THE ROLE OF ELECTRO-OSMOSIS IN THE ELECTRIC-FIELD-INDUCED MOVEMENT OF CHARGED MACROMOLECULES ON THE SURFACES OF CELLS

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ABSTRACT The surfaces of most cells bear a net negative charge. The imposition of an electric field parallel to the surface of the cell should produce, therefore, an electro-osmotic flow of fluid towards the cathodal side of the cell. Our analysis of a simple model of the cell surface indicates that a negatively charged mobile macromolecule will be swept by this electro-osmotic flow of fluid to the cathodal side of the cell if its zeta potential, ζ_1, is less negative than the zeta potential of the cell surface, ζ_2. Conversely, if ζ_2 is less negative than ζ_1, the negatively charged macromolecule will accumulate at the anodal side of the cell. Our experimental results demonstrate that concanavalin A (Con A) receptors on embryonic muscle cells normally accumulate at the cathodal side of the cell, but that they can be induced to accumulate at the anodal side of the cell by preincubating the myotubes either with neuraminidase, a treatment that removes negatively charged sialic acid residues, or with the lipid diI, a treatment that adds positive charges to the surface of the cell. Addition of the negatively charged lipid monosialoganglioside (G_{M1}), on the other hand, enhances the accumulation of Con A receptors at the cathodal side of the cell.

INTRODUCTION

Jaffe (1977) has pointed out that an electric field parallel to the surface of a cell should redistribute charged macromolecules that are free to move laterally in the plasma membrane. It has been demonstrated experimentally that an externally applied electric field does redistribute lectin and acetylcholine receptors in cell membranes; the receptors accumulate at either the negative (Poo and Robinson, 1977; Poo et al., 1978; Orida and Poo, 1978; Poo et al., 1979) or the positive (Zagyansky and Jard, 1979) sides of the cells. To account for the accumulation of concanavalin A (Con A) and acetylcholine receptors at the negative side of embryonic muscle cells, it was suggested that the majority of these molecules are either positively charged or less negatively charged than other displacable membrane components (Poo et al., 1979).

An alternative interpretation of the electrophoresis results is discussed here. Implicit in the analysis of Jaffe (1977) and Poo et al. (1979) is the assumption that an electro-osmotic flow of fluid parallel to the surface of the membrane either does not occur or, if it occurs, does not contribute significantly to the migration of membrane molecules. We suggest that an electro-osmotic flow of fluid occurs, that it exerts a hydrodynamic force on the mobile macromolecules, and that this force causes even negatively charged macromolecules to accumulate at the negative side of the muscle cell. Specifically, we assume that the surface of

the cell has a net negative charge. These negative charges cause mobile counterions (e.g., Na^+) to accumulate in an "aqueous diffuse double layer" adjacent to the surface of the cell. The imposition of an electric field parallel to the surface of the cell causes the counterions to move. The movement of the counterions induces a movement of fluid parallel to the surface of the cell. This fluid movement exerts a force on the portion of the macromolecules that protrudes from the lipid bilayer of the membrane.

In this report we analyze quantitatively a simple model that illustrates the basic phenomena we are considering and report experimental data that are consistent with the model. Our analysis predicts that a mobile, negatively charged macromolecule will move to the positive side of the cell only if the zeta potential of the macromolecule is more negative than the zeta potential of the cell surface. If the zeta potential of the macromolecule is less negative than the zeta potential of the cell surface, the macromolecule will be swept to the negative side of the cell by an electro-osmotic flow of fluid. Our experimental results demonstrate that Con A receptors can be induced to reverse their normal direction of movement and accumulate at the positive side of an embryonic muscle cell if the negative charge of the cell surface is reduced.

THE MODEL

The cell is considered to be a sphere of radius r (>1 μm) and the mobile macromolecule is represented by a pair tethered spheres (Fig. 1). The sphere confined to the bilayer is of radius a_2, the sphere confined to the aqueous phase is of radius a_1. Both the bilayer and the adjacent aqueous phase are assumed to behave hydrodynamically as ideal Newtonian fluids with viscosities η_2 and η_1, respectively. Stokes' law is assumed to be valid in both the bilayer and aqueous phases. The sphere confined to the bilayer is assumed to be uncharged, and the sphere in the aqueous phase is assumed to have a surface density σ_1 and a zeta potential ζ_1. The outer surface of the membrane is assumed to have a charge density σ_2 and a zeta potential ζ_2. Both σ_1 and σ_2 are assumed to be constant. Both r and a_1 are assumed to be much larger than the Debye length, $1/\kappa$, which is ~ 10 Å in a decimolar salt solution. The distance between the two spheres is also assumed to be $>1/\kappa$, and the torque exerted by the electric field on the macromolecule is ignored. This implies that little electrostatic interaction exists between the sphere of radius a_1 and the surface of the cell, and that their electrical double layers may be treated independently.

