ELECTROFUSION AND ELECTROPERMEABILIZATION OF CELLS

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INTRODUCTION

Biotechnology, computerscience and microelectronics are the new key technologies of the present day. Modern biotechnology is expected to solve the central problems of the human race such as sufficient supplies of energy, raw materials, food and medicine as well as the removal of pollution in our waterways, and all this in a manner that will conserve the environment (Dohmen, 1983).

The technical possibility of changing the properties of cells at the genetic level (genetic engineering) has led (or can lead) to the development of microbial strains with increased, changed or novel metabolic capabilities, to the production of microbial or animal strains for the manufacture of pharmaceuticals (e.g. monoclonal antibodies) and to the breeding of new plants which give higher yields or are resistant to diseases or saltwater. In principle, the genetic information of an organism can be changed in vitro by two complementary techniques: somatic hybridization with the aid of cell fusion and direct gene (plasmid) transfer. The conventional fusion and gene transfer techniques which largely use chemicals or inactivated virus are not always very efficient and are partly founded on an empirical basis. The development of electrical methods for in vitro cell fusion and for DNA (and protein) transfer across biological membranes may well represent an interesting alternative to the conventional methods since they allow the process of fusion and gene transfer to be monitored by optical means and to be governed by physical laws. The large scale application of these methods has thus moved into the realms of possibility (Zimmermann, 1982).

Electrofusion of cells and electrically induced movement of low molecular weight substances and macromolecules through membranes is based on the so-called reversible electrical breakdown of the cell membrane which leads to a reversible, controllable electropermeabilization of the cell membrane.

Reversible electrical breakdown was discovered by accident in 1973 (Zimmermann

et al., 1973) and contradicted the then current school of thought that high electrical fields irreversibly destroyed cells (Zimmermann, 1982). A number of scientists (Neumann and Rosenheck, 1972; Tsong and Kingsley, 1975) initially disputed the existence of such electric field effects in biological membranes and only later confirmed (Linder et al., 1977; Kinosita et al., 1978) that it is possible to make the cell membrane permeable for prolonged periods of time under reversible conditions, in order to manipulate the intracellular pool of cells by exchange with macromolecules.

A historical overview of the development of these methods can be found in Zimmermann's review (Zimmermann, 1982). A number of review articles (Zimmermann et al., 1976a; 1980; 1981; 1984a; 1984b; 1985; Zimmermann, 1982; 1983a; 1983b; Zimmermann and Vienken, 1982; Arnold and Zimmermann, 1983) have appeared on this subject, so that the authors of this review will concentrate only on the fundamental aspects of these methods in order to devote more attention to the latest developments.

ELECTRICAL PERMEABILIZATION OF THE CELL MEMBRANE

From an electrical point of view, the cell membrane represents a capacitor. Therefore, if cells are exposed to an electrical field or to an electrical field pulse, the cell membrane capacitor becomes charged by way of electrical charge separation. At a given cell radius the induced voltage is proportional to the external electrical field strength (Fig. 1), provided that the field is applied for a long enough period of time for the equilibrium potential to be established (Zimmermann, 1982; Arnold and Zimmermann, 1983). In the case of short field pulses this is not always the case, particularly if the cells are suspended in weakly conductive solutions. This is because the relaxation time for building up a voltage across the membrane depends not only on the membrane resistance and capacitance but also on the internal and external conductivity of the solutions on either side of the membrane (Zimmermann, 1982; Arnold and Zimmermann, 1983). The induced membrane potential is superimposed on the normal resting potential across the cell membrane. The resting potential is made up of ion diffusion potentials, potentials arising from so-called electrogenic pumps in the membrane, and surface potentials resulting from the net negative surface charge of the membrane.

The total potential (or field) in the membrane determines the breakdown behaviour of the cell (Fig. 2). The breakdown voltage of the membrane capacitor which is in the order of 1 V is reached at an external field strength of a kV/cm (depending on the cell radius, see Fig. 1). The membrane breaks down locally, and structural changes in the membrane lead to an increase in the electrical conductivity and permeability of the membrane. These permeability changes are reversible, i.e. the structural changes in the membrane can be reversed — as in the case of self-healing capacitors. The cell membrane regains its original high electrical resistance and its impermeability. For this reason, this phenomenon was termed reversible electrical breakdown by Zimmermann et al. (1973, 1974a), as opposed to irreversible destruction of cells by electrical fields. If, when considering these reversible electrical field effects, more emphasis were to be placed on the secondary induced process, i.e. the increase in the permeability of the cell membrane, then the term "electropermeabilization" would be a more accurate descrip-

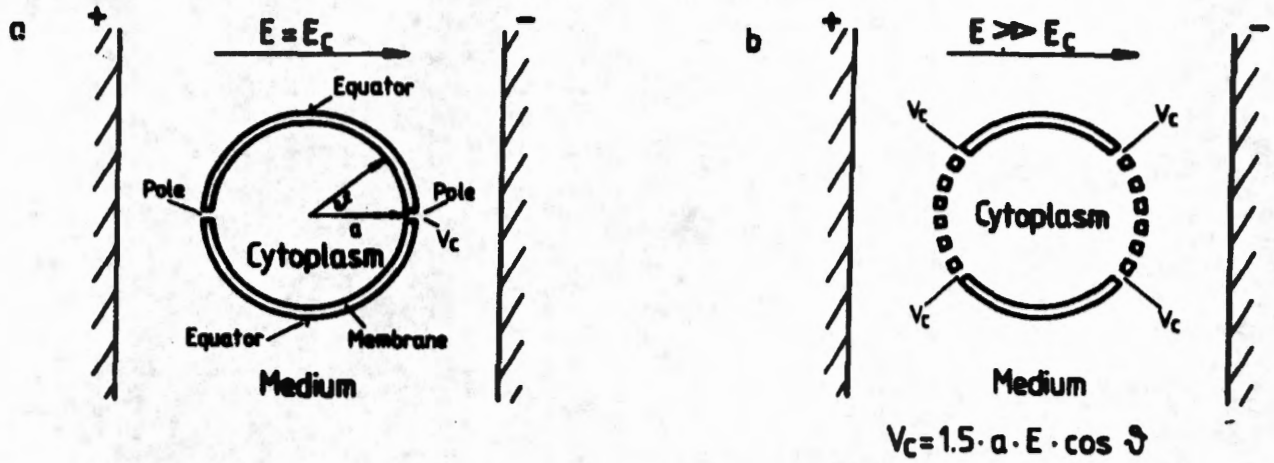

Figure 1. Schematic representation of the permeabilization of a cell in an electrical field. At a given radius, a, the induced membrane potential and hence the breakdown voltage, V_c, are proportional to the external field strength, E, (see potential equation below). At the critical external field strength, E_c, electrical breakdown of the membrane occurs at the cell "poles" (i.e. in field direction) because of the dependence on the angle (cosine term). At supercritical field strengths, $E > E_c$, the breakdown voltage is also reached in areas where $\vartheta > 0$ (represented by the formation of pores). The induced membrane potential is always equal to zero at the cell "equator", i.e. at 90° to the field direction.

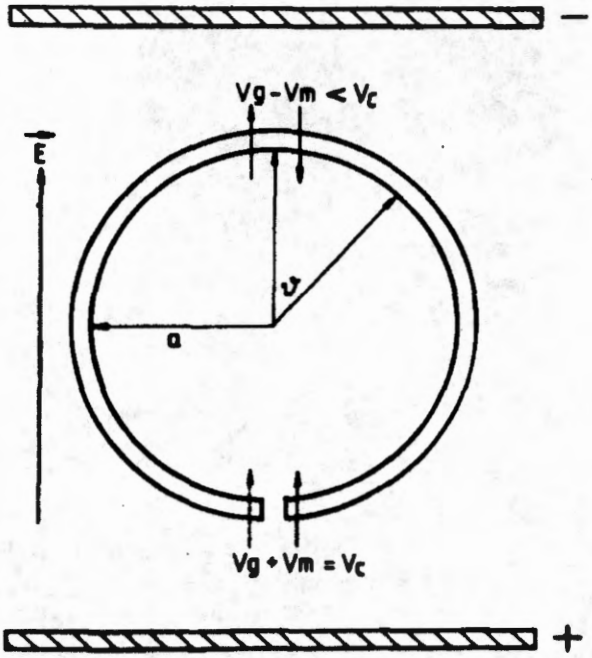

Figure 2. In contrast to Fig. 1, it is assumed that there is a resting potential, V_m, across the membrane (with the cell interior being negative with respect to the external solution) in the absence of an external electrical field. Under the simplified conditions described here (see text), the induced potential, V_g, is superimposed in parallel with the existing resting potential in the membrane hemisphere facing the anode, whereas on the cathode side superimposition of the two potentials is antiparallel. At the critical field strength, E_c, breakdown is therefore only observed on the anode side where $V_m + V_g = V_c$.

