

## Transport Equations and Criteria for Active Transport

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**SYNOPSIS.** The relation between driving forces and the flux of solutes that would be expected in a passive system is derived. This relation is a differential equation and different solutions are obtained which apply to different experimental conditions. Solutions are given for the cases of pure convective flow, diffusion, electrophoretic mobility, balance between diffusive and electrical forces, and transport in the presence of both concentration and voltage differences.

Many different criteria have been used for the definition of active transport processes across membranes. The presence of saturation kinetics and the potential for competitive inhibition suggest the formation of a chemical bond between the transported substance and something in the membrane as a preliminary step to transport. Stoichiometric coupling of transport to oxygen consumption and inhibition of transport by metabolic blocking agents suggest that transport depends on cellular supplies of energy. Comparison of the measured transmembrane movement with that expected in a non-living system can indicate the activity of a process that would not be present in a passive system. These different approaches complement one another and are often used together. The present discussion, however, will be directed entirely toward the last criterion—deviation of a measured movement from that expected on the basis of known physical relationships. This approach is somewhat more abstruse than the others, for it is necessary first to decide what we would expect. We do this by deriving and solving a transport equation which defines the passive relation between the transmembrane flux of a solute and the driving forces acting upon it. The additional difficulty involved in this approach pays dividends, for when we have the passive transport equation, we can use it, not only to determine that some substances are transported actively and to estimate the magnitude of the active process, but also to analyze the

movement of passively transported materials. If we expect that a solute obeys the passive relation, we can compare the driving forces present to the measured fluxes and thereby determine some of the properties of the membrane. Conversely, if we know the characteristics of the membrane, measurement of the flux allows us to deduce the driving forces, and, from them, cellular concentrations. Finally, the process of analyzing what we would expect, in itself, often gives us greater insight into the processes going on in a complicated biological system.

From the point of view of an anti-vitalist, a cell membrane is simply a very thin layer of specialized material. The basic laws that govern movement in a membrane should be the same as those that govern movement in free solution. Thus, we will derive laws of movement of a solute which are true anywhere, and then we shall apply them around the boundaries of a membrane. Normally, this approximation is satisfactory, and Figure 1 illustrates why this is the case. Suppose we are investigating the diffusion of an uncharged molecule, such as glucose, across a cell membrane, and suppose further that we are analyzing a steady-state condition. The rates at which glucose passes through a unit of cross sectional area of extracellular fluid immediately outside of the membrane, a unit of cross sectional area of membrane, and a unit of cross sectional area of intracellular fluid immediately inside the membrane are all equal, for that

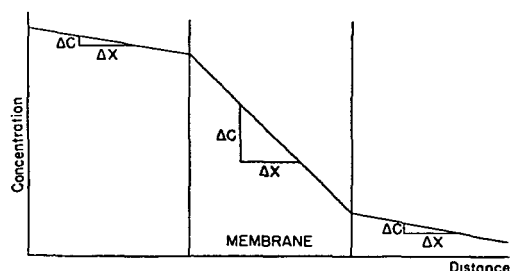


FIG. 1. Concentration gradients in external and internal aqueous solutions and through membrane during steady flow. Since the diffusion coefficients are so much higher in the aqueous phases than the membrane, the concentration gradients are much lower.

is the definition of a steady-state. In each case, the rate at which the solute passes through a unit of cross sectional area (defined as the flux, in moles/cm<sup>2</sup>-sec, and symbolized by  $J$ ) is equal to the diffusion coefficient within that medium times the rate of change of concentration with distance. Since we can equate the fluxes in each compartment and since both the extracellular and intracellular compartments are predominantly water, we can set up the relation given in Equation 1. For most solutes, the diffusion coefficient in the membrane is very much

$$\begin{aligned}
 1) \quad J &= -D_{H_2O} \frac{\Delta C_{out}}{\Delta X} \\
 &= -D_{MEMB} \frac{\Delta C_{MEMB}}{\Delta X} \\
 &= -D_{H_2O} \frac{\Delta C_{in}}{\Delta X}
 \end{aligned}$$

lower than the diffusion coefficient in aqueous solution, perhaps a million times lower. Since the product of this diffusion coefficient and the concentration gradient is the same in all compartments, the concentration gradient must be a million times higher in the membrane than in the aqueous solutions surrounding it. Normally then, we can say that the entire concentration change takes place across the membrane and assume that the conditions across the membrane are the limiting factors

in the movement. For most solutes, the concentration gradients in the aqueous solutions are so small as to be chemically undetectable. There are some exceptions to this general statement including the movement of gases or of water through very porous membranes. Here, where the diffusion coefficients are not very different in the different compartments, the concentration gradients are significant in all compartments. Analysis of transport in these conditions does require analysis of the unstirred aqueous layers adjacent to the membrane.

We start the derivation of a passive transport equation by breaking the possible modes of solute movement into two different classes. Movement of the solute particle with respect to its surrounding solvent molecules is termed interdiffusion. Movement, with respect to the observer, of the solute particle together with its immediate environment, is termed convection. The distinction between these two types of movement can be clarified by the parable of the Biologist on a Buoy in the Bosnian Straits Counting People as They Pass By (Fig. 2). Suppose you are perched on a buoy counting people as they pass you, and

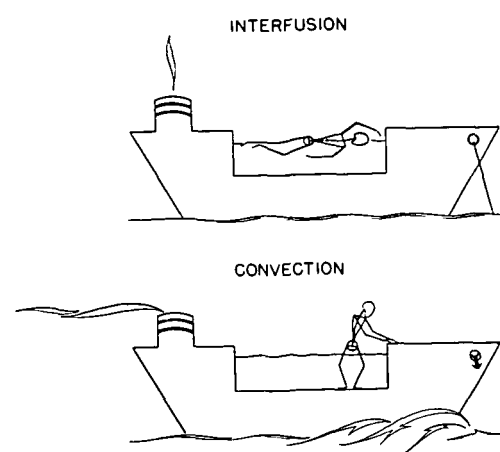


FIG. 2. Comparison of interdiffusion and convection. Upper figure: interdiffusion. The boat is anchored, but the swimmer is moving with respect to his surrounding solvent molecules. Lower figure: convection. The swimmer is fixed with respect to his surrounding solvent molecules, but the whole boat is moving with respect to the observer.

suppose further that a boat crosses your line of vision. On that boat is a swimming pool, and in the pool, a swimmer. If the boat is at anchor, but the person within the pool is swimming, he might cross your line of vision and you would count him. You would also count him if he is hanging on to the side of the pool, but the boat is moving with respect to you. If all you are doing is counting heads, you will make no distinction between these two swimmers even though the mechanism by which they pass your line of vision is different. The parable points out some way of separating the two movements. If you jump off the buoy and swim alongside the boat, matching its velocity, you eliminate the convective movement and perceive only the interfusion component. Either type of movement may occur by itself, or they may occur in combination.

