AN ANALYSIS OF THE WATER POTENTIAL ISOTHERM IN PLANT TISSUE

I. THE THEORY

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Summary

The water potential isotherm is the function relating the water potential (specific free energy of water) in a given system to its water content. The theory of this function in plant tissues, including its partition into the hydrostatic, osmotic, and matric components, is developed in three stages:

(1) The derivation of the water potential and its components from simple variables, for an “ideal”, homogeneous tissue. It is shown that solute-matrix interactions cause a possibility of ambiguity in the definitions of the osmotic and matric components.

(2) The treatment of the theoretical dependence of each component on the water content. Certain simplifying assumptions about the osmotic and the hydrostatic potentials are critically examined.

(3) The extension of the theory to the real plant tissue, which is multiphasic and heterogeneous. The components of water potential as derived from measurements on such a tissue in bulk are expressed in terms of the components in the individual phases. One of the results is that heterogeneity causes considerable difficulties in the exact interpretation of the measurable “bulk” components. The partition of water content into the “matrix-bound” and “solution” fractions, as an alternative to the partition of water potential into its matric and osmotic components, is considered.

I. INTRODUCTION

Water in the living plant as a whole is not in equilibrium under natural conditions. The understanding of the influence of water on the physiological activities of the plant requires, nevertheless, knowledge of the water equilibrium properties of the various cells and tissues.

Each water equilibrium state may be characterized by a certain value of the chemical potential of water, which is constant throughout the system (in our case a cell, tissue, or organ). As the value of this quantity in a cell changes, other quantities change also: water content, volume, solute concentration, hydrostatic pressure, gel hydration; each of these may, in turn, affect the physiological processes in the cell. The way in which these parameters in a given system (cell or tissue) depend on the chemical potential of water can be studied by measuring and analysing the “water potential isotherm” of the system. This name is given here to the function relating the water content of the system to the chemical potential of water (or the “water potential” proportional to it) at equilibrium, at constant temperature. The water potential isotherm has long been measured in soils and has been recognized as

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characterizing their water equilibrium properties (usually called "water retention curve" or "moisture characteristic"). It is only in the last few years that attention has been drawn to the same curve for plant tissues, mainly by Tyurina (1957a), Weatherley and Slatyer (1957), Slatyer (1958, 1960, 1961), Jarvis and Jarvis (1963). These authors, and lately also Gardner and Ehlig (1965) and Weatherley (1965), report measurements of water potential isotherms for leaves. In some of these papers, an attempt was made to analyse the curve into different components, and its physiological and ecological significance was discussed.

The water relations of plants have been placed on a thermodynamic basis by Taylor and Slatyer (1961), Dainty (1963), and Spanner (1964). The classical concept of the diffusion pressure deficit (or "suction pressure") as difference between osmotic and turgor pressure has been replaced (Taylor and Slatyer 1961) by the "water potential" (Ψ) as a sum of osmotic (Ψσ), hydrostatic (Ψp), and matrix (Ψτ) components. The water potential isotherm can correspondingly be partitioned into three functions, each relating one of the three components to water content (w) at constant temperature. However, a number of problems concerning both the theoretical definition of the components and their experimental evaluation are yet unsolved.

The simplest model, and the one most commonly used, treats the plant tissue as if it were a single phase of dilute ideal solution, enclosed by elastic walls, which are either very thin or have no affinity for water. This is equivalent to making the following assumptions about the components:

1. Ψσ is linear with solute concentration, i.e. with the reciprocal of water content, 1/w.
2. Ψp is linear with the relative excess water content, (w - w0)/w0.
3. Ψτ is zero.

This model may be adequate for a single, highly vacuolated, thin-walled cell, but it cannot, a priori, be extended to a whole tissue or organ, specially if it is as complex and as heterogeneous as a leaf. Inconsistencies with assumptions (1), (2), and (3) may arise for a number of reasons, the following of which are considered as most important:

(i) The osmotic potential is inconsistent with (1) whenever the solution is non-ideal, i.e. the activity coefficient of each solute varies with the concentration of solutes. Most electrolyte and polymer solutions are markedly non-ideal.

(ii) Non-linearity in the hydrostatic potential may be caused by the complex structure of the cell wall and by pressure exerted between adjacent cells. The validity of assumption (2) when Ψp falls below zero, and the possible magnitude of this "negative turgor" are still controversial (Tyurina 1957b; Slatyer 1958, 1960; Rehder 1961; Kreeb 1961, 1963).

(iii) Finally, in many plant tissues, the effect of the hydrophilic gels (mainly in the cytoplasm and the cell wall) on the water equilibrium properties of the tissue may not be negligible. There are only very few experimental data on the magnitude of this effect (Carr and Gaff 1961; Gaff and Carr 1961).
The aims of the present work are:

1. To derive an equation for the partition of water potential in plant tissue into components, in which each component is defined in terms of simple variables and is at the same time experimentally measurable.

2. To apply the equation to the measurement and analysis of the water potential isotherm of leaf tissue; to fit to the results for each component empirical functions as far as possible related to theoretical functions.

3. To test, in particular, the contributions to the isotherm of the following: non-ideality of solution, negative turgor, cell wall and cytoplasmic matrix, hysteresis.

4. To elucidate the possible eco-physiological significance of the form of isotherm and its components, especially in relation to adaptation to drought.

The work has been divided into three parts: I, the theory of the water potential isotherm; II, the methods of the analysis; III, a comparative analysis of the isotherm for leaves of different types. The present paper includes only the first part.

II. Theory of the Water Potential Isotherm

In this part we intend to examine thoroughly the thermodynamic basis of the concept of the isothermal water potential and its “components”, as it applies to multicellular plant tissues.

The water potential and its components will be defined in terms of general thermodynamic functions, from which the measurable quantities will be derived explicitly and progressively. In particular, simplifications and assumptions will be introduced explicitly, and their physical meaning indicated. Thus confusion and ambiguity, which can arise from implicit assumptions, are avoided as far as possible. The symbols used and their meanings are listed in the Appendix.

The treatment has been made in three stages:

1. Definition of the water potential in homogeneous systems and its partition into measurable components.

2. Theoretical consideration of the dependence of each component on the water content of the system.

3. Discussion of modifications required on application of the theory to heterogeneous systems.

(a) Partition of the Water Potential in a Homogeneous System

(i) Description and Definition of the System

The following treatment applies to an “ideal tissue”, i.e. a system similar to a fragment of plant tissue in that it consists of water, solutes, and matrix (insoluble substances), and in that it is able to maintain a pressure difference against the exterior. In contrast to a real plant tissue, the “ideal tissue” is assumed to be homogeneous with regard to pressure and concentration of all the substances.
The chemical potential of water, \( \mu_w \), is defined as the partial molal free energy of water:

\[
\mu_w = \left( \frac{\partial G}{\partial n_w} \right)_{X_i},
\]

where \( G \) = free energy (Gibbs), \( n_w \) = number of moles of water, and \( X_i \) = all other variables necessary for describing the state of the system.

The system is brought to a state of partial equilibrium in which it is at equilibrium with regard to water and to temperature, both internally and with an external measuring system. Then chemical potential of water and temperature are equal in all parts of the measured system (the ideal tissue) and in the measuring system. The absence of net water flow between the measured and the measuring system is a sufficient criterion for the existence of such an equilibrium, provided that two other conditions are being fulfilled:

1. There is no flow of any substance other than water between the two systems. It is reasonable to assume that such is the case, when equilibration occurs through the vapour phase.
2. There is no coupling of water flow with any chemical reactions that may occur within the tissue.

We shall consider only states of the system which are at the same temperature. Further we shall assume that no differences between states of the system are introduced by hysteresis effects. Within these limitations, the state of the system is completely defined by its pressure and by the amounts of water, solutes, and insoluble substances in it. The total differential of the chemical potential of water may then be written:

\[
d\mu_w = \left( \frac{\partial \mu_w}{\partial P} \right)_{n_i} dP + \sum_s \left( \frac{\partial \mu_w}{\partial n_s} \right)_{P,n_j} dn_s + \sum_m \left( \frac{\partial \mu_w}{\partial n_m} \right)_{P,n_j} dn_m + \left( \frac{\partial \mu_w}{\partial n_w} \right)_{P,n_j} dn_w,
\]

where

\( P \) = pressure,
\( n_s \) = number of moles of solute,
\( n_m \) = number of moles of insoluble substances, and
\( n_j \) = number of moles of any substance except the one with respect to which the differentiation is made.

