J. Membrane Biol. 110, 127–137 (1989)

The Journal of
Membrane Biology

Analysis of Ion Channels by Modeling the Osmotic Effects of Weak Acids and Bases

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Summary. This paper describes computer programs which may be used to identify and analyze cation and anion channels. Weak acids are used to increase intracellular proton concentrations and by so doing to promote the exchange of osmotically active cations with protons. The time course of cation exchange is readily identified from the changes in cell volume which accompany the net changes in osmotically active cations. Weak bases are used to identify and analyze hydroxyl/anion exchange by a comparable strategy. The model was able to produce data that agreed with experimental data in the literature with an accuracy equal to experimental error. One program, called PROPIONATE, uses the weak acid, propionic acid, to identify cation channels such as the sodium-proton exchanger or the calcium-dependent, potassium channel. A second program, called BASE, is more general because either a weak acid such as propionic acid or a weak base such as ammonia may be used individually or together. When experimental data are available, the programs may be used to calculate permeability coefficients for ion channels and the capacity of intracellular buffers. The programs may be used also in the design of experiments. Initial values may be assigned to intracellular and extracellular electrolyte and proton concentrations. Values for intracellular buffer capacity and channel permeabilities may be chosen. The program will then generate changes in ions, cell volume, and intracellular pH when either a weak acid, a weak base or combination of the two is added to the external medium.

Key Words membrane · volume regulation · permeability · cell pH

Introduction

In a series of papers, Grinstein, Sarkadi, Rothstein and their collaborators (Grinstein et al., 1984*a*,*b*; Sarkadi et al., 1985) have demonstrated the effective use of the salts of the weak acid, propionic acid to unravel the intricacies of channel transport in leucocytes, leukemic cells, and human erythrocytes by osmotic methods. With this method, complex net solute movements may be detected and analyzed by following volume changes. Much earlier, Jacobs (1940) had developed methods to analyze the osmotic effects of the salts of weak bases and their interactions with the salts of weak acids.

In the present paper, two computer programs are described which model these effects. Both have been successful in their ability to predict the results reported in the papers of Grinstein, Sarkadi, Rothstein and their collaborators. The first one, PROPIONATE, is specific for the sodium and potassium salts of propionic acid. The second program, BASE, is more general. It describes the outcome when cells are exposed simultaneously to the salts of propionic acid and to the salt of a weak base such as ammonium chloride.

These programs model a cell system which has intracellular buffering capacity and membrane ion permeabilities, but, at present, do not consider asymmetric, saturable systems such as active transport. Therefore, the programs should be applied only to those experiments where active transport has been blocked or does not contribute to transport. The program uses basic principles from Gibbs-Donnan equilibria, combined with the common assumptions that cells are isosmotic with their environment. Program logic follows the reasoning exemplified in the classical work of L.J. Henderson (1928).

The programs therefore serve as demonstration models for teaching purposes, since the model can offer a whole series of experimental options including choices of channel permeabilities, intracellular and extracellular pH, buffer capacity and pK_a of constitutive cell buffers, intracellular and extracellular electrolyte composition, and cell volumes, to name a few.

In addition, the programs may be of particular value to cell physiologists in the design of experiments because they have both analytic and predictive value. When fitted to experimental data as has been done in this paper, the model provides two important analytical parameters: the intracellular buffering capacity and the passive, unmediated membrane permeabilities of ion channels. These parameters are not always evident in the usual experimental data.

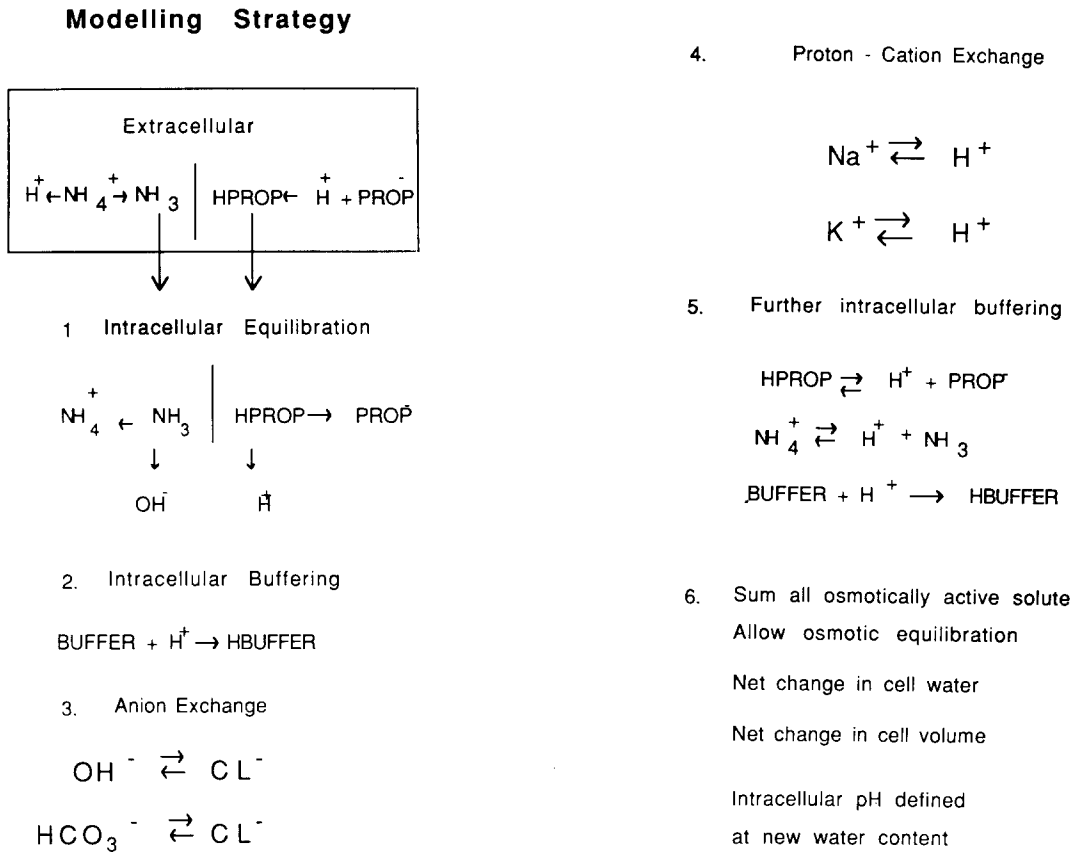

Fig. 1. Flow sheet outlining the strategy used to model the osmotic consequences which follow from the entry of weak acids or weak bases or both into cells

Theoretical Considerations

The logic in the design of the program is outlined in Fig. 1. The experimental situation being modeled is that of cells with a defined complement of solutes immersed in an extracellular medium of known composition containing either a weak acid or a weak base or both. Only electrochemical driving forces are considered. Experimentally, putative pumps must either be blocked or not contribute in the time interval under study. The starting intracellular and extracellular concentrations of the key electrolytes, Na^+, K^+, Cl^-, and HCO_3^- as well as the pH may be set at the discretion of the user. The composition of the extracellular compartment remains constant, while the concentration of electrolytes in the intracellular compartment may change.

