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Effect of Turgor Pressure on Water Permeability of *Allium cepa* Epidermis Cell Membranes

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Summary. Using onion epidermis layer a very accurate method for measuring the permeability of epidermis cells to water was standardized. In this method a 1.4 cm diameter epidermis disc was soaked in tritiated water (500 μCi/ml) for about 1 hr. Next the disc was mounted in a specially designed elution chamber where it was held flat and washed on the noncuticular side with ordinary water. A constant flow rate, high enough to minimize unstirred layer effect, was used. Permeability was calculated in the usual way after separating different exponentials from the efflux curve of tritiated water. Turgor pressure of the cell was regulated by soaking the disc in mannitol solutions containing tritiated water and washing it in the chamber with same concentration mannitol solution containing no radioactivity. Water permeability values were found to decrease less than 8% when the turgor pressure was decreased from 8 atm (full turgor) to zero. Turgor pressure had no significant effect on the water permeability of onion epidermal cells. Our results are contradictory to the findings of Zimmerman and Steudle (1974, *J. Membrane Biol.* **16**:331) but are similar to the findings of Tazawa and Kamiya (1966, *Aust. J. Biol. Sci.* **19**:399) and Kiyosawa and Tazawa (1972, *Protoplasma* **74**:257).

In hypotonic extracellular media a plant cell is under positive turgor pressure as a result of the presence of osmotically active solutes inside the vacuole. This pressure decreases in response to increasing concentration outside the cell and it becomes zero at the isotonic concentration, when the cell reaches the state of incipient plasmolysis. The effect of this decrease in turgor pressure on the water permeability[1] (K_w) has been studied in cells of various species of algae. Dainty and Ginzburg (1964) found a decrease in L_p in *Chara australia* and *Nitella translucens* with an increase in external sucrose concentration of up to 0.5 molal.

Similar results were reported by Tazawa and Kamiya (1966) for *Nitella flexilis* using mannitol solutions in a detailed and comprehensive

[1] We have used this term here instead of "hydraulic conductivity (L_p)" since "water permeability" better reflects the control function of the membrane (*see* Jacobs, 1952; p. 152).

study. When the turgor pressure of the cell was lowered to about 0.20 to 0.35 atm the water permeability value was reduced to about 80% of its original (highest turgor) value. In two cases the permeability value dropped to only 71% when the turgor pressure was lowered close to zero. These authors (Dainty & Ginzburg, 1964; Tazawa & Kamiya, 1966) explained this decrease in water permeability in terms of the effect of these tonicities on the hydration of the cell membranes and the cytoplasm. Again Kiyosawa and Tazawa (1972) obtained similar results in the case of Characean cells.

In contrast to this earlier work Steudle and Zimmermann (1974) claimed that as the turgor pressure of *Nitella* cells was decreased below 1 atm the hydraulic conductivity (L_p) increased markedly. The measurements were made after directly changing the hydrostatic pressure inside the cell; cells were punctured and transferred to artificial pond water for about 10 hr resulting in a drop of turgor pressure of the cell to 0.5 to 1 atm. It is assumed that this decrease is due to leakage of the electrolytes from the cell. With these punctured cells the authors found an increase in L_p to about 2 times compared to the L_p value for normal cells above 2 atm turgor pressure.

Again Zimmermann and Steudle (1974) reported an increase in L_p at turgor pressures below 0.5 atm in the case of *Valonia utricularis*. From their results they believe that this increase in L_p at low turgor pressure is due to folding of the membranes. In their more recent work Zimmermann and Steudle (1975) made some L_p measurements on the cell walls of *Chara* and *Nitella*. From this work they concluded that at incipient plasmolysis the L_p of the cell membrane increases so much as to exceed even the L_p of the cell wall alone, indicating that as the turgor pressure approached zero the cell wall becomes the rate limiting barrier for water flow. This finding leaves one to speculate whether these authors are dealing with living cells.

There seems to be little work done yet on the effect of extracellular concentration and turgor pressure on the L_p of the higher plant cell membranes (Steudle, Luettge & Zimmermann, 1975). The present study was undertaken to determine this effect in other higher plant cells and to compare it with the results obtained with algal cells. Another purpose was to interpret the present controversial observations on the effect of turgor pressure on water permeability.

Materials and Methods

The inner epidermis of Yellow Globe Downing onions (*Allium cepa* L.) was used for permeability measurements. The onions were stored at 5 °C and brought to room

temperature a few hours before the experiment was conducted. All the measurements were made at 20 °C under uniform light conditions.

Preparation of the Material

The upper and lower 1/4 of the onion was discarded and the middle portion was used. The third healthy scale (counting inward from the outermost fleshy scale) was selected and was infiltrated for 2 min in about 250 ml spring water with a faucet aspirator (4 mm Hg reduced pressure). Spring water contained approximately 80 ppm of Ca^{2+}, 30 ppm of Mg^{2+}, 14 ppm of Na^{+}, and 6 ppm of K^{+}, and the pH was about 7.5. The epidermis was then peeled off and circular discs of about 1.4 cm diameter were cut and floated on spring water.

Turgor Pressure Regulation

Changes in turgor pressure were induced by transferring the epidermis discs to various mannitol solutions ranging in decreasing concentration down to isotonic. The isotonic concentration was determined by transferring a separate group of 3×5 mm cuttings made from the same scale to various mannitol solutions and observing them under the microscope for incipient plasmolysis. The turgor pressure of the cell in an isotonic solution was taken as zero. For any other solution of lower concentration, its difference from the isotonic concentration was calculated. This difference in concentration when expressed in terms of osmotic pressure gave the turgor pressure of the cells in that solution.

