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# Effects of Osmotic Stress on Polar Auxin Transport in *Avena* Mesocotyl Sections

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**Abstract.** Segments of mesocotyls of *Avena sativa* L. transported [1-$^{14}$C]indol-3yl-acetic acid (IAA) with strictly basipetal polarity. Treatment of the segments with solutions of sorbitol caused a striking increase in basipetal auxin transport, which was greatest at concentrations around 0.5 M. Similar effects were observed with mannitol or quebrachitol as osmotica, but with glucose or sucrose the increases were smaller. Polar transport was still detectable in segments treated with 1.2 M sorbitol. The effects of osmotic stress on the polar transport of auxin were reversible, but treatment with sorbital solutions more concentrated than 0.5 M reduced the subsequent ability of mesocotyl segments to grow in response to IAA. The increased transport of auxin in the osmotically stressed segments could not be explained in terms of an increased uptake from donor blocks. The velocity of transport declined with higher concentrations of osmoticum. The reasons for the enhancement of auxin transport by osmotic stress are not known.

**Key words:** Auxin transport – *Avena* – Mesocotyl segments – Osmotic stress – Sorbitol.

## Introduction

This work was begun in an attempt to obtain evidence, through an investigation of the effects of plasmolysis, for or against the symplastic transport of auxin. A similar approach was adopted by Cande and Ray (1976), who found that the polar transport of auxin was demonstrable in segments of oat coleoptiles (*Avena sativa* L.) plasmolysed with 0.5 and 0.7 M mannitol. In experiments with segments of oat mesocotyls, I found that polar transport not only took place in plasmolysed tissues, but that it was strikingly enhanced. This unexpected phenomenon is described below.

## Material and Methods

### *Plant Material and Osmotic Treatment*

Seeds of *Avena sativa* L. cv. WW 16253 (Swedish Seed Assoc. Weibullsholm, Sweden) were soaked in water in the dark for 3 h and then germinated and grown in vermiculite in plastic boxes, covered with aluminium foil, in total darkness at 20° C. The seedlings were used for experiments when they were 4–6 d old. For experiments involving the pretreatment of segments with osmoticum, segments about 10 mm long were excised with a razor blade from the apical part of the mesocotyls and the basal ends were cut obliquely, to permit their subsequent recognition. These manipulations were performed in dim green light. The segments were then incubated in solutions of osmoticum (5 ml) in vials kept in a rack which was rotated for 2 h at 1 rpm around an axis inclined at 45°; control segments were similarly incubated in distilled water. The incubation took place in darkness. Microscopic examination (with Nomarski differential interference optics) of sections cut from segments which had been incubated in plasmolysing solutions (mounted in the same solutions) showed that the period of incubation was sufficient for cells throughout the segments to be plasmolysed, although plasmolysis was rather less pronounced in cells in the middle of the segments than at the ends.

### *Auxin Transport Experiments*

For transport experiments, 7-mm segments were cut from the pretreated mesocotyl segments by pressing a glass slide, on which segments had been aligned, onto razor blades mounted 7 mm apart. The segments (12 per test) were then arranged horizontally on a glass slide; they rested on a thin strip of filter paper coated with petroleum jelly. Receiver and donor agar (1%, w/v) blocks were applied and the transport assemblies were placed in Petri dishes lined with moist filter paper as described by Sheldrake (1973). Transport took place in the dark at 22° C. Donor blocks contained [1-$^{14}$C]indol-3yl-acetic acid (IAA) (Radiochemical Centre, Amersham, U.K.; 52 mCi/mM) at a concentration of 3.0 μM unless otherwise stated. Donor and receiver blocks applied to pretreated segments contained the same concentration of the same osmoticum used in the pretreatment. During the transport period water uptake into the segments may have occurred to a small extent, owing to their incubation in a saturated atmosphere, but no deplasmolysis of previously plasmolysed tissue could be detected by microscopical examination of sections cut from segments at the end of the transport period.

