A MATHEMATICAL RECONSTRUCTION OF THE FROG ATRIAL ACTION
POTENTIAL BASED ON VOLTAGE CLAMP DATA

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A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE

MASTER OF SCIENCE
ELECTRICAL ENGINEERING

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APRIL 1983
ABSTRACT

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Recent advances in cardiac electrophysiology have allowed for the preparation of viable single frog atrial cells. These cells have properties which make them ideal for voltage clamp studies. The ionic currents observed under voltage clamp conditions from single frog atrial cells are analyzed with the use of automatic methods programmed on a computer. From the analyzed data, mathematical models are formed which describe the time and voltage dependence of the various ionic currents observed under voltage clamp conditions. These models are then combined, and a membrane action potential is reconstructed based on the analyzed ionic current data. This membrane action potential is then used as input to a nonlinear least-squares fitting routine in an attempt to accurately fit the model to experimental action potential data.
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS.................................................iii

CHAPTER

I. SIMULATION TECHNIQUES IN ELECTROPHYSIOLOGY......1
   Introduction.....................................................1
   The electrical analogue model of the membrane......................4
   Ionic currents....................................................6
   Gating processes................................................10
   Organization of the thesis....................................11

II. CARDIAC ELECTROPHYSIOLOGY...........................13
   The ionic basis for the atrial resting and reversal potentials..........13
   Rate theory and gating processes.................21
   Methods of recording from excitable cells...27
   History of cardiac electrophysiology.............37
III. THE CARDIAC ACTION POTENTIAL.........................41
   Properties of single atrial cells.................41
   Response to current stimuli...................43
   Ionic substitutions........................46
   The fast transient inward current...........48
   The model of the sodium current.............52
   The slow transient inward current............66
   The model of the calcium current.............74
   The dynamic maintained outward current......88
   The model of the potassium current...........87
   The background currents......................103
   Summary of the model equations..............113

IV. EXPERIMENTAL AND COMPUTATIONAL TECHNIQUES......119
   Experimental techniques....................119
   Data analysis techniques...................121
   The model program..........................132

V. SIMULATION RESULTS.................................135
   Simulation of the dynamic ionic currents...135
   Simulation of the membrane action
   potential..................................155

VI. SUMMARY...........................................167
APPENDIX A - Cable Theory.................................172
APPENDIX B - Analysis Program Listings..................175
APPENDIX C - Model Program Listing.......................218
APPENDIX D - The Gauss-Newton Least-Squares
              Minimization Method.........................237

BIBLIOGRAPHY..............................................239
ACKNOWLEDGEMENTS

My greatest appreciation and thanks go to Drs. John Clark (Rice University) and Wayne Giles (University of Texas Medical Branch at Galveston) without whose cooperation and aid this project would not have been possible. An equal share of the acknowledgement must go to the people at the University of Texas Medical Branch at Galveston who are responsible for the experimental work and assorted advice: Drs. Donald Campbell, Joseph Hume, Richard Nathan, Erwin Shibata, and Godfrey Smith.
CHAPTER 1

SIMULATION TECHNIQUES IN ELECTROPHYSIOLOGY

1.1 Introduction

The study of the origin of the heartbeat and electrical activity of cardiac membranes has intrigued physiologists ever since the mid-19th century, when sensitive galvanometers revealed the existence of current flowing in cardiac tissues. With the improvements in both equipment and techniques within the last fifty years, it has been shown that these minute currents are caused by movements of three major ions: sodium, calcium, and potassium. The sum of the voltage drops caused by these ionic currents as they cross the membrane resistance underlie the membrane action potential. But even with all the sophisticated equipment and techniques available today, the precise effect that each ionic current component has on the shape and duration of the cardiac membrane potential is still not known. Thus mathematical models are used to bridge this gap in knowledge.
The first, and most successful model was proposed by Hodgkin and Huxley (1952); and was used to fit their voltage clamp measurements of ionic currents in squid axon. Their model was able to reproduce and predict virtually all major electrophysiological phenomena seen in squid axonal membranes. Thus the modeling formalism that they have described has been used in virtually every subsequent membrane electrophysiological simulation.

Brady and Woodbury (1960) were the first to attempt to apply Hodgkin–Huxley formalism to cardiac tissues when they modeled the repolarization process in frog ventricular muscle. Noble (1962) first developed a complete model of electrical activity applicable to cardiac membranes. His model was closely based on the Hodgkin–Huxley scheme, but contained provisions for phenomena that are unique to cardiac membranes such as rectification and a maintained plateau of depolarization.

McAllister, Noble, and Tsien (1975) proposed one of the most complete and complicated models published to date in their attempts to model their data collected from Purkinje fibers. Beeler and Reuter (1977) have also proposed a model to fit their experimental results from dog ventricular muscle. The latter two models are the ones most widely accepted by electrophysiologists since they are easily capable of mimicking published data and can be
used in a predictive manner.

One major drawback of all the above models is that all the data used to create the model equations came from multicellular preparations; and thus represents the activity of hundreds of ionic current sources, all operating in a slightly asynchronous manner. The ideal cardiac model should attempt to describe the electrical activity due to only one cell. The data from multicellular preparations is also limited by other factors such as ion accumulation in extracellular spaces, nonhomogeneous current flow, and artifactual recording voltage drops caused by the series resistance.

The existence of these technical and analytical limitations of modeling the electrical activity of cardiac muscle is the basis for my thesis. In the past few years, it has become possible to isolate single cells from the atrial region of the frog heart (Hume and Giles, 1981) which remain viable, electrically active, and possess all the ionic currents normally seen in multicellular preparations. With improved voltage clamp equipment and the use of single suction microelectrodes, it is possible to record currents which are due to sodium, calcium, and potassium ionic movements. By either pharmacologically blocking one or more of these ionic currents, or by the use of the "voltage clamp" technique, it is possible to
deduce the role each current plays in the genesis of the action potential waveform.

My thesis will involve taking data collected from single cell voltage clamp recordings, and using this data to generate a mathematical model which is based on the modeling structure used originally by Hodgkin and Huxley, and later modified by McAllister, Noble, and Tsien. As proof of the "correctness" of the model equations, I will attempt to use the model to confirm certain phenomena seen in single atrial cells. Once it has been shown that the model can reproduce the major phenomena seen in atrial muscle, the model will then be used in a predictive manner to attempt to simulate some experiments which are at present technically impossible to perform. The goal of this modeling effort is to provide further insight into the details of how ionic current waveforms influence the shape of the observed action potential.

1.2 The Electrical Analogue Model of the Membrane

To provide a quantitative basis for the discussion of electrophysiological transmembrane phenomena, it is necessary to have a working model of the membrane. One approach widely used for electrophysiological and biophysical applications is to derive an electrical analogue or equivalent circuit.
Assume that a typical axon or cardiac cell is approximately cylindrical, with its length many times greater than its diameter. This cylinder is composed of a thin resistive-capacitive sheath, representing the membrane; which surrounds a highly conductive internal medium, representing the cytoplasm. This ideal cell is assumed to be immersed in a medium of uniform conductance. The sheath has two components in parallel: a conductance whose value is a function of instantaneous trans-sheath potential, and a specific capacitance determined by the thickness and the dielectric constant of the sheath. This description of a cell is similar to that of a transmission line, or an underwater telegraph cable. The partial differential equations which describe the distribution of voltage and current in a passive cable were first derived by Thomson (1855). Modification and simplification of these equations so that they could be applied to describe the voltage and current distribution in an axon was done by Hodgkin and Rushton (1946). By utilizing the results of cable theory, the three dimensional cellular model may be described by a semi-infinite, one dimensional collection of parallel resistive-capacitive circuits, which are connected at each end by resistances.

A further discussion of the derivation and use of the "cable equations" is given in Appendix A. By the use of
cable theory, an axonal or a cardiac cell membrane may be modeled as a collection of resistors (or conductors) and capacitances connected in parallel. The results of Fricke and Morse (1925) and Cole and Curtis (1938) verified that the membrane exhibited resistive-capacitive behavior. It was also found that it is possible to model a small patch of membrane (usually 1 cm$^2$) as a resistance and a capacitance in parallel. The magnitude of the resistance measured at rest has been found to vary with preparation used. The capacitance measured is approximately 1 $\mu$F/cm in a wide variety of excitable cells. In a classic series of experiments, it was shown by Cole and Curtis (1938) that during electrical activity in squid axon, the capacitance remained constant, while resistance varied in a characteristic fashion with transmembrane potential.

### 1.3 Ionic Currents

During the late 18th century, Galvani and other investigators demonstrated that electrical activity in some tissues could be elicited by an externally imposed current flow across the membrane. Early in this century it was shown that the excitable response of a nerve preparation consisted of contributions from both a sodium and a potassium transmembrane ionic current. The work of
Hodgkin and Huxley (1952a,b,c,d) on the squid giant axon provided the first major quantitative data, as well as a theoretical framework for describing the voltage and time dependent properties of these currents. The key to the success of Hodgkin and Huxley was the development of the voltage clamping technique (cf. Hodgkin, Huxley, and Katz, 1952). Briefly, the voltage clamp may be used to control the voltage dependence of the ionic currents, thus unmasking the time dependent phenomena. By systematic variation of a command voltage (the clamp potential) Hodgkin and Huxley were able to generate a series of current vs. time relationships at various potentials.

It had been previously shown by Hodgkin and Katz (1949) that when the external ionic composition was modified by addition or removal of one ionic species, one component of the transmembrane action potential also changed. Hodgkin and Huxley (1952a,b) found that when the clamp potential was set to a certain value (the reversal or Nernst potential), one component of the transmembrane ionic current disappeared. The reversal potential is where the electric field generated by the imposed voltage gradient is equal and opposite to the electric field generated by the concentration imbalance of a certain ion. By variations of the clamp potential and/or the ionic concentration, Hodgkin and Huxley (1952b) were able to
prove that the total time and voltage dependent ionic current in squid axon was mainly due to the sum of two distinct ionic components: sodium and potassium. Ionic substitution experiments allowed subtraction of the potassium component from the total current, and made it possible to derive current vs. time curves for the sodium and potassium currents at various membrane potentials. Since the instantaneous current-voltage relationship was shown to be linear in squid axon, conductance vs. time curves for various voltages were computed by dividing net current by the voltage driving force (membrane voltage less reversal potential). By applying the results of gating theory (see 1.3), Hodgkin and Huxley (1952d) were able to describe how the conductances for sodium and potassium vary with both time and voltage. It was found that the sum of these ionic conductances corresponded well with those seen by Cole and Curtis (1939, their Figure 2), and could be described by time and voltage dependent changes in the resistance of the axon membrane model discussed above.

With these observations, it is possible to describe the electrical activity of a membrane in terms of an electrical model. This model consists of a capacitance in parallel with a number of time and voltage dependent conductances. The capacitance acts as a charge reservoir,
and represents the ability of the membrane to store or supply ionic current during activity. The conductances represent the permeability of the membrane to the various ions which make up the individual ionic currents. By writing mathematical equations to describe this circuit, and by substituting measured values for each component, it is possible to use the model to reconstruct experimental data. This reconstruction will semi-quantitatively describe the ionic current changes which underlie the action potential.

Although the voltage clamp procedure was applied to cardiac tissues as early as 1950 (Draper and Weidmann, 1951), due to problems with proper polarization of a multicellular cardiac preparation its use did not become widespread until the development of the sucrose gap voltage clamp (Deck, Kern, and Trautwein, 1964). Until that time, all descriptions of cardiac ionic currents had to be purely qualitative. Early voltage clamp data indicated that the total current in cardiac tissues was also generated mainly by sodium and potassium ionic currents. Two major differences in these currents from those in squid axon were: (1) The time dependence of the potassium current was slower and (2) The relative sizes of the sodium and potassium currents were smaller. Reuter (1967, 1968) and Rougier, Vassort, Garnier, Gargouil, and
Coraboeuf (1969) later described an additional ionic current present in cardiac muscle due to an influx of calcium ions. This calcium current is responsible for the latter portion of the upstroke of the action potential. It is analogous to the sodium current in that it is transient; but the calcium current is an order of magnitude smaller than the sodium current, and exhibits slower kinetics.

1.4 Gating Processes

The concept of voltage dependent gating was first proposed by Hodgkin and Huxley (1952d) as a means of modeling the time and voltage dependence of ionic currents in squid axon. Hodgkin and Huxley expressed each ionic current as the product of a voltage driving force and a time and voltage dependent conductance, based on the resistive and capacitive membrane model discussed in Section 1.2. The key to the Hodgkin and Huxley theory is the time and voltage dependent conductance, which is expressed as the product of a constant maximal conductance term and a voltage and time varying dimensionless gating variable, whose value lies between 0 and 1 for a given voltage and time. The gating variable is expressed as the solution to a differential equation which describes a first order two state process, where the forward and
backward rate constants are functions of voltage only. Assuming $y$ represents the value of the gating variable in time, then $y=1$ represents an "open" state (i.e. able to pass current) for an ionic channel and $y=0$ represents a "closed" state. When $y=1$, all the conducting pathways are in the open state, and the conductance assumes its maximal value. When $y=0$, all the conducting pathways are in the closed state, and the conductance is zero. A fractional value for the gating variable corresponds to a conductance which is only partially activated. A more extensive discussion of voltage dependent gating is given in Chapter 2.

The success of the method of gating variables in fitting ionic current data from squid axon encouraged researchers to attempt to apply it to cardiac tissues. Brady and Woodbury (1960) and Noble (1962) applied the Hodgkin-Huxley scheme to respectively frog ventricular and sheep Purkinje fiber preparations. Both groups were able to generate cardiac-like action potentials by making changes in the kinetics and steady-state values of the sodium and potassium gating equations.

1.5 Organization of the Thesis
This thesis consists of six chapters which attempt to cover the various aspects of using experimental data to
generate a model of the atrial membrane action potential. The organization of the chapters are:

Chapter 1: An introduction to the concepts and techniques commonly used in simulation of electrophysiological phenomena.

Chapter 2: A more detailed discussion of some of the important topics discussed in Chapter 1 which will give a better description of how a model of the electrical activity in cardiac muscle is designed.

Chapter 3: Experimental data and models used to simulate each ionic current are discussed.

Chapter 4: A discussion of experimental and programming techniques, along with a description of the model used to simulate the electrical activity of the single frog atrial cell.

Chapter 5: Simulations and results derived from the model discussed in Chapter 4.

Chapter 6: Conclusions and discussion.
CHAPTER 2

CARDIAC ELECTROPHYSIOLOGY

2.1 The Ionic Basis for the Atrial Resting and Reversal Potentials

Early observations had shown that the viability and excitable properties of both nerve and muscle preparations is intimately linked to the composition of the ionic bathing media. With the development of microelectrode techniques and the voltage clamp, it became possible for the experimenter to directly study the effects of changes in the ionic composition on the various ionic currents which generate the action potential. This section will summarize results from these investigations.

2.1.1 Resting and Reversal Potentials

In order to better understand how ionic concentration gradients may control the resting and reversal potentials in frog atrium, the mechanisms which control these potentials in squid axon should be reviewed. Curtis and
Cole (1942) demonstrated that the magnitude of the squid axon resting potential was strongly dependent on the external concentration of potassium, and that this relationship could be described by the Nernst equation:

\[
E = \frac{RT}{ZF} \ln \frac{[x_i]}{[x_o]} \tag{2.1}
\]

\[
R = \text{Gas constant} \\
T = \text{Temperature in degrees Kelvin} \\
Z = \text{Ionic charge} \\
F = \text{Faraday's constant} \\
[x_i] = \text{Internal ionic concentration} \\
[x_o] = \text{External ionic concentration}
\]

where \( E \) describes internal less external potential. Later Hodgkin and Katz (1949) were able to show that changes could be best described by a modification of the Goldman constant field equation (Hodgkin and Katz, 1949):

\[
E = \frac{RT}{ZF} \ln \frac{[K_o]_i + [Na]_o + [Cl]_o}{[K_i]_i + [Na]_i + [Cl]_i} \tag{2.2}
\]

where

\[
I = P_X Z F \frac{VP}{RT} \frac{[X_o] \exp(-\frac{ZF}{RT} V) - [X_i]}{1 - \exp(-\frac{ZF}{RT} V)} \tag{2.3}
\]

\[
P_{Na} = \text{Membrane permeability to sodium} \\
P_{K} = \text{Membrane permeability to potassium} \\
P_{Cl} = \text{Membrane permeability to chloride} \\
[X_i] = \text{Internal concentration of ion x} \\
[X_o] = \text{External concentration of ion x}
When the permeability of the membrane to sodium and chloride is small with respect to potassium permeability, this expression reduces to the Nernst equation.

Hodgkin and Huxley (1939) were among the first to investigate the electrically excitable properties of squid axon, and were able to refute the Bernstein hypothesis of electrical excitability. The Bernstein hypothesis assumed that during an action potential, there was a nonselective increase in the ionic permeability of the membrane. Transmembrane potential would drop to zero as internal and external ionic concentrations equilibrated. By use of an internal electrode, Hodgkin and Huxley (1939) were able to prove that the internal potential did not decrease to zero during an action potential, but reversed sign and became positive with respect to the external potential. Hodgkin and Katz (1949) showed that the variation of peak depolarization and magnitude of the maximum rate of rise of the action potential was proportional to the external concentration of sodium. These relationships could also be adequately described by the Nernst equation.

At the same time that investigations into ionic movements in squid axon were being performed, experiments were begun to investigate the ionic basis of the resting and reversal potentials in cardiac muscle preparations. Early investigations on frog ventricular preparations by
Luttagau and Niedergke (1958) showed that the resting potential was not significantly changed by a 50% decrease in external sodium concentration. They also showed that the slope of the relationship between potassium concentration and membrane potential was not as steep as predicted by the Nernst equation, thus indicating that the resting potential was not totally due to potassium ion movements.

The first quantitative study of resting and reversal potentials for potassium in frog atrial muscle was done by Walker and Ladle (1973). By using potassium ion sensitive microelectrodes, they were able to directly measure internal potassium activity to determine the reversal potential. Their measurements show that the potassium reversal potential lies close to \(-95\) mV. The membrane resting potential is approximately 3 mV positive to the potassium reversal potential. They also show that the membrane resting potential is not strictly dependent on the potassium gradient (and thus is not Nernstian), but must be described by contributions from sodium, potassium, and chloride fluxes. By using the Goldman constant field equation (see equation 2.2), Walker and Ladle show that the ratio of the sodium to potassium permeabilities at rest is small. The ratio of chloride to potassium permeabilities is two orders of magnitudes larger than
that for sodium to potassium, and is comparable to the permeability for potassium alone. This implies that at rest, there is a small inward background current which maintains the membrane potential positive to the potassium reversal potential.

The results of Walker and Ladle have been confirmed in single frog atrial cells by Hume and Giles (1981) who find that for external potassium concentrations above 1.5 mM, the slope of the potassium concentration versus resting potential relationship is not adequately described by the Nernst equation. The slope of this relationship is similar to that seen in intact frog atrial preparations (Walker and Ladle, 1973; Hume and Giles, 1981). There is a discontinuity in the slope at 1.5 mM, when the membrane depolarizes for external potassium concentrations below 1.5 mM. This depolarizing action may be due to the increased permeability to other ions (sodium, calcium, or chloride) when the external potassium concentration drops below a critical level. There may also be a contribution from an electrogenic transmembrane transport mechanism, which is inhibited on removal of external potassium ions. Indirect measurements of the potassium reversal potential in single atrial cells (Hume and Giles, 1982a) indicate that this potential lies in the range of -95 to -100 mV., which is in agreement with the accepted potassium
2.1.2 Ionic Currents

When a brief superthreshold current or voltage stimulus is imposed on a resting excitable membrane, a rapid all-or-none depolarization results. This is followed by a slow repolarization back to the resting potential of the cell. This time dependent voltage change is generated by transmembrane ionic currents, which are in turn functions of transmembrane voltage. The forces which drive the currents arise from the electro-chemical potential gradient created by a concentration gradient and transmembrane voltage differences. The magnitude of this driving force is commonly expressed in terms of the Nernst equation.

Ionic currents may be subdivided into classes of gated and non-gated currents. The process of gating was briefly discussed in Chapter 1. Gated currents are ones in which the current is a function of both instantaneous membrane voltage and time. Non-gated currents are ones in which the current is a function only of the instantaneous membrane voltage. Either type of current may flow inwardly or outwardly across the membrane.

Inward currents were the first ionic currents to be studied in excitable cells (Hodgkin and Huxley, 1952a,b).
There are two ions normally present in the physiological medium which may carry inward gated ionic current in atrial tissue: sodium and calcium. Though both sodium and calcium can contribute to the total inward current; their effects on the total inward current are not similar, and thus merit separate discussion.

Sodium ions were shown to be the major carrier of inward gated ionic current in squid axon by Hodgkin and Katz (1949). Hodgkin and Katz were able to show that variations in the external sodium concentration (and thus the sodium reversal potential) caused approximately proportional changes in the threshold, maximum rate of rise, and peak depolarization of the action potential. Weidmann (1955) was the first to investigate the role of sodium in cardiac tissues. Similarly, he demonstrated that the upstroke, maximal rate of rise, and overshoot all depend on external sodium concentration.

The role of calcium in the upstroke was not discovered until much later. In squid axon, calcium has little effect on the action potential, due to the fact that membrane permeability to calcium ions is small. In contrast, it was found that for cardiac muscle calcium was responsible for the slower "second inward current" which caused the latter portions of depolarization (Niedergerke and Orkand, 1966; Rougier et. al., 1969). The calcium
current in heart is activated over a different voltage range, has a different time course, and has a different pharmacological sensitivity than the cardiac sodium current.

In both squid axon and cardiac tissues, it has been well documented that most of the outward (repolarizing) gated current carried by potassium ions (Hodgkin and Huxley, 1952b) Variations in the external potassium concentration change both the duration and the rate of repolarization of the action potential. At present, there is debate exactly how many independent outward potassium current mechanisms exist in heart. It is generally accepted that the squid axon membrane possess only one outward potassium mechanism (Hodgkin and Huxley, 1952d). In cardiac membranes, the number thought to exist ranges from one (Noble, 1962) to three (McAllister, Noble, and Tsien, 1975).

Background currents are best defined as currents which show no explicit time dependence (i.e. non-gated), and are functions of instantaneous voltage only. These currents are carried by ions which are driven through the membrane by their electo-chemical driving force, and whose flux is proportional to the permeability of the membrane to that ion. A background current may be generated by any ion in the ionic medium which is in electrochemical
imbalance. Normally, these currents are small relative to the gated ionic currents, and at rest the net ion flux is zero. In most cases, only a net background current can be measured.

2.2 Rate Theory and Gating Processes

First proposed by Hodgkin and Huxley (1952d), gating variables are used to model the time and voltage dependence of ionic currents in excitable membranes. This formalism is by no means the only acceptable one. But it is usually the simplest and most straightforward of many alternative theories, and is the formalism accepted by most electrophysiologists and referred to most often in the literature.

The gating theory of Hodgkin and Huxley makes two major assumptions. It is assumed that the ionic permeability of the membrane to each ion is a linear function of that ion's voltage driving force. Also, it is assumed that the gating processes for each ionic current are completely independent of each other. With these assumptions, the ionic current may be expressed as:
\[ I(V_M, t) = g(V_M, t) \cdot (V_M - E_r) \quad (2.4) \]

\[ g(V_M, t) = \overline{g} \cdot y(V_M, t) \quad (2.5) \]

\[ g(V_M, t) = \text{the voltage and time dependent conductance for the Jth ion} \]

\[ y(V_M, t) = \text{the gating variable for the Jth ion} \]

\[ E_r = \text{reversal potential for the Jth ion} \]

\[ V_M = \text{membrane voltage} \]

The time and voltage dependence of the conductance is controlled by the time and voltage dependent gating variable \( y \). This gating variable is expressed as the solution to a first order differential equation:

\[ y(V_M, t) = \alpha(V_M) \cdot (1 - y(V_M, t)) - \beta(V_M) \cdot y(V_M, t) \quad (2.6) \]

A physical interpretation of this differential equation assumes that it describes the rate of change of the occupancy of the activated state in a first order two state chemical reaction, where the rate constants of transfer between states are functions of voltage only. These rate constants are chosen to be exponential functions of transmembrane voltage.

Under steady-state conditions, the rate of change of the gating variable \( y \) with both time and voltage is zero, and equation 2.6 can be easily solved. The value of the gating variable \( y \) at steady-state is given by:
\[ y_\infty (V_M) = \frac{\alpha (V_M)}{\alpha (V_M) + \beta (V_M)} \] (2.7)

When the voltage is suddenly changed, \( \alpha (V) \) and \( \beta (V) \) change instantaneously with voltage. The solution of 2.5 which satisfies the initial condition of 2.6 is:

\[ y (V_M', t) = y_\infty (V_M) - (y_\infty (V_M) - y_o (V_M)) \exp \left( - \frac{t}{T (V_M)} \right) \] (2.8)

Where

\[ y_\infty (V_M) = \frac{\alpha (V_M)}{\alpha (V_M) - \beta (V_M)} \]

\[ T (V_M) = \frac{1}{\alpha (V_M) - \beta (V_M)} \]

The rate constants \( \alpha (V) \) and \( \beta (V) \) are evaluated at the potential the membrane achieves at infinite time at a constant voltage.

From these equations which give a description of the processes controlling ionic conductance, it is possible to attempt to describe how the membrane controls conductance changes. Chemical and electron microscopic investigations have shown that the cell membrane is composed of a tightly packed bilaminar matrix of long chain polar lipid molecules. Inserted into this lipid matrix are proteins which span the bilayer and may be responsible for the
dynamic properties of the membrane. One theory postulates that some of the transmembrane proteins contain physical channels or pores, through which ions may travel from one side of the membrane to the other. Each channel may be either opened, closed, or partially blocked by a group of subunits within the protein which can occlude the channel. Measurements via the "patch clamp" technique suggest that some structure analogous to a channel does exist. Gating variables attempt to describe the fractional conductance of the population of channels as functions of voltage and time.

In their experiments with squid axon, Hodgkin and Huxley (1952d) found that following a step change of potential, the sodium and potassium conductances increased with a delay of approximately a few hundred microseconds. On the termination of a step, the conductance exponentially decreased to a steady state value with no delay. To model this delay of conductance increase without a delay of conductance decrease, Hodgkin and Huxley (1952d) raised the value of the gating variable to an integer power. This produces the required delay on depolarization, while retaining the single exponential character of these functions on repolarization. A common interpretation for this power is that it describes the number of simultaneous events which must occur before a
conductance channel can reach the open state.

2.2.1 Modifications Necessary for Modeling

Cardiac Cells

In squid axon, Hodgkin and Huxley (1952d) observed that the permeability of the membrane to each ion was a linear function of that ion's driving force ($V_m - E_i$). For many other preparations, including cardiac muscle, permeability is a nonlinear function of driving force. One of the best known examples of this phenomena in cardiac muscle is the background potassium current $i_{\text{K}}$ (Hutter and Noble, 1960), whose current-voltage relationship inwardly rectifies for membrane potentials more positive than the potassium reversal potential (-95 mV).

Hodgkin and Huxley (1952c) found that the time constants for inactivation and reactivation of the sodium current in squid axon were the same at a fixed potential, and concluded that these processes were controlled by one gating mechanism. It was later shown by Gillespie and Meeves (1980) that this was not true in most cases; it is possible for these time constants to be very different at the same potential in squid axon. Haas, Kern, Einwachter, and Tarr (1971) have shown that the time constants for inactivation and reactivation of the sodium current at the
same potential in frog atrial muscle are not the same; reactivation is about fifty times slower than inactivation. Since Hodgkin-Huxley theory assumes that the time constants for inactivation and reactivation at one potential are the same, some modification to the Hodgkin-Huxley inactivation variable is needed. Though a simple modification to the inactivation variable can simulate most of the experimental results (Haas, et. al., 1971), to simulate the full range of experimental results a multi-state model such as one proposed by Chiu (1977) may be needed.

In the original Hodgkin-Huxley model, there was no provision for calcium currents. It is now known that calcium is an important ionic component which affects depolarization and contraction in both cardiac and skeletal muscle cells. Reuter (1968) first attempted to apply the Hodgkin-Huxley gating formalism to explain the kinetics of the calcium current. Recent data on calcium currents (Tillitson, 1979; Standen and Stanfield, 1982) suggest that such a voltage dependent model may be inappropriate in many cases. It is now thought that the calcium current in frog atrium may not behave as a conventional Hodgkin-Huxley mechanism (Fischmeister and Horakova, 1982; also see sec. 3.6). Thus a model for this current which includes some modifications to the
Hodgkin-Huxley formalism should be developed.

2.3 Methods of Recording From Excitable Cells

There are many ways to obtain information on the excitable behavior of cells. One of the most popular methods is by monitoring the electrical activity of the cell with some type of macroscopic recording device. Most early electrode systems were external, consisting of two metal plates placed near the preparation. Current was passed or voltage recorded across these two plates. The development of the Ling-Gerard micropipette in the late 1940's gave researchers a more reliable method of recording electrical activity. Briefly, this electrode is constructed from a narrow diameter glass tube, which is heated and drawn down to a tip diameter of 1 to .1 micron. This procedure is performed in such a way that the tip of the pipette remains open. This electrode is then filled with an electrolyte, and the tip inserted into the cell. Since the majority of the ions in the cytosol are either potassium or chloride, this electrolyte is usually potassium chloride. Voltage may then be recorded or current injected into the cell. This method has a major advantage in that it is possible to record the electrical activity of small groups of cells or even single cells. It is also possible to inject current to polarize a cell.
or a well connected group of cells; which is critical for the proper application of the voltage clamp (see 2.3.2).

Despite these advantages, there are also certain disadvantages associated with this method. Problems will arise when attempting to record from preparations where the geometry of the preparation can prevent uniform polarization of the structure. One preparation where this problem arises is in cardiac trabeculae. A cardiac trabeculum is composed of a high resistance cylindrical outer sheath surrounding a group of cells; each having a very high membrane resistance. The cells are connected to each other by low resistance pathways, and this preparation has a very short space constant. When current is injected into the trabeculum from a point source (the tip of a microelectrode), the voltage decrement along the length of the trabeculum will be very large. Thus the equipotential condition for proper voltage clamp operation cannot be met. For this preparation; in principle the use of external electrodes is best, and is most commonly done with the sucrose gap (see Section 2.3.3).

2.3.1 The Voltage Clamp

Virtually all of the information on the time courses of the ionic currents has been obtained from voltage clamp studies. Thus, the development of the voltage clamp can
be considered to be the key to recent progress of membrane electrophysiology. Voltage clamping provides a reliable method for studying the ionic current changes in a membrane by eliminating the voltage dependence of the ionic currents, leaving only the time dependence.

As discussed in Chapter 1, voltage changes seen in excitable tissues arise from ionic current changes in the membrane. The maximal rate of change of voltage is roughly proportional to the magnitude of the net ionic current for a nonproporgated (membrane) action potential. The simplest way to eliminate variations of the voltage with changes in ionic current is to null the net ionic current with an external current source. This is shown in the membrane model of Figure 2.1. By injection of the proper amount of external current $I_E$, the capacitive current $I_C$ will become zero and the rate of change of the voltage will also decrease to zero. The most common criteria for determining how much current $I_E$ to inject is to monitor the difference between the measured membrane voltage and a command voltage set by the experimenter. When this difference is large, a larger amount of $I$ is injected into the preparation. A simplified diagram of a voltage clamp circuit is shown in Figure 2.2. The differential amplifier provides the voltage error signal, which is amplified, converted to current, and then
Figure 2.1  Parallel resistive - capacitive model of the sarcolemma membrane. $C_m$ represents the membrane capacitance; $R_m$ represents the variable membrane resistance; $R_g$ represents the combined series resistance of the preparation and the measuring electrode.
Figure 2.2 A simplified voltage clamp circuit. Membrane voltage, $V_M'$, is measured as the sum of voltage drops across the series resistance $R_S$ and the parallel combination of membrane resistance $R_m$ and membrane capacitance $C_m$. The difference between actual membrane potential $V_m$ and the desired membrane potential $E_c$ is simplified by a factor $K$, and then inverted. A current proportional to the magnitude of this difference is fed back into the cell to minimize the cell of this voltage error.
injected back into the cell.

Though the theory of operation of a voltage clamp is simple, there are certain requirements which must be met to obtain proper operation of the clamp. One of the most important and difficult conditions to satisfy is the requirement of uniform polarization of the preparation. As previously discussed, the electrical behavior of the cell membrane is analogous to the passive electrical behavior seen in a cable; assuming there are no radial potential variations. To achieve uniform polarization, the complete length of the cable made equipotential. In practice, this condition is very difficult to achieve and/or verify.

