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THE RESPONSE OF CHICORY ROOT TISSUE DISKS TO TREATMENT WITH 2,4- AND 3,5-DICHLOROPHENOXY-ACETIC ACIDS

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Abstract—Treatment of tissue disks from chicory root with 10^{-5} M solutions of the highly active plant growth regulator 2,4-dichlorophenoxyacetic acid (2,4-D) results in a very large water uptake. This is accompanied by extensive hydrolysis of oligosaccharides and a very marked increase in hydrolase and invertase activity. These effects are not obtained when the inactive growth regulator (3,5-D) is used. The results are discussed and compared with those already reported for tissue from the tubers of Jerusalem artichoke.[2]

INTRODUCTION

PREVIOUS studies[1] have indicated a correlation between physiological response and enzyme activity when tissue from certain inulin-storing roots and tubers were treated with the highly active growth regulator 2,4-dichlorophenoxyacetic acid (2,4-D). More detailed work using tissue from Jerusalem artichoke tubers has been reported recently and the effect of 2,4-D on the hydrolysis of oligosaccharides to simpler sugars and on the activity of some enzymes of carbohydrate metabolism described.[2]

The present study extends the work to a more detailed examination of the behaviour of a different plant tissue—chicory root. As in the case of Jerusalem artichoke, inulin is the stored polysaccharide but chicory root differs in that untreated material contains some soluble invertase whereas artichoke tubers have none (except at a very young stage of development—Flood and Rutherford, unpublished). Another difference is seen in the water uptake response to growth-regulator treatment, chicory tissue taking up nearly three times as much water as the same initial weight of artichoke tissue. It was of interest, therefore, to see whether the results obtained using Jerusalem artichoke tubers[2] would be supported when tissue from a different plant organ (a root) was used and where also there was a different initial enzyme activity.

RESULTS

The effect of duration of treatment on the magnitude of water uptake induced in disks cut from chicory roots when treated with either water or 10^{-5} M solutions of 2,4-D or 3,5-D is illustrated in Fig. 1. Each treatment induced a small water uptake during day 1, but only the 2,4-D treatment led to further water uptake which occurred during the following 2 days. This uptake with 2,4-D was very large (Fig. 1).

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[1] A. E. FLOOD, P. P. RUTHERFORD and E. W. WESTON, *Nature* **214**, 1049 (1967).
[2] P. P. RUTHERFORD, E. W. WESTON and A. E. FLOOD, *Phytochem.* **8**, 1859 (1969).

Changes in the amounts of the various sugars present in the tissue disks after treatment for 0, 1, 2, 3 or 4 days with water or 10^{-5} M solutions of either 2,4-D or 3,5-D are shown in Figs. 2–5.

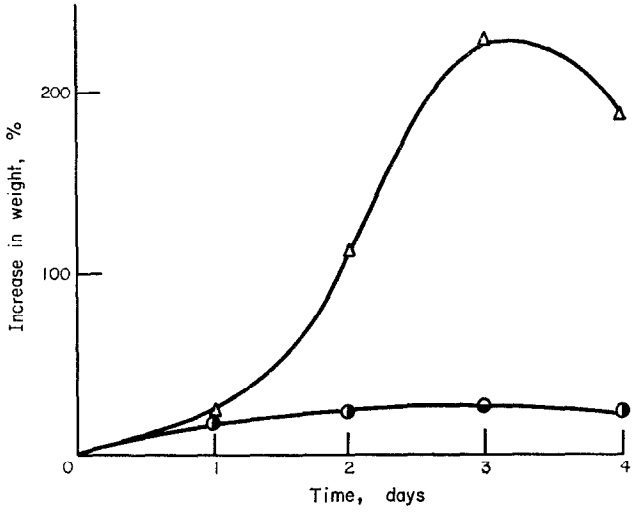

FIG. 1. THE WATER UPTAKE INDUCED IN DISKS OF CHICORY ROOT AT 25° BY TREATMENT WITH WATER OR 10^{-5} M SOLUTIONS OF EITHER 2,4-D OR 3,5-D FOR VARYING PERIODS OF TIME. △, 10^{-5} M 2,4-D; ●, 10^{-5} M 3,5-D; ○, Water.