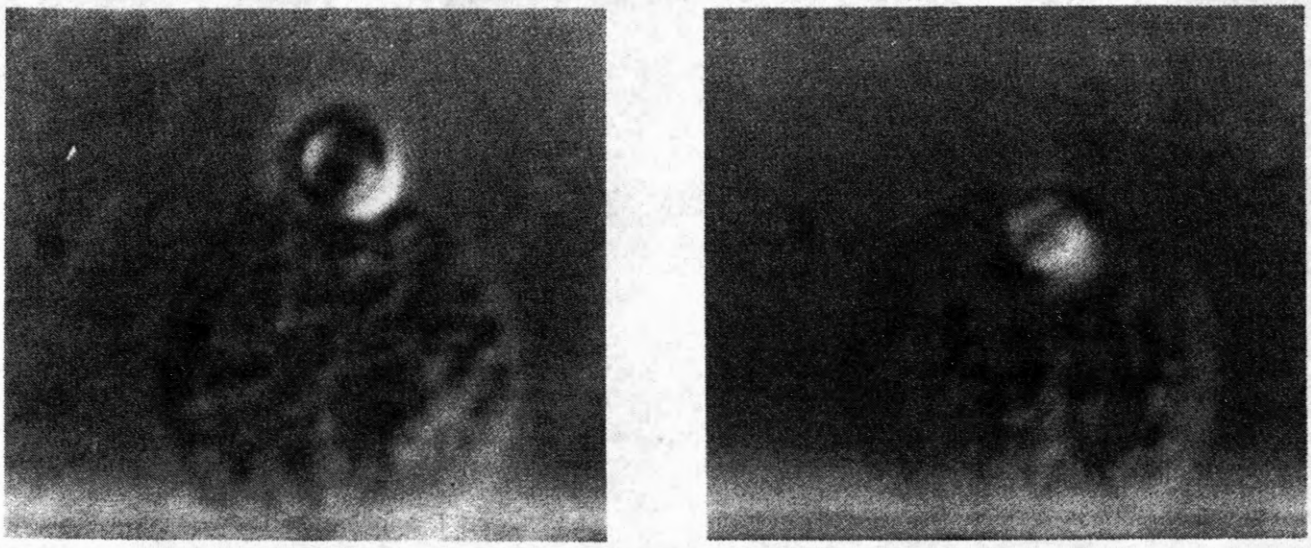

Figure 3. Field-induced uptake of an intact mouse lymphocyte by a so-called Friend cell. The lymphocyte and the Friend cell were first brought into close membrane contact with the aid of dielectrophoresis (inhomogeneous electrical alternating field, 1 MHz frequency and 200 V/cm field strength). A field pulse of 20 μs duration and 3.5 kV/cm field strength was then applied. At this field strength the breakdown voltage of the membrane of the large Friend cell is reached, but not that of the smaller lymphocyte. As a result, field-induced uptake is observed (photographed 30 seconds after the field pulse), but not fusion. In this experiment the cells were incubated in an isotonic mannitol solution.

tion. Both terms are non-committal about the molecular processes occurring during electrical breakdown. On the other hand, the term "electroporation", recently introduced by Neumann et al. (1982) for the description of the same effect, implies that pores are formed as a result of an electrical breakdown. Although the processes occuring during electrical breakdown can be well described mathematically by the formation of pores (Abidor et al., 1979; Petrov et al., 1980; Dimitrov and Jain, 1984), there is now a certain amount of evidence which cannot be reconciled with electrical pore formation (Zimmermann et al., 1982; Dressler et al., 1983; Vienken et al., 1983a; Zimmerman, 1983b; Sowers, 1984; Zimmermann et al., 1984b) Fig. 3 shows that smaller cells can be sequestered across the membrane of a large host cell with the aid of electrical breakdown, without causing irreversible damage to the host cell. If the pore model were correct, pores with a diameter of 2 μm or more would have to be assumed. With such large pores the cell membrane of the host cell would tear because of the membrane tension. The findings of Sowers (1984) and Vienken et al. (1983a) that the structural changes induced locally in the membrane of cells in close contact during electrofusion are not necessarily in the correct orientation to each other suggests that there is no pore formation.

It thus seems sensible not to use terms that imply a hypothetical mechanism.

It can be shown theoretically (Jeltsch and Zimmermann, 1979) that at a given external field strength, the breakdown voltage depends both on the cell radius and on

the orientation of any given membrane site on the cell surface in relation to the field direction (determined by the cosine term in the potential equation, Fig. 1).

This means that at a given cell radius, the induced voltage across the membrane, and hence the electrical breakdown, is dependent on angle. The membrane voltage is thus greatest in field direction and always equal to zero at sites oriented at an angle of 90° to the field direction. A successive increase in the external field strength thus initially leads to an electrical breakdown of the membrane in field direction and then — at higher field strength — in membrane areas oriented at a certain angle to the field direction. This angle dependence of the breakdown voltage is clearly illustrated in Fig. 1 by the formation of corresponding "pores" in the membrane.

The permeability increase of the membrane which is observed at supercritical field strengths and which is utilized for electrically induced transfer of macromolecules, is attributable to the fact that more and more reversible disruptions are induced in the entire membrane surface, and that the membrane perturbations progressively increase in field direction. Recent studies (Farkas et al., 1980; Mehrle et al., 1985; Stopper and Zimmermann, in preparation) have shown that an asymmetry of the electrical breakdown in the two hemispheres of the cell has to be taken into consideration in addition to the dependence of the breakdown voltage on radius and angle. Since the total potential determines breakdown behaviour, the breakdown voltage is first reached for membrane areas in the cell hemisphere where the resting potential has the same direction as the induced potential — and only at higher field strengths in the opposite hemisphere where the two potentials are antiparallel (Fig. 2). Since the natural electric field in the membrane is also influenced by the surface charges, we can expect this asymmetrical dependence of the breakdown to be governed by the net surface charge on the membrane. This can be changed both by enzymes with a proteolytic effect and by the ionic strength of the solution. This prediction has now been confirmed experimentally (Stopper and Zimmermann, in preparation).

Figure 4 illustrates studies of electrically induced uptake of a fluorescent dye into mouse myeloma cells. In solutions of low ionic strength, e.g. isotonic sugar solutions such as they are used in the conventional electrofusion method, the dye is preferentially taken up by the hemisphere facing the anode. At higher ionic strengths (isotonic sugar solutions containing about 45 mM electrolytes such as NaCl or KCl), symmetric uptake is observed, whereas in isotonic sugar solutions containing about 70 mM electrolyte, asymmetric uptake is again observed, however this time by the hemisphere facing the cathode. The symmetrical uptake observed at medium ionic strength can be explained theoretically by the assumption that the natural field in the membrane equals zero. Enzymic pretreatment of the cells with pronase leads to a more marked asymmetric uptake in non-conducting and conducting solutions.

This experimental finding shows that both the ionic strength of the solution and the enzymic pretreatment of cells have a decisive effect on the yield of electrically mediated transfer of substances across membranes in electropermeabilization experiments.

While electrofusion can be carried out at room temperature, electropermeabilization should be, if possible, carried out at lower temperature (Zimmermann et al., 1974b; 1975; 1976a; 1976b; 1980) and not at 20 °C (Neumann et al., 1982). The reason for this is that the lifespan of the electrically induced membrane permeabilization has to be

sufficiently long to enable an efficient exchange of materials to take place between cell and medium (Zimmermann et al., 1976a; 1981). At higher temperatures (i.e. at room temperature and especially at 37 °C) the resealing processes in the membrane are so rapid that sufficient transport of materials across the permeabilized membrane can no longer take place (Zimmermann et al., 1980; 1981).

We should mention that this resealing process in the membrane of individual cells cannot apparently take place in electrofusion because a cytoplasmic continuity is established between the two cells immediately after breakdown has occurred in the contact zone between adjacent cells (Zimmermann, 1982).

When performing breakdown experiments at low temperature, it should be borne in mind that the electric breakdown is strongly temperature-dependent. At about 4 °C the breakdown voltage is in the order of 2 V (Coster and Zimmermann, 1975; Benz et al., 1979), and correspondingly higher field strengths have to be applied. Below 4 °C there is an extreme increase in the breakdown voltage and, as measurements on giant algal cells (Coster and Zimmermann, 1975) and artificial planar lipid membranes (Benz et al., 1979) have shown, the changes in the membrane become irreversible. This as well as the temperature dependence of the breakdown voltage were completely overlooked by Potter et al. (1984) so that the low yield of DNA transfection reported by these authors is quite understandable.

Furthermore, it must be borne in mind with electropermeabilization experiments that there is not only an uptake of materials into the cell after electrical breakdown of the cell membrane but also a more or less marked equilibration between intracellular substances and the external solution. In particular, the exchange between intracellular potassium and extracellular sodium can seriously endanger the viability of the cells (Zimmermann et al., 1980).

