

EFFECTS OF ELECTROMAGNETIC FIELDS ON THE MOTION OF *EUGLENA GRACILIS*

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ABSTRACT The orientation behavior of *Euglena gracilis* cultures in electromagnetic fields is shown to agree with the predictions of a model involving only a passive mechanism. The increase in *Euglena* motor activity with increasing field intensity is demonstrated by measuring various motion parameters by the laser scattering technique. The effect of electric field on the speed of *Euglenas* is compared with that of temperature. We conclude that the electric field warms up the culture, thus inducing an increase in cell motility.

INTRODUCTION

Two effects can be observed when an electromagnetic field is applied to a specimen of an *Euglena gracilis* culture. Firstly, *Euglenas* take up a particular orientation and secondly, as observed by Wolken (1967) in a microscopic investigation, their mean speed increases with increasing field intensity. The orientation of motile unicellular microorganisms has been investigated by many authors (Teixeira-Pinto et al., 1960; Griffin and Stowell, 1966). For several types of nonspherical microorganisms, orientation occurs either parallel or perpendicular to the applied field, depending on the field frequency and on the electrical conductivity of the suspending medium (Griffin and Stowell, 1966).

It has been suggested that the orientation phenomenon is merely a physical effect. A theoretical deduction of the behavior of particles in alternating electric fields is possible (Saito et al., 1966); it explains the orientation as the result of the interaction between the dipole moment induced by the electric field and the field itself.

So far the theoretical results have not been compared with experimental data for any specific microorganism, because it is difficult to measure the electrical quantities involved. This paper reports such a comparison for *Euglena*. To obtain predictions about the behavior of *Euglena* in alternating electric fields, we have used the theoretical approach of Saito et al. (1966), obtaining the torque acting on an ellipsoidal particle, and studying its dependence on the medium conductivity, the cell shape, and the frequency of the applied field. These predictions are then compared with experimental results.

As regards the electrokinetic effect, we have measured the mean speed and other motion parameters (body rotation frequency and flagellar beating frequency), using laser light scattering (Ascoli et al., 1978). These measurements have been performed

at various field intensities and at various temperatures. The relation between the effect of electric field intensity and that of temperature is discussed. The conclusion is reached that an electric field warms up the culture, so inducing an increase in cell mobility.

MATERIALS AND METHODS

Euglena gracilis is a phytoflagellate, fuse-shaped protozoan whose dimensions are about $(60 \times 10 \times 10) \mu\text{m}^3$; it moves in a helicoidal path with a translation speed of about $100 \mu\text{m/s}$. The propulsion is supplied by a flagellum beating around the cell body.

The cells were grown at 24°C in an autotrophic medium (Cramer and Myers, 1952) under constant illumination of 3000 lux in a mixture of air and 5% CO_2 . Other *Euglenas*, bleached by being grown in the dark, were also used in the experiments.

To obtain samples with a suspending medium of the required conductivity, the cells were washed three or four times by gentle centrifugation; the conductivity of the suspending medium was measured by a conductimeter (Metrohm AG, Herisau, Switz.).

During the experiments, the cells were placed between two 0.17-mm thick coverglasses, about 0.2 mm apart, bounded by a ring of silicon grease about 15 mm in diameter. The chamber so obtained lay on two electrodes 2 mm apart, connected to a radiofrequency oscillator (1-35 MHz). Experiments were also made with an alternating field at 50 Hz; in this case the electrodes (platinum wires) were placed inside the sample, because the coverglass impedance is much higher at low frequencies than the suspending medium impedance.

The orienting field intensities were monitored by the peak-to-peak voltage between the electrodes (V_{pp}), which could be varied from 50 to 800 V. For each sample these voltages are proportional to the field intensities inside the sample.

Measurements of the motion parameters in oriented cultures were made by a photon beating technique which is described in detail in the companion paper (Ascoli et al., 1978). This is based on the analysis of the power spectrum of the current generated by a photodetector which collects the laser light scattered by the suspension of moving particles (homodyne technique), or, besides such scattered light, a fraction of the unperturbed laser light (heterodyne detection).

