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Evidence for Active Water Transport in a Corn Root Preparation

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Summary. Water flow was measured in a *Zea mays* root preparation consisting of a segment from which the central part had been excised. It was shown that water flow had two components, one osmotic and one non-osmotic. The non-osmotic flow was inhibited by cyanide. No correlation was found between water flow and solute flow. These findings suggest that active water transport occurred in the root preparation. The mechanism of such water movement is discussed.

In a paper to be published [6], we have described experiments on water flow in a root preparation consisting of segments from which the central stele had been excised ("sleeves"). The driving force for water flow was a given osmotic pressure difference ($\Delta\pi$). It was found that the following equation

$$J_v = -\sigma Lp \Delta\pi, \tag{1}$$

where J_v = volume flow, σ = reflection coefficient, and Lp = hydraulic coefficient, was not adequate to describe the results. Another term, J_v^*, was added:

$$J_v = -\sigma Lp \Delta\pi + J_v^*. \tag{2}$$

This equation is of the same form as that given by House and Findley [8, 9], Anderson [1], and Anderson and Reilly [2] to describe root pressure exudation in detopped root preparations. J_v^* is that fraction of the water flow which is not driven by an overall osmotic pressure difference ($\Delta\pi$); it represents non-osmotic water flow. The phenomenon of J_v^* occurs during secretion in many animal epithelia such as the kidney, toad bladder, intestine and gallbladder [4]. In all those tissues, it was found that a linear relation is obtained on plotting water flow as a function of Na or NaCl flow; at zero net solute flow, the water flow vanishes. It was concluded that water flow is passive in nature and that water moves along with the

flow of solutes. J_v^* cannot be explained by a homogeneous membran system: several models have been suggested to explain this type of flo on a structural basis. An example is the standing gradient model propose by Diamond and Bossert [5].

In contrast, in the detopped root system used by House and Findley [8, 9 by Anderson [1, 2], and in our own root preparation, the concentratio of the solution in the xylem was always much higher than that of the solutio outside, and it was assumed that water flow is secondary to solute flow More detailed analysis, however, suggested that water flow was partl driven by $\Delta\pi$ and was partly non-osmotic (J_v^*).

By using the formalism of non-equilibrium thermodynamics, Kedem [11 has written the following phenomenological equation:

$$J_v = \frac{\overline{V}_w^2 \Delta\pi}{R_{ww}} + \frac{\overline{V}_w}{R_{ww}} \sum R_{ws} J_s + \frac{\overline{V}_w}{R_{ww}} \sum R_{wr} J_r \qquad (3$$

where $\overline{V}_w$ = partial molar volume of water; J_s = solute flow; J_r = flow o chemical reactions; R_{wj} = phenomenological coefficients; and w, s, r = sub scripts for water, solute, and chemical reactions, respectively. $R_{wr} \neq 0$ implies that there is active transport of water. J_v^* is equivalent to the sun of the second and third terms in Eq. (3).

As mentioned before, it is generally accepted that $R_{wr} = 0$, i.e., tha there is no direct coupling between chemical reactions and water flow Active transport of water, as thus defined, has not been shown in biologica systems. Experiments have been done, however, to show that active wate transport occurs in insects [3]. In Eq. (3), if $R_{wr} = 0$, J_v^* could well b equated to the second term which represents that part of J_v coupled to J_s

Fig. 1 is a schematic cross-section of the primary root of *Zea mays* The cortex comprises several layers of large thin-walled cells (parenchyma bounded externally by a one-layered epidermis. The endodermis, whic lies at the border between the conducting tissues and the cortex, is alsc a single layer of cells which at maturity have a U-shaped type of thickenin with reinforced end, radial and inner tangential walls. The conductin tissues, consisting of xylem vessels, phloem tubes and parenchymatous cells, are removed in our preparation.

In studying radial movement of matter through roots, either whole or detopped, the far side of the system (i.e., the solution in the xylem tubes) is not available to the experimenter. Even detopped roots suffer from a disadvantage in that variations in parameters such as concentration, electrical potential and hydrostatic pressure can occur along the longitudinal axis.