For this reason it is necessary to provide increased potassium concentration in the medium used for the field application. Isotonic NaCl solutions such as those used by Neumann et al. (1982) should definitely be avoided. In addition, incubation times at 4 °C after electropermeabilization should be restricted to a few minutes because otherwise the viability of the cells is adversely affected. As we have already pointed out, raising the temperature to 37 °C about 10 minutes after the field application leads to rapid resealing of the induced structural changes in the membrane (Zimmermann et al., 1980; 1981). If these experimental procedures and theoretical considerations are taken into account, foreign substances can be successfully entrapped in cells with high yields, using the electrical field pulse technique developed by us. In the 1970's Zimmermann et

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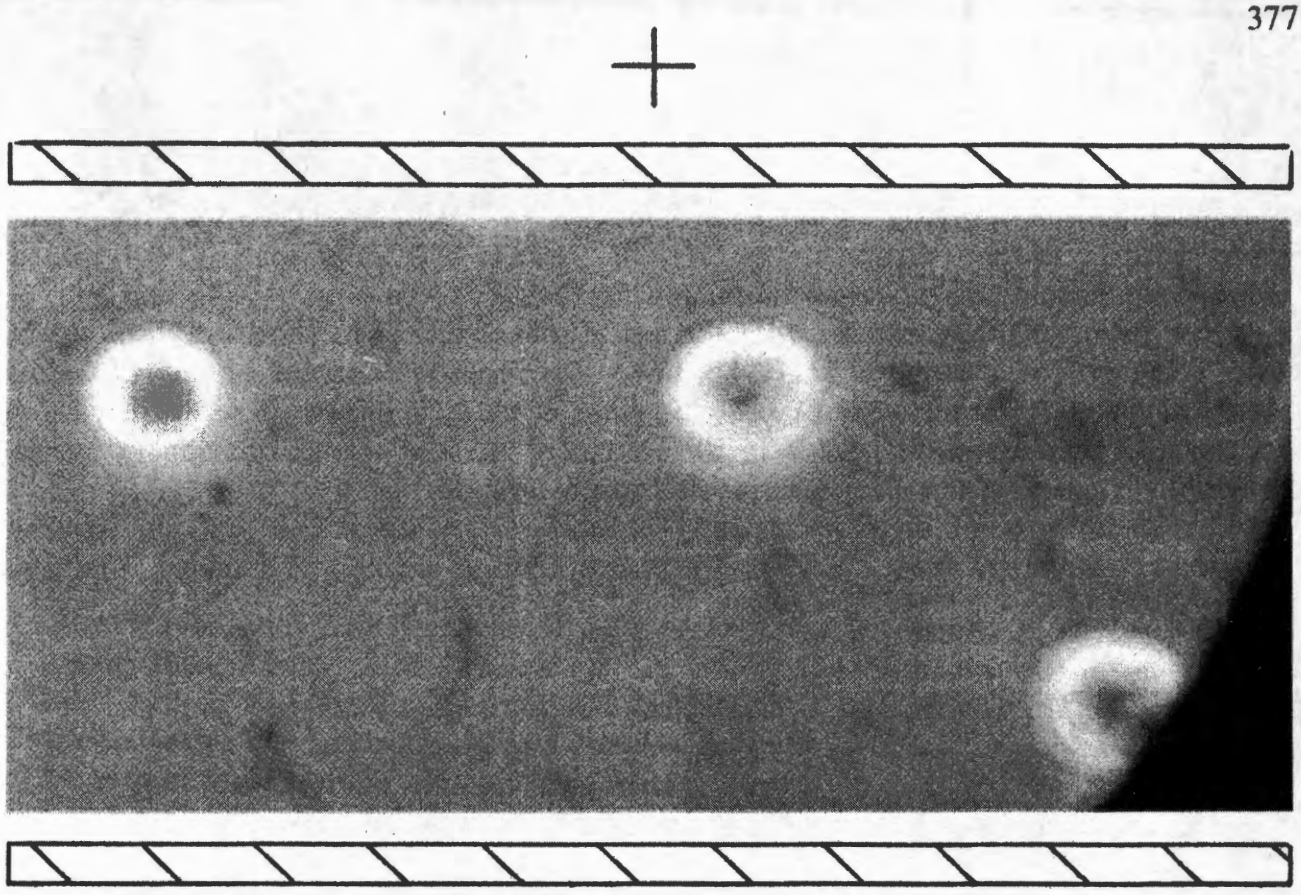
Figure 4. Asymmetric uptake of the fluorescent dye Bisbenzimid by the hemisphere of a mouse myeloma cell facing the anode side (field conditions: three pulses at an interval of 0.5 seconds, field strength 12 kV/cm, 20 μs duration. Incubation conditions: after treatment with pronase, pulses in isotonic sugar solution containing 33mM of both potassium chloride and sodium chloride).
Illustration (a) was taken under a phase contrast microscope (control), illustration (b) under a fluorescence microscope 60 seconds after the application of the field pulse. For further explanations see Fig. 2 and text.



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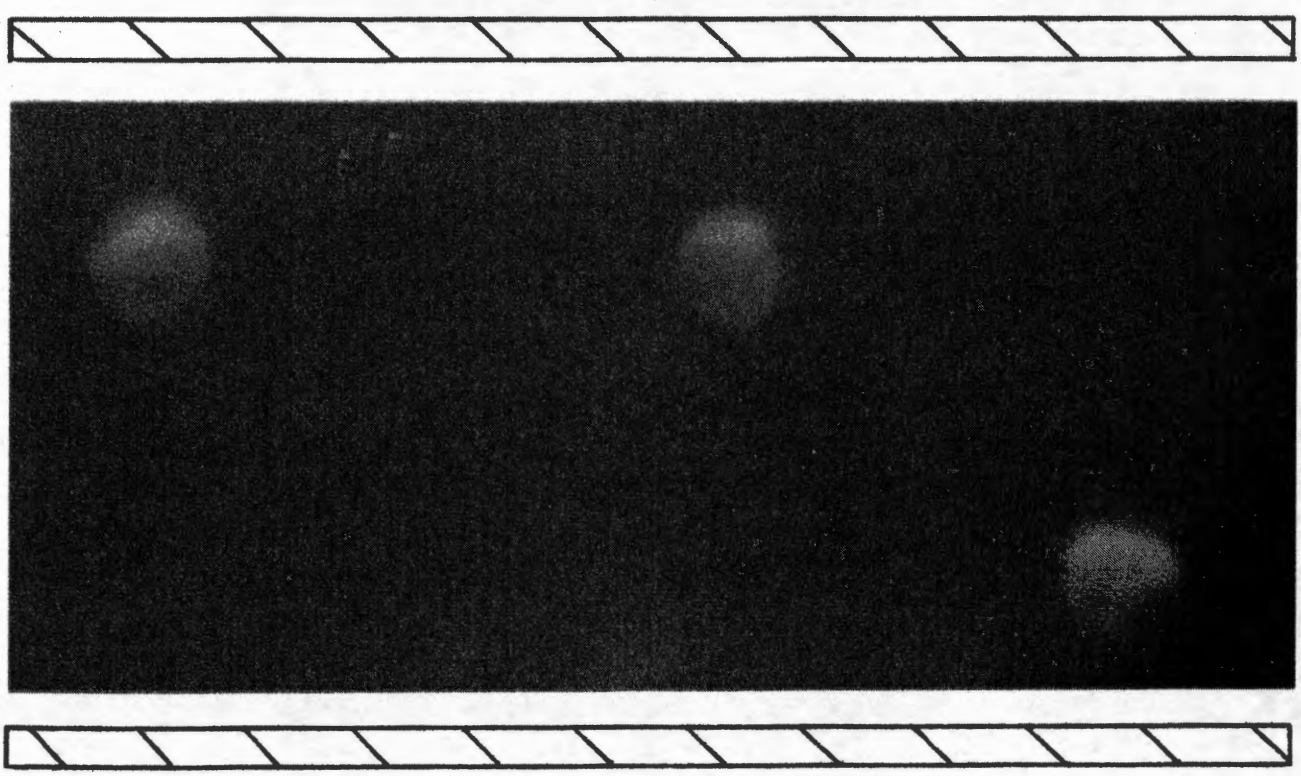

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al. (Zimmermann et al., 1974b; 1975; Zimmermann and Pilwat, 1976; Zimmermann et al., 1976b; Vienken et al., 1978; Zimmermann et al., 1980; Zimmermann, 1983a) and other authors (Auer et al., 1976; Schüssler and Ruhenstroth-Bauer, 1984) reported on the entrapment of dyes (Fig. 5), drugs, proteins, enzymes and latex particles in cells and, more recently, on the entrapment of DNA in yeast cells (Zimmermann, 1982) and of whole cells in host cells (Zimmermann, 1983b; Zimmermann et al., 1984b and Fig. 3). The first proof of DNA transfer with the aid of this electrical method was brought by Auer et al. (1976). Karube et al. (1985) have also recently reported on the successful electrical transformation of yeast cells. Stopper et al. (1985) have developed a DNA transfection protocol which leads to an extremely efficient transformation of animal cells with the aid of the electropermeabilization method.

