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# Reversible Changes in the Hydraulic Permeability of Plant Cell Membranes [1, 2]

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## Introduction

In comparison with the numerous studies into factors affecting the permeability of tissues to electrolytes, nonelectrolytes, and dyes (4), studies on changes in permeability to water have been very few in number. Moreover, much of the reported work has been based on measurements of the rates of plasmolysis and deplasmolysis (5, 7, 15). According to Myers (10) the permeability of plasmolysed cells to water is completely different from that of cells which have not been plasmolysed.

Another important criticism can be leveled at all the former work. Treatment of osmotic water flow according to the principles of irreversible thermodynamics has focused attention on a second parameter which is of equal importance with hydraulic permeability in defining water flux through the membrane (17, 8, 3). This second parameter is $\sigma$, the reflection coefficient. Its derivation and its importance in botanical studies have recently been lucidly explained by Dainty (2). This coefficient is only equal to 1 where the membrane is ideally semipermeable, or where no interaction occurs between solute and solvent as they pass through the membrane. As will be demonstrated below, effects that have been attributed to changes in permeability to water may have been caused by changes in $\sigma$. None of the earlier work on factors affecting permeability has taken this parameter into account.

Following our recent observation (6) that $CO_2$ brought about a rapid change in rate of water movement into and out of plant cells, we wished to determine whether this effect was brought about via a change in $\sigma$ or in $L_p$, the coefficient of hydraulic permeability. The distinction is of considerable qualitative importance; whereas a drop in $L_p$ indicates a decrease in permeability to water, a drop in $\sigma$ would imply an increase in permeability to solutes.

To examine this question we have had to abandon the classical equation for water uptake into plant cells based on the concept of Diffusion Pressure Deficit. Apart from its other serious defects [recently criticized by Slatyer and Taylor (16) and Ray (13)] it is inadequate for dealing with water flow when the membrane is permeable to the solutes as well as to the solvent (2). We have adopted the equation based

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on irreversible thermodynamics (8)

$$J_v = L_p (\triangle P - \sigma RT \triangle C_s) \quad \text{I}$$

where $J_v$ = volume flow of water, $\triangle P$ = hydrostatic pressure difference across the membrane, R = gas constant, T = absolute temperature and $\triangle C_s$ = difference in concentration of solutes across membrane.

We suggest we have found a criterion for distinguishing between effects on $L_p$ and on $\sigma$. We have applied this test to $CO_2$ as well as to chloroform and azide, the former known as a narcotic and suspected of influencing permeability; the latter recently implicated (1) as a factor influencing permeability to solutes.

## Material and Methods

Most of the data were obtained with sections of the root storage tissue of *Daucus carota* L. This tissue was considered more suitable than our previous experimental material, segments of sunflower hypocotyl, because the cells were not growing rapidly, and were stable in water for many days, maintaining their full turgor. A few data relating to hypocotyl segments are included. Where the latter were used, the material and methods were as described in our previous paper (6) except that 1 g samples were employed.

The carrot used was var. *Nenti*. Disks of xylem parenchyma, 7.3 mm in diameter and 2 mm thick, were washed in running tap water for 1 hour and were then transferred to aerated tap water at 18° for periods varying from 5 to 12 days till required for the experiments. Samples of 12 disks (1±0.02 g) were first allowed to equilibrate at the temperature of the experiments (25°) for 2 hours, and were then blotted to remove surface moisture and weighed.

In the case of efflux experiments the disks were next transferred to 30 ml experimental media. The latter contained osmoticum and substances to be tested, and were all adjusted to the same osmotic value. Efflux was determined by withdrawing tissue samples at varying intervals and measuring their loss in weight. Each sample was then replaced in tap water and reweighed after equilibrium had been established (90 min).

In the case of influx experiments, the tissue was first equilibrated for 90 minutes in 0.48 M mannitol, a concentration chosen because it was the highest which did not impair the capacity of the tissue for subsequent water uptake. During this period each sample lost 72±4 mg water and was still far from incipient plasmolysis (fr wt falls by 15 % at incipient plasmolysis). The tissue was then placed in 30 ml of experimental solution, samples being withdrawn and weighed after various time intervals to determine influx. Each sample was subsequently replaced in tap water as in the efflux experiments and again weighed after 90 minutes.