Cells will change volume if the water content of the cells changes. These net changes in water content will occur if the osmolarity of the intracellular compartment differs from the osmolarity of the extracellular compartment and will continue until the osmolarities on each side are equal. All solutes are assigned osmotic coefficients of 1.

Osmotic Effects of Weak Acids

As outlined in Fig. 1, the initiating event is the ready entry of the weak acid, propionic acid, designated as HPROP. The first premise in the model is that the membrane is so permeable to the weak acid that its entry is not rate limiting, and the user should establish this fact experimentally before attempting to apply the program to his data. Therefore, the intracellular concentration of the weak acid will always be equal to the concentration in the medium. This value is calculated from standard buffer equations as illustrated in Eq. (1) for an extracellular pH of 7.3

$$HPROP = PROP^-/10^{(7.3-pK)} \quad (1)$$

where HPROP is the concentration of the propionic acid and $PROP^-$ is the concentration of the anion of the salt.

The next step is the dissociation of intracellular HPROP, the weak acid, into its component parts, H^+ and $PROP^-$ and initial acidification. If considered as an isolated system, the hydrogen ion avail-

able may be calculated simply as

$$H^+ = (HPROP/10^{pK})^{1/2}. \quad (2)$$

A new pH is set by buffering with intracellular macromolecules and the $PROP^-$/HPROP buffer pair.

In contrast to the weak acid, the net transfer of the major electrolytes is determined by membrane permeability. In this model, the governing equation is based upon the behavior of an electrolyte subject to an electrochemical gradient. The derivation appears in the Appendix. An important restriction to net movement is the requirement that a cation be accompanied by an anion or that an intracellular cation exchange with an extracellular cation or an intracellular anion exchange with an extracellular anion as indicated in steps *3* and *4* in Fig. 1. The user has the option, prior to the onset of the program, to set rate constants for the net transfer of K^+, Na^+, and Cl^-. The net transfer of HCO_3^- and OH^- are coupled to the net transfer of Cl^- by a one-for-one exchange system. Similarly, the net transfer of H^+ is coupled to the net transfer of Na^+ or K^+ or both, also by a one-for-one exchange system.

Using the Euler technique, net transfer of solute is allowed to continue for a preset time interval, after which the increment change is added to the previous content of the solute being considered. After all net transfers have been completed, the total content of osmotically active solute is summed, including the contribution from the impermeant, but osmotically active, macromolecules.

Osmotic equilibrium between the intracellular and extracellular compartments defines the volume of water inside the cell. Since the extracellular medium is considered to be infinitely large, its osmolarity is held constant. Therefore, if the solute content of the cell changes, the content of cell water will change proportionately to maintain osmotic equality between the two compartments. It is the net change in water content that produces the change in cell volume. These steps are outlined in item *6* in Fig. 1.

Since net changes in cations, anions, and water will alter the concentrations of H^+ and OH^-, which in turn will lead to changes in $PROP^-$, a new intracellular pH is calculated. The cycle begins again at step *2* in Fig. 1, keeping in mind that the intracellular concentration of the weak acid, HPROP is always held constant at the extracellular value.

To illustrate the logic of the model, let us consider how it is used to analyze the swelling of cells placed in an isosmotic solution of sodium propionate, buffered at pH 7.3.

Equation (2) indicated that the dissociation of HPROP produced a dramatic increase in H^+ and a corresponding decrease in OH^-. If the anion exchange system is functional, the entry of OH^- down its gradient from an external medium maintained at pH 7.3 is coupled to the exit of Cl^- from the cell. Each OH^- which enters is available to buffer the H^+ produced by dissociation of HPROP. Therefore, each Cl^- lost is matched by the production of a corresponding $PROP^-$.

During this process, the initial low intracellular pH increases as the ratio $PROP^-$/HPROP rises while the value for HPROP remains constant because of continued rapid equilibration with HPROP outside. The process is self limiting when the intracellular pH reaches that of the extracellular pH or when anion exchange ceases. If the intracellular reserve of Cl^- is sufficient to raise the $PROP^-$ inside to a value compatible with the extracellular pH then no volume change occurs. However, if anion exchange is limited, then cell swelling will occur to the extent that the H^+ from HPROP is buffered by intracellular buffers.

A new pH develops which is determined by the balance between the buffer capacity and pK_a of the cellular buffer and the pK_a and buffer capacity of the HPROP. The latter capacity is determined in turn from the external concentration and pH of the sodium propionate solution. When anion exchange is restricted, a considerable proton gradient can develop, directed toward the downhill exit of H^+ if a cation is available in the medium for exchange. If exchange can occur, the entry of cation will reduce the H^+ ion, raise the OH^- and produce $PROP^-$. Osmotically active solute in the intracellular compartment will increase. Water will enter and the cell will swell. It is in this setting that one may unmask the presence of exchange channels. These may include one-to-one exchange channels like the amiloride-sensitive proton-Na^+ exchange system (Grinstein et al., 1984*a*). On the other hand, one may force the issue by using a protonophore like carbonilcyanide *m*-chlorophenylhydrazone (CCCP) (Sarkadi et al., 1985). If any available channel is present for a cation to cross in exchange for the now permeable proton, swelling will occur as propionate anion is formed and osmotically active cation enters. Swelling will proceed until the free energy of the proton and cation gradients are dissipated or until cell lysis occurs. The events are easily followed by monitoring changes in cell volume.

Since the user may enter different values for membrane permeability for each electrolyte, it is possible with an iterative process to obtain permeabilities for each of the exchange channels which will satisfy the volume measurements obtained experimentally.

Materials and Methods

PROPIONATE[1]: A Program that Describes the Osmotic Consequences of the Entry of the Weak Acid, Propionic Acid

The computer program was developed with the following characteristics: The intracellular complement of solutes was normalized to a starting volume of 1 liter of intracellular water and expressed in milliequivalents. Hemoglobin was used as a model for cellular buffering power. From Freedman and Hoffman (1979), the buffering power for hemoglobin is 12 to 15 meq/mM Hb/pH unit or 84 to 105 meq per liter of cell H_2O/pH unit when Hb is 7 mmol/liter H_2O. In terms of the Henderson-Hasselbalch equation, this figure converts into a total of 205 to 256 milliequivalents of buffering power.

