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Turgor Pressure Sensing in Plant Cell Membranes[1]

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HANS G. L. COSTER
Biophysics Laboratory, School of Physics, The University of New South Wales, Kensington, N. S. W. 2033 Australia

ERNST STEUDLE AND ULRICH ZIMMERMANN
Institut für Biophysikalische Chemie, Kernforschungsanlage GmbH Jülich, D 5170 Jülich, Postfach 1913, Federal Republic of Germany

ABSTRACT

Experimental evidence is reviewed which shows that the cell membrane is compressible by both mechanical and electrical forces. Calculations are given which show that significant changes in the thickness of cell membranes can occur as a result of (*a*) direct compression due to the turgor pressure; (*b*) indirect effects due to the stretching of the cell wall; and (*c*) the stresses induced by the electric field in the membrane.

Such changes in the membrane thickness may provide the pressure-transducing mechanism required for osmoregulation and growth. An important feature of the model is that this pressure transduction can occur not only in the plasmalemma (where there is a pressure gradient), but also in the tonoplast.

The regulation of turgor pressure in plant cells plays a vital role in both cell growth and homeostasis. Although this has been the subject of many studies (1, 2, 12, 14, 15, 17, 19, 25, 27, 29, 31), the mechanisms by which turgor pressure information is sensed and utilized in osmoregulation and growth remain unknown.

Some experimental studies have revealed a relationship between the turgor pressure and membrane potential and resistance as well as a regulating effect on the osmotic pressure of the cell sap and actively transported ion fluxes (13-15, 27, 29, 31, 38).

If one considers the cell membrane as a compressible structure, it seems likely that elastic deformation of the membrane or segments thereof in the plane normal to the membrane might play a role in the dependence of membrane properties on turgor pressure. Dielectric breakdown measurements on plant cell membranes, and particularly, the effect of turgor pressure on dielectric breakdown, have revealed that electromechanical forces operating in the membrane can lead to a reduction in membrane thickness (9, 33, 34).

In this communication, we present considerations of the elastic deformation of cell membranes and its possible role in the turgor-sensing mechanism of plant cells.

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ELASTIC FORCES IN CELL MEMBRANES

Transverse Stresses. The over-all compressibility and dimensional stability of a cell membrane reflects the intermolecular forces operating in this structure. Although details of the molecular architecture and forces operating are still not known, the dimensions (thickness) of the membrane must, nevertheless, reflect external forces operating on it.

When turgor pressure (P_T) is applied to the membrane, internal strain (restoring) forces are set up. For a membrane or membrane segment, which need not necessarily be structurally homogeneous, the mechanical restoring force (P_m), per unit area, is given by

$$P_m = \int_{\ell'=L_0'}^{\ell} Y_m(\ell') \frac{d\ell'}{\ell'} \quad (1)$$

Here $Y_m(\ell')$ is the rate at which the molecular restoring force increases with the change in thickness ℓ', expressed per unit thickness of the membrane, *i.e.* Y_m is the elastic, compressive modulus (subscript m for membrane). L_0' is the initial, *transversely* unstrained thickness, *i.e.* when $P_T = 0$. See Appendix A. ℓ is the final thickness.

From current notions of the structure of cell membranes, it would not be surprising if Y_m was a function of the degree of compression of the membrane. At present, we have no information on any such possible dependence of Y_m on the degree of compression; in order to proceed with the discussion, we assume for the moment that Y_m is constant, and that the membrane has linear elastic properties (*i.e.* obeys Hooke's Law). This allows us to integrate equation 1.

The restoring force is given by

$$P_m = Y_m \ell n \frac{\ell}{L_0'} \quad (2)$$

For very small compressions of the membrane, that is, when $(L_0' - \ell)/L_0' \ll 1$, this yields the more usual expression

$$P_m = -Y_m \frac{\Delta\ell}{L_0'} (\Delta\ell = L_0' - \ell \ll L_0') \quad (3)$$

When the sole externally applied stress is that due to the turgor pressure, P_T, we must have at equilibrium

$$P_T + P_m = 0 \quad (4)$$

and hence, from equation 2,

$$P_T = -Y_m \ell n \frac{\ell}{L_0'} \quad (5)$$

Whether or not significant changes in membrane thickness occur in the physiological range of turgor pressure depends directly on the magnitude of the elastic modulus Y_m.

Longitudinal Stresses. One of the possible ways in which the cell wall may play a part in osmoregulation is through its role in determining any stresses set up in the plane (*i.e.* stretching) of cell membranes when the cell turgor pressure changes. If the plasma membrane is mechanically closely coupled to the cell wall, as appears often to be the case, strains induced in the cell wall could lead to similar strains in the plasma membrane, which, in turn, would lead to changes in its thickness. It has been argued (11) that surface strains could account for the smaller thickness of the supposed lipid bilayer region in cell membranes compared with the thickness of artificial lipid lecithin-cholesterol bilayers. Long range, long time, strains in the plasmalemma might not be necessarily sustained; such strains may tend to disappear by the introduction of additional molecules into the membrane structure.

On the other hand, short range and substantial strains may remain in a membrane when it is mechanically closely connected to the cell wall at intervals equal to a few diameters of the molecular constituents of the membrane, and the cell wall is strained due to the stresses introduced by the turgor pressure. In this case, due to the pinning of the membrane to the wall, there may not be sufficient new space created in every local region to introduce additional molecules.

