A STUDY OF LIPID BILAYER MEMBRANE STABILITY USING PRECISE MEASUREMENTS OF SPECIFIC CAPACITANCE

STEPHEN H. WHITE

From the Department of Physiology and Biophysics, University of Washington, Seattle, Washington 98115. Dr. White's present address is the Physical Science Division, Fort Detrick, Maryland 21701.

ABSTRACT A method is described for measuring the specific capacitance (C_m) of lipid bilayer membranes with an estimated experimental error of only 1%. The gross capacitance was measured with an AC Wheatstone bridge and a photographic technique was used to determine the area of thin membrane. The results of measurements on oxidized cholesterol-decane membranes formed in 1×10^{-2} M KCl show that C_m depends upon temperature, voltage, time, and the age of the bulk membrane solutions. For a freshly thinned membrane (from 5 week old solution), C_m increases exponentially from an initial value of 0.432 ±0.021 (SD) $\mu F/cm^2$ with a time constant of ~15 min. A 100 mv potential applied across the membrane for 10–20 min prior to making measurements eliminated this time dependence and produced *final-state* membranes. C_m of final-state membranes depends upon applied voltage (V_a) and obeys the equation $C_m = C_0 + \beta V_a^2$ where $V_a \simeq V_{DC} + V_{AC}^{rms}$. C_0 and β depend upon temperature; C_0 decreases linearly with temperature while β increases linearly. At 20°C, C_0 = 0.559 ±0.01 (SD) $\mu F/cm^2$ and β = 0.0123 ±0.0036 (SD) $(\mu F/cm^2)/(mv^2)$ and at 34°C, C_0 = 0.472 ±0.01 and β = 0.0382 ±0.0039. These variations in C_m are interpreted as resulting from thickness changes. The possibility that they result from diffuse layer and/or membrane dielectric phenomena is discussed and found to be unlikely. The results are discussed in terms of membrane stability by constructing hypothetical potential energy vs. thickness curves.

INTRODUCTION

Lipid films 50–100 A in thickness can be formed by spreading solutions of certain surfactants in aliphatic hydrocarbons across an aperture immersed in an aqueous phase (Mueller, Rudin, Tien, and Wescott, 1962; Hanai, Haydon, and Taylor, 1964; Huang, Wheeldon, and Thompson, 1964). The spread film is initially thick (~100 μ) but spontaneously thins as lipid solution drains into the Plateau border as a result of the negative pressure caused by the border's sharp curvature (Mysels, Shinoda, and Frankel, 1959; Tien, 1968). As very small thicknesses (~150 A) are approached, London-van der Waals forces between the separated aqueous phases add a signifi-

cant compressive force (Taylor and Haydon, 1966). The end result of these processes is a stable film approximately two surfactant molecules thick. The details of the stability conditions for these lipid bilayer or "black" lipid membranes (Tien and Diana, 1968) are of basic importance to understanding cell membrane structure and the stability of colloids dispersed in hydrophobic media (Taylor and Haydon, 1966; Napper, 1967).

Since thickness is the variable of primary concern, stability is conveniently studied by measurements of membrane thickness using optical techniques (Huang and Thompson, 1965; Cherry and Chapman, 1969) or by measurements of membrane capacitance (C) using electrical methods (Hanai et al., 1964). If the lipid bilayer is considered to be a parallel plate capacitor of area A, then $C = ([\epsilon_0\epsilon_m]/\delta)A$ (mks system), where δ is thickness and ϵ_m dielectric coefficient. Since these depend upon the physical state of the thin membrane, capacitance is probably the best general measure of the membrane's state. Hanai et al. (1964, 1965 *a*) showed that the specific capacitance (C/A) of a bilayer is determined primarily by the dielectric coefficient and thickness of the hydrocarbon layer of the membrane. In a later paper, Taylor and Haydon (1966) reported the thickness of the membrane to depend upon the length (l) of the surfactant molecules used to stabilize the film and that, bascially, $\delta = 2l$. The general picture of stability which emerged from these studies was that steric interactions between surfactant molecules prevent the collapse of the film.

The *total* electrical capacitance of a lipid bilayer membrane can be increased by the application of an electric field (Babakov, Ermishkin, and Liberman, 1966; Laüger, Lesslauer, Marti, and Richter, 1967; Rosen and Sutton, 1968). The fractional increase is proportional to the square of the applied potential. Laüger et al. attributed the increase to a thickness change while Babakov et al. believed the increase results from an area change. These differing views cannot be resolved since area was not accurately measured by either group. Haydon and Overbeek (1966) suggested that the thickness should decrease because of compression by the intense ($\sim 10^5$ v/cm) electric field. The specific capacitance of lecithin-cholesterol-decane membranes was observed by Redwood and Haydon (1969) to be inversely related to temperature. This effect was interpreted on the basis of earlier work (Hanai, Haydon, and Taylor, 1965 *b*) as resulting from changes in the amount of cholesterol present in the bilayer which leads to thickness and dielectric coefficient changes. These results suggest that the stability of lipid bilayer membranes can be modified by electric fields and temperature changes.

Measurements of electrical capacitance are useful only if *specific* capacitance can be accurately determined. The major limitation in such a determination has until now been the measurement of membrane area. A technique for accurately determining specific capacitance has been developed in this laboratory and used to determine the effects of temperature and voltage. The purpose of this paper is to report the results of these measurements and to discuss them in terms of membrane stability. Preliminary reports of this work have appeared elsewhere (White, 1969 *b*, 1970).

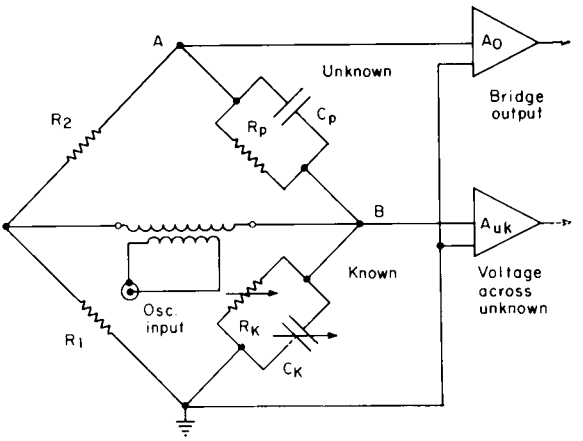


FIGURE 1 Simplified bridge circuit, which illustrates the principle features of the Wheatstone bridge used. Voltage appearing at A is a result of bridge imbalance and is detected by A_0, a high imput impedance (10^9 Ω) amplifier. The output of A_0 is displayed on a Hewlett-Packard HP-132A dual beam oscilloscope whose vertical amplifier was also used as a preamplifier for final detection by a GR-736A wave analyzer (General Radio Company). Since at balance, point A is a virtual ground, the voltage appearing at point B represents the voltage across the unknown. A_{uk}, exactly like A_0, detects the voltage at point B. The oscillator used to drive the bridge is a Hewlett-Packard HP-651B test oscillator. Generally, R_2 = 100 kΩ and R_1 = 1 kΩ. The voltage source (not shown) for applying a DC bias potential is inserted in series with the unknown. Its resistance is much smaller than the series resistance of the unknown.

METHODS

The specific capacitances of black lipid membranes were obtained from impedance measurements made with an AC Wheatstone bridge (Fig. 1). When balanced at a given frequency (f), the settings of the known arm give the parallel equivalent capacitance $C_p(\omega)$ and resistance $R_p(\omega)$ ($\omega = 2\pi f$) of the circuit connected to its "unknown" terminals. Because lipid bilayer membranes must be formed beneath an electrolyte solution and connected electrically to the bridge via metallic electrodes, the circuit of the unknown contains other elements in addition to the gross capacitance (C) of the membrane. To obtain this capacitance from measurements of $R_p(\omega)$ and $C_p(\omega)$, the total equivalent circuit of membrane, surrounding electrolyte, and electrodes must be ascertained and transformation equations developed which relate the values of the elements of the total circuit to R_p and C_p. Appropriate equivalent circuits and transform equations have been given by Hanai et al. (1964). At low frequencies (~100 cycle/sec), $C = C_p - C_s$ where C_s is the stray capacitance in parallel with the electrodes.

