FACTORS INVOLVED IN THE ORIENTATION OF MICROSCOPIC PARTICLES IN SUSPENSIONS INFLUENCED BY RADIO FREQUENCY FIELDS

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(Received May 23rd, 1961)

SUMMARY

The effects of audio and high radio frequency fields and also of a direct field on conducting and non-conducting particles suspended in various fluids have been studied. The orientation and chain formation which other workers have reported with suspensions in 10–100 megacycles/sec electromagnetic fields have been observed under suitable conditions even in direct electric fields. The effects can be explained by the attraction between dipoles induced by an electric field.

INTRODUCTION

The possible effects of electromagnetic radio fields on biological material have been discussed from time to time ever since the development of wireless telegraphy at the start of this century. The suggested health hazard to people working near high-powered radar stations has recently renewed interest in this topic. The generally accepted view, however, is that the only possible biological effect of radio waves is the simple production of heat when the waves are absorbed. But another effect that may be important has now been conclusively demonstrated by exposing suspensions of unicellular organisms, starch and other particles to intense radio frequency fields[1]. The organisms were found to orientate themselves so as to lie either along or across the field at particular frequencies in the 10–100 megacycles/sec range and the particles moved together to form remarkable series of chains. It was suggested that these phenomena could not be simply explained by the formation of electric dipoles. A number of other explanations were also ruled out.

These unexplained effects were all observed within a few millimetres of the electrodes that carried the radio frequency currents. As close as this the predominant field is the electric one; it is only at distances of the order of several wavelengths that the true electromagnetic radio field becomes of importance. We decided therefore that it might be of interest to investigate the effect of electric fields on suspensions of various sorts of particles to see under what conditions similar chain formations could be made to occur. The particles used had sizes of around 50 μ and were sus-

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pended in various fluids. They were subjected to alternating electric fields at various frequencies from 50 cycles/sec to 20 megacycles/sec and also to a direct field. The results of these experiments throw some light on the factors involved in chain formation in particle suspensions.

By extending the theory of the forces between induced electric dipoles it has been possible to give a satisfactory explanation of these effects.

APPARATUS AND MATERIALS

The apparatus was similar to that used by TEIXEIRA-PINTO *et al.*[1], but with two important differences. Smaller electrodes were used and the radio frequency output was not pulsed, since we felt that some of the effects (such as the spinning of the particles) might have arisen from the pulsation frequency.

The electrodes were two parallel copper wires 0.8 mm diameter and 1 cm long spaced 3 mm apart and cemented on to a perspex block. A drop of the suspension which was being investigated was put on a thin (No. 0) cover glass and this was placed on top of the electrodes (Fig. 1). The drop had no electrical contact with the electrodes being completely insulated from them by the cover glass. The particles suspended in the drop were influenced only through the electric field which existed between the electrodes; the heating effects with this lay-out were negligible.

A conventional 35-W output radio frequency oscillator was used to cover frequencies in the range 5–20 megacycles/sec. A 50-W audio signal was obtained from an audio frequency generator which was fed into an amplifier and was used to give

