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Membrane Permeability Equations and their Solutions for Red Cells

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Summary. The mathematical equations for the transport of nonelectrolytes across cell membranes are critically examined and cast in forms suitable for solution which involve fewer approximations than has heretofore been commonly done. For the case of red cells, the equations are developed to include the effect of the variation in apparent nonosmotic water owing to the variation in hemoglobin concentration as the cell swells or shrinks. Two methods of solution of the equations are developed and studied and sample calculations are provided. It is shown that the solutions to the linearized equations commonly found in the literature are insufficiently accurate for some purposes and this inaccuracy is avoided by the methods given here. The importance of retaining the effects of variations in apparent nonosmotic water and in solute volume in the cell is demonstrated.

The membrane permeability equations are mathematical expressions for the flow of solutes and solvents from one side of a membrane to the other when there is some driving force across the membrane. These equations are particularly useful in determining membrane properties by means of combining experimental results with some aspects of the equations which can, in principle, range from an initial flow rate to the complete solution.

In a typical experiment that is often done, cells are suddenly put in a nonisosmolal solution, and their volume change as a function of time is measured. Such a measured function contains an enormous amount of information. In principle, all of the membrane permeability parameters could be determined from such a measurement if the permeability equations could be solved. The requirement would be to find those values of the membrane parameters for which the solution of the permeability equations for cell volume *vs.* time matches the measured function. In practice, the

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ability to do this is limited by errors of three types: (1) limited accuracy of experimental measurements; (2) inaccuracy in the representation of all the cellular processes affecting membrane permeability in the form of mathematical equations; and (3) inaccuracy in the mathematical solutions of the permeability equations.

In this paper, the membrane permeability equations are critically examined and formulated in a way that minimizes errors of the second type. Then the mathematical process of solution of the equations is studied and methods of solution which minimize errors of the third type are presented along with sample calculations. The means of utilizing the equations and solution methods given here, to determine the membrane parameters from experimental measurements, will be given in a subsequent paper.

The most complete derivation of the equations for the permeability of membranes to nonelectrolytes was given by Kedem and Katchalsky [10]. In this derivation several small effects were ignored, and a complicated expression for the difference in solute free energy across the membrane was replaced by a simplified approximate form.

Since the time of the Kedem and Katchalsky derivation, the equations have been solved approximately for several cases. These approximate solutions have generally taken one of two forms. First, Solomon and his co-workers [6, 16, 17] have used the form of the Kedem and Katchalsky equations to deduce certain membrane parameters from a few particular features of the graph of cell volume *vs.* time after a red cell has been put into a nonisosmolal solution. The determination of the human red cell osmotic reflection coefficient by Goldstein and Solomon [6] has recently been questioned by Levitt [11] and by Owen and Eyring [12] who presented their own determinations of the reflection coefficient. These latter determinations have been questioned by Solomon, Milgram and Kirkwood ([18] and *in preparation*). As a result of these publications, it has become apparent that the correct determination of red cell reflection coefficients is an unsettled issue at this time and steps are being taken to resolve it. One step is the detailed analysis of the membrane permeability equations and the methods of solving them that are presented here. These methods were used by Solomon *et al.* ([18] and *in preparation*) to obtain some of their results, but the analysis and development of the methods presented here was not given in the aforementioned references.

The second form of approximate solution is the mathematical calculation of the cell volume as a function of time for an approximate form of the Kedem and Katchalsky equations. In most cases, solutions have

been obtained for the strictly linearized approximation to the equations. Johnson and Wilson [8] have given details of one method of solution of the strictly linearized equations and showed the effect of certain parametric variations on these solutions. Farmer and Macey [2, 3] have related the solutions of the linearized equations for cell volume *vs.* time to the measured volume *vs.* time function after red cells were placed in a nonisosmolal solution in order to estimate cell membrane parameters.

Hempling [7] programmed the Kedem and Katchalsky equations on an analog computer and was able to find values for the membrane parameters which gave good correspondence between the computed cell volume *vs.* time and measurements on mouse ascites tumor cells. Although Hempling's analysis contained all the approximations made by Kedem and Katchalsky, it did not require the linearized approximation of the equations used by several other investigators [2, 3, 8].

The now widespread availability of digital computers makes digital computation potentially the most convenient and accurate method of obtaining solutions to the membrane permeability equations. Not only can solutions be obtained quickly and easily, but the equations can be programmed without recourse to some of the approximations made in the past.

We have found that several effects that are normally neglected can significantly influence cell volume time histories. Therefore, the membrane permeability equations are derived here without discarding effects that are considered to be small and discarded in other treatments. Also, the exact form for the solute-free energy difference across the membrane is retained. In applying these equations to red cells, the fact that the apparent volume of solvent that does not participate in dissolving electrolytes varies with cell volume is taken into account, something which hasn't been done in previous work. This results in a pair of coupled, first order, nonlinear differential equations for the cell volume and amount of solute in the cell as functions of time.

The most straightforward method of solving these equations is by direct numerical integration on a digital computer. This method necessarily requires that the continuous process of integration be approximated by a discrete process with finite time steps. To gain confidence that one is using small enough time steps, numerical integrations are often done at a number of time step sizes with the size considered small enough when a size reduction does not result in a significant change in the solution. However, more confidence can be gained if the solution can be compared with that obtained by an entirely different method. Therefore, another

method of solution is given as well. This is a perturbation expansion of the equations which leads, in principle, to an infinite sequence of sequential sets of linear problems. For most cell membrane problems, the contribution from each succeeding set of equations diminishes rapidly enough for only the first few sets of equations to be important. The first and second order perturbation equations are developed and solved here for a restricted set of conditions. The first order equations and solutions are identical to the linearized equations and solutions given in references 2, 3 and 8. However, evaluation of the solutions for a typical case shows that the second order effects are large enough for the first order equations and solutions alone without the second order contributions to be inadequate for many purposes.

The perturbation solutions are used here as a basis for comparison with direct numerical integration solutions. The combined first and second order perturbation solutions agree well with the direct numerical integration solutions, and this comparison gives confidence in the direct numerical integration solution. Once this confidence is obtained, the direct integration method is preferred because of the broader set of cellular conditions that it can easily accommodate. Although the numerical integration is easily carried out with a digital computer, it would be very tedious without a computer. On the other hand, the first and second order perturbation solutions are obtained in closed forms that can be evaluated in a straightforward way without a computer, albeit more convenient with a computer. Therefore, if a computer is not available, the perturbation method offers a relatively convenient way of obtaining solutions.

The next sections present the derivation of the membrane permeability equations and methods of solving them. Appendix C is a description of how the equations and solutions can be used.

1. Derivation of the Membrane Permeability Equations

Consider a membrane-bound cell as shown in Fig. 1. The cell contains and is immersed in a solvent and several solutes. Some of the cell volume is not solvent, and this volume is denoted by B_w. For example, by means of drying human red cells, Savitz, Sidel and Solomon [15] found that in the isosmolal condition, 28.3% of the cell volume was not solvent. The temperature of the entire system is a constant.

It is well known that some cells appear to respond osmotically as if some of the solvent were not participating in dissolving all of the solutes.

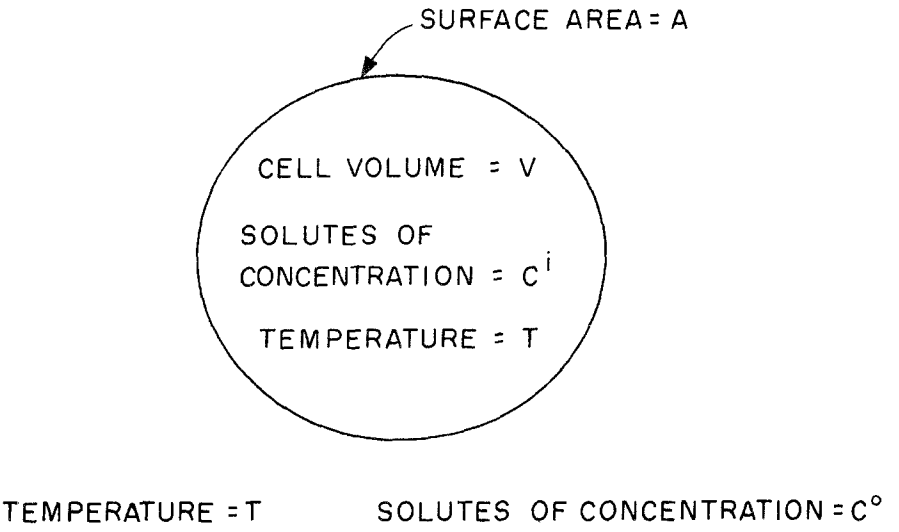

Fig. 1. Definition sketch

Some of this effect can be due to solvent bound to macromolecules in the cell as shown by Gary-Bobo [4]. In the case of red cells, however, the apparent nonparticipating solvent for apparently impermeable electrolytes in the cell is far too large to be due to bound solvent. It has been shown by Gary-Bobo and Solomon [5] that the effective net charge of the hemoglobin is concentration dependent and, as the cell volume changes, the changing hemoglobin ionization results in an actual chloride (Cl^-) transport through the membrane. This type of transport is not usually considered in the membrane permeability equations and can be handled in these equations in one of two ways. One way is to include explicitly the change in ion concentration inside the cell in the equations. The second is to follow the method of Savitz, Sidel and Solomon [15] who defined an apparent nonparticipating solvent volume which corrected the osmotic pressures for this effect. Gary-Bobo and Solomon [4, 5] have shown that this apparent nonparticipating solvent volume varies considerably with changes in cell volume in the case of the red cell. Therefore, this variation should be taken into account in the equations when this method is used. The second way will be used here with the apparent volume of solvent not dissolving the j^{th} solute called B_j. Then the apparent volume of solvent dissolving the j^{th} solute is $V\text{-}B_w\text{-}B_j$.

The jump discontinuity of any quantity, q, across the membrane is called Δq. Thus, $\Delta q = q$ outside $- q$ inside. The energy dissipation function, Φ, as derived from classical irreversible thermodynamics (*see*, e.g.,

Katchalsky and Curran [9]) is:

$$\Phi = J_w \Delta\mu_w + \sum_{j=1}^{K} J_j \Delta\mu_j. \tag{1}$$

It is shown in references 9 and 10 that

$$\Delta\mu_w = \bar{V}_w(\Delta P - \Delta\pi). \tag{2}$$

For an ideal solution the osmotic pressure is given by:

$$\pi = -\frac{RT}{\bar{V}_w} \ln\left(1 - \sum_{j=1}^{K} x_j\right) \tag{3}$$

where x_j is the mole fraction of the j^{th} solute, and for ionized solutes each ionic species is counted separately. Expanding the logarithm in a Taylor series gives:

$$\pi = RT \sum_{j=1}^{K} \frac{x_j}{\bar{V}_w} + \frac{1}{2}\frac{RT}{\bar{V}_w}\left(\sum_{j=1}^{K} x_j\right)^2 + \cdots. \tag{4}$$

The phenomenological equations introduced subsequently are most useful if π is approximated by just the first term in the Taylor series [4]. The error introduced by this approximation can be estimated by evaluating the second term. For the solute concentrations encountered in most physiological situations, this error in π is very small indeed; being only a small fraction of one percent. $x_j/\bar{V}_w$ is the concentration of the j^{th} solute in terms of molality and will be denoted by c_j. Using the molarity instead of the molality, as is commonly done with the Van't Hoff Law, leads to both practical and theoretical errors. The practical error is that for many physiological situations, the error in calculated osmotic pressure can be several percent. The theoretical error in using molarity instead of molality is that $\Delta\mu_j$ will not be a state variable in the formulations used here.

Eqs. (3) and (4) become correct for real solutions, as opposed to the restricted case of ideal solutions if concentrations are expressed in terms of osmolality, as this takes the osmotic coefficients into account. In most membrane permeability experiments, the various concentrations change during the course of the experiment. In the following theory, concentrations during an experiment are determined from initial concentrations and the amount of solute and solvent transport during the experiment. If the initial concentrations are taken in terms of osmolality and the osmotic coefficients are constants during the experiment the nonideal solution effects will cause no errors. The percentage errors due to nonideal behavior will be

of the same order of magnitude as the percentage variation of the osmotic coefficients over the course of the experiment. For example, considering some of the typical solutes used in red cell experiments we find [14] that the osmotic coefficient at 25 °C for sucrose in water increases by 0.7 % when the molality is increased from 0.2 to 0.3. For the same conditions the osmotic coefficient of a sodium chloride in water solution decreases by 0.3 %. From [19] the osmotic coefficient of urea and water solutions over molalities from 0.1 to 0.5 is constant to within a fraction of a percent. Thus, variations in osmotic coefficients can usually be neglected. Keeping in mind the fact that c_j is to be taken as osmolality:

$$\Delta\pi = \sum_{j=1}^{K} \Delta\pi_j \tag{5}$$

with

$$\Delta\pi_j = RT\,\Delta\, c_j. \tag{6}$$

References [9] and [10] use the Gibbs-Duhem equations to derive the following expression for $\Delta\mu_j$:

$$\Delta\mu_j = \bar{V}_j \Delta P + RT(\ln c_j^o - \ln c_j^i). \tag{7}$$

This can be shown to be correct if the concentrations are expressed in terms of osmolality.

