# ION TRANSPORT THROUGH EXCITABLE MEMBRANES

by

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# ABSTRACT

In this thesis an investigation is given of the dynamical electrical properties of the excitable membrane without assuming that the ionic conductivities within the membrane are dependent on the electric field or time, and the extent to which a variable conductivity can be inferred from the statistical mechanics of irreversible processes.

The dynamical theory of ionized media is applied to the semi-electrolyte component of an excitable membrane, and the adjacent electrolytes. The relevant equations are the conservation laws of charge and momentum for the ions, and Poisson's equation for the electrostatic potential. These equations are linearized and are applied first to investigate the steady state of the membrane, and then transient effects in the membrane. A dispersion equation is derived, and the characteristic modes of relaxation within the membrane are determined. These include oscillatory modes which are associated with the condition that the dispersion equation has two equal roots, and which have frequencies and amplitudes of the correct order of magnitude to account for the observed excitation phenomena.

By applying the appropriate boundary conditions at the electrodes an expression for the voltage clamp current is derived, and its dependence on the properties of the intracellular and extracellular electrolytes and also the type of excitable cell is examined. An analysis of the oscillations in the potential difference across the membrane for a subthreshold stimulus is also given

by modifying the boundary conditions at the electrodes.

By taking into account the nonlinearity of Poisson's equation, a pair of coupled nonlinear equations in the ionic potentials is derived, and these have oscillatory solutions which are similar in shape to the action potential.

Although the previous analysis can account qualitatively for excitable cell behaviour without assuming that the ionic conductivity is dependent on the electric field within the membrane, it is necessary to assume such a dependence to explain the large steady state currents of certain ions which are observed during the voltage clamp of the axon membrane. Therefore we examine the extent to which such a variable ionic conductivity can be inferred from statistical mechanics, and finally consider the effect of irreversible processes on the Hamiltonian parameters $\lambda$ and $\mu$ which appear in the Triffet-Green model of the neuron.

# STATEMENT

This thesis contains no material which has been accepted for the award of any other degree, and to the best of my knowledge and belief, contains no material previously published or written by another person except where due reference is made in the text.

Samuel Robert Vaccaro.

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# CHAPTER 1 INTRODUCTION

## 1.1 THE DEVELOPMENT OF MODELS OF EXCITABLE MEMBRANE PHENOMENA

The characterization of the equilibria of ions in solution by Nernst[1] in terms of electrical and diffusive forces, and its subsequent application by Planck[2] to the computation of the potential across a constrained boundary between electrolytes, established the theoretical framework within which subsequent electrodiffusion models of the electrical properties of excitable membranes have developed. The Nernst concepts were first applied to biological phenomena by Bernstein[3] who assumed that:

(i) the living cell is composed of an electrolytic interior surrounded by a thin membrane which has a low permeability to ions

(ii) the resting potential across biological membranes is a diffusion potential resulting from the selective permeability to potassium and the large internal concentration of potassium ions relative to that in the extracellular fluid

(iii) during excitation the membrane is depolarized by a nonspecific increase of permeability to small ions.

This membrane theory was able to account for the sign and the approximate magnitude of the resting potential, its dependence on the external potassium concentration and, in addition, the approximate equality between the resting potential and the depolarization which occurred during excitation.

Fricke (see Cole[4]) confirmed Bernstein's first assumption and also measured the electrical capacity of the membrane

(1$\mu$f/cm$^2$). From this value and the assumption of a membrane dielectric constant of value 3, he estimated the thickness of the membrane to be of the order of 33Å. Following the determination of the conductance of the cell membrane in *Nitella*, transverse alternating current impedance measurements on the axon of *Loligo* in the resting state gave data that could be represented in terms of an electrical equivalent circuit consisting of a resistance and a capacity in parallel (Curtis and Cole[5]). It was then demonstrated that the action potential is associated with a transient decrease in the equivalent circuit resistance to a value of 25$\Omega$cm$^2$, with a small decrease in the membrane capacity (Cole and Curtis[6]). The resting state axon membrane resistance was determined from a longitudinal measurement of the direct current resistance and this yielded a value of 1000$\Omega$cm$^2$ (Cole and Hodgkin[7]).

This transient decrease in the resistance of the membrane was interpreted as a measure of the increase in membrane permeability to ions, and hence was initially considered to support Bernstein's hypothesis concerning membrane excitation. However, with the advent of microelectrodes, which could be inserted in the interior of the squid giant axon, it was possible to demonstrate that the action potential exceeded the resting potential by approximately 30mV, and so was not associated with a non-specific increase of permeability to ions, as had been postulated by Bernstein (Hodgkin and Huxley, Cole and Curtis[8]).

The electrical properties of the axon membrane were further characterized by determining its longitudinal alternating current impedance over a wide range of frequencies. Below a frequency of the order of 200 c.p.s., the impedance properties of the membrane could only be reproduced by assuming that its equivalent circuit contained a capacity shunted by a resistance and conductance (approximately .2 henry-cm$^2$) in series. This circuit was also able to account for the underdamped and overdamped oscillatory phenomena which were observed to occur during measurements of the AC transverse impedance changes resulting from the application of a polarizing current (Cole and Baker[9]).

The importance of the presence of extracellular sodium ions for propagation of the nervous impulse was first recognised by Overton. Many years later, it was shown that the magnitude of the action potential was reduced by partially replacing sodium chloride by choline chloride in the external solution. In order to explain this observation, as well as the transient reversal of the potential difference across the membrane during excitation, Hodgkin and Katz[10] proposed the sodium hypothesis, which postulated that the active membrane undergoes a large selective increase in permeability to sodium. It was also suggested that during the falling phase of the action potential, the high membrane selectivity for sodium ions was replaced by a large membrane permeability to potassium. This qualitatively explained the positive phase of the action potential, which is the decrease in active membrane potential below the resting potential, and the associated refractory state of the squid axon.

An important contribution to the elucidation of the ionic processes underlying the generation of the action potential was the development of the "voltage clamp" technique. An initial step in this direction was provided by Marmont[11], who proposed a method of quantitatively controlling and measuring the current uniformly across a known area of the axon membrane. He employed a long internal needle electrode of negligible resistance and a large concentric external electrode that is guarded, so that current flow in the axial direction is minimized. At any given instant, the active membrane response to a short threshold current stimulus is identical over the controlled area, and its magnitude and wave-form are similar to that of a propagated impulse (and hence is termed a space-clamped action potential).

Cole[11] employed a similar experimental arrangement to apply a constant uniform potential difference across the membrane ("voltage clamp"), by using an electronic feedback circuit to supply a current of appropriate magnitude to the electrodes, and the latter, in the absence of axial current, was identified with the current through the membrane. Despite efforts to reduce the surface impedance of the electrodes, for large membrane currents electrode polarization introduced considerable errors in the regulation of the membrane potential, and this limited the effective duration of the clamp.

Hodgkin, Huxley and Katz[12] minimized the errors associated with this difficulty by introducing two internal electrodes; one which recorded the membrane potential and the other which supplied the current to maintain the potential difference across the membrane at a pre-determined level (after being suddenly changed from the pre-clamp level). In addition, the effect of the resistance between the potential electrodes and the membrane was reduced somewhat by use of compensated feedback.

Hodgkin and Huxley[13] applied this improved voltage clamp technique to squid giant axons surrounded by solutions with a reduced sodium concentration and, on the basis of their results, were able to conclude that when the membrane is depolarized, the resulting ionic current consists of two independent components - an early transient current carried by sodium ions and a delayed current carried by potassium ions. They estimated the magnitudes and time courses of each of these components by making the following assumptions:

(1) the time course of the potassium current is unaffected by changes in the external sodium concentration

(2) the time course of the sodium current is not greatly affected by the external sodium concentration even though its magnitude and direction may change

(3) there is a delay in the rise of the potassium current for a period about one third of that taken by the sodium current to reach its maximum.

On the basis of the observations that the early current was zero when the voltage clamp potential was equal to the sodium equilibrium potential $E_{Na}$, and that the late current vanished when $V$ was equal to $E_K$, where $E_a$ is defined by

$$E_a = \frac{RT}{F} \ln\left(\frac{[a]_i}{[a]_o}\right) \quad \text{for} \quad a = Na, K ,$$

it was assumed that the driving force for the ath ion was constant and equal to $V - E_a$. The chord conductance for the ath ion, $g_a$, defined by

$$I_a = g_a (V - E_a) ,$$

was then considered to be a measure of the membrane permeability to a. It was convenient to express the voltage clamp sodium and potassium currents in terms of $g_{Na}$ and $g_K$, respectively, because the instantaneous values of these ionic conductances are independent of the magnitude of the voltage step (but only when the external solution is physiological saline).

The voltage and time dependence of $g_{Na}$ and $g_K$ was described (Hodgkin and Huxley[13]) by assuming

$$g_{Na} = \overline{g_{Na}}\, m^3 h$$

$$g_K = \overline{g_K}\, n^4$$

where $\overline{g_{Na}}$ and $\overline{g_K}$ are constants, and the variables m, n and h are functions of V and t and are given as solutions of the differential equations

$$\frac{ds}{dt} = \alpha_s(V)(1-s) - \beta_s(V)s , \quad s=m,n,h .$$

The rate constants $\alpha_s$ and $\beta_s$ are chosen to be analytical functions of the clamp voltage which give a close fit to the experimental data. These empirical equations were given a physical interpretation by assuming that the chord conductance for a particular ion depends on the distribution of mobile charged molecules confined to the membrane, and that a change in the potential difference across the membrane results in a new distribution and hence a "gating current". Specifically, it was assumed that the variables $m$ and $n$ are associated with "activation" of the sodium and potassium ions, respectively, and that the variable $h$ is associated with sodium "inactivation".

By assuming that the total membrane current is equal to the sum of the capacity current and the ionic current

$$I = c\,\frac{dV}{dt} + I_i \;,$$

that the ionic current is composed of sodium ions, potassium ions and leakage ions, and that current is continuous at the membrane surface, Hodgkin and Huxley used the empirical expressions for ionic conductance to compute the threshold, time course and amplitude of the space-clamped (or membrane) action potential, and its associated impedance changes, the exchange of sodium and potassium ions during the impulse, the electrical response during the refractory period, the anode break response, subthreshold oscillations and the form, amplitude and velocity of the propagated action potential, and obtained good agreement with experimental results.

Early confirmation of the Hodgkin-Huxley predictions of sodium and potassium fluxes during the voltage clamp and the action potential by tracer measurements (see Hodgkin[14]) was strengthened by a series of experiments in which the magnitude and time course of the sodium flux during the voltage clamp and the action potential was determined by direct measurements with tracers using more elaborate electronic equipment.[15] Also, the Hodgkin-Huxley equations have been extended and adapted to various smooth and myelinated nerves, skeletal and cardiac muscle with modified intracellular and extracellular solutions (for a review, see Cole[4]).

Following the unsuccessful attempts by Nernst[16] and others to invoke diffusion polarization at the membrane to explain excitation phenomena, consideration was given to developing mathematical models which reproduced important electrical properties of excitable cells. One approach is to define the model in terms of a number of variables of state (or factors) which correspond to neurophysiological concepts such as excitation and inhibition (Rashevsky[17]), local potential and threshold (Hill[17]) or state of excitation (Monnier[17]). The time constants of these factors are specified by linear first-order differential equations, from which various properties of nerve may be calculated. Young[17] showed that the Rashevsky and Hill models are equivalent and are special cases of a more general model.

Another approach is to model the nerve by an electric circuit and then derive differential equations by applying conventional circuit theory and an appropriate criterion for excitation. Rashevsky[18] applied the one-factor theory of Blair to a pure-resistance network to describe the spread of excitation in a nerve, whereas Rushton[19] assumed that excitation was associated with the sudden appearance of an E.M.F. in series with nerve sheath and applied this criterion to a resistance-capacitance network. The equivalence of this particular conduction model of Rashevsky and that of Rushton was demonstrated by Weinberg[20].

Offner, Weinberg and Young[21] developed another electric circuit conduction model by considering excitation to be accompanied by a decrease in membrane permeability (Bernstein hypothesis), which was consistent with the interpretation placed upon the impedance measurements on nerve (Curtis and Cole[5]). The expression for the conduction velocity so derived was similar to that of Rashevsky and Rushton.

The similarities between the properties of the passivated iron-wire and the nerve cell axon (Lillie[22]) were made more explicit by Bonhoeffer[23], who described the kinetics of the activation of an iron-wire by a set of two first-order differential equations in the variables "degree of activation" $(x)$ and "refractoriness" $(y)$. The functional form of the equations was specified graphically on an $x$ vs $y$ plane and with this representation, many of the iron-wire properties, including its activation and response to a stimulus, were described by trajectories on the phase-plane diagram. This model is a non-linear generalization of the Rashevsky-Monnier-Hill two-factor theories of nerve excitation.

By adding appropriate terms to a pair of first-order differential equations in two variables, obtained by transforming van der Pol's equation, Fitzhugh[24] constructed a model representative of a class of non-linear systems of equations which exhibits a stable state and threshold phenomena in addition to stable oscillations. The model was recognised to have a similar phase-plane form to those of the Benhoeffer iron-wire model, Teorell's model[25] of the fixed-charge membrane oscillations and a reduced Hodgkin-Huxley model obtained by projecting the four-dimensional Hodgkin-Huxley model onto a plane. A tunnel-diode equivalent circuit for the Fitzhugh model was proposed by Nagumo, Arimoto and Yoshizawa[26].

More recently, Triffet and Green[27] developed an electrochemical model of the neuron based upon a Hamiltonian formulation of the conservation laws and expressed in terms of a dynamical variable defined to be the number of ion pairs inside the nerve cell. The resulting equations can be reduced to modified forms of the van der Pol and Fitzhugh equations, and their solutions exhibit the presence of a resting state, a threshold potential and stable oscillations with a waveform appropriate to repetitive action potentials.

The simple electrodiffusion model (homogeneous membrane without fixed charge) of excitable membranes has been successful in calculating membrane potentials across biological membranes in the resting state, and their dependence on changes of ionic concentrations in the external medium, but solutions of the electrodiffusion equations have not exhibited a steady state negative conductance region, which is observed when the squid axon is immersed in iso-osmotic potassium chloride, and have not been able to account for the voltage clamp curves of excitable cells (see Cole[4]).

An analytical solution of the steady-state electrodiffusion equations (that is, the Nernst-Planck and Poisson equations) for a 1-1 univalent electrolyte was first obtained by Planck[2] by assuming that the microscopic electroneutrality condition is satisfied in the membrane phase, that is, that the total charge density at each point is equal to zero. The general solution to this problem for electrolytes with several ionic valence types was given by Schlögl[29]. The difficulties associated with the electroneutrality assumption have been considered by Agin[30], and Arndt, Bond and Roper[30] have given a criterion for this solution to be a good approximate solution.

Goldman[31] also obtained an analytical solution of these equations by assuming that the electric field was constant within the membrane. Hodgkin and Katz[32] rederived this result by introducing explicitly for each ion a concentration partition coefficient between the membrane and aqueous phases. The validity of this assumption has been examined by Zelman, Agin, and Zelman and Shih[33]. It has also been shown that the electroneutrality and constant field assumptions are limiting cases which obtain when the ratio of the Debye length in the membrane to the membrane thickness is $<< 1$ and $>> 1$, respectively. (Cole[4], MacGillivray[34], MacGillivray and Hare[34]).

Offner[35] has solved the steady state electrodiffusion equations directly as a boundary value problem by use of a difference equation method, and has considered the effect of the spatial dependence of the mobility in the membrane on ion flow, ion distribution and boundary potentials. On the other hand, Michaelis and Chaplain[36] have obtained asymptotic solutions of an equation which was derived from the electrodiffusion equations by use of the Painlevé transformation, and these coincide with the numerical solutions in a given domain.

Walz et al., Neumcke and Läuger, and Neumcke et al.[37] have examined three mechanisms by which nonlinear current-voltage characteristics could arise in lipid bilayer membranes:

(i) the injection of ions into the membrane resulting from the dependence of the Boltzmann concentration profiles on the applied potential difference across the membrane.

(ii) the effect of an electric field on the potential energy profile of an ion in the membrane.

(iii) the Wien dissociation effect within the membrane.

However, these mechanisms are in general more appropriate to the consideration of lipid soluble ions than to the transport of ions through nerve membranes. The latter has been examined by Bass[38] in considering the significance of electrodiffusion theory in predicting both linear and nonlinear instantaneous current-voltage characteristics in squid and toad, respectively.

Although it has not been demonstrated that a steady-state negative conductance region is a consequence of the macroscopic homogeneous electrodiffusion equations, a kinetic theory approach to the transport of ions across membranes which has been adopted by Mackey[39], has shown that for certain types of interactions between ions and the membrane molecules the slope conductance becomes negative for a range of electric field strengths.

Numerical solutions to the time-dependent electrodiffusion equations (that is, the Nernst-Planck, the Poisson and the continuity equations) were obtained by Cohen and Cooley[40] by assuming microscopic electroneutrality. The response of the membrane system to a step-function rise in the current was expressed as space and time variations in the concentrations and electric potential. Their solutions were similar to the responses obtained from systems with a combination of inductive and capacitative properties. Arndt and Roper[41] considered the general voltage clamp problem and obtained numerical solutions in terms of the current densities, but they do not exhibit a transient negative resistance for electrolytes with a variety of ionic concentrations and mobilities and, in particular, for those which exist in the environment of the squid axon membrane.

The diffusion of ions across the membrane may also be described as a series of jumps across potential barriers. This description has been used by Offner[42] to obtain a numerical solution of the voltage clamp problem. By choosing a fixed charge distribution and mobility contour within the membrane, which results in an appropriate dependence of boundary potential on total membrane potential, and suitable ion permeability functions of boundary potential and external interface calcium concentration, then it is possible to model many of the observed properties of nerve cells. In addition, an attempt has been given to justify the particular choice of the boundary permeability functions in the context of the theory of rate processes.

It is clear that on the one hand the empirical Hodgkin-Huxley model of current flow across the axon membrane has been successful in explaining a wide range of electrical properties of nerve but their equations are rather cumbersome. This has been remedied to some extent by the FitzHugh model which although it has similar properties to the Hodgkin-Huxley model, its mathematical form is more tractable. It has the additional virtues of being a nonlinear extension of the Rashevsky-Monnier-Hill two-factor theories of nerve excitation and also a generalization of the van der Pol equation which describes the relaxation oscillations of the heart.[43]

On the other hand, the simplest physical model of the excitable membrane as a homogeneous regime governed by the laws of ionized media, although giving a good description of various steady state properties, has not been successful in accounting for the dynamical phenomena associated with excitable cells. The discrepancies between the analytical steady state solutions and experimental results have been attributed to the existence of a biochemical pump mechanism for certain ions (e.g. sodium). However, its operation is over a longer time scale than one millisecond and hence is not considered important in the consideration of the action potential.

An indication of a possible connection between these mathematical and physical approaches was provided by Triffet and Green[27] who formulated an expression for the Hamiltonian of the neuron and its environment and used this to derive a set of dynamical equations which had the same form as the FitzHugh equations. A more detailed consideration of the physical processes involved in the transport of ions through the membrane will show (see Chapter 4) that similar equations can be derived from the macroscopic conservation laws of charge and momentum and the laws of electrostatics and thermodynamics but the interpretation of the dynamical variable in the Triffet-Green equations as the number of ion pairs within the neuron needs to be revised. The macroscopic equations obtained in this way can be based on either classical or quantum statistical mechanics. The transition from classical to quantum mechanics can be made formally by replacing the classical velocity distribution function by a Wigner phase space distribution function. A detailed consideration of quantal effects will be given in Chapter 5.

In sections 1.2 and 1.3 we shall consider various ionic theories that have been developed to describe the equilibrium properties of electrolytes and some of the general problems in the microscopic theory of transport processes which are relevant to the statistical derivation of macroscopic equations for ionic systems. Expressions for relevant thermodynamic quantities and the conservation laws of charge, momentum and energy, which will be used to determine the normal modes of the excitable membrane in Chapter 2, are derived in 1.4 and 1.5.

## 1.2 STATISTICAL MECHANICS OF IONIC SOLUTIONS IN EQUILIBRIUM

The first successful molecular theory of electrolyte solutions was developed by Debye and Hueckel[44] by combining Poisson's equation from electrostatics with the Boltzmann factor from statistical mechanics. The resulting equation was then linearized to yield a differential equation which was solved to calculate the thermodynamic properties of the electrolyte. It was subsequently shown by Fowler and Onsager[45] that the Debye-Hueckel theory is exact in the limit of small concentrations. Although the theory has been substantiated by comparison with numerous experiments, its applicability is limited to concentrations less than .01M for aqueous 1-1 electrolytes.

This small range of application is the result of the linearization procedure as well as the inconsistency of the nonlinear Poisson-Boltzmann equation itself (Kirkwood and Poirier[46]). The inconsistency in this equation derives from equating the potential of average force between the ions in solution with the mean electrostatic potential around each ion.

An important contribution to the theory of electrolytes was provided by the reformulation of the cluster expansion theory of nonideal gases for ionic solutions by McMillan and Mayer[47]. The mathematical difficulties associated with the long-range nature of the Coulomb force were overcome by an appropriate rearrangement and cancellation of divergent terms in the cluster expansion (Mayer[48]), with the lowest order term corresponding to the Debye-Hueckel theory. The results which were obtained compared favourably with experiment in the concentration range up to .1M for 1-1 electrolytes (Poirier[49]; Meeron[49]). However, the convergence of the Mayer expansion for aqueous electrolytes is slow, so that estimates of the thermodynamic properties of an electrolyte at higher concentrations are difficult to obtain.

Further progress in the theory of electrolytes was provided by developments in the theory of fluids in obtaining integral equation approximations for the radial distribution function. The first equations of this type to be introduced were the Kirkwood and Born-Green approximations which were derived by use of the Kirkwood and Born-Green-Yvon hierarchy of equations, respectively, in conjunction with the superposition approximation (Kirkwood[50]; Born and Green[50]). By expressing the two-particle velocity distribution function as a functional of the one-particle distribution function Bogoliubov[51] also obtained the Born-Green approximation from the first hierarchy equation and used it to verify the Debye-Hueckel limiting laws.

The radial distribution function may also be determined from the hypernetted chain (HNC) and Percus-Yevick (PY) integral equations. These equations may be derived using functional Taylor series expansions (Percus[52]) or graphical methods (Morita and Hirioke[53]) but may also be based upon the Ornstein-Zernike[54] equation

$$g_{ij} - 1 = c_{ij} + \sum_{k} n_{k} \int (g_{ck} - 1) c_{kj} \, d_{rk}$$

which can be considered as a definition of the direct correlation function $c_{ij}$ (between ions $i$ and $j$) in terms of the radial distribution function $g_{ij}$ ($n_k$ is the number density of the $k$-th ion). This system of integral equations can be closed by the HNC approximation

$$c_{ij} = g_{ij} - 1 - \ln g_{ij} - \beta u_{ij}$$

or the Percus-Yevick approximation

$$c_{ij} = g_{ij}[1 - \exp(\beta u_{ij})]$$

where $u_{ij}$ is the pair potential between ions $i$ and $j$ and $\beta = 1/kT$.

These equations have been reformulated by Allnatt[55] in an attempt to overcome the difficulties associated with the long-range nature of the Coulomb face. When these modified equations (which replace the Coulomb potential by the Debye-Hueckel shielded potential) were applied to a model of the electrolyte which consists of hard spheres in a dielectric continuum (primitive model) and the thermodynamic properties were calculated and compared directly with Monte Carlo results, the modified HNC equation was determined to be more appropriate (Rasaiah and Friedman[56]).

The mean spherical model (MSM) integral equation for the primitive electrolyte (Waisman and Lebowitz[57]) is obtained by supplementing the Ornstein-Zernike equation with the approximations

$$g_{ij}(r) = 0 \ , \quad r < R_{ij}$$

$$c_{ij}(r) = -\beta e_i e_j/\varepsilon r \ , \quad r > R_{ij}$$

where $R_{ij}$ is the distance of closest approach between ions i and j. Although it is more approximate than the PY and HNC integral equations, it has the advantage of being exactly soluble for the restricted primitive model (that is, charged hard spheres of equal size) and hence yields analytic expressions for the thermodynamic quantities. Also, the osmotic and activity coefficients computed from the energy equation were in good agreement with Monte Carlo results for 1-1 electrolytes.

Another recent development is the mode expansion theory of Andersen and Chandler[58]. They avoided the divergence difficulties arising from the long-range nature of the Coulomb potential by expressing the partition function in terms of collective variables which were chosen to be Fourier transforms of the number density (c.f. the Böhm-Pines[59] method for the electron gas). Also the divergences at small r were overcome by considering the Coulomb interactions as perturbations to a reference system which is dominated by short range repulsions. The free energy was expanded in an infinite series in which each term is determined by the perturbing potentials and the n-body distribution functions for the reference system, and convergence was enhanced by a suitable choice of the perturbation within the hard core. The calculation of just the first two terms of this series for the symmetrical restricted primitive model required only the free energy and the pair distribution function for the reference system, and the results have an accuracy comparable with the modified HNC approximation for monovalent ions.

They also described a criterion for optimizing the convergence of the mode expansion which ensured that the radial distribution function inside the hard spheres was zero. When the criterion was applied to the perturbation series truncated at $n=1$ (the optimized random phase approximation) the result was shown to be equivalent to the mean spherical model if the reference system obeys the Percus-Yevick Theory exactly (Andersen and Chandler[58]). An improvement over the mean spherical model was then obtained by including the next mode (termed the ORPA+$a_2$ theory).

Although the accuracy of the ORPA+$a_2$ approximation is good for most 1-1 electrolytes, when it is applied to 2-2 electrolytes, the osmotic coefficient does not exhibit the anomalous effects at high dilution which are shown by Mayer's theory of electrolytes (Debye-Hueckel limiting law + renormalized second virial coefficient) or the modified HNC approximation (Rasaiah[60]). This has been remedied[61] by transforming the Mayer cluster series for the Helmholtz free energy and the pair distribution function into a form involving a renormalized potential which is just the shielded Debye potential if the perturbation potential is chosen to be Coulombic within the hard cores. The convergence for this choice of perturbation is prohibitively slow at higher concentrations but can be considerably improved if instead the perturbation is chosen so that the renormalized potential is zero within the physically inaccessible regions. In this case, there is good agreement with Monte Carlo calculations of the internal energy for a 2-2 symmetric primitive model electrolyte of concentration up to 2M.

