Phytochemistry, 1971, Vol. 10, pp. 1635 to 1638. Pergamon Press. Printed in England.

# WATER UPTAKE AND INVERTASE AND HYDROLASE ACTIVITIES INDUCED IN CHICORY ROOT DISKS BY TREATMENT WITH VARIOUS PLANT GROWTH-REGULATORS

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*(Received 7 August 1970)*

Abstract-Chicory disks were treated for 3 days at 25" with a range of plant growth-regulators, at a concentration close to that which induced maximum uptake of water. Growth-substance treatment of the disk8 was found to approximately double both the hydrolase activity of the soluble protein and its amount when compared to values obtained for the water treated controls. The measured increase. in invertase activity of the soluble protein which was produced by growth-substance treatment followed a similar pattern to the induced water uptake. These results are consistent with the proposal that all plant growth-regulating substances have a common function in modifying the control exerted by the cell nucleus on enzyme synthesis.

## INTRODUCTION

PLANT growth-regulators are usually divided into a number of different types, the three most important being: the auxins, the cytokinins and the gibberellins.[1] Although compounds which fall into each of these groupings are known to occur in, and have been extracted from plants, a very considerable number of synthetic compounds have been prepared which possess properties characteristic of either the auxins or cytokinins. Classification of any substance into a particular type is based on both physiological response and basic chemical structure. However, recent evidence indicates that auxins, cytokinins and gibberellins may all have a common primary function in the regulation of cell processes. It is widely considered that this primary function involves some modification of the control exerted by the nucleus on the synthesis of nucleic acid and protein, and hence enzymes within the cell.

In the work described here we have examined the effects of treating chicory tissue disks with plant growth-regulators, selected from each of the three main types-auxins, cytokinins and gibberellins, on the activities of two enzymes-invertase and hydrolase, both present in soluble protein extracted from the disks. We have also compared the water uptake induced in the disks by the growth-regulators with the increases observed in both invertase and hydrolase activities.

## RESULTS

Table 1 shows the water uptake induced in chicory tissue disks after treatment for 3 days at 25" with a number of plant growth-regulators, at concentrations just lower than those which cause the maximum uptake of water. The corresponding amounts of total soluble protein extracted from the disks after treatment, together with both the invertase

[1] M. B. WILKINS (Ed.), *Physiology of Plant Growth and Development,* McGraw-Hill, New York (1969).

TABLE 1. THE WATER UPTAKE, THE AMOUNTS OF SOLUBLE PROTEIN AND THE INVERTASE AND HYDROLASE ACTIVITIES OF CHICORY TISSUE DISKS AFTER TREATMENT FOR 3 days AT 25°

| Quantity measured / Treatment | Water* uptake | Invertase† activity | Hydrolase‡ activity | Total§ soluble protein |
|---|---|---|---|---|
| Untreated | — | 18 | 60 | 3·7 |
| Water | 27·3 | 24 | 107 | 4·0 |
| $10^{-4}$ M Gibberellic acid | 41.3 | 61 | 229 | 7·3 |
| $10^{-4}$ M Kinetin | 67·9 | 128 | 245 | 6.6 |
| $10^{-3}$ M 3-Indole acetic acid | 106.3 | 335 | 242 | 8·1 |
| $10^{-5}$ M 2,4-Dichlorophenoxy-acetic acid | 138·3 | 542 | 237 | 7·4 |

* Percentage increase in initial wet wt.
† Liberation at 25" of 2 $\mu$moles of hexose per min per mg of initial dry wt. $\times 10^6$.
‡ Liberation at 25" of 1 $\mu$mole of hexose per min per mg of initial dry wt. $\times 10^6$.
§ mg protein per mg of initial dry wt. $\times 10^3$.

and hydrolase activities of this protein are included in Table 1. Data for both untreated and water treated disks is also shown.

The induced water uptake varied from 27·3% for the water treated controls to 138·3% for disks treated with $10^{-5}$ M solutions of 2,4-dichlorophenoxyacetic acid (2,4D).

Similar amounts of soluble protein, 3·7 and 4·0 mg respectively, per gram dry tissue were extracted from the untreated and the water treated disks. The amounts, varying from 6·6 to 8·1 mg, of soluble protein extracted per gram of initial dry tissue showed no significant differences between disks treated with any of the growth-regulators, and was about twice the amount extracted from the controls. Fractionation showed that this extra protein extracted from the growth-substance treated disks was approximately equally distributed between the inactive fraction and each of the fractions which possessed either invertase or hydrolase activity.

There was no significant difference between the hydrolase activities, 229-245 units, of the soluble protein extracted from chicory disks treated with any of the plant growth-regulators. The average hydrolase activity, 238 units, of the soluble protein from treated disks was just more than twice that found in protein from the water treated controls.

The invertase activities of protein extracted from untreated and water treated disks were very similar, 18 and 24 units respectively. For each of the growth-regulators there was a highly significant increase in the invertase activity of the soluble protein extracted from the disks, and this increase varied from 61 units for $10^{-4}$ M solutions of gibberellic acid up to 542 units for $10^{-5}$ M solutions of 2,4D.