#### INTERFUSION

This is movement of the solute particles with respect to their immediate surroundings and is caused by any driving force which acts differently on the solute and the solvent molecules. When the driving force is a concentration gradient, we call it diffusion; when the driving force is a voltage gradient, we call it electrophoresis. These are the two driving forces which we normally treat in biological movement. Differences in temperature between adjacent regions can also cause interfusion movements, but we normally assume that these are minor. Differences in centrifugal force cause movement by interfusion and this principle is used in ultracentrifugal separations. Although all of these, and other forces as well, might contribute to the interfusion term, we will restrict our considerations to concentration and voltage gradients. There are three important properties of interfusion terms. The first is that the driving force is derived from the spatial rate of change of a potential energy term. Thus, for diffusion, differences in concentration are an expression of a difference in potential energy, and the driving

force is the spatial rate of change of concentration. For electric mobility, the voltage difference is the expression of potential energy difference and the spatial rate of change of voltage (the electric field), is the driving force. If, between two adjacent regions, there is no difference in potential energy, there is no interfusion. This is simply the thermodynamic criterion for equilibrium. The second property is assumed as an induction from the statement about equilibrium. We assume that the interfusion movement is proportional to the potential energy gradient. We can justify this statement by saying that it must be true if we are sufficiently close to equilibrium. From thermodynamics, the net movement is some function of the potential energy gradient. All we really know is that the movement must go to zero when the energy gradient goes to zero. We can express the movement as a series of successively higher derivatives of the energy and, if we take a sufficient number of terms, this series must describe the net transport. The argument is that, if we are close to equilibrium, only the first derivative term counts. In general, in solutions, we do not really know whether we are close enough to equilibrium. However, the approximation works for both diffusion and electric mobility taken separately. The third major property of interfusion terms is the assumption that the different driving forces add.

We can formalize these statements for both diffusion and electric mobility.

$$J_{\text{diff}} = -D \cdot \frac{\Delta C}{\Delta X}$$

2) diffusion flux	diffusion coefficient	concentration gradient
$\frac{\text{Moles}}{\text{sec-cm}^2}$	$\frac{\text{cm}^2}{\text{sec}}$	$\frac{\text{Moles}}{\text{cm}^3\text{-cm}}$

Equation 2 is the relation we use for pure diffusion, and it is simply Fick's first law. It states that the flux is proportional to the product of a driving force and a diffusion coefficient. The physical meaning of the

diffusion coefficient becomes clear; it is a measure of the amount of work that must be done to have the solute particle slip through its surrounding solute molecules. Thus, we should expect the same diffusion coefficient to appear in all interfusion terms, as indeed it does. The flux in response to a pure electric field is given by Equation 3. Here, the flux is again equal

$$3) \quad J_{\text{ELECT}} = -D \frac{ZF}{RT} C \cdot \frac{\Delta E}{\Delta X}$$

electric flux	electric mobility	voltage gradient
$\frac{\text{Moles}}{\text{sec-cm}^2}$	$\frac{\text{Moles}}{\text{volt-sec-cm}}$	$\frac{\text{volts}}{\text{cm}}$

to the product of a driving force and a mobility term. The mobility term includes the diffusion coefficient, a factor  $ZF/RT$ , which relates chemical to electrical energy, and the concentration,  $C$ . The concentration appears because the electric field acts upon each charged particle present in an element of volume, and the higher the concentration, the greater the number of particles that drift. The total interfusion term is just the sum of these two terms.<sup>1</sup>

$$4) \quad J_D = -D \left( \frac{dC}{dX} + \frac{ZF}{RT} C \frac{dE}{dX} \right)$$

<sup>1</sup> There appears to be a logical problem in adding a concentration gradient to a voltage gradient. The concentration gradient generates a net velocity from the fact that the solute particles are in continuous random movement and they tend to spread from regions where there are many of them toward regions where there are few. The electric field, on the other hand, is a classical Newtonian force and produces an acceleration, not a velocity. Anywhere outside of a vacuum, however, frictional retarding forces are generated between the moving particle and its surrounding molecules. Frictional forces increase with the velocity, and, as a particle is accelerated in the electric field, friction builds up until the frictional retarding force just balances the electric field. At this point there is no further acceleration, but a steady velocity whose magnitude depends on the electric field and the diffusion coefficient. The transient state persists only for microseconds, and once we are beyond that, it is the velocity of the movement rather than the acceleration which depends on the driving force. This same line of reasoning leads to Ohm's

If any other forces which produce interfusion are present, they would also appear in this term.

#### CONVECTION

This is movement of the whole solution with respect to the observer (or, more often in biology, with respect to the membrane, which is a fixed point in our frame of reference). Technically, we should define convection as movement of the center of mass of the solution; then we can justify the corollary that convective movement requires the application of an external force. In biological solutions, water is the overwhelming constituent of any solution and so we approximate movement of the center of mass of the solution as the movement of the solvent. If we talk about convective flow through a membrane, we make the basic assumption that there are aqueous channels or pores present. Such pores are present in some membranes, e.g., mammalian erythrocytes, but are apparently absent in others, e.g., *Valonia* plasmalemma. The magnitude of the convective flux is just what would be counted from the solution moving past the membrane, and so it depends on the product of the volume flow and the concentration. A separate relation is needed to define the volume flow. This relation will be flux-force relation for water movement and the driving force will be the gradient in water activity, either because of differences in hydrostatic pressure or osmotic activity. The convective flux is given by Equation 5.

$$5) \quad J_{\text{conv}} = C \cdot J_v$$

convective flux	concentration	volume flow per cm <sup>2</sup>
$\frac{\text{Moles}}{\text{sec-cm}^2}$	$\frac{\text{Moles}}{\text{cm}^3}$	$\frac{\text{cm}}{\text{sec}}$

law relating the driving force to the flow of electrons in a metallic conductor and also leads to a limiting velocity, rather than continuous acceleration, of a parachutist, where the gravitational field which produces an acceleration of 32 ft/sec<sup>2</sup> is balanced by the friction between the parachute and the air.

