ALIGNMENT OF MICROSCOPIC PARTICLES IN ELECTRIC FIELDS AND ITS BIOLOGICAL IMPLICATIONS

S. TAKASHIMA AND H. P. SCHWAN
Department of Bioengineering D3, University of Pennsylvania, Philadelphia, Pennsylvania 19104

ABSTRACT It is well known that electromagnetic fields cause mechanical forces. If one applies an electrical field to a suspension of microscopic particles, these particles realign themselves along the direction of the field and form pearl-chain–like aggregates. These chains are mostly single stranded but they are frequently multistranded. This phenomenon has been investigated by a number of groups. Here we discuss the dependence of threshold field strength on particle size and frequency. Also, pulsed fields have been thought to be more effective than continuous fields of the same average power in evoking biological effects. Our measurement of the threshold power requirement for the pearl-chain formation indicates that pulsed fields require as much power as continuous fields. The biological significance of pearl-chain formation is briefly discussed.

INTRODUCTION

A number of nonthermal biological responses to electrical fields of only a few volts per centimeter have been reported in recent years. These include membrane breakdown (1, 2), and a variety of field-induced force effects such as alignment of cells, their rotation, shape changes, and fusion (2, 4, 5). The mechanism responsible for membrane breakdown includes reversible and irreversible changes in membrane properties. Field-induced forces are always proportional to the square of the field strength and their time average is, therefore, finite and different from zero for alternating fields. Simple examples are the force between the two plates of a capacitor or the torque orienting an ellipsoid surrounded by a medium of different dielectric properties. A general derivation for more complex situations may be based on an appropriate use of the Maxwell stress tensor. Direct current (DC) field-induced force effects have been known for a long time. They are listed in older physics texts as "pondermotoric" or "ponderomotoric" effects. However, so far not much attention has been paid to alternating-field–induced forces and their biological manifestations.

Our earlier work on field-induced force effects on cells was motivated by the desire to find out if such forces can induce biological effects at field strength levels below those needed to cause thermal effects or, at low frequencies, excitation. Thermal considerations have been dominant for a long time in discussions of nonionizing radiation hazards. Hence, observation of nonthermal effects at lower field levels than necessary to cause noticeable heating would be significant.

Of more recent interest is the possibility of cell manipulation with alternating electrical fields. Effective cell fusion by a combination of alternating fields with electrical pulses has been demonstrated, a technique of great importance in biotechnology. Field-induced deformation can be used to study the elastic properties of membranes, and the rotation phenomena permits extraction of all sorts of cellular parameters such as membrane capacitance and cytoplasmic conductivity. The movement of cells in inhomogeneous electrical fields has been studied and termed dielectrophoresis (6). Other applications of the subjection of cells to electrically controlled forces may emerge. For further details we refer to the references quoted above.

The phenomenon that will be discussed here is pearl-chain formation (6, 7, 8). This phenomenon has been observed by many investigators (9, 10) and is currently considered to be one of the well-established phenomena caused by forces induced by electric fields. Particles of micron size suspended in aqueous media are randomly distributed in the absence of an electrical field. When a field of any frequency between DC and 100 MHz is applied, the particles realign themselves in the direction of the field. In the end, they form long chains, single or multiple, consisting of many particles in each strand. Because of the presence of the field and the presence of Brownian motion, the chains undergo a micro-Brownian motion. If the field strength is reduced below a threshold level, thermal motion disrupts the chains and the particles return to a random distribution.