A convenient technique for displaying AC impedance data introduced by Cole (1932) has been used by Hanai et al. to examine the AC properties of lipid bilayer membranes. This technique is used in the present paper. The data, obtained as R_p and C_p are converted into complex capacitance C^* ($\equiv C' - iC''$) and the imaginary part of C^* plotted as a function of the real part.

Materials

The lipid bilayers were formed from oxidized cholesterol, (Tien, Carbone, and Dawidowicz, 1966) dissolved in *n*-decane to form a saturated solution (~35 mg/ml). The oxidized choles-

terol was initially prepared in *n*-octane (Tien, personal communication) which was later evaporated off to obtain a dry powder. The membranes were created by spreading the solution across the aperture (Mueller et al., 1962) with a 0000 sable brush pruned of half its bristles.

The only electrolyte used was KCl (reagent grade) dissolved in twice-distilled water (once in metal and once in Pyrex). The solutions were buffered to pH = 7.1 with a phosphate buffer system (2×10^{-4} M). All glassware was cleansed in a chromic acid solution to insure the absence of surface active contaminants.

Measurements of Area and Specific Capacitance

The accurate measurement of the "black" area of the membrane presented a difficult problem. The method most often employed is to measure the diameter of the black area with a reticle in the microscope eye piece. In this case the membrane is observed by light reflected from the

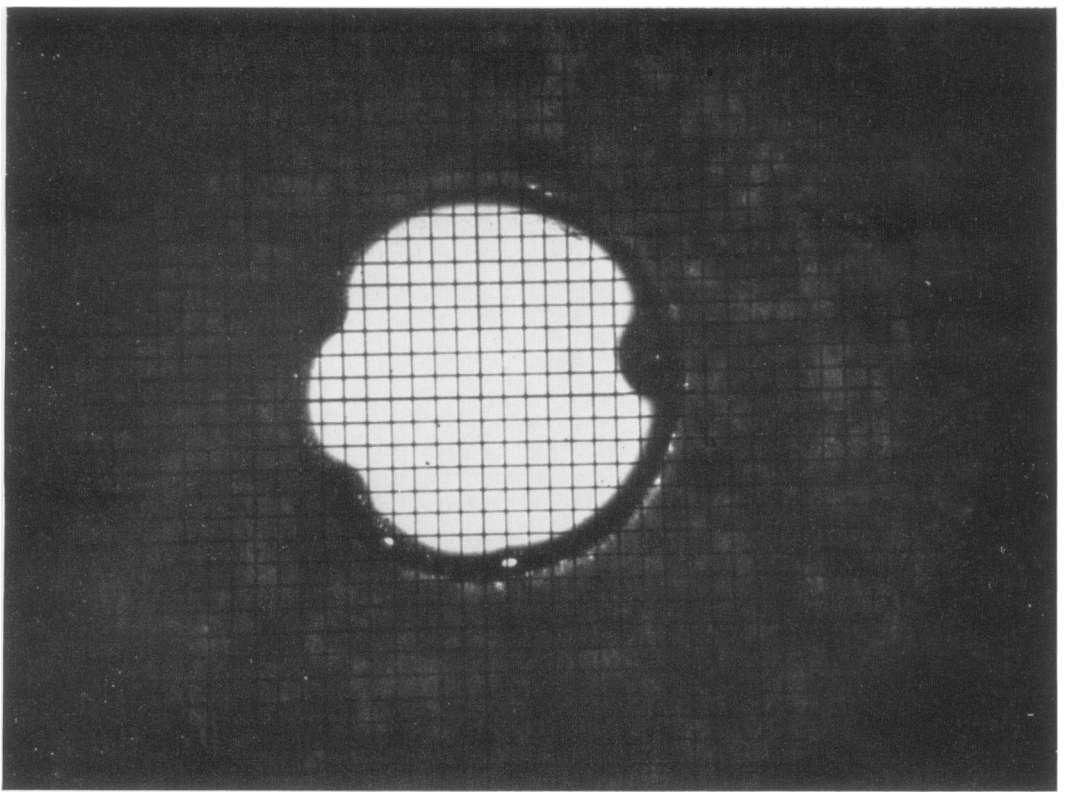


FIGURE 2 Transmitted light photograph of a black membrane. Bilayer regions appear black in reflected light because their reflectance is much smaller than that of thick membrane or surrounding supports. For the photographs made in this study, transmitted light was used so the black membrane appears to be gray. The spacing between grid lines is 8.4×10^{-3} cm. The irregular boundary (caused by bubbles trapped on the front surface) is common in lipid bilayer membranes and illustrates the difficulty of making simple visual determinations of membrane area. Magnification = ×36.

membrane's surface. However, assuming the black area to be perfectly circular, or at worst elliptical, the area can be calculated to within only 4% using the reticles available in this laboratory. Generally the black areas are *not* circular or elliptical and in reflected light the boundary is not clearly visible. The most accurate and reliable method found to measure area was from photographs (Fig. 2) made by transmitted light.

The membrane was observed through an AO Spencer series 23 steromicroscope (American Optical Corp., Scientific Instrument Div., Buffalo, N. Y.) aligned so that the optical axis of one of the monoculars was perpendicular to the membrane. A 15× eyepiece (containing a grid reticle [Edmond Scientific Co., Barrington, N. J.]) and a 3× nosepiece provided a magnification of 45 diameters. Photographs were made on Polaroid type 107 (ASA 3000) film (Polaroid Corp., Cambridge, Mass.) with a Wild photomicrography camera (Wild Heerbrugg Instruments, Inc., Farmingdale, N. Y.) mounted on an adjustable arm so that it could be easily swung into place without adjustment. The membrane was transilluminated with a standard illuminator. Crossed polaroids (one on the illuminator, the other on the nosepiece) provided fine and even control of light intensity. The teflon partition was milled to sufficient thinness (0.020 inches) to allow light to be scattered into the edges of the membrane. The abrupt change in thickness at the boundary of the black area scatters the light and clearly delineates the boundary.

After photographing the membrane, a standard area (A_S) (20 × 20 grid divisions = 2.82×10^{-2} cm²) containing the membrane image was trimmed from the film with Iris scissors and its weight (W_s) measured. The black portion of the membrane was then cut from A_S and weighed (W_M). The area of the black membrane was then calculated from $A_M = A_S \cdot (W_M/W_S)$. Comparison of W_M with W_S minimizes the effects of any nonuniformity in the photographic paper.

The method was tested by making a series of photos of the aperture alone and determining its "A_M." The standard deviation in area was only 0.7%. Since the gross capacitance of the membrane could be determined to within 0.2% (*vide infra*), the approximate experimental error in the determination of specific capacitance (C_m) was about 1%.

Chamber

The chamber was made of Plexiglas with teflon partition. The aperture in the teflon was cut with a $\frac{1}{16}$ inch twin-fluted end mill. The entire chamber was routinely washed in a dilute detergent solution, soaked in running tap water for 24 hr, and then rinsed with distilled water to insure the removal of surface active contaminants.

Tap water from a reservoir maintained near the desired temperature was pumped through a water jacket in the chamber. On-off regulation, provided by a Yellow Springs model 71 temperature controller (Yellow Springs Instrument Company, Yellow Springs, Ohio) and stainless steel encased thermistor probe, maintained any desired chamber temperature between 10°C and 50°C to within ±0.05°C.

Electrodes

Platinized platinum electrodes are frequently used for AC impedance measurements on biological tissues (Schwan, 1963) because of their low impedance over a broad range of frequencies. Unfortunately, they have no definite electrochemical potential. The potential is highly variable and often large (20–100 mv) — a feature which makes them useless for DC measurements. Silver-silver chloride electrodes, on the other hand, are excellent for DC measurements because of their small (<1 mv) and stable electrochemical potential and because of

their good low frequency characteristics. Cole and Kishimoto (1962) obtained the best features of both systems by manufacturing platinized silver-silver chloride wire electrodes. Their technique was extended in this work to flat electrode surfaces 0.625 cm^2. These electrodes were used for both AC and DC measurements resulting in a considerable savings in time and space. They had very small ($\sim\frac{1}{2}$ mv) junction potentials so that there was no risk of applying unwanted DC potentials across the high resistance membranes.