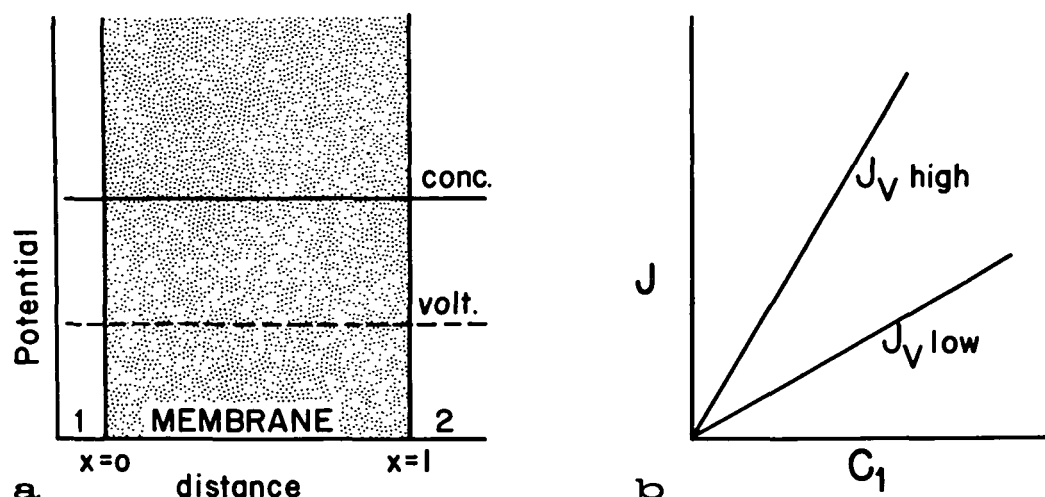


FIG. 3. Solution of the passive transport equation for pure convection. a. Concentration (solid line) and voltage profiles (dashed line) through the membrane. b. Representation of data on convec-

tion; the flux is plotted as a function of the concentration from which the transport is taking place.

The total passive movement is given by the sum of the interdiffusion and the convective terms:

$$6) \quad J = -D \left( \frac{dC}{dx} + \frac{ZF}{RT} C \frac{dE}{dx} \right) + C \cdot J_v$$

Equation 6 is the passive transport equation that we wanted to derive. If  $J_v$  is defined in terms of relative motion of the solution and an observer, Equation 6 contains no statement of requiring the presence of a membrane. This equation is not an answer of what would be expected to occur across a membrane, but rather is a relationship which must hold true at any point in space. It is a differential equation relating fluxes to driving forces, and to obtain predictions of the behavior across a membrane, we must integrate this relation through a distance equal to the thickness of the membrane. We get different solutions of Equation 6 depending on the different experimental conditions, and the criterion that we use to investigate active transport is that solution of Equation 6 that is appropriate to our conditions. The remainder of this discussion will be devoted to an examination of some of the

more commonly used solutions of the passive transport equation.

The first thing to do is to restrict the conditions under which we are going to look at solutions. Equation 6 was derived in terms of only one spatial variable,  $x$ . The same equation could be generalized to include movement in more than one direction. However, the unidirectional equation is not only much simpler, but is the proper treatment around a membrane, since the only potential gradients are directed perpendicular to the membrane. The second restriction that we shall impose is that of steady state. This means that there are no changes in concentration or voltage or flux with time. Another consequence of the steady-state condition is that the flux is constant in distance and does not vary for different values of the independent variable. Both of these restrictions limit us to consideration of reasonably simple, ordinary differential equations rather than partial differential equations.

#### Pure Convection

In this case, only the net flux and the product of water flow and concentration remain from the complete transport equation.

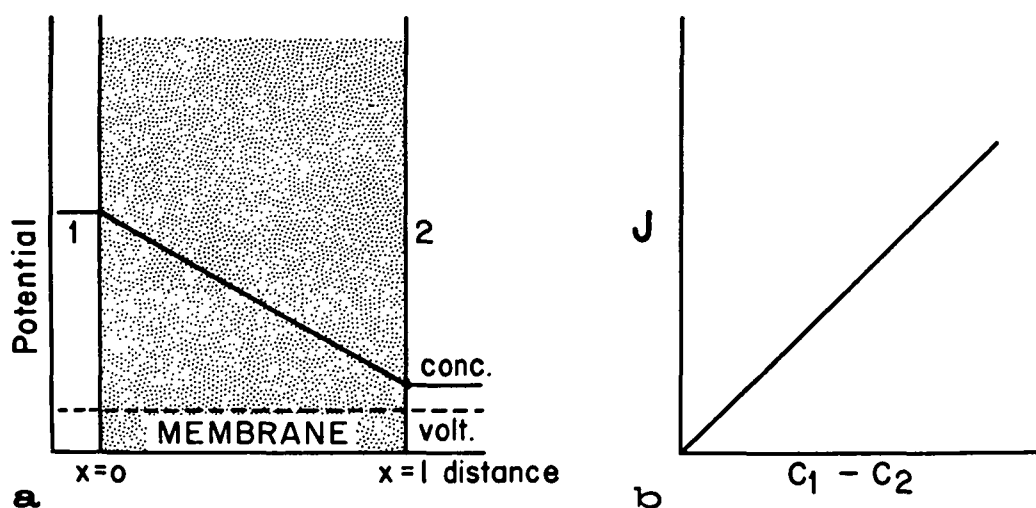


FIG. 4. Solution of the passive transport equation for pure diffusion. a. Concentration (solid line) and voltage (dashed line) profiles through the

membrane. b. Representation of data on diffusion; flux is plotted as a function of the difference in concentration across the membrane.

$$7) \quad J = -D \left( \frac{dC}{dx} + \frac{ZF}{RT} C \frac{dE}{dx} \right) + C \cdot J_v$$

In this and in subsequent cases, we shall show the complete transport equation with the terms that are present in each particular consideration emphasized. In convection, the derivative terms are all zero and the remaining terms constitute the solution in themselves. Figure 3a shows that both the

$$8) \quad J = C \cdot J_v$$

concentration and the voltage profiles are uniform as we go from compartment 1, outside the membrane, through the membrane, to compartment 2 inside the membrane. One way of plotting such data is illustrated in Figure 3b where the net transport is plotted as a function of the concentration in the compartment from which transport is occurring. The data will fall on a straight line passing through the origin with a slope equal to the volume flow. Pure convection is responsible for the movement of, say, sodium within the vascular system; no concentration or voltage gradients are present and the only factor causing movement of the sodium is the blood flow.

