Cell fusion by spark discharge and its relevance for evolutionary processes

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Electrofusion of mesophyll protoplasts of *Avena sativa* was induced by using electromagnetic coupling of energy including that from a spark discharge. The results suggest that electrofusion may have played an important role in the evolutionary process and that new species arose in this way by electromagnetic waves emitted by lightning in thunderstorms. If cell fusion had occurred in prehistory by the means described here, then the normally accepted gradual process of evolution could be leap-frogged. This would explain the existence of steps in the fossil record.

Electrofusion Spark discharge Electromagnetic waves Evolution Membrane Protoplast

1. INTRODUCTION

Electric-field-mediated fusion of cells is now a well-established technique. The results obtained with electrofusion have been extensively reviewed [1–6], and suitable apparatus is now commercially available (GCA Corp., Precision Scientific Group, Chicago, IL and Krüss GmbH, Hamburg). The electrofusion technique is based on the establishment of intimate membrane contact by dielectrophoresis and subsequent reversible electrical breakdown in the membrane contact zone by the application of a field pulse of high intensity. Until now, dielectrophoresis was achieved with the aid of a slightly inhomogeneous alternating field by connecting two parallel wire electrodes to a frequency generator and pipetting the cells into the electrode gap [7]. Electrical membrane breakdown was generated by switching in a pulse generator instead of the frequency generator.

We here report on electrofusion of cells using electromagnetic coupling of energy including that from a spark discharge. For dielectrophoresis the electrode chamber in which the fusion process was to be carried out was placed at a distance of 5 m (with no connecting wires) from the emitter of the electromagnetic waves. For fusion a distance of 10 cm from the spark discharge was used. We were able to demonstrate that fusion occurs under these generator conditions and when ore-containing pieces of rocks are used instead of the two electrodes. On the basis of these results we postulate that electrofusion may have represented an important step in the evolutionary process and that new species arose in this way by means of electromagnetic waves emitted by lightning in thunderstorms and the sun [8].

2. MATERIALS AND METHODS

The experimental arrangement is shown schematically in fig.1. The fusion chamber consists of two cylindrical Pt electrodes glued on to a microslide in parallel at a distance of 150 μm apart. The electrodes were each connected to a 50 cm long wire. One of the wires was grounded, while the other served as a receiving aerial. Pearl-chain formation of cells (dielectrophoresis) was achieved by electromagnetic waves using a frequency generator (Toellner Electronic, M. Werner, Frankfurt) which was connected to a vertical rod (about 2 m long) as a transmitting aerial (fig.1a). The frequency of the emitted electromagnetic waves was 1 MHz. The amplitude was chosen such that the field strength induced between the electrodes of the fusion chamber was high enough to

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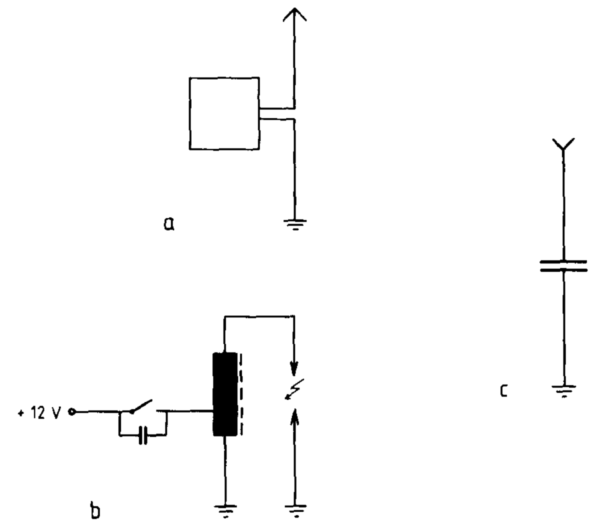