## 1.3 NONEQUILIBRIUM STATISTICAL MECHANICS OF IONIZED AND UN-IONIZED FLUIDS

Hodgkin and Huxley described their experimental results in terms of conductances which may undergo large transient variations during the voltage clamp or the action potential, and interpreted the membrane ion conductance as a measure of the ionic mobility. We shall show in Chapters 3 and 4 that even if the mobility in the membrane is assumed to be constant, the magnitude of ionic currents across the membrane may still be large during excitation because of the nonlinearity which appears in the time-dependent electrodiffusion equations. However, it is important to determine the extent to which a large variable ionic mobility can be inferred from the statistical mechanics of irreversible processes, and this investigation will appear in Chapter 5. The application of irreversible statistical mechanics to other physical systems has given no hint of such behaviour.

In this section, we shall consider the various approaches in nonequilibrium statistical mechanics for ionized and un-ionized fluids and, in particular, the derivation of kinetic and macroscopic transport equations from the equations of molecular dynamics, which will be examined in more detail for an electrolyte in Section 1.5, and the calculation of transport coefficients in terms of microscopic variables which will be applied to the resistance of the membrane in Chapter 5.

The Boltzmann equation, a kinetic equation which describes the properties of a monotomic dilute gas, was first solved by Chapman and Enskog (see Chapman and Cowling[62]) who used its normal solutions to derive the Navier-Stokes and Burnett equations. The method depends on obtaining a series approximation to the distribution function and then truncating the series when the gradients of the dynamical variables are small. Good agreement was obtained between their calculation of transport coefficients and experimental values for dilute gases.

Early attempts to generalize the Boltzmann equation by Born and Green, Bogoliubov and Kirkwood[50,51] depended on the derivation of hierarchy equations from Liouville's equation. A kinetic equation was then obtained by introducing an appropriate approximation to truncate the infinite set of equations - either the superposition approximation or the functional ansatz of Bogoliubov. An examination of the latter approximation using cluster expansion techniques has revealed divergences in the density expansions of the transport coefficients (see Cohen[63]) and these have been attributed to collision sequences occurring over an infinite volume in phase space. In order to eliminate a large class of divergences, resummation methods have been introduced and these result in a logarithmic dependence of the transport coefficients on density.

When the Boltzmann equation and its higher density generalizations are applied to plasmas the collision integral diverges because of the long range of the Coulomb force interaction. This divergence may be avoided by replacing the Coulomb potential by the equilibrium Deybe-Hueckel screened potential, but a more rigorous kinetic equation which takes into account screening effects and which can be derived from the Bogoliubov ansatz has been given by Balescu and Lenard[64]. For a plasma of low density where collisions of intermediate range are important, it can be reduced to the Landau equation.

Instead of obtaining the macroscopic equations for a fluid from a kinetic equation an alternative method is to proceed directly from Liouville's equation. This approach has been adopted by Irving and Kirkwood[65] in deriving the equations of continuity and the equations of momentum and energy conservation for a classical system of particles interacting through two-body short-range forces, and hence is not applicable to a plasma. In a similar way, Brittin[66] derived the hydromagnetic equation and Maxwell's equations for a system of charged particles interacting via the electromagnetic field. H.S. Green[67] has also obtained a complete set of macroscopic equations which overcomes some of the difficulties associated with the long-range nature of the Coulomb force, and in particular, a form of the generalized Ohm's law which is correct when thermoelectric effects are not small.

Expressions for transport coefficients based upon solutions to a kinetic equation were first obtained by Chapman and Enskog[62] by constructing normal solutions as expansions around the local equilibrium state and substituting back into the Boltzmann equation. In this way, the calculation of transport coefficients is reduced to the solution of two linear integral equations in terms of Sonine polynomials. However, the range of validity of this method is restricted to dilute gases with particles interacting through short-range forces, and hence is not suitable for plasmas.

A more recent method of deriving expressions for the transport coefficients is due to Resibois[68] and depends on establishing a one-to-one correspondence between the normal modes of a linear kinetic equation and its corresponding macroscopic equation. When applied to the Boltzmann equation, it yields the same results as the Chapman-Enskog theory, but is in general applicable to fluids of arbitrary density.

An alternative method of obtaining transport coefficients, without using a kinetic equation, was developed by M.S. Green[69] and Kubo[70]. Here, the transport coefficient is expressed as the time integral of an equilibrium time correlation function by considering the response of an equilibrium system to either an external or thermodynamic force. The equivalence between this method and the kinetic equation approach to transport coefficients has been shown by H.S. Green[71] and Resibois[72].

The Green-Kubo formulae can be used to give a density expansion of the transport coefficients by developing an integral equation for the time-correlation function from Liouville's equation in one of two ways. The first uses the lowest order hierarchy equation to obtain an integral equation in the time variable which is analogous to the generalized Boltzmann equation[73]. The other method formulates the integral equation in terms of the Laplace transform of the time variable and makes use of the binary collision expansion[74]. Each of these methods leads to divergences in the naive density expansion of the transport coefficients but a resummation of the most divergent terms to all orders in the density gives a finite contribution (Kawasaki and Oppenheim[75]). An extension of the Zwanzig method to plasmas has been given by expanding the binary-collision operator in terms of short-range binary collision operators and Coulomb interaction operators and then removing the short-range and long-range divergence by appropriate resummation techniques (Bartis and Oppenheim[76]).

An important development in the understanding of transport coefficients was the discovery by Alder and Wainwright[77] of the slow decay of the self-diffusion correlation function for a hard sphere system through molecular dynamical calculations. It had been generally assumed on the basis of simple models like Brownian motion, that the autocorrelation functions decay exponentially but the long tails of the form $t^{-d/2}$ where $d$ is the dimension of the system, have since been accounted for theoretically by a number of different approaches.

One method of describing this phenomenon uses the equations of macroscopic hydrodynamics but is internally inconsistent because it assumes the existence of transport coefficients which are later shown to diverge (Ernst et al.[78]). Another treatment applies the generalized Langevin equation and its associated mode-mode coupling ideas to the transport problem and attributes the long time decay to a nonlinear coupling among the slowly varying hydrodynamic modes (Kawasaki[79]).

The kinetic theory of dense gases has also been successful in describing this phenomenon by analyzing the relevant many-body events and considering the long-wavelength limit (Dorfman and Cohen[80]). In this case, the description of the long time tail and the calculation of the logarithmic term in the density expansion of the transport coefficients both depend on the consideration of the same class of correlated binary collision events. Recent work on the viscous flow of a moderately dense system of hard spheres and disks has indicated that it is necessary to take into account the nonlinear effects of the velocity field in order to obtain a finite correction to the Boltzmann value of the viscosity coefficient (Ernst et al.[81]).

## 1.4 STATISTICAL THERMODYNAMICS OF ELECTROLYTES

In this section, we shall derive approximate expressions for the partial pressure and the chemical potential for each ion and these will be used in Section 2.1 to develop the dynamical equations for an ionized medium.

The thermodynamic properties of a system can be evaluated from the Helmholtz free energy $A$ and for a system which may exchange both particles and energy with its surroundings, $A$ can be expressed in terms of the grand canonical partition function $Z^*$ as follows:

$$A = - kT\log Z^* \qquad (4.1)$$

where $k$ is Boltzmann's constant and

$$Z^* = \sum_N \frac{1}{\pi_a N_a!} \int \cdots \int \exp(-\beta\phi(\underline{x}_1,\ldots,\underline{x}_N) \prod_{i=1}^{N} z_i d^3 x_i \qquad (4.2)$$

where $\sum\limits_N$ denotes $\sum\limits_{N_1=0}^{\infty} \sum\limits_{N_2=0}^{\infty} \cdots$

$N_a$ is the number of particles of the a-th type,

$z_i = (2\pi\, m_i/\beta)^{3/2} \exp(\beta\mu_i)$ is the activity, $m_i$ the mass and $\mu_i$ the chemical potential of the i-th particle respectively,

$\phi(\underline{x}_1,\ldots,\underline{x}_N)$ is the total potential energy of a group of $N$ particles whose centre of masses are at the points $\underline{x}_1,\ldots,\underline{x}_N$,

$\beta = 1/(kT)$.

The equation of state may now be obtained from equations (4.1), (4.2) and the thermodynamic relation between the pressure $p$ and the free energy $A$

$$p = -\left(\frac{\partial A}{\partial V}\right)_T \tag{4.3}$$

or, alternatively, it may be derived from Liouville's equation and an appropriate definition of the partial pressure tensor (see equation (5.46) of section 1.5). The result is

$$p = \sum_a (n_a kT - \frac{1}{6} \sum_b n_a n_b\, I_{ab}) \tag{4.4}$$

$$I_{ab} = \int [g_{ab}(r_{ab})-1] r_{ab} \varphi'_{ab}(r_{ab}) d^3 r_{ab} \tag{4.5}$$

$\varphi_{ab}'(r_{ab})$ is the derivative with respect to the distance $r_{ab} = |\underline{x}_a - \underline{x}_b|$ between particles of the $a$-th and $b$-th types, of their interaction energy $\varphi_{ab}(r_{ab})$, $g_{ab}(r_{ab})$ is the radial distribution function, and $n_a$ is the number density of the $a$-th particle.

The chemical potentials $\mu_a$ may be calculated from the radial distribution functions $g_{ab}$ in two different ways. The first was proposed by Kirkwood and Buff[82] and uses the result

$$\left(\frac{\partial n_a}{\partial \mu_b}\right)_{V,T,n_c} = \frac{m_b}{kT}(n_a \delta_{ab} + \sum_b n_a n_b \int [g_{ab}-1] d^3 r) \tag{4.6}$$

where the number densities $n_a$ are regarded as functions of the $\mu_b$, $V$ and $T$. Expressions for the chemical potentials follow by inverting the matrix

$$B_{ab} = \frac{\partial n_a}{\partial \mu_b} .$$

The second method is due in principle to Güntelberg[83] and assumes that the interaction energy $\varphi_{ab}(r_{ab})$ of one particular particle of type a at the point $\underline{x}_a$, with any other particle of type b at the point $\underline{x}_b$, is reduced by a factor $\theta_a$, where $0 < \theta_a < 1$. It may then be shown that if $g_{ab}(r_{ab}, \theta_a)$ is the radial distribution function for this particle, with its charge reduced by a factor $\theta_a$, and any other particle of the b-th type, then

$$\mu_a = kT \log\left(\frac{n_a}{n_a{}^0}\right) + \sum_b n_b J_{ab} \qquad (4.7)$$

where $n_a{}^0$ is a constant, and

$$J_{ab} = \frac{1}{2}\int\int_0^1 [g_{ab}(r_{ab}, \theta_a) - 1]\varphi_{ab}(r_{ab})d\theta_a d^3 r_{ab} \qquad (4.8)$$

We shall now evaluate the above expressions for the pressure and the chemical potential by using the Debye-Hueckel approximation[44]. That is, the effect of the solvent on the interactions between the ions is accounted for by introducing a dielectric constant $\kappa$ in the interaction between two ions

$$\varphi_{ab}(r) = \frac{e_a e_b}{\kappa\ r} \quad (a \neq 0,\ b \neq 0) \qquad (4.9)$$

where $e_a$ is the charge of an ion of the a-th type and the water molecule is denoted by the subscript zero. To take account of the finite size of the ions, we assume that equation (4.9) is only valid when $r \geqslant a_I$, where $a_I$ is the ionic diameter. Also, the pair correlation function $g_{ab}$

of ions of the a-th and b-th types is approximated by

$$g_{ab}(r) = 0 \,, \quad r < a_I$$
$$g_{ab}(r) = 1 - \beta\, \psi_{ab}(r) \,, \quad r \geqslant a_I \tag{4.10}$$

where $\psi_{ab}$ is correlation energy and satisfies

$$\nabla^2\psi_{ab}(r) = -\frac{4\pi e_b}{\kappa}\sum_c e_c n_c g_{ac} \tag{4.11}$$

The function $\psi_{ab}$ may be normalized by assuming that

$$\psi_{ab}'(a_I) = \varphi_{ab}'(a_I) = -\frac{e_a e_b}{\kappa\, a_I^2} \tag{4.12}$$

Then we have

$$\psi_{ab}(r) = \frac{e_a e_b}{\kappa(a_D+a_I)} \exp[-(\frac{r-a_I}{a_D})] \tag{4.13}$$

where $$a_D = (4\pi\beta \sum_a e_a^2 n_a/\kappa)^{-\frac{1}{2}} \tag{4.14}$$

is the Debye shielding length. To determine $g_{ab}(r,\theta_a)$ we replace $e_a$ by $\theta_a e_a$ in (4.11) and obtain

$$g_{ac}(r,\theta_a) = 1 - \beta\, \theta_a\, \psi_{ac}(r) \tag{4.15}$$

With the help of these results, we find from (4.5) and (4.8):

$$I_{ab} = \frac{4\pi\beta\, e_a^2\, e_b^2\, a_D^2}{\kappa^2 (a_D+a_I)} \tag{4.16}$$

$$J_{ab} = \frac{-2\pi\beta\, e_a^2\, e_b^2\, a_D^2}{\kappa^2 (a_D+a_I)} \tag{4.17}$$

and hence, from (4.4) and (4.7), we have

$$\mu_a = kT \log\left(\frac{n_a}{n_a^o}\right) - \frac{e_a^2}{2\kappa(a_D+a_I)} \tag{4.18}$$

and

$$p = \sum_a p_a \tag{4.19}$$

where $p_a$ is the partial pressure of the a-th ion and is given by

$$p_a = n_a kT - \frac{n_a e_a^2}{6\kappa(a_D+a_I)} \tag{4.20}$$

If a system comprising a thin membrane which separates two electrolyte solutions (denoted by the subscripts 1 and 2, say) is in a state of chemical and electrical equilibrium then, for each ionic component, the electrochemical potentials in the electrolytes are equal:

$$(\mu_a+e_a\varphi)_1 = (\mu_a+e_a\varphi)_2 \tag{4.21}$$

where $\varphi$ is the electrical potential. Hence, using the expression (4.18) for the chemical potential and assuming ideal-gas behaviour (that is, neglecting the first-order Debye term) and the absence of a temperature gradient, the difference in electrical potential across the membrane is

$$\varphi_2 - \varphi_1 = \frac{kT}{e_a} \log\left(\frac{n_{a1}}{n_{a2}}\right) \tag{4.22}$$

and this is well known to physiologists as the Nernst equation.

Although it has been shown that the Debye-Hueckel theory is the correct approximation to first order of a more exact set of integral equations (Kirkwood and Poinier[84], Mayer[48], Green[85]), the agreement between the theoretical expressions for thermodynamic quantities and those obtained experimentally is only satisfactory for small electrolyte concentrations. The range of validity of the theory has been extended to electrolytes with an ionic strength of about .1M by including correction terms as follows (Guggenheim and Turgeon[86], Scatchard[87]):

$$g_{ab}(r) = 1 - \beta\,\psi_{ab}(r) + \delta g_{ab}(r) \qquad (4.23)$$

$$g_{ab}(r,\theta) = 1 - \beta\,\theta_a\,\psi_{ab}(r) + \delta g_{ab}(r,\theta_a)$$

where $\delta g_{ab}(r)$ and $\delta g_{ab}(r,\theta_a)$ do not depend strongly on the ionic number densities $n_a$. Thus, equations (4.18) and (4.20) may be replaced by

$$\mu_a = kT\log\left(\frac{n_a}{n_a{}^o}\right) - \frac{e_a{}^2}{2\kappa(a_D+a_I)} + \sum_b \delta J_{ab}n_b \qquad (4.24)$$

where

$$\delta J_{ab} = \iint_0^1 \delta g_{ab}(r_{ab},\theta_a)\,d\theta_a\,d^3r_{ab}$$

and

$$p_a = n_a kT - \frac{n_a e_a{}^2}{6\kappa(a_D+a_I)} - \frac{n_a}{6}\sum_b \delta I_{ab}n_b \qquad (4.25)$$

By defining

$$\beta_a = kT - \frac{e_a{}^2}{6\kappa(a_D+a_I)} - \frac{1}{6}\sum_b \delta I_{ab}n_b \qquad (4.26)$$

equation (4.25) becomes

$$p_a = n_a/\beta_a \tag{4.27}$$

where $\beta_a = \beta = 1/(kT)$ in the limit of small concentrations, but depends weakly on the ionic densities for the concentrations attained in the membrane environment (of the order of 100mM).

## 1.5 MACROSCOPIC EQUATIONS FOR AN ELECTROLYTE

In this section, we shall derive a set of macroscopic conservation laws for an electrolyte from Liouville's equation following the approach of Green[67]. These equations will be used in Section 2.1 to analyze the dynamics of a general ionized medium and then applied in subsequent sections to the membrane region and the surrounding electrolytes.

As we shall be considering electrolyte solutions at normal physiological temperatures it is possible to neglect quantum effects except for the justification of the existence of a lower bound to the energy of a system of charged particles of opposite sign. Similarly, relativistic effects will be small and hence radiation damping can be neglected, and also it may be assumed that the interactions between neighbouring particles are instantaneous.

Consider a system of $N$ particles such that the number of particles of the $a$-th type (with mass $m_a$ and charge $e_a$) is $N_a$ $(a=1,2,\ldots)$ and $\sum_a N_a = N$. Then, given a function $G$ of the particle velocities and coordinates, the mean value of $G$ may be defined as

$$\langle G\rangle = \sum_N \int G\, F_N\, d\Omega_N \qquad (5.1)$$

where $\sum_N$ means $\sum_{N_1} \sum_{N_2} \ldots$

$F_N$ is the $N$-particle phase space distribution function

$\Omega_N$ is the normalized volume element in phase space.

From Liouville's theorem we may write

$$\frac{\partial F_N}{\partial t} + \sum_{i=1}^{N} \frac{\partial F_N}{\partial \underline{x}_i} \cdot \xi_i + \frac{\partial F_N}{\partial \underline{\xi}_i} \cdot \eta_i = 0$$

where $\underline{\xi}_i = \frac{dx_i}{dt}$ and $\underline{\eta}_i = \frac{d\underline{\eta}_i}{dt}$ are the i-th particle velocity and acceleration, respectively.

If G does not depend explicitly on the time then

$$\frac{\partial \langle G \rangle}{\partial t} = \sum_N \int \frac{\partial F_N}{\partial t} G \, d\Omega_N$$

$$= \sum_N \int [-\sum_i (\frac{\partial F_N}{\partial \underline{x}_i} . \underline{\xi}_i + \frac{\partial F_N}{\partial \underline{\xi}_i} . \underline{\eta}_i)] G \, d\Omega_N \qquad (5.2)$$

On integrating by parts and with the use of Gauss's theorem, we obtain

$$\frac{\partial \langle G \rangle}{\partial t} = \sum_N \int F_N \sum_i (\frac{\partial G}{\partial \underline{x}_i} \cdot \underline{\xi}_i + \frac{\partial G}{\partial \underline{\xi}_i} \cdot \underline{\eta}_i) d\Omega_N \qquad (5.3)$$

By setting $$G = \sum_i \delta_{ia} \, \delta(\underline{x}-\underline{x}_i) \delta(\underline{\xi}-\underline{\xi}_i) \qquad (5.4)$$

equation (5.3) becomes

$$\frac{\partial f_a}{\partial t} + \underline{\xi} \cdot \frac{\partial f_a}{\partial \underline{x}} + \frac{\partial}{\partial \underline{\xi}} \cdot (f_a \, \underline{\eta}_a) = 0 \qquad (5.6)$$

where $$f_a = \langle \sum_i \delta_{ia} \, \delta(\underline{x}-\underline{x}_i) \delta(\underline{\xi}-\underline{\xi}_i) \rangle \qquad (5.7)$$

is the velocity distribution function of a particle of the a-th type and is defined in such a way that $f_a d^3\underline{x} \, d^3\underline{\xi}$ is the probability of finding a particle of the a-th type in the volume element $d^3\underline{x}$, with velocity in the range $d^3\underline{\xi}$, and

$$f_a \, \underline{\eta}_a = \langle \sum_i \delta_{ia} \, \underline{\eta}_i \, \delta(\underline{x}-\underline{x}_i) \delta(\underline{\xi}-\underline{\xi}_i) \rangle \qquad (5.8)$$

and $\underline{\eta}_a$ is the mean acceleration of a particle of the a-th type at the point $\underline{x}$ and moving with velocity $\underline{\xi}$.

The velocity distribution function $f_a$ satisfies

$$\int f_a \, d^3\underline{\xi} = n_a \tag{5.9}$$

$$\int f_a \, \theta \, d^3\underline{\xi} = n_a \, \langle\theta\rangle_a \tag{5.10}$$

where $n_a$ is the number density of the a-th particle, and be written as

$$n_a = \langle \sum_i \delta_{ia} \, \delta(\underline{x}-\underline{x}_i) \rangle \, , \tag{5.11}$$

$\theta$ is a function of $\underline{\xi}$, the velocity of a particle of the a-th kind at $\underline{x}$, and $\langle\theta\rangle_a$ is the mean value of $\theta$.

Now, neglecting radiation damping

$$m_i \, \underline{\eta}_i = e_i(\underline{E}_i{}' + \underline{\xi}_i \times \underline{B}_i'/c) \tag{5.12}$$

where $\underline{E}_i{}'$ and $\underline{B}_i{}'$ are the microscopic electric field intensity and magnetic induction at the point $\underline{x}_i$, respectively. The corresponding macroscopic fields are

$$\underline{E}_i = \langle\underline{E}_i{}'\rangle \quad \text{and} \quad \underline{B}_i = \langle\underline{B}_i{}'\rangle \tag{5.13}$$

and the residual fields are defined as

$$\underline{E}_i{}^r = \underline{E}_i{}' - \underline{E}_i \tag{5.14}$$

$$\underline{B}_i{}^r = \underline{B}_i{}' - \underline{B}_i$$

We can now write equation (5.8) in terms of the field variables as

$$f_a \, \underline{\eta}_a = \Big\langle \sum_i \delta_{ia} \frac{e_i}{m_i} \left( \underline{E}_i' + \underline{\xi}_i \times \underline{B}_i'/c \right) \delta(\underline{x}-\underline{x}_i)\, \delta(\underline{\xi}-\underline{\xi}_i) \Big\rangle$$

$$= \frac{e_a}{m_a} \left( \underline{E} + \underline{\xi} \times \underline{B}/c \right) f_a$$

$$+ \Big\langle \sum_i \delta_{ia} \frac{e_i}{m_i} \left( -\nabla_i \varphi_i{}^r + \underline{\xi}_i \times \underline{B}_i^r/c \right) \delta(\underline{x}-\underline{x}_i)\, \delta(\underline{\xi}-\underline{\xi}_i) \Big\rangle \qquad (5.15)$$

with the use of equations (5.13) and (5.14), where $\underline{E}$ and $\underline{B}$ are the macroscopic values at the point $\underline{x}$, $\varphi_i{}^r = \varphi_i{}' - \varphi_i$ is the residual electric potential at the point $\underline{x}_i$ and $\varphi_i{}'$ and $\varphi_i$ are the microscopic and macroscopic electric potentials at the same point, respectively.

For particles not too distant from the point $\underline{x}_i$, retardation may be neglected and hence the microscopic electric potential $\varphi_i{}'$ is given by

$$\varphi_i{}' = \sum_j \frac{e_j}{|\underline{x}_i - \underline{x}_j|} + \varphi_i{}^0 \qquad (5.16)$$

where $\varphi_i{}^0$ is the external electric potential at $\underline{x}_i$, and the corresponding macroscopic variable is

$$\varphi_i = \langle \varphi_i{}' \rangle$$

$$= \Big\langle \iint \sum_b \sum_j \frac{e_j}{|\underline{x}_i - \underline{x}'|} \delta_{jb}\; \delta(\underline{x}'-\underline{x}_j)\, \delta(\underline{\xi}'-\underline{\xi}_j)\, d^3x' \, d\xi' \Big\rangle$$

$$= \sum_b \iint \frac{e_b \, f_b{}'}{|\underline{x}_i - \underline{x}'|} \, d^3x' \; d\xi' \qquad (5.17)$$

Also, using equation (5.16)

$$< \sum_i \delta_{ia} \frac{e_i}{m_i} (- \nabla_i \varphi_i ') \delta(\underline{x}-\underline{x}_i) \delta(\underline{\xi}-\underline{\xi}_i) >$$

$$= < \sum_i \delta_{ia} \frac{e_i}{m_i} \iint [- \sum_b \sum_j \nabla_i (\frac{e_j \delta_{jb}}{|\underline{x}_i-\underline{x}'|}) \delta(\underline{x}'-\underline{x}_j) \delta(\underline{\xi}'-\underline{\xi}_j)]$$

$$\times \delta(\underline{x}-\underline{x}_i) \delta(\underline{\xi}-\underline{\xi}_i) d^3x' \ d^3\xi' >$$

$$= - \sum_b \iint \frac{\nabla \varphi_{ab}}{m_a} f_{ab} \ d^3x' \ d^3\xi' \tag{5.18}$$

where
$$\nabla \varphi_{ab} = \frac{\partial}{\partial \underline{x}} (\frac{e_a e_b}{|\underline{x}-\underline{x}'|}) \tag{5.19}$$

and

$$f_{ab} = < \sum_i \delta_{ia} \ \delta(\underline{x}-\underline{x}_i) \delta(\underline{\xi}-\underline{\xi}_i) \ \sum_j \delta_{jb} \ \delta(\underline{x}'-\underline{x}_j) \delta(\underline{\xi}'-\underline{\xi}_j) > \tag{5.20}$$

is the two-particle velocity distribution function, and is defined in such a way that $f_{ab} \ d^3\underline{x} \ d^3\underline{x}' \ d^3\underline{\xi} \ d^3\underline{\xi}'$ is the probability of finding a particle of the a-th type in the volume element $d^3\underline{x}$, with velocity in the range $d^3\underline{\xi}$, and a particle of the b-th type in the volume element $d^3\underline{x}'$, with velocity in the range $d^3\underline{\xi}'$. It satisfies

$$\int f_{ab} \ d^3\underline{\xi} \ d^3\underline{\xi}' = n_{ab} \tag{5.21}$$

$$\int f_{ab} \ \theta \ d^3\underline{\xi} \ d^3\underline{\xi}' = n_{ab} \ <\theta>_{ab} \tag{5.22}$$

where $n_{ab}$ is the two-particle distribution function, and may be written as

$$n_{ab} = < \sum_i \delta_{ia} \ \delta(\underline{x}-\underline{x}_i) \ \delta_{jb} \ \delta(\underline{x}'-\underline{x}_j) > \tag{5.23}$$

and $\langle\theta\rangle_{ab}$ is the mean value of a function $\theta$ of the velocities $\underline{\xi}$ and $\underline{\xi}'$ of two distinct particles.