Wheatstone Bridge

The Wheatstone bridge used in this work was designed and constructed specifically for these experiments since no commercial equipment was available to the author which satisfied completely the experiment's requirements. Bridges designed by others (Cole and Curtis, 1937; Schwan and Sittel, 1953) for measurements on biological systems are not completely applicable because conditions found in biological systems are quite different from those found in lipid bilayer systems. In measurements on tissues most of the information is obtained from conductivity measurements while capacitive measurements give the primary information for lipid bilayers; the design requirements for a bridge are quite different in the two cases.

The bridge finally built (White, 1969 *a*) operates between 20 cycle/sec and 10 kcycle/sec (operation to 100 kcycle/sec is possible using an appropriate bridge transformer) and is capable of detecting from a 1 part in 10^3 to 1 part in 10^5 variation in capacitance or resistance depending upon unknown, frequency, and input voltage. For low frequency capacitance measurements using small ($\sim$7 mv rms) driving voltages, the bridge is accurate to better than 0.2%. The inherent frequency dependence is negligible. Provisions were made for measuring the voltage across the unknown (at balance) and for applying DC polarizing potentials (without disturbing bridge characteristics) to a membrane during AC impedance measurements. The principal features of the bridge are illustrated by the simplified schematic diagram in Fig. 1. Current was supplied to the bridge by a Hewlett-Packard HP-651B test oscillator (Hewlett-Packard Co., Palo Alto, Calif.). The outputs from the amplifiers A_{uk} and A_0 were fed to separate channels of a Hewlett-Packard HP-132A dual beam oscilloscope which has external outputs from its vertical amplifiers. One of these amplifiers acted as a preamplifier for a GR-735A wave analyzer (General Radio Company, West Concord, Mass.) (4 cycle/sec bandwidth) which served as the final detector for the unbalance signal.

RESULTS

In early experiments, the measured values of specific capacitance, C_m, of black membranes were found to be highly variable (far outside of known experimental errors), to depend strongly on voltage, and to increase with time. Further, the sensitivity of C_m to applied voltage, V_a, depended upon the past history of the membrane: the voltage sensitivity of a given membrane always decreases with successive measurements. The initial results suggested that it might be possible to "force" the membrane into some "standard" state in which consistent results could be obtained. This was indeed found to be possible through the application of a steady DC potential at the appropriate time in the "life" of a membrane. Membranes subjected to a DC potential for a sufficient period of time ($\sim$20 min) are referred to as in a *final* state; "untreated" membranes are said to be in an *initial* state. The criterion that a state be final is that C_m at the end of series of measurements be, within experimental error, the same as at the beginning. Such a series of measurements usually took 15–45 min.

No effort was made to learn if this final state is the only possible one. Thus, the measurements reported here describe the behavior of membranes during a particular interval of their lives following a prescribed treatment.

Unless otherwise specified, the gross membrane capacitance (C) was determined using a 100 cycle/sec signal whose amplitude was 10 mv (rms) or less. Under the conditions of measurement $C \simeq C_p$ (the approximation is well within experimental error). The usual operating temperature was 27.5°C.

The Behavior of Membranes in the Initial State

The specific capacitance of the membranes was found to depend partially on the age of the bulk solution from which they were formed. This is shown by Table I which is based on data gathered over a 7 wk period from 35 membranes. The specific capacitance (C_m) of these membranes was determined when they had been black for less than 3 min. The table shows that there is a slow increase in C_m over the 7 wk period. Unless noted otherwise, the data reported below were gathered when the bulk solution was 5–7 wk old. A single 1 ml sample of bulk solution was used for the entire study.

The specific capacitance (C_m) of 14 membranes (bulk solution age = 5 wk) which had been black for 3 min or less averaged 0.432 $\pm$0.021 μF/cm² (range: 0.379–0.463). The standard deviation (~5%) represents variations inherent in the membrane since the experimental error is ~1%. The specific resistance was variable but a typical figure is 1×10^7 Ω-cm².

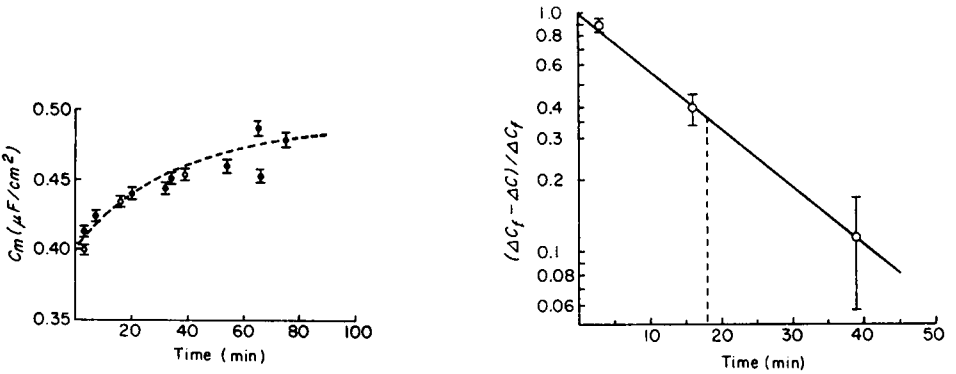
The cause of the variation in C_m is not understood. One possible explanation might be that factors which affect the equilibrium area (A_m) of black membrane also affect the capacitance. A_m varied from 1.20 to 1.44 $\times$ 10^{-2} cm² but C_m was not correlated with these variations. Using the rank difference method, the correlation was only 1.5%. C_m does not, therefore, depend totally upon details of formation which affect

TABLE I

C_m DEPENDS UPON THE AGE OF THE BULK SOLUTION. THE C_m ARE FROM INITIAL-STATE MEMBRANES WHICH HAVE BEEN BLACK FOR 3 min OR LESS. T = 27.5°C.

Age of bulk solution	Average C_m	Range of C_m
wk	*μF/cm²*	
1	0.402 (9)*	0.382–0.422
3	0.415 (5)	0.401–0.424
5	0.432 (14)	0.379–0.463
6	0.444 (5)	0.431–0.458
7	0.440 (2)	0.436 and 0.444

* Numbers in parentheses = number of membranes.



(a) (b)

FIGURE 3 The time dependence of C_m. (*a*) The specific capacitance (C_m) of freshly formed (initial-state) membranes always increases by 10–20% over a period of an hour. Zero time was taken as the time when thinning was completed. The figure is a composite of data from four membranes in 1×10^{-2} M KCl at 27.5°C. (*b*) The data from one membrane (open circles in [*a*]) plotted on a logarithmic ordinate scale to show that $C_m(t)$ is exponential. C_i is the capacitance at $t = 0$ and C_f the value at long times; $\Delta C_f = C_f - C_i$ and $\Delta C = C_m(t) - C_i$. The time constant for the increase is 18 min but varied from membrane to membrane (range: 15–20 min).

the final area. The lack of correlation also rules out current-dependent instrumental artifacts.

The specific capacitances of several membranes were followed in time for periods of up to 1½ hr. A composite of data from four membranes (Figure 3 *a*) demonstrates that C_m increases in time by about 15%. A plot of C_m vs. time (t) on semilog paper (Fig. 3 *b*) shows that the increase is approximately exponential with a time constant of ~20 min. The time constant varied from one membrane to another but was always 15–20 min.

This increase of C_m with time could result from the loss of solvent (*n*-decane) from the membrane. If the loss were to occur to the aqueous solution, the time constant should be affected by saturating the electrolyte with *n*-decane. This experiment was performed but no significant change in the time constant was observed.

