

Growth Inhibition and Metabolite Pool Levels in Plant Tissues Fed D-Glucosamine and D-Galactose

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ABSTRACT

The growth of corn (*Zea mays*) roots and barley (*Hordeum vulgare*) coleoptiles is sensitive to the presence of external D-glucosamine and D-galactose. In order to investigate this effect, tissues were fed the radioactive monosaccharides at concentrations that ranged from those that were strongly inhibitory to those that had little influence on growth. At low concentrations, D-glucosamine is converted to uridine diphosphate-N-acetyl-D-glucosamine, phosphate esters of N-acetylglucosamine, and free N-acetylglucosamine. As the external concentrations were increased, the pool levels of each of these metabolites rose several fold; and, in corn roots, two unidentified compounds, which had not been detected previously, began to accumulate in the tissues. The major products of D-galactose metabolism were uridine diphosphate-D-galactose and D-galactose 1-phosphate at all the concentrations tested. Both these compounds showed a marked increase as the external galactose concentrations were raised to inhibitory levels. The experiments indicate that efficient pathways exist in plants for the metabolism of D-glucosamine and D-galactose. These pathways, however, do not appear to be under strict control, so that metabolites accumulate in unusually high amounts and presumably interfere competitively with normal carbohydrate metabolism.

Plants, like animals, show considerable economy in the way in which they metabolize carbohydrate, and efficient pathways exist for dealing with the individual sugars that are released during hydrolytic breakdown of more complex macromolecules. The structures of L-arabinose (14), L-fucose (22), D-galactose (24), and D-glucosamine (21), for example, are conserved when supplied to plant tissues, and their carbon skeletons are incorporated as intact units into new polymeric material. Each of these compounds probably arises naturally in germinating seedlings or on other occasions when reserve substances are being broken down (1, 2, 13). Gal,¹ in particular, is an important constituent of cell walls and of sugars such as stachyose and raffinose (6). It is of interest, therefore, that above certain concentrations, exogenous Gal and GlcN are toxic and inhibit growth of many plant tissues, including roots (5, 8, 15), coleoptiles (3, 8, 20), and germinating pollen (19). Toxicity has also been observed with animal tissues and may therefore have a similar basis (17, 31).

¹ Abbreviations: Gal: D-galactose; Gal-1-P: D-galactose 1-phosphate; UDP-Gal: UDP-D-galactose; GlcN: D-glucosamine; GlcNAc: N-acetyl-D-glucosamine; UDP-GlcNAc: UDP-N-acetyl-D-glucosamine.

The metabolism of radioactive GlcN in plants, as in animals, leads to the accumulation of labeled GlcNAc, GlcNAc-phosphates, and UDP-GlcNAc, plus products of high molecular weight which are probably glycoproteins (21). Galactose metabolism probably proceeds via phosphorylation and nucleotidyl transfer to yield Gal-1-P and UDP-Gal, respectively (10, 11). There is also extensive incorporation of Gal into cell wall polysaccharide (24). In the experiments reported here, both monosaccharides have been fed to growing tissues at concentrations that ranged from the highly toxic to the noninhibitory in an attempt to determine the pool sizes of the major groups of compounds that accumulated with the onset of growth inhibition. It is assumed that one or more of these metabolites is inherently toxic or interferes with normal metabolism in some way.

MATERIALS AND METHODS

Chemical Materials. D-Glucosamine-1-¹⁴C (specific radioactivity 53 $\mu\text{C}/\mu\text{mole}$) and D-galactose-1-¹⁴C (specific radioactivity 3.4 $\mu\text{C}/\mu\text{mole}$) were obtained from the Radiochemical Centre, Amersham, England. Unlabeled sugar phosphates, nucleosides, nucleotides, alkaline phosphatase (*Escherichia coli*), phosphodiesterase, and UDP-glucose dehydrogenase were purchased from Sigma Chemical Company. DEAE-Sephadex for column chromatography was purchased from Pharmacia and was prepared in the bicarbonate form according to the manufacturer's recommendations.

Plant Materials. The corn (*Zea mays*, variety Golden Bantam) used in this study was purchased from Burpee Co., Sanford, Florida, and the barley grain (*Hordeum vulgare*, variety Larker) from Anheuser-Busch, St. Louis. Grain was sterilized with 1.25% (w/v) sodium hypochlorite solution for 15 min, transferred under sterile conditions to 0.5% (w/v) agar, and grown in darkness at 25 C for 3 days.

Growth Experiments. Coleoptile segments were surface dried with filter paper, then weighed to the nearest milligram in lots of 15 to 60 depending upon the experiment. Tissues were then transferred to small flasks (10 or 25 ml) containing 20 mM potassium phosphate buffer, pH 5.8; IAA (0.1 mM); and various concentrations of the test sugar. Flasks were sealed with rubber serum stoppers. The tissues were incubated at 30 C with continuous gentle shaking for a specific time, usually 6 hr.

