On simultaneous transport of water and solute through plant cell membranes: Evidence for the absence of solvent drag effect and insensitivity of the reflection coefficient

By

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(Received 7 December, 1979; revised 19 March, 1980)

Abstract

The simultaneous efflux of tritiated water and ^{14}C labelled ethanol from inner epidermal cells of the bulb scale of *Allium cepa* was measured with a specially designed efflux chamber. It was found that water and ethanol moved essentially independently. Rates of efflux of tritiated water and ^{14}C ethanol were essentially the same in the presence or absence of a simultaneous influx of water.

Using the same technique the efflux of tritiated water from the epidermal cells was measured during a simultaneous flow of nonlabelled ethanol. When tritiated water and ethanol moved in opposite directions, the water permeability values became slightly reduced depending upon the concentration of ethanol. When ethanol and tritiated water moved in the same direction, however, no effect on water permeability values could be detected. These results are best explained by the molecular theory of diffusion across lipid bilayer membranes, and are consistent with the above findings of lack of interaction between water and ethanol as they are transported across the cell membrane.

In another study, the solute permeability coefficients (K_s) for non-electrolytes such as urea and methyl urea were measured by plasmolyzing the epidermal cells and transferring them to equimolal solutions of urea and methyl urea. This method was also used to measure the reflection coefficient (σ) for these nonelectrolytes. The K_s values for methyl urea were 16 times greater than the ones for urea. The values of σ for both of these solutes, however, were very close to 1.

Using the K_s data available in the literature for the subepidermal cells of the *Pisum sativum* stem basis, the σ values were calculated for malonamide, glycerol, methyl urea, ethyl urea, dimethyl urea, and formamide. Again the K_s values for these nonelectrolytes varied by several orders of magnitude, whereas all σ values were found to be close to 1.

These findings point out that σ is an insensitive parameter and that K_s, the solute permeability constant, has to be used for characterizing solute transport through the membrane. The present study shows that fast (*e.g.* ethanol, formamide) as well as slowly permeating molecules do not interact with water as they are transported across the cell membrane. Aqueous pores for the simultaneous transport of water and solutes, therefore, are absent in the plant cell membranes investigated here.

Key-words: Membranes, water permeability, solute permeability, reflection coefficient, aqueous pores, *Allium cepa*, solvent drag.

Introduction

Determination of the passive permeability of a biological membrane for harmless non-electrolytes is a powerful tool to characterize the composition and structure of those membranes. Passive permeability is a sensitive indicator to differentiate membrane qualities between species and tissues and to study the changes in membrane qualities during development and externally induced alterations. Initial concepts for cell membrane composition and structure were based on such measurements (Overton 1899, Collander and Bärlund 1933, Gorter and Grendel 1925). Membrane permeabilities, however, cannot be measured directly but are determined from permeation rates and driving force and expressed as water permeability constant (K_w) and solute permeability constant (K_s). For simultaneous permeation of solute and water an interaction between both permeation processes, if it occurs, will alter their permeation rate. Interaction between those permeation processes may take place outside or inside the membrane. The first case can be taken care of by proper consideration of the resulting changes in the driving forces. When interaction occurs inside the membrane it indicates that solute and water use the same pathway in the membrane. In that case a third parameter must be devised to measure this interaction. Kedem and Katchalsky (1958) proposed to use the reflection coefficient σ as such third parameter. This coefficient was introduced by Antropoff (1911) as "Durchlässigkeitscoefficient (permeability coefficient) $\underline{\sigma}$". Later Staverman (1951) provided a theoretical basis for the derivation and the new name "reflection coefficient σ".

In order to test the existence of interaction between solute flow and solvent flow, two avenues can be fol-

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0031-9317/80/090083-09 $03.00/0

lowed. One approach has been to measure the rate of water transport when a simultaneous transport of a small molecular size solute is taking place in either the same or opposite directions (Klocke *et al.* 1972, Gutknecht 1968). This type of test is based on the "solvent drag" effect (cf. Ussing and Andersen 1956, p. 434, Christensen 1975, p. 108, 414).

The other approach derives from irreversible thermodynamics (Dainty and Ginzburg 1963, Kedem and Katchalsky 1961). When solute and solvent (water) do not interact inside the membrane as they move through it, then the reflection coefficient (σ) is given by

$$\sigma = 1 - \frac{\omega \cdot \bar{V}_s}{L_p} \text{ (Kedem and Katchalsky 1958, p. 245) (1)}$$

where σ is the reflection coefficient, ω an expression for the solute permeability, K_s solute permeability constant, L_p coefficient for hydraulic permeability, and $\bar{V}_s$ partial molar volume for solute.

