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The Actin Cytoskeleton and Polar Water Permeability in Characean Cells

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Dedicated to Professor Dr. NOBURO KAMIYA on the occasion of his 75th birthday

Summary

The hydraulic resistance was measured on internodal cells of *Chara corallina* and *Nitellopsis obtusa* using the method of transcellular osmosis described by KAMIYA and TAZAWA in 1956. The transcellular hydraulic resistances of *N. obtusa* and *C. corallina* are 2.63 and 1.11 pm^{-1} s Pa, resepectively. These values correspond to osmotic permeability coefficients (P_{os}) of 102.6 and 243.1 μm s^{-1}, respectively. Cytochalasin A, B, and E (1–30 μg ml^{-1}) increase the hydraulic resistance in a concentration-dependent manner. The order of effectiveness is: CE > CA = CB. CE increases the activation energy of water transport from 16.4 kJ mol^{-1} to 32.5 kJ mol^{-1} indicating that it increases the hydraulic resistance by eliminating a low resistance pathway. Cytochalasin B and E specifically increase the hydraulic resistance to endoosmosis; even when the driving force for transcellular osmosis is as low as 0.06 MPa. The effect of the cytochalasins is independent of their effect on cytoplasmic streaming since stopping streaming with N-ethyl maleimide or electrical stimulation has no effect on hydraulic conductivity. The possibility is discussed that a cortical actin cytoskeleton interacts with the plasma membrane in order to regulate the transport of water.

Keywords: Actin cytoskeleton; *Chara corallina*; Cytochalasin; *Nitellopsis obtusa*; Polarity; Water transport.

Abbreviations: APW artificial pond water; CA cytochalasin A; CB cytochalasin B; CE cytochalasin E; DMSO dimethylsulfoxide; NEM N-ethyl maleimide; K transcellular osmotic constant (pico m^3 s^{-1} Pa^{-1}); k′ transcellular hydraulic permeability coefficient (pm s^{-1} Pa^{-1}); R transcellular hydraulic resistance (pico m^{-3} s Pa); r_{tot} total transcellular hydraulic resistance (pm^{-1} s Pa); L_p hydraulic conductivity (pm s^{-1} Pa^{-1}); L_{pen} endoosmotic hydraulic conductivitiy (pm s^{-1} Pa^{-1}); L_{pex} exoosmotic hydraulic conductivity (pm s^{-1} Pa^{-1}); L_{pen}^{-1} endoosmotic hydraulic resistance (pm^{-1} s Pa); L_{pex}^{-1} exoosmotic hydraulic resistance (pm^{-1} s Pa); J_v rate of water flow (pico m^3 s^{-1}); $\bar{V}_w$ partial molar volume of water (pico m^3 mol^{-1}).

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1. Introduction

In 1956 KAMIYA and TAZAWA used the method of transcellular osmosis to describe quantitatively the movement of water through a single internodal cell of *Nitella*. The method consists simply of dividing a cell between two compartments and replacing the water in one compartment with a solution of nonelectrolytes like sucrose. The solution establishes a difference in the water potential gradient across the plasma membrane between the water and solution sides, and thus drives water out of one cell end (the exoosmotic end) and draws water into the other end of the cell (the endoosmotic end). Water thus moves transcellularly (OSTERHOUT 1949 a, b). The transcellular osmosis induced by subjecting the exoosmotic cell half to a solution is called forward transcellular osmosis and the transcellular osmosis induced by replacing the solution again with water is called backward transcellular osmosis.

The forward transcellular osmosis takes place in two phases; an initial rapid exponential phase and a subsequent slower linear phase which lasts indefinitely. KAMIYA and TAZAWA (1956) explained that the slowing down of water movement is a result of the establishment of a polar distribution of intracellular solutes, *i.e.*, dilution of solutes on the endoosmotic side and concentration of solutes on the exoosmotic side. But the transcellular water movement never stops as a consequence of the depolarization of these solutes by an active cy-

toplasmic streaming. In order to test this hypothesis we added cytochalasin B to cells of *Chara corallina* and *Nitellopsis obtusa* to inhibit streaming and then observed what happened to the transcellular transport of water. Contrary to our expectation, we found that CB markedly inhibits the initial rapid water movement but has a lesser effect on the continuous, linear transcellular transport of water.

KAMIYA and TAZAWA (1956) observed that transcellular water transport in *Nitella flexilis* occurs in a polar fashion. That is the hydraulic resistance to exoosmosis is greater than that to endoosmosis. This polarity is also observed in higher plant cells in that the rate constant for deplasmolysis is greater than the rate constant for plasmolysis (HÖFLER 1930, LEVITT *et al.* 1936). There has been much controversy over whether the observed polarity is real or only apparent as a result of the unstirred layers because the outflowing water on the exoosmosis side will sweep away the osmoticum and consequently reduce the driving force on the exoosmotic side (DAINTY and HOPE 1959, DAINTY 1963 a, b, KIYOSAWA and TAZAWA 1973). However, a polarity still exists, albeit somewhat reduced even after the unstirred layers are taken into consideration (DAINTY 1963 a, b, DAINTY and GINZBURG 1964 a, TAZAWA and KAMIYA 1965, 1966).

There still remained the question of whether the observed polarity is an intrinsic character of the living cell or is a result of a nonspecific dehydration by the osmoticum of the membrane on the exoosmotic side (DAINTY and GINZBURG 1964 b, KIYOSAWA and TAZAWA 1972, 1973, TAZAWA and KIYOSAWA 1973). Using the pressure probe technique, STEUDLE and ZIMMERMANN (1974) induced a water potential gradient across the membrane of *Nitella flexilis* hydrostatically thus eliminating any effect of dehydration on the membrane by an osmoticum. With this protocol they observed a polarity in the transmembrane transport of water. Furthermore the resistance to outward water movement was higher than the resistance to inward water movement. However, using smaller hydrostatic gradients STEUDLE and TYERMAN (1983) were unable to observe any polarity in *Chara corallina*, indicating perhaps that a minimum flow rate is required for the manifestation of a polarity.

Here we present our observations on transcellular water movement in *Chara* and *Nitellopsis* and confirm the suggestion made by KAMIYA and TAZAWA (1956) that the polarity is an intrinsic character of the living cell and show that actin microfilaments participate in the establishment of the cellular polarity which reduces the hydraulic resistance on the endoosmotic side.

2. Materials and Methods

2.1. Plant Materials

Chara corallina KLEIN ex WILLD., em. R.D.W. (= *Chara australis* R. BROWN) and *Nitellopsis obtusa* (Desv. in Lois.) J. Gr. were grown in a soil water mixture in large plastic buckets at 25 ± 2 C with 15 hL: 9 hD photoperiod. Internodal cells of *Chara corallina* or *Nitellopsis obtusa* were isolated and placed in artificial pond water (APW: 0.1 mM NaCl, 0.1 mM $CaCl_2$ and 0.1 mM KCl) buffered by 10 mM Mes titrated with Tris to pH 5.5. The cells were then placed on a shaker for 3 h to remove the $CaCO_3$ deposited on the wall. The cells were then transferred to APW buffered by 2 mM Hepes-NaOH (pH 7.3). The cells remained in this medium at least overnight before use.

2.2. Measurement of Transcellular Osmosis

The rate of transcellular osmosis was measured in the apparatus shown in Fig. 1. This apparatus is modeled after the apparatus designed by TAZAWA and KAMIYA (1966). Internodal cells were first treated in 1% DMSO (0.136 Osm = 0.33 MPa at 20 °C) in a covered chamber for 35 minutes and subsequently rinsed in water for 1 min. The cell was then placed in the apparatus such that the length of the cell part in chamber B was two times the length of the cell part in chamber A. Chamber A and chamber B were physically separated by a 4–7 mm (depending on the chamber) silicone seal (HVG, Toray Silicone, Tokyo, Japan) and the cell parts in both chambers A (endoosmotic side) and B (exoosmotic side) were bathed in water. Forward transcellular osmosis was initiated by replacing the solution in chamber B with 100 mM sorbitol (0.244 MPa at 20 °C) and the quantity of water movement was recorded every 10 seconds for 40–70 seconds. The magnitude of water movement was measured by following the rate of movement of a column of water in a glass capillary with an Olympus CH microscope equipped with a × 4 objective lens in the case of *Chara* or a × 10 objective lens in the case of *Nitellopsis*. The sensitivity of the apparatus is about 5 nl, which was approximately 1% of the water volume transported within the first 60 seconds. The water in the capillary was continuous with the solution in chamber A. The solution in chamber B was replaced with water

