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A MODEL FOR THE NEURON CELL MEMBRANE

by

Wolf Kohn

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Submitted in Partial Fulfillment of

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Submitted to the Department of Electrical Engineering on May 10, 1974 in partial fulfillment of the requirements for the degree of Master of Science.

ABSTRACT

During the last 30 years, a great effort has been taken in developing models that adequately represent the behavior of the neuron cell membrane, when an action potential occurs as a link in the transmission-of-information process. The best known of these models is the approximation of the membrane by a lumped parameter transmission line. The model proposed herein will permit a broader comprehension of the relation between the bioelectric properties of the membrane and the ionic and active transport processes. The membrane generates a voltage pulse as a function of the ionic concentration gradients between the solutions bathing its surfaces, and restores these gradients after an excitation has occurred.

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TO MY PARENTS,

SALOMON

AND

HILDA

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CHAPTER I

1.1 Introduction

The purpose of this thesis is to study by means of a mathematical model some of the physiological and functional aspects of a giant squid axon membrane excited by an electrical pulse. The study is directed to the case where a portion of the membrane is clamped, i.e., no potential gradient exists in the longitudinal direction of the axon. The reason for this limitation is to analyze the interrelationships of the excitatory event with the chemical characteristics of the system formed by the membrane and the surrounding solutions. We thus avoid the propagation of the excitation, which is a phenomena that depends solely on the electrical porperties of the membrane. Moreover, no study of the propagation phenomenon is possible without a careful analysis of the mechanisms involved in the generation of the electric pulse (spike), which occurs when the membrane is excited over a certain threshold. These mechanisms determine the physical characteristics of the propagation, therefore an analysis of the latter cannot be carried out without a previous careful study of the former.

The purpose in deriving a mathematical model is two fold: first, to study the characteristics of the action potential as a function of the transport of sodium and potassium ions across the membrane due to their electrochemical gradients; and second, to analyze some of the main dynamic characteristics of a possible mechanism by which these ions are transported. The first process will be referred to herein as the ionic transport process and the second, the active transport process.

Both the ionic transport process and the active transport process have been the subject of intensive research for the last 30 years [1]. In particular, the observed time course of the clamped axon membrane potential and its dependence on the flow of sodium, potassium and chloride ions have been modeled by a second order partial differential equation relating the ionic flow and the membrane potential, coupled with two empirically derived linear differential equations for the parameters of the flow equation. This model is known as the Hodgkin and Huxley model [2], [3], [4], [5] after A. L. Hodgkin and A. F. Huxley who derived it. This model is analyzed in Chapter II of the thesis. Since its appearance, many studies have been carried out in order to justify in a physical context the functional expressions for the ionic conductances that appear in [6]. To this author's knowledge, this effort has not been completely successful. This fact constituted the main motivation for developing a mathematical model for the ionic transport process based on physical principles governing the diffusion of ions across an osmotic barrier (such as the axon membrane) and its interrelations with the resulting time course of diffusion between the inner and outer surfaces of the membrane. The derivation of this model which is carried out in Chapter III, is analogous to the one followed by Goldman [7] for describing the dynamics of a system of two ionic solutions of different concentrations separated by a permeable artifitial membrane. The main difference between the model considered in this thesis and the Goldman model, resides in the role that the membrane plays in the process; in Goldman's model it is a passive barrier, in the model developed here, the membrane is assumed to present a structure that actively participates in the transport process, mainly as a regulating an ion-selecting agent.

Neuron membranes, in common with most biological cells, possess a highly complex but not completely known, built-in mechanism that enables the cell to transport sodium and potassium ions against their electrochemical gradient with the consumption of metabolic energy. This mechanism is known as the sodium pump and is synonymous with the active transport process. In most cells, the sodium pump is involved in the transport of food stuffs from the extracellular fluid to the inside of the cell. In neurons, the sodium pump is also responsible for maintaining the concentration gradients of sodium and potassium ions so that the ionic transport develops a voltage pulse when the membrane is excited. Experimental evidence strongly supports the hypothesis that ATP hydrolysis is the source of energy of the sodium pump. In Chapter V, a model for the active transport process is derived. In this model, the equation representing the process gives qualitative rather than quantitative characteristics of it. The objective of developing such a model is to study the dynamics of the process and to obtain a formal description that can help in future studies to elucidate the mechanisms that govern its behavior, to test the hypotheses that have been proposed about its structure (Engelman [8]) and also to establish the possible coupling mechanism between this process and the ionic transport process.

In order to test the validity of the ionic transport model, and to get some insight about the physical properties of the axon membrane system, a simulation of its describing equations was carried out. The results and conclusion of this simulation are given in Chapter IV.

This chapter is divided into 3 sections: 1.1 Introduction, 1.2 Historical Account of the Research in the Field, and 1.3 Brief Description of the Axon Membrane.

1.2 Summary of the Background of the Problem

The first attempt to model the neuron cell membrane based on its selective permeability to potassium and sodium ions was made by Bernstein [9].in 1902. Based on experimental evidence, using sciatic nerves of Hungarian frogs, he concluded that the resting potential that is present across the cell membrane is due to its selective permeability potassium ions. He also suggested that the action potential is brought about by a breakdown of this selectivity. In 1926, Adrian and Zotterman [10] studying sensory nerve endings, gave thermodynamic arguments to justify the action potential that was measured in nerve sensory fibers when excited by an electrical pulse. However the first to provide convincing evidence that membrane potential changes are causal agents in nervous activity was Hodgkin [11] in 1937; he developed a model based on transmission line theory for the propagation of a pulse along the axon. In 1938 Hodgkin [12] established a coherent theory for the behavior of nerve fibers for subthreshold potentials; in his paper he also established a relation between subthreshold potentials and the "all or nothing" law for the action potential, described by Katz [13], a year before.

The sodium theory of the action potential was established by Hodgkin and Katz [6] in 1949. They discovered that the membrane potential "overshoots" the zero level during the action potential, so that the inside becomes positive.

This fact suggested that the process was a rapid and specific increase in the permeability of the membrane to sodium ions. The model for the membrane potential resulting from this discovery was a lumped parameter distributed electrical circuit. In 1945 Hodgkin and Rushton [7] carried out measurements on crustacean nerve fibers in order to determine the parameters of the membrane circuit model during the action potential. A general model that considered subthreshold behavior and action potential for squid axon membranes was developed in 1952 by Hodgkin and Huxley [8]. This model interprets the properties of the axon potential in terms of a conceptual model, an electrical circuit composed of four branches in parallel: Three for representing the movement of sodium ions, potassium ions and chloride ions across the membrane, and the fourth for representing the equivalent capacitance of the membrane. The ionic branches are each composed of a battery whose electromotive force is given by the Nernst equation for that ion, and a variable conductance. These conductances represent the ease with which ions can pass through the membrane; they are very complex functions of the permeability coefficients of those ions and their mobility through the membrane. Two important factors that have been extensively studied using this model are the "sodium theory" of the action potential and the ionic movements during activity.

The sodium theory was proved by Hodgkin and Katz in 1949 [6]. They suggested that the action potential is a process characterized basically by a rapid and specific increase in the permeability of the membrane to sodium ions; in terms of the circuit model, this implies that the conductance representing the sodium permeability becomes, during the action potential, relatively bigger than those corresponding to potassium and chloride ions.

Their theory is an attempt to explain the interrelationship between the movement of sodium, potassium and chloride ions through the membrane. The magnitude and speed of propagation of the action potential from the experimental evidence can be approximated by the Hodgkin and Huxley model by considering the axon membrane as a cascade of elementary circuits such as the one described above, using resistances in series to represent the attenuation of the pulse as it travels down the axon.

Parallel to the lumped parameter model development for cell membranes (axon) during subthreshold and action potential activity, researchers in the field have tried to find models based on the biochemical reactions and the thermodynamic events that take place during the action potential. Perhaps the first results following this line were published in 1930 Tasaki [10], and later in Tasaki and Takeuchi [11] in 1941 and 1942 [12]. The basic idea is to consider the axon membrane together with the ionic solutions on both sides of the membrane as a thermodynamic closed system, and to establish a set of energy relationships between the ionic movements and the electrical, chemical and thermodynamic gradients (potentials) present in the membrane. The main parameters of this model are the different ion concentrations, ion mobility, water flow, non ionic process etc. The potential gradients responsible for these movements are the concentration gradient, the electric gradient (the two former potentials are defined for each ion type), the temperature gradient and the osmotic pressure gradient. Several researchers have considered this approach: Kinsey [13] in 1970, describing a generalized theory of ion movements in biological tissues; Dick [14] in 1971, on water movement in cells; Tasaki [15] in 1969, in electric transport of ions; Bittar [16] in regulation of ion transport by

hormones, and Gingell [17] 1971 in using cell membrane surface potential as a transducer.

While previous authors have considered particular cause-effect sequences for modeling cell membrane behavior, no attempt has been made to treat the interaction of these processes. This is the main goal of this thesis. In the next section some general anatomical characteristics of the axon membrane are described. They will be required in the derivation of the model in Chapters III and V.

1.3 Brief Description of the Axon Membrane

Nervous Systems of multicellular organisms are composed of ensembles of highly specialized cells called neurons. The neurons are arranged in complex networks; their main function is to carry information in the form of electrical pulses. While there are important differences in the general organization of nervous systems of different organisms, the neurons of a wide variety of animals present common features both in structure and function which permit a generic study of their physical characteristics.

Anatomical studies suggest that the neuron itself is a system in which 4 main components can be recognized: dendrites, the cell body or soma, the axon, and the terminal region (Fig. 1.1).

The cell body is the main part of living matter of the cell and consists of a highly organized system called the cytoplasm, which is concerned with the biological activity of the cell; in particular, with the metabolic process. The dendrites' main function is to serve as input channels for the intercommunication between cells. The axon and terminal region are concerned mainly with the transmission process.

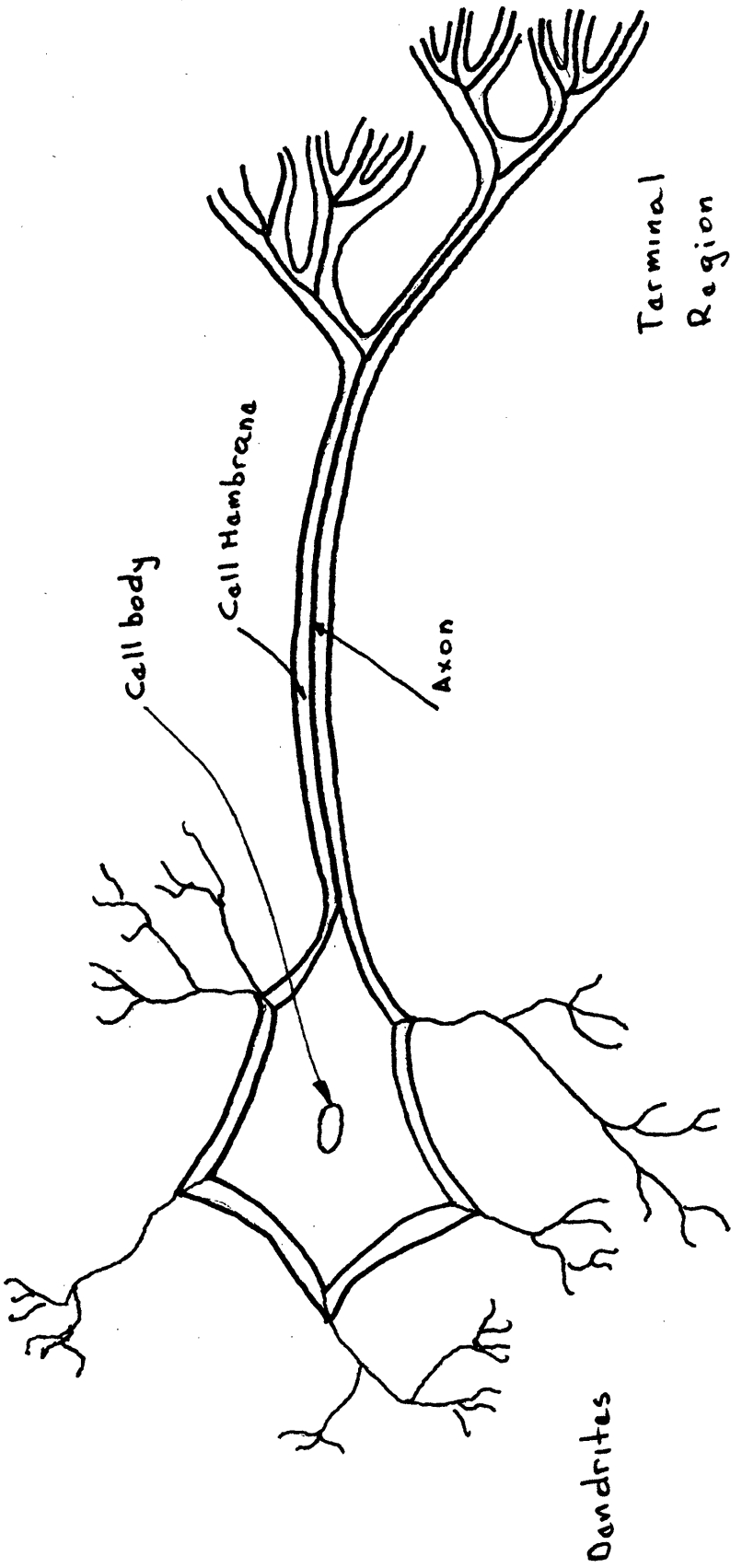
The neuron, in common with other types of cells, is surrounded by a complex plasma membrane whose thickness has been experimentally determined, and ranges between 50 and 150 Å. Using high resolution electron microscopy on sections of nerve cells, it has been shown that the cell membrane appears as two dense lines separated by a clear space; this observation agrees well with a model for the structure of the membrane developed by Davson and Danniellí [1], based on observations made by Gorter and Grendel [2]. The Danniellí model assumes that the cell membrane is a chain of phospholipoid molecules arranged in a layer two molecules thick and stabilized by a thin layer of protein molecules on each side of the lipid layer as shown in Fig. 1.2. In Danniellí's model, it is assumed that the membrane presents perforations or activation sites at regular intervals. This hypothesis is known as the "pore theory" and is a rather fundamental assumption in the development of the models considered in this thesis. Unfortunately, the pore theory has not been proved by direct observations, but rather by measuring the rate of entry of water per unit of concentration gradient, using an osmotic method. Paganelli and Solomon [3], observed that individual water molecules can pass through the membrane more easily when there is a net flow of water from one side to the other that when their movement is dependent only upon diffusion. More recently Bar et al [4], using modern methods for complete lipid extraction and accurate measurements of the membrane surface area, concluded that the lipid layer that forms the membrane is stable for ratios of layer thickness to surface cell area ranging from 2.2:1 to 1.2:1, meaning that other arrangements for the lipid layer than the one considered above are possible. Owing to the uncertainty of the molecular arrangement of the cell membrane, most of the models describing the electrical behavior

of excitable cells are based on the establishment of relationships that approximate the physical interactions between the surfaces of the cell membrane and the thermodynamic systems on both sides of it. Also, the experimental data available is based on measurements at the boundaries of the membrane. Therefore all the models for the membrane electrical behavior have as a starting assumption the characteristics of the activity inside the membrane, and in most of the cases, this is a completely arbitrary assumption.

The most important part of the neuron for the purposes of transmission is the axon (Fig. 1.1). Usually compared with the body cell, the axon is an enormously elongated process. In this work, all the models analyzed refer to the system formed by the axoplasm (i.e., the composite mixture of chemical elements in the axon), the plasmalema or membrane whose composition is assumed to be completely different from those on either side of it, and the external solution.

The transmission process is accomplished by the propagation of an electrical pulse of very specific characteristics, called the action potential, along the axon toward the terminal region (Fig. 1.1). This voltage is measured as the potential difference between the outer and the inner surfaces of the axon membrane, and in general, is a function of time (interval elapsed since excitation) and distance (along the axon, from the excitation point).

The neurons are excitable cells: this property is described as follows: when the cell and in particular the axon is in resting condition the axon membrane is polarized with constant voltage negative at the inner surface; when an electrical pulse positive at the outside surface is applied to the axon membrane, it propagates in a form analogous to the propagation of

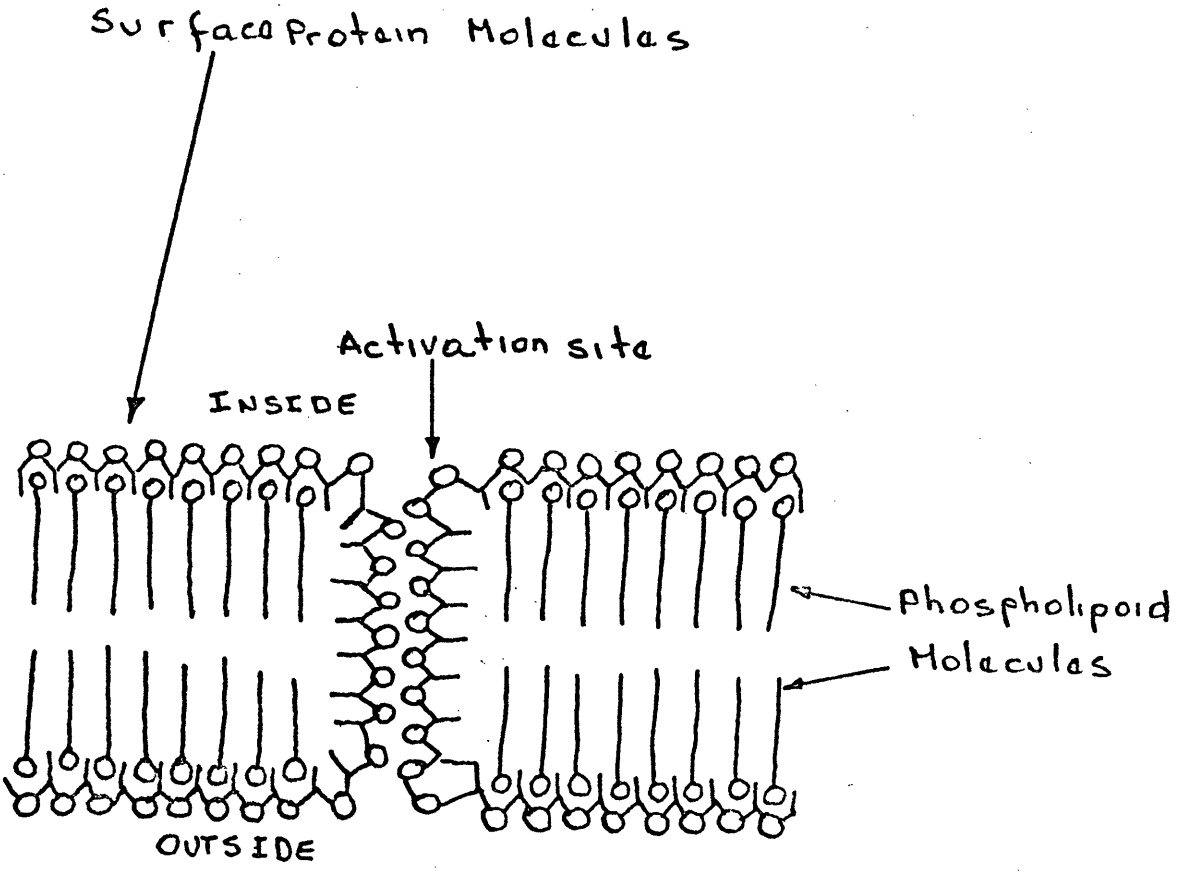


The Neuron
Fig 1.1

electrical pulses in metallic wires. If the magnitude of the excitation pulse is increased to a certain value, the cell "fires", i.e., an action potential is developed. (The general form of it is shown in Fig. 1.3). The size of the pulse which starts the action potential is called the threshold potential and is almost constant for every type of neuron. The propagation characteristics of pulses below the threshold level are passive and are described as subthreshold phenomena. The activated cell membrane potential is divided, for purposes of analysis, in two parts: The first is determined by the depolarization of the cell when the membrane potential reverses its sign (rising phase in Fig. 1.3), and the second by the recovery of the cell membrane toward the equilibrium (resting) potential (falling and subsequent phases in Fig. 1.3). The equilibrium membrane potential is known as the resting potential.

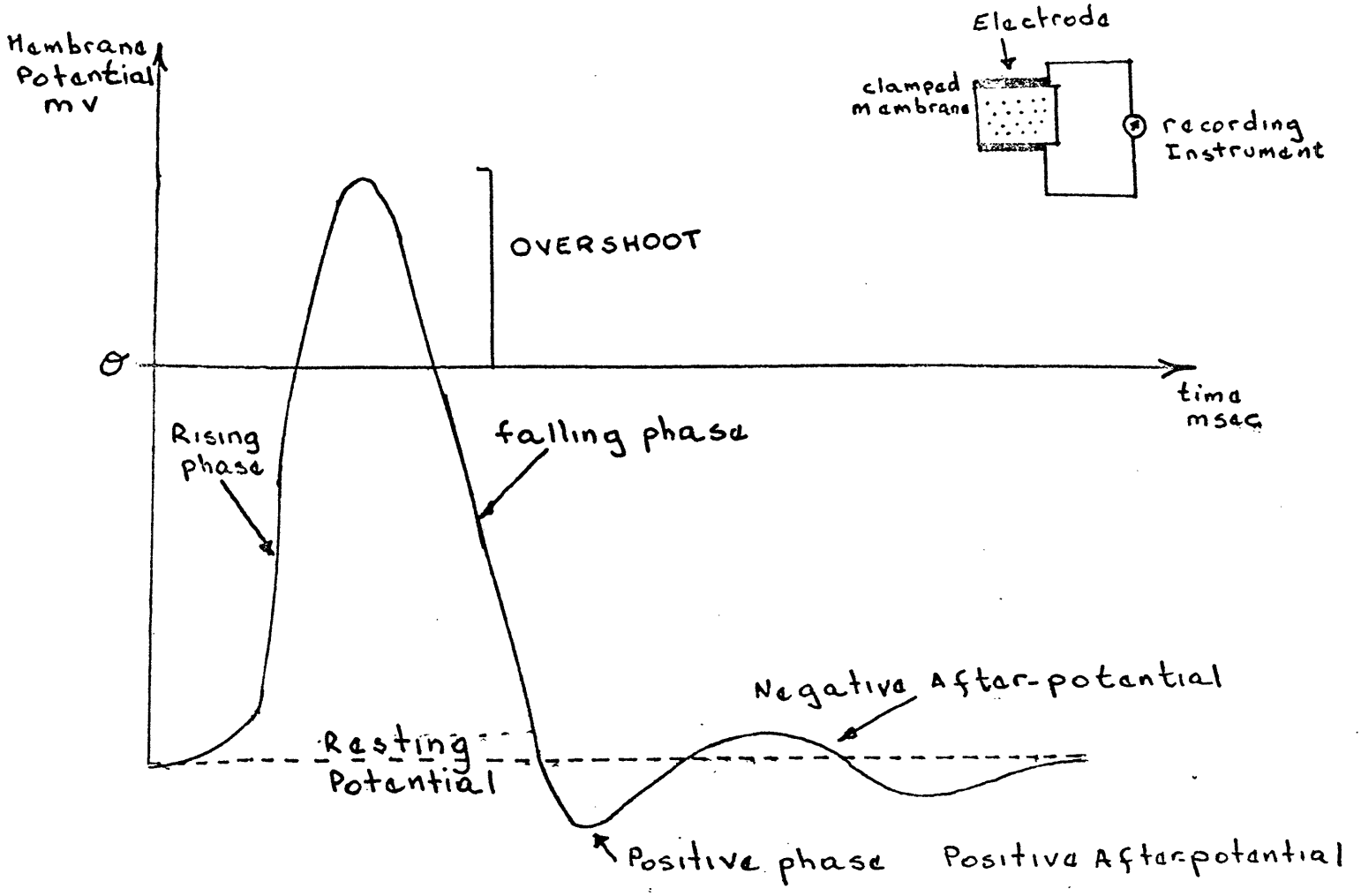
The membrane potential difference described above is caused by a complex interaction of the different ions and molecules that form part of the solutions on both sides of the membrane and the diffusion of these ions and molecules through the membrane. The purpose of this thesis is to develop two models for the cell membrane in passive and active states , and to explore the mechanism by which they regulate the propagation of an action potential along the axon.

The experimental evidence on which these models are based was obtained by Hodgkin and Katz [5], Hodgkin, Huxley and Katz [6] and Hodgkin and Huxley [7], [8], [9], [10], [11], [12], for the giant axon of the squid Loligo.



Dannielli model for the membrane
Cell structure

FIG 1.2



The Action potential on a clamped Membrane

Fig 1-3

CHAPTER II

ANALYSIS OF THE HODGKIN AND HUXLEY MODEL

2.1 Introduction

Although previous attempts were made to establish a model for the electric behavior of neuron cell membranes, (i.e. Hodgkin [1] 1937), the first relatively complete mathematical model of a nerve membrane, that of the squid giant axon, was developed by Hodgkin and Huxley [5] in 1952.

This model was obtained from the analysis of a series of experiments in which the axon membrane was excited by pair of electrodes with a pulse of magnitude and duration sufficient to trigger an action potential. The physical phenomena involved in the action potential include two coupled processes: membrane depolarization and disturbance propagation.

The first is determined by the complex interdependence between the membrane potential, the active transport of ions across it, and the geometric structure of the membrane in the direction normal to its surfaces; the second depends on the mechanism that makes the pulse travel along the axon, producing sequentially the depolarization of the membrane along the axon towards the terminal region (Fig 1.1). These processes are very difficult to observe in a coupled form, because they must be measured at points on the coordinate axis coinciding with the axis of the axon, (assuming that the axon is a perfect uniform cilinder), and at each instant of

time after the action potential starts. For this reason, Hodgkin and Huxley implemented their experimental measurements of the action potential using a technique known as "voltage clamp", developed by Cole in 1938, in which the squid axon membrane is subjected to step changes in electric potential using a pair of electrodes inside and outside the axon. These are in contact with a finite length of membrane and therefore prevent propagation to adjacent regions. Hence the potential is essentially constant for all the points of the membrane in contact with the electrodes. This fact allowed Hodgkin and Huxley to study the membrane depolarization uncoupled from the propagation phenomena.

In this chapter a re-evaluation of the experiments mentioned above (as well as the model developed from them) will be carried out. This forms the basis for the new model for the axon membrane behavior under clamped conditions to be developed in chapter 3. In section 2.2 the basic assumptions of the model are stated and a critical analysis of their validity is pursued. Their underlying physical principles are examined. In section 2.3 the dynamic equations relating the membrane state variables are stated; their predictions are correlated with the experimental evidence. In section 2.4 some modifications of the equations are proposed in order to consider aspects of the action potential and events occurring after it (refractory period), not covered by the original model. Finally in section 2.5 some general conclusions resulting from the analysis of the Hodgkin and Huxley model are given.

2.2 Basic Assumptions and Experimental Procedure

The basic assumptions on which the Hodgkin and Huxley model is based are the following:

a) In the absence of external stimulus, the clamped portion of the membrane maintains a constant potential, the resting potential. This assumption implies that there is no way to trigger an action potential by manipulating the state variables (concentrations of the ionic solutions on both sides of the membrane) of a clamped portion of the axon. This assumption is strongly supported by the available experimental evidence

b) The current that flows across the membrane during an action potential has a direction normal to the membrane surfaces. This assumption is supported by the fact that the axon is clamped and therefore no gradient is present in the longitudinal direction

c) The electrochemical force driving the transport of each ion species is produced by the difference of concentration of this ion species on the two sides of the membrane. This assumption is hardly justifiable from a physical point of view because it rules out the effect on the flow of one ion species caused by the presence of other ions in the transport process. This presence of ions of equal valence (sodium, potassium) and similar chemical activity in the solution on both sides of the membrane implies that a strong iteraction of the forces and fluxes will exist between those ions in the proximity of the membrane. In their model, Hodgkin and Huxley justify this assumption by considering that the membrane possesses specialized channels for each ion species; therefore once an ion has reached

a channel which corresponds to its species, it is transported by its own concentration gradient independently of the other ion species present. Since the membrane structure is basically unknown, this hypothesis has not been tested experimentally. In chapter 5, a possible justification of this hypothesis is analyzed in the context of carrier transport theory

d) The membrane behaves as a nonlinear passive element during the action potential

e) The driving force of each ion species, j, is represented in the model by an emf equal to the Donnan equilibrium potential

$$\bar{V}_j = \psi_{jinput} - \psi_{joutput} = \frac{RT}{F} \ln \frac{C_{jinput}}{C_{joutput}} \qquad (2\text{-}1)$$

$j = 1,2,\ldots$

where $\psi_{jinput}, \psi_{joutput}$ (V) are the values of the electric potential distribution, due to the concentration gradient of ion species j, at the inner and outer surfaces respectively; V_j is the equivalent emf in (V), R is the universal gas constant, T is the absolute temperature in °K, F is the Faraday constant, and C_{jinput}, $C_{joutput}$ are the chemical concentrations at the input and output concentrations respectively

f) The current across the membrane can be modeled as the sum of a capacitive current and an ionic current. The capacitive current represents the dynamic relative variation of the charges near the surfaces of the membrane and the ionic current is the sum of the

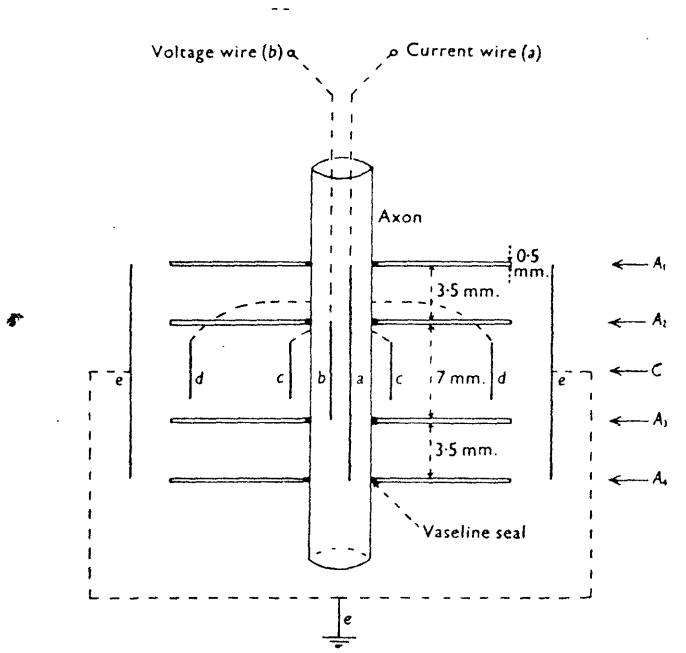
currents produced by the transport of ions. This assumption is justifiable in terms of the Gauss' Law and will be analyzed in section 2.3

g) Three different ion species are considered in the model: sodium ions, potassium ions (which participate in the active transport mechanism), and "leakage" ions (which include chloride and perhaps calcium ions, and are assumed to be transported in a purely diffusive manner). In light of the experimental evidence, there is little doubt that the nervous activity (and the active transport of molecules across any cell membrane) is strongly dependent on the mechanism of transport of sodium and potassium ions. However, the role of the leakage ions in the Hodgkin and Huxley model is obscure; the authors included them in the model mainly as parameters that allowed a better fit of the equations of the model to the experimental data

h) In the model, the complete system is assumed to be in isothermal equilibrium. The experimental data used by Hodgkin and Huxley was obtained, as stated before, using a voltage clamp technique. In Fig 2.1 a diagram ilustrates the characteristics of this technique. The squid axon is immersed in a bathing solution of known concentration. The axon is divided into 3 compartiments. In the middle compartment, 2 cylindrical electrodes are located at a determined distance from each other (electrodes c and d in the figure). The axon is penetrated by two thin silver wires (a,b). Wire (a) is driven by a current source (not shown in the figure) that is con-

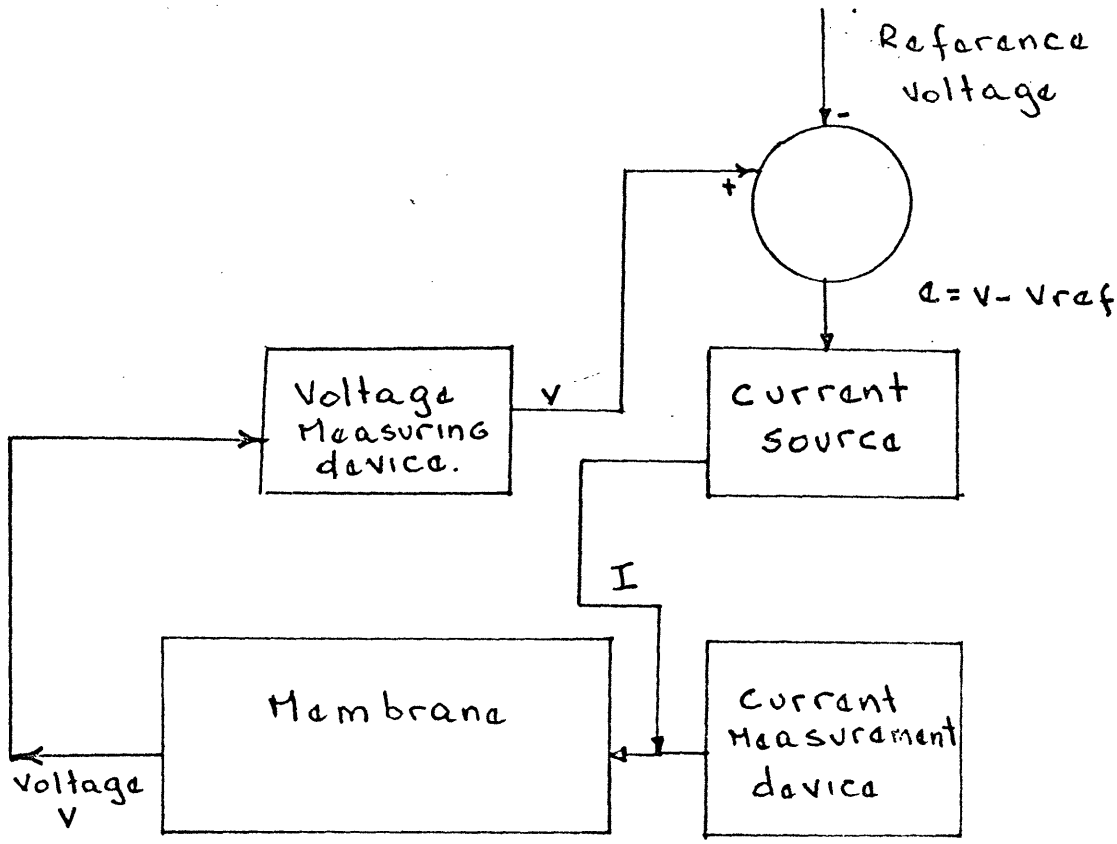
trolled by the voltage difference across the membrane: this voltage is measured between electrodes b and c. The other terminal of the current source is connected to ground (electrode e). A very important feature of the voltage clamp technique can be better visualized with the block diagram shown in Fig 2.2. The measurement system is a proportional control feedback system. The idea is to maintain the membrane potential constant at a given value Vref and to measure the resultant current at each instant of time. Since the capacitive current is proportional to the first derivative of the membrane potential, the former will vanish if the membrane potential is constant. This is very desirable since the current measured will be produced only by the transport of ions. Hodgkin and Huxley obtained a series of current measurements (as functions of time) for different membrane potentials. They also varied the external concentration of potassium and sodium ions. These data, together with the assumptions discussed above, were the basis for the identification of the parameters of their model, and also for checking its validity for different output concentrations.