Another practical difficulty in voltage clamping is the existence of series resistance. In multicellular preparations this resistance is partially due to the adherence of connective tissue to the membrane of the preparation, which causes a barrier to free ionic diffusion. Another portion of the total series resistance may be due to restricted intercellular spaces in multicellular preparations. This resistance is usually small, on the order of a few hundred ohms in cardiac tissue; smaller than the resting membrane or microelectrode resistances. Any current injected or measured in the membrane must flow through the combination
of the membrane and series resistances. From Figure 2.1 it is seen that current flow will cause a small voltage drop across the series resistance $R_S$, which will obscure the true transmembrane voltage recorded across the sum of the membrane and series resistances $R_m + R_S$. This error will cause severe problems when the voltage clamp is used, due to the fact that the amount of current injected should depend only on instantaneous membrane voltage. The addition of the series resistance voltage drop will cause too little or too much current to be injected to control the membrane voltage, resulting in an "escape" of the membrane voltage as it begins to vary with net current. This will severely limit the accuracy of any of the voltage clamp results. Series resistance will also limit clamp speed due to a slowing of the time constant of the charging curve of the membrane capacitance.

With these precautions, the voltage clamp may be applied. Hodgkin and Huxley (Hodgkin et. al., 1952; Hodgkin and Huxley, 1952a,b,c) were the first to use the voltage clamp for an experimental analysis. Their clamp for squid axon consisted of a two wire axial electrode which was longitudinally placed in the center of the axon. One wire was used to measure voltage, the other to inject current. Voltage and current were measured using an external coaxial electrode as a reference electrode. For
single or small groups of cells, it is possible to use microelectrodes to measure voltage and inject current in a method similar to that of Hodgkin and Huxley. One, two, or three microelectrode are inserted into the cell (see Figure 2.3), and the voltage and current are monitored with respect to a reference electrode.

2.3.2 The Sucrose Gap

A short discussion on the sucrose gap is necessary since much of the available data on cardiac muscle has been obtained with this method. The sucrose gap is an extracellular method of recording, which is well suited for contractile cardiac trabeculae.

Figure 2.3b shows a single sucrose gap chamber. It consists of a dish, divided into three compartments by rubber partitions. Each divider has a small diameter hole punched in it, through which the trabeculae is pulled. One end compartment is perfused with a high concentration of potassium chloride to depolarize the membrane which lies in that compartment. The center compartment is filled with a nonconducting solution of sucrose. Stimulation of the preparation occurs from the end compartment perfused with potassium chloride, and recording is done with a microelectrode in the opposite end compartment. When a stimulus is applied to one end of
Figure 2.3 Illustrations of voltage clamping methods. In all figures, $E_M$ is the membrane voltage, $E_C$ is the user-determined command voltage, $V_C$ is the clamp error voltage, and $I_M$ is the measured membrane current. A. Two microelectrode clamp. B. Single sucrose gap clamp. Striped region represents the sucrose barrier. C. Double sucrose gap clamp. D. Single suction microelectrode clamp of Hume and Giles (1981) for a single atrial cell. Dotted lines illustrate optional connections for series resistance compensation. [A, B, and C adapted from Carmeliet and Vereecke, 1979].
the preparation, current is forced to flow through the section of trabeculae which lies in the sucrose gap region. Since sucrose is nonconducting, there is little current loss into the intratrabecular spaces or into the external medium. Thus current is forced to flow through the intracellular pathways. A potential difference can be recorded across the high resistance sucrose gap. Due to the fact that the reference potential of the single sucrose gap is not at zero potential, only the difference in potential from the resting potential can be measured. To record absolute membrane potentials, the double sucrose gap (see Figure 2.3c) is utilized.

While at first glance this seems to be an ideal method to record from these tissues, the sucrose gap does have some serious difficulties. The major problem lies in current leakage across the gap region. Due to cable losses in the preparation, the gap should be very narrow, on the order of 50 microns. A gap this narrow will tend to have a small trans-gap resistance, and there may be a short circuit current flow from the stimulating chamber to the recording chamber (New and Trautwein, 1972; McGuigan, 1974). This short circuit current will tend to mask the true ionic current changes generated by the preparation.
2.4 History of Cardiac Electrophysiology

Draper and Weidmann (1952) were the first experimenters who attempted to adapt the internal microelectrode recording methods of Ling and Gerrard (1950) to cardiac muscle. These experiments investigated the resting potential and peak depolarization potential of dog ventricle and kid Purkinje fibres. The changes of these potentials with changes in extracellular sodium were also investigated in kid Purkinje fibres. Further work with this preparation by Weidmann (1955) showed that the maximum rate of rise and peak depolarization of the action potential were very sensitive to changes in the external sodium concentration. By use of a two pulse protocol similar to that used by Hodgkin and Huxley (1952c), Weidmann was also able to show that this sodium system possessed inactivation properties similar to the sodium system of squid giant axon (cf Hodgkin and Huxley, 1952c).

Brady and Woodbury (1960) presented a mathematical model of the repolarization process of frog ventricle, based on the Hodgkin-Huxley formalism. This model assumed that repolarization was due to a slowly inactivating potassium conductance, which opposed a residual amount of slowly inactivation sodium conductance.

Hutter and Noble (1960) showed that for small depolarizations from rest the total membrane conductance
decreases, and that the decrease is due to a decrease in the background potassium conductance (anomalous rectification). Based on this and other evidence, Noble (1962) presented a mathematical model of the action potential of calf Purkinje fibre, which used the Hodgkin-Huxley formalism to describe the time and voltage dependence of the ionic currents. This model assumed that there were two separate potassium conductances; one responsible for the anomalous rectification phenomena which controlled pacing along with a non-inactivation sodium conductance, the other controlled repolarization of the action potential.

Deck, Kern, and Trautwein (1964) were first to propose an improved method for voltage clamp of Purkinje fibres, which involved using two ligatures of a single long fibre to create a fibre which possessed short cable properties such that it could be easily voltage clamped. Using this method, Reuter (1967) was able to show that a calcium current exists in sheep Purkinje fibre preparation which is activated in the range of membrane potentials near the plateau of the action potential. Further investigation of this current (Reuter, 1968) showed that this current exhibited a very slow inactivation process, and that the percentage inactivation versus potential could qualitatively be described with the Hodgkin-Huxley
formalism.

By the use of a double sucrose gap voltage clamp technique Rougier, Vassort, and Stampfli (1968) were able to investigate the dynamic ionic currents in frog atrial trabeculae. They found that two major current systems existed; a fast transient inward current attributed to sodium, along with a slow outward potassium current which exhibited the anomalous rectification phenomena. Further experiments on this preparation by Rougier, Vassort, Garnier, Gargouil, and Coraboeuf (1969) showed that the fast transient inward current had both fast and slow components of inactivation. The fast component was attributed to sodium ion movements, while the slow component was due to a mixed calcium/sodium ion movement.

Noble and Tsein (1969a) proposed that two separate outward current systems existed for calf Purkinje fibres, both of which conform to Hodgkin-Huxley kinetics. These current components can be easily identified by their different kinetics, activation relationships, and reversal potentials. This observation of two separate current systems was also later observed in atrial trabeculae by Brown and Noble (1969a), who proposed that only the faster of the two components was responsible for repolarization in this preparation.

Based on previous voltage clamp data, McAllister,
Noble, and Tsien (1975) have proposed a complete model of the action potential and pacing phenomena of cardiac Purkinje fibre, based on the Hodgkin-Huxley formalism. This model incorporates transient sodium, calcium, and chloride current components, along with two maintained outward potassium currents. This model, which can reproduce many of the observed voltage clamp phenomena, is considered to be the most accurate and reliable model to date of cardiac muscle. In a fashion similar to McAllister et. al., Beeler and Reuter (1977) have proposed a model to simulate voltage clamp data from ventricular fibres.
3.1 Properties of Single Atrial Cells

Dissociated single frog atrial cells resemble tapered cylinders, with a length between 200 to 300 microns and a radius of 2 to 3 microns. A typical atrial cell is shown in Figure 3.1. Close inspection of this figure shows that this cell is relaxed, with a sarcomere spacing of about 2 microns. Using a two electrode clamp method Hume and Giles (1981) have found that the membrane capacitance of this cell is approximately 2.2 μF/cm², implying a total capacitance on the order of 100 pF. This value is somewhat larger than what is often reported in the literature (1 μF/cm²), due to the fact that the cell surface area is underestimated because of the presence of surface indentations (cavaeolae) on the sarcolemma. These indentations may increase the membrane surface area up to 50% over cylindrical estimates, which give a corrected value for membrane capacitance of 1.46 μF/cm². Assuming
Figure 3.1  The single bullfrog atrial cell
that this cell behaves like a passive R-C cable with open circuited ends at rest, a space constant of approximately 950 \text{ uM} is derived using short cable theory (Weidmann, 1952; Hume and Giles, 1982). A space constant of this magnitude implies that the decrement in voltage along the length of the cell under voltage clamp will be 2 percent or less. A space constant of this magnitude implies that there will be good voltage clamp control in this preparation, except under conditions where large and/or fast changes in membrane conductance occur.

3.2 Response to Current Stimuli

The development of the microelectrode made it possible to record intracellular potentials of in vitro cardiac preparations from both mammals and amphibians. Impalements performed by Wiedmann (1951) and Woodbury, Hecht, and Christopherson (1951) showed that the cardiac Purkinje fibre action potential elicited by short depolarizing current stimuli consists of a rapid depolarizing upstroke, a short, steady repolarizing plateau phase, followed by a slowly repolarizing return to the resting potential. Preparations taken from the pacemaker or Purkinje regions of the heart may exhibit spontaneous depolarization. The quantitative description of the mechanism of the squid axon action potential
(Hodgkin and Huxley, 1952d), provided a basis for similar explanations for cardiac muscle. Beeler and Reuter (1977) and McAllister et. al. (1975) have proposed models based on the Hodgkin-Huxley formalism to describe the electrical activity in ventricular trabeculae and Purkinje fibers respectively. These models were simple extensions of the squid axon action potential model of Hodgkin and Huxley (1952d), Both models used the Hodgkin—Huxley formalism to described the ionic currents to simulate the action potential.

A typical action potential recorded from a single frog atrial cell is shown in Figure 3.2a. On application of a depolarizing current stimulus, there is a rapid all-or-none depolarizing voltage change which causes the membrane potential to overshoot zero potential by between 30 to 40 mV. Repolarization consists of a slow decrease in membrane potential (the plateau phase) which lasts for between 400 to 600 msec; followed by a somewhat quicker secondary repolarization which begins negative to -50 mV. This action potential is similar in shape, maximum rate of rise, and duration to action potentials recorded from intact atrial preparations by microelectrode impalements (Hutter and Trautwein, 1956) or sucrose gap methods (Rougier et. al., 1968; Connor, Barr, and Jakobosson; 1975).
Figure 3.2 Action potentials from single bullfrog atrial cells, shown at two sweep speeds. A and B are under control conditions (110 mM Na⁺, 2.5 Ca⁺⁺, 2.5 K⁺), C and D are performed with 10 μg/ml TTX added to the Ringers solution. Vertical calibration 20 mV and 2.5 nA. Horizontal calibration 100 msec for A and C; 5 msec for B and D (From Hume and Giles, 1981).
3.3 Ionic Substitutions

Hodgkin and Katz (1949) discovered that the depolarizing phase of the action potential in squid axon was sensitive to the transmembrane sodium gradient, and the repolarizing phase was sensitive to the transmembrane potassium gradient. Wiedmann (1955) showed this to be also true for the cardiac action potential. Niedergerke and Orkand (1966) and Hagiwara and Nakajima (1965) found that the depolarizing upstroke of cardiac muscle was also sensitive to the transmembrane calcium gradient. The experiments of Rougier, Vassort, and Stampfli (1968) and Tarr (1971) on atrial trabeculae in the sucrose gap showed that both the maximum rate of rise and the peak depolarization are reduced when external sodium is removed or TTX (tetrodotoxin—a drug which specifically blocks the sodium conductance) is applied to the preparation. Changes in the external calcium concentration or application of manganese (Rougier, Vassort, Garnier, Gargouil, and Coraboeuf, 1969) will decrease the maximum depolarizing potential and the duration of the plateau phase. Removal of external calcium, along with application of TTX, eliminates the excitable response of the preparation. Application of TEA (Tetraethylammonium) (Connor et. al., 1975; Rougier et. al., 1968) will cause a reduction in the amount of available outward
current, which will produce an increase in the duration of the action potential.

Since the depolarizing upstroke is sensitive to both transmembrane sodium and calcium concentrations gradients, the mechanism which causes depolarization has been closely studied. Wright and Ogata (1961) were the first to note that the depolarizing phase of frog atrial muscle consists of two components. This phenomenon has been confirmed in the single atrial cell. Figure 3.2b shows the upstroke of Figure 3.2a on an expanded time scale. The depolarizing phase is composed of an early, rapidly depolarizing component (maximum rate of rise approximately 40 - 50 V/s), followed by a slower depolarizing component (maximum rate of rise approx. 2.2 V/s). Application of TTX (Figures 3.2c and 3.2d) will decrease the maximum rate of rise of the action potential (to 2 V/s), and cause the fast upstroke component seen in Figure 3.2b to disappear. This result is similar to that seen in frog ventricle by Niedgerke and Orkand (1966). From these results, along with the results of the ionic substitution discussed above; it is postulated that there are two separate mechanisms for depolarization in frog atrial muscle. These mechanisms depend on the transmembrane sodium and calcium concentrations, and correspond to the fast and slow depolarizing currents. This dual mechanism for
depolarization is also observed in mammalian cardiac tissues. Further evidence to support this hypothesis will be given in sections 3.4 and 3.6.

3.4 The Fast Transient Inward Current

3.4.1 The Sodium current in frog atrial trabeculae

The fast transient inward current was first investigated by Hodgkin and Huxley (1952d) in their studies of squid axon. Preliminary investigation of the ionic nature of this current in cardiac muscle (Wiedmann, 1955) indicated that the mechanism which caused the depolarizing phase of the cardiac action potential was similar to that of squid axon. In the period since the initial observations of Wiedmann, this inward current has been frequently studied. Due to its large size and problems with series resistance errors, little quantitative information on the kinetics of the cardiac sodium current is available.

One semi-quantitative study of the depolarization mechanism in frog atrium has been performed by Haas et. al. (1971). Under sucrose gap voltage clamp conditions, depolarization of the transmembrane potential to -30 mV activate an inward current which exhibits both fast and
slow components of inactivation. Ionic substitution experiments and application of TTX allowed Rougier et al. (1968) to identify the fast component with the movement of sodium ions across the membrane. By using a prepulse to partially inactivate the fast inward current before applying a test pulse (Hodgkin and Huxley, 1952c), Haas et al. were able to determine an inactivation vs. potential relationship for the sodium current which was similar to those found for the fast inward current of the other regions of the heart (Beeler and Reuter, 1970a; Dudel, Rudel, Peper, and Trautwein, 1967b). The inactivation process for the fast transient sodium current was described by single exponential time constant; the percentage and rate of inactivation were smooth functions of membrane potential.

One important finding of Haas et al. was that the rate of reactivation (the time necessary for the sodium current to return of full excitability following a voltage step) was also a potential dependent exponential process, with a time constant fifty times slower than that of inactivation when measured at the same potential. This discrepancy between inactivation and reactivation times was not included in the original Hodgkin - Huxley formalism. In squid axon, the time constants of the inactivation and reactivation processes have been shown
not to be the same at one potential (Gillespie and Meeves, 1980). The mechanism which is responsible for fast inactivation followed by slow reactivation in frog atrial muscle has not been identified.

3.4.2 Sodium current in single atrial cells

The transient inward current in single frog atrial cells under voltage clamp consists of two components. The early component is large (~5 nA) and exhibits a fast activation/inactivation process. The later component is much smaller (~200 pA) and exhibits much slower kinetics. This late, slow component has been identified as the calcium current (Hume and Giles, 1983) and is further discussed in the next section. On application of cadmium chloride to block the late transient calcium current, voltage clamp steps from rest (-80 mV) to membrane potentials between -60 and -20 mV elicit an inward current whose peak magnitude and kinetics are strongly dependent on membrane potential.

The current which is activated in this potential range is illustrated in Figure 3.3. The current record displayed here is recorded with 30 M TTX in the bath to reduce the peak current sizes to 300 to 400 pA. This reduction in size is necessary to eliminate voltage drops across the measuring electrode due to the series
Figure 3.3 Sodium current recorded from single bullfrog atrial cells in the presence of $1.5 \times 10^{-8}$ M TTX and $0.1$ mM CdCl$_2$. Holding potential, $-90$ mV; zero current potential $-80$ mV. Test pulses are to $+10$, 0, $-10$, $-20$, and $-30$ mV. Horizontal calibration 3 msec; vertical calibration 300 pA. (12/31/82; cell #1).
resistance errors (which can be as large as 40 mV near peak current). This will ensure adequate control of membrane potential. This current apparently peaks within 2 msec., then subsequently inactivates and returns to a steady level within 5 to 8 msec. The peak current - voltage relationship for this current is shown in Figure 3.4. The threshold for activation lies at -50 mV and peak current is reached near -20 mV. There is an apparent reversal potential for the sodium current at +35 mV.

3.5 The Model of the Sodium Current

3.5.1 Sodium Current Inactivation

To attempt to derive a description of the inactivation process, prepulses to various potentials of 50 msec duration were applied to the preparation, followed by a 10 msec test pulse to the peak current potential (-20 mV). By plotting the normalized peak values of current activated during the test pulse versus the prepulse potential, the steady-state inactivation relationship can be derived. This relationship is shown in Figure 3.5. Threshold for inactivation lies at -70 mV, and inactivation is complete by -40 mV; with a half inactivation point at approximately -55 mV. A fit of this
Figure 3.4 Maximum inward/minimum outward current-voltage relationship for the sodium current from a single frog atrial cell. $3 \times 10^{-8}$ M TTX used to reduce the peak sodium current, .1 mM CdCl$_2$ used to eliminate the transient inward calcium current. Holding potential, -85 mV.
Figure 3.5 Steady-state inactivation gating relationship \( (h_\infty) \) for the transient inward sodium current, measured in 10 M TTX and .1 mM CdCl\(_2\). Prepulse duration 500 msec, test pulse to -20 mV. Open circles are experimental data points, solid line is the fit of equation 3.1 to the experiment data where \( V_h = -54.12 \) mV and \( S = 3.486 \) mV\(^{-1}\). (4/30/81; cell #1).
data to the Boltzmann relationship (Hodgkin and Huxley, 1952d):

\[ h_\infty (V_M) = \frac{1}{1 + \exp \left( \frac{V_M - V_h}{S} \right)} \]

(3.1)

gives \( V = -54.1 \) mV and \( S = 3.486 \).

To investigate the kinetics of inactivation, a nonlinear least-squares fitting routine (DISCRETE, see Ch. 4) was used to fit the decay of current records like those of Figure 3.3 to a sum of exponential functions. One problem with this technique is that the capacitive transient will obscure the time course of decay of the current at potentials where the peak current is small or the inactivation process is fast. Thus this procedure was applicable only for potentials between +20 to -10 mV. In this range, the decay could be fit to a single exponential function with a time constant ranging from 1.6 msec at -10 mV to 0.5 msec at +20 mV. A full table of values is given in column 1 of Table 3.1.

Based on the evidence that the fast transient inward current inactivates as a single exponential process and possess a sigmoidal steady-state inactivation curve well fit by the Boltzmann distribution, it is assumed that the inactivation process can be described by the Hodgkin –
Table 3. Experimental and calculated values for the percentage activation \((h)\) and the time constant of inactivation \((\tau)\) for the sodium current.

<table>
<thead>
<tr>
<th>(h)</th>
<th>(\tau) (ms)</th>
</tr>
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<tbody>
<tr>
<td>1.0</td>
<td>0.294</td>
</tr>
<tr>
<td>2.0</td>
<td>0.494</td>
</tr>
<tr>
<td>3.0</td>
<td>0.593</td>
</tr>
<tr>
<td>4.0</td>
<td>0.692</td>
</tr>
<tr>
<td>5.0</td>
<td>0.791</td>
</tr>
<tr>
<td>6.0</td>
<td>0.890</td>
</tr>
<tr>
<td>7.0</td>
<td>0.989</td>
</tr>
<tr>
<td>8.0</td>
<td>1.088</td>
</tr>
<tr>
<td>9.0</td>
<td>1.187</td>
</tr>
<tr>
<td>10.0</td>
<td>1.286</td>
</tr>
</tbody>
</table>

\[ \tau = \frac{h}{p} \]
Huxley formalism. However, to derive values for the rate equations $\alpha_h(V)$ and $\beta_h(V)$, a full set of measurements of both the inactivation variable $h_\infty(V)$ and the time constant $\tau_h(V)$ versus potential are needed. Since it was impossible to experimentally obtain values for the time constant of inactivation more negative than $-10$ mV, the values between $-10$ and $+20$ mV listed in Table 3.1 were converted to rate constant form $(1/\tau)$, fit to a single exponential function, and then backwards extrapolated to more negative potentials. This extrapolation gives a crude estimate for the rate constant $\beta_h(V)$ where $h_\infty(V)$ is zero. Experimental and fitted values of this rate constant are given in columns 2, 3, and 4 of Table 3.1.

Since each point on the inactivation curve ($h_\infty(V)$) is a ratio of rate constants $\alpha_h(V)$ and $\beta_h(V)$, it is possible to solve for the rate constant $\alpha_h(V)$, once $h_\infty(V)$ and $\tau_h(V)$ are known. Derived values for the rate constant $\alpha_h(V)$ are given in column 5 of Table 3.1. These estimated values of $\alpha_h(V)$ and $\beta_h(V)$ were then used as input to a nonlinear least-squares routine (IACTFIT, similar to program ACTFIT listed in Appendix B) to generate a "best fit" to both the extrapolated values of $1/\tau_h$ and $h_\infty(V)$. The results of this procedure are shown in Figure 3.6, which demonstrates that the data are well fit by the model. The rate equations which best fit the
Figure 3.6 Experimental data and model results for the inactivation gating variable ('h_\text{in}') of the sodium current. Closed circles represent the experimental data, the solid line represents the fit predicted by the model equations in Table 3.5. A) Percentage inactivation versus potential. B) Rate constant of inactivation versus potential.
data were:

\[ \alpha_h (V) = 1.485 \times 10^{-6} \exp (-0.2482 (V + 8.77)) \]  

\[ \beta_h (V) = \frac{1.713}{1 + \exp (-0.061 (V + 8.77))} \]  

3.5.2 Sodium Current Activation

As seen in Figure 3.3, any attempt to derive quantitative information on either the activation relationship or the kinetics of activation from experimental records would prove futile, since the majority of the activation process occurs during, and sums with, the capacitive transient. Though a quantitative analysis is impossible, some qualitative observations can be made. For convenience, the activation process is assumed to follow Hodgkin - Huxley kinetics; and thus will exhibit a sigmoidal percentage activation versus potential relationship. From viewing the current records, it can be estimated that the activation process is very fast at most potentials, with a time constant on the order of 500 microseconds. Near the threshold of the current - voltage relationship (I-V) (-50 to -60 mV), the activation process markedly slows, with a time constant of about 1 msec. Since the threshold for the I-V lies at -50
mV, it is also reasonable to assume that the start of the activation relationship must lie in the same range.

Using these initial assumptions, the rate constants $\alpha_m(V)$ and $\beta_m(V)$ were determined by trial and error by simulating the sodium system and trying various values of $\alpha_m(V)$ and $\beta_m(V)$. The proper combination was considered to have been found when the simulated peak I-V, time course of the current, and rapid action potential upstroke matched experimental data. The rate equations derived by this method are shown in Figure 3.7. The values of the rate equations that are shown in this Figure are:

$$\alpha_M(V) = \frac{.1514 (V + 39.129)}{1 - \exp (-.392 (V + 39.129))} \quad (3.4)$$

$$\beta_M(V) = 1.6435 \exp (-.0338 (V + 39.129)) \quad (3.5)$$

3.5.3 Sodium Current Reactivation

As previously mentioned, the time constants for inactivation (time course of turn-off of current) and reactivation (time course for recovery of the current to it's peak magnitude when two pulses are given) may not be similar at the same potential in cardiac muscle. (Haas, et. al., 1971; Brown, et. al., 1981). Preliminary experiments on the time constant of recovery of sodium
Figure 3.7 Experimental data and model results for the activation gating variable ('m∞') for the sodium current. Closed circles represent the experimental data, the solid line represents the fit predicted by the model equations. Panel A: Percent activation versus recovery. Panel B: Rate constant of activation versus potential.
current in single atrial cells seem to support this observation.

Hodgkin - Huxley theory provides neither an explanation nor a model of this process, and assumes that the time course of inactivation and reactivation are the same. Haas, et. al. have suggested a simple modification of this theory which can simulate this phenomena by using a "reactivation" gating variable. The voltage dependence of this reactivation variable is the same as that for inactivation, but it possess a time constant much slower than that for inactivation. Thus the time course of inactivation will be controlled by the faster time constant of inactivation, while reactivation will be controlled by the slower reactivation time constant.

For the atrial sodium channel, this gating variable has been called "$k_\infty(V)$". The rate equations for this variable are obtained by slowing the time constant for gating variable $h_\infty(V)$ (inactivation process) while retaining the voltage dependence of $h_\infty(V)$. The time constant for this recovery variable was chosen to be 50 times that of inactivation. The voltage dependence of $k_\infty(V)$ and the rate constant $R_k(V)$ are shown in Figure 3.8. The values of the rate equations which fit these relationships are:
Figure 3.8 Model results for the recovery gating variable ('k_\infty') of the sodium current. A) Percentage recovery versus potential. B) Rate constant of recovery versus potential.
\[ \alpha_K(V) = 2.97 \times 10^{-8} \exp(-0.2482(V + 8.77)) \]  
(3.6)

\[ \beta_K(V) = \frac{0.03426}{1 + \exp(-0.061(V + 8.77))} \]  
(3.7)

3.5.4 Sodium current Instantaneous Conductance

The final portion of information needed for simulating the sodium system via the Hodgkin - Huxley formalism is the shape of the instantaneous current - voltage relationship. For the squid axon, Hodgkin and Huxley (1952b) showed that this relationship is a linear function of membrane potential. At present, no reliable data on this phenomena have been obtained from the single atrial cell preparation. It is assumed that the instantaneous current - voltage relationship follows the Goldman constant field expression (Eqn. 2.3), where \([\text{Na}_i] = 7 \text{ mM}\) and \(E = +70 \text{ mV}\). The best evidence for this assumption is the work of Campbell and Hille (1976) on the sodium system in frog skeletal muscle. The sodium system in frog muscle possess some striking similarities to the sodium system in frog atrium. Both systems seem to have similar inactivation kinetics, and similar positions for the inactivation curve. In the muscle preparation, the
permeability of the membrane is best described by the Goldman equation.

Hodgkin and Huxley (1952d) also found it necessary to raise their gating variable $m_\infty(V)$ to an integer power, since turn-on of the activation process began with an initial delay. By trial and error, they found that this power should be 3. For the same reason that it is impossible to make any quantitative statement on activation kinetics in frog atrium, it is also impossible to determine whether or not activation begins with a delay. In this case, gating variable $m_\infty(V)$ is raised to the second power, since this power seemed to work best with the chosen $m_\infty(V)$ curve. Since it is not known whether or not inactivation proceeds with a delay, the power of gating variable $h_\infty(V)$ is chosen to be one.

3.5.5 Summary of the Sodium Current Model

Experimentally derived values of gating variable $h(V)$ and time constant of inactivation were adequately fit by the inactivation model proposed by Hodgkin and Huxley (1952d), with $h_\infty(V)$ raised to the first power. Experimental results on the gating variable $m_\infty(V)$ and time constant of activation were unavailable; thus assumptions were made about their behavior and the model adjusted to agree with the experimental data. To
accurately simulate the kinetics of sodium current activation, the variable $m(V)$ was raised to the second power. Based on assumptions taken from work on skeletal muscle, the instantaneous conductance was assumed to follow the Goldmann equation, with a reversal potential at +70 mV.

3.6 The Slow Transient Inward Current

3.6.1 The calcium current in cardiac trabeculae

The "slow inward" or sodium/calcium current was first described by Reuter (1967, 1968) in mammalian Purkinje fibers. Its existence was later confirmed in ventricular (Beeler and Reuter, 1970b) and atrial (Rougier et al., 1969) preparations. Problems have arisen when attempting to define the ionic composition and time course of this current; due to its small size (approximately 10% of the magnitude of the sodium current), slow kinetics, and difficulty in accurately measuring its reversal potential. The magnitude of this transient slow inward current depends on external calcium (Beeler and Reuter, 1970b), and seems to play a vital role in control of the processes activating contraction (Horakova and Vassort, 1976)
Rougier et. al. (1969) were the first to investigate the calcium current in intact frog atrial muscle preparations. They found that this current is insensitive to TTX, and is blocked by application of manganese. Inactivation of the calcium current may be described as a single exponential process, whose rate of inactivation is dependent only on transmembrane voltage. Removal of all external calcium ions or application of manganese still permits generation of a "slow response" action potential, which exhibits a decreased peak depolarization and a shorter plateau phase. Under voltage clamp, there still was a significant net inward current in zero external calcium, which exhibited a current-voltage relationship similar to that of the calcium current. This relationship become net outward only on the additional removal of external sodium ions, and was not affected by TTX application. On this basis, Rougier et. al. postulated that the slow inward current in frog atrial muscle was a calcium current in the presence of calcium, or could be carried by sodium ions in the absence of calcium. Under normal conditions, they suggest that this current is a mixture of both calcium and sodium ions which pass through the calcium channel.
3.6.2 The calcium current in single atrial cells

Due to the small size and very positive reversal potential of the calcium current, there have been few quantitative studies to investigate this current in intact myocardial preparations. The development of single cell techniques have provided an improved preparation for the investigation of this current. On application of TTX to a single atrial cell, the fast inward sodium current is blocked and a smaller, slower inward current becomes evident. Figure 3.9 shows the currents elicited by voltage clamp steps to varying potentials during application of TTX. From this Figure, it is evident that this inward current exhibits a different voltage dependence than that for the sodium current (cf. Figure 3.3) and has slower kinetics. The apparent reversal potential for this current lies at about +60 mV. On closer inspection of Figure 3.9, it is seen that apparently this current does not fully inactivate after 100 msec. This apparent partial inactivation is due to the existence of a background component of steady inward current. This background current will be further discussed in Section 3.10.

Figure 3.10 shows the peak inward and minimum outward current-voltage relationship of this current. Threshold lies at -50 mV, and peak current is reached near 0 mV.
Figure 3.9 Calcium current recorded under voltage clamp in single bullfrog atrial cells in the presence of $3 \times 10^{-6}$ M TTX. Holding potential, $-90$ mV; zero current potential, $-94$ mV. Test pulses are to $+60$, $+50$, $+40$, $+30$, $+20$, $+10$ mV. Horizontal scale, 50 msec; vertical scale, 50 pA. (2/2/83; cell #4).
Figure 3.10 Maximum inward/minimum outward current-voltage relationship for the calcium current from a single frog atrial cell. \( \text{Ca}^{2+} \) was 2.5 mM. 3x10^{-6} M TTX applied to eliminate the transient sodium current. (2/2/83; cell #4).
This is strong evidence that this inward current is not a component of the transient sodium current which is insensitive to TTX, since the peak of this relationship is markedly different from the peak sodium current-voltage relationship (cf. Figure 3.4). There is also an apparent reversal potential for this current near +60 - +70 mV.

In a strict interpretation, it is incorrect to refer to a Hodgkin-Huxley type reversal potential when discussing the transmembrane calcium carrying mechanism in muscle cells. From studies of muscle tension (Fabiato, 1982) and intracellular measurements of free calcium (Marban, Rink, Tsein, and Tsien, 1982), it is known that at rest the intracellular free calcium levels are approximately three orders of magnitude smaller than the extracellular levels of calcium. The cell is able to maintain this gradient by sequestration of calcium, and facilitated and active transport of calcium ions out of the cell. Using the calcium sensitive luminescent protein aequorin, Allen and Blinks (1978) have shown that during a depolarization this gradient reduces by approximately two orders of magnitude. This decrease is due to calcium entry via the calcium channels increasing the intracellular calcium concentration, possibly along with calcium release from intracellular stores. The intracellular calcium levels return to their resting
levels in about one second, due to sequestration and sodium/calcium exchange.

Due to the fact that there are large changes in the transmembrane calcium gradient with time, it would seem improper to refer to a 'calcium reversal potential'; which would imply that this calcium gradient is constant with respect to both time and calcium entry. It is thought that in some species (snail neuron (Tillotson, 1979), Paramecium (Brehm, Eckert and Tillotson, 1978)) calcium influx via the calcium channel may cause a calcium mediated inactivation of the calcium current. In these preparations it has been shown that activation of the calcium current can cause large changes in intracellular free calcium levels (Brehm, et al, 1978) and pH (Ahmed and Connor, 1980); which can modulate the amount of calcium entry by an unknown phenomena (for review see Hagiwara and Bylery, 1981). Both skeletal and cardiac muscular preparations are known to have large amounts of calcium buffering capacity in the form of troponin, calmodulin, and myosin stores, which are able to control large changes in intracellular calcium. Computer simulations of calcium entry into a cylindrical cell with buffering capacity show that the internal calcium concentration can reach a steady level within 5 to 10 msec after a depolarization (unpublished observations). Thus, while it is not
possible to define a true 'calcium reversal potential' in the Hodgkin-Huxley sense (i.e. reversal is time, voltage, and current independent), it may be possible to define a 'quasi-reversal potential' ('Edf') which is constant over shorter periods of time. This 'quasi-reversal' is a function of external calcium concentration, and allows the Hodgkin-Huxley theory to be applied as a first order estimation to describe the inactivation kinetics of this current over a short (100 - 200 msec) period of time. Since there are large changes in the amount of internal free calcium during the first 5 msec of the calcium current, the Hodgkin-Huxley formalism would not be applicable for any quantitative description of the activation kinetics.