In the case of the gas treatments, a stream of the appropriate gas was led through the solution before the introduction of the tissue and continued throughout the experiment. Sodium azide solutions were adjusted to pH 6.0 with HCl.

In view of the differential effects of tap and distilled water we provide the following details regarding the tap water used: Composition in mg/liter; $Na^+$ 120, $Ca^{++}$ 60, $Mg^{++}$ 18, $Cl^-$ 217, $SO_4^{--}$ 20, $NO_3^-$ 31.

## Results

Equation I indicates that changes in volume flow of water ($J_v$) could be due to changes in $L_p$, in $\triangle P$, in $\sigma$, or in $C_s$. We believe that changes in $J_v$ can reasonably be attributed to changes in $L_p$ as opposed to changes in the other quantities only if the following conditions are fulfilled: A) The change in flux is in the same direction both in the case of entry and of exit of $H_2O$. B) The external solution used for the efflux measurements is hypotonic with regard to the cells. Influx is measured from distilled water. C) The effect on efflux is observed also when the external osmoticum is so chosen that the tissue is less permeable to it than to most of the internal solute particles. In addition it is advisable to check the following point: D) After measurement of influx or efflux, if the tissue is transferred to water its weight after equilibration should not differ from that of the control tissue.

Table I gives the effect of a number of substances on water flux. That the mannitol and raffinose solutions used in the efflux experiments were in fact hypotonic was ensured by prior cryoscopic determination of the osmotic value of the cell (which was found to be 13.4 atm). The appropriate time intervals for measurement (20 min and 10 min for efflux and influx respectively) were chosen after examination of the time course for the 2 processes: in each case the interval is slightly more than the half-time for the process.

Two osmotic controls were provided (treatments 1 and 2, 10 and 11). The osmotic concentration of the first control was equal to the initial osmotic value of the test solutions. The second control allowed for the possibility that the test substances, owing to rapid penetration, did not act as osmotica. Treatment 5 was included as a control for the $CO_2$ treatment, since this is the pH of $CO_2$-saturated $H_2O$ (6). Treatment 4 acted as a further control for the $CO_2$ treatment since the latter implies anaerobic conditions.

Table I shows that 0.02 M $CHCl_3$ and $CO_2$ significantly decreased both influx and efflux, the latter into both mannitol and raffinose solutions (treatments 3 and 7, 12 and 13). The $CO_2$ effect could not be attributed either to low pH or to anaerobic conditions (compare with treatments 4 and 5). Neither $CO_2$ nor 0.02 M $CHCl_3$ interfered with subsequent equilibration in $H_2O$. (see column D).

A concentration of 0.05 M $CHCl_3$ (treatment 6) increased efflux. Moreover, efflux continued after transfer of the tissue to $H_2O$ (column D). Influx

Table I. *Effect of $CO_2$, $CHCl_3$ and $NaN_3$ on Water Flux into and out of Carrot Disks*

| Efflux* | | | Influx** | | |
|---|---|---|---|---|---|
| | Treatment | Loss in wt (mg) | D*** (mg) | Treatment | Gain in wt (mg) | D*** (mg) |
| 1. | Mannitol 0.44 M† | 43 | — 6 | $H_2O$ | 44 | — 8 |
| 2. | Mannitol 0.41 M | 40 | — 4 | Mannitol 0.03 M | 42 | — 8 |
| 3. | $CO_2$ + mannitol 0.41 M | 26 | — 4 | $CO_2$ | 30 | — 6 |
| 4. | $N_2$ + mannitol 0.44 M | 42 | — 7 | $N_2$ | 45 | — 3 |
| 5. | $CH_3$ (pH 4.1) + mannitol 0.44 M | 45 | — 7 | $CH_3COOH$ (pH 4.1) | 41 | — 5 |
| 6. | $CHCl_3$ 0.05 M + mannitol 0.39 M | 67 | — 130 | $CHCl_3$ 0.05 M | 7 | — 138 |
| 7. | $CHCl_3$ 0.02 M + mannitol 0.42 M | 30 | — 8 | $CHCl_3$ 0.02 M | 33 | — 10 |
| 8. | $CHCl_3$ 0.01 M + mannitol 0.43 M | 46 | — 6 | | | |
| 9. | $NaN_3$ 0.05 M + mannitol 0.34 M | 40 | — 11 | | | |
| | Sig. diff.†† (P = 0.05) | 6 | 9 | Sig. diff.†† (P = 0.05) | 6 | 8 |
| 10. | Raffinose 0.40 M | 29 | — 5 | | | |
| 11. | Raffinose 0.37 M | 27 | — 4 | | | |
| 12. | $CO_2$ + raffinose 0.37 M | 18 | — 6 | | | |
| 13. | $CHCl_3$ 0.02 M + raffinose 0.38 M | 20 | — 5 | | | |
| | Sig. diff.†† (P = 0.05) | 5 | 6 | | | |