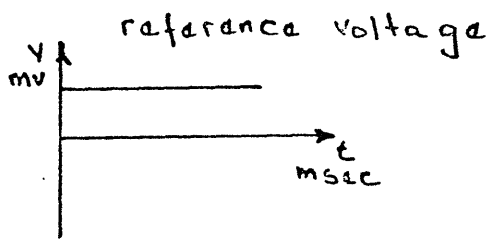
Finally, the use of giant squid axons for testing this model is justifiable from the practical point of view, since these axons have a 500-700 microns diameter (which is large enough for the size of the electrodes available), and also they are comparatively long (25-30mm), so their dissection can be carried without damaging the axon.



Arrangement of Internal and External Electrodes

Fig 2-1





Voltage - Clamp experiment equivalent Diagram

FIG 2.2

2.3 The Hodgkin and Huxley Model

As stated before, Hodgkin and Huxley considers the current density across the membrane to be composed of 2 current densities: the ionic current density and the capacitive current density.

Remark:

Since all the experiments were carried out in a definite segment of axon, all the parameters of the model are referred to a definite surface area.

The total current density J across the membrane, is given by the following expression:

$$J = C\frac{dV}{dt} + J_i \qquad (2\text{-}2)$$

where J is the total current density (at the outer surface) flowing across the membrane in mamp/cm^2, J_i is the total ionic current density in mamp/cm^2, V is the membrane potential in mV and C is the equivalent membrane capacitance in μF/cm^2. This capacitance was determined by exciting the axon membrane with a short current pulse (duration 8 μsec) using a clamped voltage arrangement, but eleminating the feedback loop, (Fig 2.2), thus allowing the membrane potential to vary with time. After the excitation current pulse has died away, the total current density function J in eq (2-2) is equal to zero and therefore

$$C\frac{dV}{dt} = - J_i \qquad (2\text{-}3)$$

Using this relationship, the value of C can be determined. It was

found that C is essentially constant with time and more or less proportional to the axon diameter. The average value of C for all the experiments they carried out is 0.91 $\mu F/cm^2$. For the low-temperature model (6°C) to be discussed below, Hodgkin and Huxley chose a value of 1 $\mu F/cm^2$.

According to the assumptions given below, the flow of each ion species is only dependent on its own potential gradient, hence the following equations for the current density of each ion species:

$$J_{Na} = g_{Na}(V-V_{Na}) \tag{2-4}$$

$$J_k = g_k(V-V_k) \tag{2-5}$$

$$J_l = g_l(V-V_l) \tag{2-6}$$

where J_{Na}, J_k, J_l are the ionic density currents of sodium, potassium and leakage ions respectively, in $mamp/cm^2$. V is the membrane potential displacement from the resting level in (mV) i.e.,

$$V = \psi_{input} - \psi_{output} - V_r \tag{2-7}$$

V_r is the resting membrane potential with the inside taken as positive (Hodgkin and Huxley considered the inside negative based on the direction of deflection of a D.C. voltmeter connected to electrodes b, c, Fig 2.1). In order to be consistent with the convention adopted in the ion transport model discussed in chapter 3,

V_{Na}, V_k, are the relative sodium and potassium equilibrium potentials respectively in mV. i.e.,

$$V_{Na} = \bar{V}_{Na} - V_r \tag{2-8}$$

$$V_k = \bar{V}_k - V_r \tag{2-9}$$

where $\bar{V}_{Na}$, $\bar{V}_k$ are computed with expression (2-1).

V_l is the relative leakage potential in mV. As will be discussed later, this variable is chosen in the model to yield V=0 when the membrane is in its resting state.

g_{Na}, g_k, are variables that govern the flow of sodium and potassium ions at the surfaces of the membrane. They have the dimensions of conductance per unit area in mho/cm^2. Their dynamic behavior constitutes the basis of the Hodgkin and Huxley model.

g_l, is a constant of proportionality between the leakage driving potential and the leakage current density, given in mmho/cm^2.

The variables g_{Na}, g_k are given by the following set of heuristic equations:

$$g_{Na} = \bar{g}_{Na}\, m^3\, h \tag{2-10}$$

$$g_k = \bar{g}_k\, n^4 \tag{2-11}$$

where

$$\dot{m} = \alpha m(V)\ (1-m) - \beta m(V)\ m \tag{2-12}$$

$$\dot{h} = \alpha h(V)\ (1-h) - \beta h(V)\ h \tag{2-13}$$

$$\dot{n} = \alpha n(V)\ (1-n) - \beta n(V)\ n \tag{2-14}$$

where m, n, h are continuous non-dimensioned bounded variables i.e.,

$$0 \leq m \leq 1 \tag{2-15}$$

$$0 \leq n \leq 1 \tag{2-16}$$

$$0 \leq h \leq 1 \tag{2-17}$$

and αm, βm, αh, βh, αn, βn are continuous functions (almost everywhere) of the relative membrane potential, V:

$$\alpha m(V) = \frac{0.1(25-V)}{\exp(0.1(25-V))-1} \qquad (2\text{-}18)$$

$$\beta m(V) = 4\exp(-V/18) \qquad (2\text{-}19)$$

$$\alpha h(V) = 0.07\exp(-V/20) \qquad (2\text{-}20)$$

$$\beta h(V) = 1/(\exp(0.1(30-V))+1) \qquad (2\text{-}21)$$

$$\alpha n(V) = \frac{0.01(10-V)}{\exp(0.1(10-V))-1} \qquad (2\text{-}22)$$

$$\beta n(V) = 0.125\exp(-V/80) \qquad (2\text{-}23)$$

these variables have the dimensions of 1/sec.

The constants in expressions (2-18) to (2-23) were determined by Hodgkin and Huxley by a trial an error procedure, in order to fit the experimental measurements made on several squid giant axons placed in solutions of different ionic concentrations, keeping the temperature of these solutions at 279K.

Schwan [18] gives an empirical factor by which the right hand sides of equations (2-12) to (2-14) have to be multiplied if it is desired to obtain the model equations at higher temperatures; this factor is given by the following expression:

$$\Delta = 3^{(T-279)/10} \tag{2-24}$$

Remark:

The model equations given above are valid for a voltage clamped axon.

The total ionic current density J_i is the algebraic sum of the ionic current densities of sodium, potassium and leakage ions. i.e.,

$$J_i = J_{Na} + J_k + J_l \tag{2-25}$$

Then, from (2-25) and 24 to (2-6) in (2-2)

$$J = C\frac{dV}{dt} + g_{Na}(V-V_{Na}) + g_k(V-V_k) + g_l(V-V_l) \tag{2-26}$$

Equation (2-26) and equations (2-10) to (2-23) constitute the Hodgkin and Huxley model for a clamped axon. In the next section an analysis of these equations is carried out in the light of the experimental evidence available.

2.4 Analysis of the Hodgkin and Huxley Model

This section is divided in two parts: In the first, the model will be represented as a dynamical system, and some considerations regarding the interrelationship between the state variables are given without any attempt to interpret their physical meaning. In the second a correlative analysis between the observed dynamical behavior of the clamped membrane and the sodium theory is carried out.

For purposes of analysis of the Hodgkin and Huxley model, the following properties will be checked

1) Global stability

2) Identifiability from the output

3) Parameter sensitivity

4) Propagation Model

This systematic analysis of a model that is known to fit the experimental data reasonably well, gives some insight on the dynamic characteristics of the membrane during the action potential. Moreover, some of the conclusions derived here will be applied to the model developed in the next chapter.

The Hodgkin and Huxley equations for the clamped axon given in the previous section can be written in the following vector notation.

From equation (2-26)

$$\frac{dV}{dt} = -\frac{1}{C} < \underline{g},\ \underline{e}V - \underline{V}_p > + \frac{J}{C} \qquad (2\text{-}27)$$

where

$$\underline{g} = \begin{vmatrix} g_{Na} \\ g_k \\ g_l \end{vmatrix} \quad \varepsilon R^3 \qquad e = \begin{vmatrix} 1 \\ 1 \\ 1 \end{vmatrix}$$

$$\underline{V}_p = \begin{vmatrix} V_{Na} \\ V_k \\ V_l \end{vmatrix} \quad \varepsilon R^3$$

and $\langle . , . \rangle$ is the standard inner product in R^3.

Let

$$\underline{S} = \begin{vmatrix} m \\ n \\ h \end{vmatrix}$$

then eqs (2-10), (2-11) can be written as follows:

$$\underline{g} = \underline{T}(\underline{S}) \tag{2-28}$$

where $\underline{T}$ is a mapping $X \to Y$ where X, Y are subsets of R^3:

$$X = \{\underline{x} \epsilon R^3 \mid |x_1| \leq 1, \quad |x_2| \leq 1, \quad |x_3| \leq 1\} \tag{2-29}$$

and the characteristics of the set Y are to be determined from the analysis and

$$T(\underline{S}) = \begin{vmatrix} \bar{g}_{Na}\, m^3\, h \\ \bar{g}_k\, n^4 \\ \bar{g}_l \end{vmatrix} \tag{2-29}$$

From equation (2-12), (2-13), (2-14) the following vector equation is obtained:

$$\dot{\underline{S}} = - \underline{\phi}(V)\underline{S} + \underline{U}(V) \tag{2-30}$$

where

$$\underline{\phi}(V) = \begin{vmatrix} +\alpha_m(V)+\beta_m(V) & 0 & 0 \\ 0 & +\alpha_n(V)+\beta_n(V) & 0 \\ 0 & 0 & +\alpha_h(V)+\beta_h(V) \end{vmatrix} \tag{2-31}$$

and

$$U(V) = \begin{vmatrix} \alpha_m(V) \\ \alpha_n(V) \\ \alpha_h(V) \end{vmatrix} \qquad (2\text{-}32)$$

Equations (2-27), (2-28), (2-30) can be represented by a block diagram as indicated in Fig 2.3. The diagram has been constructed in such a way as to indicate the functional parts that comprise the model: the ionic system that represents the dynamic interrelationships between the ionic flows and their corresponding driving forces, and the membrane control system that represents the dynamics of the control action exercised by the membrane on the ionic flow.

The dotted blocks and lines represent the external control used in the clamped experiments.

The diagram in Fig 2.3 shows the dependence of the ionic flow on the membrane voltage via two feedback loops: the first is represented in the diagram by the signal flowing between points a and b. The magnitude of this feedback signal is controlled by the flow regulator and the driving emf vector V_r. The second, represented by the signal flowing between points a and d, controls the membrane dynamics.

The inputs to the system are: the emf vector $\underline{V}_p$ which depends on the difference of concentration between the solutions on both sides of the membrane, and the current density J; in the absence of voltage clamping, this current comes from an adjacent excited region of the axon.

The analysis of the 5 dynamic properties mentioned above will be carried out next, on the basis of Figure 2.3.

1. Stability

To analyze the stability properties of the Hodgkin and Huxley model, the system will be split in two parts as indicated in Fig. 2.3. First, the stability conditions of the membrane control system are determined, and then (considering $\underline{g}$ as an input), the stability properties of the ionic system are analyzed. In the first case membrane potential is considered as a time varying parameter and the resultant quasilinear system stability is checked. For the second case the stability of the system about a particular membrane potential obtained by Hodgkin and Huxley [5] will be analyzed.

In equation (2-30) $\underline{\phi}(V)$ is a positive definite matrix for every value of V between 0 and 110 mV (the range of variation of the spike in the action potential; therefore the autonomous system

$$\dot{\underline{S}} = -\phi(V)\underline{S} \tag{2-33}$$

is asymptotically stable (Hsu and Meyer [37]).

Then assuming that V(t) is bounded everywhere for all $t>0$, (this assumption is justified below), notice that in (2-30), $\underline{\phi}(V) \equiv \underline{\phi}(V(t)) \equiv \tilde{\underline{\phi}}(t)$ and $\underline{U}(V) \equiv \underline{U}(V(t)) \equiv \tilde{\underline{U}}(t)$, therefore the solution of equation (2-30) is of the form

$$\tilde{\underline{S}}(t) = \underline{\psi}(t,0)\tilde{\underline{S}}(0) + \int_0^t \underline{\psi}(t,\sigma)\tilde{\underline{U}}(\sigma)d\sigma \tag{2-34}$$

where $\underline{\psi}(t,0)$ is the transition matrix corresponding to equation (2-33), with $V = V(t)$.

Since $\underline{S}(t; \underline{S}(0))$, the solution of (2-33), is asymptotically stable for all $\underline{S}(0)$ finite and $t>0$ the matrix $\underline{\psi}(t,0)$ is bounded (Desoer [38]). Therefore, in (2-34) the first term in the right hand side is bounded for all t. If the second term in the r.h.s. of (2-34) is bounded for all $t\geq 0$, the trajectory S(t) will be bounded for all $t\geq 0$ and the system (2-30) is said to be bounded input bounded output stable (BIBO).

From the definition of $\underline{U}(V)$ in (2-32) it can be seen that $||U(V)|| \leq M$ (M finite) for all V finite since

$$||U(V)|| = (\alpha_m^2 + \alpha_n^2 + \alpha_h^2)^{1/2} \qquad (2\text{-}35)$$

and α_m, α_n, α_h defined in (2-18), (2-20) and (2-22) respectively, are bounded for all $V\geq 0$.

Remark:

The denominators of $\alpha_m(V)$ and $\alpha_n(V)$ are zero at V = 25 mV and V = 10 mV respectively. To prove that for these values of V, α_m and α_n are bounded the limits of α_m when $V \to 25$ mV and α_n when $V \to 10$ mV are required.

Applying L'Hopital's rule:

$$\alpha_m(25) = \lim_{V\to 25} \frac{0.1(25-V)}{\exp(0.1(25-V))-1} = \lim_{x\to 0} \frac{0.1x}{\exp(0.1x)-1}$$

$$= \lim_{x\to 0} \frac{0.1}{0.1\exp(0.1x)} = 1$$

Similarly,

$$\alpha_n(10) = \lim_{V\to 10} \frac{0.01(10-V)}{\exp(0.1(10-V))-1} = \lim_{x\to 0} \frac{0.01x}{\exp(0.1x)-1}$$

$$= \lim_{x\to 0} \frac{0.01}{0.1\exp(0.1x)} = 0.1$$

therefore α_m, α_n are bounded for all V>0 finite and $||\underline{U}(\sigma)|| \leq M(V^*)$ where $M(V^*) = \sup ||\underline{U}(V)||$. In (2-34) the following inequality is valid

$$|| \int_0^t \underline{\psi}(t,\sigma)\ \underline{U}(\sigma)d\sigma|| \leq \int_0^t ||\underline{\psi}(t,\sigma)\ \underline{U}(\sigma)||d\sigma \qquad (2\text{-}36)$$

where $||\ ||$ is a suitable norm defined for a linear operator in the space $C_3(t_0, t_1, R^3 \to R^3)$.

By the Cauchy Swchartz inequality,

$$\int_0^t ||\underline{\psi}(t,\sigma)\ \underline{U}(\sigma)||d\sigma \leq \int_0^t ||\underline{\psi}(t,\sigma)||\ \ ||\underline{U}(\sigma)||d\sigma$$

$$= \int_0^t ||\underline{\psi}(t,\ 0)\ \underline{\psi}(0,\ \sigma)||\ \ ||\underline{U}(\sigma)||d\sigma$$

$$\leq \int_0^t ||\underline{\psi}(t,\ 0)||\ \ ||\underline{\psi}(0,\ \sigma)||\ \ ||U(\sigma)||d\sigma$$

Therefore the second term in the rhs of (2-34) is bounded and $\underline{S}(t)$ is BIBO.

The discussion above allows one to conclude that if the initial conditions $\underline{S}(0)$ are bounded $\underline{S}(t)$ will be bounded for all t>0 and for

all V(t) such that $0 \leq V(t) \leq 110$ m (the range of variation of the spike of the action potential. For negative values of V, a similar analysis as the one conducted above shows that S(t) remains bounded if $V(t) \geq -25$ mV ; this condition is fullfilled by the action potential function V(t).

The membrane control system output $\underline{g}$ is obtained from the mapping $\underline{g} = T(\underline{S})$ with T(.) as defined in (2-29). Since g_1 is a real finite value, $\underline{S}$ is bounded and $\underline{g}$ will be bounded.

Now, the stability of the ionic system will be considered. The ionic system as indicated in Fig 2.3 is driven by 3 inputs: $\underline{g}$, $\underline{V}_p$ and J. Therefore the stability (or instability) of the system has to be determined by showing that bounded inputs yield a bounded output, V(t).

The ionic system is described by equation (2-27), which is a quasilinear affine form in which $\underline{g}$, $\underline{V}_p$ and J are the inputs and V is the state.

For stability purposes, the membrane current density J, which is considered as the output of an adjacent excited membrane section, is assumed constant (the clamping loop is not operating).

In Fig 2.3, it can be seen that the sign of the rate of variation of the voltage, $\frac{dV}{dt}$, depends on the feedback signal flowing between a→b; given that J=0 if the signal is negative (positive feedback) $\frac{dV}{dt}$ increases, and it decreases if this signal is positive (negative feedback).

The initial value (resting state) of the voltage V is 0 and the bias vector $\underline{V}_p$ has the following constant values;

$$\underline{V}_p = \begin{vmatrix} V_{Na} \\ V_k \\ V_l \end{vmatrix} = \begin{vmatrix} 115 \\ -12 \\ +10.613 \end{vmatrix} \quad \text{in mV}$$

the vector $\underline{g}$ varies with V(t) as indicated above but it is known (and will be shown later) that the relaxation time of g_{na} (defined as the time required to attain its maximum) is about 10 times smaller than that of g_k; also $\bar{g}_{Na} \simeq 4\ \bar{g}_k$ and $g_l << g_{Na}$, $g_l << g_k$. With this semi-quantitative information, the behavior of the feedback loop in the ionic model can be determined.

It was shown above, that if V is finite $\underline{g}>0$ will be bounded; therefore, during an action potential, when the voltage is near 0, and $t < .5$ msec, the inner product

$$\xi(V) = \langle \underline{g},\ \underline{e}V - \underline{V}_p \rangle \qquad (2\text{-}37)$$

is negative (according to the previous analysis) therefore the feedback loop signal is positive; this implies that the voltage rate (consequently the voltage) increases in this region, i.e. ($\{0<t\leq 0.5\text{msec}\}$). Notice that in this region, the increase in potential is controlled mainly by the ionic sodium flow. The potassium flow goes in the opposite direction and therefore tends to decrease the potential rate; however since its relaxation time is much slower than that of the sodium, its effect (in the interval $[0\leq t\leq 0.5$ msec] does not overcome that of the sodium, and $(\partial V/\partial t)$ increases. The question is whether this growth is bounded. The solution of the following maximization problem yields the answer:

$$\max_V \xi = \max_V \langle \underline{g}\ \underline{e}V - \underline{V}_p \rangle$$

subject to

$$\underline{g} = T(\underline{S}) \qquad (2\text{-}38)$$

and

$$\dot{\underline{S}} = \phi(V)\underline{S} + \underline{U}(V) \qquad \underline{S}(0) \text{ given}$$

A numerical solution of problem (2-38) gave that a maximum is attained at V* = 72 mV and t* = 0.82 msec.

With a constant current density J = 0.8 mA/cm^2, this maximum is attained at 0.6 msec.

Next, it has to be proven that the membrane voltage V is bounded; this can be done by solving the Hodgkin and Huxley equations numerically or by checking if $\xi = \frac{dV}{dt}$ reaches a 0 value for a finite time and the sign of ξ after that time: from the expression for dV/dt (2-26), the problem can be formulated as follows

$$\langle \underline{g},\ \underline{e}V - \underline{V}p \rangle = 0$$

subject to

$$\underline{g} = T(\underline{S})$$
$$\dot{\underline{S}} = \phi(V)\underline{S} + \underline{U}(V) \qquad (2\text{-}39)$$

The approximative solution of this problem with J=0[1] gave V = 109 mV, t* = 1.2 msec; for t larger than t*, ξ<0 (up to t = 3.5 msec for t>3.5 msec the voltage V is less than 5 mV in absolute value and decreases with time)

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1 See next page

Moreover, for a sufficiently large gain k, V remains bounded for all t during the action potential; this condition along with the fact that ξ is bounded imply that the ionic system is BIBO. It is important to note that the previous analysis gives only a sufficient condition for stability.

2. Identifybility from the Output

This characteristic is probably the most critical one in a systematic study of the Hodgkin and Huxley model since the objective of the model is to determine (based on experimental measurements) the structure of the mechanism involved in the action potential. It will be seen in this section that with the available measurements, the internal parameters of the Hodgkin and Huxley model ($\underline{S}$) are unidentifiable from the output and therefore equations (2-28) and (2-30) are not only empirical (this fact will be discussed later) but also they cannot be uniquely determined from observations of the output variables (V and J_i as indicated in Fig 2.3).

Let $\underline{Y}(t)$ be the observation vector $V(t)>0$ (during the action potential) (Fig 2.1).

i.e.,
$$\underline{Y}(t) = \left|\frac{V(t)}{J(t)}\right| \qquad (2\text{-}40)$$

it is assumed that the observations are perfect, i.e., they are not corrupted by ambient noise.

Remark:

The observability of the system has to be tested with the clamping loop active since all the measurements were carried out with the membrane potential clamped.

The equations of the system then become:

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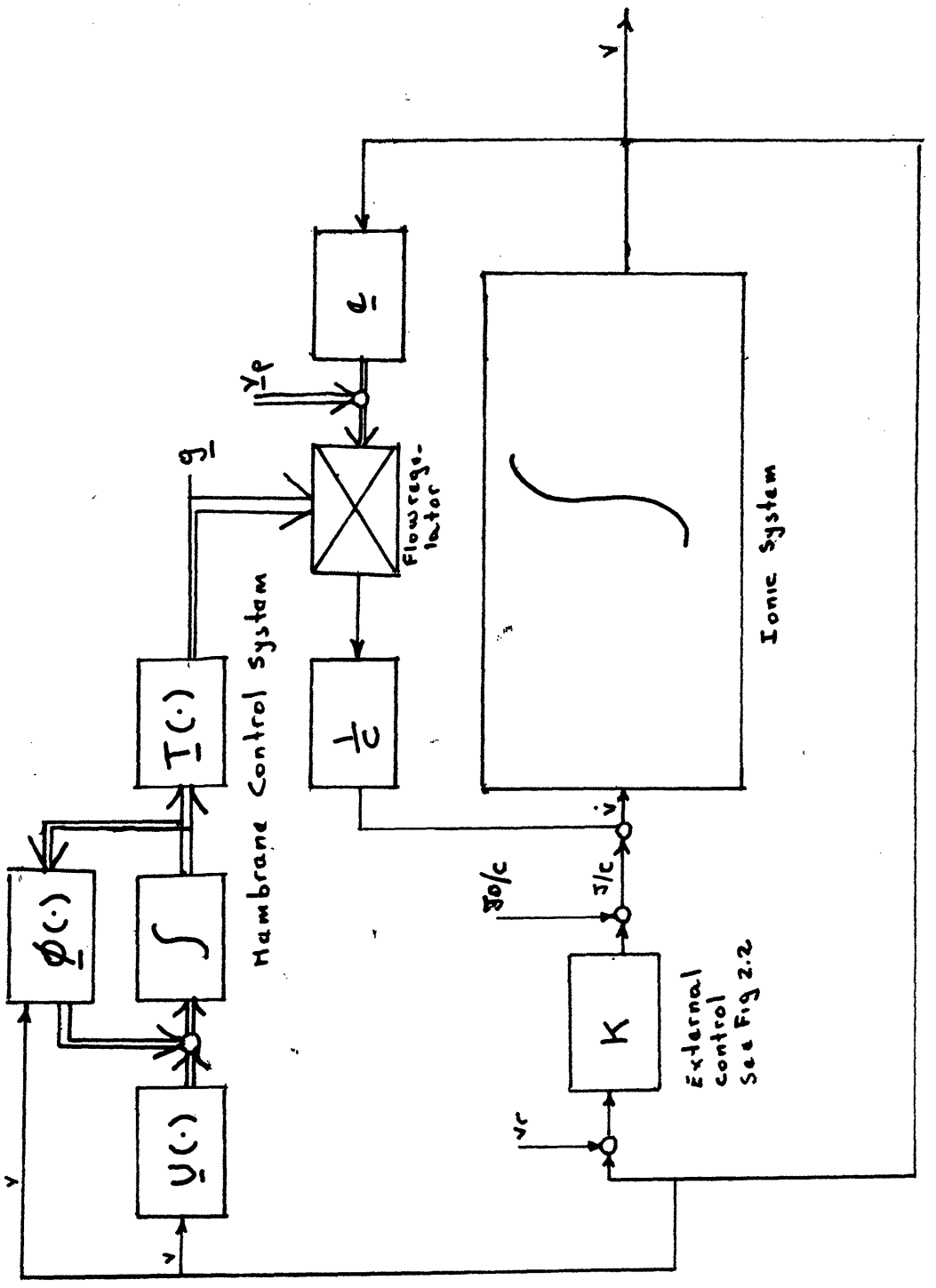
1 J=0 is the worst case condition for a depolarizing current since it subtract in (2-26) making dV/dt the biggest attainable value with respect to J ($J \geq 0$)



Hodgkin and Huxley Model of the clamped axon membrane, Block Diagram

FIG. 2.3

$$\frac{dV}{dt} = \frac{1}{C} \langle \underline{g}, \underline{e}V - \underline{V}_p \rangle + \frac{J_o}{C} + K(V - V_r) \qquad (2\text{-}41)$$

where K is a known constant (dependent on the characteristics of the current source). The objective of this arrangement is to maintain the membrane voltage more or less constant in time. A check of the graphical records obtained by Hodgkin and Huxley shows that this purpose was achieved. Therefore equation (2-41) can be simplified by the following approximations:

$$\text{(a)} \qquad \frac{dV}{dt} \simeq 0$$

$$\text{(b)} \qquad V \simeq V_r \qquad (2\text{-}42)$$

$$\text{(c)} \qquad J \simeq J_o$$

and

$$\text{(d)} \qquad \langle \underline{g}, eV_r - \underline{V}_p \rangle \simeq -J_o$$

then, given $J \simeq J_o$, and since V_r and V_p are known, the algebraic equation (2-42d) can be solved for $\underline{g}$ for each instant of time. Up to this point, the identification procedure has made use of experimental measurements and equation (2-41), which can be justified by physical arguments (Agin [19]). But no additional information is provided for determining the dynamic structure of the membrane control system; therefore any dynamical system of equations driven by $V_r - V(t)$, the relative membrane potential, such that its output vector equals the value assumed by the vector function $\underline{g}$ at each instant of time, will be equally valid, from the systems point of view, as a realization of the function $\underline{g}(V(t))$. Of course, there is an infinite number of such systems so that, without additional physical information about the membrane structure, it is not possible to determine from the feasible

realizations of $\underline{g}(V(t))$ which one corresponds to the membrane system.

3. Parameter Sensitivity

Since the appearance of the Hodgkin and Huxley model, considerable (unsuccessful) research has been conducted in order to justify physically the functional expression $\underline{T}(\underline{S})$; for this reason, a numerical analysis has been conducted in order to determine how critical is the dependence of the potential function when the exponents of m, n, h vary around their nominal values.

Let

$$\underline{p} = \begin{vmatrix} p_1 \\ p_2 \\ p_3 \end{vmatrix}$$

be the exponents of m, n, h respectively in $\underline{T}(\underline{S})$; the nominal values are $p_1 = 3$, $p_2 = 4$ and $p_3 = 1$.

Then, $\underline{g}$ can be written as a mapping of $R^3 \times R^3 \rightarrow R^3$ as follows

$$\underline{g} = \underline{T}(\underline{S}, \underline{p}) \qquad (2\text{-}43)$$

perturbing the values of $\underline{p}$ in (2-43), and expanding the resulting expression in a Taylor series neglecting variations higher than first order, the following expression for the resulting perturbation in $\underline{g}$ can be obtained:

$$\delta\underline{g} = \frac{\partial T}{\partial p}(\underline{S}^*, \underline{p})\ \delta\underline{p} \qquad (2\text{-}44)$$

where

$$\frac{\partial T}{\partial \underline{p}} = \begin{vmatrix} \bar{g}_{Na} m^{*3} h^* \ell n(m^*) & 0 & \bar{g}_{Na} m^{*3} h^* \ell n(h^*) \\ 0 & \bar{g}_K \underline{n}^{*4} \ell n(m^*) & 0 \\ 0 & 0 & 0 \end{vmatrix} \qquad (2\text{-}45)$$

and

$$S^* = \begin{vmatrix} m^* \\ n^* \\ h^* \end{vmatrix}$$

denotes the perturbed membrane control system state.