Roots from intact corn seedling were marked 1 cm from the tip with India ink and then grown in aerated solutions at various concentrations of monosaccharides for a 24-hr period. Excised roots (1 cm) were used in metabolic studies because it was advantageous to use a closed system in which CO₂ could be easily trapped and small volumes of solution employed. Control experiments have shown that the products of glucosamine metabolism were identical for both intact and excised tissues.

Measurement of Growth. After incubation, coleoptiles were blotted dry and individual lengths measured to the nearest 0.5 mm, using a binocular dissecting microscope. Each batch was

also weighed after the incubation to determine the increase in fresh weight. The growth of whole corn roots was determined by measuring the distance from the ink mark to the tip.

Isotope Incorporation Experiments. In the experiments with labeled compounds, radioactive monosaccharide was provided to each flask, and unlabeled carrier was added to increase the concentration of monosaccharide in the medium to the desired level. Aliquots of solution were removed at hourly intervals to determine the uptake. Each sample was diluted to 1 ml with water and counted in 10 ml of toluene-Triton X100 scintillant (2:1 v/v) (30). The toluene contained 0.4% (w/v) PPO and 0.01% POPOP. All measurements were carried out using a Packard automatic liquid scintillation spectrometer. Counting efficiency was about 78% but was checked routinely either by the channels ratio method or by including internal standards of toluene- ^{14}C in selected vials.

In the experiments with Gal- ^{14}C , the flasks were equipped with small cups containing 3 drops of 2 N NaOH to trap respiratory CO_2 . At intervals, the NaOH was removed from the cups and diluted to 10 ml with water, and 1-ml samples were counted as above.

After incubation, the coleoptiles or roots were washed several times with water and then killed by treatment with boiling ethanol (80% v/v) for 0.5 min. Tissues were ground using a motor-driven glass homogenizer. After centrifugation (2000g) the residue was extracted exhaustively with 80% (v/v) ethanol to remove all soluble radioactivity. Small samples of the supernatant fraction were taken for estimation of ^{14}C , and the remainder was dried under vacuum using a rotary evaporator.

After extraction with 80% ethanol, the insoluble residue was treated successively with 100% ethanol (twice) and finally air-dried. The resulting whitish powder was then hydrolyzed with 2 N HCl in an autoclave at 121 C (30 min), and the supernatant fraction was collected after centrifugation. The final acid-resistant residue was suspended in Cab-O-Sil gel for estimation of ^{14}C . The hydrolysate was dried *in vacuo* and analyzed by paper chromatography in solvents D, E, F, and G.

Analytical Procedures. After drying, the ethanol-soluble portion was redissolved in a few drops of 80% ethanol and chromatographed on Whatman No. 1 or 3MM paper. The following solvents were routinely used for identification of nucleotides and phosphate esters: A, 1 M ammonium hydroxide-isobutyric acid-0.1 M EDTA (30:50:1); B, ethanol-1 M ammonium acetate, pH 3.8 (5:2); C, ethanol-1 M ammonium acetate, pH 7.5 (5:2). Solvents D to H were used for analysis of monosaccharides; D, pyridine-ethyl acetate-water (2:8:1, v/v); E, 1-butanol-acetic acid-water (37:25:9, upper phase); F, 1-butanol-ethanol-water (52:33:18); G, 1-butanol-pyridine-water-acetic acid (5:5:3:1) in a tank pre-equilibrated with ethyl acetate-pyridine-water (8:2:1); H, 1-butanol-pyridine-0.1 N HCl (5:3:2). Solvents G and H are particularly useful in distinguishing between glucosamine and galactosamine which cannot be separated in the other systems. Compounds were also subjected to electrophoresis in pyridine-acetic acid-water (1.25:1.25:97.5, v/v), at 2000 v (40 v/cm) for 1 hr using a Gilson high voltage electrophorator (model D).

Chromatographs were examined under ultraviolet light for quenched areas indicating nucleoside diphosphate sugars. Phosphates were detected with ammonium molybdate spray reagent (9) and sugars by alkaline silver nitrate (29). Areas containing ^{14}C were detected by exposure to x-ray film. These areas were cut from the paper and counted in toluene/PPO/POPOP without addition of Triton X100 or water.

Ion Exchange Chromatography. When required, compounds were dissolved in 0.01 M NH_4HCO_3 and loaded onto a column (18 x 2 cm) of DEAE-Sephadex-A25 (HCO_3 form). After washing with 50 ml of 0.01 M NH_4HCO_3 , elution was carried out with a linear gradient of salt. The mixing flask, stirred magnetically,

contained 250 ml of 10 mM NH_4HCO_3 and the reservoir 250 ml of 0.4 M NH_4HCO_3 . A peristaltic pump maintained a constant rate of elution. Ten-milliliter fractions were collected, and samples were removed for determination of ^{14}C and absorption at 260 nm. Scans of the ultraviolet absorption spectra of nucleoside diphosphate sugars were made with a Cary 14 recording spectrophotometer.