With the help of equations (5.17) and (5.18), we can now write

$$\langle \sum_i \delta_{ia} \frac{e_i}{m_i} (-\nabla_i \varphi_i^{\,r})\,\delta(\underline{x}-\underline{x}_i)\,\delta(\underline{\xi}-\underline{\xi}_i)\rangle$$

$$= -\sum_b \iint \frac{\nabla \varphi_{ab}}{m_a} (f_{ab} - f_a\, f_b')\; d^3x'\; d\xi' \qquad (5.24)$$

$$= \sum_b \iint \frac{e_a\, E_b}{m_a} (f_{ab} - f_a\, f_b')\; d^3x' d\xi'$$

Neglecting retardation, the microscopic vector potential $\underline{A}_i'$ is given by

$$\underline{A}_i' = \sum_j \frac{e_j\, \xi_j}{c|\underline{x}_i - \underline{x}_j|} + \underline{A}_i^{\,o} \qquad (5.25)$$

where $\underline{A}_i^{\,o}$ is the external vector potential at $\underline{x}_i$. Hence, with the relation

$$\underline{B}_i^{\,r} = \nabla \times \underline{A}_i^{\,r} \qquad (5.26)$$

it may also be shown that

$$\langle \sum_i \delta_{ia} \frac{e_i}{m_i} (\underline{\xi}_i \times \underline{B}_i^{r}/c)\;\; \delta(\underline{x}-\underline{x}_i)\,\delta(\underline{\xi}-\underline{\xi}_i)\rangle$$

$$= \sum_b \iint \frac{e_a\, \underline{B}_b}{m_a} (f_{ab} - f_a\, f_b')\; d^3x'\; d\xi' \qquad (5.27)$$

Equation (5.15) now becomes

$$m_a \, \underline{\eta}_a \, e_a = \underline{E} + \underline{\xi} \times \underline{B}/c$$

$$+ \sum_b \iint \left(\frac{f_{ab} - f_a \, f_b{}'}{f_a}\right) (\underline{E}_b + \underline{\xi} \times \underline{B}_b/c) \; d^3x' \; d^3\xi' \qquad (5.28)$$

We shall now derive the conservation equations for the number density and the momentum of a particle of the a-th type. The continuity equation follows immediately from equation (5.6) by integrating with respect to $\underline{\xi}$ and using equations (5.9) and (5.10):

$$\frac{\partial n_a}{\partial t} + \frac{\partial}{\partial \underline{x}} \cdot (n_a \, \underline{u}_a) = 0 \qquad (5.29)$$

where $\underline{u}_a = \langle \underline{\xi} \rangle_a$ .

The equation of motion may be obtained by multiplying equation (5.6) by the velocity $\underline{\xi}$ of a particle of the a-th type and then integrating over $\underline{\xi}$

$$\frac{\partial}{\partial t} (n_a \, \underline{u}_a) + \frac{\partial}{\partial \underline{x}} \cdot (n_a \, \langle \underline{\xi\xi} \rangle_a) = n_a \, \langle \eta_a \rangle_a \qquad (5.30)$$

Now, the assumption that relativistic effects are small allows the force between neighbouring particles, $e_a(\underline{E}_b + \underline{\xi} \times \underline{B}_b/c)$, to be approximated by the Coulomb force alone, and so equation (5.28) becomes

$$m_a \, \langle \eta_a \rangle_a = e_a(\underline{E} + \underline{u}_a \times \underline{B}/c)$$

$$+ e_a \sum_b e_b \int \frac{\nu_{ab}}{n_a} \left(\frac{\underline{r}}{r^3}\right) d^3r \qquad (5.31)$$

where $\underline{r} = \underline{x} - \underline{x}'$ and

$$\nu_{ab} = n_{ab} - n_a n_b' \tag{5.32}$$

To determine the contribution of the force $m_a <\eta_a>_a$ to the partial pressure of the a-th particle, write

$$\nu_{ab} = \nu_{ab}^{(+)} + \nu_{ab}^{(-)} \tag{5.33}$$

where

$$\nu_{ab}^{(+)} = \tfrac{1}{2}(\nu_{ab}+\nu_{ba}) \ , \quad \nu_{ab}^{(-)} = \tfrac{1}{2}(\nu_{ab}-\nu_{ba}) \tag{5.34}$$

By expressing $\nu_{ab}^{(+)}$ and $\nu_{ab}^{(-)}$ in terms of $\underline{y} = \frac{1}{2}(\underline{x}+\underline{x}')$ and $\underline{r} = \underline{x}-\underline{x}'$ then $\nu_{ab}(\underline{y},\underline{r})$ can be expanded in a Taylor series as follows

$$\nu_{ab}(\underline{y},\underline{r}) = \nu_{ab}(\underline{x},\underline{r}) - \tfrac{1}{2}\underline{r}\cdot\frac{\partial}{\partial \underline{x}}\,\nu_{ab}(\underline{x},\underline{r}) + \ldots \tag{5.35}$$

This series may be truncated at the first order term in $\underline{r}$ by assuming that the gradients of macroscopic quantities are small relative to the microscopic correlation length. With this expansion, equation (5.31) becomes

$$\begin{aligned} m_a <\underline{\eta}_a>_a &= e_a(\underline{E} + \underline{u}_a \times \underline{B}/c) \\ &+ \sum_b \frac{e_a\, e_b}{n_a}\Big[-\frac{1}{2}\frac{\partial}{\partial \underline{x}}\cdot\int \underline{r}\,\nu_{ab}^{(+)}\Big(\frac{\underline{r}}{r^3}\Big)\, d^3r \\ &+ \int \nu_{ab}^{(-)}\Big(\frac{\underline{r}}{r^3}\Big)\, d^3r\Big] \end{aligned} \tag{5.36}$$

where we have used the odd and even function of $\underline{r}$ properties of $\nu_{ab}^{(-)}$ and $\nu_{ab}^{(+)}$, respectively. The partial pressure tensor $\underline{\underline{p}}_a$ may now be defined by

$$\underline{\underline{p}}_a = m_a\, n_a <(\underline{\xi}-\underline{u})(\underline{\xi}-\underline{u})>_a + \frac{1}{2}\sum_b e_a\, e_b \int \nu_{ab}^{(+)} \left(\frac{\underline{r}\,\underline{r}}{r^3}\right) d^3r \tag{5.37}$$

where the fluid velocity $\underline{u}$ is given by

$$\rho\underline{u} = \sum_a m_a\, n_a\, \underline{u}_a$$

and (5.38)

$$\rho = \sum_a m_a\, n_a$$

is the mass density.

Equation (5.31) may now be written

$$\frac{\partial}{\partial t}(\rho_a\, \underline{u}_a) + \frac{\partial}{\partial \underline{x}} \cdot [\underline{\underline{p}}_a + \rho_a(\underline{u}_a\, \underline{u} + \underline{u}\, \underline{u}_a - \underline{u}\, \underline{u}]$$

$$= \varepsilon_a\underline{E} + \underline{j}_a \times \underline{B}_{/c} + \sum_b e_a\, e_b \int \nu_{ab}^{(-)} \left(\frac{\underline{r}}{r^3}\right) d^3r \tag{5.39}$$

where

$$\rho_a = m_a\, n_a$$

$$\varepsilon_a = e_a\, n_a \tag{5.40}$$

$$\underline{j}_a = e_a\, n_a\, \underline{u}_a$$

By summing equation (5.39) with respect to a, the total conservation of momentum equation results,

$$\frac{\partial}{\partial t}(\rho\underline{u}) + \frac{\partial}{\partial \underline{x}} \cdot [\underline{\underline{p}} + \rho\underline{u}\underline{u}] = \varepsilon\underline{E} + \underline{j} \times \underline{B}_{/c} \tag{5.41}$$

where

$$\rho = \sum_a \rho_a$$

$$\varepsilon = \sum_a \varepsilon_a \qquad (5.42)$$

$$\underline{j} = \sum_a j_a$$

and $\underline{\underline{p}} = \sum_a \underline{\underline{p}}_a$ is the total pressure tensor.

It may be noted that an expression for the isotropic pressure may be obtained from equation (5.37) and

$$p^o = \tfrac{1}{3}(p_{11} + p_{22} + p_{33}). \qquad (5.43)$$

Hence,

$$p^o = \tfrac{1}{3} \sum_a m_a \; n_a \; <(\xi - u)^2>_a$$

$$- \tfrac{1}{6} \sum_a \sum_b \int \nu_{ab} \; r \frac{d \; \varphi_{ab}}{dr} \; d^3r \qquad (5.44)$$

Now, the temperature $T_a$ of the a-th component of the electrolyte is defined by (see Green[85](1952))

$$3kT_a = m_a \; <(\xi - u)^2>_a \qquad (5.45)$$

where $k$ is Boltzmann's constant. In equilibrium, $T_a$ is equal to the uniform temperature $T$, and so equation (5.44) becomes

$$p^o = \sum_a (n_a kT - \tfrac{1}{6} \sum_b n_a \; n_b \; I_{ab}) \qquad (5.46)$$

where

$$I_{ab} = \int (g_{ab} - 1) \; r \frac{d \; \varphi_{ab}}{dr} \; d^3r \qquad (5.47)$$

and $g_{ab}$ is the radial distribution function. If the volume viscosity can be neglected then the isotropic part of the pressure is the same as the hydrostatic pressure and hence (5.46) reduces to (4.4) in Section 1.4.

In the non-relativistic approximation, the partial conservation of momentum equation (5.39) may be simplified because $|\underline{u}_a| << c$ and so the term $\underline{j}_a \times \underline{B}/c$ may be neglected. If we further take the fluid velocity $\underline{u}$ to be equal to zero then (5.39) becomes

$$\frac{\partial(\rho_a \underline{u}_a)}{\partial t} + \frac{\partial}{\partial \underline{x}} \cdot \underline{\underline{p}}_a = \varepsilon_a \underline{E} + \sum_b e_a e_b \int \nu_{ab}{}^{(-)} \left(\frac{\underline{r}}{r^3}\right) d^3 r \tag{5.48}$$

If we define

$$\underline{F}_{ab}{}^{(-)} = e_a e_b \int \nu_{ab}{}^{(-)} \left(\frac{\underline{r}}{r^3}\right) d^3 r \tag{5.49}$$

then $\underline{F}_{ab}{}^{(-)}$ is the force per unit volume exerted by the b-th species on the a-th species, and so should be proportional to the relative velocities of the particles of type a and b (Spitzer[88], p. 28). As $\nu_{ab}{}^{(-)}$ is an odd function of $\underline{r}$, it has the general form

$$\nu_{ab}{}^{(-)} = \underline{r} \cdot (\underline{u}_b - \underline{u}_a)\ f(r)$$

By expanding the symmetric tensor $\underline{r}\,\underline{r}$ into an isotropic part and a non-divergent part and neglecting the latter, (5.49) becomes

$$\underline{F}_{ab}{}^{(-)} = \theta_{ab}\ \rho_a (\underline{u}_b - \underline{u}_a) \tag{5.50}$$

where $\theta_{ab}$ is the number of collisions in a volume per unit time between particles of types a and b.

In a strong electrolyte, the interactions between charged species are mediated by the aqueous dielectric, and so if the water molecule is denoted by the subscript zero, then for each $a \neq 0$, $b \neq 0$, $\theta_{ab} << \theta_{ao}$. We shall subsequently write $\theta_a$ for $\theta_{ao}$. With the further assumption that there is no osmotic flow, that is $\underline{u}_0 = 0$, we may write

$$\sum_b \underline{F}_{ab}^{(-)} = - \theta_a \rho_a \underline{u}_a \, . \tag{5.51}$$

Finally, if we neglect viscosity effects then

$$\frac{\partial}{\partial \underline{x}} \cdot \underline{\underline{p}}_a = \frac{\partial}{\partial \underline{x}} p_a \tag{5.52}$$

where $p_a$ is the thermodynamical partial pressure of the a-th component. Writing

$$\underline{E} = - \frac{\partial}{\partial \underline{x}} \phi \tag{5.53}$$

where $\phi$ is the electric potential, equation (5.48) becomes

$$\frac{\partial}{\partial t}(\rho_a \underline{u}_a) + \frac{\partial p_a}{\partial \underline{x}} + \varepsilon_a \frac{\partial \phi}{\partial \underline{x}} + \theta_a \rho_a \underline{u}_a = 0 \tag{5.54}$$

and this is the form of the partial conservation of momentum equation that will be used in subsequent chapters to investigate the dynamics of excitable membranes.

The partial equation of energy transport may be derived in a similar way by multiplying equation (5.6) by $\frac{1}{2} m_a \xi^2$ and then integrating with respect to $\xi$:

$$\frac{\partial}{\partial t}(\tfrac{1}{2}m_a\ n_a\langle\xi^2\rangle\ ) + \frac{\partial}{\partial \underline{x}}\cdot(\tfrac{1}{2}m_a\ n_a\ \langle\underline{\xi}\ \underline{\xi}^2\rangle)$$

$$= m_a\, n_a\ \langle\underline{\xi}\ \cdot\ \underline{\eta}_a\rangle \qquad (5.55)$$

By defining the internal energy per unit mass of the a-th particle $U_a$ by

$$U_a = \frac{1}{2}\ \langle(\underline{\xi}-\underline{u})\rangle_a^2 + \frac{1}{2}\sum_b \frac{e_a\ e_b}{\rho_a\ |\underline{x}-\underline{x}'|}\ \nu_{ab}\ d^3\underline{x}' \qquad (5.56)$$

and using the expression for $\underline{\eta}_a$ given by equation (5.28), equation (5.55) becomes

$$\frac{\partial(\rho_a U_a)}{\partial t} + \frac{\partial}{\partial t}(\rho_a\ \underline{u}_a\cdot\underline{u} - \rho_a\ \underline{u}^2/2) - \frac{1}{2}\sum_b \int \frac{e_a\ e_b\ \frac{\partial\nu'_{ab}}{t} d^3x'}{|\underline{x}-x'|}$$

$$+ \frac{\partial}{\partial \underline{x}}\ \cdot\ (\rho_a\ \langle\underline{v}\ \underline{v}^2/2\rangle)\ = \rho_a\underline{u}_a\cdot\underline{E}$$

$$+ \sum_b e_a\ e_b \int (\underline{u}_{ab}\ n_{ab} - \underline{u}_a\ n_a\ n_b')\cdot(\frac{\underline{r}}{r^3})\ d^3r \qquad (5.57)$$

The form of this equation may be simplified with the help of the partial and total conservation of momentum equations (5.39) and (5.41) and also the two-particle continuity equation:

$$\frac{\partial n_{ab}}{\partial t} + \frac{\partial}{\partial \underline{x}}\ \cdot\ (n_{ab}\ \underline{u}_{ab}) + \frac{\partial}{\partial \underline{x}'}\ \cdot\ (n_{ab}\ \underline{u}'_{ab}) = 0 \qquad (5.58)$$

This equation may be derived from equation (5.2) by setting

$$G = \sum_i \delta_{ia} \, \delta(\underline{x}-\underline{x}_i)\,\delta(\underline{\xi}-\underline{\xi}_i) \sum_j{}' \delta_{jb} \, \delta(\underline{x}'-\underline{x}_j)\,\delta(\underline{\xi}'-\underline{\xi}_j) \qquad (5.59)$$

and defining

$$\begin{aligned} \underline{u}_{ab} &= \langle\underline{\xi}\rangle_{ab} \\ \underline{u}'_{ab} &= \langle\underline{\xi}'\rangle_{ab} \end{aligned} \qquad (5.60)$$

Finally, $n_{ab}$ and $n_a \, n_b{}'$ can be expressed as functions of $\underline{y} = \frac{1}{2}(\underline{x}+\underline{x}')$ and $\underline{r} = \underline{x}-\underline{x}'$ and expanded in Taylor series around $(\underline{x},\underline{r})$ (see equation (5.38)) to obtain

$$\rho \frac{d}{dt}(\rho_a U_a/\rho) + \rho_a \underline{w}_a \cdot \frac{d\underline{u}}{dt} + (\underline{\underline{p}}_a \cdot \frac{\partial}{\partial \underline{x}}) \cdot \underline{u}$$

$$+ \frac{\partial}{\partial \underline{x}} \cdot \underline{Q}_a = (\underline{J}_a - \varepsilon_a \underline{u}) \cdot \underline{E} - \underline{u} \cdot \underline{J}_a \times \underline{B}/c$$

$$+ \sum_b G_{ab}{}^{(-)} \qquad (5.61)$$

where

$$G_{ab}{}^{(-)} = - e_a \, e_b \int \nu_{ab}{}^{(-)} (\underline{r}.\underline{u}/r^3) \, d^3r$$

$$+ \frac{1}{2} e_a \, e_b \int n_{ab}{}^{(-)} (\underline{u}_{ab}+\underline{u}'_{ab}).(\underline{r}/r^3) \, d^3r$$

$$- \frac{1}{2} e_a \, e_b \int (n_a \, n_b')^{(-)} (\underline{u}_a+\underline{u}_b').(\underline{r}/r^3) \, d^3r \qquad (5.62)$$

and

$$\underline{Q}_a = \rho_a U_a \underline{w}_a + (\underline{\underline{p}}_a - \rho_a \underline{w}_a \underline{w}_a) \cdot \underline{w}_a$$

$$+ \frac{1}{2} \rho_a \langle(\underline{v} - \underline{u}_a)(\underline{v} - \underline{u}_a)^2\rangle_a$$

$$+ \frac{1}{2} \sum_b e_a \, e_b \int n_{ab} \{\frac{1}{2}(\underline{u}_{ab}+\underline{u}'_{ab}-\underline{u}_a-\underline{u}_b').(\underline{r} \, \underline{r}/r^3)$$

$$+ (\underline{u}_{ab}-\underline{u}_a)/r\} \, d^3r \qquad (5.63)$$

and $\underline{w}_a = \underline{u}_a - \underline{u}$.

Summing with respect to $a$, we obtain the equation of energy transport

$$\rho \frac{dU}{dt} + (\underline{\underline{p}} \cdot \frac{\partial}{\partial \underline{x}}) \cdot \underline{u} + \frac{\partial}{\partial \underline{x}} \cdot \underline{Q}$$

$$= (J - \varepsilon \underline{u}) \cdot \underline{E} - \underline{u} \cdot \underline{J} \times \underline{B}_{/c} \qquad (5.64)$$

where $\underline{Q} = \sum\limits_a \underline{Q}_a$.

Now, from (5.49) and (5.62)

$$G_{ab}^{(-)} + \underline{u}.\underline{F}_{ab}^{(-)} = \frac{1}{2} e_a e_b \int n_{ab}^{(-)} (\underline{u}_{ab}+\underline{u}'_{ab}).(\underline{r}/r^3) \, d^3r$$

$$- \frac{1}{2} e_a e_b \int (n_a n_b')^{(-)} (\underline{u}_a+\underline{u}_b').(\underline{r}/r^3) \, d^3r \qquad (5.65)$$

and is the energy per unit volume transferred from the $b$-th species to the $a$-th species when the fluid velocity is equal to zero. In equilibrium, the following conditions must be satisfied:

$$\begin{matrix} \underline{u}_a = \underline{u}_b \\ T_a = T_b \end{matrix} \quad , \qquad (5.66)$$

where $T_a$ is defined by (5.45), and so we may write

$$n_{ab}^{(-)} (\underline{u}_{ab}+\underline{u}'_{ab}) - (n_a n_b')^{(-)} (\underline{u}_a+\underline{u}_b')$$

$$= f(r) (\underline{u}_a+\underline{u}_b) \underline{r}.(\underline{u}_b-\underline{u}_a) + g(r) (T_b-T_a) \underline{r} \qquad (5.67)$$

where $f(r)$ and $g(r)$ are appropriate functions of $r$. Again, by retaining only the isotropic part of $\underline{r}\,\underline{r}$, as in (5.50), we have from (5.65)

$$G_{ab}^{(-)} = \theta_{ab}\rho_a(\underline{u}_b-\underline{u}_a)\cdot\underline{u} + \omega_{ab}(\underline{u}_b^{2}-\underline{u}_a^{2}) + \psi_{ab}(T_b-T_a) \qquad (5.68)$$

where $\omega_{ab}$ and $\psi_{ab}$ can be expressed in terms of $f(r)$ and $g(r)$.

If the fluid velocity $\underline{u}$ is equal to zero in the electrolyte, then the partial equation of energy transport (5.61) may be simplified to

$$\rho\,\frac{d}{dt}\,(\rho_a U_a/\rho) + \frac{\partial}{\partial\underline{x}}\cdot\underline{Q}_a = \underline{J}_a\cdot\underline{E} - \omega_{ao}\underline{u}_a^{2} \qquad (5.69)$$

where we have assumed a uniform ion temperature.

## 1.6 OUTLINE OF THESIS

In this thesis we develop a macroscopic treatment of the dynamical process within thin lipid membranes which can account for the electrical properties of excitable cells, and in particular, the time course and magnitude of the currents which flow across the membrane during the voltage and current clamp experiments, and the action potential.

In Chapter 2, we develop a set of nonlinear equations in the ionic potentials. These equations are linearized, and following the determination of the steady states of the membrane, their solutions are obtained by deriving a dispersion equation which can be used to characterize the modes of oscillation within each ionized medium. These include oscillatory modes in the membrane which have the correct frequencies and amplitudes (when small) to explain the observed excitation phenomena.

In Chapter 3 an expression for the voltage clamp current is derived by considering appropriate boundary conditions at the electrodes. The characteristic frequencies of the currents are determined by a polynomial equation generated by the condition that the dispersion has two equal roots, and hence are dependent on the concentrations of the permeable ions in the membrane and the resistance these ions experience in their passage through the membrane phase. It is shown that the voltage clamp data for the normal squid giant axon are explicable in terms of the linearized electrodiffusion equations. The dependence of the characteristic frequencies of the voltage clamp currents on the temperature of the medium, the concentrations of ions in the intracellular and extracellular fluids and the type of excitable membrane (whether nerve, muscle, electroplaque or Chara) is in qualitative agreement with experimental data.

In Chapter 4 we investigate the nature of the singularities and associated normal modes which are relevant to observable dynamical events, and then examine the oscillations in the potential difference across the membrane for a subthreshold stimulus. When the magnitude of the stimulus which is applied to an excitable axon membrane is sufficiently large (a threshold stimulus) the character of the potential oscillations across the membrane changes dramatically. Their amplitude for the normal axon is considerably greater (approximately 100mV) than that of subthreshold oscillations (of the order of a few millivolts), and the oscillation waveform no longer resembles harmonic variation but is characterized by sharp peaks or spikes (a repetitive action potential). By taking into account the nonlinearity of Poisson's equation, the dynamical equations for an ionized medium with just two ions can be solved to yield nonlinear potential oscillations which have a similar shape to that of the axon action potential. However, these equations only exhibit threshold behaviour if the coefficient of the ionic potential in the exponential terms is sufficiently large, and this value is attained in the presence of calcium ions.

The previous analysis has shown how to account qualitatively for excitable cell behaviour without assuming that the ion mobility is dependent on the electric field in the membrane. However, it is in fact necessary to assume such a dependence in order to explain the large steady state currents of certain ions (viz. potassium) which are observed during the voltage clamp of the axon membrane, and also to describe the variations in shape of the action potential for different excitable cells. In Chapter 5 we examine the extent to which an electric field-dependent ion mobility can be inferred from the application of irreversible statistical mechanics to the Triffet-Green neuron model. We also consider the effect of irreversible processes on the Hamiltonian parameters $\lambda$ and $\mu$ which, provisionally, were assumed to be constants in the model.

# CHAPTER 2

# THE ELECTRODIFFUSION MODEL OF MEMBRANE DYNAMICS

## 2.1 THE LINEARIZED ELECTRODIFFUSION EQUATIONS

There have been many attempts to account for excitation phenomena in terms of the macroscopic equations with little success. In most cases, the starting point has been the time-dependent electrodiffusion equations[28], that is, the continuity equations, the Nernst-Planck equation (or the steady-state conservation of momentum equation) and Poisson's equation. This absence of an electrodiffusion description of the dynamical properties of excitable cells has been understood by most investigators as a confirmation of the Hodgkin-Huxley interpretation of the large trans-membrane currents during excitation in terms of electric field controlled ion-specific gating mechanisms, and as an indication of the irrelevance of the electrodiffusion equations to an understanding of excitation phenomena.

The neuron model of Triffet and Green[27] suggested, however, that the action potential could be simulated by a set of equations derivable from physical principles. The limitations of the model were that, being a microscopic quantum description of ionic events, its physical interpretation was not a priori clear and also, comparison with the numerous experiments on excitable cells was difficult. These shortcomings were overcome by a macroscopic description of ionic processes within the excitable membrane and surrounding electrolytes which included the additional effects of irreversibility (and, in particular, ionic diffusion), spatial gradients and the interactions between ions of different types.

The macroscopic equations which are necessary to describe the behaviour of an ionized medium, including the irreversible effects of heat conduction as well as chemical diffusion and viscous flow, are the continuity equation, the conservation laws of momentum and energy and Maxwell's equations. It is well-known that this set of equations, supplemented by the thermodynamic equations of state, constitutes a basis for a general theory of irreversible processes which is an alternative to the theory of irreversible thermodynamics[89]. However, in examining the physical basis for excitable behaviour in biological cells, it is not necessary to retain all of the above equations. More specifically, although rapid developments in the experimental technique of measuring magnetic effects in the central nervous system have occurred in recent years[90], the magnitude of such magnetic fields is extremely small (of the order of $10^{-12}$ Tesla). It is therefore reasonable to neglect magnetic effects to a first approximation and so, in this case, Maxwell's equations reduce to a single equation - Gauss's law or Poisson's equation.

During the action potential, heat is generated during the rising phase (approximately 20 μcal./gm for the rabbit vagus nerve) and mostly reabsorbed during the falling phase (about ninety per cent).[91] A detailed account of these changes would include the effect of temperature gradients but if we consider only isothermal changes, then it is possible to describe the dynamics of the system in terms of the conservation laws of mass and momentum, without the additional equation of energy transport.

Having decided upon which macroscopic equations are important for a description of excitable cell dynamics, a microscopic justification of these laws should be given, especially since even their applicability has been questioned to a large extent. This has been done in Section 1.5, where we presented an account of how the conservation laws of mass, momentum and energy for each ion may be derived from the microscopic Liouville equation. The derivation of the macroscopie Maxwell equations from the microscopic field equations, although originally given by Lorentz in the context of his electron theory, has also been considered in terms of statistical ensemble averages by Brittin[66] and de Groot[92].

In this section, we shall first linearize the dynamical equations for an ionized medium and in Section 2.2 the time-independent form of these equations will be considered in determining the steady states of the excitable membrane and its surrounding electrolytes. In Section 2.3, solutions of the time-dependent linearized dynamical equations are obtained by deriving a dispersion equation which can be used to characterize the modes of oscillation within each ionized medium. These include oscillatory modes within the membrane which have the correct frequencies and amplitudes in order to explain the observed excitation phenomena in various excitable cells (neurons, muscle cells and excitable cells in the algae *Chara and Nitella*). This analysis will be applied to the voltage clamp experiments in Chapter 3, and to electric potential oscillations across the membrane, both subthreshold and threshold, in Chapter 4.

