'Kelley and Carr (5) have found that the density of microfibrils appears unchanged in cotton fibers as they elongate suggesting that in this case synthesis keeps pace with elongation.

It would be of some interest to trace the route of galactose interference in cellulose synthesis more closely since this might help to illuminate the mechanism of cellulose biosynthesis.

Summary

Galactose at a concentration of 0.01 M inhibits elongation of Avena coleoptile sections quite markedly without affecting non-auxin induced respiration.

In lower concentrations which permit growth, carbon-14 derived from galactose-C^{14} is incorporated into all cell wall constituents except cellulose and protopectin as readily as in carbon-14 from glucose-C^{14}. Incorporation of carbon-14 from galactose-C^{14} into cellulose is markedly slower than is incorporation of carbon-14 from glucose C^{14}.

Galactose at 0.004 M has little effect on the incorporation of carbon-14 from glucose into cell wall fractions other than cellulose. Incorporation of glucose carbon into cellulose is strongly repressed by the presence of galactose.

Galactose is respired to carbon dioxide but at a lower rate than is glucose.

It is suggested that galactose specifically inhibits cell elongation by interfering with cellulose synthesis.

Literature Cited

1. Boroughs, H. and Bonner, J. Effects of indoleacetic acid on metabolic pathways. Arch. Biochem. Biophys. 46: 279–290. 1953.
2. Burström, H. Observations on the influence of galactose on wheat roots. Physiol. Plantarum 1: 209–215. 1948.
3. Cleland, R. and Bonner, J. The residual effect of auxin on the cell wall. Plant Physiol. 31: 350–354. 1956.
4. Hele, M. P. The phosphorylation and absorption of sugars in the rat. I. Hexokinase activity in the intestinal mucosa. Biochem. Jour. 55: 857–863. 1953.
5. O'Kelley, J. C. and Carr, P. H. Elongation of the cotton fiber. In: Growth and Differentiation in Plants, W. E. Loomis, ed. Pp. 55–68. Iowa State College Press, Ames, Iowa 1953.
6. Ordin, L., Applewhite, T. H. and Bonner, J. Auxin-induced water uptake by Avena coleoptile sections. Plant Physiol. 31: 44–53. 1956.
7. Ordin, L., Cleland, R. and Bonner, J. Influence of auxin on cell wall metabolism. Proc. Nat. Acad. Sci., U.S. 41: 1023–1029. 1955.
8. Ordin, L., Cleland, R. and Bonner, J. Methyl esterification of cell wall constituents under the influence of auxin. Plant Physiol. (In press).
9. Seegmiller, C. G., Axelrod, B. and McCready, R. M. Conversion of glucose-1-C^{14} to pectin in the boysenberry. Jour. Biol. Chem. 217: 765–775. 1955.
10. Thimann, K. V. The physiology of growth in plant tissues. Amer. Scientist 42: 589–606. 1954.
11. Thimann, K. V. Studies on the growth and inhibition of isolated plant parts. V. The effects of cobalt and other metals. Amer. Jour. Bot. 43: 241–249. 1956.
12. Wardrop, A. B. The mechanism of surface growth in parenchyma of Avena coleoptiles. Australian Jour. Bot. 3: 137–148. 1955.