We now define $\bar{c}_j$ as follows:

$$\bar{c}_j = \frac{\Delta c_j}{\ln c_j^o - \ln c_j^i}. \tag{8}$$

Eq. (7) then takes the form

$$\Delta\mu_j = \bar{V}_j \Delta P + RT \frac{\Delta c_j}{\bar{c}_j}. \tag{9}$$

In much of the literature $\bar{c}_j$ is approximated by $(c_j^i + c_j^o)/2$. This approximation is accurate when $c_j^o/c_j^i \approx /$, a condition that is not always satisfied in membrane permeability experiments.

This dissipation function can be written as:

$$\Phi = \Delta P \left(J_w \bar{V}_w + \sum_{j=1}^{K} J_j \bar{V}_j \right) + \sum_{j=1}^{K} \Delta\pi_j \left(\frac{1}{\bar{c}_j} J_j - J_w \bar{V}_w \right). \tag{10}$$

The definitions for the volume flow J_v and the solute flows with respect to the solvent flow J_{D_j}, are then made as follows:

$$J_v = J_w \bar{V}_w + \sum_{j=1}^{K} J_j \bar{V}_j \tag{11}$$

$$J_{D_j} = \frac{1}{\bar{c}_j} J_j - J_w \bar{V}_w. \tag{12}$$

As in other work [9, 10], the phenomenological equations are introduced here.

$$J_v = L_P \Delta P + \sum_{j=1}^{K} L_{Pj} \Delta \pi_j \tag{13}$$

$$J_{D_j} = L_{jP} \Delta P + \sum_{l=1}^{K} L_{jl} \Delta \pi_l, \tag{14}$$

along with the Onsager reciprocal relations

$$L_{jl} = L_{lj} \tag{15}$$

and

$$L_{Pi} = L_{iP}. \tag{16}$$

The basis of these equations is described in references 9 and 10.

As is usual, we define

$$\sigma_j = -\frac{L_{Pj}}{L_P} \tag{17}$$

so that

$$J_v = L_P \Delta P - L_P \sum_{j=1}^{K} \sigma_j \Delta \pi_j \tag{18}$$

$$J_{D_j} = -L_P \sigma_j \Delta P + \sum_{l=1}^{K} L_{jl} \Delta \pi_l. \tag{19}$$

Since the J_j's rather than the J_{D_j}'s are the quantities most readily determined in an experiment, a useful expression is:

$$J_j + \bar{c}_j \sum_{l=1}^{K} J_l \bar{V}_l = \bar{c}_j (J_{D_j} + J_v). \tag{20}$$

Eqs. (18) and (19) are identical in form to those given by Katchalsky and Curran [9], except that here all the equations have been generalized to the case of many solutes. The differences lie in consistently interpreting all concentrations as osmolality and in keeping the exact expression for $\bar{c}_j$, Eq. (8).

3. The Case of a Single Solute

The case of a single solute permeating a cell is the simplest situation to be considered. For this case $k=1$, and the solute is designated by the subscript s. The following definitions are used:

$$\sigma \equiv \sigma_1 \tag{21}$$

$$L_D \equiv L_{11} \equiv L_{SS} \tag{22}$$

$$\omega \equiv \bar{c}_S (L_D - \sigma^2 L_P). \tag{23}$$

Then Eqs. (18) and (20) become:

$$J_V = L_P \Delta P - L_P \sigma \Delta \pi \tag{24}$$

and

$$J_S(1+\bar{\phi}_S) = \bar{c}_S(1-\sigma) J_V + \omega \Delta \pi \tag{25}$$

where

$$\bar{\phi}_S = \bar{c}_S \bar{V}_S. \tag{26}$$

The term $(1+\bar{\phi}_S)$ in Eq. (25) is absent in the Kedem and Katchalsky equations (where it has the implicit value of 1). $\bar{\phi}_S$ is usually much smaller than unity so the error introduced by its absence is small. However, as will be shown subsequently, in the solutions of the equations, there is no penalty in keeping $\bar{\phi}_S$; and, since the equations are more accurate with it, it will be retained.

The membrane parameters L_P, σ and ω can depend on all the independent conditions: namely, the particular membrane, the type of solvent, all the solute concentrations, and the cell volume. The meaning of the phenomenological equations, however, [9], is that the membrane parameters do not explicitly depend on the flows, J_w, J_V, J_S or J_D.

At this point, it is appropriate to examine the nature of the dependence of L_D and ω on the solute concentration for two specific cases.

It has been pointed out by Katchalsky and Curran [9] that for an ideal semi-permeable membrane (one which passes no solute), $\sigma=1$, $\omega=0$, and $L_D=L_p$. For such a membrane, if L_p is independent of solute concentration, L_D is also independent of solute concentration.

As the second case, consider a situation where ΔP and $\Delta \pi$ are adjusted such that $J_V=0$ across a membrane that obeys Fick's Law, $J_S=D\Delta\pi$, where D is the diffusion coefficient. For this case $\omega=D$ so that

$$L_D = (\omega/\bar{c}_S) + \sigma^2 L_P.$$

It is impossible for both ω and L_D to be independent of $\bar{c}_S$ in this case. Because of the logarithmic terms in the definition of $\bar{c}_S$ (Eq. 8) the dependence of L_D or ω on permeable solute concentration can be very strong. Thus, we find that different types of membranes can have grossly different forms of the dependence of L_D or ω on solute concentration.

3. The Case of One Permeable Solute and Several Impermeable Solutes

Many cell membrane permeability experiments described in the literature are for one permeable solute and several impermeable solutes (*c.f.* References 2, 6, 8, 16 or 17). Since the only effects of an impermeable solute are contributions to the osmotic pressure and the solution volume, with no permeation of the membrane by definition; the presence of several impermeable solutes is no more complicated than the presence of one impermeable solute.

The permeable solute which is restricted here to a nonelectrolyte will be denoted by the subscript 1 and the impermeable solutes by subscripts 2 through k. For this case,

$$\sigma_1 \equiv \sigma \tag{27}$$

$$\sigma_j = 1, \quad j = 2, 3, \ldots, k \tag{28}$$

$$J_j = 0, \quad j = 2, 3, \ldots, k. \tag{29}$$

Because of the nearly identical effects of hydraulic pressure and osmotic pressure due to an impermeable solute, a modification to the general permeability equations is made for this case. The thermodynamic potential jumps are:

$$\Delta\mu_1 = \frac{RT\,\Delta c_1}{\bar{c}_1} + \bar{V}_1\,\Delta P \tag{30}$$

and

$$\Delta\mu_w = \bar{V}_w(\Delta P - \Delta\pi_m - RT\,\Delta c_1) \tag{31}$$

where

$$\Delta\pi_m = \sum_{j=2}^{k} RT\,\Delta c_j \tag{32}$$

Eq. (1) takes the form

$$\Phi = J_w\,\Delta\mu_w + J_1\,\Delta\mu_1\,. \tag{33}$$

However, the dissipation function, Φ, can be described by the sum of any pair of products of flows and forces, where the flows are any independent pair of linear combinations of J_w and J_1, and the "forces" are the natural conjugate "forces" to these flows. In particular, the most convenient flows here are J_V and J_{D_1}. The conjugate forces will be called X_V and X_{D_1}. Thus,

$$\Phi = J_v X_v + J_{D_1} X_{D_1}. \tag{34}$$

Using Eqs. (11) and (12) then gives:

$$\Phi = (J_w \bar{V}_w + J_1 \bar{V}_1) X_V + \left(\frac{1}{\bar{c}_1} J_1 - J_w \bar{V}_w\right) X_{D_1}, \tag{35}$$

and combining Eqs. (33) and (35), we have:

$$J_w(\bar{V}_w X_V - \bar{V}_w X_{D_1} - \Delta\mu_w) + J_1 \left(\bar{V}_1 X_V + \frac{1}{\bar{c}_1} X_{D_1} - \Delta\mu_1\right) = 0. \tag{36}$$

Since Eq. (36) must hold for arbitrary values of J_w and J_1, the coefficient multiplying each flow must be zero. Thus,

$$\bar{V}_w X_V - \bar{V}_w X_{D_1} = \Delta\mu_w \tag{37}$$

and

$$\bar{V}_1 X_V + \frac{1}{\bar{c}_1} X_{D_1} = \Delta\mu_1. \tag{38}$$

Solving for X_V and X_{D_1} gives

$$X_V = \frac{1}{1+\bar{\phi}_1} \left(\frac{1}{\bar{V}_w} \Delta\mu_w + \bar{c}_1 \Delta\mu_1\right) \tag{39}$$

and

$$X_{D_1} = \frac{\bar{c}_1}{1+\bar{\phi}_1} \left(\Delta\mu_w - \frac{\bar{V}_1}{\bar{V}_w} \Delta\mu_w\right) \tag{40}$$

where,

$$\bar{\phi}_1 = \bar{c}_1 \bar{V}_1. \tag{41}$$

Using the expressions for $\Delta\mu_w$ and $\Delta\mu_1$, given by Eqs. (30) and (31) gives

$$X_V = \Delta P - \frac{1}{1+\bar{\phi}_1} \Delta\pi_m \tag{42}$$

and

$$X_{D_1} = RT \Delta c_1 + \frac{\bar{\phi}_1}{1+\bar{\phi}_1} \Delta\pi_m. \tag{43}$$

The phenomenological equations for these flows and "forces" are:

$$J_V = L_P\left(\Delta P - \frac{1}{1+\bar{\phi}_1}\Delta\pi_m\right) + L_{PD}\left(RT\Delta c_1 + \frac{\bar{\phi}_1}{1+\bar{\phi}_1}\Delta\pi_m\right) \tag{44}$$

$$J_{D_1} = L_{PD}\left(\Delta P - \frac{1}{1+\bar{\phi}_1}\Delta\pi_m\right) + L_D\left(RT\Delta c_1 + \frac{\bar{\phi}_1}{1+\bar{\phi}_1}\Delta\pi_m\right). \tag{45}$$

The Onsager reciprocal relation [16] holds and σ is defined by Eq. (17). As in the single solute case, ω is defined by:

$$\omega = \bar{c}_1(L_D - \sigma^2 L_P). \tag{46}$$

Then,

$$J_1(1+\bar{\phi}_1) = \bar{c}_1(J_{D_1} + J_V) \tag{47}$$

and upon substitution for J_{D_1}, we have

$$J_1 = \frac{1}{1+\bar{\phi}_1}\left[\bar{c}_1(1-\sigma)J_V + \omega\left(RT\Delta c_1 + \frac{\bar{\phi}_1}{1+\bar{\phi}_1}\Delta\pi_m\right)\right]. \tag{48}$$

Eq. (48) is somewhat different than the corresponding Kedem and Katchalsky equation [10]. If the two denominators $(1+\bar{\phi}_1)$ in Eq. (48) are set equal to unity, the resulting expression is identical to that given by Kedem and Katchalsky. However, if $\bar{\phi}_1$ is ignored as $(1+\bar{\phi}_1)=1$ would imply, then the last term would have to be dropped, and this was not done by Kedem and Katchalsky. For a consistent expansion in $\bar{\phi}_1$, the first order approximation to Eq. (48) would have to contain all terms of the order of $\bar{\phi}_1$. This approximation is:

$$J_1 = (1-\bar{\phi}_1)[\bar{c}_1(1-\sigma)J_V + \omega RT\Delta c_1] + \omega\bar{\phi}_1\Delta\pi_m. \tag{49}$$

For many membrane permeability situations, conditions are such that the equations correct to zeroth order in ϕ_1 are quite accurate. This accounts for much of the success of the Kedem and Katchalsky equations that has been reported in the literature.