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At the end of the transport period the receiver blocks and the segments were placed in 4 ml of scintillation fluid of the following composition: 2,5-diphenyloxazole (PPO) 5 g; naphthalene 100 g; dioxane to 1 l. The quenching caused by the agar blocks was not affected by the varying concentrations of sorbitol which they contained; nor was the quenching by the mesocotyl segments affected by their pretreatment in this osmoticum. The quenching effects were in all cases small (<1%). All samples were counted on a Nuclear Chicago Unilux scintillation counter for at least 10 min and corrected for background counts (ca. 30 cpm).

Since the growth of mesocotyls is affected by exposure to red light, the effect on auxin transport of exposing the segments to daylight during the cutting of the 7-mm segments and the setting up of the transport assemblies was examined. Little or no difference was found after performing these manipulations in dim green light or daylight; they were carried out in daylight in all the experiments reported in this paper.

Ether extracts of receiver blocks were chromatographed in isopropanol-ammonia-water (10:1:1, v/v), pyridine-ammonia (4:1, v/v) and ethanol-water (7:3, v/v) on cellulose thin-layer plates; the procedures for the detection of IAA marker spots and the analysis of the distribution of radioactivity in the developed chromatograms were as described by Sheldrake (1971). Nearly all the radioactivity transported into receiver blocks applied to the basal end of *Avena* mesocotyl segments supplied with radioactive IAA was found at the $R_F$ of IAA in all three solvent systems. The transport of radioactivity can therefore be equated with the transport of IAA.

*Measurements of Segment Growth*

The influence of exposure to osmotic solutions on the ability of the mesocotyl segments to grow was examined by incubating 10-mm segments in osmotic solutions for 2 h as described above: the segments were then incubated in distilled water for 1.5 h before a 4-mm segment was cut from the apical part of each 10-mm segment and incubated (10 segments per test) in a solution of IAA (50 µg/l) under the conditions used for the *Avena* mesocotyl extension bioassay as described by Sheldrake (1971).

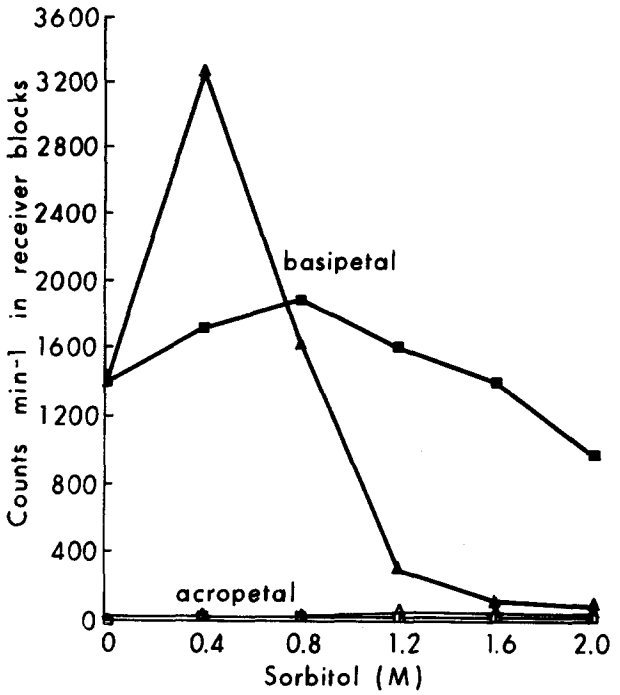


**Fig. 1.** Transport of IAA by mesocotyl segments from 4-d-old *Avena* seedlings after pretreatment with sorbitol. Transport by pretreated segments with the same concentration of sorbitol in donor and receiver blocks that was used for the pretreatments: ▲ basipetal, △ acropetal. Transport by segments pretreated with sorbitol but placed in water for 1.5 h before the transport period, with distilled water in donor and receiver blocks: ■ basipetal, □ acropetal. Concentration of IAA in donor blocks: 1.5 µM. Transport time: 3 h

## Results

### *Effects of Sorbitol Treatments on Auxin Transport*

The amount of radioactivity transported by *Avena* mesocotyl segments treated with different concentrations of sorbitol is shown in Figure 1. Over twice as much auxin was transported basipetally by segments treated with 0.4 M sorbitol as by the water-treated controls. With higher sorbitol concentrations auxin transport declined, but even with 1.2 M sorbitol there was considerable basipetal movement. In all cases very little auxin moved acropetally (Fig. 1).

