

# An Experimental Test of New Theoretical Models for the Electrokinetic Properties of Biological Membranes

## *The Effect of $\text{UO}_2^{++}$ and Tetracaine on the Electrophoretic Mobility of Bilayer Membranes and Human Erythrocytes*

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**ABSTRACT** For a large smooth particle with charges at the surface, the electrophoretic mobility is proportional to the zeta potential, which is related to the charge density by the Gouy-Chapman theory of the diffuse double layer. This classical model adequately describes the dependence of the electrophoretic mobility of phospholipid vesicles on charge density and salt concentration, but it is not applicable to most biological cells, for which new theoretical models have been developed. We tested these new models experimentally by measuring the effect of  $\text{UO}_2^{++}$  on the electrophoretic mobility of model membranes and human erythrocytes in 0.15 M NaCl at pH 5. We used  $\text{UO}_2^{++}$  for these studies because it should adsorb specifically to the bilayer surface of the erythrocyte and should not change the density of fixed charges in the glycocalyx. Our experiments demonstrate that it forms high-affinity complexes with the phosphate groups of several phospholipids in a bilayer but does not bind significantly to sialic acid residues. As observed previously,  $\text{UO}_2^{++}$  adsorbs strongly to egg phosphatidylcholine (PC) vesicles: 0.1 mM  $\text{UO}_2^{++}$  changes the zeta potential of PC vesicles from 0 to +40 mV. It also has a large effect on the electrophoretic mobility of vesicles formed from mixtures of PC and the negative phospholipid phosphatidylserine (PS): 0.1 mM  $\text{UO}_2^{++}$  changes the zeta potential of PC/PS vesicles (10 mol % PS) from -13 to +37 mV. In contrast,  $\text{UO}_2^{++}$  has only a small effect on the electrophoretic mobility of either vesicles formed from mixtures of PC and the negative ganglioside  $\text{G}_{\text{M1}}$  or erythrocytes: 0.1 mM  $\text{UO}_2^{++}$  changes the apparent zeta potential of PC/ $\text{G}_{\text{M1}}$  vesicles (17 mol %  $\text{G}_{\text{M1}}$ ) from -11 to +5 mV and the apparent zeta potential of erythrocytes from -12 to -4 mV. The new theoretical models suggest why  $\text{UO}_2^{++}$  has a small effect on PC/ $\text{G}_{\text{M1}}$  vesicles and erythrocytes. First, large groups (e.g., sugar moieties)

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protruding from the surface of the PC/G<sub>M1</sub> vesicles and erythrocytes exert hydrodynamic drag. Second, charges at the surface of a particle (e.g., adsorbed UO<sub>2</sub><sup>++</sup>) exert a smaller effect on the mobility than charges located some distance from the surface (e.g., sialic acid residues).

#### INTRODUCTION

Uranyl ions (UO<sub>2</sub><sup>++</sup>) bind strongly to the phosphate groups of phosphatidylcholine (PC) molecules in bilayers of monolayers. Low concentrations of UO<sub>2</sub><sup>++</sup> affect the electrophoretic mobility of multilamellar vesicles (De Jong, 1949; Bangham et al., 1958), the surface potential of monolayers (Shah, 1969), the carrier-mediated conductance of planar bilayers (Ginsburg and Wolosin, 1979; Ting-Beall, 1980), the infrared P=O stretching band (Chapman et al., 1974), and the phase transition temperature of vesicles (Levine et al., 1973; Chapman, 1973; Chapman et al., 1974; Sillerud and Barnett, 1982) formed from PC. Uranyl also binds strongly to membranes formed from phosphatidylserine (PS) (McLaughlin et al., 1971, 1981; Chapman et al., 1974) or phosphatidylethanolamine (PE) (McLaughlin et al., 1971).

This ability makes uranyl a useful probe of membrane structure. For example, Stamatoff et al. (1979) used low-angle X-ray scattering of membranes labeled with uranyl acetate to determine the thickness of the bilayer.<sup>1</sup> Renthal and Cha (1984) used uranyl acetate to determine the number of phosphate groups exposed at the two surfaces of purple membranes from *Halobacterium halobium*.

One of our objectives was to measure the effect of uranyl on the electrophoretic mobility of model and biological membranes in the physiological pH range; previous measurements were made in unbuffered solutions at acid pH (De Jong, 1949; Bangham et al., 1958). Another objective was to determine whether UO<sub>2</sub><sup>++</sup> adsorbs significantly to sialic acid residues, which comprise a large fraction of the negative surface charges on an erythrocyte (Levine et al., 1983). We also wanted to determine whether UO<sub>2</sub><sup>++</sup> binds cooperatively to more than one PC molecule in a bilayer membrane. Finally, and most importantly, we wanted to test experimentally new theoretical models for the electrophoretic mobility of erythrocytes and other biological membranes (Pastushenko and Donath, 1976; Jones, 1979; Donath and Pastushenko, 1979; Wunderlich, 1982; Levine et al., 1983; McDaniel et al., 1984, 1986; Sharp and Brooks, 1985; Donath and Voigt, 1986; Snabre et al., 1986).

These new theoretical models are all simple extensions of the classical model, the limitations of which have been recognized for many years (e.g., Haydon, 1961; Parsegian, 1974). In the classical model, it is erroneously assumed that the charges on an erythrocyte are located at the membrane-solution interface and that the viscous drag exerted by the sugar and protein moieties in the glycocalyx can be ignored. The Gouy equation from the theory of the diffuse double layer is combined with the Helmholtz-Smoluchowski equation to relate the electrophoretic mobility to the surface charge density and salt concentration (e.g., Aveyard and Haydon, 1973). In the new models, the charges on the outer surface of the

<sup>1</sup> However, Parsegian et al. (1981) reported that uranyl may alter the structure of bilayer membranes formed from lipids with saturated fatty acid chains.

erythrocyte are distributed in some manner throughout the glycocalyx, and the sugars and proteins exert hydrodynamic drag.

We studied bilayer membranes formed from mixtures of PC and gangliosides to test these new models (McDaniel et al., 1984, 1986). The orientation of the  $G_{M1}$  headgroup in PC/ $G_{M1}$  bilayers was determined from X-ray diffraction measurements (McDaniel and McIntosh, 1986): the charge on  $G_{M1}$  is located  $\sim 1$  nm from the PC headgroups at the membrane-solution interface, and the headgroups protrude at least 2 nm from the surface. The experimental electrophoretic mobility results we obtained with these membranes agreed satisfactorily with the predictions of the new models. Next, we wanted to examine more directly the prediction that charges at the surface exert less effect on the electrophoretic mobility than charges at the outer edge of the glycocalyx. We tested this prediction with bilayer membranes by mixing a cationic lipid with  $G_{M1}$  to produce vesicles with a layer of positive charge at the surface and a layer of negative charge 1 nm from the surface (McLaughlin, 1985). The results agreed qualitatively with the predictions of the new theoretical models. In this study, we wanted to change the charge density at the membrane-solution interface of an erythrocyte. We chose uranyl for these studies because the available evidence suggested that  $UO_2^{2+}$  adsorbs weakly to both proteins and sialic acid residues and that it should adsorb equally well to phosphate groups at the bilayer-water interface of (a) PC vesicles, (b) PC/PS vesicles, (c) PC/ $G_{M1}$  vesicles, and (d) erythrocytes. Thus, it should produce similar surface charge densities and electrostatic surface potentials in all four cases. Furthermore, we could test experimentally the prediction that it adsorbs equally well to the bilayer membranes we used. The new theoretical models predict that  $UO_2^{2+}$  will exert a smaller effect on the electrophoretic mobility in cases c and d than in cases a and b for two reasons. First, sugar moieties protruding from the bilayer surface of PC/ $G_{M1}$  vesicles and erythrocytes exert hydrodynamic drag. Second, fixed charges in the glycocalyx are more important in determining the electrophoretic mobility than the  $UO_2^{2+}$  ions adsorbed to the surface of the bilayer.