The formation of these chains is characterized most conveniently by a time constant and a threshold potential. The time constant of pearl-chain formation has been discussed in detail by Saito and Schwan (9) and then by Sher et al. (10), and appears to be proportional to E^{-2}, where E is the field strength. Here we report on the dependence of the threshold potential on particle size, frequency, and pulsing parameters of the applied fields. Saito and Schwan correlated threshold potential to the size of particles and elastic properties of particle and suspending medium. Schwan argued that a threshold field strength can be stated by equating the product of induced particle dipole moment and field strength with kT (k is Boltzmann constant, T is absolute temperature) (4). Recently, Sauer rigorously calculated the force between two particles exposed to an alternating field (11). The size dependence of E_{th}, according to all these theories, would be proportional to $R^{-1.5}$, where R is the radius of the particle. Sher measured threshold potentials with various particles (8). He observed that the threshold field strength E_{th} is indeed proportional to $R^{-1.5}$, using a variety of samples including silicon, polystyrene particles, bacteria, and erythrocytes. These particles not only have different diameters but also different shapes, dielectric constants, and charge densities. We reinvestigated the size dependence of E_{th} using only silicon particles having six different sizes. Therefore, the observed dependence of E_{th} on radius R is due only to size parameter.

It has often been stated that biological effects of a nonthermal nature may be more readily evoked by pulsing fields than by continuous fields of the same average power. However, theoretical arguments have been advanced to the contrary for ponderomotoric force effects, i.e., at least the time-averaged threshold power (E^2) needed to achieve such effects cannot, in principle, be lowered by the use of pulsing fields (9, 10). Here we present experimental data supporting this argument. We conclude, therefore, that if alternating pulsed fields can be demonstrated to be more effective than continuous fields of the same power, then the mechanism responsible for them can be neither of a thermal nature nor a nonthermal mechanism of the ponderomotoric type. We conclude furthermore that it is not possible to enhance ponderomotoric effects by the use of pulsing fields, and that their emergence from the thermal disturbance cannot be aided by pulsing.

EXPERIMENTS

The experimental procedures used here are described in detail by Sher et al. (10). Silicon emulsions were supplied by General Electric Company (Wilmington, MA). They are coated with albumin and their diameters ranged from 0.4 to 10 μm. The emulsions were fractionated by repeated gentle centrifugations. Fractions used for the size-dependence study have mean diameters: 1.8 ± 0.2, 2.2 ± 0.2, 2.5 ± 0.3, 3.5 ± 0.3, 4.7 ± 0.5, 5.0 ± 0.7, and 5.8 ± 1.0 μm, respectively. The determination of particle size was performed by the microscopic observation of 20 to 30 particles and evaluation of average and standard deviations for each fraction.

The indicated size variations do not adversely affect threshold determinations. The threshold field strength is proportional to $R^{-1.5}$ (reference 4, Fig. 1). For particles of unequal size the geometric mean of the two radii replaces R (11). Thus, an error or deviation of 10% in the radius of one particle will cause a threshold field strength change of 7 or 8%.

The determination of the threshold potential was performed using a pair of platinum wires with a diameter of 63 μm. A drop of silicon suspension was placed between two parallel wires, 0.3–0.4 mm apart, that were sealed between a microscopic slide glass and a coverslip. The wires and glass plates were glued together with epoxy resin so that no fluid could escape the microcell. Alternating current fields were applied directly from a low-frequency oscillator (3310A; Hewlett Packard Co., Palo Alto, CA) operating between 500 Hz and 50 KHz. Radio frequency fields were generated by a Hewlett Packard 606A generator (Hewlett-Packard Co.) The formation of pearl chains was determined by visual observation under the microscope by varying the field strength while keeping other parameters constant. The waiting time between changes of the field strength varied with the level of excitation. Only a few minutes were required for E to fall well below E_{th}. However, as E approached the threshold, about a 10-min wait was necessary before the field strength could be changed. The rate of voltage increment does not appear to have any significant effect on E_{th} as long as E does not exceed the threshold. The measurements were repeated many times and the values of E_{th} shown in the following figures are the mean values of at least 5–10 determinations for each size. Because of the presence of hysteresis, we had to permit enough time to allow random thermal motion to break up the pearl chains completely between measurements. The duty cycles for pulsed alternating current (AC) fields were varied from 1.0 to 0.01 by changing the duration of the square modulated pulses, keeping the total time required for pulse and pulse interval constant. The chaining process is almost an all-or-none phenomena. Many, if not most, particles are involved in chain formation if E is above the threshold but one must allow a certain interval before the completion of chaining.