Next, using equation (2-27), an equation for the resultant perturbation in the membrane potential can be obtained:

$$\dot{V} + \delta\dot{V} = -\frac{1}{C} \langle \underline{g} + \delta_{\underline{g}}, \underline{e}(V+\delta V) - \underline{V}_p \rangle + \frac{J}{C} \qquad (2\text{-}46)$$

Neglecting increment products in (2-45), the following approximate expression for $\delta\dot{V}$ is obtained;

$$\delta\dot{V} \simeq -\frac{1}{C} \langle \underline{g}, \underline{e}\delta V \rangle - \frac{1}{C} \langle \delta_{\underline{g}}, \underline{e}V - \underline{V}_p \rangle \qquad (2\text{-}47)$$

Finally, the perturbed membrane control state is given by the following expressions:

$$\underline{S}^* = \underline{S} + \delta\underline{S}$$

$$\delta\dot{\underline{S}} = -\underline{\phi}(V)\delta\underline{S} - \frac{\partial \phi}{\partial V}(V)\underline{S}\delta V + \frac{\partial U}{\partial V}(V)\,\delta V \qquad (2\text{-}48)$$

with the initial conditions

$$\delta V(0) = 0 \qquad \delta \underline{S}(0) = 0 \qquad \delta_p = \delta_{\underline{p}}(0) = \delta_{\underline{p}_o} \quad \text{given constant}$$

where

$$\frac{\partial \phi}{\partial V} = \left| \begin{array}{ccc} \frac{\partial \alpha_m}{\partial V} + \frac{\partial \beta_m}{\partial V} & 0 & 0 \\ 0 & \frac{\partial \alpha_n}{\partial V} + \frac{\partial \beta_n}{\partial V} & 0 \\ 0 & 0 & \frac{\partial \alpha_h}{\partial V} + \frac{\partial \beta_h}{\partial V} \end{array} \right| \Bigg|_{V = V_n(t)} \tag{2-49}$$

and

$V_n(t)$ = nominal membrane voltage function

and

$$\frac{\partial \underline{U}(V)}{\partial V} = \left| \begin{array}{c} \frac{\partial \alpha_m(V)}{\partial V} \\ \frac{\partial \alpha_n(V)}{\partial V} \\ \frac{\partial \alpha_h(V)}{\partial V} \end{array} \right| \Bigg|_{V = V_n(t)} \tag{2-50}$$

For testing the sensitivity of the membrane potential to variations in the parameter vector $\underline{p}$ around its nominal values,

$$\underline{p}_n = \left| \begin{array}{c} 3 \\ 4 \\ 1 \end{array} \right|$$

a computer program was written to solve numerically equations (2-44), (2-47) and (2-48), assuming constant perturbations. The results are sumarized in Fig. 2.4 below.

Surprisingly, the time course of the action potential was relatively insensitive to variations in the parameter vector $\underline{p}$. As shown in the Fig. 2.4, with a 20% variation in $\underline{p}$, the change in membrane voltage is less than 5%, on the average. For larger perturbations, the incremental model should include second order terms in the Taylor expansion.

Notice that a positive perturbation increases the rising phase of the action potential and decreases the falling phase; this fact agrees with the experimental observation that an increase of sodium concentration[1] (on the outside) increases the magnitude of the action potential while an increase in potassium concentration (on the outside) decreases it.

The important conclusion of this sensitivity analysis is that the exponents of the elements of the vector $\underline{S}$ in $\underline{T}(S)$ do not correspond to a physical (unknown) factor but rather they are more or less arbitrary and at least to a first order approximation of the perturbed model, there is an entire set of these exponents that can approximate the experimental results equally well.

4. Propagation Model

Hodgkin and Huxley assumed that the ionic transport mechanism responsible for the action potential was uniformly distributed along the axon membrane (x). Therefore they represented the membrane by a lumped parameter model of a transmission line, in which the shunt branch of the elementary circuit of the model is formed by 4 elements in parallel

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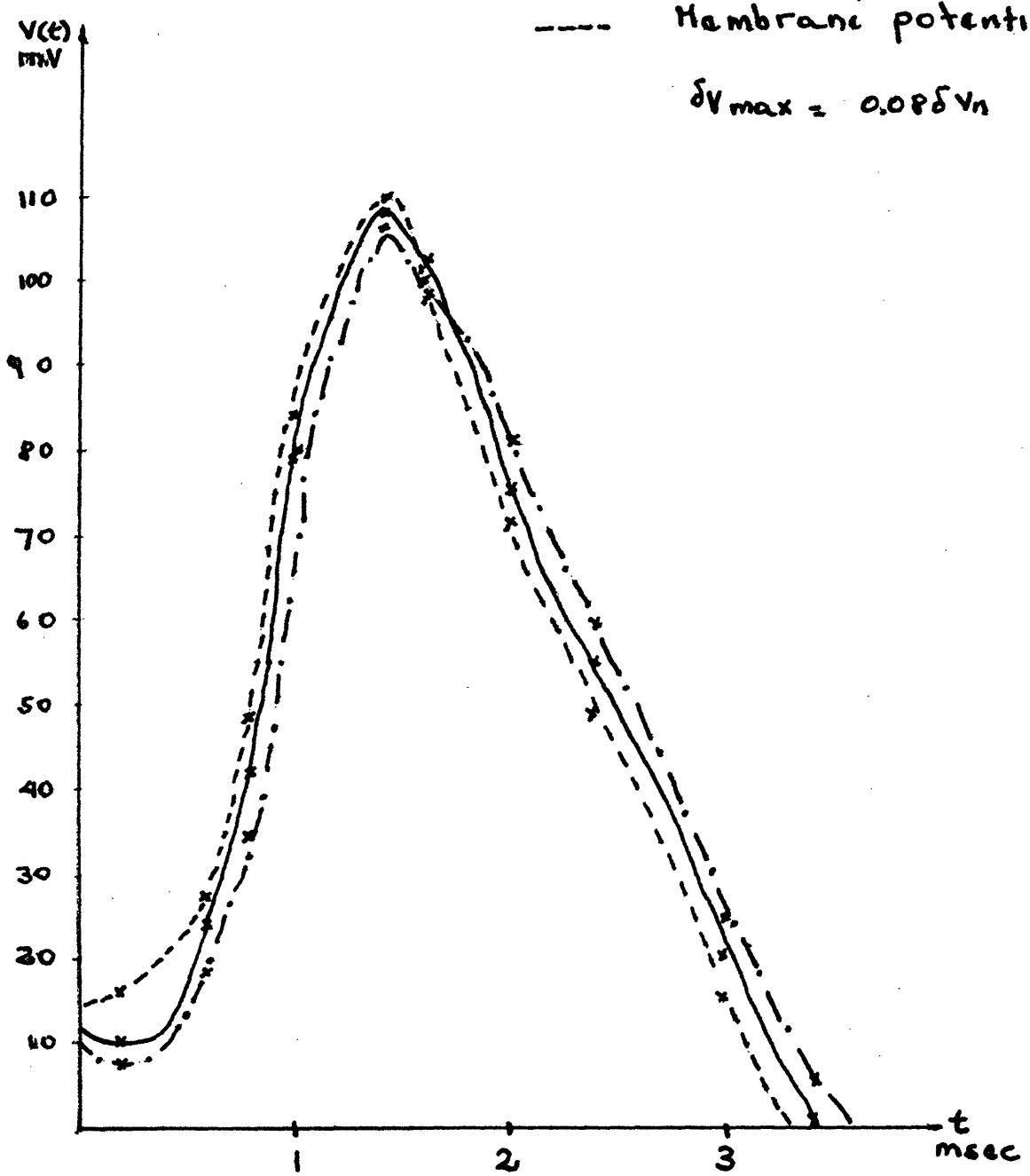
1 Notice that an increase in sodium concentration implies an increase in conductance g_{Na} see eq. (3-17) in [6].

FIG. 2.4

Changes in the Action potential spike caused by perturbations of the parameter vector $\underline{p}$



and two series resistors, as indicated in Fig. 2.5.

The series resistors represent the ohmic characteristic of the surfaces of the membrane and are assumed to be linear elements.

Applying ohm's law to the circuit of Fig. 2.5 the following relationships are obtained

$$\Delta V = (r_1 + r_2)\ J\Delta X\ 2\pi a \tag{2-51}$$

$$\Delta J = \Delta J_c + \Delta J_{na} + \Delta J_K + \Delta J_1 \tag{2-52}$$

$$\Delta J_c = C\frac{\partial(V-\Delta V)}{\partial t}\ \Delta X\ 2\pi a \tag{2-53}$$

$$\Delta J_{Na} = g_{na}(V-\Delta V-V_{Na})\ \Delta X\ 2\pi a \tag{2-54}$$

$$\Delta I_K = g_K(V-\Delta V-V_K)\ \Delta X 2\pi a \tag{2-55}$$

$$\Delta I_1 = g_1(V-\Delta V-V_K)\ \Delta X 2\pi a \tag{2-56}$$

The product $\Delta X\Delta V \leq |\Delta X|$ for ΔX, ΔV small and therefore all second order products can be neglected; then the incremental equation for the current $\Delta I(X, t)$ becomes

$$\Delta I \simeq (C\frac{\partial V}{\partial t} + g_{Na}(V-V_{Na}) + g_K(V-V_K) + g_1(V-V_1))2\pi a\Delta X \tag{2-57}$$

applying Kirchoff's current law.

Dividing both sides of (2-52) by ΔX and taking the limit as $\Delta X \to 0$:

$$\frac{1}{2\pi a}\frac{\partial I}{\partial X} = C_m\frac{\partial V}{\partial t} + g_{na}(V-V_{Na}) + g_k(V-V_k) + g_1(V-V_1) \tag{2-58}$$

In (2-51), dividing boths sides by ΔX and taking the limit as $\Delta X \to 0$:

$$\frac{1}{2\pi a}\frac{\partial V}{\partial X} = (r_1 + r_2)I \qquad (2\text{-}59)$$

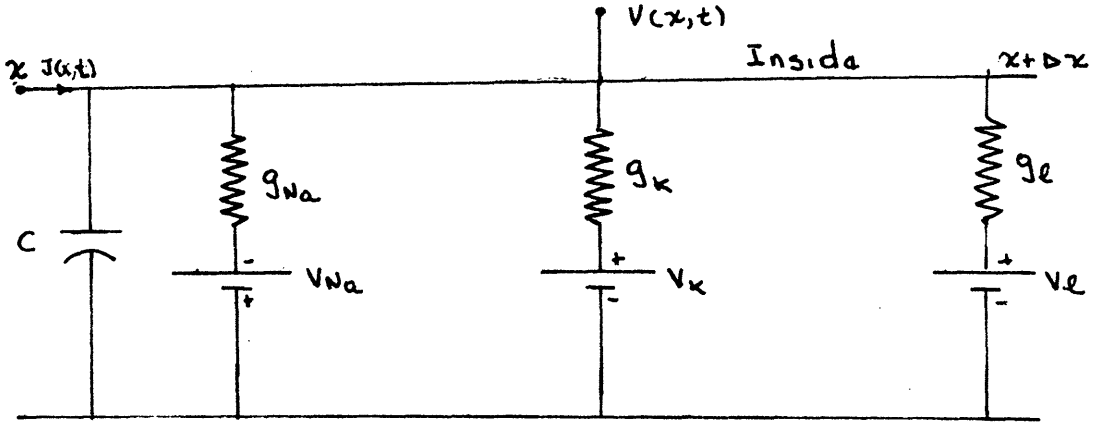
In (2-59) taking the derivative with respect to X

$$\frac{1}{2\pi a}\frac{\partial^2 V}{\partial X^2} = (r_1 + r_2)\frac{\partial I}{\partial X} \qquad (2\text{-}60$$

From (2-58) in (2-60)

$$\frac{\partial^2 V}{\partial X^2} = (r_1 + r_2)\left|C_m \frac{\partial V}{\partial t} + g_{na}(V-V_{Na}) + g_k(V-V_k) + g_1(V-V_1)\right| \qquad (2\text{-}61)$$

Equation (2-61) describes the propagated action potential. It is nonlinear partial differential equation and no closed form solution can be obtained. Numerical solutions of this equation have been obtained and they show good agreement with the experimental observations.



Lumped Parameter approximation of the axon membrane, Basic Unit

Fig 2-5

CHAPTER III

IONIC TRANSPORT MODEL

3.1 Introduction

The Hodgkin and Huxley model for grant squid axon membranes, discussed in Chapter II, shows a dynamic structure that is typical of systems involving interrelations between a potential and/or osmotic barrier (membranes in particular) and the flow of particles across it: this structure can be described by a two-subsystem model, one representing the flow of particles across the barrier and the other, the control exercised by the barrier on this flow.

In the Hodgkin and Huxley model, as stated in Chapter II, the equations describing the first subsystem can be justified by the laws of field theory, but those describing the second are completely empirical. This fact motivated the development herein of a model strictly based on well established physical laws governing the flow of charged particles though a membrane. The model equations satisfying the former requirement must also show a reasonable agreement with the observed experimental behavior of the squid axon membrane. It will be shown in this and the next chapter, that the ionic transport model to be described in this chapter partially satisfies those requirements.

Fundamentally, the ionic transport model for axon membranes is based on the determination of the relationships between the flows across the membrane of the different ion species present in the solutions of the system and the forces driving them, with the constraints imposed by

the assumed membrane structure (Danielli's model, see Chapter I).

The ionic transport model will be derived in two steps: In the first, a portion of the axon membrane will be assumed to be voltage clamped (see Fig. 2.1) and the relations between ion flows, driving forces, membrane parameters and membrane potential are derived in the second. A mechanism of propagation of the action potential is proposed.

The chapter has been organized as follows: Section 3.2 Definition of variables and parameters of the model; 3.3 Physical assumptions; 3.4 Derivation of the equations of the model; 3.5 Physical considerations about the variables of the model; 3.6 System Analysis of the model; 3.7 Conclusions.

3.2 Definition of Variables and Parameters of the Model

All the physical variables of the model are functions of 3 independent variables: the spatial variable x in the direction normal to the axon axis, bounded by the membrane surface ($x = 0$ inner surface, $x = \delta$ outer surface), the spatial variable z parallel to the axon axis, where the axon is assumed to have a constant radius (cylindrical shape) along the axis and a time variable t ($t = 0$, starting instant of the action potential). For sections 3.3 to 3.6 the model of the clamped axon is considered; therefore, the functional dependence on z of the physical variables is ignored in the definition of the variables given in this section. In section 3.7 this dependence will be indicated precisely. The following set is the domain of the functional variables of the model:

$$\Omega = \{(x,t)\epsilon R^2 \mid 0 \leq x \leq \delta,\ t \geq 0\} \qquad (3\text{-}1)$$

And any variable of the model is a mapping that can be generically represented as

$$f: \Omega \rightarrow R \qquad (3\text{-}2)$$

If additional properties of a particular variable are required, such as continuity and/or differentiability, they will be stated explicitly; otherwise, the variable is implicitly assumed to be defined as in (3-2).

The variables defining the ion transport model are:

μ_j, Electrochemical potential in J/mole defined for each of the ion species j = 1,...N involved in the transport process.

γ_j, Activity coefficient of the ion species j; indicates the affinity of a particular ion to follow a change in its chemical potential.

C_j, Chemical concentration of the ion species j in the membrane, in mole/cm^3.

z_j, Valence (with sign) of the ion species j.

ψ, Electric potential distribution across the membrane in volts. This function is assumed to be continuous for every (x,t) in Ω, once continuously (first order) differentiable in time, and twice continously differentiable in x. These mathematical properties arise from the physical characteristics of the potential distribution across the membrane, and will be justified in Section 3.2.

V, Electric potential difference between the inner and the outer surfaces of the membrane in volts. This variable is independent of x.

P, Hydrostatic pressure in Newton/cm^2.

$\underline{V}_j$, Partial molar volume of the ion species j; $\bar{V}_j = \frac{\partial V}{\partial n_j}\Big|_{n_i \; i=1,\ldots N \; i\neq j}$

where n_j is the mole number function of ion species j in cm^3/mole.

a_j, Chemical activity of ion species j in mole/cm^3.

X_j, Driving force of ion species j in volt/cm.

v_{xj}, Velocity of transport of ion species j in the x direction in cm/sec.

ϕ_j, Flux of ion species j across the membrane in ions/cm^2 sec. This variable is continuous in x and t.

E, Electric field intensity distribution across the membrane in volts/cm. This variable is continuous and continuously differentiable in x, and t for each $(x,t)\in\Omega$.

J_j, Current density distribution of ion species j in amp/cm^2. This function is continuous in x, and t for every $(x,t)\in\Omega$.

J, Driving current density in amp/cm^2. This variable is a function only of t and is piecewise continuous in t. (i.e., applied at $x = \delta$).

The parameters considered in the ion transport model which express the interrelation between the membrane structure and the ionic flow in the axon membrane are:

u_j, Mobility of the ion-species j in cm^2/volt sec. This parameter, along with other variables such as temperature, depends strongly on the structure of the membrane, especially at its surfaces, and plays a very important role in the ion transport model to be derived in Section 3.4. In the model, $u_j (j=1,...N)$ is a function of V and t but not of x.

ϵ, Electric permittivity of the membrane in F/cm. This parameter is assumed to be constant for every $(x,t) \in \Omega$. (The membrane is assumed to be isotropic in the x direction).

Finally, some constants that appear in the model equations are defined:

R, Universal gas constant (8.314 Joule/mole °K)

F, Faraday constant (96450 Coul/mole)

T, Ambient temperature: assumed constant and equal to 279°K. This temperature was chosen in order to compare the potential obtained with the ion transport model (to be computed in Chapter IV), with the one measured experimentally by Hodgkin and Katz at this temperature, Hodgkin and Katz [4].

3.3 Physical Assumptions

The membrane is assumed to have a structure such as the one described in Chapter I (Danielli's model). Except for the thin protein layer that exists at both surfaces of the membrane, it is considered that the membrane structure is passive and does not influence the

ionic flow; (in other words, the ionic flow is regulated by the protein layers on both sides of the membrane). And as will be seen later, this regulation is represented in the model by the variations of the ion mobilities of each ion species.

The flow of ions, even for the unclamped membrane, is always normal to the axon axis. This assumption is supported by the structure of Dannielli's model, in which the two layers of phospolipoids have oriented their polar terminations toward the inside of the membrane in a direction normal to the axon axis (Fig. 1.2), forming rigid channels that prevent the diffusion of the ions inside the membrane in a direction parallel to the axon axis (z coordinate).

The molecules or atoms involved in the transport process are ionized particles and the membrane potential is fully determined by them. Notice that this assumption does not exclude the possibility of diffusion of non charged molecules across the membrane but it establishes the condition that those molecules do not influence the action potential. In particular, water molecules will flow because of the presence of an osmotic gradient between the solutions on both sides of the membrane. This flow tends to maintain more or less constant the concentration of the different ion species involved in the action potential in the solutions on both sides of the membrane. This fact allows one to consider the concentration of each ion species constant in these solutions, i.e.

$$C_j(0, t) = C_{oj}$$

$$C_j(\delta, t) = C_{\delta j} \qquad (3\text{-}3)^1$$

$$j = 1,\ldots,N$$

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1 See Chapter V for a discussion about the meaning of these boundary concentrations.

Each ion species j, flows across the membrane driven only by its own force X_j and there is not any interaction force generated among ions of different species inside the membrane during the action potential. This assumption is very critical and requires further analysis. If it is assumed that the axon membrane presents holes at regular intervals, (pore theory) and all the ion species are transported across the membrane using these pores, then the hypothesis that each ion species flow is caused by its own gradient force is erroneous since the size of the pore, which has to be large enough to allow the ion species involved in the ion transport to cross the membrane, has to be sufficiently small to keep other molecules such as glucose (present in the surrounding solutions) from crossing the membrane, because the axon structure is not adapted to process them (for metabolic purposes). With this restriction in mind researchers have concluded that the pore radius has to be about 4Å (Solomon [12]). But this size implies that the distance between any two ions in the membrane is of the order of the molecular size of the ions and therefore strong forces of repulsion or attraction would be present between ion species in the membrane; therefore, the pore theory is not consistent with the hypothesis of independence of dynamic behavior among ion species.

Another hypothesis about membrane structure, that agrees with the assumption of independence, is the one that postulates that the membrane structure presents at regular intervals, specialized "activation sites" for each ion species. Each of these activation sites allows the transport of the ion species (j) that is affine to it, and no other type of ion (i, $i \neq j$) can be transported using this activation site, i.e., there

exists an exclusive one-to-one correspondence between ion species and activation sites.

The physical characteristics of activation sites will be studied in Chapter V. In this chapter, their existence will be assumed and the model equations derived accordingly.

3.4 Derivation of the Equations of the Model

In 1929, based on statistical mechanical considerations Guggenheim [13] derived an expression for the electrochemical potential distribution of an ion species j in a medium subject to composite forces; this potential, μ_j is given by

$$\mu_j = \mu_j^\circ + RT \ln \gamma_j C_j + z_j F\psi + P\bar{V}_j \qquad (3\text{-}4)$$

$$j = 1,2,\ldots,N$$

The thermodynamic principles on which expression (3-4) is based can be found in almost any book of thermodynamics; in particular, Katchalsky and Curran [14] and Spanner [15] have a comprehensive development.

In (3-4) the product $\gamma_j C_j$ gives the chemical activity of ion species j

$$a_j = \gamma_j C_j \qquad (3\text{-}5)$$

$$j = 1,2,\ldots,N$$

The activity coefficient γ_j in (3-5), has not been studied in detail in the context of biological membranes although some experimental results are available for lipoid membranes separating solutions of low concentration, (as found in the solutions on both sides of the axon membrane) and it has been found that this coefficient does not depend on the membrane structure, but rather, on the external concentration of ions (Na^+ and Cl^-), Plonsey [8]. The values of γ for sodium for concentrations ranging from 0.01M to 0.1M vary between 0.5 and 0.75. Therefore, since the model will be derived considering a constant concentration of the solutions on both sides of the axon membrane, the activity coefficients γ_j $j = 1,\ldots,N$ will be assumed to be constant.

The force driving ions of species j, X_j, can be computed as follows:

$$X_j = -\nabla\mu_j \qquad j = 1,2,\ldots,N \tag{3-6}$$

Since the flow of ions is normal to the axon axis the forces causing it are also normal to the axon axis; therefore (3-6) may be written as

$$X_j = -\frac{\partial\mu_j}{\partial x} \qquad j = 1,2,\ldots,N \tag{3-7}$$

where x is the distance from the internal surface of the membrane,

which is considered to be of constant thickness ($\delta = 150\mathring{A}$).

The velocity by which ions of species j are transported in the x direction, v_{xj}, is given by the following expression

$$v_{xj} = u_j X_j \qquad (3\text{-}8)$$

$$j = 1,2,\ldots,N$$

The latter is the key relationship in the representation of the restriction exercised by the membrane on the ionic flow.

It has been observed experimentally (using a radio-active isotope of potassium in the outer solution), Keynes et al [16], that when the membrane is in resting state, ions cross the membrane in a natural diffusion process due to the concentration gradient between the two solutions. When the membrane is excited, and an action potential is developed, this flow increases in magnitude for each of the ion species present but with widely different time constants. In particular, the experimental evidence available, (Hodgkin and Katz [3]), shows that during the action potential, there is an increase of the rate by which sodium ions are extruded from the inner solution and an increase in the rate by which potassium ions are transported from the outer solution. Indeed, the time constant of the sodium transport is roughly 4 times smaller than that of the potassium transport.

The discussion in the preceding paragraph indicates that during the action potential, there is an increase in the speed by which ions are transported; this increase, according to equation (3-8) may be due to the increase of the force X_j, driving each ion species j to the in-

crease of the mobility, u_j, or both. Additionally, after the action potential has occurred the membrane variables recover their resting condition values, so the velocity of each ion species j after the action potential will be (almost) equal to the initial value, i.e.,

$$v_{xj}(x, 0) = \lim_{t\to\infty} v_{xj}(x, t) \qquad (3\text{-}9)$$

$$j = 1,2,\ldots,N$$

Remark:

For practical purposes, $v_{xj}(x, 0) \simeq v_{xj}(x, 4\text{msec})$ $j = 1,2,\ldots,N$ (see Chapter IV.)

The physical analysis carried out above, of the time behavior of the ion velocities is based on the experimental observation of the ion transport process in axon membranes (Hodgkin and Katz [3]) and the conclusions obtained will be used later for justifying the functional expressions for the ion mobilities u_j, $j = 1,\ldots,N$ which will be derived for the model.

The flux, ϕ_j, of ions of species j, across the membrane, is given by the following expression:

$$\phi_j = v_{xj} C_j \qquad (3\text{-}10)$$

$$j = 1,2,\ldots,N$$

Then from (3-7) and (3-8) in (3-10),

$$\phi_j = - u_j \frac{\partial \mu_j}{\partial x} C_j \qquad (3\text{-}11)$$

$$j = 1,2,\ldots,N$$

And from (3-4) in (3-11),

$$\phi_j = -u_j\left(RT\frac{\partial}{\partial x}(\ln \gamma_j C_j) + z_j F\frac{\partial \psi}{\partial x} + \frac{\partial p}{\partial x}\bar{V}_j\right)C_j \qquad j = 1,2,\ldots,N \tag{3-12}$$

Applying the law of conservation of charge to each ion species j, the following relationship is obtained

$$\frac{\partial C_j}{\partial t} = -\frac{\partial \phi_j}{\partial x} \qquad j = 1,2,\ldots,N \tag{3-13}$$

Finally, the electric potential distribution inside the membrane is related to the concentrations of the different ion species by Poisson's equation

$$\frac{\partial^2 \psi}{\partial x^2} = -\frac{F}{\epsilon}\sum_{j=1}^{N} z_j C_j \tag{3-14}$$

Equations (3-12), (3-13), (3-14) give the relationships among the different variables that characterize the ionic model.

This model can be considered as a generalization of the widely used model for the mechanism of ion transport across membranes derived by Goldman in 1943. Goldman [7]. In his model, Goldman assumed that the electric field intensity E is constant across the membrane. This assumption is arbitrary from the physical point of view; moreover, as pointed out by Hodgkin and Huxley [4], it does not fit the experimental time course of the squid axon membrane ionic flows during the action potential. (In fact, the values found by using the Goldman model for

computing the flow of each ion species j are always lower than the corresponding experimental flows).

In the present model the electric field intensity E will be considered as a time varying distributed variable (across the membrane) and its relationship with the variables of the model is established by two partial differential equations based on general principles of field theory that are applicable in this case.

The electric field intensity is defined by the following expression:

$$E \equiv - \frac{\partial \psi}{\partial x} \tag{3-15}$$

Since there is no magnetic field present in the membrane, Gauss' law applied to the membrane system becomes,

$$\delta(x-\delta)J(t) - \sum_{j=1}^{N} J_j + \varepsilon \frac{\partial E}{\partial t} = 0 \tag{3-16}$$

Remark:

No flow of charges is assumed to be generated inside the membrane, $\frac{\partial J}{\partial x} = 0$.

The current density of ion species j is related to the corresponding flow ϕ_j, by the following expression:

$$J_j = z_j F \phi_j \qquad j = 1,2,\ldots,N \tag{3-17}$$

Now, some analysis of equations (3-12) will be carried out in order to simplify the expressions describing the model.

As noted before, γ_j is dependent primarily on the concentration of ion species j in the solutions bathing the membrane, therefore in (3-12) it can be treated as a constant. Also, the partial volume $\bar{V}_j$ is very small for the concentrations present in the axon system ($\simeq 10^{-9}$ cm^3/mol), therefore, even with a pressure difference of 10 atm the contribution of the third member of (3-12) to the total flow ϕ_j is less than .1% of the total, so this term can be neglected. Finally the ion mobility u_j is a function of the surface structure of the membrane, the potential difference between surfaces of the membrane, and temperature, so u_j is not an explicit function of x and can be treated as a constant in (3-12).

With the former considerations equations (3-12) become,

$$\phi_j \simeq -u_j\left(RT\frac{1}{C_j}\frac{\partial C_j}{\partial x} + z_jF\frac{\partial \psi}{\partial x}C_j\right) \qquad (3\text{-}18)$$

$$j = 1,2,\ldots,N$$

Then, from (3-18) in (3-13)

$$\frac{\partial C_j}{\partial t} = u_j\left(RT\frac{\partial^2 C_j}{\partial x^2} + z_jF\frac{\partial \psi}{\partial x}\frac{\partial C_j}{\partial x} + z_jF\frac{\partial^2 \psi}{\partial x^2}C_j\right) \qquad (3\text{-}19)$$

$$j = 1,2,\ldots,N$$

From (3-15) in (3-19)

$$\frac{\partial C_j}{\partial t} = u_j\left(RT\frac{\partial^2 C_j}{\partial x^2} - z_jFE\frac{\partial C_j}{\partial x} - z_jF\frac{\partial E}{\partial x}C_j\right) \qquad (3\text{-}20)$$

$$j = 1,2,\ldots,N$$

And from (3-15) in (3-14),

$$\frac{\partial E}{\partial x} = \frac{F}{\varepsilon} \sum_{j=1}^{N} z_j C_j \tag{3-21}$$

Finally, from (3-16)

$$\frac{\partial E}{\partial t} = \frac{1}{\varepsilon} \left(\sum_{j=1}^{N} J_j - J(t) \right) \tag{3-22}$$

The set of equations (3-20) - (3-22) is the basis of the ionic model for the axon membrane. Moreover, the model has been derived in general form and therefore is applicable to any membrane system separating ionic solutions provided that the conditions on which the activation site hypothesis is based are fulfilled by the system.

The last part of this section is devoted to determining a functional expression for the ion mobilities u_j $j = 1,\ldots,N$ and for the initial anb boundary conditions for equations (3-20) - (3-22).

The first observation regarding the determination of u_j is that according to the assumed structure of the membrane, the mobility is a variable determined by surface membrane characteristics. Therefore, it is not explicitly dependent on x. Moreover, from the quantum mechanics point of view, u_j is a phenomenological coefficient relating the velocity of an element of charge j to the force that is driving it, provided that the energy (potential plus kinetic energy) contained in that element is equal to the average (in the statistical sense) energy required for the transportation of elements of charge of the type j across the membrane and the total energy has a probability density function of the Boltzman type. Vander Ziel [17].

The discussion in the preceding paragraph requires the definition of what is meant by an element of electric charge in the context of the ionic membrane system. Since it has been assumed that the concentrations of each ion species j on the solutions on both sides of the membrane are constant (Eqs. 3-3) for all $t \geq 0$ on the average, an element of charge q_j (coulombs) for ion species j is defined by the following expression:

$$q_j = z_j F(C_{\ell j} - C_{oj})\,\mathcal{V} \qquad (3\text{-}23)$$

$$j = 1,2,\ldots,N$$

where $\mathcal{V}$ is the volume of clamped membrane in cm^3.