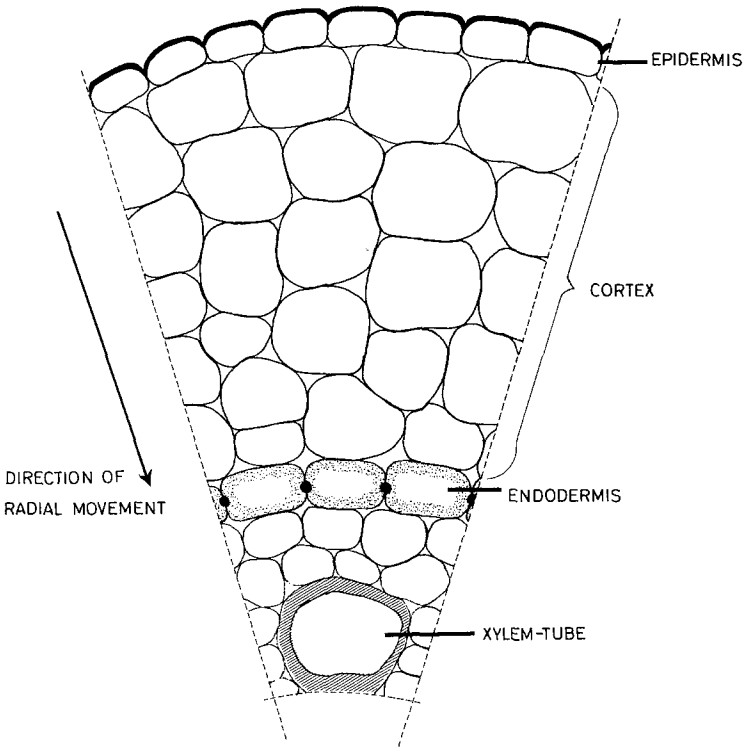

Fig. 1. Schematic cross-section of primary root of *Zea mays* seedling

We have worked on a preparation in which both faces of the system are under experimental control, and the solutions in touch with them are homogeneous. The preparation was made by pulling the stele out of a segment of corn root, leaving a hollow cylinder or a "sleeve". The inner diameter of the hollow part is 0.5 to 0.6 mm as compared with 0.01 to 0.03 mm in xylem tubes [6]. It is possible to flush solutions of known concentrations and electrical potential along both the outside and inner surfaces of the preparation.

In a following paper, the meaning of J_v^* is analyzed in our root system, to find out whether it is coupled to J_s, or whether grounds exist for the postulation of active water transport.

Methods

Seeds of *Zea mays* (var. Horse-tooth, local variety-Hazera) were soaked for 30 sec in 0.25% $HgCl_2$ solution. The seeds were rinsed for 1 hr in tap water, and then in deionized water. They were left to imbibe aerated deionized water for 24 hr. The seeds were

germinated between layers of damp filter paper laid on stainless steel mesh within plastic box. The filter paper was kept moist by dipping the edges into water. After this the seeds were kept in an incubator at 27 °C and illuminated for 12 hr each day. After 3 days, primary roots of 10 to 12 cm in length were obtained.

Preparation of "Sleeves"

A sleeve preparation consists of the epidermis, exodermis, cortex and the outer part of the broken endodermis [6]. It was prepared in the following manner. At a distance of 8 to 9 cm from the root tip, the root was bent until the cortex was disrupted. The stele was gently pulled out, breaking the endodermis, and thus became detached from the cortex at about 0.5 cm from the root tip. In some experiments, this preparation was used directly; it consisted of a hollow cylinder plugged at one end by the intact apex and is referred to as a "plugged sleeve". Alternatively, a "sleeve" was obtained from the "plugged sleeve" by cutting a suitable segment from it with a sharp razor blade. Very narrow polyethylene tubes were sealed to the two ends of the "sleeve". Details of the measurement of osmotic flow are described in a future paper [6]. In general, an osmotic pressure difference owing to the presence of solution bathing the sleeve was imposed across the "sleeve" preparation, and the resulting osmotic flow was measured.