The C_m increase might also result from small islands or lenses of thick material present initially but which eventually drift to the membrane's annulus causing the capacitance to increase. If f represents the fraction of the surface covered by islands of thickness $a\delta$ and if C_m' and C_m represent, respectively, the specific capacitance with and without islands, then

$$f = \frac{a}{a-1}\left(1 - \frac{C_m'}{C_m}\right). \quad (1)$$

The smallest fraction is obtained if $a \gg 1$ in which case

$$f = 1 - \frac{C_m'}{C_m}. \quad (2)$$

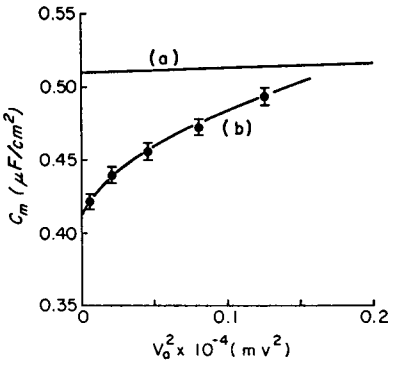


FIGURE 4

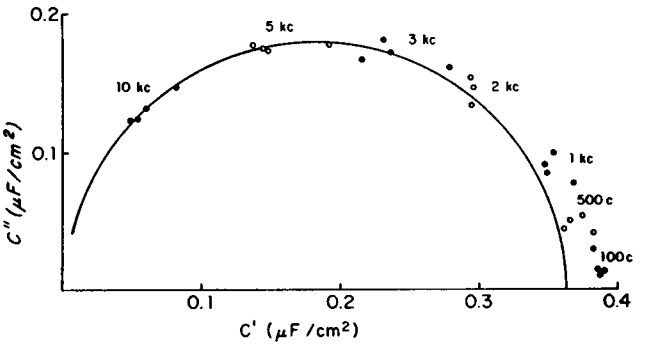


FIGURE 5

FIGURE 4 Effect of voltage on C_m for final- and initial-state membranes. Curve (*a*) is taken from Fig. 8 and represents the average of data from five final-state membranes formed from a 6 wk old bulk solution. Curve (*b*) is data from a single initial-state membrane (1 wk old bulk solution). T = 27.5°C in both cases.

FIGURE 5 Cole-Cole diagram for initial-state membranes. Data from four membranes in 10^{-4} M KCl is shown. The points scatter because each membrane was photographed at only one frequency while both A_m and C_m varied in time. The low frequency points deviate from the arc because of the strong dependence of C_m on voltage (Fig. 4). The signal across the membrane-electrolyte system was maintained at 10 mv (rms). At low frequencies, however, most of the signal appears across the membrane since the impedance of the membrane is much greater than that of the electrolyte. c = cycle; kc = kilocycle.

Since 15% increases in C_m were observed, initially ~15% of the area would need to be covered with thick lenses. This phenomenon, if it existed, should have been easily observed in the microscope but was not found.

Initial-state membranes were much more sensitive to applied voltage than final-state membranes (Fig. 4). This phenomenon can have a significant effect on Cole-Cole diagrams constructed for initial-state membranes (Fig. 5). At low frequencies where practically all of the applied voltage (10 mv rms) appears across the membrane per se, a significant deviation from the semicircle is seen. This deviation can be satisfactorily accounted through measurements of C_m vs. applied voltage.

The totality of variations seen in initial-state membranes is illustrated by Fig. 5. The scatter of points about the roughly fitted semicircle is a result of measuring A_m at only one frequency and assuming that C_m and A_m remain constant during the measurements. Since both A_m and C_m drifted during the experiment, the C_m calculated at each frequency vary. Data presented later will show that it is best to fit the data with a semicircular arc whose center is slightly depressed. This is not done here because the scatter in the points for a *particular* membrane is too great.

The Behavior of Membranes in the Final State

The specific capacitance was stabilized by applying a 100 mv DC potential for 10–20 min shortly after the thinning was complete. In four trial experiments, C_m remained

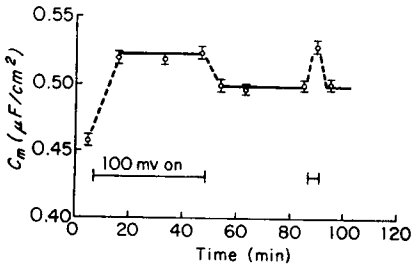


FIGURE 6

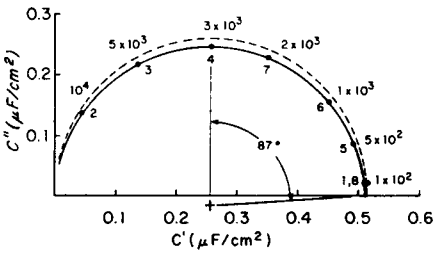


FIGURE 7

FIGURE 6 The effect of voltage on the time dependence of C_m. A 100 mv DC bias was applied to the membrane just after it had finished thinning. After removal of the bias signal, C_m remains constant in time. The reapplication and removal of the bias does not affect the zero bias value of C_m. This effect was utilized to produce *final-state* membranes. In these membranes, the zero bias C_m values are unaltered by a long series of measurements. Time was measured from the completion of thinning. T = 27.5°C.

FIGURE 7 Cole-Cole diagram for final-state membrane. The area of the membrane was determined for each frequency. Numerals below each point indicate the sequence of measurements. Points 1 and 8 are separated in time by 45 min indicating the reproducibility of measurements on final-state membranes. Note the 3° depression of the center. The frequencies are in cycle/sec.

constant for about an hour after the potential was removed (Fig. 6). Returning the 100 mv bias at a later time caused C_m to return to the previous 100 mv value. In all of the measurements which follow, C_m was measured with a 7 mv (rms) AC signal at the beginning and end of the experiment to test whether or not C_m had changed with time.

Fig. 7 is a plot of the membrane impedance at various frequencies in the complex capacitance plane. This Cole-Cole diagram shows the behavior expected from a resistance and capacitance in series. There is no low frequency deviation from the semicircle such as found for initial-state membranes. The sequence in which the measurements were made is indicated in the figure. The initial point is reproducible. Membrane area was determined at each frequency to exclude deviations caused by area changes.

The circular arc's center is depressed slightly (3°) but significantly below the C′ axis. This behavior is of the type described by Cole (1932). The depression is a result of the membrane-electrolyte-electrode system having a distribution of relaxation times rather than a single one. No explanation for this observation will be attempted here.

The specific capacitances of membranes in the final state depend linearly on V_a^2 (Fig. 1 of White, 1970). It was found that C_m responds to either AC or DC potentials. As expected, equal changes in C_m are produced by a 100 mv DC potential and a 100 mv (rms) AC potential. Since an AC signal is always present for making the capacitance measurement, the value for V_a is given approximately by $V_a = V_{DC} + V_{AC}^{rms}$ for small V_{AC}^{rms}.

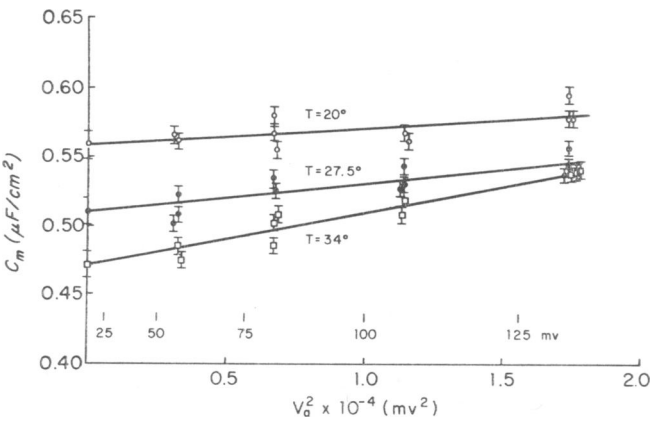


FIGURE 8 Effects of temperature on the voltage dependence of C_m. Straight lines have been fitted to the data by the method of least squares to determine C_0 and β in the equation $C_m = C_0 + \beta V_a^2$. Measurements were made on five membranes at each temperature. The value of C_0 is well approximated by measurements made at 7 mv (rms). Each C_0 is the mean of 10 measurements and the error bars for C_0 indicate the standard deviation (~2%) based on these measurements. This deviation is greater than experimental error (~1%) and represents variations in the membranes per se.

