ANOMALOUS REACTANCES IN ELECTRODIFFUSION SYSTEMS

JOHN SANDBLOM

From the Medical Physics Division, Donner Laboratory, University of California at Berkeley, Berkeley, California 94720. The author's present address is the Department of Physiology and Medical Physics, Biomedical Center, S-751 23 Uppsala, Sweden.

ABSTRACT A frequency response analysis of a constrained diffusion boundary has been made by linearizing the Nernst-Planck equations for a small applied AC current. The number of time constants and their dependence on ionic concentrations and electric field as well as membrane parameters such as dielectric constant, thickness, etc. have been evaluated by this method. Numerical solutions have been carried out for cases when the Planck charging time can be neglected and the results are presented in the form of impedance loci. These impedance loci show that if the membrane separates two univalent electrolytes with a common anion it will exhibit a combined capacitative inductive response with a 90° phase angle. The dependence of these anomalous reactances on ionic concentrations and the electric field is consistent with the behavior of the Hodgkin-Huxley axon suggesting that a homogeneous electrodiffusion regime could be adequate as a basic model for the kinetic behavior of biological membranes.

INTRODUCTION

As yet the most complete and accurate theoretical description of electrical properties of nerve cells and other excitable tissues is the Hodgkin-Huxley formulation, a set of differential equations relating electrical events to ion transfer processes. The precise physical mechanism underlying the changes in ionic conductances which the Hodgkin-Huxley theory describes has not yet been established, however, although a variety of physical models fitting either parts of or the whole of the Hodgkin-Huxley formulation have been proposed. Most of these models have been based on electrodiffusion phenomena, which is a natural consequence of the fact that all artificial membranes, whether ion-exchange membranes, organic liquids, bimolecular lipids, or solid state devices can be considered as electrodiffusion systems of one form or another. These models have been applied to biological membranes and in many cases successfully used to explain the stationary state properties (1, 2). Less successful, however, have been the attempts to relate the kinetic properties of biological membranes to those of an electrodiffusion regime, partly because of mathematical difficulties which have only permitted very special cases to be treated theoretically. One such special case, however, namely the frequency response of the system, lends itself to relatively simple theoretical analysis and will be dealt with in this paper in order to explain some important kinetic data of biological membranes.

Briefly defined, an electrodiffusion system consists of a medium containing charged particles which move under the influence of electrical and chemical gradients. The concentration of particles as a function of space and time is given by the following set of equations:

$$\frac{\partial C_i}{\partial t} = \frac{\partial}{\partial x} D_i \left(\frac{\partial C_i}{\partial x} + \frac{z_i F}{RT} \cdot C_i E \right), \qquad i = 1, \cdots, n, \tag{1}$$

$$\frac{\epsilon}{F} \cdot \frac{\partial E}{\partial x} = -\sum_i z_i \cdot C_i, \tag{2}$$

$$\frac{I}{F} = -\sum_i z_i D_i \frac{\partial C_i}{\partial x} - \frac{FE}{RT} \sum_i z_i^2 D_i C_i - \frac{\epsilon}{F} \cdot \frac{\partial E}{\partial t}, \tag{3}$$

where

C_i = concentration of particle i,
z_i = valence of particle i,
D_i = diffusion coefficient of particle i,
E = electric field, defined as the positive gradient of electrical potential,
I = electric current,
F = Faraday's constant,
R = gas constant,
T = absolute temperature,
ϵ = dielectric constant.

The first set of equations are the well-known Nernst-Planck electrodiffusion equations describing the migration of ions in ordinary electrolytes. Their validity in the space and time domain have been subject to discussion, in particular their applicability to thin biological membranes (3–5). Nevertheless, they have been applied to diffusion in thin lipid films of similar dimensions with experimental and theoretical justifications (6), and it seems reasonable to assume, therefore, that they also describe the diffusion processes taking place in biological membranes.

The second equation, called the Poisson equation relates the charge density to the divergence of the electric field and is a relationship of general validity in electrostatic theory.

The third equation, finally, can be obtained by adding equations 1, inserting them in equation 2, and then integrate with respect to x. This will yield equation 3 with I appearing as an integration constant and the last term being identified with Maxwell's displacement current. The advantage of introducing equation 3 in the theoretical formalism is the explicit appearance of the electric current, an easily measured parameter which is often applied as an constraint on the system. It is also logical, considering that equation 3 is obtained by integration, to regard the parameter I as a boundary condition of the system. The additional $2n$ boundary conditions then needed are usually given as particular constraints on the concentrations at the membrane boundaries or at the boundaries of the whole system. It is also apparent that since equation 3 is not independent of the other equations it does not introduce an independent time derivative. Therefore only n initial conditions need to be specified in order to solve the above set of equations, conditions such as the concentration profiles at a particular time.

