AUXIN-INDUCED WATER UPTAKE BY AVENA COLEOPTILE SECTIONS[1,2]

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It is known that auxin induces the uptake of water by plant tissues. Three principal suggestions have been made concerning the mechanism of such auxin-induced net water uptake. The first proposes that auxin in some manner plasticizes the cell wall. The second suggests that auxin brings about active synthesis of cell wall material. These two mechanisms suppose osmotic entry of water into the cell in response to lowered wall pressure. The third is that auxin brings about a non-osmotic transport of water into the tissue. Thimann (22) has grouped these into two hypotheses in the form of models. One model visualizes a pump (active transport) and the other a piston arrangement (cell wall pressure reduction). Such active transport has been rigorously defined by Rosenberg (21) as the movement of a substance against a chemical potential or concentration gradient, i.e., an uptake of water against a diffusion gradient as defined by Levitt (16). Active transport of water would consist then of water movement against an osmotic gradient. A fourth possibility in principle would be that an auxin-induced increase in the concentration of osmotically active solutes might occur within the cell.

The cells of plant tissues are surrounded by relatively rigid cell walls and one must discuss water movement in terms of diffusion pressure gradients rather than in terms of osmotic pressure. In the terminology proposed by Meyer and Anderson (19) and by Crafts et al (10), DPD = OP − WP where DPD = diffusion pressure deficit, OP = osmotic pressure of cell contents, and WP = wall pressure. The DPD gradient from an external solution to the inside of the tissue can then be expressed as Δ DPD = $DPD_i - DPD_e$ ($DPD_e = OP_e$) where i = internal and e = external. If Δ DPD is negative, the gradient is outward; if Δ DPD is positive, the gradient is inward. To demonstrate a net active water uptake it is necessary to show that water uptake occurs while the purely osmotic Δ DPD is negative. When the external solution is hypertonic, wall pressure becomes zero and $DPD_i = OP_i$. The net uptake of water from hypertonic solution would then involve movement against an osmotic gradient. It will be shown below that cells of Avena coleoptile sections are essentially in diffusion pressure equilibrium with the external solution over a wide range of external solute concentrations both in the presence and in the absence of auxin. There is no detectable net uptake of water from hypertonic solution by this tissue. It is concluded that water uptake by Avena coleoptile sections both in the presence and absence of auxin is a purely osmotic phenomenon.

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MATERIALS AND METHODS

The material used in this work consisted of 5-mm sections cut 2 mm below the apex of Avena (variety Siegeshafer) coleoptiles. The Avena seedlings were grown in vermiculite contained in stainless steel trays and were watered with distilled water. They were harvested when 96 hours old. Only those coleoptiles 30 ± 2.5 mm in length were used. Each part of each experiment was done using 20 sections floated on 20 ml of solution.

Changes in water content of the sections were measured as changes in section length. Net water uptake by coleoptile sections is attended by only small changes in section diameter. Kelly (12) has shown that under conditions similar to those used in elongation studies water uptake in the presence of auxin is linear with time. Measurements of section length were made under a dissecting microscope with a decimillimeter stage micrometer. The variability in elongation rates of sections under the present conditions has been discussed by McRae and Bonner (18).

Three general basal media were used: 1) potassium maleate buffer (0.0025 M, pH 4.8) with or without potassium indoleacetate (5 mg/l); 2) potassium maleate buffer, sucrose (0.09 M), $MnSO_4$ (100 mg/l), arginine (100 mg/l) and with or without potassium indoleacetate (5 mg/l); 3) redistilled water with or without indoleacetic acid (IAA) (5 mg/l, pH 5.0 to 5.5), potassium-free. All solutions were made up with redistilled water.

In the following report, the terms hypotonic and hypertonic are used in referring to solutions external to the cell. These terms are defined as follows: hypertonic = solution whose OP is greater than OP_i; hypotonic = solution whose OP is less than OP_i. It will be shown below that OP_i for freshly cut Avena coleoptile sections under the present conditions is equivalent to approximately 0.4 M mannitol.

EXPERIMENTAL RESULTS

Data on the time course of elongation of sections in media of two different osmotic concentrations are given in figure 1. In these experiments, medium 1 containing buffer alone was used. To this IAA was added as indicated. Mannitol was used as the osmotically active solute. The sections were allowed one hour to come to osmotic equilibrium with the solution before addition of auxin. It is evident that sections do not elongate in response to auxin in an external osmotic concentration of 0.5 M. The use of elevated temperatures and of forced aeration does not increase elongation under conditions of high mannitol concentration.

The respiratory rate of Avena coleoptile sections is depressed in the presence of increasing concentration of an external solute, as is shown in figure 2.

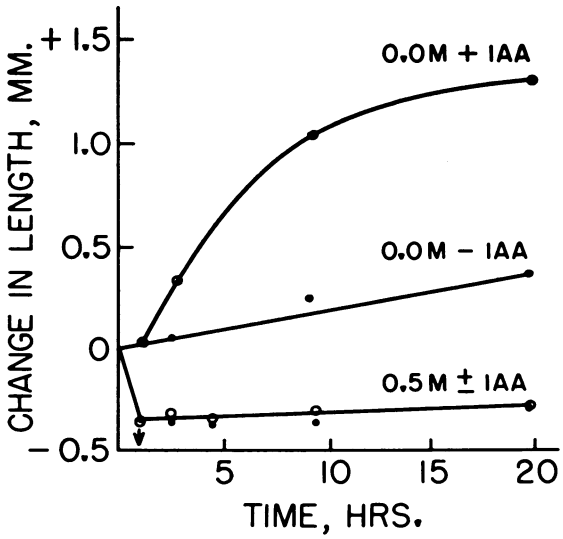

FIG. 1. Effect of external osmotic concentration on elongation of Avena coleoptile sections as a function of time. 0.0025 M potassium maleate, pH 4.8 with or without 0.5 M mannitol, and with or without 5 mg/l IAA. IAA added at arrow.

The rates of oxygen uptake were measured over a period of 3 hours. The presence of IAA increases rate of respiration at low external osmotic concentrations, as has been earlier reported by Commoner and Thimann (9), Bonner (5) and others. The auxin-induced increment in respiration diminishes with increasing external osmotic concentration, and disappears at an OP_e of 0.4 M in which elongation is reduced to a very low value. These results parallel those obtained with Jerusalem artichoke storage tissue in which the respiratory increment induced by IAA disappears as OP_e is increased (6).

