ELECTRICAL RESPONSE TO VIBRATION OF A LIPID BILAYER MEMBRANE

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ABSTRACT The discovery and characterization of a vibration response in a black lipid bilayer membrane is the topic of this paper. An electrical vibration response is obtained when the membrane is under voltage clamp and a weaker, but significant, response is obtained under current clamp. The effect arises from an induced variation in the membrane capacitance. It is further shown that the capacitance variation arises from a change in the membrane area as the membrane undergoes drumhead vibration. Possible physiological significance in mechanoreception is discussed.

INTRODUCTION

A lipid bilayer membrane of the type developed by Mueller et al. (1962 *a*, *b*) can act as a transducer under proper conditions. To date, bilayer preparations have been shown to respond to chemicals, temperature, light, and vibration. The discovery and characterization of the vibration response is the topic of this paper.

Del Castillo et al. (1966) have shown that a lipid membrane impedance can undergo a drastic change when an enzyme-substrate or antigen-antibody reaction takes place at the membrane surface. The proteolipid moiety from the electrophorus synaptic receptor complex can impart acetylcholine sensitivity to black lipid films (Parisi et al., 1971; De Robertis, 1971). The finding of an immune response has also been confirmed and extended (Barfort et al., 1968). Antigen, antiserum, and serum complement are required for a response. An important series of experiments, showing an absolute requirement for serum complement, has been performed in liposome model membranes by Haxby and his co-workers (Haxby et al., 1968; Haxby et al., 1969; Kinsky et al., 1969; Alving et al., 1969).

Finkelstein and Cass (1968) have devised a membrane preparation which they describe as "a model for a thermal receptor." A KCl gradient across a bilayer membrane treated with nystatin and valinomycin gives rise to a transmembrane diffusion potential. As the ionic selectivity of the two antibiotics varies with temperature, so does the diffusion potential. Photoresponses have been demonstrated in bilayer

membranes containing chlorophyll (Tien, 1968 *a*, *b*) or retinal (Kobamoto and Tien, 1971).

The vibration response was an accidental discovery made by us while preparing to investigate rapid conductance changes in bilayer membranes under voltage clamp conditions (Ochs and Burton, 1968). Initially, it was noticed that the membrane preparation responded to room vibrations. It was further determined that the response could be elicited by tapping or holding a struck tuning fork on the preparation and that the response arose from the bilayer itself. Quantitative experiments are described below which show that a variation in membrane capacitance is responsible for the mechanical/electrical transduction and that this capacitance change arises from a change in membrane area.

METHODS AND APPARATUS

Mechanical

Details and drawings of much of the mechanical apparatus used in these experiments have been published by Howard and Burton (1968). Photographs of the vibration chamber used for forming and vibrating the membrane are shown in Fig. 1. The vibrator, essentially the driving mechanism of a loudspeaker (Goodman Industries, Ltd., Middlesex, England; Model V15), was both magnetically and electrostatically shielded. Vibration is transmitted to the membrane through a connecting rod to the aqueous solution in the rear chamber, which was completely sealed. The front chamber was always open. The vibration amplitude and phase was routinely measured with a phonograph pickup, which was calibrated by direct observation using stroboscopic illumination.

The chamber was held in an aluminum base which was maintained at a constant temperature by flowing water. The base also served as an electrical shield. To minimize the effects of room vibrations, a tennis ball was placed under each leg of the work table. The electrometer pickup and the current-to-voltage converter were suspended from a wall so that vibration induced noise in these devices would not be coherent with the applied stimulus.

The membrane was observed through a 25-power compound microscope. As a working tool, the planarity of a membrane can be readily effected by using the voltage clamp vibration

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FIGURE 1 Vibration chamber. An exploded picture of the chamber is shown in *A*. It consists of a front (right) and rear (left) cell separated by a 10^{-2}-in thick Mylar partition. The normally clear Mylar has been painted for the photograph. A 2.5 mm diam hole was punched in the partition over which the membrane is painted. The back side of the rear cell is a semiflexible Mylar diaphragm bolted to a connecting rod. Vibration is introduced to the diaphragm by applying a longitudinal motion to the rod. The mating surfaces are sealed with paraffin, dissolved in pentane, to eliminate leaks. After the pentane evaporates, the chamber is washed in deionized, quartz-distilled water to eliminate conductance paths along the surface. The chamber is filled with aqueous solution, placed in the thermostated base, and attached to the vibrator, and the electrode cap is inserted. The silicone rubber cap, shown in *B*, completely closes the rear cell. Fluid can be added or removed with a micrometer syringe connected to the stainless steel tube in the side of the cap, distending the membrane. Any vibrations applied to the diaphragm are transmitted to the membrane through the intervening aqueous solution. The final assembly is shown in *C*.