It is perhaps not intuitively apparent why the electrophoretic mobility depends on the location of the fixed charges. To explain this dependence, we note first that the velocity of a particle in an electric field (electrophoresis) is identical in magnitude but opposite in direction to the velocity of the fluid a few Debye lengths from a fixed surface of the same material (electro-osmosis). Fig. 1A illustrates schematically the distribution of counterions and the profile of the electro-osmotic fluid velocity (length of arrows) when the fixed charges are at the surface. To calculate this velocity profile, we make all the conventional assumptions in the Gouy-Chapman theory. The most important assumptions are that (a) ions are point charges, (b) the fixed charges are smeared uniformly over the surface, (c) the dielectric constant is independent of position, and (d) image charge effects can be ignored. At equilibrium, the fixed negative charges at the surface in Fig. 1A attract counterions (e.g., Na ions) from the aqueous phase, an attraction described by the Poisson equation. These counterions form a diffuse layer because of their statistical tendency to diffuse from a region of high to low concentration. The potential profile and distribution of counterions can be described by combining the Poisson equation with the Boltzmann relation (Gouy-

Chapman theory). If we assume the potential is small ( $<25$  mV) and the solution contains only monovalent ions, the Gouy-Chapman theory predicts that the potential falls exponentially with distance from the surface:

$$\psi(x) = \psi(0)\exp(-\kappa x), \quad (1)$$

where  $1/\kappa$  is the Debye length, which is  $\sim 1$  nm in a decimolar salt solution. The surface potential,  $\psi(0)$ , is proportional to the surface charge density,  $\sigma$ :

$$\psi(0) = \sigma/\epsilon_r\epsilon_0\kappa, \quad (2)$$

where  $\epsilon_0$  is the permittivity of free space and  $\epsilon_r$  is the dielectric constant.

When an electric field ( $E$ ) is applied parallel to the surface illustrated in Fig. 1, the force exerted on the net charge in a small volume ( $Adx$ ) of fluid located a

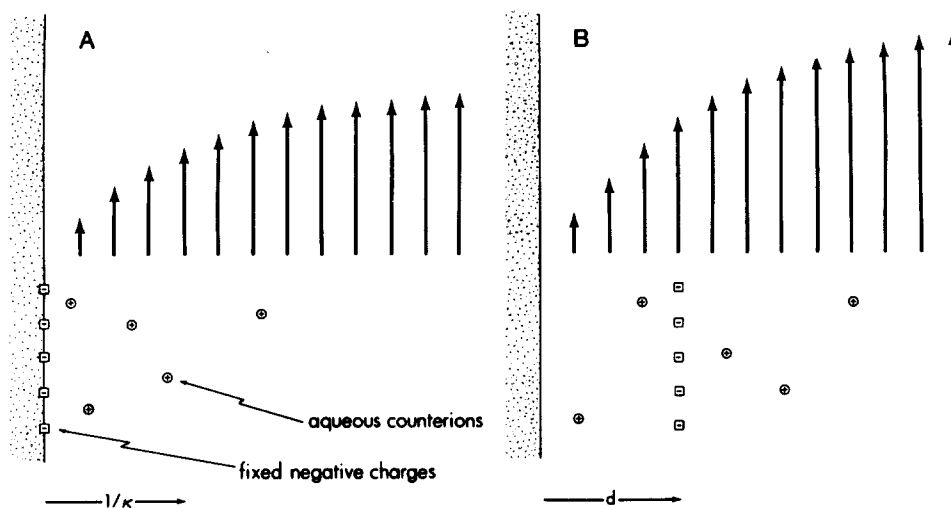


FIGURE 1. The steady state electro-osmotic velocity produced adjacent to a fixed surface when an electric field is applied parallel to the surface. The length of the arrows indicates fluid velocity. (A) The fixed negative charges (boxed minus signs) are at the surface,  $x = 0$ . According to the linearized Gouy-Chapman theory, the space charge density in the aqueous phase,  $\rho$  (number of cations – number of anions in a unit volume times the electronic charge), is maximal at the surface and decays exponentially with distance from the surface. The force exerted by the electric field on a small volume of fluid is proportional to the space charge density; therefore, it is also maximal at the surface. However, the “no slip” boundary condition states that the fluid velocity must be zero at the surface. (B) The fixed negative charges are on infinitely thin rods at a distance  $d$  from the surface. The value of  $\rho$  and the force exerted on an element of fluid is maximal at a distance  $d$  from the surface, where the fluid velocity is not constrained to be zero. Note that the fluid a few Debye lengths from the surface moves faster in B than A. Distance  $d$  is assumed to equal  $1/\kappa$  in this figure and the fluid far from the surface moves 40% faster in B than in A. Equivalently, a particle with charges a distance  $d = 1/\kappa$  from the surface will move 40% faster in an electric field than a particle with charges at the surface.

distance  $x$  from the surface is equal to  $E\rho A dx$ , where  $\rho(x)$  is the space charge density. The force is transferred to the fluid<sup>2</sup> and is balanced, in the steady state, by the hydrodynamic shear force. The Navier-Stokes equation states that:

$$E\rho dx = -\left[\eta\left(\frac{dv}{dx}\right)_{x+dx} - \eta\left(\frac{dv}{dx}\right)_x\right] = -\eta\frac{d^2v}{dx^2} dx, \quad (3)$$

where  $\eta$  is the viscosity and  $v$  is the velocity of the fluid. The Poisson equation,

$$\rho = -\epsilon_r\epsilon_0\frac{d^2\psi}{dx^2}, \quad (4)$$

can be inserted into Eq. 3 and integrated twice, and the appropriate boundary conditions can be applied. If the hydrodynamic slip plane is located at the surface, then  $\zeta = \psi(0)$  and the profile of the fluid velocity is:

$$v(x) = -\frac{E\epsilon_r\epsilon_0\zeta}{\eta} [1 - \exp(-\kappa x)] = -\frac{E\sigma}{\eta\kappa} [1 - \exp(-\kappa x)]. \quad (5)$$

This velocity profile is illustrated in Fig. 1A. Note that the velocity attains its maximum value within a few Debye lengths of the surface. The limit of Eq. 5 when  $x \gg 1/\kappa$  is termed the Helmholtz-Smoluchowski equation:

$$u_{eo} = -\epsilon_r\epsilon_0\zeta/\eta, \quad (6)$$

where  $u_{eo} = v/E$  is the electro-osmotic velocity per unit field. Eq. 6 states that the electro-osmotic velocity is proportional to the zeta potential; if we are concerned with the velocity per unit field of a particle (electrophoretic mobility,  $u$ ), we simply reverse the sign of the velocity in Eq. 6:

$$u = \epsilon_r\epsilon_0\zeta/\eta. \quad (7)$$

We note that it is not necessary to assume that Eqs. 1 and 2 are valid to derive Eqs. 6 and 7. The derivation requires only the very general Eqs. 3 and 4 and the appropriate boundary conditions: the charges are at the surface, the surface is smooth, and the radius of curvature at each point on the surface is  $\gg 1/\kappa$ . Details of the derivation can be found in Overbeek and Wiersema (1967), Aveyard and Haydon (1973), O'Brien and White (1978), and Hunter (1981).