#### Pure Diffusion

Equation 9 shows that the terms that are present are the net transport, the diffusion coefficient, and the concentration gradient. The solution for net flux in the steady state is given in Equation 10 as the product of

$$9) \quad J = -D \left( \frac{dC}{dx} + \frac{ZF}{RT} C \frac{dE}{dx} \right) + C \cdot J_v$$

the difference in concentration and the ratio of the diffusion coefficient to the thickness of the membrane,  $l$ . This ratio of diffusion coefficient to membrane thickness occurs sufficiently often to be given a special name, the permeability ( $P$ ). As shown in Figure 4a, the concentration through the

$$10) \quad J = \frac{D}{l} (C_1 - C_2) = P (C_1 - C_2)$$

membrane falls in a linear fashion from the concentration in compartment 1 to the concentration in compartment 2 on the other side. The voltage is constant in distance. Steady-state diffusion flux might be plotted as a function of the concentration difference across the membrane. The data will describe a straight line passing

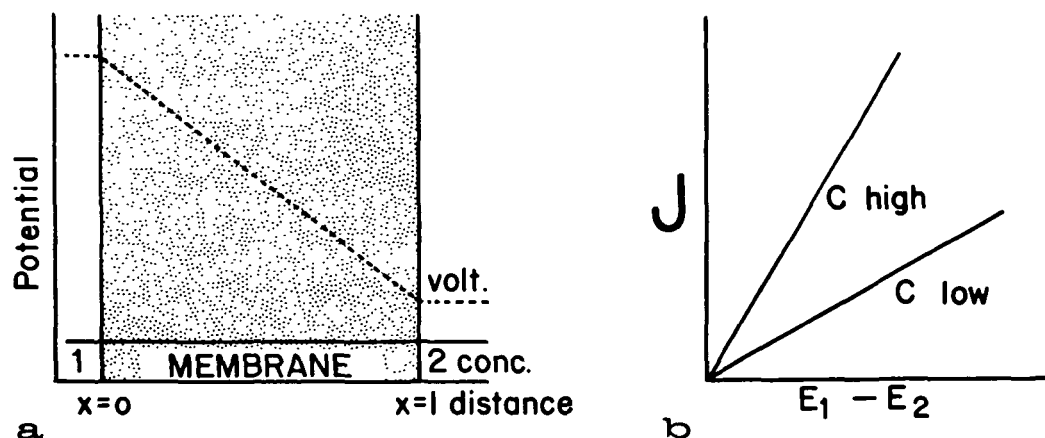


FIG. 5. Solution of the passive transport equation for pure electrophoresis. a. Concentration (solid line) and voltage (dashed line) profiles through

the membrane. b. Representation of electrophoretic data; the flux is plotted as a function of the voltage difference across the membrane.

through the origin with a slope equal to the permeability. Transport of uncharged molecules, such as urea or sugars, if they are passively transported, will follow these characteristics.

#### Pure Electric Mobility

This case is analogous to the previous one except that the driving force is the voltage gradient, rather than the concentration gradient. The terms present are the net flux, the electric mobility, and the voltage gradient.

$$11) \quad J = -D \left( \frac{dC}{dx} + \frac{ZF}{RT} C \frac{dE}{dx} \right) + C \cdot J_v$$

The solution is also analogous to that for diffusion. Figure 5A shows that

$$12) \quad J = P \frac{ZF}{RT} C (E_1 - E_2)$$

the concentration is uniform as we pass through the membrane, but that the voltage falls linearly from the outside to the inside of the membrane. The reasonable plot would be the net transport as a function of the voltage difference across the membrane. The data would fall on straight lines through the origin in which the slope would increase with increasing

permeability and with increasing concentration. This case is electrophoresis.

#### Balance of Electrical and Chemical Potentials

We come to the situation where there is no net transport, but there are driving forces of both chemical and electrical potential gradients. Equation 13 indicates which terms are present and Equation 14 gives the solution in terms of the concentrations on the two sides and the voltage differences across the membrane. This is one form of the famous Nernst equation; it is given in

$$13) \quad J = -D \left( \frac{dC}{dx} + \frac{ZF}{RT} C \frac{dE}{dx} \right) + C \cdot J_v$$

$$14) \quad \frac{C_1}{C_2} = e^{\frac{ZF}{RT} (E_2 - E_1)}$$

its logarithmic form in Equation 14a. This relation between the concentrations and voltage across a membrane at a time when there is no net transport was also derived by Boltzman and is also known as the Boltzman distribution. Relations of the same form will be generated whenever there is a balance between concentration gradients and any classical force which produces

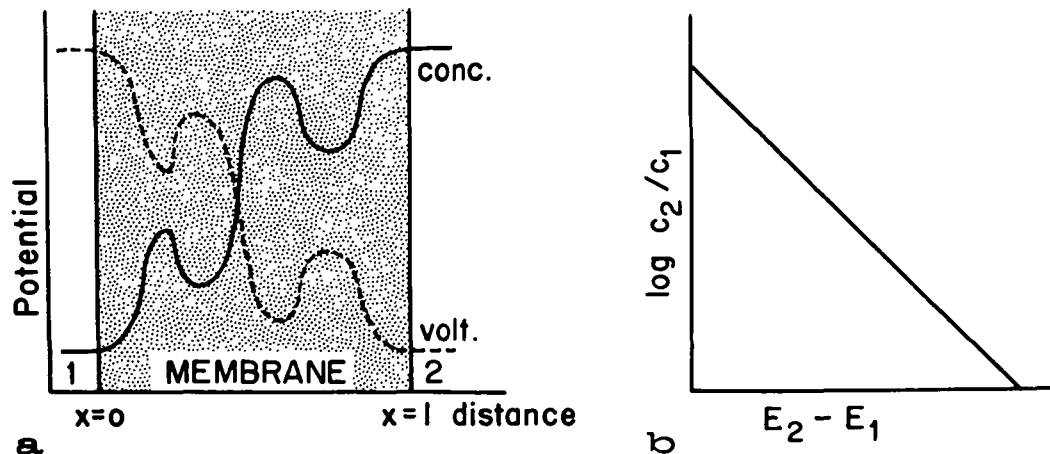


FIG. 6. Solution of the passive transport equation for balance between chemical and electrical forces. a. Possible concentration (solid line) and voltage (dashed line) profiles across the membrane. b. Representation of Nernstian data; the logarithm of the concentration ratio is plotted as a function of the voltage difference across the membrane.

$$14a) \quad E_2 - E_1 = \frac{RT}{ZF} \ln \frac{C_1}{C_2} \\ = \frac{2.3 RT}{ZF} \log \frac{C_1}{C_2}$$

Thus, the distribution of an unpumped ion across a muscle or erythrocyte membrane would be expected to fulfill the Nernst relation. In a like manner, the Nernst relation is a first approximation to the density of the air as a function of altitude. The major factors which are balanced are the density (or concentration) gradient, against the gravitational field. The final distribution is not exactly Nernstian because there are differences in temperature, too, but the basic idea is very similar. Figure 6a illustrates possible profiles of the voltage and concentration through the membrane. No statement is made about the nature of these profiles except that they must be related to each other in an exponential fashion. The Nernst equation gives the relationships at the boundaries of the membrane only. A common way of plotting this relation is shown in Figure 6b where the logarithm of the concentration ratio is plotted against the voltage difference. Solutes which obey the Nernst relation will fall on a straight

line with a slope of magnitude  $2.3 RT/ZF$ . At room temperature, this factor is about 59 mV per decade of concentration ratio (*i.e.*, equal concentrations on the two sides of the membrane are in equilibrium with 0 mV, a 10-1 ratio of concentrations is in equilibrium with 59 mV, and a 100-1 ratio is in equilibrium with 118 mV). At 39°C, the value of the factor is 61 mV. The Nernst equation not only gives the expected distribution when there is no net movement of a passively transported ion, but it also indicates, in a qualitative fashion, the expected direction of movement when the two potential terms are not in balance. Thus, in Equation 14, when the concentration ratio is numerically higher than the exponential term, there will be net movement of solute from compartment 1 to compartment 2.