Stated in the above form the electrodiffusion equations together with appropriate boundary conditions have been solved for a number of cases and various solutions have been applied to biological data. Although partly successful in this respect there are two important aspects in which the results have failed to describe the electrical properties of biological membranes.

The first is the stationary state conductance which in several excitable membranes exhibits a region of a negative slope and a dependence on voltage which rises much more steeply than the electrodiffusion equations predict. In order to account for these deviations within the framework of electrodiffusion theory it has been suggested that field effects or particular gating mechanisms (7, 8) could be responsible for these phenomena.

Secondly it has been stated that the time dependence of the equations, i.e., the transient response of a homogeneous electroduffusion regime does not agree with biological results (9, 10). From theoretical considerations of special cases it was also concluded that the number of separate relaxation processes as well as their contribution to the electrical response were not sufficient to explain the observed behavior (9). This behavior is best illustrated by Fig. 1 which shows the subthreshold transient response of a squid axon membrane and by Fig. 2 which shows the corresponding impedance locus. Both the transient and the frequency responses indicate that at least two reactances are present in the membrane.

At the time when these measurements were made it was realized that the inductance could not be of electromagnetic origin, partly because of its size and partly because it showed a strong dependence on ionic composition. Instead it was suggested by Cole (12) that the low frequency components of the impedance locus are actually the result of relaxation processes in a nonlinear system and the same rate processes that give rise to excitability. The low frequency components were therefore called *anomalous reactances* (12) and believed to be associated with dissipative processes in contrast to the conservative capacitative component of the higher dispersion region which was shown to be independent of the electric field and the ionic composition (13).

The question is then: can these anomalous reactances be due to electrodiffusion processes alone? The problem was stated and examined in detail by Cole (9) who obtained analytical solutions to the electroduffusion equations by making certain ap-

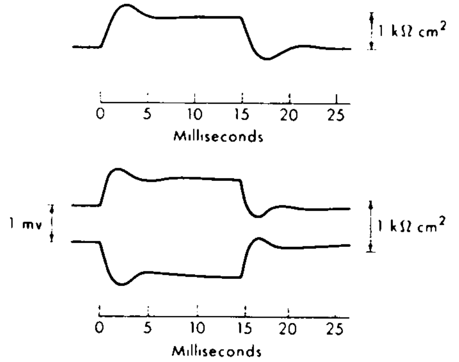

FIGURE 1

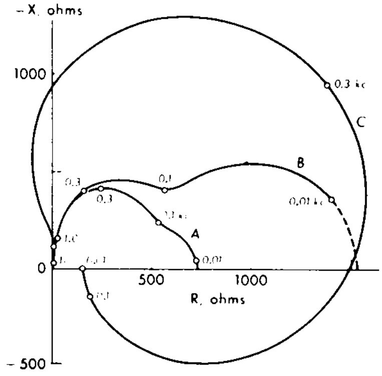

FIGURE 2

FIGURE 1 Transient electrical responses to applied current pulses; above as calculated from the Hodgkin-Huxley equations; below as recorded for a real axon. (From Cole [11]. Originally published by the University of California Press; reprinted by permission of The Regents of the University of California.)

FIGURE 2 Impedance locus of a squid axon membrane in seawater A, high calcium B, and low calcium C. (From Cole [11]. Originally published by the University of California Press; reprinted by permission of The Regents of the University of California.)

proximations. These, however, led him to conclude that there was only one anomalous reactance present in an electrodiffusion regime. Numerical solutions to the same set of equations were computed by Cohen and Cooley (14) for a constrained diffusion boundary but only in the form of responses to step changes in the electric currents. This investigation, however, indicated at least the possibility for the existence of multiple reactances in an electrodiffusion regime although no detailed information about the reactances can be obtained from transient responses which cover a nonlinear range and are difficult to interpret. Studies of the frequency response in the linear domain are therefore expected to yield more informative results. This paper presents such an analysis from which some conclusions about the nature of the anomalous reactances can be drawn.

