Effect of Turgor Pressure and Cell Size on the Wall Elasticity of Plant Cells[1]

Ernst Steudle and Ulrich Zimmermann
Institut für Biophysikalische Chemie, Kernforschungsanlage Jülich GmbH, Postfach 1913, D-5170 Jülich, FRG

Ulrich Lüttge
Fachbereich Biologie der Technischen Hochschule, Darmstadt, Schnittspahnstr. 3-5, D-6100 Darmstadt, FRG

ABSTRACT

Direct measurements of the volumetric elastic modulus, ϵ, of cells of a higher plant were performed on the epidermal bladder cells of *Mesembryanthemum crystallinum* using a pressure probe technique. Measurements on giant algal cells (*Valonia, Nitellopsis*) are given for comparison. Giant celled algae and *M. crystallinum* bladders have elastic moduli, ϵ, which depend strongly on turgor pressure, P, and on cell volume, V. The ϵ values of *Mesembryanthemum* bladders range between 5 bar at zero pressure and 100 bar at full turgor pressure (3-4 bar). ϵ increased with cell size (volume) at a given turgor pressure, and this volume dependence was pronounced more in the high pressure range. From the $\epsilon(P)$ characteristics, complete volume-pressure curves were obtained for *Mesembryanthemum* bladders and giant algal cells. The results suggest that the $\epsilon(P)$ and $\epsilon(V)$ characteristics of all plant cells are similar. The significance of the pressure and volume effects for the water relations and growth processes of plant cells is discussed briefly.

Many attempts have been made to determine water permeability of plant cells and the main pathways of water transport within a tissue (see refs. 7 and 17); however, little attention has been paid to the elastic properties of cells and tissues, although in controlling water relations and water economy of plants they are as significant as the water permeability of the cell membrane. The experimentally determinable cell wall parameter connecting changes in cell turgor pressure (dP) with the corresponding fractional changes in cell volume (dV/V) is the so-called volumetric elastic modulus, ϵ, (7, 11):

$$\epsilon = V\frac{dP}{dV} \simeq V_{ref}\frac{\Delta P}{\Delta V} \qquad (1)$$

It is shown schematically in Figure 1 that ϵ is calculated from the slope of the volume-pressure curve of a plant cell at a certain turgor pressure and the cell volume at this pressure. For most plant cells, it is valid that $\epsilon \gg P$, and the cell volume, V, will change only by a few per cent over the whole pressure range. Therefore, to a good approximation, ϵ can be estimated from equation 1 by using a constant reference cell volume, V_{ref}, instead of V. The slope of the V-P curve decreases with increasing pressure (Fig. 1). This means that the elastic modulus is a function of turgor pressure and increases with increasing pres-

sure (19, 20). As a consequence, ϵ can be determined only in small pressure intervals, where the V-P curve is assumed to be approximately linear. It should be noted that it is very difficult to obtain V-P curves of plant cells experimentally with the sensitivity required.

One possibility for measuring ϵ in higher plants was provided by the pressure bomb technique of Scholander *et al.* (12), in which twigs cut from higher plants or entire plant organs are used and over-all elastic moduli of the sample under investigation are yielded. However, with smaller samples such as individual leaves, the pressure bomb technique usually works only over large pressure intervals (2), although ϵ is a strong function of pressure (3, 9, 19, 20). A further complication which limits the determination of ϵ of individual cells from the volume-pressure relation of a tissue is the volume dependence of ϵ found 2 years ago for algal cells by us (19, 20) and reported here for *single* higher plant cells. Recently, a volume dependence of ϵ has been also suggested by Cheung *et al.* (2) for higher plant cells, but no experimental evidence was presented by these authors, because of the experimental limits of the pressure bomb technique described above. Therefore, the interpretation of pressure-volume curves of plant tissues with respect to the cell elastic moduli has to be cautious, and it is important to determine the moduli of cell wall elasticity directly.

With the pressure probe technique developed by Zimmermann and Steudle (13, 19), it is possible to measure small changes in turgor pressure in response to small fractional changes in volume of giant algal cells and thus work out this significant parameter determining water relations. In a similar way, the hydraulic conductivity L_p of the giant epidermal cells of the higher plant *Mesembryanthemum crystallinum* has been determined (15).