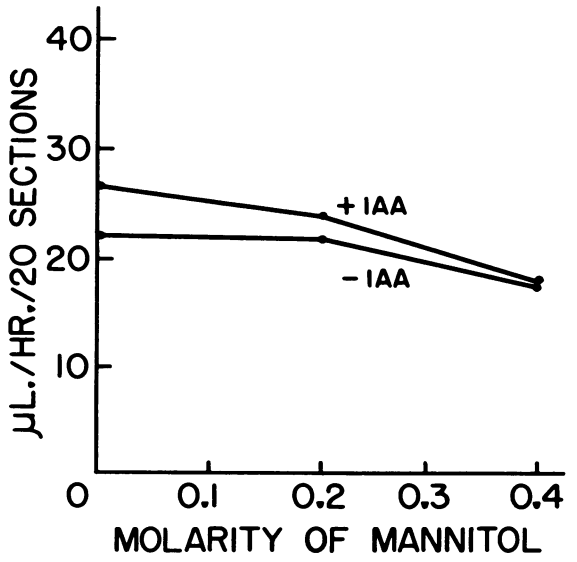

FIG. 2. Effect of external osmotic concentration and of IAA on oxygen uptake of Avena coleoptile sections. 0.0025 M potassium maleate buffer, pH 4.8, 5 mg/l IAA.

It is known that the response of coleoptile sections to IAA is prolonged in time by the addition of sucrose or certain other substances to the media. Figure 3 gives data on the time course of the elongation response to IAA in the more complete basal medium containing sucrose, manganese and arginine, and containing added mannitol to produce varying osmotic pressures. The sections were first equilibrated in the medium for one hour, after which auxin was added. In the basal medium alone ($OP_e = 0.09$ M) elongation starts at once when IAA is added. At

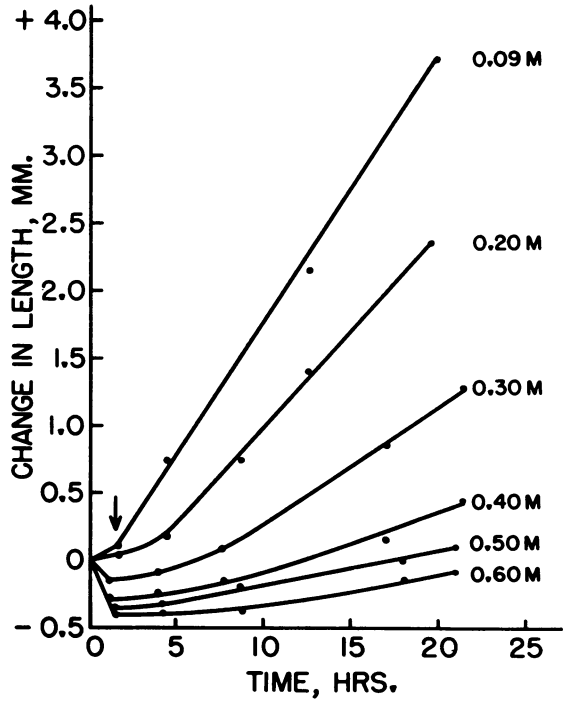

FIG. 3. Effect of external osmotic concentration on elongation of Avena coleoptile sections as a function of time. 0.09 M sucrose plus various concentrations of mannitol, 100 mg/l arginine, 100 mg/l $MnSO_4$, 0.0025 M potassium maleate, pH 4.8. IAA (5 mg/l) added at arrow.

higher values of OP_e, a lag period in attainment of steady state elongation is evident. The length of this lag period is greater the larger the value of OP_e. The final steady state elongation rates also decrease regularly as OP_e is increased. Figure 4 summarizes data on the elongation of sections in the presence or absence of IAA and in media of varying osmotic concentrations. The effects of increasing external osmotic concentration on endogenous elongation (elongation in the absence of added IAA) do not parallel effects on the auxin-induced elongation of these sections. The endogenous elongation exhibits the initial non-linear decline with increasing OP_e at a lower concentration than does elongation in response to added IAA. The two coincide at an external osmotic con-

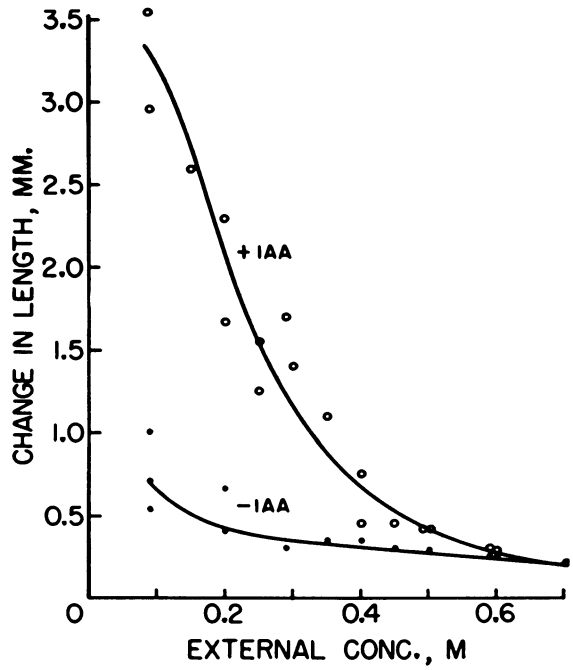

FIG. 4. Effect of external osmotic concentration and of auxin on total elongation of Avena coleoptile sections. Incubation time 20 hrs. 0.09 M sucrose plus various concentrations of mannitol, 100 mg/l arginine, 100 mg/l $MnSO_4$, 0.0025 M potassium maleate, pH 4.8, with or without 5 mg/l IAA.

centration of approximately 0.7 M. The cells of sections placed in solutions more concentrated than approximately 0.4 M are rapidly plasmolyzed, as will be discussed in detail below. After a 20-hour incubation period, however, deplasmolysis has occurred. This is true, for example, of sections placed in solutions as concentrated as 0.6 M. Elongation has then occurred in the presence of solutions initially hypertonic to the 0.4 M osmotic concentration of the initial sections.

Burström (7) has shown that the irreversible extension of wheat roots is independent of OP_e in the hypotonic region. This would suggest that the cell elongation of such roots is not due to a mere passive stretching of the wall and favors some hypothesis relating active wall synthesis to cell enlargement. In order to determine if a similar situation obtains for the Avena coleoptile, sections were placed in plasmolyzing solutions for one hour after 20 hours of elongation. The difference between initial plasmolyzed length (plasmolyzed at zero hours) and final plasmolyzed length (plasmolyzed at 20 hours) is taken as the irreversible elongation in accordance with Burström's definition. The data for these experiments are given in figure 5. The irreversible component, like the total elongation of Avena coleoptile sections, is an inverse function of OP_e. The Avena coleoptile is not, therefore, similar in behavior to Burström's wheat roots as far as irreversible extension is concerned. In the case of the Avena coleoptile, the greater the turgor pressure the greater the elongation rate. This implies that something more than intimate contact of cytoplasm with the cell wall is essential to increase in coleoptile length.