The velocity profile when the charges are some distance from the surface can be deduced by following, for example, the outline in Levine et al. (1983). We make all of the assumptions inherent in the Gouy-Chapman theory but one: we assume the fixed charges are not at the surface but are a distance  $d$  from the surface on rigid, infinitely thin rods that exert no hydrodynamic drag. To simplify the final expression, we assume that the potential profile is described by the linearized [ $\psi(x) < 25$  mV] Poisson-Boltzmann equation. Eqs. 3 and 4 can be

<sup>2</sup> In the steady state, the force exerted by the electric field on an ion is balanced by the Stoke's drag exerted by the fluid on the ion. It follows from Newton's third law that the force exerted by the fluid on the ion must be equal in magnitude but opposite in direction to the force exerted by the ion on the fluid. Thus, the force exerted by the field on the net charge in a volume is transferred to the fluid in the volume.

combined and integrated, and the potential profile (e.g., Fig. 6C) can be inserted into the resulting equation. The velocity profile is illustrated in Fig. 1B. The limiting velocity far from the surface (or the electrophoretic mobility of a particle) is given by the equation:<sup>3</sup>

$$u_{eo} = -\frac{\sigma}{\eta\kappa} [\kappa d + \exp(-\kappa d)]. \quad (8)$$

Thus, the velocity is larger than the velocity predicted by the Helmholtz-Smoluchowski equation (combination of Eqs. 2 and 6) by a factor  $\kappa d + \exp(-\kappa d)$ . This factor is equal to 1.4 for the case illustrated in Fig. 1B, where the charges are assumed to be located a distance  $d = 1/\kappa$  from the surface. If the charges on an erythrocyte are, on average,  $\sim 5$  Debye lengths from the surface (Levine et al., 1983), this location-of-charge effect should cause the erythrocyte to move about five times faster than predicted by the classical theory (combination of Eqs. 2 and 7). In fact, it moves about half as fast: the hydrodynamic drag must overwhelm the location-of-charge effect. The new theoretical models referenced above take into account both the hydrodynamic drag and the location-of-charge effect.

#### MATERIALS AND METHODS

The ganglioside  $G_{M1}$  was obtained from Supelco, Inc. (Bellefonte, PA), bovine brain PS, egg PC, and diphtanoylphosphatidylcholine from Avanti Polar Lipids, Inc. (Birmingham, AL), monoolein (glycerolmonooleate [GMO]) from Nu-Chek-Prep (Elysian, MN), cholesterol (C) and sphingomyelin (SM) from Supelco, Inc., didodecyldimethylammonium bromide (DDDA) from Eastman Kodak Co. (Rochester, NY), and monogalactosyldiglyceride (MGDG) from Lipid Products (Nr. Redhill, Surrey, England). The fluorescent probe 2-(*N*-hexadecyl)aminonaphthalene-6-sulfonic acid (2,6-HNS) was obtained from Molecular Probes (Junction City, OR) and Triton X-100 from Sigma Chemical Co. (St. Louis, MO). Uranyl nitrate was obtained from K & K Laboratories (Plainview, NY) and Fisher Scientific Co. (Springfield, NJ), 4-morpholinepropanesulfonic acid (MOPS) from P-L Biochemicals (Milwaukee, WI), Tris from Calbiochem-Behring Corp. (San Diego, CA), NaCl from Fisher Scientific Co., tetracaine from Schwarz/Mann (Orangeburg, NY), and EDTA from Baker Chemical Co. (Phillipsburg, NJ). All solutions were prepared with water purified in a Millipore Corp. (Bedford, MA) Super-Q system and then bidistilled in a Heraeus-Amersil (Sayreville, NJ) quartz still. We obtained similar electrophoretic mobility results with recrystallized uranyl nitrate and samples supplied by K & K Laboratories or Fisher Scientific Co. We checked the concentration of the uranyl nitrate stock solutions by measuring their conductivity.

Fresh human erythrocytes were obtained from a lancet puncture (S. McLaughlin). As noted by Bangham and Pethica (1959), it is necessary to wash the cells extensively to remove all traces of phosphate before adding uranyl ions. The presence of even  $10^{-6}$  M phosphate in the 0.15 M NaCl (pH 5) solution bathing the erythrocytes significantly increased the effect of  $10^{-4}$  M uranyl on the mobility. We washed the erythrocytes by hand-centrifuging the cells and resuspending them five times in 0.15 M NaCl, 1 mM

<sup>3</sup> Eq. 8 is identical to Eq. 26 of Wunderlich (1982); it can also be obtained from the more general Eq. 22 of Levine et al. (1983) by letting the viscous drag term approach zero (and correcting a typographical error of a minus sign in front of the first term in the large set of parentheses).

MOPS, pH 7.4. The original volume was diluted by a factor of  $\sim 10^7$ . This washing procedure did not significantly change the electrophoretic mobility measured at  $5 < \text{pH} < 7.4$  in 0.15 M NaCl in the absence of uranyl ions.

Multilamellar vesicles were prepared according to Bangham et al. (1974). PC, GMO, and defined  $\text{GMO}/\text{G}_{\text{M1}}$ ,  $\text{PC}/\text{G}_{\text{M1}}$ ,  $\text{PC}/\text{PS}$ , or  $\text{PC}/\text{G}_{\text{M1}}/\text{DDDA}$  mixtures were vacuum-dried with a Haake Buchler Instruments (Fort Lee, NJ) flash evaporator in round-bottomed glass flasks. We used the following molecular weights in our calculations:  $\text{G}_{\text{M1}}$ , 1,545; PS, 832; PC, 786; GMO, 356; DDDA, 463. Electrophoretic mobilities were measured at 25°C with Rank Brothers (Bottisham, Cambridge, UK) Mark I and Mark II machines as described by Cafiso et al. (1986). We focused at the stationary layer (Henry, 1938) and obtained identical results with both machines. The zeta potential was calculated from the electrophoretic mobility using Eq. 7, the Helmholtz-Smoluchowski relation. In the derivation of this equation, it is assumed that the particle is large, the surface is smooth, and the charges are located at the membrane-solution interface (e.g., Overbeek and Wiersema, 1967; O'Brien and White, 1978). Thus, the use of this equation is appropriate with PC, GMO, and PC/PS membranes, where the zeta potential is, by definition, the electrostatic potential at the hydrodynamic plane of shear (Verwey and Overbeek, 1948, p. 48). In a decimolar salt solution, the plane of shear is  $\sim 0.2$  nm from the bilayer surface (Eisenberg et al., 1979; Alvarez et al., 1983; Rooney et al., 1983). Although there is no simple relationship between the electrophoretic mobility and the electrostatic potential at the plane of shear for  $\text{PC}/\text{G}_{\text{M1}}$  vesicles and erythrocytes, we used Eq. 7 to calculate an "apparent" zeta potential from the electrophoretic mobility.

The horizontal bars through the points in Fig. 2 represent the changes in pH that occurred during the experiments. The vertical bars in all graphs represent the standard deviations of at least 20 measurements; standard deviations smaller than the size of the symbol are not illustrated. We checked that measurements made on erythrocytes and negatively charged vesicles in the absence of uranyl nitrate were not influenced by trace concentrations of divalent contaminants in the aqueous solutions by repeating the measurements in the presence of EDTA.

We measured the nonactin-induced  $\text{K}^+$  conductance of planar bilayer membranes to deduce the effect of  $\text{UO}_2^{2+}$  on the electrostatic potential at the surface of PC, PC/PS, and  $\text{PC}/\text{G}_{\text{M1}}$  membranes. The technique is described in detail elsewhere (McLaughlin et al., 1971; Cafiso et al., 1986). In brief:

$$G/G_{\text{PC}} = \exp(-F\Delta\psi/RT), \quad (9)$$

where  $G_{\text{PC}}$  is the conductance of the PC membrane in the absence of  $\text{UO}_2^{2+}$ ,  $G$  is the membrane conductance,  $\Delta\psi$  is the difference between the electrostatic potential within the membrane under consideration and the PC membrane,  $R$  is the gas constant,  $T$  is the temperature, and  $F$  is the Faraday constant.