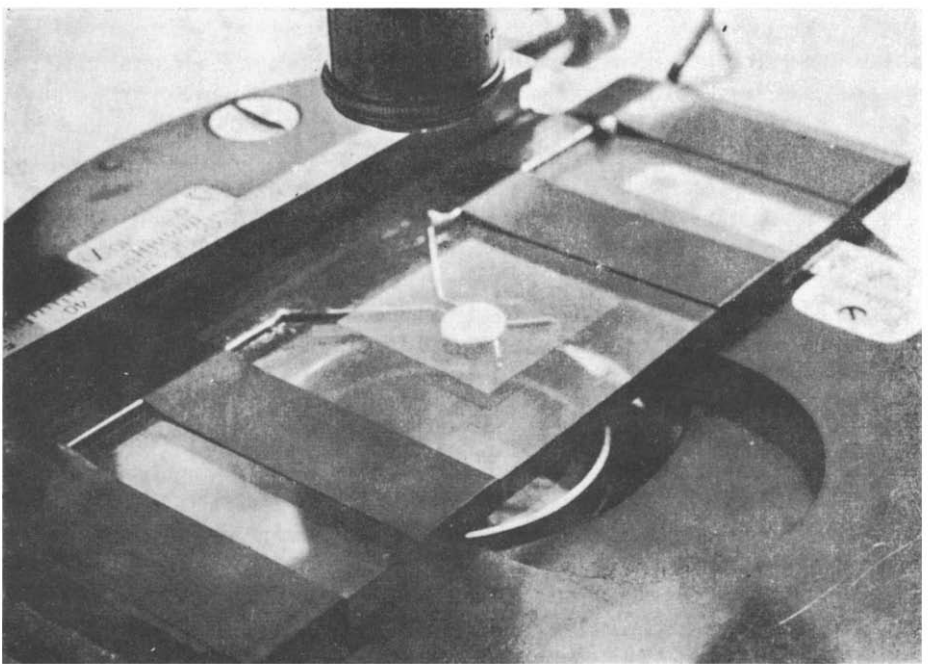

Fig. 1. The cell is shown under the microscope. A cover glass with a drop of the particle suspension lies on top of the electrodes.

frequencies of 50 cycles/sec, 1 kcycles/sec and 10 kcycles/sec. A D.C. field was obtained from a variable 0–4 kV high-tension supply. The voltages applied were up to that which caused sparking between the electrodes (about 10000 V/cm).

The particles studied were polystyrene latex spheres 0.8 μ diameter (kindly donated by the Dow Chemical Corporation, Midland, Michigan, U.S.A.); pure aluminium powder; red ferric oxide; potato starch grains (British Drug Houses Ltd.); an ion exchange resin and carbon powder obtained by rubbing pure carbon electrode rods. Suspensions of these particles were made in ion-free water, in saline (0.9% sodium chloride) or in castor oil (B.P.).

RESULTS

The results obtained are shown in Table I. The action of the electric field on the particle suspension is entered as '—' when no effect at all was observed, as $\pm$ when

TABLE I

THE EXPERIMENTAL RESULTS OBSERVED WITH DIFFERENT PARTICLES SUSPENDED IN VARIOUS FLUIDS

The symbol — indicates that no effect was observed; $\pm$ that the particles were orientated but did not form chains (Fig. 2); + that the particles formed chains (Fig. 3).

Particles	*Frequency (cycles/sec)*	*Castor oil*	*Water*	*Saline (0.9% NaCl)*
Polystyrene	D.C.	—	—	—
	50	—	—	—
	10^3	—	—	—
	10^4	—	—	—
	10^7	—	+	—
Ion-exchange resin	D.C.		—	—
	50		—	—
	10^3		—	—
	10^4		—	—
	10^7		+	—
Potato starch	D.C.	+	—	—
	50	+	—	—
	10^3	+	—	—
	10^4	+	—	—
	10^7	$\pm$	+	—
Red ferric oxide	D.C.	+	—	—
	50	+	—	—
	10^3	+	—	—
	10^4	+	—	—
	10^7	$\pm$	+	—
Carbon powder	D.C.	+	—	—
	50	+	+	—
	10^3	+	+	—
	10^4	+	+	—
	10^7	+	+	—
Aluminium powder	D.C.	+	—	—
	50	+	+	—
	10^3	+	+	—
	10^4	+	+	—
	10^7	+	+	—