We assume in our analysis that the spheres are rigid and nonconducting and that the dielectric constant is independent of position in the aqueous phase. Any variation in the charge density along the surface of the cell due to a redistribution of the mobile membrane components by the electric field is ignored. This is probably a reasonable assumption, since only a small fraction of the membrane components in embryonic muscle cells moves in the electric field (Poo et al., 1979), and the potential difference applied across the cell is usually small ($<kT/e \sim 25$ mV). The cell membrane is considered to be stationary with respect to the bulk aqueous phase, which implies that the cell is fixed in some manner or that the field is generated by the cell itself (Jaffe, 1979). The relationship between the charge density, σ, and the surface potential, ψ_0, of a phospholipid bilayer membrane can be described by the classic Gouy-Chapman theory of the diffuse double layer (e.g., McLaughlin, 1977). The zeta potential is the electrical potential at the hydrodynamic plane of shear, which is ~ 2 Å from

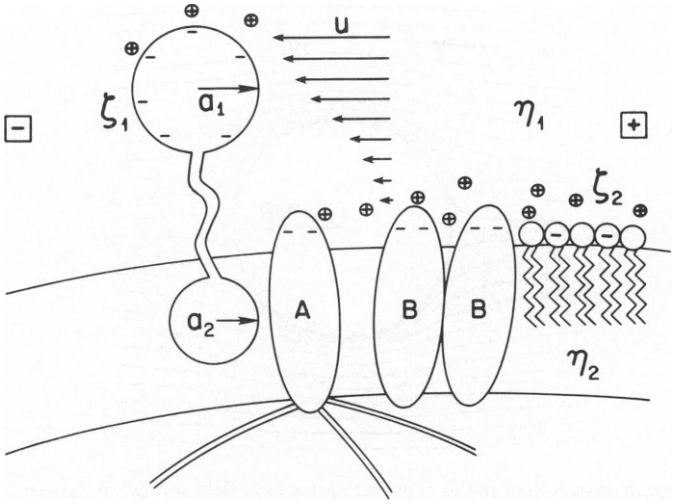

FIGURE 1 Diagram of the model. The cell surface contains lipids, proteins, and sugars, and has a net negative charge, which attracts positively charged counterions (circled plus signs) into a "diffuse double layer." The application of an electric field parallel to the surface (boxed plus and minus signs) causes these counterions to move and produces a movement of fluid, a phenomenon known as electro-osmosis. The velocity of the fluid increases with distance from the surface for a few Debye lengths, or tens of ångströms in a physiological solution, and is proportional to the length of the arrows in the figure. The mobile macromolecule is represented by a pair of tethered spheres of radii a_1 and a_2. The movement of the fluid exerts a hydrodynamic force on the macromolecule that tends to move it to the negative side of the cell. The electric field exerts an electrophoretic force on the negatively charged macromolecule that tends to move it to the positive side of the cell. The analysis indicates that the velocity of the macromolecule relative to the cell surface is proportional to the difference between the zeta potential of the macromolecule, ζ_1, and the zeta potential of the cell surface, ζ_2. A = anchored protein or glycoprotein; B = relatively immobile proteins or glycoproteins that are aggregated. See text for details.

the surface of a phospholipid vesicle (Eisenberg et al, 1979). We anticipate that more complex relationships exist between σ, ψ_0, and ζ for biological cell surfaces.

ANALYSIS

In the steady state there is no net force on a mobile macromolecule; it moves with a constant velocity, u_1, relative to the cell surface. The electrically induced flux of macromolecules, $c(\theta)u_1$, is balanced at equilibrium by a diffusional flux in the opposite direction, $D\nabla c(\theta)$:

$$c(\theta)u_1 = D\nabla c(\theta), \tag{1}$$

where D is the diffusion coefficient of the macromolecule and $c(\theta)$ is the surface concentration of mobile macromolecules at the angle θ (Fig. 2). For distances close to the spherical cell, the field tangential to the cell surface at the angle θ, E_θ, is related to the external field, E, by the formula

$$E_\theta = fE\sin\theta, \tag{2}$$

where $f = 1.5$ for a nonconducting sphere (Cole, 1972).