The “water potential”, \( \Psi \), will now be defined as

\[
\Psi = \frac{\mu_w - \mu_w^0}{V_w^0},
\]

where \( \mu_w^0 \) and \( V_w^0 \) are the chemical potential and molal volume, respectively, of pure water at atmospheric pressure and a given temperature. This definition differs from that of Taylor and Slatyer (1961) only in that here, \( V_w \) is taken at the standard state. Thus the \( V_w \) in the system, the value of which is often unknown, is eliminated from the definition of \( \Psi \). The dimensions of \( \Psi \) are energy/volume = pressure, which is convenient.

(ii) Partition by Stepwise Integration

In equation (2), \( d\mu_w \) is a total differential and its integral is independent of the path of integration. Therefore \( \mu_w - \mu_w^0 \) can be found by integration of \( d\mu_w \).
between the appropriate boundaries, by any path that we choose, and the total water potential as defined in (3) will be:

\[ \Psi = \frac{1}{\bar{V}_u} \int_{\mu_{w}}^{\Psi} d\mu_w. \]  \hspace{1cm} (4)

The upper boundary of integration is defined by the values of \( P, n_w, n_m, n_s \) (or \( P, n_s/n_w, n_m/n_w \)) in the system under consideration. The lower boundary (pure water at standard pressure) can be defined by any one of three alternative sets of conditions (\( P^0 = \text{atmospheric pressure} \)):

\[
\begin{align*}
(a) \quad & P = P^0, \ n_w = \infty; \quad \text{(any } n_m, n_s) \\
(b) \quad & P = P^0, \ n_s/n_w = 0, \ n_m/n_w = 0; \\
(c) \quad & P = P^0, \ n_s = 0, \ n_m = 0. \quad \text{(any } n_w) 
\end{align*}
\]  \hspace{1cm} (5)

We shall choose to integrate equation (4) according to set (c) as it enables the components of the water potential to be obtained most readily. Hence

\[ \Psi = \frac{1}{\bar{V}_u} \int_{P^0}^{P, n_s, n_m, n_w} P = P^0, n_s = 0, n_m = 0, n_w \quad d\mu_w. \]  \hspace{1cm} (6)

Where a variable is unspecified its value is taken to be the one at the state of the system under consideration.

The integration is now made in steps. At each step only one variable (bold type) is changed from its lower to its upper boundary, all others remaining constant. For example:

\[
\Psi = \frac{1}{\bar{V}_u} \left[ \sum_m \int_0^{P^0} P = P^0, n_s = 0, n_m, n_w \quad d\mu_w + \sum_n \int_0^{P^0} P = P^0, n_s = 0, n_m, n_w \quad d\mu_w \right. \\
+ \left. \int_{P^0}^{P, n_s, n_m, n_w} P = P^0, n_s, n_m, n_w \quad d\mu_w \right]. 
\]  \hspace{1cm} (7)

When we substitute the expression for \( d\mu_w \) from equation (2) into equation (7), then at every step all terms will vanish except the one depending on the variable which is being changed. Therefore:

\[
\Psi = \frac{1}{\bar{V}_u} \left[ \sum_m \int_0^{P^0} P = P^0, n_s = 0, n_m, n_w \quad \left( \frac{\partial \mu_w}{\partial n_m} \right) d\mu_w + \sum_n \int_0^{P^0} P = P^0, n_s, n_m, n_w \quad \left( \frac{\partial \mu_w}{\partial n_s} \right) d\mu_w \right. \\
+ \left. \int_{P^0}^{P, n_s, n_m, n_w} \left( \frac{\partial \mu_w}{\partial P} \right) dP \right]. 
\]  \hspace{1cm} (8)

We thus have divided the total water potential into three components, each of which is dependent on the change in a single variable. We may rewrite equation (8) in the form:

\[ \Psi = \Psi_t + \Psi_o + \Psi_p, \]  \hspace{1cm} (9)

where \( \Psi_t, \Psi_o, \) and \( \Psi_p \) may be called the matric, osmotic, and hydrostatic components of the water potential. Each component is defined by the corresponding integral.
The sequence of steps chosen in equation (7) is only one of several possible alternatives. The resulting total water potential will be the same in every case. For 3 variables, there are 3! (= 6) possible sequences.

We shall examine another sequence, which differs from equation (7) in that the steps \( n_s = 0 \rightarrow n_s \) precedes the step \( n_m = 0 \rightarrow n_m \). As before the last step is 
\[ P = P_0 \rightarrow P. \]
Instead of equation (8) we then have:

\[
\Psi = \frac{1}{P_0} \left[ \sum_s \int_{P}^{P^*} n_s, n_m = 0, n_w \left( \frac{\partial \mu_w}{\partial n_s} \right) d n_s + \sum_m \int_{P}^{P^*} n_s, n_m, n_w \left( \frac{\partial \mu_w}{\partial n_m} \right) d n_m + \int_{P}^{P^*} n_0, n_m, n_w \left( \frac{\partial \mu_w}{\partial P} \right) d P \right],
\]

and

\[
\Psi = \Psi_p + \Psi + \Psi_p'.
\]

Comparison of equations (8) and (10) shows that the hydrostatic component is equal in both: \( \Psi_p = \Psi' \). The osmotic and matric components in equation (10), however, are not necessarily equal to those of equation (8).

(iii) Physical Meaning of the Components

Consideration of the boundary conditions of the integrals is necessary to give physical meaning to the components. The variable of water content, \( n_w \), is equal at both boundaries of each integral. Thus, the partition of water potential at each point of the isotherm is made at constant total water content. Only those states of the system with the same \( n_w \) are comparable (except when \( n_m = 0, n_s = 0 \): then the value of \( n_w \) is unimportant).

Examination of the boundary conditions of the first term in equations (7) or (8) shows that this term is equal to the water potential (= difference in water potential from pure water) of the matrix of the system (our “ideal tissue”) when isolated and solute-free \( n_s = 0 \) at both boundaries, at \( P_0 \) and the given \( n_w \). This is a measurable quantity, which will be called \( \Psi_m \):

\[
\Psi_m = \Psi_m.
\]

The sum of the first two terms in equations (7) or (8) is the water potential of the whole system (matrix and solutes) at \( P_0 \), and the given \( n_w \); this is usually measured as the water potential of the killed tissue, which will be called \( \Psi_d \):

\[
\Psi_d = \Psi_d.
\]

The values of the three theoretical components defined in equations (7), (8), and (9) can therefore be found from the three measurements of \( \Psi \) (of the living tissue), \( \Psi_d \), and \( \Psi_m \), at constant \( n_w \):

\[
\begin{align*}
\Psi_t &= \Psi_m, \\
\Psi &= \Psi_d - \Psi_m, \\
\Psi_p &= \Psi - \Psi_d.
\end{align*}
\]
Let us now examine the physical meaning of the components resulting from the alternative partition of the water potential in equations (10) and (11). In these equations there is no term corresponding to a direct measurement of the water potential of the isolated matrix ($\Psi_m$). Instead, the first term in these equations is the water potential of the solution of the tissue, when isolated and matrix-free ($n_m = 0$) at $P^0$ and the given $n_w$. This term, designated by $\Psi_s$, can be measured on an extract of the tissue. In this case $\Psi'$ is found indirectly:

$$\begin{align*}
\Psi'_\pi &= \Psi', \\
\Psi'_s &= \Psi'_d - \Psi'_s, \\
\Psi'_p &= \Psi - \Psi'_d.
\end{align*}$$

(15)

$\Psi'$ can also be measured directly as the difference in water potential between the whole system (matrix and solution) and the solution alone (for example, by the tensiometer method used for soils). There is, therefore, a corresponding set of measurements for each of the two different sequences of integration. No matter which sequence is chosen, the sum of the first two components is the same:

$$\Psi'_\pi + \Psi'_{\tau} = \Psi'_{\pi} + \Psi'_{\tau} = \Psi'_d.$$  

(16)

However, $\Psi'$ is not necessarily equal to $\Psi'$, nor $\Psi'_\pi$ to $\Psi'_{\pi}$. Comparison of equations (8) and (10) shows that $\Psi_{\tau} = \Psi'_{\tau}$ and $\Psi'_\pi = \Psi'_{\pi}$ only if the partial derivative with respect to solutes is independent of the amount of matrix, and vice versa:

$$\begin{align*}
\left(\frac{\partial \mu_w}{\partial n_m}\right)_{n_m, P^0} &= \left(\frac{\partial \mu_w}{\partial n_s}\right)_{n_m = 0, P^0}, \\
\left(\frac{\partial \mu_w}{\partial n_m}\right)_{n_s, P^0} &= \left(\frac{\partial \mu_w}{\partial n_s}\right)_{n_s - 0, P^0}.
\end{align*}$$

(17)