THEORY

If an AC current is applied across a membrane, the potential response will in general depend on the frequency and the most convenient way of representing this frequency dependence of the potential response is to plot it in the phase plane as an impedance locus (see Fig. 2). In principle an impedance can be defined for any system, but in practice it is a parameter which is commonly used to characterize a linear system, or a nonlinear system in a linear domain. An impedance measurement must therefore be carried out in such a way that the applied AC current is small enough to maintain the response within a linear range of the system. For similar reasons the theoretical impedance locus of an electrodiffusion system is obtained by linearizing the equations with respect to small perturbations of the variables.

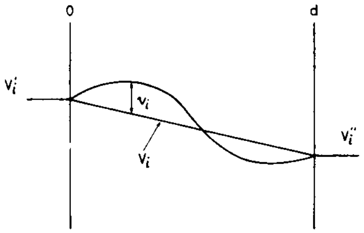

FIGURE 3 Constrained diffusion boundary with fixed (normalized) concentrations V_i and V_i at 0 and d, respectively. V_i is the steady-state concentration profile and v_i the corresponding perturbation.

If we consider a sinusoidal perturbation $i(t)$ superimposed on a constant applied electric current I_e the resulting perturbations in the concentrations and the electric field will also be sinusoidal which can be expressed as follows, using complex functions (see also Fig. 3)

$$\begin{aligned} I(t) &= I_e + i(t), & i(t) &= i_0 \exp j\omega t, \\ E(x,t) &= E(x) + e(x,t), & e(x,t) &= e(x) \exp j\omega t, \\ C_i(x,t) &= C_i(x) + c_i(x,t), & c_i(x,t) &= c_i(x) \exp j\omega t. \end{aligned}$$

$E(x)$ and $C_i(x)$ are the stationary state functions for which explicit expressions can be obtained, and $e(x)$ and $c_i(x)$ are the corresponding perturbations in general complex functions of x and ω.

In order to obtain differential equations with respect to the variables $e(x)$ and $c_i(x)$ the expressions for $I(t)$, $E(x,t)$, and $C_i(x,t)$ are inserted in equations 1–3 and second order terms are neglected. The resulting equations are then normalized in order to make the variables and the parameters dimensionless.

Normalization Table

$$\begin{aligned} V_i &= \frac{C_i}{\sum_i z_i^2 \bar{C}_i}, & \kappa &= \sqrt{\frac{F^2 \sum_i z_i^2 \bar{C}_i}{\epsilon RT}}, \\ \bar{E} &= \frac{Fd}{RT} \cdot E, & y &= \frac{F^2 e}{RT i_0} D_n \sum_i z_i^2 \bar{C}_i, \\ \frac{D_i}{D_n} &= r_i, & v_i &= \frac{F D_n c_i}{i_0 d}, \\ s &= j\omega \frac{d^2}{D_n}, & D &= d \frac{d}{dx}, \end{aligned}$$

d is the membrane thickness, κ is the inverse of the Debye length, and $\bar{C}_i$ is the mean concentration in the membrane.

After performing these operations the electrodiffusion equations 1–3 are transformed into a new set of equations

$$D^2 v_i + z_i D\bar{E} v_i - \frac{s v_i}{r_i} = -z_i D V_i y, \qquad i = 1, \cdots, n, \tag{4}$$

$$Dy = -(\kappa d)^2 \sum_i z_i v_i, \tag{5}$$

$$\frac{sy}{(\kappa d)^2} + y \sum_i z_i^2 r_i V_i = -1 - \sum_i r_i z_i D v_i - \bar{E} \sum_i z_i^2 r_i v_i. \tag{6}$$

This constitutes a linear set of ordinary differential equations which can be numerically solved when two n boundary conditions are given.[1]

The advantage of reducing the electrodiffusion equations into the linear form is that each relaxation process represented in the complex plane will give rise to a separate dispersion region and thereby more clearly indicate the influence of ionic concentrations and the electric field on the behavior of the relaxation processes. This will provide further physical insight into the system and point to some biologically interesting properties of electrodiffusion systems, as will be shown below for a few cases of interest.