We now consider the dynamical equations of an ionized medium in more detail. It can be characterized generally by a dielectric constant and a resistance to the passage of the various kinds of ions present. The charge density and the current density associated with the a-th kind of ion are denoted by $\varepsilon_a$ and $\underline{j}_a = \varepsilon_a \underline{u}_a$, respectively (a-1,2,...), so that, to secure conservation of ions of the a-th type,

$$\frac{\partial \varepsilon_a}{\partial t} + \nabla . \underline{j}_a = 0 \tag{1.1}$$

This equation is just (5.29) of Section 1.5.

It may be shown from Maxwell's equations that the electric potential $\phi$ satisfies the nonhomogeneous wave equation

$$\frac{1}{V^2} \frac{\partial^2 \phi}{\partial t^2} - \nabla^2 \phi = \frac{4\pi}{\kappa} = \frac{4\pi}{\kappa} \sum_a \varepsilon_a$$

where $\kappa$ is the dielectric constant of the medium. As the velocity of propagation, V, of the electric potential is large, and as we are concerned primarily with low frequency oscillations, then this equation reduces to

Poisson's equation

$$\nabla^2 \phi = - \frac{4\pi}{\kappa} \varepsilon \tag{1.2}$$

Now, if we neglect the effects of viscosity, heat conduction and the magnetic field, the ions of the a-th kind are subject to forces of three types: a force $-\nabla p_a$ per unit volume due to the partial pressure $p_a$, $-\varepsilon_a \nabla\phi$ due to the electric field, and a resistance $-\theta_a \rho_a \underline{u}_a$, where $\rho_a$ is the mass density of the hydrated ions, and $\theta_a$ the friction constant associated with the motion of the ions relative to the solvent, or the material of the membrane. This is expressed by the equation of the conservation of ions of the a-th kind, which was derived in Section 1.5 as (5.54), and is written here as

$$\frac{\partial(\rho_a \underline{u}_a)}{\partial t} + \nabla p_a + \varepsilon_a \nabla\phi = -\theta_a \rho_a \underline{u}_a \tag{1.3}$$

As Spitzer[88] has recognized, this conservation law may be used to derive a correct generalization of Ohm's law for ionized media. If $m_a$ is the effective mass and $e_a$ the charge of the a-th kind of ions,

$$\rho_a = m_a \varepsilon_a / e_a, \quad \rho_a \underline{u}_a = m_a \underline{j}_a / e_a \tag{1.4}$$

and on account of the Gibbs-Duhem relation, which in the absence of a temperature gradient is

$$\nabla p_a = \rho_a \nabla \mu_a , \tag{1.5}$$

where $\mu_a$ is the chemical potential of the a-th kind of ion, equation (1.3) may be written as

$$\theta_a \underline{j}_a = - \varepsilon_a \nabla(\mu_a + e_a \phi / m_a) - \frac{\partial \underline{j}_a}{\partial t} \tag{1.6}$$

This yields the usual form of Ohm's law in a steady state with $\frac{\partial \underline{j}_a}{\partial t} = 0$. As we are concerned with transient phenomena we shall retain the inertial term, though, because of the large resistance, the associated relaxation effects are of very short duration in the membrane.

Still another form of the equation of conservation of momentum results from expressing the partial pressure, on the chemical potential, in terms of the number density $\varepsilon_a/e_a$, thus:

$$\beta_a \nabla p_a = \nabla \varepsilon_a / e_a \tag{1.7}$$

where $\beta_a = \beta = 1/(kT)$ in the limit of small concentrations, but depends weakly on the ionic densities for the concentrations attained in the electrolytes surrounding the membrane (see equation (4.26) of Section 1.4). In this form, the Nernst-Planck expression for the current $\underline{j}_a$ is obtained by neglecting the inertial term.

The resulting equations are nonlinear, but for small amplitude solutions may be linearized without serious error by neglecting quadratic deviations from equilibrium, thus:

$$\frac{1}{\varepsilon_a} \frac{\partial \varepsilon_a}{\partial t} + \nabla . \underline{u}_a = 0$$

$$\frac{\partial \underline{u}_a}{\partial t} + \frac{\nabla \varepsilon_a}{\beta_a m_a \varepsilon_a} + \frac{e_a}{m_a} \nabla \phi = -\theta_a \underline{u}_a \tag{1.8}$$

By elimination of the diffusion velocity $\underline{u}_a$, we obtain

$$\beta_a m_a \left( \frac{\partial^2 \phi_a}{\partial t} + \theta_a \frac{\partial \phi_a}{\partial t} \right) + \nabla^2 (\phi - \phi_a) = 0 \tag{1.9}$$

where $\phi_a$ is the ionic potential, defined by

$$\beta_a e_a \phi_a = -\log(\varepsilon_a / \varepsilon_a{}^o) \tag{1.10}$$

and $\varepsilon_a{}^o$ is a constant which depends on the ionized medium, and is chosen in such a way as to minimize the magnitude of the potentials $\phi_a$. This usually implies that $\phi_a$ will be small throughout the medium, and under such circumstances Poisson's equation

$$\kappa \nabla^2 \phi = -4\pi \sum_a \varepsilon_a{}^o \exp(-\beta_a e_a \phi_a) \tag{1.11}$$

may also be linearized as follows:

$$\kappa \nabla^2 \phi = 4\pi \sum_a \beta_a e_a \varepsilon_a{}^o \phi_a \tag{1.12}$$

The linearized version of the equation will generally be used in this and subsequent chapters, except when the nonlinearity is considered to be important, as it is during the action potential and for large perturbations from the holding potential during the voltage clamp.

The pair of equations (1.9) and (1.11) or (1.12) are sufficient to determine the ionic and electric potentials, with the help of suitable boundary conditions. If the ionized medium under consideration is the membrane phase of a biological cell, then at the membrane surfaces, $\phi$ and the normal component of $\kappa\nabla\phi$ must be continuous, and, at the microscopic level, $\varepsilon_a$ must also be continuous, so that $\phi_a - \log\varepsilon_a{}^o/\beta_a e_a$ is also continuous, though $\phi_a$ has a discontinuity.

When $\phi_a$ and $\phi$ have been determined, the ionic currents may be obtained with the help of the relation

$$\frac{\partial \underline{j}_a}{\partial t} + \theta_a \underline{j}_a = \frac{e_a \varepsilon_a}{m_a} \nabla(\phi_a - \phi) \qquad (1.13)$$

Within the membrane phase, the time derivative is usually negligible.

## 2.2 STEADY STATES OF THE CONDUCTING MEMBRANE

In this section we determine the steady states of the excitable membrane and the surrounding electrolytes by considering the time-independent form of the equations of the previous section. Excitable cells have a large variation in shape, size and ionic composition but the excitable membrane is generally composed of an ultra-thin lipid bilayer membrane (50Å-100Å) within which are embedded large protein molecules. When these molecules extend across the thickness of the membrane, it is thought that they provide a passage through which ions (and other molecules) may diffuse from one side of the membrane to the other.

The determination of the steady states of a membrane will clearly depend on the geometry of the cell, but in what follows we shall generally restrict our attention to the simplest case – that of a plane membrane. However, with a cylindrical geometry, the solution of the time-independent forms of equations (1.9) and (1.12) are easily enough obtained in terms of the modified Bessel function $I_0(\nu r)$, where $\nu$ is the inverse of the Debye shielding length, given by

$$\nu = (4\pi \sum_a \beta_a e_a \varepsilon_a{}^{0}/\kappa)^{\frac{1}{2}} \tag{2.1}$$

and for physiological concentrations this has a value of about 5Å. Now, this is considerably less than the diameter of the smallest mammalian nerve fibres (of the order of 1000Å). We may therefore neglect the curvature of the membrane in considering ion transport which is uniform over the membrane surface. However, the diameter of the axon is an important variable in the analysis of the conduction of the nervous impulse longitudinally down the axon, and this will be discussed in Chapter 4.

The membrane phase (denoted by $R_m$), which is comprised of essentially rigid components as well as the various kinds of ions which are able to diffuse through it, is considered to be an infinite slab of uniform thickness $\delta$ which separates the electrolyte interior of the cell (denoted by $R_n$) from the electrolyte exterior to the cell (denoted by $R_\ell$). Each of these regions is characterized by a different dielectric constant and a different resistance to the flow of ions through it. If we measure radial distances outwards from an origin midway between the membrane surfaces at $x = \pm\delta/2$, and if flat electrodes are positioned symmetrically on each side of the membrane so that their separation is $\rho$ (see figure 2.1), then the solutions for the external region, the membrane and the interior of the cell are of the form

$$\phi_{a\ell} = \chi'_\ell(\exp[-\nu_\ell(x-\delta/2)] - \exp[\nu_\ell(x-\rho+\delta/2)])$$

$$\phi_\ell = \chi'(\exp[-\nu_\ell(x-\delta/2)] - \exp[\nu_\ell(x-\rho+\delta/2)]) + V'$$

$$\phi_{am} = \chi'_m \exp[-\nu_m x] + \chi_m \exp[\nu_m x] + V_a(\tfrac{1}{2}-\tfrac{x}{\delta}) + V'_a(\tfrac{1}{2}+\tfrac{x}{\delta})$$

$$\phi_m = \chi'_m \exp[-\nu_m x) + \chi_m \exp[\nu_m x] + V(\tfrac{1}{2}-\tfrac{x}{\delta}) + V'(\tfrac{1}{2}+\tfrac{x}{\delta})$$

$$\phi_{an} = \chi_n(\exp[\nu_n(x+\delta/2)] - \exp[-\nu_n(x+\rho-\delta/2)])$$

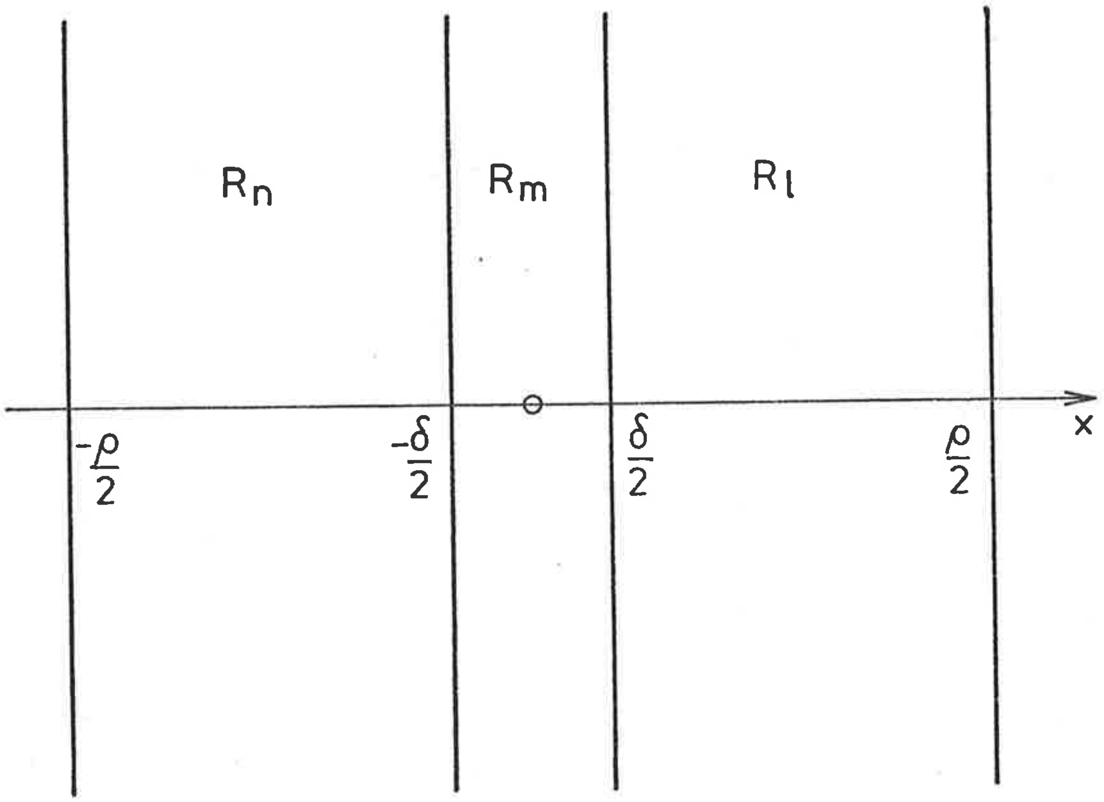

FIGURE 2.1: The geometry of the membrane.

$$\phi_n = \chi_n(\exp[\nu_n(x+\delta/2)] - \exp[-\nu_n(x+\rho-\delta/2)] + V \qquad (2.2)$$

where

$$V_a = (\log \varepsilon_{am}^{o}/\beta_{am} - \log \varepsilon_{an}^{o}/\beta_{an})/e_a$$

$$(2.3)$$

$$V_a' = (\log \varepsilon_{am}^{o}/\beta_{am} - \log \varepsilon_{a\ell}^{o}/\beta_{a\ell})/e_a$$

and $\varepsilon_{an}^{o}$ and $\varepsilon_{a\ell}^{o}$ have been chosen so that $\phi_a = 0$ at $x = \pm\rho/2$, whereas $\varepsilon_{am}^{o} = \frac{1}{2}(\varepsilon_{an}^{o} + \varepsilon_{a\ell}^{o})$.

The continuity of $\phi$ at $x = \pm\delta/2$ requires that

$$\chi_m \exp[-\nu_m\delta/2] + \chi_m' \exp[\nu_m\delta/2]$$

$$= \chi_n(1 - \exp[-\nu_n(\rho-\delta)]$$

$$\chi_m \exp[\nu_m\delta/2] + \chi_m' \exp[-\nu_m\delta/2]$$

$$= \chi_\ell'(1 - \exp[\nu_\ell(\delta-\rho)]) , \qquad (2.4)$$

and the continuity of $\kappa\nabla\phi$ at $x = \pm\delta/2$ requires that

$$\kappa_m\nu_m(\chi_m \exp[-\nu_m\delta/2] - \chi_m' \exp[\nu_m\delta/2]) + \kappa_m(V'-V)/\delta$$

$$= \kappa_n\nu_n\chi_n(1 + \exp[-\nu_n(\rho-\delta)]$$

$$\kappa_m\nu_m(\chi_m \exp[\nu_m\delta/2] - \chi_m' \exp[-\nu_m\delta/2]) + \kappa_m(V'-V)/\delta$$

$$= - \kappa_\ell\nu_\ell\chi_\ell'(1 + \exp[\nu_\ell(\delta-\rho)]) \qquad (2.5)$$

If the separation between the electrodes, $\rho \gg$ the membrane thickness, $\delta$, then the terms involving $\exp[-\nu_n(\rho-\delta)]$ and $\exp[-\nu_\ell(\rho-\delta)]$ can be neglected, and equations (2.4) and (2.5) have the solution

$$\chi'_\ell = \frac{\bar{\kappa}_m(V-V')[(c-1)\bar{\kappa}_m+s\bar{\kappa}_n]/\bar{\delta}}{(\bar{\kappa}_m^2+\bar{\kappa}_n\bar{\kappa}_\ell)s+\bar{\kappa}_m(\bar{\kappa}_n+\bar{\kappa}_\ell)c}$$

$$\chi_n = -\frac{\bar{\kappa}_m(V-V')[(c-1)\bar{\kappa}_m+s\bar{\kappa}_\ell]/\bar{\delta}}{(\bar{\kappa}_m^2+\bar{\kappa}_n\bar{\kappa}_\ell)s+\bar{\kappa}_m(\bar{\kappa}_n+\bar{\kappa}_\ell)c}$$

$$\chi_m + \chi'_m = \tfrac{1}{2}(\chi_n+\chi'_\ell)/\cosh(\tfrac{1}{2}\bar{\delta})$$

$$\chi_m - \chi'_m = \tfrac{1}{2}(\chi'_\ell-\chi_n)/\sinh(\tfrac{1}{2}\bar{\delta}) \qquad (2.6)$$

where

$$s = \sinh(\bar{\delta}),\ c = \cosh(\bar{\delta}),\ \bar{\delta} = \delta\nu_m$$

$$\bar{\kappa}_\ell = \kappa_\ell\nu_\ell,\ \bar{\kappa}_m = \kappa_m\nu_m,\ \bar{\kappa}_n = \kappa_n\nu_n \qquad (2.7)$$

If we take the dielectric constant within the membrane, $\kappa_m = 10$, which is intermediate between the value for an aqueous electrolyte ($\approx 80$) and that for a lipid phase ($\approx 5$), then for physiological concentrations of ions in the surrounding electrolytes, $\nu_m^{-1} \approx 2$Å and as $\delta \approx 80$Å, then $\bar{\delta} \approx 40$, and so the following approximations are adequate

$$\chi'_\ell = \frac{\bar{\kappa}_m(V-V')}{\bar{\delta}(\bar{\kappa}_m+\bar{\kappa}_\ell)}$$

$$\chi_n = -\frac{\bar{\kappa}_m(V-V')}{\bar{\delta}(\bar{\kappa}_m+\bar{\kappa}_n)} \tag{2.8}$$

The currents in the steady state, according to equation (1.13) with the help of (2.2), are given by

$$j_a = -\frac{e_a \varepsilon_{am}^o}{m_a \theta_{am} \delta} (V'-V-V_a'+V_a) \tag{2.9}$$

and this is in agreement with the result obtained by integrating (1.13) across the thickness of the membrane. We note here that (2.9) can be used to recover the Nernst equation by assuming $j_a = 0$, whence

$$\Delta V = V-V' = \frac{1}{\beta e_a} \log\left(\frac{\varepsilon_{a\ell}^o}{\varepsilon_{an}^o}\right) \tag{2.10}$$

where we have used (2.3) and the approximations

$$\beta_{an} = \beta_{a\ell}$$
$$\beta_{am} = \beta$$

Experimental measurements in the steady state indicate a certain degree of variation of the resistances $\theta_{am}$ with the potential difference across the membrane, $\Delta V = V-V'$, but on a scale which is quite unable to explain the generation of the action potential on the transient currents of the voltage clamp, even though it is important in accounting for the shape of the action potential. We shall therefore treat the $\theta_{am}$ as constants in most of the following, and seek some other dynamical explanation of these phenomena.

## 2.3 TRANSIENT EFFECTS IN THE MEMBRANE

There is at present a considerable amount of experimental data (see Cole[4]) concerning the transient currents at the membrane arising either from small changes of potential similar to those occurring naturally in excitable cells, or from the artificial conditions imposed by the voltage clamp. It is known that there are currents of very short duration associated with changes in the polarization of the membrane surfaces, and also currents persisting for several milliseconds which are accompanied by the transfer of ions across the membrane under a variety of circumstances. In this section, it will be shown in a preliminary way how to account for such currents without invoking the large variations in the ionic permeabilities of the membrane postulated by Hodgkin, Huxley and Katz[12]. These transient currents will in fact be attributed to plasma oscillations of the type which are known to occur in other ionized systems.

The time-dependent behaviour of the potentials can be studied in linearized approximation by first eliminating the electric potential $\phi$ between equations (1.9) and (1.12), thus:

$$\beta_a m_a\left(\frac{\partial^2\phi_a}{\partial t^2} + \theta_a \frac{\partial\phi_a}{\partial t}\right) - \nabla^2\phi_a + \sum_b \nu^2\phi_b = 0 \qquad (3.1)$$

where

$$\nu_b^2 = 4\pi\beta_b e_b \varepsilon_b^0/\kappa \qquad (3.2)$$

takes different values $\nu^2_{b\ell}$ , $\nu^2_{bm}$ and $\nu^2_{bn}$ in the three regions $R_\ell$, $R_m$ and $R_n$, and the same applies to $\theta_a$, and, to a lesser extent, to $\beta_a$. Suppose $\phi_{as}$ is a solution of equation (3.1) for the steady state approached asymptotically as $t \to \infty$, of the type obtained in the previous section, and write

$$\phi_a = \phi_{as} + \frac{1}{2\pi i}\int_{-\infty}^{\infty}\psi_a(\lambda)e^{\lambda t}d\lambda \qquad (3.3)$$

so that $\psi_a(\lambda)$ satisfies

$$\nabla^2\psi_a = w_a^2(\lambda)\psi_a + \sum_b \nu_b^2\psi_b \qquad (3.4)$$

where

$$w_a^2 = \beta_a m_a \lambda(\lambda+\theta_a) \qquad (3.5)$$

As $\phi_{as}$ already has the required discontinuity at the membrane surfaces, $\psi_a$ is continuous at the common boundaries of the three regions. The solution within each region can be obtained in the form

$$\psi_a = \sum_{j,k}\frac{c_k^{(j)}f_k^{(j)}}{\mu_k^2-w_a^2(\lambda)} \qquad (3.6)$$

where the $f_k^{(j)}$ (j=1,2,...) are independent solutions of

$$\nabla^2 f_k^{(j)} = \mu_k^2 f_k^{(j)} \qquad (3.7)$$

and the $\mu_k^2$ are the roots of the algebraic equation

$$\sum_b \frac{\nu_b^2}{\mu_k^2 - w_b^2(\lambda)} = 1 \qquad (3.8)$$

The last equation may be regarded as a dispersion equation, relating the imaginary wave number $\mu_k$ to the imaginary frequency $\lambda$. There are clearly as many solutions as there are different kinds of ions, and we can identify a particular root $\mu_k^2$ uniquely as an analytic function of $\lambda$ by supposing that $\beta_1 m_1 < \beta_2 m_2 < \ldots < \beta_N m_N$ (where $N$ is the number of different kinds of ions) and requiring that

$$w_1^2(\lambda) < \mu_1^2 < w_2^2(\lambda) < \mu_2^2 < \ldots < w_N^2(\lambda) < \mu_N^2 \qquad (3.9)$$

for sufficiently large values of $|\lambda|$.

Within the membrane, $f_k^{(j)}$ depends only on the coordinate $x$ measuring distances in a direction perpendicular to the outer membrane surface, and there are only two independent solutions for each value of $k$, corresponding to opposite values of $\mu_k$. So

$$\psi_a = \sum_k \frac{c_k(\lambda)\exp(\mu_k x) + c_k'\exp(-\mu_k x)}{\mu_k^2 - w_a^2(\lambda)} \qquad (3.10)$$

We shall see in Chapter 3 that, in the regions $R_\ell$ and $R_n$ the corresponding solutions are determined by the initial conditions at $t = 0$ and the boundary conditions at the electrodes under the conditions of the voltage clamp. We may therefore suppose that the values $\psi_a(\pm\delta/2)$ of $\psi_a$ at the membrane surfaces $x = \pm\delta/2$ are known, so that the values of $c_k(\lambda)$ and $c_k'(\lambda)$ are to be determined by the solution of the linear equations.

$$\sum_k \frac{c_k(\lambda)\exp(\pm\mu_k\delta/2)+c_k'(\lambda)\exp(\mp\mu_k\delta/2)}{\mu_k^2-w_a^2(\lambda)} = \psi_a(\pm\delta/2) \qquad (3.11)$$

These equations can, in fact, be solved explicitly in the form

$$c_k = \sum_a\left(\frac{d_{ak}+d_{ak}'}{2}\right)L_{ak} \;;\; c_k' = \sum_a\left(\frac{d_{ak}-d_{ak}'}{2}\right)L_{ak} \qquad (3.12)$$

where

$$d_{ak} = [\psi_a(+\tfrac{\delta}{2}) + \psi_a(-\tfrac{\delta}{2})]\operatorname{sech}(\tfrac{\mu_k\delta}{2})$$

$$d_{ak}' = [\psi_a(+\tfrac{\delta}{2}) - \psi_a(-\tfrac{\delta}{2})]\operatorname{cosech}(\tfrac{\mu_k\delta}{2})$$

$$L_{ak} = (\mu_k^2-w_a^2)\prod_{\ell\neq k}\left(\frac{w_a^2-\mu_\ell^2}{\mu_k^2-\mu_\ell^2}\right)\prod_{b\neq a}\left(\frac{\mu_k^2-w_b^2}{w_a^2-w_b^2}\right) \qquad (3.13)$$

It should be noticed that the time dependence of the complex integral in equation (3.3), when evaluated by the theory of residues is determined by the singularities (poles and branch points) of $c_k(\lambda)$ and $c_k'(\lambda)$ in the left half of the complex $\lambda$-plane, excluding the origin. For large values of $\lambda$, $\mu_k$ is of the order $(\beta_k m_k)^{\frac{1}{2}}\lambda$, when chosen in accordance with equation (3.9), so that the contour along the imaginary axis can certainly be closed with a large semi-circle in the left half of the complex plane, when $t/x$ is greater than each of the small quantities $(\beta_k m_k)^{\frac{1}{2}}$. Also, as $\phi_{as}$ has been defined as the limiting value of $\phi_a$ as $t \to \infty$, the pole at $\lambda = 0$ is to be excluded by deforming the path of integration in equation (3.3) to the left of the origin in the $\lambda$-plane. Finally, it is clear that the denominators $\mu_k^2 - w_a^2(\lambda)$ in equation (3.10) are removed by one or other of the factors of $c_k(\lambda)$ and $c_k(\lambda')$, so that only the singularities of $c_k(\lambda)$ and $c_k'(\lambda)$ need be considered. These singularities are of four types:

(1) There are branch points in the integrand of equation (3.3) associated with the denominators $\mu_k^2 - \mu_\ell^2$ in $L_{ak}$. We shall study the effects of these singularities in Chapter 3, and show that they correspond to plasma oscillations, which can produce transient currents in the membrane over periods of several milliseconds (with physiological concentrations in nerve cells). They are consequently implicated as responsible for such phenomena as the action potential and the voltage clamp. For the present, we note that the characteristic frequencies are determined by the condition that the dispersion equation should have two equal roots $(\mu_k^2 = \mu_\ell^2)$.

(2) There are two infinite sequences of simple poles, at the values $\lambda_{kn}$ of $\lambda$ given by $\mu_k \delta = 2n\pi i$ and $\mu_k \delta = (2n+1)\pi i$ (where $n$ is any integer), associated with the factors $\mathrm{cosech}(\mu_k \delta/2)$ and $\mathrm{sech}(\mu_k \delta/2)$ of (3.13), respectively. The first of these sequences yields a contribution

$$\sum_{k,n} \exp(\lambda_{kn} t)(\alpha_{akn}\, e^{2\pi i n x/\delta} + \alpha_{akn}\, e^{-2\pi i n x/\delta})$$

to the potential $\phi_a$, in the form of a Fourier series. The term with $n = 0$, corresponding to $\mu_k^2 = 0$, requires separate consideration. The remaining terms make no contribution to the currents $j_a$ derived from equation (1.13), when averaged over the cross-section of the membrane, because their period is the same as the thickness $\delta$ of the membrane. The associated currents are therefore capacity currents, whose only effect is to polarize the membrane. The $\lambda_{kn}$ are easily computed from the dispersion equation (3.8), and are real and negative, with assorted magnitudes corresponding to time constants the largest of which is 10msec. The occurrence of capacity currents with similar characteristics is well known experimentally[4].