The dependence of C_m on V_a may be expressed by the equation

$$C_m = C_0 + \beta V_a^2, \tag{3}$$

where C_0 is the zero voltage capacitance. The parameters C_0 and β depend upon temperature in a striking way. C_m as a function of V_a was determined for five membranes at each of three temperatures. DC bias voltages were used for these measurements. The data were fitted to equation 3 by the method of least squares on a PB-440 computer (Packard-Bell Electronics Corp., Los Angeles, Calif.). The results are plotted in Fig. 8 (these results are given in tabular form in White, 1970).

The data show that as the temperature is increased C_0 decreases. At the same time the slope, β, of C_m vs. V_a^2 increases. The initial impression is that the membrane "expands" and becomes less "stiff" as the temperature is raised. If the capacitance changes because thickness changes, this effect can be interpreted as thermal expansion. Plotting C_0 and β as functions of temperature (Fig. 9) shows that for a 10°C drop in temperature. C_0 increases by ~13% while β decreases by 51%. Over the range of temperatures used both β and C_0 are linear (within experimental error) functions of T. Whether or not this is true at extremes of temperature was not determined. By extrapolation, $\beta = 0$ at $T = 15$°C. One attempt was made to make measurements at 10°C but the membranes would not thin properly to blackness and were unstable. Rather than having a propagating black region, the membrane seemed to become irregularly thin all over with numerous black lenses or perhaps particles trapped in them.

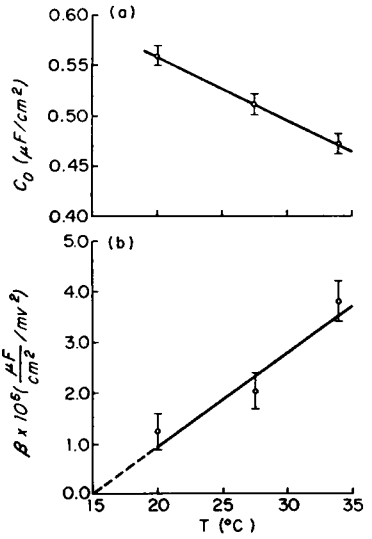


FIGURE 9 C_0 and β as functions of temperature. (*a*) The experimental values of C_0 fall on a straight line with negative slope. If the capacitance change is a result of a change in the thickness of the membrane, this effect can be interpreted as thermal expansion. (*b*) As temperature increases, β increases. The points do not significantly deviate from a straight line but there is an indication of a nonlinear relationship. A linear extrapolation to $\beta = 0$ indicates that C_m should be independent of voltage below 15°C.

An interesting phenomenon was observed when an AC signal was used to vary C_m. The bridge was balanced at the fundamental frequency and then the wave analyzer was tuned to the frequency of the third harmonic. A signal which depended upon the amplitude of the fundamental frequency was found. This is apparently not an artifact (e.g. third harmonics from the oscillator) because it was not seen in dummy circuits at similar signal levels. Harmonic generation is expected since C_m is a nonlinear circuit element.

DISCUSSION

The results reported in this paper show the dependence of specific capacitance on time, temperature, and voltage. The time course of C_m and the effects of voltage on this time course undoubtedly contain information important for understanding the dynamics of the thinning process. The membrane apparently thins to a quasistable state in which changes in composition and structure occur with a 15 min time constant. Changes might be explained by the loss or gain of oxidized cholesterol and/or decane from the thin region but the loss of decane to the aqueous solution cannot be responsible. Suppose that material is exchanged with the annulus by diffusion and treat the membrane as a thin cylindrical section with a radius (r_0) of ~0.1 cm. The equation for the time constant (τ) of the equilibration is given by Jost (1952) as

$$\tau = \frac{r^2}{(2.405)^2 D}, \qquad (4)$$

where D is the diffusion coefficient. If $\tau = 15$ min, then $D \sim 2 \times 10^{-6}$ cm²/sec. As a comparison, the diffusion coefficient of O_2 gas in vulcanized rubber is 2.1 ×

10^{-6} cm²/sec (Jost, 1952). There is apparently no reason to assume diffusion of surfactant or solvent from thin membrane to annulus to be unlikely. This simple calculation shows that a very modest diffusion coefficient would explain the data. The data are inadequate to draw any further conclusion about this effect. Hence, this discussion is directed primarily to the effects of temperature and voltage on membranes in the final state.

The Determinants of Capacitance

The use of the parallel plate capacitor formula in studies of the thickness or dielectric coefficient of lipid bilayers might be questioned since (*a*) the membrane may have fixed surface charge, (*b*) the "capacitor plates" are actually an electrolyte and hence the charge layer is diffuse rather than localized, and (*c*) polar groups of surfactant create a triple-layered dielectric. Further, the dielectric is very thin and highly oriented raising the distinct possibility of nonlinear and anisotropic behavior (Ohki, 1968).

The capacitance of the polar groups in series with the hydrocarbon moiety should have little influence on the measured capacitance. For example, the polar group capacitance of lecithin-decane membranes would be expected to be $\sim$50 μF/cm² assuming a nominal polar group thickness of 7 A and a dielectric constant of $\sim$80 (Hanai et al., 1965 *a*). This would have a negligible effect on the measured capacitance since the hydrocarbon capacitance is only $\sim$1 μF/cm². However, the work of Rosen and Sutton (1968) indicated that polar groups might still play some role. They found the capacitance of lecithin in octane membranes increases with applied field. The per cent increase depends upon electrolyte concentration. There is a minimum in the curve of per cent increase vs. concentration which occurs at an electrolyte concentration corresponding to a Debye length of $\sim$10 A, approximately the length of the polar group. Bilayers made from oxidized cholesterol which has only a diminutive polar group shows no such minimum. For oxidized cholesterol membranes, it appears reasonable to assume that the polar groups have no effect on the measured capacitance.

The diffuse charge at the surface of the membrane can also cause the measured specific capacitance, C_m, to differ from the geometrical capacitance $C_g = \epsilon_m\epsilon_0/\delta$, if the characteristic thickness (Debye length) of the diffuse layer is not negligible compared to the thickness of the membrane. Since there are two diffuse layers in series with the geometric capacitance, the measured capacitance may be written, as a first approximation,

$$C_m = \frac{C_g}{1 + \frac{2C_g}{C_d}}, \tag{5}$$

where C_d is the specific capacitance of the diffuse layer. C_d is given approximately by

the parallel plate capacitor formula taking the dielectric constant to be that of water (ϵ_w) and the thickness to be the Debye length ($1/\kappa$) of the diffuse layer. Thus, $C_d = (\epsilon_0\epsilon_w)/(1/\kappa)$ where $\kappa = (8\pi F^2)/(\epsilon_w RT)c$ (F is Faraday's number and c is the concentration of the 1:1 electrolyte). This is only an approximate equation. The complete equation has been worked out by Laüger et al. (1967) for the case of no fixed charges on the membrane surface:

$$C_m = \frac{\sinh \alpha}{\sinh \alpha + \frac{2\alpha C_g}{C_d}} C_g. \tag{6}$$

α is determined from the transcendental equation

$$\frac{C_d}{C_g} \sinh \alpha + 2\alpha = \frac{V_a}{2RT/F}, \tag{7}$$

where V_a is the applied potential. If, as is usually the case, $\alpha \ll 1$, then $\alpha = \sinh\alpha$ and equation 6 reduces to equation 5.

Assuming a 10^{-2} M electrolyte and a membrane of $\epsilon_m = 2.3$ and $\delta = 40$ A, $C_m = 0.95\ C_g$ using equation 5. Thus, the measured capacitance would appear to be about 5% lower than C_g if there are no fixed charges. As the electrolyte concentration decreases, the effect becomes more pronounced. Everitt and Haydon (1968) have performed calculations which include the effects of fixed surface charges. Their general conclusion is that fixed charges can nullify the effect of the diffuse layer if the surface concentration is great enough. They estimate that in general the charge density for lipid bilayers will be sufficient to have this effect.