A special lucite chamber was constructed for measuring osmotic flow. The water movement was measured by following the movement of the meniscus in precision-bore capillary tubing of 0.5 mm inner diameter attached to the closed chamber. The movement of the meniscus was observed with the aid of a travelling microscope which could read changes in volume as small as 5×10^{-7} cm^3. The polyethylene tubing of one side of the sleeve was attached to a perfusion pump (Unita I, Braun-B. Melsungen). The perfusion rate of the inner compartment was 6.8×10^{-3} cm^3/min, which was sufficient to change the content of the inner compartment at least once every minute. After mounting the preparation, a solution of 1×10^{-5} M $CaCl_2$ was introduced into both outside and inside compartments. About 15 min was allowed for the sleeve to equilibrate. The outer compartment was stirred by means of a magnetic bar. Depending on the plan of the experiment, the solution of either the inside or the outside compartment was changed to a solution of known osmotic pressure, and a reading of the meniscus was made after 20 min. Readings were taken every 30 to 60 sec for three successive periods of 4 to 10 min. This means that every point is a mean of not less than 30 readings. At the end of the experiments, the outer diameter of the sleeve was measured, and the volume (or water) flow) was expressed as cm^3 H_2O/sec $\times$ cm^2. The area refers to the outer surface. Osmotic pressures were determined cryoscopically by use of a semi-micro electronic osmometer (Knauer, type M), which gives values accurate to 1%. The values of $\Delta\pi$ were read from standard NaCl solutions.

The "plugged sleeves" were treated as follows: a narrow glass capillary was attached to the open end and sealed in position with hot vaseline. The plugged sleeve was fixed vertically into a Perspex holder. The whole preparation was immersed in a solution which included 10^{-5} M $CaCl_2$ and KCl at a given concentration. The solution was vigorously aerated, and kept at 23 ± 1 °C. Net movement of solution into the plugged sleeve was measured by observing the change of height of a water column within the capillary attached to the top of the plugged sleeve. These experiments usually lasted 45 to 70 hr. At the end of the experimental period, the solution in the capillary was transferred carefully into a weighing bottle, weighed and diluted for K^+ analysis. K^+ was measured with an Eppendorf flame-photometer. The outer surface of the roots was calculated from measurements of the average diameter and length, to an accuracy of ± 2%. Several attempts were made to seal the lower part of a sleeve, to determine if water and solute flows differed from those measured in plugged sleeves, and hence gauge the effect of the

root tip. It was difficult to make a successful seal capable of lasting for the duration of the experiment inside a stirred solution. The few successful preparations showed that root tips did not contribute significantly to the flow into the plugged sleeve. We have shown that the root preparation immersed in 10^{-5} M $CaCl_2$ behaved as if at a quasi-stationary state, when fluxes of ions or water were measured throughout a period of at least 50 to 70 hr.

Results

Water Flow as a Function of Osmotic Pressure Difference

Fig. 2 presents a series of measurements of water flow (measured as volume flow J_v) when an osmotic pressure difference ($\Delta\pi$) is imposed across the sleeve system. The bathing solutions were either sucrose or polyethylene glycol (PEG) with a molecular weight of 2,000. The line of linear regression for both solutes gives practically the same slope and intercept on the J_v axis. The line of regression was obtained from a series of measurements on more than 30 roots. It can be seen that the variability of the root population with respect to osmotic flow is very large. The great variability occurs partly because of the preparative procedure, but also because of the inherent inhomogeneity of the root population. When a similar line of regression is

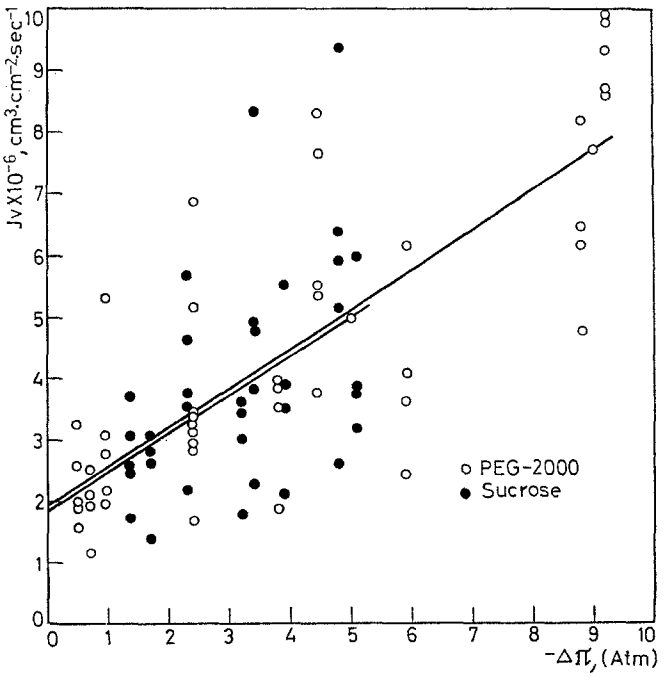

Fig. 2. Volume flow J_v as a function of osmotic pressure difference $\Delta\pi$. Line drawn by least-squares method. • sucrose; ○ PEG. Sleeves used for experiments. For experimental details, *see* text