Introducing in this equation for

$$\omega = \frac{K_s}{RT} \text{ (House 1974, p. 51)}$$

and for

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

equation (1) becomes:

$$\sigma = 1 - \frac{K_s \cdot \bar{V}_s}{K_w \cdot \bar{V}_w} \quad (2)$$

where K_w is permeability constant for water, R gas constant, T absolute temperature, $\bar{V}_w$ partial molar volume for water. Dainty (1961) and Dainty and Ginzburg (1963) have shown that in the case of water filled pores in a membrane where solute and water interact during permeation the reflection coefficient becomes:

$$\sigma = 1 - \frac{\omega \cdot \bar{V}_s}{L_p} - \frac{k_s \cdot f_{sw}}{f_{sw} + f_{sm}} \quad (3)$$

where f_{sm} is the mechanical friction coefficient of the solute with the membrane, f_{sw} the mechanical frictional coefficient of the solute with water in the membrane, and k_s the partition coefficient of the solute between water filled pores and external solution. The last term expresses an interaction between water and solute in the pores within the membrane. Thus by comparing the experimentally determined values of σ with those calculated from equation (1), the existence of interaction between solute and water inside the membrane can be tested. This approach was applied by Gutknecht (1968) and Forster (1971). Dainty and Ginzburg (1963) using formula (1) suggested that if the measured value of σ is less than $1 - \omega \cdot \bar{V}_s/L_p$ it indicates the presence of aqueous pores in the membrane. Using this criterion Gutknecht (1968) found the absence of pores in *Valonia* cell membranes, and Klocke *et al.* (1972) found the absence of "solvent drag" in the human erythrocyte membrane. On the other hand, in artificial porous membranes Andreoli *et al.* (1971) observed coupling of solute and water flows.

The present study was conducted to test the interaction between solute and water transport through onion epidermal cell membranes using both of these approaches. Another purpose of this study was to determine the significance of the reflection coefficient as a necessary parameter for characterizing membrane transport properties.

Materials and methods

Plant material

The inner epidermis of Downing Yellow Globe onions (*Allium cepa* L.) was used for this study. 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.

Solutions

All solutes used were reagent grade. The tritiated water and ^{14}C labelled ethanol were obtained from New England Nuclear Co. and from American Radiochemical Corp., respectively. Solutions were prepared in spring water containing approximately 80 ppm of Ca^{2+}, 30 ppm of Mg^{2+}, 14 ppm Na^+ and 6 ppm of K^+ and the pH was about 7.5. Distilled water was not used because of its known damaging effect on living cells (cf. De Haan 1933, p. 307, Kaczmarek 1929, p. 289).

Preparation of 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). The epidermis was then peeled off and floated in spring water.

Permeability and reflection coefficient measurements for urea and methyl urea

Permeability was measured using a plasmometric method (Höfler 1918, Stadelmann 1951, 1966). A similar procedure was used for reflection coefficient measurement. For this purpose 0.5 × 1 cm epidermal layer cuttings were prepared as described above. The cells were plasmolyzed by transferring these cuttings either to a mannitol solution or a salt solution (mixture of 9 parts of 1 *M* KCl solution and 1 part of 1 *M* $CaCl_2$ solution; the resulting salt solution was diluted with distilled water to get the desired osmolarity of the plasmolyticum; cf. Url 1971). The cut-

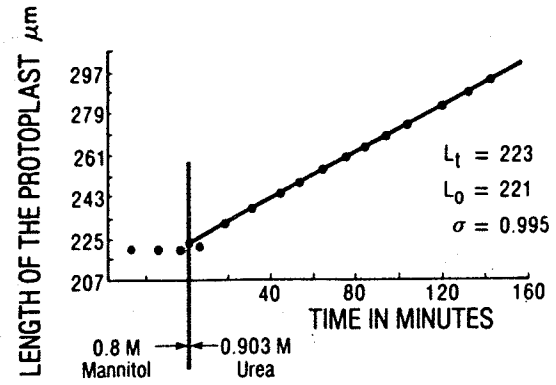

Figure 1. *Changes in the length of the protoplast with time for adaxial epidermis cell of Allium cepa bulb scales.* Cell transferred from 0.8 *M* mannitol to equiosmolal urea (0.903 *M*) solution.

tings were always transferred first to a plasmolyticum of low osmolarity to avoid osmotic shock. The final concentration of the plasmolyticum was adjusted either to 0.903 osm (0.8 *M*) mannitol or 0.74 osm salt solution. The osmolarities were measured using an Advanced Digimatic Osmometer Model 3D.

