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REVIEW

Membrane Permeability Modeling: Kedem–Katchalsky vs a Two-Parameter Formalism

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The analysis of experiments for the purpose of determining cell membrane permeability parameters is often done using the Kedem–Katchalsky (KK) formalism (1958). In this formalism, three parameters, the hydraulic conductivity (L_p), the solute permeability (P_s), and a reflection coefficient (σ), are used to characterize the membrane. Sigma was introduced to characterize flux interactions when water and solute (cryoprotectant) cross the membrane through a common channel. However, the recent discovery and characterization of water channels (aquaporins) in biological membranes reveals that aquaporins are highly selective for water and do not typically cotransport cryoprotectants. In this circumstance, sigma is a superfluous parameter, as pointed out by Kedem and Katchalsky. When sigma is unneeded, a two-parameter model (2P) utilizing only L_p and P_s is sufficient, simpler to implement, and less prone to spurious results. In this paper we demonstrate that the 2P and KK formalism yield essentially the same result (L_p and P_s) when cotransporting channels are absent. This demonstration is accomplished using simulation techniques to compare the transport response of a model cell using a KK or 2P formalism. Sigma is often misunderstood, even when its use is appropriate. It is discussed extensively here and several simulations are used to illustrate and clarify its meaning. We also discuss the phenomenological nature of transport parameters in many experiments, especially when both bilayer and channel transport are present.

Key Words: Kedem and Katchalsky; modeling; membrane; permeability coefficients; sigma.

As cryobiologists we often wish to know the membrane permeability of cells or tissues. Specifically, we are usually interested in the water (solvent) and cryoprotectant (solute) permeability of membranes. Using these permeability parameters, it is possible to model optimal protocols for the addition and removal of cryoprotective solutes or optimal cooling rates for cell cryopreservation (2, 13, 29). In other circumstances, the objective may be to characterize the fundamental physical mechanisms of solute and water transport across a cell membrane, e.g., channels versus lipid bilayer transport (15, 33, 47). A number of formalisms exist for determining these permeability parameters. These include a one-parameter (solute permeability) model introduced by Mazur and colleagues (30, 31), a classic two-parameter (water and solute permeability) model, (e.g., 22, 23), and a three-parameter model developed by Kedem and Katchalsky (23), which adds a solute–solvent interaction term (sigma). The Kedem–Katchalsky (KK) formalism is the one in common use by cryobiologists today.

The KK interaction term, sigma,[2] was introduced specifically to deal with solvent and solute transport through a common channel. However, the KK formalism is quite general and applies to any simple transport situation with or without cotransporting channels. Its applicability in the general case when cotransporting channels are present has been well demonstrated in work on artificial membranes (1, 20). In these studies nystatin or amphotericin B pores of 8Å diameter were found to readily cotransport water and small solutes, e.g., urea and glycerol.

Although the KK formalism is the most gen-

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[2] The interaction (or reflection) coefficient was first introduced by Staverman (39).

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eral of the three mentioned, it is not without its drawbacks. The introduction of the interaction parameter, sigma, significantly increases the complexity of the transport formalism. Unfortunately, as a result of this complexity, sigma is often misunderstood, misinterpreted, and improperly calculated by cryobiologists. Fortunately, the KK formalism is often unnecessary. Recent discoveries in molecular biology suggest that, in natural biological membranes, cotransport is often unlikely and/or negligible. Specifically, the discovery and characterization of water channels (aquaporins) in natural membranes has established that water channels are highly selective, typically exclude cryoprotectants, and thus infrequently act as common transport channels for solute and water (37, 44). The discovery and characterization of these water-specific channels has revolutionized our understanding of membrane water transport. They are reviewed here with emphasis on their relevance to the various formalisms and on experimental methods for identifying and characterizing them.

In light of these recent discoveries, we argue that sigma and the Kedem–Katchalsky formalism are often unnecessary. Frequently, a classic two-parameter formalism is both appropriate and simpler. To demonstrate that the two-parameter (2P) formalism is appropriate, we show that it gives essentially the same results as the KK formalism when a common channel for solute and solvent is not present. This occurs in a number of common circumstances including (i) bilayer transport in which the solute and water diffuse across the lipid bilayer, (ii) transport in which the solute diffuses across the bilayer and water, only, passes through a selective channel, and (iii) transport in which water and solute use separate channels.

To support the assertion of equivalency when solute and solvent are independently transported, we will review the 2P and KK formalisms, apply them to a number of simulated experiments, and show that the two formalisms yield essentially the same results. The simulation approach is a powerful one which yields many useful insights. In addition to examining independent transport of solute and solvent (to demonstrate 2P–KK equivalency), we will examine cotransporting channels, high solute concentrations, and transport activation energy (E_a). Special consideration will be given to sigma in an effort to clarify its meaning, as it is frequently misunderstood.

There is a second circumstance in which cryobiologists may find the simplicity of the 2P formalism useful and more appropriate than the KK formalism. When multiple transport pathways are present, the 2P and KK formalism yield only phenomenological parameters. In this case, there is little justification for the added complexity of the KK formalism if the data are adequately fit with the 2P model. The phenomenological nature of the simple 2P or KK formalism becomes apparent when we consider the complexity of transport involving both channels and a bilayer. As we will demonstrate later, a permeable bilayer containing cotransporting channels requires up to five permeability parameters to characterize. Unraveling the details of such transport is a complex process as illustrated, for instance, by the recent analysis of the UT3 urea transporter (47). In such circumstances, it may be both adequate and necessary to settle for a phenomenological description of transport. If this is the case, economy argues that we choose the simplest transport model which yields a good phenomenological description, as has often been argued by Mazur (32).

In summary, we will use simulated experiments to demonstrate that the KK and 2P formalism yield essentially the same results in a large number of common situations of interest to cryobiologists. In this circumstance, the simpler formalism is to be preferred.

TRANSPORT THEORY

Kedem–Katchalsky Transport Formalism

The Kedem–Katchalsky formalism (23) was developed specifically to handle situations where solvent (water) and solute transport across a membrane is physically coupled, typically by cotransport through a single species of

pores.[3] In this case, the flux of water and solute physically interact, with the degree of interaction being characterized by a reflection coefficient, sigma. The KK formalism may be used in situations where the solvent and solute fluxes do not interact, but in such cases sigma and the added complexity of the KK equations are not needed, as we will demonstrate. In this paper we consider a typical experimental situation involving water, a nonpermeating solute, and a single, non-ionic, permeating solute. In this case three transport parameters are utilized to characterize membrane permeability. They are the membrane water permeability or hydraulic conductivity (L_p), the solute mobility (ω), and the reflection coefficient, sigma (σ). Typically the solute mobility is expressed as a solute permeability, $P_s = \omega RT$, where R is the gas constant and T the absolute temperature. Under these circumstances, and assuming dilute solutions, the KK formalism simplifies to three equations (essentially Eqs. [39], [41], and [66] in the original (23)). Throughout this paper we will refer to this formalism as the simple KK formalism. The equations follow.

The change in cell water and solute volume (V_{w+s}) with time resulting from water and solute fluxes is given by

$$dV_{w+s}/dt = -L_pART\{(M_n^e - M_n^i) + \sigma(M_s^e - M_s^i)\} \quad [1]$$

where V_{w+s} is the water and solute volume, A is the area of the cell, and M is osmolality with the superscripts denoting the solution internal (i) and external (e) to the cell and the subscripts denoting nonpermeating (n) and permeating solutes (s), respectively.

The change in intracellular, permeating solute (expressed in osmoles) is given by

$$dN_s/dt = (1 - \sigma)(1/2)(M_s^e + M_s^i)dV_{w+s}/dt + P_sA(M_s^e - M_s^i) \quad [2]$$

[3] The term 'pore' generally means a nonselective pathway and 'channel' a selective pathway, although the terms are often used interchangeably.

where N_s is the number of osmoles of solute in the cell and P_s is the membrane solute permeability. The quantity of interest experimentally, is the observed total cell volume (V_c),

$$V_c = V_{w+s} + V_b, \quad [3]$$

which is found by adding the cell solids volume (V_b) to the water and solute volume (V_{w+s}).

Finally, σ is constrained by the condition

$$0 \leq \sigma \leq 1 - P_s\bar{V}_s/RTL_p, \quad [4]$$

where $\bar{V}_s$ is the partial molar volume of the solute. In order to prevent σ from becoming negative when P_s becomes large relative to L_p, we simply interchange solvent and solute designations. We also note that σ is identically equal to 1 only in the uninteresting case that P_s is zero and the membrane is completely impermeable to the solute. Considered as a reflection coefficient, a σ of one means that all of the solute is reflected 'back' from the membrane. For convenience, the parameters above as well as those to follow are gathered together in Table 1.