In summary, all the results presented above seem to indicate that the slow inward current in single frog atrial cells is primarily due to calcium ion movements. The magnitude, threshold potential, apparent reversal potential, and voltage dependence are consistent with those previously observed in intact myocardium.

There probably also is a small contribution from sodium ions, which may pass through the calcium channel at potentials more negative than 0 mV. Assuming that the net current which passes through the calcium channel can be described by the Goldman – Hodgkin – Katz equation
(equation 2.3), a net reversal potential of +80 mV for this mixed calcium/sodium current would indicate a sodium/calcium permeability ratio of 0.0033 (assuming a surface potential of 0 mV). At potentials near the calcium zero current (reversal) potential, the current observed under voltage clamp may be carried by fluxes of potassium ions which pass outwardly through the calcium channel (Lee and Tsien, 1982).

3.7 The Model of the Calcium Current

3.7.1 Calcium current inactivation

The inactivation versus potential relationship for the calcium current is obtained via a two pulse protocol similar to the one described in Section 3.5.1. A plot of the normalized values of test pulse current versus prepulse potential is given in Figure 3.11. A listing of these values is given in column 1 of Table 3.2. This relationship is sigmoid in shape, much like that for the sodium inactivation variable (Figure 3.5). This data is well fit by the Boltzmann relationship (Eqn 3.1), where \( V = -29 \text{ mV} \) and \( S = 6.23 \). Threshold for inactivation of the calcium current lies at -40 mV, with inactivation being complete at -10 mV.
Figure 3.11 Steady-state inactivation gating relationship ('f∞') for the transient inward calcium current measured in a blocking dose of TTX (3x10^{-8} M). Prepulse duration 100 msec, test pulse 0 mV. Open circles are experimental data points, solid line is the fit of equation 3.1 to the experimental data, where $V_b = -29$ mV and $S = 6.23$ mV$^{-1}$.
Table 3.2 Experimental values for the percentage inactivation \( f_\text{inactiv.} \) and the time constant of inactivation \( \tau_f \) for the calcium current.
Investigation of the kinetics of inactivation of the calcium current may be somewhat more difficult than that for the sodium current. This is because inactivation is a slow process, and may be obscured by the activation of the potassium current (see Section 3.8) at potentials more positive than +40 mV. Using the non-linear least-squares fitting program DISCRETE (Provencher, 1976), it was found that the time course of inactivation of the calcium current was well fit by one exponential for potentials more negative than 0 mV. For potentials more positive than 0 mV, the inactivation time course becomes bi-exponential; and for very positive potentials it is distinctly non-exponential. For the situations where the current is adequately fit by one exponential, the time constant shows little potential dependence ( = 30 - 40 msec). In some cases the time constant becomes slower at potentials negative to 0 mV. For potentials positive to 0 mV where two or more exponential components are needed, the time constants do not vary smoothly with potential; irregular changes in either time constant occur at each potential. For potentials where time constants are non-exponential, it is assumed that activation of the outward current contaminates these records.

Many competing theories have been proposed to explain the single/double exponential time course and the lack of
potential dependence of the inactivation time constant often seen in cardiac calcium systems. One of the simplest hypothesis is that above a certain internal calcium concentration, internal calcium ions can somehow block further calcium entry by binding into or near the calcium channel mouth, or they may inactivate the voltage dependent calcium carrying system via a special site. The bi-exponential decay of the current will be the sum of a voltage and a current dependent inactivation, which proceed on differing time courses. This type of behaviour has been postulated to occur in insect muscle (Standen and Stanfield, 1982). This mechanism probably does not dominate inactivation in frog atrial cells for the following reasons. Lanthanum (La^{+++}) has been shown to reduce the peak magnitude of calcium currents in single atrial cells (Nathan and Giles, 1983). Application of .1 M La^{+++} will reduce peak current size by 20%, thus reducing the amount of calcium ion entry via the calcium channel. This dose of La^{+++} has no effect on the time course of inactivation of the calcium current (unpublished observations). Computer simulations of calcium entry into a cylindrical cell also show that there is not a great enough accumulation of calcium ions within the specified time to markedly affect either the peak current or the time course of inactivation of the calcium current.
(unpublished observations, also see Fischmeister and Horackova, 1983).

At present, there is no suitable formalism for describing the kinetics of the calcium current which can also account for other phenomena (reactivation, tension generation) associated with this current. As a starting point, since this current does possess a conventional voltage dependent Hodgkin - Huxley inactivation gating variable, it is assumed that the inactivation process can be modeled using the Hodgkin - Huxley formalism (see Section 3.6.2 for a discussion of this topic). Time constant information was obtained by fitting the time course of inactivation to a single exponential whenever this was reasonable. A listing of the chosen time constant values is given in column 2 of Table 3.2. Figure 3.12 displays the inactivation relationship ("f") and the time constant ("\(\tau_f\)") versus potential and the fits to this data. Rate equations \(\alpha_f(V)\) and \(\beta_f(V)\) were derived, and the fit to the data is given by:

\[
\alpha_f(V) = 9.46 \times 10^{-6} \exp(-.1657(V - 18.44))
\]

\[
\beta_f(V) = \frac{.067318}{1 + \exp(-.0244(V + 6.257))}
\]
Figure 3.12 Experimental data and model results for the inactivation gating variable (\(f_\infty\)) of the calcium current. Closed circles represent the experimental data, the solid line represents the fit predicted by the model equations in Table 3.5. A) Percentage inactivation versus potential. B) Rate constant of inactivation versus potential.
3.7.2 Calcium Current Activation

The same problems which prevent a quantitative analysis of the activation parameters of the sodium system also prevent analysis of the calcium system. Even though the activation of this current is slower, the capacitive transient still obscures a major portion of the activation process. As previously discussed (see Section 3.6.2), Hodgkin-Huxley kinetics probably not apply to the activation process, due to large and rapid changes in the transmembrane calcium gradient. Since no quantitative information on this process is available, a reasonable first approximation is to assume that Hodgkin-Huxley kinetics can be used to describe the activation process. This will allow derivation of some qualitative information about the kinetics of activation. From the plot of the calcium current data (Figure 3.9), it is seen that the currents apparently peak in 5 to 7 msec. Since the capacitive transient obscures a major portion of the activation phase, it is assumed that this current peaks in 2 to 4 msec. To derive an estimate for the shape and position of the percent activation versus potential relationship, the peak current - voltage relationship shown in Figure 3.10 was corrected for the presence of the persistent inward background current (Section 3.10), which is assumed to be a current channel distinct from the
Figure 3.13 Experimental data and model results for the activation gating variable (\(d_\infty\)) for the calcium current. Closed circles represent the experimental data, the solid line represents the fit predicted by the model equations. Panel A: Percent activation versus recovery. Panel B: Rate constant of activation versus potential.
The time constants for the process of inactivation over the range -30 to +10 mV have been discussed in a previous section (Section 3.7.1). Experiments have been carried out to investigate the time course of calcium current recovery at potentials near the resting potential.

Two pulses of 100 msec duration and spaced at various intervals are applied to the preparation. For short interpulse intervals, the current activated by the second pulse will be negligible. As this interval lengthens, the peak current activated by the second pulse will increase in size until it equals the peak current activated by the first pulse. The time constant of recovery is defined as the time required for the peak current activated by the second pulse to reach 67% of the peak current of the first pulse. This time constant is a function of the interpulse potential. At present, it is not known whether this relationship is a linear or exponential function of membrane potential. One experiment performed at -30 mV seems to indicate that recovery slows even more at this potential. Shimoni (1981) has indicated that the time constant for reactivation in frog atrial trabeculae at potentials positive to 0 mV is indeed slow, with time constants on the order of seconds.

The Hodgkin - Huxley formalism cannot account for this behaviour. Following the suggestions of Kohlhardt,
Krause, Kubler, and Herdy (1975) and Fischmeister and Horackova, (1982), a second reactivation variable has been used. This variable ('\(e_\infty\)') has a voltage dependence identical to the inactivation variable '\(f_\infty\)', but has slower kinetics. Values of this time constant were derived by trial and error, and found to be about three times as slow as the inactivation time constant. The voltage dependence and rate constant of this reactivation variable are shown in Figure 3.14. The rate constants of this variable are given by:

\[
\alpha_e(V) = 1.8696 \times 10^{-6} \exp\left(-0.1657(V - 18.44)\right)
\]

\[
\beta_e(V) = \frac{0.0133}{1 + \exp\left(-0.0244(V + 6.257)\right)}
\]

3.7.4 Calcium Current Instantaneous Conductance

There is at present no experimental data which can be used to define the shape of the membrane permeability relationship for the calcium ion. A recent theoretical study on calcium currents in frog atrial trabeculae (Fischmeister and Horackova, 1983) assumed that this relationship was linearly related to membrane voltage. Experimental evidence of the instantaneous calcium conductance in insect muscle (Ashcroft and Stanfield,
Figure 3.14 Model results for the recovery gating variable (\(e_\infty\)) of the calcium current. A) Percentage recovery versus potential. B) Rate constant of recovery versus potential.
1982) indicate that this relationship is fit by the Goldman equation (Eqn. 2.3). On the basis of this experimental evidence, the background conductance of the calcium system is modeled by a permeability mechanism, as expressed by the Goldman equation. The internal calcium concentration was chosen to match the observed calcium reversal potential of +70 mV in normal (2.5 mM) calcium.

Since quantitative experimental data on activation kinetics is unavailable, it is not known whether the calcium activation process proceeds with a delay. Working with dog ventricular muscle, Beeler and Reuter (1970b) found that the activation and inactivation processes were best fit by raising gating variables d and f both to the first power. There is evidence that in snail (Aplysia) (Brown, et al, 1981), the activation gating variable must be raised to the second power. Experiments on insect muscle (Ashcroft and Stanfield, 1982) indicate that the gating variable should be raised to the third power. Due to the disparity in results between different experimenters, the simplest method was chosen where both the d and f gating variables are raised to the first power.

3.7.5 Summary of the Calcium Current Model

Experimentally derived values for the percentage
inactivation ('$f_\infty(V)$') and time constant of inactivation ('$\tau_f(V)$') were fit to $\alpha_f(V)$ and $\beta_f(V)$ equations, assuming that Hodgkin–Huxley gating theory was applicable. Since no quantitative data was available on the activation process, it was also assumed to follow the Hodgkin–Huxley formalism, and values for rate equations $\alpha_d(V)$ and $\beta_d(V)$ were estimated. Due to the further lack of quantitative information on the background conductance, this was assumed to have a shape dictated by the Goldmann equation, with a reversal potential located at +70 mV. The activation and inactivation processes are assumed to occur with no initial delays, which eliminates the need to raise either $d$ or $f$ to a power.

3.8 The Dynamic Maintained Outward Current

3.8.1 Potassium currents in atrial muscle

The outward current(s) have been extensively studied in cardiac trabeculae from the frog atrium. Rougier et al. (1968) were the first to investigate this current under voltage clamp conditions. They reported that two components of outward current are evident upon voltage clamp depolarizations to potentials positive to 0 mV. The first component is an instantaneous outward current
"jump", which exhibits anomalous (inward) rectification. The second component is a slowly developing outward current, which exhibits rectification after a time delay. This delayed current is not affected by either TTX or manganese application, which indicated that it was mainly carried by potassium ions. One intriguing finding of Rougier et. al. (1968) is that the "tail" currents seen on repolarization after a 1200 msec depolarization (which give an indication of the magnitude of the membrane chord conductance at that time) are all negative, and the magnitude of these negative "tails" increase for larger amounts of delayed rectifier current flow (see Rougier et. al., 1969 their Figure 4). For an outwardly directed current, it is expected that the polarity of the "tail" currents would be positive. Rougier et. al. tentatively attribute this phenomena to accumulation of potassium ions in the intercellular spaces of the trabeculae.

Brown and Noble (1969a,b) made an extensive study of the properties of the outward current system in atrial trabeculae. Using long duration voltage clamp pulses, they observed the delayed rectification phenomena. In contrast to the observations of Rougier et. al. (1968), Brown and Noble observed both outward and inward tail currents on repolarization. The polarity of the tail seemed to depend on both the magnitude and duration of the
preceeding depolarization. In this study the time course of the tail for depolarizations of short duration may be described by a single exponential, while the time course for longer durations is distinctly non-exponential. After graphically subtracting out this non-exponential component, the remaining current decay was well described as the sum of two single exponentials. Brown and Noble found that the fast time constant which results from this separation was similar to the single time constant seen during depolarizations of short duration. With this information, Brown and Noble (1969a) proposed that two independent outward current pathways (ix1 and ix2) exist in atrial muscle. These are distinguishable by their different reversal potentials and their time constants of decay near the resting potential. The third slow non-exponential component was attributed to potassium accumulation effects.

Further analysis of the slower of the two exponential current components (Brown and Noble, 1969b) showed that it was possible to describe this current in terms of the Hodgkin-Huxley formalism. It was found that this current exhibited conventional Hodgkin-Huxley kinetic behavior, but had a steady-state current-voltage relationship which was inwardly rectifying and exhibited a reversal at about -40 mV. The slow kinetics of this current with respect to
action potential duration indicated that this current plays no role in the normal action potential generation mechanism.

Following the discovery of two independent outward current components which are associated with repolarization in frog atrial muscle, other groups attempted to produce a quantitative explanations of the repolarization process. De Hemptine (1971a) analyzed the faster of the two components described by Brown and Noble (1969a), and found that this current exhibited delayed rectification, which necessitated raising the Hodgkin-Huxley potassium gating variable to the second power to properly describe this current. On performing a mathematical reconstruction of the repolarization process, De Hemptine found that repolarization is adequately described using only the quickly decaying component described by Brown and Noble (1969a). Maughan (1973) has reported that there were apparently two distinct reversal potentials; one near the potassium Nernst potential at -95 mV, and the other lying between -20 and -30 mV. This dual reversal potential had also been observed by De Hemptine (1971a). The sensitivity of the more negative reversal potential to the external potassium concentration is only 2/3 of that expected for a perfect potassium electrode. Ojeda and Rougier (1974) reported that some
atrial preparations display two outward current components, while others only display one outward component. The preparations with two components possessed kinetics and reversal potential similar to those previously seen by Brown and Noble (1969). The preparations which possessed only a single outward current component exhibited kinetics similar to that seen in the fast component ix1. Ojeda and Rougier also presented arguments supporting the assumption that analytical errors caused by the accumulation current are minimal. They claimed that the accumulation current had little or no effect on the measured time constant of the fast and slow outward current components. This conclusion contrasts with the conclusions of others (Brown and Noble, 1969b; Maugan, 1973; Brown, Clark, and Noble, 1976) who find that ignoring the contribution of the accumulation current causes large changes in the apparent time constants of the fast and slow outward currents.

3.8.2 Potassium current in single atrial cells

The above section indicates that there is much disagreement over how many outward currents exist in frog atrial muscle, what their kinetics are, and what, if any, role they play in the repolarization process. To investigate the role of potassium ions in single atrial
cell preparations, the potassium reversal potential has been studied under voltage clamp conditions. Figure 3.15 illustrates a determination of the reversal potential after two and ten second conditioning depolarizations to +40 mV. Comparing 3.15a and 3.15b, it can be seen that in both cases the potassium reversal potential lies between -95 and -100 mV. Due to the stability of the reversal potential using two and ten second depolarizations, it is assumed that accumulation effects (Brown and Noble, 1969a; Brown, Clark, and Noble, 1976) are either minimal or nonexistent. A different reversal potential experiment is displayed in Figure 3.16a, where a ten second conditioning depolarization is followed by a five second test pulse. Figure 3.16b illustrates selected tails from Figure 3.16a displayed semi-logarithmically. Except for large depolarizations and short times, the tails are approximately single exponentials. On this evidence, it is assumed that the outward current system in single frog atrial cells is: 1.) Carried mainly by a net outward flux of potassium ions and 2.) Controlled by a single Hodgkin-Huxley outward current system. This conclusion differs from that of Brown et. al. (1969a,b; 1976).

Another test for the existence of accumulation effects is to perform an "envelope of tails" experiment. According to Hodgkin and Huxley (1952b), conductance is a
Figure 3.15 Reversal of the slow outward $K^+$ current in normal (2.5 mM) potassium. A) Reversal after a 2 second depolarization to +40 mV. B) Reversal determined in the same cell after a 10 second depolarization to +40 mV. Vertical scaling is 250 pA. (4/13/82; cell #4).
Figure 3.16 Reversal of the slow outward current in normal (2.5 mM) potassium following a depolarization to +40 mV for 5 seconds. A) The reversal potential $E_r$ is -97 mV. B) Selected tails from panel A plotted semilogarithmically. (4/13/82; cell #1).
continuous function of membrane voltage. Thus, a step change in voltage will produce a gradual change in conductance. Since the outward current sigmoidially increases as a function of time, it is possible to monitor this current change by comparing the scaled peak magnitude of the tail currents evaluated at each time with the original outward current activation. If potassium accumulation were to occur, the size of this tail current evaluated immediately after the voltage change would not be proportional to the amount of current previously activated. An example of this type of experiment is shown in Figure 3.17a. Depolarizing voltage clamp steps of ten seconds duration to +60 mV are applied to activate an amount of outward current. The cell is then repolarized after varying amounts of time, and the magnitude of the tail current immediately after the step is measured for each time. A comparison of the scaled envelope of these magnitudes with the original depolarization is shown in Figure 3.17b. The correspondence between the envelope of the tails and the original depolarization over a ten second period is excellent, which gives further evidence that accumulation effects are minimal and this current acts as a single Hodgkin-Huxley mechanism.

Having confirmed that this current is due to transmembrane potassium movements, and that accumulation
Figure 3.17 An "envelope of tails" protocol for the slow outward potassium current. A) Test pulses to +40 mV for various durations are followed by a return to -70 mV. Pulse durations: .2, .4, .6, .8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.5, 3, 4, 6, and 8 seconds. Horizontal scaling, 2 seconds; vertical scaling, 500 pA. B) Analysis of panel A. Open squares denote activation phase of the current in A. Open circles are sampled values of the instantaneous current recorded at termination of the test pulse. Solid line through circles is a fit by eye. Solid line through the squares is the same as the line through the circles, scaled to agree with the sampled values of the test pulse. (4/20/82; cell #1).
is negligible; it is possible to test whether the single cell outward current system obeys the Hodgkin-Huxley formalism. Figure 3.18 shows the outward current activated on ten second depolarizations to varying potentials. The outward current exhibits a sigmoidal activation with a substantial delay. For depolarizations longer than five seconds, the magnitude of the current reaches an approximate steady-state, and shows no tendency to "sag" or inactivate with time. Figure 3.19 shows the instantaneous current-voltage relationship for this current, constructed from tail experiments similar to the ones discussed above. This relationship is linear over a 50 mV range of potentials, and indicates that the potassium conductance is a linear function of membrane driving potential. The fully activated current-voltage relationship, measured from current onsets of 10 seconds duration, is shown in Figure 3.20. This current exhibits outward rectification; with an activation threshold potential of approximately -40 mV. Figure 3.21 illustrates the normalized steady-state activation curve described by the variable n. This curve has a sigmoid shape, and exhibits an activation range from -40 to +40 mV. A listing of the values of this relationship is given in column 1 of Table 3.3 This curve may analytically described by the Boltzman relationship (Eqn. 3.1) where
Figure 3.18 Activation of the slow outward current in normal (2.5 mM) potassium. TTX used to block the early transient sodium current. Holding potential -90 mV. The test pulse potential was 10 seconds duration and varied from -30 to +60 mV. All test potentials return to -60 mV. Series resistance compensation was used in this experiment. (4/16/82; cell #2).
Figure 3.19 Instantaneous current-voltage relationship for the potassium current from a single atrial cell. Experiment performed in the presence of 2.5 mM potassium and 10^5 M La++. Test pulse of 5 second duration, currents measured immediately after termination of the test pulse. Slope of the line is 17.98x10^-12 mho, y intercept 1762.2x10^-1 A. (11/18/82; cell #5).
Figure 3.20 Fully activated (10 second) current-voltage relationship for the potassium current from a single atrial cell. Experiment performed in the presence of 2.5 mM potassium. Line is fit by eye to the data points (3/12/82; cell #1).
Figure 3.21 Steady-state activation gating relation ($n^*$') for the slow outward potassium current measured in normal (2.5 mM) external potassium. Test pulse duration of 10 seconds. Open circles represent experimental data, solid line is the fit of equation 3.1 to the experimental data, where $V_1 = 21.57$ mV and $S = 13.68$ mV$^{-1}$. 

s=13.68 mV is the slope and V = -21.57 mV. This activation curve is similar to ones described by Brown and Noble (1969a), De Hemptine (1971a), and Ojeda and Rougier (1974) for atrial trabeculae.

3.9 The Model of the Potassium Current

As discussed in the previous section, the outward current system of single frog atrial cells corresponds to a single outward potassium system of the Hodgkin-Huxley type. Thus this system has been analyzed and modeled following the formalism of Hodgkin and Huxley (1952d).

The steady-state activation curve has been mentioned in connection with Figure 3.21, and needs no further discussion. To determine the time constants of activation and decay of this current at different potentials, two separate methods were used. For potentials more negative than -60 mV, the tail currents from reversal potential experiments such as Figure 3.15 were fit to single exponential relationships. The time constant was then determined from the exponential fit. For potentials more positive than approximately -30 mV, the time constant was determined by fitting the activating phase of outward current at each potential. This was done by assuming the activation could be described as (Hodgkin and Huxley, 1952d):
<table>
<thead>
<tr>
<th>$V_m$</th>
<th>$% \text{ activ.} = n_\infty^2$</th>
<th>$\tau_n \text{ (msec \pm SD)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ 60</td>
<td>1.</td>
<td>1148.5 \pm 229.</td>
</tr>
<tr>
<td>+ 50</td>
<td>1.</td>
<td>1200.3 \pm 184.8</td>
</tr>
<tr>
<td>+ 40</td>
<td>.995</td>
<td>1287.1 \pm 211.4</td>
</tr>
<tr>
<td>+ 30</td>
<td>.979</td>
<td>1643.1 \pm 331.1</td>
</tr>
<tr>
<td>+ 20</td>
<td>.948</td>
<td>2021 \pm 376.8</td>
</tr>
<tr>
<td>+ 10</td>
<td>.9</td>
<td>2470.4 \pm 490.5</td>
</tr>
<tr>
<td>0</td>
<td>.836</td>
<td>2881.8 \pm 530</td>
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<td>- 10</td>
<td>.701</td>
<td>3579.3 \pm 517.6</td>
</tr>
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<td>.529</td>
<td>3587.7 \pm 543.9</td>
</tr>
<tr>
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</tr>
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<td>- 40</td>
<td>.223</td>
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</tr>
<tr>
<td>- 50</td>
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</tr>
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<td>- 60</td>
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<td>925.9 \pm 386.2</td>
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<td></td>
<td>621.9 \pm 219.6</td>
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<td>395.1 \pm 125.8</td>
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<td>- 90</td>
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<td>-100</td>
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<td>199.08 \pm 62.48</td>
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<tr>
<td>-120</td>
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</tr>
</tbody>
</table>

Table 3.3 Experimental values for the percentage activation ($n_\infty$) and time constant of activation ($\tau_n$) for the potassium current.
where $T(v)$ is the time constant at a certain potential, and $p$ is an integer power. The power $p$ may be interpreted as the number of consecutive events which must occur to create an "open" conduction pathway in the membrane (see section 1.3). Table 3.4 shows the least squares estimates for the value of this power for five cells. Over the activation range of the outward current ($-30$ to $+30$ mV) this power has an average value of 1.88. Since $p$ must be an integer in the Hodgkin-Huxley formalism, $p=2$ was chosen. The time constants for voltages positive to $-30$ mV were then determined by fitting equation 3.14 to the data with $p=2$. For potentials between $-60$ and $-30$ mV, little current was activated and reversal potential tails were distinctly non-exponential due to problems with series resistance errors. Thus, these tails were fitted to a single exponential function to get an estimate of the time constants in this range. A listing of the values of the time constant of activation is given in column 2 of Table 3.3. Figure 3.22 compares the analytic equations with the measured percentage activation and rate constant relationships. The analytic equations used to generate these fits were:

$$I_K(v, t) = (1 - \exp\left(-\frac{t}{T(v)}\right))^p$$

(3.14)
Figure 3.22 Experimental data and model results for the activation gating variable ('n_\infty') for the potassium current. Closed circles represent the experimental data, the solid line represents the fit predicted by the model equations. Panel A: Percent activation versus recovery. Panel B: Rate constant of activation versus potential.
Table 3.4 Best least-squares fit of the power $p$ to the 10 second activation of the outward potassium current at various potentials from four cells. The model used to fit the power was (Hodgkin and Huxley, 1952d):

$$I = g (1 - \exp(-t/T))^p$$

The average value for $p$ for all potential considered is 1.818. Over the activation range of the potassium current (+30 mV to -20 mV) the average value if this power is 1.88.
\[ \alpha_n (V) = \frac{1.4 \times 10^{-5} (V + 5.172)}{1 - \exp (-0.0525 (V + 5.172))} \] (3.15)

\[ \beta_n (V) = 9.37 \times 10^{-5} \exp (-0.0428 (V + 5.172)) \] (3.16)

3.10 The Background Currents

3.10.1 Background currents in cardiac muscle

The background or "leakage" current relationships are important in cardiac muscle since they occupy a large role in maintaining the resting potential positive to the potassium reversal potential. In isolated atrial cells, the magnitude of this current is probably about 50 pA at rest. The background current relationship may also be important in the generation of spontaneous pacemaker activity.

The classic work on the background current-voltage relationship was done by Hodgkin and Huxley (1952c) on squid axon. In addition to the active sodium and potassium currents, there is a small current component due to ions which cross the membrane by other means than the gated ionic pathways. Due to the small size of this background current relative to the active sodium and
potassium currents, Hodgkin and Huxley were unable to perform a quantitative analysis of this current. To include this background current in their simulation, Hodgkin and Huxley assumed that its current-voltage relationship was linear, and that the channel possessed no ionic selectivity.

For many years, technical limitations prevented an analysis of the background current-voltage relationships in cardiac preparations. McAllister et al. (1975) were the first to attempt to use a set of analytic equations to describe the background current-voltage relationship in cardiac muscle. They proposed that the total background current may be divided into components representing each ion. Sodium, calcium, chloride, and one portion of the potassium current were assumed to have linear current-voltage relationships, in a manner following Hodgkin and Huxley (1952c). A second component of the background potassium current, $I_k$, was found to display an inwardly rectifying current-voltage relationship. The resulting total background current relationship was linear for potentials positive to $-50 \, \text{mV}$, and exhibited a slight nonlinear behavior for potentials negative to this value (see Carmeliet and Vereecke, 1979 Figure 27B). In tandem with experiments on accumulation, Noble (1976) has determined the background current-voltage relationship in
frog atrial trabeculae.

3.10.2 The background current in single atrial cells

Figure 3.23 shows the background potassium current-voltage relationship determined in single atrial cells. In agreement with Noble (1976) and Brown, DiFrancesco, Noble, and Noble (1980) these relationships inwardly rectify and display "crossover" for increasing external potassium concentrations. These relations exhibit inward rectification over the physiological range of potentials. One feature not shown here is is a steep negative slope region for potentials positive to the potassium Nernst potential as seen by Noble (1976). external potassium. These relationships also do not exhibit the crossback phenomena observed by Noble (cf. Noble, 1976 their Figure 12). The reasons for these discrepancies are not known.

Consulting the background current-voltage relationship in 2.5 mM potassium shown in Figure 3.23, it is seen that there is little current flow for potentials between -60 and +10 mV. Positive to +10 mV, the current linearly increases. Since the slope and initial potential of this upswing are similar in many different potassium concentrations, it is assumed that this deviation is an artifact due to current leak around the measuring
Figure 3.23 Time independent background current in a single atrial cell. I-V measured at 10 msec, in the presence of 3x10^{-6} M TTX and 1 mM Cd^{2+} to block transient and persistent inward currents. Open symbols measured in 2.5 mM K^+. Closed symbols measured in 5 mM K^+ (From Hume and Giles, 1983).
electrode. If this leakage current is carried by a combination of all the ions present in the bathing media; and if the magnitude of the leak is directly proportional to the electrode seal resistance (approx. 2 gigaohms); it is possible to estimate how much of the observed current is due to "leakage" around the electrode. Using this correction factor, the peak magnitude of this current-voltage relationship is approximately 40 pA at potentials near the resting potential. This correction also produces a steep negative slope in this background relationship between -80 and -40 mV.

Based on the evidence discussed in section 2.1.1, it is known that there is a component of steady inward current which acts to maintain the resting potential above the potassium Nernst potential. Little information exists on this current's current-voltage relationship; it's current-voltage relationship is assumed to inwardly rectify. This current is also assumed to be due only to sodium ion movements, though there may be a significant contribution from calcium ions. The magnitude of this background sodium current is chosen so that there is no net contribution from the background potassium and sodium currents at rest (-80 mV).

The existence of what appears to be a second background current has also been discovered (Hume and
Giles, 1983). The steady-state current-voltage relationship of this current is shown in Figure 3.24. There is much uncertainty in the value of the reversal potential of this current, due to the fact that there is usually a small, but significant amount of outward current activated during the measurement interval. The occurrence of the peak current at hyperpolarizing potentials, and the large inward current activated in this region, would seem to indicate that this current is at least partially carried by an inward flux of sodium or calcium ions. At present, there is no consistent experimental data on the ionic nature of this current.

