# VOLTAGE-DEPENDENT CAPACITANCE IN LIPID BILAYERS MADE FROM MONOLAYERS

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ABSTRACT Electrocompression has been measured in lipid bilayers made by apposition of two monolayers. The capacitance $C(V)$, as a function of membrane potential, $V$, was found to be well described by $C(V) = C(0)\,[1 + \alpha(V + \Delta\psi)^2]$ where $C(0)$ is the capacitance at $V = 0$, $\alpha$ is the fractional increase in capacitance per square volt, and $\Delta\psi$ is the surface potential difference. In lipid bilayers made from monolayers $\alpha$ has a value of 0.02 $V^{-2}$, which is ca. 500-fold smaller than the value found in solvent containing membranes. In asymmetric bilayers made of one neutral and one negatively charged monolayer, $\Delta\psi$ values were found to be those expected from independent measurements of surface charge density. If the fractional increase in capacitance found here is a good approximation to that of biological membranes, nonlinear capacitative charge displacement derived from electrostriction is expected to be less than 1% of the total gating charge displacement found in squid axons.

## INTRODUCTION

Ionic permeability of excitable cell membranes is regulated by the membrane potential. As pointed out by Hodgkin and Huxley (1952), this voltage dependence should involve the movement of charged molecules or molecules with a dipole moment in the membrane field. "Gating currents" due to this charge movement have been observed in squid axon membrane and correlate well with changes in sodium membrane permeability (Armstrong and Bezanilla, 1973; Keynes and Rojas, 1973). In this type of study, voltage-dependent capacitance is taken as a measure of charge or dipole displacement and the lipid part of the membrane is assumed to be an ideal capacitor. The artificial lipid bilayer membrane is a good model system to estimate to what extent lipids deviate from ideal dielectrics. Several investigators have found that the capacitance of these membranes varies greatly with membrane potential, increasing ca. 10% for a potential of 100 mV (Rosen and Sutton, 1968; White, 1970; Wobschall, 1972; White and Thompson, 1973). This increase in capacitance of the bilayer is proportional to the square of the membrane potential, and arises as a consequence of a force generated

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across the dielectric when the membrane is charged. This nonlinear capacitance can influence the measurements of gating currents, as discussed by Blatt (1977). In artificial bilayers the mechanisms by which this nonlinear capacitance arises are complex and could involve *a*) formation of new membrane from the surrounding torus, *b*) exclusion of solvent from the membrane into the torus or microlenses, and *c*) membrane thinning at constant volume of the membrane. Among these mechanisms only the third would appear to have physiological relevance since biological membranes neither have solvent nor are in equilibrium with a torus. It is necessary therefore, to develop a technique to measure capacitance changes in which the solvent flow effects are minimal. Wobschall (1972) measured the voltage-dependent capacitance of cholesterol-hexadecyltrimethylammonium-dodecane membranes as a function of a sinusoidal voltage. The rationale of this method was to measure the change in membrane capacitance due to electrostriction before other changes can take place. He found that the capacitance changes were smaller with increasing the frequency, but over 10 Hz he found a constant limiting value ca. 0.06% of the capacitance increase at 100 mV.

Another method to minimize the interference of solvent flow is to prepare membranes by apposition to two lipid monolayers by the technique introduced by Montal and Mueller (1972). These membranes have a voltage-dependent capacitance below the sensitivity of the methods used previously (Benz et al., 1975; Benz and Janko, 1976). Since bilayers formed by the Montal and Mueller (1972) technique seem to be better models for biological membranes, we developed a more sensitive method to measure capacitance changes.

We present here the results of voltage-dependent capacitance measurements in membranes made by apposition of two monolayers. We show that the capacitance increases as a linear function of the square of the membrane potential, the same as in solvent-containing membranes, but the changes are much faster and three orders of magnitude smaller. Furthermore we show that the minimum of the capacitance occurs at zero potential in symmetric membranes, and at the difference in surface potential in asymmetric membranes. These facts provide a novel, model-independent method to estimate surface potential difference in membrane with asymmetric surface charges densities. Finally, we calculate that, if axon membranes behave like bilayers, the error introduced by electrocompression in measurements of gating charge displacement is not more than 1% in the conditions of the experiments of Armstrong and Bezanilla (1974).

## METHODS

### *Membrane Formation*

All membranes were formed by apposition of two separate monolayers spread at the air-solution interface by the technique described by Montal and Mueller (1972). The aqueous solutions in both compartments were equal and consisted of unbuffered NaCl or KCl at the indicated concentrations. All experiments were performed at 23 ± 2°C. The lipids were glycerol-monooleate (GMO) from Nu-Check Prep., Inc., Elysian, Minn.; bovine phosphatidylserine (PS) and bacterial phosphatidylethanolamine (PE) from Supelco, Inc., Bellefonte, Penn.

Lipids were stored at −25°C in $CHCl_3$ solutions. A portion of this solution was evaporated with a stream of nitrogen and redissolved in pentane at a concentration of 12.5 mg of lipid/ml. These pentane solutions were prepared fresh every day.

The Teflon chamber to form the bilayer consists of two pools separated by a thin Teflon partition. This Teflon partition was made of "fluorofilm," 19 μm thick, manufactured by Dielectrix Corporation, Farmingdale, N.Y. A circular hole was punched in the Teflon partition with a hypodermic needle with its tip cut at a straight angle and sharpened as a borer. The hole in the partition was treated with a saturated solution of white petrolatum USP in pentane (white petrolatum from Day-Baldwin, Inc., Hillside, N.J.). The pools on the chamber had a surface of 4 $cm^2$. Monolayers were spread on the surface of the electrolyte solution in 10 μl of the appropriate lipid solution. Membranes were formed by adding solution simultaneously to both compartments, and lifting the liquid until the hole was completely covered. Membrane conductance was usually $\leq 10^{-8}$ S/$cm^2$.

