Osmotic Behavior of Oat Coleoptile Tissue in Relation to Growth

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ABSTRACT Efforts were made to estimate the water potential difference that is required, between rapidly growing oat coleoptile cylinders and dilute medium, to support the rate of water uptake involved in elongation, (*a*) by the traditional method of determining the concentration of mannitol in which the tissue neither gains nor loses water, and (*b*) by measuring the rates of osmotic exchanges induced by treating the tissue with different hypotonic mannitol concentrations. Both methods indicated large water potential differences (3 to 10 atm), in some cases approaching the osmotic pressure of the cells. However, indication was obtained that the rates of osmotic exchanges induced by mannitol solutions, and presumably also the equilibrium response sought in (*a*), are governed by the rate of diffusional exchange of mannitol with the free space rather than by the permeability of the tissue to water. Osmotic swelling of the tissue measured by immersing it in water after its turgor pressure had been reduced by evaporation, was at least two to four times more rapid than when mannitol was involved. The permeability to water estimated by the evaporation-immersion method indicated that rapidly elongating cylinders have water potentials between −0.8 and −2.5 atm, or between 10 and 25 per cent of their osmotic pressure.

INTRODUCTION

Absorption of water is essential to the enlargement of plant cells during growth. Efforts to assess the osmotic gradient that is required to bring about this water uptake are not only of intrinsic interest, but are of importance as a means of estimating the turgor pressure of the growing cell. This is of importance in the rheology of growth, since turgor pressure provides the driving force for the processes by which the cell wall expands during growth.

Concerning oat coleoptile tissue, which has been widely employed in studies on plant growth, extremely divergent views have been published in regard to water uptake, ranging from that of Pohl (12) that the rate of cell enlargement is controlled by the permeability to water, to the views of Ordin and Bonner

(6, 7) that the permeability to water is so large that absorption of water does not govern the rate of growth.

Ordin and Bonner (7) found that the half-time for diffusion of DHO into or out of coleoptile cylinders is about 8 min., whereas, during growth, about 16 hrs. are required for a *net* water uptake of 50 per cent. From this they concluded that during growth the cells must remain virtually in osmotic equilibrium with the medium, although they did not show this consequence explicitly. To the contrary, if one computes, from their DHO diffusion data, the water potential difference (or suction force difference; see Methods) between tissue and medium that should be required to maintain the observed rate of cell enlargement, one finds that a very large water potential difference (about 10 atm) appears to be needed. However, diffusion of DHO through tissue is probably governed mainly by parameters other than permeability of the membranes (4). Moreover, in principle, diffusion experiments do not afford a measurement of the permeability that governs osmosis (4, 13). To approach quantitatively the problem of osmotic uptake of water during growth, it is necessary to make osmotic measurements, either of permeability, or of the water potential of the tissue.

The traditional method of determining water potential is to transfer the tissue into different media containing an impermeant solute, and determine in what concentration the cells neither gain nor lose water at the time of transfer from original medium into test medium. This may be called the *equilibrium method*, since it seeks to determine the water potential of medium in which the tissue would just find itself in osmotic equilibrium. With growing tissue the experiment must be designed so as to distinguish continued growth from transitory elastic changes in volume, which the method depends upon detecting. This is not possible if changes in length are measured only after many hours, as in the original experiments with mannitol performed by Thimann and Schneider (18), who interpreted their results in a manner that implied that a very large water potential difference is involved in the growth of oat coleoptile tissue.

Ordin, Applewhite, and Bonner (6) made determinations by the equilibrium method, by a procedure that attempted to exclude the interfering effect of continued elongation on interpretation of the results. They concluded that coleoptile tissue was at all times essentially in osmotic equilibrium with the medium. But they listed determinations only on coleoptile cylinders that had been kept, before transfer to test media, in mannitol concentrations of 0.25 M and greater, in which coleoptile cylinders do not grow at an appreciable rate; so the reported results do not afford information about the water potential differences that may be involved in growth. Bennet-Clark (2), on the contrary, showed results of equilibrium experiments that indicated a water potential difference of 5 to 10 atm between growing coleoptile tissue and a dilute medium.

This paper presents the results of efforts to measure the osmotic permeability of oat coleoptile tissue and to relate these measurements to its growth rate, as well as an examination of the equilibrium method. These measurements were made using an apparatus that can measure accurately small changes in length of coleoptile cylinders over short periods of time, permitting a more precise investigation of the osmotic behavior of this tissue than has been possible heretofore.

METHODS

Measurement of Changes in Length Measurements of changes in length due to growth and to osmotic exchanges were made on coleoptile cylinders, from 3 day old oat seedlings (*Avena sativa*, variety Victory), by the technique previously described (14). Briefly, a coleoptile cylinder 6 to 8 mm long was mounted in a horizontal position upon a No. 23 syringe needle held in a plexiglas trough through which the test solution flowed continuously. The free end of the cylinder was observed with a compound microscope against an ocular micrometer scale, so that changes in length could be measured with a sensitivity of 1.5 microns per micrometer scale division. Cylinders were transferred from one test solution to another simply by causing the new solution to begin flowing through the trough; complete replacement of solution in the trough occurred within 15 seconds. The length of each cylinder was measured, at the end of the experiment, from the free end of the cylinder to the point where it had been tied onto the mounting needle with a fine thread.

Relation between Length and Volume Changes It was necessary to know the relation between change in cylinder length and volume (*a*) during elastic expansion or contraction of the cells caused by changes in turgor pressure during osmotic exchanges, and (*b*) during irreversible elongation of the cells (growth).

(*a*) The volume/length relation for elastic changes in size was determined by measuring the decrease in length of thirty individual cylinders, by the above method, when transferred from water to 0.31 *m* mannitol, and comparing this with the loss in weight of four lots of fifteen cylinders each, when transferred from water to 0.31 *m* mannitol.