Sonicated unilamellar vesicles were prepared for the fluorescence experiments as described by Eisenberg et al. (1979) and Barenholz et al. (1977). Triton X-100 micelles containing 1 mol % 2,6-HNS were formed by drying a mixture in chloroform under nitrogen, adding a solution containing 0.15 M NaCl, 0.01 M MOPS, pH 5, and mixing vigorously. Fluorescence intensities were measured (Spex Fluorocomp, Metuchen, NJ) at 305-nm excitation and 410-nm emission wavelengths. Quenching experiments were performed at 25°C. A cuvette containing sonicated vesicles (25  $\mu\text{M}$ ) or micelles (0.5 mM) was titrated with aliquots of uranyl nitrate from stock solutions; NaOH was added when necessary to maintain a pH of  $\sim 5$ . The effect of uranyl nitrate on fluorescence is expressed as

$$\% \text{ quenching} = [(F_0 - F)/F_0] \times 100\%, \quad (10)$$

where  $F_0$  and  $F$  are the observed fluorescence intensities in the absence and presence of  $\text{UO}_2^{++}$ , respectively.

### RESULTS

Fig. 2 illustrates the effect of uranyl nitrate on the zeta potential of multilamellar vesicles formed from egg PC. In the absence of uranyl nitrate (circles), the zeta potential of PC vesicles is zero for  $4 < \text{pH} < 8$ . In the presence of 0.1 mM uranyl nitrate (squares), the zeta potential is positive for  $\text{pH} < 6$  and negative for  $\text{pH} > 6$ . We obtained similar results with 0.15 M NaCl solutions containing 10 mM Tris (solid squares), 0.01 mM MOPS (open squares), or no buffer (data not

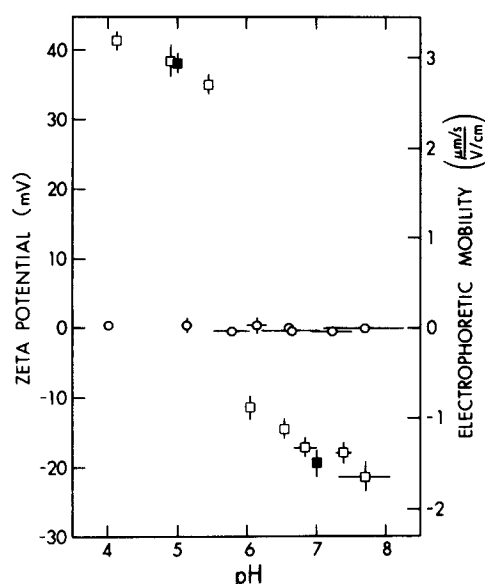


FIGURE 2. The effect of uranyl nitrate on the electrophoretic mobility of multilamellar vesicles formed from egg PC. The aqueous solutions contained 0.15 M NaCl (circles) or 0.15 M NaCl and 0.1 mM uranyl nitrate (squares). The solutions were buffered to the indicated pH at 25°C with either 0.01 mM MOPS (open squares) or 10 mM Tris (solid squares). The vertical bars through the experimental points indicate the standard deviations of at least 20 measurements.

shown). Lower concentrations of uranyl nitrate also produce positive zeta potentials for PC vesicles in acid solutions (see Appendix I).

The positive zeta potentials observed at  $\text{pH} < 6$  are due to the adsorption of the  $\text{UO}_2^{++}$  ion to the phosphate groups of PC. In Appendix I, we present evidence that the strong adsorption of  $\text{UO}_2^{++}$  to PC is due to the cooperative binding of one  $\text{UO}_2^{++}$  ion to the phosphate groups of more than one PC molecule.

The surprising feature of Fig. 2 is the negative zeta potential produced by 0.1 mM uranyl nitrate for  $\text{pH} > 6$ . We do not understand this result. We note, however, that uranyl nitrate exists in many forms in aqueous solutions (Chernyaev, 1966; Baes and Mesmer, 1976) and the result could be due to a



negatively charged complex of uranyl and hydroxide ions adsorbing or precipitating onto the vesicles.

We also studied the effect of uranyl nitrate on the electrophoretic mobility of vesicles formed from mixtures of PC and PS. The zeta potential of PC/PS vesicles (9.6 mol % PS) is  $-13.8 \pm 0.5$  ( $n = 20$ ) mV at pH 5; the addition of 0.1 mM uranyl nitrate produces a zeta potential of  $+36.9 \pm 1.3$  ( $n = 20$ ). Thus,  $\text{UO}_2^{2+}$  affects the electrophoretic mobility of PC and PC/PS vesicles similarly.

We had used membranes containing the ganglioside  $\text{G}_{\text{M1}}$  to mimic the electrophoretic properties of biological membranes (McDaniel et al., 1984, 1986) and were interested in the effect of uranyl nitrate on vesicles containing this lipid. We suspected that  $\text{UO}_2^{2+}$  would not adsorb significantly to  $\text{G}_{\text{M1}}$  because the ganglioside has no phosphate groups. Although we could not study vesicles formed from the ganglioside alone—it forms micelles rather than vesicles—we could study vesicles formed from mixtures of the ganglioside and GMO. The charged forms of uranyl do not adsorb to GMO: the zeta potential of GMO vesicles (zero at pH 5 and 7.5) is unchanged by addition of 0.1 mM uranyl nitrate (data not shown). The apparent zeta potentials of GMO/ $\text{G}_{\text{M1}}$  (5:2 wt/wt) vesicles are identical at pH 5 ( $-13.2 \pm 1.5$  mV) and 7.4 ( $-13.0 \pm 1.8$  mV) and are unchanged by the addition of 0.1 mM uranyl nitrate, which demonstrates that the charged forms of uranyl do not adsorb to  $\text{G}_{\text{M1}}$ .

The zeta potential of PC/ $\text{G}_{\text{M1}}$  vesicles (16.9 mol %  $\text{G}_{\text{M1}}$ ) is  $-11$  mV at both pH 5 and 7.5 (Table I). The addition of 0.1 mM uranyl nitrate reverses the mobility of the PC/ $\text{G}_{\text{M1}}$  vesicles at pH 5 (Table I). This reversal does not depend on the nature of the buffer and is due to the adsorption of the  $\text{UO}_2^{2+}$  ion to the phosphate group of PC. In Appendix II, we present evidence that  $\text{UO}_2^{2+}$  adsorbs comparably to PC, PC/PS, and PC/ $\text{G}_{\text{M1}}$  bilayer membranes and produces similar changes in the electrostatic potential within these membranes ( $\Delta\psi = 66, 74,$  and  $63$  mV, respectively). Table I and the data reported above illustrate that  $\text{UO}_2^{2+}$  ions adsorbed to the bilayer surface have a much smaller effect on the mobility of PC/ $\text{G}_{\text{M1}}$  vesicles than either PC or PC/PS vesicles.

We measured the effect of uranyl nitrate on the electrophoretic mobility of fresh human erythrocytes. The cells were washed extensively to remove phosphate from the solution (see Materials and Methods). In the absence of uranyl nitrate, the apparent zeta potential of the red blood cells in 0.15 M NaCl is similar at pH 5 ( $-12.4 \pm 0.7$  mV,  $n = 30$ ) and 7.4 ( $-14.0 \pm 0.9$  mV,  $n = 20$ ). This observation agrees with the results previously obtained with human (Heard and Seaman, 1960; Seaman and Heard, 1960) and sheep (Bangham et al., 1958) red blood cells. The addition of 0.1 mM uranyl nitrate to the 0.15 M NaCl solution changes the apparent zeta potential of the erythrocytes to  $-4.1 \pm 1.5$  mV ( $n = 60$ ) at pH 5, but has no significant effect at pH 7.4 ( $-14.7 \pm 1.7$  mV,  $n = 20$ ).