Uniform Case

In a completely uniform system with equal concentrations on both sides of the membrane and in the absence of an externally applied electric field ($\bar{E} = 0$) the solutions to equations 4–6 become particularly simple. All V_i, and hence all the coefficients, are constants and the solutions are exponential functions given by

$$v_i = z_i V_i \sum_j A_j \frac{\sqrt{\lambda_j}}{\lambda_j - \frac{s}{r_i}} \sinh \sqrt{\lambda_j}\,\frac{1}{d}\left(x - \frac{d}{2}\right), \tag{7}$$

$$y = -\frac{1}{\sum_i z_i^2 r_i V_i + \frac{s}{(\kappa d)^2}} - \sum_i A_i \cosh \sqrt{\lambda_i}\,\frac{1}{d}\left(x - \frac{d}{2}\right). \tag{8}$$

In these equations the constants A_i can be determined by imposing symmetrical boundary conditions and the constants λ_i are roots of the following equation.

$$\sum_i \frac{z_i^2 V_i}{\lambda - \frac{s}{r_i}} = \frac{1}{(\kappa d)^2}. \tag{9}$$

From the number of roots of this equation we can conclude that at least n time con-

---

[1] Note that only $n + 1$ equations of the set 4–6 are necessary in order to obtain a solution.

stants corresponding to the n components are present in the system. In systems with electroneutrality on the other hand, i.e. when $(\kappa d)^{-1} = 0$, the number of time constants reduce to $n - 1$ as can be seen from equation 9. It is further apparent that the time constants are dependent on the concentrations as well as on the diffusion coefficients, the dielectric constant, and the thickness of the membrane.

Equation 8, which describes the behavior of the electric field in response to the applied AC current is seen to be composed of $n + 1$ terms. The physical significance attached to the first of these terms is a space change rearrangement and the time constant corresponding to this process is usually referred to as the Planck charging time (9). This phenomenon gives rise to the measured conservative capacitance. The remaining n terms in equation 8 are associated with the rearrangement of concentration profiles, and the time constants of these relaxation processes are called redistribution times (9). We therefore conclude from these results that although the electrodiffusion equations do not contain more than n time derivatives and n time constants it is physically meaningful to separate the corresponding processes into a charging time and n redistribution times, the latter giving rise to the anomalous reactances.

That such a statement does not lead to contradictory conclusions, however, can easily be shown for a particular case, namely the single ion case. The expression for λ given by equation 9 will yield a time constant which coincides with the charging time obtained from equation 8 when $n = 1$. For this case therefore the two processes are one and the same.

Constrained Diffusion Boundary

In order to examine the nature of the anomalous reactances and how they depend on the concentration of ions as well as the electric field, it is necessary to specify the boundary conditions. The case most commonly studied in artificial membranes and most often applied to biological membranes is that of fixed boundary concentrations, or the so called constrained diffusion boundary. The conditions specifying such a system can be stated mathematically, using the notations previously defined:

$$V_i(0) = V_i', \qquad V_i(d) = V_i'',$$
$$v_i(0) = 0, \qquad v_i(d) = 0.$$

Since our purpose is to investigate the anomalous reactances or the diffusion redistribution processes we shall neglect the charging time and assume that the quantity $1/\kappa d \approx 0$. This is equivalent to assuming that the membrane is thick in comparison with the Debye length.

Considering that a biological membrane possesses at least two anomalous reactances our calculations will be carried out for a system containing two electrolytes with a common anion as shown in Fig. 3. For further comparison with biological data we shall assume that one electrolyte is NaCl located on the outside (prime) and that the other is KCl located on the inside (double prime). The potassium ion is

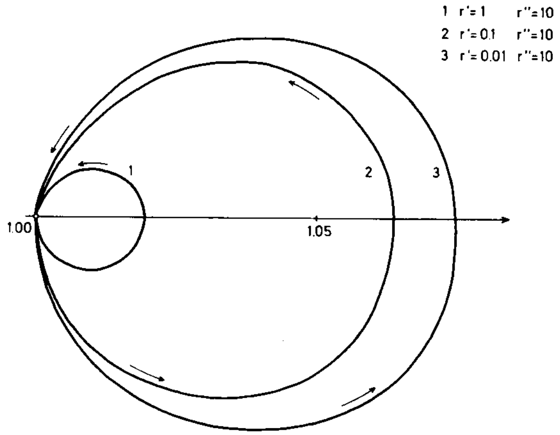

FIGURE 4 Impedance loci for equal external concentrations and with different values of the diffusion coefficients. r' and r'' are indicated in the figure and the arrows indicate the direction of increasing frequency. The bias current is set equal to zero.

assumed to be faster than the sodium ion and the chloride ion is arbitrarily chosen to be the nth ion (see the normalization table). The mobility of potassium and sodium ions are therefore denoted by r'' and r', respectively.