To determine the exact effect of surface strains in the cell membranes on its thickness, we need to know the detailed mechanical properties of the membrane. As we lack this information at present, we proceed, for illustrative purposes, by making the assumption that the density of the membrane material remains constant under surface strain (*cf.* ref. 28 for lipid bilayer membranes). Before any transverse compressive stresses of any kind are applied, the thickness of the membrane will vary with its area—much like a liquid film.

Under these conditions,

$$AL_0' = A_0L_0 \tag{6}$$

where A_0 is the unstrained surface area (*i.e.* when $P_T = 0$); A is the strained surface area; L_0' and L_0 are, respectively, the longitudinally strained and unstrained thickness of the membrane, in the absence of any transverse compressive stresses (*i.e.* longitudinal stress effects only). See also Appendix A.

The surface area of the cell is related to its volume, which, in turn, is related to the turgor pressure and the elastic modulus of the cell wall. The Philip equation (21) relating these quantities is:

$$v = v_0 \cdot \exp\,(P_T/Y_w) \tag{7}$$

where v is the cell volume; v_0 is the cell volume when $P_T = 0$; Y_w is the cell-volumetric-elastic modulus of the cell wall (subscript w for cell wall).[2]

Assuming a spherical cell, equation 7 can be also written in terms of the radius of the cell. This yields

$$r = r_0 \cdot \exp\,(P_T/3Y_w) \tag{8}$$

where r is the cell radius and r_0 is the radius when $P_T = 0$.

Equations 7 and 8 imply that the elastic modulus of the wall is independent of pressure for a given cell over a large pressure range. This is a rough simplification of the real situation, where Y_w will increase strongly with pressure (30, 32, 38). The assumption of constant elastic modulus will not restrict the general validity of the relations derived, as is shown in Appendix B, where the pressure dependence of Y_w is taken into account.

The exact relation between the cell volume and its surface area depends in a complicated way on the cell shape and the elastic moduli of the cell wall. For a homogeneous spherical cell, the relation is trivial, and the area, using equations 7 and 8, is given by

$$A = A_0 \cdot \exp\,(2P_T/3Y_w) \tag{9}$$

Assuming that the membrane is tightly connected to the cell wall, the strained thickness of the membrane is then given by (equation 6)

$$L_0' = L_0 \cdot \exp\,(-2P_T/3Y_w) \tag{10}$$

Resultant Effect of Turgor on Membrane Thickness. As discussed earlier, the turgor pressure also has an effect on the thickness by direct transverse compression of the membrane material. When the elastic modulus for transverse compression of the membrane is independent of the longitudinal strain, the combined effect of the turgor pressure on the membrane thickness can be obtained from equations 5 and 10. Thus, again assuming a one-to-one relationship between strains in the cell wall and cell membrane,[3] equations 5 and 10 yield

$$\ell = L_0 \cdot \exp\,(-P_T/\gamma) \tag{11}$$

where

$$\frac{1}{\gamma} = \frac{2}{3 \cdot Y_w} + \frac{1}{Y_m} \tag{12}$$

γ, therefore, represents the effective membrane modulus for the cell as a whole, being the fractional rate at which the membrane thickness decreases with increasing turgor pressure. In some cells, for instance in the giant cells of some species of algae, it is possible to obtain a direct measurement of Y_w, the elastic modulus of the cell wall, as well as Y_m.

ELECTROMECHANICAL STRESSES

The presence of an electric field in the membrane, like the field in a parallel plate capacitor, creates stresses, which, like the turgor pressure, will also lead to a compression of the membrane.

The local stress P_e (per unit area) created by the electric field is given by

$$P_e = -\frac{dW_e}{d\ell} \tag{13}$$

where W_e is the energy stored in the field per unit area, given by

$$W_e = {}^1/_2 \int_{x=0}^{x=\ell} \epsilon\epsilon_0 E(x)^2\,dx \tag{14}$$

Here, $E(x)$ is the electric field intensity, at a position x (along the normal to the membrane); ϵ, the dielectric constant; and ϵ_0 the permittivity of free space.

If the field is constant (*i.e.* independent of x) in the membrane or in a segment thereof, and if ϵ is also independent of x, the integral in equation 14 yields

$$W_e = \frac{\epsilon\epsilon_0 V^2}{2\ell} \tag{15}$$

[2] In much of the published literature concerned with the elastic modulus of the plant cell wall, the elastic modulus is denoted by "ϵ." In this paper, however, ϵ is used (in keeping with the usual convention) to denote the dielectric constant of the material. For consistency with our use of Y_m for the transverse elastic modulus of the membrane, we use Y_w for the elastic modulus of the cell wall.

[3] When the strains in the cell wall are not reflected in an identical strain in the cell membrane, equations 10 through 12 are no longer valid. A coupling coefficient should then be introduced and this would modify equations 11 and 12. This is considered elsewhere (33).

where V is the total potential difference across the membrane.