3.11 Summary of the Model Equations

The motivations for and the structures of the equations which model the various ionic currents were discussed in a previous chapter. Analysis of voltage clamp data has shown that there are at least five separate ionic currents present in frog atrial muscle. A large, fast transient inward sodium current ('Ina') provides the initial rapid depolarization of the action potential. The transient inward calcium current ('Ica'), which is 20 times smaller than the transient sodium current, is responsible for the second slower depolarization and the plateau phase. In the absence of the sodium current, this
Figure 3.24 Current-voltage relationships for the persistent inward current of a single atrial cell. Experiment performed in the presence of $3 \times 10^{-6}$ M TTX to eliminate the transient inward current. Curve is fit to the data points by eye. (2/2/83; cell #4).
current may also give a "slow response" action potential of normal height and duration. A slow maintained outward potassium current (\('Ik'\)), which activates with a considerable delay, controls the early portion of the repolarization process. An inwardly rectifying potassium current (\('Ik_B'\)) exists, and is responsible for speeding the repolarization process negative to \(-60\) mV. A second inward sodium background current (\('Inab'\)) holds the resting potential positive to the potassium reversal potential (\(-98\) mV). No useful role was found for the background current (\('Ipi'\)). Addition of this current usually prevented the action potential from repolarizing, due to the existence of a large region of inward current near \(0\) mV. Thus in further simulations performed with this model, this current component has been set to zero. Table 3.5 summarizes the model equations.
Table 3.5 The Model Equations

FAST TRANSIENT INWARD (SODIUM) CURRENT

Activation: \( a_M (V) = \frac{.1514 (V + 39.13)}{1 - \exp (-.0338 (V + 39.13))} \)

\( \beta_M (V) = 1.6435 \exp (-.0338 (V + 39.129)) \)

Inactivation: \( a_h (V) = 1.485 10^6 \exp (-.2482 (V + 8.77)) \)

\( \beta_h (V) = \frac{1.713}{1 + \exp (-.061 (V + 8.77))} \)

Recovery: \( a_k (V) = 2.97 10^{-8} \exp (-.2482 (V + 8.77)) \)

\( \beta_K (V) = \frac{.0342}{1 + \exp (-.061 (V + 8.77))} \)

\[ I_{Na} = P_{Na} m^3 n k [Na^+] F \frac{\exp VF}{RT} \frac{RT}{\exp RT} \frac{1}{1 + \exp (.1 (V + 45))} \]

Where \([Na^+] = .007 M\)

\[ F = 1.9059 \text{ cm}^3 /\text{sec} \]

\( m, h, \text{ and } k \) are the solution to:

\[ \frac{dx}{de} = a_x (V) - (a_x (V) - \beta_x (V)) X \]

Where \( x = M, h, \text{ or } k \)

SLOW TRANSIENT INWARD (CALCIUM) CURRENT

Activation: \( a_d (V) = \frac{.1654 (V - .07)}{1 - \exp (-.1619 (V - .07))} \)

\( \beta_d (V) = .3612 \exp (-.0714 (V - .07)) \)

Inactivation: \( a_f (V) = 9.46 10^6 \exp (-.1657 (V - 18.44)) \)

\( \beta_f (V) = \frac{.067}{1 + \exp (-.0244 (V + 6.257))} \)
Recovery: $\alpha_e (V) = 1.869 \times 10^{-6} \exp (-.1657 (V - 18.44))$

$\beta_e (V) = \frac{.0133}{1 + \exp (-.0244 (V + 6.257))}$

$I_{Ca} = 4P_{Ca} d f e \frac{VF}{RT} \cdot \frac{[Ca_i] \exp \left(\frac{2F}{RT} V\right) - [Ca_o]}{\exp(\frac{2F}{RT} V) - 1}$

Where $[Ca_i] = 1.069 \times 10^{-5}$
$[Ca_o] = .0025 \text{ M}$
$E_Ca = +70 \text{ mV}$
$P_{Ca} = .5406 \text{ cm}^3/\text{sec}$

d, f, e are the solutions to

$$\frac{dx}{dV} = \alpha_x (V) - (\alpha_x (V) - \beta_x (V)) \cdot x$$

Where $x = d, f, or e$

GATED OUTWARD (POTASSIUM) CURRENT

Activation: $\alpha_n (V) = \frac{1.4 \times 10^{-5} (V + 5.17)}{1 - \exp (-.0525 (V + 5.17))}$

$\beta_n (V) = 9.37 \times 10^{-5} \exp (-.0128 (V + 5.17))$

$I_k = g_k n^2 (V = E_k)$

$I_k = \bar{g}_k n^2 (V = E_k)$

Where $\bar{g}_k = 9.5 \text{ nmho}$

$E_k = -98 \text{ mV}$

n is the solution of $\frac{dn}{dt} = \alpha_n (V) - (\alpha_n (V) + \beta_n (V)) \cdot n$

OUTWARD BACKGROUND (POTASSIUM) CURRENT

$I_{k_2} (V) = \bar{g}_{k_2} \cdot \frac{\exp (.07606 (V - E_k)) - 1}{\exp (.1248 (V + 59.55)) + \exp (.0543 (V + 30.34))}$

Where $\bar{g}_{k_2} = 1.9595 \text{ nmho}$

$E_k = -98 \text{ mV}$
INWARD BACKGROUND (SODIUM) CURRENT

\[ I_{Na_B}(V) = P_{Na_B} \cdot \text{sinh} \left( \frac{V - E_{Na_B}}{S_{l_B}} \right) \]

Where

\[ P_{Na_B} = 8.02 \times 10^{-6} \]
\[ E_{Na_B} = +40 \text{ mV} \]
\[ S_{l_B} = 8 \text{ mV}^{-1} \]

TOTAL MEMBRANE CURRENT

\[ I_I = I_{Na} + I_{Ca} + I_{K} + I_{K_2} + I_{Na_B} \]

MEMBRANE EQUATION

\[ \frac{dv}{dt} = \frac{I_M - I_I}{C_M} \]

Where

\[ I_M = \text{Membrane current} \]
\[ I_I = \text{Ionic current} \]
\[ C_M = \text{Membrane Capacitance} \]
4.1 Experimental Techniques

Adult bullfrogs were pithed, and the hearts rapidly removed and placed in cold Ringer's solution to wash excess blood from the chambers. Under a 10X dissecting microscope, the right atrium was opened and small strips of tissue were cut from the interior wall. To dissociate single atrial cells, these strips are then transferred to a 50 ml flask, which contains collagenase and trypsin in calcium free Ringer's solution (for details see Hume and Giles, 1981). A small stirring bar is used to agitate the tissue in the enzyme solution at room temperature for a total of approximately one to two hours. Frequent changes of bathing enzyme solution are made until the solution becomes cloudy. Microscopic inspection of small samples of this solution show that the solution contains a mixture of single cells, multicellular clusters and cell debris. At this point, aliquoits of cells are removed with a
pipette and placed into the experimental chamber for study. Approximately fifteen minutes is allowed for the cells to settle to the bottom of the dish before experimentation begins.

These cells are impaled and voltage-clamped using a single suction microelectrode technique (Hume and Giles, 1981). Electrodes are pulled from capillary glass on a standard microelectrode puller, and backfilled with one molar potassium glucanate. This electrode is then mounted in a half-cell with a suction port, and under a microscope at 400X the tip of the electrode is touched against the surface of a chosen cell. Using a syringe connected to the suction port, a short intense pulse of negative pressure is applied to the electrode. This causes the cell membrane to be drawn up inside the tip of the electrode, forming a high resistance (Giga-ohm) seal between the membrane and the electrode tip. Additional negative pressure will cause the membrane inside the tip to burst, providing access to the interior of the cell while retaining the high resistance seal around the electrode tip.

The voltage and current clamp methods use a high input resistance amplifier connected to the microelectrode to simultaneously record voltage and inject current into the preparation. Membrane current is measured either
differentially between the measuring electrode and bath ground, or as the voltage drop across a 1 to 6 megohm resistor placed between the electrode half-cell and the measuring amplifier. Membrane potential is measured differentially between the microelectrode and a reference electrode in the bath. This membrane potential is then compared with a command potential, and the error voltage is used to control the magnitude and polarity of the current to be injected into the preparation to drive membrane potential to the command potential (negative feedback). This voltage clamp apparatus is schematically shown in Figure 4.1. As noted above, in the single suction electrode technique, voltage is measured and current is injected simultaneously; switching between voltage measuring and current injection modes is not done. In a few cases, series resistance compensation was used (Hodgkin, Huxley, and Katz, 1952), by summing a filtered portion of the measured current signal into the command voltage signal (Figure 4.1).

4.2 Data Analysis Techniques

A schematic diagram of the steps used during the analysis of a set of voltage clamp records is shown in Figure 4.2. The design of the experimental setup has been discussed in Section 4.1. Voltage clamp data from the
Figure 4.1 Experimental set-up for the single suction microelectrode technique of Hume and Giles (1981, 1983). Membrane potential \( V_M \) is measured differentially between the suction microelectrode and ground; and is then differentially compared with the rectangular command potential. The 'error current' is injected into the microelectrode. Membrane current is usually recorded as the voltage drop across \( R_T \).
Figure 4.2 A flow sheet illustrating the steps involved in voltage clamp data analysis. Operations are illustrated in small letters, computer programs used are illustrated in capital letters. Detailed explanation of this Figure is given in the text.
experimental setup is recorded on FM tape. Data is taken on four channels, with a bandwidth set between DC and 20 KHz, depending on experimental protocol. Voltage, current, and trigger information are continuously recorded during the course of an experiment. The fourth channel on the recorder is reserved for either a voice track or flutter compensation. To record the current signal, a preamplifier is used as a current-to-voltage converter, and is usually set at a gain of 1 pA = 1 mV. In some cases, this current monitor signal may be both filtered and amplified to improve the signal-to-noise ratio before going to tape. The voltage data is usually taken at unity gain. The trigger signal, generated by an external stimulator (TTL output) is also recorded at unity gain.

Analysis of data recorded on FM tape can be carried out by one of two methods. For qualitative analysis such as computation of current-voltage relations, reversal potential analysis, or percentage inactivation/activation versus potential, data is replayed from FM tape onto a two channel chart recorder. Further analysis is then carried out by hand. For quantitative analysis of ionic current kinetics, computer processing of the data is used. The data is replayed from FM tape into a two channel digital oscilloscope (Norland 3001). Depending on the type of experiment to be analyzed, the digitizing rate ranges from
100 Hz to 100 KHz; with the channel gains set between .2 and 2 V. The parameters of this oscilloscope are set such that digitizing begins on receipt of a negative edge of the trigger pulse, and continues at the chosen digitizing rate until 1024 points (at 12 bits/point) are taken. Channels A and B are digitized simultaneously at the chosen rate, and a total of 64 pair of records (2048 points/pair) may be taken and stored in oscilloscope memory.

After an experiment has been digitized, the collection of records may be stored on a floppy diskette. Norland program 05 is used to transfer these records from memory to diskette. A listing of this program is given in Table 4.1. A collection of these diskettes may be stored, and then played back into the Norland using program 10, also listed in Table 4.1. In most cases, once the data has been taken from FM tape and stored in memory, it is immediately sent to the departmental PDP 11/70 for processing and storage. Blocks of data are sent from the Norland memory to the 11/70 memory via an RS232 communications link. Norland program 10 controls the data transmission on the Norland, while fortran program NORLD controls data reception on the 11/70. Program NORLD was provided by Dr. Burgess Christensen, University of Texas Medical Branch at Galveston, and is listed in Appendix B.
Table 4.1 Norland Programs 05 and 10

Program 05: DMX to Disk

64512 ==> A
43.000 ==> H
1.0000 ==> D
DPLY I A DPLY
0.0000 ==> I
100.00 ==> B
H IF ==> I
DMX A ==> Q1
DMX A ==> Q2
B + I ==> C
DPLY Q1 ==> DISK C
INCI
I + B ==> C
DPLY Q2 ==> DISK C
A - 1024.0 ==> A
INCI
GOTO 7
END

Program 10: Disk to RS232

380 ==> RS232
0.000 ==> I
100.00 ==> A
DPLY A DPLY
44.00 IF ==> I
I + 100.00 ==> A
DISK A ==> Q1
DPLY Q1 ==> RS232A
INCI
I + 100.00 ==> A
DISK A ==> Q2
DPLY Q2 ==> RS232A
INCI
GOTO 4
END
Files transferred between these two programs consist of channel A and channel B data encoded as 2048 4 digit ASCII characters. Program NORLD accepts the data on the RS232 line, and writes it into a file NORLD.DAT on the system disk. Up to 64 pairs of records may be transferred and read into a file at one time. Once a full experiment has been digitized and transmitted to the 11/70, all further processing is done on the 11/70.

To convert the ASCII file NORLD.DAT to a more convenient format, program NORNIC is used. Program NORNIC was also provided by Dr. Christensen and is listed in Appendix B. This program accepts the sequential ASCII file NORLD.DAT, puts a header on each record, and converts these records to ITPLOTS (see below and Table 4.2) readable format. Each set of channel A and channel B integer records is now accessed by a 'tag' number, which is unique for each A-B record pair. Thus each file is divided into a group of 'tags', each of which contains one 1024 point current sample and a corresponding 1024 point voltage sample.

Once the data is in ITPLOTS compatible format, the program ITPLOTS may be used to process the file. Program ITPLOTS was provided by Dr. Douglas Eaton, University of Texas Medical Branch at Galveston. Due to the large size of this program, a listing is not included. This program
Files to be read or written by ITPLOTS consist of an arbitrary number of sequential unformatted binary records. All data in the file is assumed to be type integer. Each record ('tag') contains both voltage (A channel) and current (B channel) data arranged in the following manner:

\[ TAG1, TAG2, NPTS, (LABEL (5)), (IDATA (NPTS)) \]

TAG1 and TAG2 are integer variables which are set by the user as indices to each record in the file. TAG1 is usually used as an index for each experiment, while TAG2 is usually used as an index to individual voltage/current records within an experiment.

NPTS is the number of points in the vector IDATA. This is usually chosen to be 2048; 1024 voltage data points and 1024 current data points.

LABEL is a vector which allows character information to be stored within each record.

IDATA contains the sampled data points, arranged in an A-B-A-B---fashion.
allows the user to easily manipulate files consisting of an arbitrary number of current and voltage records, by the use of the program's command language. This program was most commonly used for plotting and bookkeeping tasks on files digitized on the Norland. This program was also often used to preprocess files in the preparation of using one of the data analysis programs described below.

Once a file had been preprocessed with program ITPLOTS, one of two nonlinear least-squares analysis programs was used to obtain time constant data. The majority of the time constant data was obtained by using program EST2; which is based on subroutine provided by Bevington (1969). This program is a nonlinear minimization of least-squares routine commonly used to fit single exponential and other functions directly to experimental data. The theory on which this and the other fitting programs are based is explained in Appendix D. A listing of this program is given in Appendix B. EST2 allows the user to open and read ITPLOTS files, and select certain 'tags' for processing. In all cases, only the current data (channel B) was used in the fitting process. Each 'tag' current record is read into the program data buffer; and the user may use the terminal cursors to set zero time, the first point to fit, last point to fit, and a baseline value for each record. Any one of five
separate functions may be fit to the data. Program output is either to a Textronix 4010 graphics terminal or hardcopy.

As an independent check on the accuracy of EST2, as well as a separate method for analyzing time constant data, program DISCRETE was used. This program was provided by Provencher (1976), and utilizes a Fourier transform method to compute initial estimates for the number of exponential components; and their magnitudes and time constants from the input data. These estimates are then transformed into the frequency domain, and the transform of the estimates are least-squares fit to the transform of the data. Due to the length of this program, a listing is not provided here but is available from the author. To get files into a format acceptable to DISCRETE, preprocessing program DISCRE is used. A listing of this program is given in Appendix B. This program allows the user to set the zero time, first and last points, and baseline value of each current record in the file. Each record is then scaled and output into a new file acceptable to DISCRETE. The advantage of using this program is that predictions of the number of exponential components, their magnitudes, and their time constants may be derived without providing initial guesses of these values. In some cases, experimental data was fit using
only this program, and in other cases both EST2 and DISCRETE were used on the same data and the results compared. There was no significant difference in the accuracy of the fits generated by either DISCRETE or EST2.

After the time constant data has been obtained using EST2 or DISCRETE, it was collected and tabulated by hand. The results of analysis of many experiments using either EST2 or DISCRETE are tabulated and averaged, and yield tables of values for time constants versus voltage (see Table 3.3). At this point, one of two methods were used to derive the rate equations like those presented in Table 3.5. One method was to derive sampled values for rate constants (ie $\alpha$ and $\beta$) versus voltage by taking the ratio of the percentage activation ($n_{\infty}(V)$ or $1-n_{\infty}(V)$) to the time constant ($\tau_n(V)$) at the same potential. This will create a table of values of $\alpha_n(V)$ and $\beta_n(V)$ which are functions of membrane voltage. Then program ESTIMATE was used to fit various forms of equations to the $\alpha$ and $\beta$ data. ESTIMATE is a nonlinear least-squares routine based on the Bevington (1969) subroutines, and is very similar to program EST2. Thus a listing of this program is not provided. The other method commonly used was to convert time constant to rate constant data versus potential ($R = 1/\tau$). Then program ACTFIT was used to fit both the $n$ and rate equations to the $\alpha_n(V)$ and $R_n(V)$
data. This program is also a nonlinear least-squares routine, and is listed in Appendix B. Use of either of these methods will produce a set of $\alpha(V)$ and $\beta(V)$ equations similar in form to Equations 3.15 and 3.16. These equations may then be used to simulate experimental data, by solving the equation system given in Table 3.5.

4.3 The Model Program

The program written to implement the equations discussed in Chapter 3 is listed in Appendix C. This program (FROGIE), written in DEC FORTRAN 4+, is an interactive routine designed to simulate both voltage and current clamp experiments. The internal command language of this program permits the user to select various tasks to be carried out either independent of or concurrent with the simulation computations.

The design of this program assumes that the format of the equations used in the simulation follow the Hodgkin-Huxley formalism (see Table 3.5). The user supplies all the values of the variable parameters for these equation in a sequential formatted file APRATE.DAT. The format of this input file is shown in Table 4.3. Using the program command language, the user may change program parameters from their default values. Command 'HELP' prints a listing and short explanation of each
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<td></td>
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<td>1.485E-6, -0.2482, -8.77</td>
<td></td>
<td></td>
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<tr>
<td>Sodium inactivation beta</td>
<td>1.713, -0.061, -8.77</td>
<td></td>
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<td></td>
<td></td>
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<td>Calcium activation alpha</td>
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</tr>
<tr>
<td>Potassium activation beta</td>
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<td></td>
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<tr>
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<td></td>
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<tr>
<td>Background potassium</td>
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</tr>
<tr>
<td>Capacitance in pF</td>
<td>7.2E-11</td>
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command. 'HP' allows either the holding potential and/or the simulated run time to be modified. 'SHIFT' allows the user to shift the position of any of the m, h, d, f, or n curves along the voltage axis. 'RS' allows the user to eliminate or change the amount of series resistance to be used in the computation. 'DEBUG' open a file into which intermediate output will be sent for debugging or hand analysis purposes. 'PAR' prints out the current values of all the parameters. 'DUMP' changes the output mode of the program from plotting to numerical output of the data. 'RATE' plots the steady-state inactivation and activation curves for the model. 'BACK' will plot the components of the background I-V. 'RESET' will reset all the program variables back to their default values, along with re-reading the parameter values. 'QUIT' terminates the program, and calls the plotting processor subroutines.

The command most often used is 'DEP', which simulates either voltage or current clamp results. By means of prompts, the user may design either a 1, 2, or 3 pulse protocol to simulate in either mode. The pulse durations are also set individually.
CHAPTER 5

SIMULATION RESULTS

5.1 Simulation of the Dynamic Ionic Currents

5.1.1 Simulation of the transient sodium current

The simulation of the sodium current under voltage clamp, based on the data discussed in Section 3.4.3 (also see Table 3.5), is shown in Figure 5.1. This simulation is carried out assuming a normal external sodium concentration (110 mM Na\(^+\)), with no blocking of sodium current by TTX. The series resistance is assumed to be zero. This current is computed from -50 mV (threshold) to +70 mV (beyond reversal) in 10 mV steps. Under most experimental conditions, there is substantial series resistance present, arising from the preparation and the electrode measurement system. This will limit the accuracy of recording the peak value and time course of the sodium current under voltage clamp. Figure 5.2
Figure 5.1 Simulated sodium currents from the atrial model. Parameter values are the same as those in Table 4.3, model equations are given in Table 3.5. No series resistance included.
Figure 5.2 Simulated sodium current-voltage relationships with various amounts of series resistance. Parameters are the same as in Figure 5.1. Relationships computed with $R_s = 0$, $R_s = 3 \text{ M} \Omega$ and $R_s = 6 \text{ M} \Omega$. 

CURRENT IN pA $\times 10^3$

VOLTAGE IN mV $\times 10^1$
illustrates the effects of varying amounts of series resistance on the peak inward/minimum outward current-voltage relationship (I-V). Elimination of all series resistance ($R_s=0$) produces an I-V which possess a smooth transition from threshold to peak current; where this peak lies at about -20 mV. With increasing amounts of series resistance, the transition from threshold to peak current becomes steeper, as voltage clamp control of membrane potential progressively fails. The peak sodium current becomes smaller as the peak of the I-V relationship shifts to more negative potentials when the series resistance is increased.

The above figure illustrates why series resistance compensation and/or reduction of peak transient current is essential for accurate analysis of this current. Assuming that the series resistance is approximately 3 megohms, a peak sodium current of 5 nA will cause a voltage measurement error of 15 mV. When the sodium current is reduced by 90% by application of 30 nM TTX, this voltage error is limited to 1.5 mV. Thus with a 90% block of sodium current, the loss of control is smaller, but still is not negligible.

Another problem which arises when attempting to record the transient sodium current in single frog atrial cells involves changes in the space constant during activation
of the sodium current. In Appendix B, it was shown the
the space constant is proportional to $\sqrt{R_m}$, where $R_m$ is
the membrane resistance. The resting membrane resistance
is about 220 megohms (Hume and Giles, 1981). During
sodium activation, the membrane resistance may decrease 10
to 20 times from its resting value. This implies a
reduction in the space constant of 3 to 4 times of the
resting value. A reduction of this magnitude will
compromise the ability of the single electrode clamp to
uniformly polarize the cell membrane, thus making analysis
of the voltage dependent kinetics of the system more
difficult to analyze.

5.1.2 Simulation of the transient calcium current

Figure 5.3 illustrates the simulation of the transient
inward calcium current, based on the data presented in
Section 3.6. This current has a threshold at
approximately $-60$ mV, a peak value at approximately $0$ mV,
and reverses near $+70$ mV. The maximum inward/minimum
outward current - voltage relationship (I-V) is shown in
Figure 5.4. This I-V has a smooth, rounded shape, with a
peak near $0$ mV similar to the experimental calcium I-V
shown previously (Figure 3.10). To further investigate
the behavior of this model, the isochronal I-V of this
model and experimental data are compared. Isochronal
Figure 5.3 Simulated calcium currents from the atrial model. Parameter values taken from Table 4.3. Model equations from Table 3.5. No series resistance is included.
Figure 5.4 Simulated calcium current-voltage relationship. Parameters taken from Table 4.3. Model equations from Table 3.5.
I-V's are derived by measuring the peak current at many voltages at a fixed time (peak transient I-V's measure peak current at variable times). A comparison between the model and experimental isochronal I-V's is shown in Figure 5.5. These I-V's are similar when comparing peak current size and overall shape. One striking difference is the lack of a persistent inward component in the model I-V. This can be seen by comparing the 120 msec isochronal I-V's. At present, the reasons for the discrepancy are unclear partially due to the nature and origin of this persistent inward current (Hume and Giles, 1983).

One very important shortcoming of this calcium current model is its inability to accurately reproduce experimental currents elicited by a two pulse protocol such as recovery or inactivation experiments; especially where membrane potential is more negative than -70 mV. Since experimental data on the kinetics of activation and inactivation of this current were limited, values of the time constant outside of this potential range are subject to large errors. The Hodgkin - Huxley equations were used in an attempt to extrapolate this data to more negative potentials. Unfortunately this approach fails at these negative potentials; the estimated time constants of both inactivation and recovery become very fast in this model (on the order of 10's of microseconds). This drastic
Figure 5.5 Experimental and simulated isochronal current-voltage relationships for the calcium current. A) Current values measured in 10 mV intervals from an experimental record (2/2/83 Cell #4). Legend: 1 = 12.4 msec, 2 = 14.4 msec, 3 = 19.6 msec, 4 = 31.2 msec, 5 = 41.6 msec, 6 = 49.2 msec, 7 = 130 msec.
B) Current values measured in 10 mV intervals from simulated calcium currents. Parameter values and model equations same as Figure 5.4. Legend: 2 = 2 msec, 4 = 4msec, * = 10 msec, + = 20 msec, # = 30 msec, Ø = 50 msec.
increase in the time constants is not experimentally observed. Thus some improved and/or different formalism is necessary to properly describe the inactivation/recovery kinetics of this current. Many theories have been recently proposed (Standen and Stanfield, 1982; Fischmeister and Horackova, 1983) which can explain some, but not all the observed phenomena.

5.1.3 Simulation of the dynamic potassium current

The simulation of the slow outward potassium current in normal (2.5 mM) potassium, based on the data discussed in Section 3.8 is illustrated in Figure 5.6. This figure shows the activation phase of this current, calculated at various membrane potentials. Threshold for current activation lies at -30 mV, with steady-state current being reached in one to two seconds. On return of the membrane potential to a value close to the reversal potential (-98 mV), a 'tail' current is generated. This 'tail' current has an initial amplitude which is not dependent on the amount of current activated during the preceeding depolarization; the time constant of inactivation of the 'tail' current is dependent only on membrane voltage. Figure 5.7 illustrates the simulation of a reversal potential experiment. Here the potassium current is activated by depolarization to +40 mV, and the 'tail'
Figure 5.6 Simulated potassium currents from the atrial model. Parameter values taken from Table 4.3. Model equations are taken from Table 3.5. First pulse is from -40 mV to +60 mV for seconds, followed by a pulse to -70 mV for 5 seconds. Vertical scale: 500 pA. Horizontal scale: 5 seconds.
Figure 5.7 Simulated potassium currents. Conditions are as given in Figure 5.6. First pulse is to +40 mV for 10 seconds, and is followed by pulses to -40 mV to -120 mV for 5 seconds. Vertical scale: 500 pA. Horizontal scale: 5 seconds.
current is measured at various potentials. Positive to the potassium reversal potential, the 'tail' is a decaying outward current, negative to this potential the 'tail' is a decaying inward current. Note that the time constants of inactivation of these 'tails' are functions of membrane potential.

The simulated peak outward current - voltage relationship (I-V) of this current at 1, 2, 5, and 10 seconds is shown in Figure 5.8. This I-V is sigmoidal from -30 mV to +30 mV (the current activation range), and becomes linear for potentials more positive than +30 mV. Addition of series resistance will cause an apparent shift of the I-V to more positive potentials. This effect will become important for potentials more positive than +10 mV, where large amounts of outward current are activated.

Since the potassium current system was the most thoroughly analyzed current system in frog atrium, the model on which the simulation is based can be considered to be much more accurate and complete than those for the sodium or calcium current. Thus it was decided to attempt simulations of more complex protocols, and attempt to use this model in a predictive manner. Figure 5.9 shows a simulation of an envelope of tails experiment (c.f. Figure 3.17). The results of this simulation are very similar to experimental results carried out with a similar
Figure 5.8 Simulated peak isochronal current-voltage relationships for the potassium current. Conditions the same as in Figure 5.6. Peak current measured at 1, 2.5, 5 and 10 seconds.
Figure 5.9 Simulation of an 'envelope of tails' experiment for the potassium current. Parameter values the same as in Figure 5.6. First pulse to +40 mV for .5, .8, 1, 2, 3, 4, 5, 8, and 10 seconds. Second pulse is to -60 mV. Vertical scale: 500 pA. Horizontal scale: 5 seconds.
One phenomenon which is of great interest in single atrial cells is the observance on the 'false reversal' potential. 'Tail' currents recorded positive to -50 mV begin to markedly deviate from single exponential behaviour; and at very positive potentials a second 'reversal' potential is observed (DeHemptine, 1971; Maughan, 1973). In an attempt to simulate these observations, current reversal was computed at potentials ranging from -120 mV to +40 mV, following a 10 second depolarization to +30 mV. The result is shown in Figure 5.10. In this simulation, positive to -40 mV the 'tail' currents begin to deviate from single exponential behaviour and at approximately +30 mV a current 'reversal' occurs. The change in the shape of the 'tail' currents is due to the fact that the n gating variable, which is set to 1 during the 10 second depolarization to +40 mV does not return to 0 positive to -40 mV (the foot of the activation curve). Hodgkin - Huxley theory predicts that in this case the resulting current relaxation (ie 'tail') may be bi-exponential, where the two exponential components may have different initial magnitudes. The 'false reversal' is due to the fact that the n gating variable, which is set to 1 during the 10 second depolarization, remains at 1 for a 'tail'
Figure 5.10 Simulation of the potassium 'false reversal' potential. Parameter values the same as in Figure 5.6. First pulse to +40 mV for 10 seconds, second pulse to −120 mV to +30 mV in 10 mV steps for 5 seconds. The potassium reversal potential lies at −98 mV, the 'false' reversal potential is near +40 mV. Vertical scale: 500 pA. Horizontal scale: 5 seconds.
current evaluated at +30 mV. Since \( V \) is small, little or no current relaxation is observed, resulting in the apparent reversal phenomenon.

5.1.4 Simulation of the background currents

Figure 5.11 illustrates the simulated current-voltage relationships (I-V) for the background (time independent) currents, based on the equations presented in Table 3.5. The rectifying background current \( I_{k_1} \) is assumed to possess a negative slope region between -80 and -40 mV; where the amount of current decreases for increasing potential. This phenomena has been previously observed in cardiac Purkinje fibres (Hutter and Noble, 1960). This outward background current has been shown to be almost entirely due to potassium ions (Hume and Giles, 1983). Thus the reversal potential for this current was chosen to be the same as that for the slow potassium current (-98 mV).

The inward background current \( I_{Na} \) was also assumed to rectify. Initial trials with the model assuming a linear (conductance) relationship for this current showed that a linear function could not reproduce the proper time course of repolarization of the action potential. The next trial used a rectifying function based on the Goldman equation (Equation 2.3), with \([Na] = 6 \text{ mM}\). This relationship also
Figure 5.11 Simulated background current-voltage relationships for the atrial model. Parameter values taken from Table 4.3. Model equations are taken from Table 3.5.
failed to produce a properly shaped repolarization phase. Finally the function given in Table 3.5 was chosen to describe this current. A background I-V relationship of this shape has not been previously described in the literature. Yet, this choice is a reasonable one for the following reason. It was mentioned in Chapter 2 that the membrane potential is not a function solely of potassium; but that there is a substantial contribution from other ions due to the presence of an inward background current component. This current may arise from time independent ion 'leakage' across the membrane, ATP dependent electrogenic pump currents, and possibly gradient dependent transport mechanisms. Thus, there is no strong reason to believe that the I-V of the net current which is simulated would be linear. The function chosen to describe this background current was a simple formulation in an attempt to simulate this current.

5.2 Simulation of the Membrane Action Potential

Action potential simulation experiments were carried out using the model program discussed in Section 4.3 and the model equations in Table 3.5. After some initial adjustments of the parameters derived in Chapter 3, what will be known as the 'standard' action potential was produced. This action potential is shown in Figure 5.12,
Figure 5.12  A simulated action potential and the related ionic currents from the atrial model.  A) Simulated action potential, resting potential -88 mV, stimulus current = 300 pA for 1 msec. B) Ionic currents underlying the action potential in A. Four of the five ionic current components which generate the action potential are illustrated: $I_{Ca}$, $I_K$, $I_{Ks}$, INAB. $I_Na$ is not visible on this time scale. Action potential duration 540 msec, maximum rate of rise 41.5 v/sec, peak depolarization 35.3 mV.
and is computed using the parameter values displayed in Table 4.3. The peak depolarization, duration, and plateau shape are all similar to action potentials recorded experimentally (c.f. Figure 3.2) from a single atrial cell. The ionic currents associated with this action potential are also shown in Figure 5.12. This action potential is computed based on the contributions from five ionic currents (Table 3.5): dynamic sodium, calcium, and potassium currents; and background potassium and sodium currents. The sodium and calcium currents produce the rapid upstroke of the action potential in response to a stimulus. The sodium current is not shown in this figure since it's large size and rapid activation time constant would make it indistinguishable from the current axis. The height and duration of the action potential plateau positive to 0 mV is controlled by the interaction between inactivation of the calcium current and activation of the potassium current. Repolarization of the action potential is controlled by the activation and subsequent decay of the dynamic potassium current, along with the background potassium current. The speeded late repolarization phase is due to a rapid increase in this outward background potassium current. The increase in current with repolarization is due to the negative slope of this relationship (see Figure 5.11). The resting potential is
due to the balance between the outward potassium background current and the inward sodium background current.

Figure 5.13 shows the same simulated action potential as in Figure 5.12 on an expanded scale. The shape and maximum rate of rise of this action potential upstroke are also similar to an experimentally recorded action potential (c.f. Figure 3.2). The biphasic action potential upstroke is now visible, where the rapid phase is due to activation of the sodium current and the second slower phase is due to activation of the calcium current. The ' +' symbol lies at the time of maximum rate of rise of this simulated action potential, which in this case is about 41 V/sec. Also shown in Figure 5.13 are the ionic currents which underlie this action potential. The rapid decrease of the potassium background current on depolarization can be clearly seen, which is due to the negative slope (current decreases for depolarized potentials) of this relationship. The activating phase of the calcium current can also be observed.

The behaviour of this model under simulated voltage clamp conditions at three clamp potentials is shown in Figure 5.14. The clamp potential is assumed to have a rise time of 100 microseconds, and no series resistance is assumed to be present. Again the sodium current is not
Figure 5.13 The same action potential as Figure 5.12 on an expanded scale to illustrate the initial depolarization, or upstroke. All parameters are the same as in Figure 5.12.
Figure 5.14 Simulated ionic currents under voltage clamp conditions. Assumed clamp rise time of 100 microseconds, clamping to -30 mV, 0 mV, and +30 mV from a 'holding potential of -85 mV. On this time scale, only currents $i_{Ca}$, $i_{KB}$ and $i_{K}$ are visible.
displayed since its rapid activation/inactivation kinetics and large size cause it to merge with the calcium activation process at this time scale. At $-30\, \text{mV}$, little calcium current is activated, and the only current evident is a contribution from the outward background potassium current. At $0\, \text{mV}$ the calcium current is at its peak value. At $+30\, \text{mV}$, the calcium current has decreased as membrane potential approaches the zero current potential for calcium (at $+70\, \text{mV}$). Near the end of the pulse, activation of the dynamic potassium current is evident.

In an attempt to produce a more accurate simulation of the action potential, a nonlinear least-squares fitting routine was utilized to fit the model directly to an experimental action potential. This program (APFIT) is listed in Appendix B. The theory behind the minimization routines used are described in Appendix D. Initially, it was assumed that kinetics of the ionic current (see Chapter 3) were correct; and that to accurately fit the action potential only the various ionic conductances and permeabilities needed to be adjusted. Trials with this assumption showed that varying only the conductances and permeabilities would not work. The next approach allowed the shape of the background current-voltage relationships to change, along with a selected set of the above parameters. PNa was not varied since the sodium current
is so fast that it makes no significant contribution to any segment of the action potential after the initial rapid upstroke. Calcium permeability usually was not varied since the resulting I-V that was obtained did not correspond in size to experimental data. Thus only the conductance of the slow outward potassium current $g_s$, sodium background permeability $P_{Na}$ and slope factor $S_{IB}$, and the inwardly rectifying potassium conductance $g_K$ were varied. The result of one of these fitting trials is shown in Figure 5.15. The experimental and fitted action potentials are overlaid for comparison. Except for the initial 100 msec of the action potential, this program is able to produce an excellent fit to the data.
Figure 5.15 Model fit to an experimentally recorded action potential, using program APFIT. Five parameters were varied: PNAB, GKB, SLB, GK, and PCA (see Table 3.5). Starting values for these parameters were: .000018, 7.5, 2.32, 11.5, and .5406. For this iteration the parameter values were: .00000526, 7.789, 2.9, 11.5, and .245.
CHAPTER 6

SUMMARY

The goal of this modeling effort has been to design and implement a model of the electrophysiological responses in the atrial region of the frog heart, fully based on experimental voltage clamp data obtained from single, enzymatically dispersed cells. After this model was formulated, attempts were made to use this model in a predictive manner to analyze the effects of the kinetics of the ionic currents on the membrane action potential. The work presented in the previous chapters has realized some of these goals. In Chapter 3, the analysis of voltage clamp data from each of the three voltage and time dependent current systems was discussed. Models which conform to the Hodgkin-Huxley formalism were used for each ionic current system (sodium, calcium, and potassium), based mainly on the available voltage clamp data. In Chapter 5, the results from Chapter 3 were used to compute a membrane action potential, which has characteristics
similar to an experimentally recorded action potential. It was also shown that with the use of an automatic parameter adjustment method, it was possible to get very good agreement between the simulation and the experimental data by adjustment of a few selected parameters.