### *Capacitance Measurements*

VOLTAGE-DEPENDENT CAPACITANCE The basis of the capacitance measurements is to make a step change of membrane potential and measure the transient charging current. The total charge stored in the capacitor is obtained by integration of the transient current. The solutions surrounding both sides of the membranes were connected to a voltage source through a low impedance ammeter by means of two large (>1 $cm^2$) Ag/AgCl electrodes. Since we are interested in measuring changes in membrane capacitance, rather than the absolute capacitance, we used a differential ammeter, which subtracts the charging current of the membrane from the charging current of a resistance-capacitance (RC) network equivalent to the membrane (see Fig. 1). It consists of two identical current-to-voltage converters. One measures

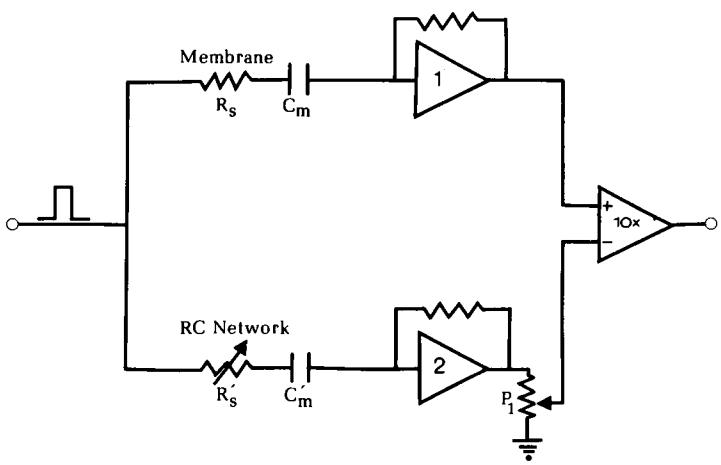



FIGURE 1 Differential ammeter. This circuit consists of two current-to-voltage converters connected to a differential amplifier with a gain of 10. The potential step from the pulse generator is attenuated and filtered before it is applied to the membrane and the RC network. Rise time of the voltage is 1 μs. $C_m$ and $R_S$ are the membrane and series resistance. $R'_S$ and $C'_m$ are their equivalent in the RC network. $C'_m$ is a fixed capacitor. Matching of the two arms of the circuit was accomplished by adjusting $R'_S$ to get the same time constant and the potentiometer $P_1$ to get the same amplitude of the charging transient currents. The differential amplifier subtracts from the current of the membrane arm, the current of the RC network arm. The sensitivity of the ammeter is $10^{-6}$ A/V and the rise time 10 μs.

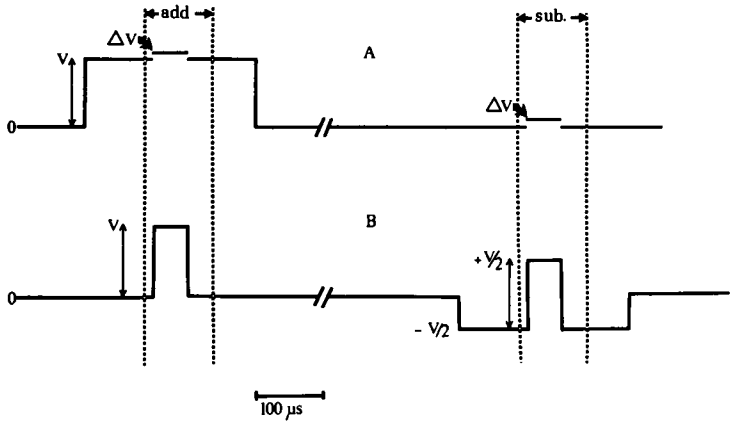



FIGURE 2 Pulse paradigms. These are the two sets of pulses used to measure changes of capacitance. The current is recorded during the time intervals marked "add" and "sub"; the latter is subtracted from the former. To measure the tangent capacitance $C_T$, we used the set *A*, where the difference between the charging current when a small pulse of amplitude $\Delta V$ (usually 10 mV) is applied from a holding potential $V$ and from zero is measured. $\Delta V$ duration was typically 50 $\mu$s, the delay from the holding potential beginning was 100 $\mu$s, and the repetition rate 20 $s^{-1}$. To measure the cord capacitance $C_C$, we used set *B*, where the difference between the current during a large pulse of amplitude $V$ and the same pulse from a holding potential $-V/2$ is measured.

the current passing through the membrane and the other measures the current passing through the equivalent RC network. The output of these two current-to-voltage converters is subtracted by a differential amplifier. To balance the circuit, pulses of 10 mV are applied simultaneously to the membrane and to the RC network, and the resistance and capacitance components of the RC network are trimmed until the output of the differential amplifier is minimal. In this condition the passive network is the best analogue of the membrane circuit. All amplifiers are wide-band fast-settling operational amplifiers (48 K, Analog Devices, Inc., Norwood, Mass.). The sensitivity of the system is $10^{-6}$ A/V and its bandwidth 100 KHz. The output of the differential ammeter is further amplified 1,000-fold by the vertical amplifier of an oscilloscope (model 502A, Tektronix, Beaverton, Ore.) and fed to a transient recorder (model 610B, Biomation, Cupertino, Calif.). This instrument was set to take 256 samples of the current signal at a rate of $2 \times 10^6$/s with a resolution of 6 bits. Successive current transients were accumulated in the memory of a signal averager (model 1070, Nicolet Instrument Corp., Madison, Wis.) to improve signal-to-noise ratio.

To measure voltage-dependent capacitance changes of the membrane, we used the following protocol (see Fig. 2): The membrane potential is displaced from zero to $V$. After a delay, usually 100 $\mu$s, a potential pulse of a small amplitude, $\Delta V$, and duration of 50 $\mu$s is added on top of $V$. The transient current of the charging and discharging during the small pulse is recorded. Membrane potential is returned to zero and again the same small pulse is applied and the current transient recorded. Subtraction point by point of these two records gives the difference in charging current, when the membrane potential is changed from $V$ to $V + \Delta V$, as compared with a change from 0 to $\Delta V$. The total charge difference is obtained by integration, and the capacitance difference is obtained by dividing this charge by $\Delta V$. Usually this protocol is repeated 512 times to improve signal-to-noise ratio. At high values of $V$ the differences can be clearly seen with only one repetition (see Fig. 3). Sequence of pulses were generated with a pulse generator built in our laboratory.

