A COMPUTER EVALUATION OF EQUATIONS FOR PREDICTING THE POTENTIAL ACROSS BIOLOGICAL MEMBRANES

STEVEN A. ROSENBERG

From the Biophysical Laboratory, Harvard University, Cambridge, Massachusetts 02138

ABSTRACT The Goldman, Henderson, and Planck junction potential equations can be used to describe the potential across the resting giant squid axon. These equations are used to calculate the relative Na, K, and Cl permeabilities of the squid axon using the experimental measurements of Hodgkin and Katz. The equations all provide excellent agreement with the observed data and yield similar permeability values.

The junction potential is the potential existing at the boundary between two solutions due to the different mobilities of the oppositely charged ions in the solutions. Three equations have been theoretically formulated to describe the magnitude of this junction potential between two solutions. These are the equations formulated by Planck in 1890 (1), Henderson in 1907 (2), and Goldman in 1943 (3). Widely different assumptions about the nature of the junction between the solutions are made in deriving the three equations. In 1949 Hodgkin and Katz used the equation formulated by Goldman to describe the potential across the membrane of the resting giant squid axon (4). The Goldman equation was very successful in fitting this experimental data and since then has been widely applied in studies of a variety of membrane systems.

Probably because of the success of the Goldman equation and the greater complexity of the equations of Planck and Henderson few attempts have been made to test whether the latter two equations can be used to describe the potential across a biological membrane. We have used a digital computer to study the application of these equations to a biological membrane system.

THEORY

The general differential equation for the junction potential, E, across a membrane is given by,

$$E = -\frac{RT}{F}\int_x \sum_i \frac{t_i}{z_i}\, d\ln a_i \qquad (1)$$

where t_i, z_i, a_i are the transference number, valence, and activity of the ith ion respectively, x is the width of the membrane, and R, T, F have their usual values. To solve this equation for the potential, E, by performing the necessary integration across the width of the membrane it is necessary to make certain assumptions about the physical properties of the membrane and the ions within it. The details of the integrations by Goldman, Henderson, and Planck may be found in the original papers (1–3) or in most standard textbooks of electrochemistry (5).

Henderson assumed that the membrane consisted of a continuous series of solution layers, each layer produced by mixing the two solutions on either side of the membrane. Thus if C_i and m_i are the concentration and mobility of the ith ion and "in" and "out" refer to the two sides of the membrane we result with,

$$E = \frac{RT}{F} \frac{(u_1 - v_1) - (u_2 - v_2)}{(u_1 + v_1) - (u_2 + v_2)} \ln \frac{(u_1 + v_1)}{(u_2 + v_2)} \tag{2}$$

in which,

$$u_1 = \sum_i C_i^{+\text{in}} m_i^{\text{in}} \qquad \text{(inside cations)}$$

$$v_1 = \sum_i C_i^{-\text{in}} m_i^{\text{in}} \qquad \text{(inside anions)}$$

$$u_2 = \sum_i C_i^{+\text{out}} m_i^{\text{out}} \qquad \text{(outside cations)}$$

$$v_2 = \sum_i C_i^{-\text{out}} m_i^{\text{out}}. \qquad \text{(outside anions)}$$

Goldman assumed the membrane was homogenous with a constant electric field across it. The potential is then given by,

$$E = \frac{RT}{F} \ln \frac{\sum_i C_i^{+\text{in}} m_i + \sum_i C_i^{-\text{out}} m_i}{\sum_i C_i^{+\text{out}} m_i + \sum_i C_i^{-\text{in}} m_i}. \tag{3}$$