A second set of mesocotyl segments was incubated in sorbitol solutions at the same time and in the same way as those used in the experiments above, but before being tested for their ability to transport auxin they were rinsed and placed in water for 1.5 h, during which time cells that were previously plasmolysed underwent deplasmolysis. The results (Fig. 1) showed

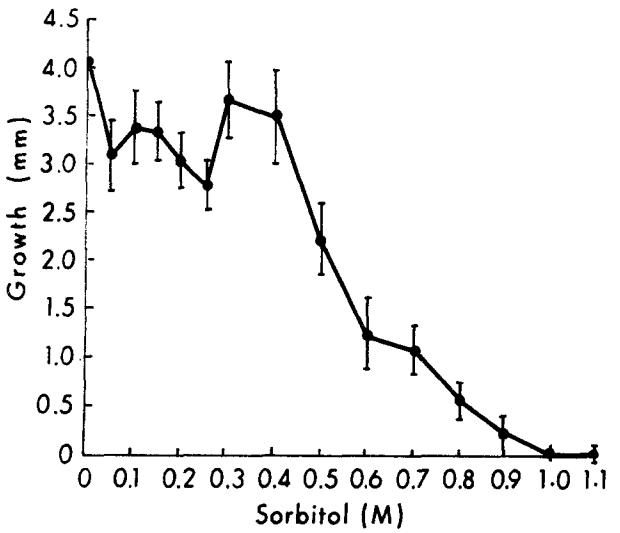


**Fig. 2.** Growth of mesocotyl segments (4 mm) from 5-d-old *Avena* seedlings. The segments were pretreated with sorbitol solution for 2 h, then placed in water for 1.5 h before incubation in the dark for 20 h at 20° C in a medium containing 50 µg/litre IAA

that the effects of osmotic stress on basipetal auxin transport were more or less reversible. Again there was very little acropetal movement of auxin.

### *Effects of Pretreatment with Sorbitol on the Ability to Grow*

The effects of pretreatment with sorbitol followed by a 1.5 h period in water on the ability of mesocotyl segments to grow in response to IAA are shown in Figure 2. Pretreatment with sorbitol solutions more concentrated than 0.4 M resulted in reductions in growth, and no growth occurred after pretreatment with 1.0 M sorbitol.

### *Effects of Other Osmotica*

The experiments described above were repeated with mannitol as the osmoticum, with similar results.

The effects of a number of other osmotica on basipetal auxin transport were compared with sorbitol at concentrations of 0.5 M. Quebrachitol resulted in increases similar to those with sorbitol (100–110% more transport than in controls). Only small increases (20–30%) were observed with glucose and sucrose, perhaps because these sugars were taken up and metabolized, thus reducing their osmotic effect. If auxin transport was increased because the osmotica acted as a carbon source, larger increases would have been expected with glucose and sucrose than with the sugar alcohols sorbitol, mannitol and quebrachitol. Moreover all three sugar alcohols brought about similar increases and it would seem improbable that all three entered the cells and were metabolized to similar extents, if they were metabolized at all. These results therefore indicate that the increased auxin transport brought about by sorbitol was primarily owing to its osmotic effect.

### *Further Observations on the Effects of Different Concentrations of Sorbitol*

More detailed observations on basipetal auxin transport by mesocotyl segments treated with sorbitol showed that increased auxin transport became apparent at concentrations above 0.15 M (Fig. 3C). Slightly less transport than in the control occurred at concentrations of 0.05 and 0.10 M sorbitol; this effect was observed in each of four separate experiments. The amount of radioactivity in the segments at the end of the transport period decreased with increasing concentrations of sorbitol, although the total counts in segments plus receiver blocks remained more or less constant up to sorbitol concentrations of 0.5 M (Fig. 3B). The growth of the segments which took place during the transport period is shown in Figure 3A.