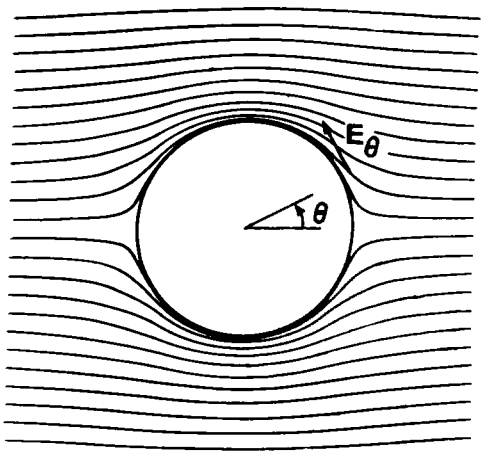

FIGURE 2 Diagram of the cell of radius r, indicating the local field parallel to the sufrace, E_θ, and the angle θ. Modified from Cole (1972).

We now consider the motion of the fluid adjacent to the cell. It is easy to show (e.g., Overbeek and Wiersema, 1967) that the velocity of the fluid just outside the double layer adjacent to the cell surface is:

$$u = \frac{-\epsilon_r \epsilon_0 \zeta_2 E_\theta}{\eta_1}, \tag{3}$$

where ϵ_0 is the permittivity of free space and ϵ_r is the dielectric constant of the aqueous phase. The Stokes force on the sphere in the aqueous phase is:

$$F_1 = 6\pi\eta_1 a_1 (u - u_1), \tag{4}$$

where u_1 is the velocity of the macromolecule relative to the fixed cell.

The Stokes drag exerted on the sphere in the bilayer is:

$$F_2 = -6\pi\eta_2 a_2 u_1. \tag{5}$$

The net force exerted by the local field E_θ on the charged sphere of radius a_1 is calculated in the following manner. Assume for the moment that the charged sphere is not connected to the second sphere and that it is free in the bulk aqueous phase. When a field, E, is applied, the velocity the sphere acquires, v, is given by the Helmholtz-Smoluchowski equation (Overbeek and Wiersema, 1967):

$$v = \frac{\epsilon_r \epsilon_0 \zeta_1 E}{\eta_1}. \tag{6}$$

(In the derivation of Eqs. 3 and 6 the "relaxation" effect is ignored. Corrections due to this effect have been determined numerically by Wiersema et al. [1966]. They are negligible in decimolar salt solutions [Eisenberg et al., 1979].) In the steady state the Stokes' drag, $6\pi a_1 \eta_1 v$, balances the net force exerted by the electric field on the sphere. This net force is the sum of the force exerted by the field on the charge within the plane of shear and the

"electrophoretic retardation" force exerted by the ionic atmosphere (Overbeek and Wiersema, 1967). Thus, the net force exerted by the field is $6\pi a_1 \epsilon_r \epsilon_0 \zeta_1 E$ (Eq. 6). When the sphere is tethered in the vicinity of the cell surface, E must be replaced by E_θ and the net force produced by the electric field is:

$$F_3 = 6\pi a_1 \epsilon_r \epsilon_0 \zeta_1 E_\theta. \tag{7}$$

In the steady state the sum of all the forces on the macromolecule must be zero:

$$F_1 + F_2 + F_3 = 0. \tag{8}$$

By inserting Eqs. 3–5 and 7 into Eq. 8, the following expression is obtained for u_1, the velocity of the macromolecule:

$$u_1 = \frac{\epsilon_r \epsilon_0 a_1 E_\theta (\zeta_1 - \zeta_2)}{a_1 \eta_1 + a_2 \eta_2}. \tag{9}$$

The expression can be simplified if the radii of the two spheres are similar, $a_1 \simeq a_2$, and the viscosity of the membrane is much greater than the viscosity of the aqueous phase, $\eta_2 \gg \eta_1$. In this case:

$$u_1 = \frac{\epsilon_r \epsilon_0 (\zeta_1 - \zeta_2) E_\theta}{\eta_2}. \tag{10}$$

This is identical to the Helmholtz-Smoluchowski equation for the electrophoretic mobility of a charged particle in a medium of viscosity η_2, with the zeta potential of the particle replaced by the difference between the zeta potentials of the particle and the cell.