4. The Form of the Equations for Cells

Rand and Burton [13] have indicated that ΔP is negligibly small for red cells. It seems likely that this is the case for most membrane bound cells, and ΔP will be set to zero here. The cells will be considered to be immersed in a solution containing a single permeable solute and one or

more impermeable solutes. Eqs. (44) and (48) are first multiplied by the cell surface area, A; so they become the equations for dV/dt, the rate of change of cell volume; and dS/dt, the rate of change of permeable solute in the cell. V_0 is an arbitrary constant volume, and c_0 is an arbitrary constant concentration used to partially nondimensionalize the equations. Then, Eqs. (44) and (48) become:

$$\frac{d\underline{V}}{dt} = K_1 \left[-\frac{1}{1+\bar{\phi}_1} \Delta \underline{\pi}_m - \sigma \Delta \underline{c}_1 - \sigma \frac{\bar{\phi}_1}{1+\bar{\phi}_1} \Delta \underline{\pi}_m \right] \tag{50}$$

and

$$\frac{d\underline{S}}{dt} = \frac{1}{1+\bar{\phi}_1} \left[\bar{\underline{c}}_1 (1-\sigma) \frac{d\underline{V}}{dt} + K_2 \left(\Delta \underline{c}_1 + \frac{\bar{\phi}_1}{1+\bar{\phi}_1} \Delta \underline{\pi}_m \right) \right] \tag{51}$$

where

$$\underline{V} = V/V_0 \tag{52}$$

$$\underline{c}_1 = c_1/c_0 \tag{53}$$

$$\bar{\underline{c}}_1 = \bar{c}_1/c_0 \tag{54}$$

$$\underline{\pi}_m = \pi_m/RTc_0 \tag{55}$$

$$\underline{S} = S/V_0 c_0 \tag{56}$$

$$K_1 = L_P A RTc_0/V_0 \tag{57}$$

$$K_2 = \omega A RT/V_0 . \tag{58}$$

It will be assumed that the B_j's are the same for all the impermeable solutes, and this value will be called B_2. If B_2 serves as a correction for ion transport as explained in § 1, it will be a function of cell volume in general as shown by Gary-Bobo and Solomon [5]. Nondimensional quantities $\underline{a}$ and $\underline{b}$ are defined here such that the apparent volume of solvent dissolving the permeable solute is $V - \underline{a} V_0$, and the apparent volume dissolving the impermeable solute is $V - \underline{b} V_0$. This defines $\underline{a}$ and $\underline{b}$ as:

$$\underline{a} = \frac{V}{V_0}(1-\phi_w) + \phi_w \frac{B_w + B_1}{V_0} \tag{59}$$

and

$$\underline{b} = \frac{V}{V_0}(1-\phi_w) + \phi_w \frac{B_w + B_2}{V_0} . \tag{60}$$

In general, $\underline{a}$ and $\underline{b}$ depend on the various solute concentrations and the cell volume. By taking the reference concentration, c_0, as the sum of the impermeable solute concentrations outside the cell, the permeability equations take the most easily used form. The values of $\underline{a}$ and $\underline{b}$ when the cell volume is V_0 are called a and b. Then,

$$\underline{\pi}_m^i = \frac{1-b}{\underline{V}-\underline{b}} \tag{61}$$

and Eqs. (50) and (51) become:

$$\frac{d\underline{V}}{dt} = K_1 \left[\frac{1+\sigma\bar{\phi}_1}{1+\bar{\phi}_1} \frac{1-b}{V-\underline{b}} + \frac{\sigma S}{\underline{V}-\underline{a}} - \frac{1+\sigma\bar{\phi}_1}{1+\bar{\phi}_1} - \sigma \underline{c}_1^O \right] \tag{62}$$

and

$$\frac{d\underline{S}}{dt} = \frac{1}{1+\bar{\phi}_1} \left[\underline{c}_1 (1-\sigma) \frac{d\underline{V}}{dt} + K_2 \left(\underline{c}_1^0 + \frac{\bar{\phi}_1}{1+\bar{\phi}_1} - \frac{S}{\underline{V}-\underline{a}} - \frac{\bar{\phi}_1}{1+\bar{\phi}_1} \frac{1-b}{\underline{V}-\underline{b}} \right) \right]. \tag{63}$$

Equilibrium is the condition for which both $d\underline{V}/dt$ and $d\underline{S}/dt$ are zero. Calling the dimensionless volume at equilibrium $\underline{V}^{eq}$, and the values of $\underline{a}$ and $\underline{b}$ at equilibrium a^{eq} and b^{eq}, we have:

$$\underline{V}^{eq} = b^{eq} + \frac{1-b}{\underline{\pi}_m^i} \tag{64}$$

and

$$\underline{S}^{eq} = (\underline{V}^{eq} - a^{eq})\, \underline{c}_1^O. \tag{65}$$

Here, the most convenient choice for V_0 is V^{eq} for which:

$$\underline{V}^{eq} = 1, \tag{66}$$

$$b^{eq} = b \tag{67}$$

and

$$S^{eq} = (1-a)\, \underline{c}_1^O. \tag{68}$$

Eqs. (62) and (63) are a pair of coupled, first-order, nonlinear differential equations. An unique mathematical solution for these equations exists, and this is discussed in Appendix A. Two methods of finding solutions will be described in the following sections.

The most straightforward method of solving the equations, if a digital computer is available, is by direct numerical integration by means of a Runge Kutta method or a variation of such a method. This can be done for any initial conditions and any functional dependence of the membrane parameters (L_p, σ and ω) and $\underline{a}$ and $\underline{b}$ on the cell volume and the various concentrations. However, it is not known *à priori* if the method will converge for acceptable integration time steps. Even if it appears that numerical convergence has been achieved, it is still not certain that convergence to the correct solution has occurred.

Eqs. (62) and (63) can also be solved by means of a perturbation series of successive linear problems. A solution to each higher and higher order problem exists in closed form, but it is not certain that the sum of these solutions converges to the actual solution. However, if the first order perturbation solution converges to the direct integration solution as the perturbation from equilibrium is made arbitrarily small, and if, for larger perturbations, the sum of the first and second order solutions is much closer to the direct integration solution than is the first order solution alone, one attains enough confidence in the methods to believe the solutions so found are accurate approximations to the correct solution.

The aims of deriving and solving the perturbation equations here are: (1) to demonstrate the perturbation method as simply as possible; (2) to compare solutions of the perturbation equations with direct numerical solutions to confirm the validity of both methods; (3) to show that the linearized equations and their solutions alone are inadequate for many purposes; and (4) to provide solutions in forms that can be evaluated by computer or by hand which are accurate for a variety of red cell permeability situations.

These aims are best met here by deriving and solving the perturbation equations for the restricted conditions of constant membrane parameters and small enough solute volume fractions to allow ϕ_w, the volume fraction of solvent to be approximated by unity in Eqs. (59) and (60). Approximating ϕ_w by unity does not necessarily set $\underline{a}$ and $\underline{b}$ to constants because B_1 and B_2 may vary during the course of an experiment. However, it has been shown by Gary-Bobo [4] that B_1, which is the solvent volume not participating in dissolving the permeable solute, is constant (and nearly zero) for red cells. On the other hand, B_2 is not constant for red cells as shown by Gary-Bobo and Solomon [5]. Fig. 2 shows the relationship between B_2 and cell volume. This figure is based on the nonsolvent volume measurements of Savitz, Sidel and Solomon [15] and the apparent nonparticipating solvent measurements of Gary-Bobo and Solomon [5].

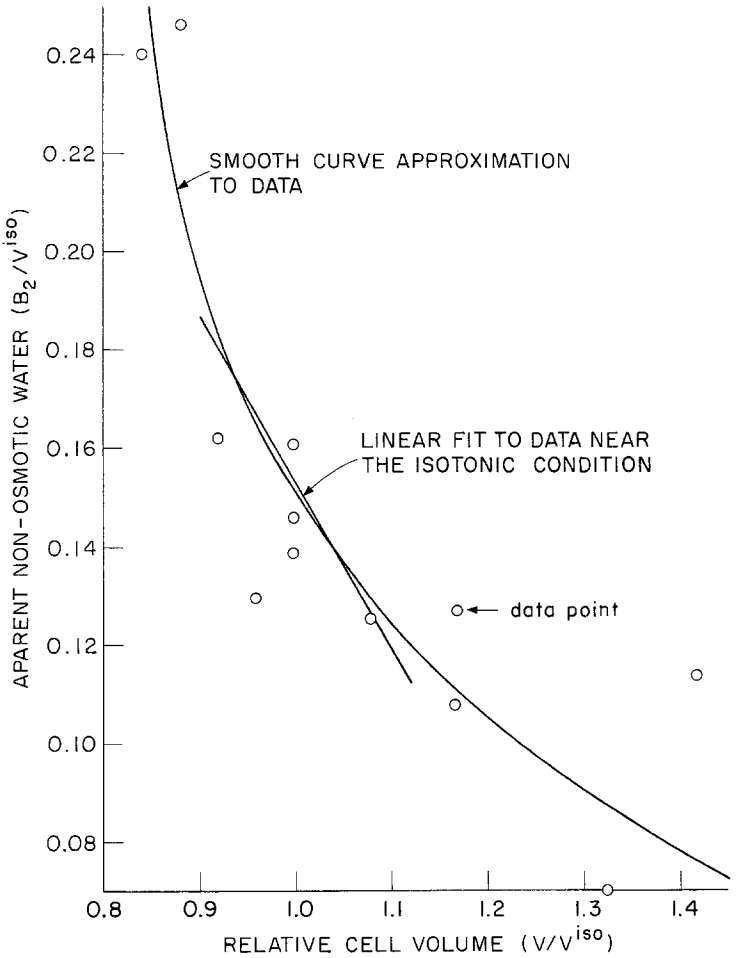

Fig. 2. Apparent nonosmotic water *vs.* cell volume for human red cells. Data taken from Reference 5

Fig. 2 shows that B_2 depends on V in a nonlinear way for the range of cell volumes encompassed by the figure. Typically, the cell volume varies by about 20% in a cell permeability experiment. Over such a volume range, the relationship between B_2 and cell volume can be approximated accurately by a linear function, as shown in Fig. 2, but it cannot be approximated accurately by a constant. A linear relationship can be accommodated very easily into the perturbation equations. With ϕ_w approximated by unity and B_2 linearly dependent on V, $\underline{b}$ is a linear function of $\underline{V}$ [Eq. (60)], and since $\underline{V}^{eq}=1$, $\underline{b}$ can be expressed as:

$$\underline{b}=b-b'(\underline{V}-1). \tag{69}$$

In the permeability Eqs. (62) and (63), $\underline{b}$ appears only in the expression $(1-b)/(\underline{V}-\underline{b})$. With substitution of Eq. (69) this takes the form $(1-b_c)/(\underline{V}-b_c)$ where:

$$b_c = \frac{b-b'}{1-b'}. \tag{70}$$

Therefore, replacing both the constant b and the variable $\underline{b}$ in Eqs. (62) and (63) by the constant b_c is an accurate simplification when $\phi_w \cong 1$.

The normalizing volume used here is the volume of the cells at equilibrium in the final solution into which they have been placed. Often, however, the cell and nonsolvent volumes are not initially known at this condition, but rather are known at some other equilibrium condition. This will be called the isosmolal condition, designated by the superscript $^{\text{iso}}$, because it is often the physiological isosmolal condition. In order to relate the normalizing volume, $V_0 = V^{\text{eq}}$, to the isosmolal cell volume, V^{iso}, the relation between B_2 and V must be known. Here it will be assumed that this can be approximated by the following linear relationship over the range of cell volumes for the experiment,

$$B_2 = B_2^{\text{iso}} + B'(V - V^{\text{iso}}). \tag{71}$$

Then the required relation between V^{eq} and V^{iso} is:

$$V^{\text{eq}} = \left\{ \left[\frac{c_m^{\text{iso}}}{c_m^{\text{eq}}} (V^{\text{iso}} - B_2^{\text{iso}}) - B' V^{\text{iso}} + B_2^{\text{iso}} \right] [1 + \phi_1^{\text{eq}}] + B_w^{\text{iso}} - \bar{V}_1 S^{\text{iso}} - B_1^{\text{eq}} \phi_1^{\text{eq}} \right\} \Big/ \{1 - B' - B' \phi_1^{\text{eq}}\} \tag{72}$$

where:

$$S^{\text{iso}} = c_1^{\text{iso}} (V^{\text{iso}} - B_1^{\text{iso}}). \tag{73}$$

To solve Eq. (72) for V^{eq} requires specification of $B_1^{\text{eq}} \phi_1^{\text{eq}}$. Gary-Bobo [4] has shown that for most red cell experiments, B_1 is very small. Since most of B_1 is the solvent bound to macromolecules in the cell, and the number of these do not change during a permeability experiment, the variation in B_1 during an experiment will be even smaller than the mean value of B_1. Hence, if B_1^{iso} is known, this value can be used for B_1^{eq} in Eq. (72). Generally, an accurate approximation for V^{eq} results even if B_1^{eq} is set to zero in Eq. (72) inasmuch as the term $B_1^{\text{eq}} \phi_1^{\text{eq}}$ is exceedingly small.

B_w includes the volume displaced by the solutes in the cell. B_w^{iso} is usually determined by drying cells initially in the isosmolal condition.

5. First and Second Order Perturbation Equations and Solutions

These equations are most easily derived in terms of deviation of cell volume and amount of internal solute from their equilibrium values. Let,

$$G = \underline{V} - \underline{V}^{eq} \tag{74}$$

$$H = \underline{S} - \underline{S}^{eq}. \tag{75}$$

Then, the permeability equations with $\underline{a}$ and $\underline{b}$ given the values a and b_c have the form:

$$\frac{dG}{dt} = K_1 \left[-\frac{1-\sigma\bar{\phi}_1}{1+\bar{\phi}_1} \frac{G}{1-b_c+G} + \sigma \frac{H - G\underline{c}_1^o}{1+G-a} \right] \tag{76}$$

and

$$\frac{dH}{dt} = \frac{1}{1+\bar{\phi}_1} \left[\bar{\underline{c}}_1 (1-\sigma) \frac{dG}{dt} + K_2 \left(\frac{\bar{\phi}_1}{1+\bar{\phi}_1} \frac{G}{1+G-b_c} + \frac{G\underline{c}_1^o - H}{1+G-a} \right) \right]. \tag{77}$$

The problem to be considered is that of a cell with arbitrary values of G and H at time $=0$, called G_0 and H_0, and with constant values of all external concentrations. If $G_0 = H_0 = 0$, the concentrations are in equilibrium and $dG/dt = dH/dt = 0$. For small values of G_0 and H_0, linearized approximations of (76) and (77) can be expected to be accurate, being more and more accurate as G_0 and H_0 are made smaller and smaller. The solutions to the linearized approximations of (76) and (77) are called first-order solutions and are denoted by $G^{(1)}$ and $H^{(1)}$. They will have terms proportional to G_0 and terms proportional to H_0.

As G_0 and H_0 are increased, the first order solutions will have reduced accuracy. Usually, improved accuracy can be achieved by adding second order solutions to the first order solutions. The second order solutions, called $G^{(2)}$ and $H^{(2)}$ have terms proportional to G_0^2 and H_0^2 and $G_0 H_0$. This procedure can be carried out indefinitely with the equations for any order being derived by a systematic procedure from Eqs. (76) and (77).