This is equivalent to saying that the effects of solutes and matrix on water potential are independent; or that the cross-derivative is zero:

$$\left(\frac{\partial^2 \mu_w}{\partial n_m \partial n_s}\right)_{P^0} = 0.$$

(18)

In this special case

$$\Psi'_m + \Psi'_s = \Psi'_d,$$

(19)

and therefore

$$\Psi'_{\tau} = \Psi'_{\pi}; \quad \Psi'_{\pi} = \Psi'_{\pi}.$$

(20)

Equations (17)–(20) do not hold when the water potential is affected by interactions between the solutes and the matrix. Such interactions may well exist. If so, different values of the osmotic and matrix components are obtained from each of the two ways of partition. These concepts are therefore ambiguous unless referred to a particular sequence of integration, or method of measurement. Neither method can claim to give the "real" osmotic and matrix potentials. The difference between them lies in the fact that the "interaction" effects are included in the osmotic
component in the first way [eqn. (8)], and in the matric component in the second [eqn. (10)]. A complete analysis requires the separate determination of $\Psi_d$, $\Psi_m$, and $\Psi_s$; then the "solute–matrix interaction potential", $\Psi_{sm}$, can be found from the equation:

$$\Psi_d = \Psi_s + \Psi_m + \Psi_{sm}. \quad (21)$$

The sign and magnitude of $\Psi_{sm}$ depend on the cross-derivative $\left(\frac{\partial^2 \mu_w}{\partial n_w \partial n_\ell}\right)$.

The hydrostatic term has been the last step in both of the sequences of integration studied so far. We may choose another sequence which begins with integration of the hydrostatic variable:

$$\Psi = \frac{1}{\bar{V}_w} \int P, n_s = 0, n_m = 0, n_w \left(\frac{\partial \mu_w}{\partial P}\right) dP + \sum \int P, n_s = 0, n_m = 0, n_w \left(\frac{\partial \mu_w}{\partial n_s}\right) d\bar{n}_s$$

$$+ \sum \int P, n_s = 0, n_m = 0, n_w \left(\frac{\partial \mu_w}{\partial n_m}\right) d\bar{n}_m. \quad (22)$$

If $\frac{\partial \mu_w}{\partial P} (= \bar{V}_w)$ depends on $n_s$ and $n_m$ (which is quite likely), then this method of partition will give a different result from the two former methods. But it is impossible to evaluate the terms in equation (22), or any other sequence in which $n_s$ or $n_m$ change, while $P$ remains at its original value. $n_s$ or $n_m$ cannot be brought to zero without changing $P$, except at $P^0$; in other words neither solute nor matrix can be separated from a living tissue without destroying the turgor pressure. Neither is there as yet any technique for the direct measurement of hydrostatic pressure in intact tissue. Thus the four sequences of integration, in which $P$ is changed in the first or second step, yield terms which are mathematically defined, and even physically interpretable (in terms of hypothetical operations), but which cannot be measured experimentally.

The most interesting conclusion from the explicit treatment in this part is the following: when a quantity such as water potential depends on several variables, there is more than one way of partitioning it into components dependent on each of the variables separately. This ensues from the possible existence of terms which depend on the interaction between two, or more, variables. These must either be defined as separate components or else included in one of the "single-variable" components, the definition of which then depends on the choice made in this inclusion.

(b) Dependence of the Water Potential Components on Water Content

(i) Definition of the Water Content Parameter

In this part we shall treat each of the components separately, and try to predict, on the basis of current theories, the form of the functional relationship between each component and the water content of the system.

The water content in a system may be expressed in terms of the number of moles of water, $n_w$, or of any extensive parameter directly proportional to it. But measurements made on various samples (for example, samples of disks from a leaf)
are comparable only if the water content is related to some measure of the "size" of the sample. In this work we have chosen dry weight of the sample as the basis of comparison (details in Part II). The water content parameter \( w \) is therefore defined as:

\[
\begin{align*}
  w &= (W_w/W_d) = (n_w M_w/W_d) = (P_{w0}^0/W_d) n_w, \\
  \text{where} \\
  W_w &= \text{weight of water in the sample}, \\
  W_d &= \text{dry weight of the sample}, \\
  M_w &= \text{molecular weight of water}, \\
  \rho_w &= \text{density of water, and} \\
  \bar{V}_w^0 &= \text{molal volume of pure water at } P^0.
\end{align*}
\]

The density of water at usual temperatures is very near to unity (at 25\(^\circ\)C, \( \rho_w = 0.997 \)). Therefore, \( w \) is numerically almost equal to \( (P_{w0}^0/W_d) n_w \). Dimensionally, \( w \) is a pure number (g/g).

(ii) The Hydrostatic Potential, \( \Psi_p \)

As defined by equations (8)--(11), the hydrostatic component of water potential is:

\[
\begin{align*}
  \Psi_p &= \frac{1}{\bar{V}_w^0} \int_{P = P^0}^{P} \left( \frac{\partial \mu_w}{\partial P} \right) dP = \frac{1}{\bar{V}_w^0} \int_{P = P^0}^{P} \bar{V}_w (n_a, n_m, n_w, P) dP. \\
\end{align*}
\]

\( \bar{V}_w (n_a, n_m, n_w, P) \) is the partial molal volume of water in the system, and may be a function of its composition and pressure. As the compressibility of water is low, we may assume that \( \bar{V}_w \) is independent of \( P \) when the range of pressures is not too wide. Then the integration is simple, and we have:

\[
\begin{align*}
  \Psi_p &= (\bar{V}_w/\bar{V}_w^0)(P - P^0) = (\bar{V}_w/\bar{V}_w^0)\Delta P. \\
\end{align*}
\]

Here \( \bar{V}_w \) is the partial molal volume of water in the tissue at the given composition \((n_m, n_a, n_w)\). It may differ markedly from \( \bar{V}_w^0 \) especially at high concentrations of matrix. However, since exact information is lacking, it will henceforth be assumed that \( \bar{V}_m = \bar{V}_w^0 \), bearing in mind the possible error resulting from that. On this assumption, we arrive at the simple equation

\[
\Psi_p = \Delta P, \quad (26)
\]

i.e. the hydrostatic potential is equal to the excess hydrostatic pressure (turgor pressure) in the system.

If the system is enclosed within ideally elastic walls:

\[
\Psi_p = \Delta P = \epsilon' [(V-V^0)/V^0], \quad (27)
\]

where

\[
\begin{align*}
  \epsilon' &= \text{the elasticity modulus of the walls,} \\
  V &= \text{volume, and} \\
  V^0 &= \text{the volume when } \Delta P = 0.
\end{align*}
\]
If we assume the non-aqueous volume constant \((V - V^0 = V_w - V^0_w)\), then

\[
\Psi_p = \epsilon' \left( \frac{V_w - V^0_w}{V^0_w} \right) = \epsilon' \left( \frac{V^0_w}{V^0_w} \right) = \epsilon' \phi_w \left( \frac{n_w - n^0_w}{n^0_w} \right),
\]

or

\[
\Psi_p = \epsilon \left[ (n_w/n^0_w) - 1 \right] = \epsilon \left[ (w/w^\theta) - 1 \right],
\]

where

\[
V_w = \text{volume of water},
\]
\[
V^0_w = \text{volume of water at } \Delta P = 0,
\]
\[
n^0_w = \text{number of moles of water at } \Delta P = 0,
\]
\[
\phi_w = \text{volume of fraction of water at } \Delta P = 0 \quad (= V^0_w/V^0),
\]
\[
\epsilon = \epsilon' \phi_w, \text{ and}
\]
\[
w^\theta = \text{water content at } \Delta P = 0.
\]

When elasticity is non-linear (the elasticity modulus is not constant), the equations may be modified by expressing \(\epsilon\) as a function of \(w\).

In principle an equation like (29) may be valid both when \(w > w^\theta\) (then \(\Psi_p\) is positive) and when \(w < w^\theta\) (negative \(\Psi_p\)). But the magnitude of the excess negative pressure may be limited by the ability of the system (in this case, the plant cells) to maintain this pressure. In this case the negative pressure will increase (in absolute value) with decreasing \(w\), up to a "critical point", where there will be a "collapse", and the negative pressure will fall abruptly from its maximal value to zero.