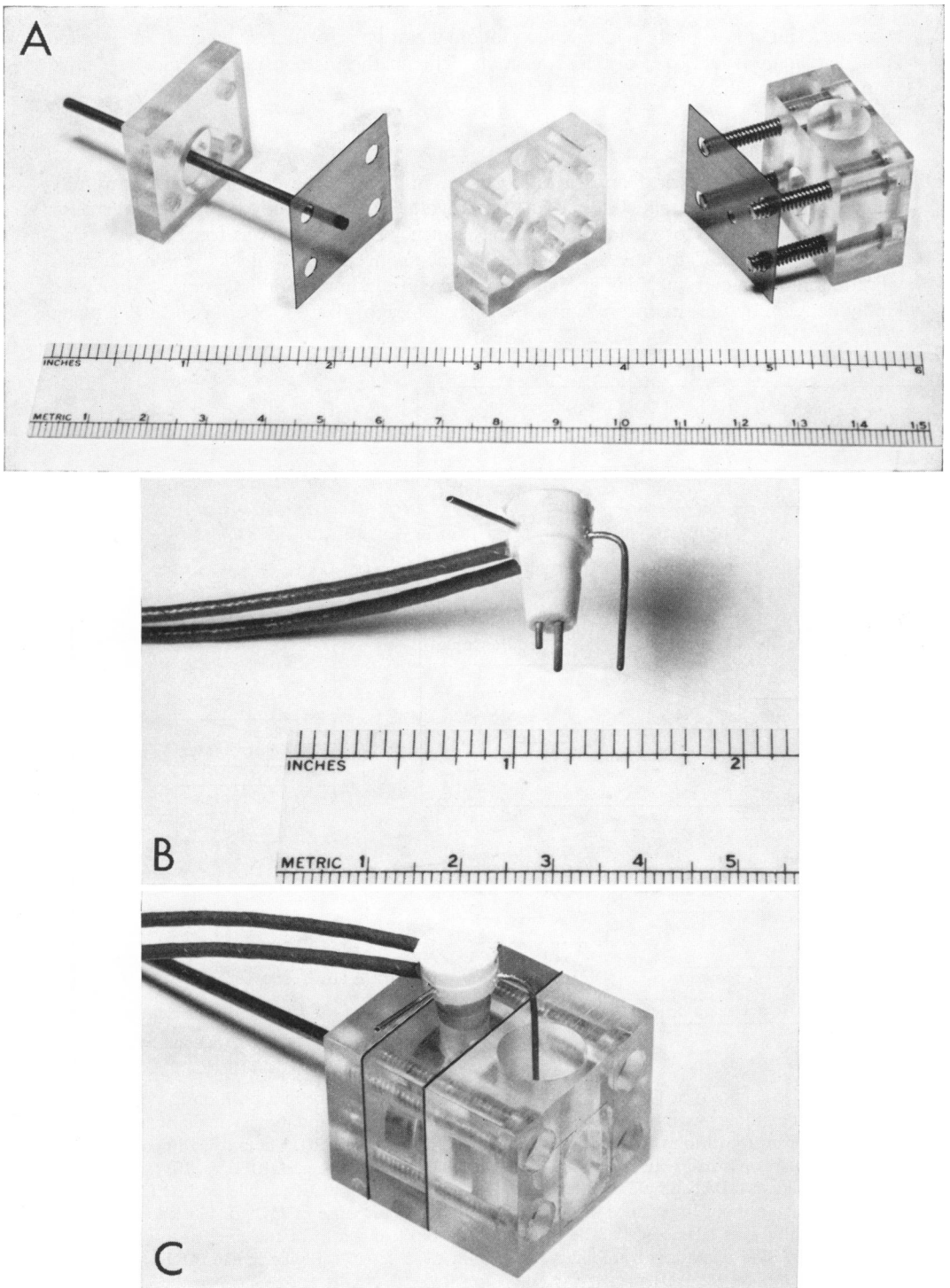

FIGURE 1

response as an index. The response due to random vibration is minimized when the membrane is made planar. It has been our experience that this method is considerably more sensitive than optical methods.

Electrical

A diagram of the electrical apparatus is shown in Fig. 2. The equipment was carefully shielded within a screen cage. Ground and guard voltages were employed for internal shielding where appropriate to obtain an adequate frequency response. It was found necessary to use "low noise cable" for the electrode leads since conventional shielded cable generates vibration-induced currents in high impedance circuits. Samples of low noise cable were kindly donated by the Amphenol Cable Division, Chicago, Ill. (no. 21-541) and the Hitemp Wire Co., Westbury, N. Y. (no. 50-29CWSTJ).