Osmotic Quantities during Elongation

In order to ascertain to what extent purely osmotic considerations govern auxin-induced water uptake in the Avena coleoptile it is necessary to have measurements of DPD and of OP_i during the elongation process. The simplified method of Ursprung (25) was used for determination of the DPD_i of Avena coleoptile tissue. The method consists in measuring the lengths of a group of sections which are then placed in a graded series of mannitol concentrations. The sections are held submerged in stainless steel baskets and argon bubbled through the solutions to maintain anaerobic conditions. A separate group of sections is used for each mannitol concentration. After one to two hours, the sections are removed and their lengths quickly measured. That group which shows no change in length is said to have been in a solution whose $OP = DPD_i$. The maintenance of anaerobic conditions during the DPD determination insures to a high degree that metabolic processes such as are essential to IAA induced net water uptake are kept at a minimum and that any such metabolic components of DPD are not measured.

According to classical osmotic theory, the simplified method of Ursprung should also be capable of yielding values of OP_i for the tissue measured. The cytoplasm should pull away from the cell wall at incipient plasmolysis and as OP_e is further increased, the cytoplasm should continue to contract without

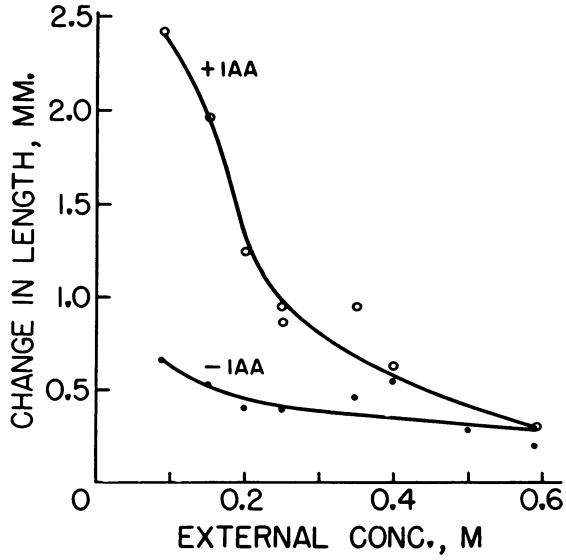

FIG. 5. Effect of external osmotic concentration and auxin on irreversible elongation of Avena coeloptile sections. Incubation time 20 hrs followed by plasmolysis in 1 M mannitol. Same experimental conditions as figure 4.

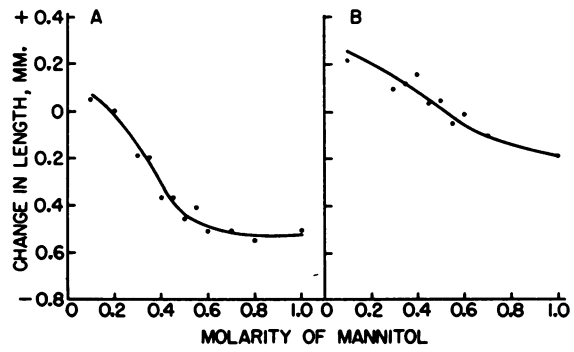

FIG. 6 A. Change in length of Avena coleoptile sections as a function of external mannitol concentration. Incubation for 1 to 2 hours under anaerobic conditions. Freshly cut sections.

FIG. 6 B. Change in length of Avena coleoptile sections as a function of external mannitol concentration. Incubation for 1 to 2 hrs under anaerobic conditions. Sections previously grown 20 hrs in complete medium of osmotic concentration 0.5 M (mannitol + sucrose) solution and containing 5 mg/l IAA.

further cell shrinkage. Hence, the curve which relates tissue length (or change in length) to OP_e should show a sharp inflection at the OP_e of incipient plasmolysis, and should become a straight line of zero slope for higher values of OP_e. Ketellapper (13) has previously attempted to determine the osmotic concentration of Avena section tissue in this way. His data appear to follow closely the expectations of classical osmotic lore. This is not true in the present experiments. Figure 6 A presents data on the length of freshly excised Avena sections after one hour of anaerobic incubation in media of varying OP_e. The length of the sections decreases with increasing OP_e up to an external concentration of approximately 0.6 M. Although there is an inflection in the curve between 0.4 and 0.6 M, there is no sharp change in slope. OP_i apparently lies between 0.4 and 0.6 M but cannot be determined more precisely by this method. The same is true for tissue which has been previously incubated in a medium of high OP_e. Figure 6 B gives data on tissue incubated anaerobically for one hour in media of varying OP_e after 20 hours in solution containing IAA in 0.5 M solution (mannitol plus sucrose). There is no evident inflection in the curve of figure 6 B. It is necessary to conclude, therefore, that OP_i cannot be measured in this way for Avena coleoptile section tissue. This method is, however, satisfactory for DPD determinations, and yields a precision of determination of approximately ± 0.05 M. Table I gives DPD values for sections after varying pretreatment. The data of table I show that DPD_i as measured by the short term anaerobic incubation method is always equal to the OP_e of the external solution in which the section has previously been incubated. Equilibration of tissue DPD with external OP appears to take place within one hour over a wide range of OP_e values. Equilibration of DPD_i and OP_e is maintained through a 20-hour period both in the presence and absence of added auxin. There is no evidence in these data for any component of DPD_i dependent on aerobic metabolism and measurable by the present method. This important point was further studied by experiments in which argon was bubbled through solutions in which sections had previously been elongating under the influence of auxin. The change of section length with time in argon for

TABLE I

DIFFUSION PRESSURE DEFICIT (DPD) OF AVENA COLEOPTILE SECTIONS AFTER INCUBATION FOR 1 HR AND 20 HRS IN MEDIA OF DIFFERENT OSMOTIC CONCENTRATIONS

EXTERNAL CONC, M	DPD OF SECTION TISSUE, M		
	AFTER 1 HR	AFTER 20 HRS	
		+IAA	−IAA
0.25	0.25	0.27	0.28
0.35	0.36	0.39	0.40
0.40	0.37	0.41	0.46
0.50	0.47	0.52	0.45
0.59	0.60	0.54	0.56