Efflux Measurements

The permeability of epidermis cells to water was determined by using a special chamber (Figs. 1 and 2) designed for a rapid and continuous exchange of the liquid in contact with the epidermis. Chambers for similar purpose with or without discontinuous change of the liquid on one side of the membrane were described by Ussing and Zerahn (1951), and Dainty and House (1966). It essentially consists of two pieces cut from a 4.4 cm diameter solid plexiglas rod. In the center of the flat surface a circular disc 1 cm in diameter and 3 mm deep was removed, leaving a chamber when the two pieces were put together. An epidermis disc 1.4 cm in diameter was held flat between the male and female joints of the chamber (Fig. 2 enlarged view). A distance of 0.1 mm between the male and female joints when put together facilitated the retention of the epidermis disc without injuring the cells held between the joints. A groove 0.5 mm deep on each side of the joints was filled with petroleum jelly to make it water tight. The enclosed chamber was connected to an inlet and outlet tube by radially drilled holes 3 mm in diameter.

The flow rate was kept constant during an efflux experiment by using an inverted Mariotte flask reservoir. Different flow rates were obtained by changing the height of the Mariotte flask and adjusting a screw-type clamp fixed on the tube connecting the flask and the chamber.

To make a permeability measurement the epidermal discs were transferred from spring water to the mannitol solutions containing tritiated water (500 μCi per ml) and left there for 1 hr. During this period tritium moved into the epidermis cells and equilibrium was established. The turgor pressure of the cell was regulated at the same time by changing the concentration of the mannitol solution while keeping the concentration of tritium the same. Next, the cutting was removed from the solution and the excess liquid was blotted with filter paper. The epidermis disc was immediately sealed in the chamber by coating the joints with petroleum jelly and washed at a constant flow rate (2 ml/sec) with the same concentration mannitol solution but without tritium.

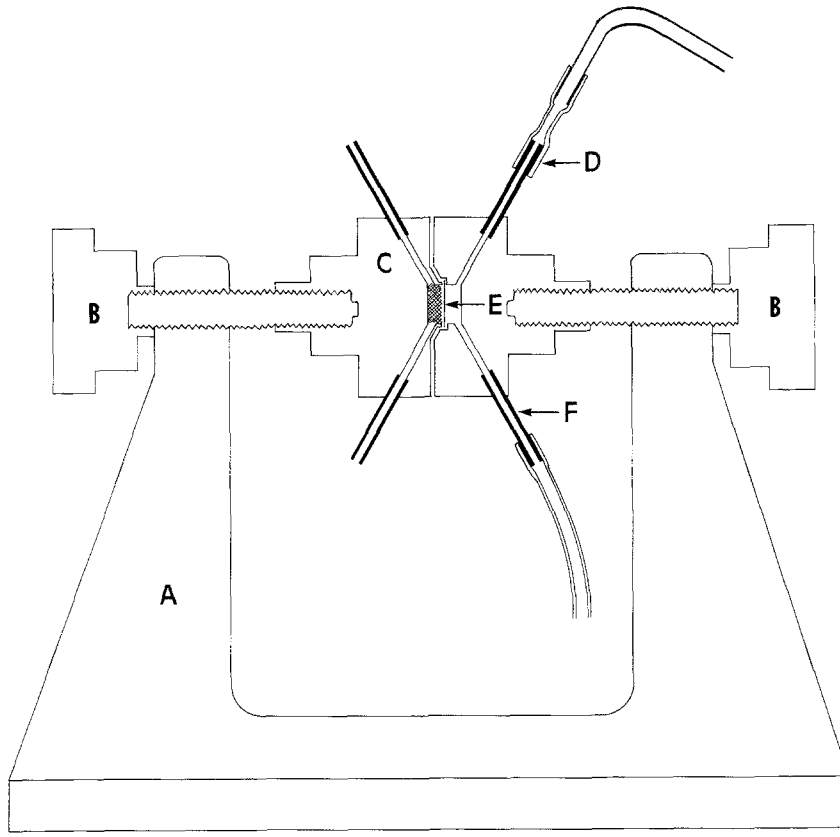

Fig. 1. Diagram of the apparatus used for efflux experiments. *A*: stand; *B*: screw to hold chamber in place; *C*: plexiglass chamber; *D*: outlet; *E*: epidermis disc; *F*: inlet connected to Mariotte flask reservoir

The efflux of THO from the inner and outer (i.e., cuticular) side of the epidermis was determined in preliminary experiments. The contribution of the cuticular side to the efflux was found to be almost zero. This result agrees with the absence of water loss observed in epidermis parts where the cuticular side is exposed to air (Wattendorff, 1964).

The washing solution was collected in counting vials at 5 sec intervals up to 150 sec and 10 sec intervals up to 600 sec. From each vial 1 ml of solution was removed and its radioactivity counted in a scintillation counter after mixing it with cocktail (Handiflour). Using the flow rate of 2 ml/sec the washing solution in the chamber was replaced 9 times every sec. A higher flow rate than this did not yield any increase in water permeability values, indicating that the unstirred layer effect, if any, was very small.

Calculation of Water Permeability (Hydraulic Conductivity) and Analysis of Efflux Curve

The efflux of tritiated water from epidermis discs, previously equilibrated with tritiated water was followed. If c is the concentration of labelled water in the cells (mainly cell