The differential equations 4–6 for this system are solved numerically and the impedance, which is simply given by the integral of y from 0 to d:

$$z = \int_0^d y \, \mathrm{d}x,$$

is computed and plotted in the form of an impedance locus. We also assign a special symbol p to the total membrane potential:

$$p = \frac{1}{d} \int_0^d \bar{E} \, \mathrm{d}x.$$

RESULTS

Fig. 4 shows the impedance loci for a membrane having equal concentrations on the two sides. Each locus corresponds to a particular set of diffusion coefficients as indicated in the figure. The bias current is set equal to zero. In this case the high frequency and low frequency resistances are equal and the locus shows the presence of an inductive and a capacitative reactance. The two reactances are also seen to increase as the differences in diffusion coefficients increase but an optimum is reached at about K:Cl:Na = 1:0.5:0.01.

Apart from the diffusion coefficients, however, there are two more parameters that can be varied in the system, namely the concentration difference on the two sides of the membrane and the applied electric field. Fig. 5 shows the effect of varying the

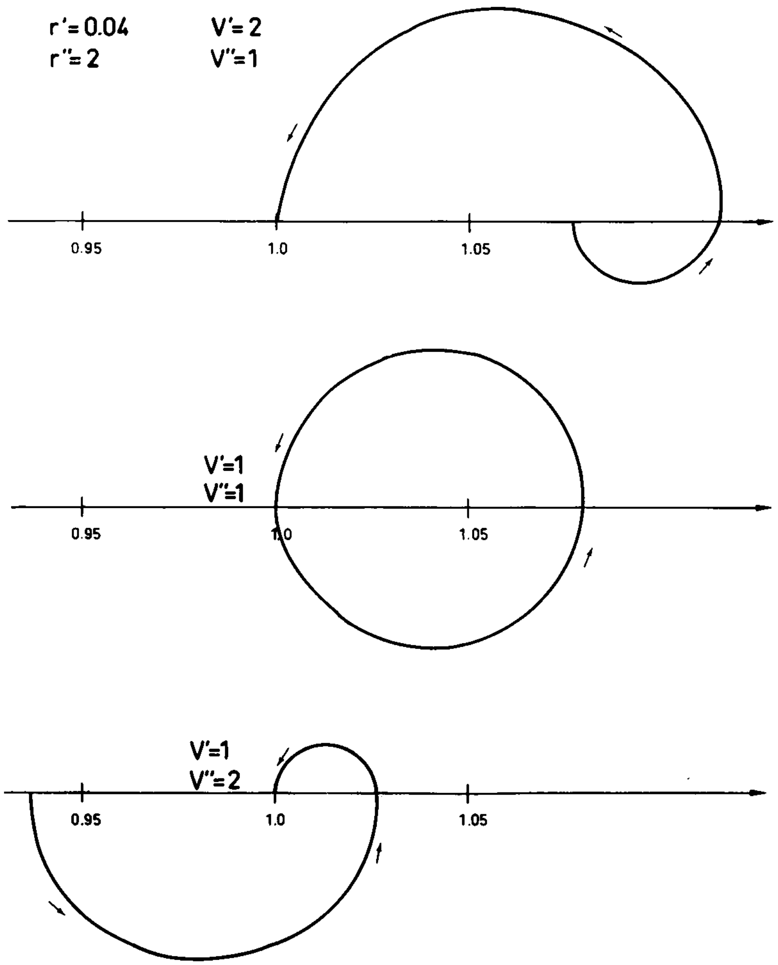

FIGURE 5 Impedance loci for different values of external concentration differences as indicated in the figure. The bias current is set equal to zero.

concentration difference while keeping the bias current equal to zero. It is seen that a decrease in potassium with respect to sodium increases the capacitative component, whereas an increase in potassium relative to sodium increases the inductive component.

This effect of ionic composition is entirely analogous to that observed in the squid axon membrane where a large amount of potassium on the outside increases the membrane inductance (11, p. 303) and where a decrease in potassium on the same side is usually followed by a decrease in the inductance with a gradual shift to a capacitative component. The transition takes place at the equilibrium potential for potassium, and the inductance in the squid axon is therefore normally attributed to