These authors obtained electrically induced DNA uptake at 4°C in isotonic solutions containing 30 mM KCl and a corresponding amount of inositol. The cells

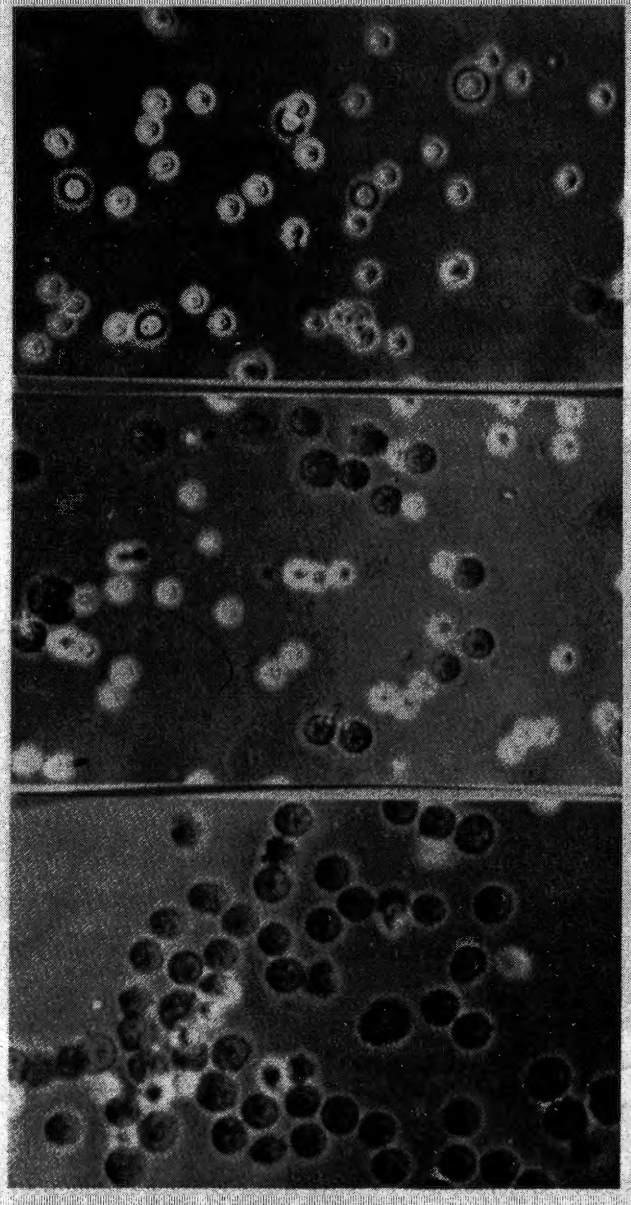


Figure 5. Introduction of the dye Eosin into mouse lymphocytes after pulse application (from top to bottom: 6, 12 and 18 kV/cm field strength, 0.5 μs pulse duration) at 4°C. The dye was added to the isotonic solution before pulse application. The number of lymphocytes, that take up the dye, increases with the field strength.

(mouse L-cells) were pretreated with dispase so that optimal breakdown conditions were provided (see above). Electric transfection was carried out with the plasmid pSV2-neo which confers resistance to the antibiotic G-418. Under these conditions about 500 clones of transformed cells (in relation to about 6×10^6 cells subjected to the treatment) could be obtained, with the yield apparently depending on the field strength and on the number of breakdown pulses.

CELL MEMBRANE CONTACT BY PHYSICAL FORCES

Electrical breakdown of cell membranes triggers fusion of cells, provided that the cells have close membrane contact (Fig. 6). As mentioned above, a number of reviews on the various aspects of electrofusion in which the individual stages in electrofusion are discussed in detail, have been published to date (Zimmermann et al., 1976a; 1980; 1981; 1984a; 1984b; 1985; Zimmermann, 1982; 1983a; 1983b; Zimmermann and Vienken, 1982; Arnold and Zimmermann, 1983). In this article the authors will therefore restrict themselves to a brief summary and concentrate more on new methods for establishing the required membrane contact for fusion between different cells.

In the conventional electrofusion method, cell membrane contact is achieved by the phenomenon of dielectrophoresis (Fig. 7). This phenomenon is based on the fact that a dipole is created in an electric field as a result of charge separation (see above). If the field is inhomogeneous, as is the case between two parallel cylindrical electrodes, a force is exerted on the dipole in the cell which moves the dipole (or cell) in the direction of the higher field strength (i.e. in the direction of the electrodes). In contrast to electrophoresis, this migration of cells is also observed in an alternating field, provided that the field is inhomogeneous. If the cells approach each other during their migration towards the electrodes, they attract each other up to a distance of a few micrometers because of their strong dipole forces (see Fig. 7) and, at the appropriate field strength, to a distance of a few nanometers. Electrical breakdown in the contact zone leads to fusion

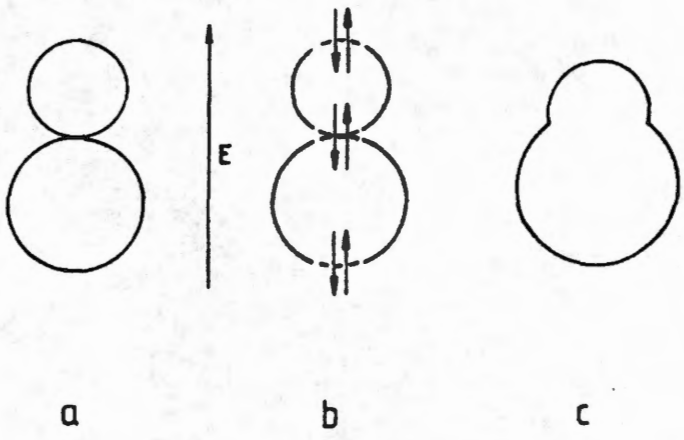


Figure 6. Representation of two cells oriented parallel (a) to the electric field (E) undergoing membrane breakdown (b) and cell fusion (c). The opposed arrows indicate exchange of material through the temporarily permeable membrane.

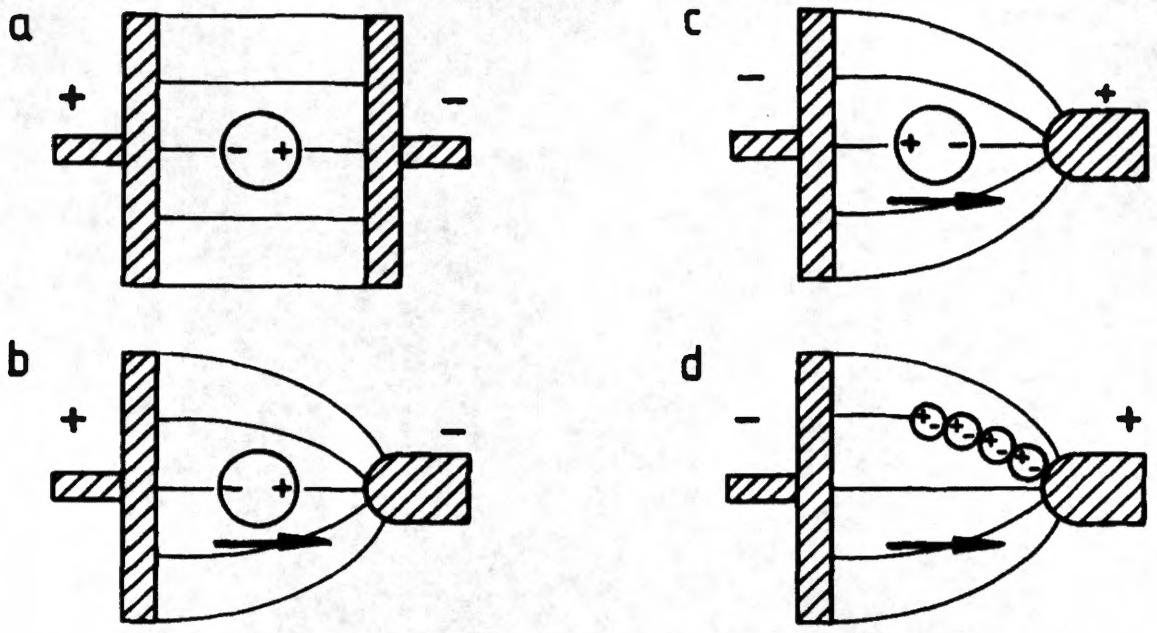


Figure 7. Pearl chain formation of cells in an inhomogeneous alternating field between two electrodes (cross section). (*a*) Polarization of the cells in the field. (*b*) Migration (dielectrophoresis) in the direction of the right-hand, negatively charged electrode. (*c*) Reversal of the external field direction: the cells still migrate towards the right-hand electrode which is now positively charged. (*d*) The cells attract each other and form chains.

of the cells. The entire process of electrofusion can be monitored under the microscope (Fig. 8), and the number of cells to be fused can be controlled by way of the suspension density and appropriate electrode chambers. A large number of cells can be fused and high yields of hybrids can be obtained if the commercially available helical and rotation chambers are used (GCA Corporation, Chicago, USA; see Figs. 9 and 10).