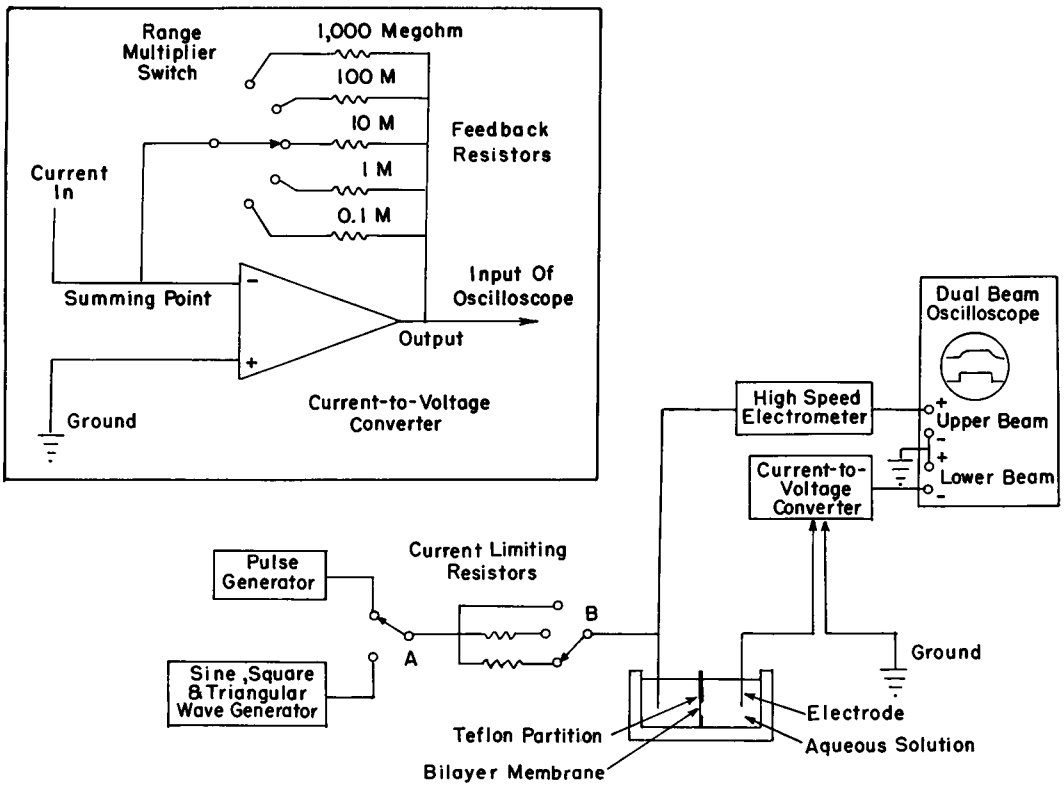

FIGURE 2 Diagram of the electronic apparatus. The switches are in a position to perform a current clamp experiment. Removing the current-limiting resistors by placing switch *B* in the uppermost position will create a voltage clamp circuit (see text). The output impedance of all sources is small with respect to the membrane resistance (i.e., $\simeq 10^5\ \Omega$ vs. $10^8\ \Omega$). The electrometer is a BAK amplifier (Electronics for Life Sciences, Inc., Rockville, Md.; ELSA-1) which has an input impedance of $>10^{10}\ \Omega$, a frequency response of DC to 400 kilocycles, and a unity gain (Bak, 1958). The inset shows a block diagram of the current-to-voltage converter. The operational amplifier has a gain of 2×10^5 (Philbrick Researches Inc., Dedham, Mass.; P25AU).

Reversible silver chloride electrodes were used rather than liquid junction electrodes because of their superior electrical properties at high frequencies. The AgCl was deposited electrolytically onto polished silver wire. Polarization differences were compensated with an opposed DC voltage. The electrode impedance was typically 10^5 Ω.

In this study, both voltage and current clamp techniques were employed. Current clamping was achieved in the standard fashion by using a current-limiting resistor whose value was much higher than the membrane resistance. Voltage clamping was achieved by connecting the membrane to a low impedance source. The membrane voltage was thus equal to the source voltage. Cole and Moore (1960) have pointed out that the effect of the standard voltage clamp feedback system is to lower the equivalent source impedance to a value much below that of the membrane impedance. Since the voltage clamp method will reveal details of fast changes in membrane impedance, we suggest that this simple technique may be useful in studying receptor analogs.

The circuits were calibrated and the upper frequency limits determined by replacing the membrane with several precision resistors whose nominal value had been confirmed with an accurate digital ohmmeter. The resistors were voltage- and current-clamped over a range of audio frequencies. The frequency at which the measurements differed by more than 2% was taken as the upper frequency limit for the system.

Chemical

The membrane-forming solution (MFS) was made from egg L-α-phosphatidyl choline (Pierce Chemical Co., Rockford, Ill.; Type II) and cholesterol (Sigma Chemical Co., St. Louis, Mo.; standard for chromatography) dissolved in redistilled *n*-decane (Matheson, Coleman, and Bell, Norwood, Ohio). The proportions used were 1×10^{-2} M phosphatidyl choline (assumed average mol wt = 752) and 2×10^{-2} M cholesterol (mol wt = 387).

Aqueous solutions were made from analytical grade reagents buffered with Tris-chloride buffer (tris[hydroxymethyl]aminomethane, Sigma Chemical Co.). The water used was distilled in Pyrex glass and passed through a deionizer. Its purity was better than 0.1 ppm expressed as NaCl. In the most recent experiments, the deionized water was further distilled in a quartz double distillation unit to remove contamination by monomers of the ion exchange resin. However, no change in the experimental results was observed on taking this added precaution. Two aqueous solutions were employed: (*a*) 10^{-3} M NaCl solution, buffered to pH 7.0 with 0.5×10^{-3} M Tris chloride; and (*b*) 10^{-1} M NaCl solution, buffered to pH 6.1 with 10^{-3} M Tris chloride.

RESULTS

To quantify the early results obtained by tapping on the table and touching a tuning fork to the preparation, the vibration chamber shown in Fig. 1 was constructed. Fig. 3 shows records of a voltage clamp experiment illustrating the basic phenomenon. The membrane's current-voltage relationship was determined in its stationary, planar state and then remeasured with a vibrating stimulus applied. The vibration stimulus caused an alternating current to flow whose phase sense is dependent on the membrane voltage polarity. This current was symmetrical about the unstimulated direct current line and the lower peaks show current flowing against the applied voltage gradient. The amplitude is a linear function of the applied transmembrane potential between ±100 mV. Slight deviations from linearity were seen. These