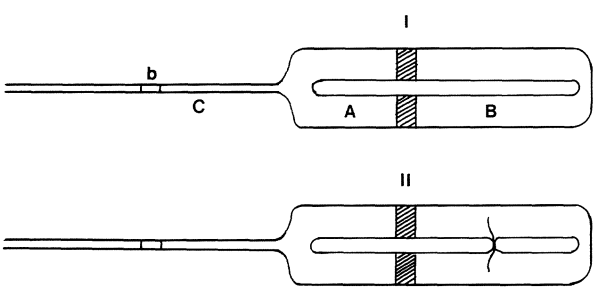

Fig. 1. A diagram (top view) of the apparatus used for transcellular osmosis. The initial condition where the cell part in chamber *A* is one-half the length of the cell part in chamber *B* is shown in *I*. After ligation, the cell lengths in chambers *A* and *B* that can contribute to transcellular osmosis are equal (*II*). The volumes of chambers *A* and *B* were 750 and 2250 µl, respectively. The volume of the capillary (*c*) was 615.3 nl mm^{-1}. The flux of water was determined by following the movement of an air bubble (*b*) in the capillary. When electrical stimulation was given to the cells, silver wires were placed along the length of chambers *A* and *B*

to initiate backward transcellular osmosis and the cells were allowed to reach their original equilibrium conditions during the next 10 minutes except when stated otherwise. Subsequently, the cell part in chamber B was ligated (KAMIYA and KURODA 1956) at its middle so that the length of the cell in chamber B that could participate in transcellular osmosis was equal to the length of the cell part in chamber A. Again transcellular osmosis was initiated with 100 mM sorbitol and was recorded every 10 seconds for 40–70 seconds. At the end of the experiment, the cell end distal to the ligation was cut off to ensure that the ligation was complete.

The cell could also be placed asymmetrically in the apparatus in a way that the length of the cell part in chamber A was equal to one-half of the length of the cell part in chamber B and the water flow was measured. Then the cell was removed and reset so that the length of the cell part in chamber B was equal to one-half the length of the cell part in chamber A and again water flow was measured. Experiments done in this manner yielded values of L_{pen}^{-1}, L_{pex}^{-1} and r_{tot} similar to those obtained with the ligation method (data not shown). In order to eliminate any interference from the small volume change that occurs during the onset of transcellular osmosis (0–5 sec) (TAZAWA and KAMIYA 1966) or the buildup of unstirred layers (more than 40 seconds after the onset of transcellular osmosis) the amount of water movement between 10 and 40 seconds and 10 and 30 seconds was used to calculate the rate of transcellular osmosis in *Chara* and *Nitellopsis*, respectively. Experiments were performed at room temperature (20–26 °C) unless stated otherwise.

2.3. Determination of the Total Hydraulic Resistance Using Transcellular Osmosis

The rate of transcellular osmosis was measured in the apparatus described above. Internodal cells were first treated in 1% DMSO (0.136 Osm = 0.32 MPa at 20 °C) in a covered chamber for 35 minutes and subsequently rinsed in water for 1 min. The cells were then placed symmetrically in water in the apparatus. Forward transcellular osmosis was initiated by replacing the solution in chamber B with 100 mM sorbitol (0.100 Osm = 0.237 MPOa at 20 °C) and the quantity of water movement was recorded every 10 seconds for 40–70 seconds by measuring the rate of movement of an air bubble as detailed above. The total transcellular hydraulic resistance was determined from the following equation (KIYOSAWA and TAZAWA 1972, TAZAWA 1980):

$$r_{tot} = \frac{A\Delta\Pi}{J_v}$$

where J_v is the observed flow of water movement in pico m^3/s; A is the surface area (in pico m^2) of the cell part in each chamber; and $\Delta\Pi$ is the osmotic gradient (in Pa) used to drive transcellular osmosis. The total hydraulic resistance is given in pm^{-1} s Pa.

2.4. Analysis of Results

The hydraulic conductivities for endoosmosis (L_{pen}) and exoosmosis (L_{pex}) were calculated using the following equations. The general equations can be found in KAMIYA and TAZAWA (1956) and they are based on the following assumptions: (1) The membranes form the main resistance to water permeation; and (2) The resistance to endoosmosis and the resistance to exoosmosis are independent, both work in series and can be summed.

The initial rate of water flow (J_v, in pico m^3 s^{-1}) is proportional to the osmotic gradient ($\Delta\Pi$, in Pa) used to initiate transcellular osmosis.

$$J_v = K\Delta\Pi \quad (1)$$

The transcellular osmosis constant (K) (TAZAWA and KAMIYA 1965) was calculated by dividing the initial flux of transcellular water movement by the magnitude of the osmotic gradient.

$$K = J_v/\Delta\Pi \quad (2)$$

K (pico m^3 s^{-1} Pa^{-1}) is a constant that is given as $A_{en}A_{ex}L_{pen}L_{pex}/(A_{en}L_{pen} + A_{ex}L_{pex})$. The transcellular osmotic constant (K) is equal to the transcellular hydraulic permeability coefficient (k′, in pm s^{-1} Pa^{-1}) times the surface area of half of a cell (A, in pico m^2) when the cell is equally partitioned.

$$K = k'A \quad (3)$$

where k′ is given as $L_{pen}L_{pex}/(L_{pen} + L_{pex})$.

In the initial transcellular osmosis before ligation $A_{en} = A_{ex}/2 = A$ and the transcellular osmotic constant (K_1) equals:

$$K_1 = \frac{2A\,L_{pen}\,L_{pex}}{(L_{pen} + 2L_{pex})} \quad (4)$$

After ligation, $A_{en} = A_{ex} = A$ and the transcellular osmotic constant (K_2) equals:

$$K_2 = \frac{A\,L_{pen}\,L_{pex}}{(L_{pen} + L_{pex})} = k'A \quad (5)$$

The polarity of water movement (α) is defined as the ratio of L_{pen} to L_{pex}. That is:

$$\alpha = L_{pen}/L_{pex} \quad (6)$$

We can substitute L_{pen} in Eqs. (4) and (5) with α L_{pex} to get the following equations:

$$K_1 = \frac{2\alpha\,L_{pex}}{\alpha + 2} \quad (7)$$

$$K_2 = \frac{\alpha A\,L_{pex}}{\alpha + 1} \quad (8)$$

From Eqs. (7) and (8) we get:

$$\alpha = \frac{2(K_1 - K_2)}{2K_2 - K_1} \quad (9)$$

Rearranging Eq. (8) we can solve for L_{pex}

$$L_{pex} = \frac{K_2(\alpha + 1)}{\alpha A} \quad (10)$$

We can now calculate L_{pen} from Eq. (6) as α L_{pex}

$$L_{pen} = \alpha\,L_{pex} = K_2(\alpha + 1)/A \quad (11)$$

The reciprocal of L_{pen} and L_{pex} are the transcellular hydraulic resistances for endoosmosis and exoosmosis, respectively. When the cell is partitioned in equal halves ($A_{en} = A_{ex} = A$),

$$R = (L_{pen}^{-1} + L_{pex}^{-1})A^{-1} \quad (12)$$

Then we can introduce the total transcellular hydraulic resistance (r_{tot}) in units of pm^{-1} s Pa.

$$r_{tot} = L_{pen}^{-1} + L_{pex}^{-1} \quad (13)$$

Therefore

$$r_{tot} = AR = A/K = 1/k' \quad (14)$$

where r_{tot} is in units of pm^{-1} s Pa, A is in units of pico m^2, R is in units of pico m^{-3} s Pa and K is in units of pico m^3 s^{-1} Pa^{-1}. Assuming that there is no polarity, or that $L_{pen} = L_{pex} = L_p$, we get from Eq. (13)

$$L_p = 2r_{tot}^{-1} \quad (15)$$

where L_p is the hydraulic conductivity across a single membrane.

2.5. Measurement of Cellular Osmotic Pressure

Cellular osmotic pressure was measured by the turgor balance method (TAZAWA 1957). Solution osmotic pressures were periodically tested by the turgor balance method and the vapor pressure osometer (Wescor 5100C) making sure to pay attention to the ill effect of DMSO on the osmometer chamber.

2.6. Measurement of Membrane Potential (E_m), Membrane Resistance (R_m), and Membrane Excitability During Transcellular Osmosis

Figure 2 shows the experimental setup for measuring E_m and R_m during transcellular osmosis. The setup was similar to that described by HAYAMA *et al.* (1979). The cells were equally partitioned into two 1 cm diameter pools that were separated by a 1 cm vaseline seal. The cells were bathed in APW containing 1% DMSO or APW containing 1% DMSO and 30 μg ml^{-1} CE. A glass Ag−AgCl microcapillary electrode filled with 3 M KCl was inserted into the vacuole of the cell half in chamber B. An agar bridge containing 100 mM KCl was used as the reference electrode. The electrical resistance of the membrane was determined by measuring the change in the membrane potential after applying rectangular 0.2 μA current pulses through Ag−AgCl wires placed in the two pools. The resistance due to components other than membranes was small (HAYAMA *et al.* 1979) and was neglected. E_m and R_m of the endoosmotic side during forward osmosis was measured by replacing the solution in chamber A with APW containing 0.4 M sorbitol. E_m and R_m during backward osmosis of this same side was measured by replacing the sorbitol solution with the original solution. E_m and R_m of the exoosmotic side during forward osmosis was measured by replacing the solution in chamber B with APW containing 0.4 M sorbitol. E_m and R_m of this same side during backward osmosis was measured by replacing the sorbitol solution with the original solution.