Both cations and anions were allowed to exchange according to their relative permeabilities and driving forces. The equation used was an integration of the Hodgkin-Katz derivation (Hodgkin & Katz, 1949) based on a net transport under an electrochemical gradient and is derived in the Appendix. Since the net transfer of electrolytes uses a H^+/OH^- exchange channel, this gradient is calculated as a Gibbs-Donnan potential difference and is inserted into the Hodgkin-Katz equation. The Gibbs-Donnan potential difference was calculated, in turn, from the distribution ratio of the proton and was calculated from $10^{(pH_i-pH_o)}$.

Permeability coefficients were set at the start of the simulation and could be changed at a selected interval after the initiation of the simulation. For example, to simulate an experiment in which cells were exposed to SITS initially and then followed by nigericin, permeability coefficients for K^+, Na^+, Cl^-, and HCO_3^- were set at 0 because SITS is a blocker of anion exchange channels. At the point when nigericin was introduced, the value for K^+ was set while all others were left at 0 because nigericin selectively promotes proton/K^+ exchange. On the other hand, when SITS was omitted, finite values were entered for Cl^- and HCO_3^-.

Certain limitations were placed on values for the intracellular solutes. If the gains and losses of ions depleted the intracellular anion of the weak acid, its value was set equal to the value of hydrogen ion when only the weak acid was present. Further, any negative values were treated as a loss of free hydroxyl ions. Accordingly, the protonated form of the macromolecular buffer was increased and its anionic form was decreased, to simulate the shift in the buffer ratio as hydroxyl ion was lost and excess hydrogen ion was buffered. The program was written in BASIC for use on the Apple IIe, Apple II+, or Macintosh.

Osmotic Effects of Weak Acids and Weak Bases Combined: The Program BASE[2]

When ammonium chloride and sodium propionate are present together in the medium, the extracellular concentrations in mmol/liter of the weak acid and the weak base are calculated from the Henderson-Hasselbalch equations.

$$NH_3 = NH_4^+ \cdot 10^{pH-pK_a} \quad (3)$$

$$HPROP = PROP^- / 10^{pH-pK_a}. \quad (4)$$

As noted in Fig. 1, both NH_3 and HPROP enter the cell water and equilibrate with the external medium, which is constant. Therefore, both weak acid and weak base inside the cell remain constant. Permeability of the membrane to the weak acid and weak base is not rate limiting.

Inside the cell both weak base and weak acid dissociate:

$$H^+ + OH^- + NH_3 = NH_4^+ + OH^- \quad (5)$$

and

$$OH^- = (NH_3 \cdot 10^{-3} \cdot k_b)^{1/2} \quad (5a)$$

while

$$HPROP = H^+ + PROP^- \quad (6)$$

and

$$H^+ = (HPROP \cdot 10^{-3} \cdot K_a)^{1/2}. \quad (6a)$$

The initial values of intracellular $PROP^-$ and NH_4^+ are set equal to H^+ and OH^-, respectively, as defined in Eqs. (5a) and (6a).

The H^+ produced from the dissociation of HPROP is allowed to interact with the OH^- produced from the dissociation reaction between NH_3 and water. The excess of either OH^- or H^+ is added to the H^+ already set by the initial intracellular pH and a new intracellular pH is calculated.

The program then models the interaction of the three buffer systems: Hb^-/HHb, $PROP^-$/HPROP, and NH_3/NH_4^+. Hb^-/HHb was chosen to represent the combined intracellular buffers because of its use with the human erythrocyte where its pK_a and concentration are known. However, the user may vary both concentration and pK_a to fit any other intracellular, constitutive buffer.

The first pair of interacting buffers are $PROP^-$/HPROP and NH_3/NH_4^+. In a series of iterative steps, the present intracellular pH is subtracted from the previous intracellular pH and the difference is subtracted from $PROP^-$ and from the NH_4^+. Both procedures will alter the pH defined by $PROP^-$/HPROP in one direction and that of the NH_3/NH_4 system in the opposite direction until they converge to a limit of 0.01 pH units. After convergence of this buffer pair, Hb^-/HHb is compared to $PROP^-$/HPROP by another series of iterative steps until this pair of buffers converges. When the concentration of the NH_3/NH_4 buffer far exceeds that of the $PROP^-$/HPROP buffer, such that the value for $PROP^-$ drops below 0, then Hb^-/HHb is matched against NH_3/NH_4^+ until the pH values for each buffer pair converge within a limit of 0.01 pH unit.

When buffering has been completed, the total amount of osmotically active solute has been increased or decreased by either $PROP^-$ or NH_4^+ or both. At this point, the total amount of osmotically active solute is summed and compared to the osmolarity of the external medium. The volume of cell water is then calculated relative to the initial isotonic volume, taken as 1.

In preparation for the calculation of net fluxes of anions and cations, using the derivation from the Hodgkin-Katz equation summarized in the Appendix, the distribution ratio for the permeable anions was calculated from the Gibbs-Donnan relation.

$$Cl_i^-/Cl_o^- = 10^{(pH_i-pH_o)}. \quad (7)$$

[1] Both of these programs have been written in BASIC for the Apple II+ or IIE computers and for the Macintosh Plus. Discs containing the programs or their listings may be obtained from the author upon request.

[2] *See* footnote 1.

When cations behave according to the Gibbs-Donnan relation, then the distribution ratio becomes the reciprocal of the chloride distribution ratio. The distribution ratios are important components in the calculation of the fluxes of the permeable anions and cations down their electrochemical gradients since they define the electrical component of the driving force under passive, nonmediated conditions.

Having defined the electrochemical driving forces for the permeable anions and cations, net fluxes for each ion are calculated during a time interval chosen by the user. The user also may vary the permeability coefficient for each ion. The concentration in meq or mmol/liter of cell water at the end of the time interval chosen is calculated according to Eq. (A5) as derived in the Appendix. The product of this concentration times the normalized water content of the cell at that time interval becomes the current content of the particular anion or cation under consideration. The net change in anion or cation content is calculated from the difference between the present content and that from the previous time interval.

Since anions are hypothesized in this model to exchange with OH^- by an anion exchange system with a defined rate constant, a gain in intracellular chloride is equivalent to a loss in intracellular OH^-, while a loss in intracellular chloride is equivalent to a gain in OH^-. The second premise of the model is that cations exchange by a proton-cation exchange system. When a cation is gained, such as Na^+, it is in exchange for H^+. Therefore, a gain in Na^+ is equivalent to a gain in OH^-, while a loss in Na^+ is equivalent to a loss in OH^-.