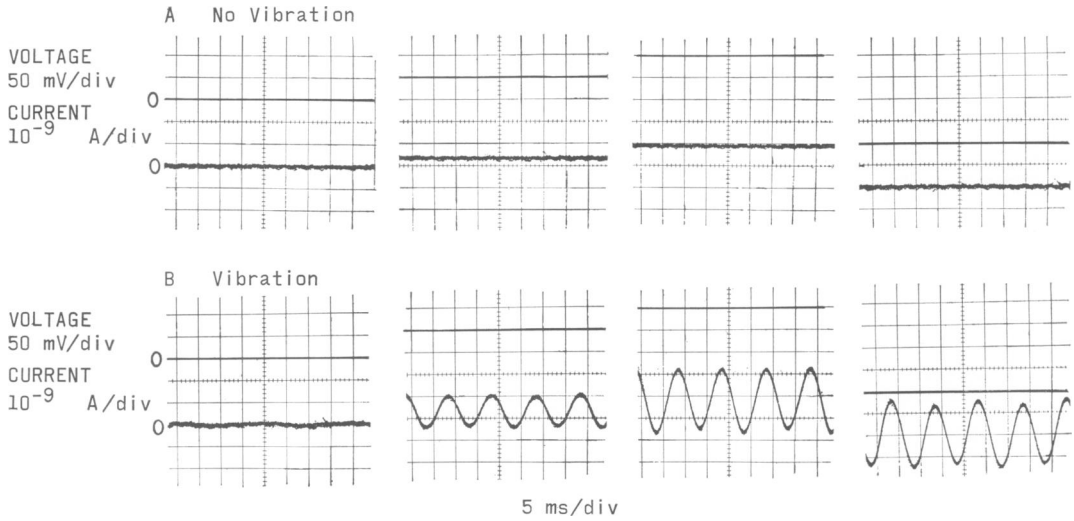

FIGURE 3 Voltage clamp experiment. From left to right, the membrane was clamped at: 0 V, 50 mV, 100 mV, and −100 mV. In these experiments the shutter was held open for 10 sweeps after the membrane capacitance became charged. The trace was synchronized to the 50 cycle oscillator in both series *A* and *B*. The vibration amplitude was 1.75 μm peak to peak, measured at the connecting rod. The membrane was planar. The temperature was 30°C.

are best explained by contamination from vibration-induced noise and inherent inaccuracies in oscilloscopic measurements. For Fig. 3, the peak-to-peak responses are: 1.4, 1.6, 2.8, and 3.0 $\times$ 10^{-9} A at +50, −50, +100, and −100 mV. Notice the 0.2 $\times$ 10^{-9} A of subharmonic noise at 0 mV.

Fig. 4 illustrates the behavior of a current-clamped planar membrane undergoing vibration. In this case the relative magnitude of the response is significantly reduced (i.e., $\Delta E/E \gg \Delta I/I$). Again, the output phase sense is dependent on the membrane voltage polarity. In this case, the phase lags that of the voltage clamp response by 90°.

To investigate the possibility that an artifactual response was generated by the equipment, control experiments were performed with a broken membrane under current clamp, and no membrane hole in the partition under voltage clamp. The experimental conditions of Figs. 3 and 4 were utilized except that the vibration stimulus was four times as strong (7 μm). The artifactual response was negligible.

The vibration response is dependent on the shape of the membrane, whether planar or rounded. By adding or removing aqueous solution from the sealed rear chamber, the membrane can be distended in either direction. The membrane position was controlled with a micrometer syringe capable of being adjusted to approximately ½ μl about its 1 μl markings. Fig. 5 illustrates the results of a typical experiment. The response to vibration was determined from a rounded membrane.

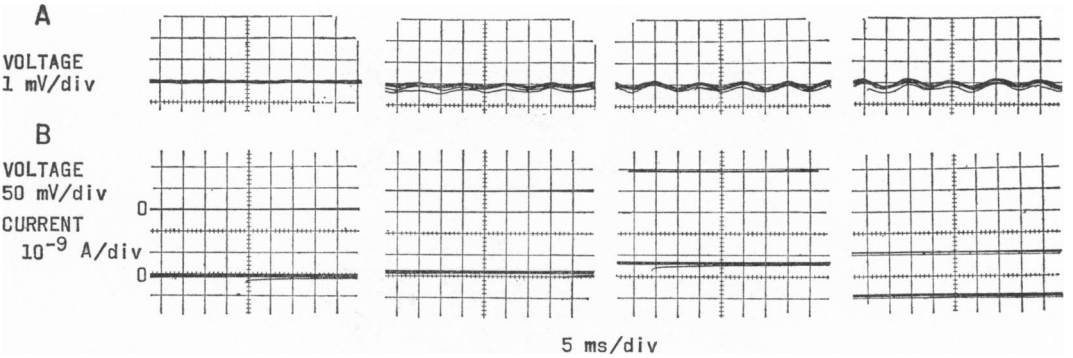

FIGURE 4 Current clamp experiment. These photographs were taken as in Fig. 3 after the membrane capacitance had reached full charge. In Series *B* the clamped current was adjusted such that the nominal transmembrane voltage approximated Fig. 3. That is, from left to right: 0 V, 50 mV, 100 mV, −100 mV. Series *A* consists of separate photographs of the voltage trace AC coupled and taken at a higher gain. The transients are interference from the electrical relays in the camera.

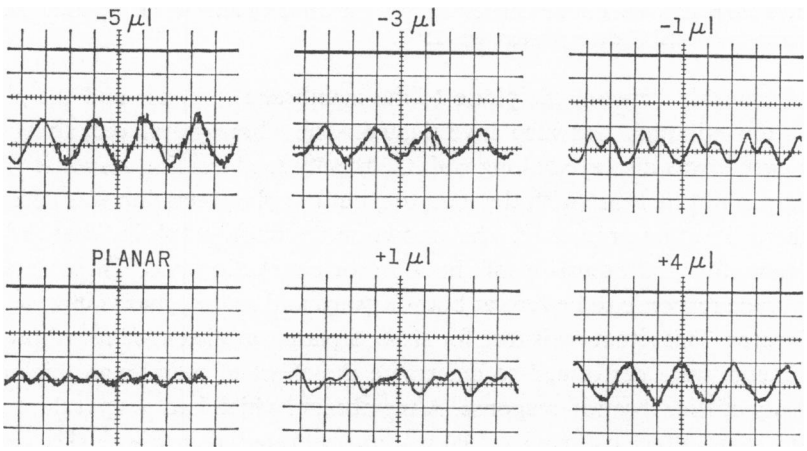

FIGURE 5 Effect of static distention. The membrane was voltage clamped at 40 mV. The temperature was 25°C. Using a micrometer syringe, aqueous solution was removed or added to the rear chamber. The accuracy of the syringe is approximately ½ μl which accounts for the lack of symmetry around the planar membrane. The vibration level was 7 μm peak to peak, at the connecting rod. The oscilloscope sweep was synchronized with the vibration oscillator. Current sensitivity is 10^{-8} A per major division.