In the weighing experiment the cylinders, 8 mm long, were handled in a nearly water-saturated atmosphere, to retard evaporation. Water-saturated air was blown through the central cavity of each cylinder to expel droplets of water, and the external surface was carefully blotted. The lots of cylinders were weighed to 0.01 mg, on a microbalance, in small closed weighing bottles that prevented changes in weight due to evaporation. Each lot was weighed first after removal from water, then was immersed in 0.31 *m* mannitol for 1 hr., which is adequate for complete osmotic equilibration (the cylinders do not grow in this medium), and the cylinders were removed and reweighed by the same procedure. The per cent loss in weight of the four lots of cylinders (each weighing initially about 95 mg) was 2.12, 2.20, 2.24, and 2.30, with a mean of 2.21 per cent.

Decrease in length of each of thirty individual cylinders when transferred from water to 0.31 *m* mannitol was followed to completion (which occurred within half an hour in almost all cases). In this and other experiments it was observed that the elastic change in length of coleoptile cylinders upon transfer to a given concentration of impermeant

solute varies greatly from cylinder to cylinder but is consistent if repeated with any given cylinder. The mean decrease in length in 0.31 *m* mannitol was found to be 1.55 per cent with a standard deviation of 1.24 per cent and a probable error of the mean of 0.15 per cent. Dividing into the mean per cent decrease in weight (2.21) gives 1.43 as the ratio of relative change in volume to relative change in length ($l\Delta v/v\Delta l$), which will be designated ψ.

It may be noted that this result indicates that the cell walls are considerably less elastic in the transverse than in the longitudinal direction. If transverse and longitudinal per cent changes in elastic extension were equal, ψ would be 3.0, whereas if the wall were completely inextensible in the transverse direction ψ would be 1.0.

(*b*) The ratio between length and volume changes during elongation was determined by weighing groups of cylinders before and after they had elongated by a measured increment in 3 mg/liter indoleacetic acid. It was found that cylinders which had elongated 71 per cent had increased in fresh weight by 78 per cent. The ratio of relative increases in volume and length during elongation (called ϕ) is, therefore, 1.10.

Evaporation Experiments For reasons to be given below, it was found necessary in certain experiments to measure expansion of coleoptile cylinders after their turgor pressure had been reduced by evaporation of water from them. After growth rate in water or indoleacetic acid solution had been measured, the measuring trough was emptied of liquid by stopping the inflow, the measuring microscope was raised, air was blown briefly through the hypodermic needle upon which the coleoptile cylinder was mounted, so as to remove water from the central cavity, and water droplets on the external surface of the cylinder were removed by absorbent tissue. A stream of air at room temperature was directed over the cylinder from a gun-type hair dryer, and the measuring microscope was repositioned to follow the shrinking of the cylinder as water evaporated from it.

Cylinders were allowed to shrink not more than about 200 microns (3 per cent of their length), which required 5 to 20 min. depending upon the cylinder. Through the trough, water containing 10^{-3} M KCN was then caused to flow at the usual rate; as rapidly as possible (5 to 10 sec.) a focus point, at the end of the cylinder, for measuring change in length was located under the microscope, and the swelling that occurred as water was absorbed by the cylinder was followed. The purpose of KCN was to inhibit growth (irreversible elongation) of the cylinder, which would otherwise add appreciably to the osmotic swelling and would prevent precise interpretation of the results. In some experiments the cylinder was also immersed in 10^{-3} M KCN *before* evaporation of water from it, but this had no apparent effect on the results.

Measurement of Osmotic Concentration of Tissue Osmotic concentration was determined at the end of the experiment, by the plasmometric method. The coleoptile cylinder was cut in half lengthwise and immersed in 0.6 *m* mannitol, which is hypertonic, for 45 to 60 min. In the same medium the halves were mounted external surface upward on a microscope slide under a coverglass. Under 430 magnification, measurements of protoplast and cell volume were obtained for at least twenty cells in the layer just beneath the epidermis, by measuring cell and protoplast lengths with an ocular micrometer (cells almost always plasmolyzed from the ends, in convex form), and

correcting for curvature of the protoplast surface in the standard manner. Osmolal concentration of the tissue before plasmolysis was calculated as 0.6 times the mean ratio of plasmolyzed protoplast volume to cell volume.

Diffusion of Labeled Mannitol out of Free Space Twenty coleoptile cylinders 8 mm long, with a total fresh weight of 110 mg, were immersed for 60 min. in 0.5 ml of water containing 5 μc of uniformly C^{14}-labeled mannitol (1.4 μc per μ mole). Using absorbent tissue the outside of each cylinder was carefully blotted dry. In one experiment (Fig. 2, curve A) the central cavity of each cylinder was rinsed for about 1 sec. with a stream of water, followed by air to expel all water from the cavity. In a second experiment (Fig. 2, curve B) the central cavity of each cylinder was carefully blotted dry using a fine wick rolled from absorbent tissue. The cylinders were then placed in 5.0 ml of water, which was continuously stirred by agitation, and from which 0.2 ml aliquots were removed at intervals to planchets, dried, and counted in a thin window gas flow counter with a counting efficiency of 25 per cent.

Expression of Osmotic Quantities We follow Slatyer and Taylor (15, 16) in expressing the thermodynamic state of water in tissue and medium, the resultant of effects of solute, pressure, and matrix, as the *water potential* (μ). The specific definition of water potential used here is that given by Dainty (4), with units of atmospheres, and corresponds numerically with traditional scales of suction force or diffusion pressure deficit multiplied by -1. Osmotic pressure is calculated for 23°C (the temperature of the experiments) as RT times the osmolal concentration. Concentrations of experimental solutions are given in molal (m) units.

RESULTS

Equilibrium Method Experiments We shall describe first our observations on the estimation of water potential of growing coleoptile cylinders by the conventional method of transferring the tissue to different concentrations of impermeant solute so as to determine the concentration in which the cells neither gain nor lose water.