The second infinite sequence of poles does not contribute to the net current through the membrane and it will be seen in Chapter 3 that the term with $n = 0$ makes no contribution to the voltage clamp currents.

(3) There are also, in general, some poles associated with the denominators $w_a^2 - w_b^2$ in $L_{ak}$. Apart from the pole at the origin, which, as already explained, does not contribute to the value of the integral of equation (3.3), there are poles for

$$\lambda = \frac{(\beta_a m_a \theta_a - \beta_b m_b \theta_b)}{\beta_b m_b - \beta_a m_a} \tag{3.14}$$

These are, however, either in the right half of the complex plane, or, if in the left, so far from the origin that they have no effect on observable phenomena. For example, the resting nerve membrane resistance to potassium ions can be obtained from radioactive flux data[93,94] and yields a value for $\theta_K$ of the order of $10^{21}\text{sec}^{-1}$. The resulting extremely small time constants evidently correspond to the times required for the ions to respond to an abrupt change in the forces acting on them.

(4) Since the factors $\psi_a(\pm\delta/2)$ are influenced by ionic relaxation effects in the electrolyte outside the membrane, they are functions of $\lambda$ and therefore have poles and branch points corresponding to such effects. As the external resistances are very much smaller than those in the membrane (by a factor of about $10^8$), the time constants associated with these singularities are in general much smaller than those considered under (1) and (2) above, so that they have little or no effect on the action potential or voltage clamp.

The contributions to the voltage clamp currents from the singularities of type (1) and (2) above, will be considered in Chapter 3, but for the remainder of this section we shall examine how ionic processes within the membrane affect the currents in the external electrolytes and hence the potential difference across the membrane, both of which are amenable to experimental determination. In particular, we shall show that time-dependent processes are transmitted from one region to another through their effect on the electric potential.

As in equation (3.3) we may write the electric potential $\phi$ as

$$\phi = \phi_s + \frac{1}{2\pi i}\int_{-\infty}^{\infty} \psi(\lambda)\ e^{\lambda t}\ d\lambda \qquad (3.15)$$

where $\phi_s$ is a steady state solution of the type obtained in Section 2.2. It then follows from equations (1.12) and (3.3), with the help of (3.8) and (3.10), that

$$\nabla^2\psi = \sum_k [c_k(\lambda)\exp(\mu_k x) + c_k'(\lambda)\exp(-\mu_k x)] \qquad (3.16)$$

within the membrane, so

$$\psi = \sum_k [c_k(\lambda)\exp(\mu_k x) + c_k'(\lambda)\exp(-\mu_k x)]/\mu_k^2$$
$$+ c(\lambda)(\tfrac{1}{2} - \tfrac{x}{\delta}) + c'(\lambda)(\tfrac{1}{2} + \tfrac{x}{\delta}), \qquad (3.17)$$

where $c(\lambda)$ and $c'(\lambda)$ are integration constants.

The corresponding solutions in the external regions $R_\ell$ and $R_n$ are, assuming that the linear x-dependence can be neglected for the low-resistance electrolyte solutions,

$$\psi_\ell = \sum_k \left(\frac{c_{k\ell}(\lambda)\exp(\mu_{k\ell}(x-\delta/2)) + c_{k\ell}'(\lambda)\exp(-\mu_{k\ell}(x-\delta/2))}{\mu_{k\ell}^2}\right)$$
$$+ c_\ell'(\lambda)$$

$$\psi_n = \sum_k \left(\frac{c_{kn}(\lambda)\exp(\mu_{kn}(x+\delta/2)) + c_{kn}'(\lambda)\exp(-\mu_{kn}(x+\delta/2))}{\mu_{kn}^2}\right)$$
$$+ c_n(\lambda) \qquad (3.18)$$

The boundary conditions require the continuity of both $\psi$ and $\kappa\nabla\psi$ at $x = \pm\delta/2$, and are sufficient to determine not only $c(\lambda)$ and $c'(\lambda)$, but also $c'_\ell(\lambda)$ and $c_n(\lambda)$, in terms of $c_k(\lambda)$ and $c'_k(\lambda)$ and their external counterparts. So the time dependence of the external electric potential has features determined by the ionic potentials within the membrane, as might be expected. The external current is given by equation (1.13), and is therefore also determined in part by processes within the membrane. It is worth noticing also that the integrand of equation (3.15) has singularities for $\mu_k = 0$, and these are associated with relaxation processes within the membrane.

To complete the above analysis, it is necessary to determine $c_{k\ell}$, $c'_{k\ell}$, $c_{kn}$ and $c'_{kn}$ from the boundary conditions at the electrodes and the initial conditions, and clearly these depend on the particular experimental situation that is under consideration - the voltage clamp, subthreshold oscillations or the action potential. However, the singularities of $c_k(\lambda)$ and $c'_k(\lambda)$ of type (1) and (2) above are clearly not affected by the boundary conditions, and in the next chapter they will be used to compute the contributions to the currents which are observed during the voltage clamp, but, as will be seen in Chapter 4, they are equally relevant to a consideration of subthreshold oscillations or the action potential.

# CHAPTER 3

# THE VOLTAGE CLAMP

## 3.1 THE VOLTAGE CLAMP CURRENT

The "voltage clamp" is the term which has been used to describe the experimental techniques in which the potential difference across an excitable cell membrane is changed in a very short time from one value to another and maintained at this value (by use of an electronic feedback system) for a period of time during which the measured ionic current relaxes towards a new steady state. Hodgkin and Huxley[13] separated this current into its sodium and potassium components for the squid giant axon, and used empirical expressions for these components to reconstruct the action potential and other properties of the axon. It has been recognised (Cole[4]) that despite the success in describing most of the important electrical phenomena associated with squid axon, the Hodgkin-Huxley analysis is only a sufficient description of membrane behaviour.

In this section an expression will be derived for the current of each ionic species which depends on the concentrations of all the permeable ions within the membrane phase and the resistance these ions experience in their passage through the membrane. With the aid of this expression, it will be shown in Section 3.2 that the voltage clamp data obtained from the squid giant axon with physiological intracellular and extracellular solutions, are explicable in terms of the linearized electrodiffusion equations. However, quantitative agreement between theory and experiment for large deviations of the clamp potential from the holding potential is only obtained when nonlinear effects are taken into account.

Measurements of asymmetries in the capacity current for voltage clamps of equal and opposite magnitude from a given holding potential have been interpreted by most investigators as an experimental manifestation of the hypothetical ionic gates proposed by Hodgkin and Huxley[13]. In Section 3.2 it is also shown that such experiments are amenable to a simple alternative explanation which is consistent with the electrodiffusion model of excitable cells. This is followed by a brief consideration of how the effect of temperature on the time constants of the voltage clamp currents can be accounted for by the present model.

In section 3.3 the previous analysis is applied to the calculation of the time constants of the currents observed from the squid giant axon with perfused solutions. We first consider an axon with large concentrations of external calcium ions and an internal solution of cesium fluoride and then one with an intracellular fluid diluted with sucrose. Finally, there is a discussion of the theoretical analysis of voltage clamp data from other excitable cells - muscle fibres and the excitable cells in the electric eels and certain freshwater algae.

The clamping procedure described above makes use of electrodes on either wide of the membrane, and these may be idealized by supposing that they are plane and have the effect of clamping the ionic, as well as the electric potentials, at the points $x = -\rho/2$ and $+\rho/2$, relative to an origin midway between the (plane) membrane surfaces at $x = \pm\delta/2$. Consequently, the potentials $\phi_a$ and $\phi$ between the electrodes at the initial time $t = 0$ are everywhere those ($\phi_{a0}$ and $\phi_0$) characteristic of a steady state, but that following this time the potentials at the electrodes are held steady at values ($\phi_{as}$ and $\phi_s$) characteristic of some other steady state. As time progresses, the potentials will then approach $\phi_{as}$ and $\phi_s$ everywhere between the electrodes.

We shall first determine the solution in regions $R_\ell$ and $R_n$. The general solutions of equation (3.4) of Section 2.3 for these regions are:

$$\psi_{a\ell} = \sum_K \left(\frac{c_{K\ell}(\lambda)\exp(\mu_{K\ell}(x-\delta/2))+c_{K\ell}'(\lambda)\exp(-\mu_{K\ell}(x-\delta/2))}{\mu_{K\ell}^2 - w_{a\ell}^2}\right) \tag{1.1}$$

$$\psi_{an} = \sum_K \left(\frac{c_{Kn}(\lambda)\exp(\mu_{Kn}(x+\delta/2))+c_{Kn}'(\lambda)\exp(-\mu_{Kn}(x+\delta/2))}{\mu_{Kn}^2 - w_{an}^2}\right)$$

The boundary conditions at the electrodes $x = \pm\delta/2$ imply that

$$\begin{aligned} c_{K\ell}(\lambda) &= -\,c_{K\ell}'(\lambda)\exp(\mu_{K\ell}(-\rho+\delta)) \\ c_{Kn}'(\lambda) &= -\,c_{Kn}(\lambda)\exp(\mu_{Kn}(-\rho+\delta)) \end{aligned} \tag{1.2}$$

These expressions can be used with the steady state solutions (2.2) of Section 2.2, to specify the initial conditions in $R_\ell$ and $R_n$, thus:

$$\frac{1}{2\pi i}\int_{-i\infty}^{i\infty}\sum_{K}\left(\frac{c'_{K\ell}[\exp(-\mu_{K\ell}(x-\delta/2))-\exp(\mu_{K\ell}(x-\rho+\delta/2))]}{\mu_{K\ell}^{2}-w_{a\ell}^{2}}\right)d\lambda$$

$$= \delta\chi'_{\ell}[\exp(-\nu_{\ell}(x-\delta/2))-\exp(\nu_{\ell}(x-\rho+\delta/2))]$$

$$\frac{1}{2\pi i}\int_{-i\infty}^{i\infty}\sum_{K}\left(\frac{c_{Kn}[\exp(\mu_{Kn}(x+\delta/2))-\exp(-\mu_{Kn}(x+\rho-\delta/2))]}{\mu_{Kn}^{2}-w_{an}^{2}}\right)d\lambda$$

$$= \delta\chi_{n}[\exp(\nu_{n}(x+\delta/2))-\exp(-\nu_{n}(x+\rho-\delta/2))] \tag{1.3}$$

where

$$\begin{aligned}\delta\chi'_{\ell} &= (\chi'_{\ell})_{0} - (\chi'_{\ell})_{s}\\ \delta\chi_{n} &= (\chi_{n})_{0} - (\chi_{n})_{s}\end{aligned} \tag{1.4}$$

With the solutions to the equations of (1.3) (see Appendix A) it is then possible to determine $c_K$ and $c'_K$ from the continuity of $\psi_a$ at $x = \pm\delta/2$ (see equation (3.11) of Section 2.3).

Denote the initial potential difference between $x = \pm\rho/2$ (internal potential relative to an external ground) by $V_h$ and the clamped potential difference by $V_c$. The time derivative of the ionic current can be neglected in equation (1.13) of Section 2.1 because of the large value of $\theta_a$ for the membrane (approximately $10^{21}$), and so with the help of (3.3) and (3.15) of Section 2.3, an expression for the ionic current of the a-th component can be obtained as follows:

$$\underline{j}_a = \underline{j}_{as} + \frac{e_a \varepsilon_a}{\theta_a m_a} \left( \frac{1}{2\pi i} \int_{-i\infty}^{i\infty} \nabla(\psi_a - \psi) e^{\lambda t} d\lambda \right) \tag{1.5}$$

where

$$\underline{j}_{as} = \frac{e_a \varepsilon_a}{\theta_a m_a} \nabla(\phi_{as} - \phi_s) \tag{1.6}$$

Now from equations (3.10), (3.12) and (3.17) of Section (2.3) we may write (with $\hat{x}$ the unit vector in the positive $x$ direction)

$$\nabla\psi_a = \sum_{K,b} [(d_{bK} \sinh\mu_K x + d_{bK}' \cosh\mu_K x) \frac{\mu_K L_{bK}}{\mu_K^2 - w_a^2}] \hat{x} \tag{1.7}$$

$$\nabla\psi = \sum_{K,b} [(d_{bK} \sinh\mu_K x + d_{bK}' \cosh\mu_K x) \frac{L_{bK}}{\mu_K}] \hat{x} - e_m \hat{x} \tag{1.8}$$

where

$$e_m = \frac{c(\lambda) - c'(\lambda)}{\delta} \tag{1.9}$$

and may be determined from the continuity of $\kappa \nabla\psi$ at $x = \pm\delta/2$:

$$\begin{aligned} \kappa_n \nabla \psi_n &= \kappa_m \nabla \psi \\ \kappa_\ell \nabla \psi_\ell &= \kappa_m \nabla \psi \quad , \end{aligned} \tag{1.10}$$

where, from (3.18) of Section 2.3 and (1.2), the left hand side of each equation may be determined from

$$\nabla\psi_{\ell} = \sum_{K} \left(\frac{-c_{K\ell}'(\lambda)\,[\exp(-\mu_{K\ell}(x-\delta/2))+\exp(\mu_{K\ell}(x-\rho+\delta/2))]}{\mu_{K\ell}}\right)$$

$$\nabla\psi_{n} = \sum_{K} \left(\frac{c_{Kn}(\lambda)\,[\exp(\mu_{Kn}(x+\delta/2))+\exp(-\mu_{Kn}(x+\rho-\delta/2))]}{\mu_{Kn}}\right) \qquad (1.11)$$

The solution to the equations in (1.10) may be written as

$$e_m = \sum_{K,b} \left(\frac{f_{bK}(\lambda)\quad L_{b\ K}}{\mu_K \ \sinh\ (\frac{\mu_K\delta}{2})}\right) \qquad (1.12)$$

where $f_{bK}(\lambda)$ depends on $V_h$ and $V_c$.

If the total current density is denoted by $\underline{j}$ then

$$\underline{j} = \sum_{a} \underline{j}_a - \frac{\kappa}{4\pi}\frac{\partial}{\partial t}(\nabla\phi) \qquad (1.13)$$

where $\sum_{a} \underline{j}_a$ is the total ion current density and

$\frac{-\kappa}{4\pi}\frac{\partial}{\partial t}(\nabla\phi)$ is the displacement current density.

From equations (1.1) and (1.11) of Section 2.1, it is clear that

$$\nabla.\underline{j} = 0 \qquad (1.14)$$

By approximating $w_a^2$ by $\beta_a m_a \theta_a \lambda$ (as $\theta_a >> \lambda$ for low frequency oscillations in the membrane), we obtain from (1.13), with the help of (3.8) and (3.15) of Section 2.3, and (1.5) and (1.12) of this section:

$$j = \sum_a j_{as} + \frac{1}{2\pi i}\int_{-i\infty}^{i\infty} \sum_{K,b} \left(\frac{f_{bK}(\lambda)\ L_{bK}}{\mu_K \sinh\left(\frac{\mu_K \delta}{2}\right)}\right)\left(\lambda + \sum_a \left(\frac{\nu_a^{\,2}}{\gamma_a}\right)\right)\frac{\kappa e^{\lambda t}}{4\pi}\, d\lambda \tag{1.15}$$

where

$$\gamma_a = \beta_a m_a \theta_a$$

The only singularities of the integrand in equation (1.15) which are associated with observable currents occur at those values of $\lambda$ for which

(1) $\mu_K^{\,2} = \mu_\ell^{\,2}$ , $K \neq \ell$ (1.16)

(2) $\mu_K \delta = 2n\pi i$, $n$ is a non-zero integer. (1.17)

Note that the non-zero value of $\lambda$ for which $\mu_K^{\,2} = 0$ is

$$\lambda_0 = -\sum_a \left(\frac{\nu_a^{\,2}}{\gamma_a}\right) \tag{1.18}$$

and hence is not a singularity.

Two Ionic Components

If there are only two ionic components in the membrane the condition for equal roots of the dispersion equation

$$\frac{\nu_1^{\,2}}{\mu^2 - w_1^{\,2}} + \frac{\nu_2^{\,2}}{\mu^2 - w_2^{\,2}} = 1 \tag{1.19}$$

reduces to a quadratic equation in $\lambda$ (if we approximate $w_a^{\,2}$ by $\beta_a m_a \theta_a \lambda$) and this can be solved to yield complex conjugate branch point singularities $\lambda = \alpha \pm i\beta$.

By denoting the contribution to the integral in equation (1.15) from the singularities of type (i) by $S_i$, $i=1,2$, we may write

$$S_1 = \frac{1}{2\pi i}\int_{-i\infty}^{i\infty} \frac{H(\lambda)e^{\lambda t}}{[\lambda-(\alpha+i\beta)]^{\frac{1}{2}}[\lambda-(\alpha-i\beta)]^{\frac{1}{2}}}\, d\lambda \qquad (1.20)$$

The contour can be closed with a large semi-circle in the left half of the complex plane and hence, by the definition of $S_1$, it can be reduced to an integration around the branch cut joining $\alpha+i\beta$ and $\alpha-i\beta$. Equation (1.20) then becomes

$$S_1 = \frac{1}{2\pi}\int_{-i\infty}^{i\infty} \frac{H(\alpha+i\gamma)e^{(\alpha+i\gamma)t}+H(\alpha-i\gamma)e^{(\alpha-i\gamma)t}}{(\beta^2-\gamma^2)^{\frac{1}{2}}}\, d\gamma \qquad (1.21)$$

By defining $u(\gamma)$ and $v(\gamma)$ by

$$H(\alpha+i\gamma) = u(\gamma) + iv(\gamma) \qquad (1.22)$$

equation (1.21) becomes

$$S_1 = \frac{e^{\alpha t}}{2\pi}\int_{-\beta}^{\beta} \frac{u(\gamma)\cos(\gamma t)-v(\gamma)\sin(\gamma t)}{(\beta^2-\gamma^2)^{\frac{1}{2}}}\, d\gamma \qquad (1.23)$$

Let $\gamma = \beta \sin\theta$ and change the variable of integration to $\theta$:

$$S_1 = \frac{e^{\alpha t}}{\pi}\int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} [u(\beta \sin\theta)\cos(\beta t \sin\theta)-v(\beta \sin\theta) \sin(\beta t \sin\theta)]d\theta \qquad (1.24)$$

By expanding $u(\beta \sin\theta)$ and $v(\beta \sin\theta)$ as Fourier series in the variable $\theta$ over the interval $(-\frac{\pi}{2}, \frac{\pi}{2})$ and noting that $u$ and $v$ are even and odd functions of $\theta$, respectively, we have

$$u = u_0 + \sum_{n=1}^{\infty} u_n \cos n\theta \qquad (1.25)$$

$$v = \sum_{n=1}^{\infty} v_n \sin n\theta \qquad (1.26)$$

The Bessel function of the first kind of the n-th order $J_n(x)$ can be written as:

$$J_n(x) = \frac{1}{\pi} \int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} \cos(n\theta - x \sin\theta)\, d\theta \qquad (1.27)$$

Hence, equation (1.24) becomes

$$S_1 = e^{\alpha t} \sum_{n=0}^{\infty} z_n J_n(\beta t) \qquad (1.28)$$

where

$$z_n = \begin{cases} u_n, & n \quad \text{even} \\ -v_n, & n \quad \text{odd} \end{cases}$$

To a first approximation, we may write

$$H(\lambda) = u_0 + i \frac{v_1 \gamma}{\beta} \qquad (1.29)$$

Then equation (1.28) becomes

$$S_1 = e^{\alpha t} (z_0 J_0 + z_1 J_1) \qquad (1.30)$$

We shall now consider the contribution to the integral in equation (1.15) from type (2) singularities. In the two component, the dispersion equation (1.19) may be written

$$\frac{\nu_1^2}{\mu^2-\gamma_1\lambda} + \frac{\nu_2^2}{\mu^2-\gamma_2\lambda} = 1 \tag{1.31}$$

where

$$\mu^2 = -\frac{4n^2\pi^2}{\delta^2}$$

Solving for $\lambda$ in equation (1.31) we have

$$\lambda = -\frac{1}{2\gamma_1}\left(\nu_1^2 + \frac{4n^2\pi^2}{\delta^2}\right) - \frac{1}{2\gamma_2}\left(\nu_2^2 + \frac{4n^2\pi^2}{\delta^2}\right) \pm \frac{1}{2}\left\{\left[\left(\frac{\nu_2^2}{\gamma_1} - \frac{\nu_2^2}{\gamma_2}\right) + \frac{4n^2\pi^2}{\delta^2}\left(\frac{1}{\gamma_1} - \frac{1}{\gamma_2}\right)\right]^2 + \frac{4\nu_1^2\nu_2^2}{\gamma_1\ \gamma_2}\right\}^{\frac{1}{2}} \tag{1.32}$$

When

$$\frac{4n^2\pi^2}{\delta^2} << \nu_1^2 , \quad i=1,2$$

then

$$\begin{aligned} \lambda_{2n-1} &\approx -\left(\frac{\nu_1^2}{\gamma_1} + \frac{\nu_2^2}{\gamma_2}\right) \\ \lambda_{2n} &\approx -\frac{4n^2\pi^2}{\delta^2}\left(\frac{\nu_1^2+\nu_2^2}{\nu_1^2\gamma_2+\nu_2^2\gamma_1}\right) \end{aligned} \tag{1.33}$$

and when

$$\frac{4n^2\pi^2}{\delta^2} >> \nu_1^2 , \quad i=1,2$$

then

$$\lambda_{2n-1} \approx -\frac{4n^2\pi^2}{\delta^2\gamma_1}$$

$$\lambda_{2n} \approx -\frac{4n^2\pi^2}{\delta^2\gamma_2} \qquad (1.34)$$

By evaluating the residue of the integrand in (1.15) for each of the poles $\lambda_n$, $n=1,2,\ldots$ we may write

$$S_2 = \sum_{n=1}^{\infty} R_n e^{\lambda_n t} \qquad (1.35)$$

Hence, there is a sequence of time constants associated with the terms in $S_2$, and is analogous to that obtained from the one-dimensional heat equation (Cole[4], p. 188).

If we consider unit area of the membrane and denote current by the symbol $I$, equation (1.15) may be written as

$$I = \sum_{n=1}^{\infty} R_n e^{\lambda_n t} + (\sum_a I_{as})$$

$$+ e^{\alpha t}(z_0 J_0(\beta t) + z_1 J_1(\beta t)) \qquad (1.36)$$

### N Ionic Components

In the case where there are three ionic components in the membrane then the condition for equal roots of the dispersion equation

$$\frac{\nu_1{}^2}{\mu^2-w_1{}^2} + \frac{\nu_2{}^2}{\mu^2-w_2{}^2} + \frac{\nu_2{}^2}{\mu^2-w_3{}^2} = 1 \qquad (1.37)$$

is a sextic polynomial in $\lambda$ (its form will be examined in Chapter 4). The roots of this polynomial will include two pairs of complex conjugate branch point singularities

$$\lambda_i = \alpha_i \pm i\beta_i, \quad i=1,2$$

and a pole singularity at $\lambda = 0$ (see Appendix B). If there are four ionic components in the membrane then the condition for equal roots of the dispersion equation

$$\sum_{b=1}^{4} \frac{\nu_b{}^2}{\mu^2-w_b{}^2} \tag{1.38}$$

is a polynomial of twelfth degree in $\lambda$ with roots which include three pairs of complex conjugate branch point singularities

$$\lambda_i = \alpha_i \pm i\beta_i \ , \quad i=1,2,3$$

and also a pole singularity at $\lambda = 0$ (see Appendix B).

In general, for an $N$ component electrolyte within the membrane, the equal root condition will generate $(N-1)$ pairs of complex conjugate branch point singularities

$$\lambda_i = \alpha_i \pm i\beta_i \ , \ i=1,2,\ldots N-1$$

Hence, the generalization of (1.36) for an electrolyte with $N$ ionic species is

$$I = I_c + I_i \tag{1.39}$$

where

$$I_c = \sum_{n=1}^{\infty} R_n e^{\lambda_n t} \tag{1.40}$$

$$I_i = \sum_{a=1}^{N} I_{as} + \sum_{b=1}^{N-1} e^{\alpha_b t} [(z_0)_b J_0(\beta_b t) + (z_1)_b J_1(\beta_b t)] \tag{1.41}$$

Here $R_n$, $n=1,2,\ldots$ is the sequence of residues of the integrand in equation (1.15) for the poles $\lambda_n$ which are obtained from the $N$ component dispersion equation

$$\sum_{b=1}^{N} \frac{\nu_b^{\,2}}{\mu^2 - w_b^{\,2}} \tag{1.42}$$

with

$$\mu^2 = -\frac{4n^2\pi^2}{\delta^2} \quad .$$

$I_c$ may be interpreted as the capacity current which is the term that has been used to describe the current observed in voltage clamp records when the trans-membrane ionic current has been eliminated by the application of various chemical agents, whereas $I_i$ may be identified with the observable ionic current.

## 3.2 THE SQUID GIANT AXON

In this section we consider the voltage clamp currents which flow across a squid giant axon membrane surrounded by physiological electrolyte solutions. It will be shown that the expression for the ionic current derived in the last section (equation (1.41)) may be reduced to a sum of two terms having a magnitude and time course that are in qualitative agreement with those which appear in the interpretation of the voltage clamp experiments by Hodgkin and Huxley[13]. The expression for the capacity current is also in agreement with experimental observations and can be used to explain the presence of "gating" current in experiments where the ionic current is considerably reduced. Finally, consideration is given to the temperature dependence of the time constants of the voltage clamp currents.

Suppose physiological concentrations of $K^+$, $Na^+$ and $Cl^-$ are present in a squid giant axon and its environment (without $Ca^{++}$ or other polyvalent ions) and choose

$$\theta_K : \theta_{Na} : \theta_{Cl} : \theta_{Ca} = 1 : 10 : 12.5 : 100 \qquad (2.1)$$

with $\theta_K = 5 \times 10^{20}$ $\sec^{-1}$. These are representative values for the resistance of the membrane to these ions and are obtained from steady-state ionic flux data (Hurlbut[95]; Lakshminarayanaiah[93]). We can now solve the sextic equation obtained from the condition for equal roots of equation (1.37) and, to a first approximation, the branch point singularities specified by $\alpha_1 \pm i\beta_1$

and $\alpha_2 \pm i\beta_2$, may be associated with the sodium and potassium currents respectively. If $\beta_1$ and $\beta_2$ are determined from the concentrations which are present at the outer and inner membrane boundaries, respectively, then we obtain

$$\beta_1 = 1 \text{ msec}^{-1} \quad \text{and} \quad \beta_2 = .5 \text{ msec}^{-1} \tag{2.2}$$

and so the time constants for the sodium and potassium currents are in good agreement with those observed experimentally. The electronic feedback system ensures that the electric field oscillations in the membrane are more strongly damped than in the case of free subthreshold oscillations, and this may be represented by choosing correspondingly larger inverse time constants:

$$\alpha_1 = -1 \text{ msec}^{-1} \quad \text{and} \quad \alpha_2 = -1 \text{ msec}^{-1} \tag{2.3}$$

In comparison, the damping constants for undamped subthreshold currents are less than (in magnitude) $.1 \text{ msec}^{-1}$.