After plasmolyzing, the epidermis cuttings were placed in a perfusion chamber (Werth 1961, Stadelmann 1959) fixed on the stage of a Reichert Zetopan microscope equipped with a long focus condenser. The plasmolyzed protoplast takes the form of a cylinder with two hemispheres at its ends. The length of the protoplast was recorded using an eye piece micrometer. When the length of the protoplast remained constant for about 30 min., the perfusing solution was changed to a solution of the permeator (urea or methyl urea) having the same osmolarity as the plasmolyticum. A time record of the changes in the length of the protoplast was kept, and a plot of this was made on graph paper (Figure1 and Figure 2). A regression line was then fitted to these points. From this line the length of the protoplast at zero time (time when perfusing solution was changed to a permeating solute) was obtained by extrapolation. This extrapolated value (L_t) was compared to the length (L_o) of the protoplast recorded just before the perfusing solution was changed to the permeating solute and the reflection coefficient was then calculated using the equation (Goldstein and Solomon 1960, Stadelmann 1966).

$$\sigma = \frac{V_t}{V_o} = \frac{L_t - \frac{b}{3}}{L_o - \frac{b}{3}} \qquad (4)$$

where b is the inner width of the cell, V_o the volume of the protoplast in a non-permeating plasmolyticum just before the external solution was changed to a permeating solution, and V_t the volume of the protoplast at zero time by extrapolation from the protoplast dilatation in a solution of a permeator.

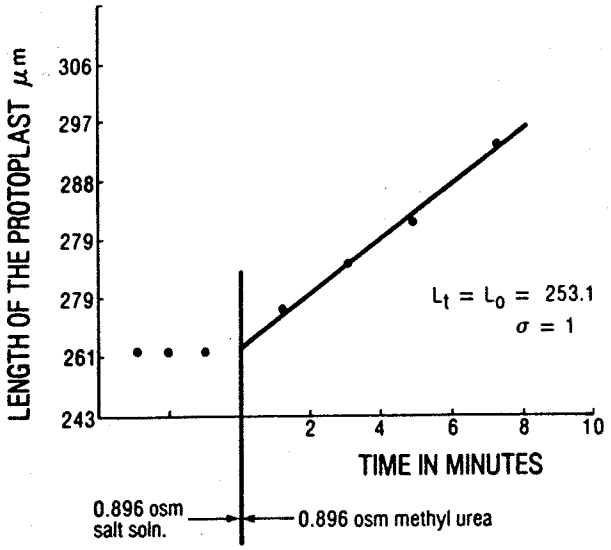

Figure 2. *Changes in the length of the protoplast with time* (adaxial epidermis cell of *Allium cepa* bulb scale). Cells transferred from 0.896 osm salt solution to equiosmolal methyl urea solution.

Measurement of simultaneous transport of water and ethanol

The diffusional permeability constants for water and ethanol were determined by a radio tracer method. In this method an epidermal disc 1.4 cm in diameter was saturated in water containing tritiated water (500 μCi/ml) and/or ethanol labelled as ^{14}C (125 μCi/ml) for ½ h to 1 h. The efflux of tracer from these loaded cells was measured by washing the noncuticular side of the epidermal disc with a solution without the tracer. In order to hold the epidermal disc flat during the wash a specially designed chamber described by Palta and Stadelmann (1977b) was used.

To illustrate the experiments clearly, the schematic diagram of the chamber used is shown in Figure 3. The chamber consisted of two pieces of plexiglass cut out of a solid rod 4.4 cm in diameter. In the center of these two pieces a circular disc 1.4 cm in diameter and 3 mm deep was removed, leaving a cylindrical chamber (6 mm long and 1 cm in diameter, with a 2 mm rim for holding and tightening of the epidermis) when the two pieces were put together. This chamber was connected to an inlet and outlet tube by radially drilled holes 3 mm in diameter. The surface of the epidermal disc held in this chamber was washed at a constant flow rate maintained by using an inverted Mariotte flask reservoir. The washing liquid was collected at 5 s and later at 20 s intervals. From the activity in the washing solution a curve was plotted for tritium efflux on a semilogarithmic scale. Permeability was calculated in the usual way by subtracting two expo-