It will be demonstrated that for typical values of G_0 and H_0 in membrane permeability experiments, fairly large errors can result if only first order solutions are used, but if second order solutions are used as well, errors will be reduced to a few percent.

Let

$$G(t) = \varepsilon \underline{G}^{(1)}(t) + \varepsilon^2 \underline{G}^{(2)}(t) + \cdots \tag{78}$$

and

$$H(t)=\varepsilon \underline{H}^{(1)}(t)+\varepsilon^2 \underline{H}^{(2)}(t)+\cdots \tag{79}$$

where

$$\varepsilon^{\eta} \underline{G}^{(\eta)}(t)\equiv G^{(\eta)}(t) \tag{80}$$

and

$$\varepsilon^{\eta} \underline{H}^{(\eta)}(t)\equiv H^{(\eta)}(t). \tag{81}$$

It is assumed that there will always be some apparent solvent available to the solutes in the cell so that the terms $1+G-a$ and $1+G-b$ appearing in Eqs. (76) and (77) are always positive. Then the perturbation expansions will be regular perturbations from equilibrium. The equations for such an expansion are determined by substituting the expressions (78) and (79) for G and H into the differential equations and then collecting terms in like powers of ε. A solution is sought for arbitrary ε so that equality must hold between terms of each power of ε separately.

Equations and solutions for the first and second order variables, $G^{(1)}$, $H^{(1)}$, $G^{(2)}$ and $H^{(2)}$ will be given here for the restricted case of constant L_P, σ and ω. To facilitate writing these, the following definitions are made:

$$\lambda^{(0)}=\underline{c}_1^{O} \tag{82}$$

$$\lambda^{(1)}=\frac{1}{2}\underline{c}_1^{O}\left(\frac{H^{(1)}}{\underline{S}^{\mathrm{eq}}}-\frac{G^{(1)}}{1-a}\right) \tag{83}$$

$$\beta^{(0)}=\frac{1}{1+\phi_1^{0}} \tag{84}$$

$$\beta^{(1)}=-\frac{c_0 \bar{V}_1 \lambda^{(1)}}{(1+\phi_1^{O})^2} \tag{85}$$

$$\delta_{11}=-\frac{\sigma+\beta^{(0)}(1-\sigma)}{1-b_c}-\frac{\sigma\lambda^{(0)}}{1-a} \tag{86}$$

$$\delta_{12}=\frac{\sigma}{1-a} \tag{87}$$

$$\delta_{21}=\beta^{(0)}K_2\left(\frac{1-\beta^{(0)}}{1-b_c}+\frac{\lambda^{(0)}}{1-a}\right) \tag{88}$$

$$\delta_{22}=-\frac{\beta^{(0)}K_2}{1-a} \tag{89}$$

$$\alpha^{(0)}=\beta^{(0)}\lambda^{(0)}(1-\sigma) \tag{90}$$

$$\alpha^{(1)}=(1-\sigma)(\lambda^{(0)}\beta^{(1)}+\lambda^{(1)}\beta^{(0)}). \tag{91}$$

Using the procedure described above for the first and second order equations gives:

$$\frac{dG^{(1)}}{dt} - K_1(\delta_{11} G^{(1)} + \delta_{12} H^{(1)}) = 0 \tag{92}$$

$$\frac{dG^{(2)}}{dt} - K_1(\delta_{11} G^{(2)} + \delta_{12} H^{(2)}) = f_v(t) \tag{93}$$

$$\frac{dH^{(1)}}{dt} - \alpha^{(0)} \frac{dG^{(1)}}{dt} - \delta_{21} G^{(1)} - \delta_{22} H^{(1)} = 0 \tag{94}$$

$$\frac{dH^{(2)}}{dt} - \alpha^{(0)} \frac{dG^{(2)}}{dt} - \delta_{21} G^{(2)} - \delta_{22} H^{(2)} = f_s(t) \tag{95}$$

where:

$$f_v(t) = K_1 \left[\frac{\sigma + \beta^{(0)}(1-\sigma)}{(1-b_c)^2} G^{(1)^2} - \frac{1-\sigma}{1-b_c} \beta^{(1)} G^{(1)} + \frac{\sigma}{1-a} \left(\frac{\lambda^{(0)} G^{(1)^2}}{1-a} - \frac{H^{(1)} G^{(1)}}{1-a} \right) \right] \tag{96}$$

and

$$f_s(t) = -\beta^{(0)} K_2 \left[\frac{1}{1-a} \left(\frac{\lambda^{(0)} G^{(1)2}}{1-a} - \frac{H^{(1)} G^{(1)}}{1-a} \right) + \frac{1-\beta^{(0)}}{(1-b_c)^2} G^{(1)2} + \frac{\beta^{(1)} G^{(1)}}{1-b_c} \right] + \alpha^{(1)} \frac{dG^{(1)}}{dt} \beta^{(1)} K_2 \left[\frac{1-\beta^{(0)}}{1-b_c} G^{(1)} - \frac{1}{1-a} (H^{(1)} - \lambda^{(0)} G^{(1)}) \right]. \tag{97}$$

To solve these equations, the first order equations for $G^{(1)}$ and $H^{(1)}$ are solved subject to the initial conditions:

$$G^{(1)}(0) = G_0 \tag{98}$$

and

$$H^{(1)}(0) = H_0 \tag{99}$$

Then, $G^{(1)}$, $H^{(1)}$, $\beta^{(1)}$, $\lambda^{(1)}$ and $\alpha^{(1)}$ are known so that $f_v(t)$ and $f_s(t)$ are known and the second order equations can be solved.

6. Solution of the First Order Equations

The first order Eqs. (92) and (94) are identical to the equations of Macey and Farmer [1, 2] and Johnson and Wilson [8] if $\bar{C}_1$ in their treatments is given the value $\underline{C}^0$. A means of solution to the first order

equations is given here which forms a consistent basis for solution of the second order equations in § 7.

The first order equations will be solved by use of Laplace Transforms and the superscript* will be used to represent transformed quantities. For example,

$$\int_0^\infty G(t)\, e^{-pt}\, dt \equiv G^*(p). \tag{100}$$

Taking transforms of the first order equations gives:

$$G^*(K_1\delta_{11}-p)+H^*(K_1\delta_{12})=-G_0 \tag{101}$$

$$G^*(\alpha^{(0)}p+\delta_{21})+H^*(\delta_{22}-p)=\alpha^{(0)}G_0-H_0. \tag{102}$$

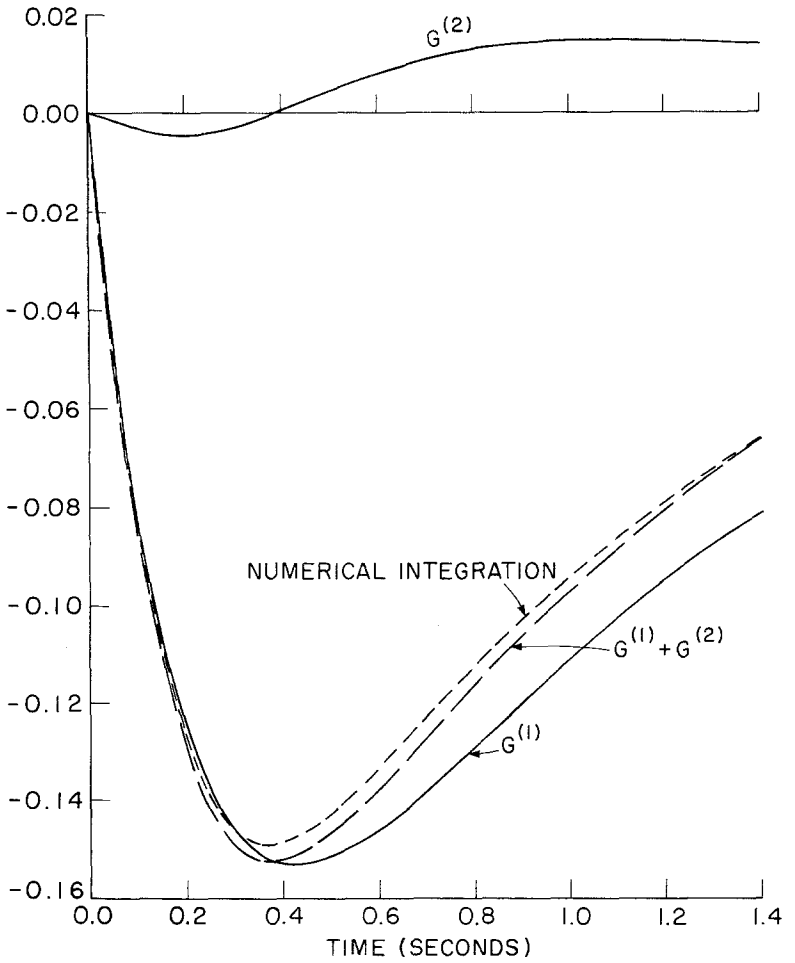

Fig. 3. Cell volume *vs.* time for first sample case, calculated by perturbation theory and by direct numerical integration. The quantities shown are the differences between normalized cell volume and normalized cell volume at equilibrium

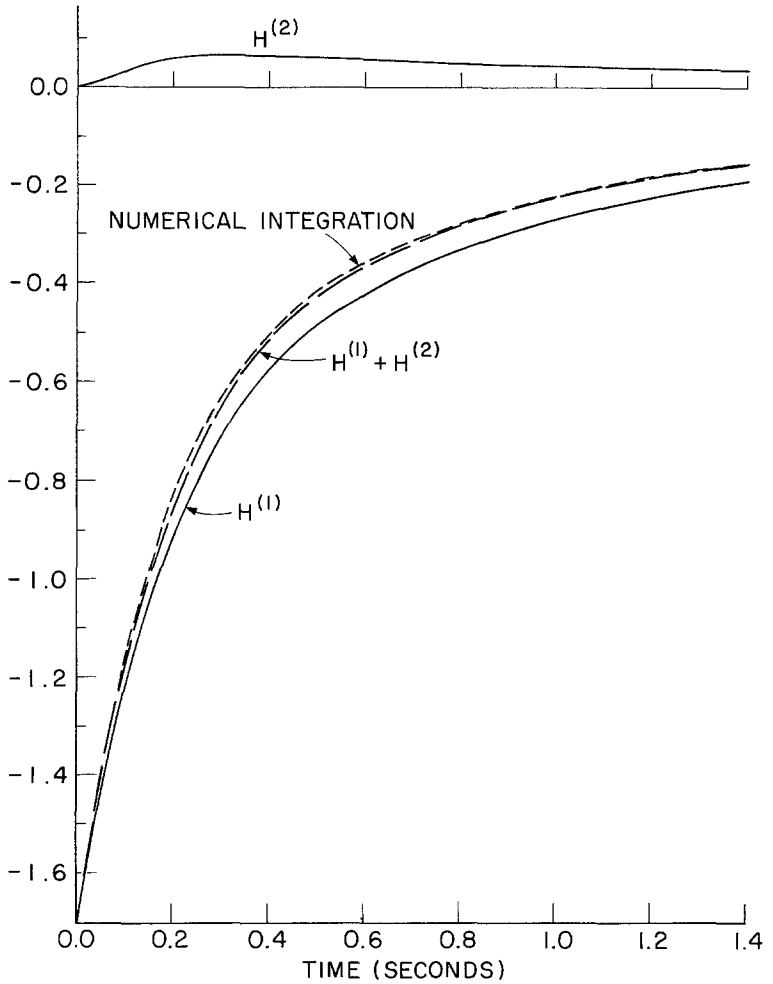

Fig. 4. Quantity of permeable solute in cell *vs.* time, calculated by perturbation theory and by direct numerical integration. The quantities shown are the differences between normalized amount of solute in cell and its value at equilibrium

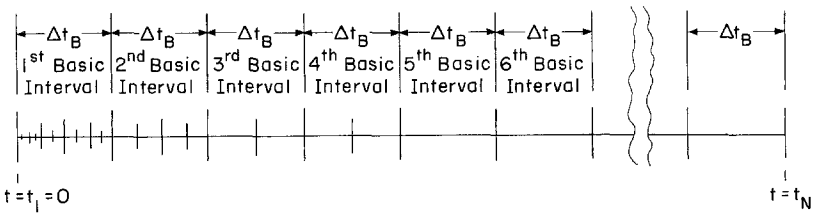

Fig. 5. Time steps for direct numerical integration. Δt_B = Basic time interval

We now make the definitions:

$$\eta_{\frac{1}{2}} = \tfrac{1}{2}(K_1\,\delta_{11} + \delta_{22} + \alpha^{(0)} K_1\,\delta_{12}) \pm \sqrt{\tfrac{1}{4}(K_1\,\delta_{11} + \delta_{22} + \alpha^{(0)} K_1\,\delta_{12})^2 - K_1(\delta_{11}\,\delta_{22} - \delta_{12}\,\delta_{21})} \tag{103}$$

$$\zeta_g = K_1\,\delta_{12}\,H_0 - (K_1\,\delta_{12}\,\alpha^{(0)} + \delta_{22})\,G_0 \tag{104}$$

$$\zeta_h = (\alpha^{(0)} K_1\,\delta_{11} + \delta_{21})\,G_0 - K_1\,\delta_{11}\,H_0. \tag{105}$$

Then solving for G^* and H^* gives:

$$G^*(p) = \frac{G_0\,p + \zeta_g}{(p-\eta_1)(p-\eta_2)} \tag{106}$$

$$H^*(p) = \frac{H_0\,p + \zeta_h}{(p-\eta_1)(p-\eta_2)}\,. \tag{107}$$

Taking inverse transforms then gives the first order solutions

$$G^{(1)}(t) = \begin{cases} 0, & t<0 \\ \dfrac{\eta_1\,G_0 + \zeta_g}{\eta_1 - \eta_2}\,e^{\eta_1 t} + \dfrac{\eta_2\,G_0 + \zeta_g}{\eta_2 - \eta_1}\,e^{\eta_2 t}, & t>0 \end{cases} \tag{108}$$

$$H^{(1)}(t) = \begin{cases} 0, & t<0 \\ \dfrac{\eta_1\,H_0 + \zeta_h}{\eta_1 - \eta_2}\,e^{\eta_1 t} + \dfrac{\eta_2\,H_0 + \zeta_h}{\eta_2 - \eta_1}\,e^{\eta_2 t}, & t>0 \end{cases}. \tag{109}$$

Figs. 4 and 5 show graphs of these functions for a sample case.