In the meantime this standard electrofusion method has been successfully used by many laboratories.

With this method, cells are usually fused in weakly conductive solutions (i.e. solutions with a low ionic strength) in order to avoid excessive heat development by the alternating field. Such solutions do not harm the cells as long as the incubation time is relatively short. However, fusion in these solutions assumes a fine balance between the apparatus being used, the fusion and postfusion media selected and the time course of the entire fusion process up to transfer of the fusion products to nutrient or selection medium. This problem has often been overlooked in the application of this method, particularly in the fusion of animal cells (e.g. the production of hybridoma cells by fusion of lymphocytes with myeloma cells), or when self-designed equipment or other commercially available apparatus has been used.

In some investigations, in particular in the membrane fusion of animal cells, it may be advantageous to establish membrane contact by other physical means.

The simplest way is to expose cells at high suspension densities to an electrical breakdown pulse of about 20 μs duration. Under these conditions, the mean distance between the individual cells is so small that dielectrophoresis occurs during field pulse

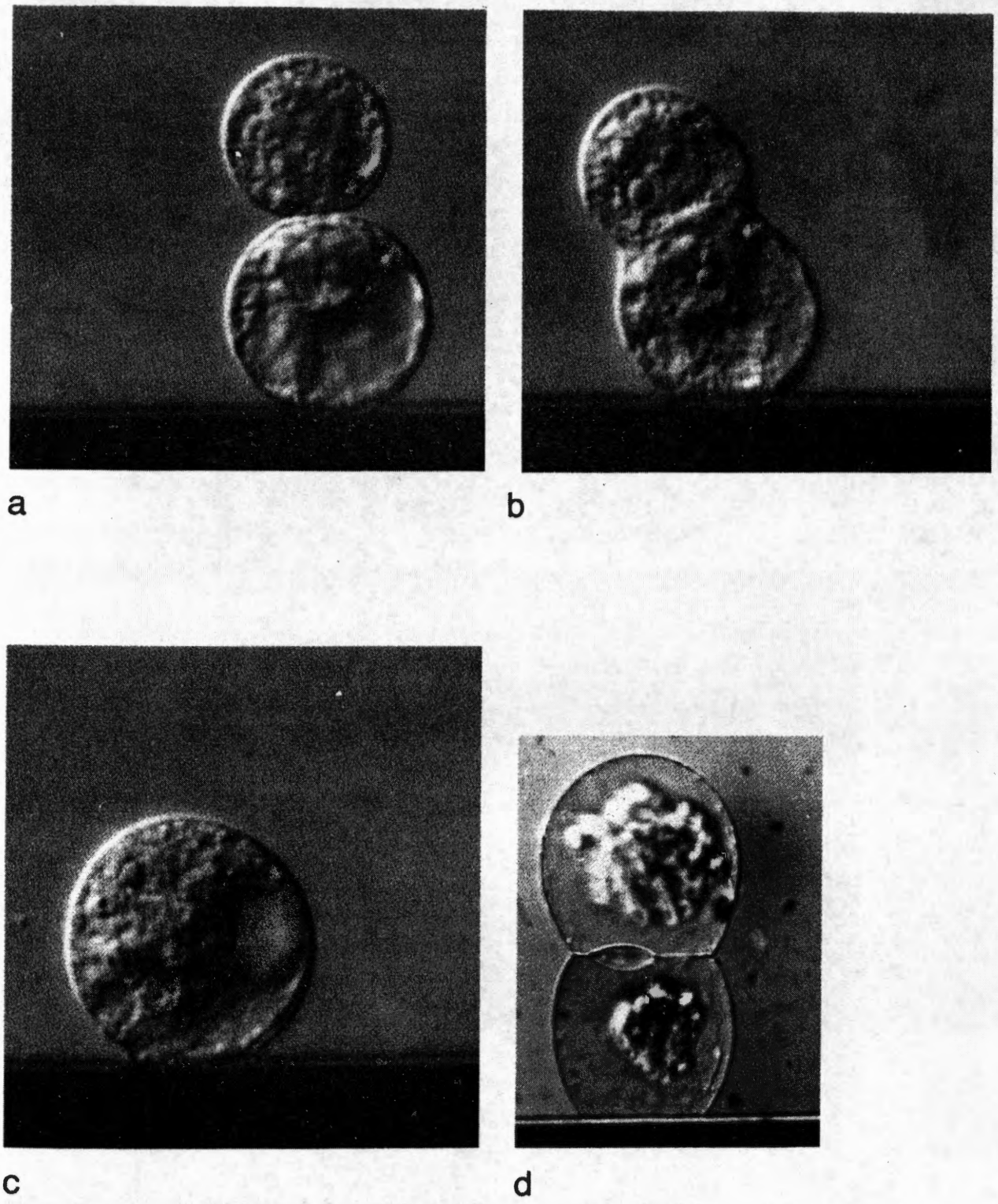


Figure 8. Electrofusion between two mesophyll protoplasts of Avena sativa. The cells were suspended in an isotonic sorbitol solution. Illustrations from top to bottom: (*a*) Chain formation by dielectrophoresis (1 MHz frequency, 75 V/cm field strength). (*b*) 30 seconds after application of a field pulse (750 V/cm, 20 μs duration). (*c*) 10 minutes after field application. The cell contents of the individual cells do not appear to mingle immediately in the fused cell. (*d*) Vesicle formation in the contact zone during the fusion of two mesophyll protoplasts of Kalanchoë daigremontiana (taken 5 minutes after application of a field pulse-1 kV/cm, 15 μs duration).

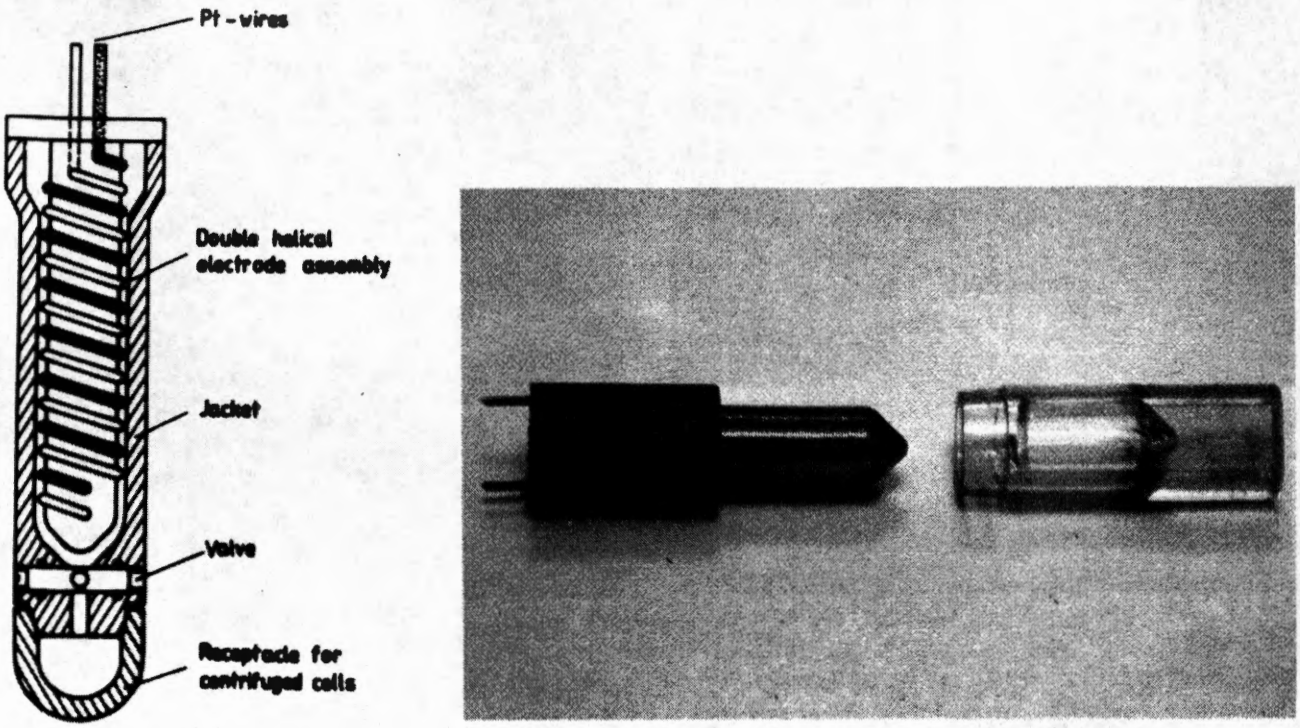


Figure 9. Helical fusion chamber. The chamber is made up of two parts: an external casing into which the cell suspension is pipetted, and an inner cylindrical tube. Two parallel platinum wires (length about 1 m) which serve as electrodes are wound round the tube thus providing a large electrode space in which large quantities of cells can be fused. The tube is carefully inserted into the outer casing. The cells and the solution rise up in the space between the inner and outer tube well. Electrofusion is then carried out under standard conditions. When the fused cells have become rounded, they are either pipetted off by removing the tube, or centrifuged into a vessel containing nutrient medium by opening a valve in the bottom of the chamber.