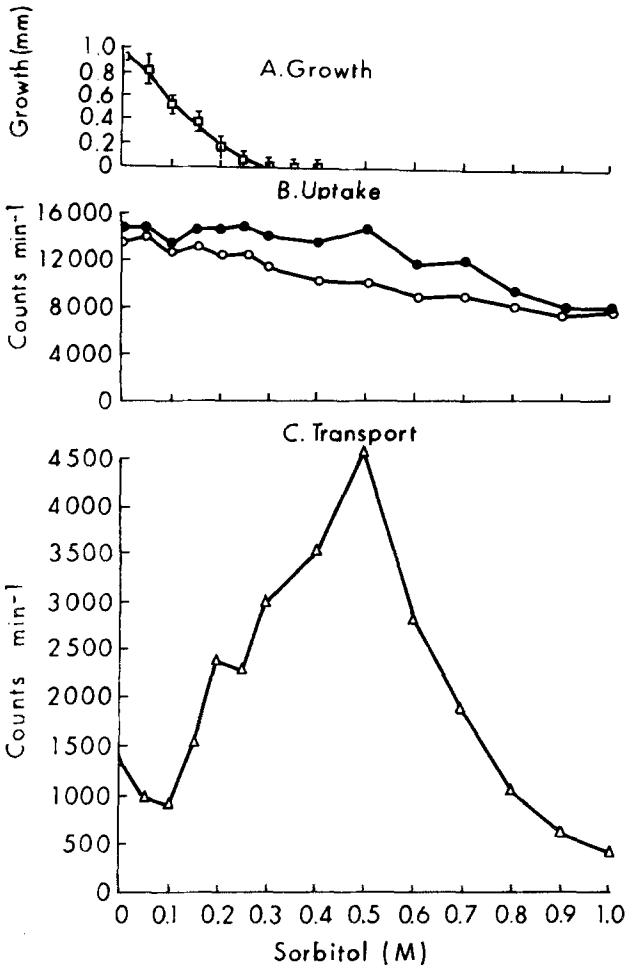


**Fig. 3A–C.** Basipetal transport of IAA by mesocotyl segments, from 4-d-old *Avena* seedlings, pretreated with sorbitol. Transport time: 3.5 h. **A** Growth of segments during the transport period. **B** Total radioactivity in receiver blocks and segments at the end of the transport period: ●; radioactivity in segments at the end of the transport period: ○. **C** Radioactivity in receivers

Microscopical examination of sections of mesocotyl segments after incubation in sorbitol solutions showed that cortical and epidermal cells were plasmolysed in concentrations of 0.4 M sorbitol and above, in such a way that the protoplasts had pulled away from one or both of the end walls of the cells. It was difficult to observe plasmolysis in cells within the stele; in some of the cells protoplasts appeared to have remained in contact with both end walls and retracted from the side walls. No plasmolysis was observed in 0.3 M sorbitol. Increased auxin transport occurred with concentrations of sorbitol lower than those necessary to plasmolyse the cells or to suppress growth completely (Fig. 3).