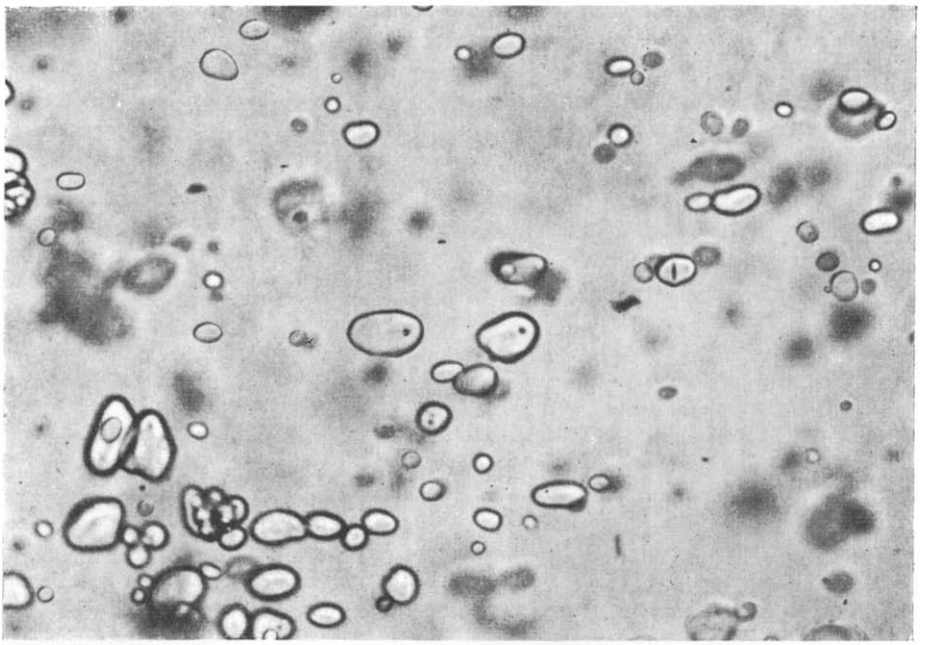

Fig. 2. Starch particles orientated but not lined-up in an electric field.

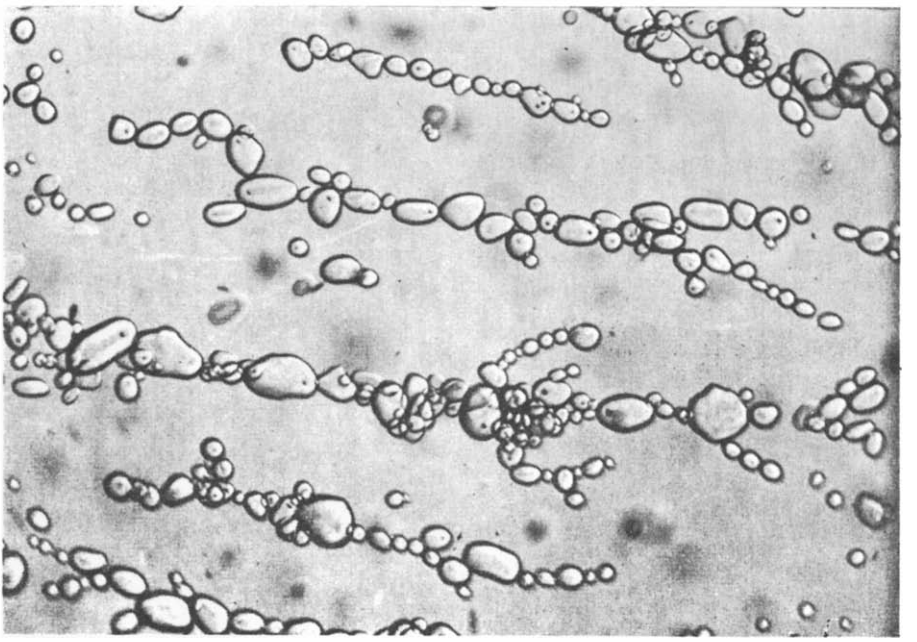

Fig. 3. Starch particles after forming chains in an electric field.

the particles were turned so that their longest axis was parallel to the electric field, but without chain formation (Fig. 2) and as + when the particles rapidly formed into long chains (Fig. 3).

It will be seen from Table I that, with the exception of the polystyrene and resin particles, the particles suspended in castor oil formed chains in D.C. and low frequency A.C. fields just as well as in the high radio frequency field. The phenomena observed at the different frequencies appeared to be identical in every respect. When suspended in water, none of the suspensions formed into chains in a D.C. field, but the particles that were good conductors of electricity formed chains at audio frequencies and all the particles formed chains in a 10 megacycles/sec field. The chain formation was instantly destroyed when a small amount of salt was added to the drop and no chain formation could be produced with any of the particles suspended in saline (0.9 % sodium chloride).