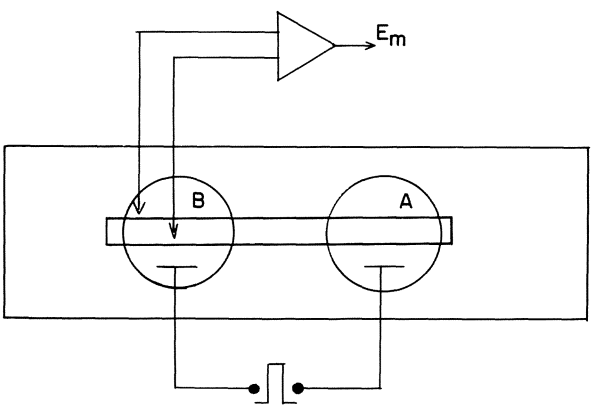

Fig. 2. A diagram of the experimental setup used for measuring membrane potential (E_m) and resistance (R_m) during transcellular osmosis. See text for details

2.7. Extraction and Measurement of Intracellular ATP Content

Cells were rapidly frozen in liquid nitrogen after a 35 minutes treatment in either 1% DMSO or 30 μg ml^{-1} CE. ATP was extracted in a boiling buffer which included 25 mM K^+-Hepes, 10 mM K^+-EDTA and 0.3% H_2O_2 (pH 7.4). The extract was analysed by the firefly-flash method with an ATP photometer (Chemglow photometer J 4-7441; Aminco, Silver Spring, Md., U.S.A.) following the method of MIMURA *et al.* (1984).

2.8. Chemicals

In all experiments DMSO was used as a carrier. Cytochalasin A, B, and E, N-ethyl maleimide (NEM) and colchicine were all dissolved in DMSO (so that the final concentration of DMSO always was one percent) and the cells were treated with these solutions for 35 minutes. Preliminary experiments showed that the rates of osmosis were similar whether the drugs were present or absent in the chambers during transcellular osmosis. As an economic measure, therefore, we eliminated the drugs from the chambers during transcellular osmosis.

Cytochalasins A, B, and E, and colchicine were obtained from Sigma. Cytochalasin B was also obtained from Aldrich. NEM was obtained from Nakarai Chemical Co.

3. Results

3.1. Hydraulic Resistance

Internodal cells of *Nitellopsis* and *Chara* have a total transcellular hydraulic resistance (r_{tot}) of 2.63 and 1.11 pm^{-1} s Pa, respectively. This corresponds to a hydraulic conductivity (L_p) of 0.76 pm s^{-1} Pa^{-1} for *Nitellopsis* and 1.80 pm s^{-1} Pa^{-1} for *Chara*. If the volume flow is expressed as the flow of water molecules, then the units of L_p can be converted to the units of the osmotic permeability coefficient (P_{os}) by multiplying L_p with $RT/\bar{V}_w$ (DAINTY 1963 b). P_{os} for *Nitellopsis* is 102.6 μm s^{-1} and 243.1 μm s^{-1} for *Chara* (Table 1). Although these values are similar to values

Table 1. *The total transcellular hydraulic resistances, hydraulic conductivities and osmotic permeability coefficients of Nitellopsis obtusa and Chara corallina*

	r_{tot}	L_p	P_{os}
	pm^{-1} s Pa	pm s^{-1} Pa^{-1}	μm s^{-1}
Species			
N. obtusa (n = 47)	2.63 ± 0.11	0.76	102.6
C. corallina (n = 51)	1.11 ± 0.07	1.80	240.54

The total transcellular hydraulic resistances (r_{tot}) were determined for the cells which were firstly treated with 1% DMSO for 35 min and then rinsed with distilled water for 1 min. After temperature equilibration cells were subjected to transcellular osmosis using a driving force of 100 mM sorbital. The hydraulic conductivities (L_p) and the osmotic permeability coefficient (P_{os}) were calculated from the following formulas: $L_p = 2(1/r_{tot})$ and $P_{os} = L_pRT/\bar{V}_w$ (where L_p is the hydraulic conductivity in pm s^{-1} Pa^{-1}, R is the gas constant), T is the absolute temperature in K (set to 298 K) and $\bar{V}_w$ is the partial molar volume of water (HANSSON MILD and LØVTRUP 1985). Please note that the total transcellular hydraulic resistance represents the total resistance to both endoosmosis and exoosmosis; therefore L_p represents the average hydraulic conductivity. The value for the total transcellular hydraulic resistance represents the mean ± SEM for measurements made from May 1987 to February 1988

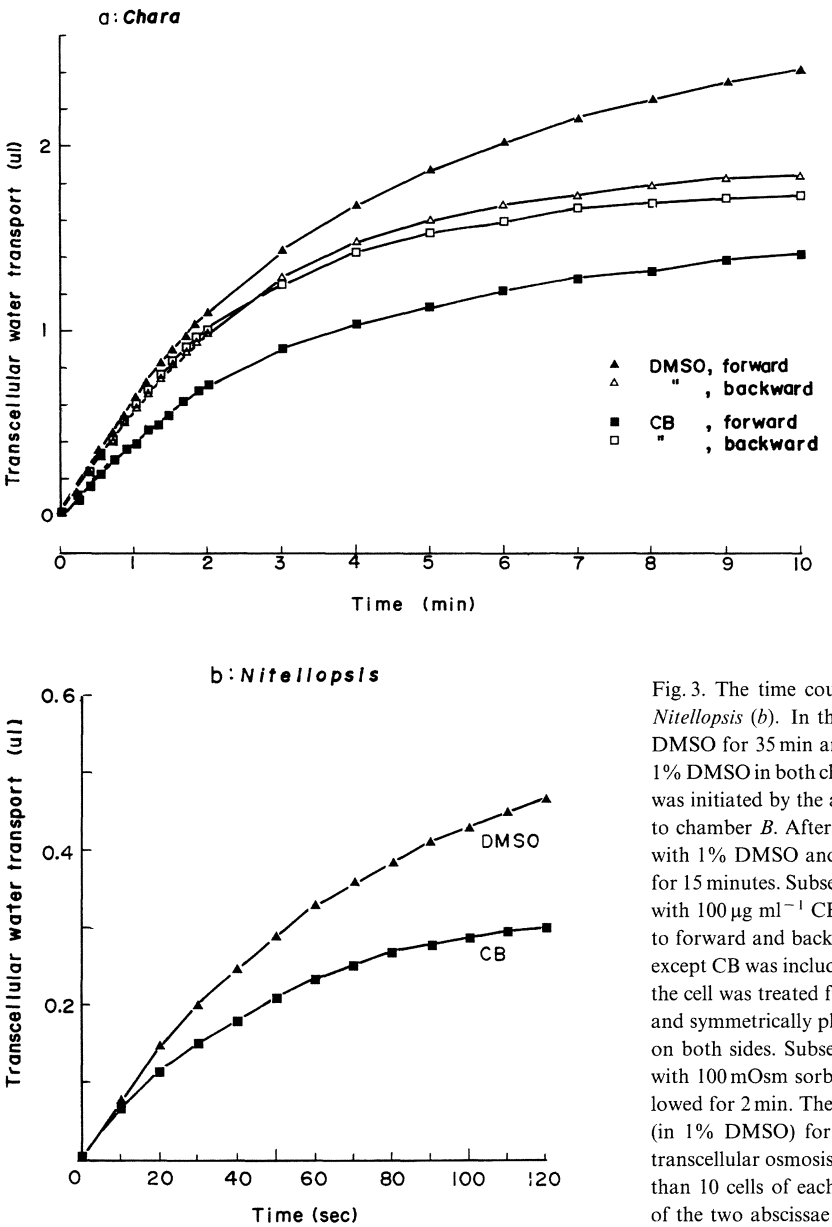

Fig. 3. The time course of transcellular osmosis in *Chara* (*a*) and *Nitellopsis* (*b*). In the case of *Chara*, the cell was treated with 1% DMSO for 35 min and placed symmetrically in a chamber that had 1% DMSO in both chambers *A* and *B*. Forward transcellular osmosis was initiated by the addition of 1% DMSO plus 100 mOsm sorbitol to chamber *B*. After 15 minutes, the sorbitol solution was replaced with 1% DMSO and backward transcellular osmosis was observed for 15 minutes. Subsequently, the same cell was taken out and treated with 100 μg ml^{-1} CB (in 1% DMSO) for 35 minutes and subjected to forward and backwards transcellular osmosis as described above except CB was included in all the solutions. In the case of *Nitellopsis*, the cell was treated first with 1% DMSO for 35 min, rinsed in water and symmetrically placed in the apparatus which had distilled water on both sides. Subsequently, the water in chamber *B* was replaced with 100 mOsm sorbitol and forward transcellular osmosis was followed for 2 min. Then the same cell was treated with 30 μg ml^{-1} CB (in 1% DMSO) for 35 minutes, rinsed and subjected to forward transcellular osmosis. Each cell is a representative experiment. More than 10 cells of each species were used. Notice the different scales of the two abscissae

obtained with characean cells by other workers they are approximately 20–300 times higher than the values obtained with other plant cells (KAMIYA and TAZAWA 1956). The difference in the hydraulic resistances of *Chara* and *Nitellopsis* are reproducible throughout the year.