7. Solution of the Second Order Equations

The first order equations can be written as:

$$\mathscr{L}_{11}\,G^{(1)} + \mathscr{L}_{12}\,H^{(1)} = 0 \tag{110}$$

$$\mathscr{L}_{21}\,G^{(1)} + \mathscr{L}_{22}\,H^{(1)} = 0 \tag{111}$$

where,

$$\mathscr{L}_{11} = \frac{d}{dt} - K_1\,\delta_{11} \tag{112}$$

$$\mathscr{L}_{12} = -K_1\,\delta_{12} \tag{113}$$

$$\mathscr{L}_{21} = -\alpha^{(0)}\frac{d}{dt} - \delta_{21} \tag{114}$$

$$\mathscr{L}_{22} = \frac{d}{dt} - \delta_{22}. \tag{115}$$

Whereas, the first order equations are homogenous linear equations, the second order equations are inhomogenous linear equations containing the same linear operators as the first order equations, namely,

$$\mathscr{L}_{11} G^{(2)} + \mathscr{L}_{12} H^{(2)} = f_v(t) \tag{116}$$

$$\mathscr{L}_{21} G^{(2)} + \mathscr{L}_{22} H^{(2)} = f_s(t), \tag{117}$$

subject to the initial conditions,

$$G^{(2)}(0) = H^{(2)}(0) = 0. \tag{118}$$

$G^{(2)}$ and $H^{(2)}$ will be found by use of the set of Green's Functions R_{11}, R_{12}, R_{21} and R_{22} such that

$$G^{(2)}(t) = \int_0^t R_{11}(t, \tau) f_v(\tau)\, d\tau + \int_0^t R_{12}(t, \tau) f_s(\tau)\, d\tau \tag{119}$$

$$H^{(2)}(t) = \int_0^t R_{21}(t, \tau) f_v(\tau)\, d\tau + \int_0^t R_{22}(t, \tau) f_s(\tau)\, d\tau. \tag{120}$$

The R's satisfy the same initial conditions as do $G^{(2)}$ and $H^{(2)}$, namely,

$$R_{ij}(0, \tau) = 0, \tag{121}$$

and the R's satisfy the equations:

$$\mathscr{L}_{11} R_{11} + \mathscr{L}_{12} R_{21} = \delta(t - \tau) \tag{122}$$

$$\mathscr{L}_{21} R_{11} + \mathscr{L}_{22} R_{21} = 0 \tag{123}$$

$$\mathscr{L}_{11} R_{12} + \mathscr{L}_{12} R_{22} = 0 \tag{124}$$

$$\mathscr{L}_{21} R_{12} + \mathscr{L}_{22} R_{22} = \delta(t - \tau) \tag{125}$$

where $\delta(t)$ is the "Dirac Delta Function".

The reason for solving the equations in this way is that the equations for the R's are nearly identical to those already solved for $G^{(1)}$ and $H^{(1)}$. The only difference between the equations for the transforms of the R's and those for the first order solutions, Eqs. (101) and (102), is that instead of initial conditions on the right hand side, the equations for the transforms of the R's have right hand sides that are either zero or the transform of the

delta function which is simply $e^{-P\tau}$. Carrying through the solution gives:

$$R_{11}(t,\tau)=\begin{cases}0, & t<\tau\\ \dfrac{\eta_1-\delta_{22}}{\eta_1-\eta_2}\,e^{\eta_1(t-\tau)}+\dfrac{\eta_2-\delta_{22}}{\eta_2-\eta_1}\,e^{\eta_2(t-\tau)}, & t>\tau\end{cases} \tag{126}$$

$$R_{21}(t,\tau)=\begin{cases}0, & t<\tau\\ \dfrac{\alpha^{(0)}\eta_1+\delta_{21}}{\eta_1-\eta_2}\,e^{\eta_1(t-\tau)}+\dfrac{\alpha^{(0)}\eta_2+\delta_{21}}{\eta_2-\eta_1}\,e^{\eta_2(t-\tau)}, & t>\tau\end{cases} \tag{127}$$

$$R_{12}(t,\tau)=\begin{cases}0, & t<\tau\\ K_1\,\delta_{12}\left[\dfrac{1}{\eta_1-\eta_2}\,e^{\eta_1(t-\tau)}+\dfrac{1}{\eta_2-\eta_1}\,e^{\eta_2(t-\tau)}\right], & t>\tau\end{cases} \tag{128}$$

$$R_{22}(t,\tau)=\begin{cases}0, & t<\tau\\ \dfrac{\eta_1-K_1\,\delta_{11}}{\eta_1-\eta_2}\,e^{\eta_1(t-\tau)}+\dfrac{\eta_2-K_1\,\delta_{11}}{\eta_2-\eta_1}\,e^{\eta_2(t-\tau)}, & t>\tau\end{cases}. \tag{129}$$

From the expressions for $f_v(t)$ and $f_s(t)$, it can be seen that they have the forms:

$$f_v(t)=F_{11}\,e^{2\eta_1 t}+F_{12}\,e^{2\eta_2 t}+F_{13}\,e^{(\eta_1+\eta_2)t} \tag{130}$$

$$f_s(t)=F_{21}\,e^{2\eta_1 t}+F_{22}\,e^{2\eta_2 t}+F_{23}\,e^{(\eta_1+\eta_2)t}. \tag{131}$$

Expressions for the F's are given in Appendix B.

Carrying out the integrals indicated in Eqs. (119) and (120) yields the second order solutions which are naturally zero for $t\leqq 0$. For $t>0$, they are:

$$\begin{aligned}G^{(2)}(t)=&\frac{\eta_1-\delta_{22}}{\eta_1-\eta_2}\left[\frac{F_{11}}{\eta_1}(e^{2\eta_1 t}-e^{\eta_1 t})+\frac{F_{12}}{2\eta_2-\eta_1}(e^{2\eta_2 t}-e^{\eta_1 t})\right.\\ &\left.+\frac{F_{13}}{\eta_2}(e^{(\eta_1+\eta_2)t}-e^{\eta_1 t})\right]+\frac{\eta_2-\delta_{22}}{\eta_2-\eta_1}\left[\frac{F_{11}}{2\eta_1-\eta_2}(e^{2\eta_1 t}-e^{\eta_2 t})\right.\\ &\left.+\frac{F_{12}}{\eta_2}(e^{2\eta_2 t}-e^{\eta_2 t})+\frac{F_{13}}{\eta_1}(e^{(\eta_1+\eta_2)t}-e^{\eta_2 t})\right]\\ &+\frac{K_1\,\delta_{12}}{\eta_1-\eta_2}\left[\frac{F_{21}}{\eta_1}(e^{2\eta_1 t}-e^{\eta_1 t})+\frac{F_{22}}{2\eta_2-\eta_1}(e^{2\eta_2 t}-e^{\eta_1 t})\right.\\ &\left.+\frac{F_{23}}{\eta_2}(e^{(\eta_1+\eta_2)t}-e^{\eta_1 t})-\frac{F_{21}}{2\eta_1-\eta_2}(e^{2\eta_1 t}-e^{\eta_2 t})\right.\\ &\left.-\frac{F_{22}}{\eta_2}(e^{2\eta_2 t}-e^{\eta_2 t})-\frac{F_{23}}{\eta_1}(e^{(\eta_1+\eta_2)t}-e^{\eta_2 t})\right]\end{aligned} \tag{132}$$

and

$$H^{(2)}(t)=\frac{\alpha^{(0)}\eta_1+\delta_{21}}{\eta_1-\eta_2}\left[\frac{F_{11}}{\eta_1}(e^{2\eta_1 t}-e^{\eta_1 t})\right.$$
$$+\frac{F_{12}}{2\eta_2-\eta_1}(e^{2\eta_2 t}-e^{\eta_1 t})+\left.\frac{F_{13}}{\eta_2}(e^{(\eta_1+\eta_2)t}-e^{\eta_1 t})\right]$$
$$+\frac{\alpha^{(0)}\eta_2+\delta_{21}}{\eta_2-\eta_1}\left[\frac{F_{11}}{2\eta_1-\eta_2}(e^{2\eta_1 t}-e^{\eta_2 t})+\frac{F_{12}}{\eta_2}(e^{2\eta_2 t}-e^{\eta_2 t})\right.$$
$$+\left.\frac{F_{13}}{\eta_1}(e^{(\eta_1+\eta_2)t}-e^{\eta_2 t})\right]+\frac{\eta_1-K_1\delta_{11}}{\eta_1-\eta_2}\left[\frac{F_{21}}{\eta_1}(e^{2\eta_1 t}-e^{\eta_1 t})\right. \tag{133}$$
$$+\frac{F_{22}}{2\eta_2-\eta_1}(e^{2\eta_2 t}-e^{\eta_1 t})+\left.\frac{F_{23}}{\eta_2}(e^{(\eta_1+\eta_2)t}-e^{\eta_1 t})\right]$$
$$+\frac{\eta_2-K_1\delta_{11}}{\eta_2-\eta_1}\left[\frac{F_{21}}{2\eta_1-\eta_2}(e^{2\eta_1 t}-e^{\eta_2 t})+\frac{F_{22}}{\eta_2}(e^{2\eta_2 t}-e^{\eta_2 t})\right.$$
$$+\left.\frac{F_{23}}{\eta_1}(e^{(\eta_1+\eta_2)t}-e^{\eta_2 t})\right].$$

Figs. 4 and 5 show graphs of the second order solutions for a sample case.

8. Solution by Direct Numerical Integration

The equations to be solved are (62) and (63) subject to the initial conditions at $t=0$,

$$\underline{V}(0)=\underline{V}_0 \tag{134}$$

$$\underline{S}(0)=\underline{S}_0. \tag{135}$$

To carry this out numerically, the time interval over which the solution is to be found is partitioned at times $t_1, t_2, \ldots t_N$; where $t_1=0$ and t_N is the largest time at which a solution is to be found. This gives $N-1$ time intervals of time span,

$$\Delta t_i=t_{i+1}-t_i, \qquad i=1, 2, \ldots, N-1. \tag{136}$$

The time intervals are not necessarily equal. The values of $\underline{V}(t_i)$ and $\underline{S}(t_i)$ will be called $\underline{V}_i$ and $\underline{S}_i$. Since $V(t)$ and $S(t)$ are differentiable functions,

$$\underline{V}_i=\underline{V}_{i-1}+\left\langle\frac{d\underline{V}}{dt}\right\rangle_{i-1}\Delta t_{i-1} \tag{137}$$

where $\langle d\underline{V}/dt\rangle_{i-1}$ is the mean value of $d\underline{V}/dt$ in the time interval Δt_{i-1}. Similarly,

$$\underline{S}_i = \underline{S}_{i-1} + \left\langle \frac{d\underline{S}}{dt} \right\rangle_{i-1} \Delta t_{i-1} \tag{138}$$

If V_{i-1} and $\underline{S}_{i-1}$ are known, then $d\underline{V}/dt$ and $d\underline{S}/dt$ are known at time t_{i-1}, but $\langle d\underline{V}/dt\rangle_{i-1}$ and $\langle d\underline{S}/dt\rangle_{i-1}$ are not known and must be estimated in order to be able to estimate $\underline{V}_i$ and $\underline{S}_i$. The same procedure is then used for the next time interval and so forth.

There are various means for estimating the mean values of the derivatives and thereby obtaining numerical estimates for $\underline{V}$ and $\underline{S}$ at each of the times t_i. In general, they all become more accurate as the step sizes, Δt_i, are reduced. The simplest one is Euler's Method which estimates the mean value of a derivative in an interval by the value of that derivative at the beginning of the interval. In this method,

$$\left\langle \frac{d\underline{V}}{dt} \right\rangle_{i-1} \simeq \left(\frac{d\underline{V}}{dt} \right)_{t=t_{i-1}} \tag{139}$$

$$\left\langle \frac{d\underline{S}}{dt} \right\rangle_{i-1} \simeq \left(\frac{d\underline{S}}{dt} \right)_{t=t_{i-1}} . \tag{140}$$

More complicated methods can give better estimates for the mean values of the derivatives, for fixed interval size, but require more numerical evaluations of $(d\underline{V}/dt)$ and $(d\underline{S}/dt)$. For example, the commonly used fourth-order Runge-Kutta procedure requires four calculations of both $d\underline{V}/dt$ and $d\underline{S}/dt$ for each integration step, as opposed to the single evaluations of each quantity required by Euler's Method. Thus, when numerically integrating ordinary differential equations one must usually choose between simpler methods which use relatively little computer time per integration step, but which require relatively many steps, and more complicated methods which require more computer time per step, but which require less steps.