RESULTS

Growth of Roots. The growth of corn roots was strongly inhibited by GlcN and Gal (Fig. 1A). Elongation was almost completely suppressed by concentrations of monosaccharide as low as 5 mM, and inhibition was evident even at 0.5 mM. The inhibited roots were slightly swollen in the region where growth normally occurs (3–7 mm from the tip) compared with the control samples, but otherwise they showed no signs of necrosis or browning. Of the other monosaccharides tested, D-glucose, L-arabinose, and L-fucose had no visible effects on the growth of roots when supplied at a range of concentrations extending from 0.1 mM to 0.1 M. D-Mannose, however, inhibited growth to about the same extent as Gal.

Growth of Excised Coleoptiles. In preliminary experiments, barley coleoptile segments were incubated at various concentra-

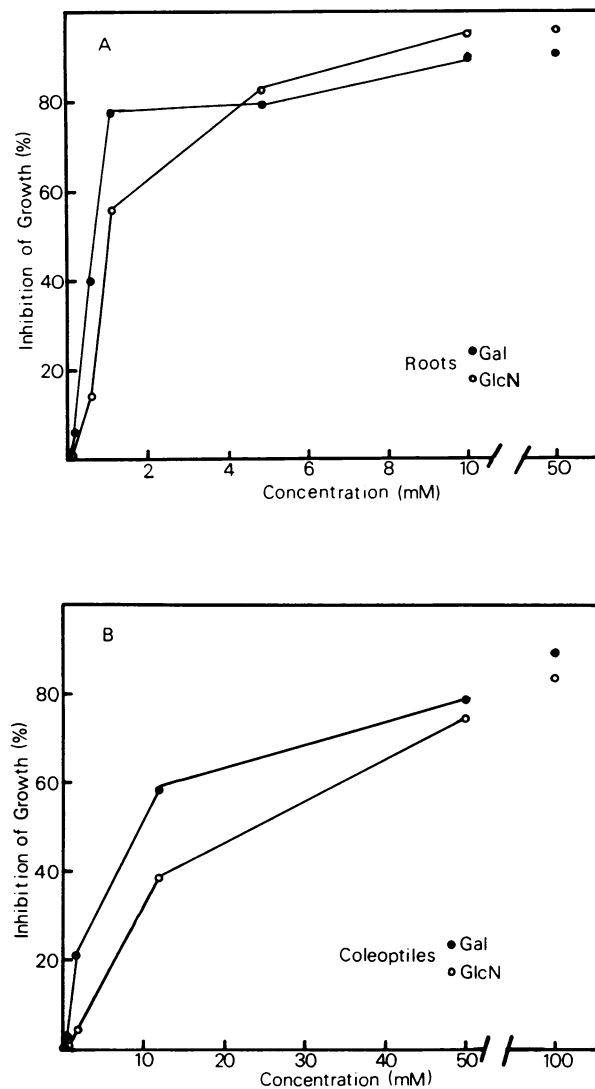


FIG. 1. Growth of corn roots (A) and excised barley coleoptiles (B) in presence of glucosamine and galactose.

tions of IAA to determine the best growth. Maximal rates of elongation were observed at 0.1 mM. Under these conditions, the coleoptiles increased their fresh weight by approximately 100% and their lengths by 74% in 6 hr. Tissues incubated without IAA showed only a 45% increase in fresh weight and an 18% increase in length.

The effects of GlcN and Gal were investigated under conditions at which the hormone stimulated coleoptile growth to a maximum. GlcN below 1 mM caused no significant inhibition of elongation or of increase in fresh weight (Fig. 1B). At the highest concentration tested (0.1 M) the amino sugar was clearly toxic, as the tissues failed to grow and some browning was observed. Gal was similar in its effects to GlcN, but, again, relatively high con-

centrations of sugar were required to inhibit growth completely (Fig. 1B).

Metabolism of GlcN by Corn Roots. Because the growth inhibition observed with both monosaccharides is assumed to be caused either directly or indirectly by the formation or accumulation of some product(s) of their metabolism, the radioactive monosaccharides were provided to the tissues at concentrations which ranged from the nearly toxic to the noninhibitory. After a period of metabolism which, in the experiments reported here, was either 3 or 6 hr the tissues were killed, and the ethanol-soluble products were analyzed in detail. The disappearance of the labeled compound from the medium was also determined at hourly intervals throughout the incubation to ensure that uptake was a continuous process.

GlcN was readily taken up by the roots and even at the highest concentration continued to be accumulated throughout the 6-hr incubation. Only small quantities of labeled CO_2 were evolved, and in no case did this radioactivity trapped in KOH amount to more than 0.3% of the ^{14}C taken up. Therefore, even though the sample of GlcN- ^{14}C used in these experiments was more than 98% radiochemically pure, it is conceivable that this $^{14}\text{CO}_2$ originated, not from the GlcN supplied, but from small quantities of impurity present (Table I).