RESULTS

Size Dependence of Threshold Potential

Silicon SM 70 was used throughout this work with further fractionations as discussed earlier. The threshold potential was measured for each fraction between 1 and 70 MHz where the frequency dependence of E_{th} is very small (see Fig. 3). The results of these measurements are illustrated in Fig. 1. This figure clearly demonstrates a marked dependence of threshold potential on the particle size,

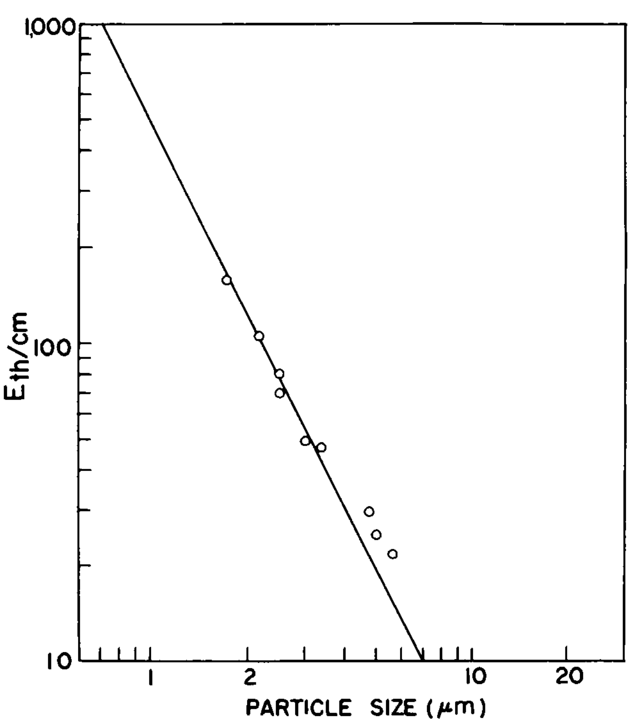

FIGURE 1 Size dependence of threshold potential with silicon suspension in water. Frequency: 1 MHz, continuous wave. The solid line is a theoretical curve with a slope of −1.5 (4). Standard deviation as indicated in Fig. 2.

keeping other parameters (dielectric properties) constant. The determination of the slope of the double logarithmic plot indicates that the threshold potential is proportional to $R^{-1.5}$ if one disregards the slight deviation from linearity for very large particles. The small deviation from linearity has not been observed before and may be due to an artifact. It is statistically not proven, but appears to be too systematic to be caused by random error. In spite of this slight deviation, the figure supports that the threshold potential has a $R^{-1.5}$ dependence as predicted by theory. As mentioned before, Sher (8), observed a $R^{-1.5}$ proportionality of the threshold potential using a variety of particles with different sizes, different shapes, and chemical compositions that would indicate that particle properties do not significantly influence the threshold field strength.

Frequency Dependence of E_{th}

Many electrical phenomena in biological systems are frequency dependent and, in general, low-frequency fields are more effective in evoking biological processes than high-frequency fields. The purpose of this experiment is to demonstrate frequency dependence of the threshold potential and, in addition, extract a quantitative correlation between E_{th} and frequency. The frequency range studied was from 50 to 70 MHz. The mean particle diameter of the particles used for these experiments was 2.2 μm. The results are shown in Figs. 2 and 3. As shown in Fig. 2, the threshold potential decreases markedly below 100 KHz and becomes as small as 20 V/cm at 500 Hz. The threshold determination below 500 Hz was found to be difficult because electrophoretic movement begins to interfere with the measurement at low frequencies. Replotting the value of E_{th} against frequency using a double logarithmic scale, we find that the limiting slope of this plot is 0.5, indicating that the low-frequency threshold potential is proportional