(iii) The Osmotic Potential, \(\Psi_\pi\)

We shall use in the following treatment that definition of the osmotic potential which does not include solute–matrix interaction effects; from equations (10) and (11):

\[
\Psi_\pi = \frac{1}{\nu_0} \sum_{s} \int_{P_s, n_s, n_m = 0, n_w}^{P_s, n_s = 0, n_m = 0, n_w} \left( \frac{\partial \mu_s}{\partial n_s} \right) dn_s = \frac{1}{\nu_0} \sum_{s} n_s \left( \frac{\partial \mu_s}{\partial n_s} \right) P_s, n_m = 0, n_w \quad \text{dn}_s. \tag{30}
\]

From fundamental thermodynamics there follows:

\[
\left( \frac{\partial \mu_s}{\partial n_s} \right) = \left( \frac{\partial n_s}{\partial n_w} \right) = RT \left( \frac{\partial \ln n_s}{\partial n_w} \right), \tag{31}
\]

where \(a_s\) = activity of solute. If we use the definition of activity in terms of molality \((m_s)\) and molal activity coefficient \((\gamma_s)\), then

\[
a_s = m_s \gamma_s. \tag{32}
\]

Therefore

\[
\left( \frac{\partial \mu_s}{\partial n_s} \right) = RT \left( \frac{\partial \ln (m_s \gamma_s)}{\partial n_w} \right) = RT \left( \frac{\partial \ln m_s}{\partial n_w} \right) + RT \left( \frac{\partial \ln \gamma_s}{\partial n_w} \right), \tag{33}
\]

and by substitution in (30)

\[
\Psi_\pi = \frac{R T}{\nu_0} \sum_{s} \int_{n_s = 0}^{n_s} \left( \frac{\partial \ln m_s}{\partial n_w} \right) dn_s + \frac{R T}{\nu_0} \sum_{s} \int_{n_s = 0}^{n_s} \left( \frac{\partial \ln \gamma_s}{\partial n_w} \right) dn_s. \tag{34}
\]
The first term, which is dependent on \( n_s \), is very nearly equal to the potential of a dilute ideal solution, and will be called the "ideal" osmotic potential, \( \Psi_{\pi l} \). The second term, which is dependent on \( \gamma_s \), consists mainly of the deviations from ideality, and will be called the "additional" osmotic potential, \( \Psi_{\pi r} \).

(An exact separation between ideal and non-ideal terms at all concentrations can be achieved by defining activity as the product of molar ratio and rational activity coefficient, instead of equation (32). But the result is less convenient for further treatment, while the difference is small over the considered concentration range.)

We may therefore rewrite equation (34) as:

\[
\Psi_{\pi} = \Psi_{\pi l} + \Psi_{\pi r}. \tag{35}
\]

The first term depends on the total number of solute molecules; the second one on the specific properties and relative amount of the different solute species. We shall briefly treat each of these further.

(1) The Ideal Osmotic Potential (\( \Psi_{\pi l} \)).—From equation (34)

\[
\Psi_{\pi l} = \frac{RT}{V_w} \sum_s n_s \left( \frac{\partial \ln m_s}{\partial n_w} \right) dn_s. \tag{36}
\]

A general solution is possible if we assume that the amount of solutes, \( n_s \), is independent of water content, \( n_w \). This assumption seems justified for a killed tissue or a tissue extract. But it may not apply to a living tissue, where the balance between hydrolytic and synthetic processes is liable to change with changes in water content. Thus, this assumption should be experimentally checked before the isotherms of living and killed tissue are compared. When the assumption is acceptable, i.e. \( (dn_s/dn_w) = 0 \), then

\[
(\partial \ln m_s/\partial n_w) = -(1/n_w), \tag{37}
\]

and

\[
\Psi_{\pi l} = -\frac{RT}{V_w} \sum_s n_s \frac{dn_s}{n_w} = \frac{RT}{V_w} \frac{\Sigma n_s}{n_w} = -RT \rho_w \Sigma n_s. \tag{38}
\]

\( \Psi_{\pi l} \) is always negative.

On the introduction of the water content parameter \( w \), as defined in equation (23):

\[
\Psi_{\pi l} = -RT \rho_w \frac{\Sigma n_s}{W_d} \frac{1}{w} = -\frac{a}{w}, \tag{39}
\]

where \( a \) is a constant (since we have assumed \( \Sigma n_s \) constant). Its value is given by

\[
a = RT \rho_w (\Sigma n_s/W_d). \tag{40}
\]

Equation (39) may also be written in the form

\[
-\Psi_{\pi l}w = a. \tag{41}
\]
(2) The Additional Osmotic Potential ($\Psi_{\text{osm}}$).—From equation (34)

$$\Psi_{\text{osm}} = \frac{RT}{\mu_0} \sum_{s} \int_{n_s}^{n_s} \left( \frac{\partial \ln \gamma_s}{\partial n_w} \right) dn_w.$$  \hspace{1cm} (42)

The exact solution requires knowledge of the partial derivative ($\partial \ln \gamma_s / \partial n_w$) for each of the solutes. This is almost impossible for a multi-component solution such as exists in plant cells. Still, some information about the general form of function to be expected may be deduced from data obtained with pure solutions. The variation of activity coefficient, $\gamma_s$, with molality, as measured for many solutions (such as solutions of sugars, proteins, and not too dilute electrolyte solutions) can be approximated, over a wide range, by a linear expression of the form:

$$\ln \gamma_s = B_s m_s,$$  \hspace{1cm} (43)

where $B_s$ is a constant. Theoretical interpretations of this equation exist for electrolyte and polymer solutions. Substitution of (43) in (42) and integration yields

$$\Psi_{\text{osm}} = \frac{-RT \Sigma (B_s n_s^2)}{2(\mu_0)^2} \frac{1}{m_w^2} = -\frac{b}{m_w^2},$$  \hspace{1cm} (44)

where $b$ is a constant. In dilute electrolyte solutions the Debye–Hückel law requires introduction of another term in equation (43) which is approximately proportional to $I^4$ ($I =$ ionic strength) and therefore to $m_s^4$:

$$\ln \gamma_s = B_s m_s - k_s (m_0)^4,$$  \hspace{1cm} (45)

and therefore instead of equation (44) we get

$$\Psi_{\text{osm}} = -(b/w^3) + (c/w^{3.2}).$$  \hspace{1cm} (46)

The second (Debye–Hückel) term is positive, while the first one is usually negative.

In the preceding treatment of the osmotic potential, solute–matrix interaction effects have not been taken into account. If these are not negligible, and if, by definition, they are included in the osmotic potential, they will probably cause further deviation from ideality.

(iv) The Matric Potential, $\Psi_z$

If we define the matric potential so that it does not include the solute–matrix interactions, then according to equations (8) and (9):

$$\Psi_z = \frac{1}{\mu_0} \sum_{m} \int_{m}^{m} \left( \frac{\partial \mu_w}{\partial m_w} \right) m_{m},$$  \hspace{1cm} (47)

The matric potential is then equal in magnitude and opposite in sign to the "swelling pressure" of the isolated matrix in pure water. A number of different empirical and theoretical equations for the swelling pressures of gels have been obtained (reviewed by Katchalsky 1954). Most of these include an "osmotic" term, contributed by the free energy of mixing of the polymer molecules with water, and an
“elastic” term, contributed by the free energy of stretching of the polymer network:

$$\Psi_r = \Psi_{\text{rmix}} + \Psi_{\text{str}}$$  \hspace{1cm} (48)

The first term is negative, and the second positive. The total $\Psi_r$ is always negative.

For non-electrolytic gels the following equations have been theoretically derived:

$$\Psi_{\text{rmix}} = -RTn_m[(1/n_w) + (k/n_w^2)]$$  \hspace{1cm} (49)

$$\Psi_{\text{str}} = RTn_m[d/(n_w)^4]$$  \hspace{1cm} (50)

where $k$ and $d$ are constants. In polyelectrolytes the term proportional to $1/n_w^2$ usually becomes less important, while a term proportional to $1/(n_w)^4$ appears.

(c) Effects of Heterogeneity

(i) “Bulk” Components of Water Potential

The preceding treatment considered the partition of water potential of a homogeneous system, which is uniform with regard to pressure, and to concentration of the different substances. In such a system at equilibrium the value of each of the components of the water potential (which depend on the abovementioned variables) as well as the total water potential, is the same in all parts of the system. Not so in a plant tissue. This is a very heterogeneous system, where pressure and concentration of soluble and insoluble substances vary markedly (and discontinuously) between “subsystems” which, for physiological purposes, have to be considered separately (different cells, or different parts of the cells). At equilibrium, though the total water potential is equal throughout the tissue, its hydrostatic, osmotic, and matric components, as defined above, are not. Their values, as obtained by measurements on the whole tissue, are then in the simplest case (see below) averages over all the water in the tissue. The local partition of the water potential in a small part of the system (the cytoplasm of a mesophyll cell, the wall of an epidermal cell, or the water in a xylem vessel) may be quite different from the average partition. The latter gives only general information about the whole tissue. More measurements are needed to evaluate the local variation. This point seems obvious, but it is mentioned here because it may be overlooked in the interpretation of the components measured on the bulk of the tissue.