The preceding discussion may be summarized by saying that the measured capacitance of the lipid bilayer membrane may contain contributions from the capacitance of (*a*) the membrane hydrocarbon, (*b*) the surfactant polar groups, and (*c*) the diffuse layer. The contribution of the polar groups is probably negligible while that of the diffuse layer may or may not be depending upon fixed charge and electrolyte concentration. Considering the electrolyte concentration used in these experiments and the arguments of Everitt and Haydon, there is no clear need for correcting C_m for the degrading effect of C_d.

Even if C_d is included, it probably is not responsible for the voltage and temperature effects. Using equations 6 and 7 it is estimated that a 40 v potential would be required to decrease C_m by 4%. Increasing the temperature from 20°C to 34°C would cause only a 0.4% decrease in C_m. Other changes (Haydon, 1964) resulting from dielectric saturation and finite ionic radii will also have a negligible effect.

It thus appears there is little error in assuming that C_d has no effect on C_m and that

$$C_m \equiv C_g = \frac{\epsilon_0 \epsilon_m}{\delta}, \tag{8}$$

where δ is taken as the hydrocarbon thickness.

Either δ or ϵ_m or both could depend on temperature and voltage. The effects of temperature and voltage on dielectric coefficients are well known. Thickness could change through a thermal expansion phenomenon (White, 1970) and by compression of the membrane by the electric field (Haydon and Overbeek, 1966).

The dielectric coefficient is an expression of the polarizability per unit volume of a medium (Debye, 1929) originating from permanent and induced dipoles in the constituent molecules. A permanent dipole can "rotate" in an applied field and contribute a polarizability proportional to μ^2/T where μ is the dipole moment and T the temperature. The induced dipole moment results from field-induced distortions in the electronic cloud and is independent of temperature.

The polarizability of a molecule i is

$$\alpha_i = \alpha_0{}^i + \frac{\mu_i^2}{3KT}, \tag{9}$$

where $\alpha_0{}^i$ is the electronic polarizability. For a dilute solution of polar molecules in a nonpolar medium, the α_i are related to the dielectric coefficient by

$$\frac{\epsilon - 1}{\epsilon + 2} = \frac{4\pi}{3} \sum_i n_i \alpha_i, \tag{10}$$

where n_i is the number of i molecules per unit volume and cgs units are used. Assuming that the temperature effect observed with the lipid bilayers is a result of the phenomena described by equations 9 and 10, a plot of $(\epsilon_m - 1)/(\epsilon_m + 2)$ vs. $1/T$ should yield a linear curve. This is found to be the case if one assumes that thickness remains constant. If these assumptions are valid, then it should be possible to calculate $\alpha_0{}^i$ and μ_i for the oxidized cholesterol molecules in the bilayers.

This calculation is complicated because the membrane has a minimum of four components: n-decane and at least three oxidation products of cholesterol (Tien et al. 1966)., These products are much the same so average "cholesterol" values are calculated. The exact composition of the membrane is unknown but two fairly extreme cases resolve the problem. Based on data for phospholipid-decane membranes (Cook, Redwood, Taylor, and Haydon, 1968; Henn and Thompson, 1968), α_0^{chol} and μ_{chol} were calculated assuming either 1.4 or 10 decanes per "cholesterol." In both cases α_0^{chol} was found to be negative! Thus, the variation of ϵ_m with temperature is too great to be caused by a $\mu^2/3KT$ term alone. The use of equation 10 for a highly condensed system can be questioned but the results still strongly suggest that the temperature effect does not result from rotating permanent dipoles. This might be expected a priori since the membrane is highly organized and the surfactant molecules are under high pressure (Tien, 1968).

A 100 mv potential difference across a 40 A membrane creates an electric field

of 2.5×10^5 v/cm. A field this size can affect ϵ_m through dielectric saturation. However, saturation generally *decreases* the dielectric coefficient and such changes usually are of the order of 1 part in 10^4. These facts (Böttcher, 1952) suggest that the electric field does not directly affect ϵ_m.

The most reasonable conclusion from the foregoing arguments is that temperature and voltage have no *direct* or large effect on the dielectric coefficient. By exclusion, hydrocarbon thickness is the quantity affected. However, the possibility that the change in thickness has a secondary effect on ϵ_m through a composition change cannot be excluded. If the membrane's density and composition remain constant, ϵ_m should also remain constant.

The conclusion that hydrocarbon thickness is the affected variable is compatible with equation 3 if it be supposed that the membrane is a compressible elastic system. The force exerted on the dielectric of a parallel plate capacitor (1 cm^2) by an electric field is given by

$$F_v = \frac{C_m^2 V_a^2}{2\epsilon_m \epsilon_0}, \tag{11}$$

(Corson and Lorrain, 1962). C_m is very accurately linear in V_a^2 which strongly supports the compression hypothesis (Haydon and Overbeek, 1966). Since $C_m = 0.524$ $\mu F/cm^2$ at 30°C and $V_a = 100$ mv, the pressure calculated from equation 11 is 0.7×10^5 dynes/cm^2 or 0.07 atm. Rosen and Sutton (1968) point out that this would have a negligible effect if the membrane has a compressibility typical of organic liquids (10^{-4}/atm). However, the lipid bilayer is a highly organized interfacial system and the concept of bulk compressibility is probably not applicable.

If only the thickness of the membrane varies, the thickness at a given temperature and voltage can be calculated from the data in Fig. 8 by using equation 8. A reference capacitance, dielectric constant, and thickness are needed, however, to carry out the calculation. Tien et al. (1966) give the thickness of oxidized cholesterol membranes as 40 ± 10 A at 30°C. Assuming $\delta = 40$ A and using the value of $C_m = 0.496$ $\mu F/cm^2$ at 30°C from Fig. 9, the dielectric coefficient is found to be 2.24. These reference values have been used to calculate membrane thickness as a function of temperature and voltage. The results of these calculations have been plotted elsewhere (White, 1970) as δ vs. T with V_a as a parameter. The rate of thermal expansion decreases as voltage increases. The coefficient of thermal expansion, $\theta = (1/\delta) \cdot (\partial\delta/\partial T)$. For $V_a = 0$, θ is found to be 1.3×10^{-2}/°C. An organic liquid such as pentane has a $\theta \sim 0.05 \times 10^{-2}$/°C. Thus, θ is approximately two orders of magnitude greater than expected if the membrane behaves as a simple liquid.

It is quite clear that the observed effect cannot be explained on the basis of simple bulk properties. A physical basis for the observations can be found by examining the details of membrane stability.

The voltage and temperature effects appear to be closely linked. A possible explanation for this linkage can be found by examining semiquantitatively the potential energy vs. thickness curves for this system. If the relationship between potential energy and thickness is affected by temperature and voltage, then changes in thickness would be expected; that is, the stability conditions would be modified. In the discussion which follows, the basic concept of stability suggested by Taylor and Haydon (1966) will be followed. It will be assumed that the basic force driving the monolayers of the bilayer together is that arising from the London-van der Waals forces between the separated aqueous phases. If an electric field is present, there is an additional force in the same direction proportional to the square of the applied potential (equation 11). Also assume that steric repulsive interactions between the monolayers oppose this compression. Finally, assume that the Plateau-Gibbs border suction (Overbeek, 1960) has an insignificant effect. Experimentally, this last assumption seems justified since there is no correlation between C_m and area (i.e. curvature of meniscus and therefore border suction).

Let $U(\delta)$ represent potential energy of a membrane of thickness δ relative to a very thick (unthinned) membrane. $U(\delta)$ may be written

$$U(\delta) = U_L + U_V + U_S, \tag{12}$$

where U_L represents potential energy between separated aqueous phases, U_V the term due to the electric field and U_S the term due to steric interactions. Consider each of these terms in turn.