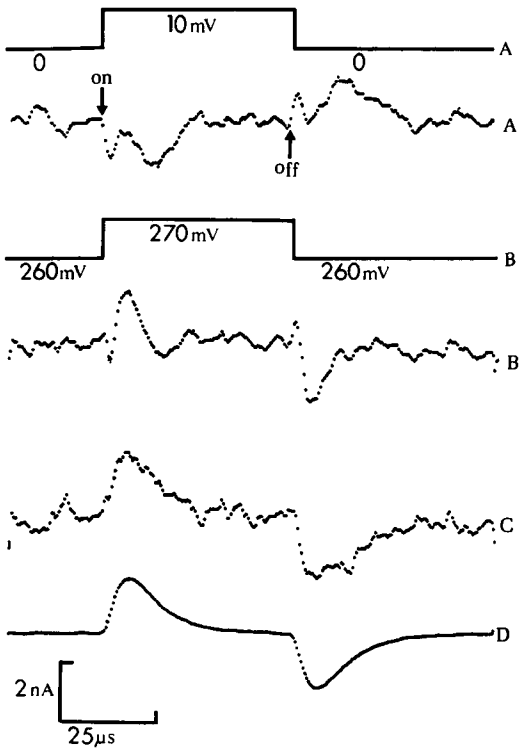
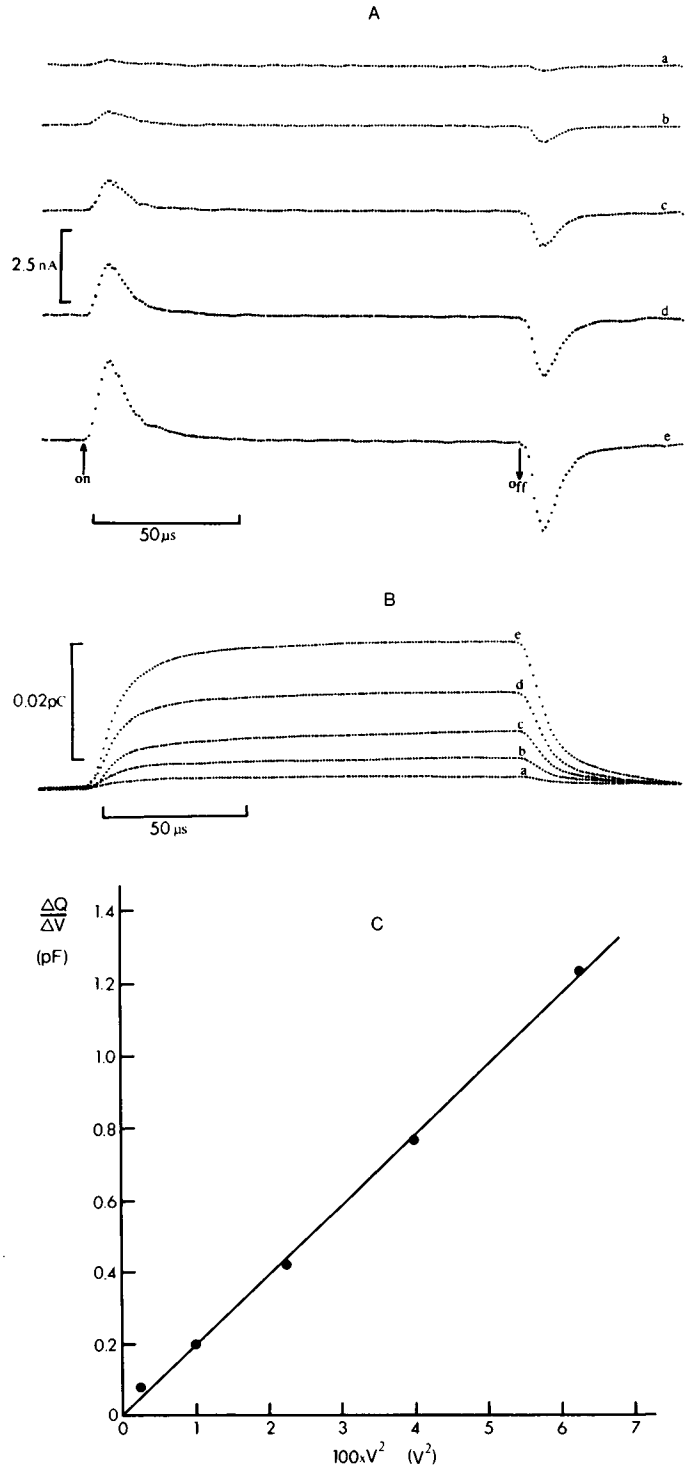



FIGURE 3 Currents measured during a small pulse experiment. Part A is the output of the differential ammeter during a pulse of 10 mV, the current of this record represent the mismatching of the membrane and the RC network shown in Fig. 1. Part B is the current recorded when the same pulse of 10 mV is on top of a holding potential of 260 mV. Part C is the difference point-to-point of B – A; integration of this curve gives the charge taken by the nonlinear part of membrane capacitance. Part D is the result of 512 determinations of the curve C. The membrane was made of one monolayer of PS and one monolayer of PE in 1 M KCl. $C(0)$ = 324 pF.

An alternative protocol is to record first the transient current during the charge and discharge of the membrane capacitor from 0 to $V$ and back to zero (see Fig. 2 B). After a delay the transient current during the charge and discharge from $-V/2$ to $+V/2$ is recorded. Subtraction point by point of these two currents and integration gives the charge difference, which divided by $V$ gives the change in membrane capacitance under these conditions.

We stored the current waveform in digital form and the critical step of analog to digital conversion was done by a six-bit transient recorder. This set a maximum resolution of the current record to 1 part in 64, or 1.6%. This is a serious source of error since we expect changes of less than 1%. We overcame this difficulty by using a differential ammeter, which subtracts the linear part of the capacitance; therefore a high resolution analog-to-digital converter is not needed since we will record only the charging current for the voltage-dependent part of the capacitance. In addition, quantization error is minimized because the original analog current signal has an amount of noise much larger than one bit (see Figs. 3 A and B). This results in an approximation to random sampling of the analog signal and the averaged result of several repetitions is a smooth curve (see Fig. 3 D).