After carrying out this procedure for K^+, Na^+, HCO_3^-, and Cl^-, in that order, the algebraic sum of OH^- generated or diminished by the contributions of the several permeable ions is calculated. The extra OH^- or H^+ produced by ion movements is buffered by the two buffering systems, $PROP^-$/HPROP and NH_3/NH_4^+. The apportionment is carried out by calculating the available H^+ from each weak acid, viz: HPROP and NH_4^+ according to the equation $H^+ = (\text{concn. of weak acid} \cdot K_a)^{1/2}$ and then dividing up the total buffering capacity according to the relative ability of each buffer pair to provide or absorb H^+.

Since the amounts of $PROP^-$ and NH_4^+ may have increased or decreased because of the movement of cations and anions, a new intracellular pH is calculated from the weighted average of the pH values defined by each of the buffer pairs. If only one buffer had changed, the pH value for that particular buffer pair is used. Since the gain or loss of cations and anions and the addition or subtraction of $PROP^-$ and/or NH_4^+ changes the osmotic content of the cellular compartment, it is necessary to recalculate the volume of osmotically active water, normalized to an isotonic value of 1 in order to reflect the new concentrations of the buffer constituents. Once this procedure is carried out, the new intracellular pH value is calculated.

Calculations for the particular time interval are now complete. A new cycle begins as the three buffer pairs deal with a new intracellular pH set by the cation and anion movements.

Results

The Use of the Model Program, PROPIONATE

The paper of Grinstein et al. (1984*a*) was used to test the model because it provides enough data from thymocytes to compare with the output produced by the computer program. The only significant data not readily available are the buffering power and the combined pK_a of the constitutive intracellular buffers. Therefore, a family of curves was generated using the following concentrations in meq/liter H_2O for intracellular electrolytes for thymocytes: $K^+ = 140$; $Na^+ = 30$; $Cl^- = 80$; $HCO_3^- = 0$, and the following experimental conditions of Grinstein et al. (1984*a*): intracellular pH = 7.0; extracellular pH = 6.7 and 5 meq/liter KCl and 140 meq/liter Na^+ propionate in the extracellular medium. The change in intracellular pH appears in Fig. 1*A* of the paper of Grinstein et al. (1984*a*). This graph could be modeled with a buffer concentration ranging between 1.0 and 1.5 mM at a pK_a of 6.8. The output from the computer program is graphed in Fig. 2 using a buffer concentration of 1.25 mM and may be compared to their Fig. 1*A*.

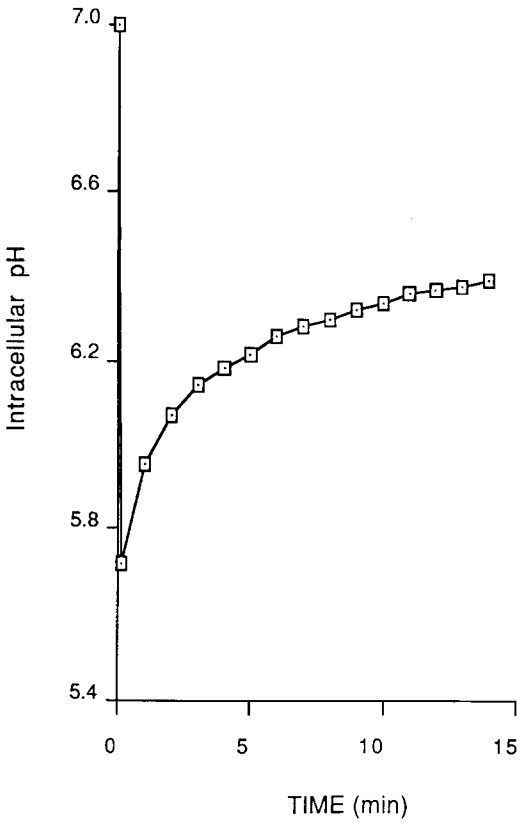

Fig. 2. Computer-generated data from program, PROPIONATE, which illustrates the change in intracellular pH when rat thymocytes at an initial intracellular pH of 7.0 were exposed to 140 mM Na^+propionate; buffered at pH 6.7. Intracellular buffer concentration set at 1.25 mM and a pK_a of 6.8. This figure may be compared to Fig. 1*A* from Grinstein et al. (1984*a*)

A similar approach was taken for sodium fluxes. Reference is made to Fig. 2 of Grinstein et al. (1984*a*). The ordinate is in nmol/10^6 cells. Since the isotonic volume of the rat thymocyte was 110

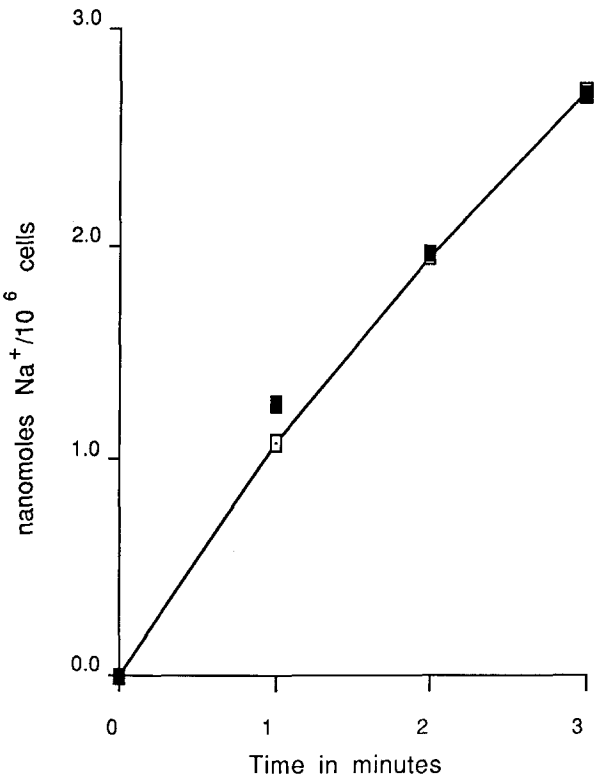

Fig. 3. Computer-generated Na^+ fluxes from program: PROPIONATE, which compares the model (open squares) with the experimental data from rat thymocytes from Grinstein et al. (1984*a*) (filled squares). Permeability for putative Na^+-H^+ exchanger set at 3.8×10^{-6} cm/min