* Efflux was measured over 20 min.
** Influx was measured over 10 min. Loss in wt during the previous 90 min in 0.48 M mannitol was 72 mg.
*** Difference between final wt after return to tap $H_2O$ for 90 min and initial wt.
† All solutions except for 2 and 11 were equiosmolar as determined cryoscopically.
†† All treatments were carried out in quadruplicate.

was much reduced, and on transfer to $H_2O$ the tissue scarcely gained in weight. These facts point to permanent damage to the selectively permeable membranes.

A concentration of 0.01 M $CHCl_3$, $N_2$, and 0.05 M $NaN_3$ had no effect on efflux.

*Possible Influence of Osmoticum.* Iso-osmotic solutions of mannitol and of raffinose (treatments 1 and 10) caused different rates of efflux. This suggested that the latter was influenced by the rate of diffusion of the osmoticum. The question arose whether the effects of $CO_2$ and of chloroform could conceivably be due to an influence on the rate of diffusion of the osmoticum into the free space of the tissue.

We therefore investigated the effect of $CO_2$ on efflux when the osmoticum was very low in mass. With NaCl as osmoticum efflux was more rapid. It thus seems likely that the diffusion rate of the osmoticum was in fact a factor determining the rate of water flux. The $CO_2$ effect, however, was of the same order as in our previous experiments.

Strong evidence that the effects are exerted principally on permeability to water rather than on the diffusion of the osmoticum comes from experiments on influx into air-dried tissue, when no osmoticum is present. We earlier showed (6) that the $CO_2$ effect is very marked under these conditions for both sunflower and carrot tissue (see also our present fig 3).

We have now checked that the effect of $CHCl_3$ is also pronounced for both tissues under these conditions. The effect of azide on influx into air-dried hypocotyls may be seen in table III.

*Time Course of Permeability Changes.* The fact that 0.02 M $CHCl_3$ decreased efflux, while 0.05 M $CHCl_3$ on the contrary increased it, recalls similar observations by Lepeschkin (9), concerning the effects of narcotics on permeability to dyes. Lepeschkin held that intermediate concentrations existed which would be without influence. Figure 1, however, shows that this is not the case for permeability to water. The effect of a given concentration of chloroform on efflux depends on the length of the treatment period. The slope of the curves for 0.02 M and 0.03 M $CHCl_3$, for instance, though initially less than that for the control, indicating decreased permeability to water, exceeds that for the control in the later periods.

The extent to which the chloroform treatments (fig 1) affected the capacity of the tissue for subsequent water uptake on transfer to water was also investigated. Table II shows that even 0.02 M $CHCl_3$ did in fact reduce subsequent water uptake if treatment continued 60 minutes (though it was without effect when applied for shorter periods). This suggests that solutes had leaked from the tissue.