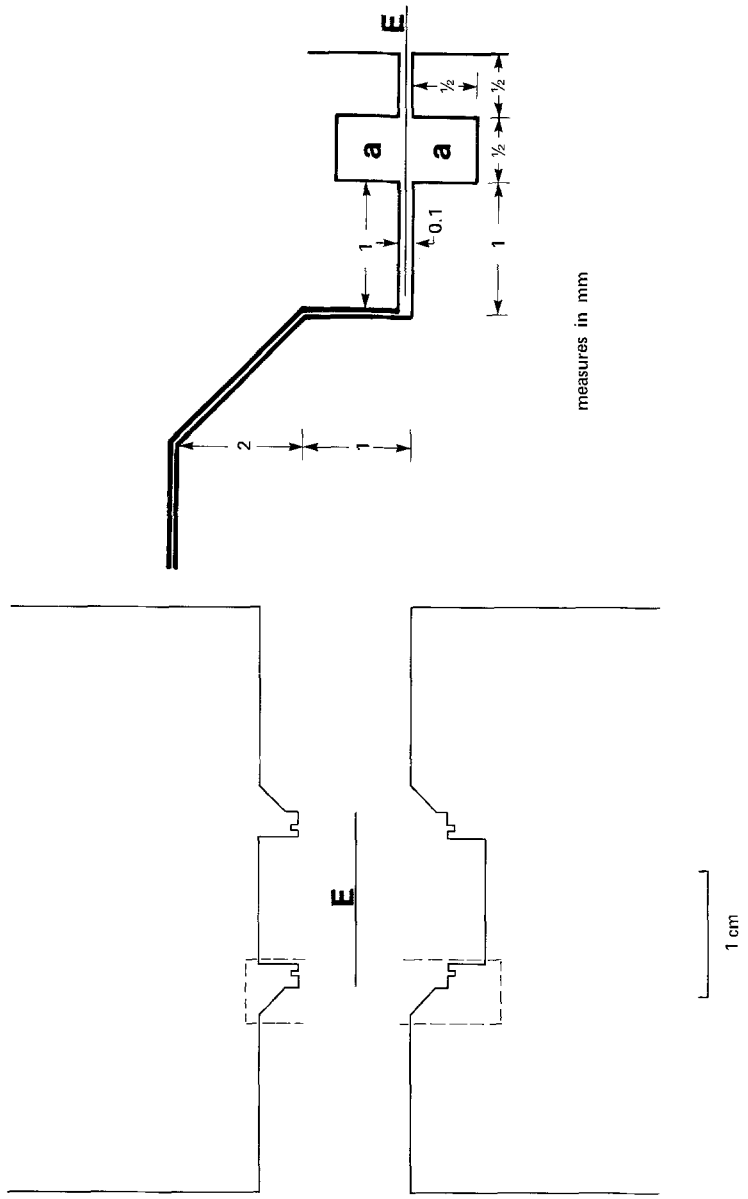

Fig. 2. Edge views of the chamber showing mounting rim and seal in detail. *E*: epidermis; *a*: groove for application of petroleum jelly

sap in the vacuole) the rate of the decrease of c is given by the usual equation:

$$\frac{dm}{dt} = -K_w \cdot A \cdot (c - c_e). \tag{1}$$

where dm/dt is the amount of tritiated water permeating per unit time from the cell interior to the external solution (wash liquid), A is the surface area of the protoplast of the cells effective in the exchange with the external solution, and c and c_e is the concentration

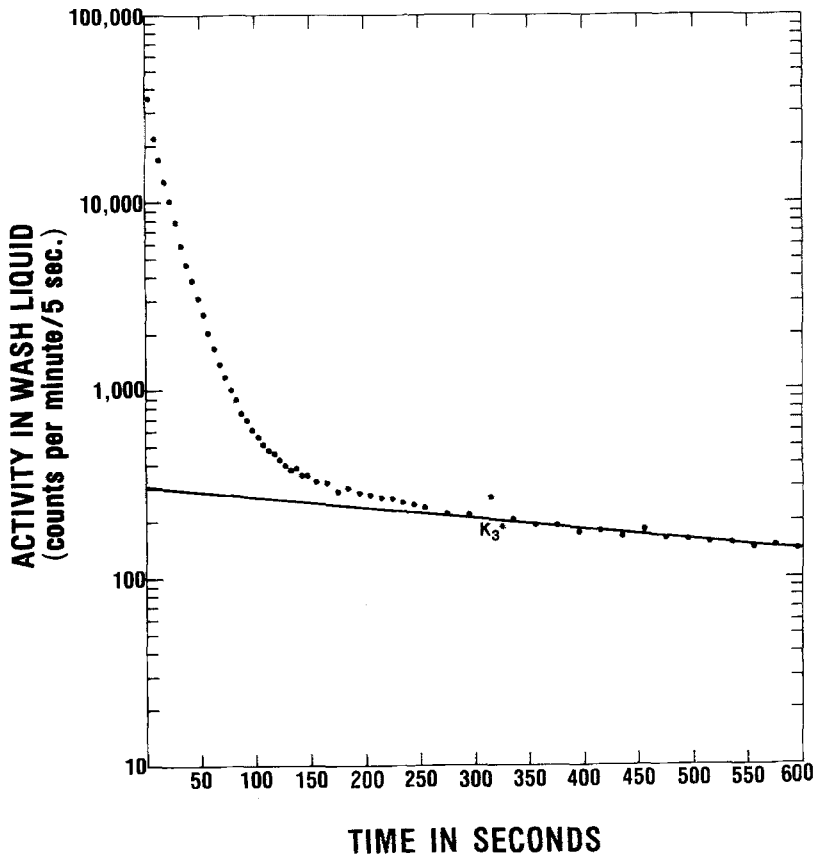

Fig. 3. Typical efflux curve. The logarithm of the activity in wash liquid is plotted as a function of time. A line drawn through the later part of the curve is a regression line for all the points after the time 250 sec (*see* Appendix)

of labelled water in the cell sap and the external solution respectively. Since the wash liquid was continuously replaced, c_e was constantly maintained at zero. Also, dm in the above equation can be expressed as $V \times dc$, where V is the (constant) volume of all the vacuoles of the cells of the washed part of the epidermal disc. Thus, Eq. (1) can be written as:

$$\frac{dc}{dt} = -K_w \cdot \frac{A}{V} \cdot c. \tag{2}$$

Integration of (2) yields solution

$$c = c_0 \cdot e^{-K^* t} \tag{3}$$

where

$$K^* = K_w \cdot \frac{A}{V} \tag{4}$$

c_0 = concentration of the labelled water for $t = 0$.