The meaning of the water and solute permeabilities (L_p and P_s) are relatively self evident. However, σ is often misunderstood. Two very different situations are possible (23). When solute and solvent cross the membrane via independent pathways, e.g., water via channels and solute via lipid bilayer diffusion, the right hand equality in Eq. [4] holds. We refer to this as the noninteracting case and σ is given by

$$\sigma_{NI} = 1 - P_s\bar{V}_s/RTL_p. \quad [5]$$

In this situation σ is *not* an independent parameter; it is completely determined by L_p and P_s.

On the other hand, when solvent and solute are cotransported through the same channel, the possibility of physical interference between the two fluxes arises and the inequality in Eq. [4] holds. The greater the degree of interference between the two fluxes, the greater the reduction in σ below σ_{NI}. We refer to this as the interacting σ case

$$\sigma_I < 1 - P_s\bar{V}_s/RTL_p. \quad [6]$$

TABLE 1
Definition of Symbols

Symbol	Meaning
w, s, n, o	Subscripts (w, water; s, permeating solute, n, nonpermeating solute; o, initial value)
e, i, c, b	Superscripts (e, external to cell; i, internal to cell; c, channel; b, bilayer)
A	Cell area
V_o	Isosmotic cell volume
V_c	Total cell volume (at time t)
V_n	V (normalized) = V_c/V_o
V_b	Cell solids (and nonosmotic water) volume
v_{bf}	Fractional value of cell solids = V_b/V_o
$\bar{V}$	Partial molar volume
L_p	Water permeability (hydraulic conductivity)
P_f	Water filtration coefficient ($P_f = RTL_p/\bar{V}_w$)
P_s	Solute permeability
σ	Reflection coefficient, sigma
σ_{NI}	Sigma noninteracting (no solute–solvent flux interaction present)
σ_I	Sigma interacting (solute–solvent flux interactions present)
R	Universal gas constant
T	Absolute temperature
M	Osmolality
π	Osmotic pressure ($\pi = RT\Delta M$)
Δ	Difference (outside − inside)
N_s	Osmoles of solute inside cell
E_a	Arrhenius activation energy

Sometimes it is mistakenly believed that any value of σ less than one implies a solute–solvent interaction with its attendant complexity. This is not true, as demonstrated in Table 2, which shows σ values for typical values of L_p and P_s with *no* flux interaction. In order to make the example specific, water and glycerol are assumed to be the solvent and solute, respectively. The values of σ shown in Table 2 are σ_{NI} values computed using Eq. [5]. Depending on the relative magnitudes of L_p and P_s, σ can be made arbitrarily small without implying *any* interaction between the solute (glycerol) and the solvent (water) flux. If the water and glycerol are being cotransported through a common channel, then the value of σ will be *less* than those shown in the table.

Two-Parameter Transport Formalism

The two-parameter transport formalism utilizes the parameters L_p and P_s to characterize membrane permeability when water, a permeable solute, and a nonpermeable solute are present. Throughout this paper we will refer to this as the simple 2P formalism. It is not a new formalism. Jacobs used it in 1933 (22) and Kedem and Katchalsky reviewed it in their original 1958 paper (23). In this formalism the water flux into a cell is given by

$$dV_w/dt = -L_pART(M^e - M^i) \qquad [7]$$

and the solute flux is given by

$$dN_s/dt = P_sA(M_s^e - M_s^i), \qquad [8]$$

where M^i and M^e are the intracellular and extracellular osmolality, respectively, and the remaining symbols are as previously defined (Table 1). To obtain the total cell volume it is necessary to know the solute volume,

$$V_s = N_s\bar{V}_s, \qquad [9a]$$

or in differential form

$$dV_s/dt = \bar{V}_s dN_s/dt. \qquad [9b]$$

TABLE 2
The Reflection Coefficient, σ

L_p[a] (μm min^{-1} atm^{-1})	P_f (cm min^{-1})	P_s (cm min^{-1})	σ_{NI}
1.0	0.134	0	1.00
1.0	0.134	0.001	0.97
1.0	0.134	0.01	0.71
1.0	0.134	0.02	0.41
1.0	0.134	0.03	0.11

Note. Sigma was computed for a range of L_p and P_s values assuming that there was no interaction between solute and solvent fluxes, Eq. [5]. Thus we refer to it as a noninteracting sigma (σ_{NI}). The solute was assumed to be glycerol with a partial molar volume of 0.071 L/mol and the temperature was 293 K.

[a] Sometimes it is useful to express L_p as a water filtration coefficient (P_f). The filtration coefficient is given by $P_f = RTL_p/\bar{V}_w$, where $\bar{V}_w$ is the partial molar volume of water. They are different but equivalent ways of expressing the membrane water permeability. Here it allows for an easier comparison of the solute and solvent permeabilities because P_f and P_s have the same units. In terms of P_f, Eq. [4] for σ becomes $0 \leq \sigma \leq 1 - P_s\bar{V}_s/P_f\bar{V}_w$.

Then the total cell volume is just the sum of the water, solute, and solids volume:

$$V_c = V_w + V_s + V_b. \qquad [10]$$

These equations assume no physical interaction between the water and solute fluxes which would occur if cotransporting channels were present. This point should not be confused with the fact that these equations are coupled through the definitions of the intracellular osmolalities (M^i, M^i_n, and M^i_s) and thus each flux indirectly affects the other. The same coupling occurs in the KK formalism.

Subsidiary Equations

Several subsidiary equations are required in order to complete these transport models. Specifically, it is necessary to compute the various intracellular osmolalities which appear in the equations. These are (i) the intracellular solute osmolality,

$$M^i_s = N_s/V_w, \qquad [11]$$

where V_w is the volume of intracellular water; (ii) the intracellular nonpermeating solute osmolality

$$M^i_n = M^i_{no}(V_{wo}/V_w), \qquad [12]$$

where a Boyle van't Hoff relationship is assumed, M^i_{no} is the initial intracellular nonpermeating solute osmolality, and V_{wo} is the initial volume of intracellular water; and (iii) the total intracellular osmolality

$$M^i = M^i_s + M^i_n. \qquad [13]$$

Finally, it is often convenient to express volume in normalized terms as

$$V_n = V_c/V_o, \qquad [14]$$

where V_o is the isotonic cell volume.

Multiple Transport Pathways

Both the simple KK and 2P transport formalisms presented above assume that there is no more than one pathway for solute and one for solvent. In actual practice, multiple pathways are often present. In cells, lipid bilayer diffusion of low-molecular-weight solutes and water is always present to some degree when a concentration gradient is present. Then, if channels are added, a second path is opened by the channel transport. A proper transport model must account for both pathways (10, 15, 33, 47). Lipid bilayer transport can be characterized using the 2P formalism as diffusion of solute and solvent due to a concentration gradient are generally considered to occur independently (10, 27, 40). For a channel, the KK formalism is required if the channel cotransports solute and solvent. Otherwise the 2P formalism may be used for the channels. If there are multiple channel types present, each must be accounted for. Basically, the membrane fluxes for each pathway must be added. We briefly consider two cases.

First, we will consider a membrane with lipid bilayer transport and a cotransporting channel. Utilizing the 2P formalism for the lipid bilayer and the KK formalism for the channel, the volume flux is given by

$$dV_{w+s}^{c+b}/dt = dV_{w+s}^{c}/dt + dV_{w}^{b}/dt + dV_{s}^{b}/dt, \quad [15]$$

where the superscript c refers to channel transport and the superscript b refers to lipid bilayer transport. Thus, the total volume flux into the cell is given by the water and solute flow through the channel plus the water flow through the bilayer plus the solute flow through the bilayer. Similarly, the solute flux in osmoles is

$$dN_{s}^{c+b}/dt = dN_{s}^{c}/dt + dN_{s}^{b}/dt, \quad [16]$$

where the solute flux into the cell is just the sum of the flux through the channel plus the flux through the bilayer.

The various terms in Eqs. [15] and [16] may be expanded using Eqs. [1], [2], [7], [8], and [9b]. For simplicity, we introduce several abbreviations: $\Delta\pi = RT(M^{e} - M^{i})$, $\Delta\pi_{n} = RT(M_{n}^{e} - M_{n}^{i})$, $\Delta\pi_{s} = RT(M_{s}^{e} - M_{s}^{i})$, $\Delta M_{s} = (M_{s}^{e} - M_{s}^{i})$, and $\bar{M} = (1/2)(M_{s}^{e} + M_{s}^{i})$. Then Eqs. [15] and [16] may be expressed as

$$dV_{w+s}^{c+b}/dt = -L_{p}^{c}A(\Delta\pi_{n} + \sigma^{c}\Delta\pi_{s}) - L_{p}^{b}A\Delta\pi + P_{s}^{b}A\Delta M_{s}\bar{V}_{s} \quad [17]$$

and

$$dN_{s}^{c+b}/dt = -(1 - \sigma^{c})\bar{M}L_{p}^{c}A(\Delta\pi_{n} + \sigma^{c}\Delta\pi_{s}) + (P_{s}^{c} + P_{s}^{b})A\Delta M_{s}. \quad [18]$$

Note that five permeability parameters are required to describe this membrane: L_{p}^{c}, the channel water permeability; P_{s}^{c}, the channel solute permeability; σ^{c}, the channel reflection coefficient; L_{p}^{b}, the bilayer water permeability; and P_{s}^{b}, the bilayer solute permeability. This formalism is essentially that used by Yang and Verkman to analyze *Xenopus* oocytes expressing cotransporting water and urea channels except that their volume flux expression assumes that the bilayer solute flux is small and omits it (47).