It becomes clear that, though measurement of efflux over a single 60-minute period would have suggested that 0.03 M $CHCl_3$ was without effect on per-

Table II. *Effect of Treatment with $CHCl_3$ and $CO_2$ on Final Weight of Carrot Disks after Subsequent Equilibration in Water*

| Treatment | Difference between final and initial wt* (mg) | | | |
|---|---|---|---|---|
| | 20 min | 40 min | 60 min | 120 min |
| $H_2O$ | — 5 | — 8 | — 4 | — 6 |
| $CHCl_3$ 0.02 M | — 7 | — 6 | — 22 | — 55 |
| $CHCl_3$ 0.03 M | — 12 | — 40 | — 70 | — 135 |
| $CHCl_3$ 0.04 M | — 120 | — 150 | — 148 | — 152 |
| $CO_2$ | — 2 | — 8 | — 7 | — 10 |
| Sig. diff.** (P = 0.05) | 9 | | | |

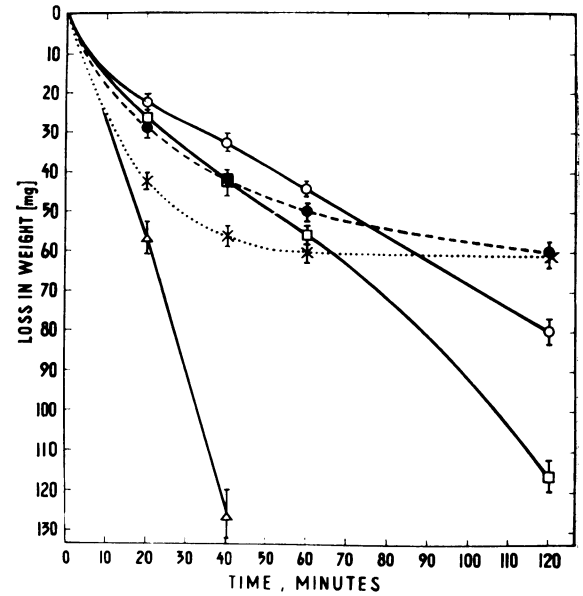
* The tissue was first treated for varying periods as indicated in the table, then transferred to tap water for 90 min.

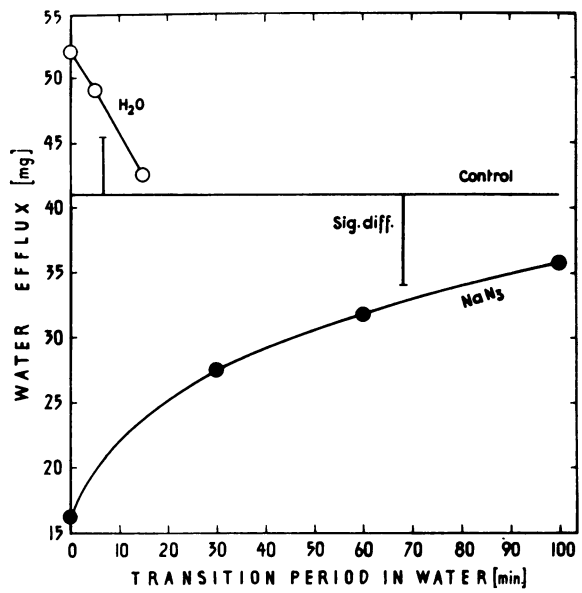
** All treatments were carried out in quadruplicate.

meability to water in accordance with Lepeschkin's views (fig 1), the conclusion would have been an erroneous one. Both table II and the gradually increasing slope relative to the control in figure 1 show that towards the end of the 60-minute period permeability had been raised.

By contrast the curve for $CO_2$ in figure 1 shows that the treatment decreased permeability to water throughout the 2-hour period. The figures in table II lend additional support to the conclusion that within this period, at least, there was no transition to a state of increased permeability.

*Pretreatments with Azide and with Distilled Water.* The observation that 0.05 M azide was not effective in carrot tissue was interesting in that the