#### *Net Transport from Combined Interfusion Terms*

The Nernst relation is the proper solution when there is no net transport and no convective term. However, there are many situations in which there is a steady net transport of solute and the question of whether or not there is any active transport cannot be answered from application of the Nernst equation. Investigation of ionic movements in intestinal or renal ep-



ithelium or across amphibian skin are examples of this more general situation. Equation 15 indicates the terms of the full transport equation which are present in these cases.

$$15) \quad J = -D \left( \frac{dC}{dx} + \frac{ZF}{RT} C \frac{dE}{dx} \right) + C \cdot J_v$$

The only difference between the present case and that considered previously is that there is a net flux in the present case. However, this minor modification changes a simple mathematical problem into a very formidable one. In a simple solution, such as a binary solution of univalent electrolytes, it is possible to solve the system comprising transport equations for each of the solutes together with the basic laws of electrostatics which define the electric potential in terms of the net charge density in an element of volume. However, the solutions that are generated, even in this simplest case, are so complicated as to be virtually useless to the experimenter. There are two commonly used techniques for obtaining simplified relations. The most direct is simply to assume that we know the voltage profile as we pass through the membrane and, having chosen a voltage profile which renders integration easy, to integrate the transport equations directly. We shall discuss this approach below. A second approach is to look at the ratio of one-way movements whose difference makes up the net flux. Suppose we have the possibility of adding isotopic tracers of the solute we wish to investigate, first to one compartment only and then to the other compartment only. We might analyze the movement of sodium by adding  $^{24}\text{Na}$  to compartment 1 and  $^{22}\text{Na}$  to compartment 2. So long as we wash the membrane with sufficient vigor that the concentration of  $^{24}\text{Na}$  never gets high in compartment 2, we can use the rate at which this isotope appears in compartment 2 as a measure of the one way flux from compartment 1 to compartment 2 ( $J_{12}$ ). We can use the appearance of  $^{22}\text{Na}$  in com-

partment 1 as a measure of the reverse flux ( $J_{21}$ ). Then, if the transport equation for the tracer is the same as that for the stable isotope and if the membrane makes no distinction between the different isotopes, we can solve Equation 15 without further assumptions. The result is given in Equation 16 which states that the ratio of one-way fluxes is proportional to the ratio of concentrations from which the fluxes are occurring weighted by an exponential function of the transmembrane voltage difference. This equation was derived by Ussing and provided a major breakthrough in the analysis of ionic transport in non-equilibrium conditions. Equation 16 is generally called the Ussing criterion. Examination of Equation 16 shows

$$16) \quad \frac{J_{12}}{J_{21}} = \frac{C_1}{C_2} e^{-\frac{ZF}{RT}(E_2 - E_1)}$$

that it reduces to the proper simpler relations. Thus, if there is no voltage difference, or if we are looking at an uncharged molecule, the ratio of one-way fluxes is just the ratio of concentrations from which the fluxes are derived. If the two one-way fluxes are equal, their ratio is one, and the Ussing criterion reduces to the Nernst equation. Of course, when the two one-way fluxes are equal, there is no net flux and the Nernst equation is the correct solution. Figure 7a illustrates that, like the Nernst equation, the Ussing criterion does not have any specific requirements about profiles as we pass through the membrane, but depends solely on the concentrations and voltages at the boundaries. The normal way of presenting such data is given in Figure 7b in which the flux ratio is plotted against the voltage-weighted concentration ratio. Passive movement produces a straight line passing through the origin with unity slope.

Although the Ussing criterion allows one to determine whether an individual solute fits the passive transport equation, it does not allow predictions of the magnitude of the passive flux. In order to obtain simple solutions which possess this property, it is

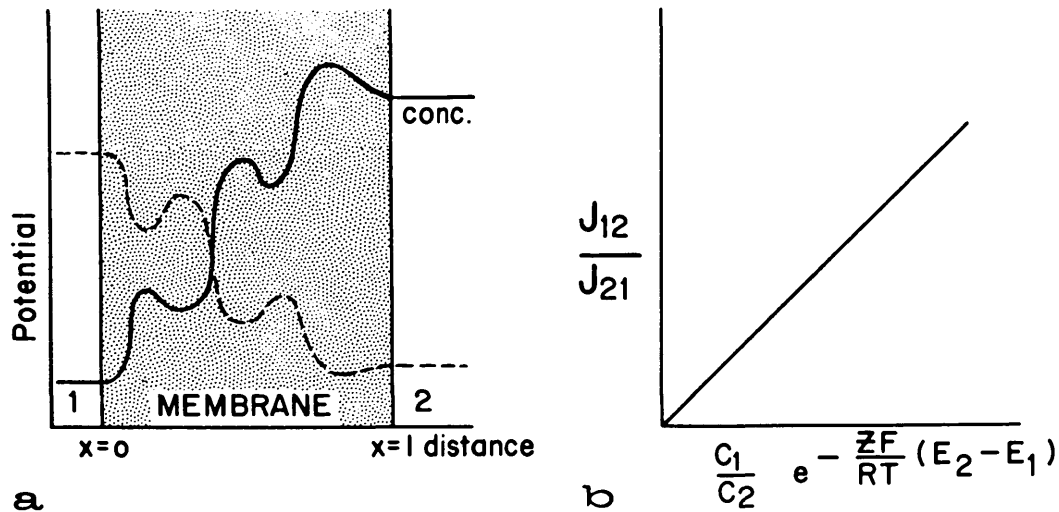


FIG. 7. Solution of the passive transport equation for flux ratios. a. Possible concentration (solid line) and voltage (dashed line) profiles across the

necessary to assume either the concentration profile or the voltage profile through the membrane. Both approximations have been made at different times and the choice of which to use depends on how accurate is the resulting solution and on how fruitful the solution is to the experimenter. The assumption that seems best for the treatment of thin membranes is the assumption that the voltage falls in a linear fashion from one side of the membrane to the other. This is known as the constant field assumption and was first applied to membrane ionic movement by Goldman in 1943. The main reason for using this assumption is that it simplifies the problem of obtaining a solution to Equation 15 which can give absolute values for net fluxes. Strong justification for the constant field assumption comes from a consideration of the physical situation around a membrane. The presence of a voltage across a membrane means that there is a slight excess of positive charges on one side of the membrane balanced by an equivalent number of negative charges on the other side. The charges remain separated (else there would be no voltage) and are held in place by the electrostatic attraction of their counterions. In terms of the thickness of a cell membrane, the dis-

membrane. b. Representation of the Ussing equation; the flux ratio is plotted as a function of the voltage-weighted concentration ratio.