Planck assumed that diffusion of ions occurred in an area of "constrained diffusion" until a stationary state was reached. This integration led to,

$$\frac{\xi u_2 - u_1}{v_2 - \xi v_1} = \frac{\ln \frac{C_{\text{out}}}{C_{\text{in}}} - \ln \xi}{\ln \frac{C_{\text{out}}}{C_{\text{in}}} + \ln \xi} \cdot \frac{\xi C_{\text{out}} - C_{\text{in}}}{C_{\text{out}} - \xi C_{\text{in}}} \tag{4}$$

in which C_{out} and C_{in} refer to the total ionic concentrations on either side of the membrane and ξ is defined by,

$$E = \frac{RT}{F} \ln \xi. \tag{5}$$

In all three equations it is implicitly assumed that the activities of the ions are equal to their concentrations, the ions are all univalent, the two boundary solutions are infinite and well stirred, and that the mobilities of the ions are independent of concentration. The Gibbs-Donnan equilibrium was not considered in the original discussion of these equations and is not included in our calculations. Though it does effect the results obtained, the presence of relatively high concentrations of salt on both sides of the membrane probably make this effect a small one.

RESULTS AND DISCUSSION

To evaluate the applicability of these three equations to describing a biological membrane we have used the original data of Hodgkin and Katz on the giant squid axon (4). In these experiments the potential across the axonal membrane was measured when the axon was bathed in nine solutions of varying Na, K, and Cl ion concentrations. Since the ion concentrations within the nerve are measurable only the relative mobilities (permeabilities) of the ions are unknown. These mobilities may be determined by selecting those values for which each of the above equations gives the best fit with the experimental measurements. This method was used by Hodgkin and Katz to demonstrate the applicability of the Goldman equation. They found, by trial and error, a pair of relative permeabilities for which all nine calculated potential measurements agreed closely with the experimentally determined potentials.

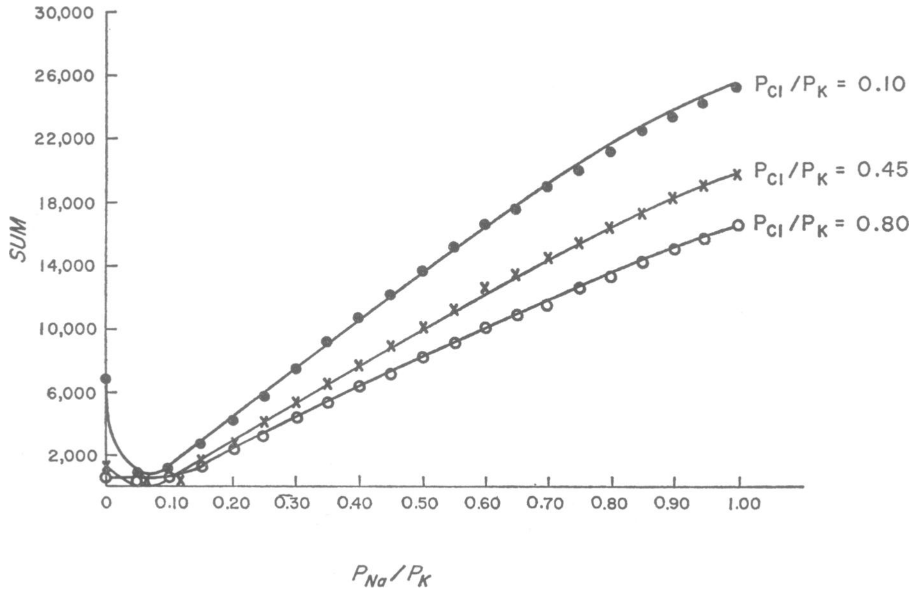

FIGURE 1 Least-square fit of P_{Na}/P_K at three different values of P_{Cl}/P_K. An illustration of the curves generated as the computer calculates the least-squares SUM for varying values of P_{Na}/P_K and P_{Cl}/P_K in the Goldman equation.

Though Hodgkin and Katz used a trial and error method to find the two relative permeabilities that best fit the Goldman equation it is possible to calculate these two values analytically. The method which is outlined in the Appendix involves minimizing the least-squares sum by taking its partial derivative with respect to each of the unknown parameters. This procedure is very lengthy and tedious and it is easier for the computer to perform a random least-squares search over a wide range of relative permeabilities to find those values of the permeability constants that, when used in the Goldman and Henderson equations, provide the best over-all fit to all nine experimental measurements. To evaluate each pair of permeability values the measured potential, E_{m_i}, and the calculated potential, E_{c_i}, for each test solution, i, were used to calculate,

$$\text{SUM} = \sum_{i=1}^{9} (E_{m_i} - E_{c_i})^2. \tag{6}$$

SUM thus serves as a least-squares measure of correlation of the measured to the calculated values of the potential.