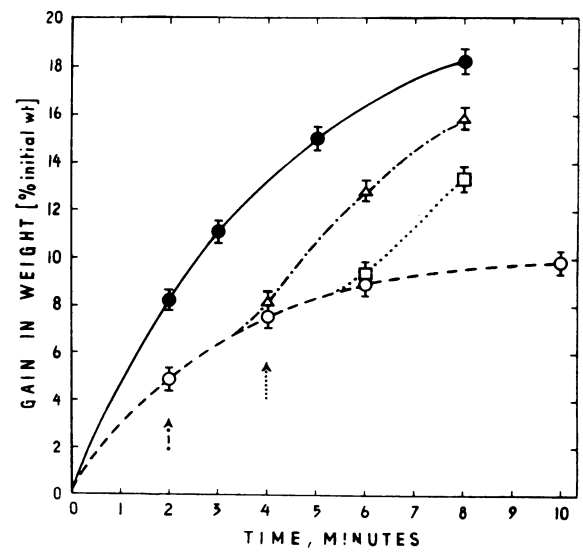




FIG. 1 (*upper*). Effect of $CHCl_3$ and of $CO_2$ on the time course for efflux of water from carrot disks into 0.44 M mannitol solution. ×, control (dotted line); ○, 0.02 M $CHCl_3$; □, 0.03 M $CHCl_3$; △, 0.04 M $CHCl_3$; ●, $CO_2$ (dashed line). In the figures, each point represents the mean of quadruplicates. Their range is indicated where this extended beyond the symbol drawn.

FIG. 2 (*middle*). Reversibility of the effects of $NaN_3$ and of distilled water ($H_2O$) on water efflux from carrot disks. The latter were treated with 0.05 M $NaN_3$ or with distilled water for 90 min, and were then immersed in tap water for a transition period of varying duration before transfer to 0.44 M mannitol for 20 min for the efflux measurements. The control remained in tap water till the efflux measurement.

FIG. 3 (*lower*). Reversibility of the effect of $CO_2$ on water influx into segments of sunflower hypocotyl. The latter had first lost approx. 35 % of their initial water content while drying in air. They were then placed in water through which air (●) or $CO_2$ (○) was bubbling. At the points indicated by the arrows samples of tissue were transferred from $CO_2$ solution to aerated water. △, transferred after 2 min; □, transferred after 4 min.

inhibitor had a marked immediate effect on both efflux and influx into sunflower hypocotyl tissue at much lower concentration (table III). Raising the azide concentration in the case of carrot could not be tried, since the inhibitor would then add considerably to the osmotic value of the external solution. As the rate of penetration of azide into the tissue is not known, there could be no effective osmotic control. It was possible, however, to allow the azide longer time to act. Table III shows that after 90 minutes of pretreatment with 0.05 M azide there was a significant effect on both influx and efflux. Return to original weight after transfer to water was not affected (column D).

The figures for the control in this experiment suggested higher rates of water flux than were normally obtained. This led us to investigate the effect of pretreatment in distilled $H_2O$. The interesting result was obtained (table III, 3) that 1.5 hour's immersion in distilled water, as compared with a similar period in tap water, significantly increased subsequent water flux in both directions. This effect was probably connected with the presence in tap water of one or more of the ions $Na^+$, $Ca^{++}$, and $Cl^-$, since pretreatment in a solution of these ions in the concentrations in which they are present in our tap water gave results very close to those for tap water. Pretreatment with $CaCl_2$ alone also appeared to give results close to those for tap water, but the data were not statistically significant.

The effects of combinations of effective treatments on efflux can be seen in table IV. In this experiment pretreatment in distilled water raised efflux by 17 % (compare 1 and 3). $CO_2$ was equally effective whether applied after pretreatment in distilled water or in tap water (treatments 5 and 7). In other words, the $CO_2$ effect and the distilled water effect do not cancel each other out. Similarly, azide was

Table III. *Effect of $NaN_3$ and of Distilled Water on Water Flux into and out of Segments of Sunflower Hypocotyl and Carrot Disks*

| Tissue | Efflux: Pretreatment (90 min) | Treatment (20 min) | Loss in wt (mg) | D** (mg) |
|---|---|---|---|---|
| 1. Sunflower | None | Raffinose 0.22 M*** | 42 | + 6 |
| | " | $NaN_3$ $10^{-3}$ M + raffinose 0.22 M | 34 | + 2 |
| | " | $NaN_3$ $10^{-2}$ M + raffinose 0.22 M | 51 | − 98 |
| | | Sig. diff. (P = 0.05) † | 5 | 8 |
| 2. Carrot | Mannitol 0.1 M | Mannitol 0.44 M | 49 | − 6 |
| | $NaN_3$ 0.05 M | Mannitol 0.44 M | 26 | − 9 |
| | | Sig. diff. (P = 0.05) † | 6 | 7 |
| 3. Carrot | Dist. water | Mannitol 0.44 M | 51 | − 6 |
| | Tap water | Mannitol 0.44 M | 41 | − 5 |
| | | Sig. diff. (P = 0.05) † | 6 | 4 |