Three experiments in which elongation of an auxin-treated coleoptile cylinder was followed before and after transfer from dilute medium to different mannitol concentrations are shown in Fig. 1. When transferred to 0.1 m mannitol the tissue did not lose water but continued to elongate at a slower rate, suggesting that 0.1 m mannitol had not reduced the potential difference between tissue and medium to zero; *i.e.*, the water potential of the tissue was lower than -2.44 atm. When higher concentrations were tried, a point was reached (0.15 m in A and 0.20 m in B and C) at which the tissue either did not change in length (C) or shrank very slightly immediately after transfer (A and B), but then began to elongate at a slow rate.

According to classical concepts the water potential of the solutions just mentioned was slightly less than (more negative than) that of the tissue in A and B so that the cylinders lost water, and just equal to that of the tissue in C.

That the cells began to elongate again can be explained by the fact that growth processes tending to weaken the cell wall and reduce the turgor pressure were still going on, with the result that the water potential of the tissue dropped until it became lower than that of the medium, whereupon uptake of water was again observed.

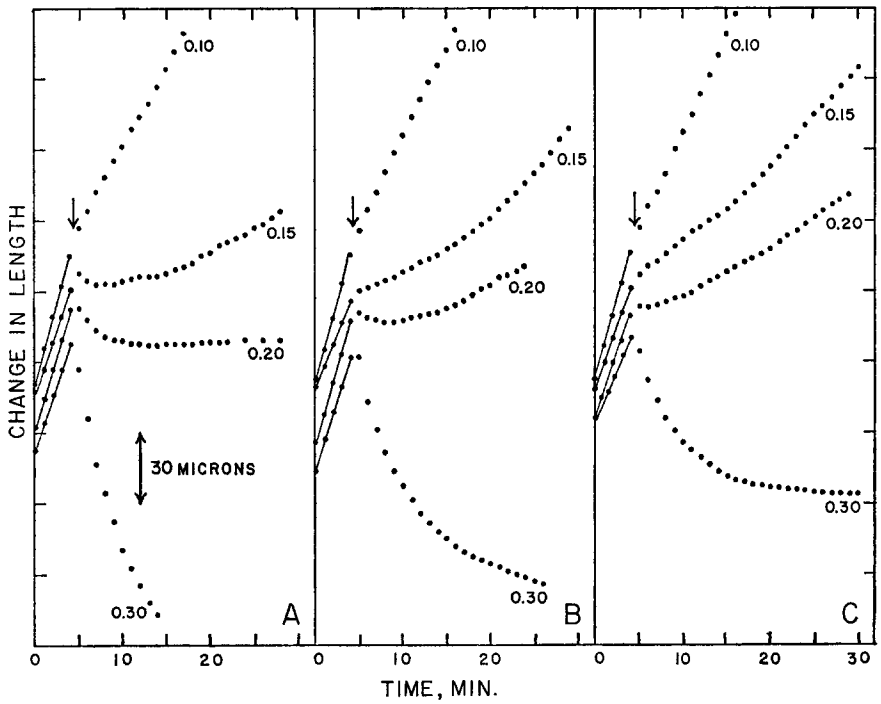

FIGURE 1. Effect of transferring elongating coleoptile cylinders from 3 mg/liter indoleacetic acid (IAA) to different concentrations of mannitol (containing IAA). Transfers were made at times shown by arrow; after the response to a given mannitol concentration was determined, the cylinder was returned to IAA alone and was followed until a steady elongation rate was observed. It was then transferred to the next higher mannitol concentration, and so forth, as in the successive curves shown from top to bottom in each part of the figure. The figure placed by each curve gives the molal concentration of mannitol in that test. Concentration of the cell contents, determined at the end of each experiment, was (A) 0.37, (B) 0.40, (C) 0.41 osmolal. Length of each cylinder, 7.2 mm. Vertical scale is 30 microns per scale unit. Rate of elongation of cylinders in IAA alone was about 720 microns, or 10 per cent, per hr.

By interpolation of the data it may be estimated that the concentration of mannitol in which coleoptile cylinders A and B would neither have gained nor lost water just after transfer would have been about 0.14 *m* (A) and 0.17 *m* (B); as previously mentioned the concentration for C was found to be 0.2 *m* directly. Since the solute concentration of the cells was about 0.4 osmolal,

these data indicate that the growing tissue is not close to osmotic equilibrium. That is, the turgor pressure of the growing cells appears by the equilibrium method to be some 3 to 5 atm or 30 to 50 per cent below the value which would be attained at osmotic equilibrium with a dilute medium (30 to 50 per cent less than the osmotic pressure of the cell contents).

Osmotic Exchange Measurements with Solutes In these experiments the elongation rate of a coleoptile cylinder was determined, and then the time course of osmotic exchange was followed when the cylinder was transferred from one concentration of mannitol to another. The concentrations chosen were great enough to inhibit elongation and ensure osmotic equilibrium at the beginning and end of the exchange measurement, but were below the concentration of the cells so that the latter retained turgor pressure and did not plasmolyze (transfer from 0.2 to 0.3 *m* mannitol was most often used). We assumed that, if the rate of exchange after such a transfer were governed by the osmotic permeability of the cells, it should be possible to calculate the permeability along the lines suggested by Philip (9–11) and thereby to estimate the water potential difference that would be required to support the previously measured rate of elongation.

The half-times observed for osmotic exchanges induced by mannitol were generally between 5 and 8 min. (examples are given in Fig. 3 and Tables I to III), in agreement with data of Ordin and Bonner (7), although occasional cylinders gave half-times of up to 15 min. Calculations from the data indicated that large (3 to 10 atm) water potential differences were needed to account for the rate of elongation in indoleacetic acid.

However, various observations led us to investigate whether the rate of swelling or shrinking of a coleoptile cylinder, when transferred from one concentration of mannitol to another, might be governed by the rate of diffusion of mannitol into or out of the free space of the tissue rather than by its permeability to water.

