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SQUID AXON: CONDUCTING CHANNEL APPROACH

Guy Roy
(Ph. D. Thesis)

July 1969

LAWRENCE RADIATION LABORATORY
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A MODEL FOR ELECTRICAL CONDUCTIVITY OF THE SQUID AXON:

CONDUCTING CHANNEL APPROACH

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ABSTRACT

The purpose of this work is to explain in terms of physical principles the behavior of nerve cells. The most detailed and reliable experiments made on nerve cells to elucidate their mechanism are those with the voltage clamp method. The functioning of nerve cells can be explained in terms of ionic currents across their membrane. A model is being developed to explain the measured ionic currents.

1. The basic hypothesis of this model is that there are conducting channels in the membrane. There is a chemical reaction between some membrane components and several ions. Vacancy formation and diffusion in the ionic complex results in conduction. This part of the model can be called the "conducting channel" (C-C) hypothesis.

2. A second hypothesis is introduced to describe the experimentally observed dynamics of the conductances. It is assumed that the reaction between ions and membrane substrates is an autocatalytic reaction. The complete model can be called the "autocatalytic-conducting-channel" (A-C-C) model.

3. The A-C-C model is compared with the experimental data of Hodgkin and Huxley (1952). The unknown constants are determined by curve fitting. Using the cable equation, stationary and propagating action potentials are generated.

4. The model accounts for various properties of axons like threshold, refractory period, accomodation, impedance change, strength-duration characteristic, repetitive firing and hyperpolarization. It is also shown that the model for the axon can be used with some modifications for the dendrites and soma of nerve cells.

5. Many recent experiments have been performed on squid axons, changing the external and internal medium of the axons. The observed effects produced by changing calcium, sodium, potassium and chloride ions have been explained satisfactorily by the C-C model. Even some quantitative aspects of the effect of drugs on the axon can be approximated.

UN MODELE DE LA CONDUCTIVITE ELECTRIQUE DE L'AXON DU SQUID:

METHODE DES CANAUX CONDUCTEURS

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ABREGE

Ce travail a pour but d'expliquer, en se basant sur des principes physiques, le comportement des cellules nerveuses. Les expériences les plus précises et les plus fiables faites sur les cellules nerveuses pour élucider leur fonctionnement sont celles faites avec la méthode dite "voltage clamp". Le fonctionnement des cellules nerveuses peut être expliqué à partir des courants ioniques à travers leur membrane. Un modèle a été développé pour expliquer les courants ioniques mesurés.

1. L'hypothèse de base du modèle suppose l'existence de canaux conducteurs dans la membrane. Il se produit une réaction chimique entre certains composés de la membrane et plusieurs ions. La formation de sites vacants et la diffusion à travers ces sites vacants amène la conduction. Cette partie du modèle a été appelée l'hypothèse du " canal conducteur " (C-C).

2. Une deuxième hypothèse est introduite pour décrire les observations expérimentales sur la dynamique des conductances. On suppose que la réaction entre les ions et les composés de la membrane est autocatalytique. Le modèle au complet est dénommé "canal conducteur auto-catalytique" (C-C-A).

3. Le modèle C-C-A a été comparé aux résultats expérimentaux de Hodgkin et Huxley (1952). Des constantes inconnues ont été déterminées par un ajustement optimal des courbes théoriques et expérimentales. En se servant de l'équation du câble coaxial, des potentiels d'action stationnaires et propagés ont été calculés.

4. Le modèle explique les propriétés des axones, telles que le seuil, la période réfractaire, l'accommodation, la variation d'impédance, la caractéristique d'amplitude vs durée du courant de stimulation, les décharges répétées et l'hyperpolarisation. Le modèle de l'axone a aussi été appliqué avec quelques modifications au corps et dendrites des cellules nerveuses.

5. Plusieurs expériences récentes ont été faites sur les axones de squid en introduisant des variations du milieu ionique intérieur et extérieur. Les effets observés à la suite de changement de la concentration du calcium, du sodium, du potassium ou du chlore ont été expliqués de façon satisfaisante par le modèle C-C. Même quelques aspects quantitatifs des effets des molécules biochimiques sur l'axone ont été introduits.

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Mon écriture tout à fait illisible a donné beaucoup de mal à plusieurs secrétaires. Je les remercie toutes de leur patience, sans laquelle cette thèse n'aurait pû être complétée.

Mes remerciements vont aussi au Gouvernement des Etats-Unis qui, par l'intermédiaire de NIH et de NASA-AEC a permis à ma famille de vivre convenablement grâce à son généreux support financier.

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INTRODUCTION

A. Cable equation

Since it was found that nerve cells were generating electrical potentials, models have been developed to explain them. An important contribution was Hermann's local circuit theory inspired from the theory of the submarine cable. A quantitative model was developed to calculate the potential as a function of the distance along the axon and as a function of time. A recent review of this model has been made by Taylor (1963).

The axon was considered as a leaky cable along which the potential propagated. The circuit was as shown below
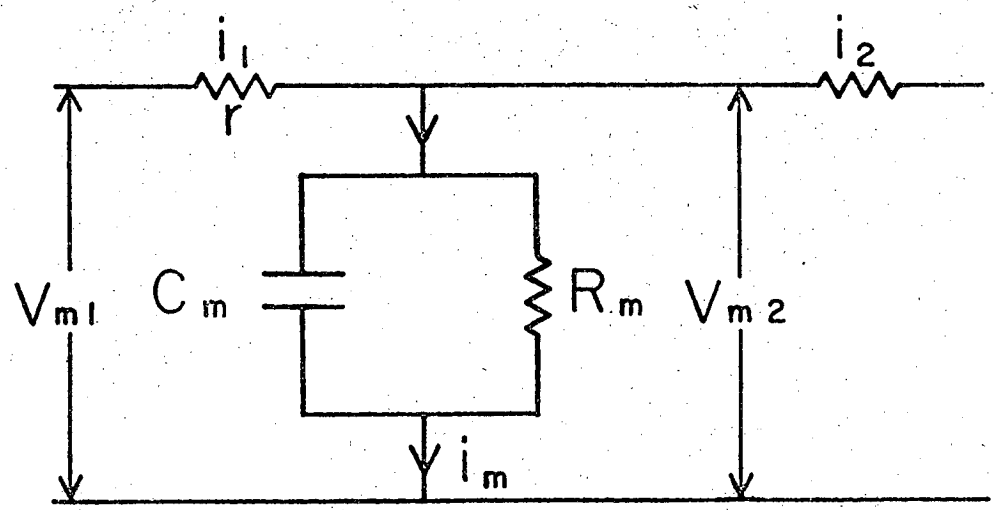
V_m	is the membrane potential difference
r	is the resistance per cm of axoplasm
i_1, i_2	are the current in the axoplasm
i_m	is the membrane current
x	is the distance along the axon

The sum of potentials around a closed path gives

$$V_{m2} - V_{m1} = r\, i_1\, \Delta x$$

$$\frac{dV_m}{dx}\, \Delta x = r\, i_1\, \Delta x \tag{1-1}$$

The sum of currents gives

$$i_2 - i_1 = i_m$$

Dividing by Δx ,

$$\frac{i_2 - i_1}{\Delta x} = \frac{i_m}{\Delta x}$$

Introducing $\qquad I_m = \dfrac{i_m}{2\pi b\, \Delta x}$

where b is the axon radius. I_m is called the current density across the membrane.

Taking the limit of

$$\frac{i_2 - i_1}{\Delta x} = \frac{di}{dx}$$

$$\frac{di}{dx} = 2\pi\, b\, I_m \tag{1-2}$$

Taking the derivative with respect to x of (1-1),

$$\frac{d^2 V_m}{dx^2} = \frac{r\, di}{dx}$$

$$2\pi\, b\, I_m = \frac{d^2 V_m}{dx^2} \tag{1-3}$$

Expressing r in terms of the resistivity ρ of the axoplasm

$$\rho = r\pi\, b^2$$

$$2\pi b I_m = \frac{\pi b^2}{\rho}\, \frac{d^2 V_m}{dx^2}$$

$$I_m = \frac{b}{2\rho}\, \frac{d^2 V_m}{dx^2} \tag{1-4}$$

In the case of the steady state and with a constant membrane resistance R_m, we have

$$I_m = \frac{V_m}{R_m}$$

That gives an exponential function of x for V_m. For the transient situation

$$I_m = C_m \frac{dV_m}{dt} + I_R \tag{1-5}$$

where I_R is the resistive current and C_m the membrane capacitance. The result is a partial differential equation with x and t, called the cable equation

$$\frac{b}{2\rho} \frac{\partial^2 V_m}{\partial x^2} = C_m \frac{\partial V_m}{\partial t} + I_R \tag{1-6}$$

B. Bernstein theory

Another important contribution to the understanding of the action potential of the nerve was made by Bernstein (1902). He postulated that the resting potential of the nerve cell was caused by the concentrations of the different ions on each side of the membrane and their respective permeability through the membrane. If one ion, for example potassium, has a greater permeability than the others, it will tend to flow down its concentration gradient. But this flow will establish an electrostatic potential which will prevent further flow of the K ions, if the negative ions cannot cross the membrane. The value of the electrostatic potential which prevents any net current is given by the Nernst equation

$$V_{Ck} = \frac{RT}{ZF} Ln \frac{[K_i]}{[K_o]}$$

V_{Ck} is called the concentration potential of the potassium ions

Bernstein could predict approximately the amplitude of the resting potential with the value of V_{ck}. To explain the action potential, he simply assumed that the stimulation of the nerve cell increased the permeability to all the other small ions, like Na and Cl, thus bringing the resting potential to zero. In the framework of the cable theory, it meant a change of I_R with time.

This was supported by the experiments of Cole and Curtis (1939) who measured the impedance of the membrane of the squid axon during the spike, and found that it decreased to about 25 ohm/cm^2. The resting value was 1000 ohm/cm^2. The capacitance was found to be rather constant and remarkably uniform over most membranes ranging only from .5 to 2.0 uf/cm^2; for the squid it was 1.0 uf/cm^2.

The Bernstein theory was kept for almost 40 years until Hodgkin and Huxley (1939) showed that the action potential reversed the membrane potential instead of bringing it to a zero value. The sodium ions have a very high concentration outside, which then could produce a positive membrane potential, if they are allowed to flow inside until the electrostatic force stops them. If after a stimulation, the sodium permeability increases very much, sodium will flow inside bringing the membrane potential to the sodium equilibrium potential, thus reversing the membrane potential.

C. Voltage clamp

Then Hodgkin and Huxley (1952) made experiments to determine the details of the ions movements during the stimulation of the nerve cell. Their experimental method was based on a technique developed by Cole (1949). They used the giant axon of the squid into which two

longitudinal electrodes were inserted. Through one electrode and another

one outside they could pass a current and using a feed-back amplifier,

the potential across the membrane could be maintained constant. This very

ingenious technique, called voltage-clamp, permitted measurement of the

flow of ions at different step values of the membrane potential. It was

found earlier that the current density through a portion of the membrane

is given by

$$I_m = C_m \frac{dV}{dt} + I_R$$

Keeping V constant, $I_m = I_R$. By stimulating with negative potentials or

making the membrane potential more negative almost no current flows, but

by making the membrane potential less negative or increasing it toward

zero, the current was much larger, being inward then reversing outward

to attain a constant amplitude. Hodgkin and Huxley (1952a) made experi-

ments to identify the ions that were producing such a current. From our

previous considerations, they had reason to suspect that sodium was flo-

wing inward and potassium outward. By replacing Na in the outside medium

by choline the inward current disappeared, and when Na is reintroduced,

the inward current reappears. Besides, the steady outward current is not

modified by removing Na. That supports the fact that the Na current is

only temporary and produced by the high concentration gradient of sodium.

They found that the outward current is mostly produced by potassium ions,

chloride providing only a very small part of it. By recording the current

in sea water with and without sodium at different voltage step amplitudes,

they obtained the time curve of sodium current and potassium current sepa-

rately for each voltage step. During those experiments they also measured

the membrane potential at which the Na current was reversing for different

external Na concentration. From the Nernst equation the membrane potential

at which the Na current should be zero can be calculated; the calculated
and measured values compared within the limit of experimental accuracy.
Then they assumed that the ionic currents could be expressed as a pro-
duct of the conductance times the total potential; we call concentra-
tion potential the value of the potential given by the Nernst equation.
The total potential is the difference between the electrical and the
concentration potential; for Na we have $V_m - V_{CNa}$ and $I_{Na} = g_{Na}$
$(V_m - V_{CNa})$ and a similar expression for I_k. They assumed then that the
time and potential dependance of the currents were caused by the conduc-
tance variations. This hypothesis was supported by the Bernstein's model,
which was assuming changes in membrane permeability during the action
potential and by the experiments of Cole and Curtis (1939) who measured
an impedance change during the action potential. In order to prove that
these relations were correct they made experiments with a double step
of voltage. A first step of voltage is applied, for example 25 mv.,
then after different intervals of time, the step is reduced to 10 mv.
If the change is made at around 1 msec., when the sodium current is high,
we see a rapid instantaneous surge of sodium current. The surge is much
smaller if the change occurs at earlier or later times. Making many such
experiments at various initial and final voltages, curves can be made
showing the relation between the instantaneous change of current and the
step change in voltage. The curves are all straight lines with a slope
that depends on the value of the conductance at the moment of the step
change. These experiments are a very strong demonstration of the current-
voltage relations given above. From their data on the sodium and potas-
sium currents, they could obtain the conductances as function of time
and voltage. The sodium conductance presented a particular problem:

It was only temporary, but had a small steady state value at applied

potentials close to the resting potential. That disappearance of the Na

following an initial increase

conductance was called inactivation. This problem had to be investigated

in more details. They found that by applying a hyperpolarizing voltage

followed by a depolarizing voltage, the amplitude of the sodium current

was larger for the same depolarizing voltage when no hyperpolarization

was applied. By making many such experiments, they found that the inac-

tivation was potential dependant; it was about zero for membrane potential

of 40 mv below the resting potential and complete for 40 mv above the

resting potential. They also determined the time course of inactivation

for a series of applied potentials.

D. Hodgkin and Huxley model

With all these experimental results in hand, Hodgkin and

Huxley (1952d) developed a theory to explain their results. From the

potassium conductance curves, a first order differential equation seems

to be a good starting point; but the rise and amplitude do not correspond

to a linear differential equation. The amplitude of the conductances are

voltage dependent and their increase is quite steep. From a semi-log plot

of g_K vs V, we see that for e-fold increase in g_K, the voltage changes by

only 5 or 6 mv. Although it should be possible to apply the Goldman (1943)

constant field theory to derive g_K (V), the argument in the exponential

will have to be about 4 times smaller which means that 4 charges are

involved in the process. Besides the rise of the conductance is slow

initially and then becomes rapid. By using the solution of a linear first

order differential equation, $(1 - e^{-at})$ and putting it to a fourth power

$(1 - e^{-at})^4$ they could obtain the correct shape for the g_K curves.

When g_K returns to its resting value, the time course is linear; their theory satisfies that requirement too.

$$g_K = \left[g_{K\infty}^{1/4} - (g_{K\infty}^{1/4} - g_{Ko}^{1/4}) \ e^{-t/\tau} \right]^4$$

For the fall of g_K we have only an exponential to the fourth power. This is the total equation used to fit the data. From each curve a value for τ and $g_{K\infty}$ were obtained. The model was that four events had to occur simultaneously in the membrane in order to have the ions cross. Each event had a time dependent probability n given by

$$\frac{dn}{dt} = \alpha_n \ (1-n) - \beta_n$$

and

$$g_K = \bar{g}_K \ n^4$$

By identifying the two formulations for g_K, we have a correspondance between $g_{K\infty}$ and τ_n and α_n and β_n

$$\tau_n = \frac{1}{\alpha_n + \beta_n} \qquad g_{K\infty} = \frac{\alpha_n}{\alpha_n + \beta_n}$$

From all the values of τ_n and $g_{K\infty}$ corresponding values of α_n and β_n could be obtained. Then α_n and β_n are plotted against V. An empirical formula was used to fit these curves, so that the potassium conductance curves could be derived theoretically. For the sodium curves the same method was used, assuming a similar model. The probability equations were given by the product of activation times inactivation equations

$$\frac{dm}{dt} = \alpha_m \ (1-m) - \beta_m$$

$$\frac{dh}{dt} = \alpha_h \ (1-h) - \beta_h$$

$g_{Na} = \bar{g}_{Na} \ m^3 h$. A cubic was found to fit best the rise of sodium conductance. From the inactivation experiments they had the voltage dependance

of inactivation, so that h_o and h_∞ were known. Because g_{Nao} is very small

$$g_{Na} = g_{Na\infty}1/3 \cdot (1-e^{-t/\tau})^3 \cdot h_o e^{-t/\tau_h} + g_{Na\infty}1/3 \, (1-e^{-t/\tau})^3 \, h_\infty(1-e^{-t/\tau_h})$$

By fitting the data curves to the equation and identifying with the

theory they again obtain curves for α_m, β_m, α_h and β_h for which empi-

rical formulas, similar to the one for potassium could be obtained. The

procedure was not as easy as for the potassium conductance curves because

of the inactivation dependance on potential.

Cole and Moore (1960) made experiments with high hyperpolarizing

potentials and found that a twenty-fifth power was more adequate to fit

their data for the potassium conductance but in the normal range of

potential variation, the fourth power is accurate enough.

Taking the cable equation developed in the introduction

$$\frac{b}{2\rho} \frac{\partial^2 V_m}{\partial x^2} = C_m \frac{\partial V_m}{\partial t} + I_R$$

where

$$I_R = I_{Na} + I_K + I_L$$

I_{Na} and I_K have been determined and can be calculated. I_L is called the

leak current and is also assumed to be of the form $I_L = g_L \, (V_m - V_{CL})$

Hodgkin and Huxley (1952b) made a few experiments to determine g_L and V_{CL}.

They concluded that g_L was constant; it has a rather small value.

Experiments were made to obtain space clamped action potentials,

where the potential is forced to be the same on all the length of the

fiber; that simplifies the cable equation because $\frac{\partial^2 V_m}{\partial x^2} = 0$. The numerical

integration of the four differential equations can be made and theoretical

action potentials are obtained. They compare them with experimental ones;

we see that they are identical within the range of experimental accuracy.

With the space-clamped action potential they could verify many fundamental

properties of action potentials, like threshold and refractory period.

At more than one centimeter from the point of stimulation the action potential propagates at constant speed. Then the cable equations are again simplified because

$$\theta^2 \frac{\partial^2 V_m}{\partial x^2} = \frac{\partial^2 V_m}{\partial t^2}$$

That simplification gives an ordinary differential equation again. But its integration presents a difficulty. If θ is either too large or too small, the calculated action potential will blow up to plus or minus infinity. By trial and error, more and more accurate values of θ can be obtained until the action potential is completely determined. The value of θ compares well with the experimental value of the speed of propagation. This problem of integration is caused by the simplification of the cable equation. If the partial differential equation is integrated no such difficulty occurs, as shown by Cooley and Dodge (1966).

Hodgkin, Huxley and Katz (1952) also made experiments to determine the effects of temperature on the ionic currents. The results were that the time dependance of the ionic currents were greatly modified but not their amplitudes. They could account rather well for the change in the time course of the action potential by supposing a Q_{10} of 3 applied to all the rates coefficients α_n, β_n, α_m, β_m, α_h and β_h.

The series of experiments performed by Hodgkin and Huxley to determine the detail process of the nerve action potential were very well designed and gave very interesting results. Their model, although working well, is rather empirical. The physical basis they tried to give to it does not seem very probable.

Because these theoretical results were not giving any mechanism for the change of conductance of the membrane, an intensive search for

possible mechanism was started.

In chapter five of Cole (1968) there is a review of many different models that were tried to explain the squid axon data. Also in Tasaki (1968) many of the physico-chemical approaches to membrane phenomena are reviewed. In Moore (1968) there is a review of the necessary features that a model of the nerve membrane must have to be acceptable. From these different reviews we see that many models have been tried, but have not succeeded in explaining the voltage-clamp data. Most of the models have attempted to describe the conduction mechanism using the hypothesis that the current goes through pores in the membrane. The pore model encounters serious difficulties when it comes to explain the transient nature of the sodium current and the independence of the sodium and potassium currents. Because the pore model did not succeed in explaining the conduction mechanism of the membrane, we thought a different approach was necessary.

Before doing so we would like to state the necessary requirements of any model, if the model is going to behave as well and even better than the H-H empirical system.

The first part of a model is the steady state relation between current and voltage. From the experiments of Hodgkin and Huxley described above, it seems necessary that current-voltage relation should be expressed as I = g.V with g being time and potential dependant. There is no reactive elements in the ionic currents in voltage clamp. Then to satisfy the conductance dependence on potential, the model must provide for a very sharp rise of conductance in a short range of potential; for the sodium conductance the increase is 10^3 from 0 - 20 mv. We know from our own experience that the fit of the theory with the experiments must be very good, if the model is going to work.

The next major problem comes from the kinetic data. The model must reproduce quite well the rise of the conductances with time. A suitable hypothesis must explain the non-linear rising of the conductance and the potential dependance of the rate constants. And again the fit must be quite good to achieve suitable results. Also the recent data on voltage clamp of squid axon has brought some other difficulties and requirements; the most interesting are those where ionic concentrations inside and outside the axon are modified. The results can be interpreted as a shift of the conductance steady state curve to the left or to the right. That summarizes the most important points that must be explained by a model and we will attempt to satisfy them in our model.

We will suppose that a macromolecule extending across the membrane reacts with many cations along its chain and those ionic sites are used for conduction. Vacancy formation at those sites will permit a transfer of ions across the membrane. We will develop steady state and time dependent equations for the conductances and compare them to the data of Hodgkin and Huxley (1952 a, b, c, d) and of many others after them.

E. Recent experiments

Since the invention of the voltage clamp by Cole and the detailed studies by Hodgkin and Huxley, a great many quantitative investigations of the voltage current relationships were made in the squid axon as well as in crab nerve and in other experimental test objects. These are admirably reviewed in the detailed monograph by Kenneth Cole(1968)

and by Katz (1966) Tasaki (1968), and Moore (1968). Quantitative compa-
risons in this thesis are made with the original set of data on the squid
axon by Hodgkin and Huxley, and with some of the more recent data. It is
important to note, that a number of additional facts have emerged during
the last 20 years about the behavior of excitable membranes. Any good
model must furnish at least qualitative explanations for these and hope-
fully suggest new experiments. The most salient points with regard to
the behavior of nerve axon membranes are listed here only briefly.

An important progress in investigating squid axons was the advent
of perfusion of the axon brought by Baker,Hodgkin and Shaw (1961); that
technique permits the experimenter to vary the internal concentration
and composition of the ions inside the axon. A long line of experiments
were started with this technique; the experimenter can select any ion for
the outside and inside solution and study the conductivity of the membrane
for these ions selectively.

We must mention that the effect of changing the outside solu-
tion was studied without the need for perfusion, but the control of the
experiment is better with perfusion. Hodgkin and Huxley (1952) had
studied the effect decreasing the sodium ion concentration on the mem-
brane current and conductances. Then Frankenhaeuser and Hodgkin (1957)
modified the calcium ion concentration and found that the result was a
shift of the conductance voltage curves for the sodium and potassium
ions and the inactivation of the sodium conductance. Then Moore (1959)
worked on the effect on increasing the potassium ion concentration in
the outside solution and found an important effect in the I-V charac-
teristic. Later on the results of Ehrenstein and Gilbert (1966) and
Lecar et al (1967) showed that the effect of increasing the potassium
ion concentration could be explained by a shift in the potassium

conductance vs. voltage curve.

Meanwhile Tasaki et al (1965) started to study the effect of ions other than those normally found in the squid axon. They found that they could substitute ammonium and lithium ion for sodium, and rubidium for potassium, while cesium does not substitute too well for potassium. Neither cesium or rubidium substitute for sodium.

Also another series of experiments conducted by many investigators, studied the effect of different drugs on the axon potential. Detailled voltage clamp experiments were performed by Hille (1967) to work out the effect of tetraethylammonium chloride (TEA) and that of tetrodotoxine by Moore (1967). Many other drugs were also experimented and the few experimenters who worked with voltage clamp found that the effect of the drugs were to shift the conductance voltage curve, in a way similar to that produced by changes in ion concentrations. Specific organic dyes which interact with the membranes and render it susceptible to ultra-violet light were introduced in the outside solution and voltage clamp studies were made by Pooler (1969). It seems that the effect is to shift the inactivation of the sodium conductance.

There still is many experiments to perform in this line of investigations to obtain the precise effect of each component on each of the membrane conductances.

There are also experiments that measure electrical and electromagnetic phenomena during the action potential or in the resting membrane. Squid and lobster axons appear to carry a net negative charge as measured by Segal (1968) This finding tends to prove the presence of negative charges in the membrane and thereby explain the cation selectivity of the membrane.

It was shown recently by Cohen, Keynes and Hilley (1968), that there are rapid structural changes accompanying action potentials measurable by light scattering and birefrigerance changes. These appear to arise from the radially oriented molecules associated with the membrane and are an indication that considerable rearrangement in molecular membrane structure occurs.

Frazer and Frey(1968) have neasured the emission of electromagnetic waves at microwave frequencies from active nerves (Biophysical Journal, 8:731, 1968). It has been shown that heat changes occur during initiation and passage of action currents in the membrane which are greater than those that would be encountered by ohmic heating by the currents. The heat changes are probably associated with chemical interactions at the membranes and with allosteric changes of macromolecules. And finally we have the work performed on artificial bylayers. They have been produced by a number of investigators and it has been shown by them that electrical conductivity across such membranes can be radically altered by specific proteins, particularly antibiotics. Among these are Nystatin and Valinomycin; ion selectivities have been claimed for some of these substances Colacicco et al(1968). The status of this field was recently reviewed by Finkelstein and Cass (1968) who have also shown that the glycoprotein molecules responsible for decreased conductivity are also specific to certain cations. Muller and Rudin (1968) assume that the protein and antibiotics which have the specific membrane affinities are capable of interacting with bylayer membranes and form protein bridges across them allowing conductivity to take place. The recent finding of Bean et al (1968) that membrane active factors can cause a stepwise increase of transverse conductivity of lipid bylayers is particularly interesting. It may be assumed that each step corresponds

to the punch-through action of a single macromolecule. Muller and Rudin (1963), (1968) also demonstrated induced excitability in reconstituted cell membrane structure and could obtain "resting" and "action" potentials from these. Current voltage relationships in some of these systems are very similar to those found in electronic semiconductor tunnel diodes.

F. Other models

We have already commented previously that the phenomenological theory supplied by Hodgkin and Huxley accompanying the original investigations, while admirably describing the experimental data, has not been particularly helpful in the development of physical and molecular models for ion conduction across the membranes. The theory proposed by Mullins (1961) on pore activity, does not appear to agree with experimental facts since this model assumes that potassium and sodium ions each travel in the same pore and has so far failed to account for the effects of hyperpolarization of the membrane. The most elaborate investigations have been conducted on the assumption that the phenomenon of electrodiffusion will explain all the active phenomena. Another set of ideas include the assumption that conductivity changes depend on significant structural reorganization of the macromolecules in the membrane and that the molecular arrangement is a bistable configuration. Finally, several authors have proposed that the conduction of electricity in living membranes is analagous to solid state conduction phenomena found in semiconductor crystals.

The physical basis of the electrodiffusion theory has been reviewed by Finkelstein and Mauro (1963). Much interest was raised in this form of the theory by the experiments and calculations of Teorell

and his group on artificial ion exchange membranes. However, the model did not adequately explain the peculiar changes in conductivity encountered in living membranes during the excitation process. In order to provide a better approach to this problem, Katchalsky, working with Kedem (1963), and later with Richardson (1967), has presented the transport equations for multilayer membranes and initiated work on mosaics. None of these models has as yet quantitatively accounted for all the essential properties of the squid axon membrane as pointed out by Kenneth Cole in his exhaustive review (1965).

Goldman (1965) initiated a model of nerve membrane excitation by considering the role of calcium ion and assuming that calcium enters into chemical interaction with the surface of the membrane, then by causing the reactivity of chemical components in the membrane to be altered. Changeux, et al (1967), have attempted to give a more generalized form to the alteration of properties of membranes composed of macromolecules with more than one allosteric state. It was assumed here that as the molecules of the membrane interact with ions outside of the membrane, interaction forces between neighboring macromolecules also change. These authors arrived at a description of membrane properties which allows bistable mode of operation and abrupt transition from one state to another. Very recently, Terrell Hill (private communication) gave a more generalized description of the thermodynamic properties of the bistable membrane. Also Katchalsky and Spangler(1968) have developed equations based on irreversible thermodynamics to explain facillated diffusion in terms of allosteric states of a "carrier".

Adams (1968) based his recent calculations on a model developed by Tasaki (1963) and assumes that protein subunits bind calcium ions in the resting state; upon depolarization these are freed and the active sites

combine with K or Na ions. When these chemical reactions occur there is surface cooperation between neighboring macromolecules; this has an influence on ion binding. From thermodynamic considerations Adams derives expression for a membrane bistable with respect to electrical conduction. In its present state the theory would appear to predict an abrupt transition from ont to the other state in the voltage clamp: this appears to contradict the voltage clamp experiments which fail to show such rapid transitions.