We also consider a cell with lipid bilayer transport, a water exclusive channel (c1), and a solute exclusive channel (c2). The human red blood cell, with urea as the solute, is an example of such a cell. Adding the volume and solute fluxes as in the above example, we obtain

$$dV_{w+s}^{c1+c2+b}/dt = -(L_{p}^{b} + L_{p}^{c1})A\Delta\pi + (P_{s}^{b} + P_{s}^{c2})A\Delta M_{s}\bar{V}_{s} \quad [19]$$

and

$$dN_{s}^{c2+b}/dt = (P_{s}^{b} + P_{s}^{c2})A\Delta M_{s}. \quad [20]$$

In this case only four permeability parameters are required to characterize the membrane permeability.

When a simple phenomenological description of transport is desired, it may be appropriate, or even necessary, to lump together some of the transport parameters which appear in Eqs. [17] and [18] or [19] and [20]. For instance, in Eq. [20], we could let $P_{s} = P_{s}^{b} + P_{s}^{c2}$, yielding a phenomenological coefficient characterizing the membrane solute permeability resulting from both channel and bilayer transport.

Activation Energy

Frequently membrane permeability is measured as a function of temperature. The temperature dependence of transport parameters is generally expressed as an activation energy (E_{a}) which is related to permeability through the Arrhenius relationship:

$$P = P_{o}\exp(-E_{a}/RT) \quad [21]$$

where P is the permeability parameter of interest and P_{o} is a constant determined when fitting the experimental data. The activation energy is generally found by making an Arrhenius plot of the data (ln[P] vs $1/T$), least squares fitting the slope, and solving for $E_{a} = -R^{*}$slope.

MODEL CELL AND SIMULATION METHODS

Model Cell and Hypothetical Test Conditions

To compare the KK and 2P formalism we consider a model (hypothetical) cell of 50 μm radius[4] which is moved from isotonic buffer into 2 osmolal glycerol in isotonic buffer. In such an experiment the model cell will exhibit a typical shrink–swell response in which water initially rushes out of the cell in response to the

[4] A large cell was assumed to yield a convenient response time for kinetics plotting. The cell size has no effect on the 2P-KK comparison.

TABLE 3
Model Cell and Hypothetical Experiment

Parameter	Value	Comments
Model Cell		
r_o =	50 μm	Initial cell radius
A =	3.14×10^4 μm^2	Cell area[a]
V_o =	5.24×10^5 μm^3	Initial cell volume
v_{bf} =	0.25	Osmotically inactive cell fraction
Model Experiment		
M^i_{no} =	0.3 osmolal	Initial cell osmolality
M^e_n =	0.3 osmolal	External nonpermeating solute osmolality
M^i_{so} =	0.0 osmolal	Initial intracellular solute (glycerol) osmolality
M^e_s =	2.0 osmolal	External solute (glycerol) osmolality; $t > 0$
$\bar{V}_s$ =	0.071 L/mol	Partial molar volume of solute (glycerol)
T =	293 K	Temperature

Note. A spherical cell of radius 50 μm is moved from isotonic buffer (0.3 M) to 2 osmolal glycerol in isotonic buffer (0.3 M).

[a] It is common to assume area (A) fixed during modeling or fitting (32); however it may easily be varied if that is more appropriate for the cell under consideration, e.g., for a sphere, $A = (36\pi)^{(1/3)} V^{(2/3)}$.

hyperosmotic medium and then reswells as solute and water reenter the cell. Details of the hypothetical cell and test conditions are listed in Table 3. Unless otherwise specified, the examples to follow will utilize a cell characterized by a single L_p, P_s, (and σ).

Simulation Procedures and Software

The simulations and other data manipulations presented in this paper were performed using the Windows program 'Scientist', Ver. 2.01, from Micromath Software (Salt Lake City, UT). With this software it is possible to either simulate the response of a cell when permeability parameters are known or to determine permeability parameters by fitting to known experimental data. The 'Stiff Episode' integrator option was used to integrate the differential equations in the transport models. Hypothetical (simulated) experimental data were fit using the Powell variant of the Levenberg–Marquardt method. Other commercial software packages with similar capabilities include MatLab (Mathworks, Natick, MA) and MLab (Civilized Software, Bethesda, MD).

When fitting data using the KK formalism, it is necessary to impose the constraint on σ given in Eq. [4]. Scientist (Ver. 2.01) does not appear to directly implement such a constraint although it does permit setting an upper limit on parameters. Thus it was necessary to manually iterate fits, recomputing the upper limit (or equality) for σ after each iteration, until Eq. [4] was met. Typically, three to four iterations were necessary.

When generating hypothetical data for subsequent fitting, consideration must be given to the temporal spacing of the data points. When fitting shrink–swell data, L_p is primarily determined by the initial portion of the curve and P_s by the reswell portion of the curve. If either portion of the shrink–swell curve contains too many points relative to the other, fits will be biased to that portion of the curve at the expense of the opposite portion. In order to avoid this problem, the hypothetical reswell data are more widely spaced than the hypothetical shrink data.

We note parenthetically that the permeability units in common use, μm min^{-1} atm^{-1} for L_p and cm min^{-1} for P_s, are inconsistent in that L_p uses μm for length and P_s uses cm for length. When actually setting up a model for simulation

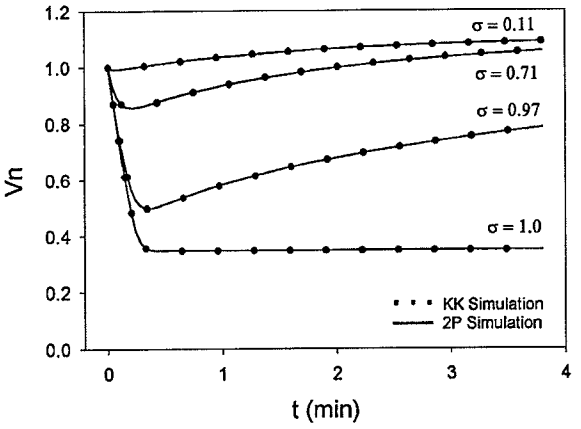

FIG. 1. Comparison of the Kedem–Katchalsky and two-parameter transport formalism in the limit of no solute–solvent flux interactions. The KK (●) and 2P (—) normalized volume vs time simulations yield essentially the same results. This is demonstrated for a model cell moved from isotonic buffer to 2 M glycerol in isotonic buffer (Table 3). For these simulations, four curves were generated with L_p = 1.0 μm min^{-1} atm^{-1} and P_s varied from 0 to 0.03 cm min^{-1}, yielding a σ of 1.0 to 0.11 (Table 4). Sigma (σ) is given by $\sigma_{NI} = 1 - P_s\bar{V}_s/RTL_p$.

or fitting, it is necessary to use a self consistent set of units which means that scaling factors have to be introduced, for instance, to convert cm to μm.

SIMULATION EXPERIMENTS

Comparison of 2P and KK Transport when No Solute–Solvent Flux Interaction is Present

A central thesis of this paper is that the 2P and KK formalisms yield the same results when applied to membranes without cotransporting channels. We demonstrate this by simulating the volume vs time response of a hypothetical cell with known L_p and P_s using both the 2P and KK formalisms, Fig. 1. In these simulations, a model cell (Table 3) of known permeability (Table 4) is moved from buffer to 2 osmolal glycerol in buffer, eliciting a shrink–swell response. The KK simulations require a value for σ. Because no solute–solvent flux interaction is present, σ is not an independent parameter, and is determined from L_p and P_s using Eq. [5].

At the scale of the figures, the cell response modeled by the KK formalism is indistinguishable from that modeled by the 2P formalism, Fig. 1. The essential equality of the two methodologies is shown here for a typical shrink–swell experiment. However, the result is generally true, and holds, for instance, in swell–shrink experiments in which the cell is preloaded with cryoprotectant which is then washed out (simulations not shown). This latter protocol is often used for permeability measurements made with electronic particle counters (14).

Note that for the $\sigma = 1$ case, the cell shrinks but does not reswell because in this case the cell is impermeable to the solute, Fig. 1. In the remaining curves, σ diminishes as a result of the increase of P_s. As P_s increases, the initial inward flux of solute counter balances the initial outward flux of water and the degree of cell shrinkage diminishes.

