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Electro-Osmotic and Biopotential Measurements on Phloem Strands of *Nymphoides*

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Summary. Electroosmotic measurements on the excised vascular strand from the petiole of the water plant *Nymphoides peltatum* have been carried out, and the Onsager coefficients obtained. About 120 water molecules per ion are carried electroosmotically when the strand is in 10^{-4} M KCl, about 30 in 10^{-1} M KCl. Potential measurements made on an intact and functioning petiole are discussed in the light of the pressure-flow and electroosmotic theories of translocation.

Introduction

The electro-osmotic theory of phloem transport suggested by the present authors (Fensom, 1957; Spanner, 1958) has received little serious attention from plant physiologists, in spite of the fact that electrokinetic phenomena have been repeatedly demonstrated in plants (Fensom, 1958, 1962, 1963; Fensom and Dainty, 1963; Fensom, Meylan and Pilet, 1965; Fensom, Ursino and Nelson, 1967); and that the application of irreversible thermodynamics has provided new ways of analysing transport phenomena, including electroosmosis. It seems likely that the present impasse in our understanding of phloem mechanism will only be overcome by a more complete and minute analysis in quantitative terms of the transport process itself, and any attempt to do this, however great the obstacles in its way, is therefore worthwhile. That is the justification we believe for the present attempt which is recognised as exploratory, inconclusive about mechanism, yet an advance both in technique and theory.

The experimental work was undertaken on the water plant *Nymphoides peltatum* (Gmel.) O. Kunze, whose long uniform petioles, of simple anatomy, terminate in a floating leaf like that of a small water lily. This was chosen because of its availability, and because it has been the subject of physiological work (Spanner and Prebble, 1962) and electron microscopical investigation (Mehta and Spanner, 1962; Johnson, 1968, 1969). The central bundle is surrounded by air spaces and so is easily excised. It contains a substantial amount of xylem as well as phloem, and these tissues cannot be readily separated.

Methods

In the measurements of the Onsager coefficients a length of 18 mm of the central vascular strand was carefully dissected out and mounted in the vaselined split rubber bung of an electro-osmometer (FENSOM and DAINTY, 1963). The strand had a diameter of about 0.5 mm which included some of the ground tissue external to the endodermis. The cross section of the strand being small relative to the size of the electrodes (1 cm^2) no allowance was made for the resistance between the latter and the tissue. Both chambers of the apparatus were filled with a dilute solution of KCl, usually 10^{-4} molar. Potentials of 5 to 10 volts were applied to the Ag-AgCl electrodes and the flow rate measured by observation of the movement of an index bubble in a precision capillary of 0.2 mm diameter. Zero drift of the latter was allowed for, the waterbath in which the apparatus was immersed being at laboratory temperature and unthermostated.

Measurements of biopotentials along conducting bundles were made as follows. A potted plant was accomodated in a pail of water and the petiole of a vigorous leaf was run horizontally in a groove milled in a length of polymethacrylate. The lamina was enclosed in a chamber of similar material and kept well moistened. The uppermost segment of the horizontal petiole was carefully shaved off; this left the endodermis almost exposed and made the phloem readily accessible. Silver wire electrodes were sharpened to a fine point, chloridised and as far as possible inserted lightly into the phloem. Owing to twisting of the petiole however it was impossible to be sure of the orientation of the strand, and in any case the scale of the electrodes was hardly fine enough to discriminate accurately between xylem and phloem. A reference electrode terminating in a salt bridge was immersed in the root stock pail. The petiole between electrodes was covered with black polyethylene film. Potential measurements were made with a valve milli-voltmeter of input impedance 10^{12} ohms.

Results

Measurements on Excised Strands

The measurements of electroosmotic efficiencies (the meaning of this term is explained below) are shown in Table 1 and suggest the following remarks. Firstly, the flow was always towards the cathode and there was no evidence of any morphological polarity. In view of their similar mobilities in open solution it is reasonable to interpret the results as indicating that owing to a negative charge on the walls of the conducting channels more current was being carried by the K^+ ions than by Cl^- ions. The electroosmotic efficiency, or water movement per faraday under zero pressure gradient, was calculated on the approximation that all volume flow represents water electroosmotically conveyed, i.e. neglects the volume of the hydrated ions. Further, when the efficiency is interpreted in molecular terms (water molecules per K^+ ion) the implication is that all the current was carried by the cations. Strictly cation-selective membranes have channels of very small width (40 A in the case of the resin membrane of MACKAY and MEARES, 1959). The channels in the sieve plate pores, even allowing for the development of injury callose, are likely to be appreciably more than this, so that an