The above theories are in qualitative agreement with some observed changes in membrane properties; e.g. birefrigerance, local heat and microwave emission during the excitation process; they have not as yet been sufficiently developed to provide a quantitative description of the Hodgkin-Huxley phenomena.

During the last few years a number of authors have pointed out that certain analogs exist in the behavior of excitable biological membranes and conductivity current relationships in inorganic semi-conductors; particularly rectifiers and transistors.

Mauro (1962) observed that fixed charges in ionic membranes are analogous to impurity ions in semi-conductors. Muller and Rudin (1962, 1968) noted the ressemblence of the behavior of reconstituted membranes to tunnel diodes. Wei (1966) points to qualitative agreement between membrane and solid state conductivity phenomena. However, no attempt has been made to quantitatively account for the Hodgkin-Huxley findings using a solid state model.

The current investigation was initiated (Roy and Tobias (1968)) with the realization that steady state conductivities obtained in the squid axon by the voltage clamp method for potassium ion current as well as for sodium ion current corrected for inactivation, resembled the

dependence of conductivity on voltage in inorganic semiconductor devices. A close examination of the correspondence, however, revealed that the conductance properties behaved like solid state rectifiers only when the membrane potential differed from the resting potential by a greater amount than about 30 millivolts depolarization. When the change of potential on the membranes differed from the resting potential by less than about 30 millivolts, the rapid rise of conductivity upon depolarization could not be accounted for by the assumption of a constant number of charge carriers. One could, however, account for the entire change of steady state conductivity by the assumptions that the first 30 millivolts of "change" from the resting potential results in a rapidly increasing number of channels (charge carriers) available for conduction of currents across the membrane by ions and that above 30 millivolts the number of conductor channels would be constant.

An examination of the complex behavior of nerve membranes (as described above) would lead to a fairly plausible assumption that initial steps of depolarization as well as the later step of inactivation probably involves chemical interaction of ions with macromolecules, and could, therefore, be also associated with surface cooperative phenomena.

Early in this work, it was realized that the analogies to solid state crystal semiconductors are very tenuous. In the first place, most of our knowledge of inorganic semiconductors comes from studies on crystals where electric current is made up of opposite migration of electrons and of vacancy sites. The knowledge of ion conduction in crystals is incomplete and the behavior of organic semiconductors has not been studied in sufficient detail as yet. Agin (1967) discussed some of the pitfalls of applying semiconductor theory. For example, the time

constant for response to an applied field in a membrane can be near 100 μsec whereas, inorganic semiconductors may respond 10^8 times faster. It is also questioned whether ion tunneling may occur in a membrane in a manner analogous to electron tunneling in transistors.

Our current idea of the molecular structure of membranes is still very vague and non-specific. The analog as far as it can be carried in this paper, therefore, only extends to general formalisms. The somewhat surprising goodness of fits, however, suggests that it may be well worth while to undertake a detailed investigation of the basic physics of the electrical conductivity behavior in organic semiconductors with structure akin to proteins, glycoproteins and lipids.

- II -

STEADY STATE EQUATIONS

A. Current-Voltage Relation

The Nernst-Planck equation gives the relation between the current and the electrical and chemical potentials. Considering it for one dimension and for a single ion involved in conduction, we have the current density

$$I_R = - (RT \; \mu \frac{dC}{dx} + C \; \mu z_v \; \frac{d\Psi}{dx}) \tag{2-1}$$

The above equation can be rearranged to give

$$I_R = - \mu z_v FC \left[\frac{RT}{z_v FC} \frac{dC}{dx} + \frac{d\Psi}{dx} \right]$$

$$I_R = - \mu z_v FC \frac{d}{dx} \left[\frac{RT}{z_v F} \; LnC + \Psi \right]$$

We introduce

$$\frac{RT}{z_v F} \; LnC + \Psi = \varphi$$

this is the electrochemical potential

$$I_R = - \mu z_v FC \; \frac{d\varphi}{dx} \tag{2-2}$$

Assuming that I is independant of x and integrating across the membrane

$$I_R \int_o^d \frac{dx}{\mu z_v FC} = - \Delta \varphi \tag{2-3}$$

$$\Delta \varphi = - (V_m + V_c)$$

$$V_m = - \Delta \Psi \qquad\qquad V_c = \frac{RT}{z_v F} \; Ln \; \frac{C_o}{C_1}$$

C_o is the concentration outside the axon and C_i inside the axon, V_c is called the concentration potential. The integral in equation (2-3) gives the resistance of the membrane R_m or its inverse the conductance g

$$\frac{1}{g} = R_m = \int_o^d \frac{dx}{\mu z_v FC} \tag{2-4}$$

$$I_R = g \ (V_m + V_c) \tag{2-5}$$

This transformation is quite convenient, because it modifies the problem of the current across the membrane into a problem of the resistance of the membrane. If the parameters μ and C in equation (2-4) are constant within the membrane, the resistance R is constant and we have Ohm's law. But the parameters μ and C can be variable and that will give a non-linear relation between current and voltage.

Equation (2-5) is general and restricted only by the assumption that I is independent of x and by the applicability of the Nernst-Planck equation. For example the result of the integration of equation (2-4) might give a conductance g which is a function of V_c and V_m. We could also have V_c and V_m vary with time. It is possible then that the conductivity g, defined in (2-4), is also time dependent. Hodgkin and Huxley (1952b) found that the relation between current and voltage was linear when they measured the instantaneous change of current produced by a step change of the potential V_m. But if the current is measured when it has reached a steady state, the current voltage relation is not linear any more. Equation (2-5) can explain such results if g is time dependent; if g is a slowly changing function, the instantaneous current change will be directly proportional to the potential but the steady state current will not; if g is a rapid function of time, then both instanta-

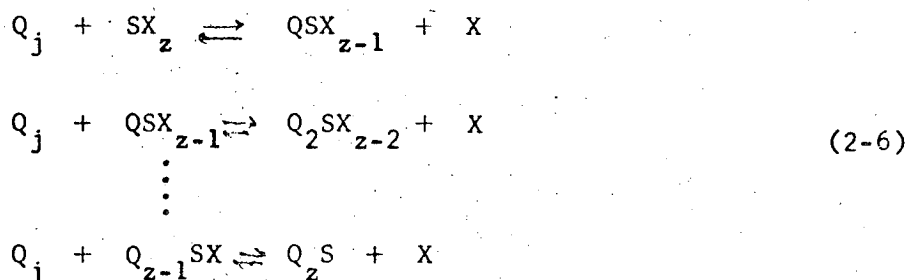neous and steady-state currents will have non linear characteristics.

B. Conducting Channels

Our problem will be to develop a model for the conduction of ions in the membrane. The basic hypothesis is that there are molecular components in membranes reaching from one side to the other which form complexes with several Na or K ions; there is a specific membrane component for each ion. The assumption for the existence of selective membrane components that bind Na^+ or K^+ does not have any direct experimental support because there is not yet sufficient data on the membrane structure. However phospholipids as well as proteins are known to bind cations; the only requirement for the model is that there are at least two different membrane components forming channels across the membranes in different locations and that one has a high preference for Na^+ or for ions that can substitute for Na^+ and the other one for K^+ or its substitutes. The assumption of separate channels is supported by the experiments of Moore et al. (1967) who blocked the K currents with TEA without affecting the Na current. Although phospholipids cannot be ruled out as possible substrates, we are inclined to favor proteins or polypeptides, because experiments by Rojas and Tobias (1965) on phospholipid reactions with K and Na ions have failed to show preferred binding for one ion by any of the usual membrane phospholipids. On the other hand, experiments by Muller and Rudin (1963, 1968) have shown that the resistance of artificial membranes drop by a factor of about 10^4 when a certain protein called EIM is introduced on the membrane; also this protein permits them to obtain I-V curves similar to those observed on squid axons in isoosmotic potassium concentrations. Additional evidence supporting

the view that EIM provides the channel through the artificial membrane was found by Bean et al. (1968). They found that the conductance increased by small and regular steps when the membrane reacted with EIM. Also, many polypeptides were found to reduce the membrane resistance, especially Valinomycin and its family. Valinomycin was found by Colacicco et al. (1968) to have a high preference for K ions when the latter were in concentration of 0.5 M or more. Barfort et al. (1968) have shown that the introduction of insulin on one side of an artificial membrane and its antiserum on the other side could decrease the resistance of the membrane by as much as 10^3. All these decreases of resistance bring the artificial membrane resistance to a value much closer to the natural membrane resistance; the lipid bilayer alone has a much too high resistance compared to those of natural membranes. A possible implication of the results is that the proteins or the polypeptides form bridges across the lipid bilayer, thus providing channels for the ions. The usual membrane models assume a continuous lipid bilayer coated with proteins; but recent experiments by Lenard and Singer (1966, 1968) have suggested that proteins, because of their structure, must form bridges across the membrane, some parts of the proteins are hydrophobic and would associate with the hydrophobic portion of the phospholipids, the other parts are hydrophylic and remain on the surface of the membrane.

The hypothesis of protein bridges or channels across the membrane is gathering more evidence in its favor, not only from the above mentioned experiments, but also from many others, specially in the field of active transport. This model will use this hypothesis, but will make it more specific to explain the experimental results on axonal membranes. Our hypothesis is that the protein channels must first react with the ions

to form an ionic complex before they could become conducting channels. The complexes are formed when the ions react with a receptor molecules SX_z. The name "receptor molecule" was used to differentiate between the "conducting channel" concept and the "carrier" models. The "receptor molecule" remains in stationary position, whereas the "carrier" is sometimes assumed to move from one side of the membrane to the other. X is assumed to be an atom other than Na or K, prevalent in the form SX_z in the membrane when the membrane is not conducting. Since we do not know the identify of X, it is used initially only in a formal sense. In mitochondrial membrane oscillations, X corresponds to hydrogen, which can be involved in exchange reactions with potassium. The following chemical reactions can take place:

$$Q_j + SX_z \rightleftharpoons QSX_{z-1} + X$$

$$Q_j + QSX_{z-1} \rightleftharpoons Q_2SX_{z-2} + X \tag{2-6}$$

$$\vdots$$

$$Q_j + Q_{z-1}SX \rightleftharpoons Q_zS + X$$

The subscript j will be i or o depending on whether the ions Q come from the inside or outside the cell. Ionic concentrations are different on each side of the membrane and the changes in concentration on either side of the membrane will affect the equilibrium constants. In equations (2-6) it is assumed that the interaction between Q and S is specific for Q. This is the simplest case; later it will be seen that S can also react with other ions e.g. inactivators, inhibitors or substitutes.

Using the mass law, relations between concentrations, C, can be established, *to be valid near steady state*

$$\frac{C_{SX_{z-1}} Q\, C_X}{C_{Q_j} C_{SX_z}} = K_1$$

$$\vdots$$

$$\frac{C_{SQ_z} C_X}{C_{Q_j} C_{SQ_{z-1}}X} = K_z$$

(2-6a)

Because S is assumed to be present in the membrane in a limited amount, the total concentration of substrate S equal

$$C_{ST} = C_{Q_z S} + C_{Q_{z-1}SX} + \cdots C_{QSX_{z-1}} + C_{SX_z}.$$

(2-6c)

However Q is available in ample quantity outside the membrane and C_Q is not appreciably modified by its incorporation in C_{QS}. Using equations (2-6a) and (2-6c), the concentrations $C_{SQ_i X_{z-i}}$ of the products of the various steps of the reactions can be calculated as functions of K_i, C_X, C_Q and C_{ST}. The results of calculating C_{SQ_z} is

$$C_{SQ_z} = \frac{C_{ST}}{1 + \frac{1}{K_z}\frac{C_X}{C_{Q_j}} + \frac{1}{K_z K_{z-1}}\left(\frac{C_X}{C_{Q_j}}\right)^2 + \cdots + \frac{1}{K_z K_{z-1} \cdots K_1}\left(\frac{C_X}{C_{Q_j}}\right)^z}$$

(2-6b)

The value of j will be either i or o; for example there might be z_i ions from inside and z_o from outside; in the last term that would give

$$\frac{C_X^{\,z}}{C_{Q_i} C_{Q_i} \cdots C_{Q_o} C_{Q_o} \cdots} = \frac{C_X^{\,z}}{C_{Q_i}^{\,z_i} C_{Q_o}^{\,z_o}} \qquad z_i + z_o = z$$

For most purposes it is sufficient to use the value of z in obtaining
fits to observed I-V relationships. However to account for experiments
where the inside and outside concentrations of Q are changed, it was
necessary to introduce z_i and z_o. The authors realize that the kinetics
of the contributions of ions Q from both sides of the membrane is a com-
plex problem. z_i and z_o denote average contributions; eventually experi-
mental results should lead to a verification of this treatment. From this
general model to calculate the concentration of conducting components in
the membrane, a series of simplifying assumptions will be made in order
to obtain formulas easier to utilize and containing less arbitrary
parameters. The first simplification is that only the complex having
the form SQ_z is conducting; it is possible that the other forms are also
conducting, but it seems that they should be much less usefull for con-
duction because the mobility of the ions in them will be smaller and also
because they should be present in lower concentration than SQ_z.
A second simplification can be introduced by assuming that the concentra-
tions of the intermediate components in (2-6c) are low enough to be
neglected. These simplifications reduce the reactions (2-6) to their
simplest form

$$z_i Q_i + z_o Q_o + SX_z \; \rightleftharpoons \; SQ_z + zX \qquad (2\text{-}7)$$
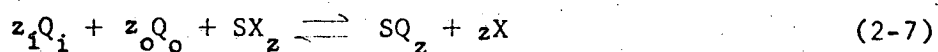
and $\quad C_{ST} = C_{SX_z} + C_{SQ_z}$ $\qquad\qquad$ (2-7a)

Eliminating the intermediate concentrations by taking the product of each
equilibrium equation in (2-6a), we have

$$C_{SQ_z} = K_1 \, K_2 \, K_3 \; \text{---} \; K_z \left(\frac{C_{Q_j}}{C_X} \right)^z C_{SX_z} \qquad (2\text{-}7b)$$

and with (2-7a)

$$c_{SQ_z} = \frac{c_{ST}}{1 + K^{-1} c_X^{z} c_{Qi}^{-z_i} c_{Qo}^{-z_o}}$$

(2-7c)

$$K = K_1 K_2 \text{---} K_z$$

This result is equivalent to neglecting all the intermediate terms in (2-6b) for c_{SQ_z}. One obtains a result equivalent to (2-7c) if K_z is large and K_1 very small and the other K_i have intermediate values in (2-6b) The factor containing $1/K_1$ must be larger than any other.

In reactions (2-6) and (2-7), Q is displacing X in the receptor molecule. An equivalent treatment would assume instead a simple association reaction between Q and S without displacing any atom or molecule, but producing only a rearrangement of the molecules. Then we would have

$$Q_j + S \rightleftharpoons SQ$$

$$Q_j + SQ \rightleftharpoons SQ_2$$

$$\vdots$$

$$Q_j + SQ_{z-1} \rightleftharpoons SQ_z$$

(2-8d)

$$\frac{c_{SQ}}{c_S c_Q} = K_1$$

$$\frac{c_{SQ_2}}{c_{SQ} c_Q} = K_2$$

$$\vdots$$

$$\frac{c_{SQ_z}}{c_{SQ_{z-1}} c_Q} = K_z$$

(2-8f)

$$c_{ST} = c_S + c_{SQ} + \text{---} c_{SQ_z}$$

(2-8g)

With (2-8g) and (2-8f) we obtain

$$C_{sq_z} = \frac{C_{ST}}{1 + K_z^{-1} C_{Q_j} + K_z^{-1} K_{z-1}^{-1} C_{Q_j}^{-2} + \cdots K^{-1} C_{Q_j}^{-z}}$$

$$K = K_z \, K_{z-1} \cdots K_1$$

Using the same simplifying assumption as before: C_{SQ_z} is the only conducting component, and approximating

$$C_{ST} = C_S + C_{SQ_z} \qquad\qquad (2\text{-}8b)$$

One has,

$$z_i \, Q_i + z_o \, Q_o + S \quad SQ_z \qquad\qquad (2\text{-}8)$$

$$C_{SQ_z} = K \, C_S \, C_{Q_i}^{z_i} \, C_{Q_o}^{z_o} \qquad\qquad (2\text{-}8a)$$

Using (2-8b) and (2-8a)

$$C_{SQ_z} = 1 + K^{-1} \, C_{Q_o}^{-z_o} \, C_{Q_i}^{-z_i} \qquad\qquad (2\text{-}8c)$$

Two similar results have been obtained for C_{SQ_z}; they are given by equations (2-7c) and (2-8c). Either of them could be used for later calculations; choosing one of them is rather arbitrairy. But the results obtained with any one of the two say (2-8c), can be transformed very simply into the results that would be obtained using (2-7c); introducing the factor C_X^z into (2-8c) transforms it into (2-7c). This is why all later calculation can be made with (2-8c) without loosing any generality. If experiments show the necessity of having component X, it

can be introduced easily. To simplify the writing of equation (2-8c)
we introduce an equivalent value for the product

$$C_{Q_i}{}^{z_i} \; C_{Q_o}{}^{z_o} \;=\; C_Q{}^z \tag{2-9a}$$

Introducing (2-9a) into equation (2-8c)

$$C_{QS} \;=\; \frac{C_{ST}}{1 + K_o{}^{-1} \, C_Q{}^{-z}} \tag{2-9c}$$

It must be remembered that $C_Q{}^z$ does not have any experimental corres-
pondance and its equivalent should always be used if we want to introduce
into equation (2-11) the experimental values of C_{Q_o} and C_{Q_i}. C_{QS} has the
same meaning as C_{SQ_z}.

C.   Vacancy Formation and Diffusion

There is always vacancy formation in any solid at a temperature
above 0° K because the free energy $F = E - TS$ must remain at its minimum.
The number of vacancies can be calculated. Following Kittel (1967) we have

$$n \;=\; \frac{N}{1 + e^{E_v/RT}}$$

where n is the number of vacancies, N the total number of sites available
for ions and E_v the energy of vacancy formation.

The diffusion of ions in alkali halide crystals has been studied

and the mobility of the ions in these crystals was calculated. It is
assumed that the ions can jump to the next site if they have enough energy
to do so; the number of trials per second is equal to the frequency of
vibration υ in the crystal. The number of successful trials per second, p,
is given by υ times the proportion of ions that have an energy greater than E_v.

$$p = \frac{\upsilon n}{N} = \frac{\upsilon}{1 + e^{E_v/RT}} \qquad (2\text{-}10b)$$

If E_v is much greater than KT, equation (2-10b) reduces to the more fami-
liar case $p = \upsilon e^{-E_v/KT}$. But if E_v is small, equation (2-10b) must be
used. If there is a concentration gradient across the crystal, a net flux
of these ions will be obtained. The net flux can be calculated. Applying
the definition of the flux, we have

$$J_+ = pM \qquad\qquad J_- = p\left(M + \frac{adM}{dx}\right)$$

where M is the number of ions per unit of surface, and "a" is the lattice
distance. If there is a gradient of ions in the crystal, the increase in
M from one lattice site to another is given by a first order approximation.
The net flux is

$$J = J_+ - J_- = -p\,a\,\frac{dM}{dx}$$

$\frac{dM}{dx}$ is transformed into a concentration gradient, by using $C = aM$, where C
is the concentration.

$$\frac{dM}{dx} = a\,\frac{dC}{dx}$$

$$J = pa^2\,\frac{dC}{dx}$$

Introducing (2-10b)

$$J = \frac{-\upsilon a^2}{1 + e^{E_v/RT}} \cdot \frac{dC}{dx}$$ (2-10c)

This result is equivalent to Fick's law of diffusion where the diffusion

constant D is

$$D = \frac{\upsilon a^2}{1 + e^{E_v/RT}}$$

If an electrical gradient is introduced in equation (2-10), it gives the

Nernst equation (2-1) that was used at the beginning of this chapter.

From Einstein's relation, the mobility μ is calculated from the diffusion

constant.

$$\mu = \frac{z_v FD}{RT} = \frac{z_v F \upsilon a^2}{1 + e^{E_v/RT}}$$ (2-10d)

It is well known experimentally that the diffusion constant D for many

types of molecules diffusing through a membrane is an exponential func-

tion of the free energy. In Stein (1967) a large number of experiments

have been put together by assuming a lattice model for the diffusion of

molecules through the membranes. The diffusion is assumed to take place

between hydrogen bonds of the membrane and those of the diffusing mole-

cules. We have assumed in this case a lattice model for the diffusion

of ions, but through a protein structure rather than through the lipid

lattice and by specific bonds with the protein. Once the ions have

reacted with their respective receptor molecules they will have formed

ionic complexes which may serve as matrices for the conduction of ions.

Vacancies can be formed at the ionic sites; the ions jump from one site

to the next and this can result in the diffusion of ions through the

membrane.

It is likely that "impurities" would hinder such a diffusion process; hence the requirement that S be "saturated" with z atoms of Q before ionic conduction becomes significant. The presence of even one atom of X or of some other type of inactivator or inhibitor might conceivably cause a drastic reduction in the rate of diffusion of ions through the membrane. Equation (2-10d) is used to calculate the mobility of ions through the ionic complexes. The value of "a" is the distance between ionic sites on the receptor molecule. F, z_v, υ are known quantities, E_v will be determined later.

D. Membrane Surface Barriers

The equilibrium constant K in equation (2-8) can be calculated with the partition function, ζ, of each of the molecules in the reaction, including ζ for transfer of Q into and out the membrane. Referring to REIF (1965)

$$K = \zeta_{QS}^{+1} \; \zeta_{S}^{-1} \; \zeta_{Q_i}^{-z_i} \; \zeta_{Q_o}^{-z_o} \qquad\qquad \zeta = \sum_{\ell} e^{-E(\ell)/KT}$$

Since it is not always simple to calculate the partition functions, K is expressed in terms of the free energy change of the reaction, ΔF

$$K = e^{-\Delta F/RT} \tag{2-11}$$

The value of ΔF is made of the sum of many energies; the ones that we want to determine more precisely are those for the ions penetrating the membrane.

$$\Delta F = \Delta F_o + z_i \, \Delta F_{Q_i} + z_o \, \Delta F_{Q_o} \tag{2-11a}$$

For the reaction to occur, z_i ions will have to change their energy by ΔF_{Q_i} and z_o ions by ΔF_{Q_o}. ΔF_{Q_o} and ΔF_{Q_i} will depend on the difference in the energy of the ions between inside and outside the membrane. ΔF_o includes all the other energy terms of the partition function of the chemical reaction.

Davson and Danielli (1952) have proposed a model for the membrane permeability and they applied it to the case of the ionic diffusion across the membrane. Their model assumes a local potential energy jump for a molecule to cross the membrane water interface; this energy is E_{wi} or E_{wo}, E_{wi} for the jump from axoplasm into the membrane and E_{wo} for the jump from the outside medium into the membrane. In ionic solutions, there will also be an induced dipole field at the interface; Davson and Danielli (1936) have shown that the dipoles in membranes should be oriented with their positive end in the membrane phase and their negative end in the liquid phase. The energies required to overcome the dipole field are E_{Di} and E_{Do}.

For the moment we assume $E_{Wi} = E_{Wo} = E_W$ and $E_{Di} = E_{Do} = E_D$. We shall see later that in some cases $E_{Wi} \neq E_{Wo}$ and $E_{Di} \neq E_{Do}$.

$$\Delta F_{Q_i} = \Delta F_{Q_o} = E_W + E_D \tag{2-11b}$$

with (2-11)

$$K = K_o e^{-z(E_W + E_D)/RT} \tag{2-11c}$$

where

$$K_o = e^{-\Delta F_o/RT}$$

with (2-9c)

$$C_{QS} = \frac{C_{ST}}{1 + K_o^{-z} C_Q \, e^{z(E_W + E_D)/RT}} \tag{2-11d}$$

The next problem is to evaluate the energy E_v in equation (2-10d) needed by an ion to jump into a vacant site. From the energy barriers proposed above for the membrane, it is very likely that the highest energies are those at the interfaces. The diffusion constant will have a much smaller value for the crossing at interfaces than inside the membrane. The value of E_v is approximated by $E_v = E_w + E_D$

with (2-10d)

$$\mu = \frac{\nu a^2 \, Fz_v}{RT \left(1 + e^{(E_w + E_D)/RT}\right)} \qquad (2-12)$$

The dipole potential E_D can be evaluated. When there is a potential difference across a capacitor, a number of positive charges accumulate on one side and an equal number of negative charges accumulate on the other side. But when the capacitor is in a solution containing negative and positive mobile charges, dipoles are formed one each side and the potential difference is produced by the difference in the concentration of dipoles between the two sides. This increase in the concentration of dipoles on each side increases the dipole potential E_D near the surface (the zeta potential) and E_D becomes a function of V_m, the potential across the membrane. For each charge on the surface, there will be a new dipole;

$$E_D = FV_m + E_D^o$$

E_D^o is the dipole potential when $V_m = 0$.

It must be noted here that the above proposed potential barriers are only local barriers and do not produce any net current. The potential difference V_m across the membrane is the only one to produce a net current; the above proposed potential energies influence the amplitude of the currents by their action on the conductances. A more detailed and more

fundamental treatment of the membrane surface barriers will be done in future work.

Equations (2-11d), (2-12) and (2-13) can be used to calculate the membrane conductance g. Repeating equation (2-4), for the membrane resistance R_m

$$R_m = \int_0^d \frac{dx}{\mu z_v FC} = 1/g \qquad (2\text{-}4)$$

The value of C, the concentration of conducting charges, is actually given by C_{QS} in equation (2-11d). It is independant of x because in this model the conducting charges are evenly distributed in the membrane. The value of μ has been calculated for the interface regions in the membrane where it is lowest and where it will have a limiting value for the diffusion of ions. The mobility μ of equation (2-12) is thus also indépendent of x The membrane conductance becomes,

$$g = \frac{z_v F \mu \, C_{QS}}{d}$$

z_v is the valence of K^+ and Na^+ ions

Using equation (2-11d), (2-12) and (2-13)

$$g = \frac{g_m}{(1 + e^{(E_o + FV_m)/RT}) (1 + K_o^{-1} C_Q^{-z} e^{z(E_o + FV_m)/RT})} \qquad (2\text{-}17)$$

$$E_o = E_w + E_D^o$$

$$g_m = \frac{F^2 \nu a^2 C_{ST}}{RTd} = \frac{F^2 \nu d \, C_{ST}}{z^2 RT}$$

$$a = d/z \qquad\qquad d = \text{membrane thickness}$$

Equations (2-17) and (2-17a) represent the main results of the conductivity

calculations in conducting channels.

E. Applications to K^+ and Na^+ conductance

The above calculations can be used to obtain the concentration of conducting potassium and sodium ions inside the membrane, their mobility and thereby their conductivity through the membrane.

For the potassium ions, equation (2-11d) becomes

$$C_{KS_\infty} = \frac{C_{KST}}{1 + K_{oK}^{-1} \, C_K^{-z} \, e^{-z_k (E_{WK} + E_{DK})/RT}} \tag{2-18}$$

and equation (2-12) becomes

$$\mu_{K_\infty} = \frac{F \, \nu_K \, a_K^2}{1 + e^{(E_{WK} + E_{DK})/RT)}} \tag{2-20}$$

and equation (2-17) becomes

$$g_{K_\infty} = \frac{g_{mK}}{(1 + e^{(E_{oK} + FV_m)/RT}) \; (1 + K_{oK}^{-1} \, C_K^{-z_k} \, e^{z_K (E_{oK} + FV_m)/RT})} \tag{2-21}$$
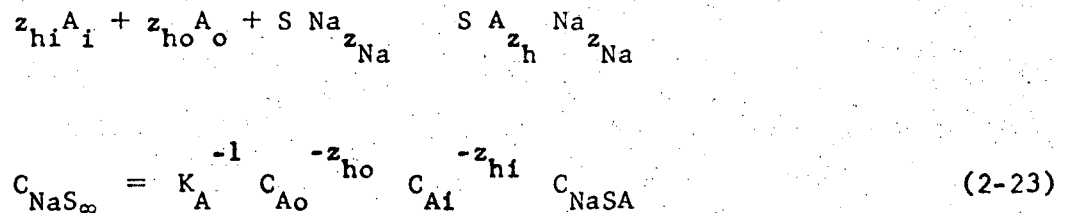
$$E_{oK} = E_{WK} + E_{DK}^o$$

$$g_{mK} = \frac{F^2 \, C_{KST} \, \nu_K \, a_K^2}{RT \, d} = \frac{F^2 \, C_{KST} \, \nu_K \, d}{z_K^2 \, RT}$$

$$d = \text{membrane thickness} \qquad a_K \, z_K = d$$

For the <u>sodium</u> ions, the conductance presents an additional problem which does not occur in the potassium conductance: its increase is only temporary and it disappears even under a constant potential.