tances tangential to the membrane surface are so enormous that a membrane can be considered as a charged parallel plate capacitor with no edge effects. The field within such a parallel plate capacitor is constant and the voltage does change in a linear fashion from one plate to the other. Using this assumption, then, Equation 15 can be solved to give the net flux in terms of the concentrations and voltages on each side of the membrane. Equation 17 gives this solution which is often called the Goldman current equation. Although the

$$J = \frac{P \frac{ZF}{RT} C_1 (E_1 - E_2) \left( 1 - \frac{C_2}{C_1} e^{\frac{ZF}{RT} (E_2 - E_1)} \right)}{1 - e^{\frac{ZF}{RT} (E_2 - E_1)}} \quad (17)$$

solution looks more formidable than previous solutions, it really contains the same terms. The flux is the product of the electric mobility times the voltage differences times a Nernstian term. When the Nernst condition is satisfied, and there is no net transport, the factor

$$1 - \frac{C_2}{C_1} e^{\frac{ZF}{RT} (E_2 - E_1)}$$

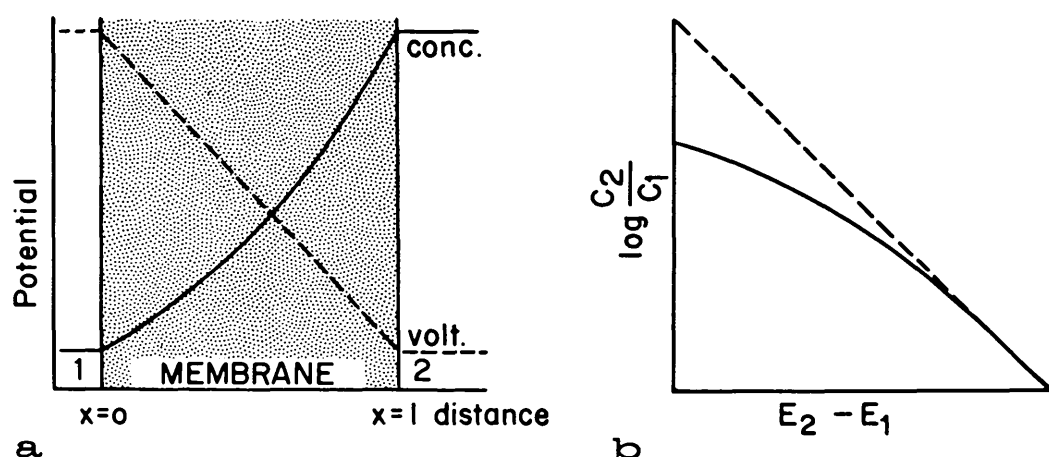


FIG. 8. Solution of the passive transport equation with the constant field assumption. a. Concentration (solid line) and voltage (dashed line) profiles across the membrane. b. Representation of constant

field data; the logarithm of the concentration ratio is plotted as a function of the voltage difference across the membrane.

is zero. When transport is present, this factor assumes the sign of the net transport. The current equation can be used to derive the steady voltage that would be expected to develop across a membrane when several ions can cross it. If a membrane is investigated under steady-state open-circuit conditions, where there is no net current, the total charges transferred by the net transport of cations must be just equal to the total charges transferred by the net transport of anions. If the ions move independently and passively and if there is no convective flow, the flux of each should obey the current equation. Thus, we have a set of relations of the form of Equation 17, one for each ion present, each with its own flux and each with its own pair of concentrations. The relations are not completely independent though, for the sum of the cationic fluxes must be equal to the sum of the anionic fluxes and the transmembrane voltage is common to all of these relations. These fluxes can be added up and the voltage factored out to give an expression of the stable voltage as a function of the concentrations of all of the permeable ions present and the membrane permeability to each of them. When all of the ions present are univalent, the result is fairly simple and is given in Equation 18, which

is

$$18) \quad E_2 - E_1 = \frac{2.3 RT}{F} \log \left( \frac{\sum P_{cat} C_1 + \sum P_{an} A_2}{\sum P_{cat} C_2 + \sum P_{an} A_1} \right)$$

usually termed the Goldman voltage equation. Here, the voltage is expressed as the logarithm of a fraction which includes a summation of all of the cationic concentrations on side one, each times its own permeability factor plus all of the anionic concentrations on side two, each times its permeability factor divided by the summation of the concentrations on the opposite side, each weighted by its permeability factor. Equation 18 is a generalization of the logarithmic form of the Nernst equation for, if there is only one permeant ion, it goes to the Nernst equation for that ion. Figure 8a shows the voltage and concentration profile through the membrane under the constant field assumption. The voltage drops in a linear fashion, as is required by the initial assumption. The concentration changes in an approximately exponential fashion through the membrane. The normal manner of plotting such data is given in Figure 8b and is identical to the plot of the Nernst equation.

TABLE 1.

Condition	Equation	Requirements for validity
Convection	$J = C \cdot J_v$	No concentration gradients present. No voltage gradients present or no charged particles in solution.
Diffusion	$J = P (C_1 - C_2)$	No convective term. No voltage gradients present or no charged particles in solution.
Electrophoresis	$J = P \frac{ZF}{RT} C (E_1 - E_2)$	No convective term. No concentration gradients present.
Nernst relation	$\frac{C_1}{C_2} = e^{\frac{ZF}{RT} (E_2 - E_1) *}$	No convective term. No net transport.
Ussing criterion	$\frac{J_{12}}{J_{21}} = \frac{C_1}{C_2} e^{-\frac{ZF}{RT} (E_2 - E_1) *}$	No convective term.
Goldman current equation	$J = \frac{P \frac{ZF}{RT} (E_1 - E_2) C_1 \left( 1 - \frac{C_2}{C_1} e^{\frac{ZF}{RT} (E_2 - E_1)} \right) *}{1 - e^{\frac{ZF}{RT} (E_2 - E_1)}}$	No convective term. Constant field across the membrane.
Goldman voltage equation	$E_2 - E_1 = \frac{2.3 RT}{F} \log \left( \frac{\sum P \text{ cat } C_1 + \sum P \text{ an } A_2}{\sum P \text{ cat } C_2 + \sum P \text{ an } A_1} \right) *$	No convective term. Constant field across the membrane. All ions with significant net transfer are included. All ions with significant transfer are univalent.

