NONLINEAR ELECTRICAL EFFECTS IN LIPID BILAYER MEMBRANES

III. THE DISSOCIATION FIELD EFFECT

B. NEUMCKE, D. WALZ, *and* P. LÄUGER

From Fachbereich Biologie, Universität Konstanz, 7750 Konstanz, Germany

ABSTRACT In the course of an analysis of nonlinear electrical effects in lipid bilayer membranes, the influence of the dissociation field (or Wien) effect on the membrane conductivity is investigated. It is shown that the theory of Onsager for the Wien effect in a macroscopic phase can be applied to a thin membrane when the proper boundary conditions at the membrane-solution interface are introduced. It is assumed that an activation energy is associated with the passage of the ion across the interface. The mathematical treatment of the model is restricted to the case for which cations and anions have identical properties except for the charge sign. The resulting differential equations for the ion concentration within the membrane are integrated numerically. The analysis shows that the influence of the Wien effect on the membrane conductivity is appreciable only if the energy barrier at the interface is sufficiently high, i.e. if the rate limiting step for the ion transport is the passage of the ion across the interface.

INTRODUCTION

In the third part of this series (Walz, Bamberg, and Läuger, 1969; Neumcke and Läuger, 1969), we investigate the possible influence of the dissociation field (or Wien) effect on the nonlinear current-voltage characteristic of lipid bilayer membranes. The interior of these membranes which is formed by the hydrocarbon tails of the lipid molecules is a medium of low dielectric constant. In such a medium ion association becomes predominant, i.e. most ions are present in the form of neutral ion pairs which cannot contribute to the electric current. The equilibrium between ion pairs and free ions is described by a dissociation constant which is a function of the electric field. In high electric fields (such as are present in a bilayer membrane under the usual conditions of a current-voltage experiment) the dissociation of ion pairs is substantially increased. In this way the number of charge carriers and hence the conductivity becomes a function of the applied voltage. This effect was first investigated by Wien, and is now well established for solutions of electrolytes in organic solvents (for detailed references, see Harned and Owen, 1958).

The dissociation field effect was already envisaged by Miyamoto and Thompson

(1967) as a possible source of the nonlinear current-voltage characteristic of thin lipid membranes, but without a quantitative analysis. An interesting application to biological membranes was proposed by Bass and Moore (1968) who developed a model of the nervous excitation in which a change in the electric field produces a pH change in the nerve membrane via the Wien effect.

The mathematical analysis of the dissociation field effect is due to Onsager (1934) and is based on Bjerrum's treatment of ion association (1926). Onsager developed his theory for a macroscopic phase; when the theory is applied to a thin lipid film interposed between two aqueous solutions, certain boundary conditions at the film-solution interface enter into the calculation. Unfortunately, these boundary conditions lead to a considerable complication in the numerical treatment of the problem. It seemed, therefore, advisable to restrict the analysis to a special situation, namely the case in which all permeable ions have identical properties, except for the charge sign. The analysis of this case makes it possible to define the conditions under which the membrane conductivity is influenced by the Wien effect. The main result will be that only when the passage of the ions across the solution-membrane interphase is restricted by sufficiently high activation energy barriers, will it be possible for the dissociation field effect to make a sizable contribution to the conductivity of the membrane.

THEORY OF THE DISSOCIATION FIELD EFFECT IN A THIN MEMBRANE

As in parts I and II of this series, we consider the membrane as a thin film of thickness d and dielectric constant ϵ_m interposed between two aqueous phases of identical composition. The aqueous phases contain the ions A^+ and B^- which have a very low but finite solubility in the membrane. In addition to the free ions, neutral ion pairs A^+B^- exist in the membrane. In the absence of an external voltage, an equilibrium

$$A^+ + B^- \underset{k_D}{\overset{k_R}{\rightleftarrows}} A^+B^- \tag{1}$$

is set up in the membrane which may be described by an equilibrium constant

$$K = \frac{k_D}{k_R} = \frac{\bar{c}_+ \cdot \bar{c}_-}{\bar{c}_\pm}. \tag{2}$$

($\bar{c}_+$, $\bar{c}_-$, $\bar{c}_\pm$ = equilibrium concentrations of cations, anions, and ion pairs respectively, in the membrane).