To explain such a phenomena within the framework of this model we use the possibility mentioned previously, that another ion can be involved in the complex formation. It is assumed that another ion can react with the sodium protein complex. The new complex can be such that the sodium ions are much more strongly bound to the proteins and are not able to form as much vacancies. If their vacancy energy is only 0.1 ev., the sodium conductance will be very small. The reaction will be the following

$$z_{hi}A_i + z_{ho}A_o + S\,Na_{z_{Na}} \qquad S\,A_{z_h}\,Na_{z_{Na}}$$

$$C_{NaS_\infty} = K_A^{-1}\,C_{Ao}^{-z_{ho}}\,C_{Ai}^{-z_{hi}}\,C_{NaSA} \tag{2-23}$$

Applying equation (2-8c) to the case of the sodium ions we have C_{NaSO}, the concentration of conducting complexes when there is no inactivation.

$$C_{NaSO} = \frac{C_{STNa}}{1 + K_{Na}^{-1}\,C_{Nao}^{-z_{Nao}}\,C_{Nai}^{-z_{Nai}}} \tag{2-24}$$

When inactivation takes place a certain quantity of NaS is transformed into NaSA. The maximum amount of inactivation is

$$C_{NaSO} = C_{NaS_\infty} + C_{NaSA} \tag{2-25}$$

Using (2-23), (2-24) and (2-25)

$$C_{NaS\infty} = K_A^{-1} C_A^{-z_h} (C_{NaSO} - C_{NaS\infty})$$

Using the usual short

$$C_A^{z_h} = C_{AO}^{z_{ho}} C_{Ai}^{z_{hi}}$$

$$C_{Na}^{z_{Na}} = C_{NaO}^{z_{Nao}} C_{Nai}^{z_{Nai}}$$

$$C_{NaS\infty} = \frac{C_{STNa}}{(1 + K_A C_A^{z_h})(1 + K_{Na}^{-1} C_{Na}^{-z_{Na}})} \qquad (2\text{-}26a)$$

$$\frac{C_{NaS\infty}}{C_{NaSO}} = \frac{1}{1 + K_A C_A^{z_h}} \qquad (2\text{-}26)$$

The expression for K_A and K_{Na} are

$$K_A = K_{Ao} \, e^{-z_h (E_{oh} + FV_m)/RT} \qquad (2\text{-}27)$$

$$K_{Na} = K_{Nao} \, e^{-z_{Na} (E_{oNa} + FV_m)/RT} \qquad (2\text{-}28)$$

$$E_{oNa} = E_{WNa} + E_{DNa}^{o}$$

$$E_{oh} = E_{Wh} + E_{Dh}^{o}$$

With the value of $C_{NaS\infty}$ we can calculate the amplitude of the sodium conductance. The details of that calculation will be given in the next chapter.

$$g_{Na\infty} = g_{NaO} \left[h\,(V_m) + h\,(V_R) \right] \tag{2-29}$$

$$V_R = \text{resting potential}$$

$$h\,(V_m) = \frac{C_{NaS\infty}}{C_{NaSO}}$$

$$g_{NaO} = \frac{g_{mNa}}{(1 + e^{(E_{oNa} + FV_m)/RT})\,(1 + K_{Nao}^{-1}\,C_{Na}^{-z_{Na}}\,e^{z_{Na}(E_{oNa}+FV_m)/RT})} \tag{2-30}$$

$$h_\infty(V_m) = \frac{1}{1 + K_{Ao}\,C_A^{z_h}\,e^{-z_h\,(E_{oh} + FV_m)/RT}} \tag{2-31}$$

$$g_{mNa} = \frac{F^2\,C_{STNa}\,\upsilon_{Na}\,d}{z_{Na}^2\,RT}$$

The identification of this inactivator is still uncertain; we know it can't be Ca or Mg since their removal increases inactivation as was shown by Frankenhaeuser and Hodgkin (1957). The potassium current has been shown to be independent of the sodium current. Chandler et al (1965) have shown that anions are not without importance in the inactivation process: when inside KCl is reduced to 50 mM and sucrose is introduce, the inactivation curve is shifted by 20 mv. If the sucrose is replaced by choline chloride, the inactivation curve is restored to

its normal position. We could interpret these results as showing that Cl is the inactivator.

To compare our theoretical calculation with experimental results, we have chosen to use the data of Hodgkin and Huxley (1952d); we take the steady state potassium conductance for the different voltage clamp values of the potential and also the amplitude of the sodium conductances and compare them to our equations. A computer program, which minimizes chisquare, determines the best values for the parameters. The calculations were made by CDC 6600 of the Lawrence Radiation Laboratory and the program was developed by the personnel of the mathematics and computing group of the laboratory.

We will transform equations (2-21), (2-29), 2-30) and lump many parameters together to make them simpler to handle.

$$g_K = \frac{g_{mK}}{(1 + A e^{-V/V_o})(1 + B e^{-z_K V/V_o})}$$

$$V_m = V_R - V$$

V is the applied potential

V_R is the resting potential

$$V_0 = RT/F$$

$$g_{Nao} = \frac{g_{mNa}(h(V) - h(0))}{(1 + C e^{-V/V_o})(1 + D e^{-z_N V/V_o})}$$

$$h(V) = \frac{1}{1 + E e^{z_h V/V_o}}$$

$$A = e^{(E_{oK} + FV_R)/RT}$$

$$B = K_{oK}^{-1} \, C_K^{-z_K} \, e^{z_K \, (E_{oK} + FV_R)/RT}$$

$$C = e^{(E_{oNa} + FV_R)/RT}$$

$$D = K_{oNa}^{-1} \, C_{Na}^{-z_{Na}} \, e^{z_{Na} \, (E_{oNa} + FV_R)/RT}$$

$$E = K_{Ao} \, C_A^{z_h} \, e^{-z_h \, (E_{oh} + FV_R)/RT}$$

We obtain a very good fit to the data, as seen in Fig. 1 and 2. The values of the parameters are given in Table 1.

<u>Table 1</u>

| A	B	z_K	g_{mK}
4.6	11.0	4.0	21.0
C	D	z_{Na}	g_{mNa}
6.0	1000.	8.0	66.0
	E	z_h	$h(0)$
	0.67	4.0	0.66

The values of $z_K = 4.0$ and $z_{Na} = 8.0$ mean that four K ions are involved in forming the potassium protein complex and eight Na ions form the sodium receptor protein complex. The value $z_h = 4.0$ gives us the number of charges required for the inactivation.
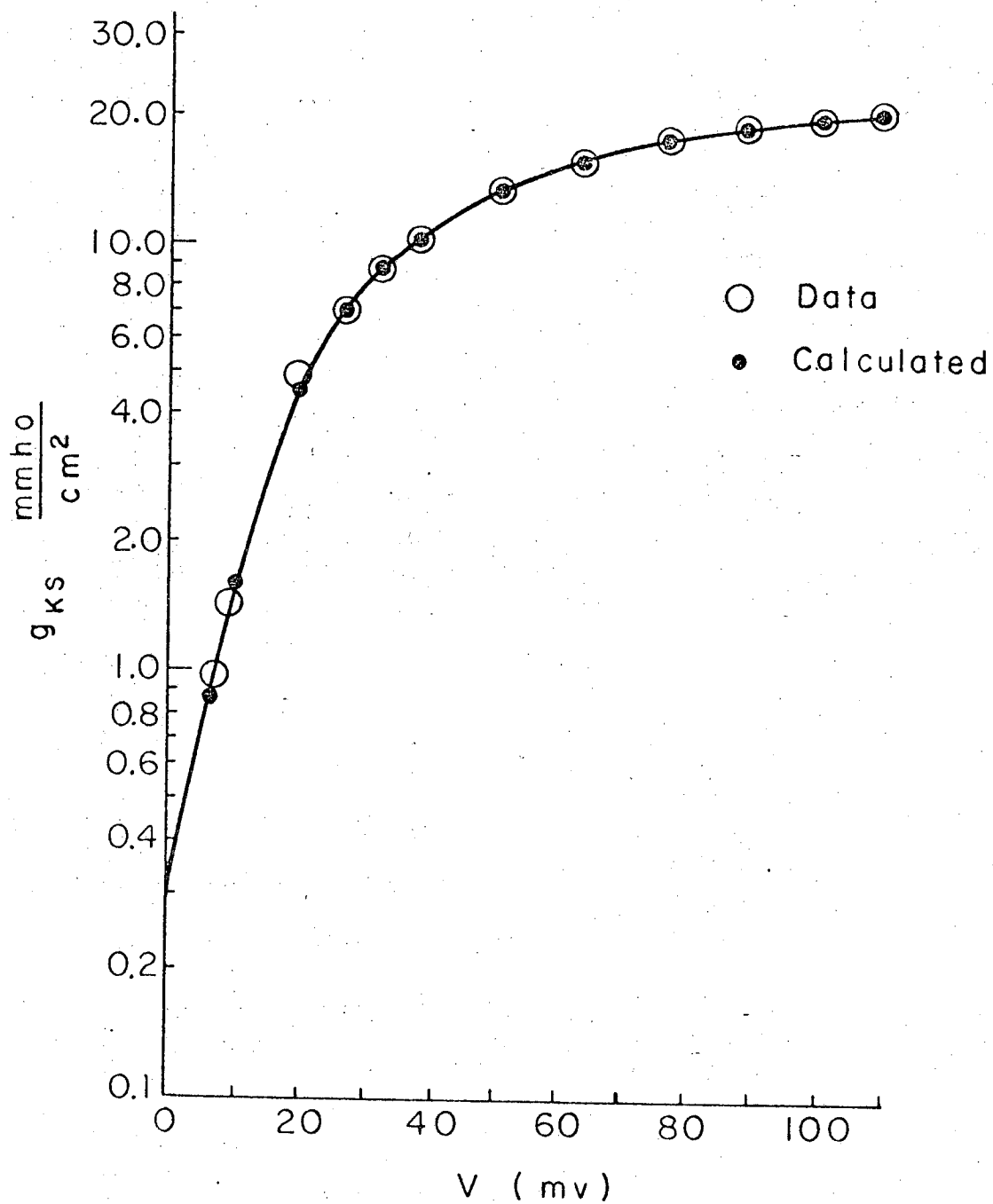
Figure 1 - Steady State Potassium Conductance

Vs Applied Potential in Voltage Clamp - Temp = 6°C.
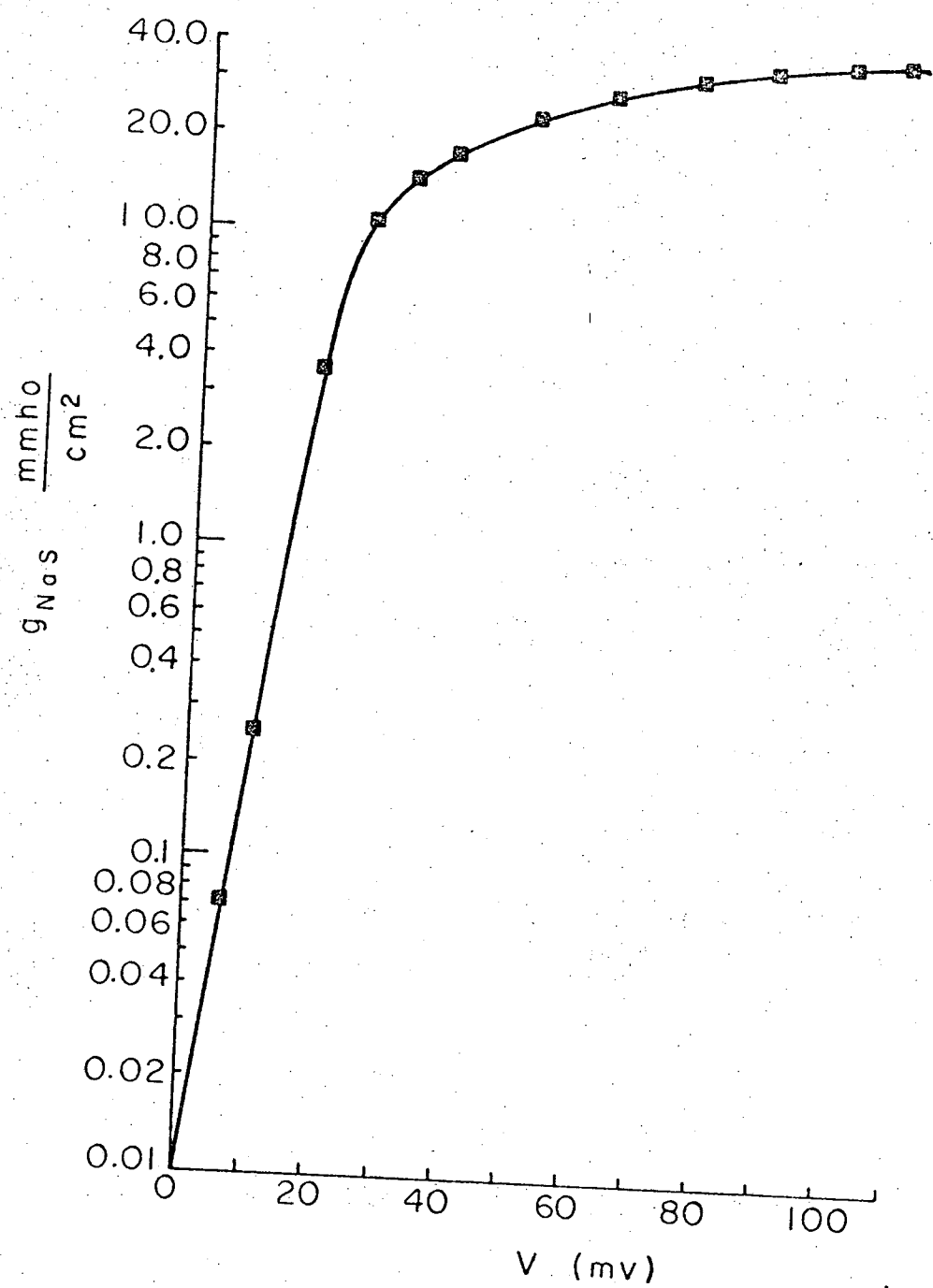
Figure 2 - Amplitude of sodium conductance vs Applied Voltage in Voltage-Clamp - Temp. 6° C.

F. Leakage Current

The proposed model to explain the ionic currents through the membrane does not exclude the possibility that ions could cross the membrane through other regions outside the specific channels. For example there could be a diffusion of ions through the interstitial positions in the lipid lattice. The leakage conductance is calculated from equation (2-4) where the mobility μ and the concentration C of conducting charges is independent of x.

$$g_L = \frac{\mu_L \, z_L \, FC_L}{d}$$

The concentration C_L of conducting charges is given by the concentration interstitial positions in the lattice. The mobility μ has the same expression as before

$$\mu = \frac{F \, \upsilon_L \, a_L^2 \, z_L}{RT \, (1 + e^{(E_{WL} + E_{DL})/RT})}$$

$a_L =$ the distance between interstitial positions. The value of C_L is probably constant, although it could vary with the membrane potential; more interstitial positions could be created when the membrane potential becomes large.

A leakage conductance g_L was found by Hodgkin and Huxley (1952b) and also a concentration potential V_{CL}. They found that g_L was small and they assumed it to be constant. Adelman and Taylor (1961, 1962) made more detailed experiments on the leakage current and found a rectification in it. Gilbert and Ehrenstein (1969) and Lecar et al (1967) have found a linear (possibly leakage) current that was very large when Ca and Mg ions were removed from the outside medium. When Ca and Mg are introduced the linear current is reduced very much. It is known that Ca and Mg ions

diffuse very little through the membrane. If they take many of the interstitial positions in the lipid lattice, the value of C_L will be greatly reduced; this can explain the observed effects of Ca and Mg ions on the linear current.

The treatment of the leakage conductance has not been developed very much yet in the framework of this model. We reserve more developments for the future.

- III -

DYNAMIC EQUATIONS

A. Mobility kinetics

There are two processes taking place together during the change in the conductances of the membrane. The first one is the mobility increase or decrease. The second one is the variation in the ion-complex concentration. The experimental results we have on the time dependence of the conductances permit us to have only an approximate evaluation of the first process, the mobility variation; the kinetics of the conductance variations will clearly be dominated by the variations in C_{QS}, when the membrane is depolarized from its resting state. But there is a situation where we can get a clear picture of the kinetics of the mobility. When the membrane has been depolarized by more than 30 mv from its resting state for a long time (more than 5 msec) the value of C_{QS} is saturared and does not increase any more. If then another increase in potential is produced, it will show the kinetics of the mobility. Unfortunately there is only one such experiment available in Hodgkin and Huxley (1952c); but its results are interesting because they show a linear exponential increase with time, in contrast with the very non-linear increase observed for the first depolarizing step. The time constant is about 0.5 msec. It would be very interesting to have more experimental results with this method, in order to demonstrate better the existence of two separate processes that regulate the variation in the membrane conductance.

From the detailed kinetics of conductance changes, we could have argued that the mobility variations could not be much slower or

faster than the chemical kinetics. If it is much faster, let us say about 10 μsec or less, it would have increased at the same time as the potential and it would have shown a non-linear relation between the instantaneous current and the membrane potential; we know from the Hodgkin-Huxley experiments that it is not the case for the squid axons. But it might happen in other axons; for example, in the frog's node, there is a non-linear relation between the instantaneous current and the potential.

If the mobility time constant is larger than 10 μsec or lower than one msec we would see a first increase in the conductance, a saturation, and then the second increase; in other words we would see two saturation levels. Finally if the time constant is longer than 10 msec, we would also observe two levels of saturation, at least for high depolarization. All these arguments apply to the kinetics of the potassium conductance; they will not appear as well with the sodium conductance kinetics because of their transient character. We can conclude then that the time constant for the mobility variation should be between one and ten msec for the potassium conductance and between 0.1 and one msec for the sodium conductance; the sodium conductance variations are about 10 times faster than the potassium conductance variations. The only experimental results (the one mentioned above) we have for the sodium conductance is within this range and is about 0.5 msec.

When the membrane is depolarized from its resting state, the increase in conductance produced by the increase in mobility is small compared to the one produced by the increase in complex formation. It seems also that with the actual experimental results we cannot separate the two processes. Because of these reasons we will not introduce any time dependent equations for the mobility variations. But it is an

approximation that we are making to keep the model as simple as possible; for a more general model there should be a differential equation for the mobility.

B. Ion-complex kinetics

Now we come to the kinetics of the complex formation. They will be taken from chemical kinetics. The only basic theory that we have for chemical kinetics is derived for gas-phase reaction from the collision theory or the activation theory.

The results are linear differential equations which for this case would be like this:

$$\frac{d\,C_{QS}}{dt} = R_4\,C_Q^{\,Z}\,C_S - R_3\,C_{QS}$$

$$= \frac{R_4}{K}\left[KC_Q^{\,Z}\,C_S - C_{QS}\right] \qquad\qquad K = \frac{R_4}{R_3}$$

For the steady state situation

$$\frac{d\,C_{QS}}{dt} = 0$$

and $KC_Q^{\,Z}C_S = C_{QS}$ as we have used it to develop the steady state relation between C_{QS} and membrane potential. When the membrane potential is changed, the value of K is changed and there will follow a time dependant change in C_{QS} until equilibrium is obtained again. We can replace the factor $K\,C_Q^{\,Z}C_S$ in the differential equation by the new equilibrium value of C_{QS} called $C_{QS\infty}$. We have already developed a function for $C_{QS\infty}$. We might say that $C_{QS\infty}$ is the input and C_{QS} is the output, although the

real input is the membrane potential. This transformation is made to eliminate the equilibrium constant which has already been calculated and leaves only the rate constant.

$$\frac{dC_{QS}}{dt} = R_3 \left[C_{QS\infty} - C_{QS} \right]$$

(3-1)

If we attempt to fit this equation to the data on the kinetics of the potassium conductance, we can easily see that it does not work. This equation gives a simple exponential function of time; the data shows an inflexion initially which the equation above cannot give as it is. That differential equation has been developed for very simple cases of chemical kinetics and it could not be expected to work for reactions occuring in liquid-solid phases. Many chemical reactions occuring in solution are found to be non-linear and there is no general theory that we can use. The phenomena of catalysis and the problems of liquid-solid interface reaction give the theoretical developments complicated and unsolved difficulties. But we can temporarily solve the situation by making empirical modifications of the fundamental differential equation. Among the non-linear chemical kinetics equations, we found one type that was fitting the data very well and which also was logically plausible within the framework of our model. This type of reaction is the autocalytic process; it means that the new complex formed from the substrates can serve as a catalyser for the formation of the other complexes. At the begining the reaction will proceed slowly and as more complexes are formed it will proceed faster until it has reached its equilibrium state. This kind of kinetics is precisely the one we have for the increase in the ionic conductances. Then it is assumed that the protein ion complex is a catalyser for its own formation. We might say that the substrate is an

enzyme in its inactive state and when ions are bound to it, it becomes active and catalyze its own activation. Such a case is well known for the enzyme pepsinogen which becomes pepsin when activated; HCL in the stomach can activate the enzyme but at a very slow rate. But as soon as some of the pepsinogen is turned to pepsin, then pepsin itself catalyses the activation of pepsinogen.

It is possible that we have a similar case with the activation of the membrane conductances. Changeux et al (1967) have assumed that as the molecules of the membrane interact with ions outside the membrane, interaction forces between neighboring macromolecules may also change. This hypothesis is similar to what we have called autocatalysis. The kinetic equations for such a process can be expressed simply; the rate constant is proportional to the concentration of the complex.

$$R_3 = R_{31} C_{QS} + R_{32}$$

The term R_{32} is the rate of the reaction when $C_{QS} = 0$. It represents the others ways of increasing C_{QS} when there is no autocatalysis. The differential equation would then be

$$\frac{dC_{QS}}{dt} = (C_{QS\infty} - C_{QS}) (R_{31} C_{QS} + R_{32}) \tag{3-2}$$

Since the conductance g is directly proportional to C_{QS} and since we are neglecting the kinetics of the mobility variations, we will have

$$\frac{dg}{dt} = (g_\infty - g) (R_1 g + R_2) \tag{3-4}$$

g_∞ is the previously developed equation (2-17) for the steady-state value of the conductance; R_1 and R_2 are unknown constants that will be obtained by curve fitting. Their only requirements is that they are

time-independent; but they could depend on many other variables like, temperature and membrane potential. Equation (3-4) can be integrated when the membrane potential is maintened constant.

$$\frac{dg}{(g_\infty - g)(R_2 + R_1 g)} = dt$$

$$\frac{1}{(g_\infty - g)(R_2 + R_1 g)} = \frac{1}{R_1} \left[\frac{1}{(g_\infty - g)} + \frac{1}{\left(\frac{R_2}{R_1} + g\right)} \right] \frac{1}{\left(\frac{R_2}{R_1} + g_\infty\right)}$$

$$\frac{1}{(R_2 + R_1 g_\infty)} \left[\frac{dg}{(g_\infty - g)} + \frac{dg}{\left(\frac{R_2}{R_1} + g\right)} \right] = dt$$

$$Ln \left(\frac{g_\infty - g_o}{g_\infty - g} \right) + Ln \left(\frac{R_2/R_1 + g}{R_2/R_1 + g_o} \right) = (R_2 + R_1 g_\infty) \, t$$

$$Ln \, \frac{(g_\infty - g_o)}{(R_2 + R_1 g_o)} \frac{(R_2 + R_1 g)}{(g_\infty - g)} = (R_2 + R_1 g_\infty) \, t$$

We can see that the time constant is dependent of g_∞ and then it is potential dependent. It is also obvious from the differential equation, that the conductance will increase slowly and then faster. Both of these features are seen in the time dependence of the Na and K conductances in Hodgkin and Huxley (1952d).

$$g(t) = \frac{R_2 g_\infty + R_1 g_\infty \, go - (R_2 g_\infty - R_2 \, go) \, e^{-(R_2 + R_1 g_\infty) \, t}}{R_2 + R_1 \, go + (R_1 g_\infty - R_1 \, go) \, e^{-(R_2 + R_1 g_\infty) \, t}} \qquad (3-5)$$

g_o = initial g

Equation (3-4) and (3-5) can be applied to calculate the potassium con-
ductance kinetics

$$\frac{dg_K}{dt} = (g_{K_\infty} - g_K)\ (R_{1K}\ g_K + R_{2K})$$

<div align="right">(3-4a)</div>

The integration gives the same result as in equation (3-5) when the po-
tential V_m is constant. But the sodium conductance presents an additional
problem, it is only transient. In developing the steady-state equations
we introduced an additional hypothesis into our model to explain such a
fact. We assumed that some other ions, possibly chloride, could also
react with the protein substrate for sodium and thereby reduce the diffu-
sion of sodium ions; that process was called inactivation. We have an
increase in C_{NaS} and then a decrease. We make a first calculation with
linear differential equations

$$\frac{d\ C_{NaS}}{dt} = R_1\ C_{Na}^{\ Z}\ C_S - R_2\ C_{NaS}$$

$$\frac{d\ C_{NaSA}}{dt} = R_3\ C_{NaS}\ C_A^{\ Y} - R_4\ C_{NaSA}$$

$$C_S + C_{NaS} + C_{NaSA} = C_{ST}$$

Replacing d/dt by the symbol D we have for C_{NaS}

$$D\ C_{NaS} = R_1\ C_{Na}^{\ Z}\ (C_{ST} - C_{NaS} - C_{NaSA}) - R_2\ C_{NaS}$$

$$D\ C_{NaSA} = R_3\ C_{NaS}\ C_A^{\ Y} = R_4\ C_{NaSA}$$

$$D\ C_{NaS} = R_3\ C_{Na}^{\ Z}\ (C_{ST} - C_{NaS} - \frac{R_1\ C_{NaS}\ C_A^{\ Y}}{D + R_2}) - R_4\ C_{Na}$$

$$C_{NaS} \left[D^2 + (R_4 + R_2 + R_1 \, C_{Na}^{\,Z}) \, D + R_2 \, R_4 + R_4 \, R_1 \, C_{Na}^{\,Z} + R_1 \, R_3 \, C_{A}^{\,Y} \right]$$

$$= (D + R_4) \; R_1 \, C_{Na}^{\,Z} \, C_{ST} \qquad\qquad (3\text{-}6)$$

This a linear second order differential equation. It could have a solution which is a simple exponential increase and decrease, or it could show oscillations of decreasing amplitudes, depending on the constants. From the experimental data for the sodium conductance, it is easy to see that there is no oscillations. Then the solution will be of this type

$$C_{NaS} = B_1 e^{-\gamma_1 t} - B_2 e^{-\gamma_2 t}$$

where γ_1 and γ_2 can be related to the R's and B_1 and B_2 depend on initial conditions. Rearranging the terms we have

$$C_{NaS} = B_1 e^{-\gamma_1 t} \left[1 - \frac{B_2}{B_1} e^{-(\gamma_2 - \gamma_1) t} \right] \qquad (3\text{-}7)$$

This result has the form of an increasing expotential times a decreasing one. It is as if we had multiplied the solution of two first order differential equations; one giving an increase of C_{NaS} to its steady-state and the other one decreasing C_{NaS} from its maximum to its resting value. Since we will have to introduce non-linearities in our differential equation for C_{NaS}, as we did for the potassium conductance we will use two independent differential equations to describe the increase and decrease of C_{NaS}. We have shown that it is quite justifiable to do so in This case because there is no oscillation in the experimental results. That method is much more simple to handle and will give correct results.

For the increase of C_{NaS} we will have the same autocalytic process as we had for the potassium case. For the decrease in C_{NaS} the

experimental results on inactivation show a linear process. Then for inactivation we will use a simple first order linear differential equation.

For the increase of C_{NaS} we have

$$\frac{dC'_{NaS}}{dt} = (C_{NaSO} - C'_{NaS}) \ (R_5 \ C'_{NaS} + R_6) \qquad (3\text{-}8)$$

For the decrease we will use the following

$$\frac{dh}{dt} = R_h \ (h_\infty - h) \qquad (3\text{-}10)$$

h represents the proportion of C_{NaS} remaining after the inactivation

$$h_\infty = \frac{C_{NaS\infty}}{C_{NaSO}} \qquad (3\text{-}9)$$

$C_{NaS\infty}$ and C_{NaSO} are in equation (2-26) developed in the steady state discussion.

The total value of C_{NaS} will be given by the product of $C'_{NaS} \cdot h$ For the case of constant membrane potential the solution for C'_{NaS} has already been calculated and the one for h is

$$h \ (t) = h_\infty - (h_\infty - h_o) \ e^{- R_h t}$$

h_o is the initial value of h

$$h \ (t) = h_\infty \ (1 - e^{-R_h t}) + h_o \ e^{-R_h t} \qquad (3\text{-}10a)$$

The amplitude of C_{NaS} will depend on the product of $\overline{C_{NaSO}} \ (h_\infty + h_o)$. It means that this is the maximum value of C_{NaS}; it could be smaller depending on the time constants of the two processes. In most cases h_∞ is zero, but since for small depolarization h_∞ is not negligible, it must be introduced. The initial value of C_{NaS} has been neglected because it is negligible at rest. Because g_{Na} is directly proportional to C_{NaS} we will

transform the above differential equation for C_{NaS} into one for g_{Na}

$$\frac{dg_{Na}'}{dt} = (g_{NaO} - g_{Na}') \ (R_{1Na} \ g_{Na}' + R_{2Na}) \tag{3-11}$$

$$g_{Na} = g_{Na}' \cdot h \tag{3-12}$$

and the amplitude will be $g_{NaO} \ (h_\infty + h_o)$

Putting all the equations together for the potassium and for the sodium conductance we have

$$\frac{dg_K}{dt} = (g_{K\infty} - g_K) \ (R_{1K} g_K + R_{2K}) \tag{3-13}$$

$$\frac{dg_{Na}'}{dt} = (g_{NaO} - g_{Na}) \ (R_{1Na} g_{Na} + R_{2Na}) \tag{3-11}$$

$$\frac{dh}{dt} = R_h \ (h_\infty - h) \tag{3-9}$$

$$g_{Na} = h g_{Na}' \tag{3-12}$$

The value of $g_{K\infty}$, g_{NaO} and h_∞ are given by the steady state equations (2-21), (2-30), (2-31).