The model of the atrial action potential which results from this work is significant from two points of view. This model is the first published attempt at forming a complete mathematical description of the ionic currents which underlie the action potential in frog atrial tissue. Besseau (1972) has presented a partial analysis of the ionic currents of frog atrium, based on his sucrose gap voltage clamp data. But he did not attempt to reconstruct an action potential based on this information. This model is also unique since it is the only model for cardiac muscle where the simulated ionic currents are either derived from, or based fully on experimental measurements. Experimental data came from two main sources. Analysis of the cable properties of the single cell (see Section 3.1) provided the parameters on which the passive properties of the model were based. Analysis of the available voltage clamp data for the sodium, calcium, potassium, and background currents were the basis for the design of the model equations (see Chapter 3 and Table 3.5). Though quantitative data for the dynamic sodium and calcium
currents is incomplete, it has been shown that it is possible to design models for these currents which can qualitatively reproduce some of the observed voltage clamp phenomena. Since there was more extensive and reliable data available for analysis on the slow outward potassium current, the potassium current model can successfully mimic many experimentally observed phenomena.

As with virtually every model, this atrial action potential model also has definite limitations. Since the available experimental data from the sodium and calcium systems was incomplete, the equations which are based on the analysis of this data may be in error. It has been mentioned that the present model for the calcium current does not properly reproduce many of the experimentally observed frequency dependent phenomena. Modeling failures such as these are most likely due to the fact that it was impossible to experimentally confirm whether or not these current systems could be adequately described by Hodgkin-Huxley kinetics. In addition, there are other limitations which could prevent derivation of an accurate model. The single suction microelectrode technique is restricted to recording small currents. With this technique it is virtually impossible to ensure voltage control of the preparation during activation of large and/or fast ionic current transients. Also, lack of
detailed knowledge of the mechanisms of transmembrane ion transport, along with the lack of knowledge of the cellular mechanisms for buffering and extrusion of ions, has prevented any detailed study of the calcium current in cardiac muscle.

With these limitations in mind, the predictive value of the model may be considered. Horackova and Vassort (1976) have concluded that the calcium current is an important current component which initiates and controls the repolarization process. Simulations using the present atrial model indicate that the calcium current plays no significant role in the repolarization process, and that the kinetics of activation of the slow potassium current initiate and control much of the repolarization phase. Another finding is that the outward background potassium current is partially responsible for the increased rate of repolarization observed negative to -50 mV. Previously, this current was assumed to only be important for maintenance of the resting potential of atrial trabeculae (Noble, 1976). A final result which arises from this simulation is that the inward background current in frog atrium is nonlinear.

Though the results of this model strictly apply only to frog atrium, the techniques used to design this model have wider applications. Based on the evidence from the
design of this model, it should be possible to apply the technique of analysis of voltage clamp data, formation of ionic current models, and design of a membrane action potential model to other cell types. The further development of cellular dispersion techniques and the single suction microelectrode technique will provide the experimenter with a preparation which is ideal for application of the voltage clamp, and eliminate many of the difficulties associated with quantitative analysis of voltage clamp data.
Appendix A Cable Theory

Assume that a cell can be represented as a cylinder, with its length much greater than its diameter. This cylinder is of uniform thickness and composition, immersed in a medium of uniform conductance. The passive impedance of a small segment (i.e., 1 cm²) of this membrane can be represented by a linear capacitance parallel to a linear resistance.

Define:

- \( r_i \) = internal resistance per unit length
- \( r_o \) = external resistance per unit length
- \( I_i \) = internal current flow
- \( I_o \) = external current flow
- \( i_M \) = transmembrane current
- \( V_i \) = internal potential
- \( V_o \) = external potential

As \( x \to \infty \) along the cable, \( I_i \to 0 \) due to current loss \( i_M \) per unit length. This:

\[
\frac{\partial I_i}{\partial x} (x,t) = -i_M (x,t) \quad \text{(A.1)}
\]
\[
\frac{\partial I_o}{\partial x} (x,t) = +i_M (x,t) \quad \text{(A.2)}
\]

Current flows \( I_i \) and \( I_o \) cause voltage drops between points \( x \) and \( x + \Delta x \):

\[
\frac{\partial V_i}{\partial x} (x,t) = I_i (x,t) \cdot r_i \quad \text{(A.3)}
\]
\[
\frac{\partial V_o}{\partial x} (x,t) = I_o (x,t) \cdot r_o \quad \text{(A.4)}
\]

Define the transmembrane potential \( V_m \) to be the difference between external (\( V_o \)) and internal (\( V_i \)) potentials.

\[
V_m (x,t) = V_o (x,t) - V_i (x,t) \quad \text{(A.5)}
\]
Differentiate $V_m$ with respect to $x$ and substitute A.3 and A.4 into A.5

$$\frac{\partial V_m}{\partial x} (x,t) = r_0 \cdot I_o(x,t) - r_i \cdot I_i(x,t) \quad (A.6)$$

Differentiate A.6 and substitute A.1 and A.2 into A.6

$$\frac{\partial^2 V_m}{\partial x^2} (x,t) = (r_o + r_i) \cdot i_m(x,t) \quad (A.7)$$

Since the passive membrane properties are that of a parallel resistor and capacitor the transmembrane current may be described by:

$$i_M(x,t) = C_m \frac{\partial V_m}{\partial t} (x,t) + \frac{V_m(x,t)}{r_m} \quad (A.8)$$

Substitution of A.8 into A.7 gives:

$$\frac{\partial^2 V_m}{\partial x^2} (x,t) = (r_o + r_i) \cdot C_m \frac{\partial V_m}{\partial t} (x,t) + \frac{V_m(x,t)}{r_m} \quad (A.9)$$

Rearrange A.9 into the more familiar form:

$$\frac{r_m}{r_o + r_i} \cdot \frac{\partial^2 V_m}{\partial x^2} (x,t) = r_m C_m \frac{\partial V_m}{\partial t} (x,t) + \frac{V_m(x,t)}{r_m} \quad (A.10)$$

Define:

$$\lambda^2 = \frac{r_m}{r_o + r_i} \quad \text{the space constant: the distance necessary for } V_m(x,t) \text{ to drop to } \frac{1}{e} \text{ of its initial value}$$

$$\tau_m = \frac{r}{C} \quad \text{Membrane time constant: the time required for } V_m(x,t) \text{ to approach } \frac{1}{e} \text{ of its steady-state value after a step change of } I_M(x,t)$$

Thus:

$$\lambda^2 \frac{\partial^2 V_m}{\partial x^2} (x,t) = \tau_m \frac{\partial V_m}{\partial t} (x,t) + V_m(x,t) \quad (A.11)$$

For a step change in $I_m$ of magnitude $I_o$ occurring at $t = 0$, the voltage response will be (Jack, Noble, and Tsien, 1975).
\[ v_m(x,t) = \frac{(r_0 + r_i) I_o \lambda}{4} e^{-x^*} \text{erfc} \left( \frac{x^* - 2t^*}{t^*} \right) - \]
\[ e^{x^*} \text{erfc} \left( \frac{x^* + 2t^*}{t^*} \right) \]  \hspace{1cm} (A.12)

where \( x^* = \frac{x}{\lambda} \) and \( t^* = \frac{t}{\tau_m} \)

During a "membrane" action potential, the complete length of the cell is equipotential ("voltage clamped"), thus \( I_i = I_o = 0 \) and \( \frac{\partial v_m(x,t)}{\partial x} = 0 \)

\[ i_m(t) = C \frac{dv_m}{dt} + \frac{v_m}{r_m} \]  \hspace{1cm} (A.13)

The two solutions A.12 and A.13 of A.11 are applicable only for cases where \( r_m \) is not a function of membrane voltage or time. This will be true in the region where \( v_m = v_{\text{rest}} \) where little of dynamic current is activated. In the more general case:

\[ \lambda^2 \frac{\partial^2 v_m(x,t)}{\partial x^2} = \tau_m \frac{\partial v_m(x,t)}{\partial t} + \frac{v_m(x,t)}{r_m(v_m,t)} \]  \hspace{1cm} (A.14)

Equation A.14 has no analytical solution due to the fact that \( r_m(v_m,t) \) is a highly nonlinear function of both \( v_m \) and \( t \). A numerical solution to A.14 has been computed (Hodgkin and Huxley, 1952d), where the membrane resistance may be expressed as:

\[ r_m(v_m,t) = \frac{1}{g_{Na}(v_m,t) + g_K(v_m,t) + g_L(v_m,t)} \]

and where the velocity of propagation along the cell is constant.
APPENDIX B

Program ACTFIT

C
C 03/03/83 NONLINEAR LEAST-SQUARES ROUTINE TO
SIMULTANEUSLY
C   FIT ACTIVATION AND 1/TAU DATA TO A H-H TYPE EQUATION
C
C******************************************************************************
C MAIN PROGRAM ACTFIT - CALLS FIT(*), FUNC(*), AND
FCHI(*)
C******************************************************************************
C
REAL V(50) DATA(50) TEMP(50) W(50) XM(2),
+ YM(2) DATA2(50) TEMP2(50) U2(50)
REAL*8 A(5) LAMBDA, OLDCHI, NEWCHI, ERR, ERMIN, PMIN(5)
BYTE STR(10) STR2(10)
LOGICAL YES, FNAM(12) LP
DATA XM/-120., 60./, YM/0., 2./
NPAR = 5
CALL TIPR('INPUT FILENAME ', FNAM, 12)
OPEN (UNIT=1, NAME=FNAM, ACCESS='SEQUENTIAL',
+ TYPE='OLD')
NPTS = 0
DO 10 K = 1, 50
   READ(1,8,END=20) V(K), DATA(K), W(K)
   NPTS = NPTS + 1
10 CONTINUE
8 FORMAT(F5.1,F10.5,F4.2)
20 CALL TELL('THE NUMBER OF POINTS IS ', NPTS)
NITR = 1
DO 15 K = 1, 5
   CALL TELL('ENTER PARAMETER ', K)
   CALL FETCHR( ' ', K)
   A(K) = TT
15 CONTINUE
CALL FUNC(NPTS, V, TEMP, NPAR, A)
OLDCHI = FCHI(NPTS, TEMP, DATA, W)
25 CALL FIT(NITR, NPTS, V, DATA, W, NPAR, A,)
LAMBDA, OLDCHI, NEWCHI)
ERR = (NEWCHI-OLDCHI)/NEWCHI
IF (NITR.EQ.1) ERMIN = ERR
WRITE(5,1) NITR, OLDCHI, NEWCHI
WRITE(5,2) (SNGL(A(M)), M=1,15)
1 FORMAT(/,N='i3,5X,' OLDCHI IS ',d12.5,
+ NEWCHI IS ',d12.5)
2 FORMAT(' PARMS ARE: ',5(E12.5,2X))
IF (NITR.10).NE.0) GO TO 21
IF (YES('CONTINUE FOR 10 MORE ITERATIONS? '))
+ GO TO 21
DO 22 KK = 1, NP
A(KK) = PMIN(KK)
22 CONTINUE
NEWCHI = ERMIN
GO TO 42
21 NITR = NITR + 1
IF (ERR) 24,24,25
24 OLDCHI = NEWCHI
ERMIN = ERR
DO 33 KK = 1, NP
PMIN(KK) = A(KK)
33 CONTINUE
IF (ERR.LE.-.001) GO TO 25
30 IF (.NOT.YES('PLOT OUT DATA? ')) GO TO 40
26 M = 1, NPTS
XI = U(M)
AL = A(1)*(XI-A(3))/(1. -EXP(A(2)*(XI-A(3))))
BE = A(4)*EXP(A(5)*(XI-A(3)))
WRITE(IU,13) U(M),W(M),DATA(M),TEMP(M),AL,BE
CONTINUE
WRITE(IU,16)
WRITE(IU,1) NITR, OLDCHI, NEWCHI
WRITE(IU,2) (SNGL(A(M)), M=1,15)
11 FORMAT(/,5X,'DATA FROM FIT RUN OF ',9(A1),3X,B(A1))
12 FORMAT(/, 'VOLTAGE WEIGHT',7X,'DATA ',6X,'FIT ',
+ 7X,'ALPHA',7X,'BETA')
13 FORMAT(1X,F6.1,5X,F4.2,7X,F10.5,5X,F10.5,5X,F10.5,
+ 3X,F10.5)
16 FORMAT(/,8X,'ALPHA = A(1)*(U-A(3))/(1-EXP(A(2))*',
+ '(U-A(3)))',/,'8X,
+ 'BETA = A(4)*EXP(A(5)*(U-A(3)))')
30 IF (.NOT.YES('PLOT OUT DATA? ') GO TO 40
CALL GETPLT(I)
IF (I.EQ.0) GO TO 40
IF (I.EQ.1) CALL VERON(2)
IF (I.EQ.2) CALL TEKON(2)
CALL SCAN(XM, YM, -2, 441)
CALL AXES(7.2, 'VOLTAGE', 20.2, 'N INFINITY AND TAU N')
C SEPARATE THE TWO DATA SETS
I = 1
DO 31 K = 11, NPTS
   V2(I) = V(K)
   DATA2(I) = DATA(K)
   TEMP2(I) = TEMP(K)
   I = I + 1
31 CONTINUE
I = I - 1
CALL NOTE(V, DATA, 110, -10)
CALL NOTE(V2, DATA2, 116, -1)
CALL DRAW(V, TEMP, 10, 441)
CALL DRAW(V2, TEMP2, I, 441)
CALL DRAW(0, 0, 1, 9000)
CALL DRAW(0, 0, 0, 9999)
40 CLOSE (UNIT=1)
STOP
END

SUBROU FIT(*) - CALLS FUNC(*), MATINU(*), JCOMP(*), FCHI(*)
- CALLED BY MAIN
NOTE: FOR LISTING OF PROGRAM MATINU(*), SEE UNDER
'MATINU' IN THIS APPENDIX
SUBROUTINE FIT(NITR, NPTS, XDATA, YDATA, WEIGHT,
+ NPAR, ALPHA, LAMBDA, OLDCHI, NEWCHI)
REAL XDATA(1), YDATA(1), !INPUT DATA
+ YTEMP1(100), !WORK VECTOR
+ YTEMP2(100), !WORK VECTOR
+ YTEMP3(100),
REAL WEIGHT(1), !WEIGHTING VECTOR FOR INPUT DATA
REAL*8 ALPHA(1), !PARAMETER VALUES (UP TO 10)
+ DIAG(10), !VALUES OF DIAGONAL OF
Jjt
+ DELTA(10), !PARAMETER CHANGES
+ Jjt(10, 10), !WORK MATRIX
+ TEMP(10), !WORK VECTOR
+ LAMBDA, !LEVENBURG PARAMETER
+ SUM, SUM2, !TEMPORARY SUM
+ FCHI, !CHI-SQUARED ERROR
+ OLDCHI, NEWCHI !OLD _NEW CHI ERROR
CALL TYPE('** SUBROUTINE FIT **) CALL FUNC(NPTS,XDATA,YTEMP3,NPAR,ALPHA)

DO 7 N1 = 1, NPAR
    CALL JCMP(N1,XDATA,YTEMP1,NPTS,NPAR,ALPHA)

SUM2 = 0.
DO 11 NCNT = 1, NPTS
    SUM2 = SUM2 +
        (DBLE(YTEMP3(NCNT)) - DBLE(YDATA(NCNT)))*
        DBLE(YTEMP1(NCNT))**(WEIGHT(NCNT))
11 CONTINUE

TEMP(N1) = SUM2
DO 7 N2 = N1, NPAR
    IF (N1.EQ.N2) GO TO 8
    CALL JCMP(N2,XDATA,YTEMP2,NPTS,NPAR,ALPHA)
    GO TO 9
8 DO 5 NC = 1, NPTS
    YTEMP2(NC) = YTEMP1(NC)
5 CONTINUE
    SUM = 0.
    DO 6 NCNT = 1, NPTS
        SUM = SUM +
        DBLE(YTEMP1(NCNT))**DBLE(YTEMP2(NCNT))
        *(WEIGHT(NCNT))
6 CONTINUE
    JJT(N1,N2) = SUM
7 CONTINUE

C COPY UPPER TRIAG PORTION INTO LOWER TRIAG PORTION.
C SAVE THE DIAGANOAL VALUES OF JJT FOR SCALING PURPOSES
DO 12 N1 = 1, NPAR
    DIAG(N1) = SQRT(JJT(N1,N1))
12 CONTINUE
IF (NITR.EQ.1) LAMBDA = DBLE( .01 )

C SCALE JJT AND J*E BY THE DIAGANOAL FACTORS
C AND ADD LEUVENBURG PARM
DO 4 N1 = 1, NPAR
    TEMP(N1) = TEMP(N1)/DIAG(N1)
7 CONTINUE
DO 4 N2 = N1, NPAR
    JJT(N1,N2) = JJT(N1,N2)/(DIAG(N1)**DIAG(N2))
4 CONTINUE

C do 61 nm2=1,npar
CALL MATINV(JJT,NPAR,DET)

DO 15 K1 = 1, NPAR
    SUM = 0.
    DO 14 K2 = 1, NPAR
        SUM = SUM + JJT(K1,K2)*TEMP(K2)
    CONTINUE
14    CONTINUE

C call tellr( ' parameter changes are : ',sum)
    ALPHA(K1) = ALPHA(K1) - SUM
    DELTA(K1) = SUM
15    CONTINUE

CALL FUNC(NPTS,XDATA,YTEMP1,NPAR,ALPHA)
NEWCHI = FCHI(NPTS,YTEMP1,YDATA,WEIGHT)
call tellr( 'Oldchi = ',sngl(oldchi),
+ ' newchi = ',sngl(newchi))
IF (NEWCHI-OLDCHI) 20,20,21
 20 LAMBDA = LAMBDA/10.
  GO TO 22
21 LAMBDA = LAMBDA*10.
  DO 28 K1 = 1, NPAR
      ALPHA(K1) = ALPHA(K1) + DELTA(K1)
  CONTINUE
28 call tellr('New lambda is ',sngl(lambda))
  RETURN
END

C+++++++-----------------------------------
C SUBROU JCOMP(*) - CALLS NO OTHER PROGRAMS
- CALLED BY FIT(*)-
C+++++++-----------------------------------
C
SUBROUTINE JCOMP(NROW,XDATA,YOUT,NPTS,NPAR,ALPHA)
REAL XDATA(1), YOUT(1), Y1(100), Y2(100)
REAL*8 ALPHA(1), T !PARAMETER VECTOR

  T = ALPHA(NROW)
  ALPHA(NROW) = .995 * T
  CALL FUNC(NPTS,XDATA,Y1,NPAR,ALPHA)
  ALPHA(NROW) = 1.005 * T
  CALL FUNC(NPTS,XDATA,Y2,NPAR,ALPHA)
  DO 12 M = 1, NPTS
      YOUT(M) = (Y2(M)-Y1(M))/.01*SNGL(T)
  CONTINUE
  ALPHA(NROW) = T
  RETURN
END
SUBROUTINE FCHI(*) - CALLS NO OTHER PROGRAMS
- CALLED BY MAIN AND FIT(*)

FUNCTION FCHI(NPTS,Y1,Y2,W)
REAL Y1(1), Y2(1) ! USER SUPPLIED DATA
REAL*8  R, SUM, FCHI
REAL W(1)

CALL TYPE('******** SUBROUTINE FCHI ********')
SUM = 0.
DO 1 M = 1, NPTS
   R = DBLE(Y1(M)) - DBLE(Y2(M))
   SUM = SUM + R * R * W(M)
1 CONTINUE
FCHI = SUM
RETURN
END

SUBROUTINE FUNC(NPTS,XIN,OUT,NPAR,A)
REAL XIN(1), OUT(1)
REAL*8  A(1), ALP, BET

DO 1 N = 1, NPTS
   U = DBLE(XIN(N))
   ALP = A(1)*(U-A(3))/(1.-EXP(A(2)*(U-A(3))))
   BET = A(4)*EXP(A(5)*(U-A(3)))
   IF (N.GT.10) GO TO 2
   OUT(N) = SNGL(ALP/(ALP+BET))
   GO TO 1
2 OUT(N) = SNGL((ALP+BET))
1 CONTINUE
RETURN
END

End of listing for program ACTFIT
Program APFIT

C++++++++++++++++++++++MAIN PROGRAM APFIT - CALLS FIT(*), FCHK(*), FUNC(*)++++++
C
C
C
REAL Y(1024), !EXPERIMENTAL DATA
+ TEMP(1024), !MODEL GENERATED DATA
+ DDT(7), !GATING VARIABLES
+ MSH, HSH, DSH, FSH, NSH, !SHIFT
VOLTAGES
+ IM, II !MEMB, IONIC CURRENTS

REAL PM(6), PH(6), PK(6), !SODIUM PARAMETERS
+ PD(6), PF(6), PE(6), !CALCULUM
PARAMETERS
+ PN(6) !POTASSIUM
PARMS
REAL PNA, PCA, GK, GNAB, !PEMEABILITIES
+ ENA, ECA, EK, ENAB, !REVERSAL
POTS
+ INA, ICA, IK, IK2, INAB, IPI !IONIC
CURRENTS

REAL*8 ALPHA(10), LAMBDA, OLDCHI, NEWCHI, FCHI, ERR
INTEGER WEIGHT(1024)
LOGICAL YES, SR, DUMP, SEC, DEBU

COMMON /RATE/ AM, BM, AH, BH, AK, BK, AD, BD, AF, BF,
+ AE, BE, AN, BN
COMMON /PARMS/ SEC, RS, IM, II, CM
COMMON /DATA/ PNA, ENA, PCA, ECA, PNAB, ENAB, SLB,
+ GK, EK, GKB, GKI, SPI, EPI
COMMON /DEBUG/ INA, ICA, IK, IK2, INAB, IPI
COMMON /BACK/ A1, A2, A3, A4, A5, A6
COMMON PM, PH, PK, PD, PF, PE, PN

C
OPEN (UNIT=3, NAME='APRATE.DAT', ACCESS='SEQUENTIAL',
+ TYPE='OLD')
C
C
C DYNAMIC SODIUM PARAMETERS: M, H, K
READ (3,1) (PM(I), I=1,3) !ACTIVATION ALPHA
READ (3,1) (PM(I), I=4,6) !ACTIVATION BETA
READ (3,1) (PH(I), I=1,3) !INACTIVATION ALPHA
READ (3,1) (PH(I), I=4,6) !INACTIVATION BETA
READ (3,1) (PK(I), I=1,3) !REACTIVATION ALPHA
READ (3,1) (PK(I), I=4,6) !REACTIVATION BETA
READ (3,1) PNA, ENA
READ (3,1) PNAB,ENAB,SLB !BACKGROUND SODIUM
C DYNAMIC CALCIUM PARAMETERS: D, F, E
READ (3,1) (PD(I),I=1,3) !ACTIVATION ALPHA
READ (3,1) (PD(I),I=4,6) !ACTIVATION BETA
READ (3,1) (PF(I),I=1,3) !INACTIVATION ALPHA
READ (3,1) (PF(I),I=4,6) !INACTIVATION BETA
READ (3,1) (PE(I),I=1,3) !REACTIVATION ALPHA
READ (3,1) (PE(I),I=4,6) !REACTIVATION BETA
READ (3,1) PCA,ECA
C DYNAMIC POTASSIUM PARAMETERS: N
READ (3,1) (PN(I),I=1,3) !ACTIVATION ALPHA
READ (3,1) (PN(I),I=4,6) !ACTIVATION BETA
READ (3,1) GK,EK,GKB
C PERSISTANT INWARD (NOT USED)
READ (3,1) GPI, SPI, EPI
C BACKGROUND POTASSIUM (IKB) PARAMETERS
READ (3,1) A1,A2,A3
READ (3,1) A4,A5,A6
READ (3,1) CM !MEMBRANE CAPACITANCE
1 FORMAT (3F10.6)
CLOSE (UNIT=3)
CALL FILE(2,'INPUT FILENAME? ','IB')
READ(2) N1,N2,NPTS,(IS,ME=1,50),
+ (WEIGHT(K),ISWIL,K=1,1024)
CLOSE (UNIT=2,DISP='KEEP')
NPTS = NPTS/2
CALL TELLK('NUMBER OF POINTS ARE ',NPTS)
CALL FETCHR('ENTER SCALE FACTOR FOR DATA ',SCFAC)
DO 6 K = 1, NPTS
   Y(K) = SCFAC * FLOAT(WEIGHT(K))
   WEIGHT(K) = 1
6 CONTINUE
IF (.NOT.YES('WEIGHT ANY PORTION OF THE DATA ',
+ 'TO ZERO? ')) GO TO 10
CALL FETCHR('ENTER NUMBER OF MSEC TO WEIGHT TO 0 ',
+ 'ZERO)
NZERO = INT(ZERO/0.5)
CALL TELLK('NUMBER OF PTS TO ZERO ARE ',NZERO)
DO 9 K = 1, NZERO
   WEIGHT(K) = 0
9 CONTINUE
C USER SELECTS WHICH PARAMETERS ARE TO BE VARIED
10 NPAR = 4
ALPHA(1) = PNAB
ALPHA(2) = SLB
ALPHA(3) = GKB
ALPHA(4) = GK
CALL TYPE('VARIED PARMS ARE: PNAB SLB GKB GK ')  
WRITE(5,91) (ALPHA(IJ),IJ=1,NPAR)  
91 FORMAT(' THE PARMS ARE: ',B(E12.5,2X))  
CALL FILE(3,'OPEN SENSITIVITY PARM FILE ','OB')  
CALL FILE(2,'ENTER ITPLOTS DATA OUTPUT FILE ','OB')  
NITR = 1  
CALL FUNC(NPTS,TEMP,NPAR,ALPHA)  
OLDCHI = FCHI(NPTS,TEMP,Y,WEIGHT)  
CALL TELLR('OLDCHI IS ','OLDCHI')  
20 CALL FIT(NITR,NPTS,Y,WEIGHT,NPAR,ALPHA,  
+ LAMBDA,OLDCHI,NEWCHI)  
NITR = NITR + 1  
ERR = ABS(NEWCHI-OLDCHI)/NEWCHI  
OLDCHI = NEWCHI  
CALL TELLR('CHI-SQUARED ERROR IS ','NEWCHI')  
CALL TELLI('ITERATION ','NITR-1,  
+ THE PARAMETERS ARE ')  
WRITE(5,3) (SNGL(ALPHA(K)),K=1,NPAR)  
3 FORMAT(10(E12.5,2X),/)  
IF (ERR.LT.0.001) GO TO 50  
IF (NITR.GT.6) GO TO 50  
C IF (YES('QUIT?')) GO TO 50  
50 CLOSE (UNIT=3,DISP='KEEP')  
CLOSE (UNIT=2,DISP='KEEP')  
OPEN (UNIT=2,NAME='FINAL.FIT',ACCESS='SEQUENTIAL',TYPE='NEW')  
WRITE(2,3) (SNGL(ALPHA(K)),K=1,NPAR)  
CLOSE (UNIT=2,DISP='KEEP')  
STOP  
END  

C SUBROU FIT(*) - CALLS MATINU(*), FCHI(*), FUNC(*),  
JCOMP(*) - CALLED BY MAIN  
C NOTE: FOR PROGRAM MATINU, SEE UNDER 'MATINU' IN THIS APPENDIX  
C NONLINEAR LEAST-SQUARES ROUTINE TO SIMULATE AND  
C FIT ACTION POTENTIAL - REQUIRES MAIN ROUTINE  
C MAINFIT.FTN  
C SUBROUTINE FIT(NITR,NPTS,YDATA,WEIGHT,NPAR,ALPHA,
REAL YDATA(1), !INPUT DATA
+ YTEMP1(1024), !WORK VECTOR
+ YTEMP2(1024), !WORK VECTOR
+ YTEMP3(1024),
+ PM(6), PH(6) !PARAMETER VALUES
INTEGER WEIGHT(1), !WEIGHTING FOR INPUT DATA
+ IVARY(10) !PARAMETER INDICATOR
REAL*8 ALPHA(10), !PARAMETER VALUES (UP TO 10)
+ DIAG(10), !JIT DIAGONAL VALUES
+ DELTA(10), !PARAMETER CHANGES
+ JJT(10,10), !WORK MATRIX
+ TEMP(10), !WORK VECTOR
+ LAMBDA, !LEVENBURG PARAMETER
+ SUM,SUM2, !TEMPORARY SUM
+ FCHI, !CHI-SQUARED ERROR
+ OLDCHI, NEWCHI !OLD_NEW CHI ERROR
COMMON PM, PH, IVARY

CALL TYPE('** SUBROUTINE FIT **')
CALL FUNC(NPTS, YTEMP3, NPAR, ALPHA)

COMPUTE NOMINAL VALUE OF VM FOR PARAMETER SENSITIVITY
OUTPUT
VSUM = 0.
DO 24 NZ = 1, 1024
   VSUM = VSUM + YTEMP3(NZ)
24 CONTINUE
VSUM = VSUM/1024.
DO 7 N1 = 1, NPAR
   CALL JCOMP(N1, YTEMP3, YTEMP1, NPTS, NPAR, ALPHA)
C OUTPUT RELATIVE SENSITIVITIES EACH 3RD ITERATION
IF (MOD(NITR,3).NE.0) GO TO 25
FAC = ALPHA(N1)/VSUM !NORMALIZING MULTIPLIER
WRITE(3) 1, NITR, 2048, (FAC*YTEMP1(NP), NP=1,1024)
25 SUM2 = 0.
DO 11 NCNT = 1, NPTS
   SUM2 = SUM2 + (DBLE(YTEMP3(NCNT)) -
+ DBLE(YDATA(NCNT)))*DBLE(YTEMP1(NCNT))
   TEMP(N1) = SUM2
11 CONTINUE
DO 7 N2 = N1, NPAR
   IF (N1.EQ.N2) GO TO 8
CALL JCOMP(N2,YTEMP3,YTEMP2,NPTS,NPAR,ALPHA)
GO TO 9

8      DO 5 NC = 1, NPTS
5           YTEMP2(NC) = YTEMP1(NC)
           CONTINUE

9      SUM = 0.
     DO 6 NCNT = 1, NPTS
6           SUM = SUM + DBLE(YTEMP1(NCNT)) * DBLE(YTEMP2(NCNT))
           CONTINUE

JJT(N1,N2) = SUM
CONTINUE

DO 12 N1 = 1, NPAR
   DIAG(N1) = SQRT(JJT(N1,N1))
12      CONTINUE

IF (NITR.EQ.1) LAMBDA = DBLE(.1)

DO 4 N1 = 1, NPAR
   TEMP(N1) = TEMP(N1)/DIAG(N1)
DO 4 N2 = N1, NPAR
   JJT(N1,N2) = JJT(N1,N2)/(DIAG(N1)*DIAG(N2))
   JJT(N2,N1) = JJT(N1,N2)
   IF (N1.EQ.N2) JJT(N1,N1) = 1. + LAMBDA
4      CONTINUE

CALL MATINV(JJT,NPAR,DET)

DO 15 K1 = 1, NPAR
   SUM = 0.
   DO 14 K2 = 1, NPAR
14           SUM = SUM + JJT(K1,K2)*TEMP(K2)
            CONTINUE

   SUM = SUM/DIAG(K1)
   ALPHA(K1) = ALPHA(K1) - SUM
   DELTA(K1) = SUM
15      CONTINUE

CALL FUNC(NPTS,YTEMP1,NPAR,ALPHA)
NEWCHI = FCHI(NPTS,YTEMP1,YDATA,WEIGHT)
call tellr('Oldchi = ',sngl(oldchi),
+ ' newchi = ',sngl(newchi))
IF (NEWCHI-OLDCHI) 20,20,21
20      LAMBDA = LAMBDA/10.
GO TO 22
21      DO 28 K1 = 1, NPAR
28          ALPHA(K1) = ALPHA(K1) + DELTA(K1)
          CONTINUE
      LAMBDA = LAMBDA*10.
22      call tellr('New lambda is ',sngl(lamba))
WRITE(2) 1,NITR,2048,(IR,IB=1,50),
+ (INT(100.*YTEMP1(IB)),
+ INT(100.*YDATA(IB)),IB=1,1024)
RETURN
END

C*****************************************************************************
C SUBRO JCOMP(*) - CALLS FUNC(*)
C - CALLED BY FIT(*)
C*****************************************************************************
C
SUBROUTINE JCOMP(NROW,IN,Y,NPTS,NPAR,ALPHA)
REAL Y(1),!WORK VECTOR
+ IN(1),!INPUT VOLTAGES
+ TKEEP
REAL*8 ALPHA(10) !PARAMTER VECTOR
CALL TELLI('**** SUBROUTINE JCOMP: N =',NROW)
TKEEP = ALPHA(NROW)
ALPHA(NROW) = 1.01 * TKEEP
CALL FUNC(NPTS,Y,NPAR,ALPHA)
ALPHA(NROW) = TKEEP
DO 12 M2 = 1,NPTS
 Y(M2) = (Y(M2) - IN(M2))/(.01*TKEEP)
12 CONTINUE
RETURN
END

C*****************************************************************************
C SUBRO FCHI(*) - CALLS NO OTHER PROGRAMS
C - CALLED BY MAIN AND FIT(*)
C*****************************************************************************
C
FUNCTION FCHI(NPTS,Y1,Y2,W)
REAL Y1(1),Y2(1)
REAL*8 R,SUM,FCHI
INTEGER W(1)

CALL TYPE('******** SUBROUTINE FCHI ******')
SUM = 0.
DO 1 M = 1,NPTS
 R = DBLE(Y1(M))-DBLE(Y2(M))
 SUM = SUM + R * R * FLOAT(W(M))
1 CONTINUE
FCHI = SUM
RETURN
END
SUBROUTINE FUNC(NPT,OUT,NP,A)
    REAL OUT(1), DDT(7), MSH, HSH, DSH, FSH, NSH, SHIFT
    REAL VOLTAGES, IM, II
    REAL PNA, PCA, GK, ENA, ECA, EK, GNAB, INA, ICA, IK, IK2, INAB, IPI
    CURREN
    REAL*8 A(1)
    LOGICAL YES, SR, DUMP, SEC, DEBU
    COMMON /RATE/AM, BM, AH, BH, AK, BK, AD, BD, AF, BF, AE, BE, AN, BN
    COMMON /PARMS/SEC, RS, IM, II, CM
    COMMON /DATA/PNA, ENA, ECA, EK, GNAB, ENAB, SLB, GK, EK, GKB, GPI, SPI, EPI
    COMMON /DEBUG/INA, ICA, IK, IK2, INAB, IPI
    COMMON /BACK/Al, A2, A3, A4, A5, A6
    DATA MSH, HSH, DSH, FSH, NSH/S*0.7

    T = 0.
    ICNT = 0
    HP = -90.
    TPP = .5
    RUNT = 500.
    RS = 3.0E+6
    SR = .FALSE.
    TIME PER POINT IN MSEC
    SERIES RESISTANCE
    SEIRES RESISRANCE STATE
    P1 = 3500.
    P1DUR = 1.
    NP1 = INT(P1DUR/TPP)
    P2 = 0.
    P2DUR = 1000.
    NP2 = INT(P2DUR/TPP)
    P3 = 0.
    P3DUR = 0.