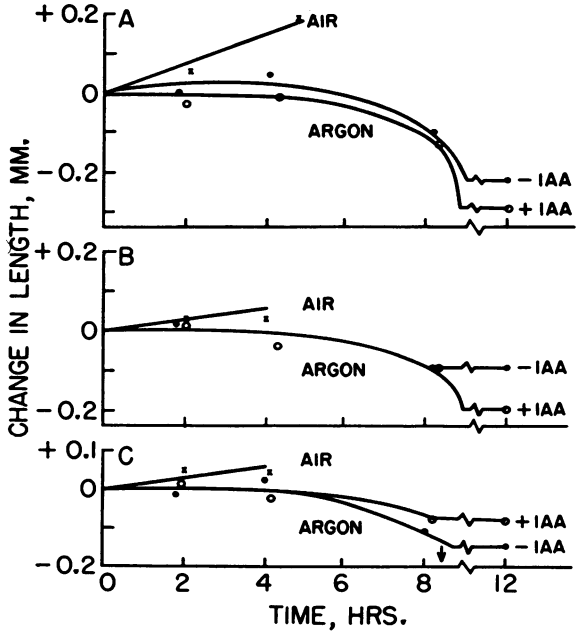

FIG. 7 A. Effect of anaerobic conditions (argon) on change in length of Avena coleoptile sections in 0.4 M solution (0.0025 M potassium maleate, pH 4.8, 100 mg/l arginine, 100 mg/l $MnSO_4$, 0.09 M sucrose, mannitol, with or without 5 mg/l IAA) following 20 hrs in same solution under aerobic conditions. Sections transferred to distilled water at arrow indicated in figure 7 C.

FIG. 7 B. Effect of anaerobic conditions on change in length of sections in 0.5 M solution. Conditions same as in figure 7 A except for mannitol concentration.

FIG. 7 C. Effect of anaerobic conditions on change in length of sections in 0.6 M solution. Conditions same as in figure 7 A except for mannitol concentrations.

sections in solutions of several different values of OP_e are plotted in figure 7 A, B and C. No change in section length occurs during the first four hours after conditions are made anaerobic. After 4 hours the sections begin to shrink. This is because the tissue is killed by the prolonged anaerobic incubation as shown by microscopic examination and lack of section expansion after transfer to distilled water. Two hours of anaerobiosis are, however, evidently without deleterious effects. These data tend to confirm the conclusion that the tissue is in DPD equilibrium with the external solution at all times, and that IAA-induced elongation occurs in the absence of any apparent DPD gradient over the entire range of external osmotic concentrations used. Since water enters the cell under the influence of auxin it must do so under the influence of a positive DPD gradient. This is apparently small and not detectable by the present methods of measurement.

When sections are placed in hypertonic solution, their cells are rapidly plasmolyzed. The cells of such sections subsequently deplasmolyze under aerobic conditions. One might, therefore, conclude that absorption of solutes or production of solutes within the tissue has taken place. According to this view, the sections deplasmolyze because OP_i is increased and the solution is no longer hypertonic. At the same time, wall pressure increases above zero so that DPD remains constant while OP_i increases. Le Gallais (14) has shown that the OP of sap expressed from Avena coleoptile section tissue increases proportionally to external osmotic concentration regardless of solute used and both in the presence and absence of auxin. Le Gallais' work was done with the cryoscopic method of osmotic pressure determination. His results suggest, however, that the cryoscopic values for OP_i are erroneously high because the sap expressed includes plasmolytic solution which has entered the tissue without entering the vacuole. The cryoscopic technique is not, therefore, useful for the present purpose. That an increase in OP_i does take place during incubation of sections in initially hypertonic solutions can however be shown by the plasmolytic method of OP determination. This method consists in the examination of coleoptile sections from the DPD determination solutions under the high power of the microscope. Cells are counted through the median region of the layer of cells just below the outer epidermis. OP_i is taken as equal to the OP_e of that solution in which sections have 50 % of their cells plasmolyzed.

The initial OP_i of freshly cut Avena coleoptile cells was found to be 0.42 M. Sections incubated for 20 hours yield various values of OP_i, depending on the solution used for incubation. Results of typical experiments are summarized in table II. In the first place, sections incubated in water alone (no solute) decrease in OP_i if IAA is present. This effect is apparently due to dilution of cell contents by the water taken up under the influence of auxin as has been previously described. Sections in mannitol alone show small increases in OP_i over the 20-hour period. Since some elongation occurs in mannitol, a small amount of mannitol must have entered the tissue. The addition of sucrose to the medium, however, permits very large increases in OP_i to take place. This is true both for solutions which are initially hypotonic and for those which are initially hypertonic (with added mannitol). The osmotic pressure increase in mannitol plus sucrose is larger than that in sucrose alone in the presence of IAA. This is undoubtedly due to the greater dilution of the cell contents in the latter case as pointed out by Hackett (11) for potato tissue. The presence of sucrose in the medium evidently permits the tissue to make an extensive adjustment of OP_i.

TABLE II

INTERNAL OSMOTIC PRESSURE OF AVENA COLEOPTILE SECTIONS AFTER INCUBATION IN VARIOUS SOLUTIONS

TREATMENT	INTERNAL OSMOTIC PRESSURE (OP_i), M	
	+IAA	-IAA
Redistilled H_2O	0.32	0.42
0.0813 M Sucrose	0.53	0.68
0.59 M Mannitol	0.44	0.45
0.5 M Mannitol + 0.0813 M sucrose	0.69	0.69
0.09 M Mannitol	0.43	0.47
0.09 M Ethylene glycol	0.42	0.52
0.045 M NaCl	0.45	0.60

Incubation: 10 hrs for H_2O treatment, 20 hrs for other treatments. Initial $OP_i = 0.42$ M.

The data of figure 8 further concern the question of the role of sucrose in the elongation of Avena coleoptile sections. Sections were incubated for 29 hours in initially hypertonic solutions made up of mannitol or sucrose and mannitol, and with or without IAA. The sections were initially plasmolyzed by the hypertonic solutions in all cases. Deplasmolysis only occurred with sections incubated in the presence of sucrose. Sucrose must therefore contribute to the

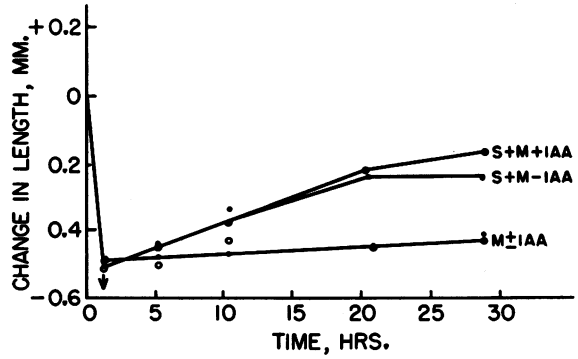

FIG. 8. Effect of 0.0813 M sucrose on change in length of Avena coleoptile sections in initially hypertonic solution containing mannitol in concentrations such that total molarity is 0.59 M. IAA added at arrow. 5 mg/l K-free IAA, no buffer. Final pH 5.5.

increase in OP_i of the tissue. Sections which have deplasmolyzed elongate more slowly than those which have never been plasmolyzed in agreement with the similar findings of Ketellapper (13). Plasmolysis itself apparently causes some irreversible damage.