We have fitted these equations to the Hodgking and Huxley (1952d) data. They work very well for the potassium conductance as can be seen on Fig. 3. The values for the constants are given in Table 2. They also work quite well for the sodium conductance for depolarization of 15 mv or higher. For lower depolarizations the experimental rate of increase becomes larger; it becomes smaller in the model. Also the rate of increase for inactivation is approximately constant for depolarization larger than 15 mv. But for lower depolarization it becomes
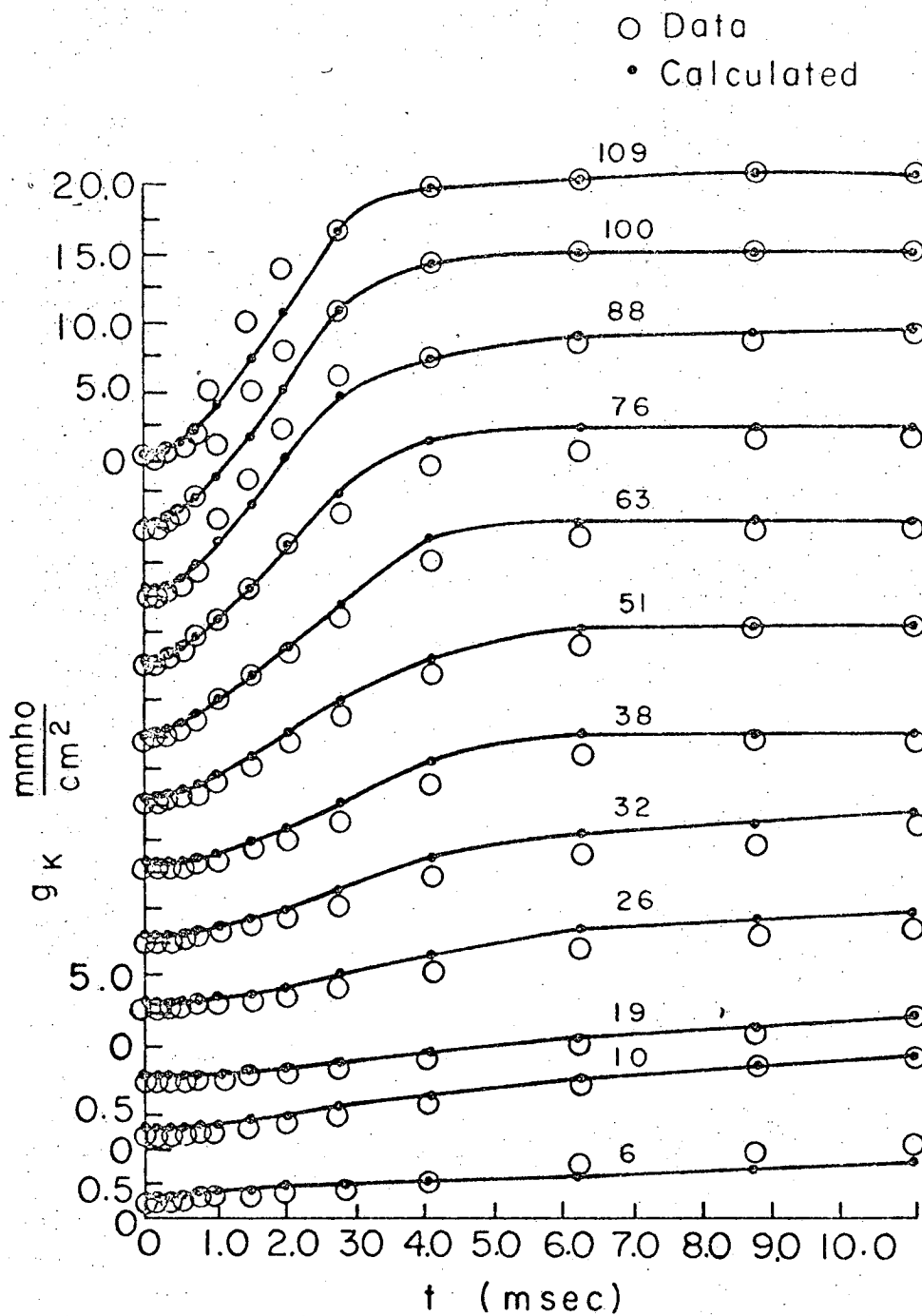
Figure 3 - Time dependance of potassium conductance in Voltage-clamp

Temp. = 6°C.

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suddenly much lower. As we said before, we do not have a fundamental theory to calculate the rate constants for all type of reactions. Their formulation are rather empirical; we introduced a special formulation for an antocalytic process, containing certain constants. These constants will certainly depend on energy and temperature. If their energy dependence is not influenced by the membrane potential changes, they will remain constant during the action potential or during a voltage clamp. We found that it is the case for the potassium conductance and for most of the sodium conductance. But it seems that the rate constants for the sodium conductance is changing when the membrane is depolarized between 0 and 15 mv and also the same thing occurs for inactivation.

Since this modification in the time constant is rather sharp and happens for both activation and inactivation of the Na conductances at the same potentials, we assume that the conformation of the proteins are somewhat modified by the decrease in membrane potential. This modification of the proteins will change the rate constants with both its reactants. The proportions of modified proteins compared to the unmodified ones being given by the Boltzman factor

$$\frac{P_1}{P_2} = e^{-Z_{Na} (45 - V_m) F/RT}$$

$$P_1 + P_2 = 1.0$$

$$P_1 = \frac{1}{1 + e^{Z_{Na} (45 - V_m)F/RT}} \qquad (3-13)$$

The rate constant for the activation of the Na conductance is increased; the rate constant for inactivation is decreased or the time constant τ_h is increased.

$$R_{2Na} = \frac{R_{21Na}}{1 + e^{z_{Na}(45 - V_m) F/RT}} + R_{20Na}$$

$$\tau_h = \frac{\tau_{1h}}{1 + e^{z_{Na}(45 - V_m)F/RT}} + \tau_{oh}$$

(3-14)

$$\tau_h = 1/R_h$$

We have tried to ignore this refinement and we made calculations to obtain an action potential without these potential dependant rate constants. We found the curious fact that we were getting a series of well graded reponses, just about like those obtained when synaptic or soma membranes are stimulated, in contrast with the axonal response which is all or none. When we introduced the potential dependance on the rate constants, we get the all or none response. This modification of the rate constants is important only for depolarizing potentials below 15 mv. The change in R_{2Na} does not seem too important on the experimental voltage clamp record because of the small amplitude of g_{Na}, but we have just shown that it is an important detail.

There is still the repolarization of the conductances to be formulated. When the potential in voltage clamp is returned to its resting or any other value, the kinetic equations are not necessarily the same, especially for the autocatalytic process.

The decrease of the K and Na conductance does not have to be autocatalytic. Experiments by Hodgkin and Huxley (1952b) show that the decrease of both conductances seems to be linear. Both processes were tried and it was found that the linear one was fitting better. It was also found that the linear process is also better for the action potential; the time it took the membrane potential to return to its resting

state was too long for the non linear decay. Then the autocatalytic rate is replaced by a constant rate in the equation when the conductances are decreasing. The potential dependent rates stay the same; inactivation has the same kinetics for increase or decrease. For the decrease we have,

$$\frac{dg_K}{dt} = (g_{K\infty} - g_K)(R_{3K} + R_{2K}) \tag{3-16}$$

$$\frac{dg'_{Na}}{dt} = (g_{Nao} - g'_{Na})(R_{3Na} + R_{2Na}) \tag{3-17}$$

We must point out that the introduction of different rates into the differential equation (3-13) and (3-11) is not absolutly necessary. The improvement of the fit on the data is rather small and it might not be worthwhile to use equation (3-16) and (3-17) with some other data. The same computer programm has been used to find the best values of the parameters to fit the data taken from Hodgkin and Huxley (1952d) by introducing the integrated equations (3-5) and (3-10a) and the steady state equations (2-21), (2-30). The fit is quite satisfying as shown in Fig. 3 and 4. The values of the constants are given in Table 2.

<u>Table 2</u>

Kilo-ohms-cm^2/msec	msec^{-1}		msec^{-1}
R_{1K}	R_{2K}		R_{3K}
0.08	0.05		0.5
R_{1Na}	R_{20Na}	R_{21Na}	R_{3Na}
0.25	0.25	5.0	3.0
	τ_{oh}	τ_{1h}	
	1.0	8.0	
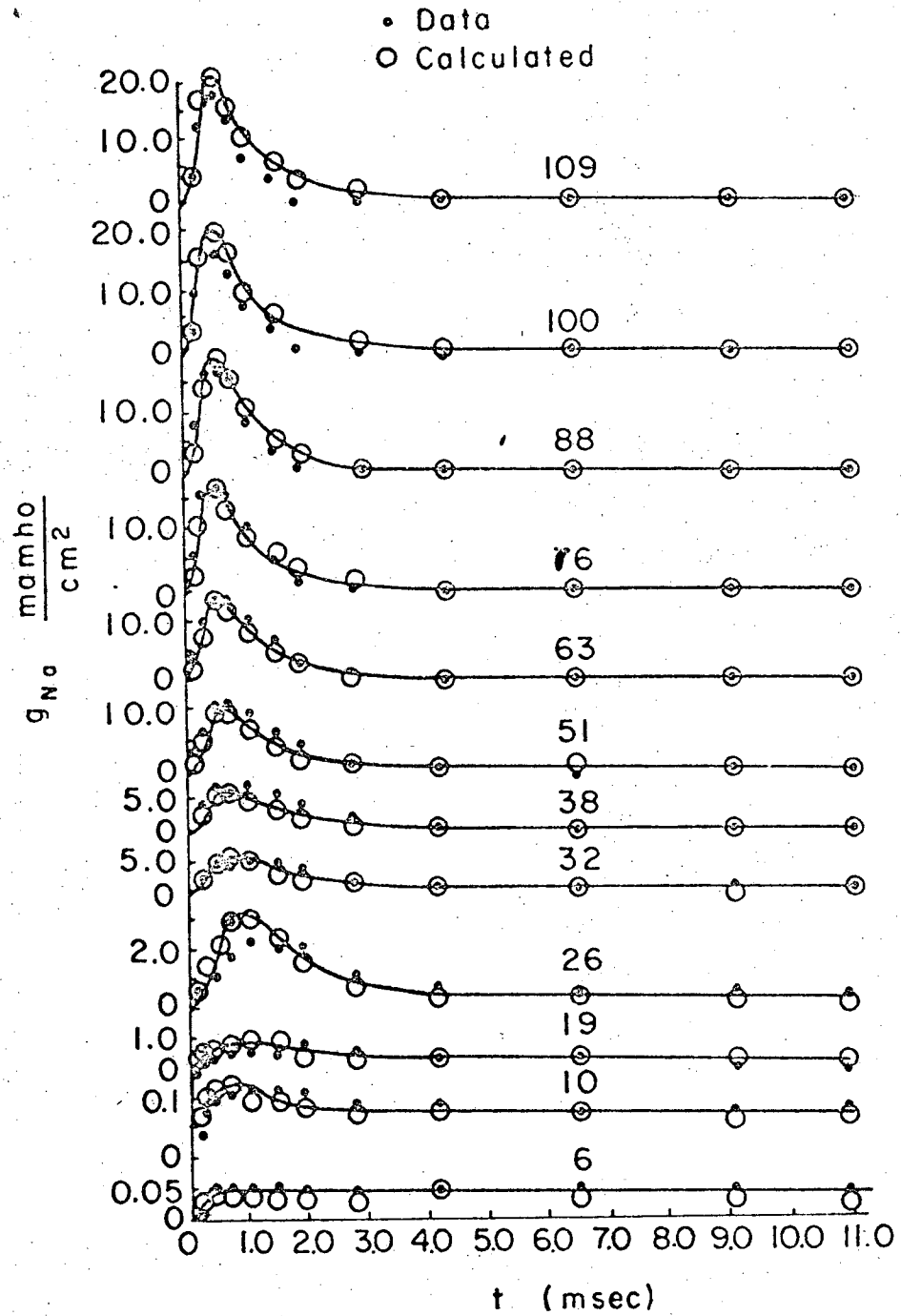
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Figure 4 -   Time dependance of sodium conductance in Voltage-clamp

Temp. = 6°C.

C. Hyperpolarization

There are important results that were obtained by Cole and Moore (1960) and which should also be explained by our model. They hyperpolarized the squid axon membrane in voltage-clamp and depolarized it to the sodium potential, in order to eliminate the sodium current. The potassium current was then obtained directly as a function of time. The stricking difference with the normal potassium current is its longer initial delay. Using the formulation developed by Hodgkin and Huxley (1952d) they couldn't fit their data; the fourth-power or sixth-power expotentials cannot possibly even approach these results. Cole and Moore (1960) had to modify the fourth-power to a twenty fifth power in order to fit their data. These results are really a hard blow to whatever model could be behind the H-H formulation. A different approach is needed to explain such kinetics. Our model brings a different idea to explain the kinetics of conductance changes. The initial rate of increase is given by $(R_{1K}g_K + R_{2K})$. If $R_{1K}g_K$ is very small initially, then the rate is given by R_{2K} until $R_{1K}g_K$ becomes larger than R_{2K}. We see that by adjusting the constants R_{1K} and R_{2K} we can explain about any length of delay. From the curve fitting we have made with the H-H data, we determined the constants R_{1K} and R_{2K}, we have $R_{1K} = 0.08$ Kohm-cm^2/msec and $R_{2K} = 0.05$ msec^{-1}. When the membrane is at rest the initial value of g_K is around 0.3 mnho/cm^2. The initial delay for depolarization at the sodium potential is short, about 0.2 msec. We can see that as soon as g_K has increased to 3 mnho/cm^2, R_{2K} is negligible.

Now if the membrane is hyperpolarized to -120 mv below the resting potential, the resting value of g_K is brought down to 10^{-6} mnho/cm^2. That means $R_{1K}g_K$ is about zero initially. The initial rate

of increase of g_K is

$$\frac{dg_K}{dt} = R_{2K}g_{K\infty} = 0.05 \times 24 = 1.2 \text{ mmho/msec} - \text{cm}^2$$

From that we can evaluate how long it will take for g_K to reach the resting value of 0.3 mmho/cm^2. We are justified to use the above equation, because $R_{1K}g_K$ is still smaller than R_{2K} when g_K reaches its normal resting value. It will take 0.25 msec to g_K to reach the resting value. From the data of Cole and Moore (1960), we see that this is just about the difference of delay observed between the hyperpolarized and the normal state.

There is another important fact demonstrated by these experiments: the sodium current does not seem to be affected by the hyperpolarization. Our rate of increase for the sodium conductance is $(R_{1Na}g_{Na} + R_{2Na})$, the value, we found for the constants by fitting our equation to the H-H data are $R_{1Na} = 0.25$ Kohm-cm^2/msec and R_{2Na} between 5.0 and 0.25 msec^{-1} depending on the value of the depolarization. The initial rate, when the membrane is depolarized to any potential beyond 30 mv, is $0.25 \times 10^{-2} + 0.25$; that means the initial rate is 0.25 msec^{-1}. Using the same method as for g_K to calculate the time it takes for g_{Na} to come back to its resting value of 10^{-2} mmho/cm^2 after it has been reduced by hyperpolarization, $dg_{Na}/dt = R_{2Na}g_{Na\infty}$ taking $g_{Na\infty} = 10$ mmho/cm^2 for $V = 30$ mv, we have $10^{-2} = 0.25 \times 10t$ and $t = 4 \times 10^{-3}$ msec. The value of t obtained in this way gives the difference in delay between a normal and a hyperpolarized conductance; we see that the delay will not be observed, as it was found by Cole and Moore (1960). These results bring additional support to our kinetic theory, which at first might seem rather arbitrary. The kinetic situation for the potassium conductance is

well explained by our model. The situation for the sodium conductance is more complicated because of inactivation and also because of these potential dependant rates that we had to introduce. Although we believe the basic theory to be correct, there is a need for more refinements and more fundamental calculations. We reserve this for future work.

D. Action Potentials

To show the performance of the model nerve in the normal situation where the membrane potential is free to modify itself depending on the currents, we will use the well known cable equation

$$\frac{b}{2\rho} \frac{d^2V}{dx^2} = C_m \frac{dV}{dt} + g_k(V - V_k) + g_{Na}(V - V_{Na}) + g_L(V - V_L)$$

$$V_m = V_R - V \qquad V_{CNa} = V_{Na} - V_R \qquad V_{CK} = V_K - V_R \qquad C_m = 1.0 \ \mu f/cm^2$$

$$V_{CL} = V_L - V_R \qquad b = \text{axon radius} \qquad \rho = \text{resistivity of axoplasm}$$

We will first examine the space-clamped action potential which simplifies greatly the cable equations because

$$\frac{d^2V}{dx^2} = 0.$$

Introducing the functions for g_{Na} and g_K, or rather taking the four differential equation, and integrating them numerically we obtain the space-clamped action potential. We use an initial value for V, and the initial values for the conductances.

$$-C_m^* \frac{dV}{dt} = g_K \ (V-V_K) + hg_{Na}^{\prime} \ (V-V_{Na}) + g_L \ (V-V_L)$$

$$\frac{dg_{Na}^{\prime}}{dt} = (g_{Na_o} - g_{Na}^{\prime}) \ (R_{K1Na} \ g_{Na}^{\prime} + R_{2Na}) \ , \quad \frac{dh}{dt} = R_h^{\cdot} \ (h_\infty - h)$$

$$\frac{dg_K}{dt} = (g_{K\infty} - g_h) \ (R_{1K}g_K + R_{2K})$$

The integration was made with a computer program whose numerical integration procedure was based on a fourth-order Adams-Moulton predictor-corrector with starting procedure based on Zonneveld's formula. This starting procedure is of Runge-Kutta type, but provides an estimate of the truncation error at each step. The program was developed by L.P. Meissner of the mathematics and computing group of the Lawrence Radiation Laboratory at Berkeley. The calculations were made by the CDC 6600. Introducing a simple printer plot routine, we obtain a plot of the action potential directly with the numerical results; we also have the sodium and potassium currents or conductances at the same time. It is a simple and fairly rapid program to use; it takes about four seconds for an action potential lasting 10 msec in real time. The results are quite good as shown on figure 5. We found that an initial depolarization of 13 mv was the one that compares the best with the data; the latter has an initial depolarization of 20 mv which decreases to about 15 mv before firing, while ours does not decrease before firing: we can say that the experimental initial potential is 15 mv, which is fairly close to our value of 13 mv. The space-clamped action potential gives us a fairly good idea of the action potential produced at the point of stimulation; we get an action potential which is much more like the one obtained experimentally on an ordinary axon when we use an initial current
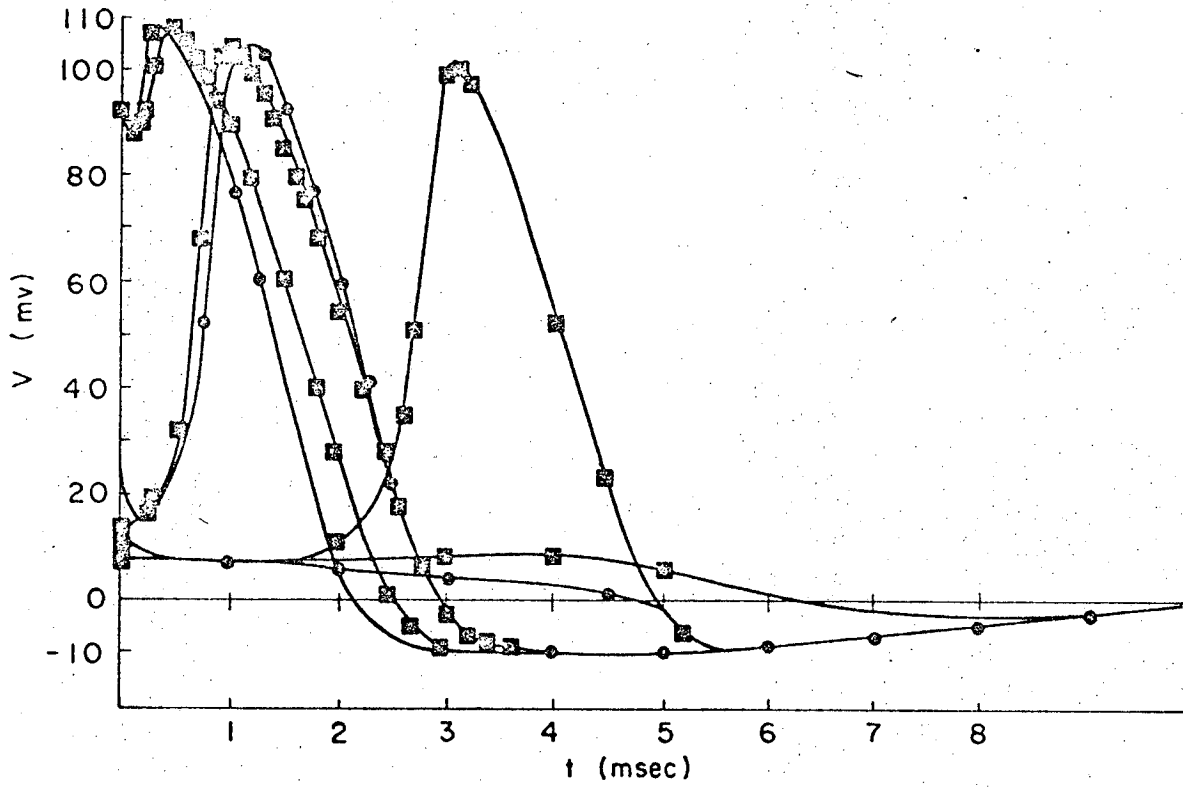
Figure 5 - Space-clamped action potentials. Temp. 6°C.

● Data ▦ Calculated

instead of an initial potential, with the same equations. We see the
subthreshold response and the threshold potential and currents; but
except for the initial rise of the action potential, both results are.
similar.

It has been shown experimentally that the action potential
propagates at constant speed; this can be introduced into the cable
equations to simplify them again.

$$\theta^2 \frac{d^2V}{dx^2} = \frac{d^2V}{dt^2} \qquad \theta = \text{the speed of propagation}$$

$$\frac{b}{2\rho \theta^2} \frac{d^2V}{dt^2} = C_m \frac{dV}{dt} + I_{Na} + I_K + I_L$$

Using the same computer program we can integrate this equation and
obtain the propagated action potential. The procedure is complicated by
the known fact that the voltage V goes to plus or minus infinity if θ
is not very accurately determined. So we have to go by trial and error,
to find a value of θ which stabilizes the potential. Six to eight
digits in θ are usually sufficient to obtain the action potential until
its point of undershoot. It compares well with the data taken from
Hodgkin and Katz (1949) as seen on figure 6. The speed we found was
12.6 m/sec. The calculated action potential will have different ini-
tial delays when we use different initial depolarizations.

The complete cable equations were integrated numerically
with a computer program developed by Cooley and Dodge (1966). They
showed the theoretical action potential does indeed propagate at cons-
tant speed at a distance of one centimeter from the point of stimulation.
The action potential obtained is essentially the same as the one obtained
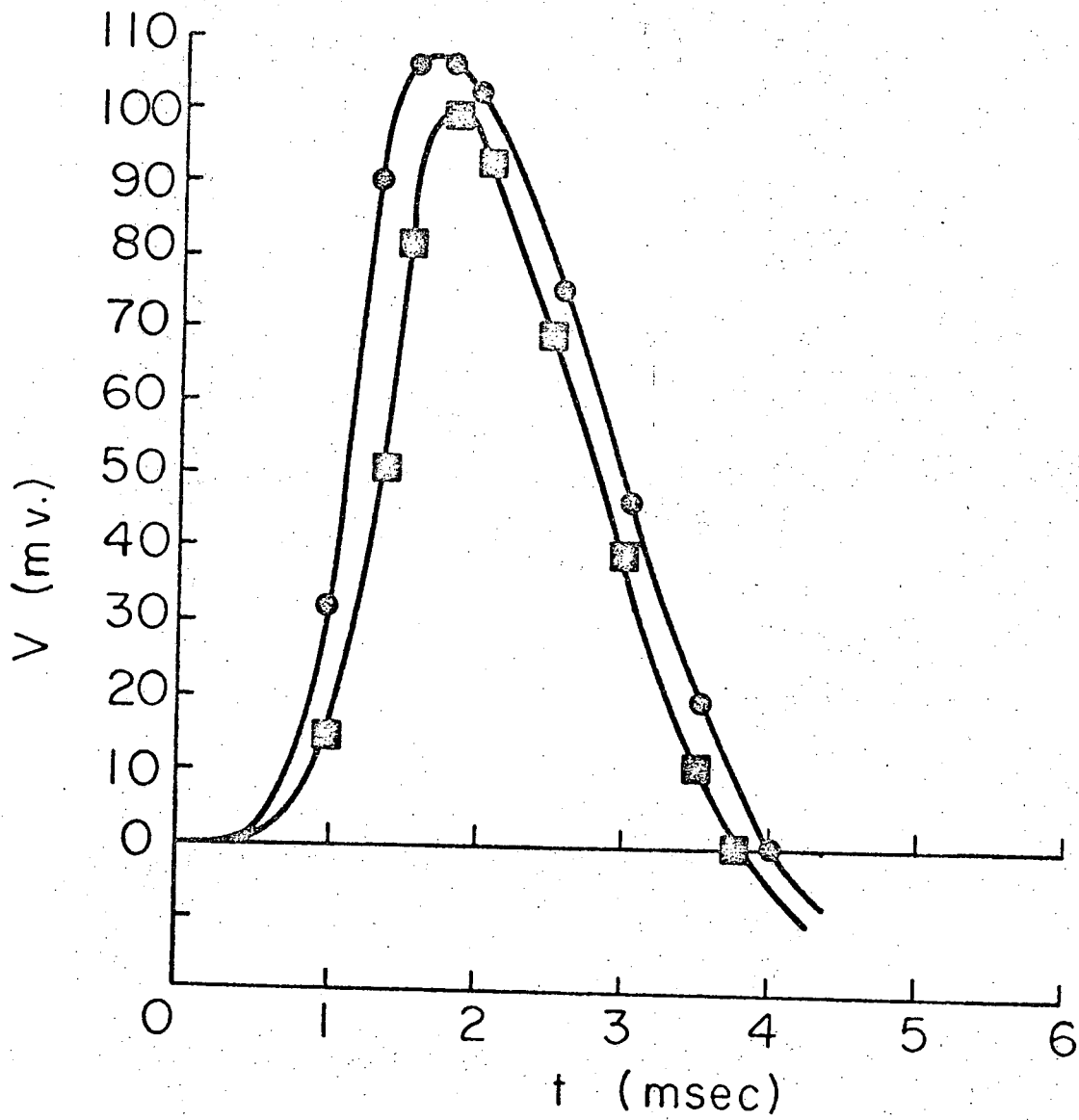
Figure 6 - Propagated action potential. Temp. = 6°C. θ = 12.6 m/sec.

● Data ▨ Calculated

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from the approximate cable equation for the propagated action potential.
Their action potential at x = 0 is similar to the one obtained from
current stimulation with the space-clamped axon. Thus we can rely quite
satisfactorily on these two ordinairy differential equatiens as approxi-
mations. They are much simpler and shorter to use, than the whole cable
equation.

E. Temperature Variation

The steady-state amplitudes of the conductances will vary with
temperature, as can be seen from their formulas. If the resting potential
is maintained and the temperature increased, we should observe an increase
in the resting value of the conductances. For any potential we should
observe a difference between the conductance at a low and high temperature;
when the membrane is around zero, the exponential factors are negligible,
and the amplitude of the conductances should increase much less with tem-
perature. If we take the plot of g versus V_m, we should have a shift of
the curve horizontally and vertically. The vertical shift is caused by
the increase of the maximum value g_m; the horizontal shift is caused
by the exponential factors. We can make some predictions of these changes
for the potassium conductance.

For temperature increases, at the resting potential we should
multiply the potassium conductance by a Q_{10} of a least 1.5. It could be
more than that because there are many undetermined constant in our theory.
That value is calculated from the exponential functions of temperature
that are appearing in the steady state equation for the conductance.

For temperature increases where the membrane potential is
between 0 and -30 mv, we will observe a smaller increase in the conductance,

because the chemical reaction is saturated and there remains only the increase in the mobility. For membrane potentials of about 25 mv, the Q_{10} is around 1.2, and it will be about 1.1 for zero membrane potential. We have very little data on the temperature dependance of the conductance; Hodgkin, Huxley and Katz (1952) gave a few results and say that the amplitudes of the conductance can be corrected for temperature changes by using a $Q_{10} = 1.3$. They found Q_{10} ranging from 1.0 to 1.5, this is quite in accordance with this theory.

Hodgkin, Huxley and Katz (1952) found that the rates of increase and decrease of the conductances were very much dependent on the temperature; they obtained an average $Q_{10} = 3.0$., with variations going between 2.7 and 3.5.

