UPTAKE OF MANNITOL INTO THE SHOOTS OF INTACT BARLEY PLANTS

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Summary

Barley plants grown in mannitol solutions wilted much more severely than those grown in sodium chloride solutions. Recovery from wilting after transference to a basal nutrient solution was rapid.

Following transference to a basal nutrient solution mannitol was excreted from the leaves in the guttation water.

The physiological importance of this uptake of mannitol is briefly discussed.

I. Introduction

Mannitol is used frequently as an osmotic substance in short-term experiments on water relations of plant tissues. For such experiments it is presumed to be physiologically inert, and its permeation is considered to be very slow (Collander and Bärlund 1933; Allsopp 1955). Collander and Bärlund studied the permeation of non-electrolytes in the cell sap of *Chara ceratophylla*. They assessed the half saturation time‡ for mannitol to be greater than 35 days.

The use over a period of some days of an osmotic substance not absorbed by the plant would be advantageous in work on water relations of intact plants and in salinity studies. In salinity studies the use of mannitol could help to distinguish between osmotic effects, caused by the reduced water availability in the substrate, and effects of ions absorbed by the plant.

Such a comparison between the effects of sodium chloride and mannitol on the growth of young barley plants was made. In this paper evidence is presented that mannitol was absorbed during the course of the experiment by the shoots of the intact plants.

II. Methods

The results described in this paper were obtained in an experiment which was part of an investigation regarding salinity effects on plant growth. In this work plants are subjected to a brief salinity stress. After subsequent salt removal from the substrate a study is made of the recovery in growth.

Barley seeds, cv. Chevron, were germinated in river sand. When the first leaf had fully expanded the seedlings were transplanted into basal nutrient solution of Arnon (cf. Hewitt 1952). This solution had a pH of 4·5, and 1 m-equiv/l of sodium chloride was supplied.

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‡ The half saturation time is defined as the time in which the concentration of the cell sap had attained 50 per cent. of the concentration of the substrate.

Treatments were applied at the sixth day after transplanting. They consisted of basal nutrient solution, and of the same solution to which either mannitol or sodium chloride was added. In the mannitol and sodium chloride treatments the osmotic pressure of the solution was increased each day by 2 atm. The highest concentration (8 atm) was attained at the fourth day of the treatment. The plants were then transferred to basal nutrient solution on the morning of the fifth day after treatment application.

To prevent fungal development in the mannitol solutions the roots of all treatments were washed thoroughly each day with half a litre of the appropriate solution. This rinsing and replacement of solution was also carried out 2 and 4 hr after mannitol removal, and then again each morning. No noticeable fungal development took place during the experiment.

Twelve replicates of each treatment were harvested on three occasions: at treatment application (H_1), at date of mannitol removal (H_2), and 4 days later (H_3).

Mannitol was detected by the paper-ionophoresis method of Frahn and Mills (1959).

III. Results

(*a*) *Growth and Wilting Response*

The dry weight and relative growth rate of the mannitol-treated plants was depressed during the mannitol treatment and also, more strongly, in the period following mannitol removal (Table 1). Slight wilting was observed in the mannitol-treated plants after the concentration was increased to 4 atm. Wilting phenomena in solutions with an osmotic pressure of 8 atm are shown in Plate 1, Figure 1. All the leaves of mannitol-treated plants wilted severely directly after transference to the 8 atm solution. At this concentration only the youngest leaf of the sodium chloride-treated plants showed slight wilting. After mannitol and sodium chloride removal all plants recovered rapidly.

The wilting in the mannitol cultures was presumably mainly due to the high osmotic pressure of the substrate. This was indicated by the sudden increase in wilting phenomena which was observed directly after an increase in the concentration of the solution. It is of interest that sodium chloride solutions of the same osmotic strength showed much later and less severe wilting.

(*b*) *Excretion of Crystalline Substance*

Mannitol-treated plants showed a necrotic burn of the leaf tips at the date of mannitol removal. This burn extended, in the old leaves, about 1–1½ cm from the tip.

During the night following mannitol removal, all mannitol and control plants guttated vigorously. The following morning a white crystalline substance was found on all the leaves of the mannitol-treated plants. The substance was usually found on the leaf tips (Plate 1, Fig. 2), but sometimes guttation water fell either on the cardboard covers or on the leaf blades. In all cases the crystalline substance was found in these locations, causing necrotic burns in the case of the leaf blades. The substance was collected from the leaf tips only.

(c) *Nature of the Crystalline Substance*

The solid left on evaporation of the guttation water was dissolved in water to a concentration of 35 mg/ml. This solution was used for paper ionophoresis in borax at pH 9·2, sodium arsenite at pH 9·6, and basic lead acetate (Frahn and Mills 1959). In each electrolyte, the unknown afforded only one component, migrating at the same rate, and reacting in the same way with spray reagents as pure D-mannitol run on the same paper strips. The substance had m.p. 164°C, not depressed by mixing it with pure D-mannitol of m.p. 166°C. Condensation of 2 mg of the solid with excess *cyclo*hexanone containing sulphuric acid gave, in 55 per cent. yield, a crystalline

TABLE 1

DRY WEIGHT AND RELATIVE GROWTH RATE OF WHOLE BARLEY PLANT

Treatment	Dry Weight (g)			Relative Growth Rate (g/g/day)	
	At Treatment Application (H_1)	At Date of Mannitol Removal (H_2)	4 Days after Mannitol Removal (H_3)	H_1–H_2	H_2–H_3
Control	0·0471	0·083	0·155	0·142	0·139
Mannitol	—	0·078	0·120	0·126	0·108
Sodium chloride	—	0·079	0·126	0·130	0·115

derivative, shown by melting point and mixed melting point determinations to be identical with 1,2: 3,4: 5,6-tri-*O-cyclo*hexylidene-D-mannitol (Bourne, Corbett, and Erilinne 1950). This derivative is very suitable for the chemical identification of small quantities of D-mannitol.