In order to examine the precise meaning of the components of water potential which are measured on an entire tissue, it is desirable to obtain an explicit expression for them. If we try to use the same method of derivation followed in Section II(a) for a homogeneous system, we get into difficulties from the beginning. Equation (2), the total differential of water potential in a homogeneous system, cannot be applied to a heterogeneous one simply by using the average or total values of $P$, $n_\text{s}$, $n_\text{m}$, $n_w$. This is because the state of the heterogeneous system is not completely defined by the average values of these variables, but it depends also on their distribution between the different phases of the system. This distribution is determined by the existence and nature of the internal barriers (membranes, walls) between the phases. If these barriers change during the operations necessary for the measurement of the water potential components, the ensuing changes in internal distribution may affect the components being measured.
As the direct derivation is impossible, we shall have to express the measurable water potential components for the "bulk" of the tissue in terms of the components for the individual phases. Each "bulk" component is the change in water potential of the system, which is measured when one of the variables \( (P, n_m, n_p) \) is brought to zero in all phases, while the total water content remains constant. The distribution of the water content between the phases may change in the course of that operation; the water content in each phase is therefore not constant. Thus in each phase one of the water potential components is indeed brought to zero, but the other components, which are functions of the water content, do not necessarily remain constant.

(In the following discussion the superscripts \( i, j \), refer to any individual phase, and * to the bulk of the tissue.)

Let us consider the situation when the osmotic potential of the tissue is measured directly on the tissue extract. The matric potential is then the difference between the water potential of the extract and that of the whole dead tissue. The hydrostatic potential is the difference between the water potentials of the dead and the living tissue.

\[
\Psi_i^* = \Psi_i, \\
\Psi^*_j = \Psi_j - \Psi_i, \\
\Psi^*_p = \Psi - \Psi_d. \\
\]

(51)

The three "bulk" components of course fulfil the equation:

\[
\Psi = \Psi_p^* + \Psi_j^* + \Psi^*_p. \\
\]

(52)

A similar equation may be written for each phase in the living tissue:

\[
\Psi = \Psi_p^i + \Psi_j^i + \Psi^*_p. \\
\]

(53)

The total potential is not superscripted because it is equal in all phases. Each of the three "phase" components is defined by the corresponding integral in equation (10), for the \( i \)th phase. The "phase" components cannot be measured, and may be different in each phase.

We shall now multiply equation (53) by the water content of the phase, \( w_i \):

\[
\Psi w_i = \Psi_p^i w_i + \Psi_j^i w_i + \Psi^*_p w_i. \\
\]

(54)

The products \( \Psi w, \Psi_p w, \Psi_j w, \Psi^*_p w \) have the dimensions of energy. Each of them is an extensive quantity, which can be added over all phases:

\[
\Sigma \Psi w_i = \Sigma \Psi_p^i w_i + \Sigma \Psi_j^i w_i + \Sigma \Psi^*_p w_i. \\
\]

(55)

The total potential on the left side may be put before the sign of summation, and the whole equation then divided by the total water content \( \Sigma w_i \):

\[
\Psi = \frac{\Sigma \Psi_p^i w_i}{\Sigma w_i} + \frac{(\Sigma \Psi_j^i w_i)}{\Sigma w_i} + \frac{(\Sigma \Psi^*_p w_i)}{\Sigma w_i} \\
= \Psi_p + \Psi_j + \Psi^*_p. \\
\]

(56)
The quantities \( \Psi_p, \Psi_s, \) and \( \Psi_\pi \) defined by this equation are averages of the respective components over all phases in the living tissue, weighted by the water content of each phase.

In the dead tissue the hydrostatic potential is zero in all phases and therefore for each phase:

\[
\Psi_d = \Psi_i^j + \Psi_\pi^j. \tag{57}
\]

The superscript \( j \) is used here instead of \( i \), because the relaxation of pressure and the killing of the tissue may involve a reduction in the number of phases, so that the phases in the dead tissue are not identical with those in the living tissue.

In the same way in which equation (56) was derived from equation (53) there may be derived from (57) the equation:

\[
\Psi_d = (\sum_j \Psi_i^j w_j) + (\sum_j \Psi_\pi^j w_j). \tag{58}
\]

The matrix-free solution, on which \( \Psi_s \) is measured, is already a single homogeneous phase, therefore:

\[
\Psi_s = \Psi_\pi^* = \Psi_\pi^j w_j/\sum_j w_j. \tag{59}
\]

By combining equations (56), (58), and (59) with (51) we can express the relation between the measurable bulk components and the phase components as defined in the first section:

\[
\Psi_\pi^* = \frac{\sum_i \Psi_i^* w_i}{\sum_i w_i} = \left( \frac{\sum_i \Psi_i^* w_i}{\sum_i w_i} \right) + \left( \frac{\sum_j \Psi_\pi^j w_j}{\sum_j w_j} \right), \tag{60}
\]

\[
\Psi_i^* = \frac{\sum_i \Psi_i^* w_i}{\sum_i w_i} - \left( \frac{\sum_i \Psi_i^* w_i}{\sum_i w_i} \right) - \left( \frac{\sum_j \Psi_\pi^j w_j}{\sum_j w_j} \right), \tag{61}
\]

\[
\Psi_\pi^* = \frac{\sum_i \Psi_i^* w_i}{\sum_i w_i} - \left( \frac{\sum_i \Psi_i^* w_i}{\sum_i w_i} \right) - \left( \frac{\sum_j \Psi_\pi^j w_j}{\sum_j w_j} \right). \tag{62}
\]

The total water content is constant, therefore

\[
\sum_i w_i = \sum_j w_j = w^*. \]

The sum of the products of \( \Psi_i \) and \( \Psi_\pi \) and water content over all existing phases will be called \( \Sigma(\Psi_i w) \) and \( \Sigma(\Psi_\pi w) \). The changes in these sums brought about by killing the tissue and by separating solute from matrix will be designated by \( \Delta_m \) and \( \Delta_x \), respectively. Equations (60)–(62) will then be simplified to:
\[ \psi^*_p = \frac{\sum \Delta_m (\psi^*_i w_i)}{w^*} + \frac{\Delta_m \sum (\psi^*_i w_i)}{w^*} = \bar{\psi}_p + \psi^m_p + \psi^m_z, \]  
\[ \psi^*_\tau = \frac{\sum \Delta_m (\psi^*_i w_i)}{w^*} + \frac{\Delta_m \sum (\psi^*_i w_i)}{w^*} = \bar{\psi}_\tau + \psi^m_\tau + \psi^z_\tau, \]  
\[ \psi^*_\pi = \frac{\sum \Delta_m (\psi^*_i w_i)}{w^*} + \frac{\Delta_m \sum (\psi^*_i w_i)}{w^*} = \bar{\psi}_\pi + \psi^m_\pi - \psi^z_\pi. \]  

Here again \( \bar{\psi}_p, \bar{\psi}_\tau, \bar{\psi}_\pi \) are the average hydrostatic, matric, and osmotic potentials in the tissue in its living state. The other terms are connected with the reduction of heterogeneity in the tissue and the following redistribution of water, and may be called "heterogeneity potentials". Thus \( \psi^m_\tau, \psi^m_\pi \) are equal to the changes in the average matric and osmotic potentials when the turgor is cancelled and the cell solutions mixed. \( \psi^z_\pi \) is the change in the average osmotic potential when the solutes are separated from the matrix of the dead tissue.

We may evaluate the osmotic and matric potentials in a way different from that indicated by the set of equations (51): the matric by direct measurement of the water potential of the isolated matrix, and the osmotic by subtraction of the matric potential from the total potential of the dead tissue. In that case we obtain equations similar to equations (63)–(65) but with the positions of \( \psi^*_i \) and \( \psi^*_\pi \) interchanged.

In some special cases all or some of the "heterogeneity potentials" may be equal to zero, so that the measured bulk components are real averages of the original phase components. Two of these cases are:

1. If the value of each component \( \psi^*_k \) is equal for all phases, and if this value is not changed by bringing any other component to zero. In that case the sum of products \( \sum \psi^*_i w_i \) remains constant for each component \( k \) for any operation on the system. This system is practically equivalent to a homogeneous one as the phase components, the average components, and the measurable bulk components are all equal.