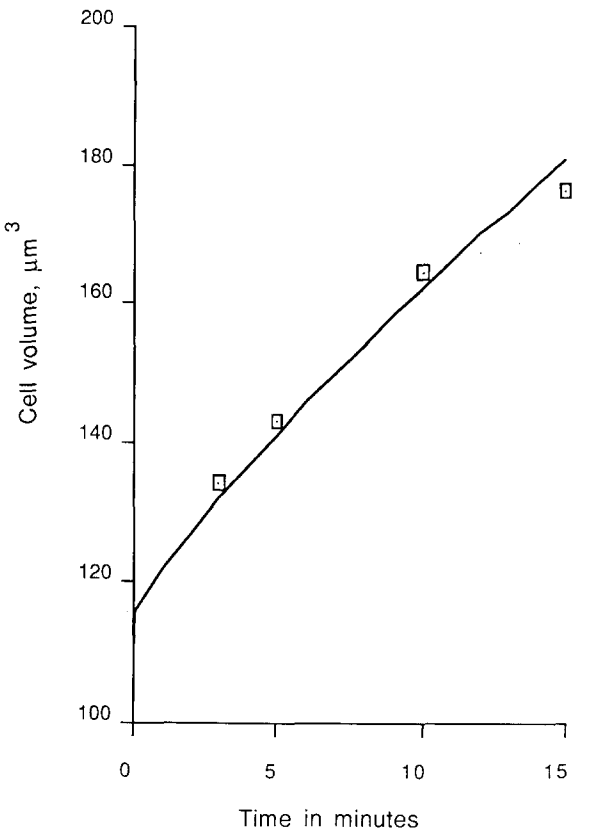

Fig. 4. Computer-generated changes in cell volume from program, PROPIONATE, which compares the model (curve) with experimental data from rat thymocytes (squares) from Grinstein et al. (1984*a*). Volume of osmotically inactive material set at 0.3; permeability for putative Na^+-H^+ exchanger set at 3.0×10^{-6} cm/min

μm^3 from their Fig. 3*B*, each increment of 1 nmol/10^6 cells corresponds to approximately 10 mmol/liter of cells or 13.3 mmol/liter of cell water since lymphocytes average 75% water by volume. Therefore, the output from the computer program was converted to nmol/10^6 cells in order to compare the data with their Fig. 2*B*.

A first estimate of the appropriate rate constant was determined from their Table I, which listed initial influxes from isotope data and net influxes in the presence of ouabain from chemical data. These data were converted from pmol/min mm^2 surface area to moles/liter of isotonic cell water/min. Since these influxes were essentially unidirectional, when they are divided by the external Na^+ concentration, the resulting value is an initial estimate of the influx rate constant. The contribution of the electrical component, of course, is being neglected in this initial estimate, but is subsequently included in the final curve fit.

The model defines the rate constant by PA/W = (liter/min)/liter (*see* Appendix). By an iterative procedure, the model generated the best fit to the data in Fig. 2*A* of Grinstein et al. (1984*a*) with a value of 3.8×10^{-6} cm/min for P, the permeability of the putative Na^+-proton exchange channel. This fit is shown in Fig. 3. The filled squares are experimental data; the open squares are model calculations. The Na^+ flux was affected by the choice of buffer concentration and reflected the slight differences in the H^+ gradient produced. When the buffer concentration was 1.5 mM, the lowest pH at $t = 0$ was 5.86; at 1.25 it was 5.80; and at 1.0 mM it was 5.72. As expected, the Na fluxes increased slightly with a decrease in pH, reflecting the proportionate increase in the driving force with an increase in the proton gradient. In Fig. 3, the model calculations used a buffer concentration of 1.25 mM and a pK_a of 6.8. These were the same values that gave the best fit for changes in intracellular pH.

The swelling of cells which accompanies the entry of Na^+ and the production of propionate anion was also approximated closely by the model. Figure 4 compares the smooth curve of the model with the experimental squares from Fig. 3*C* of Grinstein et al. (1984*a*). Since the data were not specified, a volume of osmotically inactive material of

30% or a b value of 0.3 was chosen, in keeping with the studies of Hempling, Thompson and DuPre (1977). A value of 3×10^{-6} cm/min was used for P, the permeability of the putative Na^+-proton exchange channel. Since the fractional volume changes reported by Grinstein et al. (1984*a*) in their Table I ranged between 0.049 to 0.077/min or a span of 57%, a difference in the model parameter for P between 3×1^{-6} cm/min, which was used here, and 3.8×10^{-6} cm/min, which was used for the fit of Na^+ fluxes, a difference of 27%, might be expected from biological variation. Much of that variation may derive from the choice of b value which, for the same change in water content of the cell, will produce a smaller or larger change in cell volume.

The model was used also to compare changes in the volume of erythrocytes that were suspended in an isosmotic solution of sodium propionate, buffered to pH 7.3 with HCl. Volume measurements were made every 5 min. The data collected demonstrated that under these conditions the cells shrank. At first glance this was a surprising finding, because one would have expected some swelling associated with the production of osmotically active propionate anion when the weak acid was buffered with intracellular buffer, in this case hemoglobin. Therefore, several hypotheses were posed and the model was used to generate anticipated findings.

The following data were entered into the model: Cell volume: 92.6×10^{-12} cm^3; b value = 0.6. This b value is quite high when compared to leucycotes or lymphocytes and represents a considerable volume of osmotically inactive water. However, this unusual situation is well known and Ponder (1948) had reported as much as 50% of the cell water behaved as if it were osmotically inactive. The choice here was based on actual measurements and the use of a Boyle-Van't Hoff plot. Surface area was set at 133×10^{-8} cm^2 and maintained constant, since one is dealing with a biconcave disc which increases in volume without an increase in surface area (Ponder, 1948). Intracellular ion concentrations in meq normalized to 1 liter of isotonic water were: $K^+ = 140$; $Na^+ = 20$; $Cl^- = 112$; $HCO_3^- = 0$; Hb = 7 at a pK_a = 6.8; other osmotically active, impermeant solute = 41. This value is used to establish an intracellular concentration isosmotic with that of the medium. Extracellular concentrations in meq/liter medium: $Na^+ = 160$; propionate = 160. pH outside = 7.3 and pH inside = 7.14.

The permeability for Cl^--OH^- exchange was calculated in the following way. At 25°C, the rate constant reported by Tosteson (1959) was 3 sec^{-1}. However, this value holds for conditions where the concentrations of the exchange species is not limited.