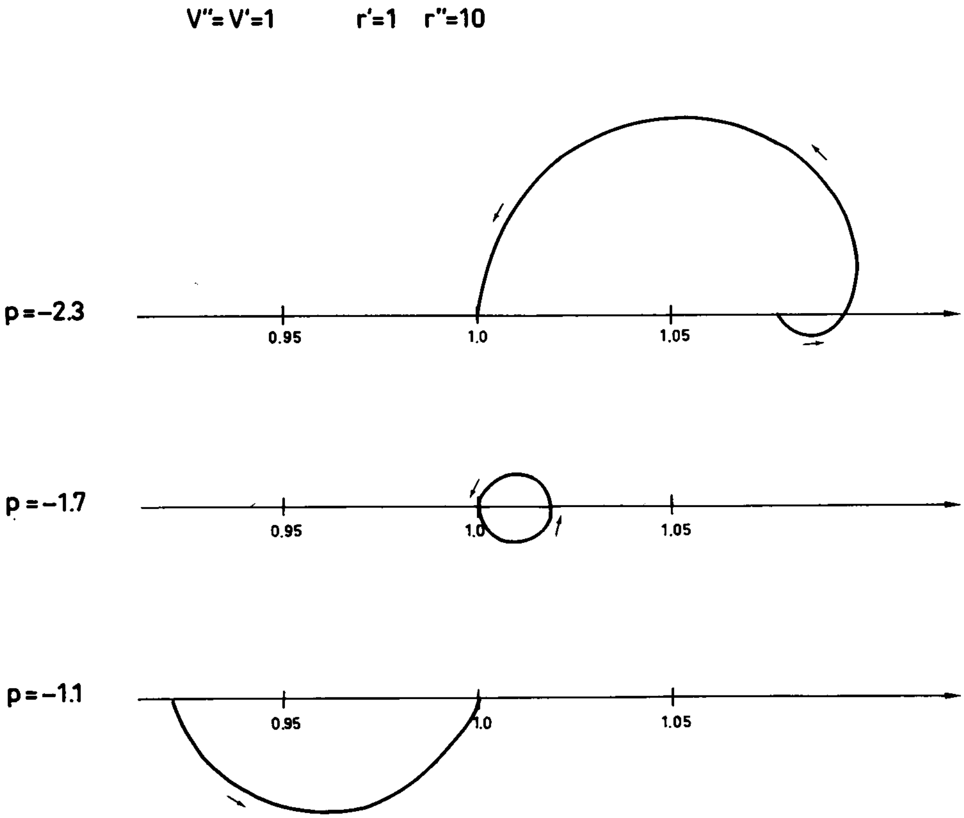

FIGURE 6 Impedance loci showing the effect of varying the electric field (expressed by p) by varying the bias current. The external concentrations are equal and the mobilities are indicated in the figure.

potassium. Of course it is not possible to attach such a significance to the properties of a simple electrodiffusion regime where the slower ion is always associated with the low frequency reactance, but the electric behavior with respect to ionic compositions is nevertheless exactly similar to the two systems.

The effect of varying the applied electric field, i.e. varying the bias current and keeping other parameters constant, is shown in Fig. 6. From this figure it is concluded that a *hyperpolarization* (making the double prime side more positive with respect to the prime side) has the effect of increasing the capacitative component whereas a *depolarization* (making the double prime side more negative with respect to the prime side) has the effect of increasing the inductive component. Again the behavior coincides with that of a squid axon membrane where a hyperpolarization increases the low frequency capacitative component whereas a depolarization leads to the appearance of a low frequency inductive component. Needless to say these impedance properties also agree with the Hodgkin-Huxley formulation (15).