We measured the diffusion of C^{14}-labeled mannitol out of the free space of coleoptile cylinders as described in Methods. Results of two experiments are shown in Fig. 2. About one-fifth of the radioactivity that came out of the tissue (which had previously been equilibrated with C^{14}-mannitol) appeared in the external medium within 10 sec. and probably came from surface films or solution entrapped in broken cells at the cut ends of the cylinders. The remaining radioactivity emerged on a nearly exponential time course with half-times of 5 to 8 min. The half-time for diffusion of mannitol out of interior free space resembles suspiciously the typical half-time for osmotic exchanges as measured with mannitol, suggesting that the latter might be determined by the former. It may be noted that diffusion out of the free space of coleoptile cylinders is markedly slower than has been observed for free space exchange in tissues that lack a cutinized epidermis (8, 17).

One test of whether penetration into the free space governs the rate of osmotic exchange is to determine the half-time for osmotic exchange using solutes of different molecular weight, which might be expected to diffuse in at different rates. If the rate of osmotic exchange were governed by osmotic permeability it should be independent of the solute used (provided that cell membranes are impermeable to the solute and the solute does not alter osmotic permeability). Table I records results of experiments in which coleoptile

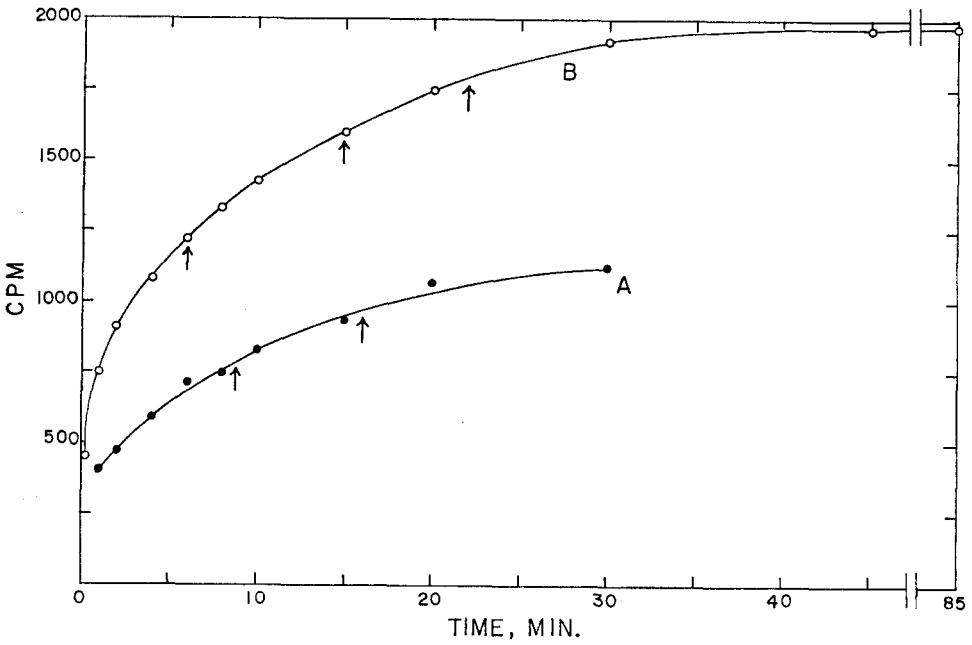

FIGURE 2. Diffusion of labeled mannitol out of free space of oat coleoptile cylinders. Procedure is explained in Methods; ordinate shows counts per minute in 0.2 ml aliquots removed from the water into which diffusion was occurring. Curve A is for cylinders whose central cavity was rinsed momentarily with water after equilibration with labeled mannitol; curve B is for cylinders which were simply blotted dry inside and out. Arrows show successive half-times figured beyond the first point on each curve. From the total radioactivity obtained, the mannitol free space was calculated as 3.3 per cent of tissue volume (curve A) and 5.7 per cent (curve B).

cylinders were transferred from water to equiosmolal solutions of different solutes, or from 0.25 *m* mannitol to 0.25 *m* mannitol plus NaCl or an equiosmolal additional concentration of mannitol. The half-time was found to be noticeably shorter for NaCl than for mannitol, and noticeably longer for sucrose, about in inverse proportion to the square root of the masses of the diffusing species. This suggests, therefore, that the rate of osmotic exchange is indeed governed by diffusion of solute into or out of the free space.

If this is the case, then in order to measure true water permeability of the

tissue it would be necessary to devise a method that does not involve treatment with an impermeant solute. We considered that a possible method would be to reduce the turgor pressure of the cells by evaporation of water from the tissue, and then to measure the rate of osmotic swelling when the tissue is suddenly immersed in water.

TABLE I

HALF-TIMES FOR OSMOTIC EXCHANGES INDUCED BY DIFFERENT SOLUTES

Results for each cylinder are given in the order in which the tests were performed, to show that the differences were not due to changes in properties of the cylinders with time. Half-times listed for cylinders 2 and 3 are the means for shrinking and swelling exchanges upon transfer from initial medium to the second medium and, upon attainment of equilibrium with the latter, back to the initial medium.

Cylinder No.	Test No.	Initial medium	Transfered to	t_{50}
				min.
1	1	Water	0.3 *m* mannitol	5.0
	2	Water	0.15 *m* NaCl	3.0
	3	Water	0.3 *m* mannitol	6.5
	4	Water	0.3 *m* sucrose	9.0
2	1	0.25 *m* mannitol	0.35 *m* mannitol	6.5
	2	0.25 *m* mannitol	0.25 *m* mannitol plus 0.053 *m* NaCl	3.7
	3	0.25 *m* mannitol	0.35 *m* mannitol	5.5
	4	0.25 *m* mannitol	0.25 *m* mannitol plus 0.053 *m* NaCl	3.0
3*	1	0.25 *m* mannitol	0.35 *m* mannitol	2.9
	2	0.25 *m* mannitol	0.25 *m* mannitol plus 0.053 *m* NaCl	1.5

* The external epidermis was removed from this cylinder just before the experiment was performed.