However, it must be remembered and emphasized that, in the experiments being analyzed, the rate constant for chloride transport is limited by the concentration of the *trans* species. Further, if the exchange is to have an osmotic effect, the *trans* species must be other than Cl^- and must be capable of interacting with intracellular H^+ in a one-to-one exchange

$$\text{efflux of } Cl^- = \text{influx of } OH^- \text{ and } r_{Cl}[Cl_i^-] = r_{OH}[OH_o^-].$$

The value r for Cl will be decreased if the [OH] or any other *trans* species is less than [Cl]. In the experiment being modeled, $[OH]_o = 10^{-6.7}$ since the extracellular pH was 7.3. $[Cl_i]$ was 0.112 M. Therefore, the rate constant for Cl^- OH^- exchange should be reduced by $10^{-6.7}/0.112$. However, these experiments were carried out in room air and Sarkadi et al. (1985) report that the concentration of HCO_3^- in the medium may be as high as 100 μM, because of the dissolved CO_2. If one uses a Cl^--HCO_3^- exchange, then the rate constant is reduced by $10^{-4}/0.112$ or approximately 10^{-3}.

From the appendix,

$$\text{rate constant, in min}^{-1} = (P)(\text{cell area})/(\text{volume of cell water}).$$

From this equation, the permeability, P, in cm/min can be calculated for a given rate constant and set of cell dimensions. For those chosen above, a value of 10^{-5} cm/min was calculated.

Figure 5 compares the changes in cell volume of human erythrocytes, predicted from the model, with the actual measurements of cell volume, using the Coulter particle size analyzer. The closed squares denote the actual measurements. Cell volume decreased from 92.6 to 82.5 μm^3 in 15 min. At first this was a surprising finding. The entry of propionic acid into the cells should generate osmotically active propionate anion as the weak acid is buffered by intracellular hemoglobin. If chloride exchanged with bicarbonate, some of the buffering could be accomplished by the interaction of the entering bicarbonate anion with the intracellular proton and in so doing reduce the total amount of propionate generated by the hemoglobin. The open triangles illustrate how the model predicted this set of events. After a transient swelling, cell volume decreased and approached the original cell volume. However, if the cells were to shrink, an additional loss of osmotically active solute must occur. This loss could occur if a potassium-proton exchange was activated. The open squares are the model calculations when chloride exchanges with bicarbonate with a P value of 10^{-5} cm/min and when potassium exchanges with proton with a P value of 10^{-6} cm/min.

Figure 6 graphs additional data generated by the model for ion concentrations and pH changes. What

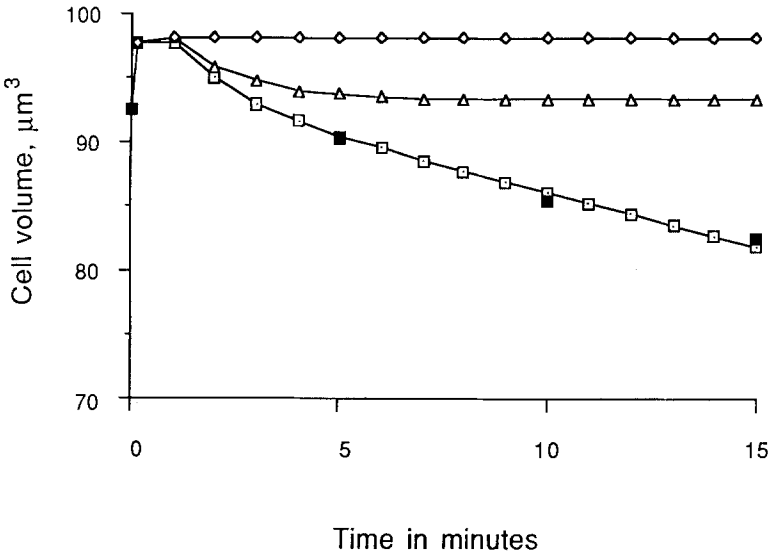

Fig. 5. The model predicts that the volume changes which were measured in the human erythrocyte suspended in 160 mM Na^+ propionate at pH 7.3 could be attributed to a K^+-proton channel with a permeability of 10^{-6} cm/min and a Cl^--bicarbonate permeability of 10^{-5} cm/min (open squares). Anion exchange alone returns the cell to the isotonic volume (triangles). Absence of channels should produce cell swelling (diamonds). Intracellular concentrations in meq/liter set at K^+ = 140; Na^+ = 20; Cl^- = 112; bicarbonate = 0; Hb = 7 mM at pK_a = 6.8. Intracellular pH set at 7.15. Measured volume = filled squares

is interesting is that the exit of potassium in exchange for proton does not influence the pH transient until 10 min after the cells were suspended in the sodium propionate solution. Apparently intracellular buffering by hemoglobin together with the generation of base by chloride exchange were sufficient to offset the addition of proton from potassium exchange.

It is obvious that apart from the actual measurements of cell volume, all the graphical data are model generated and therefore speculative. Yet, that is the model's most valuable asset. As a result of an analysis such as this, the investigator is in a position to test the model hypothesis by measurements of cell potassium and chloride. Measurements of pH, which are usually more difficult to do technically, will test for the buffering capacity, but would not help to identify the presence of a potassium-proton exchange.

The Use of the Model Program, BASE

The model program, BASE is more general than the model program, PROPIONATE because it will predict the outcome when cells are suspended in solutions of weak acids and weak bases combined. However, it is possible to get the same results with BASE as those with PROPIONATE by choosing a value for the salt of the weak base which is very low compared to that of the weak acid. For example, it was possible to obtain the same data for the response of cells to isosmotic solutions of sodium propionate by choosing a value for NH_4Cl which was only 0.01 mM.

To illustrate its applicability to the analysis of experimental data, the program was applied to some of the elegantly simple experiments of Jacobs (1940) on erythrocytes. In that classical paper, Jacobs foresaw the advent of ion channels and their osmotic significance when he distinguished between the entry of weak electrolytes as molecular entities and as ions. The program data plotted in Fig. 7 exemplify the distinction. In the upper graph, cells were placed in 160 mM solutions of NH_4propionate or NH_4Cl. In the presence of NH_4proprionate, cells swelled rapidly and hemolysis occurred almost immediately. How fast would depend upon the rate of entry of the weak acid, propionic acid and/or the weak base, NH_3. In the model it is not considered a rate-limiting step. In contrast, when anion permeability becomes a rate-limiting step, the rate of cell swelling is determined by the choice of permeability of the anion exchange channel. In this graph, the choice for Cl^- was 10^{-5} cm/min, equal to that used in the PROPIONATE model, and comparable to experimental conditions where bicarbonate in the medium was 100 μM. Under these conditions, hemolysis of erythrocytes will occur in 7 to 10 min. If the anion is totally impermeant, only a small degree of swelling occurs associated with the entry of NH_3 and its buffering by hemoglobin. Jacobs illustrated this principle in his Fig. 3 (Jacobs, 1940) in which he increased the rate of swelling of erythrocytes in NH_4Cl from minutes to seconds by increasing the concentration of bicarbonate in the medium. It is interesting that when he used phenyl urethane, which he considered an inhibitor of the anion exchange system, the rate of swelling was slowed.