    CALL RATE(HP, MSH, HSH, DSH, FSH, NSH, A)
    DDT(1) = AM/(AM+BM)  ! COMPUTE M INFINITY
    DDT(2) = AH/(AH+BH)  ! COMPUTE H INFINITY
    DDT(3) = AD/(AD+BD)  ! COMPUTE D INFINITY
    DDT(4) = AF/(AF+BF)  ! COMPUTE F INFINITY
    DDT(5) = AN/(AN+BN)  ! COMPUTE N INFINITY
    DDT(6) = AK/(AK+BK)  ! COMPUTE K INFINITY
    DDT(7) = AE/(AE+BE)  ! COMPUTE E INFINITY
    VM = HP
IM = P1
PEAKII = 0.
PEAKT = 0.
STKEEP = IM

DO 22 NOUT = 1, 1024

IF (T.LT.10.) TINC = .005
IF (T.GE.10. AND T.LT.75.) TINC = .025
IF (T.GE.75. AND UMT.GE.-50. AND UMT.LT.-75.)
   TINC = .025
IF (T.GE.75. AND UMT.LE.-75.) TINC = .01
IF (UMT.LE.-80. AND ABS(DUDT).LE.0.001) TINC = TPP

NTTIMES = INT(TPP/TINC)

DO 26 MM = 1, NTIMES
   CALL RATE(UM, MSH, HSH, DSH, FSH, NSH, A)
   CALL APMOVE(UM, DDT, T, TINC)
   II = CURR(UM, DDT, A)
   DUDT = (IM-II)*1.E-12/CM
   UM = UM + TINC * DUDT
   T = T + TINC
   CONTINUE

OUT(NOUT) = UM + IM*1.E-9*RS

IF (NOUT.GE.NP1.AND.NOUT.LT.NP1+NP2) IM = P2
IF (NOUT.GE.NP1+NP2) IM = P3

WRITE(5,36) T, UM, II, INA, ICA, IK, IK2, INAB, IPT
WRITE(5,38) (DDT(IR),IR=1,5)
WRITE(5,340) DUDT, TINC

FORMAT( 'T = ',F8.3, 'UM = ',F7.2,
+ ' TOTAL CURRENT = ',F9.2,'/','INA = ',
+ F8.2,'ICA = ',F7.2,'IK = ',F6.2,
+ IK2 = ',F6.2,'INAB = ',F6.2)

FORMAT(3X,'DUDT IS ',F6.3,
+ 'CURRENT STEPSIZE IS ',F8.6)
WRITE(5,39) AM/(AM+BM),AH/(AH+BH),AD/(AD+BD),
+ AF/(AF+BF),AN/(AN+BN),AK/(AK+AK),AE/(AE+BE)
FORMAT( 'CURRENT VAL. INF: ',7(F8.5,1X))
WRITE(5,39) 1./(AM+BM),1./(AH+BH),1./(AD+BD),
+ 1./(AF+BF),1./(AN+BN),1./(AK+AK),1./(AE+BE)
C FORMAT( 'STEADY-STATE TAU:','F6.4,1X,3(F7.4,1X),/
+ 3(F8.3,1X),/)

CONTINUE
SUBROUTINE RATEC(U, MSH, HSH, DSH, FSH, NSH, A)
REAL U, PM(6), PH(6), PK(6), PD(6), PF(6),
+ PE(6), PN(6)
REAL MSH, HSH, DSH, FSH, NSH
REAL*8 A(1)
COMMON PM, PH, PK, PD, PF, PE, PN
COMMON /RATE/ AM, BM, AH, BH, AK, BK, AD, BD,
+ AF, BF, AE, BE, AN, BN
AM = PM(1)*((U-PM(3)-MSH)/
+ (1.-EXP(PM(2)*((U-PM(3)-MSH))))
BM = PM(4)*EXP(PM(5)*((U-PM(6)-MSH)))
BH = PH(1)*EXP(PH(2)*((U-PH(3)-HSH)))
AK = PK(1)*EXP(PK(2)*((U-PK(3)-HSH)))
BK = PK(4)/(1.+EXP(PK(5)*((U-PK(6)-HSH))))
AD = PD(1)*((U-PD(3)-DSH)/
+ (1.-EXP(PD(2)*((U-PD(3)-DSH))))
BD = PD(4)*EXP(PD(5)*((U-PD(6)-DSH)))
AF = PF(1)*EXP(PF(2)*((U-PF(3)-FSH)))
BE = PE(1)*EXP(PE(2)*((U-PE(3)-FSH)))
AE = PE(4)/(1.+EXP(PE(5)*((U-PE(6)-FSH))))
AN = PN(1)*((U-PN(3)-NSH)/
+ (1.-EXP(PN(2)*((U-PN(3)-NSH))))
BN = PN(4)*EXP(PN(5)*((U-PN(6)-NSH)))
RETURN
END

FUNCTION CURR(U, GATE, A)
REAL U, M, H, K, D, F, E, N, GATE(1)
REAL INA, ICA, IK, IK2, INAB, IPI
REAL*8 A(1)
COMMON /DATA/PNA, ENA, PCA, ECA, PNAB, ENAB,
+ SLB, GK, EK, GKB, GPI, SPI, EPI
COMMON /DEBUG/ INA, ICA, IK, IK2, INAB, IPI
COMMON /BACK/ A1, A2, A3, A4, A5, A6
PARAMETER FRT = .03896,
+ NA0 = .1106,
+ CA0 = .0025, CAI = 1.069E-5
M = GATE(1)
H = GATE(2)
D = GATE(3)
F = GATE(4)
N = GATE(5)
K = GATE(6)
E = GATE(7)

T1 = PNA * M*M*H*K * NAO * 96500.*FRT
W = .6 + 4/(1.+EXP(.1*(U+45.)))
IF (ABS(U).GT..01) T2 = U*(EXP(FRT*(U-ENA))-1.)/
+ (EXP(FRT*U)-1.)
IF (ABS(U).LE..01) T2 = (EXP(-FRT*ENA)-1.)/FRT
INA = T1*T2*W

T3 = PCA * D*F*E * 4. * 96500. * FRT
IF (ABS(U).GT..01) T4 = U*(CAI*EXP(2.*U*FRT) - CAO)/
+ (EXP(2.*FRT)-1.)
IF (ABS(U).LE..01) T4 = (CAI-CAO)/(2.*FRT)
ICA = T3 * T4

IK = A(4) * N*N *(U-EK)
IK2 = A(3) * (EXP(A1 *(U-EK)) - 1.)/(EXP(A2*(U-A3)) +
+ EXP(A4 *(U-A5)))

T2 = (U - ENAB)/ A(2)
INAB = A(1) *(EXP(T2) - EXP(-T2))/2.

IPI = GPI * (U-ENAB) / (1. + EXP(SPI*(U-EPI)))

CURR = INA + ICA + IK + IK2 + INAB + IPI
RETURN
END

End of listing of Program apfit
Program DISCRE

This program makes files compatible with DISCRE.
The input file is assumed to have
the following structure:
ITAG1, ITAG2, NPTS, ISPARSE(50), NARRAY(2048).

These files are similar to those made from the
Nicolet or Norland data acquisition systems. The
user is required to pick the first acceptable point
using the 4010 X-cursor. The user must then pick
the last acceptable point which will be printed on
the screen. The program allows for
a maximum of 2048 points. However, DISCRE
accepts only 512 points. A skip factor is
calculated and only every Nth skipped point is
written into the output file.

6/4 Program modified to take ITPLOTS files
directly,
and to process multiple tags from the same file.
6/5 Mod added to allow user to subtract a baseline
current from the current record to be fit.
10/20 Statements for NPTS and time calibration moved

REAL TIME(1024), RBUF(1024)
INTEGER SPARE(50), ARRAY(2048)
LOGICAL YES
BYTE BUFF (80)
EQUIVALENCE (ARRAY(1), TIME(1))

105 CALL FILE (3, 'ENTER FILENAME: ', 'IB')
CALL FETCHR ('^ENTER TIME CALIBRATION IN ',
+ 'MICROSECS: ', 'TMIC', '')
99 CALL FETCHI ('ENTER TAG1: ', ITAG1,
+ 'ENTER TAG2: ', ITAG2, '')
100 READ(3, END=120) ITG1, ITG2, NPTS, (SPARE(I), I=1, 50),
+ (ARRAY(I), I=1, NPTS)
IF (ITAG1 .GT. ITG1) GO TO 101
IF (ITAG2 .GT. ITG2) GO TO 101
IF (ITAG1 .EQ. ITG1 .AND. ITAG2 .EQ. ITG2) GO TO 101
120 CALL TYPE('END OF FILE REACHED')
REWIND (3)
GO TO 99
101 ICT=1
DO 102 I=2, NPTS, 2
RBUF (ICT) = FLOAT (ARRAY(I))
ICT=ICT+1
102 CONTINUE
NPTS=NPTS/2
DO 115 I=1,NPTS
   TIME(I)=FLOAT(I-1)
115  CONTINUE
CALL TEKON (1)
CALL TERASE
CALL SCAN (TIME,RBUF,-NPTS,6440)
CALL DRAW (TIME,RBUF,NPTS,6440)
CALL DRAW (0.,0.,,1,9000)
CALL TYPE ('POSITION X-CURSOR AT ZERO TIME AND Y ' + 'CURSOR AT BASELINE VALUE')
CALL TEKON
CALL TKCURS (XBEG,YBASE,1)
NBEG=XBEG
CALL DRAW (0.,25,1,9000)
CALL TYPE ('POSITION X-CURSOR AT FIRST ', + 'ACCEPTABLE POINT')
CALL TKCURS (XSTART,Y,1)
NSTART=XSTART
103 CALL DRAW (0.,5,1,9000)
CALL TYPE ('POSITION THE X-CURSOR AT THE LAST ', + 'ACCEPTABLE POINT')
CALL TKCURS (XEND,Y,1)
NEND=XEND
104 CALL TERASE
CALL DRAW (0,0,9999)
CALL TEKOFF
DO 114 NI=1,NPTS
114  RBUF(NI)=RBUF(NI)-YBASE
NPTS1=XEND-XSTART+1.
CALL TELLI ('TOTAL NUMBER OF POINTS ',NPTS1)
CALL FILE (2,'ENTER OUTPUT FILENAME: ','OL')
IF (NEND .LE. 512) GO TO 106
IF (NEND .GT. 512 .AND. NEND .LE. 1024) GO TO 107
IF (NEND .GT. 1024 .AND. NEND .LE. 1536) GO TO 108
IF (NEND .GT. 1536 .AND. NEND .LE. 2048) GO TO 109
IF (NEND .GT. 2048) GO TO 110
106 IDEL=1
GO TO 111
107 IDEL=2
GO TO 111
108 IDEL=3
GO TO 111
109 IDEL=4
GO TO 111
110 CALL TYPE ('THERE ARE MORE THAN 2048 POINTS ', + 'IN THE BUFFER')
CALL CLOSE (2)
GO TO 112
111 NPLOT = NPTS1!
NPTS1=NPTS1/IDEL !NUMBER OF POINTS TO FIT
TEND=TMIC*(1.E-6)*(XEND-XBEG) !TIME AT LAST POINT
BPTS=(XSTART-XBEG)*TMIC*(1.E-6) !TIME AT FIRST POINT
DO 80 NCLR=1,80
80            BUF(NCLR) = ' ' !CLEAR BUFF
CALL TIPR ('TITLE: ',BUF,80,L)
WRITE (2,1002) BUF
WRITE (2,1003)4,1,5
WRITE (2,1005) NPLT
WRITE (2,1004)1
WRITE (2,1000) BPTS,TEND,NPT51
WRITE (2,1001) (RBUF(I),I=NSTART,NEND,IDE1)
CALL CLOSE(2)
IF (YES('DO ANOTHER TAG FROM THE PRESENT ',
   'INPUT FILE?')) GO TO 99
CALL CLOSE(3)
IF (YES('DO YOU WANT A NEW INPUT FILE?')) GO TO 105
1000       FORMAT (2D15.6,I5)
1001       FORMAT (5E15.6)
1002       FORMAT (80A1)
1003       FORMAT ('TTTTTTTT',3I3)
1004       FORMAT (I5)
1005       FORMAT (I3,'TTT')
112       CONTINUE
   END

End of listing of program DISCRE
Program EST2

program est2
update 06/28/82
update 09/23/82 - add automatic baseline correction for
exponential fits; add some input checking statements
in key places

Purpose: To take data generated by the Nicolet or
Norland digital oscilloscope and least-squares minimize
the parameters of a user-supplied function so
that they fit the chosen input data

Input Format: The input is assumed to be in the form:
a) Sequential, unformatted binary files containing
an arbitrary number of records. Each record
corresponds to one oscilloscope trace, which
displays one voltage and one current trace.
b) Each record has the form:
itag1, itag2, npts, (label(i), i=1,50),
(idata(k), k=1,npts)
where
itag1, itag2 are integer values that identify
the experiment number and record number.
Values of itag1 represent the group of
records from a single experiment,
while values of itag2 represent individual
records within a group.
npts is an integer equal to the number of
data points. This number must be between 2
and 2048. 2046 is the assumed standard
record size in this program. To increase
the record size, change all the relevant
variables in the declaration statements
below.
label is an integer vector size 50 which
usually holds identifying alphanumeric
information describing each trace
idata is an integer vector which contains the
y-axis magnitudes for each record. Each
point represents a sequential time point.
Even points represent current values, while
odd points represent voltage values.
Notes:

1) This program is set up to fit only current data (even points). To modify it to process either/or current and voltage data, change the index counter of do loop 288 at line 56.

2) The structure of this program is quite general, the user is not restricted to the fitting functions given in FUNCTN. To add more fitting functions, or to change the type of fitting functions, two modifications to the program are necessary:
   a) Change the format statements (labels >1000) which write out the model's on unit 5 to match the new fitting functions.
   b) IMPORTANT Change the number of parameters assignment statement in lines 30 thru 34 so that npar matches the new number of parameters.

3) The way this program is written, it requires the use of a Tektronix 4010 graphics terminal, which has X and Y cursors. It is possible to rewrite this program to operate without cursors by entering the values of variables ix1, ix2, and ix3 for each record.

4) Subroutines which are system specific:
   All the below are concerned with the system plotting package VERSAPLOT:
   call tekon(n) - Assign unit n to the Tektronix terminal
   call terase - Erase Tektronix screen
   call scan(*) - set scaling factors for plot
   call axes(*) - consult scaling factors, and draw axes with specified labels
   call note(*) - write specified string or value onto plot
   call tkcurs(*) - illuminate 4010 cursors, and wait for user to reposition cursors and press any key to continue
   call draw(*) - plot the data according to the scale factors set in call scan(*)

Main routine of EST2

Variables section
x(1024), y(1024) - vectors holding the time and current
data of the points to be fit

a(9) - vector of parameter values
input: contains the user supplied guesses for
the parameter values
output: contains the least-squares estimates
for the parameter values
delta(9) - vector of stepsize values corresponding
to each parameter value
siga(9) - vector of uncertainties corresponding
to each parameter value
lambda - Levenburg parameter used in initial
iterations of the fit
npar,npts - number of parameters and data
points to be used in fit
chisq,oldchi - values of the chi-squared error
at each iteration
fname(15) - name of input file
model - number to select which equation
to use in fit
ixl,ix2,ix3 - cursor locations returned by
the 4010 cursors
yb - correction for current baseline, value returned
by subroutine PLOT2 using the
4010 cursors
off2,offset - correction for steady currents, value
returned by subroutine PLOT2 using
the 4010 cursors
open - flag indicating whether an input file
has been opened
itplt - flag indicating whether the data is in
Nicolet format

Variables available, but not used in this program

yfit(*) - output - vector of calculated values of
best-fit using the parameters a(*)
sigmay(*) - input - vector of standard deviations of
the input points

Subroutines and function subprograms required:
curfit() - The Marquardt fitting routine
plot1() - Plotting routine for input data and
fitting function
plot2() - Intermediate plotting routine

real x(1024),y(1024),a(9),delta(9),siga(9),
+ lambda,chisq,oldchi
integer npts,npar,itr,data(2048)
User selects fitting function for input data by entering an integer value for model. Model is passed via blank common to function FUNCTN, which is then used in a computed GO TO to select the correct fitting function.

```
logical*1 fname(15), open, itplt
equivalence (data(1), x(1))
common model, off2, cmax, p1, p2, p3
common /loc/ ix1, ix2, ix3, yb, offset

open = .false. ! set file flag to indicate closed file

User selects fitting function for input data by entering an integer value for model. Model is passed via blank common to function FUNCTN, which is then used in a computed GO TO to select the correct fitting function.

write(5,999)
write(5,1000)
write(5,1005)
write(5,1001)
write(5,1002)
write(5,1003)

888 format(' Is this data in ITPLOTS readable form?')
999 format(4x,'model=10
g*a(1)*(1.-exp(-x(i)/a(2)))',
      '***3.*exp(-x(i)/a(3))')
1000 format(4x,'model=11
a(1)*(1.-exp(-x(i)/a(2)))',
      '*exp(-x(i)/a(3))')
1005 format(4x,'model=12 a(1)*exp(-x(i)/a(3))'
1001 format(4x, 'model=13
a(1)*exp(-x(i)/a(2))+a(3)',
      '*exp(-x(i)/a(4))')
1002 format(4x, 'model=14
a(1)*(1.-exp(-x(i)/a(3)**2'))
1003 format(4x, 'model=15
a(1)*1.-exp(-x(i)/a(2))**a(3)' )
636 write(5,100)
read(5,101) model
if (model.lt.10.or.model.gt.15) go to 636

Select number of parameters associated with each fitting function
npar = 3
if (model.eq.12) npar=2
if (model.eq.13) npar=4
if (model.eq.14) npar=2
write(5,110) npar, model

100 format(1x,'enter number of model equation desired')
101 format(i3, i2)
110 format(1x, 'NUMBER OF PARAMETERS ARE ', i1,
      ' MODEL IS ', i2)
```

Read in the data points from an external file, if no file is open, get filename of unformatted binary file.
if (.not. open) write(5,200)
if (.not. open) read(5,201) nch,(fname(jj),jj=1,nch)
200 format(1x,'enter the sequential data file name')
201 format(q,15a1)

if (.not. open) open (unit=2,name=fname,type='old',
    + form='unformatted')
  if (.not. open) read(2,end=37) itag1,itag2,npts,
    + (iw,j3=1,50), (data(jj),jj=1,npts)
write first tag1 and tag2 values
277 write(5,227) itag1,itag2
write(5,208) c user entered tag1 and tag2 values
read(5,101) it1,it2
208 format(' Enter tag1 and tag2 value to fit ')
227 format(3x,' Tagl is ',i2,' Tag2 pointer ',
    + is now at ',i2)

In the event the new tag2 value is the same as the one
used in a previous iteration of the program, back up
one record and reload the data buffer with the data
from this tag2 value

if (it1.eq.itagl.and.it2.eq.itag2) backspace

Read each record in the file until a match between
it1 and itag1 and it2 and itag2 is found

read(2,end=37) itag1,itag2,npts,
  + (iw,j3=1,50), (data(jj),jj=1,npts)
write(5,229) itag1,itag2,npts
229 format(' Tag1 is ',i2,' Tag2 is ',i2,' npts is ',i4)
if (it1.ne.itag1.or.it2.ne.itag2) go to 223

Convert integer data representing digitized current
record (even points of each tag2 value) to real
numbers
so they can be used by ESTIMATE. Write real values
into vector y(*) so that they now lie in consecutive
locations i.e. y(1)<= data(2); y(2)<= data(4);
... y(npts/2)<= data(npts)

222 iy=1
do 228 j7=2,npts,2
  y(iy)=float(data(j7)) !convert to real
  iy iy+1
228 continue

readjust of data points to reflect packing
npts=npts/2
Branch to this segment if user input itag2 value is not found. Rewind file and request new itag2 value.

Branch to this segment if user input itag2 value is not found. Rewind file and request new itag2 value.

Enter time and current scale factors per point. These are found by measuring the analog records and comparing them to the digitized records e.g. if a 1 msec, 1 nA calibration pulse extends from 0 to 500 on the time axis, and from -400 to +600 on the current axis when digitized, then the scale factors are 2 usec/pt for time and 1 pA/pt for current. These scale factors may be chosen in any convenient units the user desires i.e. sec, msec, hrs, fA, pA, A. The results will be scaled in the same units.

If the data is noisy, clean it up with an averaging filter.

Interim plotting routine
Subroutine to allow user to select first and last points in record to be processed.
Offset to remove any steady-state currents. This is
useful when fitting exponential tails on top of a steady baseline current

c current

c 454 off2=offset
   if (ix2.lt.ix1) ix2=ix1 !no points before zero time
   if (ix1.eq.1) go to 666

c Discard all points before zero time by repositioning c the current vector y(*) until the first point chosen by c the cursor in PLOT2 is moved to y(1),
c the second point to y(2), ect.
c c ir=1
   c shift first pt to zero time
      do 446 mk=ix1,npts
         y(ir)=y(mk)
         ir=ir+1
      446 continue
   c Readjust npts to reflect discarded points, and c correct cursor position for discarded points
   npts=npts-ix1+1
   ix2=ix2-ix1+1
   ix3=ix3-ix1+1

c Put first point to be fit by CURFIT into y(1), and move c first time point to x(1). This is done to avoid passing c offsets ix1, ix2 and ix3 to CURFIT since CURFIT assumes c the first point to be considered resides in y(1). The c time vector is also shifted to correspond to c the shift into y(1)

c 666 ir=1
   c shift first acceptable pt into y(1) and subtract c baseline value
      do 447 mk=ix2,ix3
         y(ir)=y(mk)-yb
         x(ir)=x(mk)
         ir=ir+1
      447 continue
   c Readjust number of points to reflect above shifts
   if (ix2.ne.ix3) npts=ix3-ix2+1
   if (ix2.eq.ix3) npts=1

c CURFIT requires an initial guess for the values of the c parameters to be estimated. Depending on how
well-behaved the error domain is, and how sensitive
the function is to that parameter, it is possible to
be one or two orders of magnitude off and still
reach the "correct" value for that parameter.
Obviously, the speed of convergence of the method
depends on how accurate the initial guesses for
the parameters are:
136  write(5,102) npar
102  format(6x,'enter the guesses of values for the ', +
     103  format(9f12.4)
     write(5,112) (a(ih),ih=1,npar)
112  format(6x,'GUESSES FOR THE PARAMETERS ARE ', 9(f10.4,1x))

Add on one extra parameter for the baseline value of
fit
if (model.eq.14.or.model.eq.15) go to 308
npar = npar + 1
a(npar) = offset

delta(*) are the increments for the parameter values,
which are used to numerically compute the partial
derivatives of the fitting function in subroutine
FDERIV. Set initial size of these increments to
1% of the parameter value
308  do 104 nl=1,npar
    delta(nl) = a(nl)/100.
104   continue

do the least squares fit
indicate that this is the first iteration
itr=-1
call CURFIT(x,y,npts,npar,a,delta,siga, +
     lambda,chisq,itr)
now use as an iteration counter
itr=0
31  oldchi = chisq      !previous value of
     chisquare error
     call CURFIT(x,y,npts,npar,a,delta,siga, +
                     lambda,chisq,itr)

Compute relative error between present and previous
iteration. If the relative error changes by less
than .1%, assume the "best" parameter values have
been found
err=abs(oldchi-chisq)/chisq
if (err.lt..001) go to 399  !stop computation
itr=itr+1
write(5,130) itr,oldchi,chisq
write(5,107) (a(m),m=1,npar)
130 format(//,1x,'iteration ',i2,
+   ' oldchi, chisq ',2(e12.5,2x))
c Recompute increments for computing partial derivative
do 230 ni=1,npar
   delta(ni) = a(ni)/100.
230 continue
c Continue until 100 iterations or convergence
if (itr.lt.100) go to 31
c 105 format(//,1x,'sigma = ',9(e12.5,2x))
106 format(1x,'chi-square error = ',e12.5)
107 format(3x,'parameters ',9(e12.5,2x))
c optional plotting routines
c Section to plot out both input data and fitting function
c for visual comparison of fit. This uses the VERSAPLOT
c plotting package to create plots (see above).
c
399 write(5,106) chisq
c Remove extra parameter for the baseline value
if (model.eq.14.or.model.eq.15) go to 309
   off2 = a(npar)
   npar = npar - 1
309 write(5,107) (a(m),m=1,npar)
write(5,400)
400 format(//, 'Do you wish a plot of the estimate',
+   ' result (yes=1)?')
read(5,101) iansw
if (iansw.ne.1) go to 455
35 call plotl(y,x,a,chisq,npar,npts,itag1,itag2,fname)
call draw(0.,0.,1.,9000)
write(5,400)
write(5,107) (a(m),m=1,npar)
read(5,101) iansw
if (iansw.eq.1) go to 35
455 write(5,455)
read(5,101) iansw
456 format(,'Do another plot run?')
if (iansw.eq.1) go to 2
write(5,457)
read(5,101) iansw
close (unit=2, disp='save')
457 format(,'Open another input file?')
if (iansw.eq.1) go to 1
9999 call draw(0,0,6,9999)
stop
end
SUBROUTINE CURFIT

REFERENCE:

A modified version of curfit with the mode call removed (mode implicitly is 0 here) and the matrix weight removed (weight=1 for all calculations)

PURPOSE - MAKE A LEAST-SQUARES FIT TO A NON-LINEAR FUNCTION WITH A LINEARIZATION OF THE FITTING FUNCTION

SUBROUTINES AND FUNCTIONS CALLED
FUNCTN(X,I,A) - USER SUPPLIED FUNCTION FOR EVALUATION
FCHISQ(Y,SIGMAY,NPTS,NFREE,MODE) - EVALUATES CHI-SQUARE ERROR
FDERIV(X,I,A,DELTAA,NTERMS,DERIV) - EVALUATES THE DERIVATIVES OF FITTING FUNCTION WITH RESPECT TO THE Ith PARAMETER TERM
MATINV(ARRAY,NTERMS,DET) INVERTS AND CALCULATES THE DETERMINANT OF DEGREE NTERMS
NOTE: SEE THIS APPENDIX UNDER 'MATINV' FOR A LISTING OF THIS PROGRAM

CURFIT. PROGRAM FOR MARQUADT FITTING FROM PAGE 238 OF BEVINGTON'S "DATA REDUCTION AND ERROR ANALYSIS."

SUBROUTINE CURFIT(X,Y,NPTS,NTERMS,A,DELTA,A,
1 SIGMAA,FLAMDA,CHISQR,nitr)

C DOUBLE PRECISION ARRAY
DIMENSION X(1),Y(1),A(1),DELTA(1),SIGMAA(1)
DIMENSION YFIT(1024)
DIMENSION ALPHA(10,10),BETA(10),DERIV(10)
DIMENSION ARRAY(10,10),B(10)

C NFREQ = NPTS - NTERMS
11 IF (NFREQ) 13,13,31
13 CHISQR = 0.0
GO TO 110

C EVALUATE WEIGHTS
20 DO 30 I=1,NPTS
21 IF(MODE) 22,27,29
22 IF(Y(I)) 25,27,23
23 WEIGHT(I) = 1./Y(I)
GO TO 30
WEIGHT(I) = -1./Y(I)
GO TO 30
WEIGHT(I) = 1.
GO TO 30
WEIGHT(I) = 1. / SIGMAY(I)**2
CONTINUE

EVALUATE ALPHA AND BETA MATRICES

DO 34 J=1, NTERMS
BETA(J) = 0.0
DO 34 K=1, J
34 ALPHA(J,K) = 0.0
DO 50 I=1, NPTS
CALL FDERIU(X,I,A,DELTAA, NTERMS, DERIU)
DO 46 J=1, NTERMS
BETA(J) = BETA(J) + (Y(I) - FUNCTN(X,I,A,1))*DERIU(J)
DO 46 K=1, J
46 ALPHA(J,K) = ALPHA(J,K) + DERIU(J)*DERIU(K)
CONTINUE
DO 53 J = 1, NTERMS
DO 53 K = 1, J
53 ALPHA(K,J) = ALPHA(J,K)
CONTINUE

EVALUATE CHI SQUARE AT STARTING POINT

DO 62 I=1, NPTS
YFIT(I) = FUNCTN(X,I,A,2)
CONTINUE
CHISQ1 = FCHISQ(Y, NPTS, NFREE, YFIT)

compute the optimal starting value for lambda. Make
lambda 10 times the sum of the largest column of alpha(*,*)
This is done to ensure that lambda will be large enough
to force fitting routine to act like a gradient method
if (nitr.ne.-1) go to 71
do 132 j=1, nterms
132 FORMAT(5,133) (alpha(j,k), k=1, nterms)
133 FORMAT(3x,5(f9.4,1x))
amax = 0.
do 130 k=1, nterms
  cmax = 0.
do 131 j=1, nterms
    if (j.eq.k) go to 131
    atest = abs(alpha(j,k))/
             sqrt(alpha(j,j)+alpha(k,k))
           cmax = cmax + atest !sum columns
  continue
if (cmax.gt.amax) amax=cmax  
continue  
flamda=10.*amax  
c write(5,'(a5,134) amax,flamda ')  
c134  
format(9.4) amax is ',f9.4,' flamda is ',f9.4)  
C  
C INVERT MODIFIED CURVATURE MATRIX TO FIND NEW  
PARAMETERS  
C  
71 DO 74 J=1, NTERMS  
DO 73 K=1, NTERMS  
C Statements such as the one below are inserted to  
prevent  
c the value of sqrt from becoming small or zero, and  
c causing a floating zero divide error. These were  
c inserted in the test version of this program,  
c and may be removed  
t1 = SQRT(abs(ALPHA(J,J)*ALPHA(K,K)))  
if (t1.lt.1.e-12) t1=1.e-12  
73 ARRAY(J,K) = ALPHA(J,K) / t1  
74 ARRAY(J,J) = 1.0 + FLAMDA  
80 CALL MATINV(ARRAY, NTERMS, DET)  
81 DO 84 J=1, NTERMS  
B(J) = A(J)  
DO 84 K=1, NTERMS  
t2 = SQRT(abs(ALPHA(J,J)*ALPHA(K,K)))  
if (t2.lt.1.e-12) t2=1.e-12  
84 B(J) = B(J) + BETA(K)*ARRAY(J,K) / t2  
C  
C IF CHI SQUARE INCREASED, INCREASE FLAMDA AND TRY AGAIN  
C  
92 DO 92 I=1, NPTS  
93 CHISQR = FCHISQ(Y, NPTS, NFREE, YFIT)  
95 FLAMDA = 10.0*FLAMDA  
GO TO 71  
C  
C EVALUATE PARAMETERS AND UNCERTAINTIES  
C  
101 DO 103 J=1, NTERMS  
A(J) = B(J)  
t3 = alpha(j,j)  
if (t3.lt.1.e-12) t3=1.e-12  
103 SIGMAA(J) = SQRT(abs(ARRAY(J,J)/t3))  
FLAMDA = FLAMDA/10.  
110 RETURN  
END  
C
function fchisq

purpose
evaluate required chi square for fit to data:

\[ fchisq = \sum \left( \frac{(y-yfit)^2}{\sigma^2} \right) / \text{nfree} \]

usage
result = fchisq(y, sigme, npts, nfree, mode, yfit)

description of parameters

y - array of data points
sigmay - array of standard deviations for data points
npts - number of data points
nfree - number of degrees of freedom
mode - determines method of weighting least squares
  +1 (instrumental) \( \text{weight}(i) = 1/\text{sigmay}(i)^2 \)
  0 (no weighting) \( \text{weight}(i) = 1 \)
  -1 (statistical) \( \text{weight}(i) = 1/\text{y}(i) \)
yfit - array of calculate values of y
fchisq - chi-square error of calculated and actual data points

subroutines and function subprograms called
none

function fchisq(y, npts, nfree, yfit)
double precision chisq
dimension y(i), yfit(i)

11 chisq = 0.
12 if (nfree) 13, 13, 20
13 fchisq = 0.
go to 40
20 do 30 i = 1, npts
30 chisq = chisq + (y(i) - yfit(i)) * (y(i) - yfit(i))
31 free = float(nfree)
32 fchisq = chisq / free
40 return
end

FDERIV.FTN

PURPOSE:
EVALUATES DERIVATIVES FOR CURFIT FROM A DIFFERENCE EQUATION.