To complete the analysis in a parallel manner to Jaffe (1977) and Poo et al. (1979), the ratio u_1/E_θ is defined to be m, the "effective electrophoretic mobility." From Eq. 9:

$$u_1/E_\theta = m = \frac{\epsilon_r \epsilon_0 a_1 (\zeta_1 - \zeta_2)}{a_1 \eta_1 + a_2 \eta_2}. \tag{11}$$

From Eqs. 1, 2, and 11:

$$\frac{mfEc(\theta) r \sin \theta}{D} = \frac{dc(\theta)}{d\theta}. \tag{12}$$

The appropriate boundary condition is that the integral of the concentration over the surface must equal $4\pi r^2 C_0$, where C_0 is the uniform surface density of the molecules before the application of the field. It follows that:

$$c(\theta) = \alpha \exp [-\beta(1 + \cos \theta)], \tag{13}$$

where α and β are constants, $\beta = mfEr/D$, and $\alpha = 2\beta C_0/(1 - e^{-2\beta})$.

It is apparent from Eq. 13 that when $\beta = 0$, $c(\theta) = C_0$. In terms of the analysis of Jaffe (1977) and Poo et al. (1979), this occurs for a mobile charged macromolecule only if the field, E, is zero. In terms of our extended model, β is also zero if $\zeta_1 = \zeta_2$. In the previous model, negatively charged macromolecules always accumulate at the positive side of the cell. In our extended model, negatively charged macromolecules accumulate at the positive side of the cell when $\zeta_1 < \zeta_2$, but at the negative side of the cell when $\zeta_1 > \zeta_2$.

EXPERIMENTAL RESULTS AND DISCUSSION

The role of electro-osmosis in the field-induced migration of Con A receptors in embryonic *Xenopus* muscle cells was examined by performing several experiments designed to modify the charge density and zeta potential of the cell surface. The results were all consistent with the hypothesis that electro-osmosis plays a significant role in the redistribution of Con A receptors in this cell.

Treatment with Neuraminidase

Pretreatment of 2-d-old *Xenopus* muscle cells with neuraminidase reversed the direction of field-induced migration of Con A and acetycholine receptors (Poo et al., 1979; Orida and Poo, 1978). In control cells these receptors accumulate at the cathodal side of the cell; in cells pretreated with neuraminidase these receptors migrate towards the anodal side of the cell. In the present study, similar results were obtained with 1-d-old *Xenopus* muscle cells (Figs. 3 and 4). A parsimonious interpretation of this result is that neuraminidase cleaves negatively