Another source of error is nonlinearity of the pulse generator or the amplifiers used. This was tested with a linear circuit in the place of the membrane and in this situation no voltage-


A
a
b
c
2.5 nA
d
e
on
off
50 μs
B
0.02pC
e
d
c
b
a
50 μs
C
ΔQ/ΔV
(pF)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
6
7
100xV² (V²)

dependent capacitance was observed. We conclude that any nonlinearity in the circuit results in currents smaller than the background noise of the averaged signal. We can easily measure changes of 0.01% in membrane capacitance, using pulses of 10 mV on top of the holding potential.

SPECIFIC CAPACITANCE To measure capacitance of the membrane, $C(0)$, the equivalent RC network of the lower current to voltage converter was disconnected (amplifier 2 in Figure 1) and a 5 KHz, 10-mV peak-to-peak triangular voltage waveform was applied to the membrane. The amplitude of the charging current was measured with a digital multimeter (Hewlett-Packard Co., Palo Alto, Calif., model 3476 B). The system was calibrated after the experiment with a known capacitor. Calibration has an accuracy of 5%.

The area of the membrane was estimated from the area of the hole in the Teflon partition. It was measured from photographs taken through a microscope with an overall magnification of 200×. The contour of the hole was drawn on tracing paper, cut, and weighed out. Specific capacitance is the ratio between membrane capacitance and membrane area.

## RESULTS

### *Membrane Capacitance Is Voltage-Dependent*

We will describe first a representative experiment that illustrates the voltage dependence of membrane capacitance. Original records are shown in Fig. 3. Trace A is the output of the differential ammeter shown in Fig. 1 when a pulse of 10 mV is applied simultaneously to the membrane and to the equivalent passive network. Trace B is the output of the differential ammeter when a 10-mV pulse on top of 260 mV is applied simultaneously to the membrane and to the RC network. Traces A and B are different as the result of the change in membrane capacitance when 260 mV are applied across the membrane. Trace C is the difference of the two previous records and is the difference between the current needed to change the membrane potential from 260 to 270 mV and that needed to change it from 0 to 10 mV. This waveform is independent of the matching of the two current-to-voltage converter circuits, as long as all the points in records A and B are within the range of the analog-to-digital converter. Integration of this curve gives the difference in charge, $\Delta Q$, and the ratio $\Delta Q/\Delta V$ is a measure of the effect of transmembrane potential on membrane capacitance. Trace D is the average of 512 repetitions of curve C, at a rate of 20/s. Comparison between curves C and D indicates that the curves are essentially equal except for the signal-to-noise ratio. This means that at this repetition rate there are no effects of the previous pulses on the result of a given particular pulse. In this experiment the quotient $\Delta Q/\Delta V$ at 260 mV is 2.6 pF, or 0.8% of the membrane capacitance at zero potential.

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FIGURE 4 Dependence of $\Delta Q/\Delta V$ on applied potential. A: original records of the current measured with small pulses of 20 mV on top of different holding potentials. Traces *a–e* show progressively larger holding potentials: 50, 100, 150, 200, and 250 mV, respectively. B: The integrals of the current records shown in A. C: Plot of $\Delta Q/\Delta V$ as a function of $V^2$. The plot is well fitted by a straight line, the slope of which corresponds to an $\alpha$ of 0.022 $V^{-2}$. These are results from a symmetric bilayer made of GMO and PS at a molar ratio of 4:1 in 0.1 M NaCl. $C(0)$ = 380 pF.

*The Ratio $\Delta Q/\Delta V$ Is Proportional to the Square of Membrane Potential*

Results of a representative experiment in which $\Delta Q/\Delta V$ was measured as a function of membrane potential are shown in Fig. 4. Original records of current and integral of current are shown in Fig. 4 A and B, respectively. Fig. 4 C shows that $\Delta Q/\Delta V$ is proportional to the square of the potential. This result is comparable to those of others and is the expected relationship if the changes in capacitance were produced by electrocompression of the membrane (Rosen and Sutton, 1968; White, 1970). We will describe membrane capacitance with the following equation:

$$C(V) = C(0)[1 + \alpha V^2], \tag{1}$$

where $C(0)$ is the capacitance at $V = 0$ and $\alpha$ is a constant of proportionality.

*Calculation of $\alpha$ from Small and Large Voltage Pulse Experiments*

The charge, $Q$, stored in a capacitor of a capacitance $C$, at an electrical potential $V$, is by definition

$$Q \equiv CV. \tag{2}$$

Introducing Eq. 1 into 2 gives:

$$Q = C(0)(V + \alpha V^3). \tag{3}$$

If the potential is suddenly changed from a value $V = V_1$ to a value $V = V_2$ it is clear that

$$Q_2 - Q_1 = C(0)[(V_2 - V_1) + \alpha(V_2^3 - V_1^3)]. \tag{4}$$

Eq. 4 can be expressed in a more convenient way:

$$Q_2 - Q_1 = (V_2 - V_1)[C(0) + C(0)\alpha(V_1^2 + V_1V_2 + V_2^2)]. \tag{5}$$

In Eq. 5, $C(0)$ is the linear part of the capacitance and $C(0)\alpha(V_1^2 + V_1V_2 + V_2^2)$ is the voltage-dependent part of the capacitance.