After extraction of the tissues with ethanol, some radioactivity was found to be associated with the insoluble residue. This radioactive material is known to be of high molecular weight and is readily solubilized by treatment with alkali or proteases (21). At the highest concentration of GlcN, about 3% of the ^{14}C taken up was recovered in the ethanol-insoluble residue. This amounts to a flow of approximately 0.1 μmole of GlcN into macromolecular product in 3 hr.

Figure 2 shows an autoradiograph prepared from a chromatogram of the ethanol-soluble material from roots that had been

Table I. Uptake and Metabolism of *D*-Glucosamine- ^{14}C by Corn Roots

Excised corn roots (20; 0.4 g) were supplied with different concentrations of GlcN- ^{14}C in 2.5 ml of water. At the end of 3 hr, tissues were killed and extracted with 80% ethanol. Radioactivity in respired CO_2 was negligible and is not recorded here.

GlcN Supplied			Radioactivity Recovered from Roots		
Concn	Amount	Radioactivity	Ethanol-soluble	Ethanol-insoluble	Total
mM	μmoles	μc		μc	
10	25	5.0	0.61	0.019	0.629
2	5	5.0	1.74	0.063	1.803
1	2.5	5.0	1.73	0.069	1.799
0.2	0.5	5.0	2.78	0.131	2.911
0.13	0.33	5.0	3.05	0.210	3.260
0.07	0.18	5.0	3.05	0.286	3.336

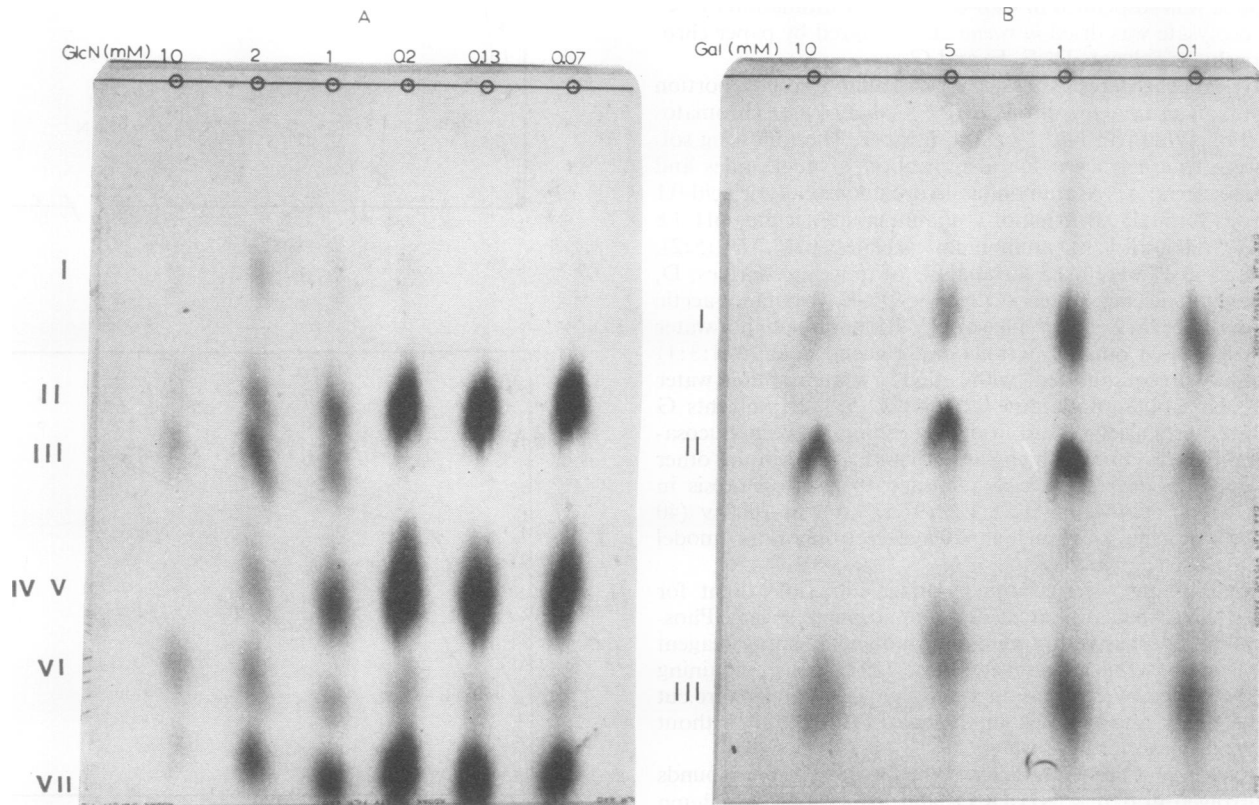


FIG. 2. Autoradiographs of chromatograms run in solvent B: 80% ethanol extract from corn roots fed GlcN- ^{14}C (A) and Gal- ^{14}C (B). Experiments A and B represent duplicates of experiments reported in Tables I and V, respectively.