Hodgkin and Katz used microelectrodes filled with sea water and corrected their measured potential values for the liquid junction potential between the microelectrode solution and the axoplasm (4). They used 11 mv for the microelectrode junction potential correction because this value provided the best fit of the Goldman equation to their measurements. Other authors have used different values (6) and in fact it is theoretically not possible to measure this correction exactly. To calculate

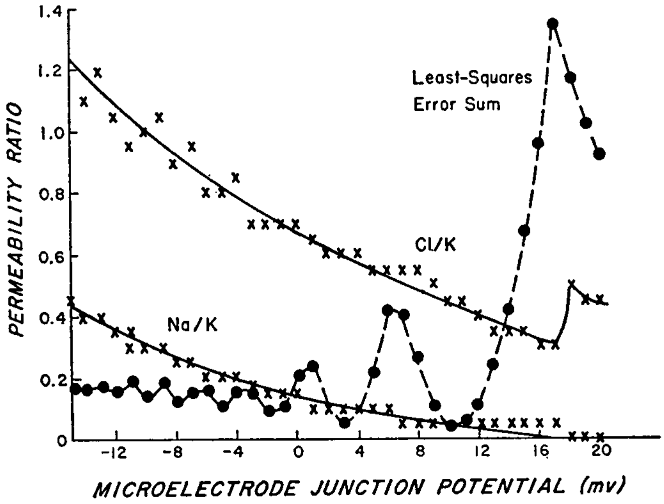

FIGURE 2 Fit of Goldman equation to Hodgkin and Katz data. For each microelectrode potential correction the values of the relative ion permeabilities that best fit the Goldman equation are plotted. In addition the least-squares SUM is plotted to show the correction between the observed and measured values of the potential for each set of permeability values.

the liquid junction potential exactly we need to know the exact single ion activities involved which, as has often been pointed out, is not possible (5).

Fit of Goldman and Henderson Equations

We have calculated the best permeability values for both the Goldman and Henderson equations for microelectrode potential corrections varying from −15 to 20 mv. For each correction value a unique set of permeabilities exists that best fits each equation to the experimental data. Fig. 1 shows an example of the kind of curves generated using the Goldman equation to find one permeabilty value as the other is varied. The calculated results using the Goldman and Henderson treatment are given in Figs. 2 and 3.

As can be seen, permeabilities of the ions can be found that provide an excellent fit (low SUM) of the experimental data to both the Goldman and the Henderson equations. If the microelectrode junction potential is zero the potential predicted by both equations is equally good, differing from the measured values by less than an average of 2 mv per measurement. For the case of the Goldman equation this

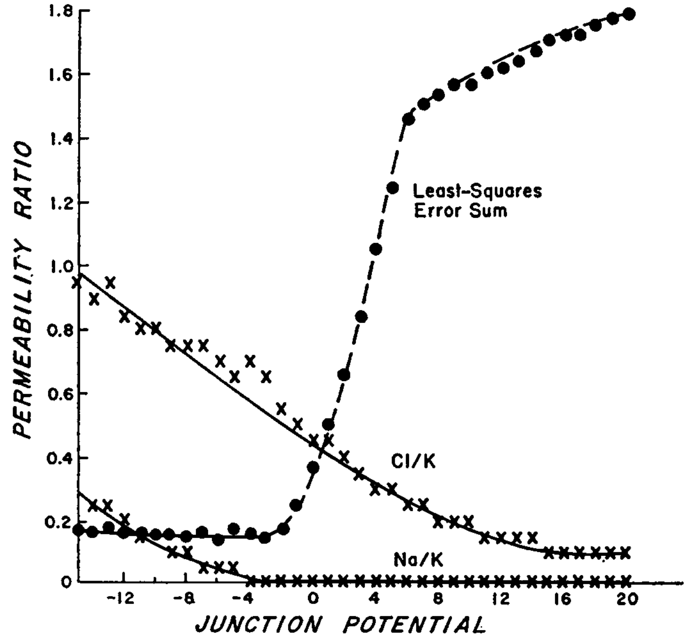

FIGURE 3 Fit of Henderson equation to Hodgkin and Katz data. For each microelectrode potential correction the values of the relative ion permeabilities that best fit the Henderson equation are plotted. In addition the least-squares SUM is plotted to show the correction between the observed and measured values of the potential for each set of permeability values.