We summarize our  $\text{UO}_2^{2+}$  results obtained with model membranes and erythrocytes at pH 5 in Fig. 3, which represents the electrophoretic mobility as an apparent zeta potential calculated from Eq. 7. In the absence of uranyl nitrate, the electrophoretic mobility of erythrocytes can be mimicked by mixing either 17 mol %  $\text{G}_{\text{M1}}$  or 10 mol % PS with PC (striped bars). (The charge per area of

TABLE I

*Effect of Uranyl Nitrate on the Apparent Zeta Potential of Vesicles Formed from a Mixture of Egg PC and 17 Mol %  $G_{M1}$  in 0.15 M NaCl at 25°C*

[Uranyl nitrate]	Apparent zeta potential		Buffer
	pH 5	pH 7.5	
<i>mM</i>	<i>mV</i>	<i>mV</i>	
0	-11.1±1.1	-11.4±0.8	10 mM Tris
0	-11.2±1.4	-11.1±1.0	10 mM Tris, 0.1 mM EDTA
0	-11.7±1.6	-11.5±1.0	0.01 mM MOPS
0	-11.8±1.4	-11.3±1.3	0.01 mM MOPS, 0.1 mM EDTA
0.1	+5.3±1.0	-15.3±1.4	10 mM Tris
0.1	+5.5±0.7	-11.1±1.2	0.01 mM MOPS

Data are expressed as means  $\pm$  standard deviation for 20 determinations.

erythrocytes [Levine et al., 1983] is similar to that of the PC/ $G_{M1}$  vesicles.) It is apparent from Fig. 3 (stippled bars) that the addition of 0.1 mM uranyl affects the electrophoretic mobility of the PC and PC/PS vesicles strongly, the mobility of PC/ $G_{M1}$  vesicles moderately, and the mobility of erythrocytes only weakly.

We did two control experiments to demonstrate that this result is due to the location of the positive charge and not to some peculiar chemical property of

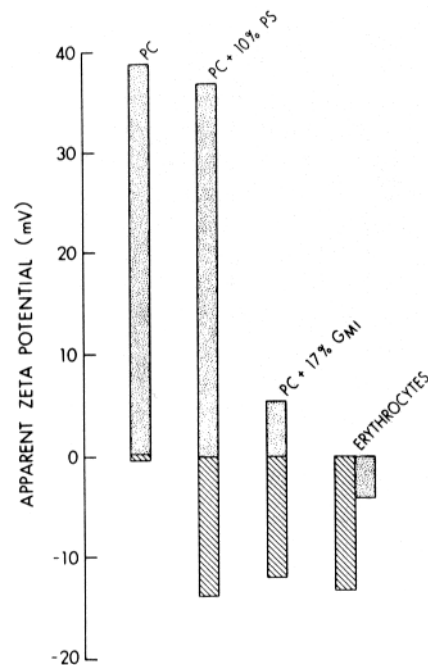


FIGURE 3. The effect of uranyl nitrate on the electrophoretic mobility of PC, PC/PS, and PC/ $G_{M1}$  vesicles and human erythrocytes at pH 5,  $T = 25^\circ\text{C}$ . The solutions contained 0.15 M NaCl, 0.01 mM MOPS, and either no uranyl (striped bars) or 0.1 mM uranyl nitrate (stippled bars). Electrophoretic mobility is expressed as apparent zeta potential, calculated from Eq. 7.

the  $\text{UO}_2^{2+}$  ion. First, we added positive charge to the surface of the model membranes by mixing in the positive lipid DDDA. The results (Fig. 4) are similar to those produced by the adsorption of  $\text{UO}_2^{2+}$ . Specifically, the addition of DDDA to PC or PC/PS vesicles produces a large increase in the zeta potential, but the addition of DDDA to PC/ $G_{M1}$  vesicles produces only a moderate increase in the apparent zeta potential.

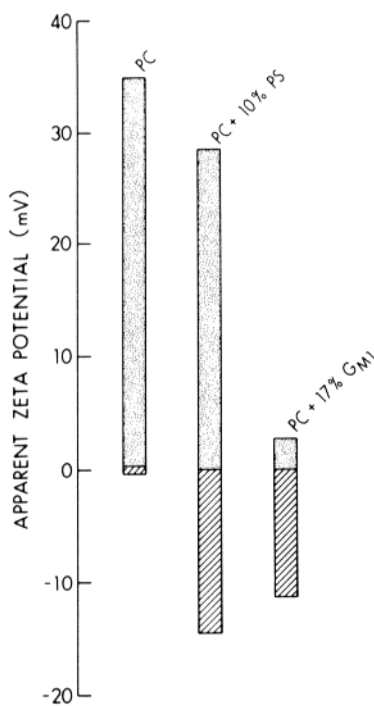


FIGURE 4. The effect of 40 mol % positive lipid (DDDA) on the electrophoretic mobility of vesicles formed from PC, PC/PS, or PC/ $G_{M1}$ . The aqueous solutions contained 0.15 M NaCl buffered to pH 7 with 0.01 M MOPS at 25°C. The results illustrate the apparent zeta potentials when the vesicles are formed from the indicated mixture of lipid (striped bars) or from a mixture where 40 mol % PC is replaced by DDDA (stippled bars).

Second, we exposed the vesicles or erythrocytes to tetracaine. This cation adsorbs hydrophobically to bilayer membranes (e.g., McLaughlin, 1975). The results (Fig. 5) are similar to those produced by  $\text{UO}_2^{2+}$ . Specifically, the addition of tetracaine produced a large increase in the zeta potential of PC and PC/PS vesicles, a moderate increase in the apparent zeta potential of PC/ $G_{M1}$  vesicles, and only a 0.5-mV increase in the apparent zeta potential of the erythrocytes. The last result agrees quantitatively with the predictions of the new theories: positive charges at the surface of the erythrocyte should have very little effect on the electrophoretic mobility.

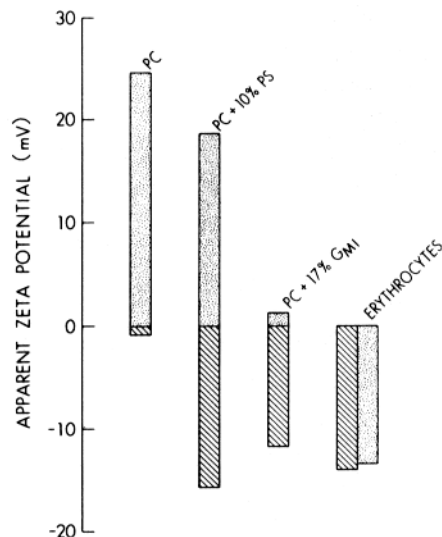


FIGURE 5. The effect of tetracaine on the electrophoretic mobility of PC, PC/PS, and PC/G<sub>M1</sub> vesicles and human erythrocytes at pH 7.4,  $T = 25^\circ\text{C}$ . The solutions contained 0.15 M NaCl, 1 mM MOPS, and either no tetracaine (striped bars) or 10 mM tetracaine (stippled bars). Electrophoretic mobility is expressed as apparent zeta potential, calculated from Eq. 7.

#### DISCUSSION

Our main results are summarized in Fig. 3. On the one hand, 0.1 mM  $\text{UO}_2^{++}$  has a large effect on the electrophoretic mobility of PC and PC/PS vesicles, producing zeta potentials of about +40 mV. There is a simple relationship (Eq. 7) between the mobility of phospholipid vesicles and the zeta potential, the potential at the plane of shear, because all the charges are in a plane at the membrane-solution interface and no groups protrude from the surface to exert hydrodynamic drag. On the other hand, 0.1 mM  $\text{UO}_2^{++}$  has only moderate or weak effects on the electrophoretic mobility of PC/G<sub>M1</sub> vesicles and erythrocytes, producing apparent zeta potentials of +5 and -4 mV. There is no simple relationship between the electrophoretic mobility of these particles and the potential at the surface of the membrane.