Here, we report ϵ measurements of these cells and compare them with those of giant algal cells (*Valonia, Nitellopsis*). The epidermal bladder cells of the halophyte *M. crystallinum* were chosen because their large size allows ready insertion of the pressure probe. Although these epidermal bladders are not cells *within* a tissue, first measurements of ϵ in higher plant cells are considered an important step toward a model resolving water relations of higher plant tissues. These measurements are also of great significance in all pressure-driven processes that occur in plant cells, such as osmotic regulation of ion transport (22) and extension growth.

MATERIALS AND METHODS

Materials. Stem bladder cells of *M. crystallinum* plants which were grown for 2 to 4 months in the greenhouse in a mixture of
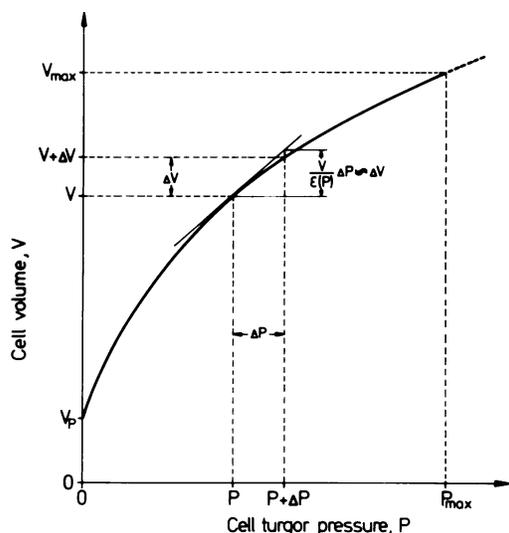
FIG. 1. Schematical representation of a typical volume-pressure curve of a plant cell. Cell volume increases with turgor pressure from the plasmolytic point (V_p) to a maximum pressure, V_{max}, which will be reached if the cell is placed in distilled H_2O. From the slope of the curve at a given pressure and from the cell volume at this pressure, the elastic modulus, ϵ, of the cell can be calculated according to equation 1.

soil-sand, 1:1, have been used (15). Cells selected for pressure and elasticity measurements were elliptically shaped. The length (1.1-2.7 mm), the width (0.5-1 mm), and the height (0.6-0.9 mm) of cells used in the experiments were measured under the magnification of a stereo microscope. Cell volumes (0.3-1.2 μl) were calculated from these measurements by treating the cells as semiellipsoids. The error in the determination of cell volumes was in the order of 10 to 15% at most. Cells at the upper end of the cell volume range were taken from plants which were watered regularly with 40 mM NaCl in a nutrient solution (15), because plants had larger bladder cells when they were grown in the presence of salt.

Nitellopsis obtusa was grown in artificial pond water (1 mM NaCl; 0.1 mM KCl, CaCl₂, and MgCl₂, respectively) from plants originally obtained from a pond near Strasboürg. Adult internodal cells were used in the experiments that varied between 47 and 244 mm in length at a constant cell diameter ($\emptyset$ = 0.86-1.03 mm). Prior to the measurement, internodes were freed from branches and adjacent internodes and fixed in a small chamber (14). Turgor pressure was changed by varying the osmotic pressure of the medium (addition of sucrose). Similarly, the measurements on the marine alga *Valonia utricularis* were performed as already described previously (22).

ϵ **Measurements.** Measurements of ϵ and turgor pressure were performed using the pressure probe already described (13, 19). The procedures and experimental conditions presented in previous papers were followed (13-15, 19, 20). The diameter of the probe tip was 10 to 20 μm for measurements on *Mesembryanthemum* and about 60 μm for giant algal cells. For the smaller cells of *Mesembryanthemum* in the range of high turgor pressure (*i.e.* at high ϵ), the measured volume changes were corrected for the compressibility of the oil in the pressure chamber connected with the microcapillary. This was performed by measuring the response to small volume changes with the tip of the capillary sealed off with resin.

ϵ was determined in small pressure intervals (0.1-0.5 bar) from full turgor to zero turgor pressure by measuring changes in turgor pressure (ΔP) in response to small changes of cell volume (ΔV) with the aid of the pressure probe. To estimate ϵ according to equation 1, the cell volume was taken as constant over the whole pressure range, and V_{ref} was approximated by the cell

volume at full turgor pressure. From the $\epsilon(P)$ curves obtained in this way, the $V(P)$ curves of the cells were estimated by stepwise graphical integration of the differential equation, which defines ϵ (equation 1).