The time course of the average velocity of the element of charge q_j can be computed with the following expression:

$$\bar{v}_{xj}(t) = \frac{1}{\delta} \int_o^\delta v_{xj}(x,t)dx \qquad (3\text{-}24)$$

$$j = 1,2,\ldots,N$$

From (3-8) in (3-24)

$$\bar{v}_{xj}(t) = \frac{1}{\delta} \int_o^\delta u_j X_j(x,t)dx \qquad (3\text{-}25)$$

$$j = 1,2,\ldots,N$$

Since u_j $j = 1,2,\ldots,N$ does not depend explicitly on x, (3-25) can be written as follows

$$\bar{v}_{xj}(t) = u_j\left(\frac{1}{\delta}\int_0^{\delta} X_j(x,t)dx\right) \qquad (3\text{-}26)$$

$$j = 1,2,\ldots,N$$

In (3-26), the term in brackets in the right hand side is the average driving force of ion species j at each instant of time. Then (3-26) becomes:

$$\bar{v}_{xj}(t) = \overline{u_j X_j}(t) \qquad (3\text{-}27)$$

$$j = 1,2,\ldots,N$$

or

$$\bar{v}_{xj}(t) = u_j \bar{X}_j(t) \qquad (3\text{-}28)$$

$$j = 1,2,\ldots,N$$

Since it was assumed that the only force acting on each ion species j during the action potential was its own driving force, the total average energy $W_j(t)$, of an element of charge q_j crossing the membrane at some time t during the action potential is given by the following expression:

$$W_j(t) = \frac{1}{2} m_j^* \bar{v}_{xj}^2 + q_j \bar{X}_j \delta \qquad (3\text{-}29)$$

$$j = 1,2,\ldots,n$$

where m_j^*, is the equivalent mass of the element of charge q_j and is determined by the net interaction between this element and the membrane structure during the transport process (frictional forces).

The transport process is assumed to be at constant temperature and the membrane system is thermodynamically (in the ionic model) a closed

system; therefore, the total average energy of each ion species j is constant for all $t \geq 0$, i.e., $W_j(t) \equiv$ constant, $j = 1,2,\ldots,N$. Then taking the time derivative on both sides of (3-29),

$$0 = \frac{m_j {}^*\bar{v}_{xj} u_j \dot{\bar{X}}_j}{2} + q_j \dot{\bar{X}}_j \delta \qquad (3\text{-}30)$$

$$j = 1,2,\ldots,N$$

or

$$u_j = -\frac{2 q_j \delta}{m_j {}^*\bar{v}_{xj}} \qquad (3\text{-}31)$$

$$j = 1,2,\ldots,N$$

From (3-10) in (3-24), another expression of $\bar{v}_{xj}$ can be computed:

$$\bar{v}_{xj} = \frac{1}{\delta} \int_o^\delta \frac{\phi_j}{C_j} \, dx \qquad (3\text{-}32)$$

$$j = 1,2,\ldots,N$$

And from (3-18) in (3-32),

$$\bar{v}_{xj}(t) = -\frac{u_j}{\delta} \int_o^\delta \left(RT \frac{1}{C_j} \frac{\partial C_j}{\partial x} + z_j F \frac{\partial \psi}{\partial x}\right) dx \qquad (3\text{-}33)$$

$$j = 1,2,\ldots,N$$

Performing the integration in the right handside of (3-33),

$$\bar{v}_{xj}(t) = -\frac{u_j}{\delta}\left(RT \ln\left(C_{1j} \,|\, C_{oj}\right) + z_j F(\psi(\delta) - \psi(0))\right)$$

$$j = 1,2,\ldots,N$$

but

$$V(t) = \psi(\delta) - \psi(0) \tag{3-35}$$

the membrane potential difference. Then (3-34) becomes:

$$\bar{v}_{xj}(t) = -\frac{u_j}{\delta}(RT \ln(C_{1j}/C_{oj}) + z_j FV(t)) \qquad j = 1,2,\ldots,N \tag{3-36}$$

From (3-36) in (3-31), the following expression for u_j is obtained:

$$u_j = \left(\frac{q_j \delta^2}{m_j^*(RT \ln(C_{1j}/C_{oj}) + z_j FV(t))}\right)^{1/2} \qquad j = 1,2,\ldots,N$$

or (3-37)

$$u_j = \frac{\sqrt{q_j}\ \delta}{(m_j^*(RT \ln(C_{1j}/C_{oj}) + z_j FV(t))^{1/2}} \qquad j = 1,2,\ldots,N$$

From (3-23) in (3-37),

$$u_j = \left(\frac{z_j F(C_{1j} - C_{oj})\ \mathscr{V}}{m_j^*(RT \ln(C_{1j}/C_{oj}) + z_j FV(t))}\right)^{1/2} \delta \qquad j = 1,2,\ldots,N \tag{3-38}$$

Remark:

The volume $\mathscr{V}$ is given by the following expression:

$$\mathscr{V} = (\pi\delta^2 + 2\pi a\delta)X \quad cm^3$$

since the axon is assumed to have a regular cylindrical shape.

Now, the boundary conditions for equations (3-20) - (3-22) will be discussed.

For equations (3-20), the boundary conditions are given by the concentrations of the solutions on each side of the membrane, eqs. (3-3).

Now it has to be shown that those conditions define also the boundary conditions for equations (3-21), (3-22) for the electric field intensity: since equation (3-21) has to be satisfied for all x, the boundary conditions for E can be computed as follows:

$$\frac{\partial E}{\partial x}(0,t) = \frac{F}{\varepsilon} \sum_{j=1}^{N} z_j C_j(0;t) \qquad (3\text{-}39)$$

or from (3-3) in (3-39)

$$\frac{\partial E}{\partial x}(0,t) = \frac{F}{\varepsilon} \sum_{j=1}^{N} z_j C_{oj} \qquad (3\text{-}40)$$

Similarly,

$$\frac{\partial E}{\partial x}(\delta,t) = \frac{F}{\varepsilon} \sum_{j=1}^{N} z_j C_j(\delta,t) \qquad (3\text{-}41)$$

or from (3-3) in (3-41)

$$\frac{\partial E}{\partial x}(\delta,t) = \frac{F}{\varepsilon} \sum_{j=1}^{N} z_j C_{1j} \qquad (3\text{-}42)^1$$

Since C_{oj}, C_{1j} are constant with time, $\frac{\partial E}{\partial x}(0,t)$, $\frac{\partial E}{\partial x}(\delta,t)$ are also constant.

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1 This boundary condition is not needed for the integration but is used for checking the integration routine in the next chapter.

Finally, the initial conditions for eqs. (3-20) - (3-22) are determined.

At resting conditions, the time variation of the concentrations of ions in the membrane, vanishes; therefore, the concentration of ion species j, C'_j, which is a function only of x, has to satisfy the following differential equation:

$$RT \frac{d^2C'_j}{dx^2} - z_j F E' \frac{dC'_j}{dx} - z_j F \frac{dE'}{dx} C'_j = 0 \qquad j = 1,2,\ldots,N \tag{3-43}$$

where $E' \equiv E'(x)$ is the electric field intensity distribution across the membrane at resting conditions and satisfies equation (3-21):

$$\frac{\partial E'}{\partial x} = \frac{F}{\varepsilon} \sum_{j=1}^{N} z_j C'_j \tag{3-44}$$

Applying the continuity equation for the system at resting conditions, ($J \equiv 0$),

$$\sum_{j=1}^{N} J'_j(x) = 0 \tag{3-45}$$

where $J'_j(x)$ $j = 1,2,\ldots,N$ are the current density distributions at resting conditions.

The boundary conditions for the system of equations (3-43) - (3-45) are given by conditions (3-3) i.e.,

$$C'_j(0) = C_{oj}$$

$$j = 1,2,\ldots,N \tag{3-46}$$

$$C'_j(\delta) = C_{1j}$$

Then, the initial conditions for the dynamic system, equations (3-20) - (3-22) are,

$$C_j(x,o) = C'_j(x) \qquad j = 1,2,\ldots,N \tag{3-47}$$

$$E(x,o) = E'(x) \tag{3-48}$$

which are the solutions of the system of equations (3-43) - (3-45).

3.5 Some Properties of the Variables of the Model

In this section some additional properties that the variables of the model must satisfy are considered, in particular, those conditions that these variables must satisfy in order to be consistent with the physical entities they represent.

The concentration $C_j(x,t)$, of each ion species j, is a continuous function on x and t. This condition arises from the fact that the mass is assumed to be continuously distributed for the membrane system; therefore the density ρ_j of each ion species j is a well defined continuous function for each point in Ω

$$C_j(x,t) \geq 0 \qquad j = 1,2,\ldots,N \tag{3-46}$$

since a negative concentration has no physical meaning.

The electric potential distribution ψ is a continuous function for every point in Ω. Moreover, it has continuous first and second order derivatives in x and first derivative in t for every $(x,t)\varepsilon\Omega$. The phy-

sical basis of this condition arises from the fact that ψ is a phenomenological variable whose gradient is a "force" proportional to the ionic flow, and since this flow cannot be discontinuous (by the continuity law of matter), $\frac{\partial \psi}{\partial x}$ has to be continuous; moreover by Poisson's Law the second derivative of ψ with respect to x is proportional to the dynamic volumetric charge distribution in the membrane which is continuous (by the continuity law of charge distribution in the context of field theory).

The three conditions mentioned above have to be satisfied by the solution of eqs. (3-20) - (3-22) and their associated boundary conditions (eqs. 3-3) in order to ensure consistency with their physical meaning.

Assuming that C_j $j = 1,2,\ldots,N$ and ψ are continuously differentiable in x from equation (3-18) it may be concluded that the flow ϕ_j of each ion species j is continuous (almost everywhere) in x and t, and consequently, the current density functions J_j $j = 1,2,\ldots,N$ are also continuous (Eqs. 3-17).

The conditions of continuity on C_j and J_j (in Ω) imply by observation of equations (3-21) and (3-22) that $\frac{\partial E}{\partial t}$ are continuous functions and since $\frac{\partial^2 \psi}{\partial x^2} = - \frac{\partial E}{\partial x}$ this implies that the second derivative of the potential distribution is continuous, as was stated above.

3.6 System Analysis of the Model

The model is defined by 3 kinds of variables from the system point of view: the state variables C_j $j = 1,2,\ldots,N$, the output varia-

bles, E, $\sum_{j=1}^{N} J_j$, ψ, V and the control variables u_j $j = 1,2,\ldots,n$ and J(t).

The dynamic equations of the system are:

$$\frac{\partial C_j}{\partial t} = u_j L_j(E,C_j) \qquad j = 1,2,\ldots,N \tag{3-49}$$

where

$$L_j(E,C_j) = RT\frac{\partial^2 C_j}{\partial x^2} - z_j FE\frac{\partial C_j}{\partial x} - z_j F\frac{\partial E}{\partial x} C_j \qquad j = 1,2,\ldots,N \tag{3-50}$$

is a nonlinear **differential operator.** Notice that the system model possesses an inherent output **feedback law, since E is an output variable** and the state depends on it. Later, in this section, an analysis of the characteristics of the operators $L_j(\cdot,\cdot)$ $j = 1,2,\ldots,N$ will be carried out.

The output equations of the system are:

$$\frac{\partial E}{\partial x} = \frac{F}{\varepsilon} \sum_{j=1}^{N} z_j C_j \qquad \text{(eq. 3-21)}$$

From Eqs. (3-17) in Eq. (3-22) the following expression is obtained:

$$\frac{\partial E}{\partial t} = \frac{F}{\varepsilon} \sum_{j=1}^{N} z_j \phi_j - \frac{1}{\varepsilon} J(t) \tag{3-51}$$

From Eqs. (3-18) in Eq. (3-51),

$$\frac{\partial E}{\partial t} = \frac{F^2}{\varepsilon}\left(\sum_{j=1}^{N} u_j z_j C_j\right)E - \frac{F}{\varepsilon}\sum_{j=1}^{N} u_j z_j RT\frac{\partial C_j}{\partial x} - \frac{1}{\varepsilon}J(t) \qquad (3\text{-}52)$$

Assuming that the functional form of the state $C_j(x,t)$ $j = 1,2,\ldots,N$ and the control u_j $j = 1,2,\ldots,N$, and $J(t)$ (inputs) are known it has to be proven that Equations (3-51) and (3-52) are sufficient to determine the output variables, E, $\sum_{j=1}^{N} J_j$, ψ, V

If E is an exact differential, (physically the electric field intensity is an exact differential, dE, it has to be proven that in the equations of the model E is an exact differential) the knowledge of $\frac{\partial E}{\partial x}$, $\frac{\partial E}{\partial t}$ together with the initial condition $E'(x)$ is sufficient to determine E; i.e., if dE can be expressed as follows

$$dE = M(x,t)dt + N(x,t)dx \qquad (3\text{-}53)$$

with M, N satisfying the following condition

$$\frac{\partial M}{\partial x} = \frac{\partial N}{\partial t} \qquad (3\text{-}54)$$

where $M \equiv \frac{\partial E}{\partial t}$ and $N \equiv \frac{\partial E}{\partial x}$ (3-55)

then, dE is an exact differential.

Assuming that (3-55) holds it has to be proven that (3-54) holds:

From (3-52)

$$\frac{\partial M}{\partial x} = \frac{F^2}{\varepsilon}\left(\sum_{j=1}^{N} u_j z_j \frac{\partial C_j}{\partial x}\right)E + \frac{F^2}{\varepsilon}\left(\sum_{j=1} u_j z_j C_j\right)\frac{\partial E}{\partial x}$$

$$+ \frac{F}{\varepsilon}\sum_{j=1}^{N} u_j z_j RT \frac{\partial^2 C_j}{\partial x} \qquad (3\text{-}56)$$

From (3-21)

$$\frac{\partial N}{\partial t} = \frac{F}{\varepsilon}\sum_{j=1}^{N} z_j \frac{\partial C_j}{\partial t} \qquad (3\text{-}57)$$

From (3-20) in (3-57),

$$\frac{\partial N}{\partial t} = -\frac{F}{\varepsilon}\sum_{j=1}^{N} z_j u_j \left(RT\frac{\partial^2 C_j}{\partial x^2} - z_j FE\frac{\partial C_j}{\partial x} - z_j F\frac{\partial E}{\partial x} C_j\right) \qquad (3\text{-}58)$$

Then comparing (3-56) with (3-58) it is seen that (3-54) holds; therefore, dE is an exact differential and E can be expressed by the following integral equation

$$E(x,t) = \int_o^t M(x,\tau)d\tau + \int_o^x N(\xi,t)d\xi - \int_o^x \int_o^t \frac{\partial M}{\partial \tau}(\eta,\tau)d\tau d\eta \qquad (3\text{-}59)$$

where M, and N are defined by equations (3-55).

Since E can be determined if $C_j(x,t)$ $j = 1,\ldots,N$ are known for every $(x,t)\varepsilon\Omega$, it remains to be seen if the rest of the output variables are also determined:

V(t) is obtained by the following expression:

$$V(t) = -\int_{o}^{\delta} E(x,t) + V_r \qquad (3\text{-}60)$$

where V_r is the membrane resting potential.

$\psi(x,t)$, the potential distribution can be obtained by integrating eq. (3-15):

$$\psi(x,t) = -\int_{o}^{x} E(x,t)dx + V(t) \qquad (3\text{-}61)$$

finally from expression (3-22), $\sum_{j=1}^{N} J_j(x,t)$ can be computed:

$$\sum_{j=1}^{N} J_j = \epsilon\frac{\partial E}{\partial t} + J(t) \qquad (3\text{-}62)$$

Remark:

The choice of which variables of the model are output variables is largely motivated by the fact that these variables are measurable by standard laboratory procedures, as indicated in section 2.2.

The control variables u_j, j=1,2,...,N determine a multiplicative output feedback as can be seen by observing eqs. (3-38) and (3-52). It is important to stress that u_j $j = 1,2,...,N$ are the mathematical representation of the interaction of the membrane on the ionic flow; in particular, m^*_j $j = 1,2,...,n$, the effective masses of the ion species, are determined by the membrane structure (assumed), as will be shown in Chapter IV. Notice also, that u_j is a well defined real number for each value of V(t), only if the expression inside the parenthesis in (3-38), is positive and $m^*_j(RT \ln(C_{1j}/C_{oj}) + z_jFV(t)) \neq 0$. In Chapter IV it will be shown that for the squid axon membrane, those conditions

are always met. J(t) is the input variable; it directly controls the ionic flow by regulating the electric field intensity, (eq. (3-22)).

J(t) is assumed to be the output current density of an adjacent portion of membrane to the clamped region under study, it provides the system with energy enough to trigger an action potential. In Chapter IV, a correlation analysis between the membrane response (voltage) and the current density excitation is carried but, in particular, it will be seen that a short pulse of enough magnitude (about .1 msec of duration), triggers an action potential, but if the pulse duration is increased as to be comparable with the action potential spike duration, (about 3 msec) a train of spikes is produced; this fact agrees with the experimental observations reported by Katz, [3].

The questions that must be answered by the analysis of the system are the following:

a) Are the equations developed in section 3.5 together with the restrictions on the variables of the model established in section 3.6 sufficient for ensure the existence of a solution?

b) Is this solution unique?

c) Is the system stable?

d) Is the system observable from the output?

e) Does the control action excercised by the membrane on the ionic flow (u_j $j = 1,\ldots,N$) drive the system in such a way as to satisfy conditions 3-9

a) This question is intimately related with the characteristics of the operator equation (3-49). By the existence of a solution to the system of equations (3-49) is meant to find the mappings,

$$C_j: \Omega \to \Sigma_j \qquad j = 1,2,\ldots,N$$

and (3-63)

$$E: \Omega \to R$$

where Σ_j $j = 1,2,\ldots,n$ are bounded subsets of the real positive line; such that $C_j(x,t)$ $j = 1,2,\ldots,N$ and E satisfy the following conditions:

i) $C_j \varepsilon C^2(\Omega)$ $\quad j = 1,2,\ldots,n$

ii) $C_j \varepsilon C(\Omega \cup \partial\Omega)$

iii) $E \quad \varepsilon C^2(\Omega)$

iv) $E \quad \varepsilon C(\Omega \cup \partial\Omega)$

v) Eqs. (3-49) and Eq. (3-59) are satisfied by C_j and E for every $(x,t) \varepsilon \Omega$

vi) Eqs. (3-43) - (3-45) are satisfied by $C_j(x,o)$ $j = 1,2,\ldots,n$, $E(x,o)$ for every $x, \varepsilon (0,\delta)$ Lieberstein [20].

where $C^2(\Omega)$ is the space of the continuous function square integrable for every $(x,t) \varepsilon \Omega$ and $C(\Omega \cup \partial\Omega)$ is the space of the continuous functions in every $(x,t) \varepsilon \Omega \cup \partial\Omega$ ($\partial\Omega = \bar{\Omega} - \Omega$) and $C^2(\Omega)$ and $C(\Omega \cup \partial\Omega)$ are the space of continuous functions once integrable for (x,t) in Ω and its boundaries, respectively.

Remark:

Notice that in the interest of finding a solution, the conditions on $C_j(x,t)$ $j = 1,2,\ldots,N$ and $E(x,t)$, have been weakened, i.e., instead of asking that $C_j(x,t)$ be twice differentiable in x and once differentiable in t ($j = 1,2,\ldots,N$), and $E(x,t)$ be once differentiable in x, t

it is asked that $C_j(x,t)$ $j = 1,2,\ldots,N$ be square integrable in Ω and continuous on its boundary, and $E(x,t)$ be once integrable. It happens that those conditions are enough for proving existence of a solution, Lieberstein [20], i.e., the physical restrictions on the variables of the model are stronger than the mathematical restrictions.

To prove that the solution of (3-49) satisfies conditions i - vi the following procedure will be followed: Assuming iii, iv are satisfied v, vi will be checked; then their validity will be used to show that conditions i, ii are satisfied, then it will be proved that if those condition are fulfilled, iii and iv are satisfied closing the implication chain.

Notice that (3-50) can be rewritten in the following form:

$$L_j(E,C_j) = \frac{\partial}{\partial x}\left(RT\frac{\partial C_j}{\partial x} - z_j F E C_j\right) \qquad (3\text{-}64)$$

$$j = 1,2,\ldots,N$$

And from (3-18), the terms in brackets are equal to $-\frac{\phi_j}{C_j}$, then

$$RT\frac{\partial C_j}{\partial x} - z_j F E C_j = -\frac{\phi_j}{u_j} \qquad (3\text{-}65)$$

$$j = 1,2,\ldots,N$$

The flow, ϕ_j of each ion species is bounded because otherwise a consumption of infinite energy by the system, would be implied; this is not physically feasible. u_j is different from zero for almost all $t\in\Omega$ as long as the concentration of ion species j at the outer solution is different from the concentration at the inner solution; therefore the term in the right hand side of (3-65) is bounded (almost everywhere).

The solutions of equations (3-65) can be written explicitly:

$$C_j(x,t) = \exp\left(\frac{z_j F}{RT}\int_0^x E(\xi,t)d\xi\right) C_j(0,t)$$

$$- \frac{1}{u_j}\int_0^x \exp\left(\frac{z_j F}{RT}\int_0^\sigma E(\xi,t)d\xi\right) \phi_j(\sigma,t)d\sigma \qquad (3\text{-}66)$$

$$j = 1,2,\ldots,N$$

Since by assumption $\int_0^x |E(\xi,t)|d\xi < \infty \quad x,t\in\Omega$

the term

$$\exp\left(\frac{z_j F}{RT}\int_0^x E(\xi,t)d\xi\right)$$

is bounded, for every j, $C_j(0,t)$ $j = 1,2,\ldots,N$ are bounded by definition.

The second term in (3-66), can be considered as a Linear operator $T: C(\Omega) \to C(\Omega)$ i.e.,

$$I_j(x,t) \equiv T_j(\phi_j)$$

$$\text{where } T_j(\phi_j) = -\frac{1}{u_j}\int_0^x \text{Exp}\left(\frac{z_j F}{RT}\int_0^\sigma E(\xi,t)d\xi\right)\phi_j(\sigma,t)d\sigma \qquad (3\text{-}67)$$

$$j = 1,2,\ldots,N$$

since the inner integral in (3-67) is bounded, and since the following inequality holds (Douglas [39]),

$$||I_j||_C \leq ||T_j||_{C^*}\ ||\phi_j||_C \qquad (3\text{-}68)$$

$$j = 1,2,\ldots,N$$

where $||\ \ ||_C$ is the usual norm in $C(\Omega)$ the space of piecewise continuous functions on Ω and $||\ \ ||_{C^*}$ is the norm in its dual space. Therefore, since ϕ_j and T_j, $j = 1,2,\ldots,N$ are bounded, by (3-68) I_j, $j = 1,2,\ldots,n$ will be bounded and by (3-66), $C_j(x,t)$ $j = 1,2,\ldots,N$ is bounded, therefore

$$\int_o^x C_j(x,t)dx < \infty \qquad x\varepsilon\Omega \qquad (3\text{-}69)$$

$$j = 1,2,\ldots,N$$

In (3-66), the first term is bounded for all $t \geq 0$ and so is the second term by the previous argument; moreover by conditions (3-9), the second term goes to zero as t goes to infinity, so

$$\lim_{t\to\infty} \int_o^t C_j(x,t)dt < \infty \qquad x\varepsilon(0,\delta) \qquad (3\text{-}70)$$

$$j = 1,2,\ldots,N$$

Combining (3-69) and (3-70), the following conditions for $C_j(x,t)$ $j = 1,2,\ldots,N$ are obtained

$$\int_\Omega |C_j(x,t)|d\Omega < \infty \qquad (3\text{-}71)$$

$$j = 1,2,\ldots,N$$

and since $C_j \geq 0 \qquad x,t\varepsilon\Omega \qquad (3\text{-}71)$

can be strengthened to

$$\int_{\Omega} |C_j(x,t)|^2 d\Omega < \infty \qquad (3\text{-}72)$$

$$j = 1,2,\ldots,N$$

Therefore, conditions i), ii) are satisfied and the solutions (3-66), satisfy condition v) and vi) for C_j.

The former condition implies that equation (3-59) for E(x,t) holds and therefore conditions iii), iv), v) and vi) hold for E(x,t).

Therefore, the existence of a solution to the equation of the model is guaranteed.

b) The uniqueness of the solution can be established by the observation of (3-64): the expression in brackets which equals $-\frac{\phi_j}{u_j}$ is a linear differential operation and therefore, if two solutions exist, the sum of them will be also a solution and the resultant flow $(\phi^I{}_j - \phi^{II}{}_j)$ operated on by the linear operator $-u_j \frac{\partial}{\partial x}(\ )$ will equal the sum of the derivatives of the concentrations $C^I{}_j$ and $C^{II}{}_j$ corresponding to the two solutions, and therefore eqs. (3-49) are satisfied. Additionally, the two solutions must satisfy the boundary conditions (eqs. 3-3) then, from (3-3) in (3-66), for $C^I{}_j(\delta,t)$ and $C^{II}{}_j(\delta,t)$ the following expressions are obtained

$$C_{1j} = \exp\left(\frac{z_j F}{RT} \int_o^{\delta} E^I(\xi,t)d\xi\right) C_{oj}$$

$$- \frac{1}{u^I{}_j} \int_o^{\delta} \exp\left(\frac{z_j F}{RT} \int_o^{\sigma} E^I(\xi,t)d\xi\right) \phi^I{}_j(\sigma,t)d\sigma$$

$$C_{1j} = \exp\left(\frac{z_j F}{RT}\int_o^\delta E^{II}(\xi,t)d\xi\right)C_{oj}$$

$$- \frac{1}{u^{II}_j}\int_o^\delta \exp\left(\frac{z_j F}{RT}\int_o^\sigma E^{II}(\xi,t)d\xi\right)\phi^{II}_j(\sigma,t)d\sigma \qquad (3\text{-}73)$$

$$j = 1,2,\ldots,N$$

The two solutions and their differences must satisfy eq. (3-21), i.e. i.e.,

$$\frac{\partial E^I}{\partial x} = \frac{F}{\varepsilon}\sum_{j=1}^{N} z_j C^I_j$$

$$\frac{\partial E^{II}}{\partial x} = \frac{F}{\varepsilon}\sum_{j=1}^{N} z_j C^{II}_j \qquad (3\text{-}74)$$

and

$$\frac{\partial (E^I - E^{II})}{\partial x} = \frac{F}{\varepsilon}\sum_{j=1}^{N} z_j (C^I_j - C^{II}_j)$$

In particular, for x=0 and x=δ: $C^I_j = C^{II}_j$; therefore

$\frac{\partial (E^I - E^{II})}{\partial x}(0,t) = \frac{\partial (E^I - E^{II})}{\partial x}(\delta,t) = 0$ for every $t \geq 0$ then

$E^I(0,t) = E^{II}(0,t)$, $E^I(\delta,t) = E^{II}(\delta,t)$ and by the continuity of the electric field this property can be extended to all $x\varepsilon(0,\delta)$. This implies in turn that $u^I_j = u^{II}_j$ (since u^I, u^{II} are functions only of V^I and V^{II} respectively which in turn are functions only of E^I and E^{II} respectively. See Eqs. (3-38) and (3-60)). Then in (3-73) substracting the two equations and combining the integral term, it can be concluded

that $\phi^I = \phi^{II}$ or the solution to the equations of the model is unique.

Remark:

The procedure followed to prove uniqueness gives only a sufficient condition for the solution of the model to be unique. The necessity can be proven using the techniques of "test" functions applied to the linearized equations of the model and showing that the solution of this system satisfies the condition

$$||\underline{\psi}|| \leq k||\underline{\theta}||$$

where $\underline{\psi} = (C_1, C_2, \ldots, C_N, E)^1$, $\underline{\theta}$ is the vector test function and k is a positive constant. The norm here is the standard L_p norm. Lieberstein [20].

c) For the ionic model, the stability question can be answered by showing that the solution to the system of equations (3-43) - (3-45) is an equilibrium point and that this equilibrium point is stable. i.e.,

$$\left.\begin{array}{l} L_j(E,C_j) \\ j = 1,2,\ldots,N \end{array}\right| \begin{array}{l} = 0 \\ E = E^1 \\ C_j = C_j^{\,1} \quad j = 1,\ldots,N \end{array}$$

with

$$\frac{\partial E}{\partial x} = \frac{F}{\varepsilon} \sum_{j=1}^{N} z_j C_j \;\Bigg|\; \begin{array}{l} E=E^1 \\ C_j=C_j^{\,1} \quad j = 1,\ldots,N \end{array} \qquad (3\text{-}75)$$

and

$$\sum_{j=1}^{N} J_j \;\Big|\; \begin{array}{l} = 0 \\ J_j=J_j^{\,1} \end{array}$$

and a "small" perturbation of the control variable J; δJ drives the system to a neighboring solution C''_j $j = 1,2,\ldots,n$, E'' and u_j'' $j = 1,2,\ldots,N$ such that

$$\begin{aligned} C''_j &\to C_j^1 \text{ as } t \to \infty \\ E'' &\to E^1 \\ u^1_j &\to u_j'' \quad j = 1,2,\ldots,N \end{aligned} \tag{3-76}$$

Provided that the perturbation in J is small enough as to prevent the system to develop an action potential. As a first step, the solution of (3-75) is characterized:

From (3-64) in (3-75):

$$u^1_j L_j(E^1, C^1_j) = \frac{\partial}{\partial x}\left[u^1_j\left(RT\frac{\partial C^1_j}{\partial x} - z_j F E^1 C^1_j\right)\right] = 0 \qquad j = 1,2,\ldots,N \tag{3-76}$$

Therefore the term in brackets in (3-76) is constant in x; and from (3-18), this term is equal to $-\phi^1_j$ $j = 1,2,\ldots,N$ i.e., $(\phi^1_j(x) \equiv \phi^1_j$ constant)

$$u^1_j\left(RT\frac{\partial C^1_j}{\partial x} - z_j F E^1 C^1_j\right) = -\phi^1_j \qquad j = 1,2,\ldots,N \tag{3-77}$$

Multiplying both sides of (3-77) by Fz_j; and adding all the equations of the form of (3-77) the following expression results

$$FRT \sum_{j=1}^{N} u^1{}_j z_j \frac{\partial C^1{}_j}{\partial x} - F^2\left(\sum_{j=1}^{N} u^1{}_j z^2{}_j C^1{}_j\right) E^1 = F \sum_{j=1}^{N} z_j \phi^1{}_j \qquad (3\text{-}78)$$

By (3-17) in the right hand side of (3-78) and from the last equation in (3-75), (3-78) becomes

$$FRT \sum_{j=1}^{N} u^1{}_j z_j \frac{\partial C^1{}_j}{\partial x} - F^2(\Sigma u^1{}_j z^2{}_j C^1{}_j)E^1 = 0 \qquad (3\text{-}79)$$

then, from (3-79);

$$E^1 = (RT \sum_{j=1}^{N} u^1{}_j z_j \frac{\partial C^1{}_j}{\partial x})/F \sum_{j=1}^{n} u^1{}_j z^2{}_j C^1{}_j \qquad (3\text{-}80)$$

Notice that as in the dynamic case, the knowledge of the concentration distribution of each ion species, in resting conditions, fully specifies the electric field intensity E^1.

The equilibrium point is specified by $C^1{}_j(x)$, $E^1(x)$ for $0 \leq x \leq \delta$, and $u^1{}_j = u^1{}_j(-\int_o^\delta E^1 dx)$ = constant (see (3-38)) $j = 1,2,\ldots,N$.

Remark:

The conditions given by eqs. (3-9) imply that after an action potential occurs, the system returns asymptotically to the resting state, therefore, if this resting state is stable in the small, the ionic transport model for the axon membrane, is globally stable.