2. If the value of a component \( \psi^*_k \) is different in the various phases, but if in all phases and all states of the system that component is a function of water content \( \psi^*_i = \psi^*_i (w_i) \) of such a form that the sum of products \( \sum \psi^*_i w_i \) remains constant. For example, if \( \psi^*_i = a_i w_i \) \( a_i = \text{constant} \) for any \( i \), then \( \sum \psi^*_i w_i = \Sigma a_i \) is also a constant. \( \psi^m_\pi \) and \( \psi^z_\pi \) will be zero, and \( \psi^*_k = \bar{\psi}_k \).

(ii) Effect of Mixing of Solutions on the Hydrostatic Potential

In the following discussion of the bulk hydrostatic component we shall for simplicity neglect the effect of the matrix, i.e. assume either \( \psi^*_i = 0 \) or at least
\( \Psi^m = 0 \) and \( \Psi^m_i = 0 \). Then we have:

\[
\Psi^* = \Psi^p + \Psi^m,
\]

(66)

\[
\Psi^* - \Psi^m = \Psi^p,
\]

(67)

where

\[
\Psi^m = (\Sigma \Psi^i w_i - \Psi^* \Sigma w_i) / \Sigma w_i
\]

(68)

When the turgor pressure in the tissue is brought to zero, the osmotic pressure in each phase (cell) changes from its original value \( \Psi^m_i \) (which may be different in each cell) to the same value of \( \Psi^* \) in all cells. This equalization reduces the original heterogeneity of the tissue, and involves also a redistribution of water between the phases. It would occur even if we could relax the turgor pressure in all cells (at constant total water content) by a hypothetical operation that does not, at the same time, destroy the membranes. Then the elastic barriers would be removed, but not the diffusional ones; each protoplast and each compartment in it would be preserved as a distinct phase with a probably different solute composition. The equalization of the osmotic potential would still take place, only by means of water exchange between the phases.

Usually, however, the hydrostatic potential is brought to zero by killing the tissue rather than by the way described above. In that case the reduction of heterogeneity involved in the operation goes one step further: the diffusional barriers are removed as well as the elastic ones and the solutions of all phases (vacuoles and cytoplasms of all cells) are mixed. The resulting tissue solution is uniform not only in the total osmotic potential, but also in solute composition, and therefore in the contribution of each solute species to the osmotic potential.

We shall consider the “mixing potential” \( \Psi^m \) in this more feasible case when both elastic and diffusional barriers are removed. As we have already seen there are two important conditions in which \( \Psi^m \) as defined in equation (68) is zero:

1. When \( \Psi^i \) (and therefore also \( \Psi^m \)) is equal for all phases and also equal to \( \Psi^* \). This “quasi-homogeneous” situation will occur if the contribution from each solute to the osmotic potential, as well as the total osmotic potential, is the same in all phases. (If only the total is the same, but the partial contributions vary from phase to phase, then \( \Psi^m \) will still be zero when only the elastic barriers are removed. But when the diffusional barriers are removed as well, the mixing of solutes may cause the final \( \Psi^* \) to be different from the initial \( \Psi^i \).)

2. When the solutions in all phases and in all states of the system are approximately ideal and conform to the hyperbolic equation:

\[
\Psi^{\prime} = -a_i/w_i = -RT \Sigma n_i w_i.
\]

(69)
The same equation for the bulk solution will be:

\[ \Psi^*_\pi = -\Sigma a_i \Sigma w_i = -RT \Sigma \Sigma n_{si} / \Sigma w_i. \]  
(70)

The sum of products \( \Sigma (\Psi^*_\pi w) \) is then constant:

\[ \Sigma \Psi^*_\pi w_i = \Sigma \Psi^*_\pi w_i = -\Sigma a_i = -RT \Sigma \Sigma n_{si}, \]  
(71)

and therefore \( \Psi^m_{\pi} = 0. \)

But when neither of conditions (1) or (2) is true, i.e. when at least some of the solutes are unevenly distributed in the tissue and also show deviation from equation (69), then \( \Psi^m_{\pi} \) will not vanish. It may be calculated as a function of the properties of the solutions in the various phases for the following three cases.

*Case 1.*—A single solute is present in all phases (or several solutes in constant proportions) at various concentrations; the deviation of the solution from ideality is such that the logarithm of the activity coefficient depends linearly on molality:

\[ \log \gamma_s = Bm_s. \]  
(72)

Therefore generally

\[ \Psi^*_\pi = -(a_s m_s + b_s m_s^2), \]  
(73)

where \( a_s, b_s \) are constants. In particular for each phase:

\[ \Psi^*_i = -(a_s m_{si} + b_s m_{si}^2) = -[a_s (n_{si} / w_i) + b_s (n_{si}^2 / w_i^2)], \]  
(74)

and for the bulk solution:

\[ \Psi^*_\pi = -(a_s (\Sigma n_{si} / \Sigma w_i) + b_s [ (\Sigma n_{si})^2 / (\Sigma w_i)^2]) \]

\[ = -(a_s (\Sigma m_{si} w_i / \Sigma w_i) + b_s [ (\Sigma m_{si} w_i)^2 / (\Sigma w_i)^2]). \]  
(75)

The "mixing potential" can be shown to be:

\[ \Psi^m_{\pi} = (\Sigma \Psi^*_i w_i / \Sigma w_i) - \Psi^*_\pi \]

\[ = -\{[a_s (\Sigma n_{si} / w_i) + b_s (\Sigma n_{si}^2 / w_i^2)] - [a_s (\Sigma n_{si} / w_i) + b_s (\Sigma n_{si})^2 / (\Sigma w_i)^2]\} \]

\[ = -b_s [(\Sigma n_{si}^2 / w_i^2) - (\Sigma n_{si})^2 / (\Sigma w_i)^2] \]

\[ = -b_s \{ (\Sigma m_{si}^2 w_i - (\Sigma m_{si} w_i)^2 / \Sigma w_i) / \Sigma w_i \}. \]  
(76)

The last expression in bold parentheses can be interpreted as the variance of molality weighted by water content. It is zero when all phases have the same concentration; otherwise it is positive. The mixing potential is equal to the product of this variance and the coefficient \( b \), which is a measure of the non-ideality of the solution.
The mixing potential in this case (initially uniform solute composition) is associated entirely with the removal of the elastic barriers.

Case 2.—Several solutes, in different proportions, in the various phases. The deviation from ideality for each solute is as in equation (72); there are no effects of interaction between the solutes being mixed.

In this case each solute contributes an independent and additive term to the osmotic potential and to the mixing potential. The total is therefore the sum of the equation (76) over all solute species:

\[
\Psi_{\pi}^m = -\sum_{s} \left( \Sigma m_{s}^2 w_{i} - \left[ \left( \Sigma m_{s} w_{i} \right)^2 / \Sigma w_{i} \right] / \Sigma w_{i} \right) .
\]  

(77)

Case 3.—This case differs from case 2 in that there are also interaction effects between different solutes. If the interaction between each pair of solutes \( s \) and \( t \) is assumed to add a term of the form \( c_{st} m_{s} m_{t} \) (\( c_{st} \) = constant) to the osmotic potential, then the total mixing potential in that case can be shown to be:

\[
\Psi_{\pi}^m = -\sum_{s} \left( \Sigma m_{s}^2 w_{i} - \left[ \left( \Sigma m_{s} w_{i} \right)^2 / \Sigma w_{i} \right] / \Sigma w_{i} \right) 
+ \frac{1}{2} \sum_{i \neq s} \sum_{t \neq s} c_{st} \left[ \Sigma m_{s} m_{t} w_{i} - \left( \Sigma m_{s} w_{i} \right) \left( \Sigma m_{t} w_{i} \right) / \Sigma w_{i} \right] / \Sigma w_{i} .
\]  

(78)

The second expression in bold parentheses can be interpreted as the covariance of the concentrations of the solutes \( s \) and \( t \), weighted by water content. It is zero when both solutes are initially uniformly distributed, positive when they are initially concentrated in the same phases, and negative when they are initially concentrated in different phases. The interaction coefficient \( c_{st} \) may be either positive or negative. Therefore while the first term in equation (78) is always negative, the second may be either negative or positive, and so may be the total mixing potential.

To sum up, the hydrostatic potential as measured on the whole tissue has been shown to differ from the average hydrostatic potential of the water in the various phases by a term which may be called the mixing potential. This term is roughly proportional to the heterogeneity of osmotic potential and solute composition in the tissue, and to the degree of non-ideality and solute interaction in the solutions. It is difficult to estimate the magnitude, or even the direction, of the error introduced by this term in the evaluation of the hydrostatic potential. Any estimate needs some information about the internal variation and the properties of the cell solutions. The term is likely to be of importance only in tissues which include very different types of cells; or in tissues where the cytoplasm and the vacuole compartments in each cell are of comparable magnitude. An experimental estimation of the mixing potential requires an independent measurement (at least at some point) of the average hydrostatic potential on the living tissue.