Evaporation-Immersion Experiments We found that if water were evaporated from an oat coleoptile cylinder, by the technique described in Methods, it would undergo a rapid osmotic expansion when immersed in water, as illustrated in Fig. 3. In order to measure the amount and the time course of osmotic expansion it was necessary to suppress the growth which would otherwise occur. This was done by including in the immersion medium 10^{-3} M KCN, which rapidly causes reversible inhibition of elongation (14). Although 10^{-3} M KCN does not suppress elongation completely, inhibition is so strong that it was not difficult to distinguish the initial osmotic expansion from the slow subsequent elongation. That the rapid phase of expansion is almost entirely osmotic is indicated by the fact that the amount of expansion during the rapid phase was approximately equal to the shrinkage in length observed during evaporation (Fig. 3).

Table II gives data for a number of trials in which the half-times for osmotic expansion both after evaporation and after treatment with mannitol were determined. In all cases the half-time was conspicuously shorter by the evaporation method than when the osmotic exchange involved mannitol (KCN was

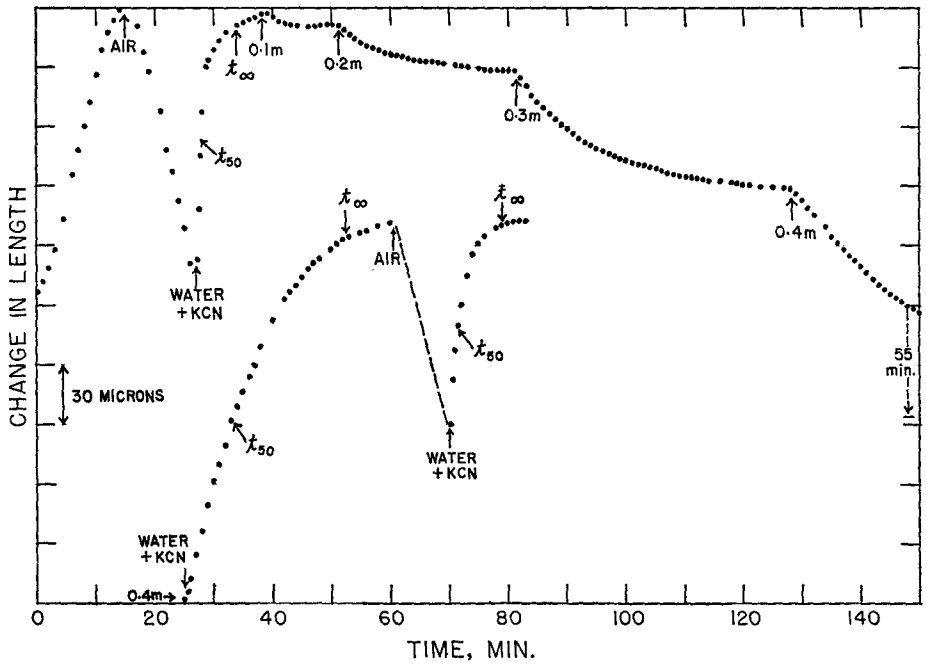

FIGURE 3. Example of evaporation-immersion experiment and accompanying measurements. The procedure is described in Methods. Cylinder was elongating in 3 mg/liter indoleacetic acid (IAA) at zero time (upper curve). At first arrow evaporation was begun; at second arrow cylinder was immersed in water containing 10^{-3} M KCN and 3 mg/liter IAA. After completion of swelling the cylinder was transferred successively to mannitol concentrations 0.1, 0.2, 0.3, and 0.4 *m* as indicated (the 0.1 *m* mannitol contained KCN to inhibit elongation; to save space the entire course of shrinking in 0.4 *m* mannitol is not included). Then, as shown in the lower curve (which has been shifted downward 90 microns relative to upper curve), the cylinder was transferred to water containing KCN and IAA. After completion of swelling the evaporation-immersion procedure was repeated. t_{50} indicates half-time; t_∞ indicates time at which it was assumed that virtual equilibrium with water had been attained. On vertical scale, one unit equals 30 microns change in length. (Final length of cylinder 6.5 mm; final internal concentration of tissue 0.38 osmolal.)

present in both, so the difference was not due to KCN). These results appear to show conclusively that the rates of osmotic exchanges involving mannitol solutions are not governed by the permeability of the tissue to water. That this might be the case was suggested recently by Dainty (4).

A somewhat similar evaporation and immersion method for measuring osmotic permeability was used earlier by Baptiste (1) for discs of potato and carrot tissue.

The present evaporation-immersion technique is difficult, due to the rapid manipulations and readings that must be made, and great precision cannot be claimed for it. Nevertheless we feel that, since the tissue absorbs water from a dilute medium just as it does during elongation, the evaporation-immersion

TABLE II

COMPARISON BETWEEN OSMOTIC EXCHANGES AND ELONGATION RATE

The sequence of measurements is illustrated in Fig. 3 and described in the text. The half-time (t_{50}) listed for mannitol applies to the swelling that occurred when the cylinder was transferred from 0.3 or 0.4 *m* mannitol to water containing 10^{-3} M KCN (and IAA, if this was present during the elongation measurement); this involved about the same amount of expansion as that which occurred in the evaporation-immersion test. Effective permeability P_e (microns change in length per minute per atm) shows the mean and average deviation for two to four values found from the evaporation-immersion swelling curve(s), as explained in the text. Water potential of the growing tissue was computed using formula (1).