In this model a Q_{10} of three is quite normal to expect since the kinetics of the conductances are dominated by chemical reactions whose Q_{10} are normally in the range of 2 to 6. The Q_{10} is directly related to the energy of activation in the theory of rate processes for simple chemical reaction. If the rate of a reaction can be expressed as

$$R_j = Ae^{-E_{ja}/RT}$$

The Q_{10} can be obtained from the ratio

$$Q_{10} = \frac{R_j (T + 10)}{R_j (T)} = e^{10 E_{ja}/T^2}$$

The product $T (T + 10)$ is approximately equal to T^2 when T is around 300°K. From the Q_{10} we can calculate the energy of activation, E_{ja} for each reaction.

For the range of Q_{10} between 2.7 and 3.5 we obtain energies of 18-23 Kcal/mole or between 0.7 ev and 1 ev. These energies are of

the order of those for chemical bonds and are much higher than the surface energies that we have introduced for the equilibrium constants. The energies involved when the membrane potential is changed are small compared to these activations energies. Nevertheless the amplitude of the depolarization should modify the Q_{10} a little.

At first we tried a $Q_{10} = 3.0$ for all rate constants.

When we introduced these Q_{10} into the equations, we found that the amplitude of the action potential at a just threshold stimulation was rather low compared with the data, although it reached its normal amplitude for stronger stimulation. We introduced a $Q_{10} = 2.5$ for inactivation and a $Q_{10} = 3.5$ for activation of Na conductances. These values are still within the experimental results and give a much higher amplitude for a just threshold stimulus. Since we did not have that problem at low temperatures, we conclude that this difficulty is due to the highly approximate determination of the temperature dependence of the conductances. A complete set of conductance curves in voltage clamp at high temperatures would be needed to determine more accurately the effect of temperature on the different rate constants. Space-clamped action potential for many initial depolarizations were calculated at $20.5^{\circ}C$. One taken from the data of Hodgkin and Huxley (1952d) was compared with them: it fits well with one of ours which has an initial depolarization of 11 mv, as shown on figure 7. The action potential from the data has an initial minimum potential of about 15 mv. The difference between them is probably caused by a difference of threshold.

A propagated action potential for a temperature of $18.5^{\circ}C$ is shown in figure 8. It is compared to an action potential taken from Hodgkin and Huxley (1952d): they are quite similar. The speed of
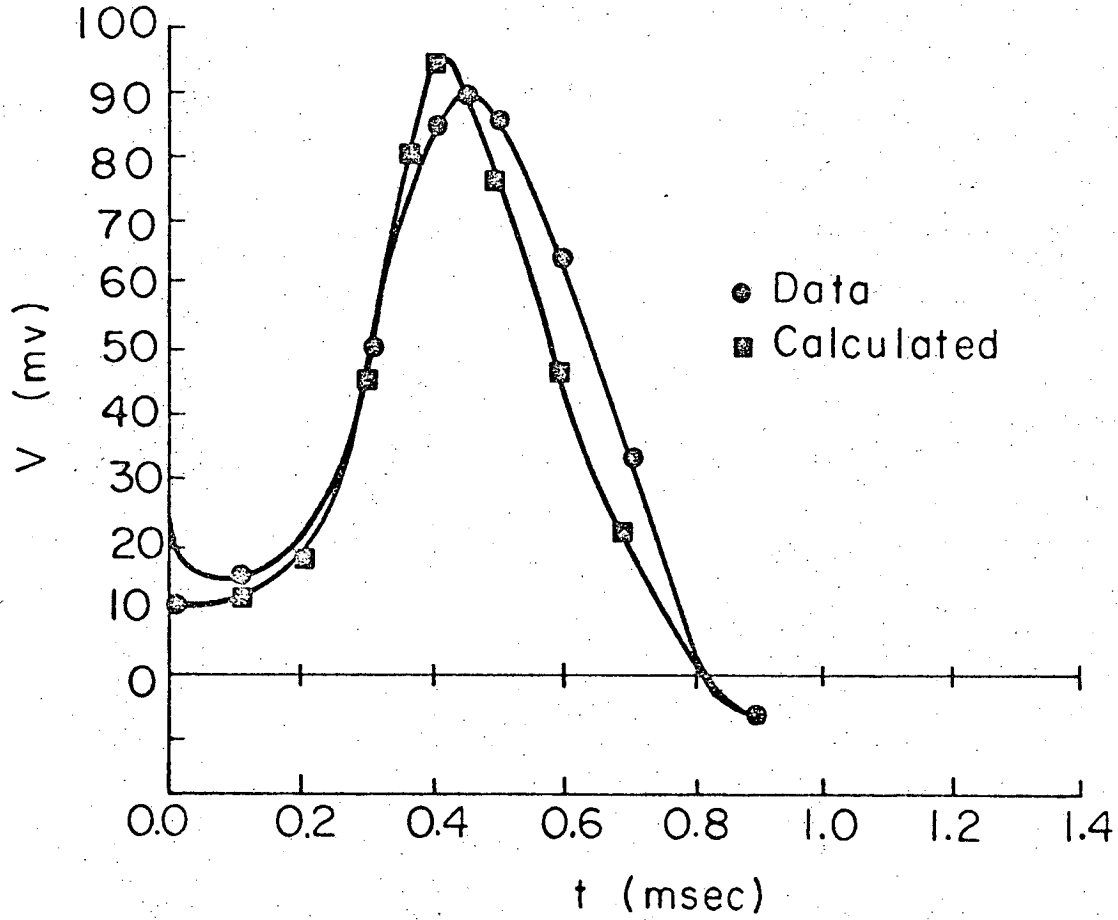
Figure 7 - Space-clamped action potential.   Temp. = 20.5°C.
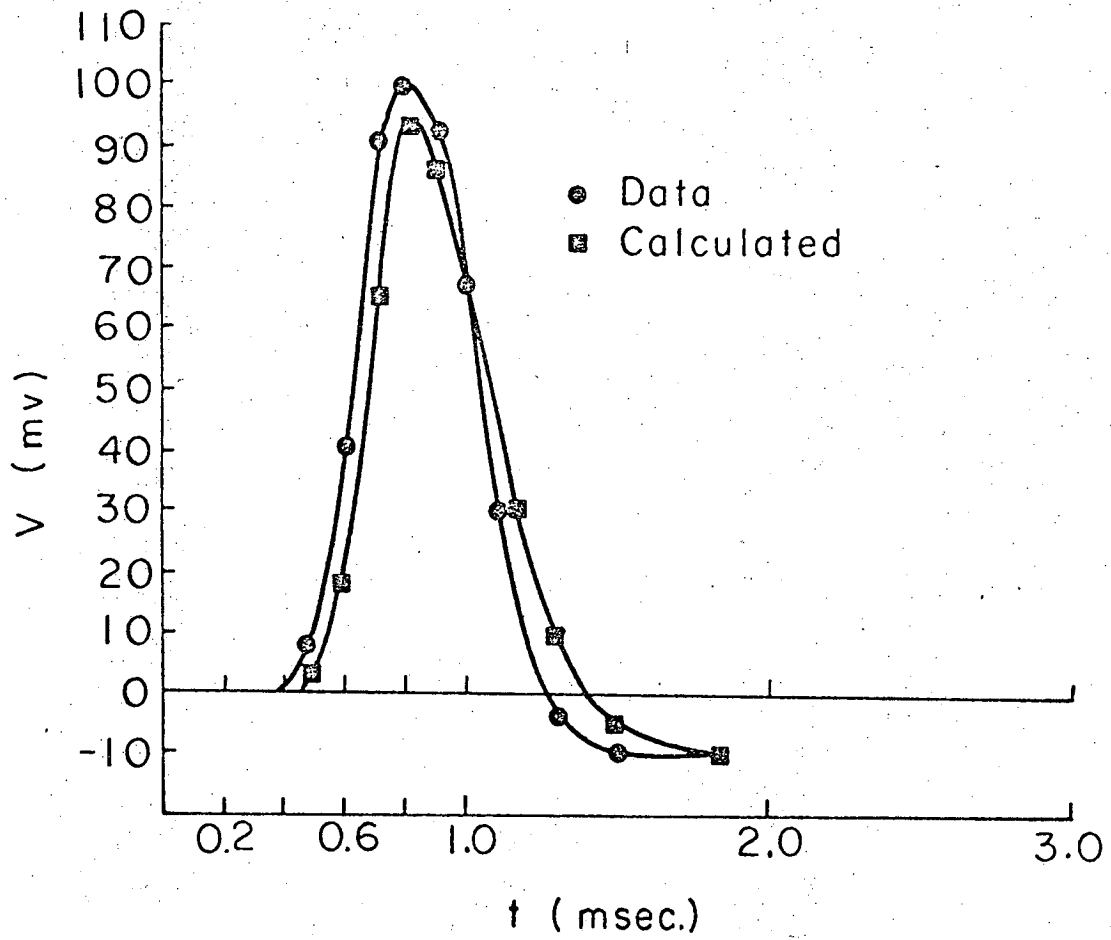
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Figure 8 - Propagated action potential. Temp. = 18.5°C. $\theta = 23$ m/sec.

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propagation is 23 m/sec for the model axon and 21 m/sec for the experimental axon. The Q_{10} for the speed of propagation is 1.5. It is in the usual range of Q_{10} observed for conduction velocity in nerve fibers. We can also calculate the propagated action potential for various temperatures. We find that the speed of propagation increases almost linearly with temperature up to $35^{\circ}C$ and then decreases. The amplitude of the action potential decreases continuously with temperature to disappear completely at $42^{\circ}C$. The average amplitudes of the action potentials for squid axons were measured for different temperatures by Hodgkin and Katz (1949). Their results with our calculations are shown on Figure 9. The agreement is quite good in spite of the fact that our parameters were calculated for one particular axon data. The temperature they reported for the disappearance of the spike is $38^{\circ}C$ which is close to the $42^{\circ}C$ we found for the model. The reason for the disappearance of the spike as suggested by Hodgkin and Katz (1949), is because the Q_{10} of the rate of rise of the action potential is smaller than the Q_{10} for the rate of its decay. The increase of potential depends very much on the membrane capacitance. Its modification will affect strongly the temperature dependence of the amplitude of the action potentials. A smaller capacitance will make the potential rise much faster and consequently it will disappear at a higher temperature. Of course modification in the Q_{10}'s for the rate process or the steady state amplitudes will also change this temperature dependence, but it is interesting to note that only a small decrease of the capacitance is sufficient to do it. We tried a capacitance of 0.5 $\mu f/cm^2$; the results in Figure 9 show a shift of $8^{\circ}C$. We also tried a capacitance of 1.5 $\mu f/cm^2$ and we found that it gave results extremely close to those given by the experimental data.

We do not have any average value of the capacitance in the experiments of
Hodgkin and Katz (1949) but values found by Curtis and Cole (1938) are
seen to be between 0.6 and 1.6 $\mu f/cm^2$ with an average of 1.1 $\mu f/cm^2$.
As mentioned by Hodgkin and Katz, their results are scattered because
of the conditions of the fibers and those with the highest amplitude
are more representative of the real situation. Our result with 1.0 $\mu f/cm^2$
comes close to the one given by fresh fibers. On figure 9b, we show the
speed of propagation against temperature; the decrease in the capacitance
from 1.0 to 0.5 $\mu f /cm^2$ has resulted in very large increase in the speed
of propagation.
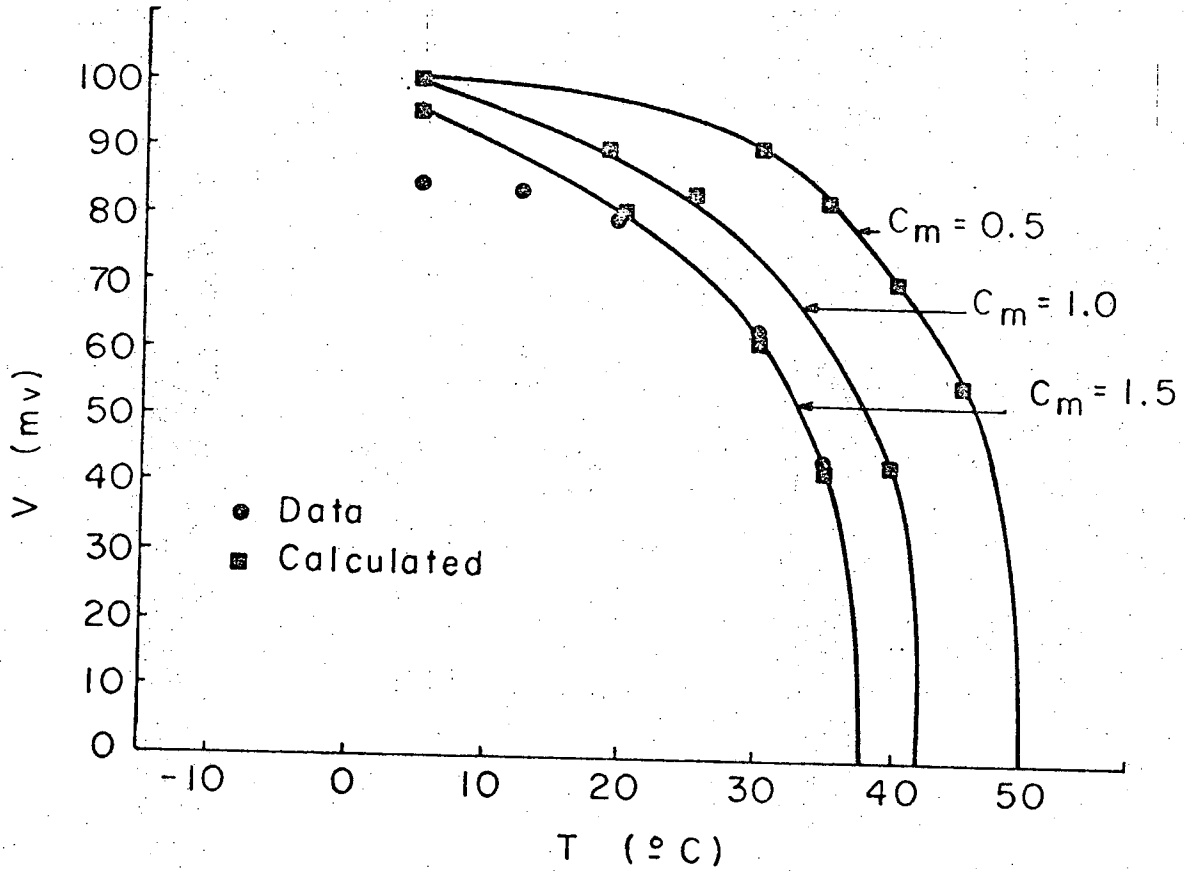
Figure 9 a - Amplitudes of propagated spikes vs temperature

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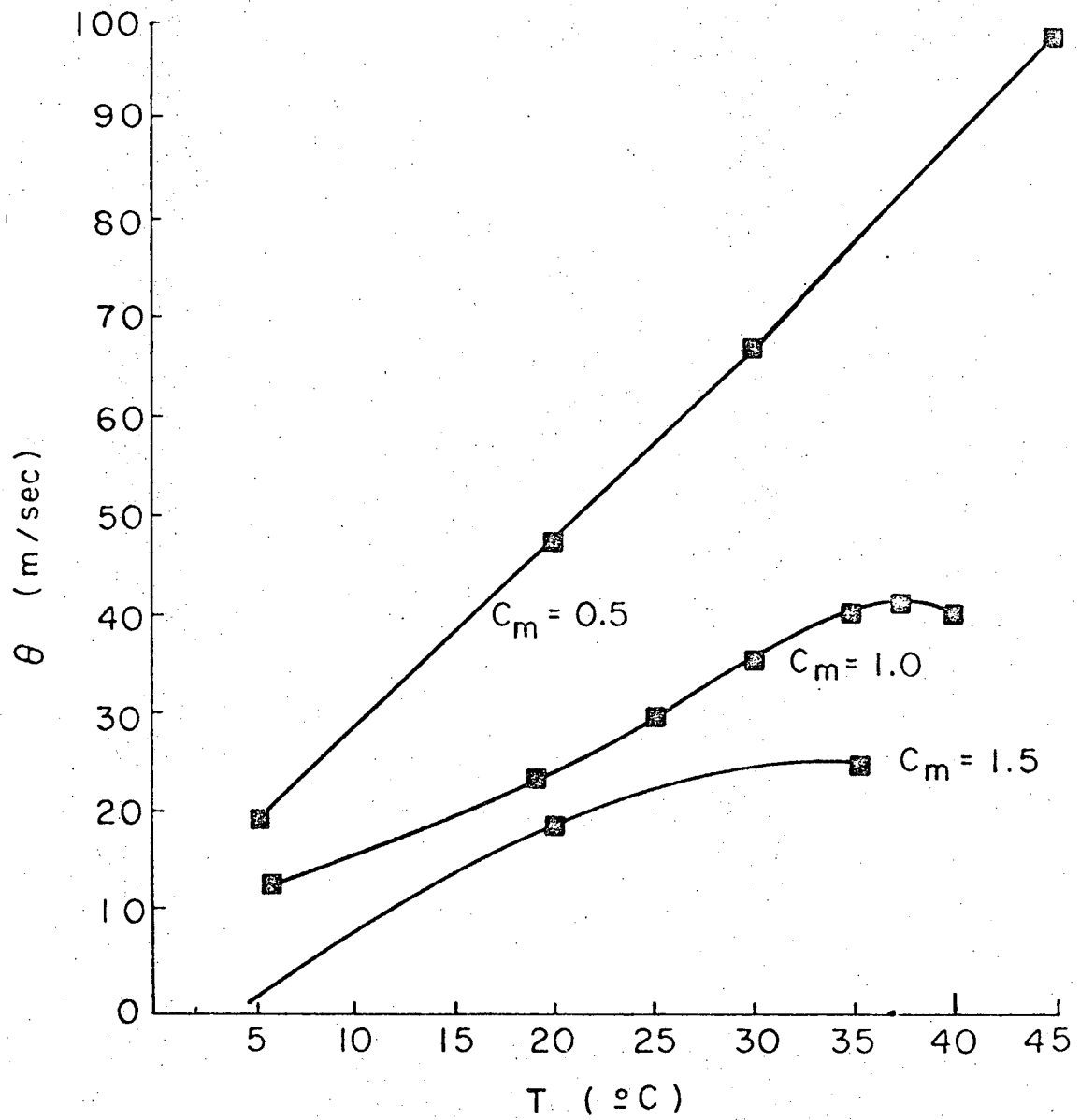
Figure 9 b -   Speed of propagation vs temperature.

- IV -

GENERAL PROPERTIES OF AXONS

There is a certain number of experimental features of the action potential which should be verified by the model axon: threshold, refractory period, accommodation, impedance change, net ionic fluxes, strength-duration characteristic and repetitive firing. Hodgkin and Huxley (1952d) used their theory to verify the first six properties and Cole et al.(1955) the last two. All calculations yielded results in accordance with experiments. Since we have the same theoretical results for the conductances, and since we used the same cable equation, we should verify all these properties without difficulty.

A. Threshold Potential

The threshold of firing can be obtained from this model. If the potential is increased to 6.7 mv, there is no firing, but if it is increased to 6.8 mv, a spike of full amplitude is obtained. A threshold of 8.5 is about the one obtained experimentally by Hodgkin and Huxley (1952d, Fig. 21). When a 12 mv potential is applied, there is a rapid capacitive surge followed by a decrease of potential to either 8 or 8.5 mv. If it decreases to 8 mv, there is no firing, but if it decreases to 8.5 mv, firing occurs. It is then assumed that the threshold is 8.5 mv. We did not include provision for an initial capacitive surge in our model. There is only a small initial decrease in potential so that the initial potential is the one that produces the spike. At a temperature of $18^{\circ}C$, the threshold of firing is higher: we obtain a spike with 7.7 mv, but not with 7.6 mv. There is no reliable experimental determination of the threshold at $18^{\circ}C$ in Hodgkin and Huxley (1952) but that value is in the normal range usually observed.

Hagiwara and Tasaki (1958) mentioned that threshold of firing was always between 10-15 mv for a temperature of 21-22°C. They considered as threshold the maximum synaptic potential amplitude for which there was no firing. We can obtain similar synaptic potentials by using a current stimulus in the space clamped equations. For a current stimulus of 8.8 $\mu A/cm^2$ we obtain a maximum subthreshold potential of 11 mv at 22°C. If we use a potential stimulus, we find that 8.1mv is the maximum initial potential stimulus before firing, and the subthreshold potential increases to 10 mv. These results show that our value of the threshold potential is well within the range of experimental thresholds.

B. Current Stimulus

If we stimulate with a constant current density we also have a threshold. At 6°C a step of 2.4 $\mu A/cm^2$ is producing a spike but 2.3 $\mu A/cm^2$ does not as shown on Fig. 10. For 18°C, a step of 6.7 $\mu A/cm^2$ will produce a spike. These threshold values of a current stimulation give us the rheobase current of the strength duration curve. It was found by Hagiwara and Omura (1958) to be about 9 $\mu A/cm^2$ for temperature around 18°C. They also give their experimental strength duration curve taken from a space clamped axon. We have calculated it for 18°C and it is plotted on relative time and current scale as usual. The relative scale curve does not change with temperature or different rheobase currents. As seen on Fig. 11, the agreement between theory and experiments is very good. The theoretical and experimental values of the constants are given in Table 3 below. I_0 is the rheobase current. At short times, the strength duration curve can be approximated by $It = Q_0$. This is the empirical equation to determine the threshold amplitude of stimulating current necessary when this
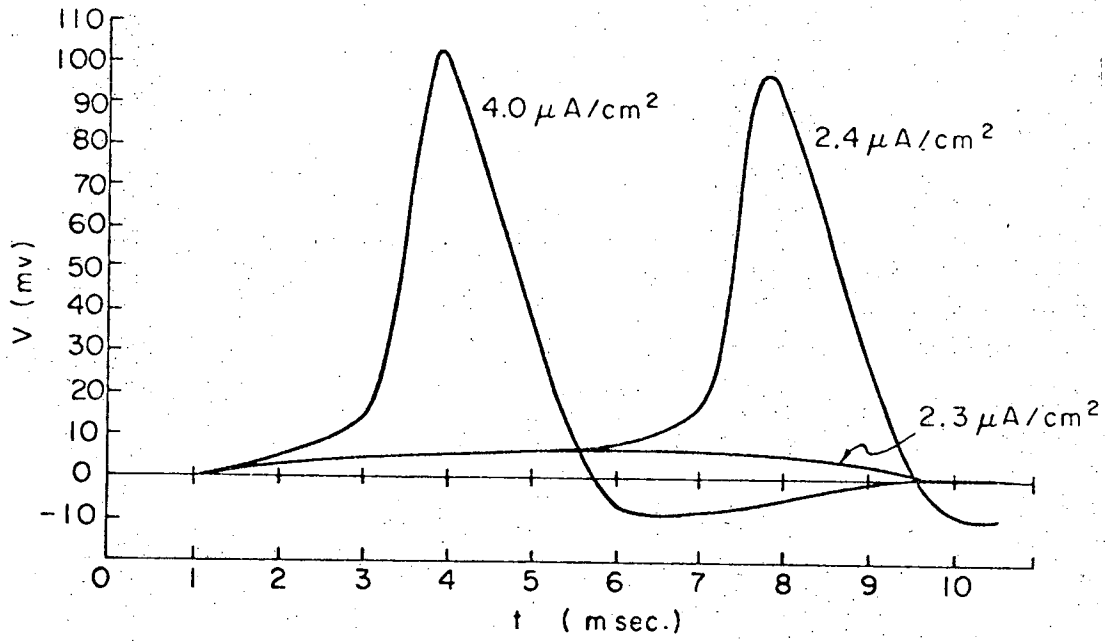
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Figure 10 -   Space-clamped action potential with current stimulus

Temp. = 6° C.
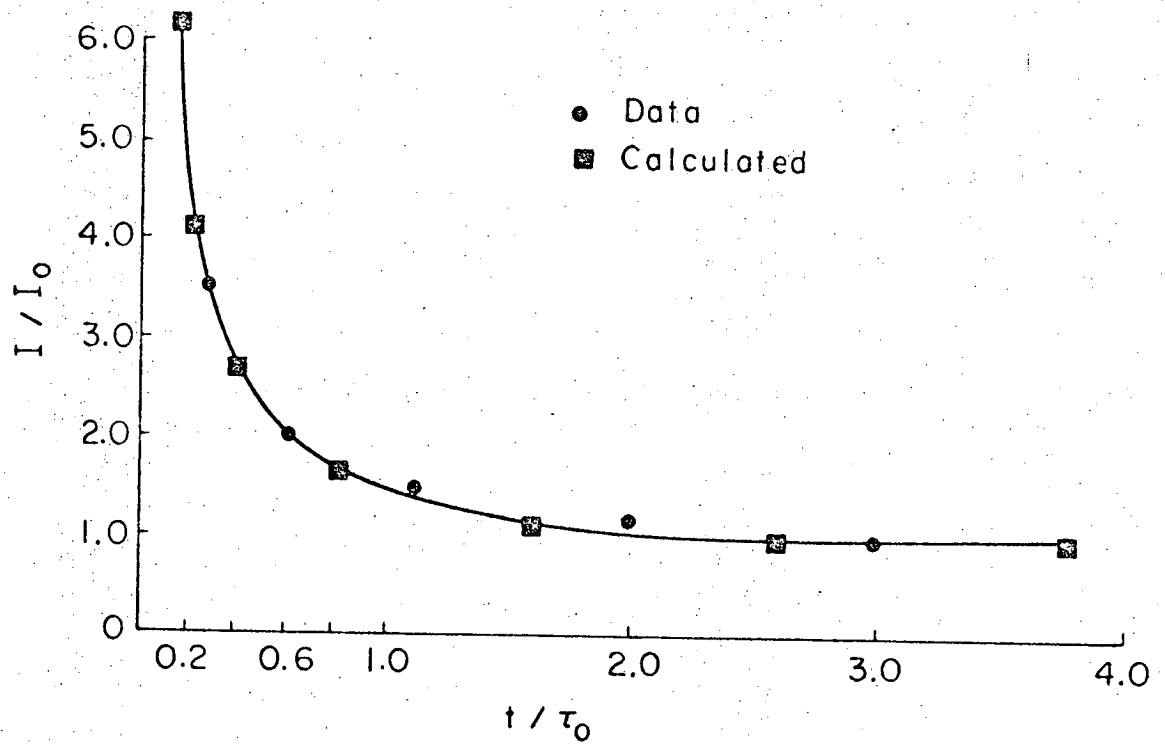
Figure 11 - Strength-duration curve. Temp. = $18^{\circ}C$.

current is kept for a time t. Q_0 is the area under the current pulse and it is constant. With the ratio of $Q_0/I_0 = \tau_0$, we have what is called the time constant of the strength-duration characteristic. The constants are not the same because the threshold for firing is higher in Hagiwara and Omura's axon than in our model axon obtained from the Hodgkin and Huxley data.

Table 3

	Current I_0	Charge Q_0	Time τ_0
Model	6.7 A/cm^2	8.5 nCoul	1.25 msec
Experimental	9 A/cm^2	18 nCoul	2 msec

C. Accommodation

Accommodation is a property of the axon that makes it unresponsive to a slowly increasing current stimulus that reaches an amplitude beyond threshold. Hagiwara and Omura (1968) tested the accommodation property of the space clamped squid with a linearly increasing stimulus. They found that the action potential disappeared when the slope was lower than a limit value. As well as we can evaluate it from these plots, it seems that a rate of 1.6 µA/msec is low enough to prevent the firing but 3.0 µA/msec gives a spike. We tried our model axon with a linear increase of current stimulation, using the space clamped equations and we found that a rate of 2.0 µA/msec was not producing a spike, but 2.5 µA/msec was giving a spike (Fig. 12). This is in close agreement with the experiments.
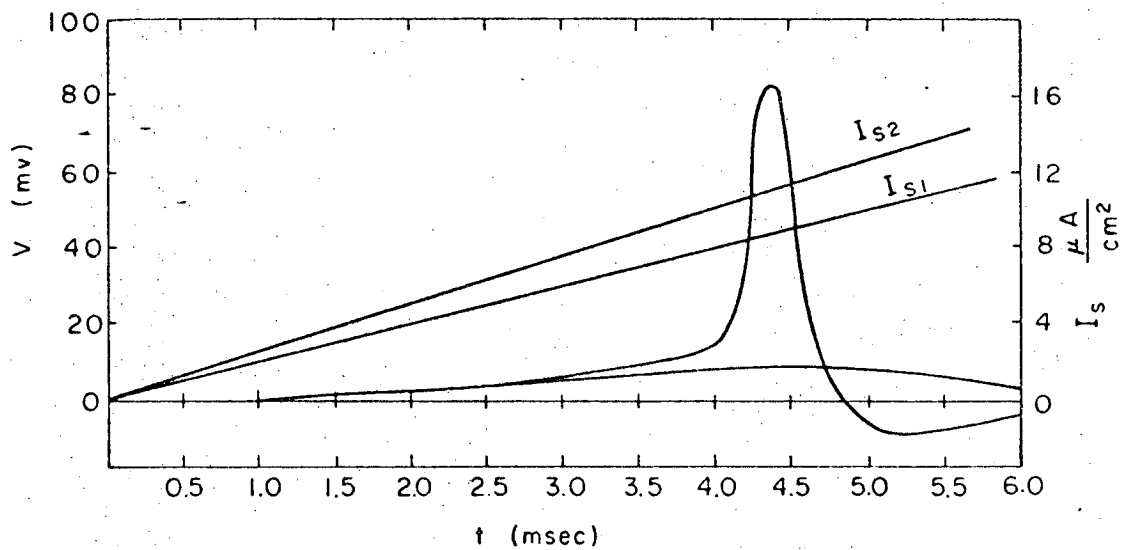
Figure 12 - Accommodation for linearly increasing current.