If an electric current flows through the membrane, the ion concentrations are no longer given by the equilibrium constant K. Instead, we have to replace equation 2 by the equation of continuity which states that for each ion species the divergence of the flux is equal to the rate of production of the ion. If Φ_+ and Φ_- are the ion fluxes, and

x is the coordinate normal to the membrane surfaces, then

$$\frac{d\Phi_+}{dx} = k_D \cdot c_\pm - k_R \cdot c_+ \cdot c_- \tag{3}$$

$$\frac{d\Phi_-}{dx} = k_D \cdot c_\pm - k_R \cdot c_+ \cdot c_-, \tag{4}$$

where c_+, c_-, and $c_\pm$ denote the concentrations in the nonequilibrium state. Onsager (1934) has shown that the dissociation rate constant k_D depends on the electric field strength, whereas the rate constant for the recombination, k_R, is independent of the field. For an electrolyte of valency type A^+B^- he obtained the result (E = field strength, e_0 = elementary charge, k = Boltzmann constant, T = absolute temperature):

$$\frac{k_D(E)}{k_D(0)} = \frac{I_1(2\sqrt{2q\,|E|\,e_0/kT})}{\sqrt{2q\,|E|\,e_0/kT}}. \tag{5}$$

I_1 denotes the modified Bessel function of the first kind. q is the so-called Bjerrum length, i.e., the distance at which the mutual electrostatic energy of the ions A^+ and B^- is equal to $-2kT$:

$$q = \frac{e_0^2}{2\epsilon_m kT}. \tag{6}$$

In Bjerrum's theory every pair of ions A^+, B^- whose distance r is less than q is considered as associated, giving no contribution to the conductance. If we assume the dielectric constant of the lipid film to be $\epsilon_m = 2$, the Bjerrum length q becomes 140 A at 25°C, a value about two times the membrane thickness. One may therefore question whether Onsager's theory of the Wien effect is applicable to the bilayer membrane. However, as Onsager pointed out, most ion pairs have a distance r much smaller than q, so that in the formal development of the theory the integration could usually be restricted to the region $r \ll q$. It seems therefore justified to apply Onsager's results to the lipid membrane even if the membrane thickness is of the same order as q.

In part I of this series, it was shown that the electric field in a lipid bilayer membrane is practically independent of position, as a consequence of the extremely low ion concentration in the membrane. We may therefore introduce into equation 5 the relation

$$\frac{E \cdot e_0}{kT} \approx \frac{u}{d}, \tag{7}$$

in which u is the external voltage (in units of kT/e_0) and d is the membrane thickness. The dissociation rate constant becomes thus a function of voltage alone (independent of position):

$$\frac{k_D(E)}{k_D(0)} \equiv f(u). \tag{8}$$

We now introduce a further approximation by the assumption that the concentration of ion pairs is much higher than the concentration of free ions. This is always true in a medium of low dielectric constant such as the interior of a lipid membrane. Therefore the concentration $c_\pm$ of ion pairs remains practically constant and equal to the equilibrium concentration $\bar{c}_\pm$ even in the case $u \neq 0$. As the ions A^+ and B^- are assumed to be identical except for the charge sign, the equilibrium concentrations $\bar{c}_+$ and $\bar{c}_-$ become equal:

$$\bar{c}_+ = \bar{c}_- \equiv c_0. \tag{9}$$

Using equations 2 and 8, we obtain the relation

$$k_D \cdot c_\pm - k_R \cdot c_+ \cdot c_- = k_R \cdot c_0^2 \cdot \left[f(u) - \frac{c_+ \cdot c_-}{c_0^2} \right], \tag{10}$$

which has to be introduced into equations 3 and 4.

In the stationary state the ion fluxes Φ_+ and Φ_- in the membrane are given by the Nernst-Planck equations

$$\Phi_+ = -D \cdot \left(\frac{dc_+}{dx} + c_+ \cdot \frac{u}{d} \right) \tag{11}$$

$$\Phi_- = -D \cdot \left(\frac{dc_-}{dx} - c_- \cdot \frac{u}{d} \right). \tag{12}$$

D is the diffusion coefficient which is assumed to be the same for both ions. In the case of a thin dielectric film, the Nernst-Planck equations have to be considered as an approximation, as long as the image force acting on the ions is not taken into account (see part II of this series). However, this complication is omitted here.