One use of the red cell permeability equations is for the determination of cell membrane parameters by means of a comparison of the solutions of the equations with experimental measurements. Doing this most efficiently requires numerical and experimental evaluations of cell volume, and solute content if it is known experimentally, at identical values of time. As a result, there are benefits to making most of the numerical time steps equal to the time interval between data points, which is typically between 5 and 50 msec. Therefore, we have chosen a numerical integration proce-

dure which is the simplest and most computationally efficient one giving good accuracy with most time intervals in the upper end of this range. Our results indicate that for most time intervals in the lower end of the range (5 to 10 msec) even the Euler Method is adequate.

The method we have chosen is a two-step predictor-corrector method which requires two evaluations of both $d\underline{V}/dt$ and $d\underline{S}/dt$ for each integration step. In the predictor step, values of $\underline{V}$ and $\underline{S}$ called $\underline{V}_i^f$ and $\underline{S}_i^f$ are calculated by equations (137) and (138) using equations (139) and (140) for $d\underline{V}/dt$ and $d\underline{S}/dt$. For the corrector step, the integration is repeated using the average of the derivatives at $(\underline{V}_{i-1}, \underline{S}_{i-1})$ and $(\underline{V}_i^f, \underline{S}_i^f)$ for the mean values in the interval Δt_{i-1}. The forms than taken by equations (137) and (138) are then:

$$\underline{V}_i \simeq \underline{V}_{i-1} + \frac{1}{2}\left\{\left(\frac{d\underline{V}}{dt}\right)_{t=t_{i-1}} + \left(\frac{d\underline{V}}{dt}\right)_{\underline{V}=\underline{V}_i^f, \underline{S}=\underline{S}_i^f}\right\} \Delta t_{i-1} \tag{141}$$

$$\underline{S}_i = \underline{S}_{i-1} + \frac{1}{2}\left\{\left(\frac{d\underline{S}}{dt}\right)_{t=t_{i-1}} + \left(\frac{d\underline{S}}{dt}\right)_{\underline{V}=\underline{V}_i^f, \underline{S}=\underline{S}_i^f}\right\} \Delta t_{i-1}. \tag{142}$$

The two steps are done sequentially before the integration in the next time interval is begun.

For most cell membrane permeability experiments, $d\underline{V}/dt$ and $d\underline{S}/dt$ are largest near the beginning of the experiment because at that time, the jumps in thermodynamic potentials across the membrane are generally the largest. Therefore, the time steps should be smaller for the first few time steps than for succeeding steps. The following arrangement of time steps, illustrated in Fig. 5, has been found to be efficient and successful. First the total time for which the solution is desired is divided into equal time intervals called basic time integrals. The first four time intervals are then further subdivided. The third and fourth intervals are each divided in half. The second interval is divided into four equal parts. The first quarter of the first interval is divided into four equal parts (sixteenths of the interval). The remaining three quarters of the first interval is divided into six equal parts (eighths of the interval). The degree of accuracy of the complete calculation depends on the size of the basic interval.

In performing the numerical integration, $d\underline{V}/dt$ and $d\underline{S}/dt$ are evaluated by Eqs. (62) and (63) for many values of $\underline{S}$ and $\underline{V}$. These equations require values of $\underline{\pi}_m^0$, $\underline{c}_1^0$, a, b, $\underline{a}$, $\underline{b}$, $\bar{\underline{c}}_1$, $\bar{\phi}_1$, σ, K_1 and K_2. $\underline{\pi}_m^0$, $\underline{c}_1^0$, a and b are constants. $\underline{a}$ and $\underline{b}$ are given by Eqs. (59) and (60). In terms of isotonic quantities and quantities known at each stage of the computation, Eq. (59) takes the

form

$$\underline{a} = -\frac{\bar{V}_1 n_1^{\text{iso}}}{V^{\text{eq}}} + \frac{B_w^{\text{iso}}}{V^{\text{eq}}} + \frac{B_1}{V^{\text{eq}}} + \underline{S}\,\bar{V}_1 c_0. \tag{143}$$

Similarly, if B_2 is assumed to have the form given by Eq. (70), Equation (60) takes the form,

$$\underline{b} = -\frac{\bar{V}_1 n_1^{\text{iso}}}{V^{\text{eq}}} + \frac{B_w^{\text{iso}}}{V^{\text{eq}}} + \frac{B_2^{\text{eq}}}{V^{\text{eq}}} + B'(\underline{V} - 1) + \underline{S}\,\bar{V}_1 c_0 \tag{144}$$

where

$$B_2^{\text{eq}} = B_2^{\text{iso}} + B'(V^{\text{eq}} - V^{\text{iso}}). \tag{145}$$

9. Sample Calculations for Cases with One Permeable Solute and One or More Impermeable Solutes

A digital computer program was prepared to evaluate Eqs. (108), (109), (132) and (133) for the first and second order perturbation solutions. Another program was prepared to carry out the numerical integration of Eqs. (62) and (63) according to the two step iterative procedure described in § 8. Calculations were then made for three sample cases having the parameters shown in Table 1.

The primary purpose of the first sample case is to compare the results of the two methods of solution of the equations. Cell membrane parameters and solute concentrations were chosen to be roughly typical of what would be encountered in an experiment with human red cells (for some typical membrane parameters, *see* reference 9, p. 123). The case considered is one where the cells initially contain none of the impermeable solute and are suddenly put in a solution containing both permeable and impermeable solutes. The external impermeable solute concentration is set to the initial value of the internal impermeable solute concentration so that the cell volume is equal to the final equilibrium cell volume for constant $\underline{a}$ and $\underline{b}$. Under such conditions, the cell first shrinks due to solvent moving out of the cell as a result of a higher external than internal total effective solute concentration. Permeable solute moves into the cell during this time, but the effect of the solvent flow on cell volume is much stronger than the effect of solute flow on volume. The solute flow into the cell and the solvent flow out of the cell each increase the internal solute concentration which slows the rate of solvent efflux. When the opposing volume flows of solvent efflux and solute influx are equal, shrinking stops

Table 1. Variables for the sample cases[a]

Variable	Value for first sample case	Value for second sample case	Value for third sample case	Units
Independent variables (specified)				
$\underline{a}$	0.15	0.15		
b_c	0.20	0.20		
B_1^{iso}/V^{iso}			0.0	
B_2^{iso}/V^{iso}			0.15	
B_w^{iso}/V^{iso}			0.29	
V^{eq}	1.0×10^{-10}	1.11×10^{-10}		cm^3
V^{iso}			0.92×10^{-10}	cm^3
A	1.67×10^{-6}	1.67×10^{-6}	1.67×10^{-6}	cm^2
L_p	1.02×10^{-11}	1.02×10^{-11}	0.85×10^{-11}	cm^3/dyne-sec
σ	0.65	0.65	0.60	
ω	8.1×10^{-15}	8.1×10^{-15}	1.4×10^{-15}	osmoles/dyne sec
$\underline{V}(0)$	1.0	0.9	$\underline{V}^{iso}/\underline{V}^{eq}$	
$\underline{S}(0)$	0.0	0.6	0.0	
c_1^0	4.0×10^{-4}	4.0×10^{-4}	4.0×10^{-4}	osmoles/dyne sec
$c_m^0=c_0$	2.0×10^{-4}	2.0×10^{-4}	2.0×10^{-4}	osmoles/dyne sec
RT	2.5×10^{10}	2.5×10^{10}	2.5×10^{10}	ergs/mole
Dependent variables (derived from specified variables)				
$\underline{c}_1^0$	2	2	2	
K_1	0.85	0.77		sec^{-1}
K_2	3.4	3.1		sec^{-1}
η_1	5.06	4.56		sec^{-1}
η_2	0.84	0.76		sec^{-1}

[a] These are typical membrane parameters and conditions drawn from references 1, 2, 3, 4, 5, 6, 9, 15, 16, and 17.

and the minimum cell volume occurs. Permeable solute continues to enter the cell (ω effect when the volume flow is zero) and the increasing total internal solute concentration results in solvent entering the cell. Thus, as time continues to increase, the cell volume increases and asymptotically approaches its final volume, which in this particular case equals the initial volume.

Fig. 3 shows the time course of cell volume given by $G^{(1)}$, $G^{(2)}$, $G^{(1)}+G^{(2)}$ together with the results of the numerical integration with a basic time interval of 0.010 sec. Fig. 4 shows the corresponding results for the amount of solute in the cell. In performing the numerical integration of Eqs. (62) and (63) for the first sample case, b and $\underline{b}$ were set equal to the constant b_c (0.20) and $\underline{a}$ was set equal to the constant 0.15 so that the results could be directly compared with the perturbation equation results.

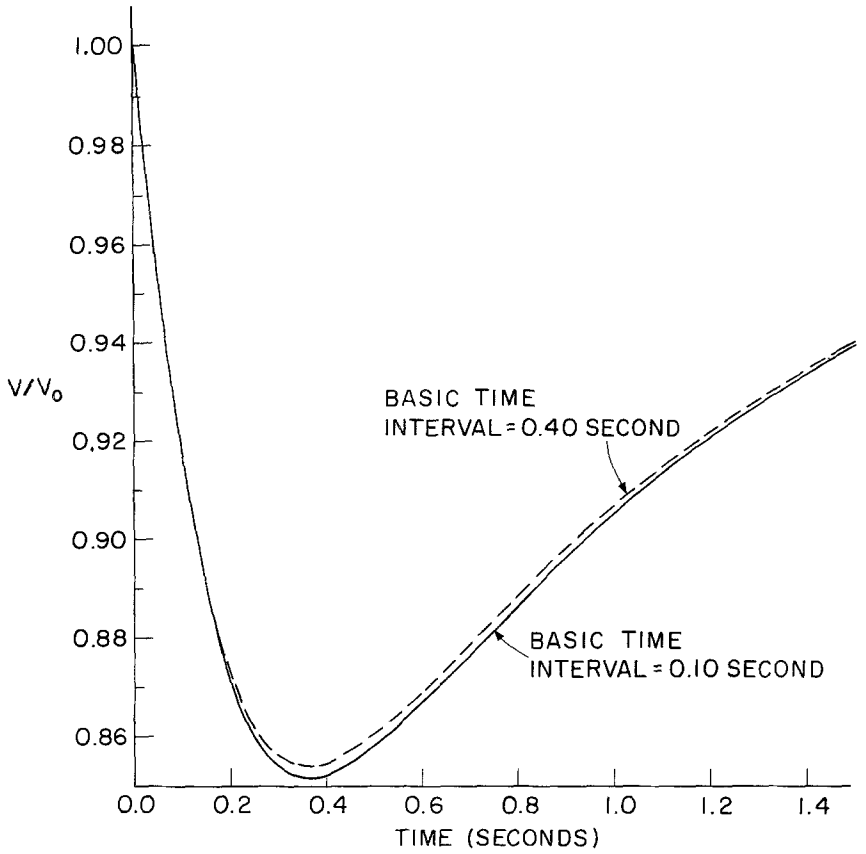

Fig. 6. Cell volume *vs.* time for first sample case, calculated by direct numerical integration for two different basic time intervals

Fig. 6 shows $\underline{V}$ as determined by numerical integration for two different basic time intervals, 0.100 and 0.400 sec. Fig. 7 shows the corresponding results for $\underline{S}$. The results for the time interval of 0.100 sec are indistinguishable from those for a time interval of 0.0100 sec, for which calculations were also made.

The case described above is a strong enough deviation from equilibrium for even the sum of first and second order perturbation solutions to be slightly different than the solutions given by numerical integration. The second sample case was chosen to determine the errors in the perturbation theory for a weaker deviation from equilibrium. For the second case, the difference in conditions from the first case were in the following parameters: $\underline{V}(0)=0.9$, $V^{eq}=1.11\times10^{-10}$ cm and $\underline{S}_0=0.6$. For this case, the results of the sum of first and second order perturbation solutions were indistinguishable from those of direct numerical integration (figures for this case are not shown).