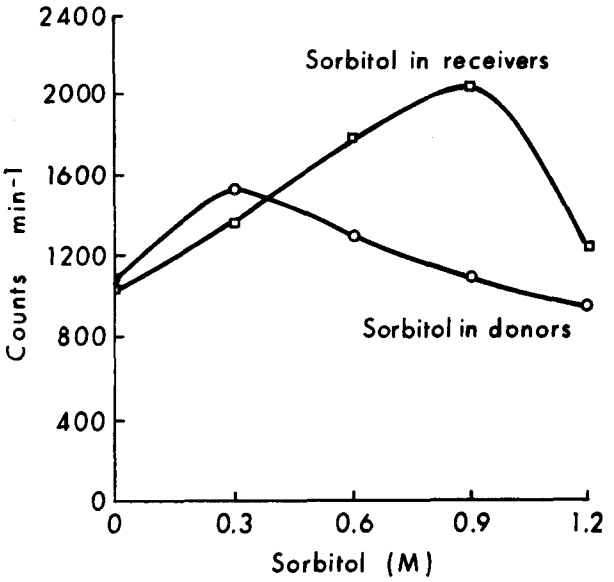


**Fig. 4.** Basipetal transport of IAA by non-pretreated mesocotyl segments from 5-d-old *Avena* seedlings. Distilled water in receiver blocks, sorbitol in donor blocks ○; distilled water in donor blocks, sorbitol in receiver blocks □. Transport time: 3.5 h

### *Sorbitol in Donor or Receiver Blocks Applied to Non-pretreated Segments*

The data in Figure 3 show that the increased transport of auxin cannot be explained in terms of an increased uptake of auxin from donor blocks into segments treated with osmoticum. The relative effects of the osmoticum on the uptake of auxin from donor blocks and on the transport of auxin through the segments were further investigated by applying donor or receiver blocks containing different concentrations of sorbitol to freshly cut, non-pretreated segments. If osmotic stress primarily affected the uptake of auxin, sorbitol in the donor blocks should have much more effect than in the receiver blocks. On the other hand, if osmotic stress modified auxin transport through the segments, the transport process should be affected by sorbitol in both donor and receiver blocks. In fact, a greater enhancement of basipetal auxin transport was observed with increased sorbitol concentrations in the receiver blocks than in the donor blocks (Fig. 4).

In this experiment, there was an efflux of water from the ends of the segments in contact with the agar blocks containing sorbitol, because these blocks were not in osmotic equilibrium with the segments. The smaller effect of sorbitol in donor than in receiver blocks may be explained at least in part by the flow of water from the segments into the blocks, diluting the IAA and also carrying some IAA from the segments back towards the donors.

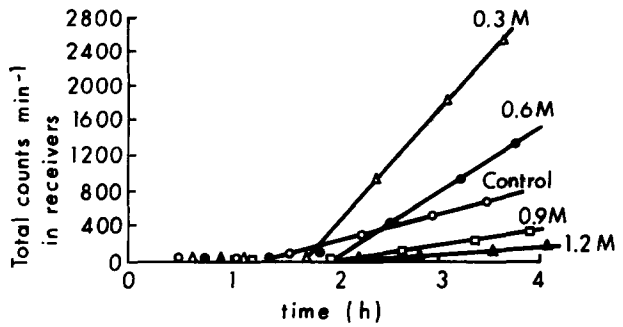


**Fig. 5.** Rates of basipetal IAA transport by mesocotyl segments (7 mm) from 4.5-d-old *Avena* seedlings. Controls (pretreated with water) ○; segments pretreated with 0.3 M sorbitol △; 0.6 M sorbitol ●; 0,9 M sorbitol □; 1.2 M sorbitol ▲. The slopes of the lines and positions of intersects were calculated by the "least squares" method. The velocities (in mm/h) calculated from these intercepts were: control, 5.3; 0.3 M sorbitol, 4.1; 0.6 M sorbitol, 3.5; 0.9 M sorbitol, 3.4; 1.2 M sorbitol, 3.1

In the other experiments described in this paper, the segments and agar blocks should have been in osmotic equilibrium, and no such flow of fluid was observed. It could perhaps be argued that nevertheless there may have been slight osmotic differences between segments and agar blocks resulting in a small flow of water into receiver blocks and thus to an increased movement of IAA into receivers. However even if this took place to some extent, it could not adequately explain the increases in auxin transport in osmotically stressed segments, for two reasons. Firstly these increases were at least as large (Figs. 1, 3) as those which occurred when sorbitol was present only in the receiver blocks (Fig. 4); the latter increases should have been much larger if water movement into receiver blocks were of primary importance. Secondly, auxin transport was enhanced when sorbitol was present only in donor blocks (Fig. 4).