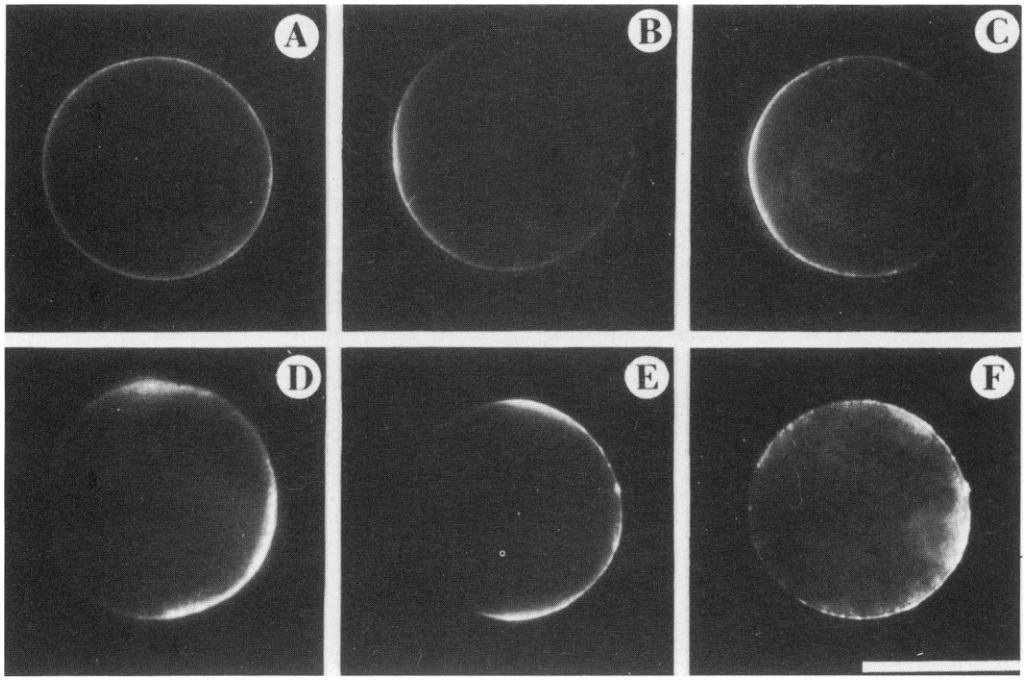

FIGURE 3 Fluorescence micrographs of representative cells shown to illustrate the migration of Con A receptors on the surface of 1-d-old, cultured myotomal cells under various experimental conditions. (*A*) Control cells labeled with Con A conjugated with fluorescein, F-Con A (25 μg/ml, Vector Laboratories, Inc., Schiller Park, Ill.). (*B*) Cells exposed to a field of 10 V/cm for 30 min, then labeled with F-Con A after the field was removed. The cells in panels *C*, *D*, and *E* were preincubated, then treated similarly. (*C*) Cells preincubated in 50 μg/ml of G_{M1} (Supelco, Inc.) for 2 h. (*D*) Cells preincubated in 0.1 μg/ml of neuraminidase (Worthington Biochemical Corp., Freehold, N.J.) at pH 6.8 for 1.5 h. (*E*) Cells preincubated with 10 μg/ml of fluorescent lipid, dil, for 20 min. (*F*) Same cell as in *E*, but fluorescence of dil photographed at 590 nm. *A-E* were photographed at 520 nm. The anode was on the right side of all the figures. The bar represents 25 μm.

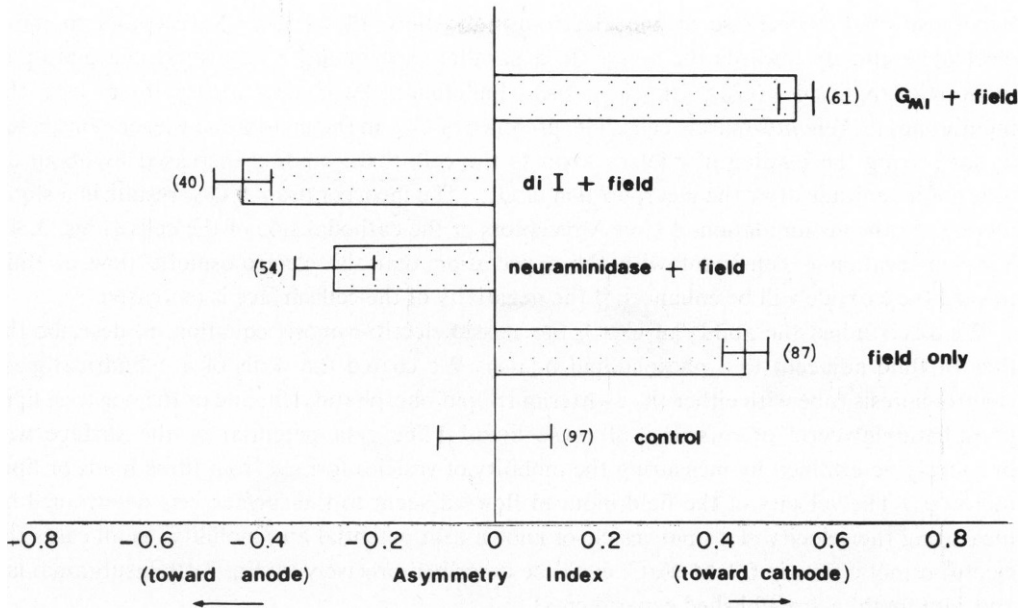

FIGURE 4 Histogram of "asymmetry indices" for the distribution of Con A receptors. The fluorescence intensities due to F-Con A at the poles of the cell facing the cathode (I_c) and the anode (I_a) were measured microfluorimetrically using a photometer (PM1, Carl Zeiss, Inc., New York) with an 8-μm aperture. The asymmetry index was defined as $(I_c - I_a)/(I_c + I_a)$. The background fluorescence from an adjacent, cell-free region was subtracted from the readings made with the cells. All cells except for the control muscle cells were exposed to a field of 10 V/cm for 30 min. (1) Control; no field. (2) Field only. (3) Preincubation with neuraminidase. (4) Preincubation with dil. (5) Preincubation with monsialoganglioside G_{M1}. The integers refer to the total number of cells measured. The error bars represent the standard error with 95% confidence level.

charged sialic acid residues from the cells' surface (e.g., Eylar et al., 1962), reducing the zeta potential and decreasing the electro-osmotic flow parallel to the surface. The Con A receptors now move "electrophoretically" toward the anode.