The distention was then reduced in a stepwise fashion through planarity, and distended in the other direction. At each step, the vibration response was recorded. The output frequency equals the input frequency when the membrane is rounded but is double the input frequency when the membrane is planar. Also, the magnitude of the response is much greater when the membrane is rounded and the phase sense of the response is dependent on the direction in which the membrane is distended.

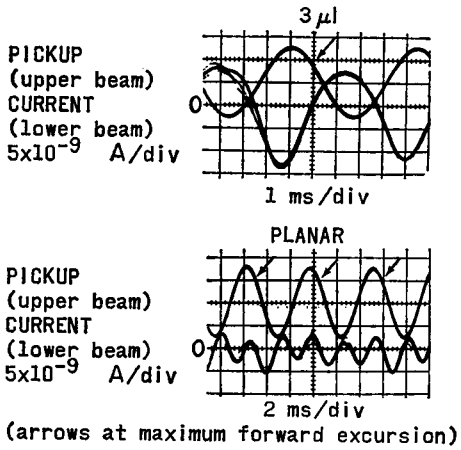

FIGURE 6 Phase measurements. The data shown are for a membrane distended by 3 μl and for a planar membrane. The membrane was voltage clamped at 50 mV and vibrated at 200 cps. The drift in the current traces is due to 60-cycle interference. The vibration amplitude was 1.75 μm peak to peak at the connecting rod. The damping filter was not used in taking these measurements. The temperature was 25°C.

The relationship between the phase of the membrane response and the phase of the vibration stimulus was also investigated. The phonograph pickup vibration monitor output was displayed along with the membrane vibration response (Fig. 6). The pickup is not in phase with the vibrator, but the phase relation was determined during the calibration procedure. The arrows on the photograph indicate the points of maximum forward excursion of the vibrator connecting rod. These data show the membrane response to be zero at both forward and reverse maximum excursions of the vibrator. This situation is true for either a planar or distended membrane.

Experiments were performed to determine the effect of membrane thickness on the intensity of the vibration response. A membrane, which had not yet thinned to a black film, was voltage clamped at 100 mV and vibrated as in Fig. 3. The response current was 5×10^{-12} A. Black films formed on the same day with the same reagents yielded an average response of 1.1×10^{-9} A. Although the magnitude of the response of both the thick and black membranes is somewhat variable, this greater than 200-fold difference in response is typical.

Shortly after beginning to use the vibration chamber, it was found that the vibration response could be erratic. The anomalous behavior was markedly frequency dependent, as though there were a resonance in the system. Waxing and waning of the output could be eliminated by partitioning the rear cell with a piece of glass filter paper to serve as a mechanical resonance damper. However, the filter would not completely eliminate other anomalous behavior, such as the appearance of harmonics at some frequencies.

From all appearances, there seems to be a mechanical resonance in the chamber

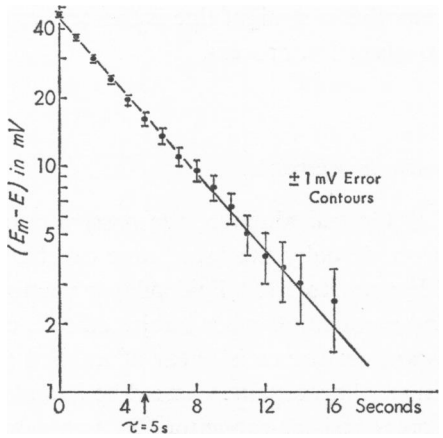

FIGURE 7 Determination of membrane relaxation process. A voltage charging curve was replotted with the ordinate as the log of $(E_m - E)$, (where $E_m = 46$ mV is the final value of the exponential rise and E is the membrane voltage at time t plotted on the abscissa). The ±1 mV error contours diverge because of the log transformation. The temperature was 40°C.

which can be set into oscillation by the driving vibration. This anomalous behavior argues against our initial assumption that the rear cell is a rigid unyielding compartment. In fact, it appears that there are hydrodynamic resonances in the rear cell which can interact with the applied vibration and present the lipid membrane with a complicated stimulus. Evidence of this resonant behavior could be found at all frequencies from 50 to 800 cps, the usable range of the vibration system. Since it proved impossible to investigate the membrane response at a frequency far from the resonance, most experiments were performed at 50 cps because the resonance was smaller and overt nonlinear behavior was not observed.

It is possible that aspects of this resonant behavior could be confused with the lipid membrane response pattern. To minimize this possibility, virtually all of the data presented in this paper were recorded with the damping filter in place, and data which showed overt signs of nonlinear behavior, e.g., marginal instability, resonance-induced harmonics, etc., were not used. It is felt that the resonance does not alter any of the qualitative aspects of the data nor will it negate any of the interpretations presented below. However, the resonance did prevent any quantitative study of the relation between the intensity of the vibration and the magnitude of the electrical response and prevented a determination of the electrical frequency response of the lipid membrane per se.

Membrane charging characteristics were obtained by the method illustrated in Fig. 2 and replotted in Fig. 7. From these data the membrane relaxation properties were obtained, yielding its resistance and capacitance. The values obtained with a 0.1 M NaCl aqueous solution were $R = 11.3 \times 10^6\ \Omega\ \text{cm}^2$ and $C = 0.42\ \mu\text{F/cm}^2$. These values implicate the membrane as a true bilayer as they compare well with

published values. It is seen that a straight line is the best representation of the data in Fig. 7, indicating one relaxation process.