Fig.1. Experimental arrangement for cell fusion using electromagnetic waves. (a) Frequency generator connected to a transmitting antenna for dielectrophoresis. (b) High voltage coil for producing a spark discharge, i.e., for transmitting the breakdown pulse leading to fusion. (c) Fusion chamber viewed as an antenna which is capable of receiving electromagnetic waves.

achieve dielectrophoresis (about $1-10\ \mathrm{V \cdot cm^{-1}}$). The distance between the aerial (acting as an emitter) and the fusion chamber was about 5 m. Breakdown in the intimate membrane contact zone of at least two dielectrophoretically collected cells was achieved by a spark discharge separated from the fusion chamber by a distance of about 10 cm. The spark discharge was generated by a spark coil (Bosch, FRG), and the spark gap was about 1 cm. During spark discharge electromagnetic waves are generated by the rapid changes in current forming the discharge, and these waves lead to damped electrical oscillations at the fusion chamber electrodes. The wires leading to the spark gap were each about 5 cm long. The voltages were measured with an oscilloscope (Tektronix 7834 Storage Oscilloscope).

3. RESULTS

Spark discharges gave rise to damped sinusoidal wave trains in the fusion chamber with an initial amplitude of about 10 V. The main frequencies range between 2.5 and 20 kHz, i.e., the length of the sine half-wave is between 25 and 200 μs. This range corresponds approximately to the lengths of the square pulses used in the conventional electrofusion procedure (3–60 μs). Higher frequencies (up to at least 100 MHz, corresponding to a pulse length of 5 ns) also occur in spark discharges, but these were either very weak or were not detectable by the oscilloscope. The existence of these high frequencies could easily be demonstrated with the aid of a radio; noise was heard over the entire frequency range (VHF, SW and MW).

It is important, particularly in view of the significance of this experiment for evolutionary processes, that the voltage induced in the chamber is solely dependent on the electrical conductivity and the relative dielectric constant of the solution. This can easily be demonstrated experimentally by, for example, removing the wire from the electrode chamber which represents the grounded part and using ground as the counter-electrode. This procedure does not alter the voltage measured between the electrodes. It is also relatively easy to show theoretically that the induced voltage is dependent only on the specific conductivity and the relative dielectric constant of the solution.

The equation for a damped oscillation (fig.2) of a given frequency is given by:

$$U = U_o \cdot e^{-t/R \cdot C} \cdot \sin\omega t \tag{1}$$

where U_o is the amplitude of the undamped oscillation and depends on the coupling coefficient of the wires, $\omega = 2\pi\nu = 2\pi/\tau$ is the angular frequency with ν = frequency and τ = time of a sinus period, respectively. If the used Pt electrode can be roughly compared to two parallel plates, the resistance R is given by:

$$R = \frac{d}{A \cdot \sigma_{sp}} \tag{2}$$

where σ_{sp} = specific conductivity of the medium between the electrodes, A = cross-section of the chamber and d = electrode distance. The capacitance C is given by:

$$C = \frac{\epsilon_o \cdot \epsilon_r \cdot A}{d} \tag{3}$$

where ϵ_o is the permittivity of the vacuum and ϵ_r the relative dielectric constant. Introduction of eqs