USAGE
CALL FDERIV(X, I, A, DELTAA, NTERMS, DERIV)
DESCRIPTION OF PARAMETERS

X - ARRAY OF DATA POINTS FOR INDEPENDENT VARIABLE
I - INDEX OF DATA POINTS
A - ARRAY OF PARAMETERS
DELTAA - ARRAY OF PARAMETER INCRIMENTS
TERMS - NUMBER OF PARAMETERS
DERIV - DERIVATIVES OF FUNCTION

SUBROUTINES AND FUNCTION SUBPROGRAMS REQUIRED

FUNCTN (X,I,A) EVALUATES THE FITTING FUNCTION FOR THE Ith TERM

SUBROUTINE FDRTV(X,I,A,DELTAA,TERMS,DERIV)
DIMENSION X(1),A(1),DELTAA(1),DERIV(1)

11 DO 18 J=1,TERMS
  AJ = A(J)
  DELTA = DELTAA(J)
  YFIT = FUNCTN(X,I,A,4)
  A(J) = AJ + DELTA
  YFIT(2) = YFIT - FUNCTN(X,I,A,5) / (2.0*DELTA)
18 A(J) = AJ
RETURN
END

Filter routines

Three point polynomial averaging filter - unity gain at d.c.

subroutine avg3(y,npts)
real y(npts)
do 6 k=2,npts-1
  y(k) = .25*y(k-2) + .5*y(k-1) + .25*y(k)
6 return
end

Five point polynomial averaging filter - unity gain at d.c.

subroutine avg5(y,npts)
real y(npts)
do 6 k=3,npts-2
  y(k) = 11111*(y(k-2) + 2*y(k-1) + 3*y(k) + 2*y(k+1) + y(k+2))
6 return
end

User supplied fitting functions
Description of parameters

- vector of data points for the independent variable
- index for accessing values in x(*)
- vector of parameter values
- used in test version to indicate which CALL statement was last executed before entry into function

Subroutines and subprograms required
none

function functn(x, i, a, n)
real x(1), a(1), offset
common model, offset, cmax, p1, p2, p3
go to (10, 11, 12, 13, 14, 15, 16), model=9

Each model corresponds to a choice for the integer model in the main program. The number of parameters for each model MUST correspond to the model given here for the program to work

10 functn = a(1)*t1*t1*t1*exp(-x(i)/a(2))+a(4)
    return
11 functn = a(1)*(1.-exp(-x(i)/a(2)))*exp(-x(i)/a(3))+a(4)
    return
12 functn = a(1)*exp(-x(i)/a(2))+a(3)
    return
13 functn = a(1)*exp(-x(i)/a(2))+a(3)*exp(-x(i)/a(4))+a(5)
    return
14 functn = a(1)*(1.-exp(-x(i)/a(2)))*(1.-exp(-x(i)/a(2)))+
          + offset
    return
15 functn = a(1)*(1.-exp(-x(i)/a(2)))*a(3)+offset
    return
16 continue
return
end

End of listing of program EST2
Program MATINV

subroutine matinv - modified for fast return for a 2x2 or 1x1 matrix

purpose
invert a symmetric matrix and calculate its determinant

usage
call matinv(array,norder,det)

description of parameters
array - input matrix which is replaced by its inverse
norder - degree if matrix (order of determinant)
det - determinant of input matrix

subroutines and function subprograms required
none

comments
dimension statement valid for norder up to 10

subroutine matinv(array,norder,det)
double precision array,amax,save
dimension array(10,10),ik(10),jk(10)

10 det=1.

fast return if order=1
if (norder.ne.1) go to 17
array(1,1)=1./array(1,1)
return

fast return if order=2
17 if (norder.ne.2) go to 11
det=1./(array(1,1)*array(2,2)-array(1,2)*array(2,1))
t=1.array(1,1)
array(1,1)=array(2,2)*det
array(2,2)=t*det
array(1,2)=-array(1,2)*det
array(2,1)=-array(2,1)*det
return

do 100 k=1,norder
find largest element array(i,j) in matrix
amax=0.
21 do 30 i=k,norder
do 30 j=k,norder
   if (dabs(amax)-dabs(array(i,j))) 24,24,30
   amax=array(i,j)
i(k)=i
   j(k)=j
30 continue

interchange rows and columns to put amax in array(k,k)

if (amax) 41,32,41
det=0.
go to 140
41 i=ik(k)
   if (i-k) 21,51,43
do 50 j=1,norder
      save=array(k,j)
      array(k,j)=array(i,j)
      array(i,j)=-save
50 continue
51 j=jk(k)
   if (j-k) 21,61,53
do 60 i=1,norder
      save=array(i,k)
      array(i,k)=array(i,j)
      array(i,j)=-save
60 continue

accumulate elements of inverse matrix

do 70 i=1,norder
   if (i-k) 63,70,63
      array(i,k)=-array(i,k)/amax
70 continue
71 do 80 i=1,norder
   do 80 j=1,norder
      if (i-k) 74,80,74
      if (j-k) 75,80,75
      array(i,j)=array(i,j)+array(i,k)*array(k,j)
80 continue
81 do 90 j=1,norder
   if (j-k) 83,90,83
   array(k,j)=array(k,j)/amax
90 continue
array(k,k)=1./amax
det=det*amax
100 continue

restore ordering of matrix

do 130 l=1,norder
   k=norder-l+1
End of listing of program MATINU
Program NORLD

C+++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
+++++++ C PROGRAM NORLD C+++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
+++++++ BYTE LINE (80)
LOGICAL EQSTR, ISW
INTEGER IPARM(6)
INTEGER IOST(2)
DATA IORUB "$10400/
DATA IOWUB "$11000/
ISW=.FALSE.
REG=.02E02
C DATA LUN /4/
CALL ASSIGN( LUN,'TT33:' )
CALL TYPE('NORLD VER. 2: USES 2 RS232 LINES ',
* '(NORLAND=TTY 33')
CALL FETCHI ('INPUT NUMBER OF TRACE PAIRS TO',
* 'BE TRANSFERRED ',NTR)
ICNT=0
C USE - SIZES IN OPEN STATEMENT TO ALLOW NON-CONTIGUOUS
EXTENSIONS. IF +, EXTEND MAY FAIL. SEE FORTRAN 4+
MANUAL FOR THIS USEFUL TIDBIT
C OPEN
(UNIT=1,NAME='NORLD.DAT',TYPE='NEW',INITIALSIZE=-35,
1EXTENDSIZE=-35,CARRIAGECONTROL='LIST',BUFFERCOUNT=2)
C I00 CONTINUE
CALL GETADR( IPARM,LINE )
IPARM(2) = 00
CALL WTQIO( IORUB,LUN,1,,IOST,IPARM,IDSW )
IF( IDSW .NE. 1 ) GOTO 200
IF( (IOST(1).AND."377") .NE. 1 ) GOTO 200
C NULL TERMINATE STRING
LINE(IOST(2)+1) = 0
NC = LENSTR(LINE)
WRITE (1,1020) (LINE(I),I=1,NC)
1020 FORMAT (80A1)
ICR = "$15"
CALL GETADR( IPARM,ICR )
IPARM(2) = 1
CALL WTQIO( IOWUB,LUN,1,,IOST,IPARM,IDSW )
200 CALL TELL('IOST=', 'IOST(1)', 'IOST(2)'),
      STOP

999 CONTINUE
   IF (ISW) GO TO 998
   ISW = .TRUE.
   GO TO 100
998 ICNT = ICNT + 1
   ISW = .FALSE.
   IF (ICNT .EQ. NTR) GO TO 110
   CALL WNORLD (REG, LUN)
   GO TO 100
110 REG = .09E02
   CALL WNORLD (REG, LUN)
   STOP 'DATA TRANSMISSION FINISHED'
   END

SUBROUTINE WNORLD (REG, LUN)
   READ (LUN, 100) ICHAR ! READ Z FROM NORLD.
   WRITE (LUN, 101) ! SEND BACK CR FROM 11/70
   READ (LUN, 100) ICHAR ! READ OPCODE FROM NORLD.
   WRITE (LUN, 101) ! SEND BACK CR
   READ (LUN, 103) FILE ! READ FILE NUMB.
   WRITE (LUN, 101) ! SEND BACK CR
   DO 1 I = 1, 7
      READ (LUN, 100) ICHAR ! SEND CR FROM NORLD.
   WRITE (LUN, 102) REG ! SEND REGISTER
1 CONTINUE
   READ (LUN, 100) ICHAR ! READ CR FROM NORLD.
   WRITE (LUN, 104) ! SEND SLASH, CR FROM 11/70
99 FORMAT (1H, +, A1)
100 FORMAT (A1)
101 FORMAT (1H+)
102 FORMAT (E11.4)
103 FORMAT ('+', E11.4)
104 FORMAT ('+'1)
Program NORNIC

THIS PROGRAM MAKES NICOLET-LIKE FILES TO BE USED EITHER BY ITPILOTS OR TRANSIENT (BNC). ITPILOT FILES ARE INTEGER AND TRANSIENT FILES ARE REAL. THE PROGRAM ASSUMES THAT IF TWO TRACE FILES ARE MADE THEY ARE DESTINED FOR ITPILOTS AND IF SINGLE TRACE FILES ARE MADE THEY ARE DESTINED FOR TRANSIENT. THE RANGE OF NORLAND NUMBERS IS 0 TO 4095. TO MAKE THEM NICOLET-LIKE 2048 IS SUBTRACTED FROM EACH NUMBER. THE RANGE IS THEN PLUS OR MINUS 2048.


IN EITHER SINGLE OR TWO TRACE DATA THE ORIGINAL MEMORY SOURCE CAN BE QUADS, HALVES, OR ALL. THE WAY IN WHICH THE PROGRAM IDENTIFIES THE DIFFERENCES IS BY EXAMINING THE FILE NUMBER. SINCE ALL DATA IS SENT TO THE 11/70 AS QUADS (IE. HALF IS TWO QUADS), IF HALF THE MEMORY IS SENT THE FILE NUMBER WILL BE THE SAME. HENCE, THE USER MUST USE DIFFERENT FILE NUMBERS EACH TIME HE/SHE SENDS A NEW TRACE OR TRACES.

THE PROGRAM ENDS BY TESTING FOR A NEW QUAD. IF NONE EXISTS IT SENSES AN EOF AND EXITS.

FOR EACH RUN THE PROGRAM ASKS FOR AN OUTPUT FILE AND STARTING TAG1 AND TAG2 VALUES. TAG2 IS INCREMENTED AND PRINTED AT THE END OF DATA PROCESSING.

DIMENSION INAR1(4096), INAR2(2048),
KOUTPT(4096), IBUF(50),
1 ARRAY1(4096), BUF(50)
EQUIVALENCE (ARRAY1(1), INAR1(1))
BYTE FILENM(40)
LOGICAL YES, I2TR, IDONE, ITPLTS, IPACK
I2TR=.FALSE. !TWO TRACE FLAG
ITPLTS=.FALSE. !ITPLTS SCHEDULED DATA FLAG
IDONE=.FALSE. !ALL DONE FLAG
IPACK=.FALSE. !PACKING TRACES/1024 PTS. FLAG
NPTS1=1024 !DEFAULT VALUE, MIN NUMBER OF POINTS
DO 1 I=1, 50
BUF(I)=0.
1 IBUF(I)=0
CALL FETCHI ('INPUT NUMBER OF POINTS/TRACE ', NPTS)
CALL FETCHI ('INPUT TAG1 ', ITG1, 'INPUT TAG2 ', ITG2)
IF(YES('ARE TRACES PACKED NTRAES/1024 POINTS? '))
+ GO TO 108
108 IPACK=.TRUE.
CALL FETCHI ('HOW MANY TRACES ARE THERE IN ',
+ '1024 POINTS? ', NTRACE)
NPTS1=1024
NCNT=1
IDX=NPTS
111 CALL FILE (3, 'OPEN OUTPUT FILE: ', 'OB', FILENM)
OPEN (UNIT=1, NAME='WORLD.DAT', TYPE='OLD',
1 INITIALIZE=35, CARRIAGECONTROL='LIST')
C SKIP OVER FIRST CODE
READ (1, 1000) AFILE, BUF(1), BUF(2), BUF(3)
1000 FORMAT (/4(E10.5/))
IF (YES('IS THIS SINGLE TRACE DATA FOR ')
+ 'TRANSIENT PROGRAM? ')) GO TO 100
IF (YES('IS THIS ITPLTS SINGLE TRACE DATA? '))
+ GO TO 105
GO TO 104
105 ITPLTS=.TRUE.
GO TO 100
104 CONTINUE
C NO, THEN IT MUST BE TWO TRACES
I2TR=.TRUE. !SET TWO TRACE FLAG
100 CALL INPUT (INAR1, IDONE, AFILE, NPTS1, BUF)
IF (ITPLTS) GO TO 106
IF (I2TR) GO TO 102 !READ NEXT QUAD
GO TO 110
106 L=2*NPTS
DO 5 I=2, L, 2
KOUTPT(I-1)=INAR1(I/2)-2048.
5 KOUTPT(I)=KOUTPT(I-1)
WRITE (3) ITG1, ITG2, L, (IBUF(I), I=1, 50),
+ (KOUTPT(I), I=1, L)
GO TO 107
110 DO 4 I=NPTS,1,-1
4   ARRAY1(I)=INAR1(I)-2048.
   GO TO 101
C OUTPUT WHAT WE HAVE AND READ NEXT QUAD
102 CALL INPUT (INAR2,IDONE,AFILE,NPTS1,BUF)
115 J=1
   L=2*NPTS
   DO 3 I=1,L,2        !STUFF ALTERNATE DATA POINTS
3   KOUTPT(I)=INAR1(I)-2048.
   KOUTPT(I+1)=INAR2(I)-2048.
   J=J+1
   CONTINUE
C MAKE NICOLET-LIKE NUMBERS
   KOUTPT(I)=INAR1(I)-2048.
   KOUTPT(I+1)=INAR2(I)-2048.
   J=J+1
   CONTINUE
C OUTPUT TWO TRACES
   WRITE (3) ITG1,ITG2,L,(IBUF(I),I=1,50),
             (KOUTPT(I),I=1,L)
   IF (IPACK) GO TO 112
   GO TO 113
112 IF (NCNT .EQ. NTRACE ) GO TO 113
   DO 114 I=1,NPTS !UNPACK DATA.
       INAR1(I)=INAR1(I+IDX)
       INAR2(I)=INAR2(I+IDX)
114 CONTINUE
C OUTPUT SINGLE TRACE
   WRITE (3), ITG1,ITG2,NPTS,(BUF(I),I=1,50),
             (ARRAY1(I),I=1,NPTS)
   ITG2=ITG2+1
   GO TO 101
2 IF(IDONE) GO TO 103        !END PROGRAM
   ITG2=ITG2+1
   GO TO 100
103 CALL CLOSE (1)
107 CALL CLOSE (3)
   CALL TELLI ('TAG1 = ',ITG1,'    TAG2 = ',ITG2,'')
END

SUBROUTINE INPUT (IDUM,IDONE,AFILE,NPTS1,BUF)
DIMENSION INARRA (1024),IDUM(1),BUF(1)

4 DO 1 J=1,64
   READ (1,1001,ERR=2) (INARRA(I), I=(J-1)*16+1,J*16)
CONTINUE
1001 FORMAT (16I4)
   J=1
   DO 5 I=N,NPTS1
      IDUM(I)=INARRA(J)
      J=J+1
   5 CONTINUE
   READ (1,1002,END=2,ERR=2) !TEST FOR ANOTHER QUAD
1002 FORMAT (/)
   C THERE IS ANOTHER QUAD
   READ (1,1004,ERR=2) BFILE,BUF(1),BUF(2),BUF(3)
1004 FORMAT (/4(E10.5/))
   C MORE DATA OF SAME TYPE
   IF (BFILE .EQ. AFILE) GO TO 6
   AFILE=BFILE
   RETURN
6 N=NPTS1+1
   NPTS1=1024+NPTS1
   READ (1,1005)
1005 FORMAT (///) !SKIP OVER HEADER
   GO TO 4
2 WRITE (5,1003)
1003 FORMAT (X,'AN END OF FILE INDICATES NO MORE DATA')
   IDONE=.TRUE.
3 CONTINUE
   RETURN
END

End of listing of program NORNIC
APPENDIX C

Program FROGIE

PROGRAM FROGIE
C 3/17 NEW AND IMPROVED VERSION WITH ACTIVATION AND I-V
C SECTIONS REMOVED. NEW COMMANDS BACK AND PARMS ADDED
C 3/18 NEW BACKGROUND CURRENTS INAB AND IPI ADDED
C 3/19 PARMS TO VARY IK2 ADDED
C 3/23 CHANGED ECA TO +70.
C 3/28 ADDED SUBROUTINE APMOVE
C 4/24 MOVE STATEMENTS INSIDE LOOP SO THAT EQUAL AMOUNTS
C OF CHARGE
C ARE APPLIED DURING A DEPOL IRREGARDLESS OF VALUE OF
C REACTIVATION VARIABLES E AND K USED HERE
C
C THIS VERSION PLOTS BOTH VOLTAGE AND CURRENT ON TEK
C SCALING: TIME IN mSEC; CURRENT IN pA; VOLTAGE IN mV
C ADDITIONAL SUBPROGRAMS:
C RATE(U) - COMPUTE THE RATE CONSTANTS (ALPHA AND
C BETA M AND H) AT POTENTIAL U
C RK4(*) - SOLVE A DIFFERENTIAL EQUATION GIVEN IN
C FUNCTION 'FN' AND RETURN THE RESULT
C INPUT : F(T(K)) OUTPUT : F(T(K+1))
C FN(*) - WHERE DIFFERENTIAL EQUATION IS EXPRESSED
C IN THE FORM: XOUT(M) = G(XIN(M))
C CURR(*) - COMPUTE THE INSTANTANEOUS MEMBRANE
C CURRENT IM FOR THE GIVEN VALUES OF
C U, M, AND H
C REAL TIME(1024), !TIME IN MSEC
* OUT(1024), !CURRENT IN PA
* XM(2), YM(2), !SCALE FACTORS FOR
C PLOTS
C VECTORS USED FOR PLOTTING AND USED IN SECTION 'RATE'
* T1(100), T2(100), T3(100),
* T4(100), X(100),
C VECTORS USED FOR INTEGRATION
* DDT(7), IDOT(1),
C M AND H SHIFT VOLTAGES
* MSH, HSH, DSH, FSH, NSH,
C MEMBRANE AND IONIC CURRENTS
  * IM, II
C PARAMETER LISTS
  REAL PM(6), PH(6), PK(6), PD(6), PF(6), PE(6), PN(6)
C PERMEABILITIES AND IONIC CURRENTS
  REAL PNA, PCA, GK, ENA, ECA, EK, GNAB, * INA, ICA, IK, IK2, INAB, IPI
  LOGICAL EQSTR, INPUT(6), PLOT, YES, SR, DUMP, DEBU, CLTY, *
  PIIND, P2IND, P3IND
C COMMON / RATE/ AM, BM, AH, BH, AK, BK, AD, BD, AF, BF, *
  AE, BE, AN, BN
C COMMON / PARMS/ SEC, RS, IM, II
C COMMON / DATA/ PNA, ENA, PCA, ECA, PNAB, ENAB, SLB, GK, EK, *
  GKB, GPI, SPI, EPI
C COMMON / DEBUG/ INA, ICA, IK, IK2, INAB, IPI
C COMMON / BACK/ A1, A2, A3, A4, A5, A6
C COMMON PM, PH, PK, PD, PF, PE, PN

C PROGRAM BEGINS
  CALL GETPLT(IU)
  IF (IU.EQ.1) CALL VERON(1) ! PLOT TO LINE PRINTER
  IF (IU.EQ.2) CALL TEKON(1) ! PLOT ON TEKTRONIX
  IF (IU.EQ.3) CALL HPON(1) ! PLOT ON H-P PLOTTER
  IF (IU.NE.0) CALL UERON(1) ! PLOT ON MODE (-2, XMAX, XMIN, XOFF)
  IF (IU.NE.0) CALL UERON(1) ! PLOT ON MODE (-3, YMAX, YMIN, YOFF)

112 OPEN (UNIT=3, NAME='APRATE.DAT', ACCESS='SEQUENTIAL'
  * , TYPE='OLD')
  READ (3,1) (PM(I), I=1,3) ! DYNAMIC SODIUM
  READ (3,1) (PM(I), I=4,6) ! PM = ACTIU
  READ (3,1) (PH(I), I=1,3) ! PH = INACTIU
  READ (3,1) (PH(I), I=4,6) ! PK = REACTIU
  READ (3,1) (PK(I), I=1,3) ! DYNAMIC CALCIUM
  READ (3,1) (PK(I), I=4,6) ! PE = REACTIU
  READ (3,1) PNA, ENA ! BACKGROUND SODIUM
  READ (3,1) PNAB, ENAB, SLB ! DYNAMIC POTASSIUM
  READ (3,1) PD(1), I=1,3 !background calcium
  READ (3,1) PD(I), I=4,6 ! PD = ACTIU
  READ (3,1) PF(1), I=1,3 ! PF = INACTIU
  READ (3,1) PF(I), I=4,6 ! PE = REACTIU
  READ (3,1) PE(1), I=1,3 ! BACKGROUND IK2
  READ (3,1) PE(I), I=4,6 ! INAB, IPI
  READ (3,1) PCA, ECA ! PESISTANT INWARD
  READ (3,1) (PN(I), I=1,3) ! DYNAMIC SODIUM
  READ (3,1) (PN(I), I=4,6) ! PN = ACTIU
  READ (3,1) GK, EK, GKB
  READ (3,1) GPI, SPI, EPI
  READ (3,1) A1, A2, A3
  READ (3,1) A4, A5, A6
READ (3,1) A4,A5,A6
READ (3,1) CM
READ (3,1) CM
!MEMB CAPACITANCE
1 FORMAT (3F10.6)
CLOSE (UNIT=3)
C
SC = 1. !SCALE FACTOR FOR PLOTS
HP = -90. !HOLDING POTENTIAL
TPP = .1 !TIME PER POINT IN MSEC
RUNT = 100.
RS = 3.0E+6 !SERIES RESISTANCE
C
CM = 8.64E-11
PIDUR = 1024.*TPP !INITIAL P1 DURATION
SR = .FALSE. !SERIES RESIS STATE INDICATOR
PLOT = .FALSE. !DATA PLOTTED FLAG
DUMP = .FALSE. !DEBUG AID
DEBU = .FALSE. !DEBUG FLAG FOR IONIC CURRENTS
C
DO 18 M=1,1024
18 TIME(M) = TPP*FLOAT(M)
C
JUMP = 0
IF (EQSTR(INPUT,'HELP',2)) GO TO 75
IF (EQSTR(INPUT,'QUIT',2)) GO TO 95
IF (EQSTR(INPUT,'DEPOL',3)) JUMP=1
IF (EQSTR(INPUT,'RTE',2)) JUMP=2
IF (EQSTR(INPUT,'RS',2)) JUMP=3
IF (EQSTR(INPUT,'HP',2)) JUMP=4
IF (EQSTR(INPUT,'DU',2)) JUMP=5
IF (EQSTR(INPUT,'SC',2)) JUMP=6
IF (EQSTR(INPUT,'SH',2)) JUMP=7
IF (EQSTR(INPUT,'DEB',3)) JUMP=8
IF (EQSTR(INPUT,'BACK',2)) JUMP=9
IF (EQSTR(INPUT,'PARM',2)) JUMP=10
IF (EQSTR(INPUT,'RESE',2)) JUMP=11
GO TO (20,40,50,60,70,80,85,90,40,97,110) JUMP
CALL TYPE('INVALID COMMAND')
GO TO 100
C
20 CLTY = .FALSE.
P1 = 0.
PIDUR = 0.
P2 = 0.
P2DUR = 0.
PP3 = 0.
P3DUR = 0.
IF (YES('GO TO VOLTAGE CLAMP MODE? ')) CLTY = .TRUE.
CALL CNDGTR('ENTER P1 LEVEL ',P1)
CALL CNDGTR('ENTER P1 DURATION IN MSEC ',PIDUR)
NP1 = INT(PIDUR/TPP)
IF (P1DUR.GE.RUNT) GO TO 21
CALL CNDGTR('ENTER P2 LEVEL ',P2)
CALL CNDGTR('ENTER P2 DURATION IN MSEC ',P2DUR)
IF (P2DUR.EQ.0.) P2DUR = RUNT
NP2 = INT(P2DUR/TPP)
PNEXT = P1DUR + P2DUR
IF (PNEXT.GE.RUNT) GO TO 21
CALL CNDGTR('ENTER P3 LEVEL ',P3)
CALL CNDGTR('ENTER P3 DURATION IN MSEC ',P3DUR)

21

T = 0.
DUDT = 50.
CALL RATE(HP,MSH,HSH,DSH,FSH,NSH)
DDT(1) = AM/(AM+BM) ! COMPUTE M INFINITY
DDT(2) = AH/(AH+BH) ! COMPUTE H INFINITY
DDT(3) = AD/(AD+BD) ! COMPUTE D INFINITY
DDT(4) = AF/(AF+BF) ! COMPUTE F INFINITY
DDT(5) = AN/(AN+BN) ! COMPUTE N INFINITY
DDT(6) = AK/(AK+BK) ! COMPUTE K INFINITY
DDT(7) = AE/(AE+BE) ! COMPUTE E INFINITY
UM = HP
IF (CLTY) VM = P1
IF (CLTY) CALL RATE(VM,MSH,HSH,DSH,FSH,NSH)
IF (.NOT.CLTY) IM = P1
PEAKII = 0.
PEAKT = 0.

XM(1) = 0.
XM(2) = 1024*TPP*SC
YM(1) = +50. ! IN pA
YM(2) = -50. ! IN pA
C PUT BOTH U AND I PLOT ON SAME SCREEN IF IN TEK MODE
C CHANGE PLOT SIZE TO .5X
IF (IU.EQ.2) CALL MODE(7,9999.,4.,9999.)
CALL SCAN(XM,YM,—2,6440)
IF (IU.EQ.2) CALL TERASE
CALL AXES(13.2,'TIME IN mSECS',13.2,'CURRENT IN pA')
NS = 1
DO 22 NOUT = 1, 1024
   IF (CLTY) GO TO 23

C SET INTEGRATION RATE DEPENDENT ON DUDT
C P1IND = .FALSE.
P2IND = .FALSE.
P3IND = .FALSE.
IF (NOUT.LT.NP1) P1IND = .TRUE.
IF (NOUT.GE.NP1.AND.NOUT.LT.NP2) P2IND = .TRUE.
IF (NOUT.GE.NP2) P3IND = .TRUE.
222

C

TINC = 1./(20.*ABS(DUDT))
IF (T.LT.10.) TINC = .005
IF (T.GE.10. AND T.LT.75.) TINC = .025
IF (T.GE.75. AND UM.GT.-50.) TINC = .05
IF (T.GE.75. AND (UM.LE.-50. AND UM.GT.-75.))
  TINC = .025
IF (T.GE.75. AND UM.LE.-75.) TINC = .01
IF (UM.LE.-80. AND ABS(DUDT).LE.0.001) TINC = TPP
NTIMES = INT(TPP/TINC)
NT2 = NTIMES

DO 26 MM = 1, NT2
  CALL RATE(UM,MSH,HSH,DSH,FSH,NSH)
  CALL APMOVE(UM,DDT,T,TINC)
  II = CURR(UM,DDT)
  DUDT = (IM-II)*1.E-12/CM
  VM = UM + TINC * DUDT
  T = T + TINC
  IF (T.GE.P1DUR.AND.T.LT.PNEXT) IM = P2
  IF (T.GE.PNEXT) IM = P3
  CONTINUE

OUT(NOUT) = UM + IM*1.E-9*RS
TIME(NOUT) = T
GO TO 27

23 CALL APMOVE(UM,DDT,T,TPP)
  II = CURR(UM,DDT)
  IM = II
C CALCULATE APPARENT MEMBRANE POTENTIAL
OUT(NOUT) = UM + IM*1.E-9*RS
TIME(NOUT) = T
  T = T + TPP
  IF (T.GE.P1DUR.AND.T.LT.PNEXT) UM = P2
  IF (T.GE.PNEXT) UM = P3
  CALL RATE(UM,MSH,HSH,DSH,FSH,NSH)