In order to check stability in the small, the output control variable J is disturbed from its resting value ($J^1 \equiv 0$) by an amount δJ, this

disturbance causes disturbances in C^1_j $j = 1,2,\ldots,N$ and in E^1 of magnitude δC_j $j = 1,2,\ldots,n$ and δE respectively. The dynamic equations of these increments are given below:

Let

$$C^{11}_j = C^1_j + \delta C_j \quad j = 1,2,\ldots,N$$

$$E^{11} = E^1 + \delta E \tag{3-81}$$

$$u^{11}_j = u^1_j + \delta u_j \quad j = 1,2,\ldots,N$$

The disturbances are produced by a small current pulse, δJ. Assuming that the disturbance δJ is small and due to the continuity of C_j $j = 1,2,\ldots,N$ and E and their derivatives, products of increments are neglected, then the approximate dynamic equations for the perturbations are:

$$\frac{\partial \delta C_j}{\partial t} = u^{11}_j\left(RT\frac{\partial^2 \delta C_j}{\partial x^2} - z_j F E^1 \frac{\partial \delta C_j}{\partial x} - z_j F \frac{\partial E^1}{\partial x}\delta C_j\right)$$

$$- u^{11}_j\left(z_j F \frac{\partial C^1_j}{\partial x}\delta E + z_j F C^1_j \frac{\partial \delta E}{\partial x}\right) \tag{3-82}$$

$$j = 1,2,\ldots,N$$

$$\frac{\partial \delta E}{\partial x} = \frac{F}{\varepsilon}\sum_{j=1}^{N} z_j \delta C_j \tag{3-83}$$

$$\frac{\partial \delta E}{\partial t} = \frac{1}{\varepsilon}\left(\sum_{j=1}^{N} \delta J_j - \delta J\right) \tag{3-84}$$

Equations (3-82) represent a second order linear system that can be written in functional form as follows

$$\frac{\partial \delta C_j}{\partial t} = Q_j(\delta C_j(x,t)) + f_j(x,t) \qquad j = 1,2,\ldots,N \tag{3-85}$$

and the boundary conditions:

$$\delta C_j(0,t) = \delta C_j(\delta,t) = 0 \qquad j = 1,2,\ldots,N \tag{3-86}$$

where

$$Q_j(\delta C_j(x,t)) = u^{11}{}_j\left(RT\frac{\partial^2 \delta C_j}{\partial x^2} - z_j F E^1(x)\frac{\partial \delta C_j}{\partial x} - z_j F\frac{\partial E^1(x)}{\partial x}\delta C_j\right) \tag{3-87}$$

$$f_j(x,t) = -u^{11}{}_j(t)\left(z_j F\frac{\partial C^1{}_j(x)}{\partial x}\delta E(x,t) + z_j F C^1{}_j(x)\frac{\partial E}{\partial x}(x,t)\right)$$

$$j = 1,2,\ldots,N$$

Since in (3-87) Q_j and t_j are bounded functions, it implies that (3-85) is a stable (in the small) system.

3.7 Conclusions

A model for the ionic transport in the clamped axon membrane was derived. The model was developed using well known laws of field theory and irreversible thermodynamics. Some assumptions about the structure of the membrane were made and analysis towards their physical justification was carried out.

CHAPTER IV

SIMULATION OF THE IONIC TRANSPORT MODEL

4.1 Introduction

In Chapter III, a model for the ionic transport model was derived. The model is based on a theoretical analysis of the physical processes involved in the transport of ions across a clamped axon membrane and the effect of this transport on the electric potential of the membrane. The equations of the model were obtained by applying well known laws of field theory and irreversible thermodynamics. Several assumptions about the structure of the membrane and its role in the regulation and control of the flow were made in the derivation. In this chapter a simulation of the model is carried out in order to test the validity of the equations and the underlying assumptions against the experimental evidence available in the literature, Hodgkin and Huxley [6], Hodgkin and Katz [5]. Additionally, some interesting aspects of the physical behavior of the axon membrane that have not received too much attention such as oscillatory behavior and calcium excitatory regulation are analyzed in a numerical context.

The simulation was carried out using a computer program in which equations (3-20) - (3-22) are integrated by discritizing them according to a Crank-Nicholson implicit scheme that is derived on appendix A1. The initial conditions are found by integrating the steady state equations (3-43)-(3-45) using the same scheme. The program was written in Fortran IV; the matrix operations in the discretized model were carried out using the IBM Scientific Library package (SL-Math).

The chapter is divided into 7 sections as follows: 4.2 Computation of ionic mobilities and analysis of their behavior, 4.3 Membrane potential time-course, 4.4 Electric field intensity in the membrane, 4.5 Current density and concentration distributions, 4.6 Oscillatory behavior of the hyperpolarized membrane, and 4.7 Conclusions.

4.2 Ionic Mobilities

As discussed extensively in Chapter III, the time varying coefficients that relate the force driving each ion species with its ensemble average velocity of transport during excitation (Eqs. (3-8) are the mobility functions. The mobility functions constitute the mechanism of regulation by which the membrane controls the flow of ions; notice that this mechanism is active only when the axon membrane is excited, therefore in this section it will be assumed that the membrane is excited and time t=0 is taken as the instant of over-threshold excitation.

Before discussing the numerical results obtained for the different ion species mobilities the procedure followed for the calculation of their equivalent masses will be analyzed, and some of the geometric properties of the sample axon membrane considered in the simulation will be stated.

Strictly speaking, the ionic transport process is a discrete phenomena since it involves the flow of particles of definite volume and mass; but in applying field theory for modeling the process it has been implicitly assumed that mass elements for each ion species crossing the membrane are differentiable; this assumption is validated by physical analysis, if the clamped region has a surface exposure to the surrounding solutions much

bigger than the surface exposure of any activation site. This condition is fulfilled in the case of squid axon membranes in particular and for cell membranes in general.

The former argument suggests that in order to justify the application of ordinary laws of mechanics to the particles (ions) involved in the transport process, (see Eqs. (3-29) and (3-31)) an effective mass for each of these particles in the membrane has to be considered, so that it accounts for the forces to which they are subjected and for the fact that the modeling has been carried out considering average particles rather than individual ions.

In this thesis the former considerations led to definition of the concept of equivalent mass, which has been extensively studied in other transport systems such as p-n junction, Vander Ziel [25]. In essence the equivalent mass concept is based on assigning a probability distribution to the event of finding a particular ion species at the respective surface-located activation-site. Since in the membrane, the ions are subject to a varying potential, a Boltzman-type of distribution is assigned to them and the equivalent mass of each ion species j is given by the ensemble average mass corresponding to this ion.

Then the equivalent mass of ion species j in the axon membrane is given by

$$m_j^* = m_j e^{-q_j \bar{v}/KT} \qquad j = 1,\ldots,N \qquad (4\text{-}1)$$

where $\bar{v}$ is the ensemble average membrane potential of the excited membrane potential and K is the Boltzman constant.

With expressions (4-1) the equivalent masses of the ion species involved in the ionic transport process can be computed. This and other numerical information about the membrane parameters utilized in the simulation, are summarized in table 4-1 below; this data was obtained from Hodgkin and Katz [3].

Table 4-1

Membrane thickness $\delta = 150$ Å

Temperature $T = 290$ K

Sodium parameters (j=1)

External (Bulk solution) concentration $C_{\delta 1} = 288 mM/cm^3$

Internal concentration $C_{o1} = 72 mM/cm^3$

Equivalent mass $m_1^* = 17.8 g/mole$

Potassium parameters (j=2)

External concentration $C_{\delta 2} = 540 mM/cm^3$

Internal concentration $C_{o2} = 203 mM/cm^3$

Equivalent mass $m_2^* = 35.1 g/mole$

Chloride parameters (j=3)

External concentration $C_{\delta 3} = 104 mM/cm^3$

Internal concentration $C_{o3} = 61 mM/cm^3$

Equivalent mass $m_3^* = 39.5 g/mole$

Calcium parameters (j=4)

External concentration $C_{\delta 4} = 30 mM/cm^3$

Internal concentration $C_{o4} = 45 mM/cm^3$

Equivalent mass $m_4^* = 20.4 g/mole$

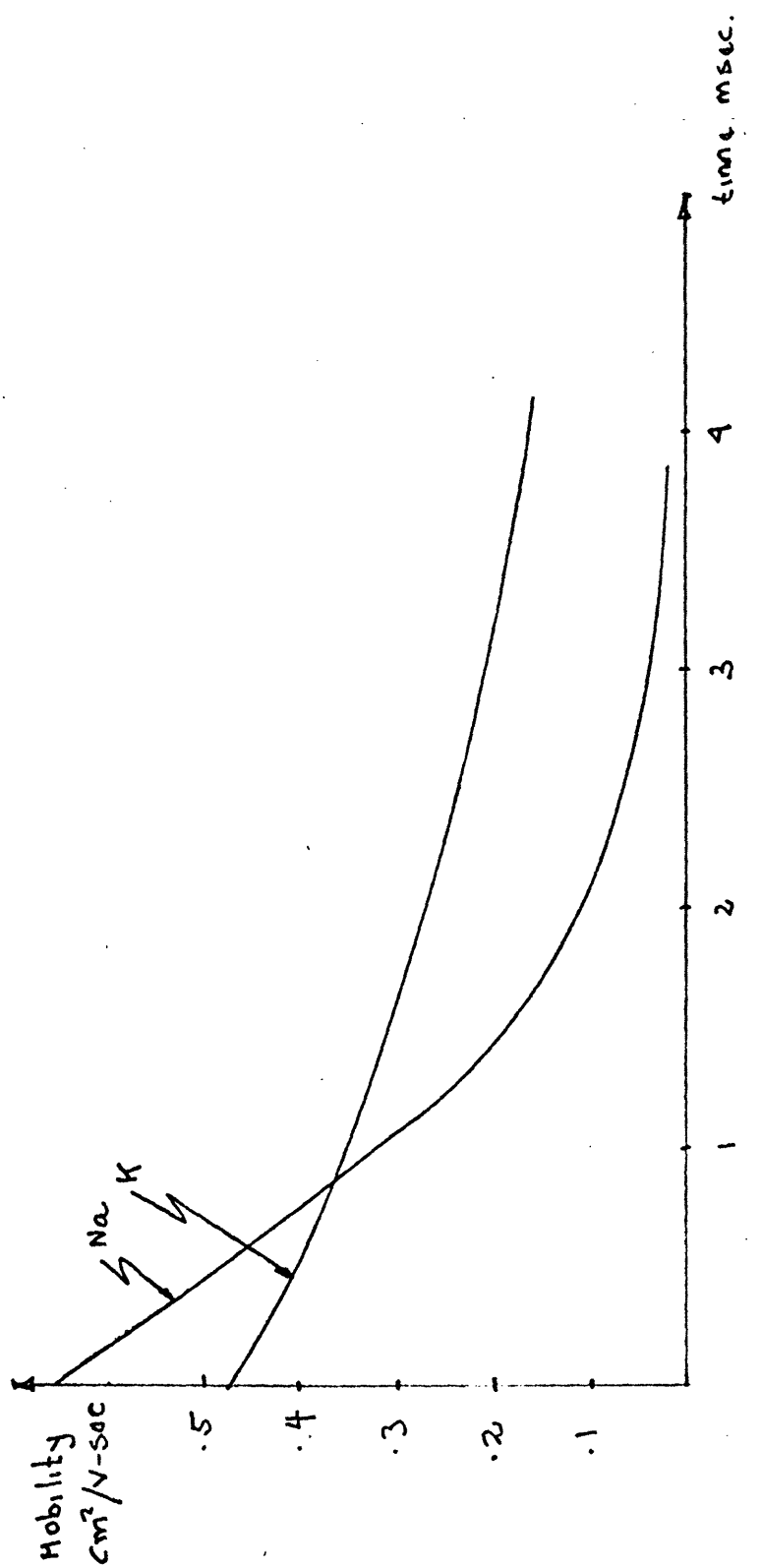
With the parameters listed in table 4-1, and using the program described in the appendix, the mobility functions of sodium, potassium, chloride and calcium ions were computed. They are shown in graphs 4-1 and 4-2 below.

In this author's opinion, the most important results towards the elucidation of the physiological characteristics of the ionic transport process are established in the so called sodium-theory of the action potential proposed by Hodgkin and Katz in 1949 (see Chapter II). It is interesting to note that the characteristics of the mobility functions shown in Figs. 4-1, 4-2 confirm the basic conclusions obtained by Hodgkin and Katz from an experimental analysis:

a) When the axon membrane is excited, it becomes initially highly permeable to the influx of sodium ions; this is represented by the high value of the initial sodium mobility with respect to the mobilities of the other ions, Fig. 4-1.

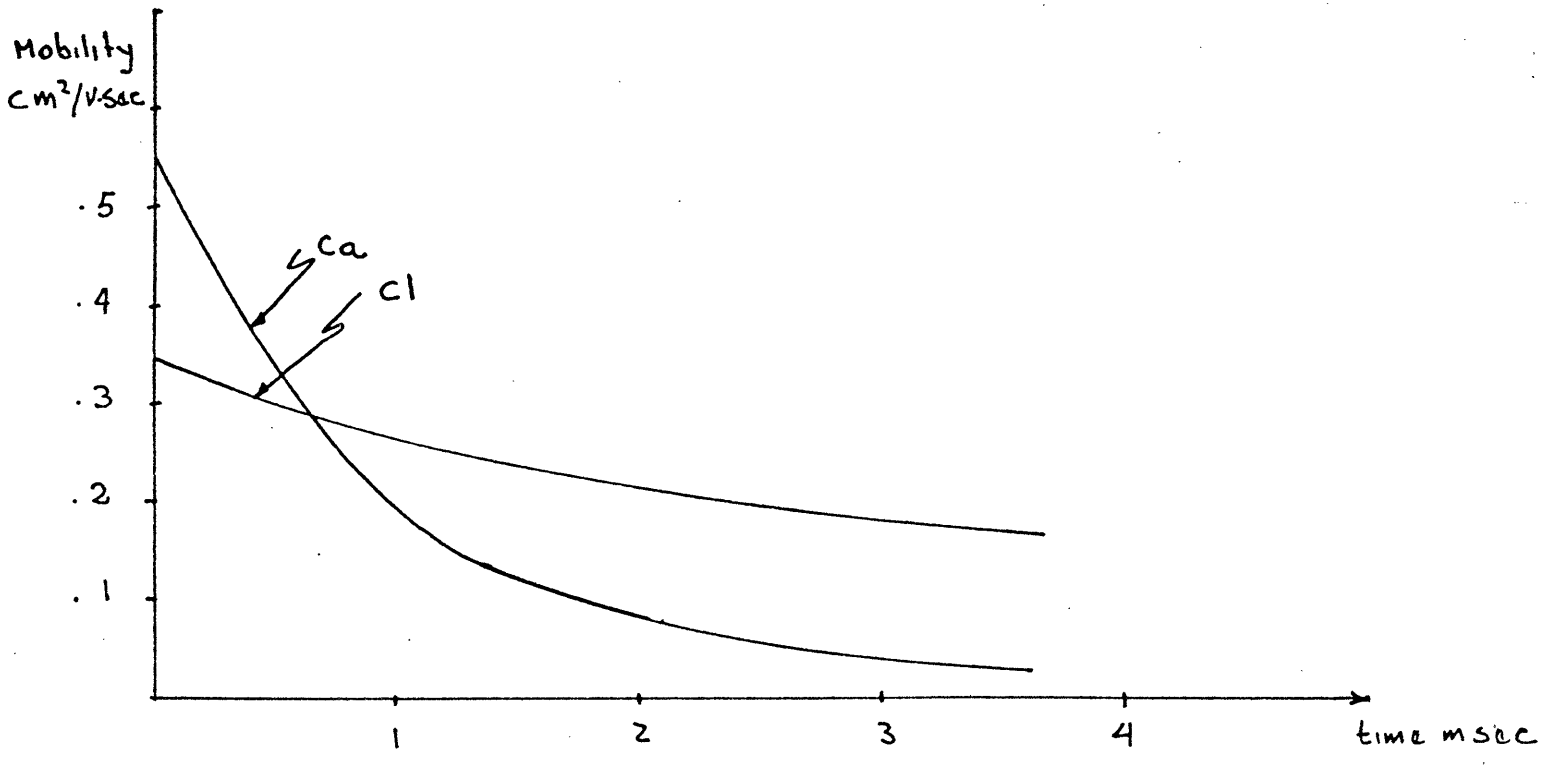
b) Roughly 1msec. after the spike starts, the sodium mobility (i.e., the membrane permeability to sodium ions) becomes smaller than that of the potassium. This condition agrees with the physiological roles assigned to sodium and potassium in the ionic transport process; namely, the rising phase of the spike is produced mainly by influx of sodium ions and the falling phase is produced mainly by efflux of potassium ions.

c) Notice that calcium ions exhibit a mobility function that initially is only slightly less than that of sodium ions but after 1msec it decays at much faster rate than sodium or potassium mobilities. This behavior is explained by the valence of calcium ions, which is twice as big as that of sodium (or potassium) ions with roughly the same equivalent charge.



Sodium and Potassium Mobilities

Fig 4-1



Calcium and chloride Mobilities

Fig 4-2

This observation shows that the role of calcium ions has to do more with the excitatory event than with the ionic transport.

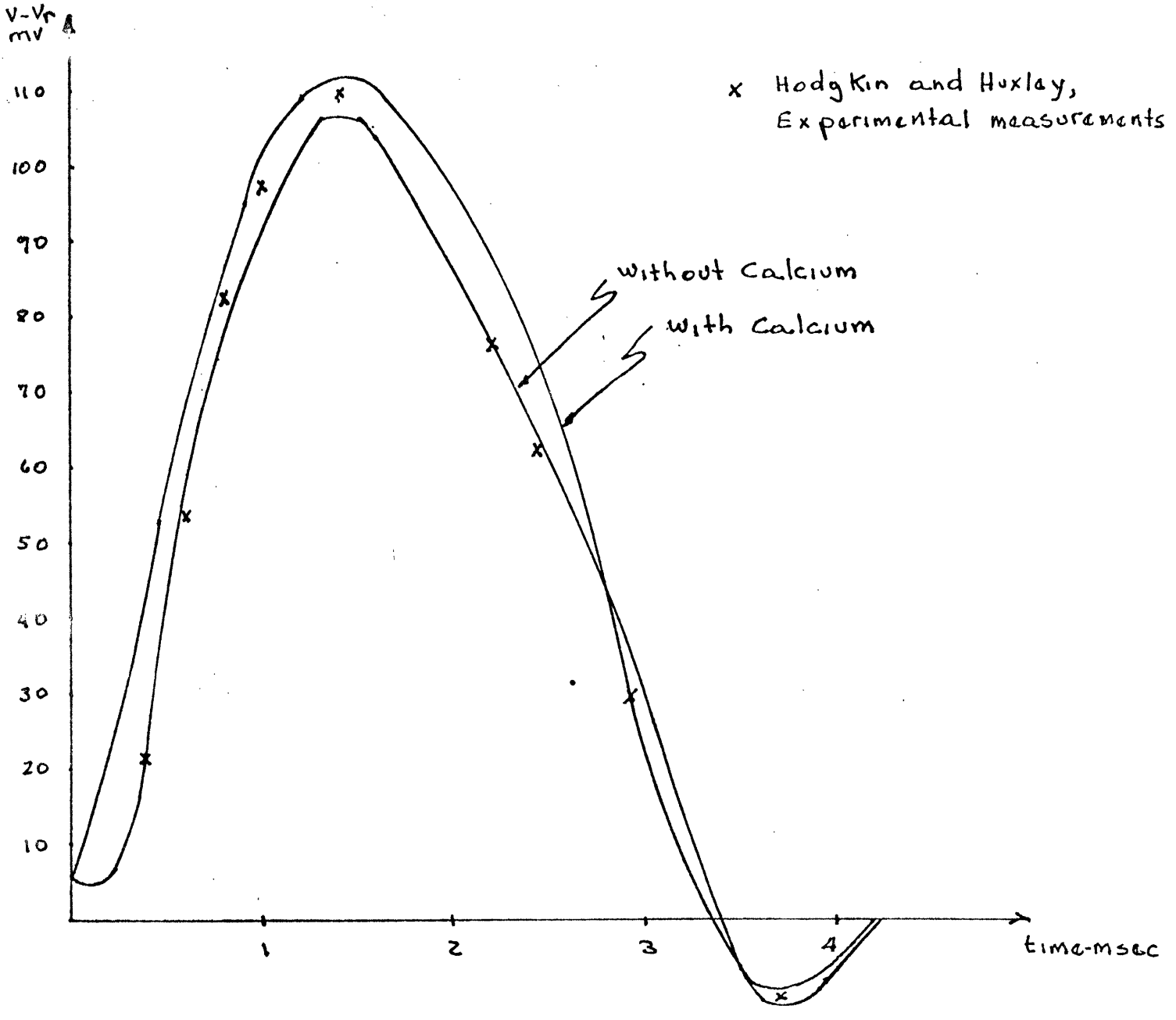
d) Finally, notice that the chloride mobility has a very slow variation during the spike-time which agrees with the conclusion of its secondary role in the ionic transport.

4.3 Membrane Potential Time-Course

In Fig. 4-3, the time course of the excited clamped axon membrane potential referred to its resting potential is shown. Also, the experimental points found by Hodgkin and Huxley (corrected to the temperature of the simulation) for the sample axon considered in this simulation are included. Additionally, in an effort to elucidate the role of calcium, in the excited membrane, two runs were carried out: one without including in the model the flow of calcium ions, and the other including its flow.

The most interesting result with respect to the simulation of the membrane potential, is its agreement with the experimental measurements, as can be seen in Fig. 4-3. The maximum deviation of the computed voltage with respect to the corresponding measurement, is of the order of 8%. Since the ionic transport model was derived on purely theoretical bases, this result backs up partially the assumptions made in its derivation and simultaneously gives an independent proof of the validity of the sodium theory.

Additionally, some conclusions can be drawn about the role of calcium in the membrane potential time course. As can be inferred from Fig. 4-3, the calcium flow increases the potential during the rising phase of the spike; this increase is of the order 10% at most.



Time Course of the Exited Clamped Axon Membrane

Fig 4-3

This observation might be interpreted as follows: the calcium ion flow, because of its relatively low equivalent mass and relatively high average charge, plays the role of starting the action potential when a pulse over threshold has excited the axon membrane and subsequently, assisting the sodium ions in building-up the depolarizing potential during the rising phase. Moreover, the threshold itself is probably a function of the calcium concentration.

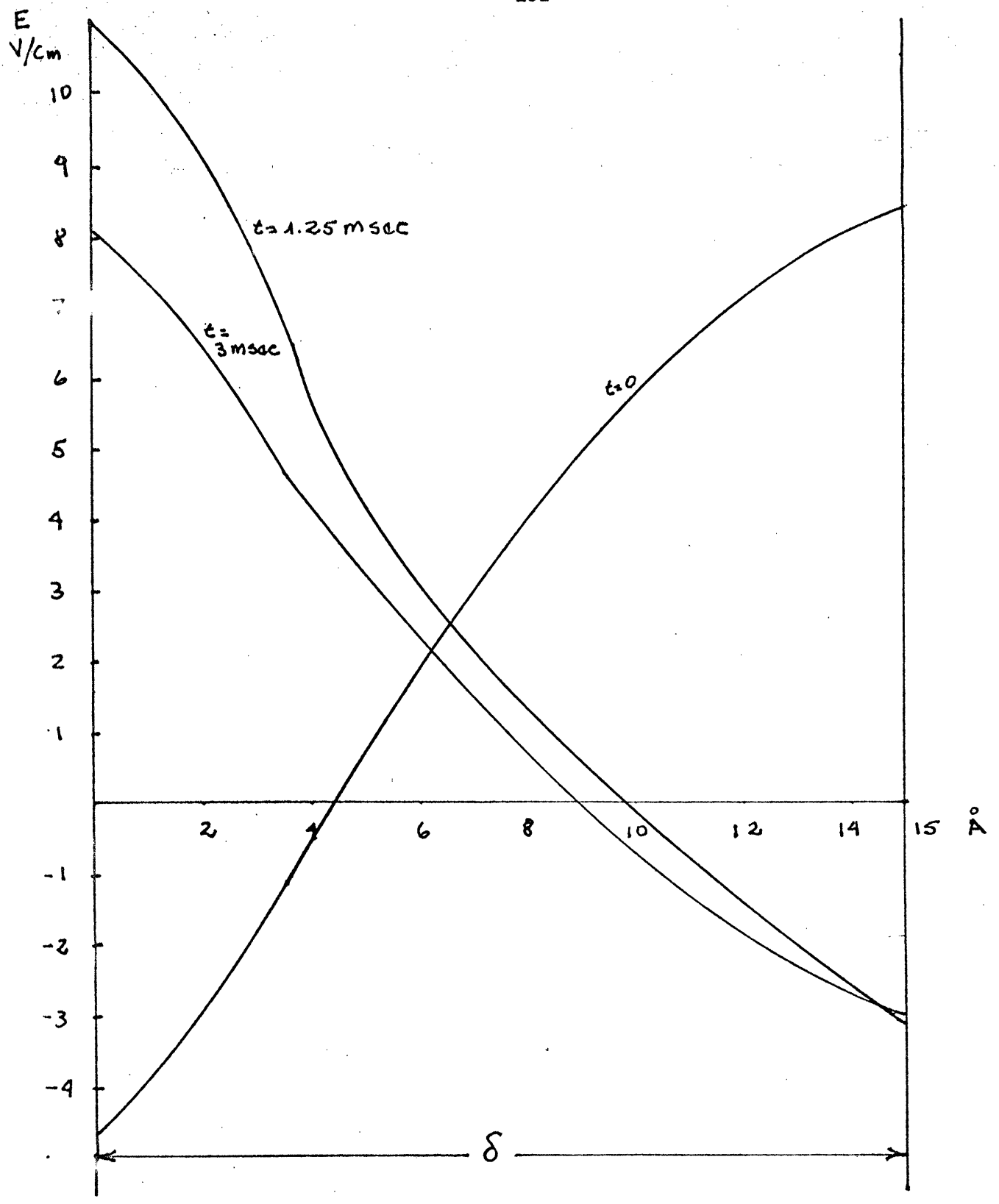
In the falling phase the flow of calcium ions induces a decrease in the rate of repolarization carried out mainly through the efflux of potassium ions.

4.4 Electric Field Intensity in the Membrane

In this author's opinion, the characteristics of the electric field intensity in the membrane is one of the most important aspects of the ionic transport model developed in Chapter III.

Most of the models developed so far consider (especially in the steady state analysis) the electric field intensity to be constant as a function of the spatial variable; this assumption is completely arbitrary. In the model developed in Chapter III this assumption was not made. The electric field intensity dynamics were found to be a function of the ion concentrantion distributions (as should be expected). In the simulation these dynamic equations were integrated simultaneously with the distribution equations, as shown in appendix A.

A sample of the spatial distribution of the electric field in the axon membrane is shown in Fig. 4-4. As discussed before, the electric field intensity is far from being constant. During the spike (t=1.25,



Electric Field Intensity distribution in the axon Membrane

Fig 4-4

t=3 msec) the electric field distribution becomes positive in the inside region and negative in the outside region. For t=1.25 msec which corresponds to the time when the membrane potential is a maximum, the sodium inflow is high as compared with the potassium efflux; this fact justifies the form of the distribution of the electric field at this time i.e., highly positive towards the outside region. At time t=3 msec the sodium flow is much smaller than the potassium flow and consequently, the electric field distribution becomes less positive towards the outside region as can be seen in Fig. 4.4.

Finally, notice that the steady state electric field distribution (t=0) is not constant. This fact suggests that at equilibrium conditions, ions are trapped inside the membrane due to the existence of at least one point of zero net driving force.

4.5 Current Density and Concentration Distributions

In the model developed in Chapter III, the excitation mechanism by which the axon membrane develops a voltage spike was assumed to be a current mechanism. The considerations behind this assumption were discussed in Chapter III.

In the simulation, it was found that an action potential occurs in the axon membrane when it is excited with a constant current density pulse of $445 \mu A/cm^2$ which is of the same order of magnitude as the one applied by Hodgkin and Huxley to the sample axon considered in this simulation (Axon # 17 in [6]). The duration of the pulse necessary for activation of the axon was found to be .15 msec. In the next section

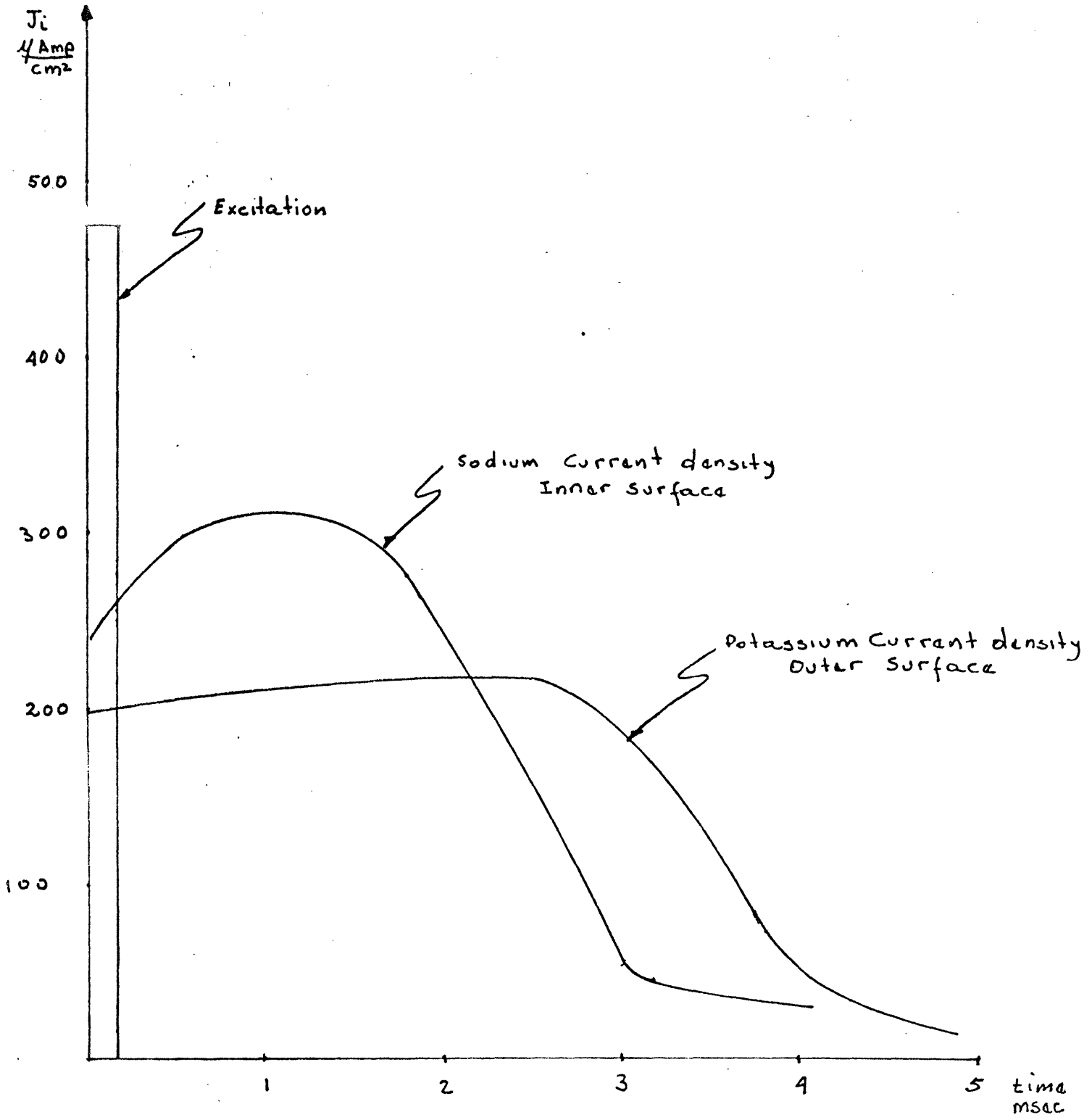
the behavior of the axon membrane when the duration of the pulse is increased to 1. msec, (a condition of hyperpolarization) will be described.

In Fig. 4-5 the sodium current density at the inner membrane surface is shown. The time duration of the simulation is 4 msec. Also in Fig. 4-5, the potassium current density at the outer membrane surface vs. time is plotted. The objective of considering sodium and potassium current densities at the boundaries is to compare the resultant graphs with those given by Hodgkin and Keynes [21]. Although no quantitative comparison can be carried out because these researchers utilized DNP as an inhibitor for potassium flow, qualitatively, the shape of the sodium and potassium current density are found to be similar; moreover the order of magnitude of the sodium current density found by them is similar to the maximum value (250 $\mu A/cm^2$) computed in the simulation of Hodgkin and Keynes vs. 310 $\mu A/cm^2$ in the present simulation. Notice the sharp decrease in sodium current in the falling phase, a fact that agrees with the sodium theory.