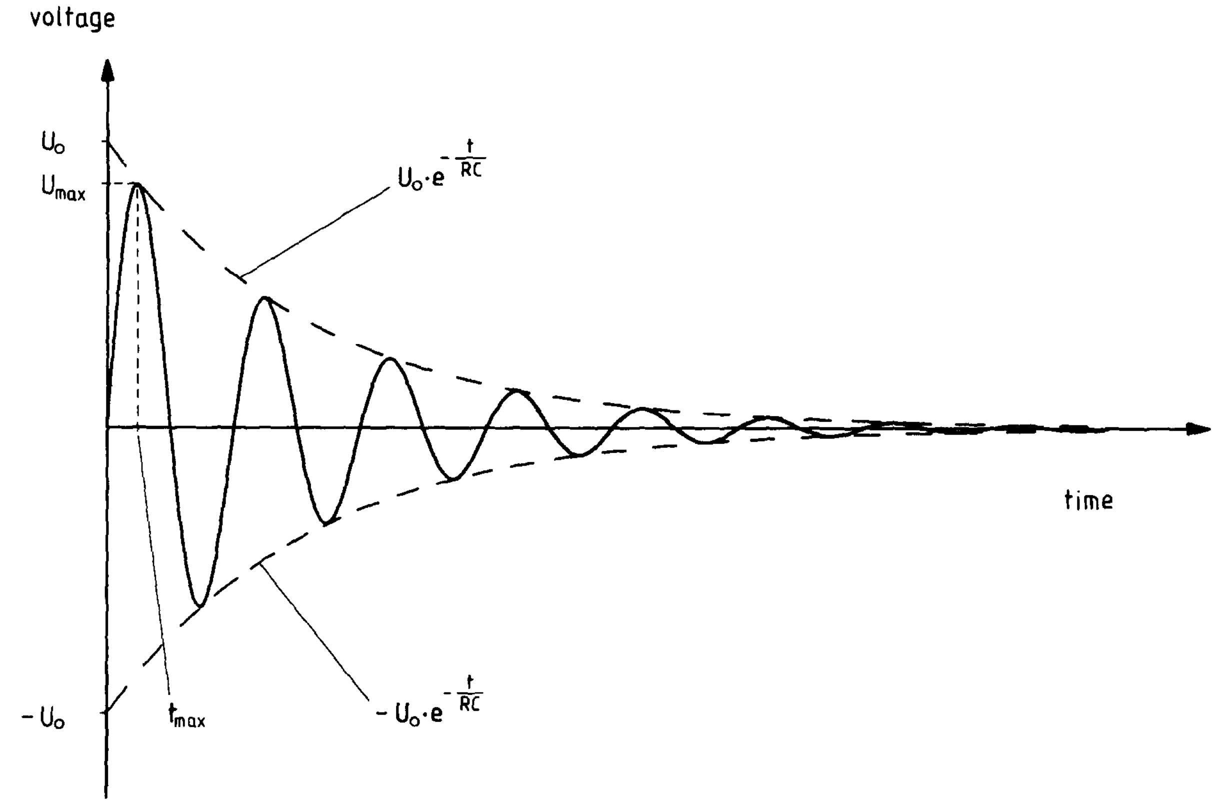

Fig.2. Time course of a damped oscillation. It is evident that for higher frequencies the first maximum of the voltage is reached when $t = \tau/4$.

2 and 3 into eq. 1 gives:

$$U = U_o \cdot e^{-\frac{\sigma_{sp}}{\epsilon_o \cdot \epsilon_r} \cdot t} \cdot \sin\omega t \tag{4}$$

As a rule, the voltage maximum, U_{max}, of the first half-oscillation is crucial for the fusion of cells, since it is only in this range that sufficiently high voltage values are achieved to induce electrical breakdown in the membrane contact zone. However, the subsequent damped oscillations can act in a fusion-stimulating manner because they may prevent the resealing process in the individual membranes and thus promote the establishment of cytoplasmic continuity and membrane bridges between neighbouring cells [3,4].

For higher frequencies or lower conductivities, the voltage maximum in the first half-wave is achieved in time

$$t_{max} \approx \frac{\tau}{4} = \frac{\pi}{2\omega} \tag{5}$$

By substituting eq. 5 into eq. 4 and taking into account that $\sin\pi/2 = 1$, we obtain eq. 6:

$$U_{max} = U_o \cdot e^{-\sigma_{sp}/(4 \cdot \nu \cdot \epsilon_o \cdot \epsilon_r)} \tag{6}$$