3.2. Effects of Cytochalasins A, B, and E on the Total Transcellular Hydraulic Resistance

Cytochalasin B inhibits transcellular water movement in the internodal cells of *Chara* and *Nitellopsis* (Fig. 3). Cytochalasin B specifically inhibits forward transcellular osmosis, but has almost no effect on backward transcellular osmosis (Fig. 3 *a*). Cytochalasin B inhibits the initial rate of forward transcellular osmosis during the exponential phase (0–40 sec). However, the slow linear phase (5–10 min) still continues indefinitely albeit at a reduced rate. The inhibition of water movement results from the ability of CB to increase the hydraulic resistance to transcellular water movement. Low concentrations of CB (3–30 μg ml^{-1} or 6–60 μM) increase r_{tot} in a concentration dependent manner (Figs. 4 and 6 *b*). After washing away CB, the effects of 30 μg ml^{-1} CB are always completely reversible (Fig. 5); the

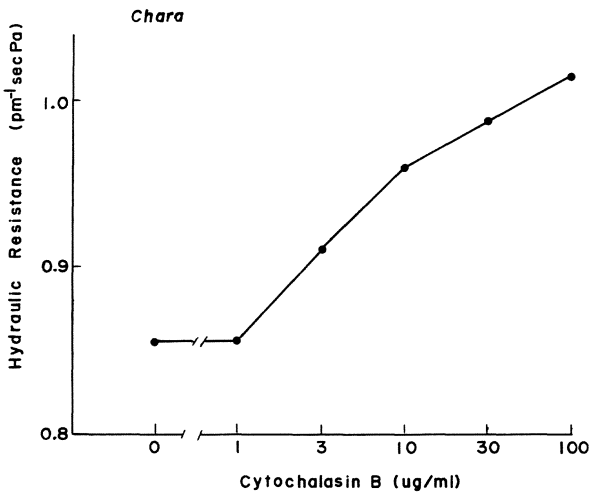

Fig. 4. The effect of cytochalasin B on the total transcellular hydraulic resistance (r_{tot}) in *Chara*. One and the same cell was treated with increasing concentrations of CB. The cell was placed symmetrically in the apparatus and water was in chambers *A* and *B*. The water in chamber *B* was replaced with 100 mOsm sorbitol to initiate forward transcellular osmosis. After a 4.5 hr wash in water, the hydraulic resistance returned to the control value (o)

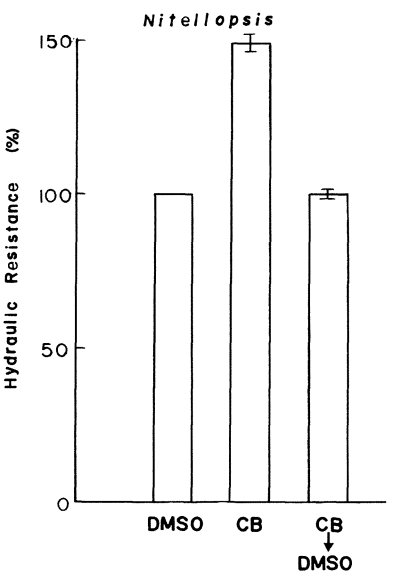

Fig. 5. The reversibility of the cytochalasin B induced increase in the total transcellular hydraulic resistance (r_{tot}) in *Nitellopsis*. Cells were sequentially treated with 1% DMSO, 30 μg ml^{-1} CB and 1% DMSO for 35 min each and after each treatment subjected to transcellular osmosis. Each bar represents the mean ± SEM for 3 cells. The 100% value is equal to 2.02 ± 0.24 pm^{-1} s Pa

effects of 100 μg ml^{-1} are sometimes reversible (Fig. 4). By contrast, STEUDLE and TYERMAN (1983) found that CB had no effect on L_p in *Chara corallina*. The reason for the discrepancy remains unknown although it may be related to the fact that their control cells show no polarity.

Cytochalasin A (CA) and cytochalasin E (CE) also increase r_{tot} in both *Chara* and *Nitellopsis* (Fig. 6 *a* and *b*). In both genera, CE is the most effective of the three tested; 1 μg ml^{-1} gives a half-maximal response. The effects of CE are irreversible. Cytochalasin A is slightly less effective than CB in *Chara* and slightly more effective than CB in *Nitellopsis*.

3.3. The Effect of Cytochalasin B on the Cellular Osmotic Pressure

The cellular osmotic pressure has an effect on transcellular water movement (KIYOSAWA and TAZAWA 1972, 1973). In order to test whether CB inhibits transcellular osmosis by altering the cellular osmotic pressure, we determined the cellular osmotic pressure of the living cell by the turgor balance method. Five cells of each species were measured. Compared to the untreated controls, 1% DMSO increases the cellular osmotic pressure from 0.253 Osm (0.617 MPa at 20 °C) to 0.292 Osm (0.712 MPa at 20 °C) in *Chara* and from 0.296 Osm (0.702 MPa at 20 °C) to 0.338 Osm (0.824 MPa at 20 °C) in *Nitellopsis*. CB does not have any effect on the cellular osmotic pressures when compared to the DMSO control. In 100 μg ml^{-1} CB, the cellular osmotic pressure is 0.292 Osm in *Chara* and 0.338 Osm in *Nitellopsis*. These data also show that CB has no noticable effect on the membrane permeability to sorbitol.

3.4. The Effect of Cytochalasin E on the Activation Energy of Transcellular Osmosis

Cells of *Nitellopsis* were treated with or without 30 μg ml^{-1} CE for 35 min and subsequently washed in water for 10 minutes while being shaken at 40 rev min^{-1} to remove all the DMSO. Then the cells were subjected to transcellular osmosis at various temperatures ranging from 5–25 °C. The activation energy (E_a) for transcellular osmosis was calculated form Arrhenius plots. The E_a for transcellular osmosis of the DMSO control in *Nitellopsis* is 16.38 kJ mol^{-1} (3.9 kcal mol^{-1}). This is similar to the values found by KIYOSAWA (1975) for *Chara* and TAZAWA and KAMIYA (1966) for *Nitella flexilis* but lower than the values found by DAINTY and GINSBURG (1964 a) for *Nitella translucens*. However in *Nitellopsis*, after treatment with CE, the activation energy increases to 32.5 kJ mol^{-1} (7.76 kcal mol^{-1}) indicating that CE causes the elimination of a low energy pathway for water (Table 2).