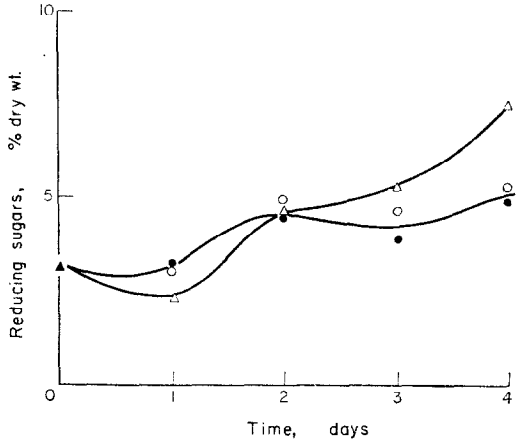

FIG. 2. CHANGES IN % REDUCING SUGARS IN CHICORY ROOT DISKS AT 25° AFTER TREATMENT WITH WATER OR 10^{-5} M SOLUTIONS OF EITHER 2,4-D OR 3,5-D FOR VARYING PERIODS OF TIME. △, 10^{-5} M 2,4-D; ●, 10^{-5} M 3,5-D; ○, Water.

Both water and 3,5-D treatment had little effect on any of the sugars present in the chicory disks. In both cases there was a slight increase in the amount of insoluble sugar during the first day of treatment which was followed by a small decrease. The small decrease in the

amounts of each soluble sugar except fructose resulted in an overall decrease in the amount of total soluble sugar present in both water and 3,5-D-treated tissues during the whole of the treatment period.

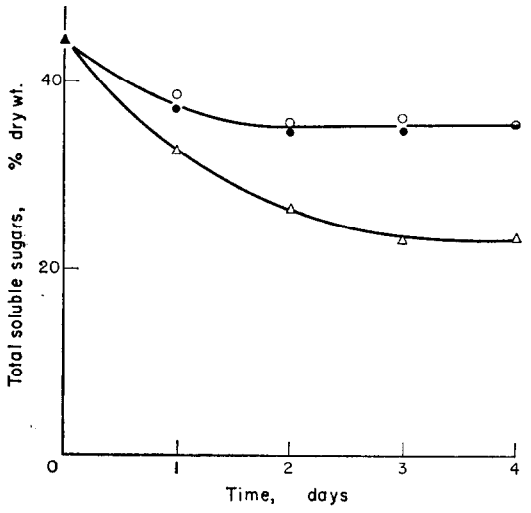

FIG. 3. CHANGES IN TOTAL SOLUBLE SUGARS AS % DRY WT. OF CHICORY ROOT DISKS AT 25° AFTER TREATMENT WITH WATER OR 10^{-5} M SOLUTIONS OF EITHER 2,4-D OR 3,5-D FOR VARYING PERIODS OF TIME. △, 10^{-5} M 2,4-D; ●, 10^{-5} M 3,5-D; ○, Water.

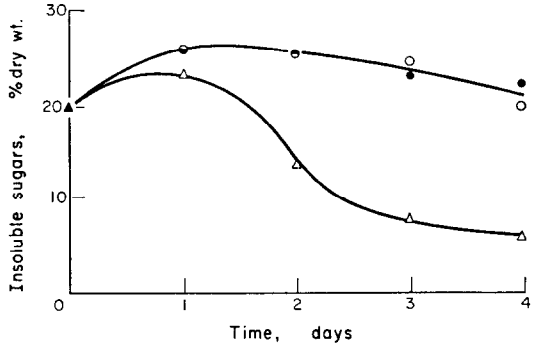

FIG. 4. CHANGE IN INSOLUBLE SUGAR AS % DRY WT. OF CHICORY ROOT DISKS AT 25° AFTER TREATMENT WITH WATER OR 10^{-5} M SOLUTIONS OF EITHER 2,4-D OR 3,5-D FOR VARYING PERIODS OF TIME. △, 10^{-5} M 2,4-D; ●, 10^{-5} M 3,5-D; ○, Water.

Treatment of the disks with 10^{-5} M solutions of 2,4-D in contrast to the water and 3,5-D treatments led to a marked increase in weight due to water uptake. There was also a decrease in each sugar with the exception of fructose and the insoluble sugars. There was a slight increase in the amount of insoluble sugar present in the 2,4-D-treated disks after 1 day but

this was followed by a considerable decrease. Although all the other soluble sugars decreased throughout the duration of the 2,4-D treatment, fructose showed some increase after 2 days of treatment with 2,4-D.