(iii) Other Effects of Heterogeneity on the Hydrostatic Potential

Even if the components of water potential measured on a heterogeneous tissue are really averages of the phase components, their dependence on total water content
may be different from that expected in a single homogeneous phase. A simple example may illustrate this: let us assume an assemblage of independent cells, each containing an ideal solution inside elastic walls. The osmotic and hydrostatic potentials in any (ith) cell will be \([equations (29) and (39)]\):

\[
\Psi^i_\pi = -RT \Sigma n_{s_i}/w_i = -a_i/w_i,
\]

\[
\Psi^p = \epsilon_i[(w_i/w_i^0) - 1].
\]

The average osmotic potential is

\[
\overline{\Psi}_\pi = \Sigma \Psi^i_\pi w_i/\Sigma w_i
\]

\[
= -RT \Sigma \Sigma n_{s_i}/\Sigma w_i
\]

\[
= -\Sigma a_i/\Sigma w_i.
\]

It is therefore a hyperbolic function of total water content, as the osmotic potential in each phase is of the water content of the phase.

The average hydrostatic potential is

\[
\overline{\Psi}_p = \Sigma \Psi^i_p w_i/\Sigma w_i
\]

\[
= \Sigma \epsilon_i w_i[(w_i/w_i^0)]/\Sigma w_i.
\]

This is a complicated function of the total water content, which is strictly linear only when the parameters \(\epsilon_i\) and \(-a_i/w_i^0\) \([= (\Psi^i_\pi)^0]\), and therefore \(\Psi^i_p\), are the same for all phases. The larger variation of these parameters in the cell population, the stronger the deviation of the function \(\overline{\Psi}_p(\Sigma w_i)\) from linearity. Thus linearity of hydrostatic potential with water content for each cell does not necessarily mean linearity for an assemblage of these cells.

Furthermore, in most tissues (except very "spongy" ones) the turgor pressures in adjacent cells cannot be expected to be independent. An effect of mutual enhancement of pressure will appear, especially in dense tissues with little intercellular space. These pressure interactions will vary with tissue geometry and water content, and prevent a simple interpretation of the pressure/water content relationship of the whole tissue in terms of elastic properties. Cutting of the tissue is liable to have a considerable effect on the pressure interactions between cells; that is the main reason why samples of tissue fragments should be compared only if size and geometry are the same.

Another effect of heterogeneity is "smoothing up" of discontinuities which might appear in isotherm curves of a single cell. For example, a discontinuity caused by a sharp change in elasticity modulus, or by the "collapse" of a negative turgor at the critical point.
(iv) Matrix–Solutes Partition: An Alternative Approach

The uneven distribution of matrix and solutes, not only between cells, but even within each cell, is perhaps the most obvious aspect of heterogeneity in the plant tissue. Our knowledge of the structure of the living plant cell indicates that in the vacuole $\Psi_\pi$ is much larger than $\Psi_\gamma$ (in absolute value), and that the opposite is true in the cell wall. In the cytoplasm the two components may be of comparable magnitude, with possible local variation (Fig. 1A). When the cell is killed there is a mixing of solutes from the three original compartments which might affect also the matrix somewhat. But the localization of the matrix remains essentially unchanged: even in the dead tissue there may be zones of dense matrix with almost no solutes in them (and therefore $\Psi_\gamma$ much greater than $\Psi_\pi$), and zones of solution which are far from any matrix surface ($\Psi_\pi$ much greater than $\Psi_\gamma$). There are, of course, in the dead as well as in the living cell, also transition zones, where the water is markedly affected by both solutes and matrix surfaces, and therefore neither $\Psi_\pi$ nor $\Psi_\gamma$ are negligible. But the water in these zones may be only a small fraction of the total water in the tissue.

If this is, indeed, the situation, it seems that the “matric potential” approach, which we have hitherto followed, may not yield very informative results. While a hyperbolic dependence of the osmotic potential on water content can be accepted as a fair approximation, the same is not true for the matric potential. Therefore the sum of products $\Sigma(\Psi_\pi \Psi_\gamma)$ will change upon any redistribution of water that accompanies reduction of heterogeneity. In equations (63)–(65) the term $\Psi_\gamma^\pi$ may be of considerable magnitude, if phases that have different solutes/matrix ratios also have different pressures.

If, alternatively, we choose to find $\Psi_\gamma^\ast$ by direct measurement on the isolated matrix (and $\Psi_\pi^\ast$ by subtraction), we may expect an even larger heterogeneity effect, mainly a $\Psi_\gamma^\pi$ term. With increasing water content, $\Psi_\gamma^\ast$ may then actually become and remain zero, while $\Psi_\gamma^\pi = \Psi_\pi^\ast + \Psi_\gamma^\ast$ only approaches zero asymptotically.

Thus, matrix–solution heterogeneity is another possible cause [besides matrix–solution interaction mentioned in Section II(a)(iii)] for the $\Psi_\pi/\Psi_\gamma$ partition being “non-commutative”, i.e. depending on whether $\Psi_\pi^\ast$ or $\Psi_\gamma^\ast$ is measured directly. In both cases the bulk components will differ from the average components by “heterogeneity potentials”, though probably more so in the second case.

Moreover, even if the averages $\Psi_\gamma$ and $\Psi_\pi$ could have been obtained, little could be inferred from them about any part of a cell in which $\Psi_\pi^\pi$ and $\Psi_\gamma^\ast$ show large variation (Fig. 1B).

These limitations of the “matric–potential” approach lead us to consider the alternative approach to the separation of the effects of matrix and solutes on the water potential isotherm of the tissue. This may be called the “matrix–water” approach. The matric–potential approach partitions the water potential into osmotic and matrix components, at constant total water content. Conversely, the matrix–water approach partitions the total water content into “solution” and “matrix–water”
Fig. 1.—The osmotic and matric components of water potential in a schematic section through a plant cell (the hydrostatic potential is assumed to be zero). The horizontal axis represents water content (w), the vertical axis the water potential (Ψ). A, a probable model of the partition in the three main compartments; the broken line indicates possible local variation within the cytoplasm. B, representation of the situation shown in A by the "matric-potential" approach: partition of the water potential into the average matric (Ψᵣ) and average osmotic (Ψₒ) components. C, representation of the same situation by the "matrix-water" approach: partition of the water content into the "matrix-water" (wₑ) and "solution-water" (wₛ) fractions.
fractions, at constant water potential. The two approaches can be expressed by two equations of the isotherm (after elimination of the hydrostatic term):

\[ \Psi(w) = \Psi_s(w) + \Psi_r(w), \]  

(83)

\[ w(\Psi) = w_s(\Psi) + w_m(\Psi), \]  

(84)

where \( w_s \) is the water content of the solution fraction and \( w_m \) the water content of the matrix fraction. Graphical illustrations of the two approaches are given in Figures 1 and 2.

![Graphical Illustration](image_url)

**Fig. 2.—**The analysis of the water potential isotherm of the whole dead tissue (A) by reference to the isotherm of the isolated solution (B). At any point \((\Psi^1, w^1)\) the “matric–potential” approach partitions \(\Psi^1\) into \(\Psi_s^1\) and \(\Psi_r^1\) on the horizontal line \(w = w_s\), while the “matrix–water” approach partitions \(w^1\) into \(w_s^1\) and \(w_m^1\) on the vertical line \(\Psi = \Psi^1\).

The solution water (or “osmotically bound water”) \(w_s\) and the matrix water (or “collooidally bound water”) \(w_m\) are defined and measured as the quantities of water “associated” with the soluble and the insoluble substances in the tissue, respectively. Obviously, this definition is exact only if the solutes and the matrix of the tissue are each confined exclusively to distinct phases, i.e. if at any point in the tissue the water potential is either totally osmotic or totally matric. This seems not to be the case in the plant tissue. At least in two of the main compartments of the living plant cell (the cytoplasm and the cell wall), and certainly in the dead tissue, solutes and matrix coexist with apparently no physical discontinuity between them. There are probably transition zones where the water potential includes both
osmotic and matrix components. The exact values of \( w_s \) and \( w_m \) depend on whether the water in these transition zones is included in the first or the last fraction, or on the way in which it is divided between them.

Thus, the partition between \( w_s \) and \( w_m \), as the one between \( \Psi_w \) and \( \Psi_e \), may be non-commutative. Different values may be obtained when \( w_s \) is defined and measured as the water content of the extracted solution, and when it is defined as \( w - w_m \), \( w_m \) being measured as the water content of the isolated matrix.