Substituting in (3) for c and c_0 from (1) we can obtain a similar relationship for the rate of loss of labelled water from the cells $R = \left(-\frac{dm}{dt}\right) = -\left(V \frac{dc}{dt}\right)$ as:

$$R = R_0 \cdot e^{-K^* t} \tag{5}$$

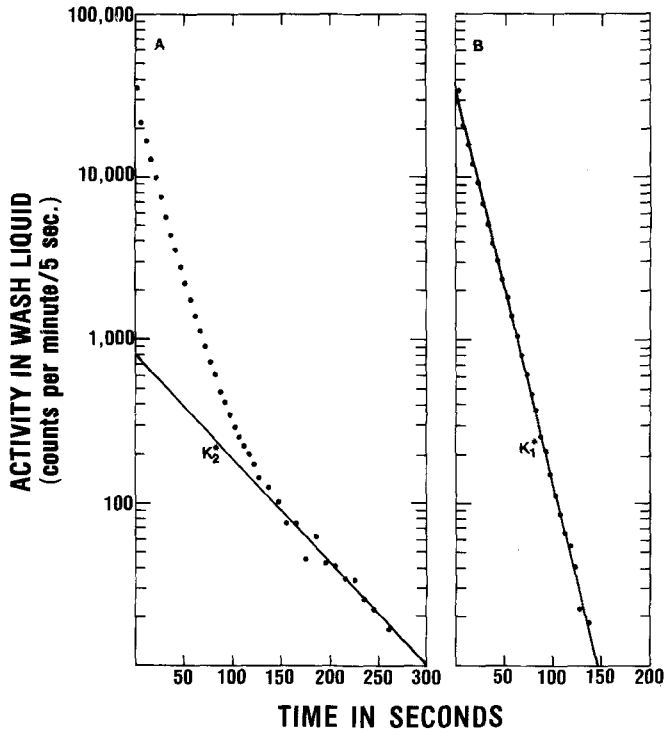

Fig. 4. Logarithm of the activity in wash liquid plotted as a function of time. *A*: The points represent the result of the substraction of line in Fig. 3 from the efflux curve in Fig. 3. *B*: The points represent the result off the subtraction of line K_2^* in 4*A* from the points in 4*A* between $t=0$ and $t=150$ sec

with $R_0=\left(\frac{dm}{dt}\right)_{t=0}$ the initial rate of loss. A semilog plot of the rate of loss against time gives a straight line with intercept R_0 and the slope K^*. The initial rate of loss R_0 is equal to $V \cdot c_0 \cdot K^*$ [Eq. (1)]. Therefore, the initial amount of labelled water present in the tissue $V \cdot c_0$ can be calculated using this relationship as:

$$V \cdot c_0 = R_0/K^*. \tag{6}$$

A typical efflux curve for onion epidermis discs is shown in Fig. 3. The log of the efflux activity is plotted as a function of time. A curve instead of a straight line indicates that several components are contributing to the efflux at different rates. At the beginning the activity eluted drops sharply during the first 10 sec and most probably can be attributed to tritiated water adhered on the inner surface of the epidermis. Between 10 and 100 sec the activity decreases at a slower rate and after 100 sec this decrease is very slow.

A graphical method can be used to separate the different components. A regression line is drawn first on the later part of this curve which gives a straight line with slope K_3^*. The activity given by this line is then subtracted from the total activity resulting in the curve shown in Fig. 4*A*. Two separate slopes are clearly evident from Fig. 4*A*. Again a regression line is drawn through the later part of this curve with slope K_2^*. Similar subtraction procedure as done in Fig. 3 is repeated and gives the curve shown in Fig. 4*B*. All data points except the first fit very well to a regression line with slope K_1^* (Fig. 4*B*). The first point at 2.5 sec represents the activity adhered to the surface of the epidermis cell walls. From the slopes K_1^*, K_2^*, K_3^*, and their intercept at time