| Tissue | Influx*: Pretreatment (90 min) | Treatment (10 min) | Gain in wt (mg) | D** (mg) |
|---|---|---|---|---|
| 1. Sunflower | None | $H_2O$ | 198 | + 10 |
| | " | $NaN_3$ $10^{-3}$ M | 154 | + 12 |
| | " | $NaN_3$ $10^{-2}$ M | 110 | − 120 |
| | | Sig. diff. (P = 0.05) † | 25 | 12 |
| 2. Carrot | Mannitol 0.48 M | $H_2O$ | 42 | − 7 |
| | Mannitol 0.48 M + $NaN_3$ 0.05 M | $H_2O$ | 27 | − 10 |
| | | Sig. diff. (P = 0.05) † | 5 | 8 |
| 3. Carrot | Mannitol 0.48 M in dist. water | $H_2O$ | 43 | − 10 |
| | Mannitol 0.48 M in tap water | $H_2O$ | 38 | − 8 |
| | | Sig. diff. (P = 0.05) † | 5 | 8 |

* The sunflower segments had previously lost 350 mg by drying in air. The carrot slices had lost 72 mg during the pretreatment.

** Difference between final wt after return to tap $H_2O$ for 90 min and initial wt.

*** This conc. is hypotonic to sunflower segments.

† All treatments were carried out in quadruplicate.

Table IV. *Effect of Combined Treatments with $CO_2$, $NaN_3$ and Distilled Water on Water Efflux from Carrot Disks*

| Pretreatment (90 min) | Treatment (20 min) | Loss in wt (mg) | D* (mg) |
|---|---|---|---|
| 1. Mannitol 0.1 M in dist. $H_2O$ | Raffinose 0.4 M with air** | 42 | − 5 |
| 2. $NaN_3$ 0.05 M in dist. $H_2O$ | Raffinose 0.4 M with air | 19 | − 8 |
| 3. Mannitol 0.1 M in tap $H_2O$ | Raffinose 0.4 M with air | 36 | − 4 |
| 4. $NaN_3$ 0.05 M in tap $H_2O$ | Raffinose 0.4 M with air | 17 | − 7 |
| 5. Mannitol 0.1 M in dist. $H_2O$ | Raffinose 0.4 M with $CO_2$** | 26 | − 3 |
| 6. $NaN_3$ 0.05 M in dist. $H_2O$ | Raffinose 0.4 M with $CO_2$ | 19 | − 8 |
| 7. Mannitol 0.1 M in tap $H_2O$ | Raffinose 0.4 M with $CO_2$ | 23 | − 4 |
| 8. $NaN_3$ 0.05 M in tap $H_2O$ | Raffinose 0.4 M with $CO_2$ | 18 | − 5 |
| | Sig. diff. (P = 0.05)*** | 4 | 6 |

* Difference between final wt after return to tap $H_2O$ for 90 min and initial wt.
** Bubbled continuously through the solution.
*** All treatments were carried out in quadruplicate.

equally effective whether applied in distilled water or in tap water (compare 2 and 4). The effects of $CO_2$ and of azide were not additive (compare 2 and 6).

*Reversibility of the Effects.* Figure 2 shows the reversibility of the effects of distilled water and of azide in the case of carrot tissue. In this experiment, we investigated the effect of interposing immersion in tap water for various lengths of time between pretreatment with distilled water or azide and the measuring period. Fifteen minutes in tap water completely reversed the increase in permeability produced by pretreatment in distilled water. The azide inhibition, on the other hand, continued for more than an hour.

When a similar experiment was performed with $CO_2$, reversal was found to be so quick that even if the measuring period followed the treatment period without interval no difference could be detected between $CO_2$-treated carrot and control. For a more detailed analysis we therefore observed influx into air-dried segments of sunflower hypocotyl (6). This technique is so delicate that it allows accurate measurements after 2-minute intervals. A clear inhibitory effect of $CO_2$ on influx was already visible after 2 minutes (fig 3). Further, complete reversal of the effect was obtained within 2 minutes after transfer of the $CO_2$-treated samples to aerated water, since the curves for subsequent water uptake parallel those for the control tissue. This is true both for the samples treated with $CO_2$ for 2 minutes and for those treated for 4 minutes.