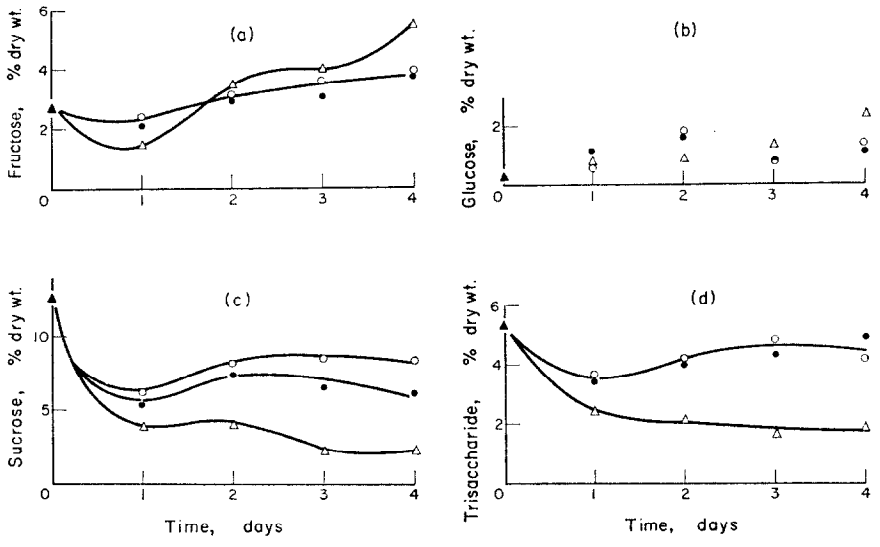

FIG. 5. CHANGES IN SOME INDIVIDUAL SUGARS AS % DRY WT. OF CHICORY ROOT DISKS AT 25° AFTER TREATMENT WITH WATER OR 10^{-5} M SOLUTIONS OF EITHER 2,4-D OR 3,5-D FOR VARYING PERIODS OF TIME. △, 10^{-5} M 2,4-D; ●, 10^{-5} M 3,5-D; ○, Water; (a) Fructose; (b) Glucose; (c) Sucrose; (d) Trisaccharide.

TABLE 1. TOTAL HYDROLASE AND INVERTASE ACTIVITIES OF PROTEIN PREPARATIONS

Treatment	Time (days)	Protein* × 10^3	Units of invertase activity × 10^6†	Units of hydrolase activity × 10^6‡
Untreated	—	12·88	43·5	107·1
Water	3	18·65	52·1	181·3
10^{-5} M 2,4-D	3	26·23	2,693	185·9
10^{-5} M 3,5-D	3	11·32	13·4	188·8

* mg Protein/mg initial dry wt.
† One unit of invertase activity represents 2 μmoles of hexose liberated/min/mg initial dry wt. at 25°.
‡ One unit of hydrolase activity represents 1 μmole of hexose liberated/min/mg initial dry wt. at 25°.

Table 1 shows the amounts of protein and both invertase and hydrolase activities for untreated disks and for disks treated with water, 10^{-5} M 3,5-D or 10^{-5} M 2,4-D. Since 3,5-D treatment did not produce any of the large effects shown by 2,4-D-treated tissue, only enzyme extracts from untreated, water, or 2,4-D-treated disks were fractionated. The data obtained are shown in Table 2. The sequence of eluents gave, in general, four peaks based on

measurements of the optical density of the column eluate at 254 nm. Such an elution profile is shown in Fig. 6.

Treatment of the chicory disks with water for 3 days resulted in an approximate 45% increase in the total amount of protein, this increase being evenly distributed amongst peaks 1, 2 and 3. Treatment with 2,4-D led to a further increase in total protein, the extra protein

TABLE 2. *INVERTASE AND HYDROLASE ACTIVITIES OF PROTEIN FRACTIONS AFTER CHROMATOGRAPHY ON DEAE CELLULOSE*

Treatment	Time (days)	Peak	Protein × 10^{-3}	Units of invertase activity × 10^{-3}	Units of hydrolase activity × 10^{-3}
Untreated	—	1	1·36	1·33	60·8
		2	1·48	0·69	8·89
		3	6·96	50·2	18·1
		4	0	0	0
Water	3	1	3·31	1·08	39·2
		2	2·10	50·7	68·1
		3	9·33	0	15·8
		4	0	0	0
10^{-5} M 2,4-D	3	1	2·90	14·8	55·7
		2	2·40	2,106	38·1
		3	14·78	672	38·4
		4	9·22	67·4	0

For units, see legend of Table 1.