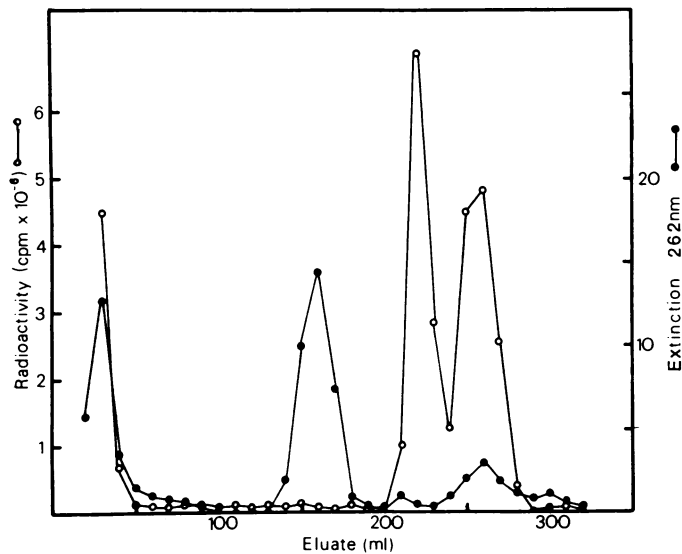


FIG. 3. Ion exchange chromatography of the ethanol-soluble extracts of corn roots fed D-glucosamine- ^{14}C .

provided with six different concentrations of GlcN. The identification of compounds VII(GlcNAc), VI(GlcN), V and IV (a mixture of GlcNAc-phosphates) has been detailed previously in experiments in which low concentrations of GlcN- ^{14}C were fed to corn roots (21). In the present experiments, V and IV chromatographed identically and so are estimated together. Compound II has been tentatively identified as UDP-GlcNAc on the basis of its electrophoretic and chromatographic mobility in a number of solvent systems, and because it was attacked by phosphodiesterase to give D-GlcNAc 1-phosphate (21). Here, a more complete analysis was undertaken. Roots (125) were incubated with 125 μC of D-GlcN-1- ^{14}C (53 $\mu\text{C}/\mu\text{mole}$) in 5 ml of H_2O for 6 hr. At the end of this time, 85% of the ^{14}C had been accumulated by the tissues. They were ground in hot 80% ethanol, and the soluble extract was dried and redissolved in 5 ml of 10 mM NH_4HCO_3 . This solution was loaded onto a column of DEAE-Sephadex pre-equilibrated with buffer, and the uncharged materials were washed through with 50 ml of bicarbonate solution (Fig. 3). Ionically bound compounds were then eluted with a linear gradient of ammonium bicarbonate. Two radioactive peaks were detected. The first of these (200–230 ml) consisted of a mixture of GlcNAc-phosphates; the second (250–280 ml) eluted in the same region as authentic UDP-GlcNAc. The latter fractions were combined and dried *in vacuo* at 35 C. The ammonium bicarbonate, which is volatile, was removed by adding

water and repeating the drying procedure three times. The radioactive compound showed no decomposition during this treatment, presumably because of the substitution on the 2-position of the pyranose ring. By contrast, UDP-glucose, which is very unstable even under mildly alkaline conditions, decomposes to its 1,2-cyclic phosphate (28). The putative UDP-GlcNAc was finally purified by paper chromatography on Whatman No. 3MM paper with solvent C. The compound had an ultraviolet absorption spectrum similar to that of uridine nucleotides at both pH 7 and pH 11 with a maximum at 262 nm. The absorption ratios measured at pH 7 were: 250/260 nm = 0.8 (0.74 ± 0.03) and 280/260 nm = 0.39 (0.38 ± 0.02). The data in parentheses are those recorded for UDP in *Specifications and Criteria for Biochemical Compounds* (27). The specific radioactivity based on a molar extinction index at 262 nm of 1×10^4 for UMP was 55 $\mu\text{C}/\mu\text{mole}$, a value very close to that of the GlcN- ^{14}C supplied. UDP-GlcNAc was also isolated after supplying 2 mM GlcN (specific radioactivity 1.0 $\mu\text{C}/\mu\text{mole}$). After purification, the nucleotide had a measured radioactivity of 0.94 $\mu\text{C}/\mu\text{mole}$. Both these experiments, therefore, indicate that there is no significant dilution of the end products of GlcN metabolism by endogenous precursors. This enables the pool size of radioactive metabolites in the tissues to be calculated from their measured ^{14}C content.

Compounds I and III have not been identified with certainty. They appear in significant amounts only at the higher GlcN concentrations when growth inhibition has already become apparent. Nevertheless, compound III is a major metabolite at 10 mM GlcN and contains over 20% of the radioactivity.