The stress due to the field given by equation 13 is then equal to

$$P_e = \frac{\epsilon\epsilon_0 V^2}{2\ell^2} \quad (16)$$

This stress leads to a mechanical strain. For dimensional equilibrium, we must then have, considering both the turgor pressure and the electrical stress in the membrane,

$$P_T + P_e + P_m = 0 \quad (17)$$

Hence, from equations 2 and 16 at equilibrium,

$$P_T + \frac{\epsilon\epsilon_0 V^2}{2\ell^2} = -Y_m \ell \mathrm{n} \frac{\ell}{L_0'} \quad (18)$$

which, by virtue of equation 10 can be written as:

$$P_T\left(1 + \frac{2Y_m}{3Y_w}\right) + \frac{\epsilon\epsilon_0 V^2}{2\ell^2} = -Y_m \ell \mathrm{n} \frac{\ell}{L_0} \quad (19)$$

Thus, both the turgor pressure, P_T, and the membrane potential, V, affect the thickness of the membrane. Equations 18 and 19 represent a fundamental control process provided that the thickness ℓ of the membrane influences the kinetics of the metabolically driven transport and other osmoregulating reactions.

DETERMINATION OF THE ELASTIC MODULUS FOR MEMBRANE COMPRESSION

No direct, purely mechanical measurements of the elastic modulus for deformation normal to the plane of the membrane have been possible. Some measurements for deformation in the plane of the membrane have been made (16, 22). The likely anisotropic[4] nature of the membrane, however, made these of dubious relevance to our present considerations.

By applying an electromechanical compression, this difficulty can be circumvented in the following way. The stress created by applying a p.d.[5] across the membrane, unlike an externally applied mechanical pressure, can lead to a mechanical instability of the membrane. As can be seen from equation 16, the electrically induced stress increases as the inverse square of the thickness of the membrane, while the internal elastic restoring forces (equation 2) increase only logarithmically with decreasing thickness. The possibility, therefore, arises that for sufficiently large compressions (*i.e.* for sufficiently large electric field strengths), a catastrophic collapse of the membrane in some localized region or segment can take place.[6] This occurs when

$$\frac{\partial P_e}{\partial \ell} = -\frac{\partial P_m}{\partial \ell} \quad (20)$$

At any given pressure it is readily shown (from equations 18 and 20) that this occurs at a critical membrane p.d., V_c, given by (ref. 8):

$$V_c = \left[\frac{0.368\,\ell_0^2 Y_m}{\epsilon\epsilon_0}\right]^{1/2} \quad (21)$$

Here, ℓ_0 refers to the membrane thickness when the electric field is zero (but the turgor pressure is present; see also Appendix A).

It is to be expected that once the critical membrane p.d. is reached and the membrane locally collapses, the membrane resistance would decrease sharply. This indeed has been found, both in studies[7] with intracellular electrodes in large cells such as *Valonia utricularis* (6–8), and with microscopic cells using external electrodes in a Coulter counter and in electrolytic discharge chambers (24, 34–37; see also Appendix C). When the turgor pressure, P_T, is not zero, this also contributes to the decrease in membrane thickness (equations 5 and 10). The turgor pressure will, therefore, affect the critical p.d. required for electrical breakdown. Thus, when the turgor pressure is P_T, the breakdown p.d. is given by (see Appendix A):

$$V_c = V_c\,(P_T = 0)\exp\,(-P_T/\gamma) \quad (22)$$

where

$$\frac{1}{\gamma} = \frac{2}{3 \cdot Y_w} + \frac{1}{Y_m}$$

Thus, measurements of the variation of the electrical potential for breakdown with turgor pressure allow us to calculate a value of γ, the rate at which the membrane thickness decreases with increasing turgor pressure. Methods for making such measurements are outlined in Appendix C. A typical result so obtained on *V. utricularis* is shown in Figure 1.

It is evident from the results shown that turgor pressure does apparently lead to a significant reduction in membrane thickness supporting our notion of the complementary effects of the stresses induced by electric fields in the membrane and those due to the turgor pressure.

A result obtained for *V. utricularis* (Fig. 1) yields a value of $\gamma = 8.5 \times 10^6\ N \cdot m^{-2}$ (85 bar) for the cell membrane. Since the elastic modulus of the cell wall of the cells used for these breakdown measurements was in the order of $3 \times 10^7\ N \cdot m^{-2}$ (300 bar) (see Appendix B), the elastic compressive modulus Y_m is calculated to be about $1.05 \times 10^7\ N \cdot m^{-2}$ (105 bar). The values of γ and Y_m so obtained for several *Valonia* cells were 6 to $7.9 \times 10^6\ N \cdot m^{-2}$ (60 to 79 bar) and 6.9 to $9.6 \times 10^6\ N \cdot m^{-2}$ (69 to 96 bar), respectively (33, 34).

It is not possible with many cell species to introduce microelectrodes or a microcapillary pressure transducer into the cell in order to measure the dependence of V_c on the turgor pressure. However, given the value of the dielectric constant, ϵ, and the thickness, L_0, of the region of the membrane in which breakdown occurs, the elastic modulus Y_m can also be calculated[8] from the breakdown p.d. via equation 21. The elastic moduli, Y_m, so derived for a number of cells, assuming reasonable values of L_0 and ϵ, are listed in Table I.