(Zimmermann, 1982) and thus leads to the establishment of the required membrane contact. Even if a geometrically homogeneous electrical field is used (e.g. if a plate capacitor is used as in the discharge method for the electropermeabilization of cells), the field is locally so strongly inhomogeneous because of the high suspension density that for a short period of time during the application of pulses of high field intensity (which is about 10-fold higher than in normal dielectrophoresis) considerable migration velocities and attractive forces between the cells can occur.

Using this method, Zimmermann and Pilwat (1978) were the first to demonstrate electrofusion between cells in the discharge experiments on erythrocyte suspensions. In principle, fusion can also be carried out in cell pellets, i.e. under conditions in which the distance between cells is practically zero (Zimmermann, 1982). This also leads to the creation of yeast hybrids and hybridoma cells, although at the moment the yields are still less than would be produced with the standard electrofusion method. On the other hand, higher yields of yeast hybrids and hybridoma cells are achieved if the cells at low suspension densities or high dilutions are first exposed to a series of electrical breakdown pulses and then centrifuged. In this case, fusion between the cells occurs in the pellet (Zimmermann et al., 1985). In contrast to the former method, this technique

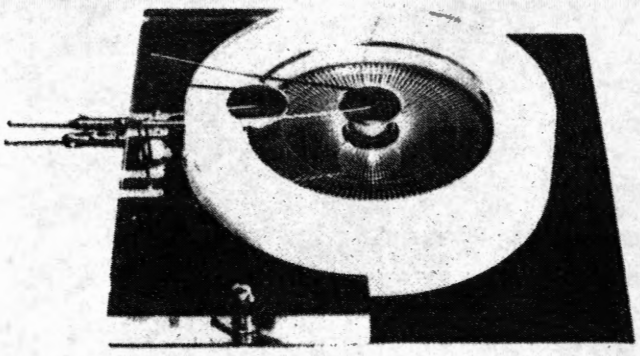


Figure 10. Rotational chamber. Radial parallel electrodes are steamed onto a translucent disc so that a large electrode space is created. Cells are introduced onto the electrode surface through an inlet and later removed from the electrode space by centrifugation through a lateral ring-shaped nut.

could be interesting from a technical point of view because it is simple as well as providing high yields. However, none of these methods allows visual control of the fusion process.

Senda et al. (1979) fused two plant protoplasts which had been brought into contact mechanically with micropipettes. However, these authors overlooked the fact that the duration of the field pulse was too long (millisecond range) so that the fused cells were not viable. This method can only be used to fuse a small number of cells.

The membrane technique (Zimmermann et al., 1985) would seem to be of more interest. In this method, the parental cells are adsorbed onto the surface of two membranes (filters), either by electrostatic or chemical means or by sucking the cells into pores with a smaller diameter. The two membranes (or filters) are brought into close contact mechanically in a plate capacitor so that the cells touch each other or are brought into contact by dielectrophoresis. The application of breakdown pulses leads to fusion of the cells with high yields of hybrids (demonstrated for yeast cells and hybridoma cells).

Magneto-electrofusion represents another method of technological interest (Kramer et al., 1984). The outer surface of the cells is magnetized by adsorption of small magnetic particles. The cells are then concentrated into a small volume between two parallel electrodes with the aid of crossed magnetic fields. The magnetic force which is directed into the center between the electrodes is sufficiently large to bring about satisfactory membrane contact between the cells. Electrofusion of erythrocytes and of yeast cells with high yields, using the magneto-electrofusion method, has been reported by Kramer et al. (1984). The advantages of this method are that fusion can be carried out in conductive solutions and that the fusion products can be isolated relatively easily with the aid of magnets. The procedure is based on the fact, that magnetic particles are taken up into the cell interior during magneto-electrofusion. Since the magnetic particles adsorbed onto the outer surface can be removed relatively easily by washing, it is possible to subsequently separate the fusion products by magnetism. The magneto-electrofusion method could be of particular interest in cases where the cells have no genetic markers (e.g. when fusing yeast strains of commercial interest). Another technique that could be of technological interest is the electro-acoustic method recently developed by us (Vienken et al., 1985). The forces arising in a sound field are analogous to those occurring in dielectrophoresis. However, in contrast to dielectrophoresis, the density differences are of importance, not the dielectric properties of the cells and the solution.

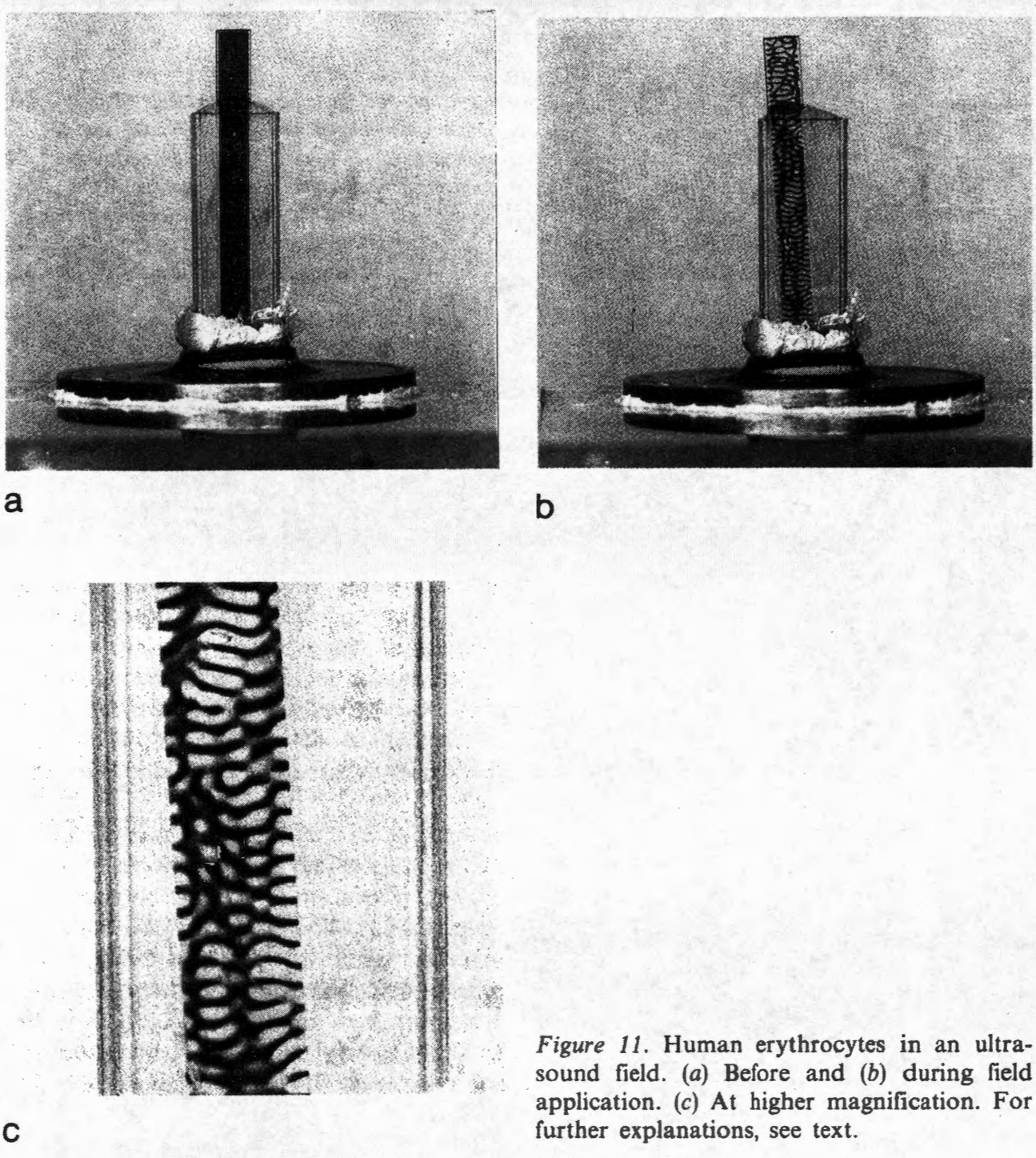


Figure 11. Human erythrocytes in an ultrasound field. (*a*) Before and (*b*) during field application. (*c*) At higher magnification. For further explanations, see text.