From Table II, it is clear that, as the GlcN concentration was increased in the medium to levels which become inhibitory to the growth of intact roots, the amount of each labeled metabolite increased markedly. In particular, the total amount of UDP-GlcNAc, the likely precursor of macromolecular material, rose from the region of 42 nmoles per 20 roots at 70 μM GlcN to 378 nmoles, 20 roots at 10 mM GlcN. Simultaneously the pool of phosphate esters, of GlcNAc, and of the unknowns increased manifold.

Metabolism of GlcN by Barley Coleoptiles. Glucosamine was taken up more slowly by the coleoptiles than by the corn roots when similar weights of plant tissue were compared. For example, at an external concentration of 70 μM GlcN, the rate of uptake by the coleoptiles was approximately one-tenth the rate shown by the roots.

At the higher concentrations, over 90% of the radioactivity incorporated into the tissues was recovered in the 80% ethanol extracts (Table III). The remainder was present in insoluble compounds of high molecular weight. As the GlcN concentration was reduced, a greater percentage of the ^{14}C taken up was converted to ethanol-insoluble material. Hydrolysis of this material released GlcN- ^{14}C .

Table II. Accumulation of Radioactive Metabolites in Corn Roots Fed Different Concentrations of D-Glucosamine- ^{14}C

Portions of the ethanol-soluble fraction from roots fed different concentrations of GlcN were chromatographed on Whatman No.1 paper in solvent B overnight. Chromatograms were placed on x-ray film for 3 days to detect radioactivity (see Fig. 2A). The radioactivity in each compound is expressed as a percentage of the total ^{14}C distributed along the solvent path.

External Concn of GlcN	Uptake into Ethanol-soluble Fraction		Unknown (I)	UDP-GlcNAc	Unknown (III)	Phosphate Esters	GlcN	GlcNAc
	%	nmoles	%	%	%	%	%	%
10 mM	12	3,000	4.5 (135) ¹	12.6 (378)	20.4 (612)	7.2 (216)	37.8 (1134)	17.6 (528)
2	35	1,750	5.0 (88)	16.7 (292)	20.8 (364)	12.6 (221)	16.2 (284)	28.6 (501)
1	35	880	2.0 (18)	18.7 (165)	10.2 (90)	29.7 (261)	10.3 (91)	29.0 (255)
0.2	56	280	0.4 (1)	25.6 (72)	0.7 (2)	37.4 (105)	4.8 (13)	31.0 (87)
0.13	61	200	0.3 (0.6)	33.6 (67)	0.4 (1.0)	33.6 (67)	3.6 (7)	29.4 (59)
0.07	61	110	0.4 (0.4)	37.5 (42)	0.5 (0.6)	33.8 (37)	3.6 (4)	24.2 (27)

¹ The figures in parentheses are the calculated amounts (in nmoles) of the various metabolites recovered from the roots.

Table III. Uptake and Metabolism of *D*-Glucosamine-1-¹⁴C by Barley Coleoptiles

Excised coleoptiles (35, 0.65 g) were supplied with different concentrations of GlcN-1-¹⁴C in 3 ml of 20 mM potassium phosphate buffer, pH 5.8, containing 0.1 mM IAA for 6 hr. Tissues were then washed and ground in 80% (v/v) ethanol. Radioactivity in respired CO₂ was negligible and is not recorded.

GlcN Supplied			Radioactivity Recovered from Coleoptiles		
Concn	Amount	Radioactivity	Ethanol-soluble	Ethanol-insoluble	Total
<i>mM</i>	μ moles	μ c	μ c		
10	30	10	0.60	0.04	0.64
1	3	10	0.83	0.03	0.86
0.17	0.5	10	0.86	0.08	0.94
0.07	0.2	10	0.90	0.22	1.12

Table IV. Accumulation of Radioactive Metabolites in Barley Coleoptiles Fed Glucosamine-¹⁴C

External Concn	Uptake into Ethanol-soluble Fraction		UDPGlcNAc	Phosphate Esters	GlcN	GlcNAc
<i>mM</i>	%	<i>n</i> moles	%	%	%	%
10	6.0	1,800	7 (126) ¹	7 (126)	33 (594)	53 (954)
1	8.3	250	13 (33)	13 (33)	28 (70)	46 (115)
0.17	8.6	43	25 (11)	23 (10)	10 (4)	42 (18)
0.07	9.0	18	28 (5)	20 (4)	14 (3)	38 (7)

¹ Figures in parentheses refer to the calculated amounts (in *n*moles) of the various metabolites recovered from the coleoptiles.

Table V. Uptake and Metabolism of *D*-Galactose-1-¹⁴C by Corn Roots

Excised corn roots (20) were supplied with different concentrations of Gal-1-¹⁴C in 2.5 ml of water. At the end of 3 hr, tissues were killed and extracted with 80% ethanol.