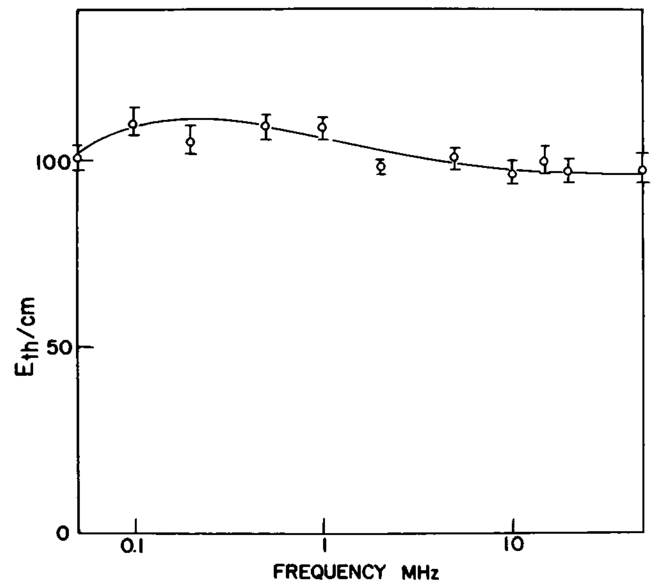

FIGURE 3 Frequency dependence of threshold potential between 50 KHz and 50 MHz. Continuous wave. Silicon particle with a diameter of 2.2 ± 0.2 μm.

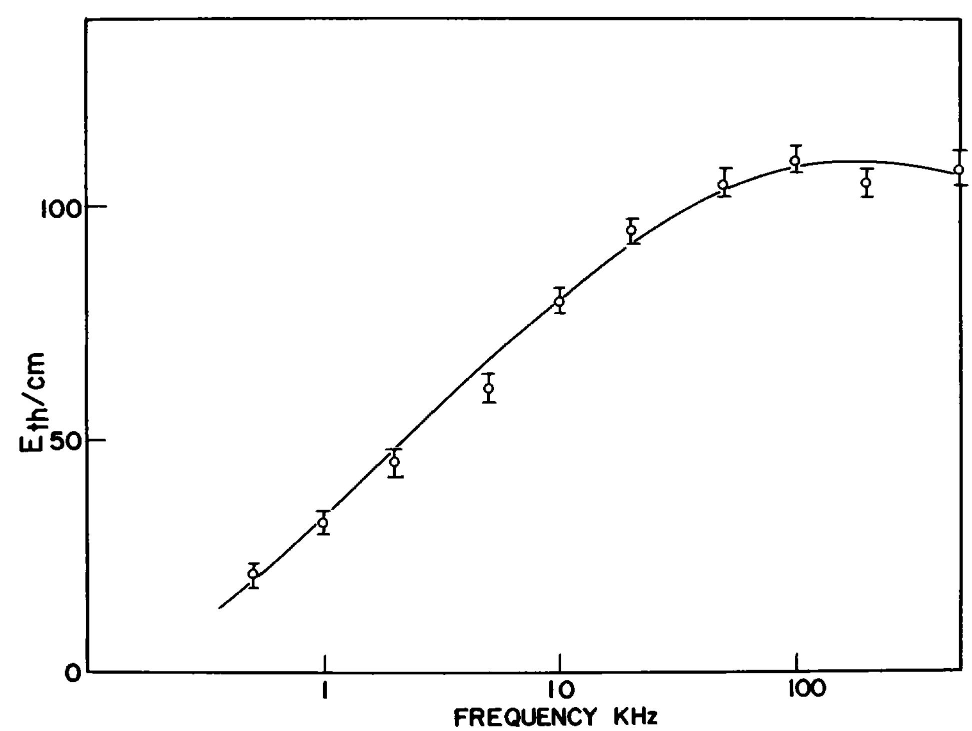

FIGURE 2 Frequency dependence of threshold potential between 0.5 and 500 KHz. Continuous wave. Silicon particle diameter 2.2 ± 0.2 μm.

to the 0.5 power of frequency, i.e.,

$$E_{th} \propto f^{0.5} . \quad (1)$$

On the other hand, E_{th} is almost independent of frequency above 1 MHz, disregarding a broad hump of small and statistically insignificant magnitude, between 100 KHz and 1 MHz.