RESULTS AND DISCUSSION

Figure 2 shows a comparison of $\epsilon(P)$ curves of two giant algae species and *M. crystallinum* bladder cells at a large range of cell volumes. The general characteristics of the volume and pressure dependence of ϵ are similar in all cases shown in the figure. Quantitative differences between *M. crystallinum* and algal coenocytes are: (*a*) the lower absolute value of ϵ in *M. crystallinum*; and (*b*) the fact that a constant value of ϵ is not reached normally at the highest turgor pressure found in the bladder cells (3-4 bar in cells grown at low salt conditions). In the pressure range where ϵ becomes independent of pressure, equation 1 can be integrated to give the $V(P)$ curves in this range. The integration yields:

$$V = V_{ref} \cdot exp \ (P \text{-} P_{ref}/\epsilon) \tag{2}$$

where V_{ref} is the cell volume at a reference pressure, P_{ref}. Usually the term $P \text{-} P_{ref}/\epsilon \ll 1$ and, therefore, to a good approximation, equation 2 can be rewritten as:

$$V = V_{ref} + \frac{V_{ref}}{\epsilon} (P \text{-} P_{ref}) \tag{3}$$

From equation 3 it follows that in the pressure ranges with constant ϵ, there will be a linear relationship between cell volume and pressure.

Conversely, in all other cases, the $V(P)$ curves are obtained only by graphical integration from the $\epsilon(P)$ curves. The results of this integration for different cell volumes of the species investigated are shown in Figure 3. The extrapolation of the $V(P)$ curves to zero pressure gives the relative volume of the cells at the point of plasmolysis. The relative changes in cell volume from full turgor to plasmolysis strongly depend on cell volume. The largest volume changes (up to 15% at a pressure difference of 3 bar) are in the smaller bladder cells of *M. crystallinum*, while for the internodes of *Nitellopsis obtusa*, the volume change (at a pressure difference of 8 bar) is only of the order of 2 to 4%.

A dependence of ϵ on cell volume or cell size as shown in Figure 2 is at the first view difficult to explain theoretically. For cells with a cylindrical shape such as *Characean* internodes or cells with an approximately spherical shape like *Valonia* or *Mesembryanthemum* bladders, ϵ should be proportional to the ratio of cell wall thickness to cell diameter and should be also dependent in a complicated manner on the Young's moduli and Poisson ratios of the cell wall (1), which in turn should be pressure dependent. The opposite finding of an increase of ϵ with increasing cell diameter (assuming constant wall thickness), as was found for *Valonia* cells (19, 22), is unexpected. Similar results were obtained with *Nitella* internodes, where, at constant diameter, ϵ was found to increase with increasing length (volume) (20). The problem of this volume dependence of ϵ was solved for *Nitella* and *Valonia* by a detailed analysis which suggests that there are at least two different cell wall regions that have different elastic properties due to alterations in the wall structure or thickness and hence different elastic constants (19, 20). This analysis shows that in the range of low volumes there is a linear relationship between volume and ϵ. The same was found for *M. crystallinum* as shown by Figure 4 which summarizes all individual measurements obtained at different turgor pressures. It is seen from the figure that the volume dependence of ϵ is the greatest at high pressures (for the giant algal cells, see Fig. 2).