The lower graph of Fig. 7 illustrates one of Jacobs' wonderful conundrums. Erythrocytes were suspended in isosmotic solutions of either sodium chloride or ammonium dihydrogen citrate. The anion dihydrogen citrate is impermeant across the

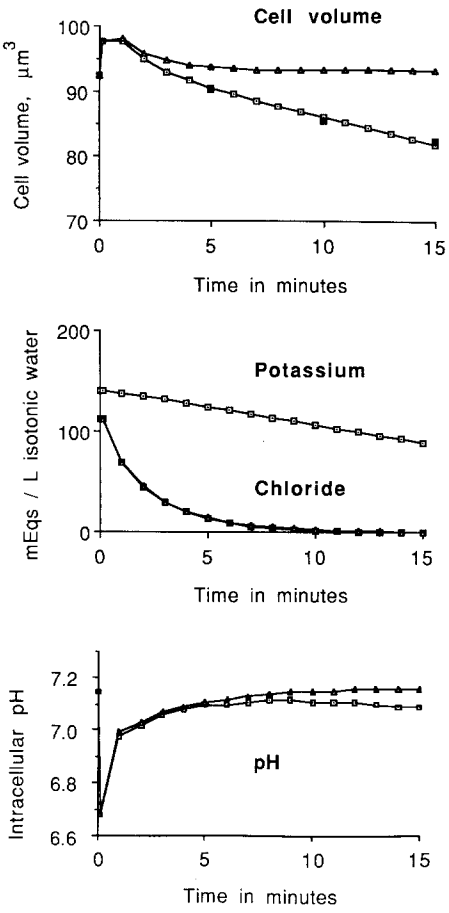

Fig. 6. Model predictions of changes in ion concentrations and intracellular pH when human erythrocytes are suspended in 160 mM Na^+propionate buffered to pH 7.3 with HCl

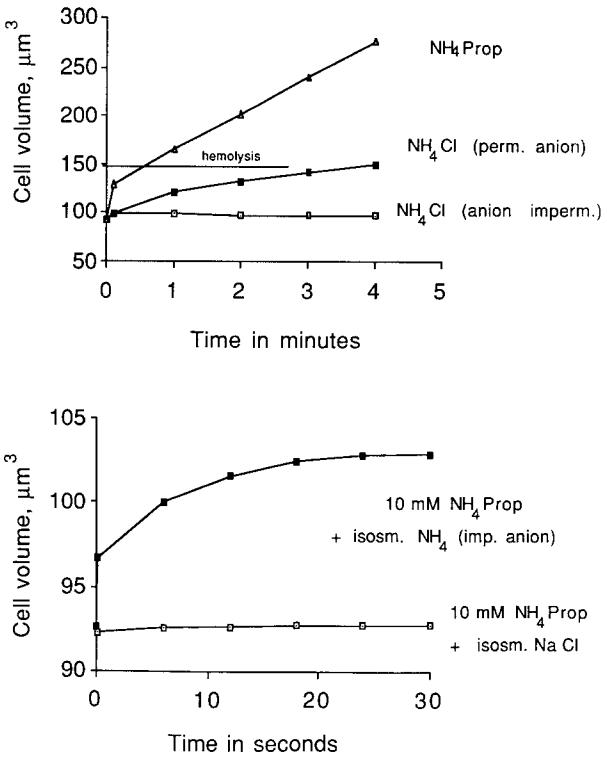

Fig. 7. *Upper graph:* Predicted changes in cell volume when human erythrocytes placed in 160 mM solutions of NH_4 propionate or NH_4Cl. Consult Fig. 5 for initial conditions. Permeability for Cl^- either 10^{-5} cm/min (permeant anion) or 0 impermeant anion). *Lower graph:* Predicted changes in cell volume in human erythrocytes placed in 160 mM NH_4Cl, with anion permeabilities = 0 to mimic an impermeant anion or placed in 160 mM NaCl with a Cl permeability = 10^{-5} cm/min. Each solution contained 10 mM NH_4 propionate. Consult Fig. 5 for initial conditions

membrane of the erythrocyte. Cells maintain isotonic volumes indefinitely under these conditions. When 10 mM of a salt of a weak acid and a weak base are added one gets totally different results in each isosmotic solution. In the model analysis, 10 mM ammonium propionate was used. When added to the isosmotic solution of sodium chloride, the cells shrank a small amount from 92.6 to 92.2 μm^3, in keeping with the slight hyperosmolarity produced by the addition of 10 mOsm solute to 320 mOsM isosmotic solution. In contrast, when the same concentration of osmotically active solute was added to the isosmotic ammonium dihydrogen citrate, rapid swelling occurred which was complete in a few seconds. Jacobs' experimental data, using ammonium acetate instead of ammonium propionate, appears in his Fig. 2 (Jacobs, 1940). According to Jacobs' inimitable style of reasoning, ammonium dihydrogen citrate is impermeant because the anion is impermeant. The only osmotic effect that the NH_4^+ could exert was a slight swelling produced by the NH_3, which entered from the medium. However, once an anion was available from the permeability of proprionic acid, then the full osmotic effect of the extracellular NH_4^+ was available, and the cells swelled as ammonium propionate was formed inside the cell.

Discussion

These model programs are useful because they can be used both as tutorials in teaching the basic principles of intracellular buffering and to design experiments and to predict the outcome. Since the model provided data that fitted experimental results very well, it is reasonable to assume that the strategy which was adopted in the development of the programs was realistic. Therefore, an investigator who makes use of the programs in the design of future experiments can be confident that he will not be wasting his time when he performs the actual experiment to test his hypotheses. However, there are limitations to the model, which any user must keep

in mind. In its present form, the model is designed to deal with coupled processes that utilize the free energy stored in the proton gradients established by the intracellular weak acids and bases after their dissociation and buffering inside the cell. The exchange between proton and cation or between hydroxyl ion and anion is electrically neutral. For this reason, an integration of the Hodgkin-Katz equation was used to calculate the concentration of the ion at any time interval after the inception of the exchange process.

Nothing in the derivation of the Hodgkin-Katz equation presupposes mechanism. The equation is a statement of the net movement of an ion across a boundary of finite thickness under an electrochemical gradient. The net movement is expressed per surface area of membrane and the membrane is assigned a permeability coefficient.