In an ultrasound field, it is also possible to form pearl chains of cells (Fig. 11). Vienken et al. (1985) used standing waves which gave rise to pressure maxima. The cells concentrated in these areas and formed close membrane contact. By using an ultrasound field of 1 MHz frequency, it was possible to fuse erythrocytes and myeloma cells in this way. At low suspension densities two-cell fusion products were preferentially formed. Fusion can be carried out in conductive solutions and be monitored under the microscope.

For the sake of completeness we should mention that some authors (e.g. Weber et al., 1981) used chemicals such as polyethylene glycol to establish membrane contact. Fusion was then initiated with a field pulse. Compared to the chemical fusion methods which usually use polyethylene glycol, this technique should have no great advantage since the fusion process cannot be controlled. The method of Lo et al. (1984) is of more interest. These authors used specific avidin-biotin binding between lymphocytes and myeloma cells and were so able to produce hybridoma cells with the application of a breakdown pulse.

ELECTROFUSION

In the meantime, so many studies of electrofusion of cells have been published, that we can justifiably say that this method is universally applicable for fusion of cells and artificial lipid systems.

Melikyan et al. (1983) reported on the electrofusion of two planar lipid membranes, and Büschl et al. (1982) on the electrofusion of liposomes of quite different compositions. Büschl (1984) also succeeded in electrically fusing cells with liposomes.

Ruthe and Adler (1985) recently described the first successful electrofusion of bacteria which resulted in hybrids with the characteristics of both parents. Yeast cells are particularly suitable for electrofusion and can be fused with high yields (Fig. 12). Schnettler and Zimmermann (1985) demonstrated that the composition of the fusion medium in particular has a determining influence on the yield of hybrids. These authors showed that low concentrations of calcium and magnesium acetate are necessary if high yields of hybrids capable of division are to be produced. In these experiments it was possible, for the first time, to transfer a plasmid by electrofusion into a cell of a different strain.

There have been a large number of studies of plant protoplast fusion (Saga et al., 1984; Bates et al., 1983; see Fig. 8). Various authors have been able to show in the meantime that electrically produced hybrids are able to regenerate to the callus stage or even into whole plants (Koop et al., 1983; Bates and Hasenkampf, 1985; Kohn et al., 1985).

In this context, Hampp et al. (1985) reported on the successful fusion of evacuolated plant protoplasts. There is justifiable hope that vacuole-free protoplasts will be able to regenerate better than vacuole-containing ones.

Various researching groups have shown in the meantime — after initial communications from Zimmermann and co-workers — that animal cells can also be fused with one another with the production of viable hybrids (Zimmermann et al., 1976a; 1980; 1981; 1984a; 1984b; 1985; Zimmermann, 1982; 1983a; 1983b; Zimmermann and Vienken, 1982; Arnold and Zimmermann, 1983; Ohno-Shosaku et al., 1984; Ohno-Shosaku and Okada, 1984). In some of these studies, chemicals were used to establish membrane contact (Chapel et al., 1984), or electrofusion was performed on confluent cells (Blangero and Teissie, 1983).

The production of hybridoma cells can be considerably increased by the electrofusion method as compared to chemical methods. Vienken and Zimmermann (1985) showed that, as in the case of yeast cell fusion, divalent cations are important and that,

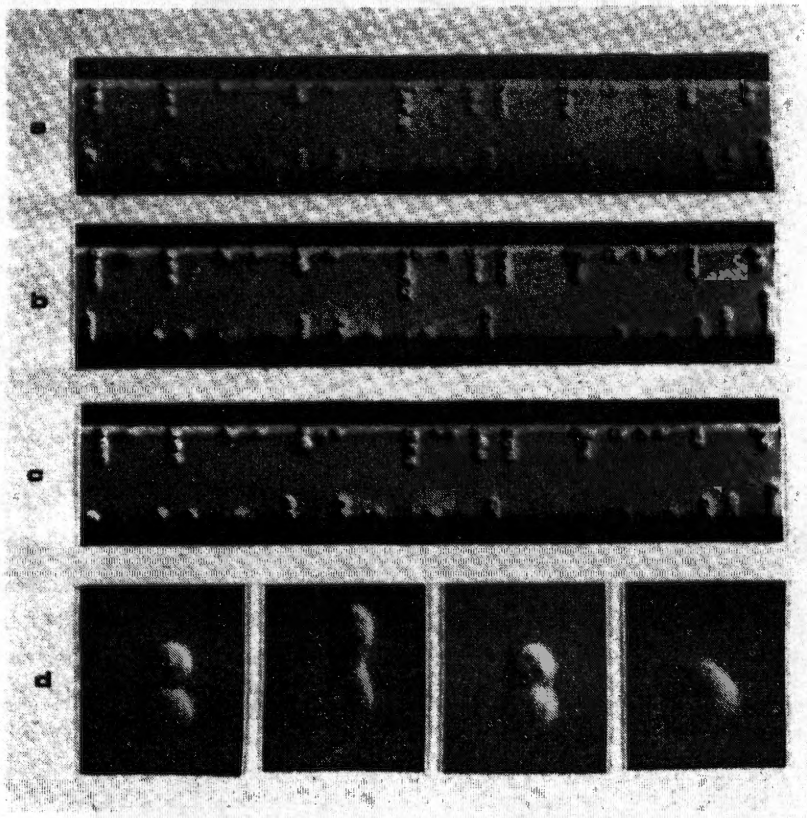


Figure 12. Electric field induced fusion of yeast protoplasts of two different strains: (*a*) A mixture of yeast protoplasts (strain 2114 and 3441) were collected by dielectrophoresis in an alternating electric field (5 V peak to peak value, 2MHz, electrode distance 28μm). (*b*) Cell fusion was induced by application of two subsequent breakdown pulses (22V, 40 μs duration) applied at an interval of about 10 s. (*c*) Photograph taken 2 min after the application of the two field pulses. Note that some hybrids have reached the rounding off stage. (*d*) Dielectrophoresis and fusion of two yeast cells at higher magnification (from left-hand to right-hand side). Same conditions as before.

in the case of electrofusion of lymphocytes with myeloma cells, it is important to incubate the cells in a postfusion medium after electrofusion (which contains an elevated potassium concentration) before adding the selection medium. There is now also proof, that these hybridoma cells produce monoclonal antibodies (Karsten et al., 1985).

Eggs can also be fused, and it is possible to observe subsequent fertilization and division (Richter et al., 1981; Fig. 13). It is also worth mentioning that it is possible to fuse thousands of cells into giant cells with the electrofusion method (Figs. 14 and 15). These giant cells could represent interesting research objects for membrane research since they are large enough for the insertion of microelectrodes.

In addition, the controlled process of electrofusion — in combination with other techniques, such as electron and fluorescence microscopy — enables the study of membrane processes and membrane properties. In this area there are some interesting studies from Sowers (1983; 1984; 1985). Since fusion occurs synchronously, individual stages of the fusion process can be monitored under the microscope. Such studies have revealed that surplus material is removed by the formation of vesicles in the contact zone (Vienken et al., 1983b; Fig. 8).