In Fig. 4-6 the concentration distributions of sodium and potassium ions in the membrane are shown. As discussed before, even at equilibrium conditions, a distribution of sodium and potassium ions is present in the membrane space. This fact should be taken into account in any study of subthreshold phenomena. Recall from the analysis in Chapter III that the ionic distributions determine the electric behavior of the membrane during excitation.

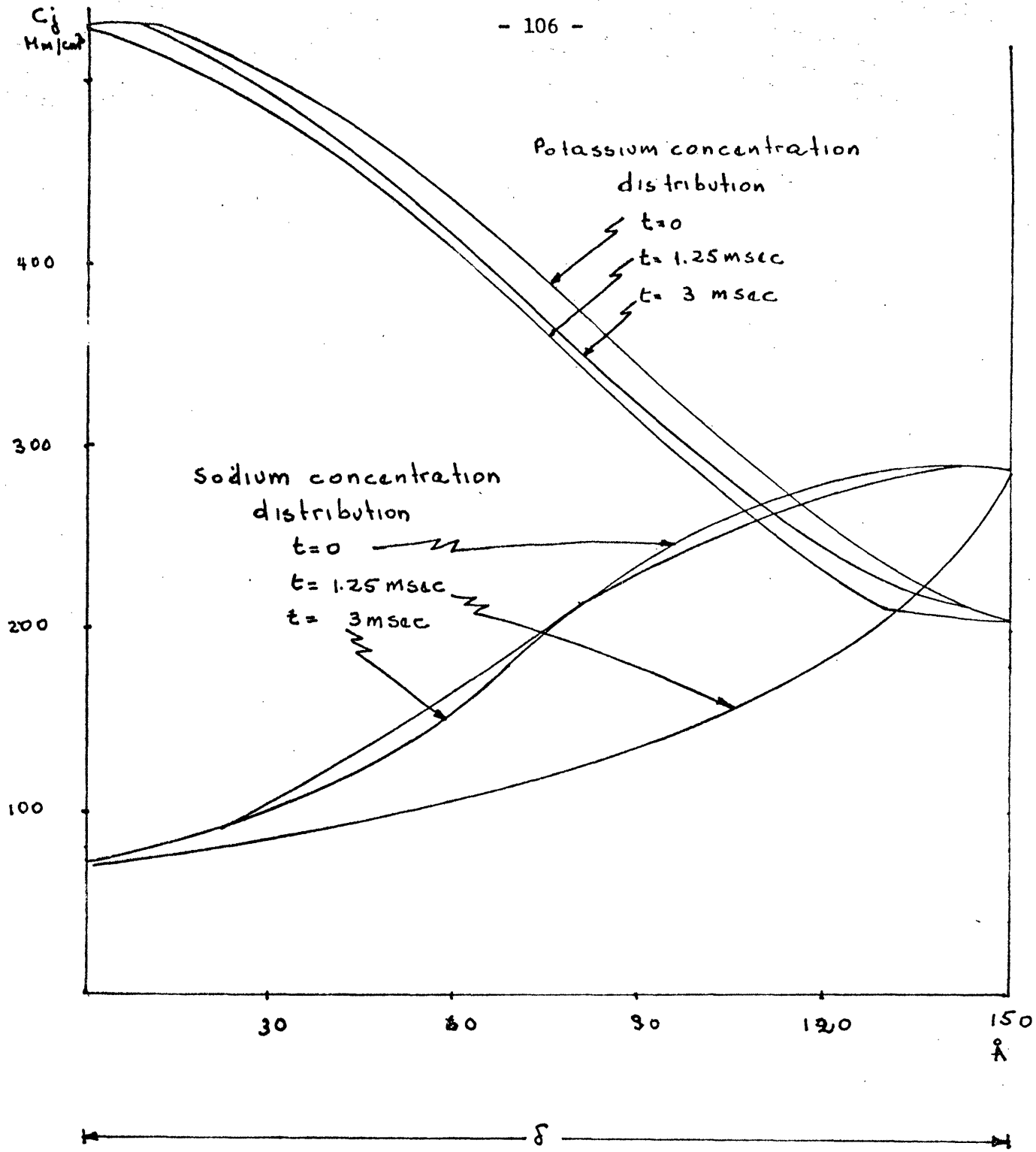
In Fig. 4-6 the following observations about the dynamic course of sodium and potassium concentration distributions can be made:

a) The variation of concentration distribution of sodium ions during the excitation period is greater than the corresponding variation of potassium



Sodium and Potassium Current densities

Fig 4-5



Sodium and Potassium concantration distributions in The membrane

Fig 4-6

concentration this condition is caused by the higher mobility of sodium ions. See Section 4-2.

b) Notice that the time constant of potassium ion flow is about 1.4 times larger than that of the sodium, where by time constant is meant the time after excitation required to recuperate 80% of the equilibrium distribution.

4.6 Oscillatory Behavior of the Hyperpolarized Membrane

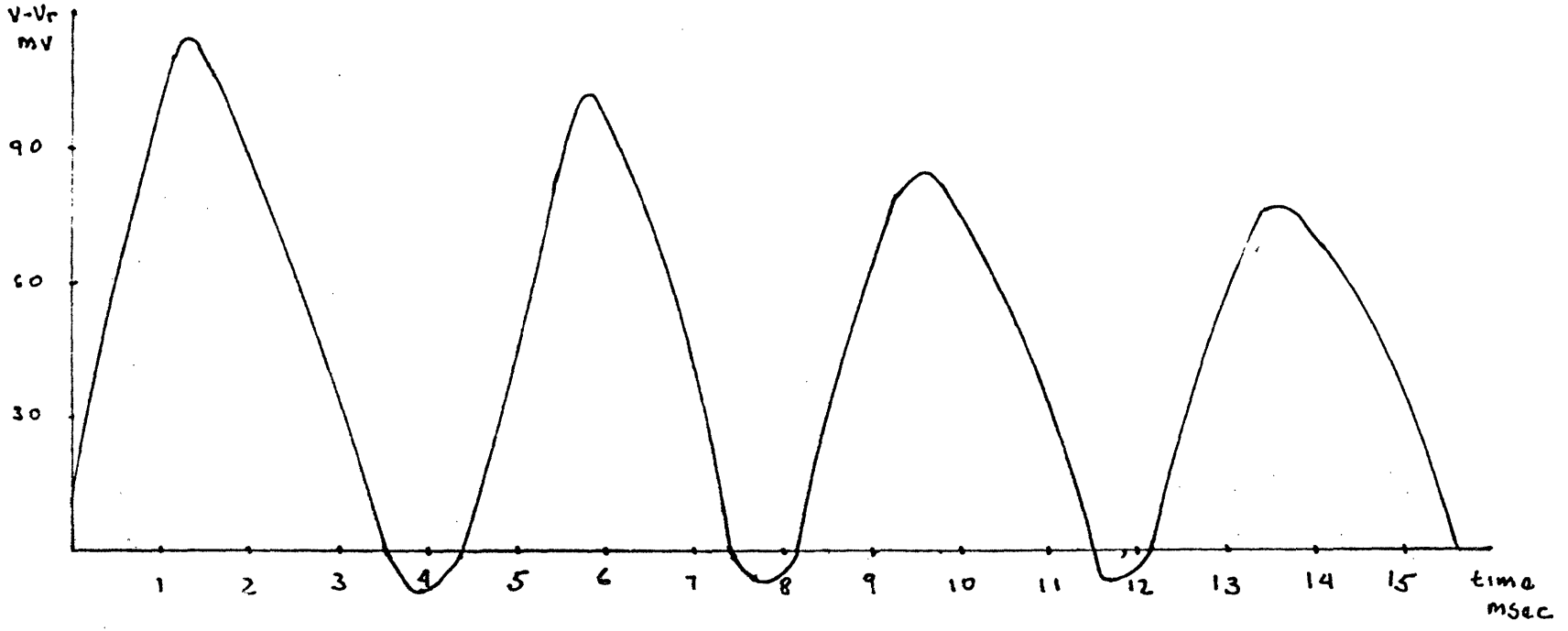
During the simulation, when the duration excitation current density pulse was increased above 1. msec the membrane potential exhibited a kind of damped oscillatory behavior with a finite number of cycles before returning to an equilibrium condition of higher resting potential than normal. The number of cycles of oscillations was found to be dependent on the duration of the current excitation pulse: for durations of 1. msec or less only one cycle was developed, for durations greater than 1 msec but less than 1.5 msec, 2 cycles; and for durations of 1.8 msec or more 4 cycles developed. This behavior of the axon membrane is rather interesting since it implies, that considering the neuron as an information module of the nervous system, the output signal i.e., the axon membrane potential, depends not only on the algebraic sum of input excitation pulses at the dendrites (see Chapter I), but also, on the duration of these excitation pulses. This condition seems to be in conflict with the "all or nothing law" of neuron excitation (Aidley [22]) in the sense that the axon membrane as an information module exhibits a non-binary behavior. Indeed, depending of the duration of the excitation (for an overthreshold excitation) 4 different dynamical responses can be

obtained. Therefore the information processing capabilities of the neuron are more complex than those of electronic modules. A deeper discussion of this information capability of the neuron is clearly beyond the scope of this thesis but this author considers that it should be fully explored in an information model of a nervous system. In Fig. 4-7 the membrane potential corresponding to a 2. msec excitation is shown.

4.7 Conclusions

A simulation of the ionic transport was carried out for a particular squid axon membrane (Loligo). The results of the simulation generally show good agreement with the experimental data. The simulation showed among other features, two very important characteristics: namely, the control action of the ionic mobilities on the transport of ions, the distribution characteristic of the electric field and the oscillatory behavior of the overexcited membrane.

Exitation : 445 μA/cm², constant
duration 2 msec



Membrane Potential Oscillatory behavior

Fig 4-7

CHAPTER V

ACTIVE TRANSPORT MODEL

5.1 Introduction

In Chapters III and IV a model for the electrical behavior of the clamped axon membrane was developed. In particular, the events of excitation and membrane potential were modeled as physically dependent processes with transport of ions across the membrane driven by their electrochemical gradients. In order for the axon to be an autonomous biological unit, it must be equipped with an active mechanism that restores the concentration gradients at the membrane surfaces after an action potential has occurred. This mechanism is known as The Sodium Pump. The reasons for this terminology will be clear later. The predominant characteristic of the sodium pump is the built-in capability for the active transport of ions against their electrochemical gradient across the membrane, with expenditure of metabolic energy. In what follows, "active transport" and "sodium pump" will be considered synonymous terms for the process sketched above.

The sodium pump mechanism has thus the essential function of building up the concentration differences on which ionic transport and therefore the conduction of electrical impulses depends.

In Chapter III, the concentrations of each of the ion species involved in the ionic transport model, were assumed to be constant in the solutions on both sides of membrane (Eqs. (3-3)). This assumption is justifiable only if a restoring process exists. Experimental evidence

carriers and surface reactions instead of the irreversible thermodynamics formalism used by Katchalsky and Spangler. This formulation allows a better qualitative and perhaps quantitative interpretation of the physical phenomena.

The chapter has been divided into the following sections: 5.2 Physical description of the active transport process, 5.3 Derivation of the equations of the model, 5.4 Qualitative analysis of the process in terms of the model, 5.5 Conclusions.

5.2 Physical Description of the Active Transport Process and Definition of the Variables of the Model

As discussed in the introduction many of the structural characteristics of the active transport process are not known. Therefore, in order to model the process, researchers in the field have established a great number of "educated hypotheses" about its structure, each of them based on the experimental study of a particular cell membrane. These hypotheses have several experimental facts in common, as listed below.

Fact 1

Almost all living cells are rich in potassium (primary cation) and poor in sodium (secondary cation) in their intercellular fluid. The reverse situation is true for the extracellular fluid.

Fact 2

The excitatory event depends upon differences in concentrations and activities of sodium as well as potassium and to lesser extent on other ion activities and concentration differences on both sides of the membrane

supports such a mechanism in most biological cell membranes. Furthermore, in particular for the squid axon membrane, constant concentrations of the ions on both sides of the membrane have been observed experimentally by Hodgkin and Keynes [21].

Although the existence of the active transport mechanism is supported by an ample variety of experiments, its structural and dynamic characteristics are not known. Therefore, a model for this process has to be developed by a deductive analysis based on some purely theoretical assumptions (e.g., fundamental physical principles) and nonconclusive observations of the process. A model that satisfies the thermodynamic principles of open systems (such as the one composed of the membrane and surrounding solutions) and agrees partially with the experimental data is the carrier-transport model proposed by Dannielli in 1954 [23]. In this model an ion combines with a specific protein present on the appropriate membrane surface, forming an electrically neutral carrier complex which flows to the opposite surface of the membrane (due to its concentration gradient) where the ion is released. This process implies chemical synthesis reactions with absorbtion of chemical energy; therefore, these reactions must be coupled with energy-releasing reactions ("down-hill" reactions) which provide the energy for the completion of the synthesis. This process is catalyzed by highly specific enzymes located in the external layers of the membrane (see Chapter I).

In this chapter, a particular model for the active transport process sketched above will be derived. The model is a generalization of the one proposed by Katchalsky and Spangler [25]. The generalization consists in formulating the model considering field theoretic laws for the flow of

The resting potential and the spike are functions of the gradient-controlled difussion of these ions across the membrane.

Remark:

Fact 2 was used as a hypothesis for the development of the ionic transport model in Chapter III.

Fact 3

The rising phase of the action potential is due to a sudden increase in permeability (i.e., ion-mobility) of the membrane to the flow of sodium ions in the direction of its concentration gradient (from the outside of the membrane towards the inside of the membrane). The repolarization is primarily dependent upon the diffusion of potassium ions in the direction of their concentration gradient. The recovery phase involves primarily the movement of the two monovalent cations against their concentration and electrical gradients, with the expenditure of energy from cellular metabolism; this is called active transport.

Remark:

The rising and repolarization phase behavior is observed in the simulation of the ionic transport model in Chapter IV. Notice that this behavior is a consequence of the flow of sodium ions and potassium ions in opposite directions, and their ion mobility time behavior differences.

Fact 4

In living cells in general, and in the squid nerve fiber cell in particular, there are several enzymes coated to the internal surface of the membrane which are activated by potassium ions and inhibited by sodium ions.

The previous facts about the mechanisms characterizing the membrane

system and the ample experimental data basis for nervous cell-membrane structure suggests the following definition for the sodium pump.

Definition

The term sodium pump is used to designate the biological system, resident in the membrane, responsible for the energy-requiring efflux of sodium (usually coupled to the influx of potassium) across the membrane. The only energy source of the pump is an ATP hydrolysis mechanism.

The previous definition involves 3 basic assumptions which have not been fully proved in practice but that agree with the experimental evidence and the theoretical analysis of the sodium pump[1]. These assumptions are:

1. The sodium pump is contained within or is part of the membrane.
2. The energy source of the pump is ATP.
3. The Active transport process is identified with the $Na^+ - K^+$ ATPase enzyme system.

The problem of modeling the sodium pump defined above has been approached in essentially 3 ways:

1. Define sodium, potassium and ATP sites on both sides of the membrane in order to elucidate the kinetic mechanisms of the coupled reactions catalyzed by the system.
2. Define the sequence, i.e., the intermediate steps that participate in the reaction leading to ATP hydrolysis.
3. Define the mechanism by which cardiac glycosides, specific inhibitors

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1 The analysis of the experimental basis for this definition is carried out by Schwartz et al [25]

of both the Na^+ and K^+ APTase, interact with the system.

The previous 3 approaches or combinations of them, have been used to derive functional models for the sodium pump. None of them can explain completely the behavior of the sodium pump, although an analysis of them has clarified to some extent the nature of the pump and has suggested the role of the experimentally observed mechanisms that characterizes it. The model considered in this chapter is based almost entirely on the first approach, the reason for this being the fact that the author's interest in the active transport process is centered on its physiological role as a "reset" of initial concentrations in the solutions on both sides of the neuron membrane, and not as a mechanism of the metabolic process, where approaches 2 and 3 have proven to be more useful for the analysis of the underlying phenomena.

Now, some of the features that characterize the active transport of monovalent cations (i.e., Na^+, K^+) will be described; they constitute the basis for the model that will be derived in the next section.

The active transport of sodium out of and potassium into the axon is coupled to the hydrolysis of ATP, according to the following scheme Skou [27].

The stoichiometry of the pump is $3Na^+:2K^+:1ATP$. These stoichiometric ratios have been observed to follow in many cells (i.e., ghost cells, Baker [28], Baker and Shaw [30]), although Keynes [29] found that such ratios vary with the conditions of the experiment, e.g., PH of the external solution. It is assumed for purposes of the model that PH in the solutions is maintained constant due to the flow of water across the membrane, driven by the osmotic pressure gradient.

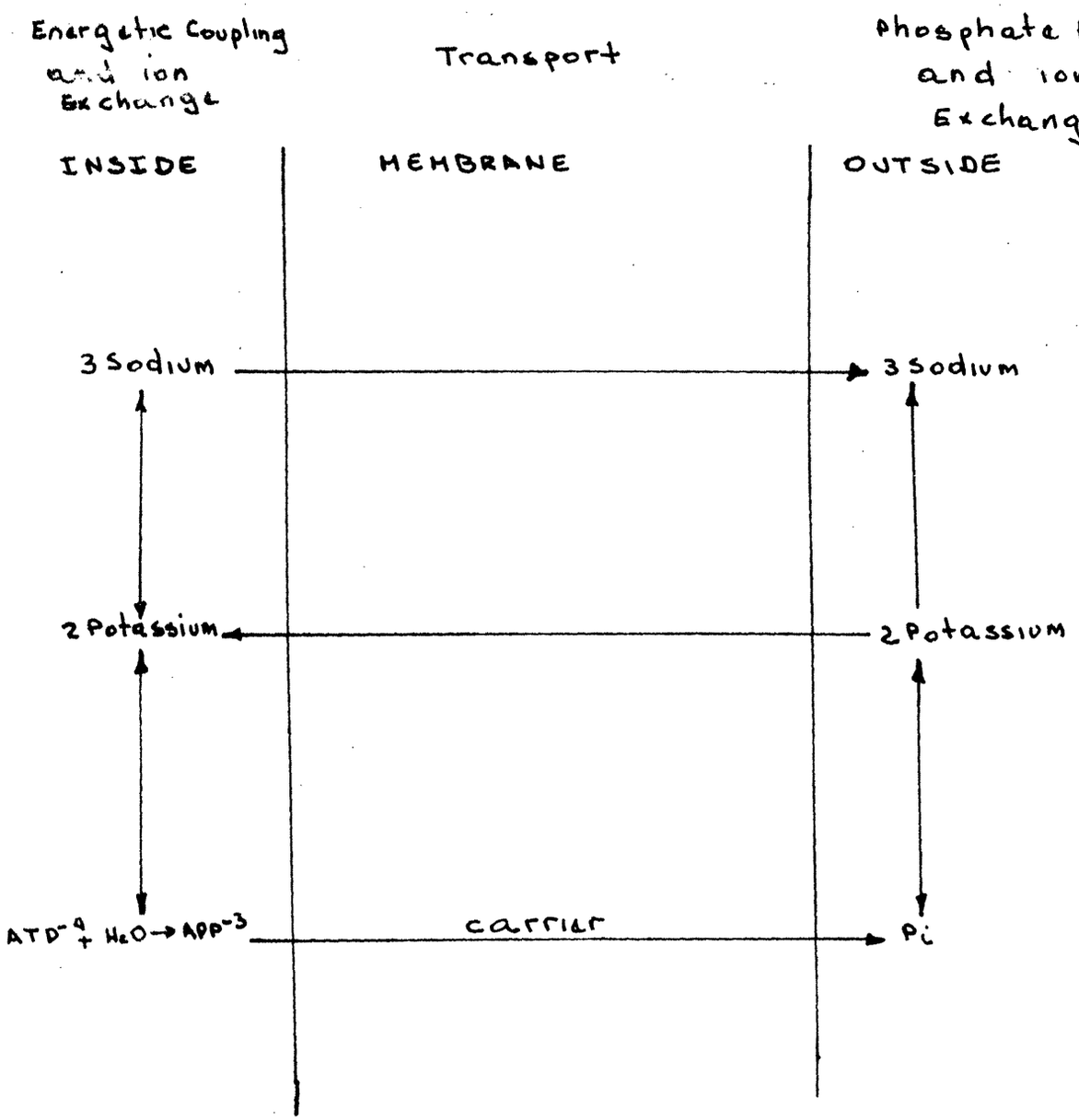
Remark:

In the Skou scheme for the sodium pump, described above, since the hydrolysis of ATP is electrically neutral, the total reaction is electrogenic because one net positive charge leaves the axon. Therefore, the pump contributes to the membrane potential. This condition can explain the resting potential of the axon membrane (negative inside).

The Na^+, K^+ - ATPase transport system described above is oriented within the membrane and has an apparent molecular weight estimated between 190000 and 5000000 Robertson [31]. This estimate suggests that assuming that the sodium pump is constituted by a single macromolecule with an assumed density of 1.3 and a molecular weight of 250000 would correspond to a spherical particle with a diameter of 85 Å, a dimension close to that estimated for axon membranes (150 Å).

It is evident from the assumed scheme for the active transport, that parts of the Na^+, K^+ - ATPase mechanism must be exposed to the internal and external membrane water surface. This condition seems to be in conflict with the bilayer-unit membrane structure discussed in Chapter I. However, the proposed configuration for Na^+, K^+ - ATPase transport system can exist at regular intervals adjacent to bilayered membrane structure and therefore does not really invalidate the Dannielli unit membrane hypothesis.

The assumed kinetic scheme for the pump and the data on concentrations in the solutions on both sides of the membrane lead to the assumption that the pump is asymetrically oriented, so that it presents a sodium activation site on the internal surface of the axon membrane and a potassium activation site on the external surface of the membrane. The sodium activation site presumably is constituted of a surface binding enzyme with the characteristic



External scheme, Sodium Pump

Basic Unit

Fig 5-1

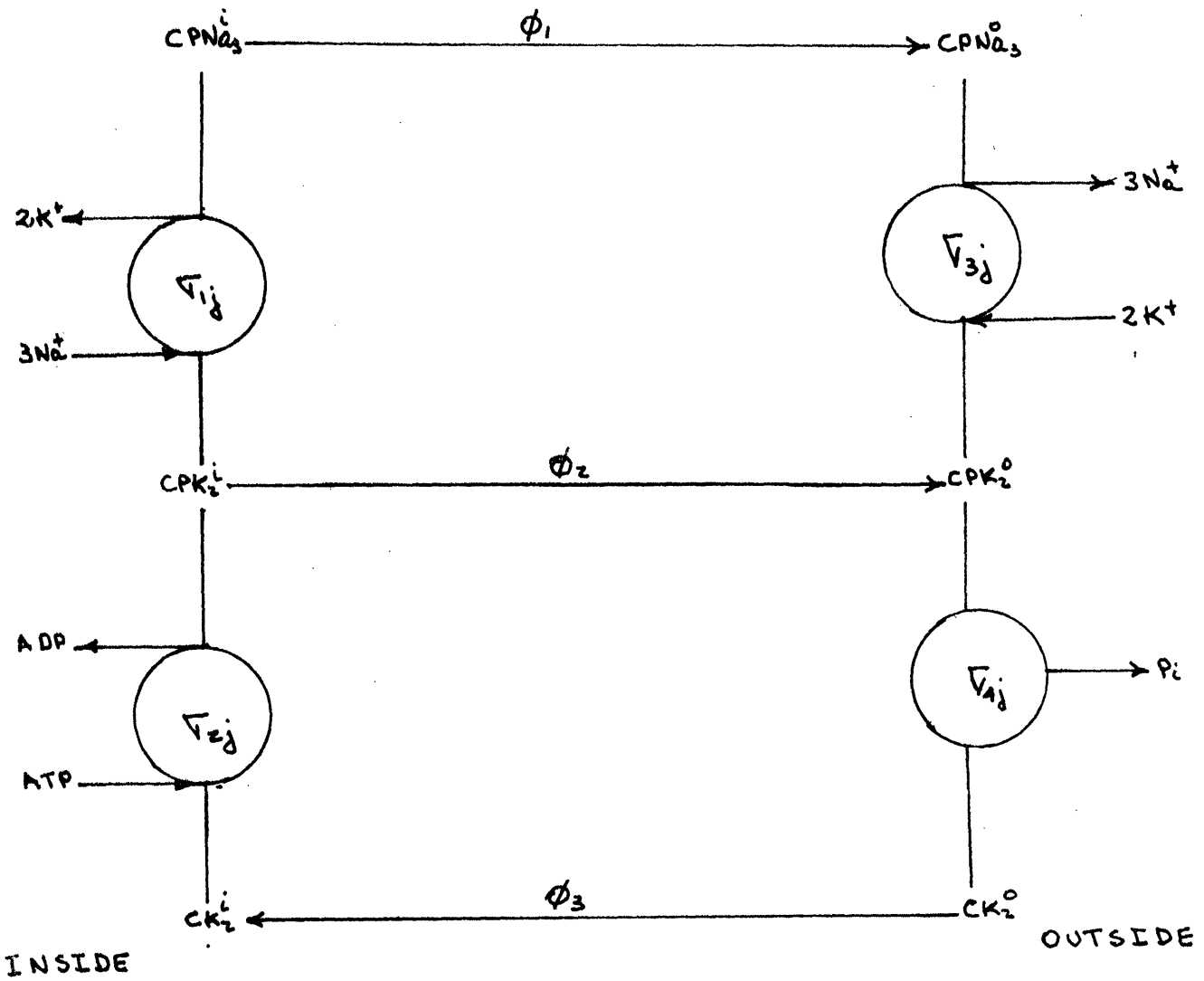
of high affinity for sodium and low (or idealistically none) affinity for potassium. Similarly the potassium activation site, located on the external surface, has high affinity for potassium and low affinity for sodium.

There are two coupled additional phenomena that have been observed in axon membranes as well as in other biological membrane systems, these are: Competitive behavior at the sodium activation site and non-ionic potassium transport from the inside to the outside (Skou [27], Post et al [32]). The first refers to the situation in which the enzyme or enzymes that form the sodium activation site might be "blocked" by potassium ions, therefore preventing the sodium carrier from being loaded. Thus the overall effect is a potassium-induced inhibition of catalysis and transport of sodium ions. Although there is no direct evidence, the equivalent process is likely to occur at the potassium activation site namely, sodium competes with potassium at the potassium activation site and if successful inhibits potassium catalysis and transport. The second additional phenomena, non-ionic potassium transport from the inside to the outside, is believed to act as a regulator of active sodium transport. Its physiological function is not very clear but because of the abundant evidence for its existence, it has been included in most of the models of active transport. In the model for the sodium pump considered below, these two phenomena are incorporated by considering partial reversibility in the chemical reactions leading to the formation of the carriers and by an additional carrier flow, respectively.

With the previous considerations, an internal representation of the basic unit of the sodium pump (Fig. 5-1) takes the following form:

In Fig. 5-2, the arrows crossing the membrane indicate flows of carriers; 3 carriers are considered, $CPNa_3$ the sodium carrier, CPK_2 the phosphrylated potassium carrier, and CK_2 the unphosphorylated potassium carrier. In the diagram, the circles indicate the 4 chemical reactions that characterize the process; σ_{1j} is the chemical reaction that characterizes the sodium activation site, σ_{3j} is the reaction occurring at the potassium activation site, σ_{2j} is the carrier phosphorilation reaction; it involves the cyclic ATP hydrolysis with the release of chemical energy; the ultimate (and only) energy source of the pump. Notice that σ_{1j} and σ_{2j} are coupled through the carrier (reactant-product respectively), CPK_2 providing thus a "path" for the transfer of energy to the carriers of the pump. σ_{4j} is the carrier dephosphorilation reaction where inorganic phosphate is released to the external solution with the production of a "light" potassium carrier CK_2. At the chemical reaction sites, arrows entering the circles are reactants and arrows leaving them are products for the <u>forward</u> reaction-direction of the pump. According to the previous discussion, σ_{1j}, σ_{2j} are (partially) reversible reactions, their degree of reversibility models the competitive behavior of sodium and potassium ions at their activation sites.

It is important to notice that the main active flow of potassium, that is the flow that has the physiological purpose of building up the potassium concentration at the axon membrane surfaces, is carried by CK_2. The dual role of CPK_2 can be explained with the aid of Fig. 5.2. The flow of carrier CPK_2 represented as ϕ_2 in Fig. 5.2, provides a mechanism of orthophosphate discharge in the outer solution and also a mechanism for regulating the active flow of sodium ions via the ion-



Sodium Pump, Basic Unit

Internal Rapresentation

Fig 5.2

exchange reactions σ_{1j} and σ_{3j}, but its main function is to serve as chemical energy transfer agent between the exogenous σ_{2j} reaction of ATP hydrolysis (in the model it is considered as an equivalent phosphate group transfer from ATP to the CK_2 carrier with products ADP and CPK_2) and the endogenous σ_{ij} reaction at the inner axon membrane surface in which a $Na^+ - K^+$ exchange takes place with the uptake of chemical energy. The process just described, is the only energy supply mechanism for the sodium pump. Although an analysis of the energetic behavior of the pump is beyond the scope of this thesis, a brief physical qualitative description of its characteristics in terms of the assumed internal representation of the active transport process (Fig. 5.2) is given. Katchalsky and Spangler showed after a thermodynamical analysis using this representation, that under a certain constant concentration ratio, α, of sodium and potassium in the solutions on both sides of the membrane, (i.e., $\alpha = [Na]_o[K]_i/[Na]_i[K]_o = 220$) which corresponds to the observed resting state (see Chapter IV) of the axon membrane, the pump exhibits a steady state condition. When the axon membrane is fired and a spike is produced the consequent induced transport of sodium and potassium ions, as discussed in Chapter III, implies that the ratio α will vary from its steady state value (220) to a new equilibrium value slightly smaller (about 219), if several spikes are produced the concentration gradients of sodium and potassium at the axon membrane will decrease further and if no mechanism is provided for a recovery the membrane would loose its excitatory ability, therefore it seems logical that the sodium pump is activated by a decrease in the α factor or some function of it such as PH of the internal solution. In the model of the sodium pump it is assumed that

the enzyme (or enzymes) catalyzing reaction σ_{2j} is (are) activated by an internal PH variation from the steady state (equilibrium) value (about 7.5), increasing the production of CPK_2^i (i stands for inside surface) with expenditure of metabolic energy (ATP). The increase in concentration of CPK_2 in turn implies an increase in the production of $CPNa_3^i$ as indicated in Fig. 5.2. The increase in the concentration of $CPNa_3$ at the inner membrane surface implies that the chemical gradient of $CPNa_3$ between the membrane surfaces increases and consequently the flow ϕ_1 of this carrier increases, producing an excess concnetration of $CPNa_3^o$ (O stants for outer surface) and as can be deducted from Fig. 5.2., this implies an increase of the σ_{3j} reaction product concentration, namely CPK_2^o. This condition has two implications for the operation of the pump; first an increase in the production of CK_2^o through reaction σ_{4j} which implies an increase in the chemical gradient of this carrier with the consequent increase in its flow ϕ_3, and second, a <u>decrease</u> in its flow ϕ_2 caused by a decrease in its chemical gradient. The second consequence constitutes the regulatory mechanism of the pump, since the decrease in flow ϕ_2 will imply a decrease in the concentration of CPK_2^o which in turn will eventually produce a decrease in CK_2^o, and therefore a decrease in CK_2 which induces a restraint in activity of reaction σ_{2j} as can be seen in Fig. 5.2. This qualitative description of the pump will be shown to be reproduce by the equations of the model in the next section.