If the results, obtained for cells as different as algal coenocytes and *M. crystallinum* bladders, may be generalized, then it follows that all turgor-driven processes should be also volume-
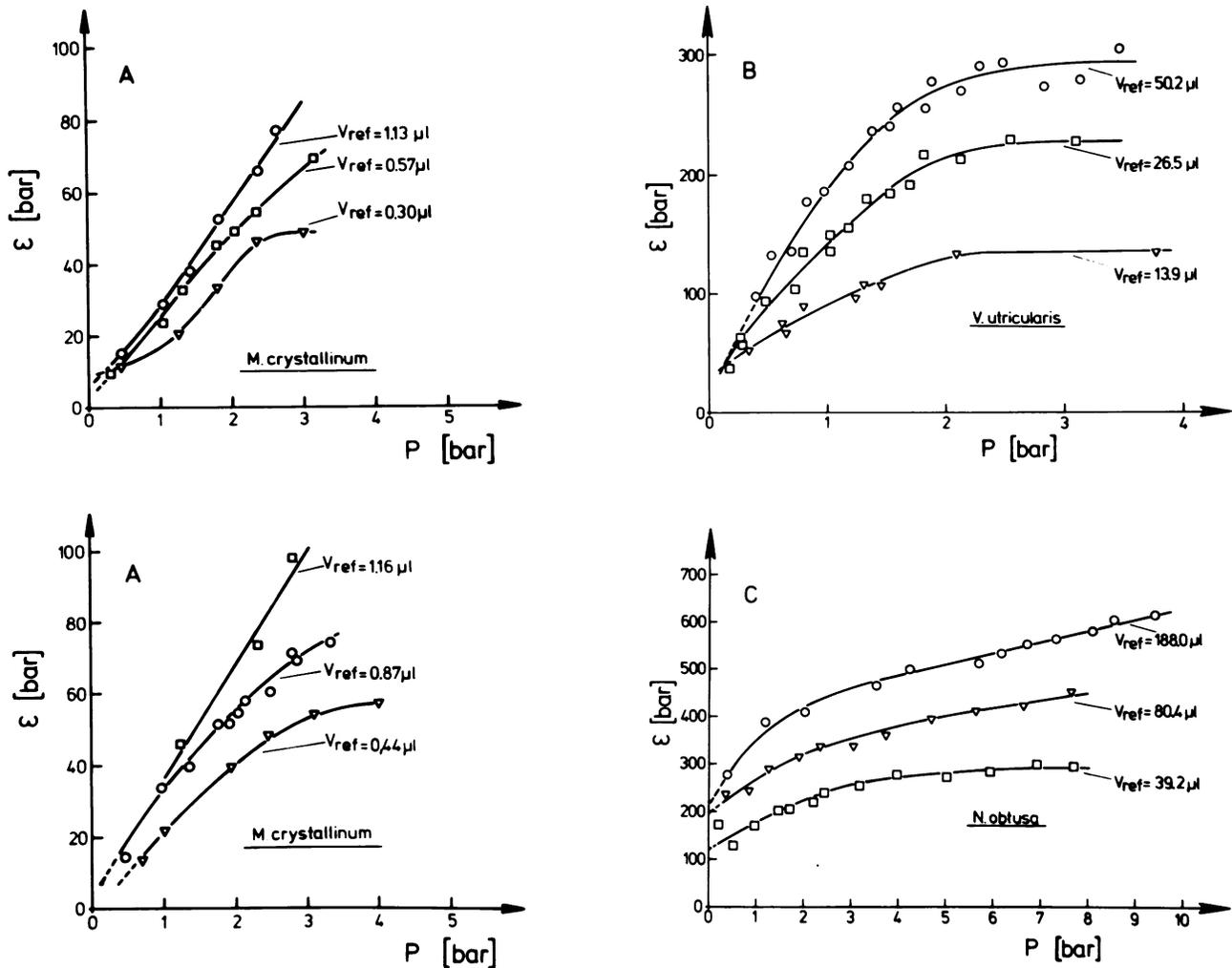
Fig. 2. Volumetric elastic moduli, ϵ, of M. crystallinum bladder cells (A) and of cells of two giant algae coenocytes (V. utricularis [B] and N. obtusa [C]) are shown in dependence on turgor pressure for cells with different cell volumes, V_{ref}. ϵ values were determined for small pressure intervals (0.1–0.5 bar) and plotted against the midsts of these intervals.

dependent, and thus the volume dependence should affect osmoregulation, water transport, and growth processes in plant cells.