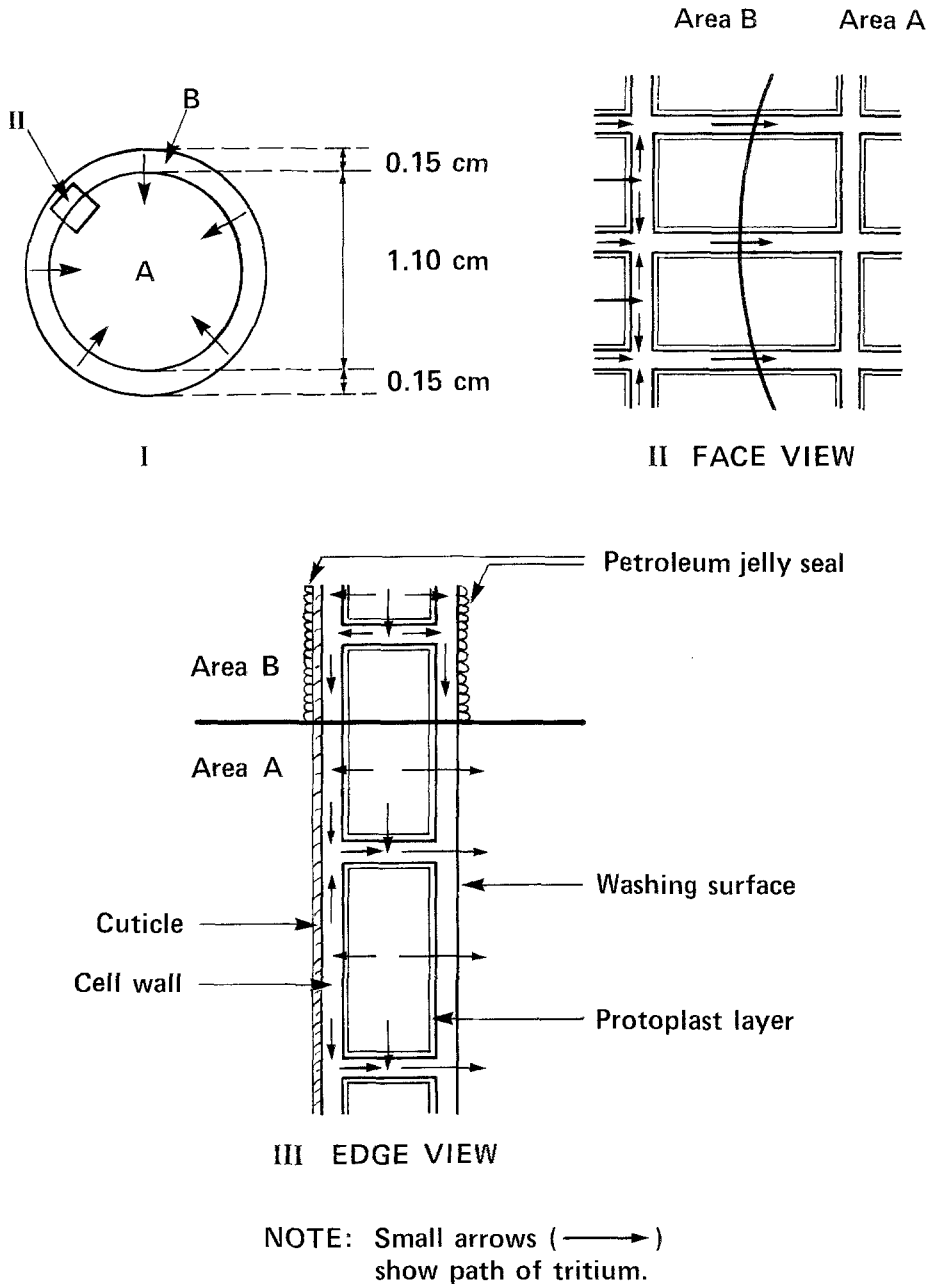

Fig. 5. Schematics of epidermis disc and epidermal cells. *I*: Epidermis disc with central area *A* exposed to the washing liquid and area *B* not exposed to washing liquid. *II* and *III*: Path of tritiated water from epidermal cells in area *B* to cells in area *A*

zero the relative amounts of the tritiated water present in these three compartments can be calculated using Eq. (6).

This way it was found that the contribution of the two slow compartments (corresponding to K_2^* and K_3) to the total efflux is about 33% and the contribution of the fast compartment (Fig. 4*B*) about 67% of the total amount prexent in the disc. About the same percentage was found in the three more experiments analyzed in this manner. In this way we find that the two slow compartments (Figs. 3 and 4*A*) contribute about 30 to 35% and the fast compartment (Fig. 4*B*) about 65 to 70% of the total relative amount of THO in the epidermis disc. The activity in the very fast first compartment was not considered since sufficiently fast measurements could not be made and it mainly concerned the water which remained adhered to the inner surface of the epidermis.

Identification of these three compartments can be made by examining the geometry of the material and the experimental procedure used. The disc is clamped between the two halves of our chamber and its main portion, the inner part of its surface (area *A*, Fig. 5, *I*; 1.1 cm diameter), is exposed to the washing solution. The ring of 0.15 cm width (area *B*, Fig. 5, *I*) which is in contact with the mounting rim of the two chamber halves, is not exposed directly to the washing solution. At the high flow rate (2 cm^3/sec) used in this study it could be assumed, however, that the washing solution elutes equally effectively an innermost ring of 0.5 mm width of the area *B*. Such an assumption, of course, does not in any way change the method for the calculation of the water permeability.

The possible pathways for tritiated water to diffuse to the washing solution from area *B* are shown in Fig. 5 *II*, *III*. Diffusion of tritiated water from area *B* is controlled by the rate at which it can diffuse centripetally from this area towards area *A*. Diffusion over a long distance through the fine capillaries of the cell wall is a slow process; therefore, tritiated water moves from area *B* to area *A* at very low rate compared to tritiated water diffusing directly from the vacuole of a cell in area *A* to the outer solution. Area *A* and *B* can be calculated as percentage of the total surface area of the epidermis disc and amounts to about 62% and 38%, respectively. This agrees very well with the experimentally found activities of THO in these compartments. Thus the slow components, slope K_3^* in Fig. 3 and slope K_2^* Fig. 4 *A*, together represent the tritiated water diffusing slowly out of the Area *B* (Fig. 5) centripetally through the cell walls, Line with slope K_1^* in Fig. 4*B* indicates the rate at which bulk of the tritiated water present in area *A* moves from the vacuoles of the cells into the washing solution through the protoplast layer. The permeability of the protoplast layer can therefore be calculated from K_1^* using Eq. (4).

The volume V in Eq. (4) is the total volume of all vacuoles for the cells in the circular area *A*. The surface *A* in Eq. (4) is in first approximation the sum of the protoplast surfaces exposed to the washing solution, i.e., πr^2, where *r* is the radius of the area *A*. Elution of THO through the other surfaces of the vacuole or the cell will be much too slow to contribute to the fast exchange of THO. Introducing this in Eq. (4) K_w can be calculated from the relationship:

$$K_w = K_1^* \cdot d \tag{7}$$

where *d* is the thickness of the epidermis disc. It follows from (7) that any inaccuracy in the estimation of the radius, *r*, i.e., the effective surface exposed to elution will not affect the value of V/A as long as *V* and *A* concern the same cylinder.

Tritiated water is reported to diffuse slightly faster than ordinary water (Kohn, 1965) and to pass through the membrane as undissociated molecules (Huebner & Wetzel, 1961). In the present calculation, however, a correction factor derived from the values reported by Kohn (1965) was used (*see* Table 1, Footnote a). The question on the applicability of such conversion factor does not change in any way the conclusions drawn in this study where permeability values are compared at different turgor pressures.