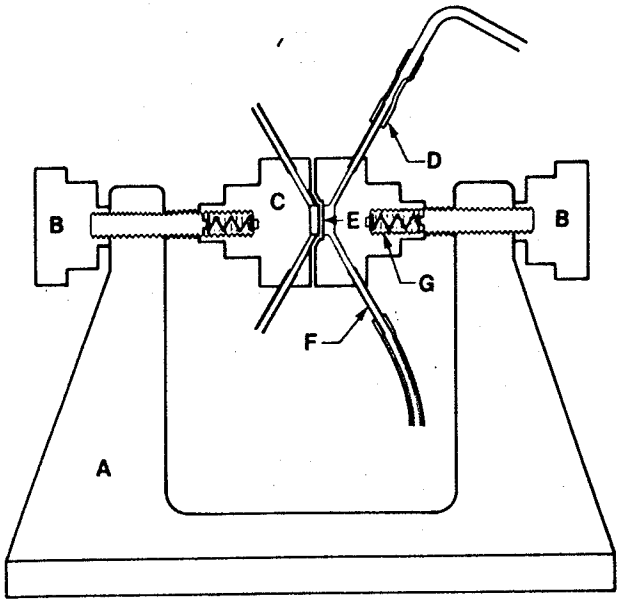

Figure 3. *Diagram of the efflux chamber used for the tracer studies.* A: Stand; B: Screw to hold chamber in place; C: plexiglass chamber; D: outlet; E: epidermis disc; F: inlet connected to Mariotte Flask reservoir. The second set of inlet and outlet was not used, since the epidermis disc was positioned in such way that the cuticular side was oriented towards the left outlet. The amount of water permeating through the cuticular side was negligible.

nentials from the main curve (for details see Palta and Stadelmann 1977b).

By changing the composition of the loading and the washing solutions, different combinations of simultaneous transport of labelled ethanol and labelled water were studied (Figure 4, cases A, B and C). To prevent fluxes arising from chemical concentration gradients, the concentration of ethanol was kept the same in the wash solution and the loading medium in these experiments (Figure 4, cases B and C).

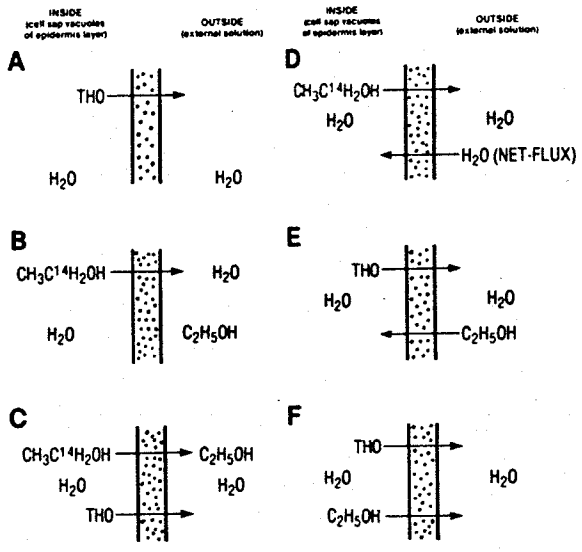

Figure 4. *Combination of the flow directions of THO and alcohol tested in simultaneous transport.*

In another set of experiments simultaneous mass flow of water into the epidermis was induced by lowering the turgor pressure of the epidermis cell in a loading solution which contained 0.4 mol/l mannitol (Figure 4, case D).

Finally a third set of measurements was made for simultaneous transport of labelled water and non-labelled ethanol (Figure 4, cases E and F). For this purpose two concentrations of ethanol (1.0 and 0.1%) were used.

Results

Permeabilities and reflection coefficients of nonelectrolytes

A typical curve for the change in length of the protoplast of one cell in isotonic solution of urea is shown in Figure 1. These measurements were made on 11 cells using 5 different onions. All the cells studied gave similar results. It is clear from Figure 1, that the reflection coefficient $\sigma = (L_o - \frac{b}{3})/(L_t - \frac{b}{3})$ (see formula 4) of urea for onion epidermis cells is very close to 1 (Palta and Stadelmann 1973). Measurements on σ were also made for methyl urea and a typical graph for the change in length of the protoplast in this case is shown in Figure 2. Again, σ for methy urea was very close to 1.