Three lines of evidence, which we discuss in more detail in Appendix II, suggest that  $\text{UO}_2^{++}$  adsorbs to the same degree and produces the same positive surface potential on the bilayer membranes that we used. First, our PC/PS and PC/G<sub>M1</sub> membranes consist mainly of PC (>80%) and it is reasonable to assume that  $\text{UO}_2^{++}$  produces the same surface potential on these membranes as on PC bilayers. For example, the addition of 20 mol % neutral lipid to a PC bilayer has little effect on the ability of  $\text{UO}_2^{++}$  to adsorb to the membrane. Second,  $\text{UO}_2^{++}$  produces similar quenching of the fluorescence from a probe located at the surface of the PC and PC/G<sub>M1</sub> membranes. Third, 0.1 mM  $\text{UO}_2^{++}$  produces similar decreases in the nonactin-induced  $\text{K}^+$  conductances of PC, PC/PS, and PC/G<sub>M1</sub> planar bilayer membranes, which indicates that it produces similar changes in the electrostatic potential within the membranes.

It is more difficult to demonstrate that  $\text{UO}_2^{++}$  produces the same positive surface potential on model and erythrocyte membranes. However, the outer monolayer of the human erythrocyte consists mainly of PC and SM (Op den Kamp, 1979; Etemadi, 1980; Van Deenen, 1981; Lin et al., 1983), and  $\text{UO}_2^{++}$  adsorbs equally well to PC and PC/SM membranes (Appendix II). Thus, the available evidence suggests that  $\text{UO}_2^{++}$  adsorbs to the same degree and produces the same positive surface potential on the one biological and three model membranes we studied. Furthermore, our experiments with DDDA and tetracaine confirm that adding positive charges to the interface of PC/ $G_{M1}$  bilayers (Figs. 4 and 5) and erythrocytes (Fig. 5) produces smaller changes in the electrophoretic mobility than adding positive charges to the interface of PC or PC/PS membranes.

The new theoretical models suggest two reasons for the differential effects of  $\text{UO}_2^{++}$ , DDDA, and tetracaine on the electrophoretic mobility of the membranes illustrated in Figs. 3–5: a hydrodynamic drag and a location-of-charge effect. Our results are consistent with these new models.

In Fig. 6, we sketch the profiles of electrostatic potential predicted by the Poisson-Boltzmann equation for the different model membranes. The solid line in Fig. 6A illustrates the potential profile adjacent to a PC membrane. The zeta potential of PC vesicles is zero for  $\text{pH} > 4$  (Fig. 2). For a simple phospholipid surface, the zeta potential is the potential at the hydrodynamic plane of shear, which is 0.2 nm from the surface (Eisenberg et al., 1979; Alvarez et al., 1983; Rooney et al., 1983); the potential is zero at distances  $> 0.2$  nm from the surface of a PC membrane. The dotted curve in Fig. 6A illustrates the potential profile adjacent to a PC membrane exposed to 0.1 mM  $\text{UO}_2^{++}$ . The profile is drawn according to the nonlinear Gouy-Chapman theory (Poisson-Boltzmann equation), assuming the surface potential is +66 mV, the value deduced from the conductance measurements illustrated in Fig. 10. The Debye length in 0.15 M NaCl is 0.78 nm and the potential decays in an approximately exponential manner with distance.<sup>4</sup> From Fig. 6A, the potential 0.2 nm from the surface is predicted to be 48 mV, a number that agrees qualitatively with the experimentally determined value of the zeta potential (see Fig. 3). A similar profile is predicted theoretically for the PC/DDDA membrane.

The solid curve in Fig. 6B illustrates the profile of the electrostatic potential adjacent to the PC/PS membrane. The dotted curve illustrates the profile upon exposure to 0.1 mM  $\text{UO}_2^{++}$ . The profiles are drawn according to the Gouy-Chapman theory assuming surface potentials of -22 and +52 mV, values deduced from the conductance measurements illustrated in Fig. 10. The predicted potentials 0.2 nm from the membrane (-17 and 39 mV) agree well with the experimentally determined zeta potentials illustrated in Fig. 3 (-14 and 37 mV).

The solid curve in Fig. 6C illustrates the profile of the electrostatic potential adjacent to the PC/ $G_{M1}$  membrane. We calculated the profile from the nonlinear

<sup>4</sup> Although all the potential profiles in Fig. 6 are drawn using the nonlinear Poisson-Boltzmann equation, there is little difference between the shape of these profiles and those deduced from the linear theory.

Poisson-Boltzmann equation by assuming all the charges on a PC/ $G_{M1}$  membrane are 1 nm from the surface, an assumption consistent with the available nuclear magnetic resonance, electron spin resonance, fluorescence, conductance (McDaniel et al., 1984), and direct X-ray diffraction (McDaniel and McIntosh, 1986) results. There are no fixed charges at the membrane-solution interface, and

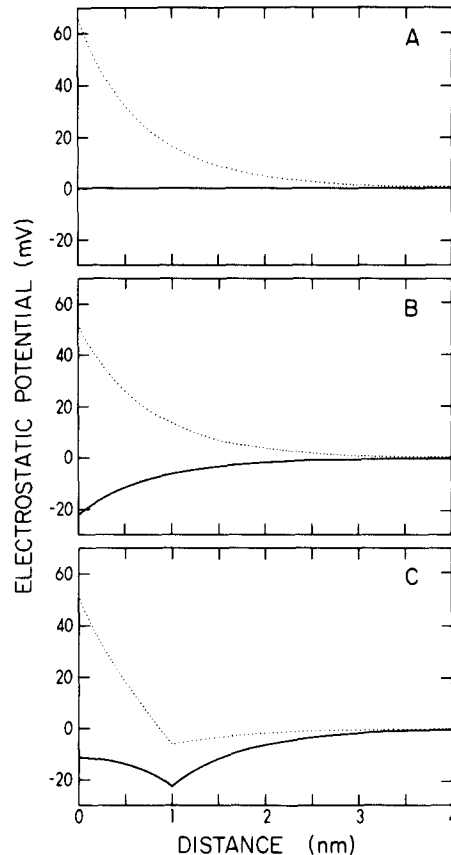


FIGURE 6. Sketches of the potential profiles adjacent to bilayer membranes predicted by the theory of the diffuse double layer (nonlinear Poisson-Boltzmann equation). The solid curves illustrate the potential profile adjacent to PC (A), PC/PS (10 mol % PS) (B), and PC/ $G_{M1}$  (17 mol %  $G_{M1}$ ) (C) membranes when the solutions contain 0.15 M NaCl. The dotted curves illustrate the effect of adding 0.1 mM  $UO_2^{++}$  to the aqueous phase. See text for details.

Gauss's law predicts that the slope of the solid curve in Fig. 6C must be zero at the surface of the membrane. The theoretically predicted value of the surface potential is  $-11$  mV. The dotted curve in Fig. 6C illustrates the profile adjacent to the PC/ $G_{M1}$  membrane upon exposure to 0.1 mM  $UO_2^{++}$ . To calculate this profile, we assumed that the charge density 1 nm from the surface corresponds to 17 mol % negative lipid and that the potential at the surface changes by 63

mV (the change in potential determined from the conductance measurements illustrated in Fig. 10) to +52 mV on addition of  $\text{UO}_2^{2+}$ . The Poisson-Boltzmann equation predicts that the potential falls in an approximately linear manner between the surface and the charge plane located at 1 nm, where it attains a negative value. It decays exponentially to zero for distances  $>1$  nm.