The whole washing out experiment was repeated with dead cells. The cells were strongly

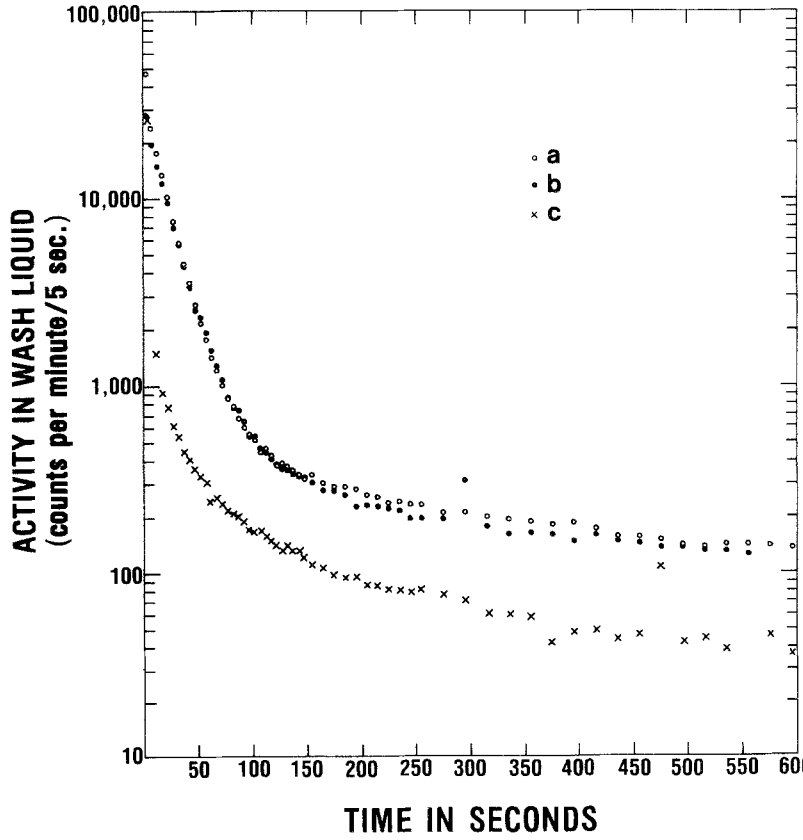

Fig. 6. Logarithm of the activity in wash liquid plotted as a function of time. Results of three experiments with the same epidermis piece. Curve (*a*): turgor pressure = 0 atm, $K_w = 2.08 \times 10^{-4}$ cm·sec^{-1}. Curve (*b*): turgor pressure = 8 atm, $K_w = 2.20 \times 10^{-4}$ cm·sec^{-1}. Curve (*c*): efflux curve with dead cells

plasmolyzed using 1.0 M mannitol solution, quickly frozen to −20 °C, then thawed very fast and checked under the microscope to make sure that no cell survived. Plasmolyzing the cells before freezing insures the complete separation of membrane from the cell wall. The epidermis piece with dead cells was loaded with tritiated water and washed out as described for the living tissue. The efflux curve (Figs. 6 and 7, curve *c*) shows a slow component with a very similar slope as in the experiment with living cells. Further analysis of the graph shows that the fast component from area A is of the order of 4–5 times the value of K^* found for living cells, indicating that the main diffusion resistance of the living tissue, the cell membrane, is nonfunctional. Furthermore, the rate of transport of THO from area B to area A remains quite the same, indicating that the slow components are controlled mainly by the diffusion through the cell walls and that the protoplasmic membranes play no role in this flow. These results support the approach used for calculating the water permeability.

The hydraulic conductivity in cm per sec per atm was calculated by using the relationship

$$L_p = \frac{K_w \cdot \bar{V}_w}{RT}.$$

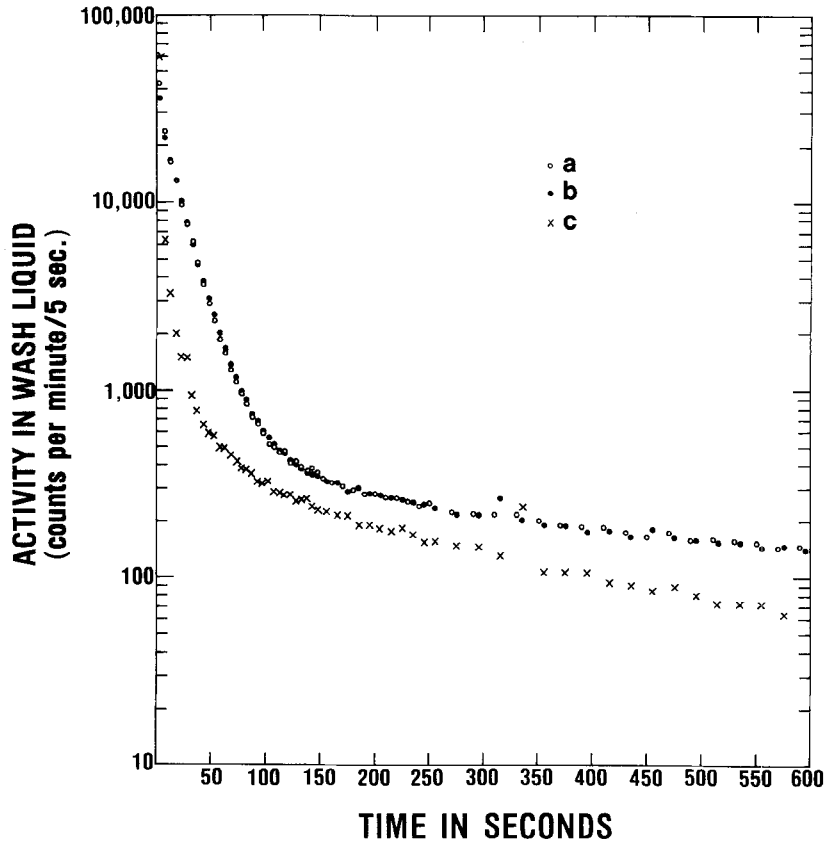

Fig. 7. Logarithm of the activity in wash liquid plotted as a function of time. Results of three experiments with same epidermis piece. Curve (*a*): turgor pressure = 0 atm, $K_w = 2.34 \times 10^{-4}$ cm·sec^{-1}. Curve (*b*): turgor pressure = 8 atm, $K_w = 2.56 \times 10^{-4}$ cm·sec^{-1}. Curve (*c*): efflux curve with dead cells

where K_w is the water permeability constant in cm per sec, $\bar{V}_w$ is the partial molar volume of water in cm^3 per mole, R is the gas constant in cm^3·atm per °K per mole and T is the temperature in °K. The quotient V_w/RT for $T = 20$ °C = 293 °K has the value of 7.498×10^{-4} per atm.