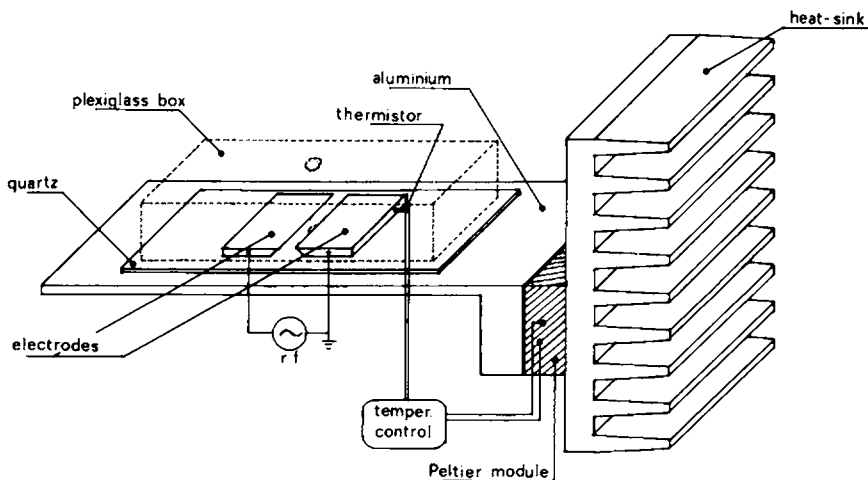


FIGURE 1 Schematic drawing of the thermostat apparatus. rf, radiofrequency oscillator.

This power spectrum offers information about the statistical distribution of the parameters of the particles.

The temperature of the electrodes supporting the sample was maintained constant by means of a Peltier module (Norton GmbH, Nurnberg, W. Germany) controlled by a NTC calibrated probe (Philips, Eindhoven, Holland). This probe measured the temperature of the ground electrode. The temperature could be varied from 5° to 45°C and reached the required value to $\pm 0.1^\circ\text{C}$ in 5–10 min. The thermostat apparatus is shown in Fig. 1. During the experiments which utilized the orienting radiofrequency field, the temperature inside the sample was not measured; all the temperatures reported in the text are those of the electrodes.

EXPERIMENTAL OBSERVATIONS ON ORIENTED EUGLENAS

In a suspending medium of low electrical conductivity, the cells orient parallel to the electrical field from very low frequencies (50 Hz) to about 10 MHz, and then at right angles from this frequency up to the frequency limit of our oscillator. The transition from a parallel to a perpendicular orientation does not occur at a single frequency, but within a frequency range of a few megahertz. Thus, the “transition frequency” may be defined as the mean frequency of the range lying between a clearly parallel and a clearly perpendicular orientation.

Far from the transition zone the lowest applied voltage necessary for a good parallel orientation was about 60 V, whereas for the perpendicular orientation a greater applied voltage—of about 200 V—is necessary. At very low frequency fields (50 Hz), with the electrodes inside the sample, an applied voltage of about 20 V is enough to orient the cells. When the field intensity is increased, the oriented cells form chains with both kinds of orientation; in both cases the chains are parallel to the electric field, as shown in Fig. 2. Near the transition zone a very high field intensity is needed to orient the cells. Nevertheless, a transition zone between parallel and perpendicular orientation is always present for each field intensity which does not damage the cells. Within the transition range, the cells behave differently in different samples. In some cases all the cells are randomly oriented, whereas in others some cells are parallel and some are perpendicularly oriented. Moreover, the transition frequency depends on the cell shape; in fact, we observed a lower transition frequency for more elongated cells.

Euglenas made nonmotile, without any significant change of shape, by adding 2% acetic acid, are oriented parallel or perpendicular at about the same frequencies as the living cells, and the two types of chains are obtained with lower field intensity. We have measured the transition frequency at several medium conductivities, as in the experiments of Griffin and Stowell (1967). When the electrical conductivity of the suspending medium is raised, the transition frequency and the width of the transition zone rise at the same time. In fact, although the width of the transition range is < 1 MHz at low conductivity ($5 \cdot 10^{-4}$ mho/m), it becomes about 10 MHz at very high conductivity (0.05 mho/m). At very high conductivities the cells remain unoriented up to the upper frequency limit of our oscillator. All this makes for poor definition of the transition frequency at very high conductivities. However, our results (reported in Table I) show an increase of the transition frequency as the medium conductivity increases, in agree-