Eq. 6 describes the dependence of the voltage maximum of the first sine half-oscillation of the voltages induced by the electromagnetic waves on the specific conductivity and on the frequency. Fig.3,4 show the frequency dependence of the voltage, U_{max}, calculated according to eq. 6, for various conductivities in the electrode gap, and the dependence on the specific conductivity at given frequencies, respectively. The described function agrees with the one measured experimentally with the oscilloscope. It is evident that an increase in the conductivity in the electrode gap leads to a sharp decrease in the induced voltage. The use of distilled water or an 'ion-free' mannitol solution already results in a 95% decrease in the induced voltage with respect to air. This theoretically expected and

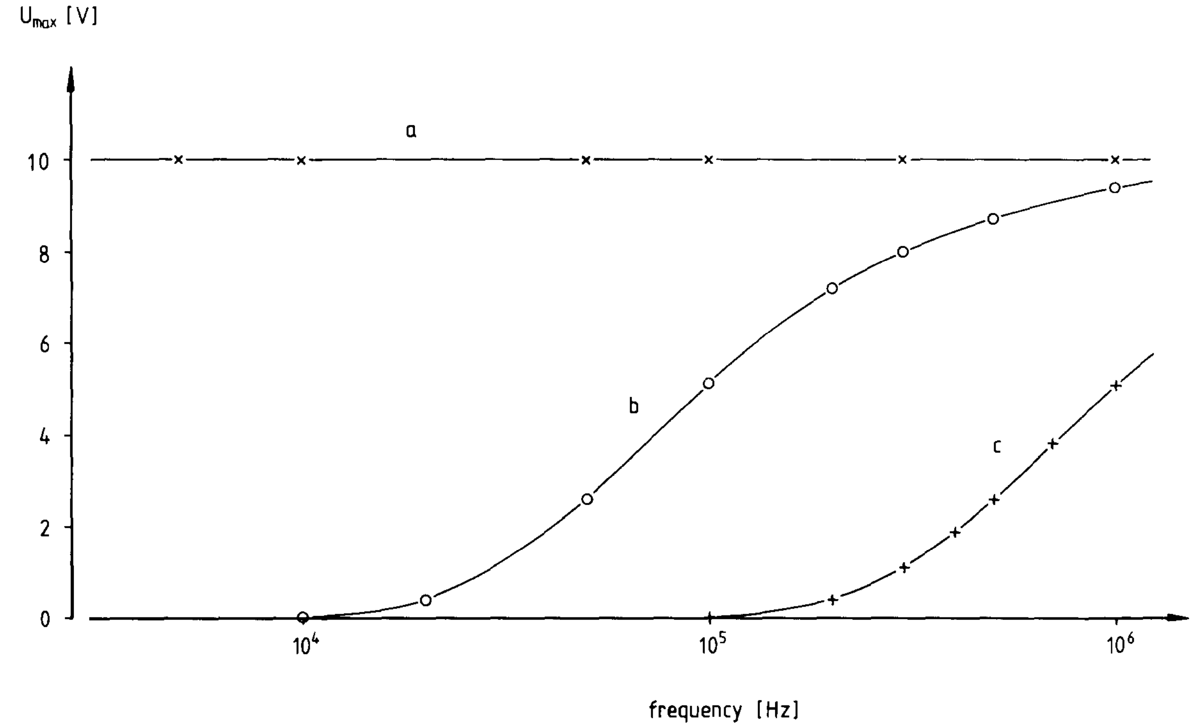

Fig.3. Dependence of U_{max}, the peak voltage of the first half wave of the damped oscillation on the frequency for different conductivities of the contents of the chamber (calculated using eq. 6). (a) $\sigma_{sp} = 2.5 \times 10^{-16}\ \Omega^{-1} \cdot cm^{-1}$, U_o = 10 V, $\epsilon_r = 1$ (air). (b) $\sigma_{sp} = 2 \times 10^{-6}\ \Omega^{-1} \cdot cm^{-1}$, U_o = 10 V, $\epsilon_r = 82$ (dest. water). (c) $\sigma_{sp} = 2 \times 10^{-5}\ \Omega^{-1} \cdot cm^{-1}$, U_o = 10 V, $\epsilon_r = 82$ (0.2 mM NaCl).

experimentally confirmed finding must be taken into account when formulating a hypothesis for the origin of life by electrofusion during the evolutionary process (see below).