Cylinder No.	Length	IAA	Rate of elongation	Osmotic exchange measurements: Mannitol t_{50}	Osmotic exchange measurements: Evaporation-immersion t_{50}	Osmotic exchange measurements: Evaporation-immersion P_e	Osmotic pressure of cells	Calculated water potential
	mm	*3 mg/liter*	*μ/min.*	*min.*	*min.*	*μ/min. atm*	*atm*	*atm*
1	6.0	−	1.2	5.0	1.6*	5.2±1.3	9.8	−0.18
2	6.0	−	2.1	4.5	1.4*	4.8±0.5	11.9	−0.44
3	5.5	+	11.4	6.0	2.7	4.8±0.02	9.0	−1.8
4	6.5	+	12.4	8.0	0.75*	11.6±4.1	9.3	−0.82
5	6.2	+	5.9	6.5	2.5	3.0±0.9	11.5	−1.5
6	7.0	+	18.7	6.0	2.2*	6.0±1.1	10.3	−2.4

* Means of two trials.

method should provide a measurement of osmotic properties that can in fact be used to compute the water potential difference required for elongation. The next section deals with the problems and results of this computation.

Interpretation of Osmotic Exchange Measurements The first question that must be decided, in order that osmotic exchange data may be applied to the growth problem, is whether water enters the coleoptile cylinder mainly through its ends or its sides. Philip (11) concluded that deuterium-labeled water diffuses into and out of oat coleoptile cylinders mainly through the ends. This was based on the finding that the time course for this diffusion was more closely in agreement with kinetics to be expected from sheets of tissue than from solid cylinders. This ignores the fact that oat coleoptiles are *hollow*

cylinders (with mean inner radius about 0.6 times mean outer radius), into which diffusion from the sides might be expected to follow kinetics more closely approximating those of sheets than of solid cylinders.

If water entered mainly through the ends, the half-time for osmotic exchanges should vary inversely as the square of the length of the cylinder (11), whereas if water entered mainly from the sides the half-time should be independent of cylinder length. Therefore, we measured the swelling of a cylinder by the evaporation-immersion method and then cut the cylinder in half transversely and repeated the measurement on each half.

TABLE III

EFFECT OF CYLINDER LENGTH ON HALF-TIME FOR OSMOTIC EXCHANGES

Cylinder No.	Entire cylinder		Apical half		Basal half	
	Length	t_{50}	Length	t_{50}	Length	t_{50}
	mm	*min.*	*mm*	*min.*	*mm*	*min.*
			Evaporation-immersion method			
1	6.5	0.65	3.2	0.70	2.8	0.5
2	6.4	1.05	3.0	0.55	3.0	0.90
3	7.0	1.10	3.4	1.45	3.2	1.10
Mean	6.6	0.93	3.2	0.90	3.0	0.83
			*Mannitol method**			
4	4.9	6.5	2.6	6.0	—	—
5	5.8	6.1	2.8	4.75	2.9	5.7
6	5.8	4.75	2.5	5.5	—	—
Mean	5.5	5.8	2.6	5.4	—	—

* Mean for shrinking and swelling exchanges (increase and decrease in length) upon transfer from 0.2 to 0.3 *m* mannitol and *vice versa*.

Results with several cylinders are summarized in Table III. There was considerable variation in behavior, but the mean half-time for half-cylinders was only slightly less than that for entire cylinders, whereas less than one-quarter would be expected if all the water entered *via* the end. The data thus suggest that most of the absorbed water enters *via* the sides.

The halving experiment was performed also for changes in length induced osmotically by mannitol, before the deficiencies of mannitol, in the measurement of osmotic permeability, had become fully apparent. The results are recorded in the lower part of Table III because they also show relatively little effect of halving the cylinder on the half-time (the half-time for loss or absorption of water in this case presumably being governed, as explained above,

by the diffusion of mannitol into or out of the free space). This suggests that mannitol enters the free space principally *via* the sides of the cylinder. This supports the opinion advanced above regarding entry of water, since it does not seem possible that polyhydric mannitol could enter the free space by a route that water could not follow also.

We examined the route of entry question from another point of view. If water entered mainly the ends of the cylinder, this would be because its epidermis is impermeable. If the epidermis were removed, the half-time for osmotic exchange should be decreased in approximately the proportion $(n_t/n_l)^2$, where n_t is the mean number of intercellular membranes that would be traversed through the transverse thickness of the tissue, and n_l is the mean number to be traversed along its length (note that in our experiments entry is possible only from one end). By microscopic examination we found that coleoptiles have a mean thickness of about seven cells, whereas the internal parenchyma of a cylinder 6 mm long averages fifty-five cells in the longitudinal direction. Thus, removal of an impermeable epidermis should reduce the half-time by a factor of about $\frac{1}{60}$, provided transverse and longitudinal cell membranes are equally permeable.

Accordingly, osmotic exchange of an intact cylinder was first measured twice by the evaporation-immersion method, half-times of 1.7 and 2.5 min. being found in the two trials. Then the cylinder, still attached to the mounting needle, was viewed through a dissecting microscope and with fine forceps the epidermis was carefully stripped off as completely as possible all the way around the cylinder down to the tie thread. Two osmotic exchange measurements on the stripped cylinder were then made, giving half-times of 0.8 and 0.9 min. Similar experiments were performed with mannitol-induced osmotic exchanges and gave half-times of 7.0 min. unstripped and 2.2 min. stripped in one case, 7.5 min. unstripped and 3.0 min. stripped in another case. These data indicate that the epidermis is not impermeable, and suggest again that the bulk of the absorbed water enters through the sides.

Another point in this connection may be mentioned. The *minimum* half-time for diffusion of mannitol into or out of the free space of a coleoptile cylinder *via* the ends can be computed (11) as $0.0475\ l^2/D$, from the diffusion coefficient of mannitol D (this assumes that diffusion within the free space is unrestricted) and the length of the cylinder l (this assumes that the pathway of diffusion within the free space is not tortuous). The diffusion coefficient of mannitol is about 0.68×10^{-5} cm^2 sec.$^{-1}$; thus the half-time for a cylinder 8 mm long should be at least 70 min. if mannitol could exchange only *via* the ends. Since the observed half-time is about one-tenth this value, it is evident that mannitol (hence presumably also water) must exchange mainly *via* the sides.