Table 1. *Electroosmotic measurements on excised vascular strands from petiole of Nymphoides*

No. of specimens	Current μamps.	Electro-osmotic efficiency	
		moles of water per faraday	cm^3 per coulomb
6	6.8	148 ± 22.7	27.6×10^{-3}
5	8.4	118 ± 7.3	22.0×10^{-3}
3	10.5	86 ± 16.5	16.1×10^{-3}
Mean	8.2	123 ± 11.8	23.0×10^{-3}

All measurements were made in 10^{-4}M KCl at 20—22° C. The specimens were of length 18 mm and approximate diameter 0.5 mm. Their mean resistance was 9.96×10^5 ohms, equivalent to a specific resistance of 1.18×10^3 ohm cm. The standard deviations in the third column refer to the means.

anionic component of the current must be expected. Disregard of this will result in an underestimate of the power of K^+ ions to drag water electroosmotically, but it is difficult to say what will be the extent of this underestimate.

Secondly, the transport of water per faraday was of the same order of magnitude as that found in *Nitella* (Fensom and Dainty, 1963; Fensom, Ursino and Nelson, 1967) but was rather greater than that found in *Lens* roots (Fensom, Meylan and Pilet, 1965). In *Nitella* the effect was independent of current over the range 3 to 60 $\mu A\ cm^{-2}$, though recent work (MacRobbie and Fensom, in press) indicates that at currents less than 0.3 $\mu A\ cm^{-2}$ the value can exceed 700 moles per faraday. It is possible from an examination of Table 1 that a similar tendency may occur here.

Table 2 shows the results of varying the concentration of KCl in the electroosmometer, and also of using a divalent anion instead of chloride. The electroosmotic efficiency clearly falls as the concentration rises; the single observation with potassium tartrate suggests no startling difference from chloride.

In Table 3 the data are recalculated in terms of Onsager coefficients. These are defined by the equations[1]

$$J = L_P \cdot \frac{\Delta P}{l} + L_X \cdot \frac{\Delta E}{l}, \tag{1}$$

$$I = L_X \cdot \frac{\Delta P}{l} + L_E \cdot \frac{\Delta E}{l} \tag{2}$$

1. In previous papers the Onsager coefficients have been written out in a fuller form, but are here shortened for convenience. Thus $L_X = L_{PE} = L_{EP}$, $L_P = L_{PP}$, $L_E = L_{EE}$, see Dainty, Croghan and Fensom (1963).

Table 2. *Effect of salt concentration on electroosmotic efficiency in excised vascular strands of Nymphoides*

Specimen	Salt	Concentration		
		10^{-4} molar	10^{-3} molar	10^{-1} molar
N 11	KCl	119	86	33
N 12	KCl	72	63	36
N 13	KCl	110	65	39
N 14	KCl	120	119	9
Mean	KCl	105	83*	29**
N 15	K tartrate	—	44	37

All values are in moles of water per faraday.

The value marked* is significantly different at the 5%, and that marked** at the 1% level from the preceding value in the same row.

where J is the volume flow per cm^2 in $cm^3 sec^{-1}$, I is the current density in amp. cm^{-2}, ΔP is the overall pressure difference in joules cm^{-3}, ΔE the difference of electric potential in volts and l the length of the specimen in cm. Of the Onsager coefficients L_P, as can be seen by writing $\Delta E = 0$, is the specific hydraulic conductivity; L_E (writing $\Delta P = 0$) is the specific electrical conductivity; and L_X is the electrokinetic cross coefficient. The electroosmotic efficiency, in the sense used here is

$$J/I = L_X/L_E, \quad (\Delta P = 0) \tag{3}$$

and can be calculated either directly or from L_X and L_E, given respectively by the relations

$$L_X = \frac{Jl}{\Delta E} \ (\Delta P = 0), \qquad L_E = \frac{Il}{\Delta E} \ (\Delta P = 0). \tag{4}$$

Biopotential Measurements on Intact Petioles

Bioelectric measurements along conducting petioles were undertaken because it was felt that under conditions of low enough transpiration the sign of the potential gradient might give information about the mechanism of phloem transport. Granted a negative zeta potential on the cell walls a pressure-flow mechanism would be expected to make the downstream direction positive.