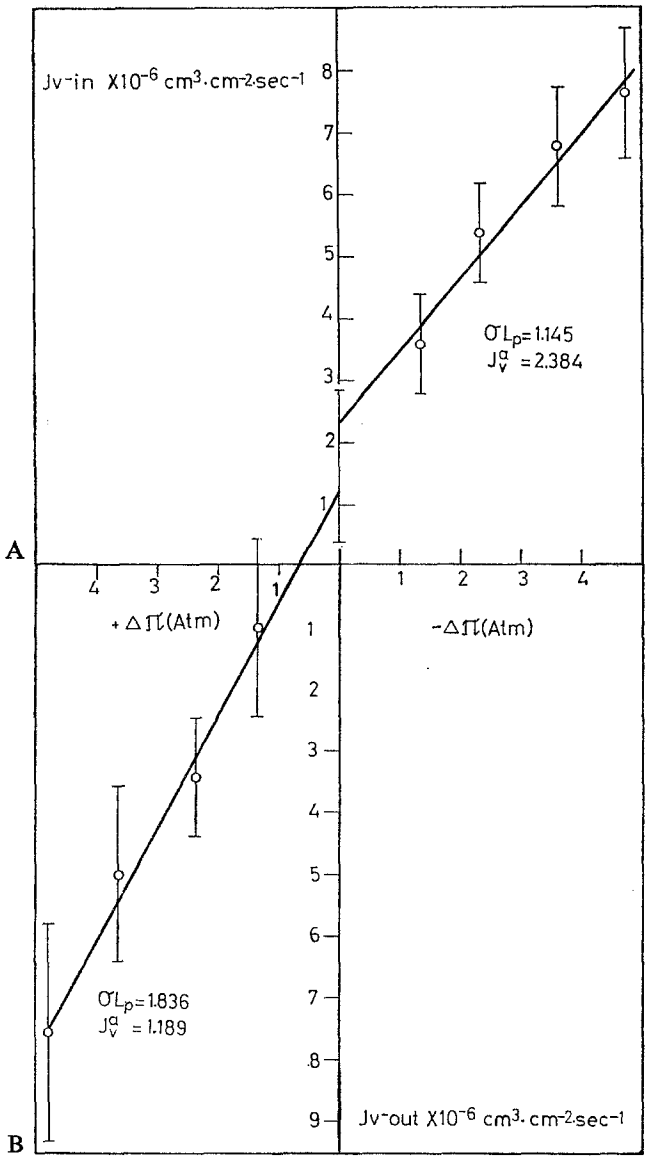

Fig. 3A and B. Volume flow J_v as a function of osmotic pressure difference $\Delta\pi$. (A) Inward flow; (B) outward flow. Sucrose solutions used throughout. Lines drawn by least-squares method. Sleeves used for experiments. For experimental details, *see* text

done on the results from single roots, it was found that the regression coefficient was very near 1.

For the lines drawn in Fig. 2, the regression coefficient (r) for PEG is 0.791 and for sucrose 0.468. Even so, the intercept on the J_v axis is shown

to be very significantly different from zero (for PEG, $t=10.73$ and $P<0.01$; for sucrose, $t=6.6$ and $P<0.01$).

It should also be noted, that when J_v vs. $\Delta\pi$ was plotted for each individual root, there was always a positive intercept when the line was extrapolated to $\Delta\pi=0$. That is another test for the statistical significance of the intercept of J_v ($\Delta\pi=0$). In Figs. 3, 4 and 5, the same plot and statistical tests were made as was described for Fig. 2. From Eq. (2), the slope of the line represents the hydraulic conductivity (Lp) multiplied by the reflection coefficient (σ). The fact that the slope is equal for both solutes, despite the sixfold difference in molecular weight of the solutes, suggests that a value of 1 be assigned to σ; the slope thus yields the value of Lp. Similar values for σ were found by another method for the same preparation [6].

Fig. 3 shows the same kind of measurement as discussed above, but with flows directed either inward or outward. These experiments were performed by changing the direction of $\Delta\pi$. The figure shows that there was no statistical difference between the values for the two intercepts for either inward or outward flow. The slopes, however, are different; the ratio of the slopes is approximately equal to the ratio of the inner and outer surface areas of the "sleeve". Since J_v represents water flow per unit area, one would expect the slopes to be proportional to the total area of each conducting surface, provided that $\sigma=1$.