\* When convective flow is present, the correct solution is obtained by replacing the term  $\left[ \frac{ZF}{RT} (E_2 - E_1) \right]$

$$\text{by } \left[ \frac{ZF}{RT} (E_2 - E_1) - \frac{J_v}{P} \right].$$

tion (Fig. 6b). When concentrations are such that one ion dominates the transmembrane movement, the results approximate those of the Nernst relation (indicated in Fig. 8b by the dashed line). As the transfer of charges by other ions becomes important, the results fall off the Nernst relation. The point at which the results deviate from the Nernst relation and the sharpness of the curvature give information about the relative permeabilities of the ions present. Equation 18 can be simplified under certain conditions to render it even more useful. The terms in the fraction are related to net transport of each of the ions present. Any ion that is in electrochemical equilibrium and for which there is no net transport does not contribute to the voltage. If, for example, Na, K, and Cl are distributed across a membrane and Cl is in electrochemical equilibrium, the numerical value of the voltage from Equation 18 is the same whether or not the Cl terms are included in the fraction. In many animal systems, these three ions are the dominant permeant ions present and Cl is so close to equilibrium that its net flux is quite low. Comparison of the transmembrane voltage to the concentrations of the two cations can give a ratio of permeabilities of the two cations directly.

Table 1 summarizes the results that have been presented here. It repeats the solutions given in the text and gives the conditions which must be filled for each solution if it is to be the expected result. In addition to the experimental conditions listed in the table, all of the solutions are valid only if the system is in a steady-state, if transport is one-dimensional, and if each solute moves independently of all others.

I have presented some of the more commonly used solutions to the passive transport equation. These are by no means the only ones, and there are many cases where more complicated solutions are required. In situations of molecular sieving, where voltage gradients are not important, solution of the equation using gradients of concentration and convective terms would

be appropriate. This is an approximation of the case for transcapillary exchange. In plant roots, concentration gradients, voltage gradients, and convective terms are all important and an even more complicated solution is required. Sometimes it is possible to design an experiment which eliminates some of the driving forces and allows one of the simpler solutions of the transport equation to be used as the criterion; sometimes, only the more complicated relations can be used.

The basic statement that can result from an experiment comparing the appropriate solution of the passive transport equation to the measured fluxes and driving forces is that the system either is or is not described by that relation. When the experimental results do fit, we feel satisfied and believe that we understand the basis for the transmembrane movement. When the experimental results do not match our predictions, the conclusion is that passive independent transport does not completely describe the system and that an additional term is needed. Rather than Equation 6, the membrane under investigation would require a relation of the form of Equation 19. The term  $J_{\text{other}}$  might be a consequence of in-file behavior in aqueous channels through the

$$19) \quad J = -D \left( \frac{dC}{dx} + \frac{ZF}{RT} C \frac{dE}{dx} \right) + C \cdot J_v + J_{\text{other}}$$

membrane, or it might be the consequence of carrier-mediated energy-independent transport, or it might be the consequence of a solute pump. Usually, different kinds of experiments are required to define the detailed mechanisms of ionic movement. The approach presented here, though, can define the magnitude of the additional term and serve as a starting point for further experiments.

## APPENDIX

### SOLUTIONS OF THE TRANSPORT EQUATION

The purpose of this section is twofold.

First, there should be a more satisfying way of obtaining the solutions given above than the authoritarian statement, "The solution is . . .". Secondly, it is hoped that illustration of the manner in which the solutions are obtained will enable the reader to derive different solutions which he may need for some particular experimental situation. The solution for pure convection will not be given inasmuch as the terms remaining from the complete transport equation are free of derivatives and constitute the solution in themselves.

#### Pure Diffusion

The equation to be solved is given as Equation 20 with the associated

$$20) \quad J = -D \frac{dC}{dx}$$

conditions that  $C = C_1$  at  $x = 0$  and  $C = C_2$  at  $x = l$ .  $E$  is constant so that  $dE/dx$  is everywhere zero and  $J$  is constant throughout the membrane. Both sides of the equation can be integrated directly between the limits of  $x = 0$  and  $x = l$  and we get the solution given in the text.

$$\int_0^l J dx = -D \int_0^l \frac{dC}{dx} dx = J l$$

$$= -D (C_2 - C_1)$$

$$21) \quad J = \frac{D}{l} (C_1 - C_2) = P (C_1 - C_2)$$

#### Pure Electric Mobility

The equation to be solved is given in Equation 22; the associated

$$22) \quad J = -D \frac{ZF}{RT} C \frac{dE}{dx}$$

conditions are that  $E = E_1$  at  $x = 0$  and  $E = E_2$  at  $x = l$ . Here, the concentration is constant so that  $dC/dx$  is everywhere zero and  $J$  is constant throughout the membrane. Again, both sides can be integrated directly and the solution given in the text is obtained.

#### Nernst Relation

The equation to be solved is just the interdiffusion portion of the transport equation and is given in Equation 23 with the values of concentration and voltage defined at the boundaries. Actually, there are two different solutions.

$$23) \quad 0 = D \left( \frac{dC}{dx} + \frac{ZF}{RT} C \frac{dE}{dx} \right)$$

One of them is the condition of zero diffusion coefficient. Although this may seem trivial, it does point out that for completely impermeable membranes, any set of conditions is possible. So long as the voltage can be expressed as a function of distance only, Equation 23 is a first order linear differential equation in concentration. If the description of voltage in distance is more complicated than first degree, the equation does not have constant coefficients, but it is still linear. The function  $\exp [(ZF/RT) E(x)]$  is an integrating factor and leads directly to the Nernst equation.

$$24) \quad \frac{ZF}{RT} E(x) \left( \frac{dC}{dx} + \frac{ZF}{RT} C \frac{dE}{dx} \right) = 0$$

$$C_2 e^{\frac{ZF}{RT} (E_2 - E_1)} - C_1 = 0,$$

$$\frac{C_1}{C_2} = e^{\frac{ZF}{RT} (E_2 - E_1)}$$

Note that it is necessary to define the integrating factor only at the boundaries, where the voltages are defined, and that nothing need be known about what happens to the voltage within the membrane.