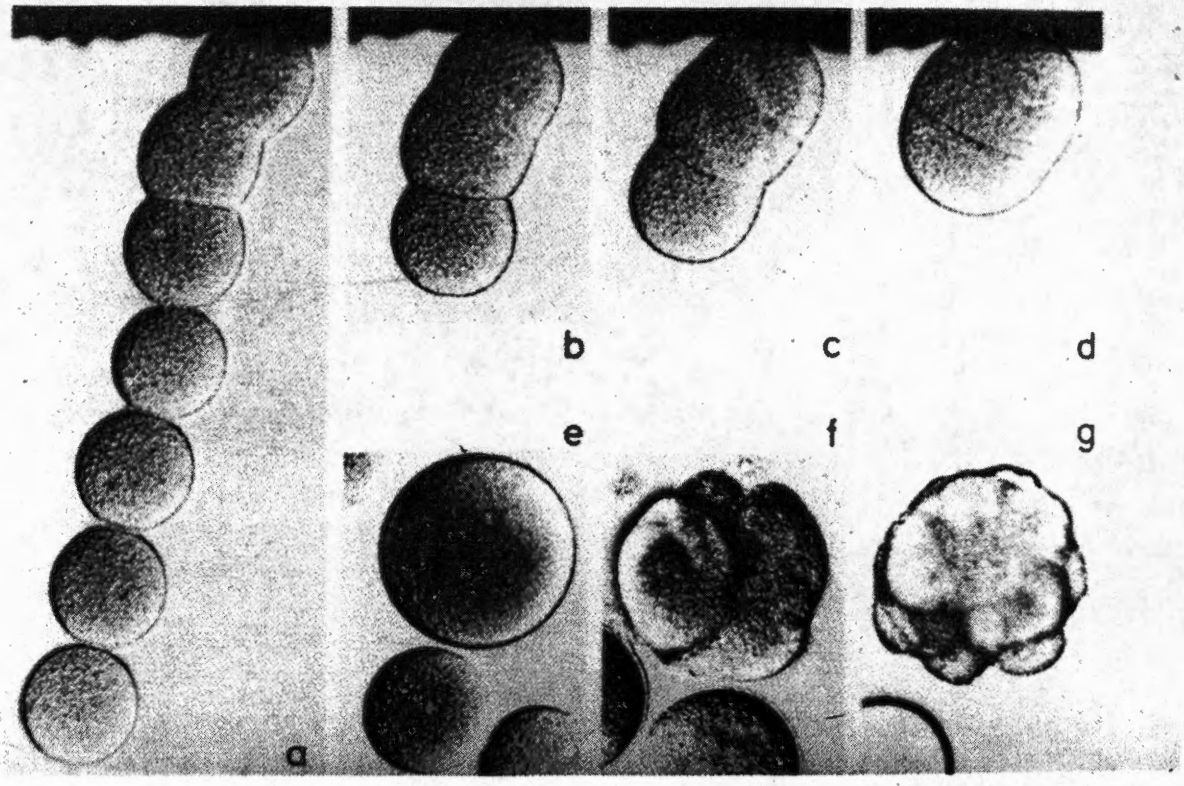


Figure 13. Time course of fusion between 3 sea urchin eggs attached to the electrode in non-conductive solution (a = 1 min, b = 2 min, c = 3 min, d = 7 min, e = 15 min after application of two field pulses (400 V/cm; 50 μs)). In Figs. e-g the eggs are transferred into ASW (artificial sea water). Figs. (f) and (g) show irregular cleavage pattern of fused two-egg stages after fertilization by sperm.

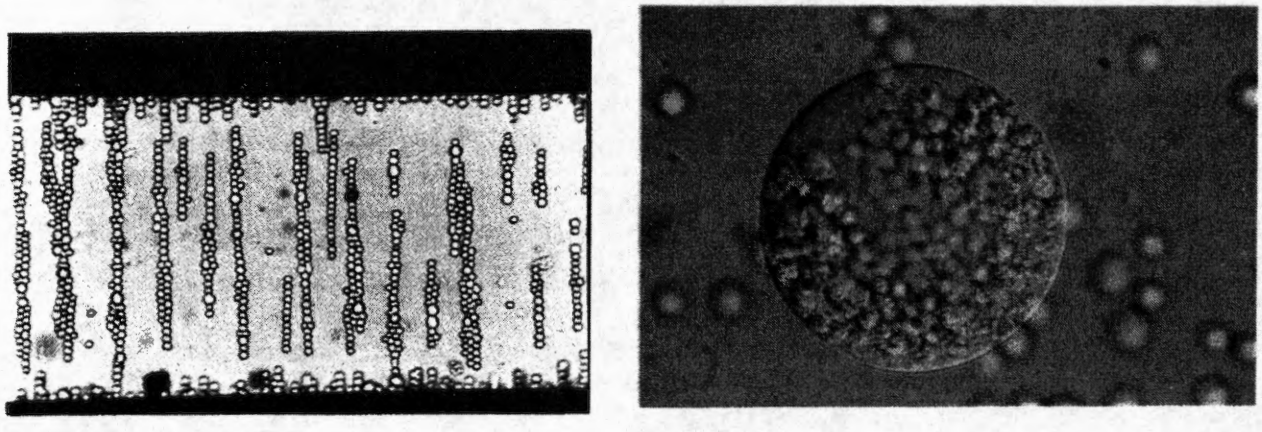
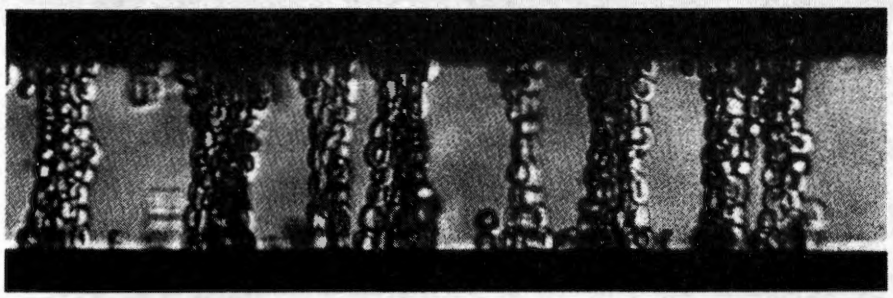
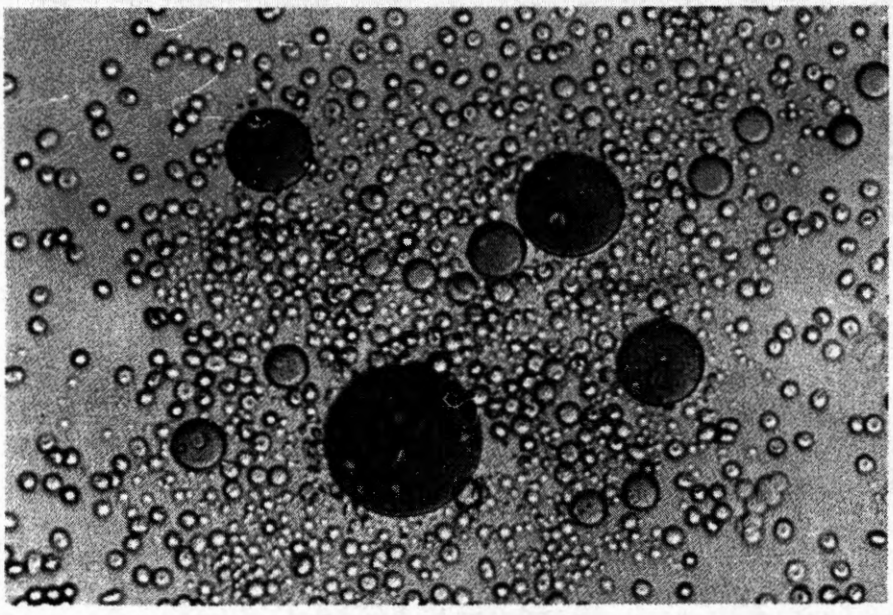


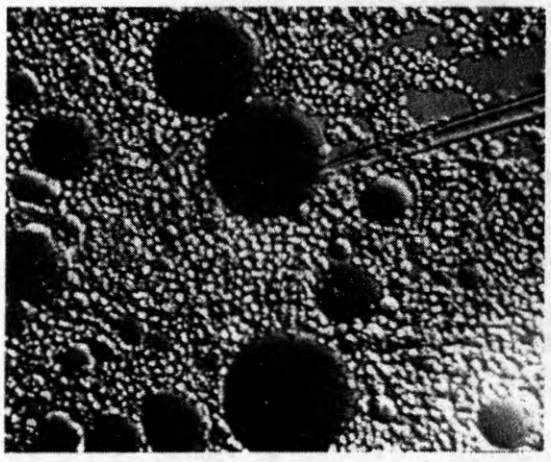
Figure 14. Electrofusion of Friend Cells. (*a*) Dielectrophoresis and cell chain formation in an inhomogeneous alternating field between two cylindrical platinum electrodes (100 V/cm, 2 MHz). (*b*) Formation of a giant cell 10 minutes after application of the field pulse (2 kV/cm, 20 μs).



a



b



c

Figure 15. Electrofusion of human erythrocytes. (*a*) Cell chain formation between two parallel cylindrical electrodes (distance 200 μm, 1 MHz frequency, 400 V/cm field strength). (*b-c*) After field application. (6 kV/cm field strength, 3 μs duration) giant cells are forming (unfused cells can be seen in the background).

CONCLUSIONS

The current state of technology shows that both the electrofusion method with its various alternatives and the electropermeabilization method are not only capable of competing with the conventional chemical methods but that they open new perspectives for biotechnology and biophysics. The fact that over a hundred research and industrial laboratories have seized upon these new methods within only two years underlines this statement. However, it is interesting to note — particularly from the point of view of promoting research — that all the theoretical foundations and the fundamental experiments go back 10 years or more in some cases, and that they are only now gaining increasing significance. One reason for this is undoubtedly the fact that the introduction of physical techniques into biology, biotechnology and medical technology, which all have a strong biochemical bias, still meets with substantial difficulties. It would be desirable if physicists could co-operate more with biologists and biotechnologists in this interdisciplinary field.

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