From the data as shown in Figure 1 and Figure 2, the solute permeability values (K_s) were determined (Stadelmann 1966). Using these values and a value of $K_w = 1.56 \times 10^{-4}$ cm $s^{-1} \times 0.868 = 1.35 \times 10^{-4}$ cm s^{-1} as an average found in earlier experiments (Palta and Stadelmann 1977b, Table 1, column 1), σ was calculated using equation (2). Table 1 gives average values of K_s and the computed values of σ.

The experimental and computed values of σ for urea and methyl urea were very close to 1 (Table 1), which agrees with the values of Dainty and Ginzburg (1964) and Gutknecht (1968). However, the permeability value for methyl-urea was about 15 times greater than that for urea.

Simultaneous transport of water and ethanol

Typical efflux results: A typical efflux curve for the transport of labelled water and ethanol across onion epidermal cell membranes is shown in Figure 5 A and B. The log of the efflux activity is plotted as a function of time. A graphical method was used to analyze these data and calculate the permeability constant by separating different compartments (for details see Palta and Stadelmann 1977b).

Transport of water and ethanol simultaneously and in the same direction: The possible existence of interaction

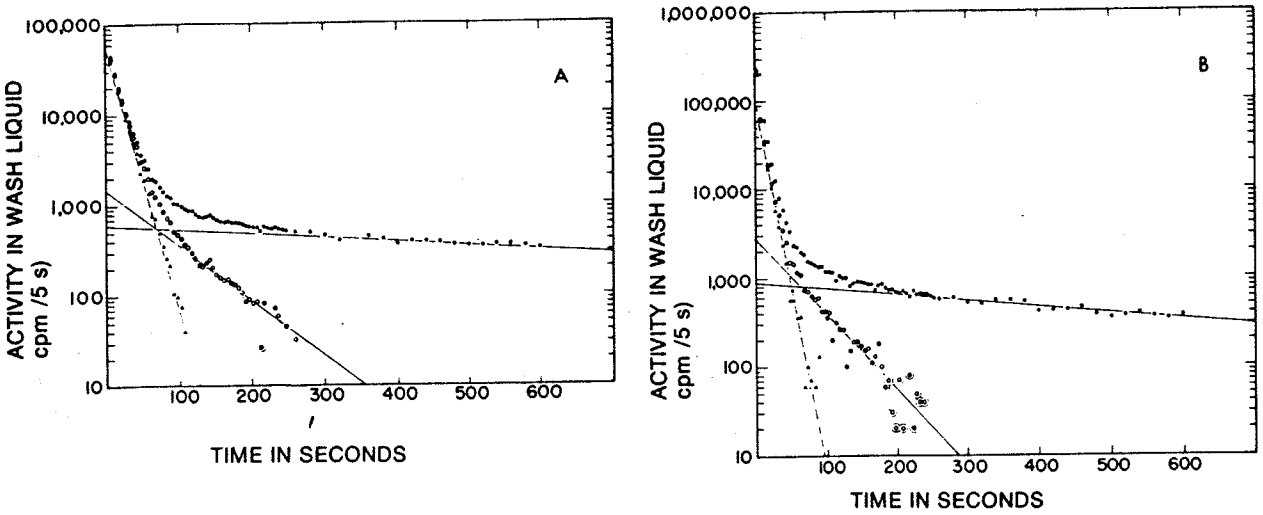

Figure 5. *Efflux curves.* Logarithm of the activity in the wash liquid is plotted as a function of time. Encircled values were eliminated for the calculation of the regression line.
(A) Efflux of water: Logarithm of the activity of tritium in the wash liquid plotted as function of time. Combination of flux directions: Figure 4 A (flow of THO from the epidermis into the wash liquid).
(B) Efflux of alcohol: Logarithm of the activity of ^{14}C in the wash liquid plotted as function of time. Combination of flux directions: similar to Figure 4 B (flow of $^{14}CCH_5OH$ from the epidermis into the wash liquid, which contained no alcohol).

between water and ethanol as they move across the membrane was tested by measuring the efflux of tritiated water during a simultaneous efflux of ethanol (Figure 4, case F compared to case A). The results from four such experiments are presented in Table 2. The water permeability constant (K_w) did not change significantly in the presence of ethanol in comparison to the control when water alone moved. The K_w values varied by less than 5% when both ethanol (0.1%) and water moved in the same direction simultaneously. Increasing the concentration of ethanol from 0.1% to 1% inside the cell gave no significant increase in K_w as compared to the control.