It has been shown above that the presence of a small amount of sucrose in the medium permits sections to deplasmolyze in an otherwise hypertonic concentration of mannitol. The presence of sucrose similarly maintains OP_i and rate of water uptake in the presence of auxin. The following type of experiment was done to find out whether sucrose pretreatment can maintain subsequent elongation rate in hypotonic mannitol solution. Sections were pretreated in 0.09 M sucrose with IAA for 4 hours and were then transferred to 0.09 M mannitol and IAA for periods up to 20 hours. The results are given in figure 9.

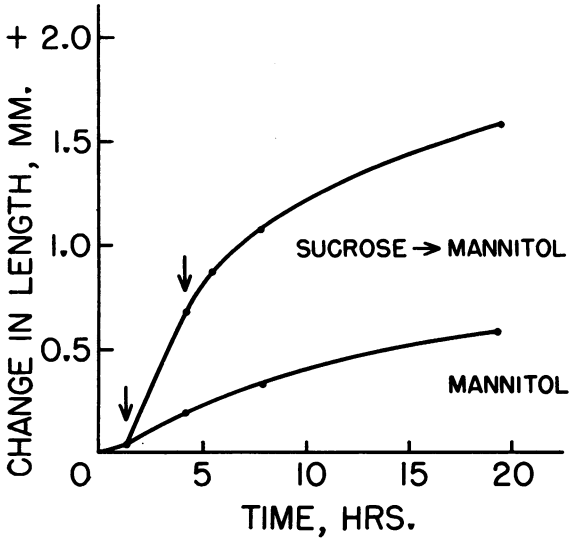

FIG. 9. Effect of 0.0813 M sucrose pretreatment on subsequent elongation of Avena coleoptile sections in 0.09 M mannitol. IAA added at first arrow. Transfer to mannitol at second arrow. 5 mg/l K-free IAA, no buffer; final pH 5.5.

The elongation rate with the latter solution drops off rapidly with time. The initial elongation rate in sucrose is greater than that in an equi-osmolar concentration of mannitol. The initial rate following transfer from sucrose to mannitol is greater than the initial rate for sections maintained in mannitol alone, but the effect does not persist. Apparently the continuing presence of sucrose is required for maintenance of elongation rate. This is consistent with the idea first presented by Thimann and Schneider (24) that sucrose acts in part to maintain OP_i at a constant high level during auxin-induced elongation.

It is of interest to know what other solutes can replace sucrose in its role of maintaining elongation rate and, apparently, OP_i. A variety of permeating substances were used, each in a concentration of 0.01 M. The results, presented as progress curves of elongation, are given in figure 10. Glucose is nearly

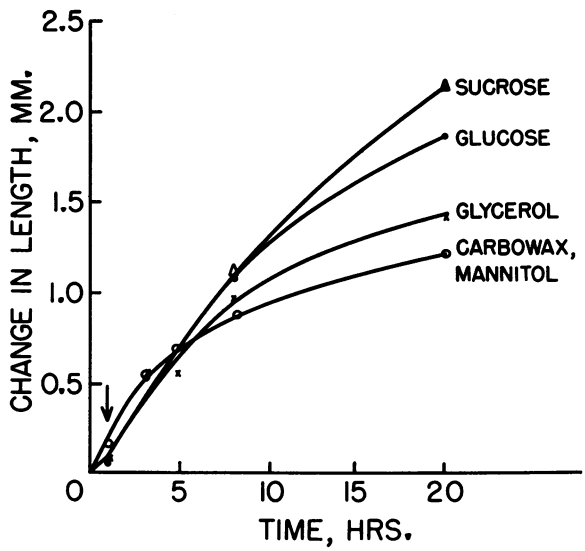

FIG. 10. Change in length of Avena coleoptile sections as a function of time and in the presence of several different solutes. Each solute given at 0.01 M concentration. IAA added at arrow. No buffer, 5 mg/l K-free IAA, final pH 5.5.

as effective as sucrose. Glycerol is but little superior to mannitol in the support of continued elongation rate. Table III gives further data on the elongation rates of sections in solutions containing various solutes, all tested at a higher concentration, 0.09 M (0.045 M for KCl and NaCl). At this concentration, sucrose and glucose are considerably more effective than any of the other solutes tested. It may be seen that the substances used form a series of varying effectiveness in replacing sucrose or glucose. Even an apparently non-metabolizable substance such as ethylene glycol is one third as effective as sucrose. NaCl and KCl possess an intermediate status.

TABLE III

EFFECT OF VARIOUS SOLUTES ON IAA-INDUCED ELONGATION OF AVENA COLEOPTILE SECTIONS

SOLUTE	ELONGATION, MM
Sucrose (0.0813 M)	2.86
D-Glucose	2.79
NaCl (0.045 M)	2.23
KCl (0.045 M)	1.75
D-Raffinose	1.16
Glycerol	1.01
Ethylene glycol	0.90
D-Ribose	0.87
Urea	0.77
L-Rhamnose	0.70
D-Xylose	0.61
L-Sorbose	0.55
L-Arabinose	0.23
D-Arabinose	0.22

Solutions 0.09 M except where noted. Incubation 20 hrs. 5 mg/l IAA throughout. Media unbuffered pH 5.4 to 6.1 for all solutions except that containing urea in which pH was 7.0. Elongation above mannitol control.

The absolute elongation of sections in 0.09 M solution is depressed as compared to that in 0.01 M solution for all solutes tested except sucrose and glucose. These two substances are more effective in increasing elongation rate in the 0.09 M concentration than at 0.01 M. This is undoubtedly due in part to the fact that sucrose and glucose are metabolizable as shown by Bonner (4). In addition, however, sections incubated in IAA sucrose-containing solutions show an increase in OP_i as compared to similar sections incubated in mannitol or in glycol, a readily absorbed solute (table II). In the absence of IAA, OP_i is increased somewhat above initial values. The relatively large increases in OP_i in the presence of sucrose may be due to the formation by the tissue of smaller molecules from the large sucrose molecules taken in. In any case, the osmotic readjustment caused by sucrose appears to be independent of the presence or absence of IAA (table II).