We have used two types of experiments to measure voltage dependent capacitance. The first type of experiment consists of changing the membrane potential from 0 to $V$; after a short delay a small voltage pulse of amplitude $\Delta V$ is added to $V$ (see Fig. 2 A). The current is recorded, covering the leading and trailing edge of the small pulse. Membrane potential is returned to zero and again the small pulse of amplitude $\Delta V$ is applied. We subtract the current records in order to obtain the difference in charge displaced due to electrostriction. This difference in charge calculated from Eq. 5 is given by:

$$\Delta Q = C(0)\Delta V(3\alpha V^2 + 3\alpha V\Delta V). \tag{6}$$

For very small values of $\Delta V$, we define a "tangent" capacitance, $C_T$:

$$(\Delta Q/\Delta V)_{\Delta V \to 0} \equiv C_T = 3C(0)\alpha V^2. \tag{7}$$

We used a $\Delta V$ of 10 or 20 mV positive for positive values of $V$, and 10 or 20 mV negative for negative values of $V$. To calculate $\alpha$ we take $\Delta Q/\Delta V$ as a first approximation of $C_T$, and compute a first approximation of $\alpha$ from a quadratic regression of $\Delta Q/\Delta V$ vs. $V$. We substract $C(0)\,3\alpha V\Delta V$ from the experimental points and new values of $C_T$ and $\alpha$ are calculated. Usually $\alpha$ did not change very much and no further corrections were necessary.

The second kind of experiment consists in applying a large voltage pulse of amplitude $V$ (see Fig. 2 B). After a delay, the membrane potential was changed to $-V/2$ and a pulse of amplitude $V$ was added to $-V/2$, displacing the potential to $+V/2$. The resulting current was subtracted from that obtained by applying a pulse from 0 to $V$. The difference of charge displaced in these two cases can be obtained evaluating Eq. 5 for $V_1 = 0$ and $V_2 = V$ and subtracting the charge calculated for $V_1 = -V/2$ and $V_2 = +V/2$. Therefore,

$$\Delta Q = \tfrac{3}{4} C(0)\alpha V^3. \tag{8}$$

From this equation we define a "chord" capacitance:

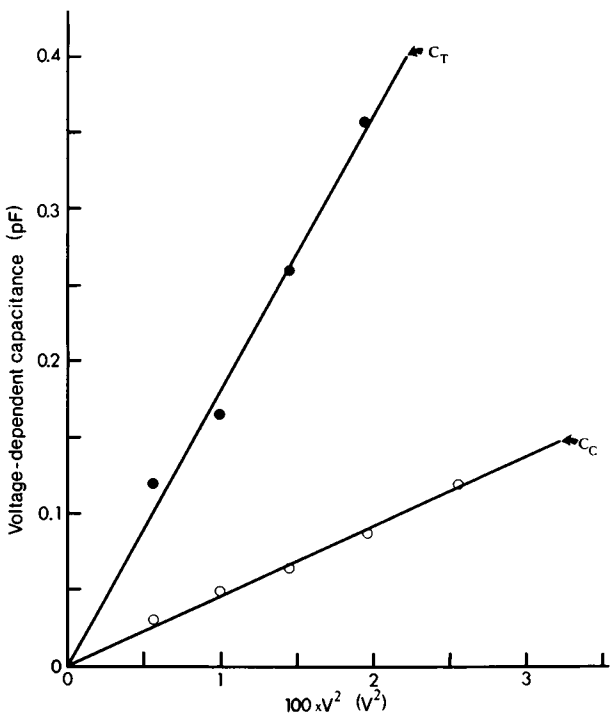


FIGURE 5 These are the results of voltage dependent capacitance with small voltage pulses (•) described in Fig. 2 A or large pulses (o) shown in Fig. 2 B. The slope of the straight line relating capacitance and $V^2$ is four times larger for $C_T$ than for $C_C$. $\alpha$ is 0.0205 $V^{-2}$ calculated from $C_T$ and 0.0203 $V^{-2}$ calculated from $C_C$. These are results of one membrane made of PE in 1 M KCl. $C(0)$ = 303 pF.

$$C_C \equiv \Delta Q/V = \tfrac{3}{4} C(0)\alpha V^2. \quad (9)$$

Eqs. 7 and 9 predict that both $C_T$ and $C_C$ depend on the square of the potential. The proportionality constant is $3\alpha C(0)$ for $C_T$ and $\frac{3}{4}\alpha C(0)$ for $C_C$.

In Fig. 5 we show that $C_T$ and $C_C$ are linear functions of the square of potential. Furthermore, it is also shown in Fig. 5 that when the appropriate pulse paradigm is applied to obtain $C_T$ and $C_C$, the slopes of the curves are exactly related as predicted: 18.3 $pFV^{-2}$ for $C_T$ and 4.57 $pFV^{-2}$ for $C_C$. Calculation of $\alpha$ from $C_C$ gives a value of 0.0203 $V^{-2}$ and calculation of $\alpha$ from $C_T$, measured 100 $\mu$s after the onset of the pulse, gives 0.0205 $V^{-2}$. These results provide good evidence that the capacitance change is instantaneous (compared with the 10-$\mu$s rise time of our measuring system) because $C_T$ is measured after a time delay and $C_C$ is measured during the voltage displacement. Comparison of the values of $C_T$ obtained at increasing elapsed times after the onset of the holding pulse can be used to determine if there is any further change in capacitance with time. In PE, GMO, or GMO + PS membranes, measurements of $C_T$ do not change significantly when measured from 25 to 250 $\mu$s after the holding potential is applied.