## DISCUSSION

A representative example was chosen from each of the three main types of plant growth-regulator: 3-indole acetic acid (IAA) as a typical auxin, kinetin, 6-furfuryl amino purine (KIN) as a typical cytokinin and gibberellic acid (GA) as a typical gibberellin. Also included was 2,4D as an example of a widely investigated synthetic plant growth-regulator.

Preliminary studies made it possible for the concentration of each plant growth-regulator to be chosen so as to induce an uptake of water in the chicory disks which was close to the

maximum for that particular growth-regulator. This choice of concentration was found to produce, in the disks, a series of water uptakes which ranged from 41·3% to $10^{-4}$ M solutions of GA up to 138·3% for $10^{-5}$ M solutions of 2,4D. The values were all significantly larger than 27·3%, the percentage of water taken up by the water control and similar to values obtained previously.[2]

The amounts of soluble protein extracted from the chicory tissue disks were always lower than in previous years.[3] Nevertheless, each growth substance approximately doubled both the amount of soluble protein which could be extracted from the disks after treatment, and also the hydrolase activity of this protein. Furthermore, fractionation, although not completely efficient, did bring about sufficient separation of the different proteins present in the soluble extract to show that each growth-substance approximately doubled the amount of protein which possessed hydrolase activity.

This present work does not enable us to distinguish between the possibilities that growth-substance treatment either caused the production of fresh protein or merely the solubilization of hydrolase protein already present. However, the very close agreement between the activities for all the treated disks indicates that each growth-substance has a similar effect upon hydrolase.

The invertase activity of the soluble protein extracted from the disks after treatment showed considerable variation between each of the growth-substances and followed a

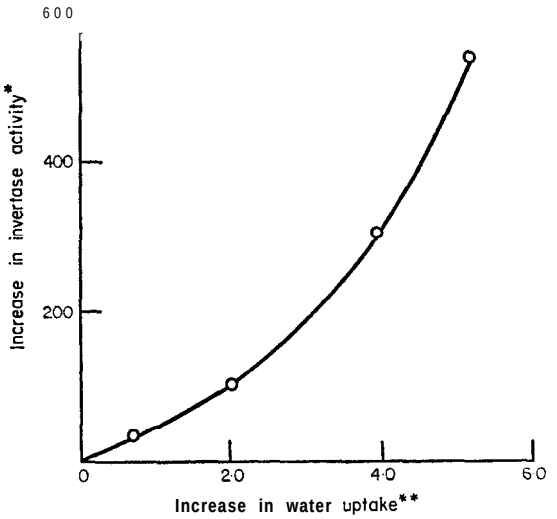


FIG. 1. THE RELATIONSHIP BETWEEN THE INCREASE IN WATER UPTAKE AND THE INCREASE IN INVERTASE ACTIVITY INDUCED IN CHICORY TISSUE DISKS AFTER TREATMENT WITH DIFFERENT PLANT GROWTH-REGULATORS AT 25".

* Increase in Invertase Activity represents the difference between the number of 2 μmoles of hexose liberated at 25" per min per mg initial dry weight × $10^6$ for the growth-substance treated disks and the water treated controls.

** Increase in water uptake represents the difference between the number of grams of water taken up per gram of initial dry weight by the growth-substance treated disks, and by the water treated controls.

[2] P. P. RUTHERFORD, C. M. GRIFFITHS and R. L. WAIN, *Ann. Appl. Biol. 58,467 (1966).*
[3] A. E. FLOOD, P. P. RUTHERFORD and E. W. WESTON, *Phytochem.* (in press).

similar variation to the induced water uptake. The relationship between the increase, over the water control, of both the water uptake and the invertase activities shown in Fig. 1, illustrates the close interdependence of these two effects.

In conclusion, both the similarity of the effect upon hydrolase activity and the relationship between water uptake and invertase activity, induced by very different plant growth-regulators, support the idea of a common primary function for all these chemicals.

## EXPERIMENTAL

### *Biological Material and Treatment*

Witloof chicory roots, variety Manila, were grown near Bath, from seed supplied by the Station de Recherches de l'etat pour l'amelioration des Plantes Fruitieres et Maraicheres, Gembloux, Belgium. The roots were lifted in November and stored in moist peat at 4 ± 2" until use the following February and March.

The following solutions were prepared: $10^{-4}$ M GA, $10^{-4}$ M KIN, $10^{-3}$ M IAA and $10^{-5}$ M 2,4D. For each of these solutions and for $H_2O$, 12 Petri dishes each containing 6 weighed chicory disks* were incubated at 25" for 3 days. Then, each disk was blotted dry, reweighed and the water uptake for each treatment was determined.[2]

### *Protein Extraction, Fractionation, Determination and Assay for Invertase and Hydrolase Activity*

The disks for each treatment were bulked and the soluble protein was extracted and fractionated by methods described previously.[3-5] A sample of 72 untreated disks was similarly treated. The invertase[4] and hydrolase[4] activities of both the unfractionated and fractionated protein were measured and the amount of soluble protein was determined by the micro-Kjeldahl estimation of $N_2$.

[4] J. EDELMAN and T. G. JEFFORD, *Biochem. J. 93,148 (1964).*
[5] P. P. RUTHERFORD, E. W. WESTON and A. E. FLOOD, *Phytochem.* **8,** 1859 *(1969).*