#### Ussing Criterion

The equation to be solved is the non-homogeneous version of Equation 23. Voltages and concentrations are defined at the boundaries and  $J$  is constant throughout the membrane. We can divide by  $-D$  to put the equation in standard form (Equation 25) and then apply the same inte-

grating factor that was

$$25) \quad \frac{dC}{dx} + \frac{ZF}{RT} C \frac{dE}{dx} = -\frac{J}{D}$$

used previously. The result shown in Equation 26 after integration constitutes a

$$26) \quad \begin{aligned} C_2 e^{\frac{ZF}{RT}(E_2-E_1)} - C_1 \\ = -\frac{J}{D} \int_0^l e^{\frac{ZF}{RT}E(x)} dx \\ = -\frac{J_{12}-J_{21}}{D} \int_0^l e^{\frac{ZF}{RT}E(x)} dx \end{aligned}$$

solution in the mathematical sense, but it is not useful. The integral of the voltage across the membrane cannot be evaluated without knowledge of the functional form of the voltage. However, we can evaluate the expected flux ratio and, in order to presage that step, the net flux has been expressed as the difference between two one-way fluxes. We could arrange conditions so that there could only be one one-way flux by setting one of the concentrations equal to zero. Thus if  $C_2$  were set to zero,  $J_{21}$  would also be zero. Conversely, if  $C_1$  were zero,  $J_{12}$  would also be zero. The ratio of these two specialized solutions gives rise to the Ussing criterion.

$$27) \quad \begin{aligned} C_1 &= \frac{J_{12}}{D} \int_0^l e^{\frac{ZF}{RT}E(x)} dx \\ C_2 e^{\frac{ZF}{RT}(E_2-E_1)} &= \frac{J_{21}}{D} \int_0^l e^{\frac{ZF}{RT}E(x)} dx \\ \frac{J_{12}}{J_{21}} &= \frac{C_1}{C_2} e^{-\frac{ZF}{RT}(E_2-E_1)} \end{aligned}$$

#### Goldman Constant Field Equations

This is another way of solving Equation 25 and one which allows direct prediction of the net flux. As discussed above, the

integral

$$\int_0^l e^{\frac{ZF}{RT}E(x)} dx$$

cannot be evaluated without knowledge of the form of  $E(x)$ . The constant field assumption forces  $E(x)$  into the form:

$$28) \quad E(x) = E_1 + (E_2 - E_1) \cdot \frac{x}{l}$$

where  $l$  is the thickness of the membrane.

The derivative term is then  $(E_2 - E_1)/l = \Delta E/l$ , the voltage difference divided by the thickness. This term can be inserted directly into Equation 25 and the integration carried out in a straightforward manner.

$$29) \quad \begin{aligned} \frac{dC}{dx} + \frac{ZF}{RT} \frac{\Delta E}{l} C &= -\frac{J}{D} \\ C_2 e^{\frac{ZF}{RT}\Delta E} - C_1 &= \frac{-J}{D} \int_0^l e^{\frac{ZF}{RT} \frac{\Delta E}{l} x} dx \\ &= \frac{-J}{D} \frac{RT}{ZF} \frac{l}{\Delta E} (e^{\frac{ZF}{RT}\Delta E} - 1) \\ 30) \quad \frac{\frac{D}{-l} \frac{ZF}{RT} \Delta E \left( C_1 - C_2 e^{\frac{ZF}{RT}\Delta E} \right)}{1 - e^{\frac{ZF}{RT}\Delta E}} \\ &= \frac{P \frac{ZF}{RT} (E_1 - E_2) C_1 \left( 1 - \frac{C_2}{C_1} e^{\frac{ZF}{RT}(E_2-E_1)} \right)}{1 - e^{\frac{ZF}{RT}(E_2-E_1)}} \end{aligned}$$

The derivation of the voltage equation comes from the statement that, in the steady state and in a situation of zero current, the sum of the cationic fluxes must

equal the sum of the anionic fluxes. This summation is only simple if all of the ions present (or which cross to any significant degree) are of the same valence. The derivation given below is for univalent ions.

$$\begin{aligned}
 31) \quad & \sum J_{\text{cation}} = \sum J_{\text{anion}} \\
 & \sum \frac{-P_{\text{cat}} \frac{ZF}{RT} \Delta E \left( C_1 - C_2 e^{\frac{ZF}{RT} \Delta E} \right)}{1 - e^{\frac{ZF}{RT} \Delta E}} = \\
 32) \quad & \sum \frac{-P_{\text{an}} \frac{ZF}{RT} \Delta E \left( A_1 - A_2 e^{\frac{ZF}{RT} \Delta E} \right)}{1 - e^{\frac{ZF}{RT} \Delta E}}
 \end{aligned}$$

In this case,  $Z$  can take values only of  $\pm 1$ , so we can eliminate  $Z$  and adjust the signs accordingly.

$$\begin{aligned}
 & \sum \frac{-P_{\text{cat}} \frac{F}{RT} \Delta E \left( C_1 - C_2 e^{\frac{F}{RT} \Delta E} \right)}{1 - e^{\frac{F}{RT} \Delta E}} = \\
 33) \quad & \sum \frac{P_{\text{an}} \frac{F}{RT} \Delta E \left( A_1 - A_2 e^{-\frac{F}{RT} \Delta E} \right)}{1 - e^{-\frac{F}{RT} \Delta E}}
 \end{aligned}$$

After multiplying the numerator and denominator of each term in the right

hand summation by the factor

$$e^{\frac{F}{RT} \Delta E}$$

this equality looks like:

$$\begin{aligned}
 & \sum P_{\text{cat}} \left( C_1 - C_2 e^{\frac{F}{RT} \Delta E} \right) \left( \frac{-\frac{F}{RT} \Delta E}{1 - e^{\frac{F}{RT} \Delta E}} \right) = \\
 34) \quad & \sum P_{\text{an}} \left( A_1 e^{\frac{F}{RT} \Delta E} - A_2 \right) \left( \frac{-\frac{F}{RT} \Delta E}{1 - e^{\frac{F}{RT} \Delta E}} \right)
 \end{aligned}$$

The last factor in each term is common and can be factored out. If we then collect the terms which contain the exponential in voltage on one side and those that lack it on the other side, we get:

$$35) \quad e^{\frac{F}{RT} \Delta E} (\sum P_{\text{cat}} \cdot C_2 + \sum P_{\text{an}} \cdot A_1) = \sum P_{\text{cat}} \cdot C_1 + \sum P_{\text{an}} \cdot A_2$$

Expressing Equation 35 in terms of the voltage as a function of the concentrations, and taking logarithms, we obtain the final result:

$$\begin{aligned}
 & \frac{F}{RT} \Delta E = \frac{\sum P_{\text{cat}} \cdot C_1 + \sum P_{\text{an}} \cdot A_2}{\sum P_{\text{cat}} \cdot C_2 + \sum P_{\text{an}} \cdot A_1} \\
 36) \quad & E_2 - E_1 = \frac{2.3 RT}{F} \log \left( \frac{\sum P_{\text{cat}} \cdot C_1 + \sum P_{\text{an}} \cdot A_2}{\sum P_{\text{cat}} \cdot C_2 + \sum P_{\text{an}} \cdot A_1} \right)
 \end{aligned}$$