corresponds to relative permeabilities for K, Na, and Cl of 1:0.15:0.70. Permeabilities selected by the Henderson equation are 1:0:0.45.

For negative microelectrode corrections the Goldman and Henderson equations fit the measured data equally well and yield similar permeabilities. At −11 mv the Goldman equation yields permeabilities of 1:0.15:0.80. At high positive correction values (e.g. 11 mv) the Henderson equation does not fit the data as well as the Goldman equation (the former averages about 7–8 mv and the latter only 1–2 mv difference between calculated and observed potentials). The permeabilities predicted by both however are similar; the Goldman equation yields 1:0.05:0.45 and the Henderson equation 1:0:0.15.

Fit of Planck Equation

The Planck equation can most easily be solved for the potentials across the squid axon by a graphical technique, though Schlögl (7) has recently described a method which makes possible an approximate integration of a slightly modified form of the Planck equation. From Equation 4 we set,

$$\eta_1 = \frac{\xi u_2 - u_1}{v_2 - \xi v_1} \tag{7}$$

$$\eta_2 = \frac{\ln \dfrac{C_{\text{out}}}{C_{\text{in}}} - \ln \xi}{\ln \dfrac{C_{\text{out}}}{C_{\text{in}}} + \ln \xi} \cdot \frac{\xi C_{\text{out}} - C_{\text{in}}}{C_{\text{out}} - \xi C_{\text{in}}} \tag{8}$$

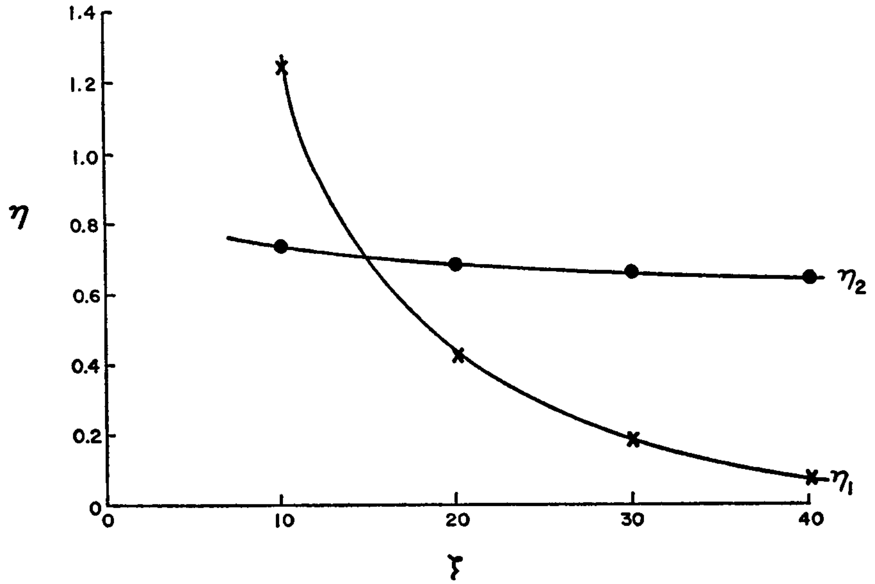

Figure 4 Fit of Planck equation to Hodgkin and Katz data. An example of the graph obtained in the graphical solution of the Planck equation.