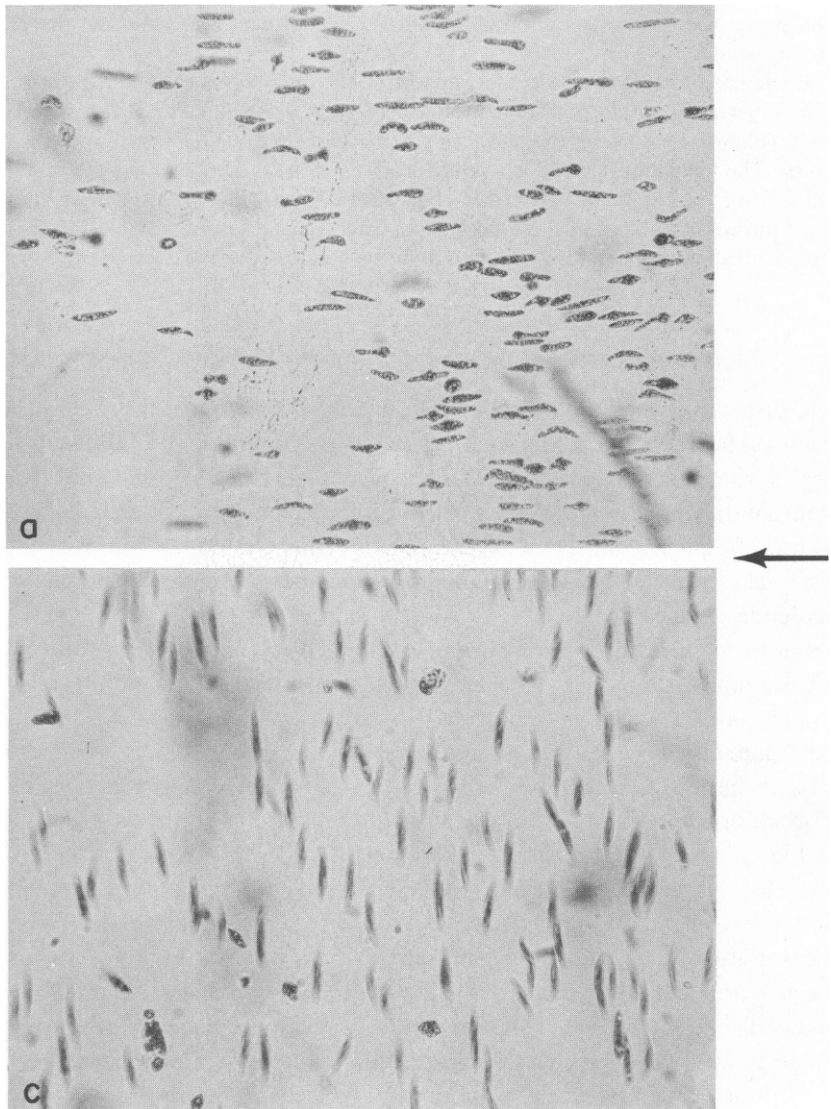


FIGURE 2 Oriented *Euglenae* in living *Euglenae* (a and c) and in nonmotile *Euglenae* forming chains (b and d). The electric field lies parallel to the major axis of the pictures.

ment with the results of Griffin and Stowell (1966). Results obtained with colorless *Euglenas* show the same features as those of green *Euglenas*.

PASSIVE ELECTRICAL MODEL AND COMPARISON WITH EXPERIMENTAL DATA

Experimental observations suggest that the orientation phenomenon is due to the interaction between the electrical dipole moment induced by the electric field in the cells

