

LETTERS TO THE EDITOR

[Brief letters to the Editor that make specific scientific reference to papers published previously in *THE JOURNAL OF GENERAL PHYSIOLOGY* are invited. Receipt of such letters will be acknowledged, and those containing pertinent scientific comments and scientific criticisms will be published.]

Use of a Fixed Charge Model to Determine the pK of the Negative Sites on the External Membrane Surface

Dear Sir:

Hille (1968) has measured the effect of the external pH on the sodium conductance of a frog node. He fit the data for the magnitude of the sodium conductance vs. pH very well with the dissociation curve of a weak acid for a pK_a of 5.2. His data for the voltage shift of the sodium conductance vs. pH were simply fit empirically. We have recently presented a surface charge model to explain voltage shifts of potassium conductance when the external divalent cation concentration is varied in a squid axon (Gilbert and Ehrenstein, 1969 *a*) and we have now applied this model to Hille's voltage shift data (Gilbert and Ehrenstein, 1969 *b*).

In essence, the surface charge model explains the shift of the conductance-voltage curve along the voltage axis as the result of an additional component of electric field inside the membrane. This electric field is produced by the double layer consisting of the fixed negative surface charge and neutralizing cations in solution. The concentration of divalent cations (or hydrogen ions) and the binding constant of the surface charge groups determine how much of the surface charge is neutralized. This, in turn, determines the magnitude of the field caused by the double layer and the resulting shift of conductance along the voltage axis.

If the concentration of the binding cation does not appreciably affect the ionic strength, then the surface charge model can be used to determine the average separation between fixed charges as a function of three parameters: the valence of the binding cation, the ionic strength of the solution, and the maximum slope of the graph of voltage shift vs. logarithm of binding cation concentration. Fig. 1 shows this relationship for solutions in which there is a uni-univalent electrolyte solution to which small amounts of the binding cation are added. Hille's data give a maximum slope of about 10 mv/e-fold change in hydrogen ion concentration for conditions corresponding to case I of Fig. 1. This corresponds to an average spacing between fixed charges of about 15 Å. The average spacing will also be determined below in the process of determining the binding constant.

The following equation was used to determine the equilibrium constant for the binding process, and is the same as equation (11) of Gilbert and Ehrenstein (1969 *a*),

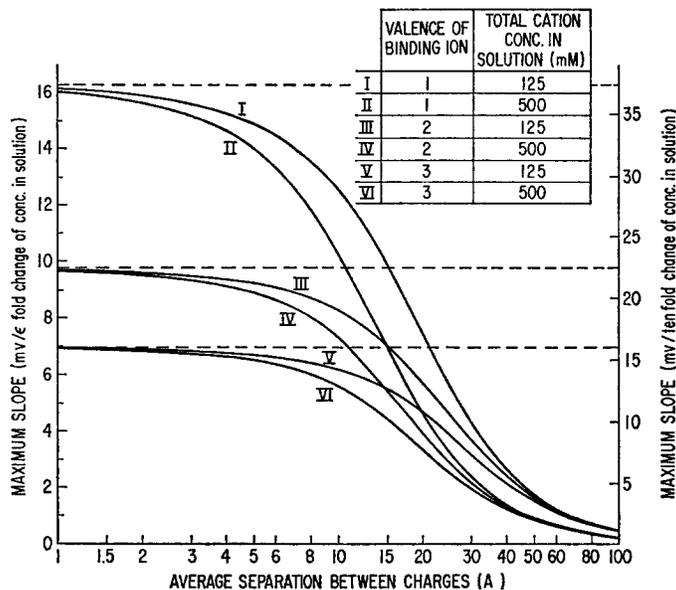


FIGURE 1. Dependence of maximum rate of change of voltage shift on average fixed charge separation.

except that here the valence of the binding cation is not specified:

$$d_i^2 [kM e^{zH(B-V_{1/2})} + 1] = \frac{G}{\left\{ \sum_{i=1}^n C_i (e^{z_i H(B-V_{1/2})} - 1) \right\}^{1/2}} \quad (1)$$

where

- | | |
|---|---|
| d_i = average spacing between fixed charges on membrane surface | $V_{1/2}$ = electrical potential at midpoint of conductance-voltage curve |
| k = equilibrium constant for binding to membrane | B = arbitrary constant determining reference for potential shifts |
| M = concentration of binding cation in the bulk solution | n = number of ionic species |
| z = valence of binding cation | C_i = concentration in bulk solution of ionic species i |
| | z_i = valence of ionic species i |

The parameter H is related to the temperature:

$$H = \frac{F}{RT} \quad (2)$$

where F = Faraday constant

R = gas constant

T = absolute temperature

The parameter G is a function of the dielectric constant and temperature:

$$G = \left(\frac{F}{N} \right) \left(\frac{2\pi}{RTDD_o} \right)^{1/2} \quad (3)$$

where N = Avogadro's number

D = dielectric constant of water

D_o = permittivity of free space

When hydrogen ions are bound to the negative sites, the pK of the negative sites equals the common logarithm of k , if the units of k are in reciprocal moles per liter.