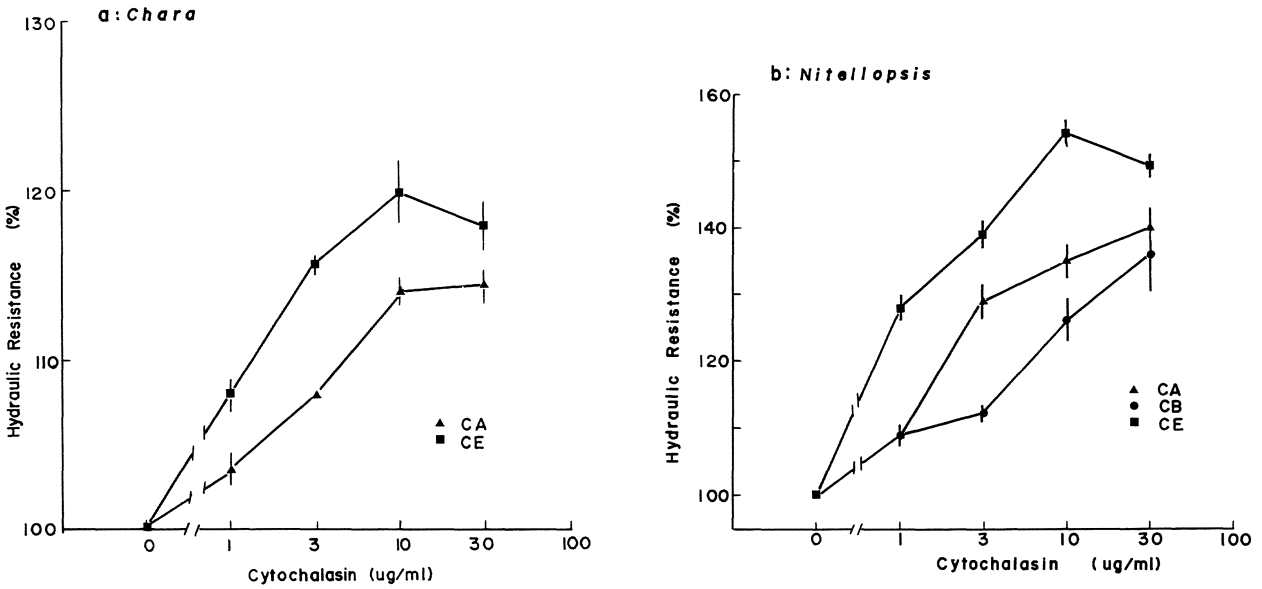

Fig. 6. The effect of cytochalasins on the total transcellular hydraulic resistance (r_{tot}) of *Chara* (*a*) and *Nitellopsis* (*b*). The abscissae represent the concentrations of CA, CB or CE. The ordinates represent the increase in the hydraulic resistance relative to the DMSO control. In the case of 6a: The controls for CA and CE were 1.1 ± 0.21 and 0.97 ± 0.19 pm^{-1} s Pa, respectively. When the cells were repeatedly exposed to 1% DMSO (5 times), the initial control value was 100% = 0.97 ± 0.21 pm^{-1} s Pa and the values varied between 96.75 and 100% (data not shown). Each point represents the average ± standard error of the mean for 3 cells. In the case of 6b: The controls for CA, CB, and CE are: 100% = 3.05 ± 0.59, 2.36 ± 0.38 and 3.06 ± 0.43 pm^{-1} s Pa, respectively. When cells were repeatedly exposed to 1% DMSO (5 times), the initial control value was 100% = 2.48 ± 0.27 pm^{-1} s Pa and the values varied between 96 and 102.3% (data not shown). Each point represents the mean ± SEM for 3 cells

3.5. Differential Effects of the Cytochalasins on Endoosmotic and Exoosmotic Hydraulic Resistances in Nitellopsis

We devised a new method to determine the endoosmotic and exoosmotic hydraulic resistances using cellular ligation to make either endoosmosis or exoosmosis limiting. We used this method to determine whether CB increases the endoosmotic resistance or the exoosmotic resistance. Figure 7 shows clearly that CB and CE specifically increases the endoosmotic hydraulic resistance which results in an increase in r_{tot} and a loss or reversal in the polarity. Similar results are observed with *Chara* (data not shown).

When we perform transcellular osmoses under various osmotic pressure gradients, we find that both r_{tot} and L_{pex}^{-1} increase as the transcellular osmotic gradient increases from 0.06 MPa to 0.50 MPa at 23 °C. By contrast L_{pen}^{-1} decreases as the osmotic gradient increases (Fig. 8). When transcellular osmosis is induced by a gradient as small as 0.06 MPa (25 mOsm), L_{pex}^{-1} is still larger than L_{pen}^{-1} indicating that a polarity still exists under physiological conditions. Although a linear extrapolation to zero indicates that a polarity exists at a zero flow rate, there is no reason to assume that a linear extrapolation is correct. We suggest that the polarity is not a function of the membrane alone but results from a physiological interaction between the membrane, the cytoplasm and the external milieu (see Discussion).

Table 2. *The effect of 30 µg ml^{-1} CE on the activation energy (E_a) for transcellular osmosis in Nitellopsis*

Cell no.	Activation Energy			
	DMSO		Cytochalasin E	
	kJ mol^{-1}	(kcal mol^{-1})	kJ mol^{-1}	(kcal mol^{-1})
1	13.83	(3.30)	32.30	(7.72)
2	15.68	(3.75)	32.60	(7.79)
3	19.62	(4.69)	—	—
4	—	—	32.73	(7.82)
$\bar{X}$	16.38	(3.91)	32.50	(7.78)
S. E.	0.99	(0.24)	0.13	(0.03)

Cells were pretreated in either 1% DMSO or 30 µg/ml CE for 35 min, washed in distilled water for 10 min and then subjected to transcellular osmosis at various temperatures ranging from 5–25 C. The difference between the DMSO control and the CE-treated cells is significant at the 0.05 level

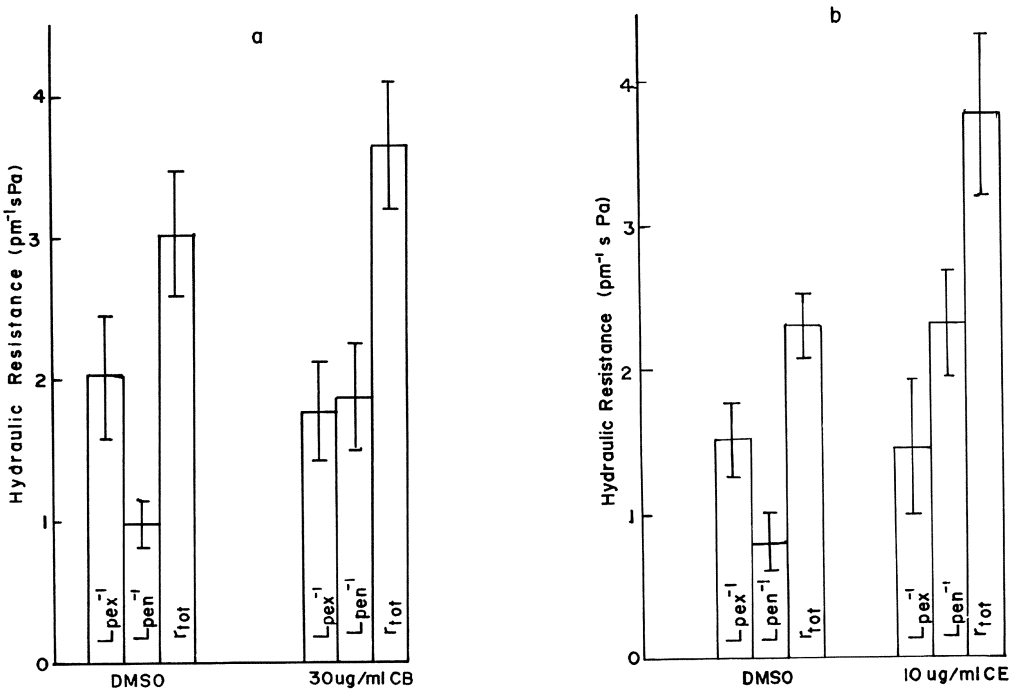

Fig. 7. The effect of cytochalasin B and E on the endoosmotic (L_{pen}^{-1}), exoosmotic (L_{pex}^{-1}) and total transcellular (r_{tot}) hydraulic resistance in *Nitellopsis*. Cells were treated in either *a* 1% DMSO or 30 µg ml^{-1} CB for 35 min or *b* 2% DMSO or 10 µg ml^{-1} CE for 35 min and then placed asymmetrically in the apparatus and subjected to the ligation method of transcellular osmosis. See Materials and Methods for details. In the case of the CB experiments, cells were temperature-equilibrated for only 5 minutes to minimize the leakage of CB. Each bars represents the mean ± SEM for 5 cells. Levels of significance obtained from t-tests are $p < 0.2$, non significant, and $p < 0.05$ for r_{tot}, L_{pex}^{-1} and L_{pen}^{-1}, respectively, of CB treated cells relative to control cells. In the case of CE treated cells, the levels of significance obtained from t-tests are $p < 0.05$, non significant, and $p < 0.05$ for r_{tot}, L_{pex}^{-1} and L_{pen}^{-1}, respectively

A particularly novel observation is that the polarity observed when the osmotic gradient is as small as 0.06 MPa is still sensitive to CE. CE eliminates or partially reverses the polarity (Fig. 9), thus providing strong support for the hypothesis that the polarity is an intrinsic but physiologically variable characteristic of the living cell (Fig. 8).

Cytochalasin B has a dramatic effect on cytoplasmic streaming (NAGAI and KAMIYA 1977). Therefore we tested whether or not CB increases the endoosmotic hydraulic resistance by interferring with streaming. We inhibited the streaming electrically by inducing an action potential with a 1.1 µA electric current reciprocally between two cell ends or chemically by treating the cell with 0.2 mM NEM in 1% DMSO (CHEN and KAMIYA 1975). This low concentration of NEM, when applied in DMSO stops streaming in 5 minutes. Two mM NEM in DMSO stops streaming instantly (data not shown). Inhibiting streaming by either electrical or chemical means does not effect the endoosmotic resistance in *Chara* and *Nitellopsis* (data not shown).