It has been noted above that when Avena coleoptile sections are placed in solutions of high external osmotic concentration there is a lag period in the attainment of steady state auxin-induced elongation. The experiments outlined above have suggested that this lag period may represent the time needed by the cells of the tissue to accumulate solutes and to increase in OP_i. The following experiments were carried out to further characterize the effects of different external solutes on the lag period. Sections were placed in hypotonic solution, 0.2 M, containing mannitol alone or mannitol with a small amount of sucrose or NaCl. All solutions contained IAA. The sections were left in these solutions for four hours to permit any osmotic readjustment to occur. The sections were then transferred to an equi-osmolar solution of mannitol, sucrose and IAA. The data of figure 11 show that sections pretreated for four hours in mannitol alone show a lag period in attainment of steady state elongation when transferred to mannitol, sucrose and IAA. If the pretreatment included sucrose or KCl in addition to mannitol the sections start elongating immediately and rapidly attain the final steady state rate. Apparently, the lag period does represent a period of readjustment of internal osmotic concentration. The length of the lag period is dependent on the presence in the external solution of readily absorbable solutes. It may be noted that NaCl behaves as does KCl in permitting shortening of the lag period.

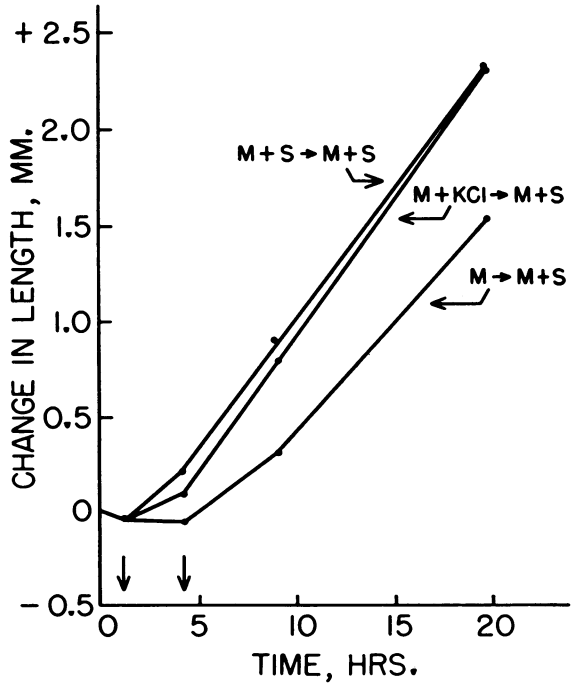

FIG. 11. Effect of pretreatment with mannitol alone or mannitol in the presence of KCl or sucrose on subsequent elongation of Avena coleoptile sections in mannitol and sucrose. Total solute concentration 0.2 M. IAA added at first arrow. Transfer to mannitol and sucrose at second arrow. 5 mg/l K-free IAA, no buffer, final pH 5.0–5.2.

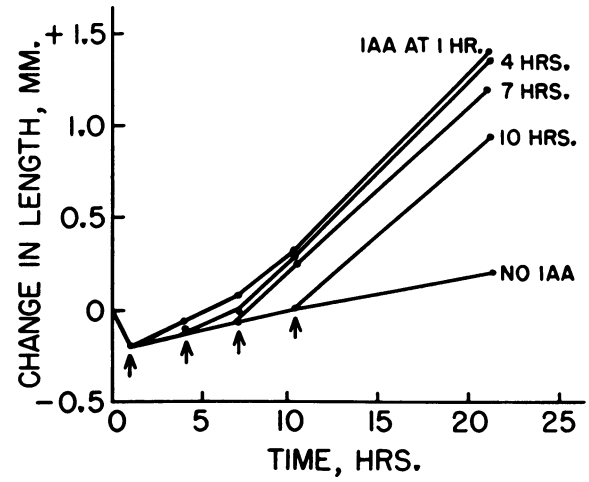

FIG. 12. Effect of time of addition of IAA on subsequent elongation of Avena coleoptile sections in solution of 0.3 M total concentration. IAA added at arrows. 0.0813 M sucrose, 0.21 M mannitol, 0.0025 M potassium maleate, pH 4.8, 5 mg/l IAA.

The osmotic regulation which takes place during the lag period in solutions of moderate osmotic concentration is not dependent upon the presence of auxin in the external medium. In order to test the effect of auxin on the lag process, sections were incubated in 0.3 M solutions containing mannitol and sucrose. Auxin was added at various times and the elongation rate after addition of auxin was followed. The data are presented in figure 12. It is apparent that the lag period extends for about 7 hours and that its duration is independent of the presence or absence of auxin in the external solution.

Discussion

An important conclusion of the present work is that the auxin-induced uptake of water by Avena coleoptile sections follows osmotic principles. It is of interest to consider these results in relation to earlier reports of metabolically maintained DPD gradients.

In order to determine whether a tissue is able to accumulate water against a diffusion pressure gradient it is necessary to possess information concerning the osmotic pressure (OP_i) of the tissue. The simplified method of Ursprung (25) has been shown above to be inapplicable because of the absence of a clear-cut inflection in the volume-OP_e relation as OP_e is increased. Coleoptile tissue as well as other tissues discussed by Crafts et al (10) continue to shrink as OP_e is increased above OP_i. The cryoscopic technique when applied to tissue sections which have been in contact with plasmolyzing solutions is attended by serious errors in assessment of OP_i as has been shown by Le Gallais (14). In the case of plasmolyzed tissues, for example, the cryoscopic method yields values of apparent OP_i which are too high. The plasmolytic method, however, can be used for OP_i determinations even with tissues which have been incubated in initially hypertonic solutions.