Sigma and Solute–Solvent Flux Interactions

When solute and solvent are cotransported via a common channel there will be a solute–solvent flux interaction. For instance, in the model experiment being considered here, water initially rushes out of the cell while solute tries to move into the cell through the same channel. The two fluxes physically oppose/impede each other (in this case) and σ becomes an independent parameter which characterizes the strength of this flux interaction, although it is still constrained by the limits shown in Eq. [4]. The effect of this interaction is illustrated in Fig. 2

TABLE 4
Simulation Parameters for 2P–KK Comparison

L_p (μm min^{-1} atm^{-1})	P_s (cm min^{-1})	σ_{NI} –
1.0	0	1.0
1.0	0.001	0.97
1.0	0.01	0.71
1.0	0.03	0.11

Note. L_p and P_s were used in the 2P simulation and L_p, P_s, and σ_{NI} were used in the KK simulation (Fig. 1). Sigma was computed assuming no solute–solvent flux interaction and thus is not an independent parameter (Eq. [5]: $\sigma_{NI} = 1 - P_s\bar{V}_s/RTL_p$).

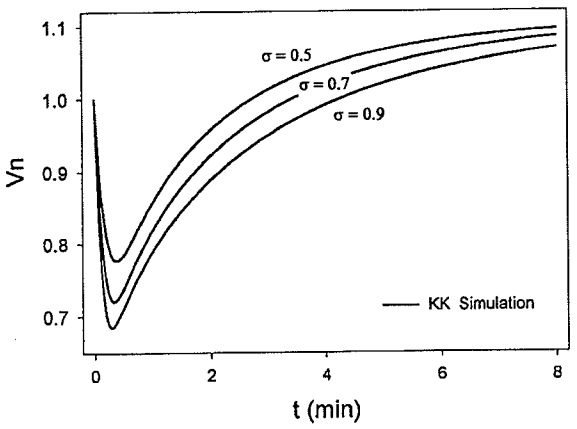

FIG. 2. Solute–solvent flux interactions. The Kedem–Katchalsky formalism is used to simulate the response (V_n vs t) of a model cell (Table 3) with differing degrees of solute–solvent flux interaction. Sigma is varied from a non-interacting value of 0.9 to interacting values of 0.7 and 0.5 (Table 5). L_p = 1.0 μm min^{-1} atm^{-1} and P_s = 0.0034 cm min^{-1}. The 2P formalism would yield a curve overlying the σ = 0.9 curve.

using the KK formalism and the permeability parameters shown in Table 5. The permeabilities, L_p and P_s, were chosen to yield a σ_{NI} = 0.9 and then these L_p and P_s values were held constant while σ was reduced to illustrate the effect of flux interactions. As the two fluxes increasingly impede/oppose each other during the initial cell shrinkage, the degree of shrinkage diminishes as expected. If a 2P simulation were plotted on Fig. 2, it would match the σ = σ_{NI} = 0.9 case.

Note that large changes in σ produce only small vertical axis (volume) shifts of the V vs t data, Fig. 2. As a consequence, high-accuracy volume measurements are required if σ is to be determined from experimental shrink–swell (or swell–shrink) data.

High Solute Concentrations

The KK formalism is derived for the case of dilute solutions of solute whereas the 2P formalism requires no such restriction. Precisely what constitutes dilute is often unclear. Using the simulation model developed here, it is easy to compare the KK and 2P formalisms at high solution concentrations. Figure 3 illustrates a comparison for our model cell with L_p = 1.0 μm min^{-1} atm^{-1}, P_s = 0.01 cm sec^{-1}, and σ_{NI} = 0.71. As previously demonstrated, the KK and 2P formalisms yield essentially the same result when the model cell is exposed to an external solute concentration of 2 M. At 4 M solute a small deviation is evident and at the highest concentration, 10 M, the deviation is moderate. In all cases the KK formalism yields a larger excursion (shrink and swell) than the 2P model. The degree of deviation between the two formalisms is dependent on the model experiment. Reducing P_s while holding the other parameters constant yields a smaller deviation (not shown). Considering the noise levels typically found in volume excursion experiments, there is no practical difference between the 2P and KK formalism up to solute concentrations of several osmolal (or molar).

Sigma Equals One Revisited

It has occasionally been noted that substituting σ = 1 into the KK formalism (Eqs. [1] and [2]) yields a pair of equations which looks remarkably like the 2P formalism (Eqs. [7] and

TABLE 5
Solute–Solvent Flux Interactions

Lp (μm min^{-1} atm^{-1})	Ps (cm min^{-1})	σ	Comment
1.0	0.0034	σ_{NI} = 0.9	$\sigma_{NI} = 1 - P_s\bar{V}_s/RTL_p$
1.0	0.0034	σ_I = 0.7	$0 \le \sigma_I \le \sigma_{NI}$
1.0	0.0034	σ_I = 0.5	$0 \le \sigma_I \le \sigma_{NI}$

Note. These transport parameters illustrate different degrees of solute–solvent flux interaction and were used along with the KK formalism to generate the simulations shown in Fig. 2. Note that L_p and P_s are the same for all cases.

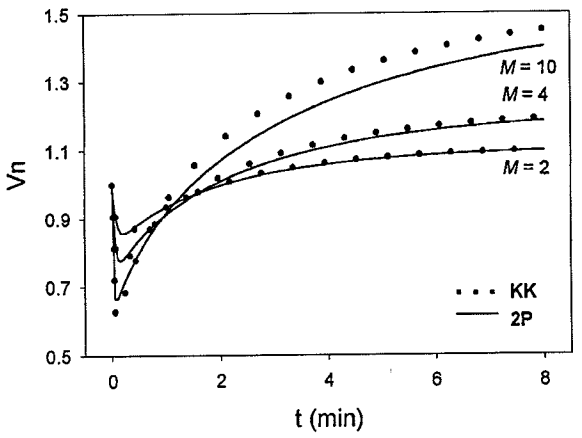

FIG. 3. Modeling with high solute concentrations. For a model cell, Table 3, the 2P (—) and KK (●) formalisms are used to simulate the cell response. At high solute concentrations, the KK simulations deviate from the 2P because the KK assumption of dilute solutions is no longer valid. Cells are exposed to 2, 4, or 10 M glycerol at time zero with L_p = 1.0 μm min^{-1} atm^{-1}, P_s = 0.01 cm min^{-1}, and $\sigma = \sigma_{NI}$ = 0.71.

[8]). Two caveats are in order. First, within the KK formalism, σ can only equal one if P_s is zero. Setting $\sigma = 1$ more generally, when $P_s \neq 0$, violates the conditions of the KK derivation and leads to incorrect results.

Second, setting $\sigma = 1$ in the KK formalism does not, in fact, reproduce the 2P formalism, as may be easily shown. The key problem occurs with Eq. [1] in the KK formalism,

$$dV_{w+s}/dt = -L_pA(\Delta\pi_n + \sigma\Delta\pi_s), \quad [1]$$

which becomes

$$dV_{w+s}/dt = -L_pA\Delta\pi, \quad [22]$$

after substituting $\sigma = 1$. Comparing this with Eq. [7] for the 2P formalism,

$$dV_w/dt = -L_pA\Delta\pi, \quad [7]$$

we see that the right hand sides are identical but the left hand sides differ. The term dV_{w+s}/dt in Eq. [22] (or Eq. [1]) is *not* the same as dV_w/dt appearing in Eq. [7]. The equations differ; one must be wrong. It is Eq. [22] because of the improper substitution of $\sigma = 1$.

To clarify further, dV_{w+s}/dt in Eq. [1] represents the cell volume change due to both the solvent *and* solute fluxes:

$$dV_{w+s}/dt = dV_w/dt + dV_s/dt. \quad [23]$$

After the improper substitution of $\sigma = 1$ into Eq. [1], we are left with Eq. [22], which contains only a term for the water flux. The solute volume flux has disappeared, which is only valid if $P_s = 0$ as previously noted.

Somewhat paradoxically, substitution of $\sigma = 1$ into the KK solute flux equation (Eq. [2]) yields the 2P solute flux equation (Eq. [8]). Thus substitution of $\sigma = 1$ into the KK formalism leads to a 'schizophrenic' equation set in which the solute volume is unaccounted for but the osmotic consequences of the solute flux are accounted for.

As a practical consequence, if $\sigma = 1$ is inserted into computer code for solving the KK equations, the solute volume disappears from the calculation and an erroneous result is obtained. This may be easily demonstrated using our model cell. Taking the case L_p = 1.0 μm min^{-1} atm^{-1}, P_s = 0.01 cm min^{-1}, and σ_{NI} = 0.71 or $\sigma = 1$ yields the results shown in Fig. 4. As expected, the 2P and KK (σ_{NI} = 0.71) simulations agree. However, for the KK ($\sigma = 1$) case, the volume at any time is low because the solute volume, which is flowing from outside to inside the cell, has been omitted.

If the solute flux were neglected in both Eqs. [1] and [2], the KK simulation would yield cell shrinkage without the reswell portion seen in Fig. 4. The shrink–swell response seen when $\sigma = 1$ occurs because the solute flux is still incorporated in the dN_s/dt equation and thus the osmotic effect of the solute flow is still present. As a consequence, the use of $\sigma = 1$ yields reasonably correct results when the solute volume flux is small compared with the water volume flux. There is, however, no need to make this approximation. Correct results may be obtained either by using the simple 2P model or by iteratively solving the KK model as described under Simulation Procedures and Software.

