EFFECT OF ULTRASOUND ON A BILAYER LIPID MEMBRANE

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ABSTRACT The effects of continuous wave ultrasound at a frequency of 1 MHz in the intensity range of 0–1.4 W/cm^2 on an oxidized cholesterol bilayer lipid membrane (BLM) were observed. Ultrasound at 1.5 W/cm^2 broke the membrane; in the range from 0.5 to 1.4 W/cm^2, it accelerated the draining of the bulk lipid solution from the annulus to the Teflon support. At all intensities it has no effect on the conductance, the capacitance, or the dependence of each on the voltage applied across the membrane. Electrical parameters were measured in the presence of aqueous solutions of NaCl, KCl, and distilled water. The motivation and results of this project are explained in relation to an overall objective of determining the specific effects of ultrasound on biological membranes.

INTRODUCTION

In recent years there has been a sharp increase in the availability and use of ultrasonic devices in medicine. As a result of this increase, there has been renewed interest in understanding the physical mechanisms involved in ultrasonically produced changes in living systems. The focus of this investigation is the site for a wide variety of life processes, the membrane. Any effects that ultrasound has on a basic membrane structure would influence many different systems, yet details of the effect of ultrasound on membrane systems have not been extensively studied. Recently Coble and Dunn (1976) have demonstrated that ultrasound at a frequency of 1 MHz can produce reversible changes in the electrical parameters of isolated frog skin. This study complements earlier work by Fry et al. (1951, 1958), in which mechanisms for reversible and irreversible changes in nervous tissue were investigated.

In the present investigation, an artificial membrane system was chosen for study to provide an unmodified membrane that has some characteristics in common with all biological membranes. If ultrasound induces changes in the unmodified membrane, then it is likely that most membranes will be influenced by ultrasound. On the other hand, if ultrasound has no effect on the unmodified membrane, then any effects appearing in modified or in biological membranes must be due to the additives, or specific

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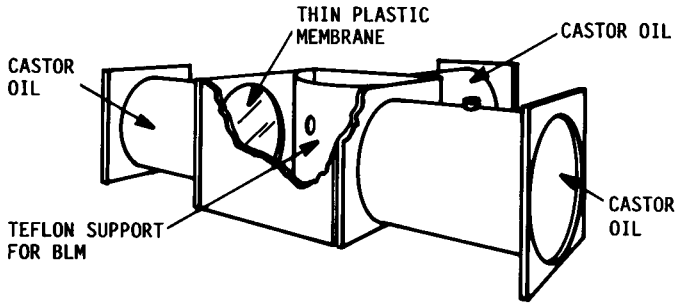

FIGURE 1 BLM chamber. The cutaway shows the hole in the Teflon where the BLM is formed and the plastic membrane separating the castor oil from the electrolyte.

molecular structures of that membrane. In fact, effects of low frequency vibration have been observed by Ochs and Burton (1974) and Pasechnik and Sokolov (1973).

Oxidized cholesterol was chosen for the bilayer lipid membrane (BLM) because oxidized cholesterol membranes can model various biological membranes (nerve, mitochondrial, visual receptor) when appropriate modifiers are added (Mueller and Rudin, 1968; Tien, 1974). This experiment consisted of monitoring electrical parameters of a BLM in the presence of NaCl, KCl, and distilled water. The membrane's reaction to continuous-wave ultrasound radiation with intensities varying from zero to 1.4 W/cm^2 was measured, the upper intensity limit being set by the bursting of the BLM.

METHODS AND APPARATUS

To measure these electrical parameters, a BLM was formed on a Teflon support that divided two aqueous solutions, as shown in Fig. 1. The hole used for the membrane support was 0.9 mm in diameter drilled through an 0.2-mm-thick region of a piece of Teflon. The experiment was

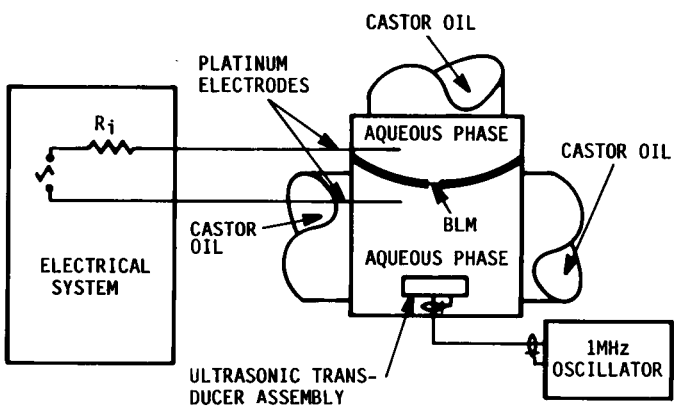

FIGURE 2 Block diagram of experimental system. From left to right: a simplified schematic of the electrical system, top view of the BLM chamber, and the ultrasound system.