Colchicine (5 mM, 60 min) has little effect on the initial rate of water movement indicating that microtubules do not participate in the regulation of hydraulic resistance (data not shown).

3.6. The Effect of Cytochalasin E on the Electrical Response Induced by Transcellular Osmosis

Transcellular osmosis induces a transcellular potential difference and differential ion movements in *Nitella flexilis* (KATAOKA *et al.* 1979, NISHIZAKI 1955, TAZAWA and NISHIZAKI 1956) indicating that other membrane properties besides hydraulic resistance become polarized. HAYAMA *et al.* (1979) showed that the potential difference results from a transcellular osmosis-induced depolarization on the endoosmotic side and a hyperpolarization on the exoosmotic side. We also find that transcellular osmosis induces a difference in the electrical properties of the endo- and exoosmotic sides in *Nitellopsis*. Transcellular osmosis induces an action potential on the endoosmotic side and a very small slow depolarization on the exoosmotic side in cells treated with APW or APW plus 1% DMSO (Fig. 10 *a* and *b*).

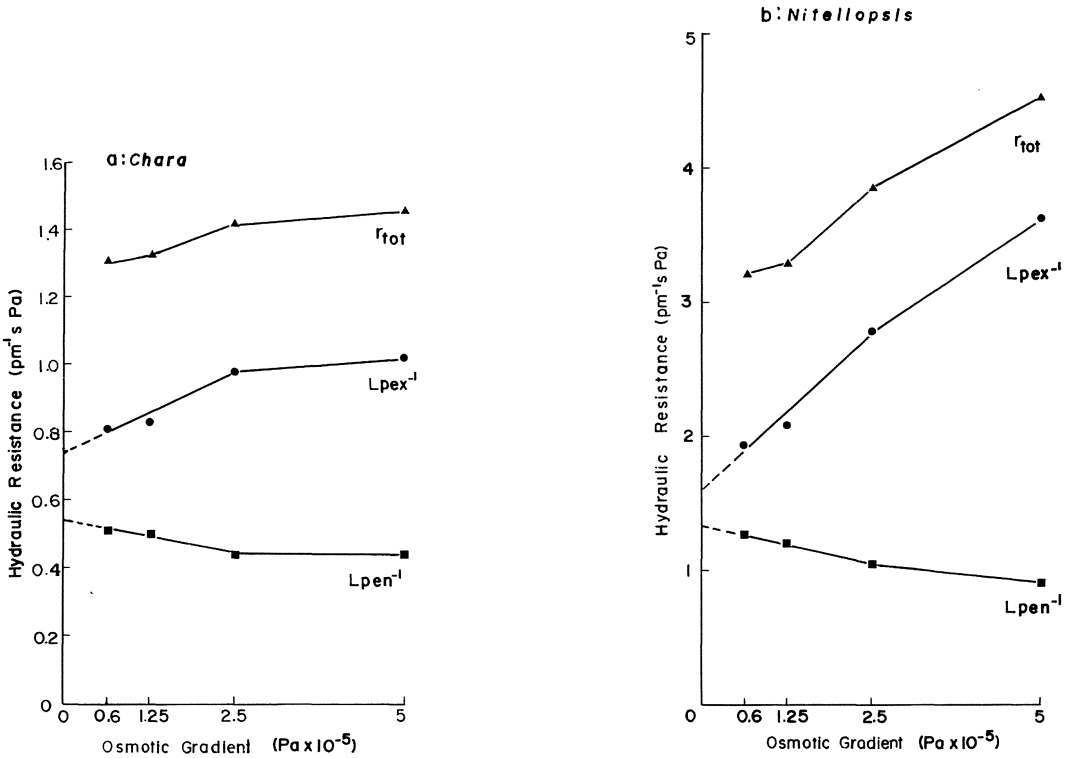

Fig. 8. The effect of the magnitude of the osmotic gradient on the endoosmotic (L_{pen}^{-1}), exoosmotic (L_{pex}^{-1}) and total transcellular (r_{tot}) hydraulic resistance in *Chara* (*a*) and *Nitellopsis* (*b*). Each graph shows one representative cell for each species. In the case of *Chara*, 9 cells were tested. In the case of *Nitellopsis*, 3 cells were tested

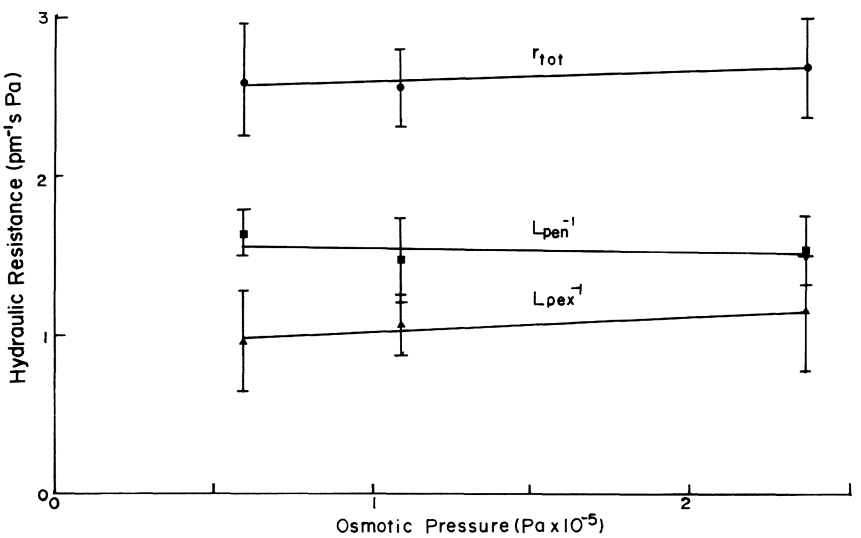

Fig. 9. The effect of the osmotic gradient on the endoosmotic (L_{pen}^{-1}), exoosmotic (L_{pex}^{-1}) and total transcellular (r_{tot}) hydraulic resistance in cells of *Nitellopsis* treated with cytochalasin E. Three cells were treated with 30 µg ml^{-1} CE for 35 min and then washed with water on a shaker (44 rev s^{-1}) to remove the CE and DMSO. The average of three individual experiments is shown with SEM

In contrast to forward transcellular osmosis, backward transcellular osmosis does not induce any electrical changes on either side. However in 7 out of 10 cells treated with 30 µg ml^{-1} CE for 35 minutes, forward transcellular osmosis was unable to induce an action potential on the endoosmotic side although the cells were still capable of generating an action potential in response to an electric current (Fig. 10 *c*). CE did not have any effect on backward transcellular osmosis. CE does not have any effect on the membrane resistance, the membrane potential or the ability of the cell to generate an action potential in response to an electrical