### *Effects of Osmotic Stress on the Velocity of Auxin Transport*

The velocity of basipetal auxin transport, measured by the classical method of van der Weij (1932), declined as the concentrations of osmoticum was raised (Fig. 5). The increased transport of auxin into the receiver blocks by osmotically stressed segments indicates that the overall capacity of the transport system was increased, either because more auxin which had already been taken up into the tissue entered the transport stream, and/or because the efflux of auxin from the segments into the receivers was enhanced.

## Discussion

An enhancement of auxin transport by osmotic stress has been observed with segments of *Phaseolus* petioles

supplied with donor and receiver blocks containing 0.2 M mannitol (McCready and Jacobs, 1967) and with segments of *Nicotiana* stem tissue with donor blocks containing 0.6 M sorbitol (Sheldrake, 1973). By contrast, treatment of *Avena* coleoptile segments with 0.5 M mannitol resulted in a slight reduction in the amount of auxin transported (Cande and Ray, 1976). These observations indicate that osmotic stress may not result in increased auxin transport in all tissues or at least that the concentration of osmoticum resulting in enhanced auxin transport may vary from tissue to tissue.

The reduced ability of *Avena* mesocotyl segments to grow in response to auxin after they had been exposed to sorbitol concentrations of 0.5 M or more (Fig. 2) may have been a result of damage to the tissue, but such damage could not have been severe since the ability to transport auxin was little affected (Fig. 1). In a given tissue the concentration of osmoticum required to increase polar auxin transport would presumably depend on the sensitivity of the tissue to damage by osmotic stress and indeed in very sensitive tissues it may not be possible to observe any increase at all.

Two negative conclusions are indicated by the results described above. Firstly, the increased auxin transport which occurred with sorbitol concentrations above 0.3 M shows that the effect cannot simply be explained in terms of a suppression of growth by the segments during the transport period (Fig. 3). Secondly, the effect does not seem to depend on the separation of the plasmalemma of the protoplasts from the cell walls, since it was observed both in segments which were not plasmolysed (in 0.20–0.25 M sorbitol: Fig. 3) and also with higher concentrations of sorbitol (0.8 M: Fig. 1) which were sufficient to plasmolyse most if not all of the cells.

One possible reason for the enhancement of auxin transport by osmotically stressed segments may be that osmotic stress somehow reduces the immobilization or metabolism of auxin within the cells, leaving more auxin available for transport.

Two further possible types of explanation are suggested by a recent hypothesis of auxin transport (Rubery and Sheldrake, 1974; Goldsmith, 1977), according to which there is a pH-dependent uptake of auxin into the cells resulting in an accumulation of auxin anions within the cytoplasm, and a carrier-mediated efflux of these anions down an electrochemical gradient. It is supposed that the carriers mediating this efflux are preferentially localized at the basal ends of the cells and that auxin moves from cell to cell by diffusion through the extracellular fluid. The rates of uptake and efflux of auxin are affected by the extracellular pH and the intracellular and extracellular concentrations of auxin (Rubery and Sheldrake, 1973, 1974). Osmotic stress results in an increase in the volume of the extracellular space and a decrease in the volume of the cells (Walter and Kreeb, 1970). These changes in relative volume could influence the rates of auxin uptake, movement and efflux from the cells during the transport process and thus influence the amount of auxin transported. However, too little is known about the kinetics of these processes to predict what these effects would be.

Alternatively, or additionally, osmotic stress could influence auxin transport through an effect on extracellular pH. In *Avena* coleptiles, osmotic stress is known to affect the excretion of $H^+$ ions from the cells into the extracellular fluid (Cleland, 1975).

Only further research will be able to distinguish between these, and other, possibilities: the phenomena described above may then be able to shed some light on the mechanism of auxin transport. At present, however, the enhancement of auxin transport by osmotic stress can only be regarded as unexplained.

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