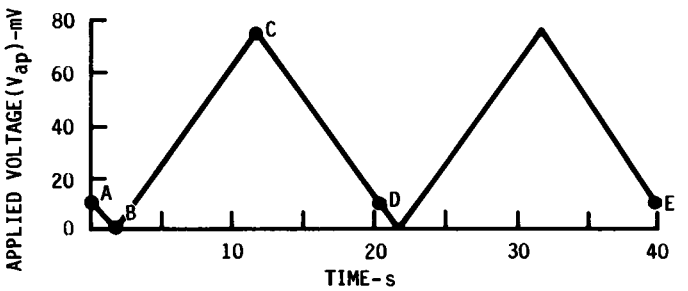

FIGURE 3 Graph of applied voltage versus time. Note that the applied voltage did not start or end at zero. The letters indicate events labeled correspondingly in Fig. 4.

repeated with distilled water, 1 mM NaCl, 10 mM NaCl, 1 mM KCl, and 10 mM KCl for the aqueous phase. An ultrasonic transducer 1.3 cm in diameter with a resonant frequency of 1 MHz was positioned in front of the BLM; to the sides and in back of the membrane were castor oil compartments for sound absorption. In this manner, the intensity at the membrane was known, because standing waves were eliminated and localized power variations were minimized. The output intensity of the transducer assembly was calibrated by radiation force measurements (Rooney, 1973) in the far field and by beam profile measurements using a small probe hydrophone. The far field for the transducer began at about 2 cm from the face of the transducer assembly, in agreement with theory (Zemanek, 1971), and the experiment was conducted with the BLM 3 cm from the transducer face.

The oxidized cholesterol membrane was formed by thinning a bulk solution of oxidized cholesterol dissolved in octane. The BLM chamber was first filled with the aqueous solution so that the BLM support hole was completely covered. A drop of the oxidized cholesterol solution was then placed in the hole with a Hamilton 50-μl syringe (Hamilton Co., Reno, Nev.). Typically, the membrane turned black in 1–10 min and remained stable for 30 min to several hours if left undisturbed.

The electrical parameters were monitored by bright platinum electrodes on both sides of the Teflon support, as illustrated in Fig. 2. A triangle wave generator set at 0.05 Hz provided a steadily increasing voltage to the electrodes during the first half cycle, as shown in Fig. 3, and a series resistor monitored the current passing from one electrode to the other. Since the voltage was increasing at a steady rate, the capacitance charging current remained approximately constant, whereas the conductance current increased with voltage. Current as a function of voltage was monitored by an *x-y* plotter, which yielded graphs of the type shown in Fig. 4. Before the

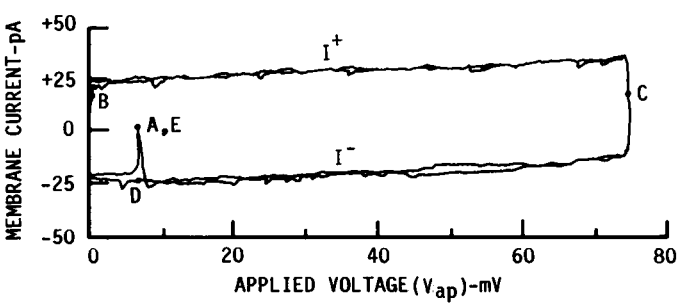

FIGURE 4 Typical data graph. The letters I^+ and I^- refer to the current present during the upward and downward (respectively) parts of the applied voltage triangle wave.

triangle wave is initiated, the resting point for the pen can be seen as a peak in the bottom line (point A). When the triangle wave is initiated, there is a sudden change in current (downward), caused by the discharge of the membrane capacitance. The sweep progresses to the left to zero applied voltage, then changes abruptly to the top line on the data graph when the capacitance of the membrane starts charging (point B). The sweep was triggered a second time before the triangle generator's resting point was reached on the return trip (point D). This established the repeatability of the system within a short time, and helped point out random noise in the curves. At the end of the second sweep, the generator stopped at about 10 mV and the current returned to its rest value for that voltage.