Effect of Metabolic Inhibitors

Should the intercept (J_v^*) represent active water movement, we might expect to reduce its value by using metabolic inhibitors.

Fig. 4 shows the effects on water flow of two metabolic inhibitors, KCN and 2,4-dinitrophenol (DNP). Both parameters, J_v^* and Lp, were reduced by the inhibitors, except that J_v^* was not reduced significantly by DNP. These results are necessary but not sufficient conditions to prove that $R_{wr}\neq 0$ in Eq. (3), i.e., that J_v is coupled directly to metabolism, since J_v could be affected indirectly through a reduction of J_s. Such effect has been demonstrated amply many times before.

Connection Between Solute Flow and Volume Flow

"Plugged sleeves" were used for these experiments. They were allowed to come to a quasi-steady state lasting for 45 to 50 hr (Fig. 5). In this system, the $\Delta\pi$ was allowed to establish itself.

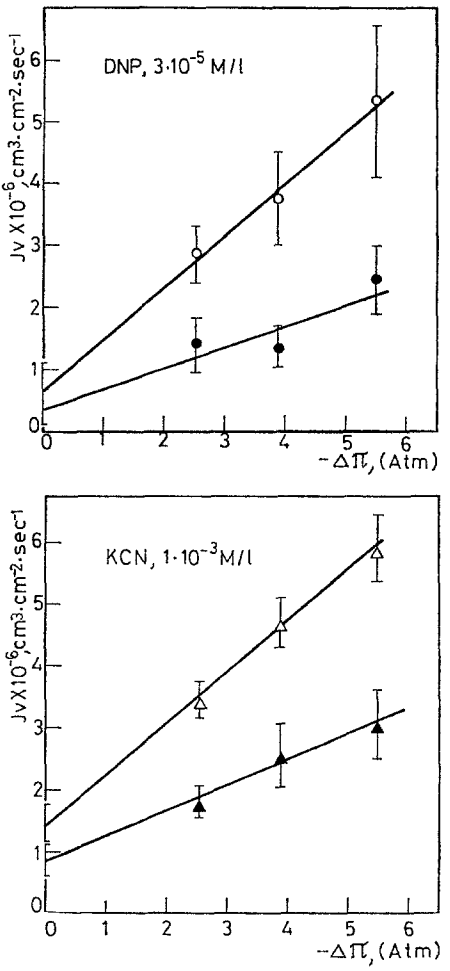

Fig. 4. Effect of DNP and KCN on J_v as a function of $\Delta\pi$. ○, △ controls; • with 3×10^{-5} M DNP; ▲ with 1×10^{-3} M KCN. "Sleeves" used for experiments. Lines drawn by least-squares method. For experimental details, *see* text

Fig. 5 shows that J_v was constant for about 45 to 50 hr. Because the amount of solute was small, J_s was estimated only at the end of the experiment, on the assumption that its rate of flow was also constant.

In Fig. 6, J_v is plotted as a function of J_s, as indicated by K^+ flow. The values of J_s at each point are means related to a given concentration of KCl in the outer solution. The use of this kind of grouping is performed so as to decrease the error of estimate of the parameter J_s which is not necessarily independent. The linear regression of the average values gives

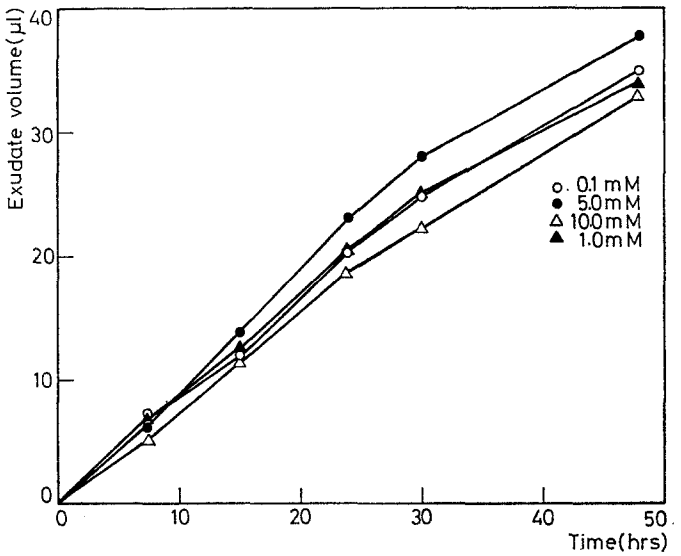

Fig. 5. Exudation volume as function of time in "plugged sleeves". Figures refer to KCl concentration in bathing solution