It is important to point out that the potential difference used in the equation is *not* the potential difference which actually exists across the membrane. Instead, it is the Gibbs-Donnan potential difference which is a *virtual* value and is defined by the proton gradient. It is the device by which we insert the free energy of the proton gradient into the net movement of the cation which is exchanging with it. For example, if we consider proton/sodium exchange and look at the integration of the Hodgkin-Katz equation, one puts in values for the intracellular and extracellular sodium concentrations. The proton gradient coupled to the sodium channel is entered as the Gibbs-Donnan potential, *not* the actual potential difference which does not participate in defining the net movement of ions by the model.

Two parameters are of particular interest and are not readily evident from experimental data: (i) the capacity and pK_a of the intracellular, constitutive buffers and (ii) the permeability of ion exchange channels. Both of these parameters define factors that contribute to volume regulation. Buffer capacity and pK_a will influence the intracellular pH and by doing so help to regulate net transport through ion channels. Net transport of ion channels, in turn, will contribute to the solute composition and osmolarity of the intracellular compartment and help to set the volume of the cell. In addition, the ion channels by exchanging cation with H^+ or anion with OH^-, will influence the intracellular pH. We have then a highly organized regulatory system.

The buffering capacity is the product of two terms: the concentration of the intracellular, constitutive buffers in mM/liter of cell water and the buffering power of the constitutive buffers in meq of protons buffered/mM of buffer/pH unit. As noted in Materials and Methods, as a first estimate, we used the buffering properties of hemoglobin because the data have been well characterized (Freedman & Hoffman, 1979). Hemoglobin has a pK_a of 6.8 and buffers 12 to 15 meq of protons/mM Hb/pH unit. The model produced data that agreed reasonably well with all of Grinstein's published data by choosing a constitutive, intracellular buffer concentration between 1.0 and 1.5 mM and a buffering power of 12 meq of protons buffered/mM of buffer/pH unit. The buffering capacity ranged from $1.0 \times 12 = 12$ meq of protons buffered/liter of cell water/pH unit to 18 meq of protons buffered/liter of cell water/pH unit. It should be noted that Grinstein had reported a buffer capacity of 25 meq of protons buffered/liter of cell water/pH unit (Grinstein, et al., 1984*b*). The data from the model are somewhat lower than the experimental data reported in that paper. However, since the experimental data were obtained from a set of experiments different from those which were used in the model, this degree of variation might be expected.

Buffer capacity and pK_a, of course, are interrelated in defining buffer effectiveness, and their interrelationship was not analyzed in this paper, but is currently under study. It appears that buffer capacity is far more sensitive to change than pK_a. If one were to use hemoglobin as an example, one might predict that the pK_a will not be far removed from the steady-state intracellular pH.

As a starting point, one might adopt an invasive method to obtain the pK_a for the intracellular buffers by permeabilizing the cell membrane to cations and anions in the presence of sucrose to maintain isovolumic conditions and noting at what extracellular pH the $[Cl^-]_i/[Cl^-]_o = 1$, according to Gibbs-Donnan theory (Freedman & Hoffman, 1979). Under the same experimental conditions, one could titrate the intracellular buffer with strong acid or base to obtain the meq H^+/pH unit and normalize it to cell number or cell volume. These data are entered into the model as a first approximation and tested to see how well it fits subsequent experimental data on pH changes in the presence of salts of propionic acid or ammonia. In this fashion it should be possible to follow changes in buffering capacity or pK_a under experimental conditions which do not allow for invasive analysis of buffering capacity or pK_a.

Finally, three options can be added to the model as it is presently constructed without altering its outline or strategy: (i) Addition of a term or terms to the equations which govern the entry or exit of cations or anions to represent active processes; (ii) making the concentration of intracellular weak acids and weak bases a variable instead of a constant and defining its value at any time, t, by an appropriate kinetic equation, probably a simple

Fick equation for nonelectrolytes; (iii) making the permeability of ion channels a continuous variable and a function of pH, for instance, or select the permeability in the present manner except to turn it on or off under preset conditions of pH. These additions have not been added yet, since it was not necessary to include them in order to test the validity of the programs with the experiments chosen. However, there are some circumstances where these additional options would be helpful or even required.

This research was supported in part by Grant #R01-HL25885 from the National Heart, Lung, Blood Institute.

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Received 20 February 1989

Appendix

$$dC_i/dt = (nFVPA/RTW)\{C_o e^{-nVF/RT} - C_i\}(1 - e^{-nVF/RT}) \quad \text{(A1)}$$

$$dC_i/C_o e^{-nVF/RT} - C_i) = [nFVPA/RTW(1 - e^{-nVF/RT})]\, dt \quad \text{(A2)}$$

Integrate between $t = 0$ and $t =$ chosen time interval

$$-\ln[C_o e^{-nVF/RT} - C_i] = \{nFVPA/RTW(1 - e^{-nVF/RT})\}t + \text{integration constant}$$

$$(C_o e^{-nVF/RT} - C_i)/C_o e^{-nVF/RT} - C_{i,0}) = e^{-\{nVFPA/WRT(1-e^{-nVF/RT})\}t} \quad \text{(A3)}$$

and

$$C_i = C_o e^{-nVF/RT} - C_o e^{-(nVF/RT) - \{nFVPA/WRT(1 - e^{-nVF/RT})\}t} + C_i e^{-\{nFVPA/WRT(1 - e^{-nVF/RT})\}t}. \quad \text{(A4)}$$

At $t = 0$

$C_i = C_{i,o}$.

At $t =$ infinity

$C_i = C_o e^{-nVF/RT} - 0 + 0$

so that

$C_i = C_o e^{-nVF/RT}$ and $C_i/C_o = e^{-nVF/RT} = r$ for anions and $1/r$ for cations.

Substituting

$$C_i = C_o r - C_o r e^{\{\ln(r)PA/(1-r)W\}t} + C_{i,o} e^{\{\ln(r)PA/(1-r)W\}t}. \quad \text{(A5)}$$

Nomenclature

C_i, C_o	concentrations of anion or cation, in meq/liter water
t	time, interval at discretion of user
n	valence of ion
F	Faraday's constant
V	cell membrane potential, volts (inside-outside)
P	permeability coefficient of membrane to ion, cm/time
R	universal constant, volt coulombs/mole °K
T	temperature, °K
W	cell water at time, t. Calculated as cell volume-volume of osmotically inactive material, cm^3
A	Area, at time t. Calculated as 4.76 (cell volume)$^{2/3}$ when cell treated as a sphere, cm^2