As a control, the same procedure just described was used with either a drop of bulk BLM-forming solution or an air bubble filling the hole in the Teflon. This established the leakage current and stray capacitance due to the leads and the chamber. The total system had a resolution of approximately ± 0.01 nmho for conductance measurements, and $\pm 3\%$ for capacitance measurements. Since the voltage dependence of conductance and capacitance was very slight, the resolution for these parameters was poor. Both dg/dV and dC/dV are reported here for order-of-magnitude comparison to other experiments. These parameters are averaged values over a change in membrane voltage from zero to 100 mV.

MATHEMATICAL ANALYSIS

The impedance of the BLM chamber with a membrane present can be represented by the circuit shown in Fig. 5, where g_m and c_m are the conductance and capacitance of the membrane, and g_l is the leakage conductance. The leakage conductance occasionally became measurable, so that the measured conductance of the cell must be represented by

$$g = g_m + g_l. \tag{1}$$

The stray capacitance was found to be immeasurably small, so that the total capacitance, C, is identical to the membrane capacitance. The capacitance of the membrane is assumed to be due to the electrolyte solutions on both sides of the membrane acting as conducting plates separated by the nonconducting hydrocarbon tails of the oxidized cholesterol. Therefore, the capacitance can be described by standard expressions for parallel plate capacitors (Tien, 1974, pp. 138–142). At the frequency used for data acquisition (0.05 Hz), any dipole rotation with dielectric relaxation time less than

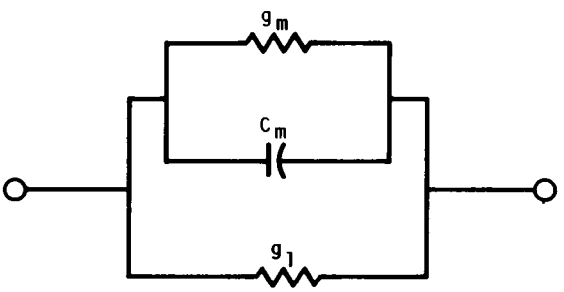

FIGURE 5 Equivalent circuit of BLM in the chamber. The lower arm of the circuit represents the leakage conductance of the chamber, and the top arm represents the BLM.

0.5 s will be adequately described by the theory, i.e. the error introduced would be less than the system's resolution. In fact the typical dielectric relaxation time for large molecules in a viscous liquid is of the order of 1 μs (Smyth, 1955).

The measured current, I, can be expressed as the sum of two terms:

$$I = gV + \mathrm{d}(CV)/\mathrm{d}t, \tag{2}$$

where V is the potential difference across the membrane, and t is time. Both terms in the derivative must be retained since most experimenters (Tien, 1974, pp. 142–145) have found the capacitance to be voltage-dependent. There is, incidentally, general agreement that conductance is voltage-dependent.

After expanding the derivative and accounting for the influence of the current measuring resistor, R_i, Eq. 2 becomes a complicated nonlinear differential equation. Since the accuracy of the data does not warrant using an exact solution, an approximation can be used, resulting in Eq. 3, accurate to within 0.2% (Rohr, 1976).

$$I = gV + (C + V \cdot \mathrm{d}C/\mathrm{d}V) \cdot (1 - R_i g - R_i V \,\mathrm{d}g/\mathrm{d}V) \cdot (\pm k). \tag{3}$$

Here $\pm k$ is the slope of the applied voltage triangle wave. Note that the voltage applied to the system is not the same as the voltage at the membrane but is changed by the voltage drop across the current-measuring resistor.

Using $I^{\pm}$ to denote the cases with values of $\pm k$, the following two equations may be written:

$$(I^+ + I^-)/2 = gV, \tag{4}$$

$$(I^+ - I^-)/2 = (C + V \cdot \mathrm{d}C/\mathrm{d}V) \cdot (1 - R_i g - R_i V \cdot \mathrm{d}g/\mathrm{d}V)k. \tag{5}$$

This analysis disregards the exponential region of the data graph at the beginning of each half cycle. Thus all data points used in the computer program were taken from the region where the I-V curve approximates a straight line. The data consisted of seven or eight pairs of values of I^+ and I^- at specified voltages (V). Eq. 4 was used to determine g at each of the seven or eight voltages. Changes in g along a given data graph were expressed as $\mathrm{d}g/\mathrm{d}V$ values and stored. This collection of values for g and $\mathrm{d}g/\mathrm{d}V$ were then used in Eq. 5 and the same data were processed again to determine C and $\mathrm{d}C/\mathrm{d}V$. Notice that there are two unknowns in this equation, so Eq. 5 would have to be written twice (at two different voltages) to calculate a single value for C and for $\mathrm{d}C/\mathrm{d}V$.