The reader may protest that Eq. [1] does not

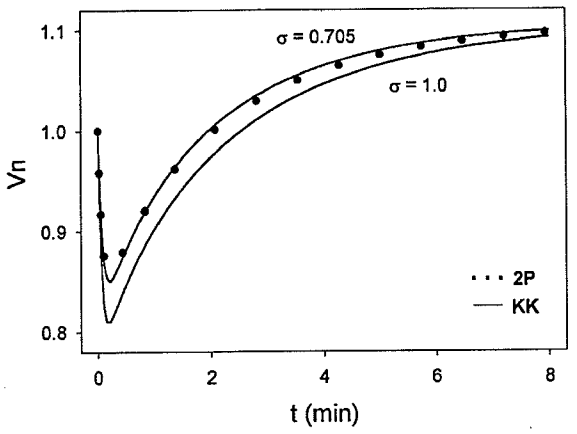

FIG. 4. Kedem–Katchalsky simulation with σ erroneously set equal to one. If σ is set equal to one when $P_s \neq 0$ in the KK formalism, the resulting model and simulation neglects the volume flux of solute and falls below the correct curve. However a shrink–swell curve is still obtained because the KK ($\sigma = 1$) model still incorporates the osmotic effect of the solute flux (see text). This is illustrated for $L_p = 1.00\ \mu m\ min^{-1}\ atm^{-1}$, $P_s = 0.01\ cm\ min^{-1}$, $\sigma = 1$ (invalid curve), and $\sigma_{NI} = 0.71$ (valid curve). For comparison a 2P (●) simulation is also shown.

contain P_s as would be expected if it included the volume flux of solute. To see that the solute volume flux is included, consider the simple case where $\sigma = 1 - P_s\bar{V}_s/RTL_p$. Inserting this into Eq. [1] yields

$$dV_{w+s}/dt = -L_pA\{\Delta\pi_n + (1 - P_s\bar{V}_s/RTL_p)\Delta\pi_s\} \quad [24]$$

which simplifies to

$$dV_{w+s}/dt = -L_pA\Delta\pi + P_sA\bar{V}_s\Delta M_s. \quad [25]$$

In this form, it is now clear that Eq. [1] includes a term for the solute volume flux. The difference in the sign of the two terms on the right side of Eq. [25] results from the fact that water flows towards regions of high osmotic strength and solute flows away from regions of high solute concentration. In the more general case when $\sigma < \sigma_{NI}$, it is necessary to appeal to the derivation of the KK formalism to see that Eq. [1] includes the solute volume flux.

Determining σ when Cotransporting Channels and Lipid Bilayer Transport Are Present

Real cells frequently have a background level of solvent and solute transport via lipid bilayer diffusion. For instance, in the well characterized human red blood cell (RBC) with a very high level of aquaporin-conferred water permeability, bilayer diffusion accounts for 10% of the total water flux (10, 27). Thus any attempt to accurately determine σ in a situation where cotransporting channels are suspected must take into account the confounding effect of lipid bilayer transport. To illustrate this we consider a hypothetical cell in which 75% of the water and solute are transported via a cotransporting channel and 25% is carried via bilayer transport (at 20°C). As previously noted, five transport parameters are required to characterize this system. We will examine the consequences of determining (fitting) transport parameters in this multiple pathway model using the simple 2P or KK formalism.

First we simulate the volume vs time response of our hypothetical cell using the multiple pathway transport model (developed above) invoking the KK formalism for the cotransporting channel and the 2P formalism for the bilayer transport. Then, these results are used as hypothetical, experimental data and fit with either the simple KK or 2P formalism. Table 6 shows the input simulation parameters and the resultant fitting parameters returned by the simple KK and 2P analysis. Both the KK and 2P fits yield good phenomenological fits to the model cell data (Fig. 5). Nominally the KK fit looks better; however, if a typical level of experimental noise were present this distinction would disappear.

This simulation experiment illustrates several points. Both the simple 2P and KK formalism return similar L_p and P_s values which are good estimates of the overall membrane water and solute permeability; i.e., they are good phenomenological parameters, Table 6. In a sense, σ is also a phenomenological parameter here. The value of σ returned by the simple KK analysis is intermediate between the channel σ and the bilayer σ_{NI}, rather than a valid measure of the

TABLE 6
Parameter Fitting with Cotransporting Channels and Lipid Bilayer Diffusion Present

	L_p (μm min^{-1} atm^{-1})	P_s (cm min^{-1})	σ	Comment
Input parameters				
Model cell channel	$L_p^c = 0.75$	$P_s^c = 2.54 \times 10^{-3}$	$\sigma^c = 0.7$	$\sigma_{NI} = 0.9$
Model cell bilayer	$L_p^b = 0.25$	$P_s^b = 0.848 \times 10^{-3}$	$\sigma^b = 0.9$	$\sigma_{NI} = 0.9$
Sum of channel and bilayer	$L_p = 1.0$	$P_s = 3.39 \times 10^{-3}$	—	—
Output fits				
KK Fit (phenomenological)	0.987	3.47×10^{-3}	0.76	$\sigma_{NI} = 0.896$
2P Fit (phenomenological)	1.05	4.06×10^{-3}	—	—

Note. A model cell (Table 3) with channel and bilayer transport (multiple pathways) was created and then analyzed using either the simple KK or 2P transport formalism. The channel was assumed to be responsible for 75% of the transport and the bilayer for 25%. The KK and 2P fits are graphically shown in Fig. 5.

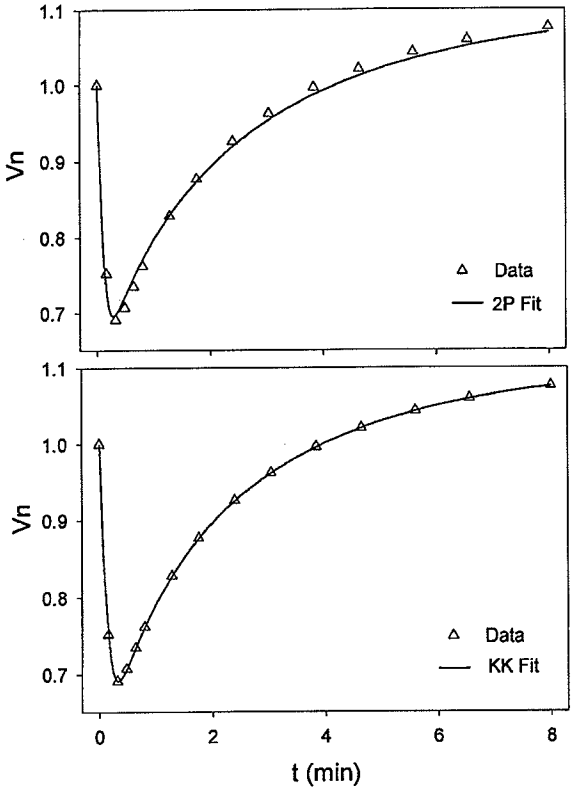

FIG. 5. Transport via bilayer diffusion and cotransporting channels. A model cell (Tables 3 and 6) is considered in which cotransporting channels carry 75% of the solute and solvent flux and bilayer diffusion is responsible for the remaining 25%. The volume vs time response of this model system is shown by the points (△). The top and bottom graphs show the simple 2P and KK fits (—) to this multiple pathway situation, respectively. The resulting fit parameters are compared with the input model parameters in Table 6.

channel σ. Also, the use of the KK formalism and the introduction of σ does not appreciably improve the quality of the fit, Fig. 5.

A determination of transport E_a also yields a phenomenological result when multiple transport pathways are present (36). This can be illustrated by extending the above example to a 0 to 30°C temperature range. We consider a typical water channel with an E_a of 4 kcal/mol (34, 44) and a typical bilayer with a water transport E_a of 15 kcal/mol (9). Then, using the previous starting values of $L_p^c = 0.75$ and $L_p^b = 0.25$ μm min^{-1} atm^{-1} at 20°C, we generate a table of permeabilities between 0 and 30°C and add the channel and bilayer L_p's to obtain a phenomenological L_p (cf. Eq. [19]) at each temperature (Table 7). These phenomenological L_p's are fit using Eq. [21] to yield an E_a of 6.2 kcal/mol, intermediate between the E_a's of the two physical pathways. Once again, we see that we have obtained a phenomenological parameter which describes the average water transport E_a of the membrane rather than the E_a's of the underlying physical processes. A similar result holds for the solute permeability.