The effects which followed when carbon powder was suspended in increasing dilutions of saline were investigated. No orientation or chain formation could be obtained at audio frequencies even with saline diluted to contain only 0.01 % sodium chloride. At 10 megacycles/sec, however, orientation was first observed with particles in 0.25 % sodium chloride and chain formation occurred in 0.01 % sodium chloride.

THEORETICAL

In this section an approximate expression is obtained for the force tending to line up the particles under various conditions. We will consider for simplicity that the particles are all spherical and that the suspensions all contain similar sized particles at the same concentration. The main limitation of this treatment is that it neglects the complex situation which can develop at the surface of the particles by taking the bulk values for conductivity and dielectric constant to apply throughout the suspension. An electric field acting in the drop will polarize the particles in the direction of the field, either in the case of conducting particles by causing electrons to flow to one end or in the case of insulators by aligning and polarizing the constituent molecules. Whatever the direction of the field, there will then be an attractive force between the particles; both an alternating and a direct field could thus produce chain-like aggregations. Chain formation will only occur, however, when the attractive forces due to the induced dipoles outbalance the repulsive forces which normally keep the particles from spontaneously aggregating. These repulsive forces arise primarily from the interaction between like charges carried on and around the surface of the particles[2].

The force of attraction between two dipoles can be obtained by differentiating the well-known expression for the energy of two dipoles[3]. For two dipoles in line and a distance d apart we can thus obtain the expression (using rationalized m.k.s. units)

$$F = \frac{6\,m^2}{4\pi\kappa_f\varepsilon_0 d^4} \qquad (1)$$

where F is the attractive force in the dipole moment induced in each particle, κ_f the dielectric constant of the fluid in which they are suspended and $\varepsilon_0 = 8.86 \cdot 10^{-12}$ (permittivity of free space).

The moment induced in a particle by an electric field E in the fluid around it is given by

$$m = 4\pi\varepsilon_0 a^3 E\kappa_f (\kappa_p - \kappa_f)/(\kappa_p + 2\kappa_f)$$

where a is the radius of the particle; κ_f is the dielectric constant of the fluid; κ_p is the dielectric constant of the particle. If either the particles or the fluid is a conductor, it can be shown (see Eqn. 3 below) that the dielectric constant κ should be replaced by $\kappa(1 - j\sigma/\omega\kappa\varepsilon_0)$ where σ is the conductivity (ohms^{-1} m^{-1}), $\omega = 2\pi f$, f being the frequency of the field, $\varepsilon_0 = 8.85 \cdot 10^{-12}$ and $j = \sqrt{(-1)}$. Thus in general the moment induced by a field E is given by

$$m = \frac{4\pi\varepsilon_0 a^3 E\kappa_f [1 - (j\sigma_f/\omega\kappa_f\varepsilon_0)] [\kappa_p - \kappa_f - (j/\omega\varepsilon_0)(\sigma_p - \sigma_f)]}{[\kappa_p + 2\kappa_f - (j/\omega\varepsilon_0)(\sigma_p + 2\sigma_f)]} \qquad (2)$$

The field E in the drop has now to be related to the voltage V_1 applied across the electrodes. An approximate equivalent circuit is shown in Fig. 4. The drop is represented by the capacity C_2 shunted by R, the resistance of the drop. C_3 and C_4 are the capacities between the drop and the electrodes and C_1 is the capacity across the electrodes. V_1 is the applied voltage across C_1, V_2 is the voltage across the drop (that is across C_2 and R) and is related to the field E in the drop by $E = (V_2/l)$ where l is the distance between the electrodes.

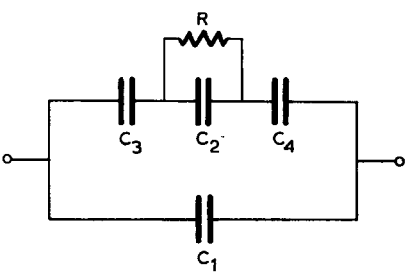

Fig. 4. The equivalent circuit diagram.

We have $V_2 = (Z_D/Z)V_1$ where Z_D is the impedance of the drop (that is the impedance of C_2 and R in parallel).