We finally introduce the dimensionless variables

$$\xi = \frac{2x}{d} \tag{13}$$

$$y = \frac{c_+ + c_-}{2c_0} \tag{14}$$

$$z = \frac{c_+ - c_-}{2c_0}. \tag{15}$$

Then, by addition of equations 3 and 4 and using equations 10–12, we obtain

$$\frac{d^2y}{d\xi^2} + \frac{u}{2} \cdot \frac{dz}{d\xi} + A[f(u) + z^2 - y^2] = 0, \tag{16}$$

$$A = \frac{c_0 \cdot d^2 \cdot k_R}{4D}. \tag{17}$$

If we express the current density J

$$J = F(\Phi_+ - \Phi_-) = -\frac{kT}{e_0} \cdot \frac{u}{R} \tag{18}$$

(F = Faraday constant, R = integral resistance of the membrane per unit area) by the relations 11 and 12, a second equation for the variables y and z is obtained:

$$\frac{2}{u} \cdot \frac{dz}{d\xi} + y = \frac{R_f}{R} \tag{19}$$

$$R_f \equiv \frac{kT}{e_0} \cdot \frac{d}{2c_0FD}. \tag{20}$$

The solution of equations 16 and 19 has to fulfill the proper boundary conditions at the membrane-water interfaces ($\xi = \pm 1$). In part I of the series we introduced the usual approximation that for each ion species the partition equilibrium at the interface is maintained even at nonzero current. In the following we replace this assumption by the more general concept that an ion moving across the interface has to overcome a certain activation energy barrier. The existence of such a barrier is probable because the polar head groups of the lipid molecules are "cross-linked" to some extent by dipole-dipole forces and hydrogen bonds. The total flux of an ion across the barrier may be represented by the difference of the net fluxes φ_i^{ms} and φ_i^{sm} from the membrane into the solution and from the solution into the membrane. φ_i^{ms} and φ_i^{sm} are proportional to the ion concentration in the membrane and in the solution, respectively. If c_s is the ion concentration in the solution[1] we obtain for the interface at $\xi = 1$:

$$\Phi_+ = \varphi_+^{ms} - \varphi_+^{sm} = l_{ms} \cdot c_+(1) - l_{sm} \cdot c_s \tag{21}$$

$$\Phi_- = \varphi_-^{ms} - \varphi_-^{sm} = l_{ms} \cdot c_-(1) - l_{sm} \cdot c_s. \tag{22}$$

As cations and anions are assumed to be identical except for the charge sign, the coefficients l_{ms} and l_{sm} are the same for both ions. Furthermore, l_{ms} and l_{sm} are re-

---

[1] In part I of this series it was shown that c_s is slightly dependent on the external voltage, because part of the potential drop occurs in the Gouy-Chapman layer at the solution side of the interface. This effect becomes noticeable only at ionic strengths below 10^{-3} M and is omitted here.

garded as independent of the external voltage. If equation 21 is applied to the equilibrium state of the system ($\Phi_+ \equiv 0$, $c_+ \equiv c_0$), the relation

$$l_{ms} \cdot c_0 = l_{sm} \cdot c_s \tag{23}$$

is obtained. We may now express the current density (equation 18) by the difference of the ion fluxes (equations 21, 22, and 15). This leads to the first boundary condition:

$$z(1) = -u \cdot \frac{R_b}{R} \tag{24}$$

$$R_b \equiv \frac{kT}{e_0} \cdot \frac{1}{2c_0 l_{ms} \cdot F}. \tag{25}$$

A second boundary relation results from the symmetry of the system:

$$c_+(0) = c_-(0), \qquad \text{or} \quad z(0) = 0. \tag{26}$$

The third boundary condition is obtained by equating expressions 11 and 21 for the flux Φ_+ at $\xi = 1$. Together with equations 19 and 23–25 this gives

$$-2\left(\frac{dy}{d\xi}\right)_{\xi=1} = u \cdot z(1) + \frac{R_f}{R_b} \cdot [y(1) - 1]. \tag{27}$$