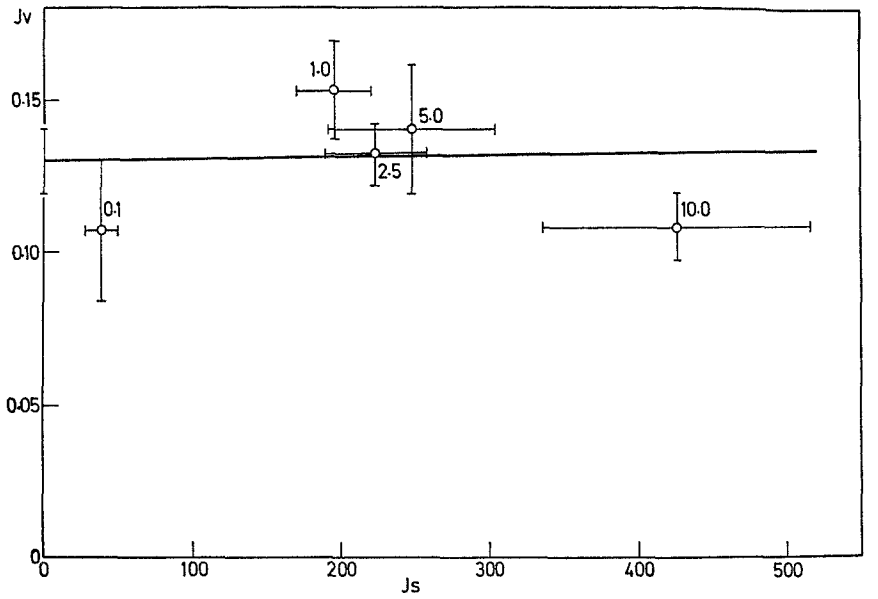

Fig. 6. Volume flow J_v as function of solute flow J_s in "plugged sleeves". Each group refers to a given outside KCl concentration (indicated near points). Bars indicate SEM

a line almost parallel to the J_s axis. The intercept thus obtained on the J_v axis has been found to be highly significantly different from zero. The points near to it represent a condition where $\Delta\pi$ is almost equal to zero

Table. *Volume flow (J_v), solute flow (J_s) and the concentration difference ($\Delta\pi$) as functio of the outer concentration (C_0) in "plugged sleeves"* [a]

C_0 (mM)	J_v ($\times 10^{-7}$ cm^3/ cm$^2 \times$ sec)	J_s ($\times 10^{-14}$ moles/ cm$^2 \times$ sec)	C_i (mM)	$C_i - C_0$ (mM)	No. of repli cates
0.01	1.40 ± 0.26	8.1 ± 1.76	0.75 ± 0.23	0.74	10
0.1	1.07 ± 0.23	3.9 ± 1.21	0.79 ± 0.12	0.69	39
1.0	1.53 ± 0.16	19.4 ± 2.45	1.70 ± 0.25	0.70	60
2.5	1.32 ± 0.10	22.2 ± 3.46	2.04 ± 0.50	-0.46	12
5.0	1.40 ± 0.21	24.7 ± 5.68	2.05 ± 0.44	-2.95	16
10.0	1.08 ± 0.11	42.6 ± 9.1	4.25 ± 0.55	-5.75	33

[a] "Plugged sleeves" from roots of *Zea mays* were immersed for 70 hr in KCl solution containing 10^{-5} M $CaCl_2$ at 23 ± 1 °C. The solution was aerated. Volume flow readings were taken every hour for several hours. The solute (K^+) was analyzed at the end of the experiment.

(Table). Thus it can be concluded that the volume flow at $J_s = 0$ does not vanish and that there is a large fraction of the total J_v independent of J_s.

The Table shows the relation of concentration difference between the inside of the "plugged sleeve" and the outer solution. Even though the concentration difference and, therefore, $\Delta\pi$ are very small, yet there is a significant flow of water across the root. When the outside concentration was above 2.5 mM, the inside solution was considerably more dilute, and a hypotonic solution was created within the cavity of the plugged sleeve. However, J_v remained unchanged. Thus the table demonstrates that J_v is independent of $\Delta\pi$ at small values of $\Delta\pi$.