Bogen (2) has shown that leaf tissues deplasmolyze under aerobic conditions in sucrose solutions in which they are initially plasmolyzed. A similar result is reported here. In the present case it has been shown that sucrose penetrates the Avena coleoptile sufficiently rapidly to permit of deplasmolysis in less than 20 hours and that the deplasmolysis is an osmotic one. Bogen's experiment would appear to be susceptible to a similar interpretation. It has been reported by Bonner et al (6) that Jerusalem artichoke discs can take up water under the influence of auxin from solutions which are initially hypertonic. Although the determinations of initial OP_i are difficult with this tissue they can be made and the values reported earlier have been confirmed. Determinations of OP_i after a period of water uptake are still more difficult to perform with Jerusalem artichoke tissue and are more questionable. Burström, (8) who used cryoscopic techniques, found that OP_i of such tissue increases with time in initially hypertonic solutions. The cryoscopic technique requires an elaborate correction if it is to be used under these conditions. Nonetheless, it is probable that the OP_i of Jerusalem artichoke increases with time in hypertonic solutions as shown in the present paper for Avena. All of the experiments of Bonner et al were done in the presence of an absorbable solute, namely potassium phosphate buffer. The water loss which takes place when Jerusalem artichokes in initially hypertonic solution (0.2 M mannitol) are transferred to anaerobic conditions may be due to progressive tissue damage as it is with Avena coleoptile sections.

The most nearly valid evidence, at least as a first approximation, of water uptake against a diffusion pressure gradient in higher plants is contained in the work of van Overbeek (20). It was found by van Overbeek that the exudate of an excised tomato root-shoot system may have an osmotic concentration 1 atmosphere less than that of the solution bathing the root. Arisz et al (1) using more refined techniques of measurement reduced this difference to 0.5 atmosphere or less. The latter workers propose that the lower OP of the exudate is due to salt absorption from the sap between the root and the stump as well as to dilution by water from tissues adjacent to the xylem. Furthermore, if the zone of water absorption exceeds the zone of salt absorption, dilution of the sap would occur.

Neither the present work, nor other work reported on water, clearly support the concept of active uptake as defined by Rosenberg (21) and Levitt (16). It is clear however that the performance of auxin-induced water uptake is attended by an increase in respiratory rate and that the auxin-induced increment is abolished by external solute concentrations which abolish net water uptake. The increase in respiration caused by auxin does not then have to do with water accumulation against a water concentration gradient but would appear rather to be related in some way to the primary act involved in cell elongation.

It has long been known that sugars (3) or potassium salts (24) act as co-factors in auxin-induced elongation of coleoptile sections. The role of these substances is in part an osmotic one. They contribute to the maintenance of OP_i. The presence of sucrose in the medium extends the period over which elongation is linear with time. This effect cannot be achieved by sucrose pretreatment as shown by experiments in which sections decrease in elongation rate after transfer to sucrose-free solutions. The effect of sucrose on rate of water uptake may be due in part to the utilization of the material in respiration and in the support of cellular syntheses. In addition however sucrose clearly provides internal osmotically active material both as intact sucrose molecules and perhaps as metabolically produced smaller molecules. The lesser effectiveness of salts and especially of other sugars is presumably due to their being less effective as food material and less abundantly converted to smaller molecules.

It has been concluded that in the Avena coleoptile DPD_i is at all times essentially in equilibrium with OP_e and that by the methods presently available no detectable differences between these two quantities can be found. Within the limits of measurement of DPD ($\pm$ 0.05 M) no movement of water against a DPD gradient appears to take place with Avena coleoptile sections. When non-absorbable solute is used to constitute a hypertonic solution, no elongation of the tissue takes place. It is therefore highly unlikely that active water uptake is a factor in auxin-induced elongation in this tissue. This agrees with the conclusions reached earlier with other tissues by Levitt (15, 17) and by Thimann (23). Adjustments

of osmotic concentration take place in the coleoptile section provided that an absorbable solute is present in the medium. These osmotic adjustments are however independent of auxin and occur in the absence as well as in the presence of added growth substance.

Since auxin-induced water uptake is a purely osmotic phenomenon in the Avena coleoptile and since auxin does not appear to directly influence internal osmotic concentration, it may be concluded that auxin must in some way decrease cell wall pressure. The fact that the irreversible component of the total elongation behaves as an inverse function of OP_e in the hypotonic region is consistent with the hypothesis that cell wall plasticization rather than cell wall synthesis is a primary cell wall effect. This is in contrast to the state of affairs with wheat roots (7) in which irreversible extension is independent of OP_e in the hypotonic region as reported by Burström. In any case it would appear that a detailed investigation of cell wall metabolism as related to the presence or absence of auxin may be required for the further elucidation of the mechanism of auxin action.

Summary

Avena coleoptile sections are in apparent diffusion pressure equilibrium with the external solution at all times, i.e., there is no detectable aerobic metabolic component of DPD in either hypertonic or hypotonic solution.

Both total elongation and irreversible elongation are inverse functions of external osmotic concentration in the hypotonic region.

Tissue placed in initially hypertonic solution deplasmolyzes and grows if a sufficient concentration of an absorbable solute such as sucrose is present in the external solution. It has been shown that the role of the permeating solutes is to cause an increase in internal osmotic concentration of the tissue.

Tissue in hypotonic solution maintains or increases in internal osmotic concentration as elongation occurs, provided that absorbable solute is present in the external solution. Tissue maintained in distilled water exhibits a decrease in internal osmotic concentration as elongation occurs.

When Avena coleoptile sections are placed in slightly hypotonic or in hypertonic solution there is a lag period in the attainment of steady state elongation rate. The duration and intensity of this lag period increases with external osmotic concentration. The length of the lag period is independent of the presence of auxin and is determined by the time required by the section to make an osmotic readjustment to the external solution by absorption of solutes.

Auxin-induced water uptake in Avena coleoptile sections does not appear to be due to the effect of auxin on maintenance of a metabolically controlled component of internal diffusion pressure deficit or to effects on the osmotic concentration of the tissue. The present evidence points to effects of auxin on the cell wall as an important factor in auxin-induced cell elongation.