TABLE I

COMPARISON OF MEASURED POTENTIAL AND THE POTENTIAL PREDICTED BY THE PLANCK EQUATION

Test solution	Calculated* E	Measured E JP‡ = 11 mv
	mv	*mv*
A	69	64
B	—	57
C	—	55
D	58	60
E	62	61
F	64	61
G	64	62
H	61	59
I	57	59

* Calculated values of the potential for each test solution using the Planck equation are compared to the potentials measured by Hodgkin and Katz. Note that for test solutions B and C ξ_1 and ξ_2 did not intersect at any value of η though a 10,000-fold range of η was tried. The correctness of the computer programs in this instance was verified by checking the programs against earlier calculations with the Planck equation by Cummings and Gilchrist on synthetic solution boundaries (13).

‡ JP: junction potential.

η_1 and η_2 are plotted vs. ξ and the ξ defined by their point of intersection is used to solve for the potential using Equation 5. Using the Planck equation with relative permeabilities of K:Na:Cl of 1:0.05:0.45 the calculated values of the potential for seven of the nine test solutions were very similar to those measured by Hodgkin and Katz (microelectrode correction of 11 mv). See Fig. 4 and Table I.

The choice of axoplasmic ion concentrations used is an important parameter in fitting the measured data to these equations. Hodgkin and Katz used the data of Steinbach et al. (8, 9) which give K inside = 345 mM, Na inside = 72 mM, and Cl inside = 61 mM. These values were used in the calculations that have been presented.

Using glass electrodes, internal ion concentrations have since been more accurately determined. The Hodgkin and Katz measurements were thus recalculated using the revised ion activities measured by Hinke (10) and Koechlin (11). The results of attempts to fit the data to the Goldman equation are presented in Table II. This data fits as well as that previously used (SUM = 77 as opposed to 41 for earlier data) though the permeability ratios determined are slightly different.

It has thus been shown that the Goldman, Henderson, and Planck equations can all be used to predict the potential across the giant squid axon and that the permeability ratio giving the best fit with all three equations are similar to one another.

TABLE II

EFFECT OF INTERNAL ION CONCENTRATION ON FITTING THE DATA OF HODGKIN AND KATZ TO THE GOLDMAN EQUATION

Source	K	Na	Cl	Best $\frac{P_{Na}}{P_K}$	Best $\frac{P_{Cl}}{P_K}$	SUM
	mM	*mM*	*mM*			
Steinbach et al. (6, 7)	345	72	61	0.15	0.70	41
Hinke (8) and Koechlin (9)	203	37	109	0.05	0.02	77

Bennett (12) has found that the predictions of the Planck and Goldman equations yield similar results in predicting the squid axon resting potential.

The Goldman equation is the simplest to calculate and its general acceptance and wide use in biological systems are thus probably justified. It is indeed surprising that the three equations all fit the experimental data as well as they do since the assumptions used in deriving the equations are very different and are all very rough approximations of the true biological state.

APPENDIX

Solution for best values of permeability ratios that fit the Goldman equation to the Hodgkin-Katz data. The Goldman equation is:

$$E = \frac{RT}{F} \ln \left[\frac{\mathrm{K_{in}} + \mathrm{P_{Na}/P_K\ Na_{in}} + \mathrm{P_{Cl}/P_K\ Cl_{out}}}{\mathrm{K_{out}} + \mathrm{P_{Na}/P_K\ Na_{out}} + \mathrm{P_{Cl}/P_K\ Cl_{in}}}\right].$$

Let

$$A = \frac{RT}{F}$$

$$C_1 = \mathrm{P_{Na}/P_K}$$

$$C_2 = \mathrm{P_{Cl}/P_K}.$$

Thus for each potential measurement, E_i, at the corresponding ion concentrations outside the axon:

$$E_i = A \ln \left[\frac{\mathrm{K_{in}} + C_1\,\mathrm{Na_{in}} + C_2\,\mathrm{Cl_{out_i}}}{\mathrm{K_{out_i}} + C_1\,\mathrm{Na_{out_i}} + C_2\,\mathrm{Cl_{in}}}\right].$$