TURGOR, MEMBRANE THICKNESS, AND OSMOREGULATION

Using the values of the moduli listed in Table I, we are now in a position to calculate examples of the variations in thickness of the membrane, or at least those segments of the membranes where dielectric breakdown can occur, in response to a change in

[4] That is, deformation in the plane of the membrane and normal to the plane of the membrane are likely to be related by different elastic moduli to the deformation stresses. The general description of such a system is quite complex (see ref. 10).

[5] Abbreviation: p.d.: potential difference.

[6] Even if the membrane elastic modulus is not constant, the possibility of a catastrophic collapse remains, provided the mechanical restoring forces increase more slowly than $\frac{1}{\ell^2}$ with decreasing membrane thickness ℓ.

[7] These experiments are done with very short current pulses (100–500 μsec) so that the excursion of the membrane p.d. to the breakdown point is very rapid; otherwise, the cells are destroyed. Breakdown can be achieved in 10 μsec and rapid breakdown, maintained for short periods (~ 1 ms) does not lead to global damage of the cell or its membranes, and the process can be repeated many times on a single cell (6–8; Appendix C).

[8] In using equation 21 with the results of the breakdown potentials, it must be borne in mind that in plant cells, the transcellular current passes through several membranes in series. The breakdown p.d. for individual membranes is thus overestimated, and hence, our values for Y_m are also overestimated. For different reasons, V_c and Y_m are also overestimated in the Coulter counter experiments (34).

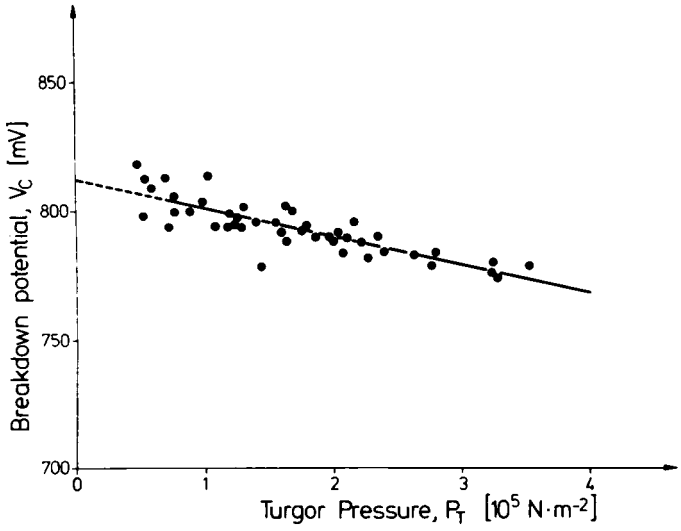

FIG. 1. Variation of the critical breakdown voltage for electrical breakdown on the turgor pressure in a cell of *Valonia utricularis*. The latter was monitored directly with an intracellular pressure transducer (see Appendix C). The curve fitted to the data is a plot of equation 22, which, over this range of pressures, is virtually linear. The denominator in the exponent yields a value of $8.5 \times 10^6\ N \cdot m^{-2}$ (85 bar) of the combined effective "modulus" γ.

the turgor pressure. The expected variation in thickness with turgor pressure, calculated from equation 5, for representative values of the elastic moduli are shown in Figure 2. It is immediately evident that the decrease in membrane thickness with increasing turgor can be relatively large. Thus, for example, from Figure 2, with $L_0 = 9$ nm and $\gamma = 5.0 \times 10^6\ N \cdot m^{-2}$ (50 bar), the membrane thickness decreases at a rate of 1.7 nm/$10^6\ N \cdot m^{-2}$ (0.17 nm/bar). While the membrane thickness would not continue to decrease at this rate with increasing turgor at large turgor pressures, it is obvious that the membrane thickness is very sensitive to the turgor pressure. It should also be noted (see also Fig. 2) that if the elastic constant of the membrane and the cell wall is sufficiently large, say $\gamma > 50 \times 10^6\ N \cdot m^{-2}$, the changes in the thickness of the membrane expected, due to the effect of turgor pressure, become very small ($\Delta\ell < 0.2$ nm for $\Delta P = 10^6\ Nm^{-2}$ (10 bar) with $L_0 = 9$ nm).

It may be argued that the elastic modulus obtained from the measurements with electrically induced stresses, which are generated internally by the field within the membrane, are not relevant to the compression of the membrane by external forces such as turgor pressure. If the membrane is thought of as a largely incompressible fluid, an external pressure applied to such a membrane would not lead to a significant reduction in its thickness.

However, this concept of the membrane is not consistent with the finding that the critical p.d. for breakdown in cells of *V.*

Table I. *Data of Elastic Moduli for Cell Membranes*[1]

The breakdown voltage, V_c, in C.C. experiments was calculated assuming ellipsoidal cell shape and an orientation of the cells with the major axis, parallel to the electrical field. In the E.D.C. experiments, the cells were randomly orientated in the field and had another cell shape (34). The precise values of V_c also depend on such factors as the effective pulse length (34), other time effects in breakdown, and the values assumed for the factors required in the calculations based on the Laplace equation (Pilwat and Zimmermann, in preparation; see also Appendix C, "Using Coulter Counters"). Our estimates of Y_m are, therefore, overestimates (Pilwat and Zimmermann, in preparation). The same is true, for different reasons, in plant cells (see footnote 8). For calculations of Y_m (see equation 21), the dielectric constant of the membrane was taken as $\epsilon = 5$ and the membrane thickness L_0 as 4 and 9 nm, respectively.