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D. Time Dependence of Currents

The time course of the currents and the conductance during an action potential are shown in Fig. 13 and 13b. The total change of conductance is seen to be similar to the data of Cole and Curtis (1939) and the results of Hodgkin and Huxley (1952d); our I_{Na} and I_K currents are quite the same as those shown in Hodgkin and Huxley (1952d). From the current curves we can obtain the net flux of Na and K ions during an action potential by integrating under each curve. For Na and K we obtain a value 3.6 p mol/cm^2 at a temperature of 18.5^{o}C. That is quite close to the experimental results of Keynes and Lewis (1951) who obtained 3.5 p mol/cm^2 for Na and 3.0 p mol/cm^2 for K at 22°C.

E. Refractory Period

The refractory period is a well known property of the action potential. If we stimulate the axon at any time during its course it will not fire again before it has reached about the middle of the positive phase. As shown by Hodgkin and Huxley (1952d), this is due to the delayed rise and fall of the potassium conductance and of the sodium inactivation. When they have partially returned to their resting value there will be a spike of reduced amplitude. This is the relative refractory period, while the absolute refractory period is when the axon does not fire at all. This model also shows the two refractory periods. Figure 14 gives the results of stimulation at different times during the space clamped action potential; they compare well with the Hodgkin and Huxley (1952d) data.
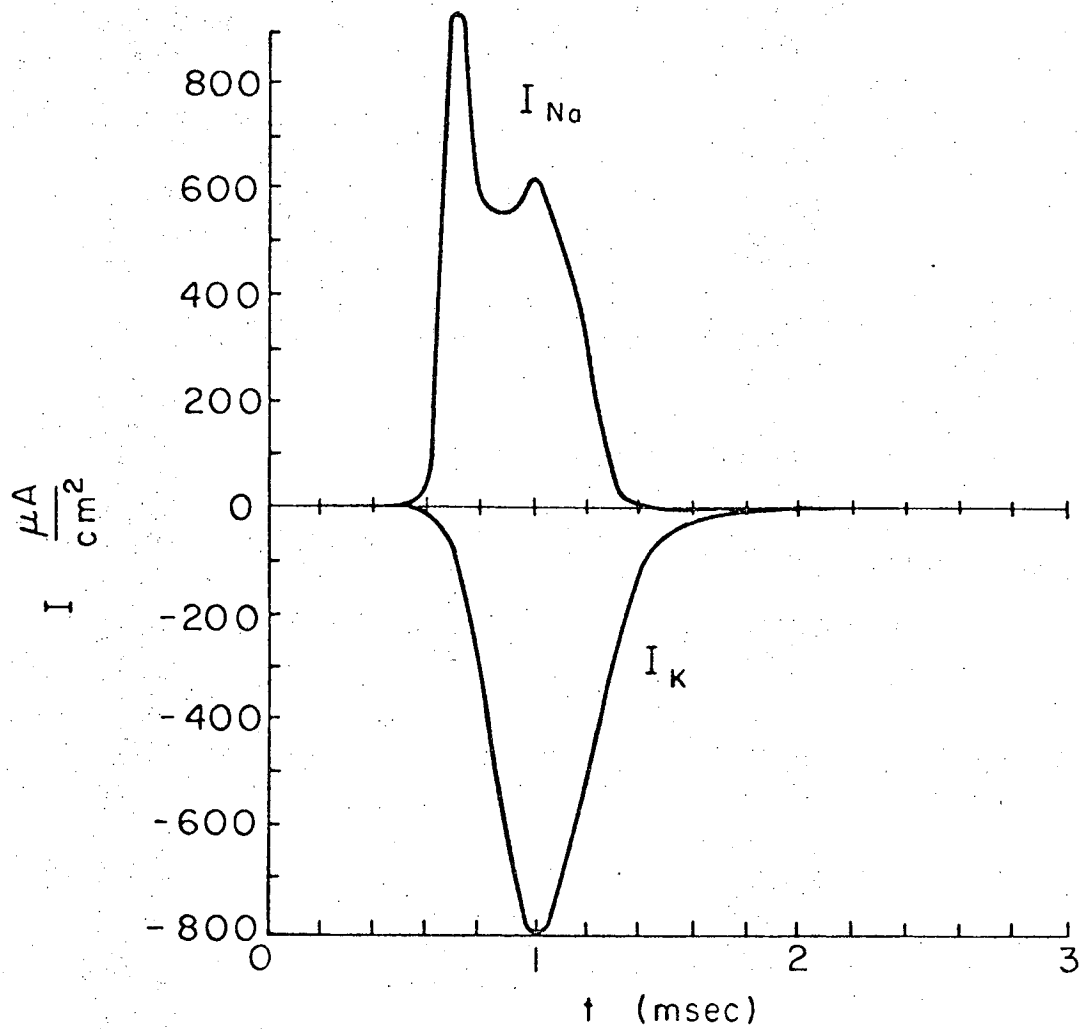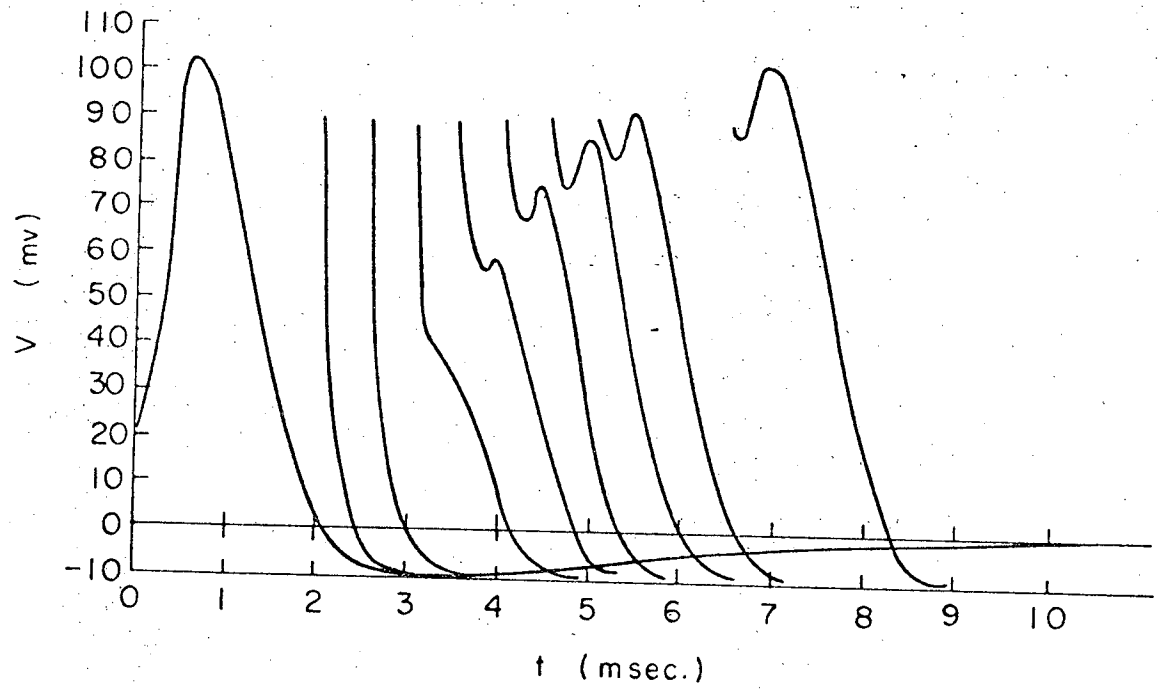
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Figure 13 a - Ionic currents during a propagated action potential
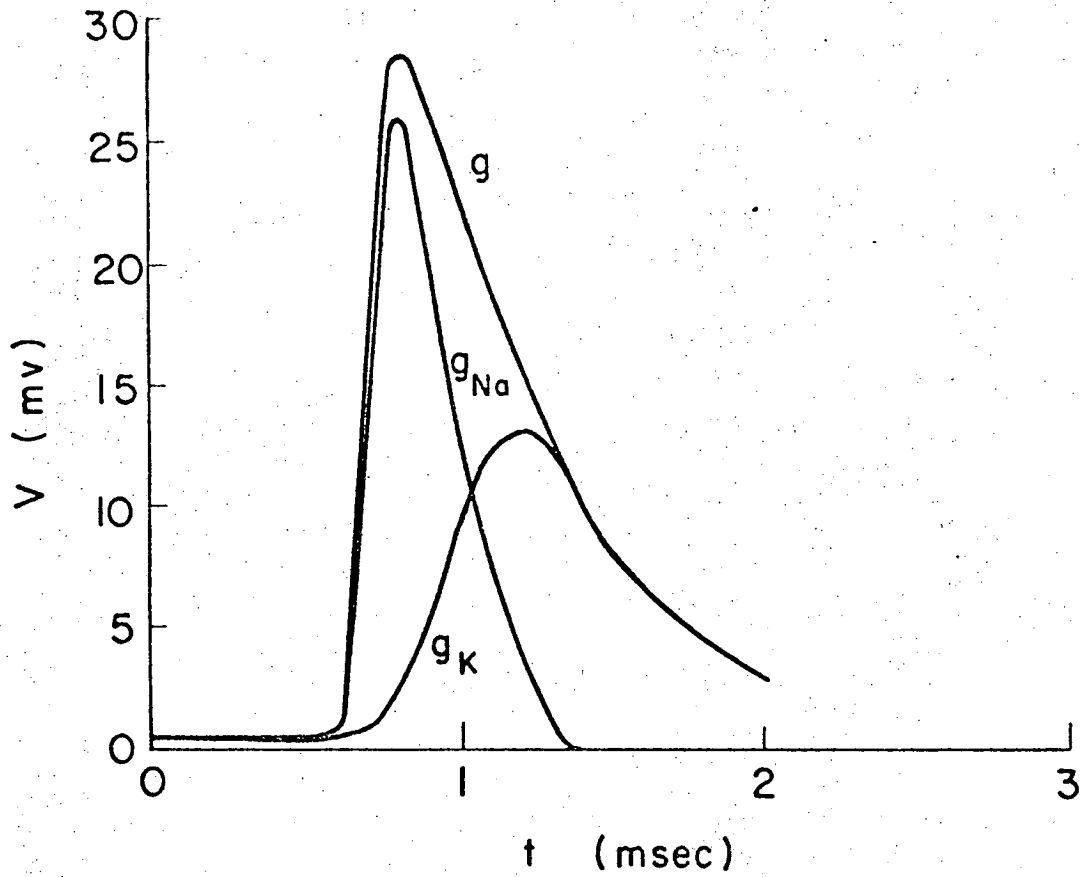
Temp. = 18.5°C.

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Figure 13 b - Ionic conductances during a propagated action potential
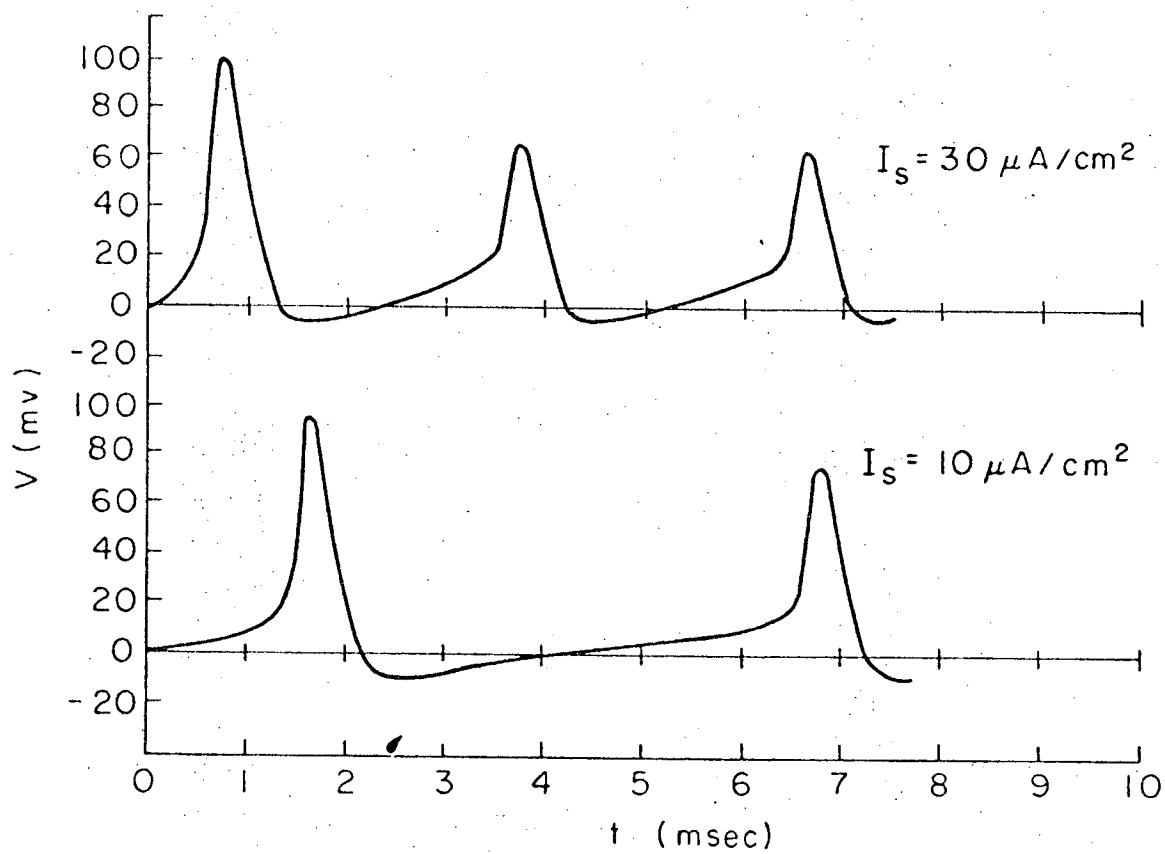
Temp. = 18.5°C.

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Figure 14 −   Refractory period for a space-clamped action potential

Temp. = 9°C.

F. Repetitive firing

Cole et al. (1955) demonstrated theoretically that an axon will fire repeatedly under a constant current stimulation. They found that a space clamped axon under a constant current will fire only once if the intensity is less than 7.5 $\mu A/cm^2$. It will produce a finite train of spikes for stimulating current between 7.7 and 8.0 $\mu A/cm^2$, indefinite trains from 8.1 to 100 $\mu A/cm^2$ and beyond that only one spike followed by oscillations. Their frequency of firing was 200/sec for 8.1 $\mu A/cm^2$ and increased to 350/sec for 50 $\mu A/cm^2$. We have tested this model for repetitive firing and we found similar results. Repetitive spikes starts with a current of 10 $\mu A/cm^2$ and have a frequency of 200/sec. At 50 $\mu A/cm^2$ the frequency reaches 400/sec. The amplitude continuously decreases as the freauency increases because the K conductance and the Na inactivation do not have time to return to their resting values. The large constant current keeps the K conductance and the Na inactivation at large amplitudes. When the current becomes to large, only the first spike will be produced because the inactivation and the K conductance are maintened at their maximum. Figure 15 gives a few examples of these results and Fig.16 gives a plot of frequency against the stimulating current amplitude.

Hagiwara and Omura (1958) made experiments with a constant current stimulation on a space clamped squid axon. They found a frequency of about 200/sec for a minimum current intensity that produce repetitive firing and for about three times that current they have a frequency of about 350/sec. In our model a current stimulus three times the minimum gives a frequency of 330/sec and the initial frequency is 200/sec. The correspondance between these theory and experiments is quite remarkable. But there remains an important problem. The experimental repetitive firing of Hagiwara and Omura is never more than a small finite train of three or four impulses. We cannot explain these results

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Figure 15 - Repetitive firing with a constant current stimulation.
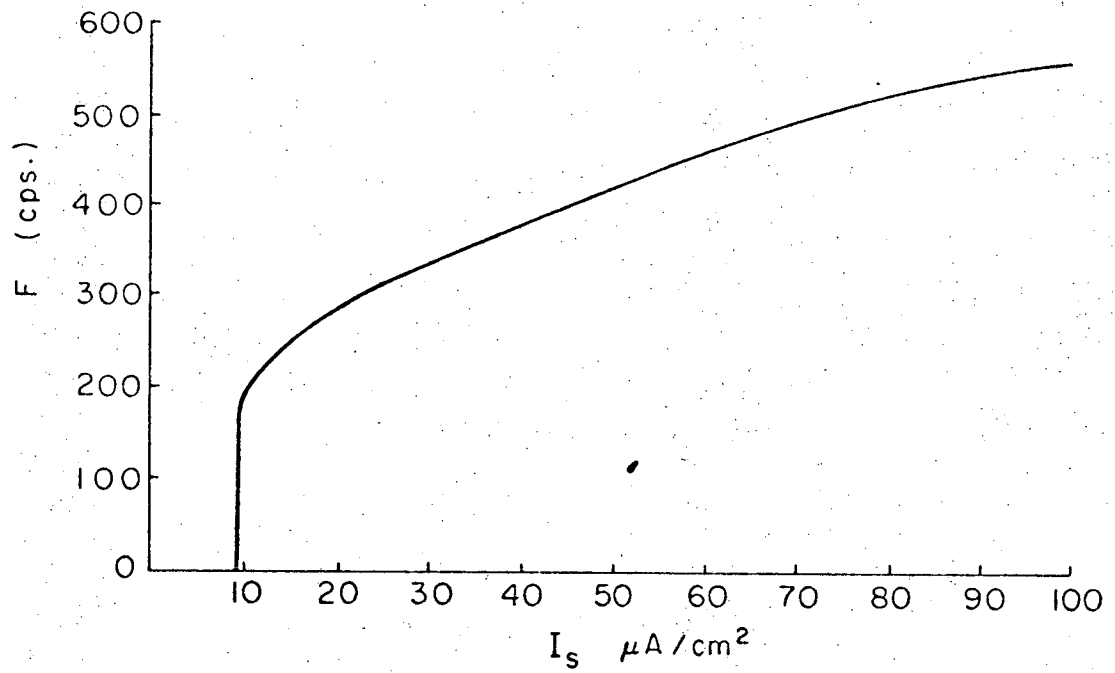
Space-clamped axon.

Temp. = 18°C.

Figure 16 - Frequency of firing with a constant current stimulation.

Space-clamped axon.

Temp. = $18^{\circ}C.$

with our theoretical model neither does the H-H model predict such behaviour.
.We can introduce a modification in this model to explain such facts. It seems
that the after effects of the action potential can produce these finite trains
of impulses.

G. After Effects

The only after effect present in the theoretical axon is the rapid-
ly disappearing low amplitude oscillation following the spike. Among the
experimentally observed after effects is the negative after potential and
the decrease in positive overshoot of the spike during repetitive responses.
Frankenhaeuser and Hodgkin (1956) investigated these phenomena and conclu-
ded that they were caused by the accummulation of K ions near the outside
surface of the axon, thereby modifying the value of V_K and the resting
potential.

The space for this diffusion process around the axon was calcu-
lated to be around 300$\overset{o}{A}$ thick. They derived an equation which gives the
time variation of the potassium concentration in that space. That function
will modify the value of V_K and of the resting potential. It will also
modify the potassium conductances which depend on concentration. The
results of Frankenhaeuser and Hodgkin (1956) can be applied to the situa-
tion of repetitive firing and explain the cessation of the firing with a
constant current step. In this model, an increase in the external K^+
concentration C_{KO} can increase the value of the potassium conductance of
the membrane g_K. About 2mM of K ions are liberated per impulse and diffuse
away quite slowly, with a time constant between 35-100 msec. When the
spikes occur every five milliseconds, almost all the K ions liberated will
remain near the membrane. Four spikes in 20 msec liberate 8 mM, and if the

time constant for diffusion is 50 msec, about 5 mM will be present near
the membrane after the last spike.

Frankenhaeuser and Hodgkin (1956) have assumed that the concen-
tration C_{KO} near the membrane was reduced following an expotential time
dependance, after each spike.

$$C_{KO} = C_{KOO}e^{-t/\tau} + C_{KOn}$$

C_{KOM} is the normal potassium concentration when the axon is at rest.
τ is the diffusion time constant, and C_{KOO} the accumulation of outside K^+
ions, released during each action potential. C_{KOO} depends on the amount
of ions, S_{KOO} liberated per cm^2 of membrane and on the thickness λ of the
space into which the ions are accumulated. $C_{KOO} = S_{KOO}/\lambda$. S_{KOO} is calcu-
lated from the potassium current for each spike. C_{KO} is continuously
introduced in the potassium conductance during repetitive firing. We have
tried many values of λ from 50Å to 300Å and many values for τ from 30 to
90 msec. Calculations were made using stimulus amplitudes from 10 to
70 $\mu A/cm^2$. We found that the relatively long time constants τ did not
influence the repetitive firing so we used the median value of 60 msec.

With a space thickness λ of 50Å we did not have any repetitive
firing. Only the first spike was obtained for all the stimulus amplitudes.
For a space thickness of 150Å, we had only one spike at 10 $\mu A/cm^2$, two
spikes at 20 $\mu A/cm^2$, three spikes for 30 and 40 $\mu A/cm^2$ and two spikes for
50-70 $\mu A/cm^2$ followed by a third one of very low amplitude. The results
are shown in Fig. 17. For larger space thicknesses we obtain longer trains
of impulses. It seems that the most interesting results are with the space
thicknesses of 50 and 150A, because they give results in good agreement
with the experiments of Hagiwara and Omura (1958).

It seems reasonable to suppose that this accumulation of K ions
is responsible for the finite train of impulses obtained when a constant
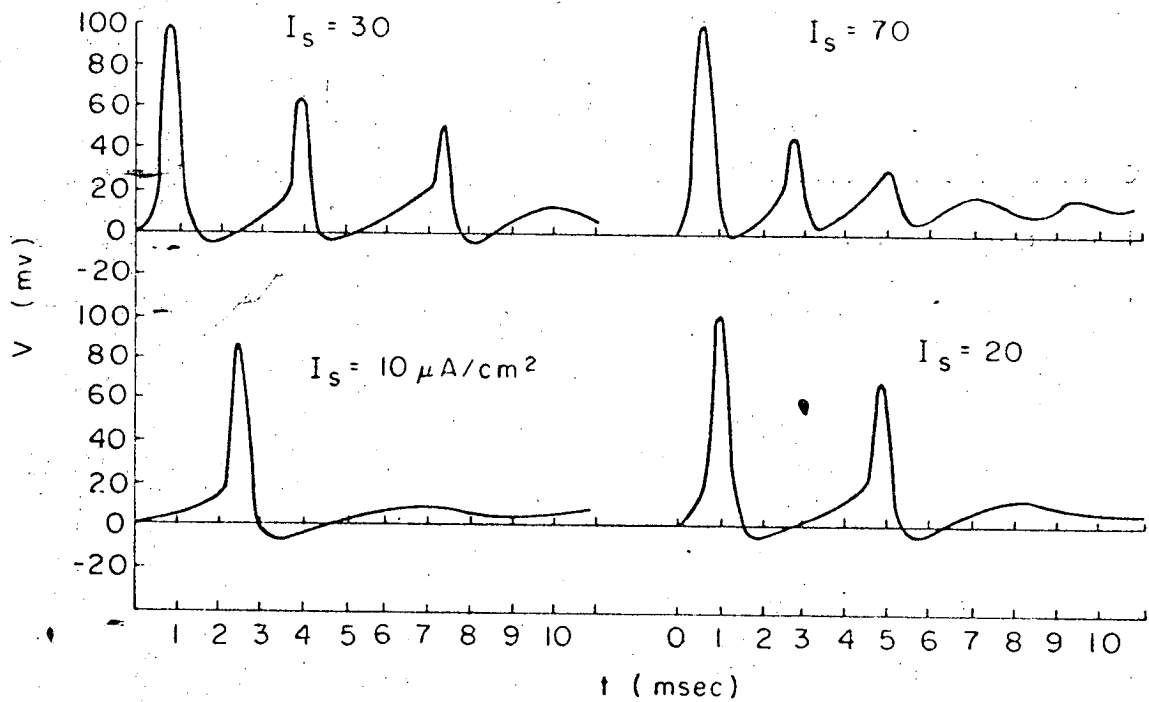
XBL687-3217

Figure 17 - Repetitive firing with K^+ diffusion space.

Constant current stimulation. Space-clamped axon.

Temp. = $18^{\circ}C$.

current is applied on a space clamped axon. But direct experiments should be made to find out if it is really the cause by measuring external potassium concentrations during and after a repetitive firing produced by a constant current on a space-clamped axon.

This high frequency of firing that is observed for a constant stimulus makes it hard to apply the model for the sensory nerve cells which usually respond with a much lower frequency to a constant stimulus. In the optic nerve of the squid, the frequency of firing recorded is 25/sec for a minimal light intensity. It increases to 75/sec when the intensity of light is increased by a factor of 10^4. These frequencies are much lower than the smallest frequency observed for a space clamped squid axon. Here again we could use the Frankenhaeuser and Hodgkin space to explain this difference. Let us use a space thickness of 50A. The K ions accumulated around the axon after an impulse, and delay the firing of the next impulse by a time long enough for the K ions to diffuse away. For a 25/sec frequency, there is 40 msec between impulses, which is about the time constant of the diffusion process of the K ions. Hagiwara and Omura (1958) found that most of their axons fired only once when stimulated with a constant current. If the K diffusion process is involved as we suppose, it should be possible to observe another impulse about 40 msec later. We can conclude that the actual model of the nerve cell can probably be applied to sensory cells. It should be possible to solve most of the questions of firing frequency and disappearance of firing by a proper knowledge of after effects. It is also necessary to know for sure if the constant stimulus on the sensory receptor is transformed into a constant current on the axon.

H. Stability of the Action Potential

An additional property of the model that we want to demonstrate
is its stability with respect to random variations of the parameters. The
value of the parameters we obtained for this model cannot be expected to be
very accurate and also not to be the same for all axons. These parameters
were determined mostly from the data on axon 17 from Hodgkin and Huxley (1952d);
if the model gives a good representation of the action potentials in many
nerve cells, a reasonable variation of the parameters should not modify it
appreciably. We calculated two space clamped action potentials, one for
20 mv and the other for 90 mv initial depolarization after having introduced
a ± 10% random variation in all the parameters. The action potentials were
very little different from the normal one. The threshold is slightly modi-
fied. Since in the living cell there are continuous fluctuations we can
expect changes to occur in the parameters and consequently a certain range
of variation of the action potential, threshold, and other properties of
the axon. The model can easily tolerate such variations.

I. Synaptic Potentials

The ionic mechanism and the conductance model have been developed
to explain the variations of potential in the axons of nerve cells following
a perturbation of the equilibrium condition. The detailed precise experi-
ments have been mostly concentrated on the axons of large nerve cells and
very few were made on the cell body and synaptic regions of the nerve cells,
using the voltage clamp method. There does not seem to be any doubt that
the change of potential observed in the synaptic regions of the nerve cells
is caused by ionic flows of sodium, potassium and chloride, down their
electrochemical gradient. Most of the experiments on synapses were made

on muscle end-plate. Takeuchi and Takeuchi (1960) made a series of experiments where they clamped the end plate at various potentials and recorded the end plate current, EPC. When the sodium or potassium concentration were modified in the external medium, the equilibrium potential for the EPC was modified but not when the chloride ions concentration was changed. There is no direct comparable evidence for the synapses of nerve cells, but introduction of monovalent anion and cations in the medium have shown that the ionic concentrations have similar effects as for the end-plate potential. Although changes in the post-synaptic potentials are certainly related to ionic currents, the conductances of the post-synaptic membranes are almost unknown.

It should be possible to use a conductivity mechanism for the soma and the dendrites that is similar to the one developed for the axon, because the cell membrane should not be too different from the soma to the axon. There is an important difference between the post synaptic responses and the axonal responses to a current stimulation: in the axon there is an all-or-none response, in the soma and dendrites the responses are continuously graded in amplitude and are propagated in the dendrites and the soma at decreasing amplitude. Lewis (1965) has suggested many possible alternations of the Hodgkin and Huxley axon to produce the observed responses of the soma and dendrites: changes in the amplitudes of the conductances or in their rate constants, or an increase in the membrane capacitance. The same arguments holds for this model. For example an increase in the membrane capacitance from 1 to 4 $\mu f/cm^2$ gives the relation between a current stimulus and the amplitude of the response shown on Fig. 18; it also eliminated the propagated action potential. Lewis (1965) had made the same modification in the H-H model and obtained similar results.