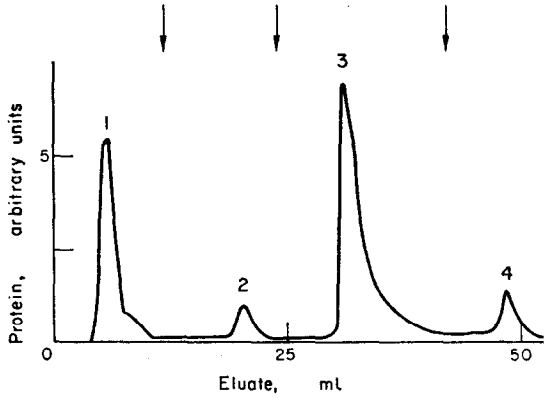

FIG. 6. CHROMATOGRAPHY OF A PROTEIN PREPARATION FROM CHICORY ROOT ON DEAE CELLULOSE. PROTEIN WAS APPLIED TO THE COLUMN IN 5 mM PHOSPHATE BUFFER pH 7·5.

Eluent was changed stepwise as shown by the arrows to 50 mM phosphate, 0·5 M NaCl in 50 mM phosphate and finally M NaCl in 50 mM phosphate buffer, all at pH 7·5.

appearing in peaks 3 and 4. Peak 4 was small and, except for 2,4-D-treated tissue, gave no indication of protein when analysed.

Total hydrolase activity increased considerably regardless of the treatment and although present in each of the first three peaks its distribution varied with the treatment to which the disks had been subjected. The main hydrolase activity in both untreated and 2,4-D-treated material appeared in the first peak whilst for water-treated disks it was concentrated in Peak 2.

Little invertase activity was found in both water and untreated material; mainly in peak 3

for untreated tissue, but in peak 2 for water-treated tissue. The massive increase in invertase activity caused by 2,4-D treatment is mainly concentrated in peak 3. Smaller amounts of invertase activity appeared in peaks 1 and 4.

DISCUSSION

Because of the possible effects of cold storage, the carbohydrate composition of untreated chicory disks was measured both at the beginning and the end of the investigation and only small differences (less than $\pm$ 10%) were found. These differences are within the experimental variation found in the measurements made using chicory roots. The variability appears to be due mainly to genetic differences among chicory roots grown from seed.

As in the case of Jerusalem artichoke[2] the amount and composition of sugar in chicory root disks and the uptake of water was hardly altered by treatment with either 10^{-5} M 3,5-D or water. 3,5-D shows no auxin activity but when 10^{-5} M solutions of the very active growth regulator 2,4-D are used the disks took up very large amounts of water.

Although the water uptake of chicory disks followed a similar pattern to disks cut from Jerusalem artichoke tubers,[2] the rate of uptake after the 1 day "lag" period was considerably more rapid and the maximum value almost three times larger. 2,4-D treatment caused a considerable breakdown of soluble sugars but, unlike the sugars in Jerusalem artichoke tissue, the rate of this decrease in the soluble sugars present in chicory disks declined markedly after 2 days of treatment.

When enzyme activities are considered, the most striking effect of 2,4-D treatment compared with either water or 3,5-D treatment is the enormous increase in invertase activity, many times greater than that which could be induced by similar treatment in Jerusalem artichoke tissue. Most of this increase occurs in peak 2 whereas the increase in protein seems to be confined to peak 3. The appearance of some invertase activity in all the peaks after 2,4-D treatment means that either the elution characteristics of the enzyme have been altered or the column has been grossly overloaded. The latter explanation is unlikely because smaller aliquots of the extracts from 2,4-D-treated material were fractionated in order to avoid overloading. Moreover, the peak heights of the elution profile were similar to those obtained with extracts from either water or untreated material.

An alternative explanation may be that the growth regulator has directly or indirectly caused the invertase protein to dissociate or associate into fractions with different elution characteristics and specific activities. It is not possible to distinguish between these two explanations because, with chicory, the elution programme does not completely separate invertase from hydrolase activity. However, the location of the protein increase in a peak different from that containing the major increase in enzyme activity suggests that specific activities must be considerably altered. This whole question is now under active investigation.