In search of an explanation of the frequency dependence of E_{th}, we investigated the dielectric constant of silicon particles (d = 2.2 μm) suspended in aqueous media. The frequency profile of the dielectric properties of the suspension is illustrated in Fig. 4. As indicated, the dielectric constant is strongly dependent on frequency reaching a value as high as 12,000 at 50 Hz. Electrode polarization was corrected by use of the electrode distance variation technique (12) and the marked frequency dependence of dielectric constant is unlikely to be due to electrode effects. Obviously, the dielectric constant of silicon suspension undergoes an anomalous dispersion in the same frequency range as that of E_{th}.

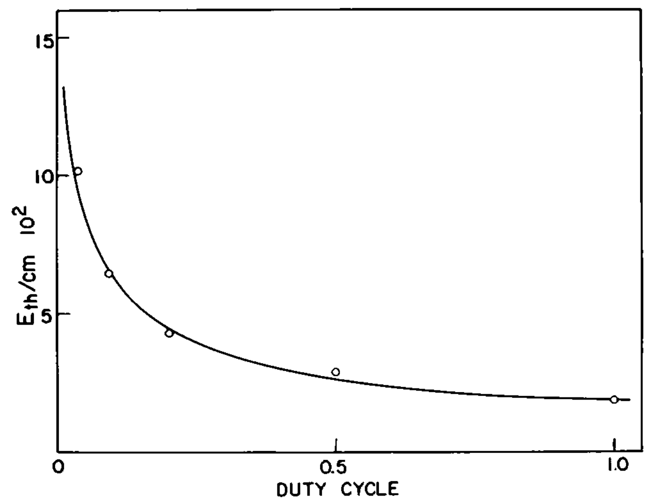

FIGURE 5 Dependence of threshold potential on duty cycles. Duty cycle is defined by pulse repetition frequency times pulse width in second. Particle size: 1.8 μm in diameter.

Threshold Potential with Pulsed Fields

Sher et al. (10) demonstrated that the time constant of pearl-chain formation with pulsed fields is the same as that of continuous waves, a clear indication that pulsed fields are not necessarily more effective than continuous waves. In the present experiment, the effect of pulsing the applied field on E_{th} is investigated. The measurement of the threshold potential was repeated with the same specimen using pulsed fields at various duty cycles ranging from 0.01 to 1.0. Fig. 5 shows a typical result obtained at 10 MHz. The threshold potential increases sharply as the duty cycle decreases. The results obtained at different frequencies are essentially the same, except for different values of E_{th} because of its frequency dependence. This result indicates that a higher field intensity will be needed for pearl chain formation as the duration of pulses becomes shorter compared to pulse interval. The total time-averaged power of pulsed fields is generally defined by

$$W = E^2 f / \rho , \quad (3)$$

where f is the duty cycle and ρ is the resistivity of the suspensions. Since we used the same specimen for each measurement, the value of ρ is constant. If we plot the value of $E^2 f$ against duty cycle f, we will be able to determine the dependence or independence of the time-