Each of the sodium, potassium and chloride currents will, in general, have contributions from the $J_0$ and $J_1$ terms in equation (1.41) (and also higher order terms). However, Hodgkin and Huxley have observed that the steady state current has a larger contribution from $K^+$ than $Na^+$, whereas the inward current consists mainly of $Na^+$. Therefore, to a first approximation, it may be assumed that the steady state current consists only of $K^+$ and the current associated with the $J_1$ terms consist only of $Na^+$. If the difference between the clamped potential and the holding potential is not too large, then one can see from equations (1.15) and (1.29) that these currents are about of the same order of magnitude and are determined by the membrane resistances to these ions.

From (2.2) and (2.3) and the above assumptions, a typical small voltage clamp record is obtained as follows:

$$I_i = I_{Na} + I_K$$

where

$$I_{Na} = - 6\ J_1(t)\ \exp(-t)$$

$$I_K = 1 - J_0(t/2)\ \exp(-t)$$

These curves are plotted in Figure 3.1. The absence of the delay in the rise of the potassium current indicates that the delay in the rise of the voltage clamp current when the clamping potential is equal to the sodium equilibrium potential may be explained by assuming that initially the sodium current is equal and opposite to that of the potassium current, with resultant mutual damping. However, for large voltage clamps, a precise determination of the magnitudes and time courses of the ionic currents requires an examination of nonlinear effects.

If we now consider the above system with increasing concentrations of $Ca^{++}$ and solve the equal root polynomial in $\lambda$ for the four component case, then the

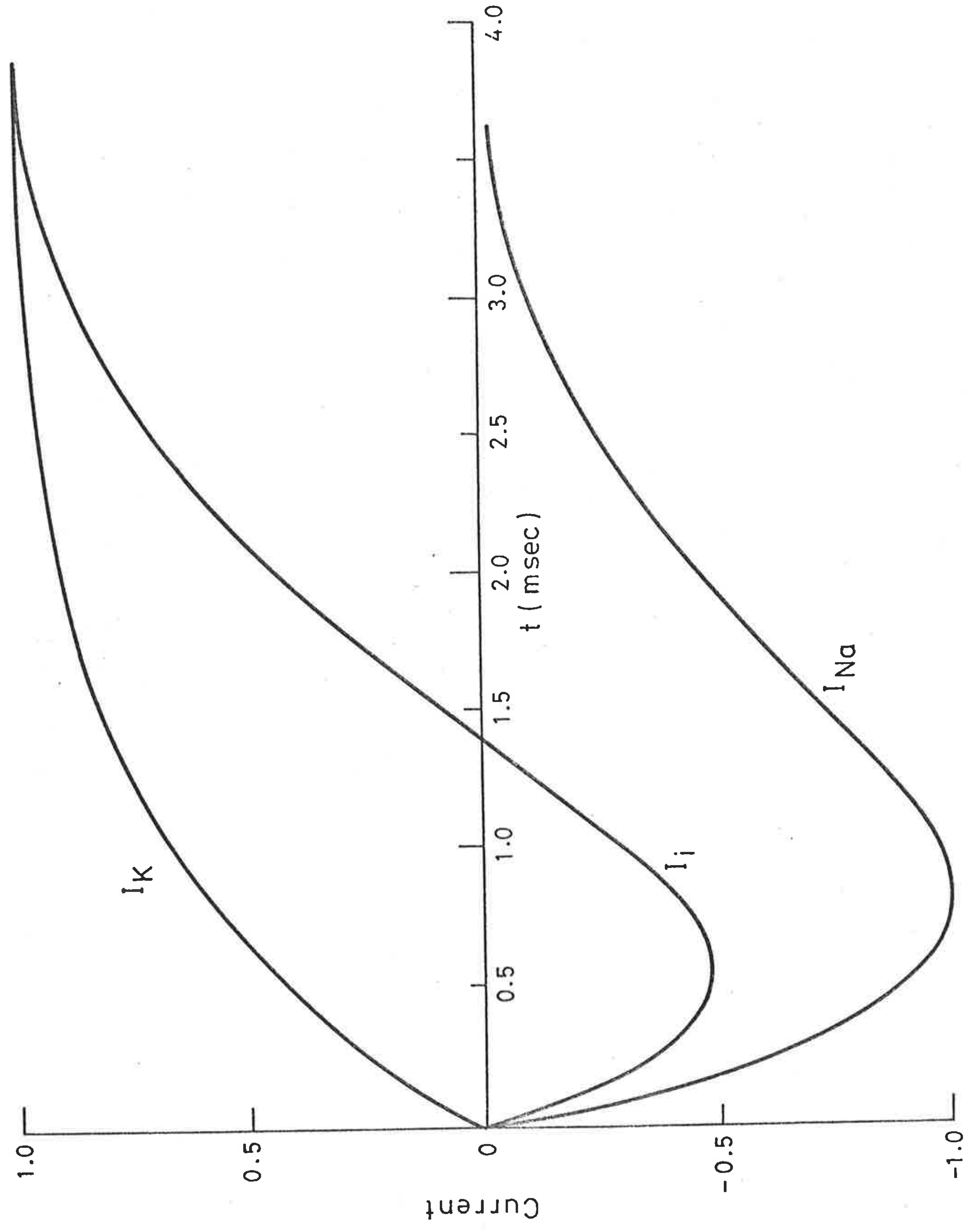

FIGURE 3.1: A typical low voltage clamp record.

result is increased damping of the voltage clamp currents. This is consistent with the experimental studies of the effects of $Ca^{++}$ on membrane excitability: these demonstrate that increasing the external calcium concentration increases the threshold for firing (Frankenhaeuser and Hodgkin; Blaustein and Goldman; Narahashi[96]) and decreasing the external calcium concentration is associated with oscillating membrane potentials. It has also been observed that when calcium ions are removed from the external solution the frog node becomes excitable (Frankenhaeuser[97]). The necessity of the presence of small concentrations of polyvalent ions in the external solution can be understood in terms of the electrodiffusion model if one assumes that the membrane has a fixed negative charge. The effect of this fixed charge is to displace the branch cut associated with the inward sodium current, somewhat to the right, and if the real part of the branch cut becomes positive then the large inward current vanishes and the cell loses its excitability. This is because, for large times, the contour along the imaginary axis in equation (3.3) of Section 2.3 can only be closed in the left half of the complex plane, and hence singularities in the right half do not contribute to the ionic potentials or currents.

We now consider the capacity current which is observed when a voltage clamp is applied to the squid giant axon. It usually has a time constant of the order of 10 μsec but under some circumstances it has a considerably larger relaxation time of the order of 10 msec (Hodgkin, Huxley and Katz[12]; Meves and Vogel[98]). This "tail" in the capacity transient is not explained by the presence of a series resistance but instead has been attributed to imperfections in the membrane capacity. In the present theory, the exponential terms have a sequence of time constants given by (1.33) and (1.34), the largest of which (about 10 msec) corresponds to small values of $n$. Also, the magnitude of the capacity current is in agreement with that obtained from RC-circuit model of the membrane, as can be seen from the expression for the voltage clamp current (equation (1.15)).

When the ionic current across the membrane is blocked by the addition of certain chemical agents (e.g. tetrodotoxin) or by the presence of impermeant ions (e.g. $Cs^+$) and hyperpolarizing and depolarizing voltage clamp pulses of equal size are applied to the axon, the resulting capacity currents exhibit asymmetry. Now, it was assumed by Hodgkin and Huxley[13] that a change in the electric field across the membrane caused a reorientation and redistribution of certain charged membrane molecules ("gates") which determined the resistance to ions, and that this was the only factor responsible for a nonlinear change in the current. Consistent with this hypothesis, the capacity current asymmetry was assumed to be a manifestation of "gating current" (Armstrong and Bezanilla; Keynes and Rojas[99]).

In the present model, we are assuming that the resistance of the membrane to ions does not depend on time explicitly but is a function of the steady state electric field within the membrane. Consequently, the time constants associated with the exponential terms in the expression for the capacity current (equation (1.40)) will also depend on the magnitude of the voltage clamp.

Let the capacity current for a positive step in the voltage be represented by a single exponential term

$$I_{c+} = R \exp(-at)$$

and similarly for an equal negative step in the voltage

$$I_{c-} = - R \exp(-bt)$$

where $R$ is a constant determined by the characteristics of the membrane phase. Then $I_{c\Sigma}$, the sum of the positive step and negative step capacity transients, is given by

$$I_{c\Sigma} = R[\exp(-at) - \exp(-bt)]$$

This is plotted in Figure 3.2 (with $a = 5$ msec$^{-1}$, $b = 6.5$ msec$^{-1}$ and $R = 1$ mamp) and may be compared with the experimental records of the "gating current" (Armstrong[100]).

Temperature

The effect of temperature on a biological variable or parameter may be described by specifying its temperature coefficient $Q_{10}$ which is defined to be

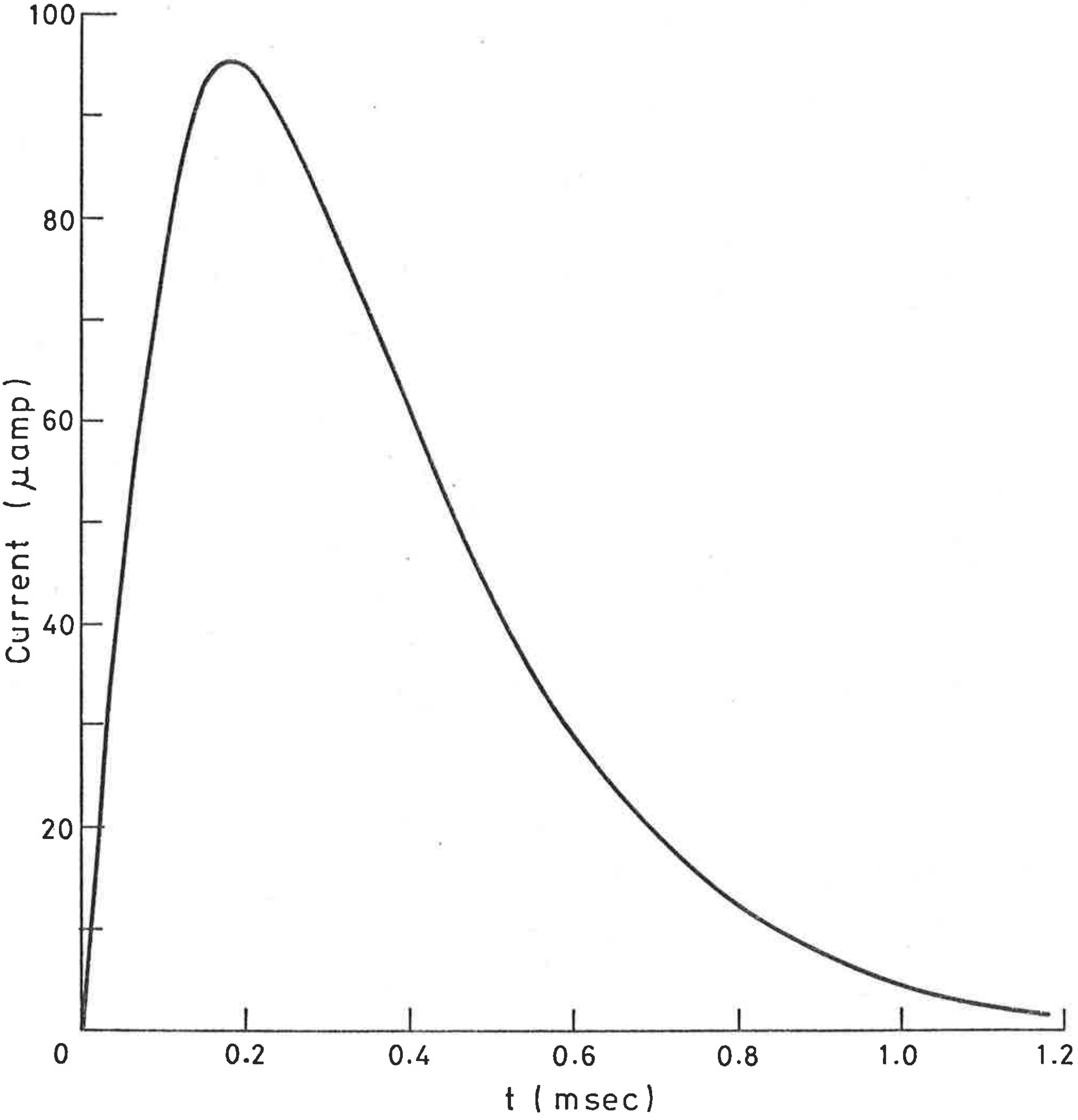

FIGURE 3.2: The sum of the capacity currents during equal positive and negative steps in the voltage.

$$Q_{10,x} = \frac{x(T+10)}{x(T)}$$

where $T$ is measured in $^{\circ}$C. The temperature coefficients for the rate constants $\alpha_s$ and $\beta_s$ of the giant axon are of the order of 3 whereas $Q_{10}$ values for $\bar{g}_{Na}$ and $\bar{g}_K$ are about 1.3, where $\bar{g}_{Na}$ and $\bar{g}_K$ are the maximum conductances for sodium and potassium (Hodgkin and Huxley[13]).

The temperature dependence of the magnitude of the sodium and potassium currents follows from the $Q_{10}$ value (about 1.4) for the steady state membrane resistance to these ions (see Cohen and Landowne[101]). If one considers equation (3.8) of Section 2.3 and notes that $\nu_b^2$ and $w_b^2$ are both proportional to $\beta_b^2$, then one can see that in the linear theory, the temperature dependence of the time constants of the ionic currents also arises from the temperature coefficients of the membrane resistances $\theta_b$, and these are too small by a factor of two. However, in a nonlinear theory one would expect that the time constants would include, in addition, an explicit temperature dependence resulting from the Boltzmann factor in the charge density (equation (1.11) of Section 2.1). In particular, if $e_b\phi_b$ had a value of about 30kT then the ionic current relaxation time would have the correct temperature coefficient of 3. Recent calculations of the electrostatic energy of ions in aqueous channels have demonstrated that the energy of $n$ ions in a channel $(n=1,2,3,\ldots)$ increases rapidly with $n$ (Levitt[102]), so that if there is a negative fixed charge within the channels, then it is reasonable to expect that $e_b \phi_b$ does in fact approach the value $30kT$.

## 3.3 THE PERFUSED AXON AND OTHER EXCITABLE CELLS

In this section we consider the application of the electrodiffusion model firstly to the squid giant axon with non-physiological solutions and then to other excitable cells.

The experimental results obtained with squid axons both in large calcium concentration solutions are important because of the significance of polyvalent ions, and in particular calcium ions, for cell excitability. If the axon is perfused with an isotonic solution of 25mM-CsF+ sucrose and placed in a solution of 100mM $CaCl_2$+sucrose then the resting potential is about -30mV. By clamping the potential difference across the axon membrane to a more positive value, a small $Ca^{++}$ inward current with a time constant of 10-20 msec is usually observed. When small amounts of $Na^+$ (10-50 mM) are added to the external solution and a small clamp is again applied to the axon then the inward current is composed of $Na^+$ (the "fast" component) and $Ca^{++}$ (the "slow" component) with time constants 2.5 msec. and 14 msec. respectively (Meves and Vogel[98]).

Now if we consider a four-component system consisting of $Na^+$, $Cl^-$, $ca^{++}$ and $Cs^+$ in the above concentrations and assume that

$$\theta_{Na} : \theta_{Cl} : \theta_{Ca} : \theta_{Cs} = 1 : 1.25 : 10 : 15 \ ,$$

which are the accepted steady state membrane resistances for these ions, then the condition for equal roots of the four-component dispersion equation can be reduced to a sextic polynomial with, in general, non-zero roots (see Appendix B). When this is solved to calculate the time constants of the voltage clamp currents, we find that those associated with the $Na^+$ and $Ca^{++}$ currents are of the same order of magnitude as those observed experimentally, that is, 1 msec and 20 msec, respectively. The "fast" component would be interpreted as the movement of $Na^+$ with respect to $Cl^-$ and the other ions present, and the "slow" component as the movement of $Ca^{++}$ with respect to these ions. This would explain why the "fast" component is never observed without the presence of NaCl in the external solution (which is not explained by the "gating" theory).

Further confirmation of the electrodiffusion model of excitable cells is obtained from experiments on squid giant axons with low ionic strength solutions. If the axons are perfused with solutions diluted with an isotonic sugar solution then action potentials can still be elicited even though the resting potential is near zero (Tasaki and Shimamura; Baker, Hodgkin and Shaw; Narahashi; Baker, Hodgkin and Meves[103]). This result was not expected because, according to the Hodgkin-Huxley theory, there should be extensive inactivation at low resting potentials. However, a shift in the sodium activation and inactivation versus membrane potential curves was observed (Moore, Narahashi and Ulbricht; Chandler, Hodgkin and Meves[104]) and, by assuming the existence of a negative fixed charge at the inner membrane surface, it was suggested that the resulting double layer potential caused a change in the kinetic characteristics of the sodium channel (Baker, Hodgkin and Meves; Chandler, Hodgkin and Meves[105]). In the present theory, if a voltage clamp is applied to an axon, the resulting sodium current depends on the difference between the holding potential $V_h$ and the clamp potential $V_c$, and not directly on the resting potential. The generation of an action potential depends on the nonlinear coupling of the ionic currents with the electric field within the membrane and this operates independently of the magnitude of the resting potential.

It has also been observed that the action potentials from axons perfused with low ionic strength solutions or axons with low external potassium concentrations have a long duration (Narahashi; Baker, Hodgkin and Meves; Adelman, Dyro and Senft[106]). This has been accounted for by assuming that a low potassium concentration is associated with incomplete sodium inactivation and an increase in the sodium inactivation time constant. By solving the equal root polynomial, we find that the effect of a decreased membrane concentration of potassium is to increase the potassium current time constant and reduce the damping of the sodium current, and this is consistent with the above observations.

The solution of the linearized electrodiffusion equations has, previously in this chapter, only been applied to squid giant axons, but we shall now give a brief consideration to its application to a number of other excitable cells.

For barnacle muscle fibres, the upstroke of the action potential is the result of an inward calcium current and its amplitude is independent of the external concentration of sodium (Hagiwara[107]). A discussion of the corresponding voltage clamp records would be similar to that already given for calcium currents across the squid axon membrane. In heart muscle fibres, depolarization is accompanied by an inward sodium current and repolarization by a slow outward potassium current which results in the characteristic cardiac action potentials (Draper and Weidmann[108]). More detailed analysis of the voltage records obtained from Purkinje fibres indicates that the transient inward current is carried mainly by sodium ions but also has contributions from calcium ions with a time constant (about 50 msec) similar to that associated with calcium currents across the axon membrane. The plateau is principally the result of an increased resistance to potassium ions as the membrane becomes depolarized (Noble and Tsien; McAllister, Noble and Tsien[109]).

The electric eel is capable of producing electric currents in the water surrounding it and these are generated by specialized cells called electroplaques. However, in contrast to the squid giant axon, the spikes are characterized by an increased sodium current but a reduced potassium steady state current (Keynes and Martins-Ferreira, Nakamura, Nakajima and Grundfest[110]).

The voltage clamp records obtained from the fresh-water plants *Chara* and *Nitella* are also similar to those obtained from the squid axon, except that the inward current corresponds to the outward flux of chloride ions, the time constants are about a thousand times larger (1 second) and the magnitude of the currents are reduced by approximately the same factor (Gaffey and Mullins; Mullins; Kishimoto[111]). From the condition for equal roots of the dispersion equation and the expression derived for the voltage clamp current (equation (1.15)), one would expect such a result if the resistance of the plasmalemma to the active ions is approximately a thousand times larger than that for the axon membrane.

Although the detailed description of the voltage clamp ionic currents varies considerably from one cell to another, and has only been accounted for in the Hodgkin-Huxley model of the squid axon and its extensions by postulating a separate gate for each class of ions with similar dynamics with respect to a given excitable membrane, this variation can be explained faithfully in the electrodiffusion model by application of the condition for equal roots of the n-component dispersion equation. This leads to the conclusion that a change in the potential difference across a cell membrane results in a capacity current and, in general, two kinds of current for each permeable ion - a damped oscillatory current (for the voltage clamp, a transient peak current) and a relaxation to a new steady state current.

# CHAPTER 4

# SUBTHRESHOLD OSCILLATIONS AND THE ACTION POTENTIAL

## 4.1 PLASMA OSCILLATIONS IN THE MEMBRANE

Plasma oscillations in an ionized medium are oscillatory motions of the charged particles which are coupled to the electromagnetic field and arise from the long-range nature of the Coulomb force. They may be generated in the cell membrane by a stimulus which is applied to the surrounding electrolytes and which then propagates to the membrane where the Debye layers are disturbed. This perturbation is accompanied by relaxation oscillations within the membrane, and with the appropriate membrane resistance to these ions and sufficiently large concentration gradients across the membrane, the amplitude of the plasma oscillations is enhanced by nonlinear coupling effects, and this in turn facilitates propagation of these oscillations to adjacent regions.

In this section we consider the normal modes of oscillation in an ionized medium of two ions, and the nature of the singularities associated with the equal root condition for the three and four-ion dispersion equation. In the next section we examine the oscillations in the potential difference across the membrane for a subthreshold stimulus, and in Section 4.3 we extend the previous analysis to the nonlinear plasma oscillations which characterize the action potential.

We begin by considering the interaction of just two different kinds of ions within the membrane. In this case the dispersion equation (3.8) of Chapter 2 becomes

$$\frac{\nu_1^2}{\mu^2-w_1^2} + \frac{\nu_2^2}{\mu^2-w_2^2} = 1 \quad . \tag{1.1}$$

As shown in Section 2.3 the normal modes of oscillation within the membrane are to be expected when the roots $\mu_1^2$ and $\mu_2^2$ are equal and in this case the condition for equal roots is

$$(w_2^2 - \nu_2^2 - w_1^2 - \nu_1^2)^2 + 4\nu_1^2\nu_2^2 = 0 \tag{1.2}$$

where

$$\begin{aligned} w_2^2 - w_1^2 &= \beta_2 m_2 \lambda(\lambda+\theta_2) - \beta_1 m_1 \lambda(\lambda+\theta_1) \\ &= \nu_1^2 - \nu_2^2 \pm 2i\,\nu_1\nu_2 \quad . \end{aligned} \tag{1.3}$$

One of the solutions of this quadratic equation is given by

$$\lambda = -\frac{\theta_1\theta_2(\gamma_2-\gamma_1)}{\gamma_2\theta_1-\gamma_1\theta_2} - \left(\frac{\nu_1^2-\nu_2^2\pm 2i\nu_1\nu_2}{\gamma_2-\gamma_1}\right) \tag{1.4}$$

and represents very strongly damped oscillations and so can be disregarded (for the squid giant axon, $\theta$ is of the order of $10^{21}$, $\gamma \approx 10^{12}$ and $\nu^2 \approx 10^{15}$ in the membrane region). The other solution is

$$\lambda = \frac{\nu_1{}^2-\nu_2{}^2\pm 2i\nu_1\nu_2}{\gamma_2-\gamma_1} \tag{1.5}$$

and corresponds to observable oscillations of frequency $10^3$Hz. for the voltage clamp, subthreshold oscillations and the action potential.

If the ions are monovalent, it is evident from equation (3.2) of Chapter 2 that, to secure electrical neutrality, $\nu_1{}^2 = \nu_2{}^2$ so that, provided $\nu_1{}^2 - \nu_2{}^2$ and $\gamma_2 - \gamma_1$ do not have the same sign, almost undamped oscillations of angular frequency

$$\omega = \mp i\lambda = \frac{2\nu_1\nu_2}{\gamma_2-\gamma_1} \tag{1.6}$$

will result.

The value of $\mu^2$ corresponding to the condition in equation (1.2) is

$$\mu^2 = 1/2(w_1{}^2 + w_2{}^2 + \nu_1{}^2 + \nu_2{}^2) \tag{1.7}$$

so that the associated normal modes of oscillation are obtained by setting

$$\frac{\partial\phi_a}{\partial t} = \lambda\phi_a \tag{1.8a}$$

$$\nabla^2\phi_a = 1/2(w_1{}^2 + w_2{}^2 + \nu_1{}^2 + \nu_2{}^2)\phi_a \tag{1.8b}$$

in equation (3.1) of Chapter 2. We thus obtain the equations

$$\begin{aligned} 1/2(w_2{}^2 + \nu_2{}^2 - w_1{}^2 - \nu_1{}^2) &= \nu_2{}^2\phi_2 \\ 1/2(w_1{}^2 + \nu_1{}^2 - w_2{}^2 - \nu_2{}^2) &= \nu_1{}^2\phi_1 \end{aligned} \tag{1.9}$$

and hence when $\nu_1{}^2 = \nu_2{}^2$ ,

$$\nu_1\phi_1 \mp i\nu_2\phi_2 = \nu\phi_{\pm} \exp(\pm i\omega t) \qquad (1.10)$$

where $\nu\phi_{\pm}$ is independent of time, though it depends on $x$ in a manner determined by equation (1.8b).

With three different kinds of ions in interaction within the membrane, the condition for equal roots of the dispersion equation (3.8) of Chapter 2 is (see Appendix B):

$$\frac{p^3}{27} + \frac{q^2}{4} = 0 \qquad (1.11)$$

where

$p=\eta_1\eta_2+\eta_2\eta_3+\eta_3\eta_1-\nu_1{}^2\nu_2{}^2-\nu_2{}^2\nu_3{}^2-\nu_3{}^2\nu_1{}^2$

$q=\eta_1\eta_2\eta_3-\nu_1{}^2\nu_2{}^2\eta_3-\nu_2{}^2\nu_3{}^2\eta_1-\nu_3{}^2\nu_1{}^2\eta_2+2\nu_1{}^2\nu_2{}^2\nu_3{}^2$

$$\eta_a = \gamma_a\lambda + \nu_a{}^2 - [(\gamma_1 + \gamma_2 + \gamma_3)\lambda + \nu_1{}^2 + \nu_2{}^2 + \nu_3{}^2]/3 \qquad (1.12)$$

and $w_a{}^2$ has been approximated by $\gamma_a\lambda$. In this case equation (1.11) is a sextic equation in $\lambda$ and there are therefore six singularities affecting the integral in equation (3.3) of Chapter 2, associated with the vanishing of the denominators $\mu_k{}^2-\mu_\ell{}^2$. It is easily shown (see Appendix B) that when $\mu_k{}^2-\mu_\ell{}^2$ is small,

$$\mu_k{}^2-\mu_\ell{}^2 = \pm\frac{(\frac{4}{3})(\frac{p^3}{27} + \frac{q^2}{4})^{\frac{1}{2}}(\omega_k-\omega_\ell)}{(2q^2)^{\frac{1}{3}}} \qquad (1.13)$$

where $(\omega_1,\omega_2,\omega_3) = (1,\omega,\omega^2)$ and $\omega$ is a complex root of unity, and that equation (1.11) has a double root at $\lambda = 0$, and so the four remaining singularities are quadratic branch points.