The interaction between water and ethanol efflux was further tested by measuring the efflux of tritiated water in the presence of ^{14}C-labelled ethanol (Figure 4, case C compared to cases A and B). The results from such experiments are given in Table 3. Again the K_w values and K_s values for water and ethanol flow were essentially the

Table 1. *Measured values of urea and methyl urea permeability and calculated and experimental values of σ for cell membranes of the adaxial epidermis of onion bulb scales of Allium cepa.* $K_w = 1.35 \times 10^{-4}$ cm s^{-1}. Since $\bar{V}_s$ of most permeators is not known, a ratio $\bar{V}_s/\bar{V}_w = 5$ was used for the calculation as maximum value which can be expected. Permeability of the whole protoplasm layer [cell membrane (= plasmalemma) + mesoplasm + tonoplast] is measured in these experiments. At present no method is available to measure the permeability of the plasmalemma alone. However, using indirect techniques (cf. Url 1971) it was demonstrated that the main resistance for water flow is the plasmalemma. We assume here this to be the case, so that we are measuring the permeability of the plasma membrane (the site for the main resistance to passive transport).

Permeant	$K_s \times 10^8$ cm s^{-1}	Reflection Coefficient σ	
		Theoretical	Experimental
Urea	6.4±0.3	0.998	1
Methyl Urea	102±14	0.992	1
*Malonamide	0.65	0.999	
*Glycerol	11.3	0.999	
*Methyl Urea	19.3	0.999	
*Ethyl Urea	119.	0.997	
*Dimethyl Urea	120	0.997	
*Formamide	1730	0.966	

* For subepidermal cells of *Pisum sativum.* $K_w = 25.6 \times$ cm s^{-1}. (From Lee 1975b).

Table 2. *Water permeability values of cell membranes of the adaxial epidermis of onion bulb scales of Allium cepa with and without simultaneous flow of ethanol.* Values on one line are measured from the same epidermis disc. – Otherwise as for Table 1.

Experiment No.	Water Permeability Constants K_w in μm/s				
	Control	With Flow of Ethanol in			
		Opposite Direction		Same Direction	
		1%	0.1%	1%	0.1%
1	2.49		2.23		2.51
2	2.49		2.35		2.60
3	2.49	2.13			
4	2.57	2.02			
5	2.59	2.27			
6	2.48			2.46	
7	2.25			2.48	

Table 3. *Permeability values of water* (THO) *and ethanol* ($^{14}C.CH_3OH$) *of cell membranes of the adaxial epidermis of onion bulb scales of Allium cepa.* Simultaneous efflux of THO and $^{14}C.CH_3OH$. Loading solutions: Ethanol alone: Ethanol 125 μCi/ml of aqueous solution. THO + Ethanol: (Ethanol 125 μCi + THO 500 μCi)/ml of aqueous solution. THO alone: THO 500 μCi/ml water. – Otherwise as for Table 1.

Experiment	Permeability constants in μm/s	
	Water	Ethanol
Epidermis disc A		
Ethanol alone		2.31
THO + ethanol	1.19	2.43
THO alone	1.07	
Epidermis disc B		
Ethanol alone		1.20
THO + ethanol	1.20	1.27
THO alone	1.37	

same when they moved simultaneously as when they moved individually.

Transport of water and ethanol simultaneously and in opposite directions: Efflux of tritiated water during an influx of non-labelled ethanol was measured (Figure 4, case E compared to case A). The results from such experiments are presented in Table 2. There was a reduction in water permeability constant values when ethanol moved in the opposite direction to the tritiated water. This reduction, however, depended upon the concentration of ethanol in the wash solution. The higher the concentration of ethanol, the greater was the reduction in K_w values. For example, data in Table 2 show that K_w values were reduced by about 20% with 1% ethanol, and 11% with 0.1% ethanol in the wash solution.

Table 4. *Permeability values of water* (THO) *and ethanol* ($^{14}C.CH_3OH$) *of cell membranes of the adaxial epidermis of onion bulb scales of Allium cepa.* Simultaneous efflux of THO and $^{14}C.CH_3OH$ from the vacuole into the external solution in the presence of an osmotic influx of water (combination D from Figure 4). The turgor was lowered during the contact of the epidermis with the loading solution by addition of mannitol (0.4 mol/l) to the loading solution. The osmotic ground value of the cells is about 0.5 mol/l mannitol. Loading solutions: Ethanol + THO for opposite water flow: (Ethanol 125 μCi + THO 500 μCi + mannitol 0.4 mmol)/ ml of solution. Ethanol + THO without water flow: (Ethanol 125 μCi + THO 500 μCi) per ml of solution (water). – Otherwise as for Table 1.