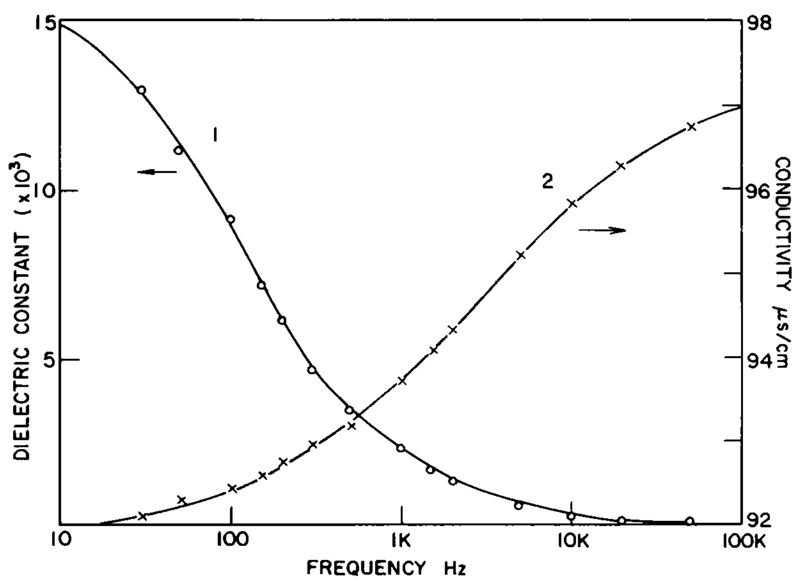

FIGURE 4 Frequency dependence of the dielectric constant (*1*) and conductivity (*2*) of silicon particle suspension (diameter of 2.2 μm). The volume fraction is 0.23 and the input voltage is 0.5 V. One high-accuracy measurement, as indicated by small deviations of experimental data from smooth curves. Both bulk dielectric constant and conductivity are needed to extract particle properties.

averaged threshold power requirement W_{th} on the duty cycle f. Fig. 6 shows the result. Clearly, the power requirement for pearl-chain formation is, within experimental error, independent of duty cycle. Hence, we conclude that pulsed field requires as much power as that for continuous fields when the pulse duration and separation are smaller than the time constants for pearl-chain formation.

DISCUSSION

As described, pearl-chain formation of microscopic particles is one of the few known in vitro examples of nonthermal effects of electromagnetic fields. Apparently, it can involve translational as well as rotary diffusion processes.

The frequency dependence of E_{th} was investigated experimentally by Sher (8). Sher observed a strong frequency dependence for silicon SM 61 particles with a diameter of 0.6 μm, whereas other particles such as polystyrene (1.17 μm) did not show any frequency dependence. For silicone particles, the threshold field strength decreased by about a factor of four as the field strength decreased from 100 KHz to 500 Hz, i.e., by an identical factor over the same frequency range as observed by us for 2.2 μm particles. Although all our measurements were performed with silicon particles that were coated with albumin and, therefore, were highly charged, in Sher's article no specification was given with regard to the coating. Whether the different results reported in Sher's article are due to the presence or absence of surface coating or due to other factors, is still unanswered. We are planning to repeat the measurement of E_{th} using uncoated silicon particles.

The theoretical formulations of Saito and Schwan (9), Schwan (4), and Sauer (11) indicated above all have in common that the dielectric constant of the suspended particles itself should not strongly influence the frequency dependence of the threshold field strength. They all state that the dielectric constant of the particle ϵ_i appears in expressions for either E_{th} or force only in the combination

$$\frac{\epsilon_i - \epsilon_a}{\epsilon_i + 2\epsilon_a},$$

where ϵ_a is the dielectric constant of the medium. Thus if ϵ_i varies from 0 to ∞, the expression in the equation varies only between $-1/2$ and 1, far less than the E_{th} results indicated in Fig. 2. We have extracted from the bulk dielectric data in Fig. 4 the effective dielectric constant of the albumin-coated silicone particles and find it to be also strongly frequency dependent in a manner reflecting the frequency dependence of the bulk data. The overall effect is quite similar to the data previously presented by Schwan et al. (13) and explained by counterion relaxation by Schwarz (14). The electrical properties of the medium are certainly frequency independent. Why the observed frequency dependence of E_{th} appears to reflect the frequency dependence of the suspended particles contrary to theory is now uncertain. Further studies in this regard are intended.