ANALYSIS

Electrical Response to Vibration

To examine the nature of the transduction, the passive electrical properties of the membrane must be known. Initially the membrane can be represented as an ideal capacitor in parallel with a resistor. The lipid bilayer membranes used here do not give rise to transmembrane potentials when subjected to a concentration difference of NaCl and the membrane resistance is linear at applied potentials less than 100 mV; thus, no batteries or nonlinear elements need be included in the equivalent circuit. The possibility of other resistor-capacitor (RC) networks is harder to answer. Hanai et al. (1965) suggested that the preparation should be represented by three parallel RC networks placed in series. These networks represent the polar head groups, the central hydrocarbon core, and the aqueous solutions. While the concept is probably valid, the effect is much too small to be measured. Our data (Fig. 7), also suggest that a single RC network is an adequate representation of the circuit. The vibration response thus arises from a change in the resistance, the capacitance, or a combination of the two.

Referring to Fig. 3, we see that the membrane current can flow against the potential gradient when the membrane is vibrating. Were the change to arise in the membrane resistance, the current could become very large, go to zero, or alternate between these two extremes. A change in an ohmic resistance cannot cause current to flow in a negative direction. On the other hand, a capacitor is an energy storage device which can cause a current flow independent of the applied potential. Consider the voltage (E) developed across a capacitance (C) by the quantity of charge (Q). In rationalized meter-kilogram-second (MKS) units:

$$Q = CE. \tag{1}$$

When voltage clamping, the capacitive current (i) is given by:

$$i = \mathrm{d}Q/\mathrm{d}t = E\,\mathrm{d}C/\mathrm{d}t. \tag{2}$$

Were the response to arise from a combined resistance and capacitance change, the response observed in Fig. 3 would not be symmetrical about the unperturbed current trace.

The analysis of the electrical effects of a capacitance change in a living or a model membrane has not been solved for an arbitrary variation in capacitance,. A solution for a constant rate of change was presented by Katz (1950) in conjunction with his work on muscle spindle receptors. This solution is unsuitable for handling periodic capacitance variations such as those of this paper. However, it is possible to trans-

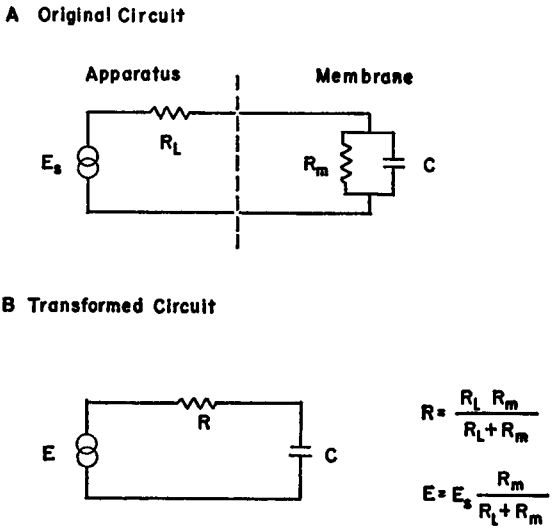

FIGURE 8 Thévenin transformation. The original circuit is shown in part *A*. Part *B* illustrates the resultant transformed circuit (Langford-Smith, 1953, p. 164). It is emphasized that the capacitance (*C*) does not represent the total membrane but simply the membrane capacitance.

form the equivalent circuits of model and living systems to a circuit for which a solution has been derived (Fig. 8). The procedure followed is to treat the membrane capacitance variation in the same manner as that of a condenser microphone (Wendt, 1917).

Assume that the membrane capacity (C) at any instant is given by:

$$C = C_0 + C_1 \sin \omega t, \tag{3}$$

where C_0 is the resting membrane capacitance; C_1, the maximum change in capacitance; ω, the frequency in radians per second; and t, the time in seconds. Using a series expansion of the differential equation for the capacitive current (i), Wendt obtained the following general solution.

$$i = \frac{EC_1}{C_0[(1/\omega C_0)^2 + R^2]^{1/2}} \sin(\omega t + \phi) + \text{higher order terms in } C_1/C_0, \tag{4}$$

where $\phi = \tan^{-1}(1/\omega C_0 R)$.

Voltage clamping of a model or living membrane is effected by letting $R_L \rightarrow 0$, yielding $R = 0$. Inserting this condition into Eq. 4, all higher terms vanish and we obtain the describing equation for a voltage clamp experiment:

$$i = EC_1\omega \cos \omega t. \tag{5}$$

Current clamping of a model or living membrane is effected by making R_L much larger than R_m, yielding $R \approx R_m$. As the sum of sources and drops around any loop must be zero, $E - iR - E_m = 0$ for the transformed circuit. The instantaneous membrane voltage (E_m) is equal to the sum of the open circuit voltage (E) and the perturbation (e) due to the capacitance change: $E_m = E + e$. These considerations yield $e = -iR \approx -iR_m$. Note, i is the capacitive current and not the total clamped membrane current which has no representation in the Thévenin transformation.

Using Eq. 4 we may solve for e. Since $C_0 \gg C_1$, we may ignore the higher order terms in C_1/C_0.

$$e = -iR_m = \frac{-EC_1R_m}{[(1/\omega)^2 + (R_mC_0)^2]^{1/2}} \sin(\omega t + \phi) \quad (6)$$

$$= -EC_1\omega Z_m \cos \omega t, \quad (7)$$

where Z_m is the impedance of a parallel RC network.

$$Z_m = R_m/[1 + (\omega\tau)^2]^{1/2}, \qquad < -\tan^{-1}\omega\tau, \quad (8)$$

and $\tau = R_mC_0$ is the membrane time constant.

We can combine Eqs. 5 and 7, yielding:

$$e = -iZ_m. \quad (9)$$

That is, we can consider the membrane as a current generator whose output is given by Eq. 3. Since the total current to the membrane is held constant, the vibration current (i) must flow through the membrane impedance to produce a change in voltage. At the vibration frequencies employed here the current generated is largely shorted out by the steady membrane capacitance.