We consider this to be fortunate, since the problem of relating osmotic measurements to elongation rate is much less difficult for lateral entry of water

than for entry from the ends, due to involvement, in the latter case, of the dependence of both water potential and elongation rate on turgor pressure. For lateral entry the next question that must be considered is how the resistance to water movement is distributed through the thickness of the tissue, and to what extent this will influence the comparison between osmotic exchanges and elongation rate.

The most extreme possibilities that seem to need consideration are (*a*) that resistance is uniformly distributed, and (*b*) that resistance is principally at the outside surface (at the epidermis); hence we investigated the problem theoretically in terms of these cases. Space precludes a detailed presentation of the analysis, but it was found that the practical results that were sought from the measurements would be relatively little affected by such differences in distribution of resistance, even though calculation of permeability itself would be strongly influenced. This is because the water potential gradients that should develop within the tissue if resistance is distributed internally, are similar in form in the case of osmotic exchange and of steady elongation, though not quite identical. Specifically, the mean water potential difference between tissue and medium required to support a given elongation rate would be 17 per cent less if computed from the half-time for osmotic exchange on the assumption that resistance is external, than if computed on the basis of uniformly distributed resistance.

The fact that removal of the epidermis sharply reduced the half-time for osmotic exchange suggests that resistance to entry of water into intact cylinders is principally at the outer surface. Since, in addition, treatment of the data on this basis is simplest and affords a minimum estimate of the water potential differences involved in elongation, we assume external resistance in the following discussion.

Unfortunately, it is not possible to use the half-time for osmotic exchange in evaporation-immersion experiments to compute the permeability directly as in formulae given by Philip (9, 11), because such formulae assume an invariant elastic modulus of the cell, whereas the elastic modulus is observed to vary conspicuously over the range of turgor pressures involved in the expansion of the cylinder during the osmotic exchange measurement (the elastic behavior of the oat coleoptile will be discussed in detail elsewhere). The method we used in evaluating the evaporation-immersion data was a graphical one, that is strictly valid only if the resistance is principally external.

At the conclusion of the osmotic swelling in 10^{-3} M KCN, the cylinder was transferred successively to 0.1, 0.2, 0.3, and 0.4 *m* mannitol, as illustrated in Fig. 3, each time allowing the cylinder to shrink until it reached equilibrium. We thus determined the relation between water potential of the tissue and elastic change in length. We then know the water potential of the tissue at certain specific points during the swelling after evaporation, namely, when the

cylinder was less than the final (water equilibrium) length by amounts corresponding to equilibrium with the given mannitol solutions. The slope of the expansion curve at each point, divided by the respective water potential difference, gives what we shall call the effective permeability P_e (microns per min. elastic change in length, per atm of water potential difference).

The effective permeability was found in this way for as many points as possible on each swelling curve, for the cylinders listed in Table II. In general the values for different points were fairly similar for a given cylinder, although they varied considerably between cylinders. There was a tendency for the values to be noticeably lower for water potentials corresponding to 0.1 *m* mannitol. Actually it is to be expected that results will be poorest for this point, because it is closest to the final (water equilibrium) length, and this latter point must be decided somewhat arbitrarily. This is because, as previously noted and as illustrated in Fig. 3, the cylinders do not *completely* stop increasing in length when the osmotic expansion is over, but continue to elongate at a slow steady rate even though KCN is present. We chose the water equilibrium length as that point at which a steady minimum rate of increase in length was first attained; this rate is so slow that one can certainly be confident that the cells are at this time indeed essentially in osmotic equilibrium with the medium.

For further computations, we ignored the permeability value corresponding to 0.1 *m* mannitol and averaged the effective permeability values for each cylinder that were found from the remaining points; we show this mean as P_e in Table II.

To compute the water potential difference $\Delta\mu_g$ required for elongation (growth) we take into account the fact that the relation between change in length and change in volume is different for elastic changes in length (in which P_e is measured) as compared with elongation, by using the factors ψ and ϕ explained in Methods. Then, if v is elongation rate in microns per minute,

$$\Delta\mu_g = \frac{\phi v}{\psi P_e} = \frac{0.77v}{P_e} \qquad (1)$$

The water potential differences found for elongation by this method (last column in Table II) are smaller by a factor of 2-4 than those indicated by either the equilibrium method or the measurement of osmotic exchanges using mannitol, and lie between about 10 and 25 per cent of the osmotic pressure of the cells for rapidly elongating cylinders. Thus it appears that coleoptile cells are indeed relatively close to osmotic equilibrium during rapid elongation, although the departure from equilibrium is by no means insignificant.

DISCUSSION

We wish first to offer some explanation for the fact that the values for water potential of growing coleoptile tissue estimated by the conventional equilibrium method were, like the results obtained from osmotic exchange measurements involving mannitol, much larger (more negative) than when the evaporation-immersion method was used to estimate permeability.

It is evident from the type of behavior that is seen when growing tissue is transferred to a solution containing a solute (Fig. 1) that, in applying the equilibrium method, the water potential can be judged only from the behavior of the tissue *immediately* after transfer to the solution, for thereafter the occurrence of processes of elongation reduces or reverses the initial response, causing the water potential difference to be judged even larger than when the initial response is used. Considering the time course for diffusional exchange of mannitol with the free space, the initial action of a mannitol solution must be exerted on the external surface of the cylinder rather than on the cellular membranes, except perhaps at the ends of the cylinder. It seems clear, from the data presented, that the external surface is to a considerable degree permeable to mannitol. Therefore, the osmotic action of a mannitol solution at the external surface must be less than the osmotic pressure of the solution, according to basic principles (4, 5, 13).