U_L may be written (Overbeek, 1952)

$$U_L = -\frac{A}{12\pi\delta^2} \cdot R, \tag{13}$$

where A is the Hamaker constant and R is the coefficient of retardation. This term may be evaluated from the data of Haydon and Taylor (1968) where R was taken as 0.8 and A was found to be 5.6×10^{-14} erg. R actually depends upon thickness but for present purposes it will be assumed constant since only small changes in δ around some equilibrium point are important.

The term U_V is most simply evaluated from the data by considering the energy stored in a capacitor at constant potential (Corson and Lorrain, 1962).

$$U_V = -\frac{\epsilon_m \epsilon_0}{2\delta} V_a^2. \tag{14}$$

U_L and $U_L + U_V$ (100 mv) have been plotted in Fig. 10.

Little is known about U_S. However, it must increase rapidly enough with decreasing thickness to cause a minimum in $U(\delta)$ (and thus a stable point) and it

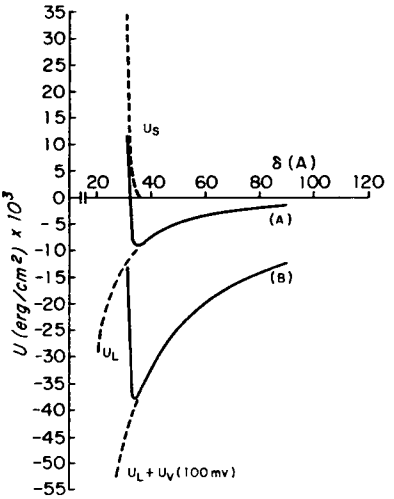


FIGURE 10 Hypothetical potential energy vs. thickness curves for oxidized cholesterol membranes. $U = U_L + U_S$ (curve A) is the potential energy when there is no applied potential. $U_L = -(A/[12\pi\delta^2]) \cdot R$ is the London-van der Waals attractive energy and $U_S = 4n\epsilon(\sigma/[\delta - 22.5])^{12}$ is the assumed steric repulsive term taken from the Lenard-Jones potential. When a voltage (V_a) is applied, the potential energy becomes more negative by $U_V = -(\epsilon_0\epsilon_m/2\delta)V_a^2$ and the potential well deepens and narrows (curve B). This simple formulation does not adequately explain the effects of temperature and voltage on membrane thickness. The increased depth, however, is consistent with experimental observations.

must equal 0 for thicknesses greater than twice the length of the surfactant molecule. Repulsive forces, such as U_S, resulting from electronic interactions between two molecules are usually taken to be proportional to $(1/r)^n$ where r is the molecular separation and n is a positive number between 9 and 12 (Hirschfelder, Curtis, and Bird, 1954). Assume for a moment that the molecules of the opposing monolayers obey this type of equation and that a molecule in one layer interacts with only one molecule in the other layer.

It makes little difference what the value of n is assumed because the curve falls so sharply that it is almost vertical on the distance scale appropriate to membrane calculations. For convenience take U_S as the repulsive term in the Lenard-Jones potential (Hirschfelder et al., 1954):

$$U_S = 4n\epsilon\left(\frac{\sigma}{r}\right)^{12}, \qquad (15)$$

where $n\epsilon$ is the total characteristic repulsive energy for the n surfactant molecules and σ is the characteristic distance for the interaction. ϵ and σ have been tabulated for a number of molecules (Hirschfelder et al., 1954); appropriate values might be those for benzene: $\epsilon \sim 5.5 \times 10^{-14}$ erg and $\sigma \sim 5$ A; n was taken as $1.6 \times 10^{14}/\text{cm}^2$ (Cook et al., 1968). These parameters have a definite meaning only for the Lenard-Jones potential since they are defined relative to the attractive potential which varies as $(1/r)^6$. The repulsive force varies so rapidly with distance that these are acceptable estimates.

U_S has been plotted in Fig. 10 as have the composite curves $U(\delta) = U_L + U_S$ and $U_L + U_V + U_S$. Since U_L is proportional to $1/\delta^2$, the shape of U changes significantly with the application of a DC potential (100 mv in this case) because U_V is proportional to $1/\delta$. The minimum becomes approximately four times lower than

in the zero voltage case, the potential well becomes narrower, but there is only a slight decrease in thickness. The deepening of the well means that the surface-free energy of the bilayer decreases when a voltage is applied. This is consistent with the observations of B. Hille and J. A. Chapman (personal communication) on small droplets trapped in bilayers. They found that the diameters of the droplets decreased when a voltage was applied. Assuming constant droplet volume, the change is explained by an additional decrease in free energy (surface tension) of the bilayer relative to a thick membrane (Haydon and Taylor, 1968). Although this simple formulation appears to be a good first approximation, it obviously does not account for the observed effects of temperature and voltage.

One way of introducing the thermal and voltage effect is to assume that the surfactant molecules are undergoing thermal vibration normal to the plane of the membrane and that the amount of thermal energy in this mode increases as the membrane thins (White, 1969 *a*). In this way thermal energy levels of the monolayers would be superimposed on the $U(\delta)$ curve. Because $U(\delta)$ is asymmetric, the time-averaged thickness (equilibrium point) would depend upon temperature. This situation would be exactly equivalent to thermal expansion in solids. The electric field would exert its effect by making the $U(\delta)$ curve more symmetric. Increased symmetry would cause the loci of the thermal equilibrium points to shift toward *smaller* thicknesses. The approach just outlined very neatly explains all of the data. However, as D. A. Haydon (personal communication) has pointed out, it is assumed implicitly that the density of the membrane changes. The density changes would unfortunately severely affect ϵ_m. Changes in density of 10–15% seem unrealistic.

An alternative approach is to assume that U_S does not increase as steeply as originally suggested and that the shape of U_S depends upon temperature and perhaps electric field. A hypothetical situation is shown in Fig. 11 which is completely

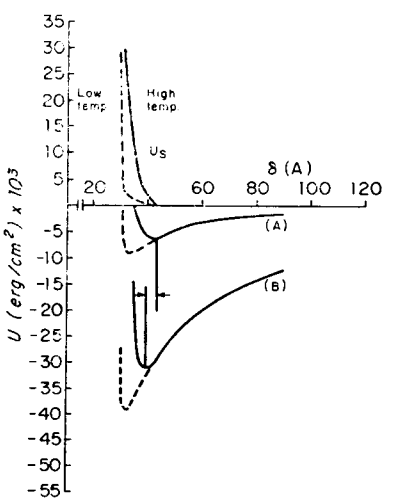


FIGURE 11 Hypothetical potential energy vs. thickness curves for oxidized cholesterol membranes. U_S is assumed to be temperature dependent and two extremes are shown. Broken lines indicate the low temperature (~15°C) case and solid lines the high temperature (~35°C) case. Curve A represents $U(\delta)$ when there is no applied voltage while curve B represents $U(\delta)$ when a 100 mv bias is present. The arrows show how much the thickness would decrease with the application of the field at high temperatures. A negligible thickness decrease is predicted at low temperatures — a result suggested by Fig. 9.

compatible with all of the data. The figure shows that as temperature is decreased, the U_S curve becomes much more steep. For the low temperature (15°C) curve, the electric field has a negligible effect on thickness. This is consistent with the data shown in Fig. 9 where, by linear extrapolation, $\beta = 0$ at 15°C.

There is some theoretical support for this hypothesis. E. W. Fischer (1958), reporting on his investigations of the stability of colloids in hydrophobic media, suggested that P. J. Flory's theory of dilute polymer solutions (see Flory, 1953) could readily explain the colloidal stability. The specific situation was to explain the change in free energy of mixing when polymer molecules in dilute solution interacted. Adapting Flory's theory to the bilayer yields the following equation for the change in free energy when the surfactant molecules of the bilayer interact:

$$\Delta F_a = 2KT(\psi_1 - \kappa_1) \frac{(\bar{v}_S^2)}{V_1} a, \tag{16}$$

where a = amount of overlap of the monolayers of the bilayer, $\bar{v}_S$ = volume fraction of membrane occupied by surfactants, V_1 = volume of solvent molecules, K = Boltzmann's constant. ψ_1 is an entropy factor—if entropy decreases, it becomes larger. When the monolayers start to overlap there is greater organization, ψ_1 increases causing ΔF_a to increase, and further overlap is not favored. κ_1 represents an enthalpy of mixing term. In effect it is the difference in interaction energy between a solvent-solvent and a solvent-solute interaction, i.e., the nonuniformity of intermolecular interactions. If surfactant molecules prefer to associate with solvents rather than one another, then a further increase in ΔF_a is favored.