With the described experimental arrangement, *Avena sativa* protoplasts [9] incubated in a 0.5 M mannitol solution were aligned in chains by emission of electromagnetic waves and fused by application of the breakdown voltage by spark discharge (fig.5). Fusion was completed after about 2 min with the rounding-off stage. The time taken is comparable with that observed in conventional electrofusion.

In order to confirm the hypothesis that such a mechanism was involved in evolution, the electrodes were replaced by FeS-containing rock samples (electrically conducting). One piece of rock was laid on sand (electrically poorly conducting, i.e., practically insulated from ground), while a second piece at an average distance of 100–200 μm was connected to ground. As predicted by the theory, dielectrophoresis (fig.6) and fusion were also observed under the same field conditions as in the experiments in which parallel Pt electrodes were used.

In this experiment, the pieces of rock were connected to 50 cm long wires. One of the wires was grounded, the other (connected to the piece of rock which was on sand) was free and acted as a receiving antenna. The natural counterpart of the experimental wires could be veins of conducting ore (e.g., FeS, PbS) extending over 50 cm or more.

4. DISCUSSION

The results reported here demonstrate that wireless transmission of electromagnetic energy can lead to electrofusion of cells. From a technical point of view, this method could represent an interesting alternative to the conventional electrofu-

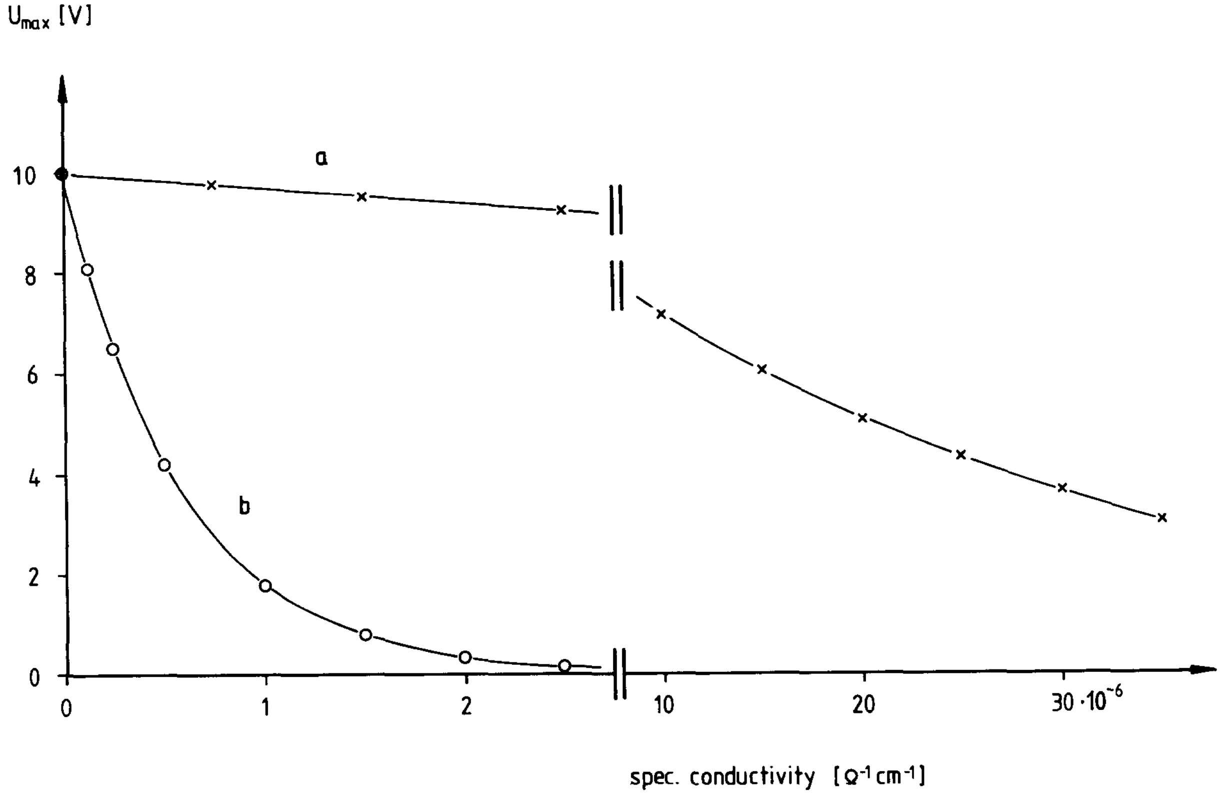

Fig.4. Dependence of U_{max} calculated using eq. 6 on the specific conductivity of the solution between the electrodes for two frequencies. (a) ν = 1 MHz (frequency of the fusion pulse produced by a pulse generator). (b) ν = 20 kHz (frequency of the electromagnetic wave, emitted by the spark discharge).

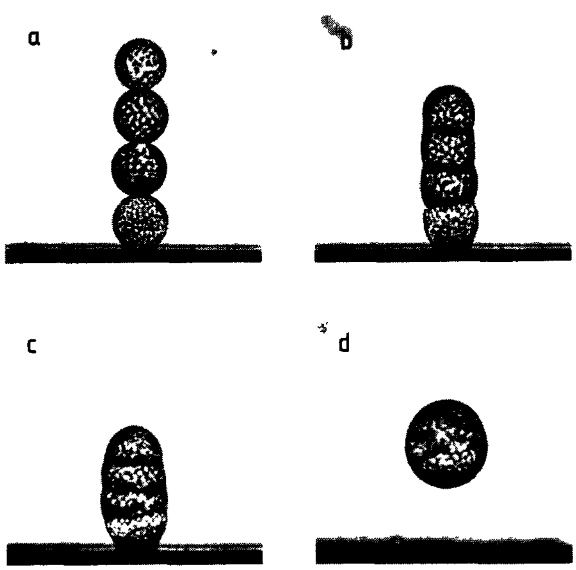

Fig.5. Fusion of mesophyll cell protoplasts of leaves of *Avena sativa* on two parallel Pt electrodes. The cells were suspended in 0.5 M mannitol and induced to form 'pearl chains' by emission of an electromagnetic wave (1 MHz) (a). Fusion was induced by application of the breakdown voltage by spark discharge (b–d).

sion procedure, because, for example, porous glass capillaries or porous hollow fibres filled with electrolyte solution could be used instead of metal electrodes. Such electrodes cannot be used for the conventional application of the alternating field and breakdown pulse, because the resistance of these capillaries is too high compared with that of the metal electrodes, so that it is impossible to establish sufficiently high voltages between the electrodes. The use of electrolyte-filled porous