Although equation (1.11) can in general be reduced to a quartic and solved exactly by quadratures, the roots can also be determined approximately by setting

$$w_2{}^2 = w^2 - \left(\frac{\delta w^2 \ \nu_3{}^2}{\nu_2{}^2+\nu_3{}^2}\right)$$

$$w_3{}^2 = w^2 - \left(\frac{\delta w^2 \ \nu_2{}^2}{\nu_2{}^2+\nu_3{}^2}\right) \qquad (1.14)$$

so that $\delta w^2 = w_3{}^2 - w_2{}^2$, and neglecting terms quadratic in $\delta w^2$, the dispersion equation then reduces to the quadratic

$$\frac{\nu_1{}^2}{\mu^2-w_1{}^2} + \frac{\nu_2{}^2+\nu_3{}^2}{\mu^2 - w^2} = 1 \qquad (1.15)$$

which is similar to equation (1.1). Two other quadratics are obtained by permuting the subscripts 1,2 and 3. In this way one sees that the roots are in general complex, and that one pair is almost pure imaginary when the condition for electrical neutrality is satisfied, and hence corresponds to almost undamped oscillations. One can also see from equation (1.15) and equation (3.2) of Chapter 2, that polyvalent cations have a more pronounced damping effect on the electrolyte system than monovalent cations (and this is confirmed by consideration of the exact equations - see Chapter 3), and this suggests why calcium ions on similar polyvalent cations are required for excitability (Frankenhaeuser[97]).

Finally, with four ionic composents in the membrane the condition for equal roots of the dispersion equation is a polynomial (D) of the twelfth degree. Again, it may be shown (see Appendix B) that when $\mu_k{}^2-\mu_\ell{}^2$ is small,

$$\mu_k{}^2-\mu_\ell{}^2 = \frac{\pm(\frac{2}{3})D\ (\omega_i-\omega_j)}{(2q^2)^{\frac{1}{3}}(\frac{q^2}{4} - \frac{2b}{3} + (\frac{q}{2})^{\frac{1}{3}})^{\frac{1}{2}}}\ , \tag{1.16}$$

where a,b, and q can be expressed in terms of $\gamma_i$ and $\nu_i{}^2$ (i=1,...,4), and that the polynomial D has a root of order 6 at $\lambda = 0$. Hence, the other six roots are again quadratic branch points.

## 4.2 SUBTHRESHOLD OSCILLATIONS

When a constant current is applied to an axon membrane then there is in general a damped oscillatory response in the trans-membrane potential difference (Sabah and Leibovic; Mauro et al.[112]). This oscillation has been described in terms of the voltage and time-dependent membrane conductances for sodium and potassium ions, which were first determined from the voltage clamp experiments by Hodgkin and Huxley[13]. In this approach the axon membrane is modelled by an equivalent electrical circuit which includes a capacitance, an inductance and a resistance, and variations in the potential difference across the membrane are attributed to voltage-dependent membrane ion permeabilities. In Chapter 3 we described the voltage clamp currents across the excitable membrane in terms of damped plasma oscillations within the membrane phase, and in this section we show that a similar treatment is also successful in accounting for subthreshold oscillations.

The geometry of the experimental system is assumed to be the same as that considered for the voltage clamp (see Figure 2.1 of Section 2.2), but in this case the electrodes maintain a constant current at the points $x = -\rho/2$ and $x = +\rho/2$ for the duration of the current clamp. Initially (at $t = 0$) the potentials $\phi_a$ and $\phi$ between the electrodes have values ($\phi_{a0}$ and $\phi_0$) characteristic of a steady state, but following the application of a constant current at the electrodes, these potentials will approach the new steady state values $\phi_{as}$ and $\phi_s$.

The steady state solutions of Section 2.2 were appropriate for voltage clamp boundary conditions but if a constant current is maintained at the electrodes then they require a minor modification in regions $R_\ell$ and $R_n$ as follows:

$$\phi_{a\ell} = \chi_\ell'(\exp[-\nu_\ell(x-\delta/2)] + \exp[\nu_\ell(x-\rho+\delta/2)])$$

$$\phi_\ell = \chi_\ell'(\exp[-\nu_\ell(x-\delta/2)] + \exp[\nu_\ell(x-\rho+\delta/2)]) + V'$$

$$\phi_{an} = \chi_n(\exp[\nu_n(x+\delta/2)] + \exp[-\nu_n(x+\rho-\delta/2)])$$

$$\phi_n = \chi_n(\exp[\nu_n(x+\delta/2)] + \exp[-\nu_n(x+\rho-\delta/2)]) + V \qquad (2.1)$$

The general solutions $\psi_a$ and $\psi$ of equations (3.4) and (3.16), respectively, of Section 2.3 for the regions $R_\ell$ and $R_n$ are given by (1.1) of Section 3.1 and (3.18) of Section 2.3, and so we may write, with the help of (1.5) of Chapter 3:

$$\sum_a \underline{j}_{a\ell} = \sum_a (\underline{j}_{a\ell})_s + \Bigg(\frac{1}{2\pi i}\int_{-i\infty}^{i\infty} \Bigg(\frac{c_{k\ell}e^{\mu_{k\ell}(x-\delta/2)} - c_{k\ell}'e^{-\mu_{k\ell}(x-\delta/2)}}{\mu_{k\ell}}\Bigg)\lambda\frac{\kappa e^{\lambda t}}{4\pi}d\lambda$$

$$\sum_a \underline{j}_{an} = \sum_a (\underline{j}_{an})_s + \frac{1}{2\pi i}\int_{-i\infty}^{i\infty} \frac{c_{kn}e^{\mu_{kn}(x+\delta/2)} - c_{kn}'e^{-\mu_{kn}(x+\delta/2)}}{\mu_{kn}}\lambda\frac{\kappa e^{\lambda t}}{4\pi}d\lambda \qquad (2.2)$$

The constant current at the electrodes $x = \pm\rho/2$ implies that

$$c_{k\ell} = c_{k\ell}' \exp(\mu_{k\ell}(-\rho+\delta))$$

$$c_{kn}' = c_{kn} \exp(\mu_{kn}(-\rho+\delta)) \qquad (2.3)$$

The initial conditions for $\phi_a$ in $R_\ell$ and $R_n$ can now be written as

$$\frac{1}{2\pi\mathbf{L}}\int_{-i\infty}^{i\infty} \sum_k \left( \frac{c_{k\ell}'[\exp(-\mu_{k\ell}(x-\delta/2)) + \exp(\mu_{k\ell}(x-\rho+\delta/2))]}{\mu_{k\ell}^2 - w_{a\ell}^2} \right) d\lambda$$

$$= \delta\chi_\ell'[\exp(-\nu_\ell(x-\delta/2)) + \exp(\nu_\ell(x-\rho+\delta/2))]$$

$$\frac{1}{2\pi\mathbf{L}}\int_{-i\infty}^{i\infty} \sum_k \left( \frac{c_{kn}[\exp(\mu_{kn}(x+\delta/2)) + \exp(-\mu_{kn}(x+\rho-\delta/2))]}{\mu_{kn}^2 - w_{an}^2} \right) d\lambda$$

$$= \delta\chi_n[\exp(\nu_n(x+\delta/2)) + \exp(-\nu_n(x+\rho-\delta/2))] \qquad (2.4)$$

where

$$\delta\chi_\ell' = (\chi_\ell')_0 - (\chi_\ell')_s$$

$$\delta\chi_n = (\chi_n)_0 - (\chi_n)_s \qquad (2.5)$$

Again, $c_k$ and $c_k'$ can be determined from the solutions to the equations in (2.4) (see Appendix A) and the continuity of $\psi_a$ at $x = \pm\delta/2$ .

Now, if we denote the constant current per unit area at the electrodes by $I_e$ and the potential difference across the membrane by $V$ (that is, take the electric potential $V'$ in $R_\ell$ to be zero) then from (1.13) of Section 3.1 we have

$$V(t) = \frac{1}{c}\int_0^t [I_e - I_i(\tau)]d\tau \qquad (2.6)$$

where

$$C = \frac{\kappa}{4\pi\delta} \qquad (2.7)$$

is the capacity of unit area of the membrane, and $I_i$ is the ionic current and is given by an expression of the form (1.41) of Section 3.1 ($(z_0)_b$ and $(z_1)_b$ will clearly depend on the boundary conditions of the current clamp).

We now show that the expression for the potential variation during a current clamp given by equation (2.6) is in agreement with experimental observation. In Chapter 3 we considered the case of three ions in interaction within the axon membrane (sodium, potassium and chloride ions) and, as the extracellular concentration of $K^+$ and the intracellular concentration of $Na^+$ are small, we assumed that the branch point singularities $\alpha_1 \pm i\beta_1$ and $\alpha_2 \pm i\beta_2$ could be associated with the sodium and potassium currents, respectively, and calculated the oscillation frequencies $\beta_1$ and $\beta_2$ from the concentrations of ions at the membrane surfaces. If instead, $\alpha_i$ and $\beta_i$, $i=1,2$ are determined from the average ion concentrations within the membrane then we find that

$$\beta_1 \approx \beta_2 = 1\text{msec}^{-1} \tag{2.8}$$

and

$$\alpha_1 = -1\text{msec}^{-1},\ \alpha_2 = -.1\text{msec}^{-1} \tag{2.9}$$

In general, the integration around each branch cut will contribute to each of the ionic currents through the membrane, but for the squid giant axon, as $|\alpha_1| > |\alpha_2|$, the contribution from the branch cut joining $\alpha_2 + i\beta_2$ and $\alpha_2 - i\beta_2$ will dominate.

If the constant current at the electrodes is small (about $1\mu\text{amp cm}^2$) then there will be little change in the steady state sodium current and so we may assume that the clamp current is equal to the steady state potassium current. Therefore, the ionic current response $I_i$ may be written as

$$I_i = I_o + I_r \tag{2.10}$$

where

$$I_o = -1.5J_1(t)\exp(-\frac{t}{10}) \tag{2.11}$$

is the damped oscillatory current and has contributions from both sodium and potassium ions, and

$$I_r = 1 - J_o(t)\exp(-\frac{t}{10}) \tag{2.12}$$

is the relaxation of the potassium current to a new steady state value. With these expressions the variation in the potential difference across the membrane is given by

$$V(t) = \frac{I_e}{c}\int_0^t [J_o(\tau)+1.5J_1(\tau)]\exp(-\frac{\tau}{10})d\tau \tag{2.13}$$

where $I_e = 1\mu\text{amp cm}^2$ and $C = 1\mu\text{farad cm}^2$. Equation (2.13) is plotted in Figure 4.1. We can see from equation (2.13) that the subthreshold oscillation about the resting potential is symmetrical with respect to positive and negative applied currents. Although approximate symmetry is certainly evident in the experimental record of small subthreshold oscillations, there are several reasons why this is not exact.

The first depends on the fact that increases in the potential difference across the membrane are associated with predominantly inward sodium currents, whereas downward excursions in the potential are associated with outward potassium currents, and the magnitude of these currents are determined by the ion concentration gradients and the resistances of these ions relative to other permeable ions. This will be shown in the next section where we analyze the action potential in an excitable cell with only two ionic species in the electrolyte solutions. In the squid giant axon this effect is manifested as a slight increase in the amplitude of the oscillation for positive current clamps as compared with those which are equal in magnitude but opposite in sign (see Mauro et al.[112]).

A second source of asymmetry is the nonlinear relation between the ionic currents and the electric field within the membrane, and this becomes more prominent as the amplitude of the subthreshold oscillations increases. When

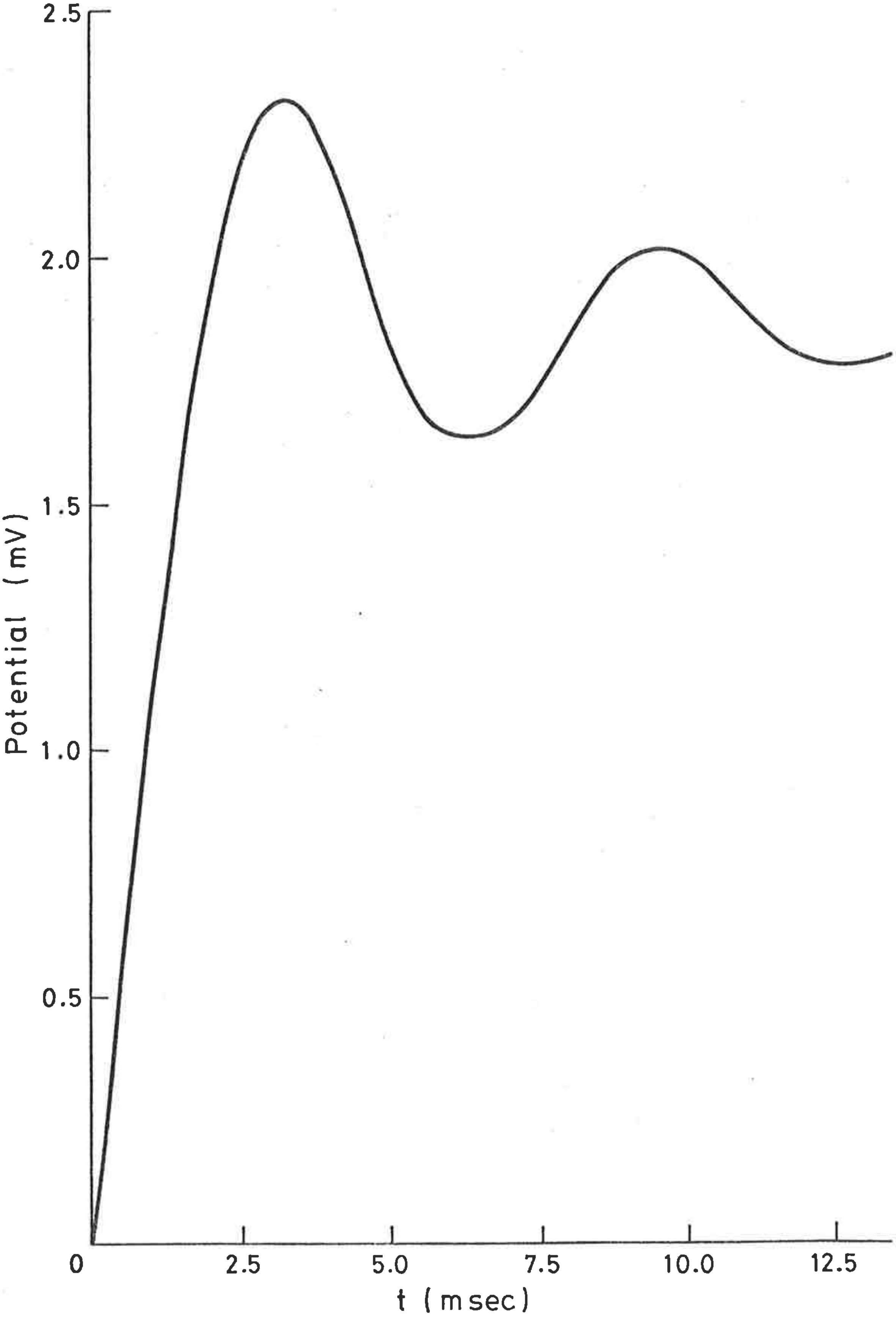

FIGURE 4.1: A subthreshold oscillation.

a large negative clamp current is applied to the resting axon, the response is characterized by a non-oscillatory relaxation to the new steady state potential. In contrast to this, if the constant clamp current at the electrode $I_e$ is progressively increased to a more positive value then, when the threshold current is attained, a large-amplitude potential variation (the action potential) will be elicited. In the next section we show that the existence of a threshold potential and the shape and amplitude of the action potential can be accounted for in terms of the nonlinear electrodiffusion equations.

## 4.3 THE ACTION POTENTIAL

In the theory of Hodgkin and Huxley[13] the mechanism of the action potential was explained in terms of the results obtained from the study of the voltage clamp, and it is clear both from an experimental point of view as well as a theoretical one, that these phenomena are indeed related. However, there are two important differences between the currents associated with the action potential and the voltage clamp. While the latter is a transient effect in which the exponential decay of the associated currents strongly suggests irreversible diffusion across the membrane, the action potential under suitable conditions can be repeated indefinitely, and, disregarding a small but noticeable assymetry in the potential profile, has many of the characteristics of a reversible process. Also, while the currents of the voltage clamp are predominantly transverse to the membrane, the most important property of the normal axon action potential from the biological point of view is its propagation without appreciable loss of amplitude in the longitudinal direction. These striking differences have not previously been reconciled satisfactorily with a common mechanism involving the transport of ions through a membrane phase governed by electrodiffusion processes. The analyses of Chapter 2, however, suggests that all excitation phenomena with relaxation times which range from a millisecond for the squid giant axon to one second for excitable plant cells, are associated with plasma oscillations in the membrane and do not require the hypothesis of large variations in the ionic permeabilities. This is particularly true for large voltage clamps and the action potential where the presence of nonlinear plasma oscillations is associated with the transfer of ions through the membrane at a rate very much greater than could be achieved steady state diffusion.

Although the possibility of undamped plasma oscillations within the membrane is necessary, it is not however sufficient to guarantee the appearance of an action potential. With only two different kinds of ions, for instance, the requirement of electrical neutrality is sufficient to prevent the generation of large amplitude oscillations within the membrane. Other factors which influence the excitability properties of biological cells are temperature, ion concentration gradients across the membrane, and the presence of small electrolyte concentrations of certain polyvalent cations (for example, calcium ions). Each of these environmental constants has an effect on the solutions of the dispersion equation (and hence on the branch point singularities which can be obtained from the condition for equal roots of this equation) and the amplitude of the relaxation oscillations within the membrane.

We have shown in Chapter 3 and the previous section that the linearized theory developed in Chapter 2 is able to account qualitatively for the observed phenomena associated with a small voltage clamp across the membrane from a given holding potential, and for subthreshold oscillations. However, it cannot account for such features as the existence of a threshold potential, the almost invariable amplitude of the action potential and the variations in the shape of the action potential for different excitable cells. We shall therefore consider the consequences of non-linearity of the exact form of Poisson's equation. Neglecting the inertial term in equation (1.9) of Chapter 2, which as we have seen is nearly always possible within the membrane, we obtain

$$\gamma_a \frac{\partial \phi_a}{\partial t} - \nabla^2 \phi_a = \frac{4\pi}{\kappa} \sum_b \varepsilon_b{}^0 \exp(-\beta_b e_b \phi_b) \qquad (3.1)$$

where $\gamma_a = \beta_a m_a \theta_a$ .

As the non-linearity is expected to affect the time dependence, rather than the spatial dependence of the solution of these equations, we adopt for $\nabla^2 \phi_a$ the expressions derived from the linearized theory. In the application to just two different kinds of ions, we substitute from equations (1.8) into equation (3.1) and obtain

$$\frac{1}{2}\alpha\nu^2 \frac{\partial \bar{\phi}_1}{\partial t} = \frac{1}{2}\nu^2 \bar{\phi}_1 + \nabla^2 \phi_{1s}$$

$$+ \frac{\nu^2}{2\beta e}(e^{-\beta e(\phi_{1s}+\bar{\phi}_1)} - e^{\beta e(\phi_{2s}+\bar{\phi}_2)})$$

$$-\frac{1}{2}\alpha\nu^2 \frac{\partial \bar{\phi}_2}{\partial t} = \frac{1}{2}\nu^2 \bar{\phi}_2 + \nabla^2 \phi_{2s}$$

$$+ \frac{\nu^2}{2\beta e}(e^{-\beta e(\phi_{1s}+\bar{\phi}_1)} - e^{\beta e(\phi_{2s}+\phi_2)}) \qquad (3.2)$$

where

$$\alpha\nu^2 = \gamma_1 - \gamma_2$$
$$\varepsilon^0 = \varepsilon_1{}^0 = -\varepsilon_2{}^0$$
$$e = e_1 = e_2 \tag{3.3}$$

and $\bar{\phi}_i$ (i=1,2) are the deviations from the steady state ionic potentials. From equation (1.9) of Chapter 2 it follows, under the same conditions, that the electric and ionic potentials are related by

$$\alpha\nu^2\bar{\phi} = c(\gamma_1\bar{\phi}_2 - \gamma_2\bar{\phi}_1) \tag{3.4}$$

where

$$c = \frac{\nu^4+\omega^2(\gamma_1-\gamma_2)^2}{\nu^4+\omega^2(\gamma_1+\gamma_2)^2} . \tag{3.5}$$

By addition and subtraction of equations (3.2), we have

$$\alpha\frac{\partial}{\partial t}(\bar{\phi}_1 + \bar{\phi}_2) = \bar{\phi}_1 - \bar{\phi}_2$$
$$\alpha\frac{\partial}{\partial t}(\bar{\phi}_1 - \bar{\phi}_2) = \bar{\phi}_1 + \bar{\phi}_2 + \frac{2}{\beta e}(e^{-\beta e(\phi_{1s}+\bar{\phi}_1)} - e^{\beta e(\phi_{2s}+\bar{\phi}_2)}) \tag{3.6}$$

where $\nabla^2(\phi_{1s}+\phi_{2s})$ has been absorbed into the exponential terms.

This pair of equations is the macroscopic counterpart of a pair of equations derived by Triffet and Green[27] in their model of the neuron, and has similar properties, but whereas the oscillation frequency of the former is of the order of $10^3$Hz, the frequency of the microscopic polarization oscillations is considerably greater (approximately $10^{12}$Hz). The effect of the non-linearity is to cause a rapid reversal in the rate of change of the potentials when the exponent of either of the exponential terms attains a magnitude appreciably in excess of 1.

Consider a two-ion membrane system with

$$V_1 = V_2 = -30\text{mV}$$

and assume that the mobility of the anion (ion 2) is greater than that of the cation (ion 1) within the membrane:

$$\theta_1 = 2\theta_2 .$$

Equations (3.2) may now be solved numerically and the solution curve for the electric potential has been plotted in Figure 4.2 (from equation (3.4) with potential changes relative to -70mV). Although its shape and time course is similar to the squid axon action potential, its amplitude is dependent on the initial conditions, and equations (3.2) only exhibit threshold behaviour if the coefficient of $\bar{\phi}_i$ (i=1,2) in the exponential terms is sufficiently large. In a physical membrane system with just two ions in the surrounding electrolytes, this value is never attained, and hence provides an explanation for the excitability of a two-ion system.

For a three-ion or four-ion membrane system which includes the calcium ion, it has been shown by Green and Triffet[113] that

$$\phi_{Ca} = c(\phi_K + \phi_{Cl})$$

where c is large for a range of calcium concentrations, and this ensures that the membrane system is excitable, and that the generalization of the pair of equations (3.2) for a four-ion axon membrane system ($K^+$, $Cl^-$, $Ca^{++}$, $Na^+$), has an action potential solution with a magnitude and waveform that is in good agreement with experiment.

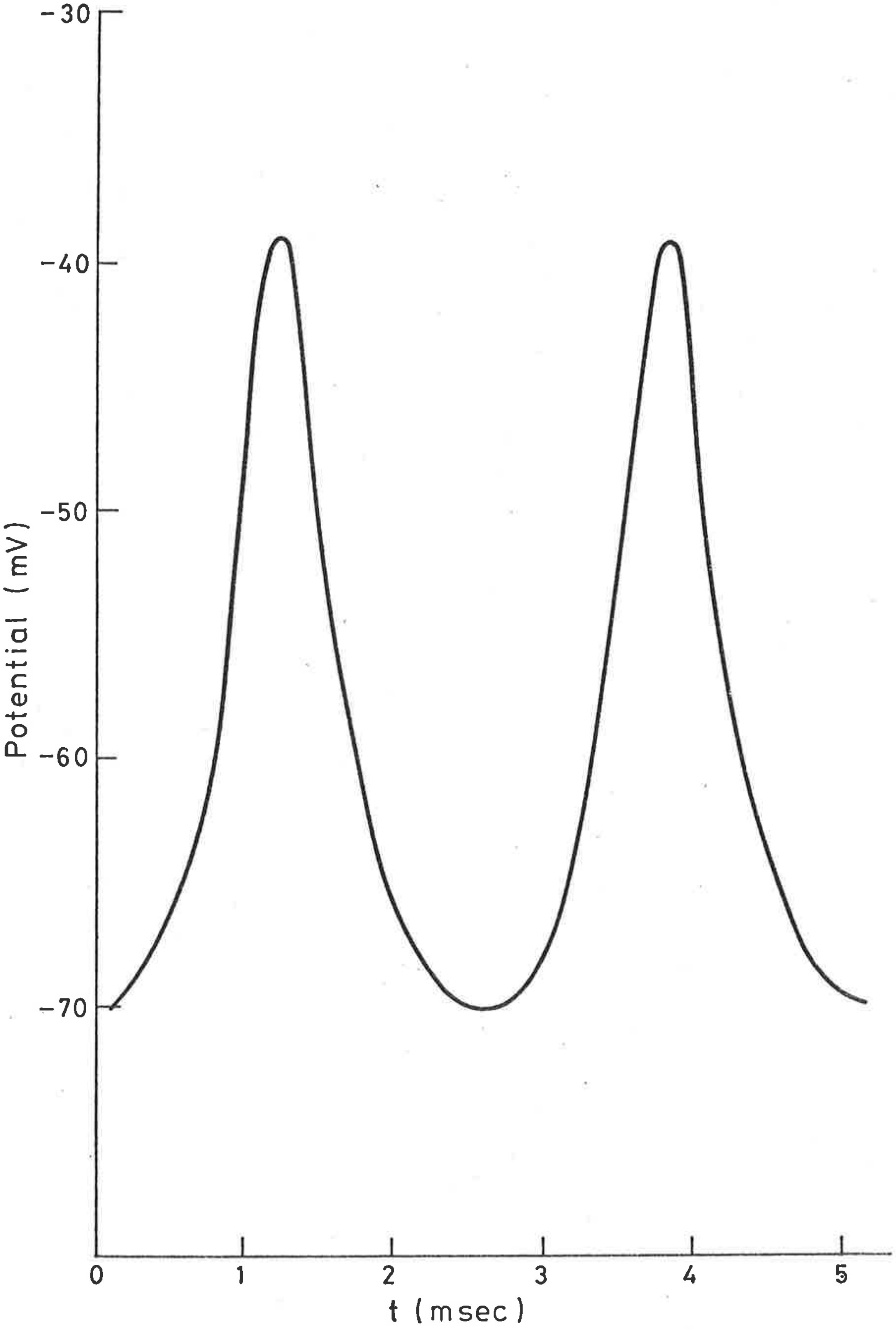

FIGURE 4.2: Nonlinear plasma oscillation.