The significance of M. crystallinum bladders as large salt and water storage compartments in osmotic adjustment of this species was discussed earlier (15). M. crystallinum is a halophilic species highly adaptable to a wide range of salinity in its root medium, growing well at 0 mM and up to about 600 mM NaCl. The epidermal bladders appear to function as a large external water and salt storage compartment (comprising up to 25% of the total volume of the plant) protecting the much smaller metabolically active cells in short term water stress. It has been argued earlier (15) that a fast loss of water from the bladder cells is facilitated by their high hydraulic conductivity ($L_p = 2 \cdot 10^{-6}$ cm$\cdot s^{-1} \cdot$bar^{-1} [15]). The ϵ values which range from 10 to 100 bar are another important parameter in water exchange. By the volume dependence of ϵ, an additional advantage of the large size of the bladders becomes clear. Since the volume dependence of ϵ compensates for the effect of an increasing volume to surface area ratio, the bladder compartment may be enlarged without affecting the availability of water. Quantitatively, this relationship is explained best by an equation which applies to an isolated (semipermeable) plant cell as follows:

$$T_{1/2} = \frac{0.693}{L_p} \frac{V/A}{(\epsilon + \pi)} \qquad (4)$$

This equation relates the half time, $T_{1/2}$, of osmotic swelling and shrinking of a plant cell to cell parameters such as the volume (V), the area (A), the hydraulic conductivity (L_p), the internal osmotic pressure (π), and ϵ. It may be used here to estimate the water exchange between a bladder cell and the underlying tissue, because the storage capacity of the tissue compartment (or of parts of it), for water should be high (15). Taking the different volume to (basal) area ratios of bladder cells of different size, their hydraulic conductivity ($L_p = 2 \cdot 10^{-6}$ cm$\cdot s^{-1} \cdot$bar^{-1} [15]), and corresponding ϵ values, the half-times of water exchange are calculated for different turgor pressures. The result is that the rates of exchange are practically independent of the cell size (at least for high turgor pressures) although the value of ϵ is doubled in this range (Fig. 5). This clearly demonstrates the regulatory function of the volume and pressure dependence of ϵ in the supply of water from bladders of different size.

The question concerning the structural basis of the volume dependence of ϵ in the bladder cells remains open. One possible explanation is the assumption of cell wall regions of different elasticity as was postulated for giant algal cells (see above). Another, perhaps more likely one, is a stress-hardening effect within the cell wall due to the tension. The tension (T) in the wall of a spherical cell is given by:
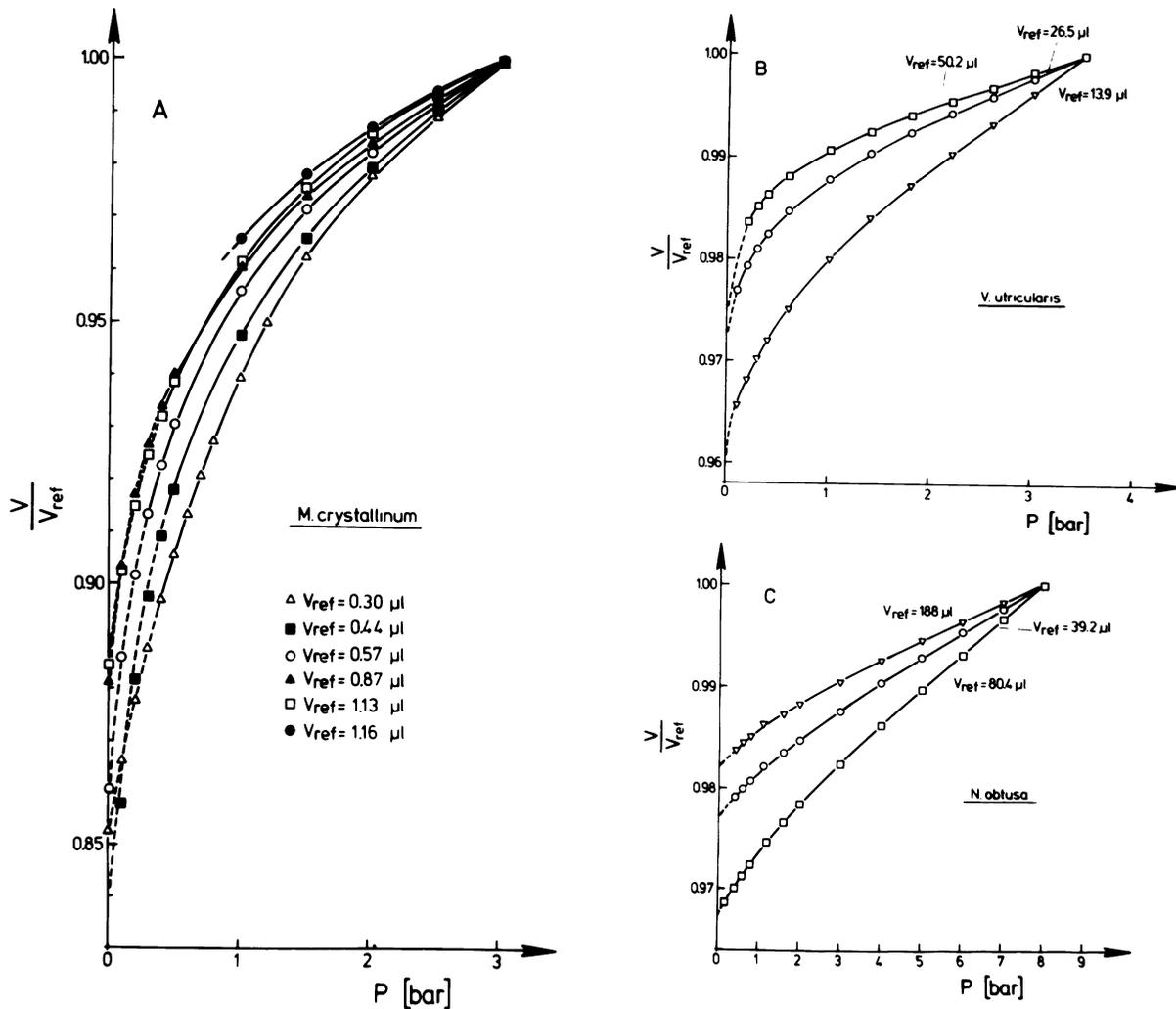
$$T = \frac{P \cdot r}{2d} \qquad (5)$$