The phenomenological L_p's in Table 7 do not follow an exact Arrhenius relationship, a fact that would be more obvious if a wider temperature range were examined. Also, note that water transport is completely dominated by the water channel at low temperatures, whereas the bilayer transport becomes comparable to the

TABLE 7
Activation Energy with Multiple Pathways

T (°C)	L_p^c (μm min^{-1} atm^{-1})	L_p^b (μm min^{-1} atm^{-1})	L_p (phenom.) ($L_p^c + L_p^b$)
30	0.941	0.585	1.526
20	0.750	0.250	1.000
10	0.588	0.101	0.689
0	0.453	0.038	0.491
E_a	4 kcal/mol	15 kcal/mol	6.2 kcal/mol

Note. In this example, water is transported across the cell membrane by a channel and lipid bilayer diffusion with differing E_a's. Adding the two permeabilities yields a phenomenological L_p with a phenomenological E_a intermediate between the two physical pathways (See Eq. [19]).

channel's at higher temperatures. This point may be useful to remember when designing experiments or modeling cell dehydration during cooling.

It might be argued that the proper way to analyze these hypothetical experiments would be with the same multiple pathway model used to generate them. However such a model contains five independent parameters (L_p channel, P_s channel, σ channel, L_p bilayer, and P_s bilayer) and would be virtually impossible to fit using typical, noisy, experimental volume vs time data. Fortunately, the phenomenological transport parameters are frequently all we require. If all five transport parameters are desired, substantially more experimental work is required and has rarely been achieved. One approach to this five-parameter problem is to measure L_p bilayer and P_s bilayer in an independent control experiment, as will be discussed later.

Activation Energy and Sigma

The temperature dependence of L_p and P_s yield valuable insights into the physical mode of solvent and solute transport, e.g., via channels or the bilayer. Occasionally an activation energy is also reported for σ and a point made of its 'unusual' negative value. We advise caution. The activation energy of σ does not lend itself to the simple physical interpretation possible with L_p and P_s. An example is considered below which illustrates why $E_a(\sigma)$ is often negative. This is a natural consequence of the temperature dependence of L_p and P_s. There is nothing unusual about this.

Consider the hypothetical cell, permeable to water and glycerol, utilized in this paper. We assign commonly found E_a values of 4 kcal/mol to L_p and 12 kcal/mol to P_s (4, 6, 34, 44) and give the cell starting permeability values of L_p = 1.0 μm min^{-1} atm^{-1} and P_s = 0.01 cm min^{-1} at 20°C. The only essential characteristic of these starting parameters is that the activation energy for water transport be less than that for glycerol, as is frequently found. For simplicity, consider the simple limiting case of $\sigma = \sigma_{NI}$. Using these conditions we generate values of L_p, P_s, and σ_{NI} as a function of temperature, Table 8. Finally, this simulated σ_{NI} data may be fit with Eq. [21] to yield an activation energy of −2.5 kcal/mol, Fig. 6.

This negative E_a is a simple consequence of the lower activation energy of L_p relative to P_s. In this example, σ is not an independent parameter, and therefore, neither is its activation energy. It is determined by the behavior of L_p and P_s. Furthermore, the Arrhenius plot (Fig. 6) does not yield a straight line relationship as may be demonstrated by analytically solving for the temperature dependence of σ_{NI} using Eqs. [5] and [21].

In the more general case where $\sigma \leq \sigma_{NI}$, the situation is more complex. Nevertheless, σ is

TABLE 8
Temperature Dependence of Sigma

T (°C)	L_p (μm min^{-1} atm^{-1})	P_s (cm min^{-1})	σ_{NI}
30	1.26	1.97×10^{-3}	0.55
20	1.00	1.00×10^{-3}	0.71
10	0.784	0.48×10^{-3}	0.81
0	0.605	0.22×10^{-3}	0.88
E_a (kcal/mol)	4.0	12.0	−2.5

Note. In a simple model cell, we assume L_p and P_s have E_a's of 4 and 12 kcal/mol, respectively. Then σ_{NI} is computed from L_p and P_s using Eq. [5] and it's E_a determined assuming an Arrhenius temperature dependence. The negative E_a of σ_{NI} follows automatically from the smaller activation energy of L_p relative to P_s.

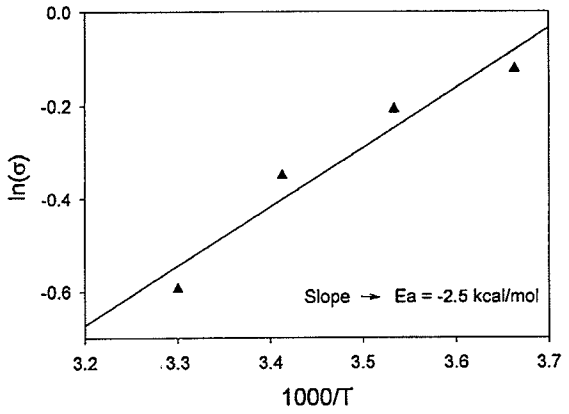

FIG. 6. The activation energy of σ_{NI}. Negative E_a's for sigma are a natural consequence of assuming a lower E_a for water transport compared to solute transport. For a model cell at 20°C, Table 3, we assume L_p = 1.00 μm min^{-1} atm^{-1} with an E_a of 4 kcal/mol and P_s = 0.001 cm min^{-1} with an E_a of 12 kcal/mol. Then L_p, P_s, and σ_{NI} are computed at 0, 10, 20, and 30°C (Table 8) and σ_{NI} plotted on an Arrhenius plot (▲). The slope of the plot yields an E_a (σ) of −2.5 kcal/mol.

still constrained by Eq. [4] and we believe will frequently demonstrate a negative E_a because of the behavior of L_p and P_s. It may be empirically useful for modeling purposes to determine the temperature dependence of σ, but a physical interpretation in terms of the solute–solvent interaction is difficult.

DISCUSSION

Simulation Experiments

The objectives of the simulation experiments were several fold; we summarize only the main points. First, we demonstrated that when solute–solvent flux interactions are absent, the two-parameter formalism yields essentially the same result as the Kedem–Katchalsky formalism, because σ is not an independent parameter. Under these circumstances the 2P formalism yields the correct results with much greater simplicity. Additionally, we demonstrated that this correspondence between the simple KK and 2P formalism occurs in the limit of $\sigma_{NI} = 1 - P_s\bar{V}_s/RTL_p$ and *not* in the limit of $\sigma = 1$. Sigma equals one only in the uninteresting case in which the membrane is completely impermeable to solute.

In situations where channels are present, they generally function in the presence of a background level of lipid bilayer transport. In this circumstance both the simple 2P and KK formalisms yield phenomenological L_p and P_s values which are the sum of the channel and bilayer values. Additionally, a KK analysis does not yield a correct value of the channel σ in the presence of confounding bilayer transport.

Even when bilayer transport is negligible, σ may be difficult to measure. The modification of a cell's volume vs time response due to flux interactions is typically small, as Fig. 2 illustrates. A large change in σ introduces only a moderate vertical (volume) shift in the volume vs time curves. Thus noise, small biases, or errors in cell volume measurements can seriously compromise the determination of σ.

Phenomenological Parameters and Reality

We noted above that both the 2P and KK formalisms yield phenomenological parameters when there are multiple transport pathways present, e.g., lipid bilayer and channels. There are other contexts in which we also measure phenomenological parameters. Averaging over morphological features frequently occurs. Mammalian sperm, for instance, have a multilayer membrane structure associated with the head, while the midpiece and tail membranes likely have very different characteristics from the head. Nevertheless, we frequently measure a single water and solute permeability for sperm, representing an average over these very different parts. Similarly, when measuring a multicellular embryo, permeability parameters are often determined assuming a simple spherical cell for fitting purposes. The parameters obtained in these examples are phenomenological averages. They are not the permeability of a specific membrane or pathway. Nevertheless they provide valuable guidance when determining cooling rates, cryoprotectant loading times, and protocols for avoiding osmotic shock during addition and removal of cryoprotectants (2, 13, 16, 32). The two-parameter formalism typically meets the needs of such modeling situations. Indeed, when P_s is small compared to P_f, the 2P

TABLE 9
Aquaporins

	AQP1 (CHIP28)	AQP2 (WCH-CD)	AQP3 (GLIP)	AQP4 (MIWC)	AQP5	AQP7
Water	+	+	+	+	+	+
Urea	−	−	+	−	−	+
Glycerol	−	−	+	−	−	+
E_a(water)	≈4	4	3			2
E_a(urea)			20			
E_a(gly)			12			5
References	19, 25, 37, 46	5, 15, 25, 46	7, 8, 25, 26, 46	18, 25, 46	46	21

Note. The water, urea, and glycerol transport properties of common aquaporins are listed. The activation energies are in kcal/mol.

model can be simplified to a one-parameter model (30, 31).

The temptation to use the KK formalism simply because it yields a slightly better fit to experimental data than the 2P formalism should be resisted. Other things being equal, three parameters will give a better fit than two. However, if the third parameter has little physical meaning, it simply becomes a 'fudge' factor with the potential for causing mischief.

