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Reversible large-scale deformations in the membranes of electrically-treated cells: electroinduced bleb formation

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Morpholigical changes in electrically-treated cells have been investigated by light and scanning electron microscopy. The application of 100-μs rectangular pulses of 1.3 kV/cm electric field to different types of cells (FBT, MEF, RAT-1, L-cells) in the physiological medium leads to the formation and growth of spherical and hemispherical protuberances of the cell membrane. The formation of such electroinduced blebs is not associated with the cells' death and is reversible. The electroinduced blebs are mainly formed at those sites of the cell membrane which are subjected to the highest voltage during the electric pulses. Increasing the tonicity of the medium by introducing 20 mM of inulin prevents the bleb formation, indicating the osmotically-dependent nature of the processes involved. When electric pulses are applied to the cells pre-treated with cytochalasin B, the formation of electroinduced blebs occurs independently from cytochalasin-induced ones originally present on such cells. Speculations are presented concerning the nature of the membrane structural changes underlying the electroinduced blebbing and their possible role in some electrically-induced processes.

Introduction

In recent years there have been a great number of reports about the use of electric pulses of high intensity for cell fusion and introduction of donor DNA into the cells [1–3]. The question about the mechanism of these phenomena remains open. It is known that, when sufficiently high voltage (hundreds of millivolts) is applied for a short time to cell or lipid membranes, local permeable structures – pores – are induced in the membranes (1,4–6]. This process, electroporation, is generally regarded as the most important step in electroinduced fusion and transfection. However, there have been data suggesting that the effect of electric field during fusion and introduction of DNA into the cells cannot be explained by pore formation only [7–10].

In the present paper we show that the application of high-intensity electric pulses to cells leads to the formation of long-lived reversible osmotically-dependent large-scale deformations of the cell membrane – blebs – localized predominantly on those sites of the cells' membrane which are subjected to the maximum voltage during the electric pulse.

Materials and Methods

Cell preparation and electrical treatment

We used fetal bovine trachea (FBT) epithelial cells of a nonsynchronized monolayer culture 2 to 3 days after reseading. The cells were taken off the glass by a trypsin/Versen mixture in the proportion of 2 : 1. Then the cells were placed in a cultural medium (medium 199 + 10% of bovine serum, Moscow), put on 22 × 22 mm cover slips and incubated at 37 °C for 35 min. The cells attached to the glass and began spreading. Before the electric field application the cultural medium was replaced by PBS (phosphate buffer: 8 g NaCl, 0.2 g KCl, 0.1 g $CaCl_2$, 0.12 g $MgSO_4$, 0.2 g KH_2PO_4, 1.15 g Na_2HPO_4 per 1 l of solution, pH 7.4) and the cover slips were placed into a chamber with two parallel 10-mm-long flat stainless steel electrodes at a fixed distance (550 μm) between them. Then the chamber was mounted on the stage of an inverted photomicroscope (Leitz Fluovert, F.R.G.) for the direct observation of morphological changes of the cell surface following electrical treatment. Three 100-μs rectangular electric pulses of 1300 V/cm intensity were applied at intervals

Abbreviations: FBT, fetal bovine trachea; MEF, mouse embryo fibroblast; RAT-1, rat fibroblast.

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of 0.4 s. During the pulse maximum voltage across the cell membrane [1,11] was 0.780–0.975 V (the diameter of rounded FBT cells was 8–10 μm). In some experiments electric pulses of 5 μs, 10 μs, 20 μs duration were applied. The control cells were parallelly subjected to all of the above procedures except the electrical treatment.

A number of experiments were performed also with the cells of primary culture of mouse embryo fibroblasts (MEF), rat fibroblasts (line RAT-1) and L-cells. These cells were taken off the glass by trypsin solution; incubation and electrical treatment were as described above. Human erythrocytes were obtained just before the experiments from apparently healthy donor, then washed in PBS and used for the electrical treatment: without incubation at 37° C (in suspension) or after 30 min incubation (they became attached to the cover slip).

Preparation of samples for the scanning electron microscopy

Cells were fixed in the chamber for 5 min by a 2.5% glutaraldehyde solution (Serva) in PBS (pH 7.4) in different whiles (1 to 20 min) after the pulses, and then postfixed for several hours at room temperature. The samples were dehydrated in acetone, critical point dried, shadowed with gold and investigated by scanning electron microscopy (Philips, PSEM-500X, The Netherlands).