Let E_{oi} be the observed potential for the ith set of concentrations. Then:

$$E_{oi} - E_i = E_{oi} - A \ln \beta$$

where

$$\beta = \left[\frac{\mathrm{K_{in}} + C_1\,\mathrm{Na_{in}} + C_2\,\mathrm{Cl_{out_i}}}{\mathrm{K_{out_i}} + C_1\,\mathrm{Na_{out_i}} + C_2\,\mathrm{Cl_{in}}}\right].$$

Then:

$$\begin{aligned}\sum(E_{oi} - E_i)^2 &= \sum(E_{oi} - A \ln \beta)^2\\ &= \sum(E_{oi}^2 - 2A \ln \beta + A^2(\ln \beta)^2)\\ &= \sum E_{oi}^2 - \sum 2A \ln \beta + \sum A^2(\ln \beta)^2.\end{aligned}$$

To find the C_1 and C_2 that minimizes $\sum (E_{oi} - E_i)^2$ we take the derivative with respect to C_1 and C_2 :

$$\frac{\delta\sum(E_{oi} - E_i)^2}{\delta C_1} = -\frac{\delta(\sum 2A \ln \beta)}{\delta C_1} + \frac{\delta\sum A^2(\ln \beta)^2}{\delta C_1}$$

$$\begin{aligned}\frac{-\delta\sum 2A \ln \beta}{\delta C_1} = &-2A \sum\left(\frac{\mathrm{Na_{in}}}{\mathrm{K_{in}} + C_1\,\mathrm{Na_{in}} + C_2\,\mathrm{Cl_{out_\mathit{i}}}}\right)\\ &- 2A \sum\left(\frac{\mathrm{Na_{out_\mathit{i}}}}{\mathrm{K_{out_\mathit{i}}} + C_1\,\mathrm{Na_{out_\mathit{i}}} + C_2\,\mathrm{Cl_{in}}}\right)\end{aligned}$$

$$\begin{aligned}\frac{\delta\sum A^2(\ln \beta)^2}{\delta C_1} = 2A^2\sum \ln \beta \Bigg(&\frac{\mathrm{Na_{in}}}{\mathrm{K_{in}} + C_1\,\mathrm{Na_{in}} + C_2\,\mathrm{Cl_{out_\mathit{i}}}}\\ &+ \frac{\mathrm{Na_{out}}}{\mathrm{K_{out_\mathit{i}}} + C_1\,\mathrm{Na_{out_\mathit{i}}} + C_2\,\mathrm{Cl_{in}}}\Bigg).\end{aligned}$$

To find the minimum with respect to C_1 we set the derivative $= 0$. Let

$$\alpha = \mathrm{K_{in}} + C_1\,\mathrm{Na_{in}} + C_2\,\mathrm{Cl_{out_\mathit{i}}}$$

$$\gamma = \mathrm{K_{out_\mathit{i}}} + C_1\,\mathrm{Na_{out_\mathit{i}}} + C_2\,\mathrm{Cl_{in}}$$

then:

$$0 = -2A \sum_i\left(\frac{\mathrm{Na_{in}}}{\alpha} + \frac{\mathrm{Na_{out_\mathit{i}}}}{\gamma}\right) + 2A^2 \sum_i \ln \beta \left(\frac{\mathrm{Na_{in}}}{\alpha} + \frac{\mathrm{Na_{out}}}{\gamma}\right).$$

Similarly for C_2:

$$0 = -2A \sum_i\left(\frac{\mathrm{Cl_{out_\mathit{i}}}}{\alpha} + \frac{\mathrm{Cl_{in}}}{\gamma}\right) + 2A^2 \sum_i \ln \beta \left(\frac{\mathrm{Cl_{out_\mathit{i}}}}{\alpha} + \frac{\mathrm{Cl_{in}}}{\gamma}\right).$$

We thus have two equations in two unknowns and can solve for C_1 and C_2.

I am very grateful to Dr. A. K. Solomon for his valuable suggestions and advice throughout the present work. Also helpful were discussions with Dr. J. Diamond, Dr. H. Meves, and Mr. B. Bunow.

This project was supported in part by the Atomic Energy Commission. The author is a Special Fellow of the United States Public Health Service, Award 1-F3-GM-23,453-01.

Received for publication 23 October 1968.

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