PHYSICAL MEANING OF THE PARAMETERS R_f, R_b, AND A

In the ohmic range ($|u| \ll 1$), the functions y and z may be represented by the series expansions

$$y = 1 + \alpha_2(\xi) \cdot u^2 + \alpha_4(\xi) \cdot u^4 + \cdots \tag{28}$$

$$z = \alpha_1(\xi) \cdot u + \alpha_3(\xi) \cdot u^3 + \cdots. \tag{29}$$

Inserting these relations into the differential equation 19 and neglecting terms with u^2 and higher, we find by integration (using equation 26):

$$z(\xi) = -u \frac{R_0 - R_f}{2R_0} \cdot \xi. \tag{30}$$

Here $R_0 \equiv (R)_{u \approx 0}$ is the ohmic limit of the membrane resistance. Combination of equation 30 with the boundary condition 24 gives

$$R_0 = R_f + 2R_b. \tag{31}$$

By inspection of equation 20 it is seen that R_f is the ohmic resistance of the lipid film without the barriers. In the limit $R_f \rightarrow 0$, the membrane resistance is determined

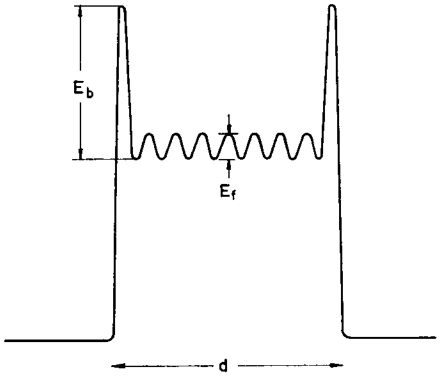

FIGURE 1 Energy barriers for the transport of an ion across the membrane. (E_b, E_f = activation energies, d = membrane thickness.)

by the barriers alone: $R_0 \approx 2\ R_b$. Therefore we may identify R_b with the electrical resistance of the barrier at the membrane-solution interface.

As will be shown below, the influence of the Wien effect on the membrane conductivity depends strongly on the ratio R_b/R_f. It is therefore interesting to make an estimate of R_b/R_f on the basis of Eyring's theory of membrane transport (Glasstone, Laidler, and Eyring, 1941; Johnson, Eyring, and Polissar, 1954). If E_b is the activation energy for the movement of the ion from membrane to solution (see Fig. 1), the coefficient l_{ms} in equations 21 and 22 is given approximately by the relation

$$l_{ms} \simeq \frac{kT}{h} \cdot l \cdot e^{-E_b/kT}, \tag{32}$$

in which h is the Planck constant and l the "jump length", i.e. the distance from which the ion may jump across the barrier. On the other hand, the diffusion of the ion in the membrane is determined by energy barriers of height E_f (Fig. 1). If we assume the jump length for both types of barriers to be approximately equal, then, after Eyring, the diffusion coefficient D becomes

$$D \simeq \frac{kT}{h} \cdot l^2 \cdot e^{-E_f/kT}. \tag{33}$$

Using equations 20 and 25 we obtain for the ratio R_b/R_f the estimate

$$\frac{R_b}{R_f} \simeq \frac{l}{d} \cdot e^{-[(E_f - E_b)/kT]}. \tag{34}$$

With a membrane thickness of $d \simeq 100$ A, the ratio l/d is in the order of 0.1. However, the barrier height E_b may be larger than E_f by several kilocalories/mole. It is therefore conceivable that the resistance of the barrier, R_b, exceeds the film resistance R_f by a factor of ten to a hundred.

In order to evaluate the physical meaning of the parameter A (equation 17), we define two relaxation times. The "diffusional relaxation time" τ_{diff} is the time in which an ion migrates a distance equal to half the membrane thickness by diffusion, and is given by the Einstein relation

$$\tau_{\text{diff}} = \frac{d^2}{8D}. \tag{35}$$

τ_{diff} is the time constant for the reestablishment of the equilibrium after a sudden perturbation of the ion concentrations in the film without barrier. The "chemical relaxation time" τ_{chem} is the time lag of the Wien effect in a macroscopic phase. If the concentration of free ions is small compared with the concentration of ion pairs, τ_{chem} is given by

$$\tau_{\text{chem}} \approx \frac{1}{2 \cdot c_0 \cdot k_R} \tag{36}$$

(Eigen and Schoen, 1955). By comparison of equations 35 and 36 with equation 17 it is seen that A is the ratio of the two relaxation times:

$$A = \frac{\tau_{\text{diff}}}{\tau_{\text{chem}}}. \tag{37}$$

With a membrane thickness of $d = 100$ A and a diffusion coefficient of $D = 10^{-6}$ cm^2 s^{-1}, the diffusion time becomes $\tau_{\text{diff}} \simeq 10^{-7}$ sec. The rate constant for the recombination of oppositely charged ions, k_R, was first calculated by Debye (1942). In a medium of low dielectric constant, k_R is given by

$$k_R \approx 16\pi N_A q D \tag{38}$$

(N_A = Avogadro's number). With $\epsilon_m = 2$, the Bjerrum length q (equation 6) becomes 140 A, so that $k_R \simeq 4 \cdot 10^{13}$ cm^3 mole^{-1} s^{-1}. Of the concentration of free ions in the membrane, c_0, not even the order of magnitude is known. If we assume $R_0 = 10^5$ ohms·cm^2 and a value of 10 for the ratio R_b/R_f, an ion concentration of $3 \cdot 10^{-11}$ moles/cm^3 is calculated from equations 20 and 31. This gives $\tau_{\text{chem}} \simeq 4 \cdot 10^{-4}$ sec, so that $A \simeq 3 \cdot 10^{-4}$.

APPROXIMATE SOLUTION FOR $A \ll 1$, $R_b/R_f \gg 1$

From the numerical example of the preceding section we may expect that under many experimental conditions A is a very small number. In this case an approximate solution may be obtained by a perturbation treatment of the differential equations. For $A = 0$ the equations 16 and 19 can be easily integrated. If we denote

$$[y(\xi)]_{A=0} \quad \text{by } y^*(\xi)$$

$$[z(\xi)]_{A=0} \quad \text{by } z^*(\xi)$$

and $[R]_{A=0}$ by R^*, the result is

$$y^*(\xi) = \frac{R_b}{R^*} \cdot u \cdot \frac{\cosh (u\xi/2)}{\sinh (u/2)} + \frac{R_f}{R^*} \tag{39}$$

$$z^*(\xi) = -\frac{R_b}{R^*} \cdot u \cdot \frac{\sinh (u\xi/2)}{\sinh (u/2)} \tag{40}$$

$$R^* = R_f + R_b \cdot u \cdot \coth (u/2). \tag{41}$$

These equations obey the boundary conditions 24, 26, and 27. For small values of A we can write

$$y(\xi) = y^*(\xi) + A \cdot \beta(\xi) \tag{42}$$

$$z(\xi) = z^*(\xi) + A \cdot \gamma(\xi), \tag{43}$$

neglecting terms which contain A^2 or higher powers of A. With these relations the differential equations 16 and 19 can be solved in closed form. We omit the details of the somewhat lengthy calculation and merely give the end result which is valid for $A \ll 1$ and $R_b/R_f \gg 1$. Introducing the membrane conductivity $\lambda = 1/R$ and the ohmic membrane conductivity $\lambda_0 = 1/R_0$ we find

$$\frac{\lambda}{\lambda_0} \approx \frac{2}{u} \cdot \tanh\left(\frac{u}{2}\right) + A \cdot \frac{R_b}{R_f} \cdot \frac{4}{u} \cdot \tanh\left(\frac{u}{2}\right) \cdot \left[f(u) - \frac{1}{\cosh^2 (u/2)}\right] \qquad (A \ll 1, R_b \gg R_f). \tag{44}$$

This relation has been found to be useful as a first approximation in the numerical integration of the differential equations.

RESULTS AND DISCUSSION

The result of the numerical integration of equations 16 and 19 is represented in Figs. 2 and 3 in which the ratio $\lambda(u)/\lambda_0$ is plotted for different values of the parameters A and R_b/R_f. The function $f(u)$ (equations 5 and 8) was calculated assuming a dielectric constant $\epsilon_m = 2$ and a membrane thickness $d = 70$ A, (see Table I). Interestingly, with certain combinations of u, A, and R_b/R_f the ratio λ/λ_0 becomes < 1. It can be shown that this is a general property of membrane models involving an activation energy barrier at the interface. Under most circumstances, however, λ/λ_0 is an increasing function of u. The main result of the numerical integration is the conclusion that the influence of the Wien effect on the membrane conductivity is appreciable only if A and R_b/R_f are not both small. $A \ll 1$ means that the rate of production of additional free ions by the field effect is much smaller than the rate by which these ions may diffuse out of the membrane, so that the ion concentration