Literature Cited

1. Arisz, W. H., Helder, R. J., and van Nie, R. Analysis of the exudation process in tomato plants. Jour. Exptl. Bot. 2: 257–297. 1951.
2. Bogen, H. J. Beiträge zur Physiologie der nichtosmotischen Wasseraufnahme. Planta 42: 140–155. 1953.
3. Bonner, J. The action of the plant growth hormone. Jour. Gen. Physiol. 17: 63–76. 1933.
4. Bonner, J. Biochemical mechanisms in the respiration of the Avena coleoptile. Arch. Biochem. 17: 311–326. 1948.
5. Bonner, J. Relations of respiration and growth in the Avena coleoptile. Amer. Jour. Bot. 36: 429–436. 1949.
6. Bonner, J., Bandurski, R. S., and Millerd, A. Linkage of respiration to auxin induced water uptake. Physiol. Plantarum 6: 511–522. 1953.
7. Burström, H. Studies on growth and metabolism of roots. IX. Cell elongation and water absorption. Physiol. Plantarum 6: 260–274. 1953.
8. Burström, H. Growth and water absorption of Helianthus tuber tissue. Physiol. Plantarum 6: 685–691. 1953.
9. Commoner, B. and Thimann, K. V. On the relation between growth and respiration in the Avena coleoptile. Jour. Gen. Physiol. 24: 279–296. 1941.
10. Crafts, A. S., Currier, H. B., and Stocking, C. R. Water in the Physiology of Plants. Pp. 1–240. Chronica Botanica, Waltham, Massachusetts. 1949.
11. Hackett, D. P. The osmotic change during auxin-induced water uptake by potato tissue. Plant Physiol. 27: 279–284. 1952.
12. Kelly, S. The relationship between respiration and water uptake in the oat coleoptile. Amer. Jour. Bot. 34: 521–526. 1947.
13. Ketellapper, H. J. The mechanism of the action of indole-3-acetic acid on the water absorption by Avena coleoptile sections. Acta Bot. Néerl. 2: 388–444. 1953.
14. Le Gallais, D. R. A study of growth of plant tissue in relation to composition and concentration of the external medium. Ph.D. Thesis, University of California, Berkeley. 1955.
15. Levitt, J. The thermodynamics of active (non-osmotic) water absorption. Plant Physiol. 22: 514–525. 1947.
16. Levitt, J. The role of active water absorption in auxin-induced water uptake by aerated potato discs. Plant Physiol. 23: 505–515. 1948.
17. Levitt, J. Further remarks on the thermodynamics of active (non-osmotic) water absorption. Physiol. Plantarum 6: 240–252. 1953.
18. McRae, D. H. and Bonner, J. Chemical structure and antiauxin activity. Physiol. Plantarum 6: 485–510. 1953.
19. Meyer, B. S. and Anderson, D. B. Plant Physiology. Pp. 1–696. D. Van Nostrand Co., New York. 1939.
20. van Overbeek, J. Water uptake by excised root systems of the tomato due to non-osmotic forces. Amer. Jour. Bot. 29: 677–683. 1942.
21. Rosenberg, T. The concept and definition of active transport. Symposia Soc. Exptl. Biol. 8: 27–41. 1954.
22. Thimann, K. V. Studies on the physiology of cell

enlargement. Growth 15 (Suppl.): 5–22. Tenth Symposium, Soc. Study Develop. & Growth, Northampton, Massachusetts. 1951.
23. THIMANN, K. V. The physiology of growth in plant tissues. Amer. Scientist 42: 589–606. 1954.
24. THIMANN, K. V. and SCHNEIDER, C. L. The role of salts, hydrogen-ion concentration and agar in the response of the Avena coleoptile to auxins. Amer. Jour. Bot. 25: 270–280. 1938.
25. URSPRUNG, A. Zur Kenntnis der Saugkraft. VII. Eine neue vereinfachte Methode zur Messung der Saugkraft. Ber. deut. bot. Ges. 41: 338–343. 1923.

PERMEABILITY OF AVENA COLEOPTILE SECTIONS TO WATER MEASURED BY DIFFUSION OF DEUTERIUM HYDROXIDE[1,2]

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The present investigation, carried out in connection with studies of the mechanism by which auxin induces net water uptake, concerns the resistance of Avena coleoptile section tissue to water movement. The factors which govern tissue permeability to water have also been examined. Heavy water has been used in order to eliminate changes in the geometry of the tissue during the measurement. Permeability constants obtained by this method are true diffusion constants in contrast to the filtration constants obtained by osmotic methods. The first use of deuterium oxide (D_2O, 30 to 50 %) solutions in the study of water permeability of plants was that of Wartiovaara (11) on the alga Tollypellopsis. Buffel (2) and Ketellapper (5) have also used 50 % D_2O to determine the water permeability of Avena coleoptiles. These workers studied the outward diffusion of D_2O from tissue previously equilibrated with the material. In both investigations cartesian diver techniques were used for determination of D_2O. The high concentrations of D_2O used exert injurious effects on the tissue after several hours. Buffel therefore allowed his sections to age in distilled water for 12 to 18 hours, a procedure found empirically to decrease such injury.

Buffel (2) has reported that indoleacetic acid (IAA) increases D_2O permeability of sections aged in this manner. Ketellapper (5) on the other hand finds that IAA has slight if any effect on D_2O permeability. It will be shown below that rate of absolute water movement in the coleoptile is rapid and is not influenced by IAA, metabolic inhibitors, or the state of turgor of the tissue.

MATERIALS AND METHODS

The material used in this work consisted of 5-mm sections cut 2 to 3 mm below the apex of the Avena coleoptile. The primary leaf was removed. The Avena seedlings were grown at 25° C in vermiculite contained in stainless steel trays and were watered with distilled water. They were harvested when 96 hours old. Only those coleoptiles 30 ± 2.5 mm in length were used. Each part of each experiment was performed using 50 or 75 sections in 10 or 15 ml of solution respectively.

Dilute solutions of deuterium hydroxide (DHO) (0.5 % D_2O) were prepared from 99.5 % D_2O obtained from Stuart Oxygen Company by authorization of the U. S. Atomic Energy Commission. The D_2O was used without further purification. Groups of sections were placed in DHO solution for varied periods of time. After the desired time interval the group of sections was dried externally with filter paper and placed in a ground glass stoppered vial. The vial was then utilized as the distilling vessel of a micro-distillation apparatus in which the tissue water was removed by lyophilization. The DHO content of the tissue water was determined by mass spectrometry. The direct determination of DHO in the mass spectrometer has been described by Washburn et al (12). The precision of measurement is greater than $\pm$ 0.01 atom percent over the range of DHO concentrations used in this work. The data are presented as mole percent DHO in the tissue water as a function of time.

Indoleacetic acid (IAA), 5 mg/l, was used as the auxin throughout. The basal medium contained potassium maleate buffer (0.0025 M, pH 4.8). All solutions were made up with redistilled water.

RESULTS

The data in figure 1 show that IAA has no influence on the course of entrance of heavy water into coleoptile tissue. The half-time of the movement, i.e., the time required for the tissue to attain a concentration of DHO equal to one half the difference between the external concentration and the initial internal concentration (that of normal water, 0.02 % DHO), is about 9 minutes. This is slightly longer than the half times found by Buffel (2) and Ketellapper (5) of 4 to 7 minutes and 4 to 6 minutes respectively. The logarithmic shape of the curve of figure 1 indicates that water enters the tissue by a simple diffusion process.

The course of the outward diffusion of water was compared with that of the inward diffusion by pretreating tissue for 2 hours in 1.1 % DHO. The tissue

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