Results and Discussion

Hydraulic conductivity was determined at different turgor pressures as described above. The results from two different experiments are given in Table 1. In these experiments each measurement represents a different piece of epidermis taken from the middle portion of the third scale of the onion bulb. Although there is variability in the L_p values, there seems to be no relationship between L_p and turgor pressure. The variability in L_p values is due to the individual differences between the epidermis

Table 1. Hydraulic conductivity values ($cm \cdot sec^{-1} \cdot atm^{-1} \cdot 10^7$ of $^1H^3HO_2$) at different turgor pressures for onion epidermis cells[a]

Onion	Turgor Pressure (atm)				
	0	3.8	6.5	10.4	11.8
1	1.80[b]	1.84	1.57		1.23
	1.45	1.52	1.73		1.35
2	1.14			1.53	
	1.86			1.48	

[a] To obtain the values for ordinary water, the numbers must be multiplied by 0.868. This value derives from the ratios of the diffusion constants (ordinary water/tritiated water) which can be interpolated for the temperature of the experiment (20 °C) from the values 0.863 and 0.873 for 15 °C and 25 °C (*cf.* Kohn, 1965).

[b] Each value represents measurement on a different piece of epidermis disc. Two epidermis discs were used for any turgor pressure treatment.

discs tested. In spite of the variability, a sharp increase in L_p as the turgor pressure approaches zero could not be verified.

To avoid the variability of L_p between different pieces of epidermis, in the next experiments, L_p measurements were made on the same piece of epidermis at different turgor pressures. The results of two such experiments are shown in Figs. 6 and 7. The efflux curves show no significant change when the turgor pressure is lowered from 8 atm to about 0–0.5 atm. The calculated values of L_p at these two turgor pressures are given in the Figs. 6 and 7. It is evident that there is no difference between L_p values determined at zero and at 8 atm turgor pressure. In a separate experiment L_p was determined 3 times repeatedly on the same piece of epidermis under the same conditions. The purpose was to evaluate the accuracy of the method used by calculating the standard deviation in L_p values. The average L_p was found to be $(2.38 \pm 0.03) \times 10^{-7}$ cm per sec per atm, indicating that our method is very precise and the results are reproducible.

The present data does not confirm the earlier findings of Zimmermann and Steudle (1974) and Steudle and Zimmermann (1974) concerning a sharp increase in L_p for *Nitella* and *Valonia* cells as turgor pressure approached zero. There are some details in their work which might be of interest for the possible interpretation of such discrepancy. Two different methods were used to lower the turgor pressure in *Nitella* by Steudle and Zimmermann (1974). One method is identical with the one used here and applies increased outside concentration of an impermeable

solute. With this method L_p decreased slightly with increasing concentration of sucrose up to 200 mM. These results are in agreement with the results of Dainty and Ginzburg (1964) and Tazawa and Kamiya (1966), and the findings here. With the other method used the turgor pressure was lowered by leaving the punctured cells in a solution of an impermeable solute of very low osmolarity for a period of 10 hr up to a day. This period, the authors report, could be shortened by artificially inducing exosmotic and endosmotic water flows alternatively. With *Nitella* cells pretreated in such a way L_p increased to about 2 times after the cells lost their turgor. The absence of any significant effect of low turgor pressure on L_p by the first method, and a sharp increase in L_p after leaching out the electrolytes indicates a possible effect of the loss of electrolytes on the membrane rather than of the turgor pressure itself.

Further, Zimmermann and Steudle (1974) conducted similar experiments with *Valonia*. They found that only exosmotic L_p increased sharply at low turgor pressure whereas no effect was found on endosmotic L_p. By applying external hypotonic osmotica L_p increased to 2–3 times whereas it increased to 8 times after electrolyte leakage. Here again electrolytic concentration in the cell seems to affect the membrane permeability to water.

Zimmermann and Steudle (1975) conclude that at turgor pressures close to zero, the cell wall becomes the rate limiting barrier because the L_p determined by the second method at low turgor pressure had a similar value to that measured for the cell wall alone using dead cells. This might, however, suggest that these authors are dealing with altered cells or the method they used produces changes in the cell membranes. The range of pressure change needed for each L_p measurement is usually 1–2 atm. Near incipient plasmolysis it is possible to considerably alter the cell membrane by instantaneously increasing or decreasing the pressure inside the cell by 1–2 atm. This may be of concern especially in cells which are difficult to plasmolyze due to a high degree of cell wall attachment of the protoplasm layer as in *Nitella* (Baker, 1972) and *Chara* (Hayashi & Kamitsubo, 1959).