Gal Supplied				Radioactivity Recovered from Roots			
Concn	Amount	Radio-activity	CO ₂	Ethanol-soluble	HCl hydrolysate	Acid-resistant residue	Total
<i>mM</i>	μ moles	μ c		μ			
10	25.0	2.2	0.007	0.123	0.036	0.003	0.169
5	12.5	2.2	0.009	0.186	0.062	0.008	0.265
1	2.5	2.2	0.023	0.825	0.258	0.034	1.140
0.1	0.25	2.2	0.038	1.278	0.450	0.040	1.806

Table VI. Accumulation of Radioactive Metabolites in Corn Roots Fed Different Concentrations of *D*-Galactose-1-¹⁴C

External Concn	Uptake into Ethanol-soluble Fraction		Gal	Gal-1-P	UDP-Gal	Others
<i>mM</i>	%	<i>n</i> moles	%	%	%	%
10	5.4	1,350	47 (634) ¹	43 (581)	8 (108)	2 (27)
5	8.5	1,063	43 (457)	40 (426)	13 (138)	4 (43)
1	37.5	938	35 (328)	38 (356)	23 (216)	4 (38)
0.1	58.1	145	35 (51)	28 (41)	34 (49)	3 (4)

¹ Figures in parentheses refer to the calculated amounts (in *n*moles) of the various metabolites recovered from the roots.

When material soluble in ethanol was chromatographed in solvents A and B, only four radioactive regions were detected by x-ray film. These corresponded with UDP-GlcNAc, GlcNAc-phosphate, GlcN, and GlcNAc. The pool size of each of these metabolites increased markedly in the tissues as the GlcN concentration was increased (Table IV). The unknowns (I and III) found in corn roots were not detected in the coleoptiles.

Metabolism of Gal by Corn Roots. When Gal was fed to corn roots, a considerable proportion of the ¹⁴C taken up was recovered in material insoluble in 80% ethanol (Table V). Most of this radioactivity could be released by hydrolysis with 2 N HCl. The rest was associated with acid-resistant α -cellulose. Analysis of the hydrolysate revealed the presence of only two major radioactive compounds, which were identified as *D*-galactose-¹⁴C and *D*-glucose-¹⁴C, respectively. The former accounted for more than 80% of the radioactivity in each of the hydrolysates as determined by paper chromatography followed by radiochromatogram scanning. In contrast to the experiments with GlcN-¹⁴C, considerable amounts of radioactive CO₂ were evolved at the expense of the Gal supplied, and up to 4.4% of the ¹⁴C taken up by the roots was recovered in the alkali traps. This is consistent with earlier work (11), although we found little evidence for extensive diversion of label into other sugars or into pathways of intermediary metabolism.

After the ethanol-soluble fraction from roots fed Gal-¹⁴C had been chromatographed in either solvent A or B, three main radioactive regions were detected (Fig. 2B). At all four external concentrations of Gal tested, these compounds accounted for more than 90% of the ¹⁴C along the solvent track. Each of the compounds appeared chromatographically homogeneous when eluted from the papers, concentrated at low temperature, and rerun in solvent B.

Compound III was identified as *D*-galactose by paper chromatography and by reverse isotopic dilution analysis with crystalline *D*-galactose. Compound II is considered to be Gal-1-P. It cochromatographed with authentic standard in solvents A, B, and C, and it was completely hydrolyzed in the presence of alkaline phosphatase or 0.1 N HCl (100 C, 10 min) giving Gal-¹⁴C. No other radioactive monosaccharides were released during these treatments.

Compound I was identified as UDP-Gal on the following evidence: (a) It had the chromatographic and electrophoretic mobility of standard UDP-Gal in solvents A, B, and C and pyridine-acetic acid buffer. (b) The purified compound (after removal of UDP-glucose with UDP-glucose dehydrogenase and rechromatography in solvent B) had an ultraviolet absorption spectrum identical with that of uridine with a maximum wavelength at 262 nm and 250/260 and 280/260 absorption ratios of 0.70 and 0.41, respectively. (c) It was attacked by snake venom phosphodiesterase, giving Gal-1-P-¹⁴C and unlabeled UMP. (d) Dilute acid (0.1 N HCl, 10 min, 100 C) completely hydrolyzed compound I, releasing Gal-¹⁴C. Glucose accounted for less than 5% of the ¹⁴C recovered after this hydrolysis procedure at all four external concentrations of *D*-Gal employed. This indicates that the amount of UDP-glucose-¹⁴C formed at the expense of the Gal supplied was very small and that there was no ready equilibration *in vivo* between the two nucleoside diphosphate sugars.

As with the experiments with GlcN, there was a marked increase in the total amounts of nucleoside diphosphate sugar and sugar phosphate which accumulated in the tissues as the external levels of Gal were raised from 0.1 mM to 1 mM, a range of concentration which coincides with the onset of inhibition (Table VI). One interesting feature of the results, however, is that the pool of UDP-Gal declined when the concentrations were increased beyond 1 mM, even though Gal-1-P, its precursor, continued to accumulate in progressively larger amounts.