Previous experience with red blood cells reveals the potential for modeling problems resulting from data artifacts and analysis errors (27). For example, there are a number of potential problems with volume and area determinations. Cell area may not be constant, as is often assumed, but may vary as a result of folding or pleating during cell shrinkage (32). Cell volumes are typically measured indirectly. With light microscopy, cell cross sectional areas are measured and converted to volumes using an assumption about the cell shape. Alternatively, particle counters can be calibrated to yield cell volumes, but many assumptions are required. It is our subjective experience that poor fits during parameter estimation typically result from measurement artifacts or failure of the cells to respond in an ideal Boyle van't Hoff fashion, possibly due to membrane damage. Thus it is common, for instance, to observe cells which do not attain their expected equilibrium volume after exposure to a test solution. Introduction of the Kedem–Katchalsky σ in such situations may improve the apparent agreement between experiment and theory, but will contribute nothing to an understanding of the biology.

Aquaporins

The discovery and characterization of aquaporins has revolutionized our understanding of membrane water transport. It was suspected for some time that water-selective channels existed (27). Work in the field accelerated rapidly with the specific identification of a water transporting protein (CHIP28) in human RBC in 1992 (37). These water-selective channels are called aquaporins and have now been discovered in a wide variety of animal and plant cells (3, 44). Table 9 summarizes the transport properties of some well characterized aquaporins found in mammalian cells. We note, however, that the field is in a rapid state of flux with new aquaporins being rapidly discovered and with frequent disagreements about their transport properties.

Several characteristics are of interest. All measured aquaporins have water transport E_a's of less than 6 to 7 kcal/mol (44). Typically, aquaporins are selective for water and exclude small non-ionic solutes (AQP1, AQP2, AQP4, AQP5). A few aquaporins do transport solutes (AQP3 and AQP7); however, there is some evidence suggesting that the glycerol and urea channel in AQP3 is distinct from the water channel (8). Further, the permeability of AQP3 to glycerol and urea is substantially less than to

water (8). Most of the literature contains transport data only for the solutes, glycerol and urea, which are used as proxies for all low-molecular-weight, non-ionic solutes. Occasionally other solutes of interest to cryobiologists are reported, as in the AQP2 study which found permeability to formamide but not to mannitol, glycerol, ethylene glycol, or urea (15).

We must also consider whether known solute transporters, such as those for glycerol, glucose, or urea, act as aquaporins. The data are mixed and incomplete. The glycerol transporter, GlpF, does not appear to transport water (28, 44), while the glucose transporters (GLUTs) appear to have a small permeability to water (11, 12). Urea transporters are generally found to be impermeable to water (45); however one recent report does find water permeability in UT3 (47). It is premature to make a final judgment, but the general consensus to date is that in most cases these other transporters make only a negligible contribution to water transport (44).

Experimental Tests for Water Channels (Aquaporins) and Cotransporting Channels

The tools of molecular biology aside, what experimental tests are there for water channels? The activation energy for water transport is the single most powerful test. All measured aquaporins have water transport E_a's less than 6 to 7 kcal/mol (44). In contrast, water transport across lipid bilayers typically has an E_a of 10 to 20 kcal/mol (9). Thus we are generally skeptical of the use of the KK formalism in cells or tissue with water transport E_a's $\geq$ 10 kcal/mol. Without cotransporting water channels, σ becomes unnecessary. However, to be cautious, we should note Verkman *et al.*'s point that there appears to be no a priori theoretical basis for the low E_a's of water channels (44).

A second test compares the water diffusion (P_d) and filtration (P_f) permeabilities.[5] Diffusional permeability is measured using labeled water with no osmotic gradient present while P_f is just the osmotically determined L_p re-expressed as $P_f = RTL_p/\bar{V}_w$. If water channels are absent, $P_f/P_d = 1$, whereas $P_f/P_d \geq 2$ is generally taken as evidence of water channels (10). For instance, AQP1, the archetypal aquaporin, has a P_f to P_d ratio of 11 in human RBC (33). The ratio is generally interpreted as the number of water molecules spanning the channel (10).

The KK reflection coefficient (σ) is a test for cotransporting channels (it provides no evidence concerning water-only channels). If $\sigma \ll \sigma_{NI}$ then a cotransporting solute and solvent channel is present (23). The literature is filled with reports of $\sigma \ll \sigma_{NI}$, however, few have been independently confirmed or have stood the test of time. The most interesting of these is the case of the human RBC membrane which was repeatedly reported to have a cotransporting water and urea channel based on σ measurements (38). These measurements are now believed to be artifactual (27, 40).

Recently, a σ of 0.3 was reported for the UT3 urea channel, suggesting cotransport of water and urea (47). Several points concerning this measurement bear note. The channel was assayed in *Xenopus* oocytes expressing cRNA for UT3 (from rat). Thus large numbers of the channels were present and, equally important, urea bilayer and water bilayer transport were separately measured in control oocytes not expressing UT3, so that the confounding effect of background bilayer transport could be compensated for. Sigma has also been measured in AQP2 for a number of solutes (15). Formamide yielded $\sigma = 0.62$, suggesting that AQP2 cotransports water and formamide. As was the case in the UT3 channel study, a number of experiments were performed to distinguish between bilayer and channel transport of the formamide.

[5] The water diffusion permeability (P_d) mentioned here should not be confused with the previously discussed lipid bilayer diffusion of water. Here we are discussing diffusion across the membrane, by any path, in the absence of an osmotic gradient. Previously we discussed diffusion of water across the lipid bilayer in the presence of an osmotic gradient. In the presence of an osmotic (concentration) gradient there is a net flow of water. When an osmotic gradient is not present, water inside the cell exchanges with water outside the cell via diffusion, but there is no net flow. A similar situation holds for solutes.

Neither of these papers compare σ with σ_{NI} directly, but rather, invoke a variety of arguments and experiments to establish cotransport. Whether these papers are the leading edge of a coming wave of cotransporting channel discoveries remains to be determined. However both papers do serve to make the point that verification of cotransport in a biological membrane requires more than a simple KK analysis.

Other Forms of Solute–Solvent Interaction

There are many circumstances in which solute and solvent interact, albeit not in the Kedem–Katchalsky sense as embodied in σ. As has been pointed out by Hammerstedt *et al.* among others, solutes diffuse into membranes and very likely alter their properties (17). There is wide-spread evidence that solutes modify the water permeability of membranes, typically reducing it (14, 35, 41, 43). Perhaps the best studied of these is the human RBC membrane in which glycerol, ethylene glycol, and urea were found to reduce L_p to less than half its solute-free value (35, 41, 42). Because we now know that 90% of human RBC water transport occurs through the water-selective channel, AQP1, this inhibition of L_p cannot be related to solute–solvent cotransport interactions. Thus, it is inappropriate to use σ as a means to characterize these solute–membrane interactions.

For completeness, we should note that Kedem and Katchalsky argued in their original paper (23) and in subsequent papers (24) that solute–solvent flux interactions (friction between the solute and solvent fluxes) can occur even for pure diffusion across the bilayer. In principle this may be possible. However, as discussed above, a review of the literature of recent years indicates that flux interactions have been found only in those rare situations where cotransport through a common channel is the dominant mode of transport.

SUMMARY

The last several years have seen many exciting developments in the field of molecular biology which have greatly expanding our understanding of facilitated water and solute transport. When a detailed understanding of the physical mechanisms of transport is desired, a number of measurements including those of transport activation energy (E_a), water P_f and P_d, background lipid bilayer transport, and the Kedem–Katchalsky reflection coefficient (σ) provide valuable information. However, in practice, it has proven difficult to establish the existence and to determine the properties of cotransporting water and solute channels in natural biological membranes. Experience to date suggests that water channels are unlikely when the E_a of water transport is $\geq$10 kcal/mol.

If we desire to phenomenologically model the response of a system to, for instance, cooling protocols or cryoprotectant addition and removal, then the two-parameter formalism is frequently appropriate and offers the advantage of simplicity. Unless there is strong evidence or reason to suspect significant levels of solute–solvent cotransport, use of the Kedem–Katchalsky formalism is unnecessary and sometimes inappropriate. Channel studies to date reveal very few instances of significant cotransport of water and solute.

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REFERENCES

1. Andreoli, T. E., Dennis, V. W., and Weigl, A. M. The effect of Amphotericin B on the water and nonelectrolyte permeability of thin lipid membranes. *J. Gen. Physiol.* **53,** 133–156 (1969).
2. Arnaud, F. G., and Pegg, D. E. Permeation of Glycerol and Propane-1,2-diol into human platelets. *Cryobiology* **27,** 107–118 (1990).