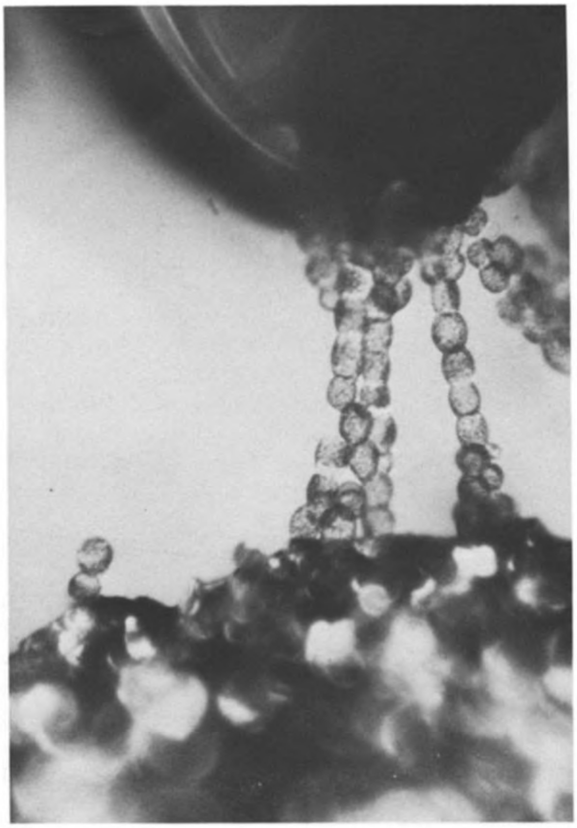

Fig.6. Dielectrophoresis of *Avena sativa* protoplasts between pieces of FeS ore by emission of an electromagnetic wave of 1 MHz frequency. For further information see text.

capillaries for fusion by electromagnetic waves would have the great advantage that fusion would still have to be carried out in weakly conducting solutions but that electrolyte would reach the vicinity of the cells to be fused by diffusion either during or immediately following field application. We now know [10] that it is essential, particularly in the production of hybridoma cells, for ions and fetal calf serum to be added as soon as possible after the onset of fusion. However, the main significance of the experiments described here lies in the possible involvement of electrofusion in evolution.

During the entire evolutionary process the Earth was exposed to electromagnetic waves from the sun which emits waves of all frequencies. The present day atmosphere, which contains regions of ionization (Appleton layer etc.) screens the Earth's surface from frequencies below 10–30 MHz typically. The lowest frequency that may pass decreases with the level of ionization. In earlier times, it is possible that the level of ionization was much lower and allowed even low MHz frequencies to reach the surface.

Furthermore, transient strong electric fields can be produced by lightning in a thunderstorm, and it is interesting to note that there are thought to have been long periods of thunderstorms while evolution was progressing. At 10^8 V potential difference [11], the field strengths are sufficiently high to achieve fusion of cells between metal-containing rocks at an appropriate distance away from a lightning strike, as has been demonstrated here in simulation experiments with spark discharge. The mean duration of a lightning flash is between 1 and 100 μs, i.e., in the range in which electrical breakdown of cell membranes is reversible (without deterioration of the cellular functions and membrane integrity). There is evidence that hybrids which were obtained by electrofusion are viable and capable of division only if the pulse duration of the field did not exceed 20 μs [4]. If the pulse durations are longer, intermingling of the membranes of adhering cells is still observed but the fusion products are dead.

Electrofusion of cells could thus have been an efficient process for producing a multitude of hybrids with new properties from existing unicellular organisms (cells or eggs) within a relatively short period of time in terms of the Earth's history. The emergence of new species could be explained in this way, which is at present impossible on the basis of Darwin's theory. If our hypothesis is correct, the origin of new species would have taken place at the cellular level or at the level of the egg, which would also explain why no missing links have ever been found between the various species. This idea, which has now been supported experimentally for the first time, was indirectly formulated by Koestler in 1978 [12].

The current school of thought cannot provide a straightforward explanation for the origin of new species by point mutation and selection from existing species. However, electrofusion by electromagnetic waves could have led to the origin of new species by the recombination of the genomes, thus allowing new genes to be expressed under these conditions.

Electrofusion leads to a macromutation, a concept which was already discussed at the beginning of this century and which is now being seriously reconsidered [13].

As shown above, the necessary voltages can probably only be established in weakly conducting solutions. We can thus conclude that the creation of new life by electrofusion would have predominantly taken place in freshwater environments.

On the other hand, the field strength of lightning may be sufficient to induce breakdown in more strongly conducting media, provided that the cells adhere to each other. As demonstrated elsewhere, cells can be brought into intimate membrane contact by proteins [including poly(ethylene glycol)] and then fused with a breakdown pulse. This may be an alternative way of electrofusion in nature.

Further experiments are undoubtedly necessary in order to lend more support to the hypotheses described here. However, results presented here should give new impetus to the discussion of the origin of life.

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