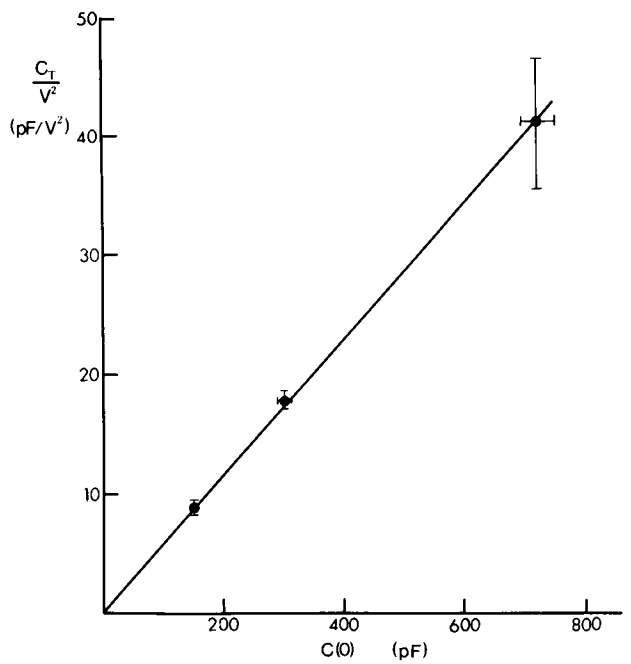


FIGURE 6 Dependence of $C_T/V^2$ on membrane capacitance. These results were obtained from three different groups of membranes. The Teflon partitions were punched with hypodermic needles of different diameters to obtain increasingly larger holes. The areas of the holes where the membranes were formed were $2.2 \times 10^{-4}$, $4.5 \times 10^{-4}$, and $10.4 \times 10^{-4}$, respectively. The solid circles are the average measurements of at least four different membranes. The bars are the SEM. The straight line connecting the points has a correlation coefficient better than 0.999. Membranes were made of PE in 1 M KCl.

Eq. 7 predicts that $C_T/V^2$ must be proportional to $C(0)$. To test this point $C_T$ was measured as described above in membranes having different areas. As shown in Fig. 6, $C_T/V^2$ is directly proportional to $C(0)$. This is an important finding since bilayers made from monolayers are supposed to be surrounded by a liquid torus, as pointed out by White et al. (1976), and therefore one might expect formation of new membrane from the plateau border when the potential is applied. We will assume that at the bilayer border there is a transition from a lipid bilayer membrane to a zone of solvent covered by two lipid monolayers. The contact angle between the monolayers and the bilayer planes, at the transition zone, is determined by the surface tension of the bilayer and the interfacial tension of the solvent-water interface covered by a monolayer of lipid, (Wobschall, 1972; White et al., 1976). This angle increases when a potential is applied, as demonstrated by studies in microlenses by Requena et al. (1975). An increase in contact angle implies an increment in membrane radius and an increase in area equal to the increment in radius times membrane perimeter. Since the relation perimeter/area is inversely proportional to membrane radius, we should expect that a curve of $C_T/V^2$ vs. $C(0)$ will bend towards the $C(0)$ axis. We found that $C_T/V^2$ is proportional to membrane area rather than membrane perimeter and therefore we conclude that there is no significant formation of new membrane at the expenses of the plateau border.

### *Surface Charge Density Can Be Measured from the Voltage-Dependent Capacitance*

Membrane capacitance is minimal when the transmembrane potential is zero. This must occur at zero applied potential for symmetric membranes. On the other hand, we expect the minimum of $C_T$ to occur at $V = \Delta\psi$ for membranes having a difference in surface potential $\Delta\psi$. If a surface potential difference exists across the dielectric when no external potential is applied, we must write $(V + \Delta\psi)$ instead of $V$ in Eq. 5 and in derived new expressions for $C_T$ and $C_C$. Thus,

$$C_T = C(0)\,(3\alpha V^2 + 6\alpha\Delta\psi V), \tag{10}$$

$$C_C = C(0)\,(\tfrac{3}{4}\alpha V^2 + 3\alpha\Delta\psi V). \tag{11}$$

$\Delta\psi$ should depend on salt concentration if the asymmetry of the membrane was due to a differential surface charge density (Latorre and Hall, 1976). We measured the voltage-dependent capacity in both symmetric and asymmetric membranes to test this prediction. Fig. 7 shows the results of three different experiments. The curve connecting the solid circles in Fig. 7 is a plot of the tangent capacitance against potential of a membrane made of PE; experimental points can be well fitted by a parabola with its minimum at the origin. The curves connecting the open circles and squares show the result of asymmetric bilayers made of one monolayer of PS and one monolayer of PE in 1 M and 0.1 M KCl, respectively. The minima occur at $-50$ mV for the membrane made in 1 M KCl and at $-115$ mV for the membrane made in 0.1 M KCl. The minus sign indicates that the surface potential of the PS side is more negative than the PE side of the membrane.

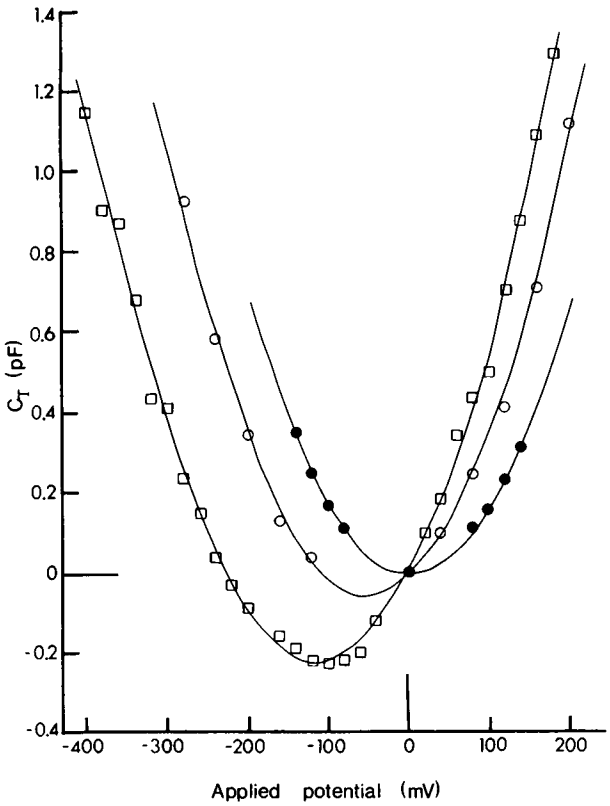


FIGURE 7 Effect of surface potential difference on voltage-dependent capacitance. This figure shows the result of three different membranes. Solid circles represent the values of $C_T$ for a symmetric membrane made of PE under 1 M KCl. The points are well fitted by a parabola with the minimum at the origin. $C(0)$ = 299 pF. Open circles are the results for an asymmetric membrane made of one monolayer of PE and the other of PS under 1 M KCl. In this case the parabola is shifted and the minimum occurs at −51 mV. $C(0)$ = 324 pF. The PS side is grounded and the PE side is where potential is applied, and since the minimum of the parabola occurs when the PE side is negative with respect to the PS side, it means that $\Delta\psi$ is 51 mV, PS side negative. Squares are the results of another PS/PE membrane but the KCl concentration is 0.1 M. In this case the parabola has the minimum at −115 mV. $C(0)$ = 308 pF.