Changes in hydrolase activity are small when compared with those produced in Jerusalem artichoke tuber disks and very much smaller than the invertase activity changes in disks from chicory root. However, as in the case of invertase, there is an alteration in the elution characteristics of the hydrolase after both water and 2,4-D treatment.

Incubation in water of tissue cut from storage organs of plants is well known to lead to profound physiological and biochemical changes (e.g. Laties)[3] and some of these effects, such as increase in overall protein content, are seen here. Chicory differs from artichoke in

[3] G. G. LATIES, *Arch. Biochem. Biophys.* **79**, 364 (1959).

that some invertase can be extracted from the untreated tissue, but, like artichoke,[2] when 2,4-D-treated tissue is compared with water-treated tissue, then further dramatic effects are seen, both in relation to a physiological response (water uptake) and biochemical characters (enzyme activities). Chicory root tissue responds to auxin treatment in a manner similar to other biological systems used as test material, e.g. wheat coleoptile, pea segments,[4] so the demonstration of a relationship between auxin treatment and altered enzyme activities in chicory root disks may have wider implications.

When discussing the very large increase in enzyme activity induced in artichoke tissue by 2,4-D treatment, Rutherford *et al.*[2] suggested that perhaps the auxin loosened enzyme bound to the cell wall and that this might account for the increased activity found in the protein preparations from treated material. The change in elution profile of enzymes after 2,4-D treatment seemed perhaps to support this view in that if treatment with a growth regulator caused dissociation of the enzyme protein into smaller units, then perhaps these would be held less firmly to the cell wall. More recent work in this laboratory suggests that at least, in the case of chicory, this is unlikely. Although the activity of extracted protein is very much greater after 2,4-D treatment so is the activity of a cell-wall preparation when incubated directly with substrate. If the growth regulator had merely loosened enzyme protein from the cell wall, then the activity obtained from the wall after treatment should have been lower than that of the control by an amount equivalent to the increase in activity of soluble enzyme protein. This is not the case and although some solubilization of protein attached to the wall cannot be ruled out, it seems very unlikely that such a concept would account entirely for the results reported here.

EXPERIMENTAL

Chicory roots, variety Magdeburg, were obtained from F. G. Harrison, Bury St. Edmunds, Suffolk, in the autumn and stored in moist peat at $3 \pm 1°$ for approx. 26 weeks. After an initial 4-week storage period, when the water uptake response had increased to a reasonably constant value, roots were removed from the store as required and disks of tissue prepared from them as described by Rutherford *et al.*[4]

Extraction and Chromatographic Examination of Sugars

The methods used were those described by Rutherford and Weston.[5]

Determination of Water Uptake and Sugars

Determination of the water uptake and the amount of sugars present were made after 0, 1, 2, 3 and 4 days' treatment. For each determination six different roots were used. Water uptake and sugar content were determined by methods already described[2] for Jerusalem artichoke.

Protein Preparation from Tissue Disks

Sufficient disks to give a fresh weight of *ca.* 40 g were used for each treatment and total protein extracts prepared as previously described.[2] After assay of total hydrolase and invertase activity,[2] the protein preparation was fractionated on a 1-g column (10 × 1 cm) of DEAE cellulose[6] but with a modified elution procedure. The sequence of eluents used was 5 mM phosphate buffer, 50 mM phosphate buffer, 50 mM phosphate buffer containing 0·5 M NaCl and finally 50 mM phosphate buffer containing M NaCl. All eluents had a pH of 7·5. Elution with each eluent was continued until no more material absorbing light at 254 nm appeared in the eluate, the latter being continually monitored at 254 nm. The column flow rate was 48 ml/hr and 4-ml fractions were collected automatically, all operations being conducted at 3°. Fractions corresponding to a single peak were combined and analysed for hydrolase and invertase activity and for protein content. The assays of enzyme activity and determination of protein were performed as described[2] except that the substrate used for measurement of hydrolase activity was obtained from chicory by a similar method to that used to prepare substrate M from artichoke tubers.[6]

Acknowledgement—We wish to thank Professor R. L. Wain for his encouragement and advice during this work.

[4] P. P. Rutherford, C. M. Griffiths and R. L. Wain, *Ann. Appl. Biol.* **58**, 467 (1966).
[5] P. P. Rutherford and E. W. Weston, *Phytochem.* **7**, 175 (1968).
[6] J. Edelman and T. G. Jefford, *Biochem. J.* **93**, 148 (1964).