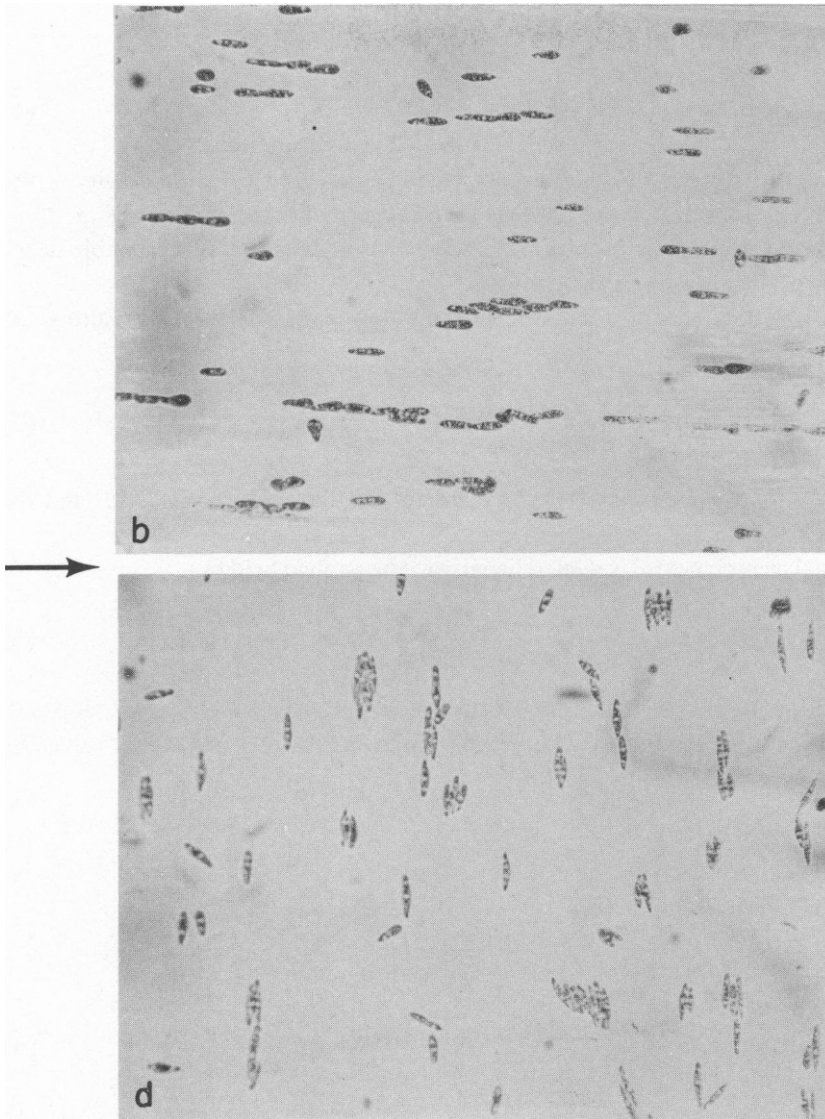


FIGURE 2 (b and d)

and the field itself. The *Euglena* cell can be schematized as an isotropic and homogeneous elongated ellipsoid of revolution, with semiaxes $a = 30 \mu\text{m}$, $b = c = 5 \mu\text{m}$.¹

Let us define the complex dielectric constants of the ellipsoid ϵ_i and the surrounding medium ϵ_e by

¹The cell actually has a membrane, but Saito et al. (1966) showed that we can account for it by assuming a homogeneous ellipsoid with renormalized electrical constants.

$$\begin{aligned}\epsilon_i &= \epsilon'_i - \frac{j\sigma_i}{\omega\epsilon_0} \\ \epsilon_e &= \epsilon'_e - \frac{j\sigma_e}{\omega\epsilon_0},\end{aligned}\quad (1)$$

respectively, where ϵ' is the real dielectric constant, σ the electrical conductivity, ϵ_0 the vacuum dielectric constant, and ω the angular frequency of the applied field $E_0 e^{-j\omega t}$.

We assume the electrical constants are independent of ω , which is reasonable in the frequency range explored in the experiments.

The mean potential energy stored within the ellipsoid is, as shown by Saito et al. (1966):

$$W = Re \frac{1}{4} \int_{V_e} \frac{\epsilon_e^*}{\epsilon_e} \mathbf{E} \cdot \mathbf{E}_0^* (\epsilon_e - \epsilon_i) dV, \quad (2)$$

where V_e is the *Euglena* volume, \mathbf{E} is the vector of the internal electric field, and the asterisk indicates complex conjugation.

The internal electric field E is related linearly to the applied field by

$$E_{x,y,z} = \frac{\epsilon_e}{\epsilon_e + (\epsilon_i - \epsilon_e)n_{x,y,z}} E_{ox,oy,oz}, \quad (3)$$

where n_x , n_y , and n_z are shape factors depending only on the geometry of the ellipsoid. Now if we choose the longest axis of the ellipsoid as x axis, n_x , n_y , and n_z are given by (Landau and Lifschitz, 1966):

$$\begin{aligned}n_x &= \frac{abc}{2} \int_0^\infty \frac{dr}{(r+a^2)^3(r+b^2)} \\ &= \frac{b^2}{2a(a^2-b^2)} \left\{ \ln \frac{a + \sqrt{a^2-b^2}}{a - \sqrt{a^2-b^2}} - 2 \frac{\sqrt{a^2-b^2}}{a} \right\}, \\ n_y &= n_z = \frac{1-n_x}{2}.\end{aligned}\quad (4)$$

Substituting the expressions (Eq. 3) into the relation (Eq. 2) and integrating, we obtain:

$$W = \frac{1}{4} V_e Re \left\{ \frac{\epsilon_e^*}{\epsilon_e} (\epsilon_e - \epsilon_i) [E_{ox}^2 \gamma_x + E_{oy}^2 \gamma_y + E_{oz}^2 \gamma_z] \right\} \quad (5)$$

where we have defined

$$\gamma_{x,y,z} = \frac{\epsilon_e}{\epsilon_e - (\epsilon_e - \epsilon_i)n_{x,y,z}}. \quad (6)$$

If we call α the angle between the applied field and the x axis, we have

$$E_{ox}^2 = E_o^2 \cos^2 \alpha, E_{oy}^2 + E_{oz}^2 = E_o^2 \sin^2 \alpha \quad (7)$$

and

$$W = \frac{1}{4} V_e Re \left\{ \frac{\epsilon_e^*}{\epsilon_e} (\epsilon_e - \epsilon_i) [\gamma_x E_o^2 \cos^2 \alpha - \gamma_y E_o^2 \sin^2 \alpha] \right\}. \quad (8)$$