For the curve-fitting procedure, it was assumed that the temperature was 8°C, the dielectric constant was 84.88, univalent cation concentration was 116 mM, univalent anion concentration was 120 mM, and divalent cation concentration was 2 mM. The best fit of equation (1) occurs for an average spacing between fixed surface charges of 15 Å, a pK of 4.6, and a value of B , the arbitrary parameter, equal to 55 mv. Equation (1) with these parameters is plotted together with Hille's experimental data in Fig. 2.

The agreement between theory and experiment provides further confirmation of the presence of negative surface charge on nerve membranes. The average spacing between fixed surface charges of 15 Å agrees with the value determined from Fig. 1 (which is independent of binding constant) and is close to the value of 11 Å we found for squid axons.

The pK of 4.6 equals the pH at the membrane surface when half the sites are bound. The apparent pK is the bulk pH for this condition and is equal to 5.3.

It might be argued that changing the external pH might affect the internal surface of the membrane. However, Spyropoulos (1960) has shown that this effect is small, especially for short periods of time.

Addition of the binding ion should decrease the surface charge and thus decrease the membrane cation concentration, resulting in a decreased sodium or potassium conductance. We previously found that the magnitude of the potassium conductance was decreased by calcium ions for squid nerve.

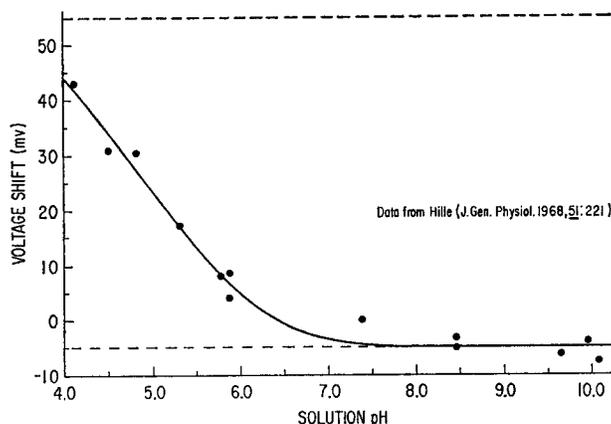


FIGURE 2. Effect of pH on voltage shift of m_{∞} .

A magnitude change has also been observed for the sodium conductance of the frog node, with an apparent pK of about 5.2 (Hille, 1968; Drouin and The, 1969). This value is in good agreement with the apparent pK of 5.3 calculated from the measurement of voltage shift.

The pK value for a free carboxyl group is 4.8 (Edsall and Wyman, 1958), which is very close to the calculated pK at the membrane surface of 4.6. This is consistent with the idea that a carboxyl group on a fatty acid chain is responsible for the surface charge.

Received for publication 16 January 1970.

DANIEL L. GILBERT
GERALD EHRENSTEIN
Laboratory of Biophysics
National Institute of Neurological
Diseases and Stroke
National Institutes of Health
Bethesda, Maryland 20014

REFERENCES

- DROUIN, H., and R. THE. 1969. The effect of reducing extracellular pH on the membrane currents of the Ranvier node. *Arch. gesamte Physiol. Menschen Tiere (Pfluegers)*. **313**:80.
- EDSALL, J. T., and J. WYMAN. 1958. *Biophysical Chemistry*. Academic Press, Inc., New York. **1**.
- GILBERT, D. L., and G. EHRENSTEIN. 1969 *a*. Effect of divalent cations on potassium conductance on squid axons: Determination of surface charge. *Biophys. J.* **9**:447.
- GILBERT, D., and G. EHRENSTEIN. 1969 *b*. A model for the determination of the pK for negative sites located on the external surface of the nerve membrane. Abstracts of the 3rd International Union for Pure and Applied Biophysics. Cambridge, Mass. **82**.
- HILLE, B. 1968. Charges and potentials at the nerve surface. Divalent ions and pH. *J. Gen. Physiol.* **51**:221.
- SPYROPOULOS, C. S. 1960. Cytoplasmic pH of nerve fibres. *J. Neurochem.* **5**:185.