Species	Experimental Method	L_0	Elastic Modulus, Y_m, in $10^6\ N \cdot m^{-2}$	Remarks	References for Y_m or V_c
		nm			
Valonia utricularis	E. B. intracellular electrodes	4	5.0		8
		9	1.0		
	Effect of turgor on E.B. voltage		10.5 (From Fig. 1)	Calculated on the basis of tight coupling between cell wall and membrane	33, 34
			8.1 (Average value)		
Ochromonas malhamensis	E.B. in C.C.	4	40.1	Cells exposed to 0.5% NaCl	36[2]
		9	7.9		
Escherichia coli B	E.B. in C.C.	4	17.4		35[2]
		9	3.4		
Bovine red blood cells	E.B. in C.C.	4	13.9		
		9	2.8		
	E.D.C.	4	12.3		24, 34, 35[2]
		9	2.4		
Human red blood cells	E.B. in C.C	4	6.8		
		9	1.3		
	E.D.C.	4	8.5		24, 34, 35[2]
		9	1.7		
Oxidized cholesterol B.L.M.[3]	E.B. films made from decane solution	4	~0.3–0.5	ϵ = 2–3	28
Lecithin B.L.M.	Capacitance *versus* p.d.; "solvent-free" membranes		~10	Effect of solvent lenses taken into account. Later measurements (3) on solvent-free membrane show that such bilayers have very much higher elastic moduli.	23

[1] Abbreviations: E.B. = electrical breakdown; C.C. = Coulter Counter; E.D.C. = electrolytic discharge chamber; B.L.M. = bimolecular lipid membrane.

[2] Shape factors taken from Pilwat and Zimmermann, in preparation.

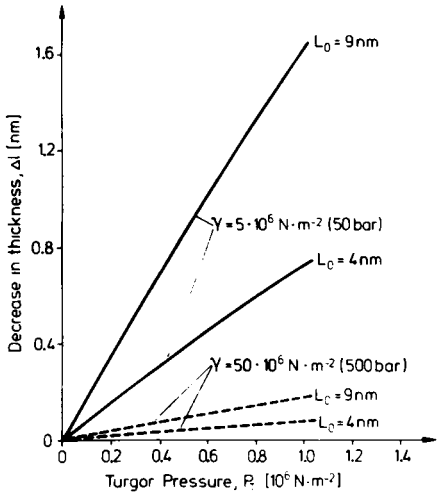

FIG. 2. Calculated variations of the changes in the membrane thickness (in the absence of a membrane potential) in response to changes in the turgor pressure. Curves are shown for values of the effective elastic modulus, $\gamma = (\frac{1}{Y_m} + \frac{2}{3Y_w})$ of the membrane of $5 \times 10^6\ N \cdot m^{-2}$ (50 bar) and $50 \times 10^6\ N \cdot m^{-2}$ (500 bar), for two values (4 nm and 9 nm) of unstressed membrane thickness, L_0. Note that for the smaller values of γ, the absolute values of the changes in membrane thickness are more sensitive to the value of the unstressed thickness, L_0, than at the higher value of γ. With $\gamma = 5.0 \times 10^6\ N \cdot m^{-2}$, the decrease in membrane thickness is very significant for both values of L_0.

utricularis decreases with increasing turgor pressure, a result expected from the electromechanical compression forces discussed in the last section if the membrane thickness decreases as the turgor pressure increases. This, therefore, suggests that the membrane structure, or at least some segments of it, are compressible both by the stresses generated by the internal field as well as the turgor pressure.

It is not difficult to link changes in membrane thickness by compression or stretching to a mechanism of osmoregulation. Evidence has recently been presented that distortion of pores by externally applied pressure in gels containing trapped trypsin and chymotrypsinogen leads to a dramatic increase in the conversion of chymotrypsinogen into chymotrypsin (4).

Similarly, the compression, for instance, of transport modules (5) imbedded in a membrane such as that envisaged in the fluid-mosaic model, would very likely alter the dynamics of the active transport processes. The rate of pumping of ions, and hence, osmotic pressure of the cell sap, could, therefore, be directly controlled in this way by the turgor pressure.

An important consequence of our hypothetical osmoregulating mechanism is that it can operate equally well in the tonoplast. Compression of the membrane should occur also as a result of an increase in the absolute pressure (as well as a pressure difference). An increase in the turgor pressure of the cell will, therefore, affect the thickness of the tonoplast. Thus, while the pressure gradient appears across the plasmalemma and not the tonoplast, the osmoregulation can, nevertheless, be located in the tonoplast.

The fact that both electric fields and the turgor pressure create stresses which compress the membrane means that the osmoregulation mechanism we have proposed will also be sensitive to the membrane potential. The latter is, in turn, of course, also dependent on the concentration of various ions in the cell sap and external solution. Control of turgor pressure by this osmoregulation mechanism may, therefore, be sensitive not only to the total osmolarity, but also to the concentration of specific ions which, in some instances, may not necessarily contribute greatly to the osmolarity of the external solution. For example, a small increase in the external K^+ concentration might reduce the absolute value of the membrane p.d. and, hence, lead to an increase in the membrane thickness similar to that which occurs when the turgor pressure is reduced. A small increase in K^+ may, therefore, compensate, or even overcompensate for a large decrease in the total osmolarity. Some evidence that this occurs in cells of *Chaetomorpha linium* has already been presented (25, 29).