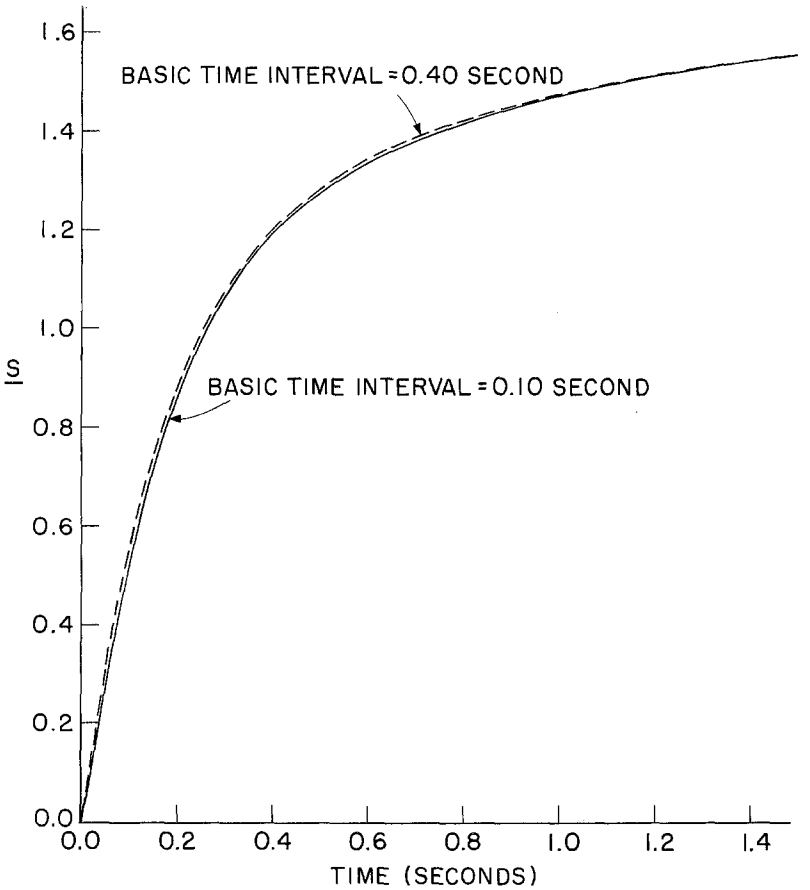

Fig. 7. Permeable solute in cell *vs.* time for first sample case, calculated by direct numerical integration for two different time intervals

The preceeding calculations confirm the validity of solutions obtained by direct numerical integration. Because of the convenience of incorporating variations of $\underline{a}$ and $\underline{b}$ during cell volume changes and of including the effect of solvent specific volume on osmotic pressure, direct numerical integration was used to evaluate these effects in the third sample case. The situation considered here is that of human red blood cells, which are initially in a salt buffer of 0.280 osmolality, being suddenly placed in a solution containing salt osmolality of 0.200 and a urea osmolality of 0.400. All conditions and parameters except for B' and $\bar{V}_1$ are given in Table 1. Four calculations were made with different combinations of values of B' and $\bar{V}_1$ as given in Fig. 8. From Fig. 2, over the anticipated range of cell volume, $B' = -0.325$. For urea, $\bar{V}_1 = 44.1\ \text{cm}^3/\text{mole}$. The numerical integrations of Eqs. (62) and (63) were done with $\underline{a}$ and $\underline{b}$ varying during the shrinking and swelling processes according to Eqs. (59), (60) and (71).

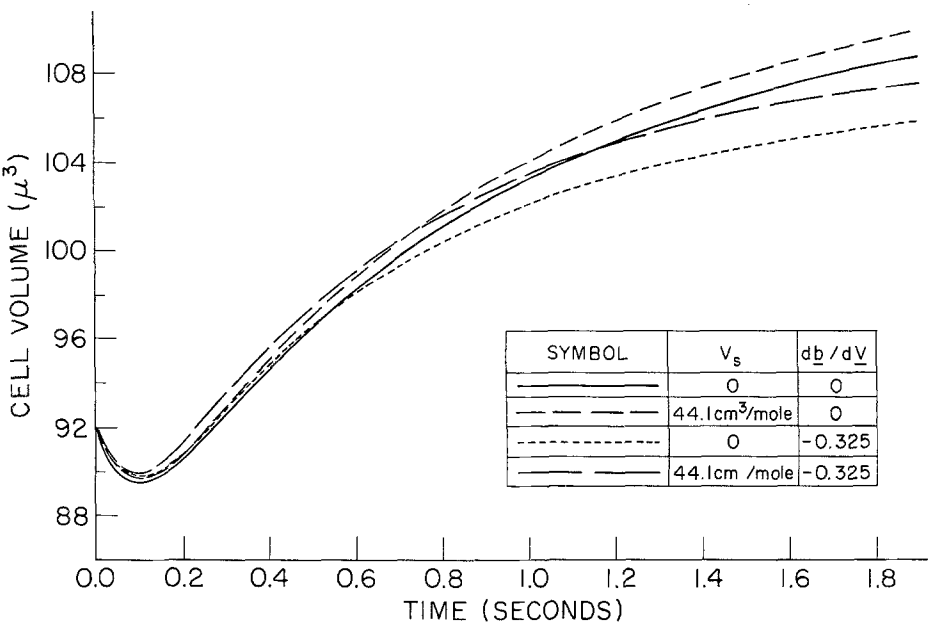

Fig. 8. Effects of solute specific volume and variation of nonsolvent water on a computed red cell shrink-swell curve. $L_p = 0.85 \times 10^{-11}$ cm^3/dyne-sec. $\sigma = 0.60$. $\omega = 1.4 \times 10^{-14}$ moles/dyne-sec. External permeable solute concentration = 0.400 Osmolal. External impermeable solute concentration = 0.200 Osmolal. The osmotic coefficient of urea is 0.96 so $\bar{V}_s$ can be taken as 44.9 cm^3/osmole

The calculated cell volumes *vs.* time for the four cases are shown in Fig. 8. The conclusions reached from these calculations are discussed in the next section.

10. Conclusions

The conclusions drawn from this work are divided into those that relate mainly to the mathematics of the red cell permeability equations and those that relate mainly to the biology of red cell shrink-swell experiments. The mathematical conclusions are discussed first.

We have shown that the solutions to the permeability equations by the two term perturbation expansion are consistent with those of direct numerical integration for small enough perturbations from equilibrium. The first term of the perturbation expansion alone (linearized theory), which has been used extensively in the literature in this field, is inadequate for some typical experimental conditions (*see* Figs. 3 and 4). Adding the second order solutions results in substantial improvement in accuracy. Assuming the results of the direct numerical integration are correct, even the sum of first and second order perturbation results has a small, but

noticeable, error for the conditions of the first sample case. Presumably third and higher order theory would improve the perturbation theory results further.

There is every indication that direct numerical integration by the two-step procedure given in § 8 yields the correct solution. The solution converges as the basic time interval is made smaller and smaller; and this solution is approached by the perturbation solution as the perturbation is made small, with the sum of first and second order perturbation results approaching more closely than the first order results alone.

If a user has the availability of a digital computer, the direct integration technique is highly recommended, both because of its accuracy and its versatility. Any functional dependence of $\underline{a}$, $\underline{b}$, L_p, σ, and ω on concentrations and cell volume can be accommodated. All that is needed is to set these parameters to their appropriate values at each point in time during the integration, including the points corresponding to $(\underline{V}_i^f, \underline{S}_i^f)$ so as to most accurately estimate the derivatives in the $(i-1)^{\text{st}}$ interval.

The calculations of the first sample case form a basis for determination of the needed basic time interval for direct numerical integration. The salient part of the system of equations has two characteristic times $1/\eta_1$ and $1/\eta_2$. $1/\eta_2$ is the smaller time, which for the sample case has the value of 1.19 sec. It was found that error due to finite time step size was negligible if the basic time step was 0.100 sec or less. This indicates that the basic time interval should be taken as $1/(12 \times \eta_2)$ or less.

Solving the permeability equations for the third sample case has led to two important biological results for red cell shrink-swell experiments. The first is the effect of the variable volume occupied by the permeable solute in the cell. The third sample case (Fig. 8) shows that for 0.4 osmolal urea, the difference in cell volume for the case in which the urea volume in the cell is taken into account and that in which it is not, is about $1\,\mu^3$. This difference is small in comparison to the total cell volume of about $100\,\mu^3$, but it is appreciable with respect to the total volume change during the shrink-swell process which is about $20\,\mu^3$. It is this change in volume and its rate of change that are affected by the membrane parameters in a shrink-swell process so that accurate results require that errors be small with respect to the volume change. For the sample case, the cell volume error caused by neglecting the volume occupied by the permeable solute is about 5% of the total volume change. This effect is directly proportional to both the permeable solute concentration and its specific volume.

The second biological result shown is the effect of the variation of B_2 during a shrink-swell experiment. For the third sample case, the effect

of the B_2 dependence reported by Gary-Bobo and Solomon [5] is about $2\,\mu^3$, which is about 10% of the total volume change in the shrink-swell process. Since some membrane parameters are determined from differences in various quantities, a 5% to 10% error in volume calculation can lead to substantially larger errors in estimated parameters. Thus, this effect must be taken into account if accurate results are to be achieved. This can be done directly in solving the equations by direct numerical integration, as was done in the sample case, or can be closely approximated in any method of solution by replacing both b and $\underline{b}$ by the quantity b_c given in Eq. (70).

These efficient and accurate methods of solving the membrane permeability equations can lead to useful results. One use is for examining the accuracy of experimental techniques. For example, determination of σ by the "zero-time slope" method of Goldstein and Solomon [6] requires knowledge of the rate of change of cell volume at the instant of mixing the cells with a nonisosmolal solution. This cannot be measured exactly. For example, Owen and Eyring [12] estimated the zero-time rate of change by the measured rate of change at times between 25 and 50 msec after mixing. We have solved the membrane permeability equations by the methods of this paper for the conditions of the experiments of Owen and Eyring and thereby have determined the error that would exist in estimates of σ by use of rates of volume change at various times after mixing in place of the zero-time rate of change. Typically, the error introduced in an estimate of σ by use of rates of change at times between 25 and 50 msec after mixing, instead of that immediately after mixing, is about 33%.

Other important questions concern the dependence of the membrane parameters on concentration, cell volume and flow direction. The kinds of questions that can be addressed include: does L_p depend on the direction of flow through a red cell membrane; is ω constant, or is L_D constant, or is neither constant as $\bar{c}_s$ varies during the course of a shrink-swell experiment; and does the red cell membrane really behave as a simple permeability barrier? Essentially any variation of the membrane parameters during the course of a shrink-swell experiment can be accommodated in the solution of the equations by direct numerical integration. This means that the accuracy of the answers to these sorts of biological questions are now limited only by the accuracy of the experimental measurements themselves.

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Nomenclature

A – cell surface area.
$\underline{a}$ – dimensionless nonsolvent volume for permeable solute.
$\underline{b}$ – apparent dimensionless nonsolvent volume for impermeable solute.
b' – $d\underline{b}/d\underline{V}$.
b_c – $(b-b')/(1-b')$.
B_j – apparent volume of the solvent in the cell which does not participate in dissolving the j^{th} solute.
B_w – volume of cell that is not solvent or solute.
c_j – concentration of the j^{th} solute (osmoles/volume).
c_m – concentration of impermeable solutes (osmoles/volume).
c_0 – normalizing concentration.
c_w – concentration of solvent (osmoles/volume).
J_j – flow of the j^{th} solute into the cell (osmoles/area × time).
J_w – flow of the solvent into the cell (osmoles/area × time).
k – number of solutes.
n_j – amount of j^{th} solute in cell (osmoles).
n_m – amount of impermeable solute in cell (osmoles).
n_w – amount of solvent in cell (osmoles).
p – Laplace Transform variable (time^{-1}).
P – hydraulic pressure (force/area).
R – gas constant (energy/°K × osmoles).
S – amount of solute (osmoles = moles × osmotic coefficient).
$\underline{S}$ – normalized amount of solute.
T – temperature (°K).
V – cell volume (length^3).
V^{eq} – equilibrium cell volume.
V^{iso} – isotonic cell volume.
$\underline{V}$ – normalized cell volume.
V_0 – normalizing volume (length^3).
V_j – partial molar volume of j^{th} solute (length^3/osmole).
V_w – partial molar volume of solvent (length^3/osmole).
x_j – mole fraction of j^{th} solute.
X – generalized "force".
i – as superscript, refers to region inside cell.
o – as superscript, refers to region outside cell.
0 – as subscript, refers to a reference quantity for normalization.
$\underline{\ }$ – as underscore, denotes a normalized quantity.
eq – as superscript, refers to the equilibrium value of a quantity.
iso – as superscript, refers to the quantity in the isotonic condition.
ε – perturbation parameter.
π – total osmotic pressure (force/area).
π_i – osmotic pressure of the i^{th} solute (force/area).
$\phi_i = c_i \overline{V}_i$ – volume fraction of the i^{th} solute inside the cell.
ϕ_w – volume fraction of solvent inside the cell.
Φ – dissipation function (energy/area × time).
μ_i – thermodynamic potential for the i^{th} solute (energy/osmole).
μ_w – thermodynamic potential for the solvent (energy/osmole).
(1) – as superscript, designates a first order quantity.
(2) – as superscript, designates a second order quantity.
* – as superscript, designates the Laplace Transform of the corresponding unstarred variable.

Appendix A

A Note on the Uniqueness of the Solution to the Membrane Permeability Equations for Cells

Eqs. (62) and (63) are a pair of coupled, first-order, nonlinear differential equations for the dependent variables $\underline{V}$ and $\underline{S}$ which depend on the independent variable t. Substitution of Eq. (62) in Eq. (63) for $d\underline{V}/dt$ allows the equations to be written in the form

$$\frac{d\underline{V}}{dt} = f(\underline{V}, \underline{S}) \tag{A 1}$$

and

$$\frac{d\underline{S}}{dt} = g(\underline{V}, \underline{S}) \tag{A 2}$$

where f is the right hand side of Eq. (62) and g is the right hand side of Eq. (63) after the aforementioned substitution for $d\underline{V}/dt$ has been made.

The question to be considered here is whether, for a given set of initial values for $\underline{V}$ and $\underline{S}$, a unique solution to Eqs. (A 1) and (A 2) exists. If the solution is not unique, integration of Eqs. (62) and (63) could lead to a solution that is not representative of the physiological situation being modeled by the equations.