Now the torque M around the direction orthogonal to the plane containing the x axis and the applied field can be obtained by taking the derivative of W with respect to α . Performing the derivation yields:

$$M = \frac{1}{4} V_e Re \frac{\epsilon_e^* (\epsilon_e - \epsilon_i)^2 (1 - 3n_x) E_o^2 \sin 2\alpha}{[\epsilon_e - (\epsilon_e - \epsilon_i)n_x][2\epsilon_e - (\epsilon_e - \epsilon_i)(1 - n_x)]}. \quad (9)$$

M vanishes for $\alpha = 0$ and $\alpha = \pi/2$; therefore two equilibrium orientations exist, the stable one corresponding to a minimum of the potential energy W , and thus to a positive value of the derivative $\partial^2 W / \partial \alpha^2 = \partial M / \partial \alpha$.

Let us write $M = K(\epsilon_i, \epsilon_e, n, \omega) E_o^2 \sin 2\alpha$; then for $\alpha = 0$ we obtain $\partial M / \partial \alpha = 2KE_o^2$, while for $\alpha = \pi/2$, we obtain $\partial M / \partial \alpha = -2KE_o^2$. Hence the parallel orientation is possible only for $K > 0$, and the transverse only one for $K < 0$, whereas the transition frequencies are the nulls of $K(\omega)$.

The nulls of $K(\omega)$ have been calculated by a computer after assuming suitable values for ϵ'_i , ϵ'_e , σ_i , σ_e , and n . Because the suspending medium is an aqueous solution, we assumed $\epsilon'_e = 80$ and used the values of σ_e corresponding to the experimental points reported in Table I. For the cell body we used n values lying between 0.05 and 0.02 corresponding to a/b ratios from 25/5 to 50/5. A reasonable estimate of σ_i was achieved by measuring the electrical conductivity of a sample before (σ') and after breaking the cells by warming (σ''), and by using the relation $\sigma_i = (\sigma'' - \sigma') / Vd$, where V is the average volume of the cell and d is the sample density. The σ_i values obtained for five different densities of the sample fell in the range 0.25 ± 0.06 mho/m. The real part of the dielectric constant ϵ'_i could not be measured; we therefore ran the program for many values of ϵ'_i . We found that for ϵ'_i in the range 2-10, theoretical inferences are in close agreement with the experimental data.

TABLE I
TRANSITION FREQUENCIES AND
MEDIUM CONDUCTIVITY

| σ_e | Transition frequency |
|----------------------|----------------------|
| <i>mho/m</i> | <i>MHz</i> |
| 2×10^{-4} | 9 |
| 10^{-3} | 9.5 |
| 10^{-2} | 14.5 |
| 2.5×10^{-2} | 21 |
| 4×10^{-2} | 29.5 |

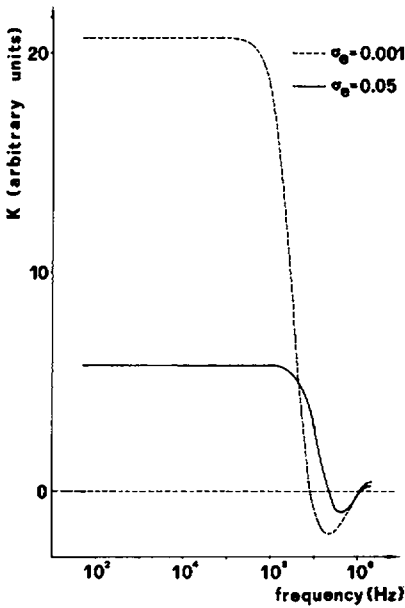


FIGURE 3

FIGURE 3 $K(\omega)$ function for the two values of the conductivity of the medium, σ_e . The other parameter values are $n = 0.04$, $\epsilon'_e = 80$, $\epsilon'_i = 4$, $\sigma_i = 0.3$.

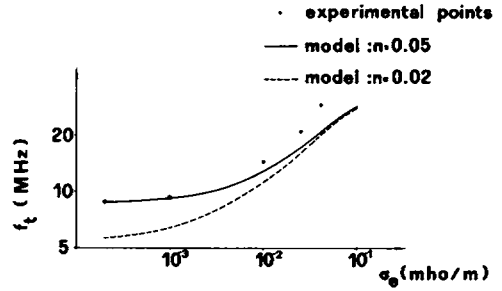


FIGURE 4

FIGURE 4 Theoretical and experimental dependence of the transition frequency on the conductivity of the medium σ_e . The theoretical curves correspond to the values of n shown; other parameters are as in Fig. 3.