It is not possible to predict theoretically, from available information, how much this reduction in osmotic action should be. But we assume that the effective osmotic action should be approximately as much less than the osmotic pressure, as the half-time for osmotic exchanges involving mannitol is greater than the half-time for swelling in the evaporation-immersion experiment. We base this on the expectation that these half-times should go roughly in inverse proportion to the initial rates of water transfer, in the two types of experiment, under comparable water potential differences. Since these half-times differed by a factor of about 3, the effective initial osmotic action of a mannitol solution should be about one-third its osmotic pressure. This reduces the water potential values of growing cylinders estimated from the equilibrium method to between -1 and -1.5 atm, like those found using the evaporation-immersion method.

It has been shown by Ordin, Applewhite, and Bonner (6), and in unpublished experiments of our own, that over a time scale considerably longer than the present experiments the membranes of coleoptile cells are essentially impermeable to mannitol. Therefore, as the mannitol concentration in the free space builds up to equal that of the external solution it will exert its full osmotic effect on the cells, and their turgor pressures must change so as to satisfy the simple laws of osmotic equilibrium. We depend, of course, upon this principle in arriving at a permeability value in the evaporation-immersion experiment.

It is of interest to consider the actual water permeability of the coleoptile

cylinder on the basis of the effective permeability obtained from the measurements. If, as we concluded, resistance to water uptake is mainly external, the permeability of the external surface in microns $atm^{-1}hr.^{-1}$ may be found as 60 $P_e\psi V/lA$, where V is volume of the tissue, l is length of the cylinder, and A is area of the external surface. V/l is essentially equal to the weight per unit length, which is 0.75 mg/mm from a number of determinations. A equals l times the external perimeter of cross-section, which we determined by measurement to be 4.2 mm. Hence permeability is 15.3 P_e/l (with l in millimeters).

The P_e values in Table II lead to permeabilities between 7 and 30 microns $atm^{-1}hr.^{-1}$ These fall within the range of values for water permeability of plant cells listed by Bennet-Clark (3). Since the penetration of water actually

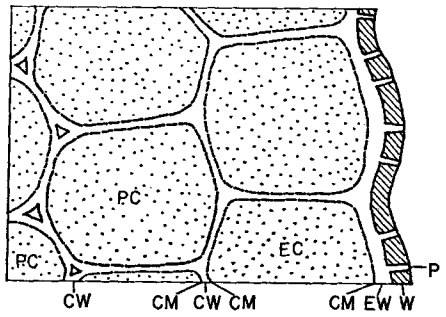

FIGURE 4. Diagram of coleoptile tissue as seen in transverse section, including features inferred from water transport experiments. *EC*, cell of external epidermis. *PC*, parenchyma cell. *EW*, outer wall of epidermis, including an external layer of wax *W* traversed by relatively large aqueous channels (pores) *P*. *CW*, cell walls of internal cells, taken with EW to constitute the mannitol-free space. *CM*, cell membranes, shown traversed by numerous narrow pores. Inner space (cell contents) is stippled area. The widths of presumed pores in *CM* and *W* are both greatly exaggerated relative to cell dimensions.

involves a number of cell layers that individually may have higher permeability than what is computed for the external surface on the assumption that all resistance is localized there, the correspondence between the latter and typical cellular permeabilities suggests that the effective permeability has not been underestimated and that the computed $\Delta\mu_g$ values are minimum estimates.

Since there may appear to be some contradiction between the conclusions (*a*) that mannitol can exert its full osmotic effect only on the cell membranes, and (*b*) that the principal resistance to water uptake is not at the cell membranes but at the external surface, we give in Fig. 4 a diagram of the structure of part of an oat coleoptile, in transverse section, that helps at least qualitatively to make understandable the observed water transport behavior.

The reduced osmotic effect of mannitol at the external surface can be ex-

plained by assuming that the aqueous channels or pores by which water can penetrate the waxy layers of the outer epidermal cell wall are large enough that mannitol also penetrates without much restriction. The pores in the cell membranes must, on the other hand, be narrow enough to exclude mannitol, so it exerts its full osmotic effect there.

The resistance to water flow should be proportional to the thickness of the membrane (*i.e.*, to the length of the pores), and inversely proportional to the total number of pores and to the square or a higher power of their diameter. Thus even though the cell membrane pores are considerably smaller in diameter, this may be outweighed, as illustrated in Fig. 4, by a much greater number of pores in the cell membranes, and by the fact that the outer epidermal wall is much thicker (about 4 microns) than a cell membrane (<0.1 micron). In this event the principal resistance to water flow will be at the outer cell wall of the epidermis, and the rate of water uptake by the tissue from a dilute medium will not be governed by the permeability of the cell membranes.

The outcome of this investigation lends support to Dainty's (4) criticisms of the efforts of one of us (13) to use data from the literature, cited above in the Introduction, to estimate the ratio of water diffusion permeability to osmotic permeability of oat coleoptile cylinders.

In view of the relatively meager information on water permeability of plant cell membranes compared with the importance of this quantity, as brought out in the discussion by Dainty (4), it would be of interest to try to compute cell membrane permeability using the *stripped* coleoptile cylinder, for which the rate of water uptake may well be governed by permeability of cell membranes. Unfortunately, the results depend drastically upon whether water passes into the interior of the tissue mainly by moving osmotically from cell to cell, or mainly around the cells *via* the free space (in the former case permeability of cell membranes would be around 250 microns $atm^{-1}hr.^{-1}$, while in the latter case, around 5 microns $atm^{-1}hr.^{-1}$). A direct test of the penetration question is needed, and therefore we leave this matter open.

This investigation was supported by National Science Foundation Grant G-8705, and was conducted in part during the tenure of a National Science Foundation Research Participation Fellowship by A. W. R.

We thank Dr. J. Dainty for sending us copy of a manuscript in advance of publication.

Received for publication, March 8, 1963.

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