Fig. 1 shows the results for a fairly typical experiment. The chloridised electrodes A, B and C were inserted into the exposed central strand under a hand lens, the petiole having previously been darkened for six hours. While the leaf was exposed to weak daylight a 10 min exposure

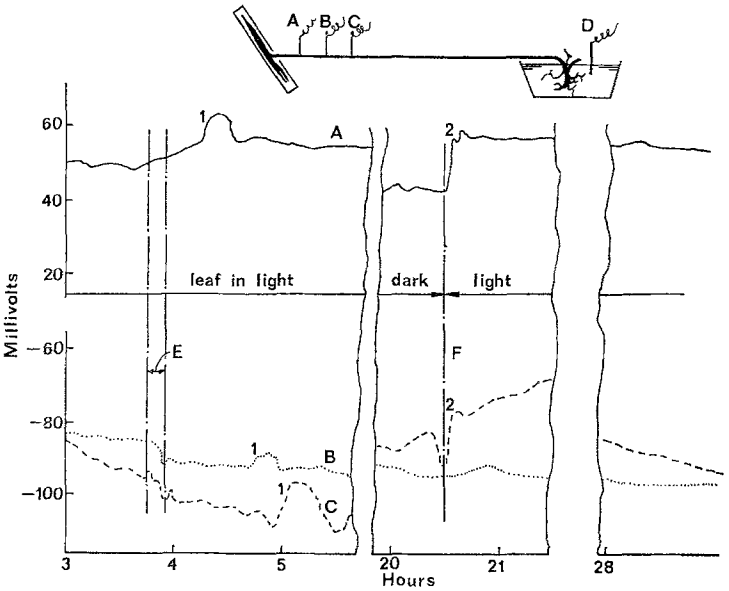

Fig. 1. Potential difference — time curves between electrodes *A*, *B* and *C* in the phloem of *Nymphoides* and reference salt bridge electrode *D*. The distances leaf —*A*, *A*—*B*, *B*—*C*, and *C*—*D* were 5,5,5 and 30 cm respectively. Petiole covered with black polythene, but leaf illuminated in the daylight at time 0. At *E* about 3000 lux additional illumination was added to the leaf for 10 min. The biopotential change possibly associated with *E* is marked 1—1—1. At *F* the leaf was again exposed to daylight after 14 hrs dark, on the following day. This time the associated potential changes seemed to occur on curves *A* and *C* at 2—2, but were not picked up on *B*

to additional light (*E*, 300 ft-candles) was given. The effects of this exposure seem to be reflected in the maxima marked 1 on the potential curves. These seem to indicate a disturbance passing down the petiole at about 12 to 14 cm hr^{-1}. After 14 hours in the dark the leaf was re-exposed to light at *F*. Both *A* and *C* became more positive; *B* strangely remained unaffected. The responses to illumination were fairly rapid, and if the maxima marked 2 correspond the velocity of propagation was of the order of 60 cm hr^{-1}.

Experiments on ten petioles were carried out, and while the pattern of potential changes was not always regular (the example quoted is a fairly representative one) it did seem to be the case that the electrode tended to become more positive on illumination. While this might indicate that a small electroosmotic contribution was acting to make basal electrodes more electropositive than apical in light, it cannot be regarded as a firm conclusion.

Discussion

The measurements of the Onsager coefficients made in the present work clearly leave much to be desired. For one thing the strands contain a large amount of xylem. Cortical parenchyma, endodermis and other cells are also present besides the sieve tubes in whose properties we are chiefly interested. Even with the latter there is the problem of callose formation resulting from the wound reaction, and of displacement of contents due to turgor release. While the association of qualitatively different channels in series and parallel arrangements does not render the use of the Onsager approach invalid (Kedem and Katchalsky, 1963) it does complicate the interpretation. All-in-all therefore we cannot be certain as to how closely these measurements represent the properties of functioning phloem. Though it seems unlikely that much of the flux of water or ions would traverse the parenchymatous cells, the presence of the xylem constitutes a real problem and so does manipulative damage to the phloem. It may even be that if damage to the terminating sieve tubes is extensive most of the current and fluid flow is along the xylem and the calculated figures really reflect the properties of the latter. These provisos must be borne in mind in interpreting the data, but in view of the great obstacles in the way of determining the flow properties of functioning sieve tubes it seemed worthwhile to publish the present results.

The data on biopotentials also need to be interpreted cautiously. If we accept the conclusions that the potential movements are due to increased translocation down the petiole, that the channels have a negative zeta potential, and that illumination swings the upper electrodes more positive, then this would favour a mechanism other than pressure flow. On the other hand in spite of the precautions taken illumination might easily increase transpiration which would promote the observed effect. Further work along these lines is necessary before reliable conclusions can be drawn.