The function $K(\omega)$ is plotted in Fig. 3 for two values of the electrical conductivity of the suspending medium. It appears that the transition frequency lies near 8 MHz for the lower conductivity value and that it increases with increasing conductivity, in agreement with the experimental findings.

The model also predicts a transition, above 100 MHz, from transverse to parallel orientation; this, too, was observed experimentally by Griffin and Stowell (1966). We note that the function $|K(\omega)|$ has lower values at the higher conductivity, which means that it becomes more and more difficult to measure the transition frequency as the conductivity rises; moreover, the function values for $K < 0$ (transverse orientation) are lower than those for $K > 0$ (parallel orientation), which explains why it is difficult to obtain good transverse orientation at high conductivities. Fig. 4 shows the transition frequency vs. the external medium conductivity σ_e for two values of the shape factor n . In agreement with our experimental observations, higher transition frequencies correspond to less elongated *Euglenas*; furthermore, the separation between the two curves could account for the experimental finding that within the transition range some cells are oriented in a parallel manner and some perpendicularly. Fig. 4 also shows the experimental data of Table I. Considering the large error affecting the transition frequency estimates, there is good agreement with the theoretic-

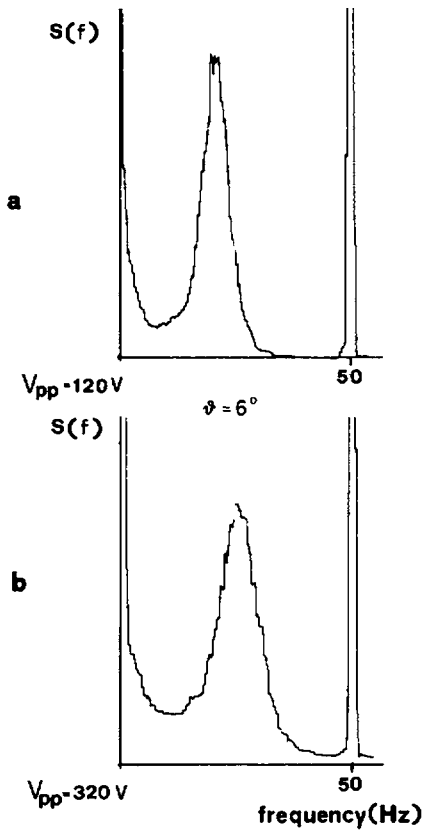


FIGURE 5

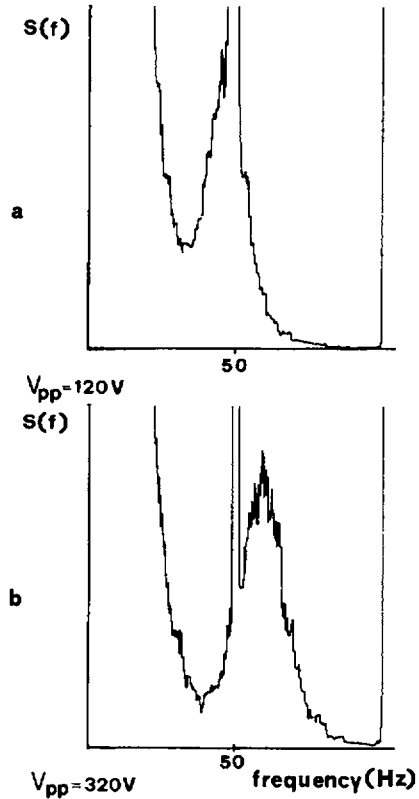


FIGURE 6

FIGURE 5 (a and b) Spectra showing the rise in translation velocity as field intensity rises. Heterodyne detection at $\vartheta = 6^\circ$. The swimming direction, at right angles to the laser beam, lies in the scattering plane.

FIGURE 6 (a and b) Spectra showing the rise in the flagellum beating frequency as field intensity rises. Homodyne detection at $\vartheta = 6^\circ$. The swimming direction is perpendicular to the scattering plane.

cal curve for $n = 0.05$. This value corresponds to the less elongated *Euglenas* ($a/b = 5$).