3. Chrispeels, M. J., and Agre, P. Aquaporins: Water channel proteins of plant and animal cells. *Trends Biochem. Sci.* **19,** 421–425 (1994).
4. Cohen, B. E. The permeability of liposomes to nonelectrolytes. I. Activation energies for permeation. *J. Membr. Biol.* **20,** 205–234 (1975).
5. Coury, L. A., Mathai, J. C., Prasad, G. V. R., Brodsky, J. L., Agre, P., and Zeidel, M. L. Reconstitution of water channel function of aquaporins 1 and 2 by expression in yeast secretory vesicles. *Am. J. Physiol.* **274** (*Renal Physiol.* **43**): F34–F42 (1998).
6. Du, J., Kleinhans, F. W., Mazur, P., and Critser, J. K. Human spermatozoa glycerol permeability and activation energy determined by electron paramagnetic resonance. *Biochim. Biophys. Acta* **1194,** 1–11 (1994).
7. Echevarria, M., Windhager, E. E., Tate, S. S., and Frindt, G. Cloning and expression of AQP3, a water channel from the medullary collecting duct of rat kidney. *Proc. Natl. Acad. Sci. USA* **91,** 10997–11001 (1994).
8. Echevarria, M., Windhager, E. E., and Frindt, G. Selectivity of the renal collecting duct water channel aquaporin-3. *J. Biol. Chem.* **271,** 25079–25082 (1996).
9. Fettiplace, R., and Haydon, D. A. Water permeability of lipid membranes. *Physiol. Rev.* **60,** 510–550 (1980).
10. Finkelstein, A. "Water Movement Through Lipid Bilayers, Pores, and Plasma Membranes: Theory and Reality." Wiley, New York, 1987.
11. Fischbarg, J., Kuang, K., Vera, J. C., Arant, S., Silverstein, S. C., Loike, J., and Rosen, O. M. Glucose transporters serve as water channels. *Proc. Natl. Acad. Sci. USA* **87,** 3244–3247 (1990).
12. Fischbarg, J., and Vera, J. C. Multifunctional transporter models: Lessons from the transport of water, sugars, and ring compounds by GLUTs. *Am. J. Physiol.* **268** (*Cell Physiol.* **37**): C1077–1089 (1995).
13. Gao, D. Y., Liu, J., Liu, C., McGann, L. E., Watson, P. F., Kleinhans, F. W., Mazur, P., Critser, E. S., and Critser, J. K. Prevention of osmotic injury to human spermatozoa during addition and removal of glycerol. *Hum. Reprod.* **10,** 1109–1122 (1995).
14. Gilmore, J. A., McGann, L. E., Liu, J., Gao, D. Y., Peter, A. T., Kleinhans, F. W., and Critser, J. K. Effect of cryoprotectant solutes on water permeability of human spermatozoa. *Biol. Reprod.* **53,** 985–995 (1995).
15. Gutierrez, A. M., Gonzalez, E., Echevarria, M., Hernandez, C. S., and Whittembury, G. The proximal straight tubule (PST) basolateral cell membrane water channel: Selectivity characteristics. *J. Membr. Biol.* **143,** 189–197 (1995).
16. Hagedorn, M., Kleinhans, F. W., Artemov, D., and Pilatus, U. Characterization of a major permeability barrier in the zebrafish embryo. *Biol. Reprod.,* **59,** 1240–1250 (1998).
17. Hammerstedt, R. H., Graham, J. K., and Nolan, J. P. Cryopreservation of mammalian sperm: What we ask them to survive. *J. Androl.* **11,** 73–88 (1990).
18. Hasegawa, H., Ma, T., Skach, W., Matthay, M. A., and Verkman, A. S. Molecular cloning of mercurial-insensitive water channel expressed in selected water-transporting tissues. *J. Biol. Chem.* **269,** 5497–5500 (1994).
19. Heymann, J. B., Agre, P., and Engel, A. Progress on the structure and function of aquaporin 1. *J. Struct. Biol.* **121,** 191–206 (1998).
20. Holz, R., and Finkelstein, A. The water and nonelectrolyte permeability induced in thin lipid membranes by the polyene antibiotics nystatin and amphotericin B. *J. Gen. Physiol.* **56,** 125–145 (1970).
21. Ishibashi, K., Kuwahara, M., Gu, Y., Kageyama, Y., Tohsaka, A., Suzuki, F., Marumo, F., and Sasaki, S. Cloning and functional expression of a new water channel abundantly expressed in the testis permeable to water, glycerol, and urea. *J. Biol. Chem.* **272,** 20782–20786 (1997).
22. Jacobs, M. H. The simultaneous measurement of cell permeability to water and to dissolved substances. *J. Cell. Comp. Physiol.* **2,** 427–444 (1932–1933).
23. Kedem, O., and Katchalsky, A. Thermodynamic analysis of the permeability of biological membranes to non-electrolytes. *Biochim. Biophys. Acta* **27,** 229–246 (1958).
24. Kedem, O., and Katchalsky, A. A physical interpretation of the phenomenological coefficients of membrane permeability. *J. Gen. Physiol.* **45,** 143–179 (1961).
25. Knepper, M. A., Wade, J. B., Terris, J., Ecelbarger, C. A., Marples, D., Mandon, B., Chou, C. L., Kishore, B. K., and Nielsen, S. Renal aquaporins. *Kidney Int.* **49,** 1712–1717 (1996).
26. Lee, M. D., King, L. S., and Agre, P. The aquaporin family of water channel proteins in clinical medicine. *Medicine (Baltimore)* **76,** 141–156 (1997).
27. Macey, R. I. Transport of water and urea in red blood cells. *Am. J. Physiol.* **246** (*Cell Physiol.* **15**): C195–C203 (1984).
28. Maurel, C., Reizer, J., Schroeder, J. I., Chrispeels, M. J., and Saier, M. H., Jr. Functional characterization of the Escherichia coli glycerol facilitator, GlpF, in Xenopus oocytes. *J. Biol. Chem.* **269,** 11869–11872 (1994).
29. Mazur, P. Kinetics of water loss from cells at subzero temperatures and the likelihood of intracellular freezing. *J. Gen. Physiol.* **47,** 347–369 (1963).
30. Mazur, P., Leibo, S. P., and Miller, R. H. Permeability of the bovine red cell to glycerol in hyperosmotic solutions at various temperatures. *J. Membr. Biol.* **15,** 107–136 (1974).

31. Mazur, P., and Miller, R. H. Permeability of the human erythrocyte to glycerol in 1 and 2 M solutions at 0 or 20°C. *Cryobiology* **13,** 507–522 (1976).
32. Mazur, P. Equilibrium, quasi-equilibrium, and nonequilibrium freezing of mammalian embryos. *Cell Biophys.* **17,** 53–92 (1990).
33. Moura, T. F., Macey, R. I., Chien, D. Y., Karan, D., and Santos, H. Thermodynamics of all-or-none water channel closure in red cells. *J. Membr. Biol.* **81,** 105–111 (1984).
34. Noiles, E. E., Mazur, P., Watson, P. F., Kleinhans, F. W., and Critser, J. K. Determination of water permeability coefficient for human spermatozoa and its activation energy. *Biol. Reprod.* **48,** 99–109 (1993).
35. Papanek, T. H. The Water Permeability of the Human Erythrocyte in the Temperature Range +25 C to −10°C. PhD thesis, MIT, Jan 1978.
36. Petcu, I., Lupu, M., and Grosescu, R. NMR study of the selective inhibition of water permeability of rat erythrocyte membrane. *Biosci. Rep.* **15,** 55–63 (1995).
37. Preston, G. M., Carroll, T. P., Guggino, W. B., and Agre, P. Appearance of water channels in Xenopus oocytes expressing red cell CHIP28 protein. *Science* **256,** 385–387 (1992).
38. Solomon, A. K. Water channels across the red blood cell and other biological membranes. *Methods Enzymol.* **173,** 192–222 (1989).
39. Staverman, A. J. The theory of measurement of osmotic pressure. *Recueil des Travaux Chimiques des Pays-Bas* **70,** 344–352 (1951).
40. Stein, W. D. "Transport and Diffusion across Cell Membranes." Academic Press, New York, 1986.
41. Toon, M. R., and Solomon, A. K. Modulation of water transport in human red cells: Effect of urea. *Biochim. Biophys. Acta* **940,** 266–274 (1988).
42. Toon, M. R., and Solomon, A. K. Transport parameters in the human red cell membrane: Solute–membrane interactions of hydrophilic alcohols and their effect on permeation. *Biochim. Biophys. Acta* **1022,** 57–71 (1990).
43. van Hoek, A. N., de Jong, M. D., and van Os, C. H. Effects of dimethyl sulfoxide and mercurial sulfhydryl reagents on water and solute permeability of rat kidney brush border membranes. *Biochim. Biophys. Acta* **1030,** 203–210 (1990).
44. Verkman, A. S., van Hoek, A. N., Ma, T., Frigeri, A., Skach, W. R., Mitra, A., Tamarappoo, B. K., and Farinas, J. Water transport across mammalian cell membranes. *Am. J. Physiol.* **270** (*Cell Physiol.* **39**): C12–C30 (1996).
45. Wintour, E. M. Brief review: Water channels and urea transporters. *Clin. Exp. Pharmacol. Physiol.* **24,** 1–9 (1997).
46. Yang, B., and Verkman, A. S. Water and glycerol permeabilities of aquaporins 1-5 and MIP determined quantitatively by expression of epitope-tagged constructs in Xenopus oocytes. *J. Biol. Chem.* **272,** 16140–16146 (1997).
47. Yang, B., and Verkman, A. S. Urea transporter UT3 functions as an efficient water channel. *J. Biol. Chem.* **273,** 9369–9372 (1998).