As we have seen neither the results of the matric-potential approach nor those of the matrix–water approach can be given a simple interpretation, except in two extreme cases, neither of which applies to most plant tissues. Each approach yields only partial information, and each involves the possibility of the partition being non-commutative. Both are legitimate, providing that these limitations, and the correct physical meaning of the results, are recognized. The choice between them depends on previous information about the structure of the system, and on the purpose of the analysis. One should consider the two extreme cases in which the matric-potential and the matrix–water approach, respectively, yield simple results:

1. Matrix and solutes are distributed uniformly through the system.
2. Matric and solutes are each confined to a discretely separated part (one phase or more) of the system.

If (1) is nearer to the truth than (2) (as it seems probable for an agar–NaCl gel or, perhaps, for the cytoplasm itself), then the matrix–potential approach is likely to give the more meaningful information. Conversely if statement (2) is a better approximation to the real situation in the system, the matric–water approach is more informative. This seems to be the case for most plant tissues, at least for the cell wall–protoplast partition. In many cases the information from the analysis of the isotherm is to be used in the context of physiological processes under study, each of which tends to be localized in a single compartment of the cell. Then there is another reason for preferring the matrix–water approach, which overemphasizes the differences between compartments, to the matric–potential approach, which conceals them.

### III. Conclusions

Several difficulties in the interpretation of the water potential components, some of them unexpected, have been revealed and clarified by an explicit theoretical treatment. Only in some special cases each of these components has a simple meaning. In general, each of them includes terms due to effects of interaction or heterogeneity or both. In principle these terms can be separated by additional measurements; but for some of them this involves considerable experimental difficulties.

These conclusions do not invalidate the current methods of partition of the water potential in plant tissues into measurable components. Rather, they show that these methods should be considered as first approximations, and are legitimate as such. These approximations will be good enough if the water potential analysis and the physiological or ecological inferences drawn from it are required to be only
rough and general. But as improved methods of measuring water potential allow refinement of the analysis and its inferences, the errors caused by neglecting the additional terms are liable to become more important.

The theoretical treatment has given also some practical indications as to the ways in which the analysis can be made more meaningful. Thus it has been shown that by combining measurements on the isolated solution and the isolated matrix, the effects of solute–matrix heterogeneity and solute–matrix interactions can be estimated. Further, that the osmotic potential measured on the solution is likely to be less distorted by heterogeneity effects than the matric potential measured on the isolated matrix; and that a “clean” evaluation of the average hydrostatic potential requires a technique which will separate from it the effects of solution mixing. It has been suggested that when there is a marked heterogeneity in the system, as between the cell wall and the protoplast, the matrix–water approach is preferable to the matric–potential approach.

Finally, there is one principle of a more general nature which this analysis seems to reaffirm: the results of any application of thermodynamic analysis to a system as complex as a biological one are significant only as far as the internal structure of the system is known and accounted for.

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V. References

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APPENDIX

LIST OF SYMBOLS

\( a, b, c, d, k \) various constants;
\( a, a_s \) coefficients relating the osmotic potential to the inverse of water content or to the molality of a solute \((s)\);
\( b_s \) coefficient relating the osmotic potential to the square of the molality of a solute \((s)\);
\( c_{st} \) coefficient relating the osmotic potential to the product of the molalities of two solutes \((s \text{ and } t)\);
\( G \) Gibbs' free energy (joule);
\( I \) ionic strength of solution;
\( \gamma_s \) molal activity coefficient of a solute \((s)\);
\( M_w \) molecular weight of water;
\( n_s \) molality of a solute \((s)\);
\( n_{i,j} \) number of moles of any substance \((i \text{ or } j)\);
\( n_m \) number of moles of an insoluble substance \((m)\);
\( n_s \) number of moles of a solute \((s)\);
\( n_w \) number of moles of water;
\( n_{w,0} \) number of moles of water at standard pressure;
\( P \) pressure (joule cm\(^{-3}\)) \((= 10 \text{ bars, } \approx 9·9 \text{ atm})\);
\( P_0 \) standard (atmospheric) pressure;
\( \Delta P \) excess of pressure over the standard pressure \((= P - P_0)\), turgor pressure;
\( R \) gas constant \((= 8·31 \text{ joule deg}^{-1} \text{ mole}^{-1})\);
\( T \) absolute temperature \((^\circ \text{K})\);
\( V \) volume (cm\(^3\));
\( V_0 \) volume at standard pressure;
\( V_w \) volume of water;
\( V_{w,0} \) volume of water at standard pressure;
\( \bar{V}_w \) partial molal volume of water (cm\(^3\) mole\(^{-1}\));
\( \bar{V}_{w,0} \) molal volume of pure water at standard pressure \((c. 18 \text{ cm}^3 \text{ mole}^{-1})\);
\( W_w \) weight of water \((g)\);
\( W_d \) dry weight of the sample \((g)\);
\( w \) water content variable; specifically, weight of water per dry weight;
\( w_0 \) water content at standard pressure;
\( w_s \) water content in the solution;
\( w_m \) water content in the matrix;
\( a_s \) activity of a solute \((s)\);
\( \epsilon \) \((= \epsilon' \delta_{w}^{0})\), product of elasticity modulus and volume fraction of water;
\( \epsilon' \) elasticity modulus (joule cm\(^{-3}\));
\[
\begin{align*}
\mu &\quad \text{chemical potential (joule mole}^{-1}) ; \\
\mu_w &\quad \text{chemical potential of water} ; \\
\mu_0 &\quad \text{chemical potential of pure water at standard pressure} ; \\
\mu_s &\quad \text{chemical potential of a solute (s)} ; \\
\varphi_w &\quad \text{volume fraction of water at standard pressure (}= V_w^0/V^0) ; \\
\rho_w &\quad \text{density of water (g cm}^{-3}) ; \\
\Psi &\quad \text{water potential [=(} \mu_w - \mu_0 \text{)/} \varphi_w \text{]} (\text{joule cm}^{-3} ; = 10 \text{ bars} ; \approx 9.9 \text{ atm}) ; \text{unless otherwise specified it is the total water potential of a system in its initial living state} ; \\
\Psi_p &\quad \text{hydrostatic component of water potential} ; \\
\Psi_o &\quad \text{osmotic component of water potential} ; \\
\Psi_i &\quad \text{matric component of water potential} ; \\
\Psi_{ni} &\quad \text{ideal osmotic potential} ; \\
\Psi_{nR} &\quad \text{additional (non-ideal) osmotic potential} ; \\
\Psi_{r,\text{mix}} &\quad \text{the part of the matric potential contributed by the energy of mixing} ; \\
\Psi_{r,\text{str}} &\quad \text{the part of the matric potential contributed by the energy of polymer network stretching} ; \\
\Psi_{m} &\quad \text{water potential of the isolated matrix} ; \\
\Psi_{s} &\quad \text{water potential of the isolated solution} ; \\
\Psi_{d} &\quad \text{water potential of the entire system in the dead state} ; \\
\Psi_{cm} &\quad \text{solute-matrix interaction component of water potential} ; \\
\Psi_i, \Psi_o, \Psi_i &\quad \text{the hydrostatic, osmotic, and matric potentials in an individual (ith) phase in the living tissue} ; \\
\Psi_i, \Psi_i &\quad \text{the osmotic and matric potentials in the jth phase in the dead tissue} ; \\
\Psi_p, \Psi_o, \Psi_i &\quad \text{the weighted averages of the hydrostatic, osmotic, and matric potentials over all phases in the living tissue} ; \\
\Psi_p, \Psi_o, \Psi_i &\quad \text{the hydrostatic, osmotic, and matric potentials as found from measurements on the tissue as a bulk} ; \\
\Psi_m, \Psi_i &\quad \text{the changes in the average osmotic and matric potentials involved in the mixing of solutions when the tissue is killed ("mixing potentials") [=} \Delta_m \Sigma(\Psi_m w), \Delta_m \Sigma(\Psi_i w)] ; \\
\Psi_p &\quad \text{the change in the average osmotic potential upon separating the matrix from the solution [=} \Delta_p \Sigma(\Psi_p w)] ; \\
\Psi_o &\quad \text{the osmotic and matric potentials in the cell wall} ; \\
\Psi_i &\quad \text{the osmotic and matric potentials in the cytoplasm} ; \\
\Psi_i &\quad \text{the osmotic and matric potentials in the vacuole} .
\end{align*}
\]

Subscripts and superscripts:

- \( i, j \) general subscripts and superscripts; any substance; any phase;
- \( m \) insoluble (matrix) substance;
- \( s \) soluble substance;
- \( w \) water.