Z is the total impedance of C_3, C_4, C_2 and R.

Now

$$\frac{1}{Z_D} = \frac{1}{R} + j\omega C_2 = j\omega C_2\left(1 - \frac{j}{\omega RC_2}\right)$$

If C_2 is considered to be a parallel plate capacitor of area A and spacing l then if σ_f is the conductivity of the dielectric and κ_f the dielectric constant

$$R = \frac{l}{\sigma_f A}$$

and

$$C_2 = \frac{\kappa_f\varepsilon_0 A}{l} \qquad \therefore RC_2 = \frac{\kappa_f\varepsilon_0}{\sigma_f}$$

Hence

$$\frac{1}{Z_D} = j\omega C_2\left(1 - \frac{j\sigma_f}{\omega\kappa_f\varepsilon_0}\right) \qquad (3)$$

and

$$Z = \frac{1}{j\omega}\left[\frac{1}{C_3} + \frac{1}{C_4} + \frac{1}{C_2\,(1 - j\sigma_f/\omega\kappa_f\varepsilon_0)}\right]$$

$$\therefore V_2 = \frac{V_1}{1 + \left(1 - \dfrac{j\sigma_f}{\omega\kappa_f\varepsilon_0}\right)\left(\dfrac{C_2}{C_3} + \dfrac{C_2}{C_4}\right)}$$

From the linear dimensions of the particular apparatus used we have

$$\frac{C_2}{C_3} = \frac{C_2}{C_4} \simeq \frac{\kappa_f}{150}.$$

$\therefore$ since $E = V_2/t$

$$E = \frac{V_1}{t\left[1 + (\kappa_f/75)\left(1 - \dfrac{j\sigma_f}{\omega\kappa_f\varepsilon_0}\right)\right]} \tag{4}$$

When the conductivity of the suspending fluid is taken into consideration Eqn. 1 becomes

$$F = \frac{6\,m^2}{\kappa_f\left(1 - \dfrac{j\sigma_f}{\omega\kappa_f\varepsilon_0}\right)4\pi\varepsilon_0 d^4} \tag{5}$$

The complete expression for F in terms of V_1 is now obtained by combining Eqns. 2, 4 and 5.

$$F = \frac{V_1{}^2 6a^6 4\pi\varepsilon_0\kappa_f\left(1 - \dfrac{j\sigma_f}{\omega\kappa_f\varepsilon_0}\right)\left[\kappa_p - \kappa_f - \dfrac{j}{\omega\varepsilon_0}(\sigma_p - \sigma_f)\right]^2}{d^4 f^2\left[1 + \dfrac{\kappa_f}{75}\left(1 - \dfrac{j\sigma_f}{\omega\kappa_f\varepsilon_0}\right)\right]^2\left[\kappa_p + 2\kappa_f - \dfrac{j}{\omega\varepsilon_0}(\sigma_p + 2\sigma_f)\right]^2} \tag{6}$$

Particular cases can now be considered. For a suspension of conducting particles $\sigma_p > 10^6$ ohms^{-1} m^{-1} and $\sigma_p/\omega\varepsilon_0$ (that is $10^{12}\,\sigma_p/8.86\,\omega$) is very large for all frequencies we are considering (ω up to 10^8). Eqn. 6 then gives for the force F_c between conducting particles

$$F_c = \frac{\beta\kappa_f\left(1 - \dfrac{j\sigma_f}{\omega\kappa_f\varepsilon_0}\right)}{\left[1 + \dfrac{\kappa_f}{75}\left(1 - \dfrac{j\sigma_t}{\omega\kappa_f\varepsilon_0}\right)\right]^2}$$

where $\beta = 24\pi\varepsilon_0 a^6 V_1{}^2/d^4 f^2$ and is a constant for a particular apparatus and type of particle.