Experiment	Permeability constants in μm/s	
	Water	Ethanol
Epidermis disc A		
Ethanol + THO with opposite water flow	1.85	2.60
Ethanol + THO without water flow	1.73	2.55
Epidermis disc B		
Ethanol + THO with opposite water flow	1.80	2.64
Ethanol + THO without water flow	1.97	2.39

Transport of water and ethanol during a simultaneous osmotic water flow: Efflux of ^{14}C-labelled ethanol and tritiated water was measured during a net osmotic water influx. The cell turgor was lowered to about 80% of its original value by adding mannitol to the loading medium. These partially dehydrated cells loaded with tritiated water and ^{14}C-labelled ethanol were washed in the efflux chamber with ordinary water. Thus, as the labelled water and ethanol diffused out, the cell regained turgor giving rise to a net influx of water. The results from these experiments are shown in Table 4. Although the values of K_w and K_s varied somewhat in the presence and absence of net water influx, there was no systematic reduction in K_w or K_s in the presence of net opposite water flux.

Discussion

The following points are of interest for the purpose of discussion:

Importance of determining σ for characterizing membrane transport

No attempt has been made so far, to determine σ for a greater number of nonelectrolytes for higher plant cell membranes. Yet the absolute necessity of determining σ continues to be emphasized in recent plant physiology texts (Nobel 1974, Dainty 1976). In the present study, values of σ were found to be very close to 1 for urea and methyl urea. Gutknecht (1968) also found σ = 1 for urea, formamide, acetamide and ethylene glycol in *Valonia* cell membranes. These results are in agreement with the values of σ predicted by equation (2), for example in case of urea,

$$\sigma = 1 - [(6.35 \times 10^{-8} \times 0.045)/(2.5 \times 10^{-4} \times 0.018)] = 0.9994$$

(see Table 1). This equation reveals a serious shortcoming of σ; for most of the nonelectrolytes which are frequently used in permeability experiments the values of σ will be very close to 1. The values of the permeability constants, however, vary up to several orders of magnitude. This shows that σ is a very insensitive parameter for these permeators. Therefore, it is safe to conclude that there is no need to determine σ for such membranes. Furthermore, the reflection coefficient does not give any additional information about the transport properties of a solution diffusion type membrane, like the plasmalemma and the tonoplast of higher plant cells. The solute permeability constant (K_s) on the other hand, is a sensitive measure of the passive transport properties of these membranes.

Serious reservations of the usefulness of σ which derive from the general limitations of irreversible thermodynamics were expressed by Ogston and Michel

(1978). These authors also cautioned about the misinterpretations of σ and other coefficients in terms of hydromechanical parameters and the inexact definition of J_D by Kedem and Katchalsky (1958).

Interaction between water and non-electrolyte transport

Experimental and computed (from equation 2) values of σ were found to be in good agreement (Table 1). This indicates that solutes like urea and methyl urea do not interact with water as they pass through the plant cell membranes. In other words the interaction term in equation 3 is essentially zero. Gutknecht (1968) found similar results for methanol transport through *Valonia* cell membranes, and argued against the existence of aqueous pores in these membranes. A similar argument can be made here.

Another approach to determine this interaction has been to measure the so called "solvent drag" experimentally. Gutknecht (1968) and Klocke *et al.* (1972) failed to detect this effect when they created a water flux, in the direction of a simultaneous solute flux, by producing osmotic changes. In the present study, however, ethanol and water moved in the same or opposite direction by simple diffusion (Table 2). No effect on K_w was found when ethanol moved in the same direction, whereas K_w values decreased to some extent with increasing concentration of ethanol when ethanol moved in the opposite direction. Since the effect of ethanol was direction dependent, membrane alterations by ethanol as well as the existence of aqueous pores can be ruled out.