Incorporation of Charged Lipids

A positively charged fluorescent lipid, 3,3' dioctadecylindocarbocyanine iodide (diI; Sims et al., 1974) was incorporated into the plasma membrane of *Xenopus* muscle cells. The successful incorporation of diI is indicated by the ring of fluorescence around the perimeter of the cell (Fig. 3). Measurements of the lateral mobility of diI in this membrane (Poo and Hamasaki, unpublished observation) suggest that diI molecules are tightly associated with relatively immobile, negatively charged membrane proteins; an applied field induces them to migrate toward the anode, and the rate of back diffusion after removal of the field is much slower than that observed for free lipids in cell membranes. In any event, the incorporation of positively charged lipids into the plasma membrane should reduce the magnitude of the negative zeta potential of the cell and, therefore, the magnitude of the electro-osmotic flow induced by the electric field. After the cells had been preincubated with diI, Con A receptors were found to migrate towards the anode (Figs. 3, 4). This result is consistent with the

hypothesis that a decrease in the electro-osmotic flow allows Con A receptors to move electrophoretically towards the anode. In a parallel experiment, a negatively charged lipid, monosialoganglioside (G_{M1}; Supelco, Inc., Bellefonte, Pa.), was incorporated into the membranes of *Xenopus* muscle cells. The presence of G_{M1} in the membrane was demonstrated by measuring the binding of cholera toxin to the cell: toxin binding increased by about an order of magnitude after the incorporation of G_{M1}. The incorporation of G_{M1} results in a slight increase in the accumulation of Con A receptors at the cathodal side of the cells (Figs. 3, 4). This observation is consistent with the expectation that the electro-osmotic flow of fluid toward the cathode will be enhanced if the negativity of the cell surface is increased.

We also studied the ability of Eq. 3, the classic electro-osmosis equation, to describe the flow of fluid adjacent to a phospholipid bilayer. We coated the walls of a cylindrical glass electrophoresis tube with either the zwitterionic lipid phosphatidylcholine or the negative lipid phosphatidylglycerol or mixtures of these lipids. (The zeta potential of the surface was previously determined by measuring the mobility of vesicles formed from these lipids or lipid mixtures.) The velocity of the field-induced flow adjacent to the surface was determined by measuring the velocity of a lipid vesicle of known zeta potential and mobility. In all cases the electro-osmotic flow in 0.1 M NaCl could be described precisely by Eq. 3 (Balasubramanian and McLaughlin, unpublished experiments).

Poo et al. (1979) observed that "medium flow alone failed to produce any significant redistribution, and that gross alteration of the medium flow produced no significant change in the field-induced asymmetric distribution of Con A receptors." This observation appears to indicate that electro-osmosis could not play a significant role in the field-induced movement of macromolecules. However, externally induced laminar fluid flow does not produce significant fluid flow at distances of a few hundred ångströms from the surface (e.g., Vetter, 1967). Thus, the negative results obtained by Poo et al. (1979) do not rule out the possible involvement of electro-osmotic flow near the charged layer of the cell surface.

In the model shown in Fig. 1, we have greatly simplified the situation at the cell surface by assuming that the charged portion of the mobile protein extends beyond and does not interact with the charged surface of the membrane. In reality, the charged mobile receptors probably exist among the other proteins and glycoproteins that contribute to the surface charge. Furthermore, we have ignored in our model the effects of a field-induced redistribution of charged groups. We postpone an analysis of more realistic models until the results of experiments in progress on simple model systems are available. We believe that complementary experiments with reconstituted systems and living cells will provide a better understanding of the role of electro-osmosis in the field-induced movement of macromolecules on the surface of cells and organelles. The field-induced movement of macromolecules is being studied by workers interested in topics as diverse as the molecular basis of morphogenesis (Woodruff and Telfer, 1980) and the structure of energy transducing membranes (Sowers and Hackenbrock, 1980).

We thank Doctors James Hall and Tazewell Wilson for helpful discussions.

This research was supported by National Institutes of Health grant GM 24971 and National Science Foundation (NSF) grant PCM 7903241 to Dr. McLaughlin and NSF grant BNS 80-12348 to Dr. Poo.

Received for publication 7 August 1980.

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