The absolute magnitude of F_c expressed in terms of β is obtainable from the expression

$$\frac{F_c}{\beta} = \frac{\kappa_f\left(1 + \dfrac{\sigma_f{}^2}{\omega^2\kappa_f{}^2\varepsilon_0{}^2}\right)^{1/2}}{\left(1 + \dfrac{\kappa_f}{75}\right)^2 + \left(\dfrac{\kappa_f}{75}\right)^2\left(\dfrac{\sigma_f{}^2}{\omega^2\kappa_f{}^2\varepsilon_0{}^2}\right)} \tag{7}$$

For a non-conducting oil this becomes for all frequencies and for a direct field

$$\frac{F_c}{\beta} = \frac{\kappa_f}{\left(1 + \dfrac{\kappa_f}{75}\right)^2}$$

The force F_i on an insulator particle is given in terms of F_c for particles suspended in water or saline (where $\kappa_f \gg \kappa_p$) by $F_i = F_c/4$.
For insulator particles suspended in a non-conducting oil the expression is

$$F_i = \frac{F_c\,(\kappa_f - \kappa_p)^2}{(\kappa_f + 2\kappa_p)^2}$$

Table II shows the calculated values of F/β for conducting particles and Table III the values for polystyrene particles. The values taken for the dielectric constant κ and for the conductivity σ (ohms^{-1}·m^{-1}) were the values quoted for the material in bulk: castor oil, $\kappa = 4.3$, $\sigma = \infty$; water, $\kappa = 80$, $\sigma = 5 \cdot 10^{-4}$ (measured); polystyrene $\kappa = 2.5$, $\sigma = \infty$, saline (0.9 % NaCl) $\kappa = 80$, $\sigma = 1.5$; saline (0.009 % NaCl) $\kappa = 80$, $\sigma = 1.5 \cdot 10^{-2}$.

If it is assumed that the force F/β required to form chains with conducting particles is about 0.5 or more and for polystyrene particles is 2.0 or more there is satisfactory agreement between these theoretical predictions and the observed effects recorded in Table I. The only disagreement is in the behaviour of conducting particles at 50 cycles/sec.

TABLE II

THE CALCULATED FORCE BETWEEN CONDUCTING PARTICLES SUSPENDED IN VARIOUS FLUIDS IN AN ELECTRIC FIELD

See text for unit.

Frequency (cycles/sec)	*Castor oil*	*Water*	*Saline (0.9 % NaCl)*	*Saline (0.009 % NaCl)*
0	3.84	0	0	0
50	3.84	0.03	0	0
10^3	3.84	0.64	0	0.02
10^4	3.84	6.15	0	0.2
10^5	3.84	20.8	0.02	2.1
10^6	3.84	18.6	0.2	16.5
10^7	3.84	18.6	2.1	15.1
10^8	3.84	18.6	16.5	18.6

TABLE III

THE CALCULATED FORCE BETWEEN POLYSTYRENE PARTICLES SUSPENDED IN VARIOUS FLUIDS IN AN ELECTRIC FIELD

For unit see text.

Frequency (cycles/sec)	*Castor oil*	*Water*	*Saline (0.9 % NaCl)*
0	0.10	0	0
50	0.10	0	0
10^3	0.10	0.16	0
10^4	0.10	1.54	0
10^5	0.10	5.2	0
10^6	0.10	4.6	0.05
10^7	0.10	4.6	0.5
10^8	0.10	4.6	4.1

DISCUSSION

The observations recorded in Table I are in good qualitative agreement with the theoretical predictions given in Tables II and III which are based on a theory of the forces between dipoles induced by a sufficiently strong electric field. The phenomena of chain formation and orientation thus seem to be explained by the classical laws of electricity. The reason why in water, and particularly in saline, the effects are only seen at high radio frequencies is because at lower frequencies the conductivity of the suspending fluid shorts out the field. We have shown, however, that exactly similar effects to those described by TEIXEIRA-PINTO *et al.*[1] can be observed even with a D.C. field if suspensions in oil are used.

TEIXEIRA-PINTO *et al.*[1] attribute the first observation of chain formation to MUTH[5] in 1928, who observed it in emulsions of fat particles. The phenomenon is, however, probably exactly similar to that reported by BRANLY[6] in 1889 who found that the electrical resistance of a pile of iron filings dropped markedly when they were subjected to the field produced by Hertzian waves. This effect was attributed to a coherence of the particles which was induced by the field. It formed the basis of MARCONI's famous "coherer" by means of which the first wireless messages to be transmitted across the Atlantic were received. Many explanations of how the coherer works were put forward before interest in it was lost with the coming of the thermionic radio valve; but it seems quite likely that its action depended on "chain formation" in the metal filings.