We shall now consider the significance of the analyses of previous sections for an understanding of the propagation of the action potential along the axon to either another nerve cell, a muscle cell or an effector organ. The propagating nervous impulse depends on the generation of sufficiently large potential oscillations across the membrane which induce adjacent regions to undergo similar changes. However although the subthreshold oscillation is rapidly attenuated in a longitudinal direction along the axon, its velocity of propagation is almost the same as that of the action potential.[114] Therefore, the study of propagation does not depend on an understanding of the ionic mechanism of the space-clamped action potential, and this has permitted attempts to estimate the propagation velocity of the nervous impulse based upon the cable properties of fibre (see Taylor[115]). Such an analysis can account for the dependence of the propagation velocity on the square root of the diameter of the unmyelinated fibre, and also its linear dependence on the diameter for the myelinated axon (Mackey[116]). The importance of the dynamical theory of excitable membranes is in calculating the oscillation frequencies of the currents across the membrane, in terms of which the velocity of propagation can be expressed.

# CHAPTER 5

# MICROSCOPIC TRANSPORT PHENOMENA AT THE EXCITABLE MEMBRANE

## 5.1 CORRELATION-FUNCTION EXPRESSION FOR THE IONIC CONDUCTIVITY OF THE MEMBRANE

In previous chapters we have shown how to account qualitatively for the voltage clamp ionic currents, subthreshold oscillations and the action potential, without assuming that the ionic conductivity is dependent on the electric field within the membrane. However, it is in fact necessary to assume such a dependence in order to explain the non-Ohmic steady state currents of certain ions (for example, potassium) which are conserved during large voltage clamps of the axon membrane, and also to describe the variations in shape of the action potential for different excitable cells. In this chapter we shall examine the microscopic basis of the electric field-dependence of the ionic conductivity within the membrane, and in view of the correspondence between the Triffet-Green[27] quantum mechanical model of the neuron and the macroscopic theory of excitable membranes developed in this thesis, this can be determined by applying correlation function methods to the Hamiltonian characterizing the energy of a nerve cell and its environment.

In this section we give a non-perturbative derivation of the time correlation function expressions for the transport coefficients, and deduce the Einstein relation between the ionic conductivity and the corresponding diffusion coefficient. By considering the time evolution of the microscopic currents across the membrane, the nonlinear dependence of the ionic conductivity on the membrane potential difference can be calculated, and may be compared with the voltage-dependence of the ion conductance as determined by Hodgkin and Huxley[13] from the voltage clamp experiments. However, the dependence of the ionic conductivity on the electric field within the membrane is expected to show only moderate deviations from linearity when the magnitude of the field is large within the membrane.

The thermodynamic properties of an equilibrium system can be evaluated from the partition function which depends on the Hamiltonian of the system and the thermodynamic properties of the reservior with which it is in contact, such as the chemical potential, pressure and temperature. When an equilibrium system is perturbed by an external or mechanical force which can be represented by an additional term in the Hamiltonian, then the evolution of the thermodynamic quantities can be described by applying Kubo's linear response formula.[117] When the perturbation is instead due to an inhomogeneity in the system, there are several alternative methods which can be applied to yield analogous expressions for the transport coefficients (M.S. Green[118], Kubo et al.[119], Mori[120], McLennan[121]). In the next section we derive a set of integral equations which can be used to determine the time-dependence of the equilibrium parameters in the Triffet-Green model.

The expressions for the transport coefficients which are obtained by applying correlation function methods are in agreement with those obtained from kinetic theory.[71,72] However, van Kampen[122] has claimed that the usual method of deriving Kubo's formula is dependent on assumptions which are not in agreement with the fundamental randomization hypothesis of statistical mechanics. More specifically, he claims that the usual derivation assumes that the microscopic motion is linearly dependent on the driving force when the latter is sufficiently small, but this condition is not satisfied for macroscopic forces.

The perturbation to a many-body system may be considered to have two distinct effects. The first is to change the microscopic orbit of each particle, and the second is to alter the density matrix which depends on the ensemble used to describe the system. Although it is not legitimate to assume that individual particle orbits are linearly dependent on a small perturbation, it is however valid to assume that a small macroscopic perturbation will result in a correspondingly small change in the density matrix describing the system, and so it appears that his objection cannot be sustained.

In response to van Kampen's critique there have been attempts to derive response formulae by alternative methods. Edwards[125] derived a formula for the electrical resistivity by expanding the electric field in terms of the current, whereas Kenkre[124] has attempted to avoid linearization of the Liouville equation by use of the projection techniques of Mori and Zwanzig. However, both derivations make a linear assumption which is equivalent to that used by Kubo.

In considering the correlation function expression for the ionic conductivity during the action potential, one cannot assume that the change in the electric field is small, and so we now derive a response formula which is independent of the magnitude of the driving force, and is similar in some respects to that used by Mori[120] in obtaining thermal transport coefficients (see also Visscher[125]).

If we write the Hamiltonian $H$ of the system as

$$H = H^0 + \int V(x)Q(x)\,dx \tag{1.1}$$

where $H^0$ is the non-electromagnetic part of the Hamiltonian, $V(x)$ is the electrostatic potential and $Q(x)$ is the charge density at a point in phase space, then the observable $A(t)$ satisfies Heisenberg's equation

$$\dot{A}(t) = \frac{1}{i\hbar}[A(t),H] \ . \tag{1.2}$$

If we consider the statistical average of $A(t)$ in the Heisenberg picture (instead of the Schrodinger picture which is used by Kubo[117]) we have

$$
\begin{aligned}
<A(t)> &= \mathrm{Tr}(\rho(0)A(t)) \\
&= <A(0)> + \frac{1}{i\hbar}\int_0^t \mathrm{Tr}\ \rho(0)[A(t'),H]dt' \\
&= <A(0)> + \frac{1}{i\hbar}\int_0^t \mathrm{Tr}\{[H,\rho(0)]A(t')\}dt' \qquad (1.3)
\end{aligned}
$$

where $\rho(0)$ is the density matrix of the system at time $t=0$, and, for a grand canonical ensemble, is given by

$$\rho(0) = e^{-\int\beta(x)[h(x)-\mu(x)n(x)-V(x)Q(x)]dx} \qquad (1.4)$$

where $h(x)$ is the total Hamiltonian density, $n(x)$ is the number density and $\mu(x)$ is the chemical potential.

Now, if $A$ is any operator, it is easily shown that

$$
\begin{aligned}
&[A,\ e^{-\beta(H^0-\mu N)}] \\
&= e^{-\beta(H^0-\mu N)}\int_0^1 e^{\lambda\beta(H^0-\mu N)}[\{\ \beta(x)[h(x)-\mu(x)n(x)-Q(x)V(x)]dx\},A] \\
&\qquad\qquad e^{-\lambda\beta(H^0-\mu N)}d\lambda \quad . \qquad (1.5)
\end{aligned}
$$

Also,

$$
\begin{aligned}
&[\int\beta(x)[h(x)-\mu(x)n(x)-Q(x)V(x)]dx,H] \\
&\quad = i\hbar\int\beta(x)[\dot{h}(x)-\mu(x)\dot{n}(x)-\dot{Q}(x)V(x)]dx \\
&\quad = i\hbar\int\nabla\beta(x)(J_h-VJ_Q)dx-i\hbar\int\nabla(\beta(x)\mu(x))J_n dx \\
&\qquad - i\hbar\int\beta\nabla V(x)J_Q dx \qquad (1.6)
\end{aligned}
$$

where we have used the operator conservation laws for the energy, number and charge densities:

$$\begin{aligned} \dot{h} + \nabla J_h &= 0 \\ \dot{n} + \nabla J_n &= 0 \\ \dot{Q} + \nabla J_Q &= 0 \end{aligned} \tag{1.7}$$

and $J_h$, $J_n$ and $J_Q$ are the corresponding currents.

If we now assume that the gradients $\nabla\beta(x)$, $\nabla(\beta\mu)$ and $\nabla V(x)$ are constant, then the average response in the dynamical variable $A$ is

$$\begin{aligned} &\langle \nabla A(t) \rangle \\ &= \nabla\beta \int_0^t \int_0^1 \mathrm{Tr}[e^{\lambda\beta(H^0-\mu N)} J_H e^{-\lambda\beta(H^0-\mu N)} A(t') \rho(0)] d\lambda dt' \\ &- \nabla(\beta\mu) \int_0^t \int_0^1 \mathrm{Tr}[e^{\lambda\beta(H^0-\mu N)} J_n e^{-\lambda\beta(H^0-\mu N)} A(t') \rho(0)] d\lambda dt' \\ &+ E \int_0^t \int_0^1 \mathrm{Tr}[e^{\lambda\beta(H^0-\mu N)} J_Q e^{-\lambda\beta(H^0-\mu N)} A(t') \rho(0)] d\lambda dt' \end{aligned} \tag{1.8}$$

where $J_H = J_h - VJ_Q$

and, if in addition, the temperature gradient is zero, the response in the current $J_Q$ is

$$\begin{aligned} &\langle \Delta J_Q(t) \rangle \\ &= \beta e(E - \frac{\nabla\mu}{e}) \int_0^t \int_0^1 \mathrm{Tr}[e^{\lambda\beta(H^0-\mu N)} J_Q e^{-\lambda\beta(H^0-\mu N)} J_Q(t') \rho(0)] d\lambda dt' \end{aligned} \tag{1.9}$$

If this is compared with the phenomenological transport law

$$\langle \Delta J_Q \rangle = -eD\nabla n + \sigma E \tag{1.10}$$

where $D$ is the diffusion coefficient and $\sigma$ is the ionic conductivity, we obtain

$$\sigma = \beta e\int_0^t\int_0^1 \mathrm{Tr}[e^{\lambda\beta(H^0-\mu N)}J_Q e^{-\lambda\beta(H^0-\mu N)}J_Q(t')\rho(0)]d\lambda dt' \quad (1.11)$$

$$D = \frac{1}{ne}\int_0^t\int_0^1 \mathrm{Tr}[e^{\lambda\beta(H^0-\mu N)}J_Q e^{-\lambda\beta(H^0-\mu N)}J_Q(t')\rho(0)]d\lambda dt' \quad (1.12)$$

and hence the relation between $\sigma$ and $D$ is given by

$$\sigma = \beta ne^2 D , \quad (1.13)$$

which is just the Einstein relation.

The above expression for $\sigma$ can now be applied to the Triffet-Green neuron model with its Hamiltonian given by

$$H = E(a^*a + A^*A) + \lambda(a^*A + A^*a) + \mu N \exp(-\alpha N) \quad (1.14)$$

where $E$ is the dissoication energy of an ion-pair, $\lambda$ and $\mu$ are parameters which determine the unit of time and the magnitude of the electrostatic energy in the model, and $\alpha$ is dependent upon the temperature and the mean potential energy associated with an ion and its screening charge. The number of excitons within the neuron is $N = a^*a$ and the number outside is $A^*A$, and so the current across the membrane is (with $\hbar=1$)

$$\begin{aligned} J_Q &= e\frac{dN}{dt} \\ &= e(-i)[N,H] \\ &= e(-i\lambda)(a^*A - A^*a) \end{aligned} \quad (1.15)$$

where $e$ is the charge associated with the exciton. In the resting state $J_Q(t')$ depends weakly on the electrostatic energy term in the Hamiltonian of (1.14), but during the action potential, although it would be difficult to evaluate precisely from the integral over the operators in equation (1.11), it is clear that there is an exponential dependence of the ion conductivity on the potential difference across the neural membrane, which has been defined in the model as

$$\phi = \phi_0 + \bar{\phi} N \qquad (1.16)$$

where $\phi_0$ is a uniform negative background potential and $\bar{\phi}$ is the mean potential associated with the exciton.

Hodgkin and Huxley[13] described the current across the squid giant axon membrane in terms of ion conductances which are also defined with respect to the potential difference across the membrane $\phi$, and from the voltage clamp experiments they were determined to have an exponential dependence on $\phi$ and $t$ which is similar to that expected from the Triffet-Green model. If, however, the electrostatic energy term is expressed, not in terms of the potential difference across the membrane, but instead in terms of the electric field within the membrane $\mathcal{E}$ (which also has an exponential dependence on time) then the extent of the variation of the ion conductivity with respect to $\mathcal{E}$ is considerably reduced. We may therefore conclude that the application of irreversible statistical mechanics to the Triffet-Green model permits the calculation of conductivities which are in agreement with experimental determinations both from excitable cells and other physical systems.

## 5.2 THE TIME-DEPENDENCE OF THE EQUILIBRIUM PARAMETERS IN THE TRIFFET-GREEN MODEL.

In this section we derive a set of integro-differential equations which can be solved to determine the time-dependence of the equilibrium parameters in the Triffet-Green neuron model. The model is characterized by the Hamiltonian

$$H = E(a^*a + A^*A) + \lambda(a^*A + a^*A) + \mu a^*a \exp(-\alpha a^*a) \qquad (2.1)$$

which describes the energy of a neuron and its environment. In general, the parameters $\lambda$ and $\mu$ are dependent on thermodynamic variables of state and hence vary with time according to the nature of the irreversible processes within the system. The above Hamiltonian must therefore be considered to have an explicit time dependence $H(t)$, say, and hence the non-equilibrium density matrix $\rho(t)$ may be written as

$$\rho(t) = \rho^0(t) + \rho'(t) \qquad (2.2)$$

where $\rho^0(t)$ is the reversible part of the density matrix which only contributes to time-dependent statistical averages of dynamical variables, and in a grand canonical ensemble it may be expressed as

$$\rho^0(t) = e^{-\beta(H^0(t)-\mu_1 N_1-\mu_2 N_2)} \tag{2.3}$$

where

$$\begin{aligned} H^0(t) &= H(t) - \mu a^*a \exp(-\alpha a^*a) \\ &= d_1 N_1 + d_2 N_2 \end{aligned} \tag{2.4}$$

$N_i = \beta_i{}^*\beta_i$, i=1,2 are quasi-particles which can be defined in terms of a and A, and $\mu_i$ are the chemical potentials of the i-th quasi-particle (i=1,2), and the $d_i$ (i=1,2) and $\mu$ are functions of $\mu_1, \mu_2$ and $\beta$.

The irreversible part of the density matrix is $\rho'(t)$ and is used to compute the irreversible currents. The non-equilibrium density matrix $\rho(t)$ satisfies Liouville's equation

$$\frac{\partial}{\partial t}\rho(t) = \frac{1}{i\hbar}[H(t),\rho(t)] \tag{2.5}$$

which has the solution

$$\rho'(t) = \int_{-\infty}^{t} e^{-i\int_{t'}^{t} H(t'')dt''/\hbar} R(t') e^{i\int_{t'}^{t} H(t'')dt''/\hbar} dt' \tag{2.6}$$

where

$$R(t') = -\frac{\partial\rho^0(t')}{\partial t'} + \frac{1}{i\hbar}[H(t'),\rho^0(t')]. \tag{2.7}$$

The average number of quasi-particles of type 1 is given by

$$\begin{aligned} \langle N_1\rangle &= \mathrm{Tr}(\rho^0(t)\beta_1{}^*\beta_1) \\ &= \frac{1}{\beta}\frac{\partial}{\partial\mu_1}\mathrm{Tr}(\rho^0(t)) \end{aligned}$$

$$= \frac{1}{\beta}\,\frac{\partial}{\partial\mu_1}\,\Big[\sum_{\substack{n^{(1)}=0\\ n^{(2)}=0}}^{\infty} e^{-\beta(d_1 n^{(1)}+d_2 n^{(2)}-\mu_1 n^{(1)}-\mu_2 n^{(2)})}\Big] \qquad (2.8)$$

$$= \frac{\exp(-\beta(d_1-\mu_1))}{[1-\exp(-\beta(d_1-\mu_1))]^2[1-\exp(-\beta(d_2-\mu_2))]} = f_1(\mu_1,\mu_2,\beta)$$

and, similarly, the average number of quasi-particles of type 2 is

$$\langle N_2\rangle = \frac{\exp(-\beta(d_2-\mu_2))}{[1-\exp(-\beta(d_2-\mu_2))]^2(1-\exp(-\beta(d_1-\mu_1))]}$$

$$= f_2(\mu_1,\mu_2,\beta)\,. \qquad (2.9)$$

The energy associated with the interaction between excitons (denoted by $H_I$) is

$$\begin{aligned} H_I &= \mu a^* a \exp(-\alpha a^* a) \\ &= F\Big(\sum_{r,s} \beta_r^{*}\beta_s\Big) \quad \text{for some function } F. \end{aligned} \qquad (2.10)$$

The average interaction energy in the grand canonical ensemble is

$$\langle H_I\rangle = \mathrm{Tr}(\rho^0\, H_I) = f_3(\mu_1,\mu_2,\beta)\ , \quad \text{say}\ . \qquad (2.11)$$

Now, the conservation laws for the number of quasi-particles and their interaction energy are

$$\frac{\partial}{\partial t}\langle N_i\rangle + \langle J_i\rangle^r + \langle J_i\rangle^i = 0\ , \quad i=1,2 \qquad (2.12)$$

$$\frac{\partial}{\partial t}\langle H_I\rangle + \langle Q_I\rangle^r + \langle Q_I\rangle^i = 0 \qquad (2.13)$$

where $J_i = \dot{N}_i$ , $i=1,2$ and $Q_I = \dot{H}_I$

and
$$\langle J_i\rangle^r = \mathrm{Tr}(\rho^0(t)J_i\rangle \quad i=1,2$$
$$\langle J_i\rangle^i = \mathrm{Tr}(\rho'(t)J_i\rangle\ , \quad i=1,2 \qquad (2.14)$$
$$\langle Q_I\rangle^r = \mathrm{Tr}(\rho^0(t)Q_I\rangle$$
$$\langle Q_I\rangle^i = \mathrm{Tr}(\rho'(t)Q_I\rangle$$

are the reversible and irreversible currents of quasiparticles and interaction energy, respectively. The conservation equations (2.12) and (2.13) are integro-differential equations in the parameters $\mu_1,\mu_2$ and $\beta$ and hence given an appropriate set of initial conditions, their time dependence may be determined. However, the complexity of the structure of the above equations would make their numerical solution somewhat difficult.

The variation of $\mu_1,\mu_2$ and $\beta$ in the resting state of the neuron will be small, but during the action potential, the irreversible process of ionic diffusion (and to some extent heat conduction) will induce a relatively much larger change in these parameters, and hence also in $\lambda$ and $\mu$. Changes in the latter parameters were shown to be associated with an increase in the information content of the system[27], and hence justified describing $\lambda$ and $\mu$ as memory parameters. Hence, it seems reasonable to conclude that the rate of change of the information content of a collection of neurons is strongly influenced by the rate of firing of the individual neurons, which in turn depends on the nature of the interconnections between them.

# CONCLUSION

In this thesis we have attempted to account for the electrical properties of excitable membranes, and in particular, the existence of a threshold potential and the large transient currents which are observed during excitation, without invoking the assumption of a large variable ionic permeability. Instead, we have assumed that the excitable membrane is an ionized medium through which each permeable ion may diffuse with a resistance to flow which depends weakly on the electric field and the extent of hydration within the membrane.

It was argued in Section 5.1 that the weak dependence of the ion conductivity on the electric field followed from the application of correlation function methods to a Hamiltonian neuron model which is a microscopic counterpart of the present theory. Also, although there is reduced shielding of the ions within the membrane during the action potential, its effect on the ion conductivity would not be sufficient to account for the large variations in ion conductances for sodium and potassium determined experimentally by Hodgkin and Huxley.

There is considerable evidence to suggest that ion transport through the membrane is mediated by isolated protein molecules which extend across the thickness of the membrane (largely composed of lipid), but the model of ion transport adopted in this thesis is to a large extent independent of the detailed structure of the membrane. The membrane resistance to each permeable ion

$$\frac{1}{2\pi i}\int_{c-i\infty}^{c+i\infty}\sum_k \frac{\bar{c}_k(\mu)\ d\mu}{[\mu^2 - w_{ak}{}^2(\mu)](s-\mu)} = \frac{\chi_a{}'}{s+\nu} \tag{A4}$$

where $0 < c < \nu$, and

$$\bar{c}_k(\mu_k) = c_k(\lambda)\ d\lambda/d\mu_k$$

$$w_{ak}{}^2(\mu_k) = w_a{}^2(\lambda)\ . \tag{A5}$$

We thus obtain a singular integral equation of Cauchy's type (Mushkhelishvili[127]) which has the obvious solution

$$\sum_k \frac{\bar{c}_k(\mu)}{\mu^2 - w_{ak}{}^2(\mu)} = \frac{\chi_a{}'}{\mu+\nu}\ . \tag{A6}$$

This is a set of linear equations from which the $\bar{c}_k(\mu)$, and hence the $c_k(\lambda)$, can be determined.

The second integral equation, obtained by subtracting (A2) from (A1), is solved in the same way, except that in applying the Laplace transformation, the integration is from $-\infty$ to $0$ instead of from $0$ to $\infty$.

# APPENDIX B

In this appendix we show that the condition for equal roots of the N-component dispersion equation for $N=3$ and $4$, can be expressed as the non-zero solutions of a polynomial of degree $2(N-1)$ (if we neglect strongly damped oscillations), and that these correspond to quadratic branch points of the integrand in (3.3) of Chapter 2.

The three-component dispersion equation is

$$\sum_{b=1}^{3} \left(\frac{\nu_b^{\,2}}{x-\gamma_b\lambda}\right) = 1 \tag{B1}$$

with solutions given by the roots of the cubic equation

$$x^3 + ax^2 + bx + c = 0 \tag{B2}$$

where

$$\begin{aligned} a &= -a_1\lambda - a_0 \\ b &= \lambda(b_1\lambda + b_0) \\ c &= -\lambda^2(c_1\lambda + c_0) \end{aligned} \tag{B3}$$

Equation (B2) has solutions

$$\begin{aligned} x_1 &= A^{\frac{1}{3}} + B^{\frac{1}{3}} - a/3 \\ x_2 &= \omega A^{\frac{1}{3}} + \omega^2 B^{\frac{1}{3}} - a/3 \\ x_3 &= \omega^2 A^{\frac{1}{3}} + \omega B^{\frac{1}{3}} - a/3 \end{aligned} \tag{B4}$$

where $\omega$ is a complex root of unity, and

$$\begin{aligned} A &= \frac{-q}{2} + D^{\frac{1}{2}} \\ B &= \frac{-q}{2} - D^{\frac{1}{2}} \\ D &= \frac{q^2}{4} + \frac{p^3}{27} \end{aligned}$$

$$p = b - a^2/3$$
$$q = c - ab/3 + 2a^3/27 \qquad \text{(B5)}$$

From equations (B3) and (B5) we may write

$$D = \sum_{n=0}^{6} \lambda^n D_n \qquad \text{(B6)}$$

and it is easily shown that $D_5 = D_6 = 0$. Now, (B1) and (B2) have equal roots when $D = 0$, and if $x_k - x_\ell$ $(k \neq \ell)$ is small then

$$x_k - x_\ell = \pm(\tfrac{4}{3}) \frac{D^{\frac{1}{2}}}{(2q^2)^{\frac{1}{2}}} (\omega_k - \omega_\ell) \qquad \text{(B7)}$$

where $\omega_k$, $k=1,2,3$ are the cubic roots of unity, and so there are four non-zero branch point singularities.

The four-component dispersion equation is

$$\sum_{b=1}^{4} \left(\frac{\nu_b{}^2}{x-\gamma_b\lambda}\right) = 1 \qquad \text{(B8)}$$

with solutions given by

$$x^4 + rx^3 + sx^2 + tx + u = 0 \qquad \text{(B9)}$$

where

$$r = -(r_1\lambda + r_0)$$
$$s = \lambda(s_1\lambda + s_0)$$
$$t = -\lambda^2(t_1\lambda + t_0)$$
$$u = \lambda^3(u_1\lambda + u_0) \qquad \text{(B10)}$$

The resolvent of the quartic equation (B9) is defined to be

$$y^3 + ay^2 + by + c = 0 \qquad \text{(B11)}$$

where

$$
\begin{aligned}
a &= -s \\
b &= rt - 4u \\
c &= 4su - r^2u - t^2
\end{aligned}
\tag{B12}
$$

The solutions of the resolvent (B11) are

$$
\begin{aligned}
y_1 &= A^{\frac{1}{3}} + B^{\frac{1}{3}} - a/3 \\
y_2 &= \omega A^{\frac{1}{3}} + \omega^2 B^{\frac{1}{3}} - a/3 \\
y_3 &= \omega^2 A^{\frac{1}{3}} + \omega B^{\frac{1}{3}} - a/3
\end{aligned}
\tag{B13}
$$

and $A,B,D,p$ and $q$ are given by equation (B5). From (B5) and (B12) we can write

$$D = \lambda^6 \left( \sum_{n=0}^{6} \lambda^n D_n \right) \tag{B14}$$

where $D_0 \neq 0$.

The solutions of the quartic (B9) are

$$
\begin{aligned}
x_1 &= \frac{-q}{4} + \frac{1}{2}(e_1 + e_2 + e_3) \\
x_2 &= \frac{-q}{4} + \frac{1}{2}(e_1 - e_2 - e_3) \\
x_3 &= \frac{-q}{4} + \frac{1}{2}(-e_1 + e_2 - e_3) \\
x_4 &= \frac{-q}{4} + \frac{1}{2}(-e_1 - e_2 + e_3)
\end{aligned}
\tag{B15}
$$

where

$$
\begin{aligned}
{e_1}^2 &= \alpha + A^{\frac{1}{3}} + B^{\frac{1}{3}} \\
{e_2}^2 &= \alpha + \omega A^{\frac{1}{3}} + \omega^2 B^{\frac{1}{3}} \\
{e_3}^2 &= \alpha + \omega^2 A^{\frac{1}{3}} + \omega B^{\frac{1}{3}} \\
\alpha &= \frac{q^2}{4} - \frac{2b}{3}
\end{aligned}
\tag{B16}
$$

Equations (B8) and (B9) have equal roots when $D=0$, and if $x_k - x_\ell$ $(k \neq \ell)$ is small then

$$x_k - x_\ell = \pm\left(\frac{2}{3}\right) \frac{D^{\frac{1}{2}}\,(\omega_i - \omega_j)}{(2q^2)^{\frac{1}{3}}\left(\frac{q^2}{4} - \frac{2b}{3} + \left(\frac{q}{2}\right)^{\frac{1}{3}}\right)^{\frac{1}{2}}} \qquad \text{(B17)}$$

and so in this case there are six non-zero branch point singularities.

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