Finally, before closing this section, the chemical equations for the 4 reactions of the sodium pump are written down and followed by a discussion of some of their chemical properties.

$$\sigma_{1j}: \quad CPK_2 + 3Na^+ \underset{S-1}{\overset{S_1}{\underset{\leftarrow}{\rightarrow}}}_{E_1} CPNa_3 + 2K^+ \tag{5-1}$$

$$\sigma_{2j}: \quad CK_2 + ATP \overset{S_2}{\underset{H_2O}{\rightarrow}} ADP + CPK_2 \tag{5-2}$$

$$\sigma_{3j}: \quad CPNa_3 + 2K^+ \underset{S-3}{\overset{S_3}{\underset{\leftarrow}{\rightarrow}}}_{E_3} CPK_2 + 3Na^+ \tag{5-3}$$

$$\sigma_{4j}: \quad CPK_2 \overset{S_4}{\underset{E_4}{\rightarrow}} CK_2 + P_i \tag{5-4}$$

In (5-1) through (5-4) S_i $i = -3, -1, 1,\ldots,4$ are reaction constants where the negative subindex indicates that the respective reaction operates also in the reverse direction and E_i $i = 1,\ldots,4$ stand for the enzyme-complexes that catalyze each reaction.

It is important to remark that in kinetic theory the l.h.s. of eqs. (5-1) - (5-4) is seen as the <u>starting state</u> and the rhs as the completion state of a very complex set of sequential reactions involving the activation of the respective enzyme, the formation of an enzyme-reactant complex, the transformation of this complex into a enzyme-product complex, and the release of the end products. This approach is beyond the scope of this thesis, rather reactions (5-1) through (5-4) are considered in their global form: starting → completion state, and in this context the reaction rates $\{S_i\}$ are phenomenological functions that indicate the rate at which products are formed at the expense of reactants. In (5-1) and (5-3) the components that appear on both sides are considered as products and as reactants due to the assumed

reversibility of these reactions.

The consideration of the pump-reaction in the global sense indicated above requires the establishment of additional assumptions on the enzymatic structure of the axon membrane, namely, in order for the reaction constants $\{S_i\}$ to make sense, the concentrations of enzymes $\{E_i\}$ must remain constant. This assumption is validated by the hypothesis of surface-membrane binding of these enzymes (see Chapter I).

Notice that reactions σ_{1j}, σ_{2j} are chemically coupled, and similarly reactions σ_{3j}, σ_{4j} but the two reaction systems are spatially separated and therefore uncoupled. Finally, recall that the reaction system is restricted to occur in the membrane space and therefore the reactions are carried out under constant volume. Furthermore, an energy balance of the pump to be developed in the next section will show that the pump operates also at an almost constant temperature.

5.3 Derivation of the Equations of the Model

In this section, the equations describing the Active Transport process are derived. The mathematical model is based on the physical representation of the pump discussed in the last section and as for the ionic transport process, its mechanisms of operation are determined by using first order principles of field theory in the context of irreversible thermodynamics. However, as opposed to the ionic transport process in which the dynamic behavior of each species was described by a generic set of equations and no direct interaction was allowed among the flow of each species, in the model for the active transport process this simplification

is not possible since the process is composed of mechanisms that are not uniformly distributed along the membrane space.

The model of the sodium pump as discussed before, is composed of 3 dynamical systems: The ion-exchange coupled to the carrier phosphorylation at the inner axon-membrane surface, the carrier flow and ion-exchange coupled to the carrier dephosphorylation at the outer membrane surface. In short, the mathematical model of the sodium pump can be described as a diffusion process with time varying boundary conditions, where the kinetics of the chemical reactions (5-1) - (5-4) constitute the dynamics at the boundaries.

As a first step towards the development of the model, equations for the diffusion of carriers are derived. Recall from the last section that the meaning assigned to ϕ_1, ϕ_2, ϕ_3 is that of the effect of transport which may or may not be caused by actual movement of molecules; with this convention, the equations of continuity for the species $CPNa_3$, CPK_2 and CK_2 at any point inside the membrane are given by

$$\frac{\partial C_1}{\partial t}(x,t) = -\frac{\partial \phi_1}{\partial x}(x,t) \qquad 0 \leq x \leq \delta \qquad (5\text{-}5)$$

$$\frac{\partial C_2}{\partial t}(x,t) = -\frac{\partial \phi_2}{\partial x}(x,t) \qquad 0 \leq x \leq \delta \qquad (5\text{-}6)$$

and

$$\frac{\partial C_3}{\partial t}(x,t) = -\frac{\partial \phi_3}{\partial x}(x,t) \qquad 0 \leq x \leq \delta \qquad (5\text{-}7)$$

where $C_1(x,t)$ represents the concentration distribution of $CPNa_3$ in the membrane and $C_2(x,t)$, $C_3(x,t)$ represent the concentration distributions of CPK_2 and CK_2 respectively.

For the purpose of simplification, it will be assumed that the reactions of the pump are restricted to occur at the boundaries i.e., at $x=0$ and $x=\delta$. This condition, of course, is not physically realizable. Nevertheless, this simplification approximates reality since each reaction dynamics develops at a specialized activation site located at the internal or external protein layer of the membrane structure (see Chapter I), and the thickness of these layers is much smaller than that of the membrane. Now, the kinetic equations of reactions (5-1) - (5-4) are established.

The rate of production (or disappearance), σ_j, of species j measured in moles/cm^3-sec, is given by the following equation (Prigogine [13]):

$$\sigma_j(t) = \sum_{i=1}^{p} \alpha_{ij} r_i(t) - C_j(t)\frac{d\ell nV}{dt} \qquad j = 1,\ldots,n \qquad (5\text{-}8)$$

where p is the number of <u>simultaneous</u> reactions, α_{ij} $i = 1,\ldots,p$, $j = 1,\ldots,n$ is the stoichiometric coefficient of species j in reaction i (positive for products and negative for reactants), $r_i(t)$ is the reaction rate of reaction i, $C_j(t)$ is the concentration in (moles/cm^3-sec) of species j and V is the "volume of reaction" i.e., the volume of the region where the reactions occur; since for the axon membrane this volume is constant, the second term in the r.h.s. of (5-7) vanishes.

In the sodium pump model, two <u>separate</u> groups of simultaneous reactions are present: Reactions (5-1) and (5-2) at the inner surface and reactions (5-3) and (5-4) at the outer surface.

The reaction rate $r_i(t)$ is given by the following expression; E.M. Eyring [26]:

$$r_i(t) = S_i \prod_{j=1}^{\ell} C_j^{\beta_{ij}} - S_{-i} \prod_{j=\ell+1}^{n} C_j^{\gamma_{ij}} \qquad i = 1,\ldots,p \qquad (5\text{-}9)$$

where S_i, S_{-i} are respectively the forward and reverse reaction constants of reaction i, $j = 1,\ldots,\ell$ are the reactants and $j = \ell+1,\ldots,n$ are the products and β_{ij} and γ_{ij} are phenomenological coefficients that must satisfy constraints imposed by the stoichiometry of the reaction, as will be shown later on in this section.

The boundary conditions for equations (5-5) through (5-7) are obtained by a flow balance at the membrane surfaces as follows:

For (5-5)

$$\frac{\partial C_1}{\partial t}(0,t) = -\frac{\partial \phi_1}{\partial x}(0,t) + \sigma_1^{\,o}(t) \qquad \text{at } x=0$$

$$\frac{\partial C_1}{\partial t}(\delta,t) = \frac{\partial \phi_1}{\partial x}(\delta,t) - \sigma_1^{\,\delta}(t) \qquad \text{at } x=\delta \qquad (5\text{-}10)$$

For (5-16)

$$\frac{\partial C_2}{\partial t}(0,t) = -\frac{\partial \phi_2}{\partial x}(0,t) - \sigma_2^{\,o}(t) \qquad \text{at } x=0$$

$$\frac{\partial C_2}{\partial t}(\delta,t) = \frac{\partial \phi_2}{\partial x}(\delta,t) + \sigma_2^{\,\delta} \qquad \text{at } x=\delta \qquad (5\text{-}11)$$

And for (5-7)

$$\frac{\partial C_3}{\partial t}(0,t) = \frac{\partial \phi_3}{\partial x}(0,t) - \sigma_3{}^{o}(t) \qquad \text{at } x=0$$

$$\frac{\partial C_3}{\partial t}(\delta,t) = -\frac{\partial \phi_3}{\partial x} + \sigma_3{}^{\delta}(t) \qquad \text{at } x=\delta \tag{5-12}$$

where the σ's are the chemical concentrations rates defined according to the generic equation (5-8), the superscript 0 or δ indicates the corresponding group of simultaneous reactions to which the chemical concentration rate is referred: 0 the group (5-1), (5-2) and δ the group (5-3), (5-4).

Before deriving expressions for the flows ϕ_1, ϕ_2 and ϕ_3, the dynamic equations for the component concentrations entering in reactions (5-1) - (5-4) are established using equations (5-8) and (5-9).

Let $C_{40}(t)$, $C_{50}(t)$, $C_6(t)$ and $C_7(t)$ be the concentrations of the species K^+, Na^+, ADP and ATP at the inside respectively; similarly, let $C_{4\delta}(t)$, $C_{5\delta}(t)$ and $C_8(t)$ be the concentrations of species K^+, Na^+, and P_i respectively (see Fig. 5.2); then from (5-8) and (5-9), the following equations are obtained

$$\frac{dC_{40}}{dt} = 2\, S_1 C_{50}{}^{\beta_{15}}(t) C_2{}^{\beta_{12}}(0,t) - 2\, S_{-1} C_{40}{}^{\gamma_{14}}(t) C_1{}^{\gamma_{11}}(0,t) \tag{5-13}$$

$$\frac{dC_{50}}{dt} = -3\, S_1 C_{50}{}^{\beta_{15}}(t) C_2{}^{\beta_{12}}(0,t) + 3\, S_{-1} C_{40}{}^{\gamma_{14}}(t) C_1{}^{\gamma_{11}}(0,t) \tag{5-14}$$

$$\sigma_1{}^{o}(t) = S_1 C_{50}{}^{\beta_{15}}(t) C_2{}^{\beta_{12}}(0,t) - S_{-1} C_{40}{}^{\gamma_{14}}(t) C_1{}^{\gamma_{11}}(0,t) \tag{5-15}$$

Recall that reaction (5.1) was assumed to be reversible. Similarly,

$$\sigma_2^{o}(t) = -\sigma_1^{o}(t) + S_2 C_3^{\beta_{23}}(0,t) C_7^{\beta_{27}}(t) \tag{5-16}$$

$$\frac{dC_6}{dt} = S_2 C_3^{\beta_{23}}(0,t) C_7^{\beta_{27}}(t) \tag{5-17}$$

Notice that $\frac{dC_6}{dt}$ is not dependent on $C_6(t)$. This is due to the fact that reaction (5-2) was assumed to be <u>completely irreversible</u> (see section 5-2); this is an idealization because in the real world no reaction is fully irreversible; nevertheless the contribution of the irreversible part to the concentration rate is negligible (i.e., $|S_2| >> |S_{-2}|$).

$$\frac{dC_7}{dt} = -S_2 C_3^{\beta_{23}}(0,t) C_7^{\beta_{27}}(t) \tag{5-18}$$

$$\sigma_3^{o}(t) = -S_2 C_3^{\beta_{23}}(0,t) C_7^{\beta_{27}}(t) \tag{5-18a}$$

Equations (5-13) - (5-18a) describe the dynamics of each species concentration at the inside surface of the axon membrane in the sodium pump.

Similarly, for the species on the outer surface

$$\frac{dC_{4\delta}}{dt} = -2 S_3 C_1^{\beta_{31}}(\delta,t) C_{4\delta}^{\beta_{34}}(t) + 2 S_{-3} C_2^{\gamma_{32}}(\delta,t) C_{50}^{\gamma_{35}}(t) \tag{5-19}$$

$$\frac{dC_{5\delta}}{dt} = 3 S_3 C_1^{\beta_{31}}(\delta,t) C_{4\delta}^{\beta_{34}}(t) - 3 S_{-3} C_2^{\gamma_{32}}(\delta,t) C_{50}^{\gamma_{35}}(t) \tag{5-20}$$

$$\sigma_1^{\delta}(t) = - S_3 C_1^{\beta_{31}(\delta,t)} C_{4\delta}^{\beta_{34}(t)} + S_{-3} C_2^{\gamma_{32}(\delta,t)} C_{50}^{\gamma_{35}(t)} \qquad (5\text{-}21)$$

$$\sigma_2^{\delta}(t) = \sigma_1^{\delta}(t) - S_4 C_2^{\beta_{42}(\delta,t)} \qquad (5\text{-}22)$$

$$\frac{dC_8}{dt} = - S_4 C_2(\delta,t) \qquad (5\text{-}23)$$

$$\sigma_3^{\delta}(t) = S_4 C_2(\delta,t) \qquad (5\text{-}24)$$

The flows $\phi_1(x,t)$, $\phi_2(x,t)$ and $\phi_3(x,t)$, and the concentrations $C_1(x,t)$, $C_2(x,t)$, $C_3(x,t)$ are considered to be distributions across the membrane; therefore, using the principles of field theory, differential equations relating each flow distribution with its corresponding concentration distribution follow a derivation analogous to the one used for this purpose in Chapter III. Notice that this is possible since no interaction among the flow of carriers inside the membrane is allowed to occur.

Let μ_j $j = 1,2,3$ be the chemical potential of carriers $CPNa_3$, CPK_2 and CK_2 respectively, then (see Chapter III)

$$\mu_j = \mu_j^{o} + RT \ln \psi_j C_j \qquad j = 1,2,3 \qquad (5\text{-}25)$$

where ψ_j is the (constant) chemical activity of carrier j in the membrane and μ_j^{o} is its chemical potential at ground state conditions.

The force x_j driving carrier j is given by

$$X_j = - \frac{\partial \mu_j}{\partial x} \qquad j = 1,2,3 \qquad (5\text{-}26)$$

and their velocities are given by

$$v_j = u_j X_j \qquad j = 1,2,3 \tag{5-27}$$

where u_j is the carrier j mobility. In Chapter III the mobility of each ion species was found to be a function of the membrane potential; since the carriers are electrically neutral the carrier mobility as opposed to the ionic mobility is only dependent on the temperature of the axon membrane which is assumed to be constant, therefore the carrier mobilities u_j $j = 1,2,3$ are constant.

From (3-10) the flow of carrier j is given by

$$\phi_j(x,t) = v_j(x,t)C_j(x,t) \qquad j = 1,2,3 \tag{5-28}$$

Then, from (5-25), (5-26), (5-27) in (5-28)

$$\phi_j = - u_j(RT \frac{\partial}{\partial x}(\ell n\ \psi_j C_j(x,t))C_j(x,t) \qquad j = 1,2,3$$

or

$$\phi_j(x,t) = - u_j RT \frac{\partial C_j}{\partial x}(x,t) \qquad j = 1,2,3 \tag{5-29}$$

Now, from (5-29) in (5-5), (5-6), (5-7), the dynamic equations for the concentration distributions of carriers $CPNa_3$, CPK_2, CK_2 respectively are obtained.

$$\frac{\partial C_1}{\partial t} = u_1 RT \frac{\partial^2 C_1}{\partial x^2} \tag{5-30}$$

$$\frac{\partial C_2}{\partial t} = u_2 RT \frac{\partial^2 C_2}{\partial x^2} \tag{5-31}$$

$$\frac{\partial C_3}{\partial t} = u_3 RT \frac{\partial^2 C_3}{\partial x^2} \tag{5-32}$$

Since the mobilities are constant, equations (5-30) - (5-32) are usual linear diffusion equations with the boundary conditions given by equations (5-10) - (5-12).

Before going further in the derivation of the model, an analysis of the "well-posedness" of the system of equations (5-30) - (5-32) with the associated boundary conditions will be carried out.

Equations (5-10), (5-11) and (5-12) can be written solely in terms of the respective concentration distributions by using (5-29):

$$\frac{\partial C_1}{\partial t}(0,t) = + u_1 RT \frac{\partial^2 C_1}{\partial x^2}(0,t) + \sigma_1{}^{o}(t)$$

$$\frac{\partial C_1}{\partial t}(\delta,t) = - u_1 RT \frac{\partial^2 C_1}{\partial x^2}(\delta,t) - \sigma_1{}^{\delta}(t) \tag{5-33}$$

$$\frac{\partial C_2}{\partial t}(0,t) = u_2 RT \frac{\partial^2 C_2}{\partial x^2}(0,t) - \sigma_2{}^{o}(t)$$

$$\frac{\partial C_2}{\partial t}(\delta,t) = - u_2 RT \frac{\partial^2 C_2}{\partial x^2}(\delta,t) - \sigma_2{}^{\delta}(t) \tag{5-34}$$

$$\frac{\partial C_3}{\partial t}(0,t) = - u_3 RT \frac{\partial^2 C_3}{\partial x^2}(0,t) - \sigma_3{}^{o}(t)$$

$$\frac{\partial C_3}{\partial t}(\delta,t) = u_3 RT \frac{\partial^2 C_3}{\partial x^2} + \sigma_3{}^{\delta}(t) \tag{5-35}$$

Recall that in order to have a physical meaning,

$$c_j(x,t) \geq 0 \qquad j = 1,2,3 \tag{5-36}$$

Comparing eqs. (5-30) - (5-32) with eqs. (5-33) - (5-35) it can be seen that the σ - functions constitute impulses in the spatial coordinate at the boundaries; therefore, in a strong sense, the problem is not well possed. Recall that the assumption of point - reaction led to the definition of the reaction flow σ. The previous analysis shows that this constraint is too "hard". Furthermore, from a physical point of view, a relaxed condition i.e., one that allows the reaction kinetics to occur in a finite volume, which implies a finite distribution of reaction in the x direction is reasonable. Let Δ_o be the length of reaction in the inner surface and Δ_1 the respective length of reaction in the outer surface; where Δ_o, $\Delta_1 << \delta$. Physically these lengths of reactions are considered to be the two thin protein layers coating the phospholipoid bilayer-axon membrane according to Dannielli's model (see Chapter I).

The following scheme is proposed for approximating the boundary equations (5-33) - (5-35) Kohn [33]:

a) Discretize the spatial-derivative terms of (5-33) - (5-35) according to the following expressions:

$$\frac{\partial^2 C_1}{\partial x^2}(0,t) \simeq \frac{1}{\Delta_o^2}\left(h_i(\Delta_o,t) - 2h_i(\Delta_o/2,t) + h_i(0,t)\right) \qquad i = 1,2,3 \tag{5-37}$$

and

$$\frac{\partial^2 C_i}{\partial x^2}(\delta,t) \simeq \frac{1}{\Delta_1^2}(h_i(\delta,t) - 2h_i(\delta - \frac{\Delta_1}{2},t) + h_i(\delta-\Delta_1,t)) \qquad (5\text{-}38)$$

$$i = 1,2,3$$

where $h_i(.,t)$ $i = 1,2,3$ are the concentration-distribution approximations in the inside reaction region at the points 0, $\frac{\Delta_o}{2}$, Δ_o and at the outside reaction points $\delta-\Delta_1$, $\delta-\Delta_1/2$, δ. According to experimental evidence (Robertson [31]), the size of Δ_o, Δ_1 is of the order of 1/50 of the axon membrane thickness.

b) By the adjacent cell-method, (Angel and Bellman [34]) each equation in (5-33) - (5-35) is approximated by 3 ordinary differential equations as follows:

$$\frac{dh_i}{dt}(0,t) = \frac{u_i RT}{(\Delta_o)^2}(h_i(0,t) + h_i(\frac{\Delta_o}{2},t))$$

$$\frac{dh_i}{dt}(\Delta_o/2,t) = \frac{u_i RT}{(\Delta_o)^2}(h_i(0,t) - 2h_i(\Delta_o/2,t) + h_i(\Delta_o,t)) + \sigma_i{}^o(t) \qquad (5\text{-}39)$$

$$\frac{dh_i}{dt}(\Delta_o,t) = \frac{u_i RT}{(\Delta_o)^2}(h_i(\Delta_o,t) + h_i(\Delta_o/2,t)) \qquad i = 1,2,3$$

and

$$\frac{dh_i}{dt}(\delta-\Delta_1,t) = -\frac{u_i RT}{(\Delta_1)^2}(h_i(\delta-\Delta_1,t) + h_i(\delta - \frac{\Delta_1}{2},t))$$

$$\frac{dh_i}{dt}(\delta-\frac{\Delta_1}{2},t) = -\frac{u_i RT}{(\Delta_1)^2}(h_i(\delta-\Delta_1,t) + h_i(\delta - \frac{\Delta_1}{2},t) + h_i(\delta-\Delta_1,t)) - \sigma_i{}^\delta(t) \qquad (5\text{-}40)$$

$$\frac{dh_i}{dt}(\delta,t) = -\frac{u_i RT}{(\Delta_1)^2}(h_i(\delta,t) + h_i(\delta-\Delta_1/2,t))$$

$$i = 1,2,3$$

c) For the system of equations (5-13) - (5-19),

$$C_i(0,t) \simeq h_i(0,t) \qquad i = 1,2,3 \tag{5-41}$$

Similarly, for the system of equations (5-20) - (5-24)

$$C_i(\delta,t) \simeq h_i(\delta,t) \qquad i = 1,2,3 \tag{5-42}$$

Notice that $h_i(.,t)$ $i = 1,2,3$ are defined only at points 0, $\frac{\Delta_o}{2}$, Δ_o, $\delta-\Delta_1$, $\delta - \frac{\Delta_1}{2}$, δ.

d) The approximate boundary conditions for the carrier-transport system, eqs. (5-30) - (5-32) become

$$\begin{aligned} C_i(0,t) &\simeq h_i(\Delta_o,t) \\ C_i(\delta,t) &\simeq h_i(\delta-\Delta_1,t) \end{aligned} \qquad i = 1,2,3 \tag{5-43}$$

The well posedness of the carrier diffusion problem with the boundary conditions given by (5-51), can be proved by carrying out an analysis similar to the one developed in Chapter III for the ionic transport model.

The next issue towards the specification of the model for the sodium pump, is the determination of initial conditions for the model dynamic equation discussed above.

The initial conditions depend mainly on two factors, steady-state behavior of the carrier-reaction system inside the membrane and the activation mechanisms of enzyme complexes (E_i $i = 1,...,4$ see section 5-2). The first factor determines the initial distribution of the

carrier flows ϕ_1, ϕ_2, ϕ_3 and consequently their respective initial concentration distributions, and the second specifies the initial surface concentrations of the species that conform to the reaction mechanisms at the membrane surfaces.

Furthermore, the steady state equations of the model will be used for identifying the reaction-parameters $\{\beta_{ij}, \gamma_{ij}\}$ and the reaction constants $\{S_{-i}, S_i\}$ that under certain restrictions prevail for the pump under dynamic operation.

Assuming that for small variations around the steady state, Gibbs law holds for the system of reactions characterizing the pump,

$$dG = - SdT + vdP + \sum_{j=1}^{N} \mu_j dN_j \qquad (5-44)$$

where dN_j $\quad j = 1,2,\ldots,N$ is the amount in moles of ion species j in the reacting system at equilibrium conditions $dG = 0$, and since the reaction system of the sodium pump operates at isothermal isobaric conditions, at equilibrium, (5-44) becomes:

$$\sum_{j=1}^{N} \mu_j dN_j = 0 \qquad (5-45)$$

Recall that the chemical potentials μ_j referred to in (5-45) are the ones associated with the corresponding species in the reacting region, for $i = 1,\ldots,p$ simultaneous reactions at equilibrium conditions the law of conservation of mass can be expressed as

$$dN_j = \sum_{i=1}^{p} \alpha_{ij} dz_i \qquad j = 1,\ldots,N \qquad (5-46)$$

where dz_i is the differential of the <u>extent of reaction</u> (moles) of the

i-th reaction.

Then from (5-44) in (5-43) and since (5-43) must be true for any arbitrary set of values of dz_i $i = 1,\ldots,p$,

$$\sum_{j=1}^{N} \alpha_{ij}\mu_j = 0 \qquad i = 1,\ldots,p \tag{5-47}$$

Condition (5-47) implies that at steady state the reacting system satisfies Hess' law i.e.,

$$K_{ci} = K_i/(RT)^{\bar{\alpha}_i} \qquad i = 1,\ldots,p \tag{5-48}$$

where K_{ci} $i = 1,\ldots,p$ are defined by the following expressions:

$$K_{ci} = \prod_{j=1}^{N} (C_{jo} + \sum_{i=1}^{p} \alpha_{ij}\xi_i)^{\alpha_{ij}} \qquad i = 1,\ldots,p \tag{5-49}$$

$\{C_{jo}\}$ is a set of equilibrium concentrations and ξ_i(mole/cm^3), $i = 1,\ldots,p$ are the volumetric extents of reaction of the simultaneous reactions occurring in the system.

$\bar{\alpha}$ is defined as

$$\bar{\alpha}_i = \sum_{j=1}^{N} \alpha_{ij} \qquad i = 1,\ldots,p \tag{5-50}$$

and K_i is the equilibrium constant of the i-th reaction and is defined by the following expression

$$K_i = e^{-\Delta G_i^{\circ}/RT} \qquad i = 1,\ldots,p \tag{5-51}$$

where ΔG_i° is the Gibbs free energy of i-th reaction in the system at standard conditions, and is defined by the following relationship

$$\Delta G_i = \Delta H_i^{\circ} - T\Delta S_i^{\circ} \qquad i = 1,\ldots,p \tag{5-52}$$

where ΔH_i° is the standard enthalpy of reaction i and ΔS_i is the standard entropy of reaction i. (5-52) might be written in the following form for steady state conditions (not necessarily standard conditions)

$$K_i = K_i^* \exp(-\Delta H_i/RT) \qquad i = 1,\ldots,p \tag{5-53}$$

where ΔH_i is the difference of enthalpy between products and reactants in the i-th reaction and K_i^* is the constant of entropy production and can be experimentally determined by a calorimetric procedure, (i.e., $K_i^* = e^{(\Delta S_i/R)}$ $\qquad i = 1,\ldots,p$).

Equations (5-57) should be interpreted in the following manner; Given initial equilibrium concentrations C_{jo} $\quad j = 1,\ldots,N$ the system atains equilibrium at concentrations C_j $\quad j = 1,\ldots,N$ through p simultaneous reactions according to the following expressions

$$C_j = C_{jo} + \sum_{\ell=1}^{p} \alpha_{\ell j}\xi_{\ell} \qquad j = 1,\ldots,N \tag{5-54}$$

Hence, given the initial concentrations $\{C_{jo}\}$ and the set of equilibrium constants $\{K_i\}$, the extents of reaction $\{\xi_{\ell} \quad \ell=1,\ldots,p\}$ can be determined using equations (5-49). It can be shown that the Jacobian $\det(\partial(K_{C_1},\ldots,K_{C_p})/\partial(\xi_1,\ldots\xi_{\ell}))$ is positive for all ξ's, so a unique solution is obtained.

Now a procedure for determining the coefficients β_{ij}, γ_{ij}, S_i and S_{-i} in equations (5-9) will be discussed.

Recall from (5-9), that the reaction rate r_i of the i-th reaction is given by

$$r_i = S_i \sum_{j=1}^{\ell} C_j^{\beta_{ij}} - S_{-i} \prod_{j=\ell+1}^{N} C_j^{\gamma_{ij}} \qquad i = 1,\ldots,p$$

At equilibrium, $r_i \equiv 0 \quad i \equiv 1,\ldots,p$ so the following relations are obtained

$$S_i \prod_{j=1}^{\ell} C_j^{\beta_{ij}} = S_{-i} \prod_{j=\ell+1}^{N} C_j^{\gamma_{ij}} \qquad i = 1,\ldots,p \qquad (5\text{-}55)$$

Since by setting the exponent β_{ij} or j_{ij} equal to zero one can make the respective product in (5-63) independent of C_j one might as well enlarge the range of products on both sides of (5-63) so that both products go from $j = 1,\ldots,N$. Then, the following relations result:

$$\prod_{j=1}^{N} C_j^{\gamma_{ij}-\beta_{ij}} = S_i/S_{-i} \qquad i = 1,\ldots,p \qquad (5\text{-}56)$$

which are the conditions of chemical equilibrium of the system. But from (5-49) and (5-56) an independent expression of equilibrium can be obtained i.e.,

$$K_{C_i} = \prod_{j=1}^{N} C_j^{\alpha_{ij}} \qquad i = 1,\ldots,p \qquad (5\text{-}57)$$

From (5-56) and (5-57) a <u>sufficient</u> condition for consistency can be obtained; i.e.,

$$\gamma_{ij} - \beta_{ij} = \alpha_{ij} \qquad i = 1,\ldots,p,\ j = 1,\ldots,N \qquad (5\text{-}58)$$

and

$$K_{C_i} = S_i/S_{-i} \qquad i = 1,\ldots,p \qquad (5\text{-}59)$$

In general, if the two sides of (5-56) were the same function of the respective sides of (5-57) then the two expressions would still be

consistent. Suppose $S_i/S_{-i} = F_i(K_{C_i})$ $\quad i = 1,\ldots,p$, then

$$\pi C_j^{\gamma_{ij}-\beta_{ij}} = F_i(\pi C_j^{\alpha_{ij}}) \qquad i = 1,\ldots,p \tag{5-60}$$

Now, this must be true for all values of C_j. So set

$C_1 = v^{1/\alpha_{i1}}$, $C_2 = C_3 = \ldots C_N = 1$, in the i-th equation of (5-68), then

$$F_i(v) = v^{n_i}$$

where $n_i = (\gamma_{i1} - \beta_{i1})/\alpha_{i1}$. Doing the same thing with $j = 2,3,\ldots,N$ shows that n_i has the <u>common</u> value,

$$n_i = \frac{\gamma_{ij} - \beta_{ij}}{\alpha_{ij}} \qquad \begin{matrix} j = 1,\ldots,N \\ i = 1,\ldots,p \end{matrix} \tag{5-61}$$

So, the <u>only</u> functional relation maintaining consistency between the two expressions for chemical equilibrium is that one should be the power of the other. But β_{ij} and γ_{ij} are phenomenological constants and have no necessary connection with α_{ij} except to satisfy the consistency relation (5-61). The constants n_i, $i = 1,\ldots,p$ are termed the <u>degrees of cooperativity</u> of the reaction-system. It has been shown experimentally in fragmented membrane preparations (Dixon and Webb [35]), using a Michaelis-Menten model for the membrane reaction system, that these coefficients (n_i) are lower than unity for low sodium concentrations, about unity for medium physiological sodium concentration and about 1.5 for high sodium concentrations. (Twice the physiological sodium concentration, see Chapter IV). Therefore, for this sodium-pump model, it will be assumed that $n_i = 1$, $i = 1,\ldots,p$.

Recall that in the case where a particular reaction of the system is assumed to be irreversible, condition (5-59) becomes,

$$K_{ci} = S_i \tag{5-62}$$

similarly (5-61) becomes,

$$n_i = -\frac{\beta_{ij}}{\alpha_{ij}} \tag{5-63}$$

with i in the subset of irreversible reactions of the system.

Notice also that in the case of reversible reactions (5-59) gives only the ratio S_i/S_{-i} so in order to determine both reaction constants one of them (usually the forward reaction constant S_i) has to be determined by experimentation and then with relation (5-59), the other may be computed.

Next, the general derivation of the steady state conditions for simultaneous reactions derived above will be specialized for the two groups of reactions of the sodium pump.

For the reaction group in the inner surface, (reactions (5-1) and (5-2)) the initial conditions satisfy relations (5-48) so,

$$\begin{aligned} K_{c1} &= K_1/(RT)^{-1} = K_1 RT \\ K_{c2} &= K_2/(RT)^{o} = K_{21} \end{aligned} \tag{5-64}$$

where K_1 and K_2 are computed using (5-59). The Gibbs-free energy of reactions (5-1) and (5-2) is not known exactly; nevertheless, several experimental measurements of this energy are available for reactions occurring in fragmented membrane preparations, which give at least bounds for the values of the free energy for the inside reactions, as will be discussed in the next section.