Quantitatively, the values obtained for the Onsager coefficients and the electroosmotic efficiencies suggest some interesting points. The latter lead directly to an estimate for the ion current density necessitated by the electroosmotic theory. For if the mean linear velocity in the sieve tube lumina is 50 cm hr^{-1} the flux of water per cm^2 is 50 $cm^3\ hr^{-1}$ or $0.8 \cdot 10^{-3}$ moles sec^{-1}. If we take the electroosmotic efficiency as 100 moles $faraday^{-1}$ (Table 2) the current density in the lumen works out at about 0.8 amp cm^{-2}, with higher values in the pores. This poses a very real difficulty for the theory. Only a much higher value for the efficiency will meet it, and though MacRobbie and Fensom (in press) have found values of up to 700 moles $faraday^{-1}$ in *Nitella* this hardly meets the objection.

It is possible also to make a rough estimate of the relationship between the linear velocity of flow and the potential gradient. At zero pressure differential we have from Eq. (1)

$$J = L_X \cdot \frac{\Delta E}{l} \tag{5}$$

where J is the velocity in cm sec^{-1} averaged over the whole cross section. If the sieve tube lumens represent 20% of the whole area the mean linear velocity in them per volt cm^{-1} of potential gradient is given by

$$\frac{J}{0.2} \div \frac{\Delta E}{l} = 5\, L_X \tag{6}$$

using (5). Taking $L_X = 10^{-4}$ cm^2 sec^{-1} $volt^{-1}$ (compare Table 3) this becomes $5 \cdot 10^{-4} \cdot 3600 = 1.8$ cm hr^{-1} $(volt\ cm^{-1})^{-1}$. For a velocity of 50 cm hr^{-1} this implies a gradient of 50/1.8 or 28 volts cm^{-1}. As a motive force, this appears physiologically out of the question, but it has to be remembered that the potential distribution in the second author's electroosmotic theory (1958) is quite different from that in the electroosmometer, and the matter clearly needs further investigation.

Table 3 indicates that the electrical conductivities in the strand are appreciably higher than those in open solution with the same salt concentration. This is a well-known effect, but the calculated factors cannot be considered to be of high accuracy owing to the difficulty of measuring the strand diameter.

Table 3. *Onsager Coefficients for excised vascular strands of Nymphoides*

Number of samples	Concentration of KCl (molar)	Mean current (μamps)	Mean e-o efficiency (cm^3 $coulomb^{-1}$)	Mean specific resistance (ohm cm)	L_E (ohm^{-1} cm^{-1})	L_X (cm^2 sec^{-1} $volt^{-1}$)
4	10^{-1}	93	5.4×10^{-3}	1.15×10	8.7×10^{-2}	4.7×10^{-4}
4	10^{-3}	18	15.5×10^{-3}	5.1×10^{2}	1.95×10^{-3}	3.0×10^{-5}
4	10^{-4}	10	19.6×10^{-3}	1.12×10^{3}	8.9×10^{-4}	1.8×10^{-5}
14	10^{-4}	8.2	24.0×10^{-3}	1.18×10^{3}	8.5×10^{-4}	2.0×10^{-5}

The ratios of L_E to the conductivities in open solution are respectively 6.7, 13.3, 59 and 57.

Conclusion

The conclusions reached in this work are necessarily very tentative. The problem of actually measuring the properties of phloem relevant to the process of translocation is a very difficult one. Not only is it rendered so by the virtual impossibility of getting sieve tubes free from vessels,

fibres and other cell types, but the sieve tube walls themselves constitute a pathway for flow presumably irrelevant to the point at issue. Further, sieve tubes react quickly and significantly (by callose deposition) to any interference. The application of the present results to the interpretation of sieve plate function is therefore very problematical. However, such is the difficulty of measuring the properties of the normal sieve plates themselves that even such an imperfect attempt as the present one seemed worthwhile reporting. Of positive value, at least, are the conclusions that electroosmosis is a property of the vascular strand, that its direction indicates a negative charge on the structure, and that the order of magnitude of the efficiency is 100 water molecules per ion. It is to be hoped that it will act as a stimulus to further and better attempts to analyse the translocation pathway in quantitative terms.

Appendix

The Hydraulic Resistance of Sieve Plates

Most calculations on the resistance of the sieve plates to fluid movement have been based on Poiseuille's equation; that is, they have assumed that flow takes place through a system of fine capillaries. Electron microscopic evidence indicates that the sieve plate pores are apparently filled loosely with axially orientated fibrils or very small tubules. The following treatment regards these as a system of threads arranged in a parallel regular hexagonal pattern (Fig. 2) with separation D. The resistance to flow parallel to the threads is calculated as follows.