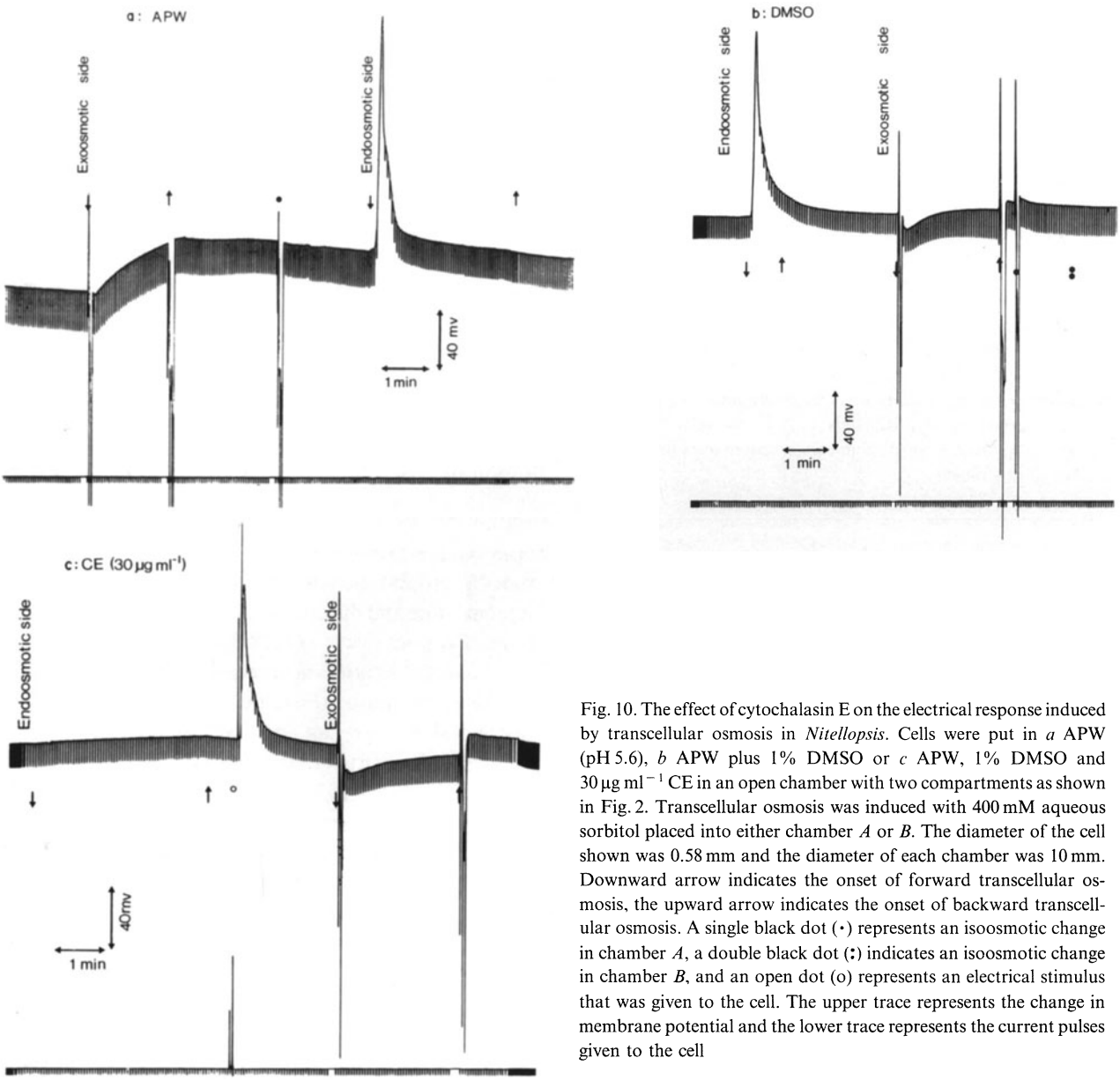

Fig. 10. The effect of cytochalasin E on the electrical response induced by transcellular osmosis in *Nitellopsis*. Cells were put in *a* APW (pH 5.6), *b* APW plus 1% DMSO or *c* APW, 1% DMSO and 30 µg ml^{-1} CE in an open chamber with two compartments as shown in Fig. 2. Transcellular osmosis was induced with 400 mM aqueous sorbitol placed into either chamber *A* or *B*. The diameter of the cell shown was 0.58 mm and the diameter of each chamber was 10 mm. Downward arrow indicates the onset of forward transcellular osmosis, the upward arrow indicates the onset of backward transcellular osmosis. A single black dot (·) represents an isoosmotic change in chamber *A*, a double black dot (:) indicates an isoosmotic change in chamber *B*, and an open dot (o) represents an electrical stimulus that was given to the cell. The upper trace represents the change in membrane potential and the lower trace represents the current pulses given to the cell

stimulus (Table 3). CE does not stop streaming or transcellular water movement by depleting the intracellular ATP levels. The concentrations of ATP found in the DMSO control cells and in cells treated with 30 µg ml^{-1} CE for 35 min are 3.10 ± 0.69 mM (4) and 3.96 ± 0.72 mM (4), respectively. Actually the treatment of cells with CE results in an increase in the intracellular ATP content, presumably by preventing ATP hydrolysis by the actomyosin system.

4. Discussion

Cells of *Chara corallina* and *Nitellopsis obtusa* exhibit a flow rectification or polarity in their hydraulic conductivity (L_p) which was first found in characean cells by KAMIYA and TAZAWA (1956). The exoosmotic resistance (L_{pex}^{-1}) is greater than the endoosmotic resistance (L_{pen}^{-1}). The polarity is enhanced by increasing the transcellular osmotic gradient. This was interpreted to be a consequence of a nonspecific dehydration of the membrane caused by the osmotic solution on the exoosmotic side as suggested by DAINTY and GINZBURG (1964 a) and KIYOSAWA and TAZAWA (1972, 1973) for characean cells and by RICH *et al.* (1968) and by BLUM and FORSTER (1970) for red blood cells (see TAZAWA 1972 for a review). However, the general dehydration effect of osmotic solutions on wa-

Table 3. *The effect of cytochalasin E on membrane resistance (R_m), membrane potential (E_m) and excitability (Excit.) in Nitellopsis*

Treatment	Electrical properties of the membrane		
	R_m (Ω m^2)	E_m (mV)	Excit. (%)
1% DMSO	1.36 ± 0.13 (32)	−180.44 ± 0.44 (32)	80 (25)
30 µg ml^{-1} CE	1.38 ± 0.19 (19)	−185.60 ± 1.79 (15)	80 (10)

Cells were pretreated in 1% DMSO-APW or 30 µg/ml CE-APW for 35 min and the membrane potential was measured by the conventional microelectrode technique. The resistance was measured by applying 0.2 µA square current pulses and measuring the change in membrane potential. Excitability was determined by applying an electrical current of approximately 1 µA to the cells. Each value represents the mean ± SEM. Numbers in parentheses represent the number of experiments

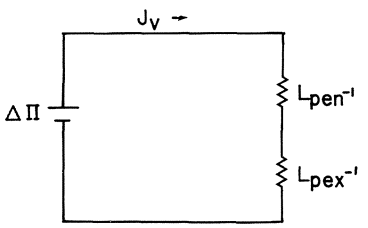

Fig. 11. The equivalent circuit for transcellular osmosis. △ Π: osmotic pressure of the sorbitol solution on the exoosmotic side. L_{pen}^{-1} and L_{pex}^{-1}: hydraulic resistances on the endoosmotic and exoosmotic side, respectively. J_v: rate of water flow

ter movement has been questioned. Using a method that minimizes the perturbation to the cell, FARMER and MACEY (1970) found that the hydraulic conductivity is independent of the osmolarity of the medium and further showed that the data of RICH *et al.* (1968) are consistent with this interpretation if we assume that a rectification of osmotic flow exists. Later, using an NMR method (PIRKLE *et al.* 1979), CHIEN and MACEY (1977) showed that the diffusional water permeability of red blood cells is independent of the osmolarity and concluded that the apparent dependence of the hydraulic permeability on osmolarity is a consequence of an intrinsic cellular polarity.

The polarity of water movement in characean cells also seems to be intrinsic since a polarity exists when the osmotic gradient used to drive transcellular osmosis is only 0.06 MPa. Furthermore, the observations of STEUDLE and ZIMMERMANN (1974) show that a polarity exists in characean cells even when the water movement is controlled by hydrostatic pressure and not by osmotic pressure. The most striking data against the idea that the polarity is a result of a general dehydration of the membrane are the reversal of polarity due to cytochalasins since it seems unlikely that the cytochalasins can act to prevent a membrane dehydration on the exoosmotic side.

The analysis of our results is based on Ohm's Law using an equivalent electrical circuit as an analog (Fig. 11). We have assumed throughout the analysis that the flow of water (J_v) is linearly related to the pressure gradient ($\Delta\Pi$) through the constant of proportionality 1/R. Experimentally we have demonstrated that this is true for osmotic gradients as small as 0.06 MPa. However, the influx of water on the endoosmotic side may remove ions etc. from the protoplasmic surface of the plasma membrane and cause a buildup at the protoplasmic surface of the plasma membrane on the exoosmotic side. A flow of water thus creates a cytoplasmic polarity that in turn may act upon the membrane and differentially create or gate channels on the two sides of the cell. If this be true, L_{pen}^{-1} and L_{pex}^{-1} may act as non-linear resistors. There is evidence that the endoosmotic resistance acts like a non linear-resistor and decreases as a consequence of an increased water flow (Fig. 8)(HAYAMA and TAZAWA 1978, KIYOSAWA and TAZAWA 1973, STEUDLE and ZIMMERMANN 1974). Therefore a linear extrapolation of the data presented in Fig. 9 to zero is unwarranted and we suggest that the polarity in water movement is a consequence of differential cytoplasmic-membrane protein interactions on the endoosmotic- and exoosmotic sides and not the presence of rectifying channels per se in the membrane.

It is generally assumed that water moves through the lipid bilayer by a solubility-diffusion mechanism (ALBERTS *et al.* 1983, FINKLESTEIN 1984). This is supported by observations that water moves through plasma membranes at a rate comparable to the rate of water movement through lipid bilayers (ZIMMERMANN and STEUDLE 1978). The water permeability coefficients of lipid membranes range from 0.2–100 µm s^{-1} (CASS and FINKLESTEIN 1967, FETTIPLACE and HAYDON 1980, FINKLESTEIN 1984, OSCHMAN *et al.* 1974); a range sufficiently large to account for the various rates of water movement in almost all cell types measured. Secondly, the activation energy of water movement across the plasma membrane (42–63 kJ mol^{-1}) is usually similar to the energy of activation of water movement across lipid bilayers (FETTIPLACE and HAYDON 1980, HANSSON MILD and LØVTRUP 1985, TOMOS *et al.* 1981).