Fig. 7 illustrates the electro-osmotic velocity profiles adjacent to fixed PC/ $G_{M1}$  membranes produced by a unit electric field parallel to the membranes. The model used to calculate the velocity profiles is described elsewhere (Levine et

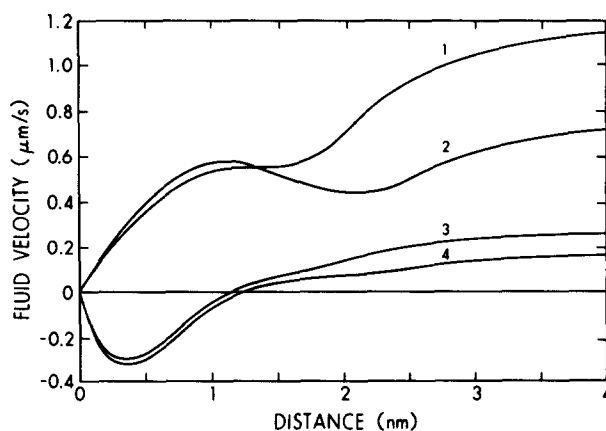


FIGURE 7. Sketches of the electro-osmotic velocity profiles adjacent to fixed bilayer membranes predicted from a combination of the Navier-Stokes and nonlinear Poisson-Boltzmann equations. The bilayer is comprised of a mixture of PC and 17 mol %  $G_{M1}$ , and the aqueous phase contains 0.15 M NaCl (curves 1 and 2) or 0.15 M NaCl plus 0.1 mM  $\text{UO}_2^{2+}$  (curves 3 and 4). The  $\text{UO}_2^{2+}$  ions impart a surface charge density of 0.39 electronic charges/nm<sup>2</sup>. We assume that: the  $G_{M1}$  headgroups protrude 2.0 (curves 1 and 3) or 2.5 nm (curves 2 and 4) from the bilayer surface, five independent spherical elements of radius 0.33 nm distributed uniformly through the headgroup region exert frictional drag, and the negatively charged sialic acid residues are located 1.0 nm from the bilayer surface. The potential profile corresponding to curves 1 and 2 is illustrated by the continuous line in Fig. 6C; the potential corresponding to curves 3 and 4 is illustrated by the dotted line in Fig. 6C.

al., 1983; McDaniel et al., 1986). In the steady state, the force exerted by the electric field,  $E$ , on a volume of fluid  $Adx$  located a distance  $x$  from the surface is balanced by the net frictional force on the volume element. The electric force is  $E\rho Adx$ , where  $\rho$  is the space charge density calculated from the potential profiles in Fig. 6C using the Boltzmann relation. The net frictional force is the sum of the shear force, which is proportional to the second derivative of the velocity (see Eq. 3), and the frictional drag exerted by the headgroups, which is proportional to the velocity. Curves 1 and 2 illustrate the velocity profiles if we assume the headgroups extend 2 and 2.5 nm, respectively, from the surface. The velocities far from the surface correspond to apparent zeta potentials (see

Eq. 6) of  $-15$  and  $-10$  mV, values that encompass the experimentally observed value of  $-11$  mV. The theoretically predicted values of the apparent zeta potential upon addition of  $0.1$  mM  $\text{UO}_2^{2+}$  are  $-3.5$  (line 3) and  $-2.2$  mV (line 4), which can be compared to the experimental value of  $+5$  mV. Thus, the model can account qualitatively for the experimental observation that  $\text{UO}_2^{2+}$  has a smaller effect on the electrophoretic mobility of PC/ $G_{M1}$  vesicles than on the mobility of either PC or PC/PS vesicles (Fig. 3).

The fixed negative charges on an erythrocyte are mainly on sialic acid residues (e.g., Levine et al., 1983), but their distribution is not known with any certainty (e.g., Viitala and Jarnefelt, 1985). Thus, one can only speculate about the profile of the electrostatic potential adjacent to these membranes (e.g., Heinrich et al., 1982). It is also not clear how one should treat the viscous drag exerted by the complicated macromolecules that constitute the glycocalyx; Wunderlich (1982) discusses some of the difficulties with the existing models. In spite of these uncertainties, the new theoretical models can account qualitatively for the effects of uranyl and tetracaine on the electrophoretic mobility of erythrocytes.<sup>5</sup> The new models predict that the apparent zeta potential should change  $\sim 0.5$  mV upon addition of uranyl or tetracaine; in fact, it changes  $8$  and  $0.5$  mV, respectively.

We have used PC/ $G_{M1}$  vesicles as models of erythrocytes. Of course, the sugars that exert hydrodynamic drag and the sialic acid residues that bear negative charges protrude farther from the bilayer-solution surface of an erythrocyte than from the surface of a PC/ $G_{M1}$  vesicle. The glycocalyx of a human erythrocyte extends  $5$ – $10$  nm from the surface (e.g., Levine et al., 1983); the headgroups of  $G_{M1}$  protrude only  $2$ – $2.5$  nm from the surface of a PC/ $G_{M1}$  vesicle. With this reservation, the results illustrated in Figs. 3 and 5 demonstrate that PC/ $G_{M1}$  membranes are useful models for studying the electrokinetic properties of biological membranes.

In conclusion, the new theoretical models are more successful than the Helmholtz-Smoluchowski equation in describing the effect of positive surface charge on the mobility of PC/ $G_{M1}$  vesicles and erythrocytes.

#### APPENDIX I

We discuss here two lines of evidence that  $\text{UO}_2^{2+}$  forms complexes with more than one PC molecule. First, if we assume that  $\text{UO}_2^{2+}$  forms only 1:1 complexes with PC, we must assume that its intrinsic association constant is  $\sim 10^6$   $\text{M}^{-1}$  to account for the effect of  $\text{UO}_2^{2+}$  on the zeta (Fig. 8) and surface potential (Fig. 10). (The PC results illustrated in Fig. 10 agree qualitatively with those obtained previously by Ginsburg and Wolosin [1979] and Ting-Beall [1980].) However, the association constant of  $\text{UO}_2^{2+}$  for  $\text{H}_2\text{PO}_4^-$  in solution is only  $3 \times 10^3$   $\text{M}^{-1}$  (Thamer, 1957). Thus,  $\text{UO}_2^{2+}$  binds two to three orders of magnitude

<sup>5</sup> If  $\text{UO}_2^{2+}$  and tetracaine adsorb to the same degree to the phospholipids in erythrocytes and phospholipid vesicles, do not adsorb to the sugars and proteins in the erythrocyte membrane, and produce no change in the structure of the glycocalyx, the new theoretical models that assume the charge is distributed uniformly throughout the glycocalyx (Levine et al., 1983; Sharp and Brooks, 1985) predict that  $0.1$  mM  $\text{UO}_2^{2+}$  and  $10$  mM tetracaine should change the apparent zeta potential by only  $\sim 0.5$  mV, from  $-15$  to  $-14.5$  mV. (C. Oliva solved numerically the combination of the nonlinear Poisson-Boltzmann and Navier-Stokes equations using an IMSL Inc. [Houston, TX] subroutine.)



more strongly to PC membranes than to isolated phosphate groups. The simplest explanation of this result is that  $\text{UO}_2^{2+}$  binds to more than one phosphate group in the PC membrane.

Second, when we replaced a significant fraction of the PC molecules in the bilayer membrane with a phosphate-free neutral lipid such as cholesterol (C), GMO, or MGDG, which do not bind  $\text{UO}_2^{2+}$ , the binding of  $\text{UO}_2^{2+}$  to the membrane decreased markedly. Fig. 8 illustrates that when membranes are formed from 1:2 (mol/mol) mixtures of PC/GMO, PC/C, or PC/MGDG instead of PC, the  $[\text{UO}_2^{2+}]$  required to produce a half-maximal effect on the electrophoretic mobility increases  $\sim 30$ -fold, from  $10^{-6}$  M to  $3 \times 10^{-5}$  M. This observation strongly suggests that several PC molecules are involved in the binding process. Sophisticated adsorption isotherms are required to describe such binding (e.g., Cohen and Cohen, 1981), and we have not attempted to describe the absorption theoretically.