The decrease in the rate of water flow by opposite flow of ethanol can probably be best explained by a molecular theory of diffusion across lipid membranes, proposed by Träuble (1971). According to this theory the hydrocarbon chains contain mobile structural peculiarities (so called 'kinks') which arise as a result of thermal rotation of the hydrocarbon chain around C – C bonds. This leads to formation of some free space in the lipid, which is sufficient to accept small molecules. Thus solute molecules in the aqueous phase may partition into the kinks near the polar region of the membrane and diffuse across the membrane interior within the mobile kink. Considering this model for membrane transport it is possible that when ethanol is moving in opposite direction to water, molecules moving in one direction along the kinks interfere with the ones moving in the opposite direction. The higher the concentration of alcohol in the outside medium, the higher will be this blocking effect. This is in agreement with the results obtained (Table 2). It is important to note that the concentration of ethanol in its 1% solution is about 10,000 times the concentration of tritiated water inside the cell. The permeability constant values for methanol and water have been found to be in the same order of magnitude for some plant cell membranes (Gutknecht 1968). It is therefore possible that under these experimental conditions about 10,000 times more ethanol molecules than tritiated water molecules cross the membrane at any given time and in opposite direction. In spite of this, the efflux rate of THO was not drastically reduced. However, when water and ethanol move in the same direction and also at about the same speed (Collander and Bärlund 1933) no such blocking effect occurred (Table 2).

In a thorough study by Oshman *et al.* (1974), the occurrence of water filled pores in cell membranes was discussed and the authors came to the conclusion that such pores are absent in cell membranes. According to Wright and Bindslev (1976) even for more complex membranes such as the toad urinary bladder membrane, the solution diffusion process seems to be sufficient as a permeation mechanism, and aqueous channels or pores do not need to be assumed. Furthermore, Lee (1975a) refers to data on activation energy of water, which indicate absence of pores in lipid bilayers. The results presented in this study support these conclusions and provide evidence that the rate determining portion of the permeation path in a higher plant cell (that is the plasmalemma and the tonoplast membrane) does not contain water filled pores wherein water and solute may interact (Palta and Stadelmann 1977a).

The authors want to thank Dr. S. Prager, Department of Chemistry, for valuable discussions.

This work was supported by the Minnesota Agricultural Experiment Station. Paper No. 8712 Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul, Minnesota 55108.

Appendix

List of Symbols and Units

- b Inner width of the cell, in cm.
- ΔC Concentration difference of the permeator, in mol cm^{-3}.
- f_{sm} Mechanical frictional coefficient per mole of the solute with membrane, in J cm^{-4} s mol^{-1}.
- f_{sw} Mechanical frictional coefficient per mole of the solute with water in the membrane, in J cm^{-4} s mol^{-1}.
- J_D Exchange flow of the solute, in cm s^{-1}.
- J_v Total volume flow (of water and solute), in cm s^{-1}.
- k_s Partition coefficient of the solute between water filled pores and external solution (no dimension).
- K_s Permeability constant for solute, in cm s^{-1}.
- K_w Permeability constant for water, in cm s^{-1}.
- L_D Phenomenological coefficient of proportionality for the exchange flow, in cm s^{-1} atm^{-1}.
- L_{Dp} Phenomenological cross coefficient, in cm s^{-1} atm^{-1}.
- L_o Length of the protoplast in a non-permeating plasmolyticum just before the perfusing solution of a non-permeating solute (osmoticum) was changed to a permeating solute (permeator), in cm.

L_p Coefficient for hydraulic permeability, in cm s^{-1} atm^{-1}.
L_{pD} Phenomenological cross coefficient, in cm s^{-1} atm^{-1}.
L_t Length of the protoplast at zero time by extrapolation from the protoplast dilatation in the solution of a permeator, in cm.
Δp Hydrostatic pressure difference, in atm.
P_s Solute permeability constant, in cm s^{-1}.
R Gas constant, in J mol^{-1} K^{-1}.
T Absolute temperature, in K.
V_o Volume of the protoplast in a non-permeating plasmolyticum just before the external solution was changed to a permeating solution, in cm^3.
$\bar{V}_s$ Partial molar volume for solute, in cm^3 mol^{-1}.
V_t Volume of the protoplast at zero time, by extrapolation from the protoplast dilatation in a solution of a permeator, in cm^3.
$\bar{V}_w$ Partial molar volume for water, in cm^3 mol^{-1}.
ω Mobility of solute, in mol cm^{-2} s^{-1} atm^{-1}.
σ Reflection coefficient (no dimension).

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Edited by A. K.

Erratum

Vol. 48. Fasc. 4. 1980, pp. 568–573.

Mounla, M. A. Kh., Bangerth, F. and Stoy, V.: Gibberellin-like substances and indole type auxins in developing grains of normal- and high-lysine genotypes of barley.

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should be V. Stoy