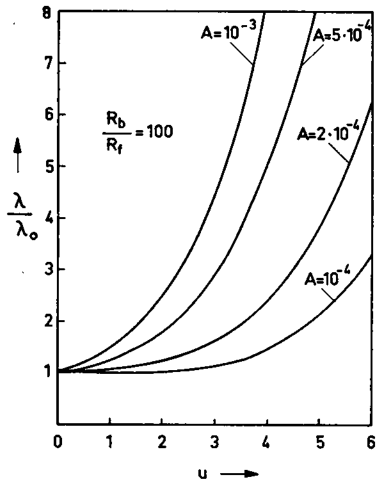

FIGURE 2

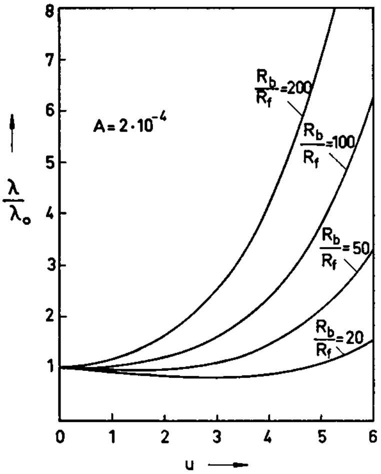

FIGURE 3

FIGURES 2 *and* 3 Membrane conductivity λ as a function of the reduced voltage $u \equiv Ue_0/kT$, as obtained by numerical integration of equations 16 and 19. λ_0 is the membrane conductivity in the ohmic limit ($u \approx 0$). The calculation was carried out with a dielectric constant $\epsilon_m = 2$ and a membrane thickness $d = 70$ A.

TABLE I

THE FUNCTION $f(u)$ (EQUATION 8), AS CALCULATED WITH $\epsilon_m = 2, d = 70$A, $T = 25°$C

u	0	1	2	3	4	5	6
$f(u)$	1.0	4.88	15.77	42.11	99.97	218.64	449.65

within the membrane remains practically unchanged. If, however, the barrier resistance is high ($R_b \gg R_f$), the ions are more or less trapped in the membrane; then even in the case $A \ll 1$ the membrane conductivity is increased by the Wien effect. As we must expect that A is always a small quantity in the case of a lipid bilayer membrane, the influence of the Wien effect on λ/λ_0 depends entirely on the existence of a barrier at the membrane-solution interface. Some indirect evidence for the Wien effect in lipid bilayer membranes comes from the fact that the nonlinearity of the experimental current-voltage characteristic can be explained only in part by the image force correction of the flux equations (see part II of the series). However, as a characteristic relaxation time is associated with the Wien effect, more direct information can be obtained from a measurement of the time dependence of the membrane conductivity after a sudden change in the voltage. Experiments of this type are now in progress.

We thank Professor Leon J. Bruner for many helpful discussions.
This work was supported by the Deutsche Forschungsgemeinschaft.

Received for publication 23 July 1969.

REFERENCES

Bass, L., and W. J. Moore. 1968. *In* Structural Chemistry and Molecular Biology. A. Rich and N. Davidson, editors. W. H. Freeman and Co. Publishers, San Francisco. 356.

Bjerrum, N. 1926. *Kgl. Danske Videnskab. Mat.-Fys. Medd.* **7**:9.

Debye, P. 1942. *Trans. Electrochem. Soc.* **82**:265.

Eigen, M., and J. Schoen. 1955. *Z. Elektrochem.* **59**:483.

Glasstone, S., K. J. Laidler, and H. Eyring. 1941. The Theory of Rate Processes. McGraw-Hill Book Company, New York. Chap. 9.

Harned, S., and B. B. Owen. 1958. The Physical Chemistry of Electrolytic Solutions. Reinhold Publishing Corp., New York.

Johnson, F. H., H. Eyring, and M. J. Polissar. 1954. The Kinetic Basis of Molecular Biology. John Wiley and Sons, Inc., New York.

Miyamoto, V. K., and T. E. Thompson. 1967. *J. Colloid Interface Sci.* **25**:16.

Neumcke, B., and P. Läuger. 1969. *Biophys. J.* **9**:1160.

Onsager, L. 1934. *J. Chem. Phys.* **2**:599.

Walz, D., E. Bamberg, and P. Läuger. 1969. *Biophys. J.* **9**:1150.