FIG. 3. Volume-pressure curves of the plant cells shown in Figure 2. Relative changes of cell volume are given with fully turgid cells as a reference. The curves were obtained by graphical integration of the $\epsilon(P)$ characteristics in Figure 2.

where d is the wall thickness and r is the radius of the cell.

From the pressure dependence of ϵ, we know that the Young's moduli of plant cell walls strongly increase with increasing wall tension. Therefore, since cells with a larger diameter exhibit a higher tension in the wall at a given turgor pressure (equation 5), their Young's moduli (and ϵ values) must be higher.

By contrast to the kinetics of the swelling or shrinking of the M. crystallinum bladders, the relative volume changes of each bladder cell when the steady state has been reached depend on the cell volume. At water flux equilibrium, the changes in the water potential of the bladders are equal to that in the adjacent tissue (subscript t). It would be valid that:

$$\Delta P - \Delta\pi = \Delta P_t - \Delta\pi_t \tag{6}$$

Using equation 3 and assuming that the cells behave like ideal osmometers, this can be written as:

$$\epsilon \cdot \frac{\Delta V}{V} + \pi \cdot \frac{\Delta V}{V} = \Delta P_t - \Delta\pi_t \tag{7}$$

or

$$\frac{\Delta V}{V} = \frac{\Delta P_t - \Delta\pi_t}{\epsilon + \pi} \tag{8}$$

From equation 8, it follows that small cells exhibit much stronger elastic changes of cell volume (and surface area) than larger ones. This should be significant for extension growth, if it is true that stretching of the cell wall is a precondition for the initiation and termination of growth processes (4, 5, 21). Furthermore, an increase of cell wall rigidity with increasing cell volume may also point to a lower cell wall plasticity, and thus the growth rate may be also retarded with increasing cell volume.

The volume dependence of the elastic cell wall stretching can be reasonably high. For Mesembryanthemum bladders in the pressure range between 2 and 3 bar, the elastic extension is doubled when the cell volume decreases by a factor of 4 (Fig. 3). Large cells require much higher changes of cell turgor pressure to reach a certain amount of cell wall stretching which is necessary to initiate extension growth, than smaller ones, and therefore, growth may be slowed as cells increase in volume. We may postulate that the volume dependence of the elastic wall stretching is one of the reasons why cells in plant tissues reach similar sizes during extension growth.