EFFECT OF THE FIELD INTENSITY ON THE MOTION PARAMETERS OF *EUGLENA*

Observations on the dependence of the *Euglena gracilis* (*E.g.*) speed on the orienting radiofrequency field intensity are reported in the literature (Wolken, 1967). We have performed the first quantitative measurements of the motion parameters of *E.g.* cells at several field intensities by the laser beating technique (Ascoli, 1978). The cells were parallelly oriented by a 2-MHz radiofrequency field. Figs. 5-7 report low frequency spectra which show the rise in translation velocity, body rotation, and flagellum beat-

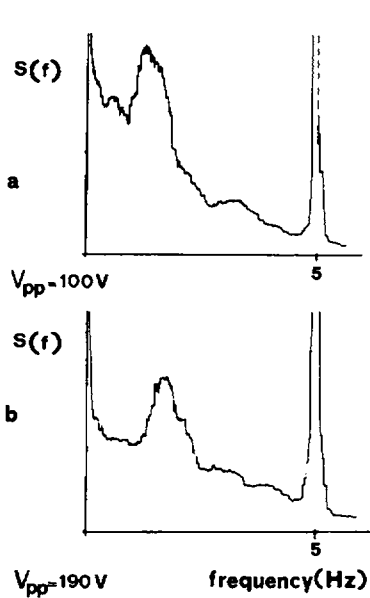


FIGURE 7

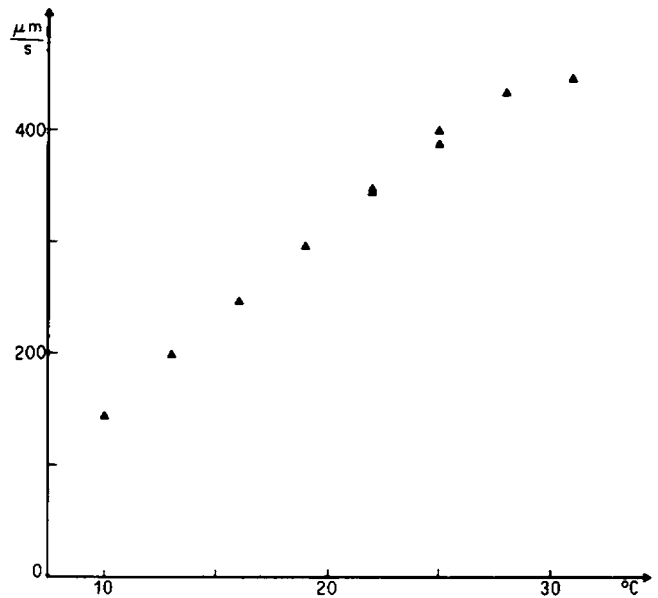


FIGURE 8

FIGURE 7 (a and b) Spectra showing the rise in body rotation frequency as field intensity rises. Homodyne detection at $\vartheta = 6^\circ$. The swimming direction is perpendicular to the scattering plane. Frequency marker at 5 Hz.

FIGURE 8 Mean speed of *Euglenas* vs. temperature. The voltage applied to the electrodes was 200 V.

ing frequency as electrical field intensity rises. The spectral line shown in Fig. 5 is homologous to the speed distribution.² The speed values can be obtained by a scale change, using the relation $f = (v/\lambda) \sin \vartheta$, where λ is the wavelength of the laser light and ϑ is the observation angle. In Fig. 4a the applied field is the lowest necessary to orientate the cells. The most probable speed (corresponding to the curve maximum) is $124 \mu\text{m/s}$; this speed shows only small changes for hours if the field remains constant. The curve reported in Fig. 5b was obtained for the highest field intensity that did not cause chain formation; the most probable speed rose to $155 \mu\text{m/s}$. The increase in speed occurs quickly (in about 1 s) and is reversible. The lines reported in Fig. 6 represent the flagellar beating frequency distributions for the voltages shown; as the field increased, the most probable frequency rose from 47 to 59 Hz. Fig. 7 refers to another sample and shows a change in body rotation from 1.4 to 1.8 Hz. These spectral lines, which are independent of the observation angle, correspond to intensity modulation of the scattered light dependent on the periodicity of cell motion. The percentage increase is about the same for all motion parameters.

As the field intensity rises, all the motion parameters increase—a phenomenon sug-

²All the lines shown in the figures are superimposed on a zero frequency band due to various effects (Ascoli et al., 1978).

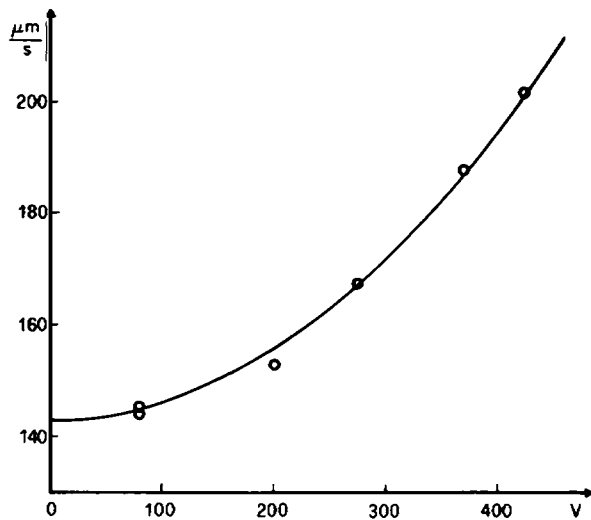


FIGURE 9 Mean speed vs. applied voltage. The temperature of the electrodes was kept at 16°C.