The influence of ion concentration on the rate of active transport of an ion can, of course, be adequately described also in terms of unsaturated carrier mechanisms. Our purpose here is to point out that the membrane p.d. itself may also exert an influence on active transport through the electromechanical compression described.

The model can, of course, readily explain the effect of turgor on the active transport of K^+ in *Valonia ventricosa* reported by Hastings and Gutknecht (15). For small changes in the total atmospheric pressure, these authors did not find an effect on the active transport. For this we would need to assume that in these cells, for small changes in the total atmospheric pressure, the indirect effect of turgor pressure on the plasmalemma and/or tonoplast thickness through in-the-plane stretching must play a dominant role. For very large changes in total atmospheric pressures, there are very significant changes in ion transport (20) as expected from our model.

The model we propose can readily accommodate the results given by Kauss (17–19) for the regulation of isofluoridoside synthesis in *Ochromonas malhamensis*. Kauss has shown that the synthesis of isofluoridoside in this species provides an osmoregulation mechanism. The mechanism requires a pressure-controlled step in the activation of the enzymes necessary for synthesis. It is assumed (19) that the regulation is mediated by the ion transport, which adjusts the ionic composition in the cell at a certain level which, in return, is sensed by the enzymes of carbohydrate metabolism. This would result if the appropriate transport modules (enzymes) in the membrane were subject to compression or stretching that occurs in either the lipid layer, or this module itself as the osmotic pressure of the medium is changed. The higher values of the compressive modulus of *Ochromonas* point to a regulation via stretching, although a quantitative description of the effects of stretching or compression would require more precise values of Y_m of both the plasmalemma and tonoplast, which, at the present, are lacking.

The effect of turgor pressure on the electrical parameters, such as the membrane resistance, can be quite complex (*e.g.* 27, 31). We could speculate on possible explanations for these complex features on the basis of our model. However, as we lack sufficient details of the parameters of the model and the way in which the compression affects the parameters of the biochemical machinery, it is too early to do so at this time.

If, in plant cells, the membrane is pinned into the cell wall, further complications would arise since the elastic properties of the cell wall are also known to be pressure- and volume-dependent (30, 32, 38).

APPENDIX A

Effect of Turgor Pressure on the Critical Breakdown Voltage. With reference to Figure 3 in the derivations, the following symbols will be used: ℓ, thickness of the membrane; ℓ_0, thickness of the membrane when the electric p.d. is zero – turgor pressure present; L_0', thickness of the membrane when no transversely impressed compressive stresses are present in the membrane. The membrane thickness may be reduced, however, due to longitudinal (*i.e.* in-the-plane of the membrane) stresses; L_0, thickness of the membrane when no strains at all are present; V_c, critical breakdown p.d.; V_c ($P_T = 0$), critical breakdown when $P_T = 0$.

From equation 18 at electromechanical equilibrium,

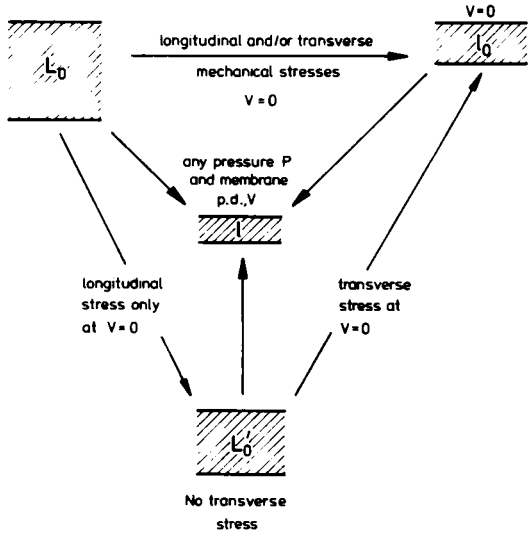

FIG. 3. Schematic diagram of transverse strains in the membrane due to transverse stress induced by the electric field, transverse stress due to the turgor pressure, and the strain due to longitudinal strains induced by stretching of the cell wall. Symbols used for the various thicknesses are also defined in Appendix A.

$$P_T + \frac{\epsilon\epsilon_0 V^2}{2\ell^2} = -Y_m \ell n \frac{\ell}{L_0'} \tag{23}$$

where ℓ is the thickness at the potential V.