*Summary of Results*

All the capacitance determinations are summarized in Table I. The parameters $\alpha$ and $\Delta\psi$ were calculated from the least square fit of a parabola to the experimental data of $C_T$ or $C_C$ measured at different values of $V$. We found that the relative change of capacitance varies in a very narrow range for all the membranes tested, both symmetric and asymmetric. This change amounts to an average value of $0.022/V^{-2}$. It is somewhat larger in GMO membranes than in phospholipid membranes. Surface potentials are in good agreement with those measured by other methods (McLaughlin et al., 1970; Hall and Latorre, 1976) and close to those predicted by the Gouy-Chapman

TABLE I

SPECIFIC CAPACITANCE, $C_s$, AND VOLTAGE-DEPENDENT CAPACITANCE MEASURED IN MEMBRANES OF VARIOUS COMPOSITIONS

| Lipid | Ionic strength | $C_s$ | $\delta$ | $\alpha \times 10^2$ | $\Delta\psi$ | $n$ |
|---|---|---|---|---|---|---|
| | $M$ | $\mu F/cm^2$ | $nm$ | $V^{-2}$ | $mV$ | |
| GMO | 1.0 | $0.75 \pm 0.03$ | 2.5 | $3.6 \pm 0.2$ | * | 26 |
| GMO + PS | 1.0 | $0.81 \pm 0.03$ | 2.3 | $2.2 \pm 0.3$ | * | 3 |
| PE | 1.0 | $0.65 \pm 0.01$ | 2.9 | $2.1 \pm 0.1$ | * | 8 |
| PE/PS | 1.0 | $0.72 \pm 0.02$ | 2.6 | $2.0 \pm 0.1$ | $64 \pm 7$ | 11 |
| PE/PS | 0.1 | $0.67 \pm 0.03$ | 2.8 | $1.8 \pm 0.2$ | $122 \pm 5$ | 3 |

$\delta$ is the thickness of the dielectric calculated from $C_s$, assuming a dielectric constant of 2.1. $\alpha$ is the constant of proportionality between voltage-dependent capacitance and $V^2$. $\Delta\psi$ is the difference in surface potential, PS side negative. Values are the mean and the SEM estimated from $n$ determinations.

*$\Delta\psi$ not significantly different from zero.

Equation with a surface charge of 1 electronic charge per 78 $A^2$ for PE/PS asymmetric membranes.

## DISCUSSION

### *General*

In the previous section we have shown the results of measurements of voltage-dependent capacitance of lipid bilayers. These changes are very small, ranging from 0.01% to 1.0% of the total membrane capacitance. We feel that the results are indeed voltage-dependent variations of membrane capacitance because they are in excellent agreement with the a priori expectations: a plot of capacitance vs. potential is a parabola, as predicted. The most significant result is that when asymmetric PE/PS membranes were tested the position of the parabola shifts exactly the amount expected from independent estimates of surface potential asymmetry.

Our measurements of voltage-dependent capacitance of bilayers made by apposition of two monolayers set an upper limit to the compression in bilayer membranes by an electric field. It is difficult to postulate a unique mechanism to explain the voltage-dependent capacitance due to the small magnitude of the changes and the poor knowledge of the composition of these membranes, but although we cannot completely rule out the formation of new membrane at the border or formation of microlens, we believe that the most probable mechanism involved is membrane thinning with increase in area, at constant membrane volume. This conclusion is supported by the following findings: First, we found that the specific capacitance of GMO membranes is 0.75 $\mu F/cm^2$, (see Table I) a value similar to that found by Benz et al. (1975) in the same membranes. Moreover, this value is comparable to the one obtained by White (1974*b*) at 1°C, using a solvent freeze-out technique for the formation of solvent-free membranes. This high specific capacitance suggests that these membranes contain very little solvent. Second, we have shown that the changes in capacitance take place in less

than 10 $\mu$s, and there is no further change up to 250 $\mu$s after the potential is applied. It has been shown that formation of new membrane at the expenses of the torus or formation of microlens are much slower processes. (Requena et al., 1975; Wobschall, 1972). Third, the change in capacitance per unit area is the same when measured in membranes of different sizes, consistent with the notion that capacitance increases due to changes in the bulk of the membrane rather than at its border.

*Comparison of $\alpha$ with Former Determinations*

Our results are the first accurate determination of voltage-dependent capacitance in lipid bilayer made with the Montal and Mueller (1972) technique. We found an average of 0.022 $V^{-2}$ of relative increase in capacitance. Measurement of voltage-dependent capacitance of membrane made by apposition of two monolayers were attempted earlier by Benz et al. (1975) and Benz and Janko (1976). They compared the total charge displaced during the on and off phase of a voltage pulse of 150 mV and a duration of 10 s. They found that membrane capacitance was independent of electrical potential within the resolution of their method (1%). This result is consistent with the results we report here, since for an $\alpha$ of 0.022 $V^{-2}$ only a 0.05% change in capacitance is expected for a 150 mV pulse, clearly below their resolution.