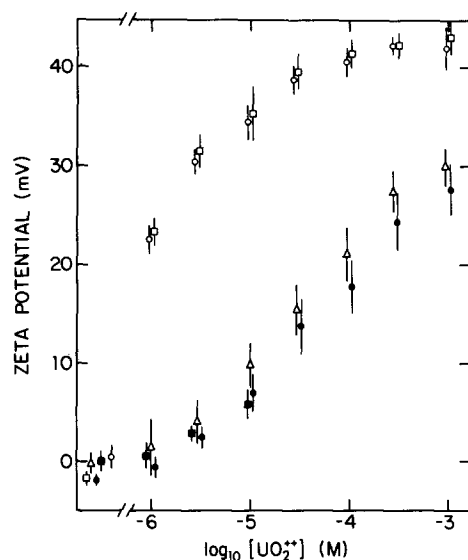


FIGURE 8. The effect of  $\text{UO}_2^{2+}$  on the zeta potential of vesicles formed from PC (open circles), PC/SM (50 mol % SM, open squares), PC/GMO (65 mol % GMO, triangles), PC/C (65 mol % C, solid circles), and PC/MGDG (65 mol % MGDG, solid squares). The aqueous solutions contained 0.15 M NaCl buffered to pH 5 at 25°C with 1 mM MOPS.

The suggestion by Barton (1968) that  $\text{UO}_2^{2+}$  interacts with four PC molecules seems reasonable to us. He noted that  $\text{UO}_2^{2+}$  combines with four phosphates in an approximately coplanar manner in the mineral autunite (Beintema, 1938) and suggested that  $\text{UO}_2^{2+}$  might interact in the same manner with phospholipids in a bilayer.

#### APPENDIX II

We present evidence that  $\text{UO}_2^{2+}$  produces similar positive surface potentials on PC membranes, PC membranes containing either 10 mol % PS or 17 mol %  $\text{G}_{\text{M1}}$ , and erythrocytes. The zeta potential results in Fig. 3 demonstrate that  $\text{UO}_2^{2+}$  produces similar electrostatic surface potentials on PC and PC/PS membranes. We made three different types of measurements which suggest that adding 17 mol %  $\text{G}_{\text{M1}}$  to PC does not greatly

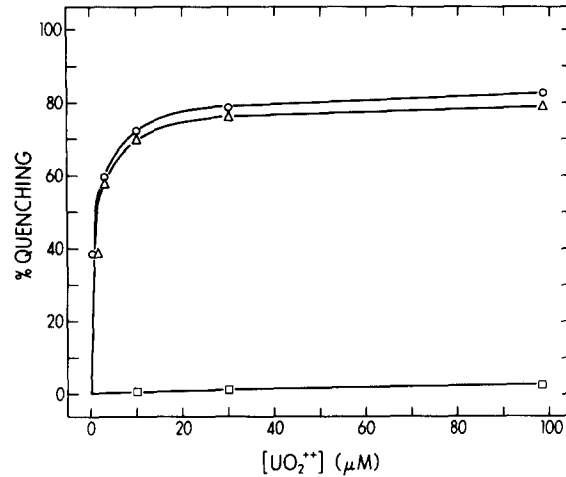


FIGURE 9. The effect of uranyl nitrate on the fluorescence of 2,6-HNS (1 mol %) in micelles of Triton X-100 (squares) and in sonicated unilamellar vesicles comprised of either PC (circles) or PC/G<sub>M1</sub> (20 mol % G<sub>M1</sub>, triangles). The percent quenching is calculated from fluorescence intensities (data not shown) using Eq. 10. The aqueous solutions contained 0.15 M NaCl buffered to pH 5 at 25°C with 0.01 mM MOPS.

reduce the adsorption of UO<sub>2</sub><sup>++</sup> ions. First, we reduced the surface concentration of PC, i.e., we increased the area per PC molecule, ~20% by adding neutral lipids that do not bind UO<sub>2</sub><sup>++</sup>. This did not greatly decrease the ability of UO<sub>2</sub><sup>++</sup> to bind to the membrane. For example, when we formed membranes by mixing 20 mol % MGDG, 38 mol % GMO,

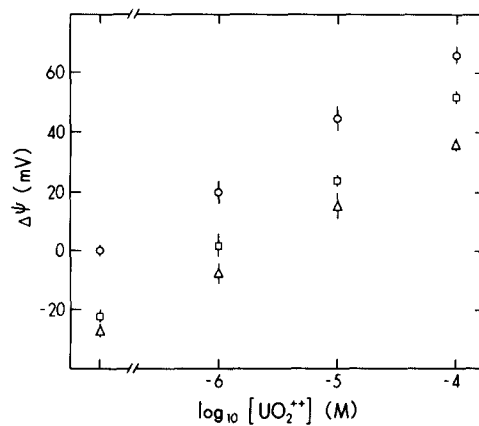


FIGURE 10. The effect of UO<sub>2</sub><sup>++</sup> on the electrostatic potential within a planar phospholipid bilayer membrane. Δψ is the potential within the membrane relative to a PC membrane in a solution without UO<sub>2</sub><sup>++</sup>. Δψ was estimated from the nonactin-induced K<sup>+</sup> conductance using Eq. 9. The aqueous solutions contained 0.1 M NaCl and 0.05 M KCl, pH 5. The decane-containing bilayer membranes were formed from PC (circles), PC/PS (10 mol % PS, squares), and PC/G<sub>M1</sub> (17 mol % G<sub>M1</sub>, triangles).

or 40 mol % C with PC and exposed them to 0.1 mM  $\text{UO}_2^{++}$ , we measured zeta potentials of  $36 \pm 2$  mV, which are only 10% smaller than the zeta potential observed with PC membranes (Figs. 3 and 8). Thus, there is no reason to suspect that 17 mol % of the negatively charged lipid  $G_{M1}$  significantly impedes the adsorption of  $\text{UO}_2^{++}$  to PC.

Second, we added a low concentration of the fluorescent probe 2,6-HNS to PC and to PC/ $G_{M1}$  membranes and studied the ability of  $\text{UO}_2^{++}$  to quench the fluorescence. The available evidence suggests that the chromophore of 2,6-HNS (our unpublished experiments) and the octadecyl version of this probe (Waggoner and Stryer, 1970) are at the membrane-solution interface. The quenching in PC membranes increases dramatically for  $0 < \text{UO}_2^{++} < 10 \mu\text{M}$  (Fig. 9); the zeta potential of egg PC multilamellar vesicles also increases markedly over this concentration range. The simplest interpretation is that the degree of fluorescence quenching reflects the amount of  $\text{UO}_2^{++}$  bound to the membrane surface, an interpretation that agrees with that of Renthal and Cha (1984). Fig. 9 illustrates that  $\text{UO}_2^{++}$  quenches the PC/ $G_{M1}$  and PC membranes about equally, which suggests that it adsorbs equally well to the two membranes. The lower curve in Fig. 9 is a control experiment which shows that  $\text{UO}_2^{++}$  does not significantly quench the fluorescent probe when it is in Triton X-100 micelles.

Third, we measured the effect of  $\text{UO}_2^{++}$  on the nonactin-induced  $\text{K}^+$  conductance of diphtanoylphosphatidylcholine (PC) membranes and PC membranes containing either 10 mol % PS or 17 mol %  $G_{M1}$ . Fig. 10 illustrates that 0.1 mM  $\text{UO}_2^{++}$  produces similar changes in the surface potential (66, 74, and 63 mV, respectively) of these membranes.

Finally, we wanted to check that a bilayer membrane formed from a mixture of PC and SM would bind  $\text{UO}_2^{++}$  to the same degree as a PC membrane, because the outer monolayer of a human erythrocyte consists of a mixture of PC and SM. The data in Fig. 8 illustrate that  $\text{UO}_2^{++}$  binds equally well to PC and PC/SM membranes.

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