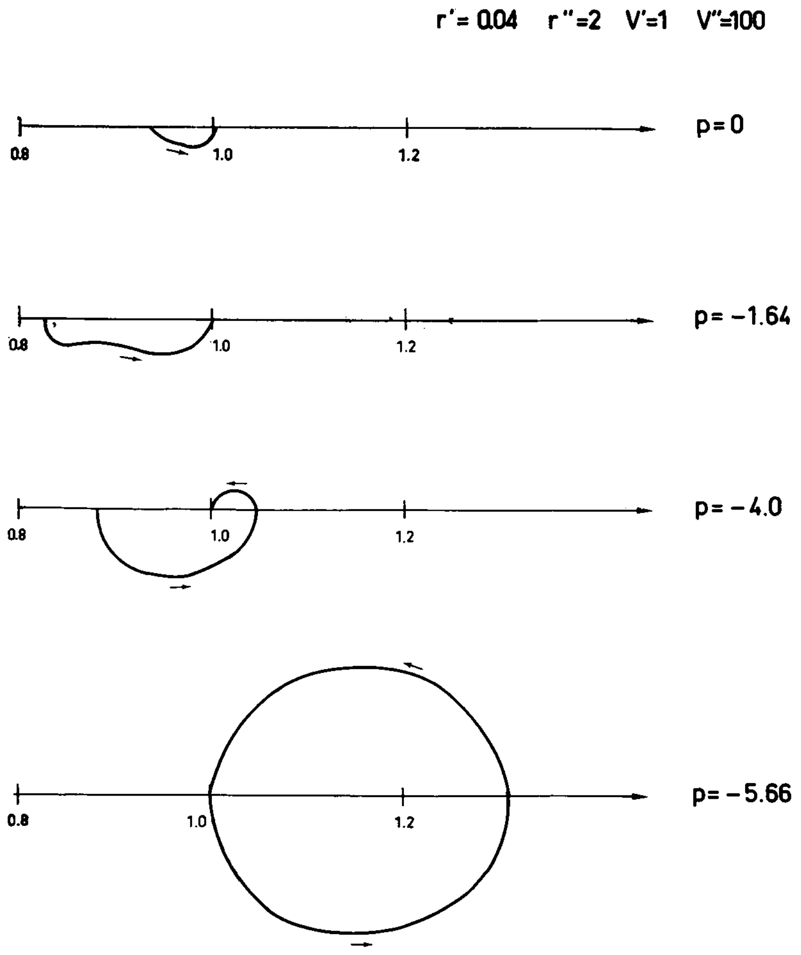

FIGURE 7 The effect of varying the electric field while maintaining a higher concentration on the right side of the membrane.

The two independent ways that we have used to alter the capacitative and the inductive components, namely by changing the ionic composition and by changing the electric field, can also be combined. Figs. 7 and 8 show the effects of changing these two parameters simultaneously and it is seen that every type of two-reactance network can be represented by this procedure. Since in addition r' and r'' determine the time constants we can conclude that a homogeneous electrodiffusion system consisting of three ions contains enough parameters to produce an electrical response which is equal to that of any given two-reactance network.

Another noteworthy feature of the anomalous reactances of an electrodiffusion regime is the 90° phase angle shown in all the figures and which is a consequence of having fixed concentrations at the boundaries. A depletion layer boundary, on the other hand, which occurs at electrode interfaces, in mobile site membranes (16), etc.,

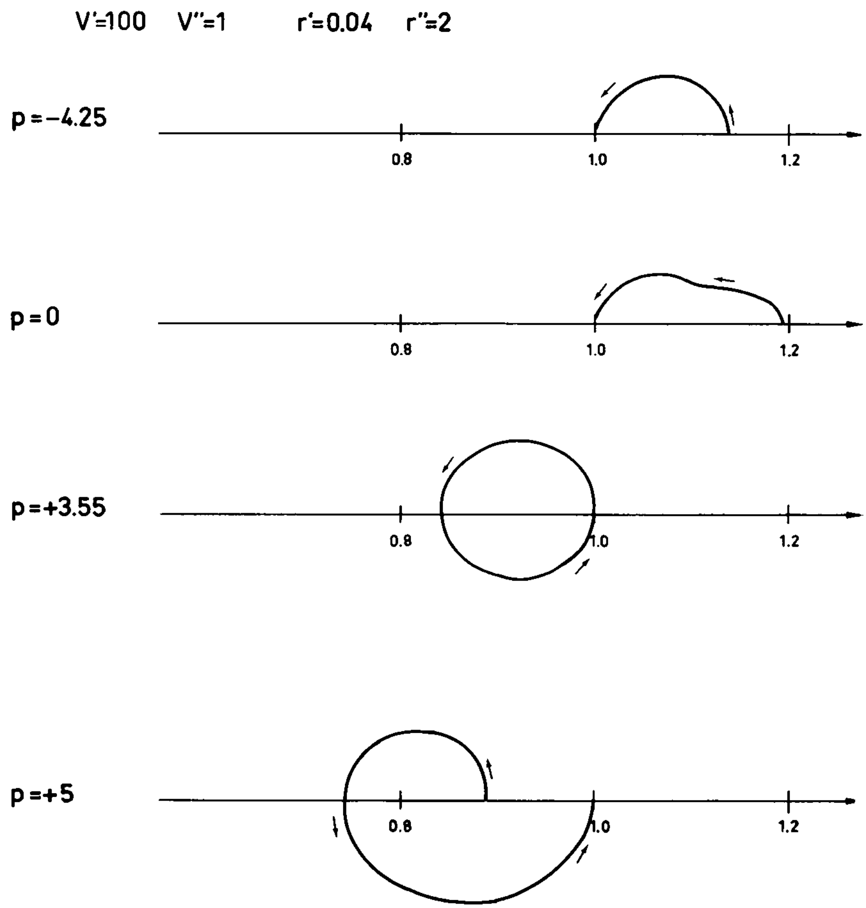

FIGURE 8 The effect of varying the electric field while maintaining a higher concentration on the left side of the membrane.