With elongated particles, the observed tendency is for the alignment of the long axis to be parallel with the electric field in accordance with dipole theory; but in the case of living unicellular organisms TEIXEIRA-PINTO *et al.*[1] describe orientation not only in the expected direction, but also report at higher frequencies a rotation of the particles through 90° so that the organisms lay across the field. They regarded existing dipole theory as deficient in explaining this change of orientation, which was only observed with living material. An explanation, in terms of dipole theory may still be possible, however, if the effect of the outer double membrane is taken into account. It is well known that this membrane acts as a capacitor[7]. In an alternating field the membrane will be charged so that dipoles form at right angles to the surface of the cell. An elongated cell will therefore be subjected to two opposing torques. The currents induced in the protoplasm, which acts as a resistance in a similar way to the particles which we have investigated, give stable equilibrium with the long axis parallel with the electric field; the charging of the capacitive membrane, however, gives stable equilibrium with the organism at right angles to the field. It is this latter position therefore which should predominate at the higher frequencies.

A possible application of these phenomena is to provide a method for investigating the electrical conductivity and the dielectric constant of living organisms without using electrodes inserted in the suspension. In fact, a rather similar technique has recently been used to determine the electrical properties of cadmium sulphate crystals by measuring the torque produced on them by a rotating electric field[8].

ACKNOWLEDGEMENTS

We are indebted to Mr. W. J. PERKINS for helpful discussion of these problems and to Mr. M. R. YOUNG who generously undertook to obtain the photographs reproduced

here. One of us (A.A.F.) wishes to thank Professor S. HESTRIN and Sir CHARLES HARINGTON, F.R.S., for their kind help in making this collaboration possible.

REFERENCES

1 A. A. TEIXEIRA-PINTO, L. L. NEJELSKI, J. L. CUTLER AND J. H. HELLER, *Exptl. Cell Research*, 20 (1960) 548.
2 E. J. W. VERWEY AND J. TH. G. OVERBEEK, *Theory of the Stability of Lyophobic Colloids*, Elsevier, Amsterdam, 1948.
3 C. A. COULSON, *Electricity*, Oliver and Boyd, Edinburgh, 1958, p. 42.
4 G. P. HARNWELL, *Principles of Electricity and Electromagnetism*, McGraw-Hill, New York, 1949, p. 75.
5 E. MUTH, *Kolloid Z.*, 41 (1927) 97.
6 E. BRANLY, *Compt. rend.*, 111 (1890) 785; 112 (1891) 90.
7 K. S. COLE, *Cold Spring Harbor Symposia Quant. Biol.*, 1 (1933) 107.
8 T. OGAWA, *J. Appl. Phys.*, 32 (1961) 583.

THE EFFECT OF LOW CONCENTRATIONS OF THIOL-GROUP-BLOCKING AGENTS ON THE OUTER MEMBRANE OF FROG SKIN

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(Received May 24th, 1961)

SUMMARY

The effect of some thiol-group-blocking agents in the solution bathing the outside of isolated frog skin was investigated by a simple method permitting of measuring two separate membrane potentials in the skin and of membrane d.c. resistances to individual ions. It was found that, while higher concentrations of these agents bring about a drop in the membrane potentials and permeability changes, similarly to mercuric chloride, lower concentrations, after a short period of incubation of the skin, cause considerable hyperpolarization of the outer membrane. The hyperpolarization is explained as a result of increased permselectivity of the outer membrane in USSING's model of frog skin. The conditions of the reaction involved are described and a crude model of the outer membrane is suggested.

INTRODUCTION

KOEFOED-JOHNSEN AND USSING's[1] model explaining the nature of the potential difference across the frog skin involves two membranes of different properties. It was only the microelectrode technique, which is less suitable for continuously following the effects of external factors, that was used recently with the result of two membrane potentials as proposed by the above model being demonstrated[2,3]. The authors of a