It will be useful to consider two frequency limiting cases of Eq. 6.

For $\omega \ll \omega_c \equiv 1/\tau$

$$e \approx -EC_1R_m\omega \cos \omega t, \quad (10)$$

and for $\omega \gg \omega_c$

$$e \approx -E(C_1/C_0) \sin \omega t. \quad (11)$$

In a manner identical to that shown above, a linear equivalent circuit of a living cell membrane (e.g., Cole, 1968, p. 272) may be transformed into the circuit of Fig. 8 B, yielding $R = R_m$. Thus the equations developed for the current clamped bilayer membrane apply to a living cell membrane, which can be thought of as clamped at zero net external current.

Physical Response to Vibration

The question now arises, how can this capacitance change come about? Vibration possibly could affect the dielectric constant, the membrane thickness, or the area. An area change seemed to be most likely and was investigated by the following procedure. If a planar membrane is vibrated, its area can only increase from its resting state but will do so twice for every cycle of vibration, resulting in the output frequency being twice the input. However, if the membrane is statically distended and then caused to vibrate, its area can increase and decrease and the output frequency will equal the input. If the membrane is distended in the opposite direction, the phase of the output will shift by 180°. The results of this experiment are shown in Fig. 5. The area effect is definitely present and is perhaps the dominant one.

To obtain a complete theoretical expression for the vibration response we must relate the membrane area (A) and its enclosed volume (V). The membrane was assumed to be representable by a segment of a sphere and the bulk lipid at the border was ignored:

$$V = \frac{1}{2}\left(\frac{A}{\pi} - \frac{D^2}{4}\right)^{1/2}\left[A - \frac{2\pi}{3}\left(\frac{A}{\pi} - \frac{D^2}{4}\right)\right], \tag{12}$$

where D is the planar membrane diameter.

We now expand Eq. 16:

$$i = (E\epsilon_0\epsilon/\delta)(\mathrm{d}V/\mathrm{d}A)^{-1}(\mathrm{d}V/\mathrm{d}t). \tag{13}$$

The displacement of the vibrator is sinusoidal and for very small displacements the volume change is also sinusoidal, yielding $\mathrm{d}V/\mathrm{d}t = K\omega \cos \omega t$. $\mathrm{d}V/\mathrm{d}A$ is obtained from Eq. 12, yielding:

$$i = \left\{\frac{(EK\omega\epsilon_0\epsilon/\delta)}{4\pi[(A/\pi) - (D^2/4)]^{1/2}}\left[A - \frac{2\pi}{3}\left(\frac{A}{\pi} - \frac{D^2}{4}\right)\right] + \frac{1}{6}\left(\frac{A}{\pi} - \frac{D^2}{4}\right)^{1/2}\right\}\cos \omega t. \tag{14}$$

Using Eq. 5, a peak area change of 12% was determined for the vibrating planar membrane of Fig. 5. This corresponds to a 1.11 μl peak volume increase. Eq. 12 was solved by successive approximations for $A = f(V)$ with $V = V_0 + 1.11\ \mu\mathrm{l} \sin \theta$, $0 \leq \theta \leq 4\pi$. The static displacement (V_0) ranged from 0 to 5 μl in steps of 0.1 μl.

Because of the resonance in the chamber, the constant K in Eq. 14 could not be evaluated. Rather, we solved for $i = (\mathrm{d}V/\mathrm{d}A)^{-1} \cos \theta$ (i.e., $K = \delta/E\omega\epsilon_0\epsilon$ in Eq. 14) and matched the amplitude of the plotted response to the data at the 5.0 μl static displacement. The results of this computation are shown in Fig. 9 and compared with the data of Fig. 5. The computation which best matched the data was chosen for comparison. The syringe used to distend the membrane is accurate to approximately ½ μl which accounts for the slight mismatch.

We can inquire as to how the membrane response will vary with thickness. From

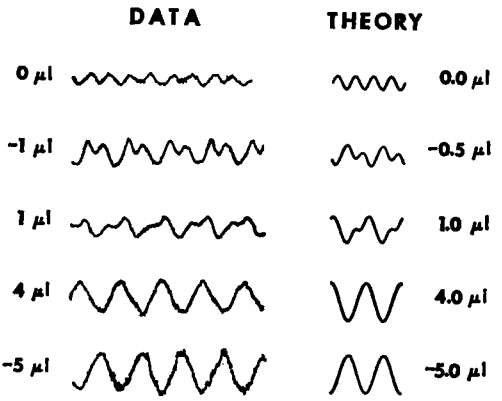

FIGURE 9 Comparison of data with theory. Data of the experiment presented in Fig. 5 are compared with theoretical plots made by the IBM 360/50 computer from Eq. 14. Computations were made at 0.1 μl intervals and the computation which best matched the data was chosen for comparison.

Eq. 3 we may write:

$$i = E\,\mathrm{d}C/\mathrm{d}t = E(\mathrm{d}/\mathrm{d}t)\,[\epsilon_0\epsilon A/\delta], \tag{15}$$

where ϵ_0 is the permittivity of free space, ϵ is the dielectric constant, A is the membrane area, and δ, the membrane thickness. When A is the only nonconstant term,

$$i = (E\epsilon_0\epsilon/\delta)\,\mathrm{d}A/\mathrm{d}t. \tag{16}$$

That is, the response is inversely proportional to the membrane thickness. We may think of the term δ^{-1} as an amplification factor. This implies that the electrical response to vibration is a significant phenomenon for only those membranes whose thickness is of the order of natural membranes.