Haydon (1970) calculated a Young's modulus of compressibility for a solvent-free bilayer.[1] He assumed that when the electric field is applied, the membrane is thinned at constant volume with increase in area. Considering that the energy for compression is a measure of the work to increase the area per molecule, the Young's modulus of the membrane is $2 \times 10^7$ N/m$^2$. Knowing Young's modulus, we can calculate the parameter $\beta$, which is the increase in specific capacitance per $V^2$, from the equation (White and Thompson, 1973)

$$\beta = C_s^2/2\delta_0 E, \tag{12}$$

where $E$ is the Young's modulus, $C_s$ the specific capacitance, and $\delta_0$ the membrane thickness. Dividing Eq. 12 by $C_s$, we obtain $\beta_{rel}$, the relative increase in specific capacitance per volt squared:

$$\beta_{rel} = C_s/2\delta_0 E. \tag{13}$$

If the membrane volume during compression does not vary, the product of area times membrane thickness is a constant and therefore, the change in total capacitance will be at least twice the change in specific capacitance (White, 1974*a*) and $\alpha$, as defined in Eq. 1, is:

$$\alpha = C_s/E\delta_0. \tag{14}$$

Using the values given in Table I for $C_s$ and $\delta$ for GMO membranes and a Young's

[1]Evans and Simon (1975) have shown that the mechanical properties of planar bilayers cannot be lumped into a single elastic constant and therefore the physical meaning of a "Young's modulus" for a bilayer is rather obscure. In our calculations the compressibility modulus is used only for the sake of the comparison between predicted and experimental results without conveying any physical meaning.

modulus of $2 \times 10^7$ N/m$^2$, we obtain an $\alpha$ equal to 0.14 V$^{-2}$, only sevenfold larger than our experimental results. We conclude that bilayer membranes are even stiffer than predicted by Haydon (Haydon, 1970). For GMO the Young's modulus is $1.4 \times 10^8$ N/m$^2$, as calculated from the data of Table I and Eq. 14.

Lipid bilayers containing solvent exhibit a large voltage-dependent capacitance that takes several milliseconds to minutes to be completed (Rosen and Sutton, 1968; White and Thompson, 1973; Benz et al., 1975). 10 s after a 150-mV voltage step has been applied, these membranes have a capacitance 5–30% larger than at zero potential (Benz et al., 1975). This effect is mainly due to formation of new membrane from the torus (White and Thompson, 1973) and squeezing of solvent into the torus or into microlenses (Requena et al., 1975). Several investigators have also detected a small (less than 1%) change 10 ms after the voltage is increased (Wobschall, 1972; White and Thompson, 1973). Wobschall (1972) was able to separate electrostriction from solvent flow, using alternating current to produce capacitance changes. From Wobschall's results we calculate an increase of 0.06 V$^{-2}$, in excellent agreement with our results. This implies that if membrane capacitance is measured a short time after a voltage is applied, solvent does not have the time to redistribute in the membrane, and solvent-free and solvent-containing membranes exhibit similar voltage-dependent capacitance.

*Voltage-Dependent Capacitance in Asymmetric Membranes*

We have shown previously that differences in surface potentials can be measured using the nonactin-K$^+$ complex as a probe (Hall and Latorre, 1976; Latorre and Hall, 1976). The present results indicate that changes in membrane capacitance provided a direct and simple method to estimate the magnitude of the difference in surface potential and therefore the surface charge density. Since the calculation of surface potential differences from capacitance changes does not require any particular assumptions about the electrochemical barrier to transport inside the membrane, it provides a very good check for the model used to interpret nonactin-K$^+$ current in asymmetric bilayers.

*Relation to Gating Current in Squid Axon*

Blatt (1977) has recently published an article discussing the contribution of electrostriction to the gating current measurements in the axon of the squid. He calculated the transient currents expected from electrostriction in the conditions used to measure gating current in axons using three different values of $\alpha$: a "maximum" value of 2 V$^{-2}$, an "average" value of 0.4 V$^{-2}$, and a "conservative" value of 0.04 V$^{-2}$. The first two values were obtained by assuming that voltage-dependent optical retardation phenomena in squid axon are due to a decrease in membrane thickness (Cohen et al., 1971). The last value of $\alpha$ is close to that calculated from capacitance measurements in cholesterol-dodecane-detergent membranes (Wobschall, 1972). Blatt concluded that, using the average estimate of $\alpha$, current arising from electrostriction has an amplitude comparable (but of opposite sign) to that of the gating currents. Using the conservative estimate of 0.04 V$^{-2}$, he finds that the currents are 10% of the amplitude of gating currents. If we use the value for $\alpha$ of 0.02 V$^{-2}$ reported here, electrostrictive currents would decrease to only 5% of the amplitude of the gating currents.

We have shown here that the membrane capacitance is minimal when the transmembrane potential is zero. Therefore to calculate charge displacement due to electrostriction in squid axons, the difference in surface potential of the membrane must be known. The best estimates of these potential differences have been obtained from the changes of electrical properties of sodium and potassium channels when the ionic composition of either the internal or external solutions are changed. The results obtained suggest that the outer surface potential of the axon membrane is of the order of −60 mV (Frankenheuser and Hodgkin, 1957) and the inner surface potential is about −15 mV (Chandler et al., 1965). The potential difference is therefore 45 mV and the minimal value of the capacitance must occur at −45 mV, the inside of the cell negative. Taking this value, together with an $\alpha$ of 0.02 $V^{-2}$ and $C(0) = 1\ \mu F/cm^2$, we can calculate the difference in charge displaced by a depolarizing pulse of 80 mV and by an hyperpolarizing pulse of the same amplitude using Eq. 5. With this set of voltage pulses, the one commonly used to measure gating currents, the charge displaced is 1 $e/\mu m^2$.

Armstrong and Bezanilla (1974) have measured gating current in the squid axon by a divided pulse technique. It consists of measuring the charge translocated during a depolarizing voltage step of amplitude $P$, and subtracting the charge translocated during four pulses of amplitude $P/4$ applied on top of a large hyperpolarizing holding potential. We can calculate the electrostriction contribution to the charge displaced with the divided pulse technique using Eq. 5 for a step from −70 to +10 mV and subtracting four times the charge displaced calculated for a step from −180 to −160 mV. The charge displacement calculated for the four small steps is four electronic charges per square micron larger than calculated for the depolarizing pulse. Armstrong and Bezanilla (1974) measured 1,000 $e/\mu m^2$ for a comparable set of pulses in squid axon. Therefore, the contribution to the total charge displaced arising from electrostriction is only 0.1% for equal and opposite voltage pulses and 0.4% for divided pulses.

We conclude that provided that the same electrocompression parameters are applicable to both artificial bilayers and biological membranes, electrostriction will have only a minor effect on the measurement of gating charge displacement in squid axon.

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