These results showed that the crystalline substance in the guttation water was essentially pure D-mannitol.

IV. DISCUSSION

The excretion of the mannitol in the guttation water is, as far as the authors are aware, the first direct evidence in the literature of mannitol absorption by the shoots of intact plants.

Some workers have presented evidence for mannitol absorption by the vacuole, in addition to the normal permeation into the free space of roots and tissue disks. Ordin, Applewhite, and Bonner (1956) found that the osmotic pressure of sections of the *Avena* coleoptile, after immersion for 20 hr in a mannitol solution, had increased slightly. Since some elongation of the tissue had taken place they concluded that a small amount of mannitol must have entered the vacuoles of the tissue. Burström

(1953) found increases in the osmotic pressure of the cell sap after immersion of disks of *Helianthus* tuber for 54 hr in mannitol solution and concluded that a substantial amount of mannitol had been absorbed by the vacuoles. Direct evidence for a mannitol permeation into the vacuole was presented by Collander and Bärlund (1933).

The data presented in this paper show that mannitol molecules are not excluded from the shoots of intact plants. It should be remembered, however, that solutions having high osmotic pressures were used in this experiment. It is possible that the roots were damaged by these concentrations, so that other results might have been obtained at lower mannitol concentrations.

It is relevant to consider the implications of the presence of mannitol in the shoots of intact plants. The absorbed mannitol might obviously have contributed significantly to the fairly small dry weight increases of the present experiment. The relative growth rates both before and after mannitol removal would be affected. That for H_1–H_2 would be increased and that for H_2–H_3 decreased.

Of more importance are the physiological implications of the presence of mannitol in the shoots of the intact plants. In studies of plant–water relations the increase of the osmotic pressure of the cell sap would be of relevance. The mannitol may be, moreover, variably distributed within the plant. For example, the presence of mannitol in the guttation water shows that mannitol must have been present in the xylem. It might, on the other hand, have been entirely excluded from the vacuoles. The presence of mannitol could have wider physiological implications which, however, it is not relevant to discuss here.

It can be concluded that mannitol is not a suitable substance to impose a water stress on intact plants for any considerable length of time.

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MANNITOL UPTAKE IN BARLEY PLANTS

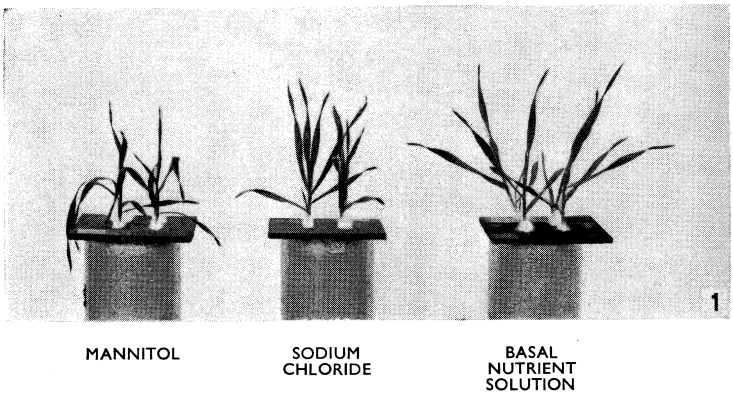

Fig. 1.—Wilting phenomena in mannitol- and sodium chloride-treated barley plants (osmotic pressure 8 atm).

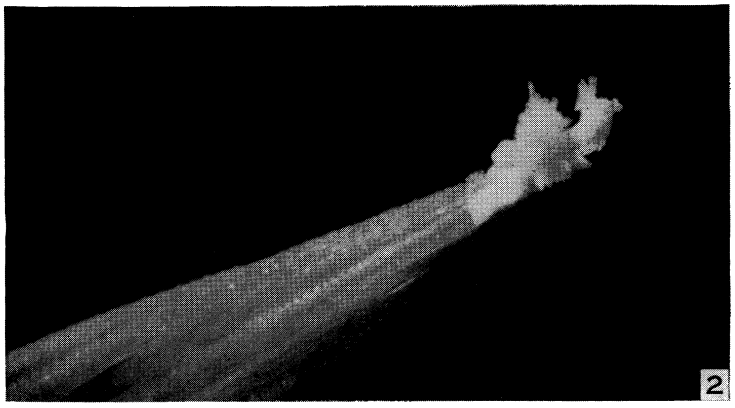

Fig. 2.—Crystalline substance on tip of barley leaf 24 hr after mannitol removal.