In a separate experiment from those described in Tables V and

VI, Gal was supplied to 100 roots for 6 hr at an external concentration of 10 mM and at a specific radioactivity of 2.3 $\mu\text{C}/\mu\text{mole}$. UDP-Gal (0.7 μmole) was isolated and purified by successive paper chromatography in solvents A and B. Any contaminating UDP-glucose, which chromatographs identically with UDP-Gal, was previously converted to UDP-D-glucuronic acid with the use of UDP-glucose dehydrogenase. The recovered UDP-Gal had a specific radioactivity of 2.2 $\mu\text{C}/\mu\text{mole}$, closely similar to that of the starting material, again indicating that the nucleoside diphosphate sugar was not significantly diluted by endogenous precursor compounds.

Metabolism of Gal by Coleoptiles. The products isolated from coleoptiles fed Gal were identical with those found in roots and showed a similar increase as growth was depressed. The rate of ^{14}C uptake, however, was much lower than that of the roots when expressed on a comparable fresh weight basis.

DISCUSSION

It is clear from the results presented here that the growth inhibition observed in both the coleoptiles and roots was proportional to the concentrations of GlcN and Gal present in the incubation medium. It is also evident that the decrease in cell elongation correlated well with a progressive increase in the internal pools of sugar nucleotides and sugar phosphates which are formed at the expense of exogenous monosaccharide. Presumably, either the monosaccharide itself or one or more of its metabolic products are responsible for the observed toxicity. Roots are probably more sensitive to inhibition than the shoot tissue because they accumulate the monosaccharides at a much faster rate.

Metabolism of Gal. Gal-1-P and galactitol accumulate in the tissues of human subjects with galactosemia or in animals maintained experimentally on a diet high in Gal (17, 25). Gal-1-P is usually considered to be the main toxic product, largely because it is known to inhibit phosphoglucomutase and therefore likely to interfere with hexose metabolism. Galactitol does not accumulate in the plant tissues tested and so cannot be responsible for the growth inhibition observed. The two main metabolites which are detected in any quantity are UDP-Gal and Gal-1-P. The concentration of the former in roots fed 0.1 mM Gal, when growth inhibition just begins to become apparent, was around 125 nmoles/g fresh weight of tissue. This is much higher than the amount recovered, for example, from untreated mung bean seedlings by Ginsburg *et al.* (14 nmoles/g) (7). In addition, the UDP-Gal isolated from roots in our experiments was of similar specific radioactivity to the Gal supplied, indicating that there was little dilution by endogenous precursors and that the pool size of this compound was therefore abnormally high.

Nevertheless, of the two main metabolites, Gal-1-P showed the most spectacular increases as the external sugar concentration was raised to levels that completely inhibited growth. Göring and Reckin have also detected Gal-1-P in tissues fed toxic levels of Gal and suggested that it interferes competitively with the formation of D-glucose 1-phosphate (8).

Metabolism of Glucosamine. GlcN is metabolized in much the same way in corn roots and barley coleoptiles as in animals, and a similar accumulation of GlcNAc, GlcNAc-P, and UDP-GlcNAc has been noted in liver supplied with high concentrations of GlcN (12, 18). It is known that UDP-GlcNAc can regulate its own synthesis from fructose 6-phosphate by specifically inhibiting the enzyme L-glutamine-D-fructose 6-phosphate amidotransferase (12, 16). Supplying GlcN, therefore, by-passes this step so that the amino sugar nucleotide and the other metabolites preceding it in the pathway continue to accumulate in an uncontrolled fashion, even though their normal *in vivo* synthesis from hexose is blocked. Corn roots, however, accumulate little GlcN-6-P at low external concentration of GlcN (21). This fact

plus the rapid formation of GlcNAc from GlcN noted in pulse chase experiments (21) suggests that *N*-acetylation might precede phosphorylation rather than vice versa.

Various explanations have been proposed to account for the cytotoxic effects of GlcN (see Ref. 31). Recently, it has been suggested that the phosphate esters formed might inhibit enzymes of intermediary carbohydrate metabolism (4). Bekesi and Winzler (31) have shown that the metabolism of GlcN by Sarcoma 180 Ascites tumor cells leads to a 4-fold increase in the pool of UDP-GlcNAc and considerable changes in the balance of the uridine nucleotide pool. In corn roots the concentration of UDP-GlcNAc increased, rising from the region of 95 nmoles/g tissue at 70 μM GlcN to almost 1 $\mu\text{mole/g}$ at 10 mM GlcN. By way of contrast, mung bean seedlings germinated in water contain approximately 1.5 nmoles of nucleotide per gram of tissue (26).

It is conceivable, of course, that the monosaccharides themselves are the toxic agents. The internal levels of both, for example, increased as their external concentrations were raised. Corn roots, however, will accumulate L-arabinose, a noninhibitor, and a structural analogue of D-galactose without ill effects (23). This sugar is also efficiently converted into the cell wall as pentosyl units, but the intermediates, L-arabinose-1-phosphate and UDP-L-arabinose, are recovered in only small amounts. Only in this respect does the general pattern of its metabolism differ from that of Gal.

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