Treatment with cytochalasin B

After taking off, the FBT cells were placed on cover slips in the cultural medium and incubated at 37° C for 35 min (as described above). Then cytochalasin B (10 μg/ml, Sigma) was added to the medium and incubation was continued for 1 h more at 37° C. After incubation, the medium was replaced by PBS, and the cells were subjected to electrical treatment. In this particular case, the whole experiment lasted no longer than 10–12 min at room temperature, since the actin skeleton recovers quite rapidly in the absence of cytochalasin.

Results

Cell surface after electrical treatment

After the application of three electric pulses to FBT cells, essential morphological changes occurred on the surface of the cells (phase contrast microscopy, 800 ×). Practically immediately (within 0 to 5 s) after the electric pulses numerous small inhomogeneities arose on the cells surface. In 15 to 30 s hemispherical and spherical protuberances, so-called 'blebs', appeared on the cell membrane. The blebs grew rather quickly within 2 to 10 min, sometimes fused with each other and in some cases became very large (up to 10 μm in diameter). According to the shape and localization on the cell surface several types of eletroinduced blebs could be distinguished. The most frequent blebs (Fig. 1b, 2a) were hemispherical protuberances appearing on the cell 'body' (that is on unspread parts of the cells or on spherical cells) This type of blebs should also include the hemispherical blebs on the 'body'-lamella boundary of the partly spread cells. The size of these blebs varied over a wide range: from 0.5 to 10 μm. Spread cells, or those which began to spread often exhibited another type of protuberances. These were shaped like bulges on the lamella and positioned either at the edge of the lamella or in any other part of it (Fig. 2b). As a rule, these blebs were of small or medium size (0.5 to 6 μm). Nearly spherical protuberances other than the first two types (Fig. 2c) formed on the lamella very seldom (on the cell's body, still more seldom).

To check the viability of the cells after electrical treatment, they were incubated with Trypan blue (0.2% solution in PBS, Serva) for 10 min. The dye was added to the medium 2–3 min after the pulses. The fraction of stained cells was on an average 20–25%. The stained cells (whether spread or not) had as a rule a lot of blebs or one (two) huge blebs. These cells were unable to recover their surface (to remove blebs), because they were, apparently, damaged irreversibly by the electric field. Among the unstained cells more than 60% had protuberances – electroinduced blebs of all types also. These blebs were generally smaller than those on the stained cells (0.5–4 μm). As for these cells, bleb formation was reversible: after 15–20 min incubation at a room temperature in PBS, most of the cells removed electroinduced blebs and began (or continued) spreading (Fig. 1c). When the cells were incubated at 37° C in the cultural medium, this process was accomplished still sooner (in 10 to 15 min). After removing blebs the cells spread (within 1–2 hours), normally lived and divided in the growth medium. 24–26 hours after the pulses the total number of cells between the electrodes was 105–115%% from electrically treated ones – some of them divided. FBT cells were normally spread, stained by Rhodamine 123 (Sigma, 5 μg/ml, 10 min) and not stained by Trypan blue. The number of dead cells (stained by Trypan blue) was 10–20%%. These cells were newly dead during 24 h, because the cells that died just after the pulses, the day after were usually removed from the glass during the procedures of cultural medium change and staining. Among the control cells, subjected to all the same procedures, except electrical treatment, the number of dead cells after 24–26 h of incubation was about 6%, the total number was 120% from initial. The low growth rate of control cells (and those under investigation) can be explained by very low cell concentration on the glass (FBT growth is sensitive to cell density); moreover, the cells were after taking off (see Materials and Methods). It is interesting to note that cells were able to recover their surface in two ways. In most cases, blebs of all types were pulled in, but some blebs could segregate in the form of closed vesicles