A well-known mathematical result (*see*, e.g., reference 1) is that the solution is unique in any domain of ranges of values of $\underline{V}$ and $\underline{S}$ for which there exists a constant K, such that for every $\underline{V}_1$, $\underline{V}_2$, $\underline{S}_1$ and $\underline{S}$ in the domain,

$$\begin{aligned}&[f(\underline{V}_1, \underline{S}_1) - f(\underline{V}_2, \underline{S}_2)]^2 + [g(\underline{V}_1, \underline{S}_1) - g(\underline{V}_2, \underline{S}_2)]^2 \\ &\leqq K^2[(\underline{V}_1 - \underline{V}_2)^2 + (\underline{S}_1 - \underline{S}_2)^2]\end{aligned} \tag{A 3}$$

(A 3) is called a Lipschitz Condition and K is called the Lipschitz constant. Examination of the terms making up f and g shows that a Lipschitz Condition is satisfied for all physically realizable conditions except for the special and important condition; $\underline{S} = 0$. This occurs when the initial condition is that of no permeable solute in the cell.

The asymptotic limits of Eqs. (62) and (63) as $\underline{S}$ approaches zero are:

$$\lim_{\underline{S} \to 0} \frac{d\underline{V}}{dt} = K_1 \left[\frac{1-b}{\underline{V}-b} - 1 - \sigma \underline{c}_1^o \right] \tag{A 4}$$

$$\lim_{\underline{S} \to 0} \frac{dS}{dt} = \bar{\underline{c}}_1 (1-\sigma) \left[\frac{1-b}{\underline{V}-\underline{b}} - 1 - \sigma c_1^o \right] + K_2 c_1^o \tag{A 5}$$

$\bar{\underline{c}}_1$ can be written in the form:

$$\bar{\underline{c}}_1 = \frac{\underline{c}_1^0 - \dfrac{\underline{S}}{\underline{V}-\underline{a}}}{\ln \underline{c}_1^0 - \ln \dfrac{\underline{S}}{\underline{V}-\underline{a}}} \tag{A6}$$

If $\bar{\underline{c}}_1$ is considered as a function of $\underline{S}$,

$$\bar{\underline{c}}_1(0) = 0. \tag{A7}$$

If $K_2 \neq 0$ when $\underline{S}=0$, $\lim_{S\to 0} \dfrac{d\underline{S}}{dt} = K_2 \underline{c}_1^0$ and Eqs. (62) and (63) have a unique solution in the mathematical neighborhood of $\underline{S}=0$ and since a Lipschitz Condition is satisfied for all other positive values of $\underline{S}$, a unique solution exists for all physically realizable values of $\underline{S}$ and $\underline{V}$.

If, on the other hand, $K_2=0$ when $\underline{S}=0$, it is easily shown that Eqs. (A4) and (A5) have an infinite number of solutions which correspond to an infinite number of solutions to Eqs. (62) and (63). The case of $K_2=0$ when $\underline{S}=0$ is of some interest because it corresponds to finite values of L_d and L_p when $\underline{S}=0$ because of Eq. (A7).

If one considers arbitrary initial values of $\underline{S}$ and requires that the solutions of Eqs. (62) and (63) be uniformly continuous functions of $\underline{S}$ for all nonnegative $\underline{S}$, including $\underline{S}=0$, the solutions satisfying this requirement are then unique. Examination of Eqs. (A5) and (A6) show that the other nonunique solutions are those for which $\underline{S}=0$ for a finite time interval, not just the initial time.

Therefore, an alternative requirement, sufficient for uniqueness, and equivalent to the aforementioned continuity requirement is that $\underline{S} \neq 0$ at any time other than the initial time. Thus, if the initial time is $t=0$, the uniqueness requirement for $K_2=0$ when $\underline{S}=0$ is:

$$\underline{S} \neq 0 \quad \text{when } t>0. \tag{A8}$$

Appendix B

Coefficients for the Inhomogenous Part of the Second Order Perturbation Equations

The form of Eqs. (96) and (97) indicate that $f_v(t)$ and $f_s(t)$ have the forms given by Eqs. (130) and (131). To determine the coefficients F_{ij}, the expressions (130) and (131) are substituted into Eqs. (96) and (97), and terms in the various exponential functions are combined.

The F_{ij}'s are given in terms of the following definitions:

$$A_{11} \equiv \frac{\eta_1 G_0 + \zeta_g}{\eta_1 - \eta_2} \tag{B1}$$

$$A_{12} \equiv \frac{\eta_2 G_0 + \zeta_g}{\eta_2 - \eta_1} \tag{B2}$$

$$A_{21} \equiv \frac{\eta_1 H_0 + \zeta_h}{\eta_1 - \eta_2} \tag{B3}$$

$$A_{22} \equiv \frac{\eta_2 H_0 + \zeta_h}{\eta_2 - \eta_1} \tag{B4}$$

$$\rho^{(0)} \equiv \frac{c_0 \bar{V}_1}{2(1+\phi_1^o)^2 (1-a)} \tag{B5}$$

$$\rho^{(1)} \equiv \lambda^{(0)}(1-\sigma)\rho^{(0)} - \frac{\beta^{(0)}(1-\sigma)}{2(1-a)} \tag{B6}$$

$$C_1 \equiv K_1 \left[\frac{\sigma + \beta^{(0)}(1-\sigma)}{(1-b_c)^2} - \frac{\rho^{(0)}(1-\sigma)}{1-b_c} + \frac{\sigma \lambda^{(0)}}{(1-a)^2} \right] \tag{B7}$$

$$C_2 \equiv \frac{K_1 \rho^{(0)}(1-\sigma)}{\lambda^{(0)}(1-b_c)} - \frac{K_1 \sigma}{(1-a)^2} \tag{B8}$$

$$C_3 \equiv \frac{K_2 \lambda^{(0)} \beta^{(0)}}{(1-a)^2} - \frac{K_2 \beta^{(0)}(1-\beta^{(0)})}{(1-b_c)^2} + \frac{K_2 \rho^{(0)}(1-\beta^{(0)})}{1-b_c} + \frac{K_2 \lambda^{(0)} \rho^{(0)}}{1-a} \tag{B9}$$

$$C_4 \equiv \frac{K_2 \rho^{(0)}}{\lambda^{(0)}(1-a)} \tag{B10}$$

$$C_5 \equiv \frac{K_2 \beta^{(0)}}{(1-a)^2} + \frac{K_2 \rho^{(0)}(2\beta^{(0)} - 1)}{\lambda^{(0)}(1-b_c)} - \frac{2 K_2 \rho^{(0)}}{1-a}. \tag{B11}$$

Then:

$$F_{11} = C_1 A_{11}^2 + C_2 A_{11} A_{21} \tag{B12}$$

$$F_{12} = C_1 A_{12}^2 + C_2 A_{12} A_{22} \tag{B13}$$

$$F_{13} = 2 C_1 A_{11} A_{12} + C_2 (A_{12} A_{22} + A_{12} A_{21}) \tag{B14}$$

$$F_{21} = C_3 A_{11}^2 + C_4 A_{21}^2 + C_5 A_{11} A_{21} + \eta_1 A_{11} \rho^{(1)} \left(A_{11} + \frac{1}{\lambda^{(0)}} A_{21} \right) \tag{B15}$$

$$F_{22} = C_3 A_{12}^2 + C_4 A_{22}^2 + C_5 A_{12} A_{22} + \eta_2 A_{12} \rho^{(1)} \left(A_{12} + \frac{1}{\lambda^{(0)}} A_{22} \right) \tag{B16}$$

$$\begin{aligned} F_{23} = {} & 2 C_3 A_{11} A_{12} + 2 C_4 A_{21} A_{22} + C_5 (A_{11} A_{22} + A_{12} A_{21} \\ & + \eta_1 A_{11} \rho^{(1)} (A_{12} - A_{22}) + \eta_2 A_{12} \rho^{(1)} (A_{11} - A_{21}). \end{aligned} \tag{B17}$$

Appendix C

How to Use the Membrane Permeability Equations for Red Cells

There are two basic uses for the equations. The first is to make theoretical predictions of cell volume and solute content as functions of time for any prescribed set of physical conditions and cell membrane parameters. The way to do this is described below. The second basic use is application of the equations to experimental results of cell volume (and/or solute content) *vs.* time to determine the cell membrane parameters, which may be constant or variable during the course of the experiment. The way to do this will be given in a subsequent paper.

The first use will now be described in sufficient detail to allow the reader to make theoretical predictions of cell volume and solute content *vs.* time for the case of one permeable solute and one or more impermeable solutes. If a digital computer is available, direct numerical integration is recommended. If the equations are to be solved by "hand" calculation, the perturbation equations should be used if the cell membrane parameters (L_p, σ, ω) are constants.

The quantities to be specified by the user are $L_p, \sigma, \omega, A, B_1, B_2, B_w$, $c_m^i(0)$, c_m^{iso}, c_m^0, S^{iso}, $S(0)$, C_1^0, R, T, $\bar{V}_1$, $V(0)$ and V^{eq}. V^{eq} (which is used for the normalizing volume, V_0, in this development) is the cell volume at the final equilibrium. If the cell volume and solute content are known at some other condition, called the isosmolal condition, Eq. (72) is to be used to determine V^{eq}. It will be assumed here that B_2 is given by Eq. (71) so that B_2 is determined by B_2^{iso} and B' which are to be specified by the user. The equations to be solved are Eqs. (62) and (63) with $\underline{\bar{c}}_1$ defined by Eqs. (8) and (54). K_1, K_2, $\underline{a}$ and $\underline{b}$ are to be determined from Eqs. (57), (58), (59) and (60).

The perturbation equations in the form given here can be used for cases where K_1, K_2, σ, and $\underline{a}$ are well approximated by constants. Both b and $\underline{b}$ should be given the value b_c (Eq. 70).

With $\underline{V}^{eq}$ and $\underline{S}^{eq}$ given by Eqs. (66) and (68), G_0 and H_0 are determined from Eqs. (74) and (75) by using $\underline{V}(0)$ and $\underline{S}(0)$ for $\underline{V}$ and $\underline{S}$ in these equations. The λ's and β's, δ's and α's are next calculated from Eqs. (82) through (91). η_1, η_2, ζ_g and ζ_h are to be determined from Eqs. (103), (104) and (105).

The first order solutions given by Eqs. (108) and (109) can then be evaluated. The first step in determining the second order perturbation solutions is determining the A's, ρ's and C's in Appendix B by use of

Eqs. (B 1) through (B 11). Next, the F's are determined by Eqs. (B 12) through (B 17). Then, Eqs. (132) and (133) for the second order solutions, $G^{(2)}$ and $H^{(2)}$, can then be evaluated.

G and H are then estimated by

$$G(t) = G^{(1)}(t) + G^{(2)}(t) \tag{C 1}$$

and

$$H(t) = H^{(1)}(t) + H^{(2)}(t). \tag{C 2}$$

Remember that G and H are the deviations of $\underline{V}$ and $\underline{S}$ from the equilibrium values $\underline{V}^{eq}$ and $\underline{S}^{eq}$ given by Eqs. (66) and (68). V and S are found by multiplication of $\underline{V}$ and $\underline{S}$ by V^{eq} and $V^{eq}\ c_m^o$ respectively.

If a digital computer is available, direct numerical integration is the preferred method of solving Eqs. (62) and (63). First the basic time interval must be determined. There are two ways this can be done. The first is to follow the procedure given above to determine η_2. As described in *Conclusions*, the error due to finite time step size was found to be negligible if the basic time interval was less than $0.083/\eta_2$. Therefore, a "safe choice" of the basic time interval is $0.05/\eta_2$. The other way is to use experimental results for the conditions being considered if they are available and if the cells did not hemolyze during the experiment. Reference to the cases given as examples shows that a "safe choice" of basic time interval is 2% of the time from the start of volume or solute change to the time at which about 95% of the total volume and solute changes have occurred.

Once the basic time interval has been determined, the partitioning times, t_i, as illustrated by the vertical bars in Fig. 5, are determined and the individual time intervals are given by Eq. (136). t_1 is the initial time (zero) and at this time $\underline{V}$ and $\underline{S}$ have the values $\underline{V}(0)$ and $\underline{S}(0)$. The solution (values of $\underline{V}$ and $\underline{S}$) is given at successive values of t_i by Eqs. (141) and (142). Application of these equations to determine $\underline{V}_i$ and $\underline{S}_i$ when $\underline{V}_{i-1}$ and $\underline{S}_{i-1}$ are known requires numerical values for $d\underline{V}/dt$ and $d\underline{S}/dt$ for two conditions: first when $t = t_{i-1}$, and second for the hypothetical condition of $\underline{V} = \underline{V}_i^f$ and $\underline{S} = \underline{S}_i^f$. Eqs. (62) and (63) give $d\underline{V}/dt$ and $d\underline{S}/dt$ for any prescribed values of $\underline{V}$ and $\underline{S}$. When determining $\underline{V}_i$ and $\underline{S}_i$ from Eqs. (143) and (144), $\underline{V}_{i-1}$ and $\underline{S}_{i-1}$ are known so Eqs. (62) and (63) yield $(d\underline{V}/dt)_{t=t_{i-1}}$ and $(d\underline{S}/dt)_{t=t_{i-1}}$. $\underline{V}_i^f$ and $\underline{S}_i^f$ are the values of $\underline{V}_i$ and $\underline{S}_i$ given by Eqs. (137) and (138). Then Eqs. (143) and (144) are used again to determine $(d\underline{V}/dt)_{\underline{V}=\underline{V}_i^f, \underline{S}=\underline{S}_i^f}$ and $(d\underline{S}/dt)_{\underline{V}=\underline{V}_i^f, \underline{S}=\underline{S}_i^f}$.

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