Discussion

The various water-flow phenomena observed in the root preparation used were unexpected and remarkable. As mentioned before [Eq. (1)]:

$$J_v = \sigma L p \Delta\pi + J_v^*$$

describes water flow in our root preparation. There are two terms in this equation; the first is an expression of a linear dependence of the volume flow on the osmotic pressure difference ($\Delta\pi$), and the second of non-osmotic water flow (J_v^*). Our aim has been to analyze this J_v^*. On comparing

Eq. (3) with Eq. (2), it is possible to write:

$$J_v^* = \frac{\bar{V}_w \Delta\pi}{R_{ww}} \sum R_{ws} J_s + \frac{\bar{V}_w}{R_{ww}} \sum R_{wr} J_r . \tag{4}$$

Eq. (4) could include other terms, should other forces be coupled to the volume flow.

We have seen that J_v^* was reduced when the root was subjected to the influence of KCN (Fig. 4). This could be caused by coupling between J_v and either J_s (the solute flow) or J_r (the flow of chemical reactions). The existence of this latter coupling would imply active water transport.

In favor of a coupling between J_v and J_s is the observation that Cl^- influx was reduced by about 70% in root "sleeves" treated with KCN [7]. This inhibition might have been the cause of the inhibition of J_v^* which was found under these circumstances. A coupling between J_v and J_s has been found in many secreting tissues or organs [4].

On the other hand, there are strong grounds for holding that, in our preparation, J_v and J_s are not coupled. The major piece of evidence in favor of this contention is that J_v was almost invariant over a wide range of J_s. It is unlikely that even a small fraction of the total J_s (say 10 to 20%) be correlated with J_v since one solute molecule would then have to drag 10^5 to 10^6 water molecules. The non-coupling of J_v and J_s is further supported by the observation that the J_v^* of whole roots and of plugged sleeves were similar, although J_s across the whole roots was 20 times larger than across the "plugged sleeves".

It can of course be argued that since measured net J_s is a mean value along the whole root, a fraction of the secreted solute could be reabsorbed at different regions of the inner surface and cause underestimation in the value of J_s. The data show that reabsorption is unlikely to occur; in the Table it is shown that, in some cases, the concentration of solutes was higher in the outside solution than in the inside compartment. Thus, the postulation of any coupling of water and solute flows while reabsorption was occurring would involve the simultaneous *inward* and *outward* pumping of ions. In fact, our own measurements of ion fluxes in this preparation [7] have revealed the existence of inward ion pumps only. It is concluded that the occurrence of much ion reabsorption is improbable.

In any event, the occurrence or not of ion reabsorption does not affect the argument that there is no connection between J_s and J_v, since J_v was almost invariant throughout the measured range of J_s. Thus the second term in Eq. (4) cannot vanish and $R_{wr} \neq 0$, a definition of active water transport

according to Kedem [10, 11]. It is concluded that active water transport does occur in our root preparation.

It is emphasized that the conclusion in favor of active water transport is based on an analysis of the overall parameters of a complex system treated as a "black box", the individual components of which are unknown. It is not implied that active water transport need exist at a cellular level, but that the cells might be organized in such a way as to make active water transport possible. Many examples of biological systems are known in which the properties of an organ are more than, and different from, the properties of the constituent cells. It is argued by us elsewhere [6] in an analysis of the hydraulic conductivity *Lp* of the root that the behavior of a whole root system and of the single root cells may be different.

Since the value of *Lp* was lowered after treatment of the root with metabolic inhibitors (e.g., KCN, DNP), it is suggested that the movement of water across the normal untreated root was cooperatively directed. This could be a part of the protoplasmic flow (cyclosis) said to occur in roots and, if so, could serve as the mechanism of active water transport.

Any mechanism of active transport consists of at least two steps, namely the loading of the transported species and its discharging at the other side of the system. In the active transport of ions, the loading step is postulated to involve ion binding, and the discharging is performed by a reversal of the binding step. It seems highly unnecessary to postulate the binding of water, so abundant in every system. Fortunately, the discharging of water is a well-known phenomenon, of which cell shrinkage is an example. One can thus envisage water reaching the endodermis or xylem tubes to be discharged by a shrinking or squeezing of the gel part of the cytoplasm in the neighborhood of the plasmadesmata.

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