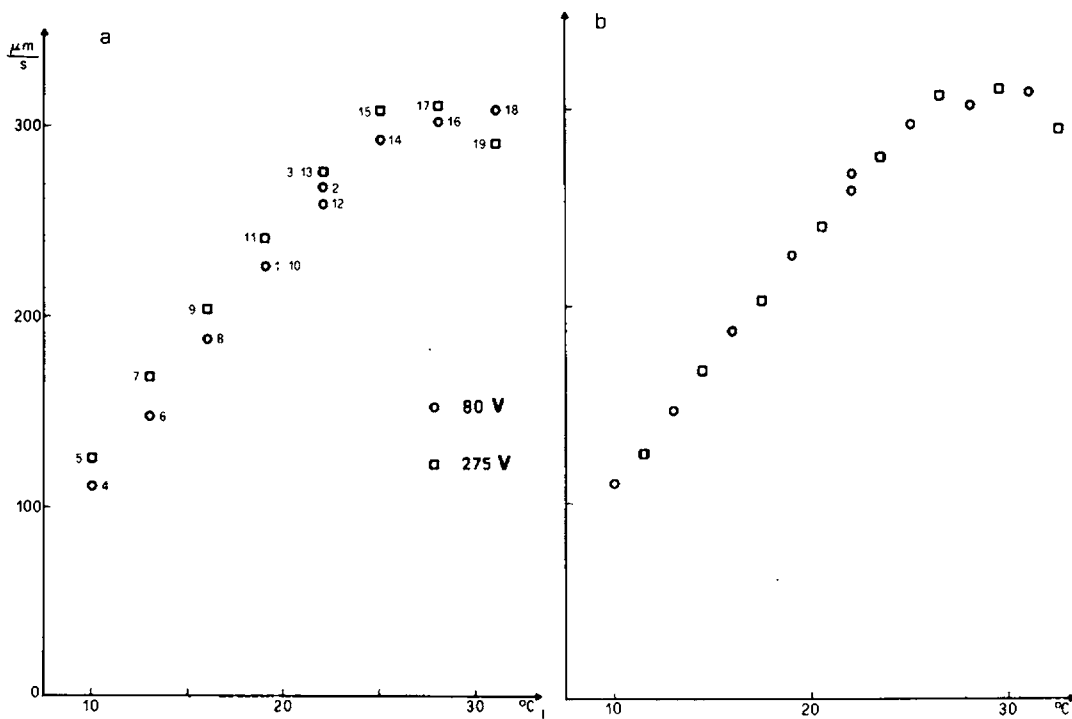


FIGURE 10 (a) Mean speed vs. temperature for the values shown of applied voltage in the same sample; (b) the same after the displacement described in the text. The numbers near the points in *a* represent the time sequence of the measurements.

gesting that the energy supplied to the motor activity of the cell is increasing too. An increase in the temperature of the sample due to the electromagnetic energy dissipation could account for this effect. On this hypothesis the temperature increase causes an increase in the motor activity of the cells. The changes in the *E.g.* speed with sample temperature observed by Lee (1954) support this view.

To test this hypothesis we have performed two kinds of experiment, measuring the mean speed of the sample as a function of the temperature and of the applied radiofrequency voltage. In Fig. 8 we report the mean speed vs. the temperature for an applied radiofrequency voltage of 80 V. In the 10–25°C temperature range the mean speed appears to depend linearly on the temperature. In Fig. 9 we report the mean speed vs. the applied radiofrequency voltage for a constant temperature of 13°C. The experimental points show a good fit against a parabola; this happened with all the samples where measurements were performed. The quadratic trend of the speed with the field intensity plus the linear trend with temperature confirm that the electrokinetic effect is related to electromagnetic energy dissipation in the sample. A further check of this hypothesis is given in Fig. 10, where we report the mean speed vs. the temperature for two values of the applied radiofrequency voltage. In Fig. 10*b* the points corresponding to the higher voltage were shifted by 1.5°C toward high temperatures. After this displacement the two groups of points show good alignment along the same curve, so demonstrating that an increase in field intensity brings about an increase in the sample temperature with respect to the environment.

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