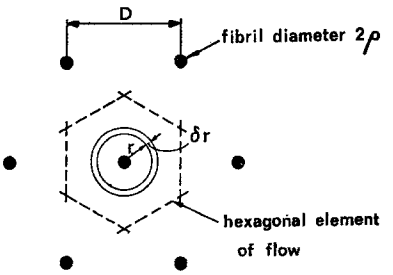

Fig. 2. Cross section of ideal array of parallel fibrils running through a sieve plate pore

To begin with, it simplifies matters enormously if the hexagonal outline of a flow unit is replaced with a circular one (radius $R = 0.525\ D$) of equal area. This reduces the problem to one in a radially-symmetrical system. Referring to the figure let v be the velocity of flow at the radius r, and consider the forces on a thin cylinder of radii r, $r + \delta r$ and length l.

The difference in the viscous drags on the internal and external faces of this will be balanced by the pressure difference on the ends. In symbols, if η is the viscosity of the fluid,

$$-\eta \cdot 2\pi r l \cdot \frac{dv}{dr} + \eta \cdot 2\pi (r+\delta r) l \cdot \left(\frac{dv}{dr} + \frac{d^2v}{dr^2} \cdot \delta r\right) = 2\pi r\, \delta r \cdot \Delta P,$$

Eliminating second-order differentials this becomes

$$r \frac{d^2v}{dr^2} + \frac{dv}{dr} = \frac{r}{\eta} \cdot \frac{\Delta P}{l}$$

or

$$\frac{d}{dr}\left(r \frac{dv}{dr}\right) = \frac{r}{\eta} \cdot \frac{\Delta P}{l}. \tag{1}$$

Neglecting slip at the fibril surface this equation can be easily integrated with the boundary conditions $v = 0$ for $r = \varrho$, and $\frac{dv}{dr} = 0$ for $r = R$. The integration gives

$$\text{volume flow per unit element} = \frac{\pi R^4}{8\eta} \cdot \frac{\Delta P}{l} \{4 \ln (1/\alpha) - (1-\alpha^2)(3-\alpha^2)\} \tag{2}$$

where $\alpha = \frac{\varrho}{R}$. Dividing by the area of the element we have for the mean linear velocity

$$V = \frac{R^2}{8\eta} \cdot \frac{\Delta P}{l} \cdot \left\{\frac{4 \ln \frac{1}{\alpha}}{(1-\alpha^2)} - (3-\alpha^2)\right\}. \tag{3}$$

In this equation $\alpha = \frac{\varrho}{R} = 1.90 \frac{\varrho}{D}$.

As an example of the use of this formula we may calculate the pressure drop over a sieve plate of *Nymphoides* using some electron microscopical measurements of R.P.C. JOHNSON (unpublished). They have been tentatively corrected for the artefact of injury callose, and agree closely with some data of U. MISHRA for *Salix*. They are as follows:

Fibrillar diameter	$2\varrho = 100$ Å.
Fibrillar spacing	$D = 300$ Å.
Sieve plate thickness	$l = 1$ μ.

The factor in braces { } in (3) works out at 2.21. Assuming a pore velocity of 200 cm hr^{-1} (equivalent to say 50 cm hr^{-1} mean in the lumen) and a viscosity of 0.015 poise the differential per plate becomes

$$\Delta P = \frac{200}{3600} \cdot \frac{8 \cdot 0.015 \cdot 10^{-4}}{(0.525 \cdot 300 \cdot 10^{-8})^2} \cdot \frac{1}{2.21} \cdot \frac{1}{1.013 \cdot 10^6} \text{ atmosphere}$$

$$= 0.12 \text{ atmosphere.}$$

With a sieve tube length of 400 μ this represents 300 atmospheres per metre. With D doubled ΔP falls to 0.014 atmos. (36 per metre); with D tripled it falls to 0.005 atmos (13 per metre). Bearing in mind the extreme thinness of electron microscopical sections (say 1,000 Å) it seems very unlikely that the image seen, even allowing for compression of the pore contents, could conceivably be interpreted as indicating a natural fibril spacing in the functioning tube of more than the limit here quoted (900 Å). Even this in a medium-sized tree requires several hundred atmospheres overall, and the micrographic evidence certainly favours the closer spacing. It is difficult to believe that in trees at least, pressure-flow is the main mechanism of movement.

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