The permeability increase was explained by Zimmermann and Steudle (1975) by the folding of membrane at low turgor pressure. There is, however, no evidence of membrane folds at low turgor pressure and electron microscopic pictures of a plasmolyzed cell showed absence of any folds in the cell membranes (Sitte, 1963). It also seems hard to visualize that folding of a liquid membrane around a highly fluid protoplasm with the central vacuole could persist and withstand the surface tension

which acts towards a minimum surface. Furthermore, the values of L_p obtained by the plasmometeric method (Url, 1971; Pedeliski, 1973; Pedeliski & Stadelmann, 1974; also *see* Stadelmann, 1963) fall in the same range as those obtained by the diffusional method applied here. If the membrane is folded at low turgor pressure the effect of folding should be much more pronounced in plasmolyzed cell. Also in their most recent work Steudle, *et al.* (1975) found no effect of turgor pressure on L_p in bladder cells of *Mesembryanthemum crystallinum*. No explanation has been given by the authors except that these cells are of a special type and perform a special function and hence the results are not the same as in *Chara* and *Nitella*. If membrane folding is the explanation, it could be expected that they should also occur in *Mesembryanthemum* bladder cell, and an increase in water permeability should result at low turgor pressure. A more probable cause for the difference between Characean cells and *Mesembryanthemum* bladder cells in water permeability changes with turgor pressure could be variations in wall attachment of the protoplasm layer of these cells. In *Mesembryanthemum* the wall attachment does not seem to be as high so that the method used by these authors does not cause membrane alterations at low turgor pressure.

In conclusion, the data found with *Allium cepa* epidermal cells does not support the concept that L_p is related to turgor pressure. It seems reasonable to assume that this is possibly the case in most higher plants. Contradictory results reported by Zimmermann and Steudle (1974) and Steudle and Zimmermann (1974) with Characean cells are probably due to some real difference in the membrane characteristics between these cells and higher plant cells but more likely due to the alterations of the membranes caused by the experimental techniques used by these authors.

The results found with *Allium cepa* epidermal cells are compatible with earlier findings of Dainty and Ginzburg (1964), Tazawa and Kamiya (1966), and Kiyosawa and Tazawa (1972) that turgor pressure has no great effect on the water permeability of the intact cell membrane. High pressure, however, may have effects on passive K^+ permeability of altered membranes; liposomes preloaded with labelled KCl became considerably leaky for K^+ after treatment with anesthetics, but a pressure of 90 atm reduced K^+ efflux to the normal value (Johnson, Miller & Bangham, 1973). Also the wellknown osmoregulation in algal cells is related to the sensitivity of the active transport systems to turgor pressure changes (Bisson & Gutknecht, 1975), and likewise hydrostatic pressure may affect active transport in animal membranes (Péqueux, 1972).

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Appendix

Calculation of water permeability constant K_w and relative amounts of tritiated water pressure in various compartments at zero time.

For the time limits t_1 and t_2 Eq. (5) can be written as:

$$\ln\left(\frac{dm}{dt}\right)_{t=t_2} - \ln\left(\frac{dm}{dt}\right)_{t=t_1} = -K^*(t_2 - t_1). \tag{A 1}$$

Introducing in (A 1) $\frac{dm}{dt} = \frac{\Delta m}{\Delta t}$ ($\frac{\Delta m}{\Delta t}$ is the amount of tritiated water washed out of the tissue per unit time in a given time interval and is measured as count/min in the wash liquid collected in that time interval and this is plotted in Fig. 3) we get an expression for K^* as:

$$K^* = 2.303 \frac{\log\left(\frac{\Delta m}{\Delta t}\right)_{t=t_1} - \log\left(\frac{\Delta m}{\Delta t}\right)_{t=t_2}}{t_2 - t_1}, \tag{A 2}$$

here K^* is the slope (K_1^*, K_2^*, K_3^*) of the lines in Figs. 3, 4*A* and 4*B*. Using (A 2) K_1^*, K_2^*, and K_3^* can be calculated as follows:

$$\left(\frac{\Delta m}{\Delta t}\right)_{t=0} = 300 \text{ count/min per 5 sec}$$

with

$$\left(\frac{\Delta m}{\Delta t}\right)_{t=600} = 145 \text{ count/min per 5 sec}$$

follows K_3^* as

$$K_3^* = 2.303 \frac{\log(300) - \log(154)}{600 - 0}$$

$$= 1.21 \times 10^{-3} \text{ sec}^{-1}.$$

Similarly with

$$\left(\frac{\Delta m}{\Delta t}\right)_{t=0} = 800 \text{ count/min per 5 sec}$$

and

$$\left(\frac{\Delta m}{\Delta t}\right)_{t=300} = 10 \text{ count/min per 5 sec}$$

follows

$$K_2^* = 1.46 \times 10^{-2} \text{ sec}^{-1}$$

and finally with

$$\left(\frac{\Delta m}{\Delta t}\right)_{t=0} = 35{,}000 \text{ count/min per 5 sec}$$

and

$$\left(\frac{\Delta m}{\Delta t}\right)_{t=146} = 10 \text{ count/min per } 5 \text{ sec}$$

follows

$$K_1^* = 5.59 \times 10^{-2} \text{ sec}^{-1}.$$

Now knowing K_1^*, K_2^*, and K_3^* and their corresponding initial rates of loss $(\Delta m/\Delta t)_{t=0}$ and using Eq. (6), the relative amounts of tritiated water present in each compartment can be calculated:

In compartment *I* with slope

$$K_1^* = (V \cdot c_0)_1 = \frac{3500}{5.49 \times 10^{-2}}$$
$$= 626{,}118 \text{ count/min.}$$

Similarly in compartment *II* with slope

$$K_2^* = \frac{800}{1.46 \times 10^{-2}}$$
$$= 54{,}794 \text{ count/min}$$

and in compartment *III* with slope

$$K_3^* = \frac{300}{1.21 \times 10^{-3}}$$
$$= 247{,}933 \text{ count/min.}$$

From this the relative amounts present in compartment *I* becomes 67.4% of the total amount. Compartment *II* and *III* contain 32.6% of the total amount in the epidermis originally present.

Using Eq. (7) we can calculate the water permeability constant K_w knowing the thickness of the epidermis disc which was found to be 0.004 cm. So compartment *I* with K_1^* gives K_w as:

$$K_w = K_1^* \cdot d$$
$$= 5.49 \times 10^{-2} \times 0.004$$
$$= 2.20 \times 10^{-4} \text{ cm} \cdot \text{sec}^{-1}.$$

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