Comparison of Experiment and Theory

The assumption that the vibration response arises from a change in membrane area, and thus its capacitance, is sufficient to explain the observations presented in this paper. The measurements were not made with sufficient accuracy to comment on a possible increase in membrane capacitance with transmembrane potential (White, 1970; Andrews et al., 1970). The slight deviation from the expected output seen in Fig. 3 is perhaps consistent with an increase in capacitance, but could also be explained by errors inherent in oscilloscopic measurements.

The current generated under voltage clamp conditions by a sinusoidal capacitance change is given by Eq. 5:

$$i = EC_1\omega \cos \omega t.$$

Likewise, the voltage generated under current clamp conditions is given by Eq. 11.

$$e = -E(C_1/C_0) \sin \omega t, \qquad \omega \gg \omega_c.$$

These descriptions are consistent with the data of Figs. 3, 4, and 6. However, the frequency dependence and the absolute magnitudes of the responses have not been experimentally confirmed because of the hydrodynamic resonances in the chamber. Given a particular voltage clamp response, the corresponding current clamp response can be calculated. For Fig. 4 the predicted peak-to-peak value is 0.53 mV as compared with the 0.4 mV seen.

The suggestion that the capacitance variation arises from a variation in membrane area is also well confirmed. Point-by-point calculations of a simple drumhead model mimic the observed response for a planar membrane and one in various stages of static distention. Moreover, a response arising from an area change was shown to be inversely proportional to the membrane thickness (Eq. 16) and this prediction is consistent with the large increase in response when the membrane thins. Thus we conclude that a change in membrane area is the primary, if not only, cause of the observed vibration response.

DISCUSSION

Since lipid bilayer membranes have many properties similar to natural membranes, the electrical response to vibration found in thin lipid membranes raises an obvious question: Does this phenomenon have any relationship to biological mechanoreception? There is no definite answer to this question. However, it is possible to comment on how the phenomenon could function in respect to what is known about the mechanism of mechanical reception and transduction.

First though, we should mention some important differences between lipid bilayer membranes and cellular limiting membranes. The vibrating film described here most likely increases its area by drawing additional material from the bulk lipid phase at the border. On the other hand, stretching a living cell would necessarily thin the membrane. A second important difference is the much lower specific resistance of cellular limiting membranes. This implies that the range of frequencies to which a cell can respond is, in general, much less than the characteristic frequency (ω_c) of the membrane. Thus the potential generated by a living cell is given by Eq. 10 rather than Eq. 11.

Katz (1950) proposed that a capacitance change could contribute to a mechanoreceptor generator potential. He had shown that the generator potential of the muscle spindle receptor has two distinct components, one arising from dynamic stretching of the muscle fiber and the other from static extension. Katz suggested that a capacitance change could be responsible for the dynamic component. Assuming that the receptor was stretched uniformly, he calculated that the potential due to a capacitance change was insufficient to account for the observed dynamic response. However, later studies have shown that the entire receptor is probably not deformed

uniformly (Katz, 1961; Karlsson et al., 1966). The nerve ending is highly branched within the muscle. These ramifications have many varicose swellings, or bulbs, which closely contact the intrafusal fibers. Katz (1961) has suggested that the mechanical to electrical transduction occurs primarily in the bulbs. Thus his earlier calculations based on stretching a uniform cylinder must be discounted. Later physiological studies of this preparation are quite consistent with the suggestion of a capacitance effect on the dynamic phase. The dynamic peak of the receptor potential is linearly related to the rate of stretch over a significant range before the potential begins to saturate. Also, the rate of rise of the generator potential and the latency decrease are linearly related to the rate of stretch (Ottoson and Shepherd, 1965).

Many other mechanoreceptors respond more dramatically to a change in the stimulus than to a steady stimulus, suggesting a possible capacitance effect. Perhaps the most dramatic example occurs in the foot withdrawal reflex of the razor clam (Olivo, 1970). The pedal nerves of this preparation will not fire except when the velocity of the mechanical stimulus exceeds a critical rate. That is, the reflex is insensitive to sustained pressure and slow rates of displacement.

Only one mechanoreceptor has been investigated under conditions which would reveal a capacitance change during stimulation. Terzuolo and Washizu (1962) measured the electrical properties of the slowly adapting stretch receptor of the crayfish in the resting and stretched state. After extension, the membrane resistance had fallen by a factor of two while the capacitance remained constant.

It would be well to examine this physiological preparation more closely. There are two types of stretch receptors in the thoracic and abdominal segments of the crayfish and lobster, termed fast and slow adapting (Kuffler, 1954; Eyzaguirre and Kuffler, 1955). On stretching a muscle, the fast adapting fibers will fire for a few seconds while the slowly adapting fibers will remain firing for a long period of time. The slowly adapting fibers are thought to provide a source of information for maintained postural reflexes. Moreover, the crayfish slowly adapting stretch receptor was later shown to lack a mechanism, capacitive or otherwise, for differentiation of the mechanical stimulus; that is, the gain vs. frequency curve is essentially flat (Terzuolo and Knox, 1971). Ironically, the only direct measurement of membrane capacitance change resulting from mechanical stimulation was made on a receptor which would not have been expected, *a priori*, to exhibit a capacitance change.

A second experiment, which relates indirectly to this question, provided measurements of nerve membrane parameters during mechanical stimulation. Julian and Goldman (1962) investigated the mechanical excitability of lobster nerve axon as a model of a mechanical receptor. In these experiments, mechanical stimulation of the axon elicited a simple conductance increase.

The most widely held hypothesis is that generator potentials in mechanoreceptors arise from a change in membrane permeability and not from a change in capacitance (Goldman, 1965; Grundfest, 1971). However reasonable this hypothesis might be,

a possible capacitance component of the receptor potential dynamic phase has not been ruled out by an accumulation of hard data. It is our opinion that a change in membrane capacitance could well be an important mechanism of transduction in mechanoreception.

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