Instability in the membrane thickness occurs when

$$\frac{\partial}{\partial \ell}(P_T + P_e) = -\frac{\partial}{\partial \ell} P_m \tag{24}$$

For a given pressure P_T this occurs when $V = V_c$ where

$$\frac{\epsilon\epsilon_0 V_c^2}{\ell^3} = \frac{Y_m}{\ell} \tag{25}$$

At zero membrane potential, the turgor according to equation 5 is given by

$$P_T = -Y_m \ell n \frac{\ell_0}{L_0'}$$

With this equation, the expression 23 yields

$$\frac{\epsilon\epsilon_0 V^2}{2\ell^2} = Y_m \ell n \left(\frac{\ell_0}{\ell}\right) \tag{26}$$

Substitution of equation 25 into 26 at $V = V_c$ then yields the following condition for breakdown:

$$\frac{\epsilon\epsilon_0 V_c^2}{\ell^2}\left(1\text{-}2\ \ell n \frac{\ell_0}{\ell}\right) = 0 \tag{27}$$

$$\ell n \frac{\ell_0}{\ell} = 0.5, \text{ or } \ell_0^2 = \ell^2 \cdot e \tag{28}$$

Equations 27 and 28 with 26 then give

$$V_c^2 = 0.3679 \frac{\ell_0^2 Y_m}{\epsilon\epsilon_0} \tag{29}$$

In equation 29, ℓ_0 represents the thickness of the membrane at any given pressure, but when $V = 0$. Since ℓ_0 is a function of pressure, this thickness is related to the thickness L_0 when no strains are present at all in the membrane (*i.e.* when $V = 0$ and $P_T = 0$) by equation 11 with $\ell = \ell_0$ (*i.e.* when $V = 0$). Thus, substituting equation 11 for the case when $\ell = \ell_0$ the expression 29 can be written as

$$V_c = V_c\,(P_T = 0) \cdot \exp(-P_T/\gamma) \tag{30}$$

where

$$\frac{1}{\gamma} = \frac{2}{3Y_w} + \frac{1}{Y_m}$$

and where

$$V_c^2\,(P_T = 0) = 0.3679 \frac{L_0^2 Y_m}{\epsilon\epsilon_0}.$$

Here, $V_c\,(P_T = 0)$ is the breakdown p.d. when $P_T = 0$ at which point $\ell_0 = L_0 = L_0'$.

APPENDIX B

Effect of the Pressure-dependence of the Elastic Modulus of the Cell Wall, Y_w, on the Membrane Thickness in the Presence of Longitudinal Stresses. If the cell-volumetric elastic modulus of the cell wall is a function of turgor pressure, equation 7 has to be written in its differential form:

$$Y_w(P_T) = v \frac{dP_T}{dv} \tag{31}$$

To integrate the equation, the function $Y_w(P_T)$ must be known. For giant algae cells, $Y_w\,(P)$ represents a saturation curve which can be fitted, to a good approximation, by the following exponential relationship between Y_w and P_T (Steudle and Zimmermann, unpublished data; see also refs. 31, 38):

$$Y_w = Y_w^0 + (Y_w^\infty - Y_w^0)\,[1 - \exp(-\alpha \cdot P_T)] \tag{32}$$

where Y_w^0 is the elastic modulus at zero pressure, and Y_w^∞ the saturation value of the modulus reached at high turgor pressures; α is a constant. Introducing equation 32 into 31 and integrating yields:

$$v = v_0 \cdot \exp(P_T/Y_w^\infty) \cdot \left[\frac{Y_w^\infty - (Y_w^\infty - Y_w^0)\exp(-\alpha \cdot P_T)}{Y_w^0}\right]^{1/(\alpha \cdot Y_w^\infty)} \tag{33}$$

or, with respect to equations 6, 7, and 8:

$$L_0' = L_0 \cdot \exp(-2P_T/3 \cdot Y_w^\infty) \left[\frac{Y_w^\infty - (Y_w^\infty - Y_w^0)\exp(-\alpha \cdot P_T)}{Y_w^0}\right]^{-2/(3 \cdot \alpha \cdot Y_w^\infty)} \tag{34}$$

This may be written as:

$$L_0' = L_0 \cdot F_1\,(P_T) \cdot F_2\,(P_T) \tag{35}$$

where

$$F_1(P_T) = \exp\,(-2P_T/3 \cdot Y_w^\infty)$$

and

$$F_2(P_T) = \left[\frac{Y_w^\infty - (Y_w^\infty - Y_w^0)\exp(-\alpha \cdot P_T)}{Y_w^0}\right]^{-2/(3 \cdot \alpha \cdot Y_w^\infty)}$$

Equations 33 and 34 differ from equations 7 and 10 by an additional factor which incorporates the pressure dependence of Y_w. The influence of this factor on the determination of γ (according to equation 7) will depend on the magnitude of α (*i.e.* on the initial slope of the $Y_w(P_T)$-curve) and on the ratio Y_w^∞ to Y_w^0.

For giant algae cells such as *Valonia*, the turgor pressure required to reach half of the value of $(Y_w^\infty - Y_w^0)$ is of the order of $0.05 \times 10^6\ Nm^{-2}$ (0.5 bar) or less, and thus, α will be of the order of $0.1 \times 10^6\ Nm^{-2}\ (1\ \text{bar})^{-1}$. On the other hand, Y_w^0 and Y_w^∞ will be about $3 \times 10^6\ Nm^{-2}$ (30 bar) and $30 \times 10^6\ Nm^{-2}$ (300 bar), respectively.

Taking these values, it can be easily shown that the determination of the effective membrane modulus, γ, (equation 2, see Fig. 1) will be influenced only at pressures smaller than 0.1×10^6

Nm^{-2} (1 bar). At higher pressures, the slope of the $V_c(P_T)$ curve will be determined by Y_w^∞ and Y_m.