C 27 IF (CLTY) T1(N5) = ICA + IK + INAB + IK2 + IPI

27 T1(N5) = ICA
  T2(N5) = IK
  T3(N5) = INAB
  T4(N5) = IK2
  X(N5) = T
  N5 = N5 + 1

  IF (ABS(II).LE.ABS(PEAKII)) GO TO 35
PEAKT = T
  !FIND PEAK VALUE
PEAKUM = OUT(NOUT)
C PROGRAM DEBUGGING AND NUMERICAL OUTPUT SECTION
35 IF (.NOT.DEBU) GO TO 42
IF (ICNT.NE.10) GO TO 37
ICNT = 0
WRITE(3,36) T,UM,II,INA,ICA,IK,IK2,INAB,IPI
36 FORMAT(' T = ',F8.3,' UM = ',F7.2,
' TOTAL CURRENT = ',F9.2,/,' INA= ',F8.2,' ICA= ',F7.2,
' IK= ',F6.2,' IK2= ',F6.2,
' INAB= ',F6.2,' IPI= ',F6.2)
WRITE(3,340) (1.E-12/CM)*(UM-II)*TINC
340 FORMAT('DUDT IS ',F6.3,'
 CURRENT STEPSIZE IS ',F8.6)
WRITE(3,38) AM/(AM+BM),AH/(AH+BH),AD/(AD+BD),
AF/(AF+BF),AN/(AN+BN),AK/(AK+BK),AE/(AE+BE)
WRITE(3,38) (DDT(IR),IR=1,7)
FORMAT(' INF:',7(F7.5/IX))
FORMAT(' STEADY-STATE TAU:'
,3(F8.3/IX)/)
37 ICNT = ICNT + 1
C PLOT OUT INTERMEDIATE CURRENT VS TIME 100 PTS AT A TIME
42 IF (N5.NE.101) GO TO 22
CALL DRAW(X,T2,100,6440) !ICA
CALL DRAW(X,T3,100,6440) !IK
CALL DRAW(X,T4,100,6440) !INAB
CALL DRAW(X,T4,100,6440) !IK2
22 CONTINUE
C PLOT OUT THE REMAINDER OF CURRENT VS TIME DATA
IF (N5.EQ.1) GO TO 46
CALL DRAW(X,T1,N5-1,6440)
CALL DRAW(X,T2,N5-1,6440)
CALL DRAW(X,T3,N5-1,6440)
CALL DRAW(X,T4,N5-1,6440)
46 IF (IU.EQ.2) CALL DRAW(0.,4,5,1,1000)
IF (IU.NE.2) CALL DRAW(0.,0.,1,9000)
IM = STKEEP
XM(1) = 0.
XM(2) = 1024.*TPP*SC
SC = 1.
YM(1) = HP
YM(2) = 50. !IN MV
CALL SCAN(XM,YM,-2,6440)
CALL AXES(12.2,'TIME IN MSEC',13.2,'VOLTAGE IN MV')
CALL DRAW(TIME,OUT,1024,6441)
C PLOT '+ SYMBOL @ PEAK DUDT
CALL NOTE(PEAKT,PEAKUM,23,-1)
PDU = -1.E-12*PEAKII/CM
C RESET X AND Y ORIGINS AND RETURN PLOTS TO 9X7 SIZE
IF (IU.EQ.2) CALL MODE(2,XMAX,XMIN,XOFF)
IF (IU.EQ.2) CALL MODE(3,YMAX,YMIN,YOFF)
IF (IU.EQ.2) CALL MODE(7.,9999.,9.,9999.)
C PUT RUN INFO ONTO PLOT
IF (IU.NE.1) GO TO 28
CALL NOTE(1.5,8.3,'Ist = ',6)
CALL NOTE(2.25,8.3,'IM 1001')
CALL NOTE(3.25,8.3,'STDUR = ',8)
CALL NOTE(4.,8.3,'P1DUR 1001')
CALL NOTE(3.,1.5,'DUDT MAX IS AT MSEC',37)
CALL NOTE(4.25,1.5,'PDU 1002')
CALL NOTE(5.4,1.5,'PEAKT 1002')
CALL NOTES(MSH,HSH,DSH,FSH,NSH)
28 CALL DRAW(0.,0.,1.,9000)
TIME2 = SECONDS(TIME1)
PLOT = .TRUE.
CALL BELL
WRITE(5,29) PDU,PEAKT
29 FORMAT('PEAK VALUE OF DU/DT IS ',F7.2,', occurring at ',F7.3,' MSEC')
CALL TELLR('ELASPED TIME IS ',TIME2,' SECONDS')
GO TO 100
C GENERATE n INFINITY (JUMP=2) OR BACKGROUND I-U (JUMP=9)
C 40 VINIT = -100.
VEND = 60.
CALL CNDGTR('START VOLTAGE ',VINIT)
CALL CNDGTR('END VOLTAGE ',VEND)
STEP = (VEND-VINIT)/100.
PLOT = .TRUE.
C UM = VINIT
FRT = .03896
DO 41 ICNT = 1, 100
IF (JUMP.EQ.9) GO TO 48
CALL RATE(UM,MSH,HSH,DSH,FSH,NSH)
R1 = 1/(AM+BM)
R2 = 1. /(AH+BH)
R3 = 1./(AD+BD)
R4 = 1./(AF+BF)
R5 = 1./(AN+BN)
R6 = 1./(AK+BK)
R7 = 1./(AE+BE)

IF (DUMP) WRITE(5,47) VM,R1,R2,R3,R4,R5,R6,R7
IF (DUMP) WRITE(5,93) AM*R1,AH*R2,AD*R3,AF*R4,
               AN*R5,AK*R6,AE*R7

* FORMA T(1X,F7.2,’ TAU S ARE:
  ’,F6.4,1X,F6.3,1X,
  * F6.4,1X,4(F9.4,1X)
  93 FORMA T(11X,’M,H,D,F,N ’,7(F6.4,1X),/)
 VM = VM + STEP
GO TO 41

C COMMAND ‘BACK’ - PRINT OR PLOT BACKGROUND I-U’S
C PLOT BACKGROUND POTASSIUM I-U
  4B T1(ICNT) = GKB * (EXP( A1*(VM-EK)) - 1.)/
        * (EXP( A2 *(VM-A3)) + EXP( A4 * (VM-A5)))
C PLOT BACKGROUND SODIUM I-U
  RECT = (VM - ENAB) / SLB
  T2(ICNT) = PNAB * (EXP(RECT) - EXP(-RECT))/2.
C PLOT BACKGROUND RESISTANT INWARD I-U
  T3(ICNT) = GPI*(VM-ENAB)/(1.+EXP(SPI*(VM-EPI)))
  X(ICNT) = VM
  IF (DUMP) CALL TELLR(’VM IS ’,VM,’ IK2 = ’,
                  * ’T1(ICNT),’ INAB = ’,T2(ICNT),
                  * ’IPI = ’,T3(ICNT))

  UM = UM + STEP
CONTINUE

IF (.NOT.DUMP) GO TO 44
GO TO 100

44 XM(1) = UINIT
XM(2) = UEND
YM(1) = 0.
YM(2) = 1.*SC
IF (JUMP.EQ.9) YM(1) = 50. !IN pA
IF (JUMP.EQ.9) YM(2) = -50.*SC !in pA
SC = 1.
IF (IU.EQ.2) CALL TERASE
CALL SCAN(XM,YM,-2,440)
IF (JUMP.EQ.2) CALL AXES(13.2,’VOLTAGE IN mV’,18.2,
                          * ’M,H,D,F,N INFINITY’)
IF (JUMP.EQ.9) CALL AXES(13.2,’VOLTAGE IN mV’,13.2,
                          * ’CURRENT IN pA’)
CALL DRAW(X,T1,100,440)
CALL DRAW(X,T2,100,440)
CALL DRAW(X,T3,100,440)
IF (JUMP.EQ.9) GO TO 49
CALL DRAW(X,T4,100,440)
CALL DRAW(X,T5,100,440)
49 IF (IU.EQ.1) CALL NOTES(MSH,HS,DSH,FSH,NSH)
CALL DRAW(0.,0.,1,9000)
CALL BELL
GO TO 100
C
50 IF (SR) CALL TELLR('SERIES RESISTANCE IS ON.',
*      'RS = ',RS)
IF (.NOT.SR) CALL TELLR('SERIES RESISTANCE IS OFF.',
*      'RS = ',RS)
IF (YES('TOGGLE STATE OF RS?')) SR = .NOT.SR
CALL CNDGTR('NEW VALUE FOR RS ',RS)
GO TO 100
C
60 CALL CNDGTR('ENTER THE NEW HOLDING POTENTIAL 
*,HP)
CALL CNDGTR('ENTER THE NEW RUNTIME IN MSEC ',RUNT)
TPP = RUNT/1000.
DO 61 K = 1, 1024
61 TIME(K) = TPP * FLOAT(K)
GO TO 100
C
70 DUMP = .NOT.DUMP
GO TO 100
C
75 CALL TYPE('********** COMMANDS ************')
CALL TYPE(' ACTIV - PLOT OUT 10 SEC ACTIVATIONS')
CALL TYPE(' DEPOL - GO TO U- OR C-CLAMP MODE')
CALL TYPE(' IU - GENERATE AND PLOT A PEAK I-U')
CALL TYPE(' RATE - PLOT OUT INFINITY VALUES')
CALL TYPE(' RS - CHANGE SERIES RESISTANCE')
CALL TYPE(' HP - CHANGE THE HOLDING POTENTIAL',
*      ' OR TPP')
CALL TYPE(' BACK - PLOT BACKGROUND I-U''S')
CALL TYPE(' DUMP - SEND PLOT OUTPUT TO SCREEN')
CALL TYPE(' SCALE - SCALE THE PLOT BY THIS FACTOR')
CALL TYPE(' SHIFT - SHIFT AN INFINITY VALUE')
CALL TYPE(' DEBUG - OPEN A DEBUG OUTPUT FILE')
CALL TYPE(' PARAM - PRINT PARM VALUES TO SCREEN')
CALL TYPE(' RESET - GO BACK TO DEFAULT VALUES')
CALL TYPE(' QUIT - EXIT PROGRAM')
GO TO 100
C
80 CALL CNDGTR(' ENTER THE NEW SCALE FACTOR ',SC)
GO TO 100
C
85 CALL CNDGTR('M SHIFT ',MSH)
CALL CNDGTR('H SHIFT ',HS)
CALL CNDGTR('D SHIFT ',DSH)
CALL CNDGTR('F SHIFT ',FSH)
CALL CNDGTR('N SHIFT ',NSH)
GO TO 100

90 IF (.NOT.DEBU) OPEN (UNIT=3, NAME='DEBUG.DAT', TYPE='NEW', ACCESS='SEQUENTIAL')
*   CALL TYPE('FILE "DEBUG.DAT" OPENED ')
IF (DEBU) CLOSE (UNIT=3, DISP='KEEP')
*   CALL TYPE('FILE "DEBUG.DAT" CLOSED')
DEBU = .NOT.DEBU
GO TO 100

97 WRITE(5,91) 'M', (PM(I),I=1,6)
WRITE(5,91) 'H', (PH(I),I=1,6)
WRITE(5,91) 'K', (PK(I),I=1,6)
WRITE(5,91) 'D', (PD(I),I=1,6)
WRITE(5,91) 'F', (PF(I),I=1,6)
WRITE(5,91) 'E', (PE(I),I=1,6)
WRITE(5,91) 'N', (PN(I),I=1,6)
CALL TELLR(' PNA = ',PNA, ', ENA = ',ENA)
CALL TELLR(' PNAB = ',PNAB, ', ENAB = ',ENAB,
*             SLB = ',SLB)
CALL TELLR(' PCA = ',PCA, ', ECA = ',ECA)
CALL TELLR(' GK = ',GK, ', EK = ',EK,
*             GKB = ',GKB)
CALL TELLR(' GPI = ',GPI, ', SPI = ',SPI,
*             EPI = ',EPI)
91 FORMAT( 'PARAMETER ',A1,3X,6(F9.5,1X))
GO TO 100

RESET COMMAND RE-READS PARM VALUES
GO TO 112

95 IF (PLOT) CALL DRAW(0,0,0,9999)
STOP
END

SUBROUTINE RATE(V,MSH,HSH,DSH,FSH,NSH)
REAL V,PM(6),PH(6),PK(6),PD(6),PF(6),PE(6),PN(6)
REAL MSH,HSH,DSH,FSH,NSH
COMMON PM,PH,PK,PD,PF,PE,PE
COMMON /RATE/AM,BM,AH,BH,AK,BK,AD,BD,AE,BF,AE,BE,AN,BN

AM = PM(1)*(V-PM(3)-MSH)/
  (1.-EXP(PM(2)*(V-PM(3)-MSH)))
BM = PM(4)*EXP(PM(5)*(V-PM(6)-MSH))
AH = PH(1)*EXP(PH(2)*(V-PH(3)-HSH))
FUNCTION CURR(U,GATE)
REAL V,M,H,K,D,F,E,N,GATE(1)
REAL INA,ICA,IK,IK2,INAB,IPI
COMMON /DATA/PNA,ENA,PCA,ECI,PNAB,ENAB,SLB,GK,
  *   EK,GKB,GPI,SP,PI
COMMON /DEBUG/INA,ICA,IK,IK2,INAB,IPI
COMMON /BACK/A1,A2,A3,A4,A5,A6
PARAMETER FRT = .03896,
  *   NA0 = .1106,
  *   CA0 = .0025, CAI = 1.069E-5
M = GATE(1)
H = GATE(2)
D = GATE(3)
F = GATE(4)
N = GATE(5)
K = GATE(6)
E = GATE(7)
T1 = PNA*M*H*K * NA0 * 96500. * FRT
W = .6 + .4/(1.+EXP(.1*(U+45.))))
IF (ABS(U).GT..01) T2 = U*(EXP(FRT*(U-ENA))-1.)/
  *   (EXP(FRT*W)-1.1,
IF (ABS(U).LE..01) T2 = (EXP(-FRT*ENA)-1.)/FRT
INA = T1*T2*W

T3 = PCA*D*F*E*4.*96500.*FRT
IF (ABS(U).GT..01) T4 = V*(CAI*EXP(2.*U*FRT)-CA0)/
  *   (EXP(2.*U*FRT)-1.1,
IF (ABS(U).LE..01) T4 = (CAI-CA0)/(2.*FRT)
ICA = T3*T4
IK = GK*N*N*(U-EK)
IK2 = GKB * (EXP(A1 * (V-EK)) - 1.0) / (EXP(A2*(V-A3)) + * EXP(A4 * (V-A5)))

T2 = (V - ENAB) / SLB
INAB = PNAB * (EXP(T2) - EXP(-T2)) / 2.

IPI = GPI * (V-ENAB) / (1.0 + EXP(SPI*(V-EPI)))

CURR = INA + ICA + IK + IK2 + INAB + IPI
RETURN
END

C  Short program to ring terminal bell
subroutine bell
  do 1 m=1,50
  call type(7)
  return
  end

C  PRINT INFO ONTO HARDCOPY PLOT ON THE PARAMETER VALUES
US ED IN FORMING THAT RUN
SUBROUTINE NOTES(MSH,HSH,DSH,FSH,NSH)
REAL MSH,HSH,DSH,FSH,NSH,
*  REAL PNA,PCA,GK,ENA,ECA,EK
LOGICAL DAY(10),TME(10)
COMMON /DATA/PNA,ENA,PCA,ECA,PNAB,ENAB,SLB,GK,
*  EK,GKB,GPI,EPI,SPI
COMMON PM,PH,PK,PD,PF,PE,PN
CALL DATE(DAY)
CALL TIME(TME)
CALL NOTE(1.5,9.,DAY,10)
CALL NOTE(3.,9.,TME,10)
CALL NOTE(.5,8.,7.,'MSHIFT:',7)
CALL NOTE(1.25,8.7,MSH,1001)
CALL NOTE(1.75,8.7,'HSHIFT:',7)
CALL NOTE(2.5,8.7,FSH,1001)
CALL NOTE(3,8.7,'DSHIFT:',7)
CALL NOTE(3.75,8.7,DSH,1001)
CALL NOTE(4.25,8.7,'FSHIFT:',7)
CALL NOTE(.5,8.7,FSH,1001)
CALL NOTE(5.5,8.7,'NSHIFT:',7)
CALL NOTE(6.25,8.7,NSH,1001)
CALL NOTE(1.,8.,'ALPHAM:',8)
CALL NOTE(1.,7.6,'ALPHAH:',8)
CALL NOTE(1.,7.2,'ALPHAD:',8)
CALL NOTE(1.,6.8,'ALPHAF:',8)
CALL NOTE(1., 6.4, 'ALPHAN: ', 8)
XP = 2.
DO 1 M = 1, 3
    CALL NOTE(XP, 8., PM(M), 1005)
    CALL NOTE(XP, 7.6, PH(M), 1005)
    CALL NOTE(XP, 7.2, PD(M), 1005)
    CALL NOTE(XP, 6.8, PF(M), 1005)
    CALL NOTE(XP, 6.4, PN(M), 1005)
XP = XP + 1.
1 CONTINUE
CALL NOTE(1., 7.8, 'BETAM: ', 7)
CALL NOTE(1., 7.4, 'BETAH: ', 7)
CALL NOTE(1., 6.6, 'BETAD: ', 7)
CALL NOTE(1., 6.2, 'BETAN: ', 7)
XP = 2.
DO 2 M = 4, 6
    CALL NOTE(XP, 7.8, PM(M), 1005)
    CALL NOTE(XP, 7.4, PH(M), 1005)
    CALL NOTE(XP, 7.0, PD(M), 1005)
    CALL NOTE(XP, 6.6, PF(M), 1005)
    CALL NOTE(XP, 6.2, PN(M), 1005)
XP = XP + 1.
2 CONTINUE
CALL NOTE(1., 5.8, 'PNA = ENA = ', 26)
CALL NOTE(1., 5.6, 'PCA = ECA = ', 26)
CALL NOTE(1., 5.4, 'GK = EK = ', 26)
CALL NOTE(1.75, 5.2, 'PNAB = GKB = ', 26)
CALL NOTE(3.75, 5.8, 'ENA = 1003)
CALL NOTE(3.75, 5.6, 'ECA = 1003)
CALL NOTE(3.75, 5.4, 'GK = 1003)
CALL NOTE(3.75, 5.2, 'GKB = 1003)
RETURN
END

Program APMOVE

C SUBRO APMOVE 04/02/83
C INPUT: U PRESENT MEMBRANE VOLTAGE
C DDT(*) VALUES OF THE GATING VARIABLES
C (M, H, D, F, N, K, E) AT TIME T
C TOUT TIME INCRIMENT
C ALPHAS AND BETAS FOR EACH PARAMETER -
THESE VALUES ARE PASSED FROM SUBROUTINE RATE(*) IN COMMON AREA /RATE/
OUTPUT: DDT(*) VALUES OF THE GATING VARIABLES AT TIME T+TOUT

NOTE: THE VALUE FOR THE OUTPUT STEPSIZE TOUT MAY BE AS
LARGE AS THE USER WISHES, THIS ROUTINE AUTOMATICALLY ADJUSTS THE INTEGRATION STEPSIZE
TO PREVENT ROUNDOFF AND/OR LOSS OF PERCISION
NOTE: THIS PROGRAM IS DIVIDED INTO THREE SECTIONS,
WHICH INDEPENDENTLY PERFORM THE INTEGRATION OF M,
H, AND D; F; AND N, K, E BASED ON THE FASTEST TIME
CONSTANT IN EACH GROUP

C SUBROUTINE APMOVECV(V, DDT, T, TOUT)
REAL V, DDT(7), M, H, K, D, F, E, N
REAL AM, BM, AH, BH, AK, BK, AD, BD, AF, AE, BE, AN, BN
REAL M1, M2, M3, H1, H2, H3, D1, D2, D3, F1, F2, F3,
   E1, E2, K1, K2, N1, N2,
   MINF, HINF, DINF, FINF, KINF, EINF, NINF,
   TAUM, TAUH, TAUD, TAUF, TAUK, TAUE, TAUEN
LOGICAL MINT, HINT, KINT, DINT, FINT, EINT, NINT
LOGICAL TESTM, TESTH, TESTD, TESTF, TESTE, TESTK, TESTN
COMMON /RATE/ AM, BM, AH, BH, AK, BK, AD, BD, AF, AE, BE, AN, BN

C ASSIGN THE GATING VARIABLES TO THE PROPER SYMBOLS
M = DDT(1)
H = DDT(2)
D = DDT(3)
F = DDT(4)
N = DDT(5)
K = DDT(6)
E = DDT(7)
MINT = .FALSE.
HINT = .FALSE.
KINT = .FALSE.
DINT = .FALSE.
FINT = .FALSE.
EINT = .FALSE.
NINT = .FALSE.
TESTM = .FALSE.
TESTH = .FALSE.
TESTD = .FALSE.
TESTF = .FALSE.
TESTK = .FALSE.
TESTE = .FALSE.
TESTN = .FALSE.

C BEGIN: INTEGRATION FOR M, H, AND D
C
CALCULATE M, H, D Derivatives

\[ \text{DMDT} = AM - (AM + BM) * M \]
\[ \text{DHDT} = AH - (AH + BH) * H \]
\[ \text{DDDT} = AD - (AD + BD) * D \]

CALCULATE TIME CONSTANTS FOR M, H, AND D

\[ \text{TAUM} = 1. / (AM + BM) \]
\[ \text{TAUH} = 1. / (AH + BH) \]
\[ \text{TAUD} = 1. / (AD + BD) \]

CALCULATE THE STEADY-STATE VALUES OF M, H, AND D

C AT THE PRESENT U

\[ \text{MINF} = AM * \text{TAUM} \]
\[ \text{HINF} = AH * \text{TAUH} \]
\[ \text{DINF} = AD * \text{TAUD} \]

C******************************************************************************

C CALL TELLR('MINF IS ',MINF,' HINF IS ',HINF,' DINF IS ',DINF)

C WRITE(5,101) V,TAUM,TAUH,TAUD,DMDT,DHDT,DDDT

C100 FORMAT(/'U=',F7.2,' TAUS:',3(F10.5,2X))

C101 FORMAT(/'M/D: ',3(F10.5,X))

C******************************************************************************

CHECK TO SEE IF ANY RATES ARE CHANGING SO SLOWLY DURING
C THE PRESENT OUTPUT STEP THAT THEY CAN BE IGNORED.
C IF SO, SET THAT GATING VARIABLE TO ITS STEADY-STATE VALUE

IF (TOUT*ABS(DMDT).LT..01) MINT = .TRUE.
IF (TOUT*ABS(DHDT).LT..01) HINT = .TRUE.
IF (TOUT*ABS(DDDT).LT..01) DINT = .TRUE.

C REPLACE INTEGRATED VALUE WITH STEADY-STATE IF X IS WITHIN
C 1% OF STEADY-STATE. OTHERWISE INTEGRATE TO NEXT VALUE

TESTM = (ABS(M-MINF) .LT. .0001)
TESTH = (ABS(H-HINF) .LT. .0001)
TESTD = (ABS(D-DINF) .LT. .0001)

IF (TESTM.AND.TESTH.AND.TESTD) GO TO 19

C******************************************************************************

C WRITE(5,182) MINT,HINT,DINT

C WRITE(5,112) TESTM,TESTH,TESTD

C102 FORMAT('/'M,H,D < .01? ','3(L6,2X))

C112 FORMAT('/'M,H,D INF > .99? ','3(L6,2X))

C******************************************************************************

CHOOSE STEPSIZE FOR INTEGRATION OF M,H,D AS MIN(TAUM,TAUH)
C OR TAUD, DEPENDING ON WHICH VARIABLES WILL BE INTEGRATED

TINC = .1 * TAUD
IF (.NOT.MINT.AND..NOT.HINT) TINC = .1 * AMIN1(TAUM,TAUH)
CALCULATE NUMBER OF ITERATIONS TO BE DONE. IF TINC IS NOT AN EVEN MULTIPLE OF TOUT, DO ONE EXTRA STEP OF SIZE TEXTRA

C DO INTEGRATION FROM T TO T+TOUT. USE A FOURTH ORDER RUNGE-KUTTA METHOD TO PERFORM THE INTEGRATION

DO 10 MM = 1, NT2
   IF (MM.EQ.NTIMES+1) TINC = TEXTRA
   IF (TESTM) GO TO 11
      M1 = TINC * (AM - (AM+BM) * M)
      M2 = TINC * (AM - (AM+BM) * (M + .5*M1))
      M3 = TINC * (AM - (AM+BM) * (M + .5*M2))
      M4 = TINC * (AM - (AM+BM) * (M + M3))
      M = M + .16666 * (M1 + 2.*(M2+M3) + M4)
   11  IF (TESTH) GO TO 12
      H1 = TINC * (AH - (AH+BH) * H)
      H2 = TINC * (AH - (AH+BH) * (H + .5*H1))
      H3 = TINC * (AH - (AH+BH) * (H + .5*H2))
      H4 = TINC * (AH - (AH+BH) * (H + H3))
      H = H + .16666 * (H1 + 2.*(H2+H3) + H4)
   12  IF (TESTD) GO TO 10
      D1 = TINC * (AD - (AD+BD) * D)
      D2 = TINC * (AD - (AD+BD) * (D + .5*D1))
      D3 = TINC * (AD - (AD+BD) * (D + .5*D2))
      D4 = TINC * (AD - (AD+BD) * (D + D3))
      D = D + .16666 * (D1 + 2.*(D2+D3) + D4)
   10  CONTINUE

C GO TO THIS SECTION IF M, H, AND D ARE ALL AT STEADY-STATE

19  IF (TESTH) H = HINF
    IF (TESTD) D = DINF
C CALL TELLR('M IS ',M,' H IS ',H,' D IS ',D)
C***********************************************************************
C END OF INTEGRATION FOR M,H,D
C************************************************************************
C BEGIN: SPECIAL SECTION FOR F INTEGRATION
C***********************************************************************
COMPUTE STEADY-STATE, TIME CONSTANT, AND DERIVATIVE FOR F  
DFDT = AF - (AF+BF) * F  
TAUF = 1./(AF + BF)  
FINF = AF * TAUF  
C IF THE RATE OF CHANGE IS TOO SLOW, SET F TO STEADY-STATE  
IF (TOUT*ABS(DFDT).LT..01) FINT = .TRUE.  
TESTF = (ABS(F-FINF) .LT. .0001)  
IF (TESTF) GO TO 23  
CHOOSE STEPSIZE BASED ON VALUE OF TAUF. IF THE STEPSIZE IS  
C NOT AN INTEGER MULTIPLE OF TOUT, DO ONE EXTRA STEP TO  
C GET TO TOUT  
TINC = .1 * TAUF  
IF (TINC.GT.TOUT) TINC = TOUT  
NTIMES = INT(TOUT/TINC)  
TEXTRA = DBLE(TOUT) - DBLE(TINC*FLOAT(NTIMES))  
IF (TINC.EQ.TOUT) NT2 = NTIMES  
IF (TINC.NE.TOUT) NT2 = NTIMES + 1  
C***********************************************************************
C WRITE(5,103) TINC,NTIMES,TEXTRA  
C WRITE(5,104) F,FINF,TAUF,DFDT  
C DO 4TH ORDER RUNGE-KUTTA TO INTEGRATE  
DO 21 MM = 1, NT2  
  IF (MM.EQ.NTIMES+1) TINC = TEXTRA  
  F1 = TINC * (AF - (AF+BF) * F)  
  F2 = TINC * (AF - (AF+BF) * (F + .5*F1))  
  F3 = TINC * (AF - (AF+BF) * (F + .5*F2))  
  F4 = TINC * (AF - (AF+BF) * (F + F3))  
  F = F + .16666 * (F1 + 2.*(F2 + F3) + F4)  
21 CONTINUE
C IF F IS WITHIN 1% OF STEADY-STATE SET F TO STEADY-STATE  
23 F = FINF  
24 CONTINUE  
C***********************************************************************
C CALL TELLR('F IS ','F')
C END OF INTEGRATION FOR F

C-----------------------------------------------
C BEGIN: INTEGRATION ROUTINE FOR K, E, N
C-----------------------------------------------

COMPUTE DERIVATIVES FOR GATING VARIABLES K, E, AND N
DKDT = AK - (AK+BK) * K
DEDT = AE - (AE+BE) * E
DNDT = AN - (AN+BN) * N

COMPUTE THE TIME CONSTANTS
TAUK = 1. / (AK+BK)
TAUE = 1. / (AE+BE)
TAUN = 1. / (AN+BN)

COMPUTE STEADY-STATE VALUES OF K, E, AND N
KINF = AK * TAUK
EINF = AE * TAUE
NINF = AN * TAUN

C****************************************************
C WRITE(5,101) U,TAUK,TAUE,TAUN,DKDT,DEDT,DNDT
C CALL TELLR('KINF = ','KINF',' EINF = ','EINF,' NINF = ','NINF')
C****************************************************

C SELECT THE STEPSIZE AS .1 * MIN(TAUK,TAUE) OR .1*TAUN
IF (TOUT*ABS(DKDT).LT..01) KINT = .TRUE.
IF (TOUT*ABS(DEDT).LT..01) EINT = .TRUE.
IF (TOUT*ABS(DNDT).LT..01) NINT = .TRUE.

C IF THE VARIABLE IS CLOSE TO IT'S STEADY-STATE VALUE,
C SET IT TO THIS VALUE
TESTK = (ABS(K-KINF) .LT. .0001)
TESTE = (ABS(E-EINF) .LT. .0001)
TESTN = (ABS(N-NINF) .LT. .0001)
IF (TESTK.AND.TESTE.AND.TESTN) GO TO 34

C****************************************************
C WRITE(5,102) KINT,EINT,NINT
C****************************************************

CHOOSE THE STEPSIZE AS TAUN OR MIN(TAUK, TAUE)
TINC = .1 * TAUN
IF (.NOT.KINT.AND. .NOT.EINT) TINC = .1 *
   AMIN(TAUK,TAUE)
   IF (NINT.AND. (KINT.AND. .NOT.EINT)) TINC = .1 * TAUE
   IF (NINT.AND. (.NOT.KINT.AND.EINT)) TINC = .1 * TAUK

CALCULATE THE NUMBER OF ITERATIONS TO BE DONE. IF TINC IS
NOT AN INTEGER MULTIPLE OF TOUT, DO ONE EXTRA STEP OF
SIZE TEXIT
IF (TINC.GT.TOUT) TINC = TOUT
NTIMES = INT(TOUT/TINC)
TEXTRA = DBLE(TOUT) - DBLE(TINC*FLOAT(NTIMES))
IF (TINC.EQ.TOUT) NT2 = NTIMES
IF (TINC.NE.TOUT) NT2 = NTIMES + 1

C**********************************************************************************************
C                   WRITE(5,103) TINC,NTIMES,TEXTRA
C**********************************************************************************************

C PERFORM INTEGRATION USING THE 2ND ORDER RUNGE-KUTTA METHOD
DO 30 MM = 1, NT2
   IF (M.EQ.NTIMES+1) TINC = TEXTRA
   IF (TESTK) GO TO 31
   K1 = K + .5 * TINC * (AK - (AK+BK) * K)
   K = K + TINC * (AK - (AK+BK) * K1)
   IF (TESTE) GO TO 32
   E1 = E + .5 * TINC * (AE - (AE+BE) * E)
   E = E + TINC * (AE - (AE+BE) * E1)
   IF (TESTN) GO TO 30
   N1 = N + .5 * TINC * (AN - (AN+BN) * N)
   N = N + TINC * (AN - (AN+BN) * N1)
30    CONTINUE
31    IF (TESTK) K = KINF
      IF (TESTE) E = EINF
      IF (TESTN) N = NINF
32    IF (TESTK) K = KINF
      IF (TESTE) E = EINF
      IF (TESTN) N = NINF

C**********************************************************************************************
C CALL TELLR('K IS ',K,' E IS ',E,' N IS ',N)
C**********************************************************************************************
C END OF INTEGRATION FOR K, E, AND N
C---------------------------------------------------------------------------------------------

C PUT THE NEW VALUES FOR THE GATING VARIABLES BACK INTO THE OUTPUT VECTOR
38    DDT(1) = M
    DDT(2) = H
    DDT(3) = D
    DDT(4) = F
    DDT(5) = N
    DDT(6) = K
    DDT(7) = E
RETURN
END
Appendix D  The Gauss-Newton Method

Assume that both model output $M(a)$ and data $d$ are available in sets of $N$ equally spaced sampled points. The model output is dependent on the $R$ parameters $a_1, a_2, ..., a_R$. To minimize differences between model and data, define an error function $e(a)$:

$$e(a) = \bar{m}(a) - \bar{d}$$

where $\bar{e}$, $\bar{m}$, and $\bar{d}$ are matrices of dimension $N \times 1$

Define the least-square error function to be:

$$\sum e(a) = \frac{1}{2} (\bar{M}(a) - \bar{d})^2 = e^T(a)\bar{e}(a)$$

For a minimum of this error to occur:

$$\frac{\partial \sum e(a)}{\partial a_1} = (\bar{M}(a) - \bar{d}) \cdot \frac{\partial \bar{M}(a)}{\partial a_1} = \bar{e}(a) \quad \frac{\partial e(a)}{\partial a_1} = 0$$

$$\frac{\partial \sum e(a)}{\partial a_2} = (\bar{M}(a) - \bar{d}) \cdot \frac{\partial \bar{M}(a)}{\partial a_2} = \bar{e}(a) \quad \frac{\partial e(a)}{\partial a_2} = 0$$

in vector form:

$$V \sum e(a) = J \bar{e}(a) = 0$$

where $J = \frac{\partial \bar{e}(a)}{\partial a}$

$$J = \begin{bmatrix} \frac{\partial \bar{e}(a)}{\partial a_R} \\ \vdots \\ \frac{\partial \bar{e}(a)}{\partial a_1} \end{bmatrix} = \begin{bmatrix} \frac{\partial e(a)}{\partial a_R} \\ \vdots \\ \frac{\partial e(a)}{\partial a_1} \end{bmatrix}$$
At a new point $\alpha + \Delta \alpha$:

$$
\nabla E(\alpha) + \Delta \alpha = J \cdot e(\alpha + \Delta \alpha)
$$

$$
= J \cdot (e(\alpha + \Delta \alpha) \cdot \frac{\partial e(\alpha)}{\partial \alpha})
$$

note that $\frac{\partial e(\alpha)}{\partial \alpha} = J^T$

Thus:

$$
\nabla E(\alpha + \Delta \alpha) = J e(\alpha) + \Delta \alpha J^T = 0
$$

Solve for the parameter adjustment $\Delta \alpha$:

$$
\Delta \alpha = -(J J^T)^{-1} J e(\alpha) \quad \text{Gauss' Method}
$$

Unluckily, the Gauss method only converges well for values of $\alpha$ in the neighborhood of the minimum. For an estimate far from the minimum point, the gradient method will converge more quickly ($\Delta \alpha = J e(\alpha)$). By use of the Levenburg adjustment parameter ($\lambda$), it is possible to smoothly change the step size estimate $\Delta \alpha$ from the gradient estimate (better global convergence) to the Gauss estimate (better local convergence).

$$
\Delta \alpha = -(J J^T - \lambda I)^{-1} J e(\alpha)
$$

where $I$ is the identity matrix

For $\lambda \gg J J^T$, $\Delta \alpha$ will be a gradient step scaled by $1/\lambda$. For $\lambda \approx 0$, $\Delta \alpha$ will be a Gauss step. The initial value of $\lambda$ is chosen to ensure the first step will be a gradient estimation.
Bibliography