has been shown to exhibit a 45° phase angle. This is inconsistent with the Hodgkin-Huxley axon and is also inconsistent with biological data. It is certainly true that the phase angle determination of the anomalous reactances in biological membranes depends on a rather uncertain method of subtracting the conservative capacitance from the total impedance but nevertheless existing impedance data, as well as the fit of the Hodgkin-Huxley theory to the early inward current of a voltage clamp experiment, seem to support the 90° phase angle approximation used by Hodgkin-Huxley. We can therefore conclude that the frequency response of a homogeneous constrained diffusion boundary is consistent with that of the Hodgkin-Huxley axon.

DISCUSSION

We do not imply by this investigation that an electrodiffusion system in the simple form considered here is an adequate model for the biological membrane as a whole.

The many aspects, for example in which simple electrodiffusion fails to describe the biological membrane phenomena, were reviewed extensively by Cole (9), and in this paper we have only examined a single aspect of this problem. It is important, however, to establish the kinetic equations of electrodiffusion as a valid basis for describing biological data. A more complete description can then be made by proper modification of the basic model, for example by assuming gate controlling mechanisms, particular profile distributions, the presence of waterflow, etc.

There have been experiments to suggest that biological membranes contain different transporting systems for sodium and potassium, and pharmacological data have strongly confirmed such a hypothesis (17). A proposed logical consequence is a heterogeneous biological membrane which cannot consist of a homogeneous diffusion regime that we have assumed in this treatment. Recently, however, it has been shown that lipid bilayer membranes containing cyclic polypeptides provide some very specific mediators for cation transport, and this is a beautiful example of a homogeneous system containing different transport mechanisms for the different cations and which could conceivably be specifically blocked by pharmacological agents. The fact that the system constitutes a homogeneous electrodiffusion regime does not therefore preclude the possibility of having independent means of transport for different ions. Consequently, the treatment in this paper does not seem severely restricted and should be of general validity with respect to biological membranes. The method and the results presented here therefore suggest a possibility to transform a kinetic description of membrane phenomena from the phenomenological variables ionic conductances and permeabilities into the physically more meaningful parameters ionic conductances and permeabilities.

I wish to thank Professor K. S. Cole for encouraging me to carry out this investigation and for his many valuable suggestions throughout the work.
I also wish to acknowledge the skillful help of Mrs. Deanna Harrell in writing the program and carrying out the calculations.

This investigation was performed while the author was a visiting Assistant Professor in the Medical Physics Division, Donner Laboratory, University of California at Berkeley, Berkeley, California, and was supported by the United States Public Health Service, Biophysics Training Grant No. GM 82906.

Received for publication 3 December 1970.

REFERENCES

1. Goldman, D. 1943. *J. Gen. Physiol.* **27**:37.
2. Hodgkin, A. L., and B. Katz. 1949. *J. Physiol.* (*Lond.*). **108**:37.
3. Agin, D. 1967. *Proc. Natl. Acad. Sci. U. S. A.* **57**:1232.
4. Schlögl, R. 1969. *Q. Rev. Biophys.* **2**:305.
5. Neumche, B., and P. Läuger. 1969. *Biophys. J.* **9**:1160.
6. Eisenman, G., S. Ciani, and G. Szabo. 1968. *Fed. Proc.* **27**:1289.
7. Goldman, D. 1964. *Biophys. J.* **4**:167.
8. Offner, F. 1969. *Bull. Math. Biophys.* **31**:359.
9. Cole, K. S. 1965. *Physiol. Rev.* **45**:340.

10. Mauro, A. 1961. *Biophys. J.* **1**:353.
11. Cole, K. S. 1968. Membranes, Ions and Impulses. University of California Press, Berkeley.
12. Cole, K. S. 1941. *J. Gen. Physiol.* **25**:29.
13. Cole, K. S., and H. J. Curtis. 1938. *J. Gen. Physiol.* **22**:37.
14. Cohen, H., and J. Cooley. 1965. *Biophys. J.* **5**:145.
15. Hodgkin, A. L., and A. F. Huxley. 1952. *J. Physiol.* (*Lond.*). **117**:500.
16. Sandblom, J., J. L. Walker, and G. Eisenman. 1972. *Biophys. J.* **12**:587.
17. Hille, B. 1967. *J. Gen. Physiol.* **50**:1287.