Equation 16 was derived assuming the concentration of surfactant to be constant. However, it is not unreasonable to expect the concentration ($\bar{v}_S$) to change with temperature. Further, since the oxidation products of cholesterol such as 7-dehydrocholesterol and 3,7-dihydroxycholesterol (Fioriti and Sims, 1966) are asymmetric molecules, one might expect ψ_1 and κ_1 to depend on thickness. Cholesterol for example has a rather solid ring structure with a flexible branched aliphatic chain attached which would be in the interior of the membrane. The chain-chain interaction at wide separation might be different from chain-ring interactions at closer separation. An electric field might also affect the ψ_1 term.

Redwood and Haydon (1969) found no temperature effect in membranes formed from lecithin in decane. When cholesterol was added to the system, they found temperature effects similar to those reported here. Their observations can be made consistent with mine by assuming that in the simpler system that U_S was independent of temperature in the range studied. Lecithin aliphatic chains are relatively more symmetric and flexible so that U_S would not depend strongly on T, δ, or V_a.

SUMMARY

The effects of voltage and temperature on the specific capacitance of oxidized cholesterol membranes have been interpreted as resulting from thickness variations. The

possibilities that these effects result from diffuse layer, polar groups, or membrane dielectric phenomena were considered but found to be quantitatively untenable. The possibility that thickness variations cause concomitant changes in the dielectric coefficient, could not be eliminated.

Assuming voltage and temperature changes cause only variations in thickness, potential energy vs. thickness curves were calculated assuming thickness equals 40 A at 30°C. It was further assumed for the calculation that an attractive force across the membrane results from London-van der Waals interaction between aqueous phases and that a repulsive force arises from steric interactions between surfactant molecules. An applied potential modifies these interactions by supplying an additional attractive force and causes a significant modification of the unperturbed potential energy diagram. The temperature effect is interpreted as resulting from the steric interaction term being temperature sensitive.

I am indebted to my friend and teacher Professor J. W. Woodbury for his constant advice and encouragement.
Discussions of this work with Drs. Bertil Hille and D. A. Haydon provided several useful insights and stimulated a number of ideas for which I am grateful.
The help and suggestions of Dr. M. C. Mackey during the initial phases of the work are sincerely appreciated.

I was supported by a training grant GM-00739 from the National Institute of General Medical Sciences and the research was supported, in part, by a grant NB-01752 from the National Institute of Neurological Diseases and Stroke, National Institutes of Health.

Received for publication 4 February 1970 and in revised form 16 July 1970.

REFERENCES

BABAKOV, A. V., L. N. ERMISHKIN, and E. A. LIBERMAN. 1966. *Nature (London).* **210**:953.
BÖTTCHER, C. J. F. 1952. Theory of Electric Polarization. Elsevier, Amsterdam.
CHERRY, R. J., and D. CHAPMAN. 1969. *J. Mol. Biol.* **40**:19.
COLE, K. S. 1932. *J. Gen. Physiol.* **15**:641.
COLE, K. S., and R. H. COLE. 1941. *J. Chem. Phys.* **9**:341.
COLE, K. S., and H. J. CURTIS. 1937. *Rev. Sci. Instrum.* **8**:333.
COLE, K. S., and U. KISHIMOTO. 1962. *Science (Washington).* **136**:381.
COOK, G. M. W., W. R. REDWOOD, A. R. TAYLOR, and D. A. HAYDON. 1968. *Kolloid-Z.* **227**:28.
CORSON, D. R., and P. LORRAIN. 1962. Introduction to Electromagnetic Fields and Waves. W. H. Freeman and Co., Publishers, San Francisco.
DEBYE, P. 1929. Polar Molecules. Dover Publications, Inc., New York.
EVERITT, C. T., and D. A. HAYDON. 1968. *J. Theor. Biol.* **18**:371.
FIORITI, J. A., and R. J. SIMS. 1966. *J. Amer. Oil Chem. Soc.* **44**:221.
FISCHER, E. W. 1958. *Kolloid-Z.* **160**:120.
FLORY, P. J. 1953. Principles of Polymer Chemistry. Cornell University Press, Ithaca.
HANAI, T., D. A. HAYDON, and J. TAYLOR. 1964. *Proc. Roy. Soc. (London).* **281A**:377.
HANAI, T., D. A. HAYDON, and J. TAYLOR. 1965 *a. J. Theor. Biol.* **9**:278.
HANAI, T., D. A. HAYDON, and J. TAYLOR. 1965 *b. J. Theor. Biol.* **9**:422.
HAYDON, D. A. 1964. Progress in Surface Science. J. F. Danielli, K. G. A. Pankhurst, and A. C. Riddiford, editors. Academic Press, Inc., New York. **2**.
HAYDON, D. A., and J. T. G. OVERBEEK. 1966. *Discuss. Faraday Soc.* **42**:66.
HAYDON, D. A., and J. L. TAYLOR. 1968. *Nature (London).* **217**:739.

HENN, F. A., and T. E. THOMPSON. 1968. *J. Mol. Biol.* **31**:227.
HIRSCHFELDER, J. O., C. F. CURTIS, and R. B. BIRD. 1954. Molecular Theory of Gases and Liquids. John Wiley and Sons, Inc., New York.
HUANG, C., and T. E. THOMPSON. 1965. *J. Mol. Biol.* **13**:183.
HUANG, C., L. WHEELDON, and T. E. THOMPSON. 1964. *J. Mol. Biol.* **8**:148.
JOST, W. 1952. Diffusion in Solids, Liquids, and Gases (Revised). Academic Press, Inc., New York.
LAÜGER, P., W. LESSLAUER, E. MARTI, and J. RICHTER. 1967. *Biochim. Biophys. Acta.* **135**:20.
MUELLER, P., D. O. RUDIN, H. T. TIEN, and W. C. WESCOTT. 1962. *Circulation.* **26**:1167.
MYSELS, K. J., K. SHINODA, and S. FRANKEL. 1959. Soap Films: Studies of their thinning. Pergamon Press, Inc., New York.
NAPPER, D. H. 1967. *Sci. Progr. London.* **55**:91.
OHKI, S. 1968. *J. Theor. Biol.* **19**:97–115.
OVERBEEK, J. T. G. 1952. Colloid Science. H. R. Kruyt, editor. Elsevier, Amsterdam. **1**.
OVERBEEK, J. T. G. 1960. *J. Phys. Chem.* **64**:1178.
REDWOOD, W. R., and D. A. HAYDON. 1969. *J. Theor. Biol.* **22**:1.
ROSEN, D., and A. M. SUTTON. 1968. *Biochim. Biophys. Acta.* **163**:226.
SCHWAN, H. P. 1963. *Phys. Tech. Biol. Res.* **6**:323.
SCHWAN, H. P., and K. SITTEL. 1953. *Trans. Amer. Inst. Elec. Eng.* **72**:114.
TAYLOR, J., and D. A. HAYDON. 1966. *Discuss. Faraday Soc.* **42**:51.
TIEN, H. T. 1968. *J. Gen. Physiol.* **52**:125s.
TIEN, H. T., S. CARBONE, and E. A. DAWIDOWICZ. 1966. *Nature (London).* **212**:718.
TIEN, H. T., and A. L. DIANA. 1968. *Chem. Phys. Lipids.* **2**:55.
WHITE, S. H. 1969 *a*. A study of the specific capacitance of black lipid membranes. Ph.D. Thesis. University of Washington, Seattle.
WHITE, S. H. 1969 *b*. Abstracts of the Third International Biophysics Congress of the International Union for Pure and Applied Biophysics. 57.
WHITE, S. H. 1970. *Biochim. Biophys. Acta.* **196**:354.