## Discussion

It follows from equation I that the equations for influx and efflux will be:

$$J_v \text{ (influx)} = L_p (\sigma_i \pi_i - \sigma_e \pi_e - P) \qquad II$$

$$J_v \text{ (efflux)} = L_p (-\sigma_i \pi_i + \sigma_e \pi_e + P) \qquad III$$

where $\pi_i$ and $\pi_e$ are the osmotic values for the cell and for the external solution respectively; $\sigma_i$ and $\sigma_e$ are the reflection coefficients for the internal and external solutes; and P is the turgor pressure. (Since there are many internal solutes, the term $\sigma_i \pi_i$ should in fact be $\sum_1^n \sigma_i \pi_i$. For the purposes of the discussion that follows, however, it will be sufficient to assign an average value of $\sigma_i$ and $\pi_i$.)

If $\sigma_i$ is approximately equal to $\sigma_e$, and both are influenced by a factor to about the same degree, then equation III can be written:

$$J_v \text{ (efflux)} = L_p (\sigma (\pi_e - \pi_i) + P). \qquad IV$$

A decrease in $\sigma$ will clearly result in a decrease in efflux into hypertonic solution.

Similarly, since influx from distilled water will be expressed by the equation

$$J_v \text{ (influx)} = L_p (\sigma_i \pi_i - P), \qquad V$$

a decrease in $\sigma$ will bring about a decrease in influx.

Thus any factor which depresses $\sigma$, even though it has no effect on $L_p$, will depress both influx and efflux under the conditions of the classic permeability investigations based on plasmolysis and deplasmolysis (5, 7, 15). The effects observed might therefore have been due to effects on $\sigma$, and not on $L_p$; in other words, to an increased permeability to solutes rather than a decreased permeability to water.

If, on the other hand, a factor decreases water flux under the conditions we have set out, it must affect hydraulic permeability. Our grounds for this conclusion are as follows: Condition A, as is readily apparent, distinguishes between effects on $L_p$ and those on $\pi_i$ or P. Inspection of equations II and III shows that, whereas a change in $\pi_i$ or in P would result in an effect on efflux opposite to that exerted on influx, a change in $L_p$ would influence both efflux and influx in the same direction. Point D is included as an additional check that any observed reduction in flux has not been due to a change in $\pi_i$, due for instance to leakage of internal solutes.

Conditions B and C distinguish between effects on $L_p$ and on $\sigma$. Consider first the case where $\sigma_i = \sigma_e$, and where the factor causes approximately the same change in both. (Equation IV) Since efflux is measured into hypotonic solution, the term $\pi_e - \pi_i$ is negative. A fall in $\sigma$ will therefore increase influx and could not account for an observed decrease in efflux. A rise in $\sigma$ is ruled out by the observed decrease in influx from distilled water (Equation V).

Now consider the case where the factor affects $\sigma_i$ and $\sigma_e$ to different extents:

$$\text{Efflux} = L_p (P - [\sigma_i \pi_i - \sigma_e \pi_e])$$

$$= L_p (P - \sigma_i [\pi_i - \frac{\sigma_e}{\sigma_i} \pi_e]). \qquad \text{VI}$$

To account for an observed decrease in efflux on the basis of changes in $\sigma$, either $\sigma_i$ must rise, which is ruled out for the reason given above, or the term in square brackets must rise, i.e., $\frac{\sigma_e}{\sigma_i}$ must fall. Since raffinose was used as external osmoticum, and since it is unlikely that the membrane would become relatively more permeable to raffinose than to the internal solutes, such a fall is extremely improbable.

Decreases in both influx and efflux under our conditions thus cannot be accounted for on the basis of changes in $\sigma$. A decrease in $L_p$ must be invoked. $CO_2$, low concentrations of chloroform, and azide were all observed to decrease flux under these conditions. It is therefore a valid deduction that they decrease the hydraulic permeability of the tissue to water. While our technique has thus enabled us to detect qualitative changes in $L_p$ unequivocably, refinements are required before quantitative statements can be made.