Similarly, for the group of reactions at the external surface ((5-3),

(5-4)),

$$K_{C_3} = K_3/(RT)$$
$$K_{C_4} = K_4/RT \qquad (5\text{-}65)$$

The extent of reaction for reactions (5-1) - (5-4) can be computed by solving 2 sets of decoupled simultaneous equations of the form of (5-55):

$$K_{C_1} = (C_{10}^{\circ} + \xi_1)(C_{20}^{\circ} - \xi_1 + \xi_2)(C_{40}^{\circ} + 2\xi_1)^2(C_{50}^{\circ} - 3\xi_1)^3$$
$$K_{C_2} = (C_{20}^{\circ} - \xi_1 + \xi_2)(C_{30}^{\circ} - \xi_2)^1(C_{60}^{\circ} + \xi_2)^1(C_{70}^{\circ} - \xi_2) \qquad (5\text{-}66)$$

where $(C_{1o}^{\circ}$ $i = 1,\ldots,7)$ are <u>any</u> equilibrium concentrations for the species reacting at the inside surface (reactions (5-1), (5-2)), ξ_1, ξ_2 are the respective extents of reactions, and $\{C_{io}\}$ can be found experimentally (i.e., see the values for C_{40}° and C_{50}° in table 4-1) and then, equations (5-66) are solved simultaneously for ξ_1, ξ_2 (recall that the solution is unique). Next, using (5-54) the equilibrium concentrations $\{C_i\}$ may be computed.

Similarly, for reactions (5-3) - (5-4), which occur at the outer membrane surface, the extents of reaction ξ_3, ξ_4 can be computed by solving the following simultaneous equations:

$$K_{C_3} = (C_{10}^{\delta} - \xi_3)(C_{20}^{\delta} + \xi_3 - \xi_4)(C_{40}^{\delta} - 2\xi_3)^2(C_{50} + 3\xi_3)^3$$
$$K_{C_4} = (C_{20}^{\delta} - \xi_3 + \xi_4)(C_{30}^{\delta} + \xi_4)(C_{80}^{\delta} + \xi_4) \qquad (5\text{-}67)$$

where $(C_{io}^{\delta}$ $i = 1,2,\ldots,8)$ are any equilibrium concentrations for the species reacting in the outside surface (reactions (5-3) - (5-4)). Also, $\{C_{io}\}$ can be determined experimentally so, knowing ξ_3, ξ_4 by (5-62), $\{C_i^{\delta}\}$ the equilibrium concentrations at the outer boundary of the axon membrane may be found.

Notice that by using (5-49), the reaction constants $(S_i$ $i = -1, -2,\ldots,4)$ for the boundary conditions of the dynamic equations of the model can be computed.

The development above indicates a procedure for computing the equilibrium concentrations at the boundaries; in order to completely specify the initial conditions, the steady state distributions of the carrier concentrations* $C_1^1(x)$, $C_2^1(x)$, $C_3^1(x)$ in the membrane must be determined. First, notice that they must satisfy the boundary conditions

$$\begin{aligned} C_1^1(0) &= C_1^o \quad , \quad C_1^1(\delta) = C_1^{\delta} \\ C_2^1(0) &= C_2^o \quad , \quad C_2^1(\delta) = C_2^{\delta} \\ C_3^1(0) &= C_3^o \quad , \quad C_3^1(\delta) = C_3^{\delta} \end{aligned} \tag{5-68}$$

Second, the carrier distributions, must satisfy the following homogeneous equations:

$$\frac{d^2 C_1^1}{dx^2}(x) = 0 \qquad 0 \leq x \leq \delta \tag{5-69}$$

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* The superscript ^{1} in C_i^1 indicates a steady state distribution.

$$\frac{d^2 C_2^1}{dx^2}(x) = 0 \qquad 0 \leq x \leq \delta \tag{5-70}$$

$$\frac{d^2 C_3^1}{dx^2}(x) = 0 \qquad 0 \leq x \leq \delta \tag{5-71}$$

Equations (5-69), (5-70), (5-71) are obtained by making the time derivatives equal to zero in eqs. (5-30) - (5-32) a condition which corresponds to a steady state, as discussed above.

Equations (5-69) - (5-71) can be integrated with the given boundary conditions (5-74):

$$\begin{aligned} C_1^1(x) &= \frac{C_1^\delta - C_1^o}{\delta} x + C_1^o \\ C_2^1(x) &= \frac{C_2^\delta - C_2^o}{\delta} x + C_2^\delta \\ C_3^1(x) &= \frac{C_3^\delta - C_3^o}{\delta} x + C_3^\delta \end{aligned} \tag{5-72}$$

With the latter, the model for the active transport process is now complete.

5.4 Qualitative Analysis of the Process in Terms of the Model

The model derived in the last section, presents several interesting mathematical characteristics that can be interpreted in physical terms and therefore presents a tool that can be used for carrying out an analysis of the operative characteristics of the pump.

First, some aspects of the dynamics of the pump will be considered.

The activation of the pump is determined by two factors: the first

is the ratio of Potassium vs. Sodium concentration in the inner solution, the second is the activation of enzyme complex E_2. As the ratio of potassium vs. sodium decreases in the inside, reaction (5-2) product (carrier $CPNa_3$) increases. This condition can be verified to follow in the model, by studying equation (5-15) corresponding to the chemical flow of $CPNa_3$ from reaction (5-1) ($\sigma_1^{\circ}(t)$); an increase in the ratio $C_{50}(t)/C_{40}(t)$ will imply an increase in $\sigma_1^{\circ}(t)$ and consequently by equation (5-28), an increase in the flow of this carrier, ϕ_1. But no increase of this ratio will increase appreciably $\sigma_1^{\circ}(t)$ if the concentration of CPK_2, the energy carrier ($C_2(0,t)$), does not increase since it enters as a positive multiplicative factor in the forward term of (5-15).

The rate increase of the concentration of CPK_2 in the inner boundary is proportional to $\sigma_2^{\circ}(t)$ (eq. (5-16)), which is directly proportional to the ATP concentration in the inner solution; therefore no activation is possible if the ATP concentration is below a certain threshold. i.e., from (5-16), for a given concentration of carrier CK_2 and a given chemical flow σ_1°, the concentration of ATP C_7, must satisfy the following inequality

$$C_7(t) > \frac{\sigma_1(t)}{S_2 C_7(t)} \qquad (5\text{-}79)$$

Consequently, the rate of flow of ATP from the organelles in the cell body to the axon must be controlled by a mechanism (which is not included in the formulation of the model) that is activated by the ATP concentration so that (5-79) is satisfied.

The form of equation (5-16) exhibits explicitly the regulation mechanism described in section 5-2. Namely an increase in $CPNa_3$ at the

inner boundary implies an increase of $\sigma_1^o(t)$ which in turn implies an increase of the flow rate of $CPNa_3$ across the membrane (Eq. (5-30)) and a decrease in the production $\sigma_2^o(t)$, since $\sigma_1^o(t)$ enters as a subtracting term in the equation defining $\sigma_2^o(t)$ (Eq. (5-16)).

Its important to notice that the system dynamics depends heavily on the initial condition that triggers the pump is not unique; this can be notice in the process by which this initial condition is computed. Given any equilibrium concentration set $\{C_{j\sigma}, C_{j\delta}\}$ for the species of the system, the initial condition for the pump will depend on the reaction extents which in turn depend on the activity of the surface reactions as measured by the reactions constants $\{K_{ci}\}$ (Eqs. (5-66) and (5-67)). This non uniqueness of initial conditions has been corroborated by experimental evidence Glynn; and shows the great flexibility of the pump for restoring the ionic concentration gradients of sodium and potassium. As discussed before, the pump will be activated by a given initial condition only if enzyme E_2-activation site is active. Pressumably it is activated by PH of the inside solution provided the concentration of ATP in the inner solution satisfies (5-79).

Finally, notice that the ionic transport model and the active transport model are coupled through the sodium and potassium boundary concentrations. In Chapter III, it was assumed that the ionic concentrations at the axon membrane surfaces were constant; in this chapter, the boundary concentrations of these ions, namely $C_{4\sigma}(t)$, $C_{5\sigma}(t)$, $C_{4\delta}(t)$, $C_{5\delta}(t)$ satisfy differential equations (5-13), (5-14), (5-19),((5-20) and therefore will not be constant in general; notice however, that the time constants for these

equations are directly proportional to the carrier mobilities u_1, u_2; and they are constant values that depend on the masses of the carriers which are much larger than the masses of the respective ions so that the ion-time constants are considerably smaller that of the carriers which implies that for practical purposes, the assumption of constant concentrations at the membrane surface is reasonable.

5.5 Conclusions

In this chapter a model for the active process in clamped axon membranes was derived. The model provides a tool for analyzing the physiological characteristics of the process and can be used also as the means for identifying the identity of the carriers operating in it and also, for determining the precise operation of the carrier mechanism and the catalytic and kinetic structure for its formation at the membrane boundaries. Also the model can be easily adapted for analyzing inhibition processes such as the produced by the cardiac glycosides.

CHAPTER VI

CONCLUSIONS

6.1 Introduction

This chapter presents a general overview of the main topics considered in this thesis with an emphasis on the main conclusions reached in the study. Also, several aspects of the dynamical operation of the axon membrane that were not covered in this study are mentioned as well as general suggestions about the possible ways by which the model developed in this thesis can be generalized or modified.

In short, the purpose of the study was to develop a mathematical model of the dynamical behavior of the axon membrane when subject to a voltage clamp in the longitudinal direction of the axon.

In the model, two processes are considered; the ionic transport process and the active transport process. The first is responsible for the generation of an electric pulse when the axon membrane is excited over a threshold and the second is the mechanism that restores and maintains operating conditions for the membrane i.e., ionic potentials.

The chapter is divided in 2 sections: 6-2 Review of the main conclusions in each of the chapters of the thesis and 6-3 Some suggestions for future research.

6.2 Review of the Main Conclusions in Each Chapter

a. Chapter I

Is an introduction to the subject of neuron cells in general, and neuron cell membranes in particular. It includes a short historical account of the research in the field and an elementary anatomical description of the neuron cell and in particular of the axon, including the bilayer unit theory of the cell membrane formulated by Dannielli.

b. Chapter II

Contains an analysis of the Hodgkin and Huxley model for the action potential from a systems theoretic point of view. It is shown that the model's dynamics presents a structure that makes it unidentifiable from input output data so that the model obtained, although it fits the experimental data measured at the output i.e., membrane potential, ionic current density, etc., cannot be guaranteed to correspond to the actual structure of the physical axon membrane system.

Also the control structure mechanism of the axon membrane on the action potential was analyzed in the context of the model; it was shown that the clamped membrane potential is the output of a dynamic system multiplicatively controlled by a time varying vector function (ionic-conductance vector) which is driven by a linear dynamical system. Although this structure of the internal representation of the control is completely empirical, it is shown in Chapter III that by physical analysis of the ionic transport process a very similar control structure is obtained.

Finally, a sensitivity analysis of the model shows that rather big variations of its parameters do not produce considerable changes in the output so instead of the parameters originally derived by Hodgkin and

Huxley, a family of parameters that agree with the experimentally measured variables, was found.

c. Chapter III

Is perhaps the most important chapter of the thesis, it describes the derivation procedure in obtaining a model for the ionic transport process. The model is derived in the context of well proven laws of field theory and irreversible thermodynamics. It is shown that the electric phenomena in the excited clamped axon membrane is a function of the flow of ions across the membrane due to their electrochemical gradients. This flow is not just passive but rather it is regulated actively by the membrane. The membrane ionic flow regulating mechanism is modelled by a phenomenological membrane potential-dependent time vector function denoted as ionic mobility (one entry for each ion species). An explicit expression for this function was derived using statistical mechanics arguments.

In the ionic transport model, each ion species has a concentration distribution inside the membrane. These distributions determine the electric field intensity distribution which in turn determines the time course behavior of the excited axon membrane potential.

The well-posedness of the equations of the model as well as the stability properties of the system were analyzed.

d. Chapter IV

Contains the main results obtained from a computer simulation of the model derived in Chapter III. It is shown that the model exhibits good agreement with the experimental data obtained by Hodgkin and Huxley.

Some interesting aspects of the role of calcium ions in the ionic transport process are analyzed.

The electric field intensity present across the membrane during equilibrium and during excitation conditions is computed and the physical implications of its dynamic course are analyzed.

Finally, the oscillatory phenomena observed in hyperpolaryzed membranes is reproduced using the model and some of its functional and physiological implications are briefly analyzed.

e. Chapter V

A model for the active transport process is derived in the context of field theory and irreversible thermodynamics. Some physiological aspects of the process are qualitatively studied using the model.

The model is developed by considering process as composed of 3 dynamical subprocess: 2 chemical mechanisms for combination of sodium and potassium ions with neutral carriers that allow these ions to be transported against their electrochemical gradients. These mechanisms are assumed to be located at the membrane inner and outer surface. The third subprocess is a diffusion of carriers across the membrane driven by their chemical gradients.

The mechanisms of activation of this process and the energy process which feeds it are studied in the context of the model.

Finally, the coupling mechanism between the ionic transport process and the active transport process is analyzed.

6.3 Some Suggestions for Future Research

One of the important aspects of the operation of the axon membrane

as a functional unit in the nervous information system, not covered in this study, is the propagation of the electric spike along the axon membrane. The ionic transport model, developed in Chapter III can be generalized to study this phenomena by considering an additional current density convective term in equation (3-16). The coefficient of this convective term, namely the axial velocity of pulse propagation is strongly dependent on the membrane surface electric properties which in this author's opinion are not well known. Therefore, in order to study the propagation phenomena a prior study of the membrane surface should be carried out.

In the active transport model some of the aspects of competitive behavior among ions different than sodium and potassium (e.g., calcium and magnessium ions) were not studied, although the complexity of the model increases when this phenomenon is considered. This increase in difficulty of the model is represented by the addition of equations to simulate this phenomenon, not in the mathematical structure of the model.

Finally, a complete simulation study of the active transport model should be carried out in order to determine how well it approximates the behavior of the real membrane system and also to obtain quantitative conclusions about the physical phenomena that characterize it.

A numerical evaluation of the parameters of the model developed in this chapter requires a considerable amount of experimental measurements as discussed in section 5-3. In [36], a dynamical model of the process is developed around an equilibrium state. This model permits the identification of the flow parameters (mobilities μ_1, μ_2, μ_3) and the reaction constants S_i, ($i = -1,-2,\ldots,4$) and therefore can be used for computing the data basis for the model developed in this chapter.

APPENDIX I

Discretization Scheme for the Dynamics Ionic Transport Model

Equations (3-33) - (3-36) that describe the dynamic behavior of ion transport model for the axon membrane during the action potential, are a set of nonlinear partial differential equations that cannot be solved using analytical techniques; therefore, a discretization technique must be used in order to get an approximate solution. The discretization scheme used in this thesis is briefly described here and a short analysis of some of the properties of the resultant difference equations is given.

General Description

The set of equations (3-33) - (3-36) is defined in a bounded region CR^2, whose boundary Γ is determined by the thickness of the membrane, δ, and T, a finite time interval (roughly 2msec) over which the major portion of the action potential takes place, therefore in set notation

$$\Omega = \{(x,t)\epsilon R^2 \mid 0\leq x\leq\delta \ , \ 0\leq t\leq T\} \qquad \text{A-1}$$

The system of equations (3-33) - (3-36) may be expressed by the following operator notation

$$\frac{\partial u}{\partial t} = \underline{L}(\underline{u}) \qquad \text{A-2}$$

where $\underline{L}(\underline{u})$ is a nonlinear partial differential operator and

$$\underline{u}(x,t) = \begin{vmatrix} C_1(x,t) \\ \cdot \\ \cdot \\ \cdot \\ C_5(x,t) \\ E(x,t) \end{vmatrix} \qquad \text{A-3}$$

The problem is to find a vector function u(x,t) that satisfies A-2 at each point of Ω with the boundary conditions;

$$\underline{\beta}_o \underline{u}(x,t)\Big|_{x=o} = \underline{u}_o(t)$$

$$\underline{\beta}_\delta \underline{u}(x,t)\Big|_{x=\delta} = \underline{u}_\delta(t) \qquad \text{A-4}$$

where $\underline{\beta}_o$, $\underline{\beta}_\delta$ is the operator notation for equations (3-38).

The solution of this problem is attempted by finding a table of approximate values of the vector $\underline{u}$ on a finite set of points $Y \subset \Omega$. The set Y: This table is called a grid and the individual points of Y are called meshpoints.

In this thesis a rectangular grid is used and a particular meshpoint of Y may be represented as $(n\Delta x, m\Delta t)$ $n = 1,2,\ldots N$, $m = 1,2,\ldots \ell$ where Δx is the spatial stepsize and Δt is the time stepsize (assumed constant).

N, ℓ are defined as follows:

$$N = [\delta/\Delta x]$$

$$\ell = [T/\Delta t] \qquad \text{A-5}$$

Thus, the number of points of the grid is equal to Nxl.

At each point of the grid a vector equation approximating A-2 is derived. The resultant discretized operator may be represented by

$$\underline{R}(\underline{u}_h(i\Delta x, k\Delta t)) = \underline{f}(i\Delta x, k\Delta t) \qquad \text{A-6}$$
$$i = 1,\ldots,l \quad , \quad k = 1,2,\ldots,N$$

$\underline{R}(\cdot)$ is a difference operator and $\underline{u}_h(i\Delta x, k\Delta t)$ is the approximate value of $\underline{u}(i\Delta x, k\Delta t)$.

Although the system of equations (3-33) - (3-36) is nonlinear, it would be advantageous if the resultant discretized operator $\underline{R}$ is linear, because the solution of equation A-6 reduces to a matrix inversion. Also, it is desirable that $\underline{R}$ be of such a form that the values of $\underline{u}_h$ at the point $i\Delta x$, $(k+1)\Delta t$ can be obtained by an equation which expresses them as a function of the values of $\underline{u}_h$ at an adjacent mesh-point say $i\Delta x$, $k\Delta t$. This condition reduces the dimension of $\underline{R}(\cdot)$ and therefore the storage requirements for implementing A-6 in a digital computer.

The boundary conditions A-4, are discretized over a set of points $Z \cup \Gamma$ giving the following operator equations:

$$\underline{r}_o\underline{u}_h(0, k\Delta t) = \underline{u}_o(k\Delta t)$$
$$\underline{r}_\delta\underline{u}_h(\ell\Delta x, k\Delta t) = \underline{u}_1(k\Delta t) \qquad \text{A-7}$$
$$k = 1,2,\ldots,N$$

where $\underline{r}_o(\ )$, $\underline{r}_\delta(\ )$ are the discretized approximations of operators A-4.

With the previous definitions the problem now is to find the table of values $\underline{u}_h(i\Delta x, k\Delta t)$ $i = 1,\ldots,l$, $k = 1,\ldots,N$ such that A-6, A-7 are satisfied.

It is assumed that the following limit holds:

$$\lim_{\substack{\Delta x \to 0 \\ \Delta t \to 0}} ||\underline{u}_h(i\Delta x, k\Delta t)||_U = 0 \qquad \text{A-8}$$

where the norm is defined on a function vector space U formed by the vector functions that satisfy A-2.

Moreover, it is assumed that for each point of the mesh Eqs A-6, A-7 have a unique solution provided that the initial conditions are properly chosen. This assumption is based on an analysis of the structure of equations (3-33) - (3-36), which exhibit a limit cycle type of dynamical behavior around the initial conditions (resting state). This behavior has profound physical implications on the characteristics of the subthreshold phenomena and will be analyzed to some extent in the thesis.

Difference Representation of the Differential Forms in (3-33) - (3-36)

The following set of equations give approximate expressions for the differentials appearing in equations (3-33) - (3-36). Their degree of approximation is studied for the model in the thesis. Here it is just important to remark that for good approximations it is necessary that

the spatial increment Δx and the time increment Δt have to be "small" as compared with δ and T respectively and also, for good convergence of the discretized equations (A-6, A-7) the following condition must hold:

$$\left|\frac{\Delta t}{\Delta x}\right| \leq 1 \qquad \text{A-9}$$

$$\left(\frac{\partial C_j}{\partial t}\right)_{i,k} \simeq \frac{1}{\Delta t}(C_j(i, k+1) - C_j(i, k))$$

$$\left(\frac{\partial C_j}{\partial x}\right)_{i,k} \simeq \frac{1}{2\Delta x}(C_j(i+1, k) - C_j(i-1, k)) \qquad \text{A-10}$$

$$\left(\frac{\partial^2 C_j}{\partial x}\right)_{i,k} \simeq \frac{1}{(\Delta x)^2}(C_j(i+1, k) - 2C_j(i, k) + C_j(i-1, k))$$

$$j = 1,2,\ldots,5$$

$$\left(\frac{\partial E}{\partial x}\right)_{i,k} \simeq \frac{1}{2\Delta x}(E(i+1, k) - E(i-1, k))$$

$$\text{A-11}$$

$$\left(\frac{\partial E}{\partial t}\right)_{i,k} \simeq \frac{1}{\Delta t}(E(i, k+1) - E(i, k))$$

where the subscripts i, k indicate the meshpoint $i\Delta x$, $k\Delta t$.

Then, using A-10, A-11 for each meshpoint i, k, equation (3-33) can be approximated by the following expression:

$$\frac{1}{\Delta t}C(i,\ k+1) = C_j(i+1,\ k)\left|-\frac{u_jRT}{(\Delta x)^2}+\frac{FZ_ju_j}{2\Delta x}E(i,k)\right| +$$

$$C_j(i,\ k)\left|\frac{1}{\Delta t}+\frac{2u_jRT}{(\Delta x)^2}+\frac{u_jFZ_j}{2\Delta x}E(i+1,\ k)\right| + \qquad \text{A-12}$$

$$C_j(i-1,\ k)\left|-\frac{u_jRT}{(\Delta x)^2}+\frac{u_jFZ_j}{2\Delta x}E(i,\ k)\right|$$

Equation (3-34), is approximated by the following difference equation:

$$\frac{1}{2\Delta x}\left(E(i+1,\ k) - E(i-1,\ k)\right) = \frac{F}{\varepsilon}\sum_{j=1}^{5} Z_jC_j(i,\ k) \qquad \text{A-13}$$

$$i = 0,1,2,\ldots,\ell+1 \qquad k = 0,1,2,\ldots,N$$

Equation (3-35) becomes:

$$J_j(i,\ k) = -\frac{Z_ju_jFRT}{2\Delta x}\left(C_j(i+1,\ k) - C_j(i-1,\ k)\right) +$$

$$u_jZ_j^2F^2E(i,\ k) \qquad \text{A-14}$$

$$j = 1,\ldots,5 \qquad i = 0,\ldots,1+1 \qquad k = 0,1,\ldots,N$$

Finally, Equation (3-36) becomes:

$$\frac{1}{\Delta t}\left(E(i,\ k+1) - E(i,\ k)\right) = \frac{1}{\varepsilon}\left(\sum_{j=1}^{5} J_j(i,\ k) - J(k)\right) \qquad \text{A-15}$$

$$j = 1,\ldots,5 \qquad i = 0,\ldots,1+1 \qquad k = 0,1,\ldots,N$$

Equations A-12, A-13, A-14, A-15 are valid for each meshpoint of the grid. The meshpoints (0, k), and (ℓ+1, k) correspond to the boundary of the region and the values of C_j. There are given by the boundary conditions: equations (3-38).

For computing purposes the variables in eqs A-12 - A-15 are organized in vector form as follows:

$$\underline{C}_j(k) = \begin{vmatrix} C_j(1, k) \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ C_j(i, k) \\ \cdot \\ \cdot \\ \cdot \\ C_j(\ell, k) \end{vmatrix} \qquad j = 1,\ldots,5 \qquad \text{A-16}$$

$$\underline{E}(k) = \begin{vmatrix} E(1, k) \\ \cdot \\ \cdot \\ \cdot \\ E(i, k) \\ \cdot \\ \cdot \\ \cdot \\ E(\ell, k) \end{vmatrix} \qquad \text{A-17}$$

$$\underline{J}_j(k) = \begin{vmatrix} J_j(1, k) \\ \vdots \\ J_j(i, k) \\ \vdots \\ J_j(\ell, k) \end{vmatrix} \qquad j = 1,\ldots,5 \qquad \text{A-18}$$

With definitions A-16, A-17, A-18, Equations A-12 - A-15 can be written as vector difference equations as follows:

$$\underline{C}_j(k+1) = \Delta t \underline{A}_j(k, \underline{E}(k))\ \underline{C}_j(k) + \Delta t \underline{B}_j(k)\ \underline{v}_j(k) \qquad \text{A-19}$$

$$j = 1,2,\ldots,5 \qquad k = 0,\ldots,N$$

$$\underline{UE}(k) = \sum_{j=1}^{5} Z_j \underline{C}_j(k) + \frac{1}{2\Delta x} \underline{Wv}(k) \qquad \text{A-20}$$

$$k = 0,\ldots,N$$

$$\underline{J}_j(k) = \underline{\alpha}_j(k, \underline{E}(k))\ \underline{C}_j(k) + \underline{\theta}_j \underline{v}_j(k) \qquad \text{A-21}$$

$$j = 1,\ldots,5 \qquad k = 0,\ldots,N$$

$$\underline{E}(k+1) = \underline{E}(k) + \frac{\Delta t}{\epsilon} \sum_{j=1}^{5} \underline{J}_j(k) - \frac{1}{\epsilon} \underline{M}\underline{J}(k) \qquad \text{A-22}$$

$$k = 0,\ldots,N$$

where $\underline{A}_j$ $j = 1,\ldots,5$ are $\ell x \ell$ tridiagonal matrices with elements defined as follows:

$$a^j_{i,i-1}(k) = -\frac{u_j RT}{(\Delta x)^2} + \frac{u_j FZ_j}{2\Delta x} E(i, k) \qquad \text{A-23}$$

$$j = 1,\ldots,5 \qquad i = 2,\ldots,\ell$$

$$a^j_{i,i}(k) = \frac{1}{\Delta t} + \frac{2u_j RT}{(\Delta x)^2} + \frac{u_j FZ_j}{2\Delta x} (E(i+1, k) - E(i-1, k)) \qquad \text{A-24}$$

$$j = 1,\ldots,5 \qquad i = 1,2,\ldots,\ell$$

$$a^j_{i,i+1}(k) = -\frac{u_j RT}{(\Delta x)^2} + \frac{FZ_j u_j}{2\Delta x} E(i, k) \qquad \text{A-25}$$

$$j = 1,\ldots,5 \qquad i = 1,2,\ldots,\ell-1$$

$\underline{B}_j(k)$ is a ℓx2 matrix with all -but b_{11} a $b_{2\ell}$- elements equal to zero and

$$b^j_{11}(k) = -\frac{u_j RT}{(\Delta x)^2} + \frac{u_j FZ_j}{2\Delta x} E(0, k) \qquad \text{A-26}$$

$$b^j_{\ell 2}(k) = -\frac{u_j RT}{(\Delta x)^2} + \frac{FZ_j u_j}{2\Delta x} E(\ell+1,k) \qquad \text{A-27}$$

$$j = 1,\ldots,5$$

$\underline{v}_j(k)$ are the boundary conditions of C_j $\quad j = 1,\ldots,5$:

$$\underline{v}_j(k) = \begin{vmatrix} C_j(0, k) \\ C_j(\ell+1, k) \end{vmatrix}, \text{ given, constant} \qquad \text{A-28}$$

$$j = 1,\ldots,5 \qquad j = 1,2,\ldots,5$$

$\underline{U}$ is the following constant ℓxℓ matrix:

$$\underline{U} = \frac{1}{2\Delta x} \begin{vmatrix} 0 & 1 & 0 & 0 & 0 & . & . & . & . & 0 \\ -1 & 0 & 1 & 0 & 0 & . & . & . & . & \\ 0 & -1 & 0 & 1 & 0 & & & & & \\ 0 & 0 & -1 & 0 & 1 & & & & & \\ & & & & & & & & & \\ & & & & & & & & & \\ & & & & & & & & & \\ & & & & & & & & & \\ & & & & & & & -1 & 0 & 1 \\ & & & & & & & & -1 & 0 \end{vmatrix} \qquad \text{A-29}$$

and $\underline{W}$ is a $2x\ell$ constant matrix with all its elements but w_{11} and $w_{2\ell}$ equal to zero

$$w_{11} = 1$$
$$w_{2\ell} = -1 \qquad \text{A-30}$$

and

$$\underline{\nu}(k) = \begin{vmatrix} E(0, k) \\ E(\ell+1, k) \end{vmatrix} \qquad \text{A-31}$$

$\underline{\alpha}_j(k, E(k))$ $j = 1,\ldots,5$ are $\ell x \ell$ tridiagonal matrices whose non zero elements are given by the following expressions:

$$\alpha^j_{i,i}(k) = u_j Z_j{}^2 F^2 E(i, k) \qquad \text{A-32}$$

$$\alpha^j_{i,i-1}(k) = - \frac{u_j Z_j FRT}{2\Delta x} \qquad \text{A-33}$$

$$\alpha^j_{i,i+1}(k) = - \alpha^j_{i,i-1}(k) \qquad \text{A-34}$$

$$j = 1,\ldots,5$$

$\underline{\theta}_j$ $j = 1,\ldots,5$ are $\ell x 2$ matrices with all their elements but θ^j_{11} and $\theta^j_{\ell 2}$ equal to zero

$$\theta^j_{11} = - \frac{Z_j u_j FRT}{2\Delta x} \qquad \text{A-35}$$

$$\theta^j_{\ell 2} = - \theta^j_{11} \qquad \text{A-36}$$

$j = 1,\ldots,5$

From A-21 in A-22 the following equation for $\underline{E}(k+1)$ is obtained:

$$\underline{E}(k+1) = \underline{E}(k) + \frac{\Delta t}{\varepsilon} \sum_{j=1}^{5} (\underline{\alpha}_j(k, E(k))\, \underline{C}_j(k) + \underline{\theta}_j \underline{v}_j(k) - \frac{1}{\varepsilon} \underline{M} J(k) \qquad \text{A-37}$$

where

$$\underline{M} = \begin{vmatrix} 0 \\ 0 \\ \cdot \\ \cdot \\ \cdot \\ 1 \end{vmatrix}, \quad \text{an } \ell \text{ vector} \qquad \text{A-38}$$

$\underline{E}(0)$, $\underline{C}_j(0)$, $\underline{v}_j(0)$ $j = 1,\ldots,5$, are given by the resting condition analysis of the system and $J(k)$ is given for every k.

Finally, the value of the potential at the inner surface of the membrane at each instant of time $k\Delta x$ $k = 0,\ldots,N$ is computed by approximating eq. (3-37) by a numerical integration procedure. Since the spatial interval is "small" as compared with the membrane thickness ($\Delta x = \delta/20$) a $^1/_3$ Simpson rule is adequate; therefore the electric potential at the inner membrane surface is given by

$$\psi(k) = -\left| \sum_{i=1}^{\ell} 2E(i, k) + 4E(i+1, k)) + E(0, k) + E(l+1, k) \right| \frac{\Delta x}{3} + V_r \qquad \text{A-39}$$

The ionic transport model has been approximated by a discretized set of equations whose solution can be obtained with a digital computer. A brief description of the logical steps followed in the implementation of the algorithm used in this thesis is given below

Step 1 Set k = 0

Step 2 Compute $\underline{E}(k+1)$ using Eqs A-22

Compute $\underline{\nu}(k)$ using Eqs A-20

Step 3 Compute $\psi(k)$ using Eq A-39

Step 4 Set J = 1

Step 5 Compute $\underline{C}_j(k+1)$ using Eqs A-19

Step 6 J←J+1 if J>5 go to step 7 otherwise go to step 5

Step 7 k←k+1 if k>N; stop otherwise, go to step 2

The initial equations (3-43) - (3-45) are integrated using the same program, by making $\underline{C}_j(k+1) = \underline{0}$ in A-19.

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