The measurement of threshold potentials was performed with pulsed fields as well as continuous fields. Although the threshold potential increases sharply with decreasing duty cycles, the time averaged power requirement for the formation of pearl chains is found to be independent of duty cycle. This is a clear indication that pulsed fields are not more efficient than continuous fields for eliciting pearl-chain formation at least for pulse parameters shorter than time constants. There has been a belief that pulsed waves may be more effective in causing biological effects than continuous fields. The results reported here clearly demonstrate that continuous waves are at least as effective as pulsed energy for pearl-chain formation. This result can be extended to other phenomena due to field-induced forces for reasons mentioned earlier (10).

The relevance of pearl-chain formation to biology has been discussed for some time. Pearl chains can be formed with biological materials such as erythrocytes or bacterial suspension. However, there is no evidence that the chain formation process is hazardous to biological systems. In biological systems, there are many processes that are elicited by electrical fields. In particular, excitation of the nerve axon membrane involves a realignment of particles of macromolecular size within the membrane. In the well-known Hodgkin-Huxley theory (15), the authors assume that at least three to four particles must undergo a voltage-dependent realignment in sodium and potassium channels. The reordering of these particles is thought to be evoked by a strong field existing in the membrane (80–100 kV/cm) and a strong voltage dependence of ionic currents and time constants is well known (16).

It is of some interest to speculate if at these field strength values, field-induced forces may be significant at the macromolecular level. The product $E_{th}R$ (R, radius) is taken from the data in Fig. 3 to be $1.1 \times 10^{-2}\ V^{1/2}$ cm. For field strength values from 80 to 100 kV/cm, radii of 10 to

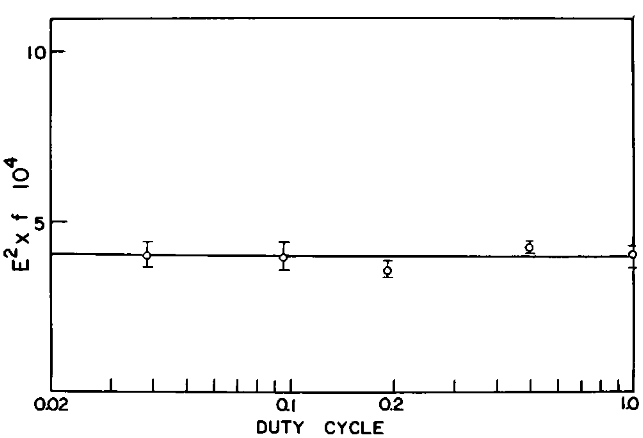

FIGURE 6 Dependence of time-averaged threshold power on duty cycle. The ordinate is $E^2 \times f$ where E is the threshold intensity and f is the duty cycle. Experimental conditions are the same as in Fig. 5.

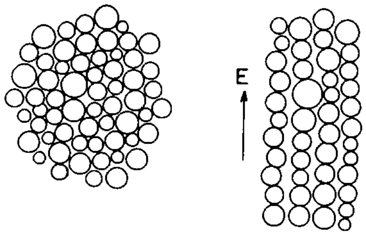

FIGURE 7 (*a*) Schematic presentation of a concentrated silicon suspension without electrical fields. (*b*) Schematic presentation of a concentrated silicon suspension with an electrical field. Arrow indicates the direction of the field.

15 Å are readily obtained. Thus, molecular membrane components larger than this could respond well to these large field strength values.

The parallelism between the realignment of macromolecular particles in nerve membranes and field-induced orientations or pearl-chain formation is, of course, speculative. However, we can perform an interesting hypothetical experiment using a concentrated silicon particle suspension. Consider a concentrated silicon suspension in water, with the particles randomly distributed as shown schematically in Fig. 7 *a*. When an electrical field of sufficient strength is applied, a drastic realignment of particles takes place (Fig. 7 *b*). The random distribution is replaced by linear arrays of particles thereby creating an anisotropic structure. Pearl chains are formed side by side with narrow interstitial spaces between them. We can surmise that current will flow more readily along the chains through the narrow channels rather than across them. Thus, chain formation due to force-field effects may have some biological relevance although no evidence has been provided so far.

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