Parpart and Rosene (12) have described effects of $7 \times 10^{-2}$ M azide on water uptake by radish root hairs. Burström (1), however, reported that he was unable to detect any change in the permeability of Rhoeo cells to water as a result of azide treatment, though he demonstrated a decreased permeability to glycerol.

Effects of $CHCl_3$ on the permeability of plant tissues to ions have previously been noted by Osterhout (11) and to dyes by Lepeschkin (9). The present paper is the first report of an effect on permeability to water. While low concentrations applied for relatively short periods decreased permeability, higher concentrations increased water flux. This increased efflux was associated with a lower water content after subsequent equilibration in water, as compared with the control. Since the latter result would be consistent with leakage of solutes from the tissue, it is possible that the increased water efflux into hypotonic solution was, in its early stages, due to a decrease in $\sigma_i$ (equation VI), i.e., to an increase in permeability to solutes. In its later stages it is probable that the semipermeability of the membranes was entirely destroyed. Figure 1 shows that whether the effect on semipermeability or that on $L_p$ predominates depends not only on $CHCl_3$ concentration but on time. The assessment of factors affecting water flux should thus not be based on measurement over a single period. A time course should rather be obtained.

An increase in water flux was also observed after 1.5 hours of pretreatment in distilled water. In this case, however, in contrast to that of 0.05 M $CHCl_3$, no leakage of solutes appeared to take place, the weight of the tissue after subsequent equilibration in tap water being the same as that of the control. Further, influx was increased as well as efflux, which was not the case with $CHCl_3$. Equation V shows that in order to account for the observed rise in influx on the basis of a change in $\sigma$, a rise in the latter must be postulated. Equation IV, on the other hand, requires a fall in $\sigma$ in order to account for the increased efflux reported in tables III and IV, since $\pi_e - \pi_i$ was negative. It is therefore more likely that the rise in flux was due to an increase in $L_p$ than to a change in $\sigma$. The loss of Ca ions from the membrane into distilled water may possibly have brought about this increase in $L_p$, but much further work is required before the point can be decided.

Our results showed that the effects of $CO_2$, of azide, and of distilled water were all reversible. In the case of $CO_2$ reversal was achieved within 2 minutes; 15 minutes and 1 hour were required in the case of distilled water and azide respectively. No data are available in the literature on the speed of reversal of similar changes in permeability for purposes of comparison.

Ray and Ruesink (14) drew the conclusion that the rate of diffusion of the external osmoticum governed the rate of water flux in experiments similar to those described here. That this factor was one of the rate-determining factors in our experiments is shown by a comparison of the control values obtained in various osmotica. If, however, this were the only limiting factor it would imply that $CO_2$ and the other treatments produced their effects owing to an influence on the diffusion of mannitol and raffinose into the free space of the tissue. The fact that $CO_2$ and the other substances also depress water influx into tissues that have lost water in air rather than by osmotic dehydration is a strong indication that this was not the case.

## Summary

Factors affecting the rate of water movement into and out of carrot disks and segments of sunflower hypocotyl have been examined. It is demonstrated that, under the conditions of the classical plasmolytic studies on permeability, effects attributed to changes in $L_p$, the coefficient of hydraulic permeability of the membrane, might have been due to changes in $\sigma$, the reflection coefficient. Conditions are defined under which effects due to a fall in $L_p$ may be distinguished from those due to a fall in $\sigma$. $CO_2$, azide, and 0.02 M chloroform have been shown to decrease

$L_p$. Higher concentrations of chloroform brought about an increase in water efflux which was associated with irreversible changes in the membrane. The effect of chloroform was shown to depend not only on concentration but on length of treatment. Ninety minutes' immersion in distilled water, as compared with a similar period in tap water, brought about an increase in $L_p$. Treatment with distilled water did not lessen the effects of $CO_2$ and of azide. The latter were not additive. The effects of $CO_2$, of azide, and of distilled water were reversible. In the case of $CO_2$ reversal was achieved within 2 minutes.

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