For simplicity, in this paper, we have referred to the value Y_w^∞.

APPENDIX C

EXPERIMENTAL DETERMINATION OF THE BREAKDOWN POTENTIAL V_c

Using Intracellular Electrodes. These experiments can be done with large cells such as those of the marine algae *V. utricularis*. For the experiments, cells are mounted in a Ag/AgCl grid which serves to fix mechanically the cell as well as the external current electrode. Current pulses are injected into the cell via a platinum/iridium (75% Pt, 25% Ir) microwire electrode introduced into the cell, through a glass micropipette with a tip diameter of ~ 5 μm, which is previously manipulated into the cell. The membrane p.d. is measured with intra- and extracellular micropipettes filled with 2 N KCl and a high impedance electrometer with capacitance neutralization.

The current pulses and responses of the membrane potential are displayed on a dual channel storage oscilloscope. To determine the breakdown p.d., a series of short current pulses (500 μsec), of increasing magnitude, are injected into the cell, and the response in membrane p.d. is measured. A typical pulsed I-V curve is shown in Figure 4.

Note that at a critical potential of 0.85 v, the current increased dramatically. At this critical potential, an increase in the current pulses (which can be achieved by adjusting the output potential of the pulse generator) did not lead to any significant change in the membrane potential response. An important feature of the breakdown phenomenon is that it does not appear to be accompanied by any global damage either to the cell or its membrane. The process may be repeated many times on a single cell with virtually identical results (see also 6–8).

To measure the variation of V_c with turgor pressure, a further micropipette filled with a silicon oil is introduced into the cell (26, 31). This micropipette is connected, via a pressure-tight seal, into a chamber also completely filled with oil. The boundary between oil and cell sap formed in the microcapillary tip can be adjusted by the insertion of a thin metal road, via pressure-right seals, using a micrometer screw. In this way, the pressure in the cell can be measured avoiding errors due to leakages in the apparatus and oil compression. The actual pressure is monitored by a small solid state pressure transducer also mounted in the oil chamber. When the pressure in the cell is suddenly increased, by changing the volume of the oil chamber communicating with the micropipette, using the micrometer screw, bulk water flows are induced through the cell membrane. The pressure, therefore, relaxes back with a time constant which depends on, among other things, the elastic modulus of the cell wall Y_w, which can be also determined with the equipment (26, 31, 32). To maintain a constant turgor pressure over longer periods, so that several measurements of V_c are possible, the osmotic pressure of the external seawater is varied, the actual turgor pressure, however, being measured with the micropipette pressure transducer.

Using Coulter Counters. In this method, cells are drawn through a capillary orifice connecting two compartments. A potential is applied between two electrodes placed in these compartments. When a cell is in the capillary tube, this causes a decrease in the current flowing between the two electrodes. A pulse height analysis of the current as cells are rapidly drawn through the capillary then yields a size distribution of the cells. When the potential applied between the two electrodes is increased, all of the current pulses increase in size. The nominal modal size of cells so obtained as a function of the potential between the two electrodes shows a sharp discontinuity which results from the electrical breakdown of the cells as they traverse the capillary. From the geometry of the capillary and cells, and the potential between the electrodes, the potential difference across the cell membrane at breakdown can then be calculated using Laplace's equation for the current flow distribution in conducting media (34–36; Pilwat and Zimmermann, in preparation).

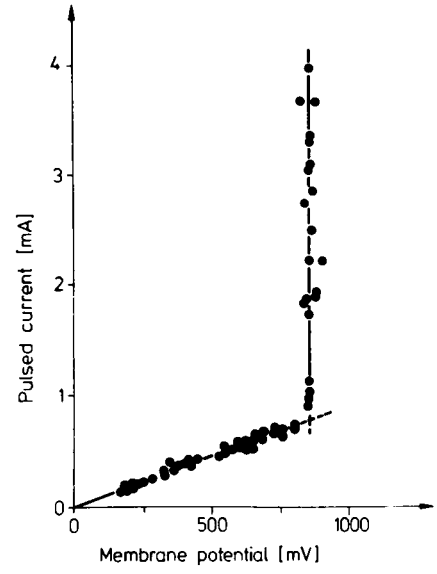

FIG. 4. Response of the membrane potential difference, inside (positive) with respect to the external solution, as a function of (pulsed) current for a cell of *Valonia utricularis*. The current was injected in 500 μsec pulses. Note the discontinuous decrease in membrane resistance at a membrane p.d. of 0.85; the cell could not be polarized beyond this value with such short current pulses. The results shown here were obtained with a single cell; the breakdown did not lead to any global damage of the cell, and the critical breakdown p.d. was reproducible. The resting p.d. of this cell was about +5 mv (inside with respect to outside).

Using Electrolytic Discharge Cells. In this method, cells are suspended in a solution between two planar electrodes (distance 1 cm). A short (~ 20 μsec) high voltage pulse (10–20 kv) is then applied to the electrodes. The high electric field during the pulse leads to an electrical breakdown of the cells provided that a critical field strength is exceeded. Breakdown is detected, in the case of red blood cells, by a loss of hemoglobin and/or a loss of K^+ and an uptake of Na^+ (24, 34, 35).

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