Lastly, in *Valonia*, the diffusional water permeability is equal to the osmotic water permeability (2.4 μm s^{-1}) and there seems to be no apparent solvent-solute interactions (GUTKNECHT 1967, 1968), a characteristic of lipid membranes without pores (CASS and FINKLESTEIN 1967). If water moves primarily through a lipid pathway by a solubility-diffusion mechanism in characean cells, it is difficult to understand how water can move in a polar fashion. However if we consider that water can also move through the protein components of the membrane, we can invoke a mechanism for the polar transport of water.

What is the evidence that water can move through channels created by membrane proteins in characean cells? The hydraulic conductivity of characean cells is markedly higher than those of most other plant cells (DAINTY *et al.* 1974, KAMIYA and TAZAWA 1956, BENNET-CLARK 1959, ZIMMERMANN and STEUDLE 1978). In fact it is comparable to the rate of water flow in red blood cells and epithelial cells (DICK 1966). In red blood cells and epithelial cells the osmotic permeability coefficient is substantially larger than the diffusion permeability coefficient indicating that water moves through aqueous pores (FINKLESTEIN 1987). Furthermore in characean internodal, red blood and epithelial cells, the activation energy for transmembrane water movement is comparable to the activation energy for the self-diffusion of water (DAINTY and GINZBURG 1964 a, KIYOSAWA 1975, TAZAWA and KAMIYA 1965, PIRKLE *et al.* 1979, WHITTEMBURY *et al.* 1984), which is smaller than that for water movement across lipid bilayer membranes. This is further evidence that water moves through water-filled channels. Additional evidence comes from the observations of solvent-solute interactions in *Chara* (STEUDLE and TYERMAN 1983). Lastly, inhibitors of protein function have been shown to increase the hydraulic resistance in red blood, endothelial and epithelial cells (BENGA *et al.* 1983, FISCHBARG *et al.* 1987, NACCACHE and SHA'AFI 1974, BROWN *et al.* 1975, LUKACOVIC *et al.* 1984, WHITTEMBURY *et al.* 1984). These studies indicate that the anion channel (band 3) and the glucose transporter may serve as water channels. Although the lipid bilayer may be the sole pathway for water transport in the majority of cells, it is likely that intrinsic membrane proteins as well as the lipid bilayer serve as pathways for water movement in cells with an inherently high hydraulic conductivity.

The intriguing possibility exists that the high hydraulic conductivities are a result of the aggregation of membrane proteins. A kinetic analysis of band 3 proteins, which were purified from red blood cells, and then inserted into lipid bilayers shows that the formation of aqueous channels is a consequence of the formation of tetramers of band 3 proteins (BENZ *et al.* 1984). Indeed the possibility that aggregates of proteins may serve as the water channel was first proposed by PINTO DA SILVA (1973) when he observed during freeze-etch experiments that sublimation of water at −100 °C occurs primarily through protein aggregates. Further support for the hypothesis that water moves through membrane protein aggregates comes from studies on amphibian bladders. Treatment of toad bladders with vasopressin, which decreases their hydraulic resistance to osmotic water flow, induces an increase in the number of protein aggregates observed by freeze fracture electron microscopy (KACHADORIAN *et al.* 1975, PARISI *et al.* 1985). In bladders, cytochalasin B inhibits both the formation of protein aggregates in the membrane and the hormone-induced decrease in the hydraulic resistance (TAYLOR *et al.* 1973, DAVIS *et al.* 1974, PARISI *et al.* 1985) indicating that the actin cytoskeleton is involved in the regulation of the hydraulic resistance. The volume of mouse C3H-2K cells is also slightly influenced by CB (IIDA and YAHARA 1986). The actin cytoskeleton is involved in other membrane events including the polarization of Ca^{2+}-channels in *Fucus* and *Funaria* (BRAWLEY and ROBINSON 1985, SAUNDERS 1986).

Cytochalasin B however also binds to the glucose transporter (LIN and SPUDICH 1974) thus making its site of action obscure. However, CE, which does not bind to the glucose transporter (JUNG and RAMPAL 1977, RAMPAL *et al.* 1980, YAHARA *et al.* 1982), increases the hydraulic resistance in characean cells, indicating that CE as well as CB acts on the actin microfilaments. The greater effectiveness of CE compared with either CA or CB in increasing the hydraulic resistance correlates with its greater effectiveness in inhibiting actin-mediated processes (YAHARA *et al.* 1982). The idea that contractile proteins may influence the hydraulic resistance of the membrane was first proposed by GOLDACRE in 1952. Indeed actin may be involved in the water regulating mechanisms of contractile vacuoles and pinocytosis (DICK 1966) and intracellular water transport (ALLEN and FRANCIS 1965). However it is important to note that CB may not always modulate hydraulic conductivity by interacting with microfilaments. For example CB inhibits water transport in corneal endothelial cells (FISCHBARG *et al.* 1987) by directly acting on the glucose transporter.

In characean cells we have demonstrated that cytochalasins A, B, and E increase the endoosmotic hydraulic resistance during forward transcellular osmosis.

Apparently, the cytochalasin-sensitive pathway does not participate in backward transcellular osmosis. The increase in the endoosmotic resistance during forward transcellular osmosis leads to a loss or reversal of polarity in cytochalasin-treated cells. The loss of the hydraulic polarity occurs in parallel with a loss in the electrical polarity. The sensitivity of the polarity to cytochalasins indicates to us that the polarity must be an intrinsic character of the living cell.

The actin bundles on the ectoplasm/endoplasm interface, which participate in force generation for streaming (KAMIYA 1959, 1981, KURODA and KAMIYA 1956, PALEVITZ and HEPLER 1975) influence membrane transport processes, including OH^- efflux (LUCAS and DAINTY 1977, LUCAS and SHIMMEN 1981) and intercellular rubidium transport (DING and TAZAWA unpublished). However, these bundles seem not to be important in the regulation of hydraulic resistance since stopping streaming by either electrical stimulation or NEM has no effect on the hydraulic resistance. The system of transverse filaments within the cortical cytoskeleton which bind anti-actin or NBD-phallacidin (NOTHNAGEL *et al.* 1981, WILLIAMSON 1985, WILLIAMSON *et al.* 1986) may contribute to the regulation of membrane activities, including water permeability and some electrical phenomena (Fig. 10). WILLIAMSON *et al.* (1986) observed that these filaments were stabilized in the presence of CB. Perhaps a dynamic action or the possibility of fragmentation is necessary for these filaments to regulate membrane properties.

Since an action potential is induced only on the endoosmotic side, and only during forward transcellular osmosis, it seems that something occurs in this localized area of the cell only during forward transcellular osmosis. The cessation of streaming, depolarization, or action potential (HAYAMA and TAZAWA 1978, HAYAMA *et al.* 1979) that occur only on the endoosmotic side and only during forward transcellular osmosis point to an increase in the intracellular $[Ca^{2+}]$ in the endoosmotic side during forward transcellular osmosis, since it is known that the cytoplasmic free $[Ca^{2+}]$ is an important regulatory factor of cytoplasmic streaming (TAZAWA and SHIMMEN 1987, TAZAWA *et al.* 1987). Perhaps a rapid influx of water washes away Ca^{2+} that is bound to the internal surface of the plasma membrane on the endoosmotic side and this results in an increase in the cytoplasmic $[Ca^{2+}]$, which subsequently acts on the microfilaments. A change in the microfilament organization in turn may induce or allow an aggregation of membrane proteins, which results in a decrease in the hydraulic resistance on the endoosmotic side only during forward transcellular osmosis. A change in the hydraulic resistance may also result from a localized change in the intracellular pH, osmolarity or ionic strength on the endoosmotic side.

The membrane proteins with which the cortical actin microfilaments presumably interact still remain unknown. KIYOSAWA and OGATA (1987) provide evidence for the presence of water channels. They showed that the electrical resistance of the cell membrane remains unchanged when the osmotic pressure of the medium is raised even though the hydraulic resistance increases. Although this data indicates that ions can not pass through at least some of the channels that water can pass through, it is still possible that water can move through ion channels (KUKITA and YAMAGISHI 1983). It seem likely that various proteins or aggregates of proteins are able to serve as water channels and open or close upon transcellular osmosis depending on the various ionic activities and osmotic pressure differences that occur in the endoosmotic end exoosmotic cell halves during forward and backward transcellular osmosis (*e.g.*, FINKLESTEIN 1987, ZIMMERBERG and PARSEGIAN 1986). In short, the flux of water, as well as ions, is subject to biological regulation as well as the laws of physics.

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