The similarities of the $\epsilon(P)$ and $\epsilon(V)$ characteristics obtained for giant algal coenocytes and the bladder cells of the higher plant M. crystallinum support the contention that these relationships also apply to cells within tissues of higher plants. There are some indications from psychrometric and pressure bomb meas-
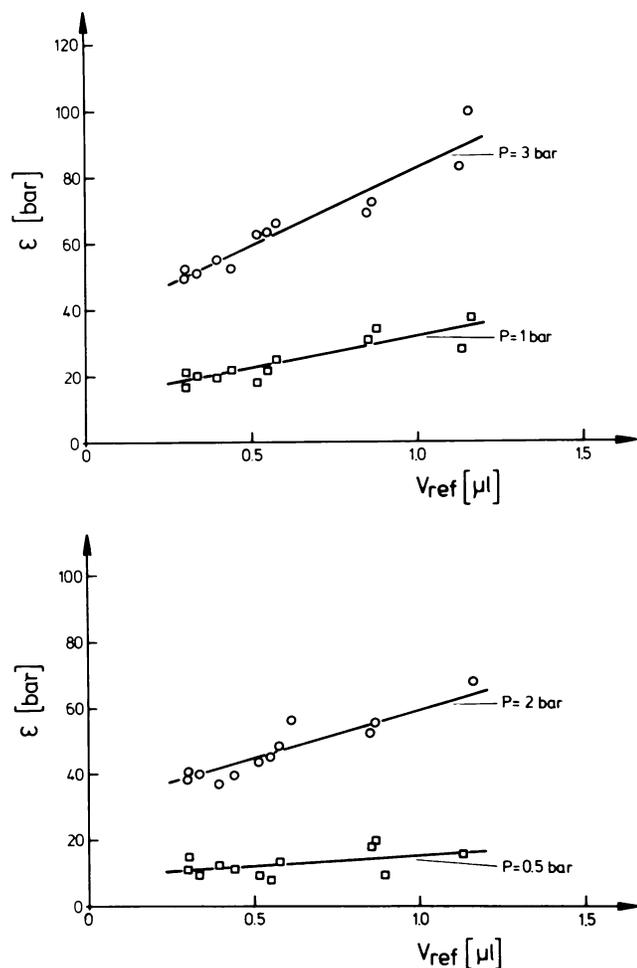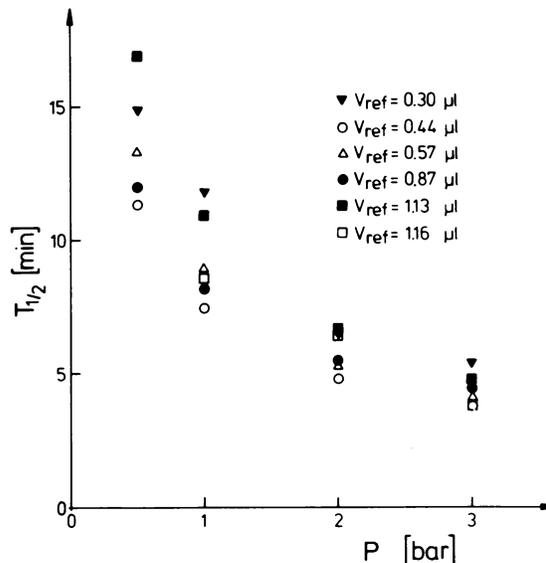
FIG. 5. Half-time $T_{1/2}$, of water exchange between bladder cells and underlying tissue in *M. crystallinum* bladder cells as a function of turgor pressure for cells of different cell volume (same cells as in Fig. 2). No dependence of the exchange rate on the cell size (volume) is observed. For calculation of $T_{1/2}$ (equation 4), the osmotic pressure of the bladder sap, π, was taken as 10 bar.

FIG. 4. Volume-dependence of the elastic modulus, ϵ, of *M. crystallinum* bladders at different turgor pressures (0.5, 1, 2, and 3 bar) obtained from $\epsilon(P)$ curves such as in Figure 2.

urements on plant tissues (3, 8–10, 16, 18 and review by Dainty [7]) that at least the pressure dependence of ϵ in a whole tissue is the same as that of the systems we have studied. Caution is needed when applying our results to cells *within* a tissue, since tissue pressure and elastic and plastic interactions between the cells may affect the elastic properties of individual cells.

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