We can also show an example with a change in the rate constants.
We have for the conductances the following differential equations:

$$dg_K/dt = (g_{K\infty} - g_K)(R_{1K} g_K + R_{2K})$$

$$dg_{NA}/dt = (g_{NAO} - g_{NA})(R_{1NA} g_{NA} + R_{2NA})$$

$$dh/dt = R_h (h_\infty - h)$$

We recall that R_{2NA} and R_h had to made potential dependant in
order to have an all or none effect in the model axon. If we remove this
potential dependance on R_{2NA} and R_h, we obtain the relation between the
amplitude of the responses and the current stimulus shown on Fig. 18.

The result is similar to the one obtained by changing the mem-
brane capacitance. We see that simple changes in the axon model can
account for that property of the dendrite and soma membranes.

The dendrites and the soma also have another well known property:
they do not have a refractory period. Excitatory and inhibitory effects
add up or substract when two stimulations are closely following each other.
Additivity is demonstrated by either of the two dendritic models, as seen
in Fig. 19 but only for a small amplitude of the stimulus. When the
membrane potential increases enough to produce a complete inactivation of
the sodium conductance, then there will be a refractory period. That will
occur when the membrane is depolarized by more than 20 mv. Dendritic depo-
larizations rarely increase to that level since the axon will fire with
much lower dentritic depolarizations.

It is also possible to use the dentritic model to introduce the
effect of the transmitter substance on the post-synaptic membrane.
Lewis (1965) as introduced transmitter substance effects in the H-H model
and he has explained many of the observed properties of the synapes, like
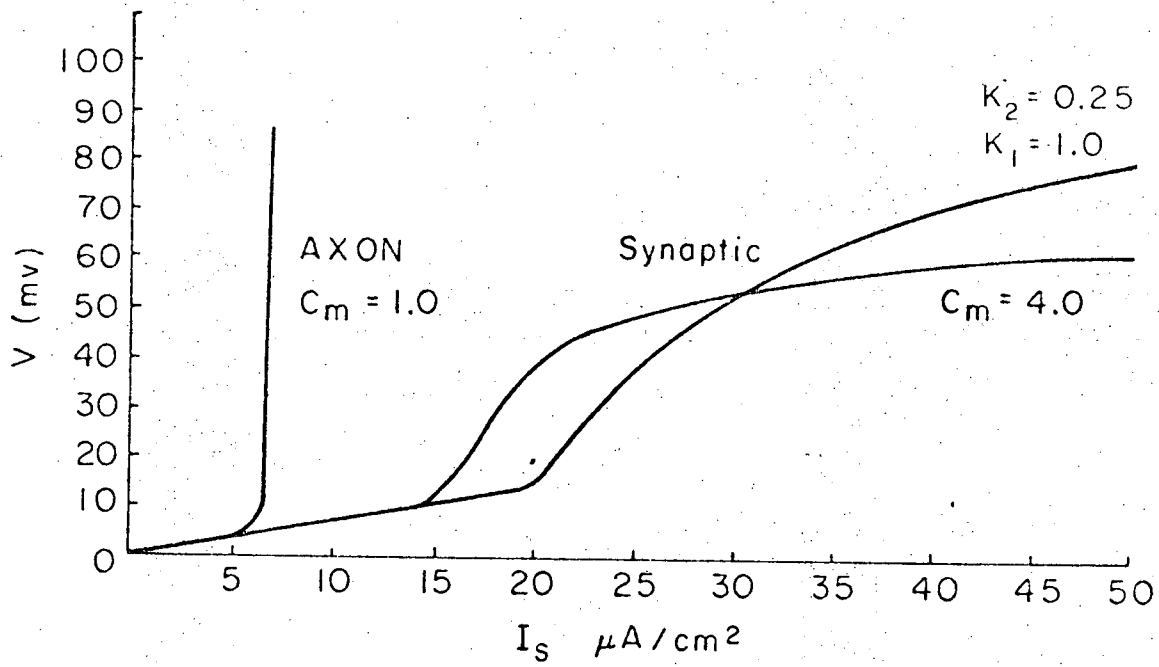
Figure 18 - Amplitude of potential relative to resting potential
Temp. = 18°C. Constant current stimulation.
Space-clamped axon.
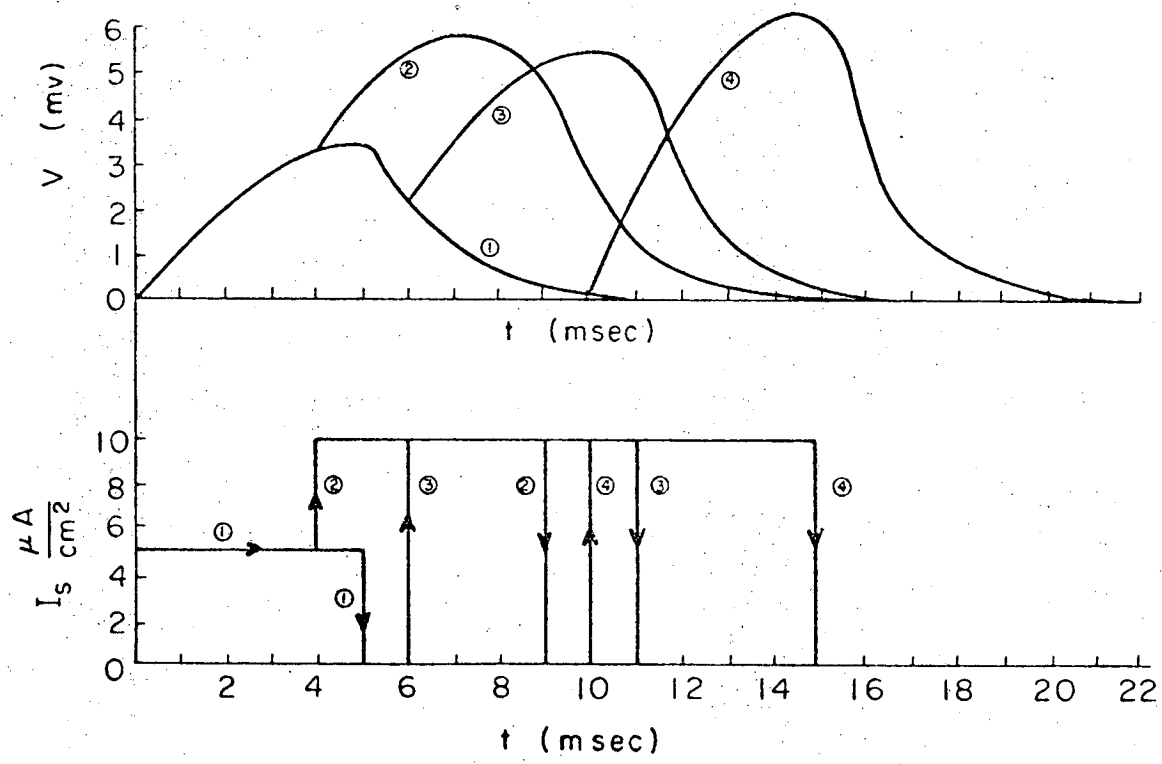
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Figure 19 - Superposition of synaptic potentials

$C_m = 4.0$ µf/cm^2 - Space-clamped axon

Temp. $= 18^\circ$C.

facillitations, anti-facillitation and no-facillitation. It is very inte-
resting to see that the axon model can be easily made to explain these
experimental facts. Here are the possible effects that the transmitter
substance can have on the post-synaptic membrane. It could modify some
parameters of the already existing membrane conductance or produce new
conducting channels in parallel with the normal ones. For example if the
transmitter substance decreases the Ca concentration near the surface or
increases theequilibrium constant for the ion protein reaction, it would
shift the conductance curves toward hyperpolarizing potentials and produce
an increase of the conductances at the resting potential. It could also
increase the value of the maximum conductance g_m. The transmitter subs-
tance can produce its own channel, by producing another voltage dependent
conductance. If the transmitter produced conductivity is different from
the normal conductance, it should be possible to separate the two and
analyse the conductance produced by the transmitter substance.

A detailed investigation with and without transmitter substance,
using the voltage clamp method has to be made in order to determine the
conductance induced by the transmitter substance. Hagiwara and Tasaki (1958)
have made experiments on the squid giant synapse, to show that it was a
chemical synapse but they do not provide a detailed investigation of the
conductance change produced by the transmitter. It is necessary that the
ionic currents are separated so that each conductance can be determined.

More experiments are needed in the field of synaptic potentials,
so that this important portion of the nerve cell might become as well known
as the properties of the axons. In large cells the voltage clamp method
appears to be an excellent way to investigate the synaptic function. We
have shown only a few examples of the possible transformations of the axon

model to obtain a dentritic model. Only detailed experiments will deter-
mine which are the needed modifications in the axon model to obtain a full
description of the conductivity behavior of the synapses.

- V -

EFFECTS OF INORGANIC ION CONCENTRATIONS

A. Calcium and Magnesium

It has been known for a long time that reducing the Ca concentration on the outside of the membrane has very important effects on the action potential. First the membrane fires repetitively and for very low concentration, and Ca free solution it does not fire at all. Frankenhaeuser and Hodgkin (1957) did a series of voltage clamp experiments to determine the effect of rediced and increased Ca in the external solution. They found that the steady-state K conductance and the amplitude of activation and inactivation of the Na conductance were given by the same curve as in the normal Ca solution except that there is a potential shift in the hyperpolarizing direction for reduced Ca and in the opposite direction for increased Ca. The amount of the shift was found to be between 10-15 mv for a five fold change of concentration. They also found a similar shift in the time dependence of the conductances. This shift of the steady state or time dependant conductance curves means that we can superpose the experimental results for different calcium concentration simply by making a horizontal translation of the coordinates axes. Huxley (1959) introduced a ΔV in the α's and β's of the H-H model to show that the H-H model was compatible with such experimental findings. The ΔV was calculated from the ratio of a new calcium concentration to the normal calcium concentration, using an exponential of ΔV. In this model, we introduce the effect of calcium by supposing that it reacts competitively with the

protein substrate:

$$z Q + S \quad SQ_z$$

$$y Ca + S \quad SCa_y$$
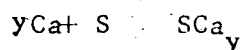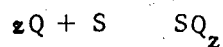
$$\frac{C_{QS}}{C_Q^z \, C_S} = K_1 \tag{5-1}$$

$$\frac{C_{CaS}}{C_{Ca}^y \, C_S} = K_{Ca} \tag{5-2}$$

$$C_{ST} = C_{QS} + C_{CaS} + C_S \tag{5-3}$$

Replacing C_S in (5-2) taken from (5-3) we have

$$C_{QS} = K_1 \, C_Q^z \, (C_{ST} - C_{QS} - C_{CaS})$$

Replacing C_{CaS}, using (5-2)

$$C_{QS} = K_1 \, C_Q^z \, (C_{ST} - C_{QS} - K_{Ca}^y \, C_S)$$

Using (5-1)

$$C_{QS} = \frac{C_{ST}}{1 + (1 + K_{Ca} C_{Ca}^y) K_1^{-1} C_Q^{-z}} \tag{5-4}$$

This revised calculation of C_{QS} will be used for C_{NaSO} and $C_{KS\infty}$

$$C_{KS\infty} = \frac{C_{KST}}{1 + (1 + K_{KCa} \, C_{Ca}^{y_K}) \, K_{IK}^{-1} \, C_K^{-z_K}} \tag{5-5}$$

$$C_{NaSO} = \frac{C_{NaST}}{1 + (1 + K_{NaCa} \, C_{Ca}^{y_{Na}}) \, K_{Na}^{-1} \, C_{Na}^{-z_{Na}}} \tag{5-6}$$

We can do a similar calculation for the inactivation process. Calcium is assumed to bind the inactivator and thereby reduce the availability of the inactivator.

$$y_h \, C_a + A_o \qquad\qquad A_o \, Ca_{yh} \qquad\qquad\qquad (5\text{-}7a)$$

$$z_{ho} \, A_o + z_{hi} \, A_i + SNa_{z_{Na}} \qquad\qquad SA_{z_h} \, Na_{z_{Na}}$$

$$C_{NaS\infty} = \cfrac{C_{NaSO}}{1 + \cfrac{K_{IA} \, C_A^{z_h}}{(1 + K_{ACa} \, C_{Ca}^{y_h})^{z_{ho}}}} \qquad\qquad (5\text{-}7)$$

We can see that equation (5-4) for C_{QS} is not much different from equation (2-8) for C_{QS}. The factor K^{-1} has been replaced by $K_1^{-1} \, (1 + K_{Ca} C_{Ca}^{y})$. When $K_{Ca} C_{Ca}^{y}$ becomes smaller than one, there will not be any more effect caused by the reduction of calcium. Gilbert and Ehrenstein (1968) made experiments with low outside calcium concentrations and found no measureable effects on the potassium conductance when C_{Ca} was reduced from 1mM to 0.1 mM. This supports our theory and permits us to evaluate $K_{KCa} \simeq 1.0$. When C_{Ca} is 10 mM or higher the factor $(1 + K_{Ca} C_{Ca}^{y})$ can be reduced to $K_{Ca} C_{Ca}^{y}$. With this approximation we can introduce the effect of calcium concentration changes by multiplying the factor K^{-1} in equation (2-8) by the ratio of the new to the normal calcium concentration $(C_{Ca}/C_{Can})^{y}$, where C_{Can} is the calcium concentration for the usual or normal external medium. We will have

$$K_K^{-1} \, (C_{Ca}/C_{Can})^{y_K} \qquad , \qquad K_{Na}^{-1} \, (C_{Ca}/C_{Can})^{y_{Na}}$$

$$K_A \, (C_{Can}/C_{Ca})^{y_h \, z_{ho}}$$

Instead of using equation (5-4), to introduce the effect of calcium, the following equation can be used

$$C_{QS} = \frac{C_{ST}}{1 + K_0^{-1}(C_{Ca}/C_{Can})^y \, C_Q^{-Z} \, e^{Z(E_W + E_D)/RT}} \tag{5-8}$$

The calcium ratio can be transformed into an exponential and introduced into $e^{Z(E_W + E_D)/RT}$ and give

$$C_{QS} = \frac{C_{ST}}{1 + K_0^{-1} C_Q^{-Z} \, e^{Z(E_W + E_D + F\Delta V)/RT}} \tag{5-9}$$

Equations (5-8) and (5-9) are equivalent if

$$\frac{C_{Ca}}{C_{Can}}^y = e^{ZF\Delta V/RT} \tag{5-10}$$

ΔV represents the voltage shift in the steady state conductance curves that has been observed by Frankenhaeuser and Hodgkin (1957). For any changes of the calcium concentration in the outside medium an equivalent ΔV can be calculated from (5-10) for the potassium conductance and the activation and inactivation of the sodium conductance.

$$(C_{Ca}/C_{Can})^{y_K} = e^{FZ_K \Delta V_K/RT} \tag{5-11}$$

$$(C_{Ca}/C_{Can})^{y_{Na}} = e^{FZ_{Na} \Delta V_{Na}/RT} \tag{5-12}$$

$$(C_{Ca}/C_{Can})^{-y_h Z_{ho}} = e^{FZ_h \Delta V_h/RT} \tag{5-13}$$

For a few calcium concentration ratios C_{Ca}/C_{Can} there is an experimental ΔV available. For example a $\Delta V = 10$ mv was found by Frankenhaeuser and Hodgkin (1957) to correspond to a 5 fold change in outside C_{Ca}. The ΔV
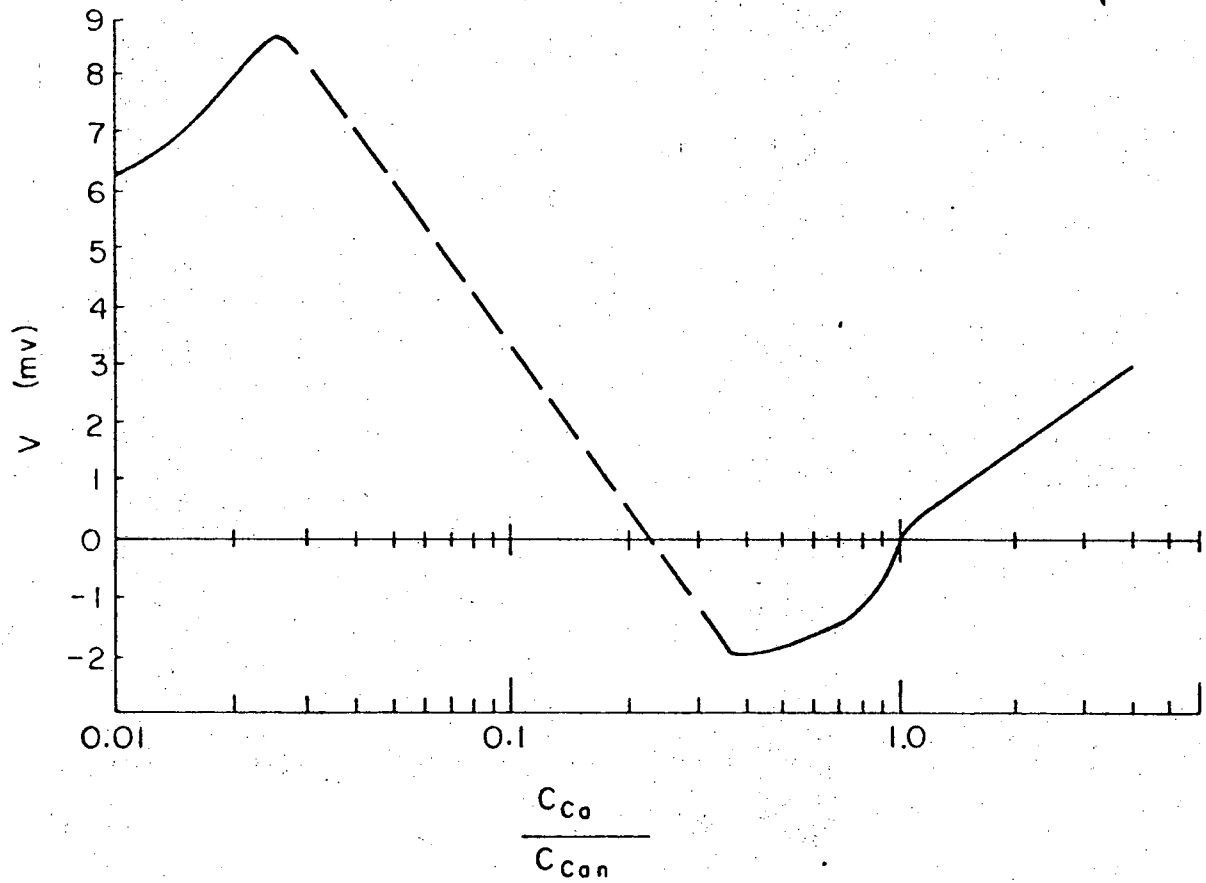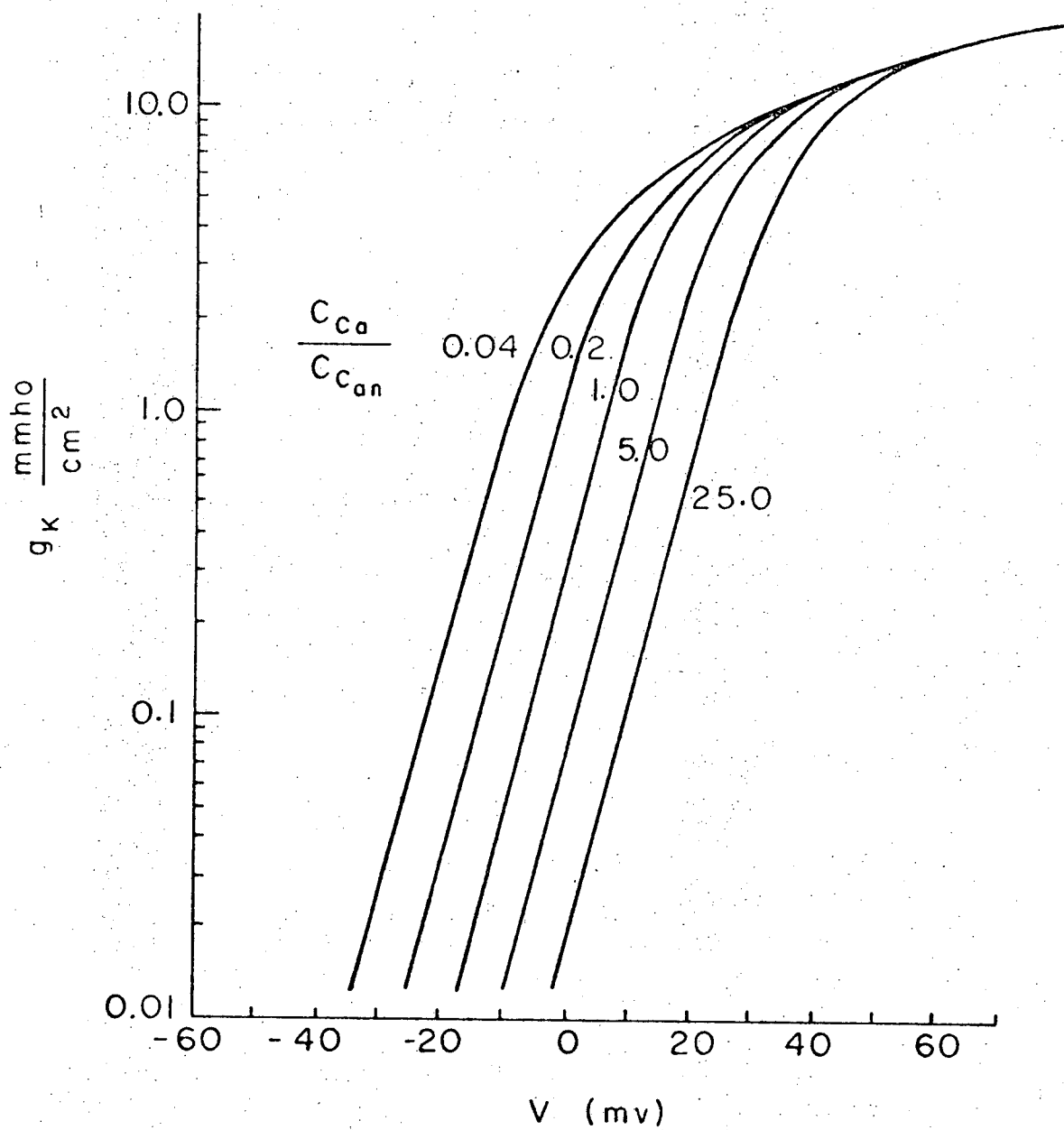
Figure 20 -   Variation of resting potential with different calcium

concentrations. Dotted Region for spontaneous firing.

Temp. $= 18^{\circ}$ C.
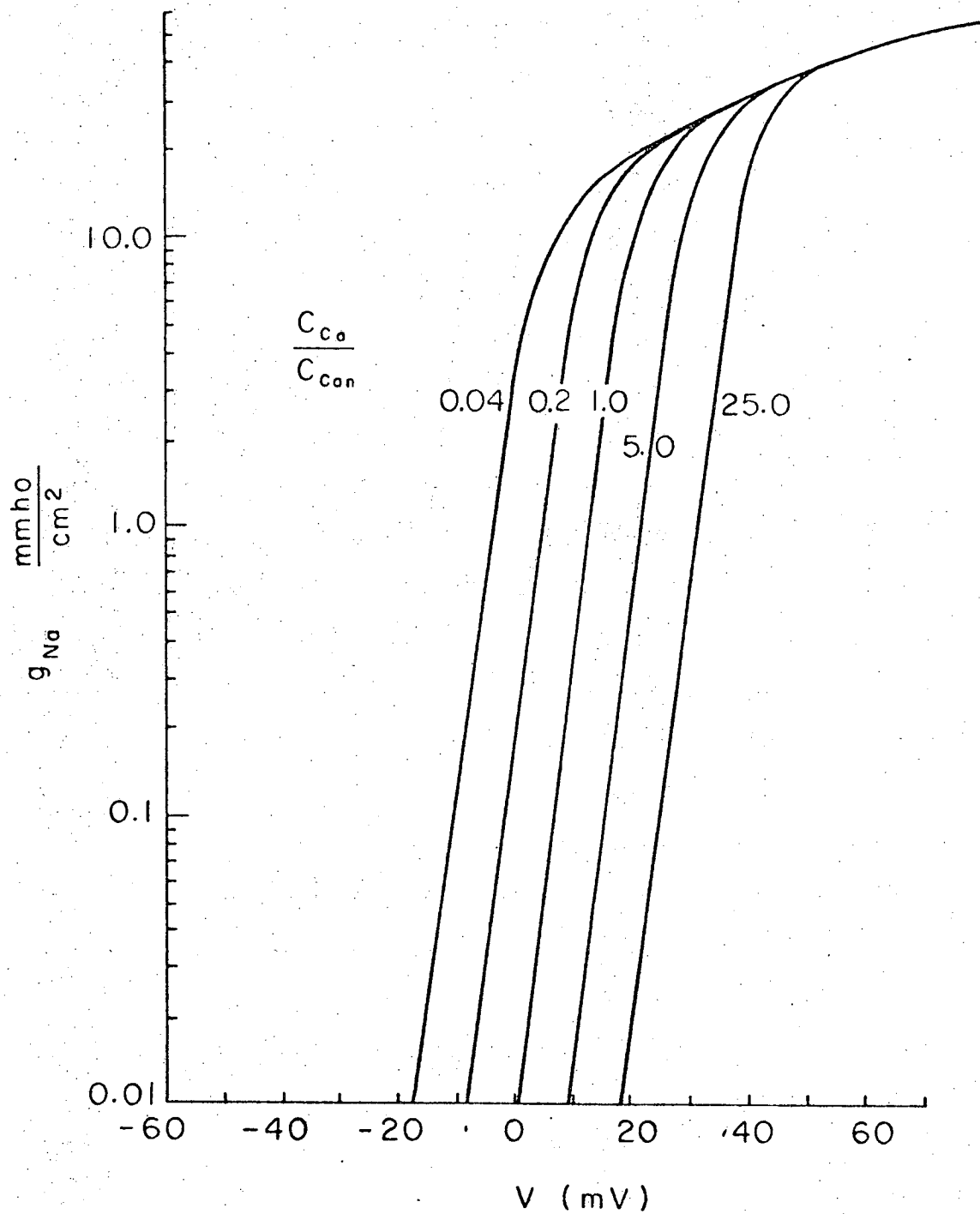
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Figure 21 a - Amplitude of sodium conductance for various calcium

concentrations. Temp = 6° C.

XBL687-3214

Figure 21 b -   Steady state potassium conductance for various calcium

concentration.   Temp = 6° C.

XBL687-3213

was the same for the potassium conductance and the activation of the sodium conductance. Introducing $C_{Ca}/C_{Can} = 5$, $\Delta V_K = 10$ mv and $\Delta V_{Na} = 10$ mv into equations (5-11) and (5-12) a value for $Y_K = 1$ and $Y_{Na} = 2$ is found. A family of $g_{K\infty}$ and g_{NaO} curves are shown in Fig. 21a and 21b for different C_{Ca}/C_{Can}. The values of Y_K and Y_{Na} means that only one Ca ion reacts with the potassium channel and two Ca ions with the sodium channel. We have found that there are four K ions in the potassium channel and eight Na ions in the sodium channel.

Having from (2-10b),

$$z_K = z_{Ko} + z_{Ki}$$

$$z_{Na} = z_{Nao} + z_{Nai}$$

we can assume that $z_{Ko} = z_{Ki} = z_K/2$ and $z_{Nao} = z_{Nai} = z_{Na}/2$.

Because the competition for the channel will be between the outside Ca ions and the outside Na ions or the outside K ions, there should be one Ca ion in the place of two Na or two K ions. And this is exactly what is found $2y_K = z_{Ko}$ and $2y_{Na} = z_{Nao}$. This justifies the assumption that $z_{Ko} = z_{Ki}$ and $z_{Nao} = z_{Nai}$.

There is also a ΔV_h in Frankenhaeuser and Hodgkin (1957), its value is not too accurately evaluated. We took $\Delta V_h = 10$ mv for a 5 fold change in C_{Ca}. This gives $y_h = 0.5$. It means, refering to (5-7a), that two atoms or molecules of the inactivator react with one Ca ion. This supports the hypothesis that the Cl ion could be the inactivator.

An important test for these values of y_K, y_{Na} an y_h is the action potentials that are obtained. Hodgkin and Keynes in Huxley (1959) show that repetitive firing occurs when the Ca concentration is reduced to a quarter

of its normal value: the frequency was one spike every 7 msec. It was still firing repetitively at $C_{Ca}/C_{Can} = 0.1$. When they removed Ca completely, the firing started to increase in frequency and decrease in amplitude until it completely disappeared. We can conclude that the repetitive firing should still be present for C_{Ca}/C_{Can} lower than 0.1. As shown of Fig. 22, all these features are present in this model and the choice of values made for y_h, y_k and y_{Na} were those which came closest to these experimental results.

The minimum frequency obtained when no initial stimulus is given is one spite per 5 msec and with a small initial stimulus we have one spike per 7 msec. The treatment for Ca ions is applicable to the Mg ions which were shown by Frankenhaeuser and Hodgkin (1957) to have effects similar to those of the Ca ions. A concentration of 50 mM Ca could replace the 11 mM Ca and 55 mM Mg present in sea water.