RESULTS AND CONCLUSIONS

The major result of this experiment is that the electrical parameters measured stay within their normal variation in the presence of ultrasound up to an intensity of 1.4 W/cm^2. Approximately 150 I-V graphs were constructed as described previously. First, a set of graphs were constructed monitoring the electrical parameters' change in time, with the ultrasonic driver off. This provided information that characterized the

TABLE I
SUMMARY OF DATA

Aqueous phase	Conductance		dg/dV	
	nmho	*nmho/cm²*	*nmho/V*	*nmho/V-cm²*
1 or 10 mM NaCl	0.7–1.0	120–160	2–7	400–1,200
1 or 10 mM KCl	0.1–0.5	32– 80	1.5–2.5	250–400
Distilled water	0.03–0.05	5– 8	<1	<150
	Capacitance		dC/dV	
	nF	*F/cm²*	*nF/V*	*F/V-cm²*
All aqueous phases	4–4.5	0.6–0.7	0.7–7	0.1–1

amount of variation in parameters and the progression of parameters in the absence of ultrasound. Second, the procedure was repeated with various ultrasound intensities. Both procedures resulted in measurements summarized in Table I.

The values shown in Table I for capacitance are in good agreement with the work done previously with similar membranes. The conductances in distilled water and in KCl are also in good agreement with values previously reported. But the conductance measurements in the presence of NaCl are an order of magnitude greater than previously reported. Tien and Diana (1967) measured conductances in the range of 10^{-8}–10^{-9} mho/cm² for oxidized cholesterol BLM's surrounded by 100 mM NaCl. We have produced other BLMs using the same procedure that have had conductances in the range reported by Tien and Diana (1967). The latter oxidized cholesterol BLMs also failed to show any changes in electrical parameters in the presence of ultrasound.

It was observed that the capacitance gradually increased in time even when the ultrasonic driver was off, although the effect was accelerated by the presence of ultrasound. This increase was always accompanied by the draining of the bulk oxidized cholesterol solution from the annulus onto the Teflon, resulting in an increase in BLM area. Direct measurement of BLM area confirmed the explanation that increase in area caused the increase in capacitance, and the acceleration in annulus draining in the presence of ultrasound was attributed to the time-independent flow of fluid normal to the transducer face called acoustic streaming (Nyborg, 1965). The total increase in capacitance was typically +15%, i.e. it changed from about 8% below average to about 8% above average as time progressed.

Because of the extremely low frequency used for capacitance measurements in this experiment (0.05 Hz), any tendency for the polar groups on the oxidized cholesterol molecule to rotate slightly under the influence of an electric field (up to 10^5 V/cm) will appear as a contribution to the capacitance. Since there was no change in capacitance during ultrasonic radiation, we can deduce that for the range of intensities used, ultrasound does not "loosen" the bilayer structure or facilitate the rotation of the polar molecules.

The dramatic increase in conductance observed when NaCl or KCl is added to the water surrounding the BLM supports Tien's belief that conductance in an unmodified

BLM is primarily ionic (Tien, 1974, p. 134). If this is true, then we can conclude that since conductance is not substantially affected by sonation, ion permeability is not substantially affected. It is important to note that this conclusion pertains to an unmodified bilayer, and that methods of conductance change radically when modifying agents are incorporated into the bilayer structure.

At first, it may seem that the fact that the capacitance remains constant contradicts the work done by Ochs and Burton (1974) and by Pasechnik and Sokolov (1973). These researchers describe effects observed in the frequency range from 50 to 150 Hz. Both groups observed a capacitance change due to change in membrane area upon sonic stretching. The acoustic power at the membrane was not calculated in either instance due to resonance in the chamber. But at similar power levels, the small amplitudes associated with ultrasonic frequencies would not provide the stretching necessary to replicate the low frequency effect.

In addition to the capacitance change in the unmodified membrane, Pasechnik and Sokolov also observed permeability changes when additives were incorporated in the BLM. They believe that stretching also caused this effect. It would be interesting to determine whether ultrasonic radiation could induce similar changes in the absence of large membrane oscillations. This information could be made specifically valuable in predicting the effects of ultrasound on various biological membranes by using additives that cause the BLM to model those membranes.

Finally, it is of great importance in theoretical modeling of biological membranes to consider the effect of ultrasound on the enzyme systems and other supporting systems that surround the membrane. If positive results are obtained at any step in this sequence, it would indicate specific investigation of biological systems with similar structures that could either suffer side effects from the use of ultrasonic devices, or benefit by refining the effectiveness of therapeutic ultrasound. The results of this experiment indicate that one must look beyond the bilayer structure to explain any observable changes in membranes caused by ultrasound at intensities less than 1.5 W/cm^2.

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