B. Sodium

In their experiments on the squid axon, Hodgkin and Huxley reduced the outside concentration of Na ions by replacing them with choline to show that the negative inward current was caused by the Na concentration gradient. There are a few records of their results, one for 30% and one for 10% of the usual external Na concentration. They developed a flux equation to calculate the ratio of the current I in the normal solution to the current I' in the modified solution; they called this the independence principle equation and it has been used by many authors to explain the effect of modified concentrations of different ions on the currents. But this method of handling the effects of the concentration of the ions is inconsistent with the equation developed for the ionic currents with the usual external medium. Even if the ionic medium is modified, the equation
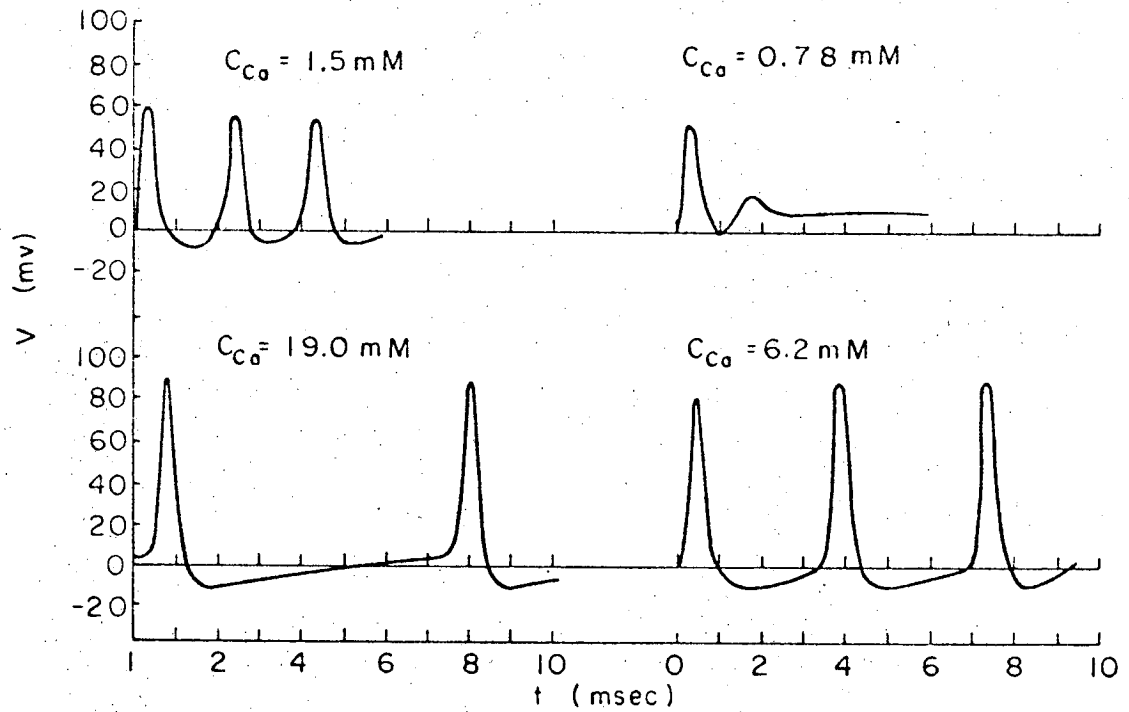
Figure 22 -   Repetitive firing with reduced external calcium

Space-clamped axon.   Temp = 18° C.

$I = g (V_m + V_C)$ should still be applicable. When the concentrations are changed the ratio of two currents I and I' is

$$\frac{I}{I'} = \frac{g (V_m + V_C)}{g' (V_m + V_C')}$$

In some cases, it is possible that $g = g'$. But in this model, we expect that $g \neq g'$, because the concentrations of ions appear in the formula for the conductances (2-17). In equation (2-30) for g_{Nao} where $C_{Na}^{z_{Na}} = C_{Nai}^{z_{Nai}} C_{Nao}^{z_{Nao}}$ and $z_{Na} = z_{Nai} + z_{Nao}$ we see that changing C_{Nao} will change g_{Nao}. Assuming again $z_{Nao} = z_{Nai} = z_{Na}/2$ we can calculate an equivalent voltage shift in the conductance in the same manner as we did for the changes in calcium concentration. Taking the ratio of the new to the usual Na concentration $(C_{Nao}/C_{Naon})^{z_{Nao}} = e^{Fz_{Na}\Delta V/RT}$ we can calculate the value of ΔV for any value of C_{Nao}. The theoretical curves have the same shape as those for the changes in calcium concentration shown on Fig.21a. For example when $C_{Nao}/C_{Naon} = 0.1$ a shift of 28 mv is calculated. The data from Hodgkin and Huxley (1952a) cannot be compared well with our curves because in their 10% Na experiment, the values of the current at low applied potentials are not sufficently accurate and for the 30% Na experiment they are not given for applied potentials smaller than 40 mv. For large values of the applied potential, the conductance becomes constant. The region of the curve where the shift will be seen is in the range of small conductance, and very accurate measurement have to be made because the current is quite small.

Variations in the inside Na concentration will also produce shifts in the conductance curve. Again experiments with high Na concentrations inside the axon are not numberous and not accurate enough. Chandler and

Meves (1965) report an I-V curve for the amplitude of the transient current
with high internal Na, but we cannot read their plot accurately enough at
low applied potential to calculate good conductance curves. But from these
few results it seems possible that we could have $z_{Nai} = z_{Nao} = 4$. Experiments
on the same axon should be performed with various inside and outside Na^+
concentration.

C. <u>Potassium</u>

The effects of modifying the potassium ion concentration on the
ionic currents has been investigated much more in the past few years than
the effect of changing the concentration of the sodium ions. Experiments
have been made on the squid axon by Moore (1959) who increased the external
potassium concentration to 400 mM; the resting potential becomes close to
zero.

With the axon in voltage-clamp, the steady state current I is
measured for different membrane potentials, V_m and a plot of I vs V_m is
obtained. It was rather unexpected to find in that plot a region of the
curves with a negative slope. As the potential V_m is increased, the current
increases and then starts to decrease as V_m keeps increasing. The current
reaches a minimum as V_m becomes larger and then goes on increasing again.
Ehrenstein and Gilbert (1966) found similar results. Lecar et al (1967)
have proposed an explanation for such results. The I-V_m plot can be sepa-
rated in two parts: a linear and a non-linear portion. The linear current
can be a leakage current. With the non-linear portion, a conductance
$g = I/V_m$ is calculated and g is plotted against V_m. The plot g-V_m is very
similar to the one obtained for g_K-V_m when the outside medium is the usual
low potassium concentration, except that the curve is shifted by a ΔV to
the left.

This finding is quite in accordance with the properties of the conductances developed in this model. We have predicted that in the case of the sodium ion a change in its concentration is supposed to produce a shift in the sodium conductances. The same thing is true for the change in the potassium ion concentration. In equation (2-21) there is a factor which depends on the concentration of potassium ions

$$C_K^{z_K} = C_{KO}^{z_{Ko}} \; C_{Ki}^{z_{Ki}}$$

It was shown in section A of this chapter that one could have $z_{Ko} = z_{Ki} = z_K/2 = 2$. Again the ratio of modified to usual potassium ion concentration C_{Ko}/C_{Kno}, where C_{Kno} is the usual K^+ concentration, can be introduced into the experimental function of V_m, to give a potential shift ΔV.

$$\left(\frac{C_{Ko}}{C_{Kno}} \right)^{z_{Ko}} = e^{Fz_K \Delta V/RT}$$

For example a ratio of

$$\frac{C_{Ko}}{C_{Kno}} = \frac{440}{10}$$

will give a $\Delta V = 47$ mv. The observed ΔV in Lecar et al (1967) is between 25-30 mv. The difference between the calculated and the observed ΔV can be explained. The value of $z_{Ko} = 2$ could be wrong. If we take $z_{Ko} = 1$, $\Delta V = 24$ mv, which is very close to the experimental ΔV. There is no direct evidence for the shift of the potassium conductance produced by the decrease in K^+ concentration inside: Chandler et al (1965) reports experiments using 300 mM and 24 mM KCl inside. They give the $I-V_m$ curve for the steady state currents, but no correction for the leakage and sodium currents is provided and we cannot measure accurately enough the data for low currents: so we cannot determine the $g_k - V$ curve to find the value of z_{Ki}.

There is also another even better explanation for the difference in the observed and the calculated ΔV when $Z_{Ko} = 2$. The observed ΔV's for changes in outside potassium concentrations are only given for two extreme of very low and very high external K^+ concentration. If the ΔV's for intermediate K^+ concentration were given, it would be easier to test the theory. At outside concentration as low as 10 mM, it is well known that the K^+ ions could be in higher concentration near the membrane surface than in the bulk solution. Hodgkin and Huxley (1952b) had found a difference of about 10 mv between the calculated and the observed potassium concentration potential V_{CK}. They proposed that it could be due to a local increase in K^+ concentration in the external medium. Later on Frankenhaeuser and Hodgkin (1956) found evidence for such a local increase. The difference between the bulk and surface K^+ concentrations will appear much more when the bulk concentration is very low. When the bulk concentration is kept a at zero, the theoretical V_{CK} should be infinite. It is impossible to have a zero K^+ concentration near the membrane because of the continuous diffusion of K^+ from the inside. When the concentration in the external medium are larger than 50 mM, the surface concentrations are much closer to bulk concentration and the calculated V_{CK} and ΔV should be much more reliable.

The experiments of Ehrenstein and Gilbert (1966) and Lecar et al (1967) have shown that the I-V_m curve in high outside K^+ concentration had a similar shape on either side of the origin. Taking only the non-linear characteristic of the I-V_m plot and plotting g-V_m, one fines that the conductance g has a maximum and decreases for either large inside positive or large inside negative membrane potentials. These results can be accounted for by this model. The dipole barrier E_D which depends on V_m will become higher as V_m is increased. It does not matter if the membrane is
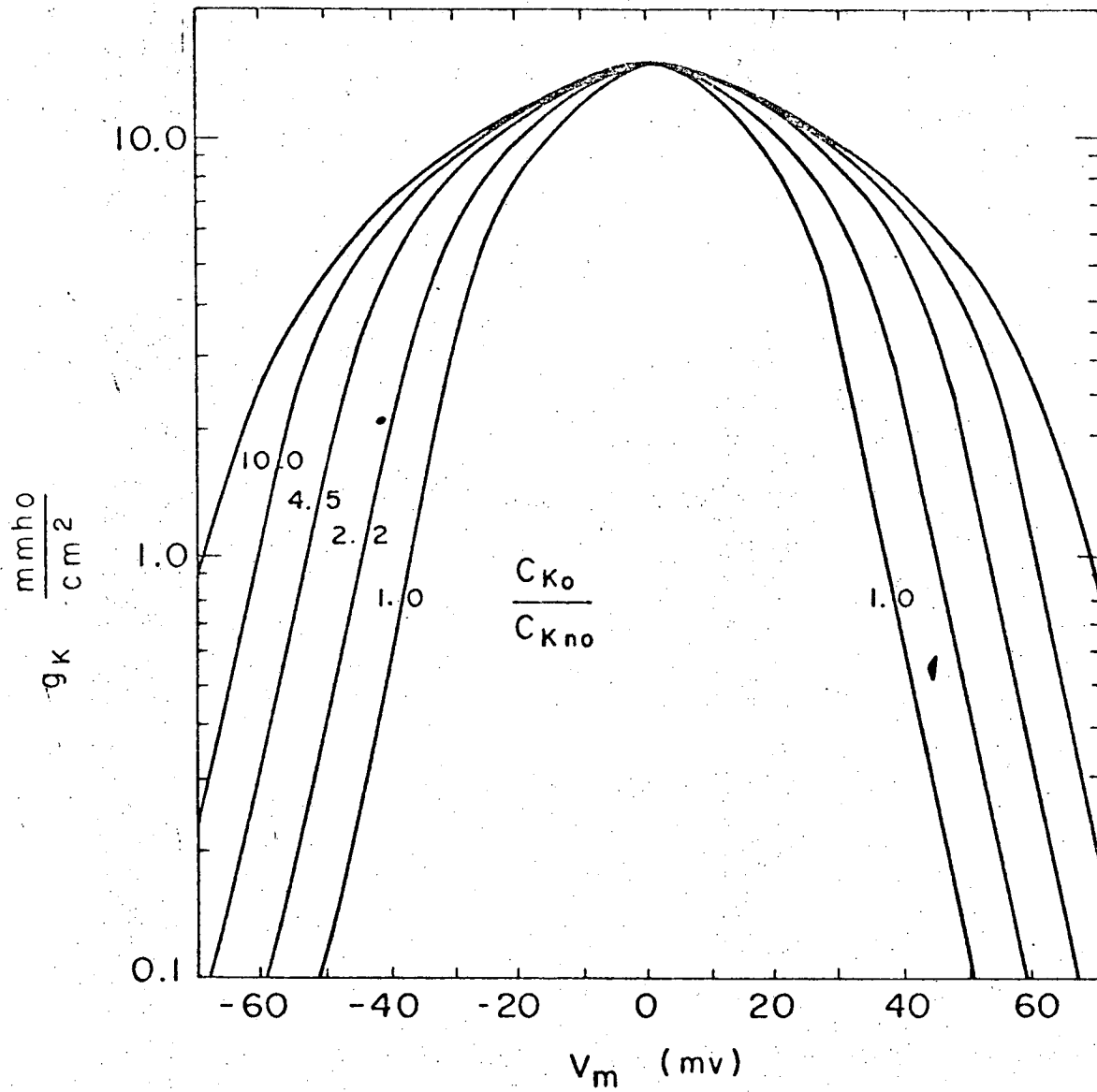
XBL687-3207

Figure 23 - Steady state potassium conductance for various potassium
concentrations - Temp = 6°C.
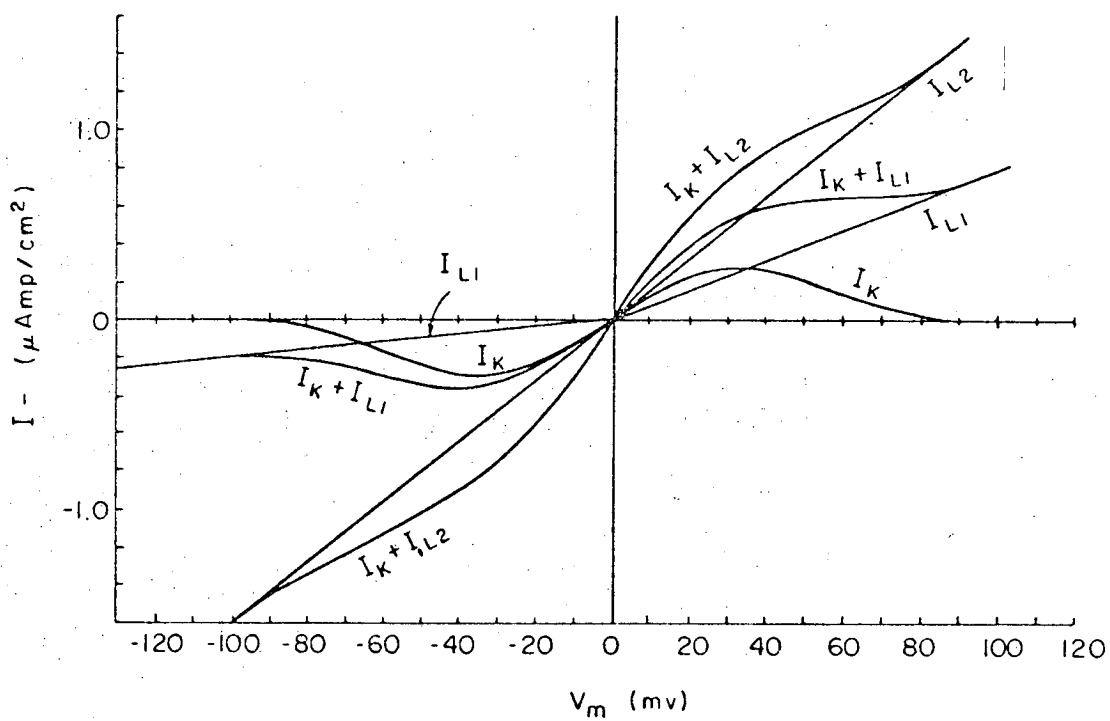
XBL 687-3211

Figure 24 - Steady state Current-Voltage - Curves for a potassium ion

concentration 10 times that of the usual concentration

charged positive inside and negative outside or vice-versa, we will always
have a layer of dipoles on each side of the membrane which will reduce the
diffusion of ions. As mentioned in Chapt. II section D, the dipoles barrier
E_D is not necessarily the same on each side of the membrane. This will bring
the result that the maximum value of g is not exactly at $V_m = 0$. There is a
family of curves for g_K vs V_m, shown on Fig. 23, for various C_{Ko}/C_{Kno}. They
were made, assuming that the conductance had its maximum at $V_m = 0$. They
are obtained by using the absolute value of V_m in equations (2-21). Using
the g_K curve on figure 23 for a $C_{Ko}/C_{Kno} = 10.0$, the potassium current, I_K,
is calculated; using the experimental linear currents I_L in Lecar et al (1967)
and adding $I_K + I_L$, a total I-V_m plot is obtained, as shown in Fig. 24.
There is a great similarity between these theoretical curves and the obser-
ved I-V_m curves. The only important difference is that the observed I_K
does not seem to be perfectly symetric with respect with the origin. The
actual theoretical I_K has been calculated for the case where the conduc-
tance g_K is perfectly symetric with the origin; this is not necessarily
a valid assumption.

D. Chlorine

We mentioned before the possibility of Cl ions being the inac-
tivator of the Na conductance. The results of Chandler and Meves (1965)
showed a shift of 20 mv in the inactivation curve for a 6 fold decrease
in the inside Cl ion concentration. Using the same method as before,
a voltage shift ΔV in the theoretical inactivation curve can be obtained

$$\frac{C_{C\ell i}}{C_{C\ell in}}^{z_{hi}} = e^{Fz_h \Delta V / RT}$$

Introducing the experimental values of $\Delta V = -20$ mv and $C_{C\ell i}/C_{C\ell in} = 1/6$

we calculate a value for $z_{hi} = 1.8$. This quite in accordance with the

assumption that

$$z_{hi} = z_{ho} = \frac{z_h}{2} = 2.$$

Unfortunately there are no experiments that show the effects of varying

external Cl ion concentrations on inactivation. Adelman and Taylor (1964)

replaced NaCl by sucrose in the external medium and they found a decrease

in steady state current curve with potential. They interpreted that as

a change in the potassium current. Even if such a correction is made

there still remains a difference between the normal and low Cl solution

curves. It could be accounted for partially if the sodium current is

increased because of reduced inactivation. But more direct experiments are

needed on this point. This model would predict a shift of 20 mv in the

inactivation curve for a 6 fold reduction in outside Cl ions concentra-

tion. Complete removal of Cl on both sides of the membrane should provide

evidence for or against Cl ions being the inactivator.

E. <u>Drugs and Other Compounds</u>

We introduce the effects of drugs in this section because we

believe most of their effects can be explained in the same way as the

effects of ionic concentrations. A wide variety of components have been

used to modify the action potential and the I-V curves in voltage clamp.

Most of them have been shown to modify either the potassium conductance

or the sodium conductance or the inactivation, or any combination of

these three processes. They could modify the sodium "on" and the sodium

"off" conductances and not the potassium conductance, or only the sodium

"on" and the potassium conductances, or only the potassium and the sodium

"off" conductances. Also they could influence them all. It is realized

that the action of each drug should be studied separately in detail.
It is possible that some of the drugs can compete with ions for the
protein substrate: in that case their action will be similar with that
of calcium. Their action can be introduced in the conductance equation
(2-17) with the factor $(1 + K_D \ C_D{}^y D)$ in the same way as the calcium
effect was introduced earlier in equation (5-4). We would have

$$g = \frac{g_m}{(1 + e^{(E_o + FV_m)/RT}) \ (1 + K_1^{-1} (1 + K_D \ C_D{}^yD) \ C_Q{}^{-Z})}$$

C_D = concentration of drug

y_D = number of molecules of the drug in the complex

K_D = equilibrium constant for the reaction with the membrane
substrate.

In the case of inactivation, the drugs might prevent inactiva-
tion by binding the inactivator. That will shift the inactivation curve.
Also they could act on the NaS complex: if they inactivate the complex,
the competition is cooperative, if not, the competition is inhibitory.
In both cases there will be a shift in one direction or the other.

Of course the drugs will influence the time dependence of the
conductance because they modify the steady state amplitudes which are
part of the time constants, except for inactivation. They could also
have some direct effect on the rate constants of the chemical reaction.

The transmitter substance can be considered as a drug having
an increasing effect on the conductances. It could shift the conduc-
tance upward and leftward and can also change the rate constants. Its
action could be to increase the equilibrium constants of the reaction.

Recent experiments by Blaustein (1968) have shown that the

effect of barbiturates and tropine esters is a shift of the Na conductance curves horizontally to the right by about 10 mv, and also a vertical down shift for Na and K conductances. The author mentioned that the effect was similar to the one produced by calcium. Also Narahashi and Haas (1968) have shown that DDT shifts the Na conductance curve by about 10 mv. We can mention again the experiments with TTX and TEA which selectively block the Na and K channels respectively. Their action is the same as the other drugs: they shift the conductance curve to the right. We know that the conductance curves are bell shaped. A shift to the right reduces also the maximum, which will become zero when the shift is large anough. In most cases, the drugs should produce a right and down shift. The effects of the drugs in this model still needs more development and closer comparison with the experimental results.

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CONCLUSION

A. Summary

The purpose of this work is to explain in terms of physical principles the behavior of nerve cells. The most detailed and reliable experiments made on nerve cells to elucidate their mechanism are those made with the voltage clamp method. The functioning of nerve cells can be explained in terms of ionic currents across their membrane. A model is being developed to explain the measured ionic currents.

1. The basic hypothesis of this model is that there are conducting channels in the membrane. There is a chemical reaction between some membrane components and several ions. Vacancy formation and diffusion in the ionic complex results in conduction. The fundamental equation for the membrane conductance is given by equation (2-17)

$$g_\infty = \frac{g_m}{(1 + e^{(E_o + FV_m)/RT})(1 + K_o^{-1} C_Q^{-z} e^{z(E_o + FV_m)/RT})}$$

This part of the model can be called the "conducting channel" (C-C) hypothesis.

2. A second hypothesis is introduced to describe experimentally observed dynamics of the conductances. It is assumed that the reaction between ions and membrane substrates is an autocatalytic reaction. The general result that describes the time dependance of the conductances is given by equations (3-4)

$$dg/dt = (g_\infty - g)(R_1 g + R_2)$$

The complete model can be called the "autocatalytic-conducting-channel" (A-C-C) model.

3. Such a model is used to be compared with the experimental data of Hodgkin and Huxley (1952). The unknown constants are determined by curve fitting. Using the cable equation, stationary and propagating action potentials are generated.

4. The model accounts for various properties of axons like threshold, refractory period, accommodation, impedance change, strength-duration characteristic, repetitive firing and hyperpolarization. It is also shown that the model for the axon can be used with some modifications for the dendrites and soma of nerve cells.

5. Many recent experiments have been performed on squid axons, changing the external and internal medium of the axons. The observed effects produced by changing calcium, sodium, potassium and chloride ions have been explained satisfactorily by the C-C model. Even some quantitative aspects of the effect of drugs on the axon can be approximated.

6. In the course of the development of the A-C-C model, we have suggested the need for some crucial experiments to demonstrate move fully the adequacy of the model. Obviously the most important test would be to identify directly the presence of specific ion binding membrane components. By using an irreversible and specific inhibitor of one of the channels, it might be possible to chemically separate and thereby identify from the membrane the inhibitor substrate components. Using the isolated component it should be possible to determine its static and dynamic properties. There has been many experiments recently on allosteric properties of enzymes and conformational changes of proteins.

The hypotheses of the A-C-C model can be considered a particular case of configuration change in proteins and research along this line will certainly yield very interesting results.

The demonstration that there is a potential dependent barrier on each surface of the membrane is also very important for the C-C model; its existence should be demonstrated experimentally. More experiments using only a single type of ion on both side of the axonal membrane are also needed, to show if the conductance equations are correct, approximately correct, or incorrect.

More experiments on dendrites and soma of large cells with the voltage clamp methods should be made to determine the membrane conductances of these important parts of nerve cells, and see if the axon model can be applied to them.

There are physical and chemical methods that could help in solving the problem of living membranes. The use of artificial membranes is getting more widespread and will undoubtedly yield further interesting results. There is also valuable research to be done on the physical ionic conductivity properties of pure organic crystals, the properties of liquid crystals, and the properties of solutes in high concentration. Work in these fields will yield fundamental results for all areas of cell biology.

B. Discussion

We have tried to keep the equations as simple as possible, and with the least number of parameters. In the normal physiological state of the axon model when all ionic concentrations are normal, the number of parameters might even be less than in the H-H model. We have fitted the

steady state amplitudes of the conductances with only three free parameters

for g_K and five for g_{Na}. Next the parameters in the kinetics equation were

fitted with the steady state parameters fixed. There are two parameters

for the potassium conductance-time curves and three for the sodium conduc-

tance-time curves, for potentials larger than 18 mv. For lower potentials,

three more parameters are needed for the configurational protein change.

Although the total number of parameters is not small, it must be remembered

that they are not all free altogether. They are adjusted a few at a time

on different experimental results.

When variations of ionic concentrations are introduced, then the

lumped parameters are split and that increases the total number of para-

meters. It must be realized that all the numerous experimental details

available cannot be explained with a very simple model. If we only want

to reproduce the shape of the action potential, it can be done with simple

mathematics and very few parameters, but not to explain all the detailed

experimental results on nerve cells. It is already quite surprising that

so many features of nerve cells can be reproduced from a model which was

derived only from the voltage clamp data. For example it was rather a happy

surprise to us when it was found that the kinetic equations (3-11) and

(3-13) could explain without any modification (not even the value of the

parameters) the experimental observations of Cole and Moore (1960) on

the hyperpolarization of the squid axon in voltage clamp. There is still

much data available on axons (squids and others) that we have not yet

tried to explain with the A-C-C model; it will be done later. We have

chosen only one type of cell-the squid axon - to develop a quantitative

model. It is quite possible that in other types of nerve cells, some

differences in the mechanism of conductance will become apparent. However

it is hoped that the basic assumptions of the A-C-C model may explain a
wide range of membrane phenomena.

The most severe criticism against membrane models is that the
hypotheses very often cannot be demonstrated. Any membrane model will come
under such a criticism because the membrane is such a thin structure that
measurements on it are limited. The development of different models,
based on different hypotheses will be usefull because it will be possible
to choose which one describes the nerve membrane most accurately. The
model that has the widest and most accurate range of applications stands
better chances of being correct. We must not forget that the purpose of
a model is to put together as many experimental results as possible and
describe them with as few hypotheses as possible. A model permits us to
predict what experimental results will be obtained given the initial con-
ditions, and hopefully point the way to crucial experiments that can verify
or reject the model. The physical sciences have made tremendous progress
in a short time because of their extensive use of quantitative models;
in physics, theory and experiments are continously progressing together.
The biological sciences will have to use a similar procedure if they are
to improve our knowledge of life.

Our purpose was to develop a model that could give quantitative
agreement with experimental data. Since our knowledge of the behavior of
membranes, their physico-chemical properties and their kinetics, is in-
complete, arbitrary assumptions had to be made. The model is not a com-
pletely detailed molecular picture without arbitrary parameters; it is not
either a totally empirical model without molecular considerations. It
stands in between these two extremes, hoping to be a fruitful step toward
a more satisfactory molecular model.

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LIST OF SYMBOLS

a	=	distance between ions sites
A_i, A_o	=	inactivator of sodium channel
b	=	axon radius
C	=	concentration
C_m	=	membrane capacitance per unit of membrane surface
C_{ST}	=	total concentration of S
C_{SQX} or C_{SQ}	=	concentration of ionic complex
d	=	membrane thickness
ΔF	=	free energy change
ΔV	=	potential shift of steady state equations
E_D	=	membrane surface dipole potential
E_D^o	=	membrane surface dipole potential at $V_m = 0$
E_{ja}	=	activation energy for chemical reactions
E_0	=	$E_D^o + E_W$
E_W	=	membrane interface energy change
F	=	Faraday's constant
g	=	membrane conductance per unit of membrane surface
g_∞	=	steady-state value of g
h	=	inactivation factor
i	=	subscript for inside of axon
I_m	=	total membrane current density
I_o	=	rheobase current
I_R	=	resistive current density
I_s	=	current stimulus
K	=	equilibrium constant
λ	=	space constant
μ	=	mobility
0	=	subscript for outside of axon

ν = vibration frequency of atoms in a lattice

φ = electrochemical potential

Ψ = electrical potential

Q = general symbol for an ion

Q_o = constant quantity for threshold

Q_{10} = temperature factor

r = axoplasm resistance per unit length

ρ = axoplasm resistivity

R = gas constant

$R_i (i=1,2,3..)$ = rate constants

R_m = membrane resistance per unit of membrane surface

S = membrane molecules

θ = speed of propagation of action potential

τ_o = Q_o/I_o

T = absolute temperature

V_c = concentration or Nernst potential

V_m = membrane potential

x = distance perpendiculer to the membrane surface

X = ion dissociated from the membrane molecules

Z = number of ions reacting with one S

Z_v = valence