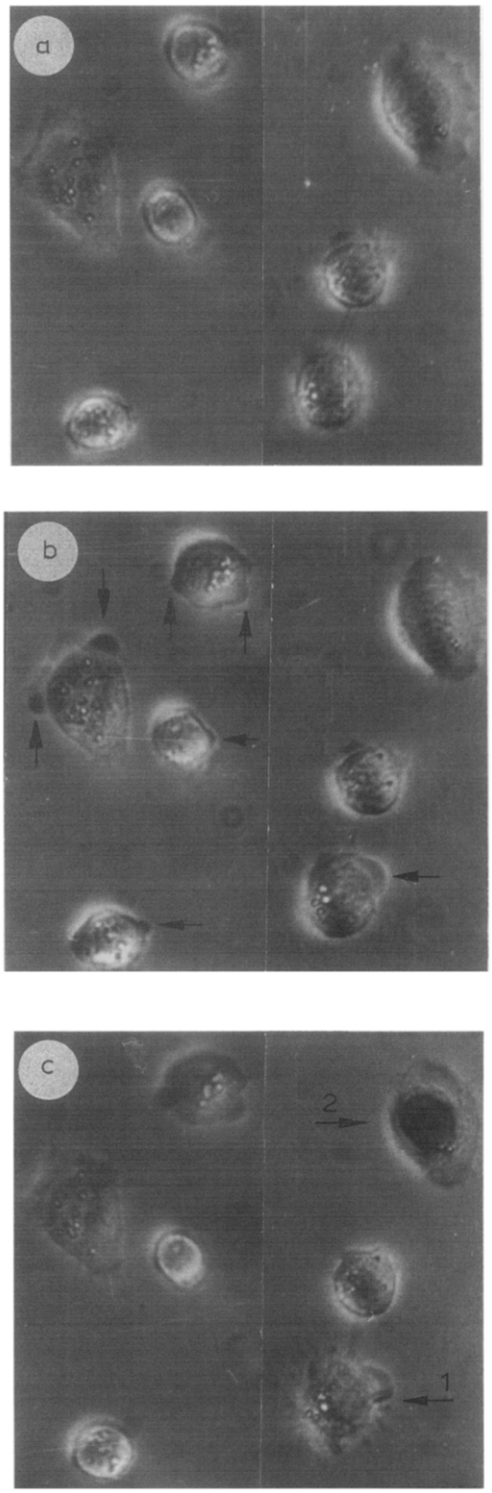


Fig. 1. Formation of electroinduced blebs after the application of three 100-μs electric pulses of an intensity of 1300 V/cm to FBT cells. (a) The FBT cells before electrical treatment. (b) The same cells 2 min after the pulses. Five of the seven cells have hemispherical protuberances on the membrane-electroinduced blebs (arrows). (c) The FBT cells 20 min after the pulses (the cells were being in PBS at room temperature). Three of the five cells 'pulled in' their electrically-induced blebs. The unspread cell began to spread (arrow 1). At the top (arrow 2) a cell that died after the electrical treatment; the nucleus is stained with Trypan blue. Magnification 800×, phase contrast.

resembling liposomes in shape (spherical and hemispherical blebs were able to segregate: the bulge-like blebs on the lamella could only pull in).

The number and size of electroindused blebs dependend on the field intensity, on the number of pulses and on the pulse duration. After three 1–1.2 kV/cm pulses of 100 μs duration blebs appeared on a few cells only. If we applied 1.5–1.7 kV/cm pulses, we observed a lot of large blebs, but the percentage of dead cells was too high. With increasing the number of pulses (1.3 kV/cm) the number and size of electroinduced blebs progressivelly increased, as well as the number of dead cells. Three 1.3 kV/cm pulses of 5 μs, 10 μs and 20 μs duration also caused the formation of electroinduced blebs on more than 60% of the cells. But these blebs were very small (0.5–1.5 μm) and were very quickly removed (in 5–7 min at a room temperature).

The surface of all types of blebs looked homogeneous in the scanning electron microscope. It was practically devoid of any projections or microvilli (Figs. 2a–c), thereby differing from the rest of the cell surface.

On the control cells, what paralleled the ones under investigation in all of the corresponding procedures of taking off, incubation, staining and fixing, blebs were vary rare. Large blebs were revealed on several dead (stained by Trypan blue) cells of the control population. The scanning electron microscope made it possible to reveal also single tiny protuberances on some (one of 20, on an average) living control cells, localized usually on the lamella (Fig. 3). It was easy to distinguish these blebs from the electronduced ones, which were mainly arising by several blebs at a time on one cell and were found on most of the cells after electrical treatment (cf. Fig. 3 and Figs. 2a, b).

The cells' viability after electrical treatment was dependent on the field intensity during the pulses, on pulse duration, on the number of pulses and the interval between them. So, 20 to 25% of the FBT cells were killed by three pulses (1.3 kV/cm) applied at an interval of 0.4 s (the standard procedure), whereas 60 to 70% of the cells dead after the same three pulses when applied at an interval of 3 s. A separate pulse applied 5 min after three standard pulses (see above) caused death of 95% of FBT cells. After three 10 μs pulses (1.3 kV/cm) at an interval of 0.4 s the percentage of dead cells was only 5–8%.

Distribution of electroinduced blebs and the voltage on the membrane

It is known that the voltage across the cell membrane during the eletric pulse is different in different sites of the membrane. The maximum voltage drops on those sites that are located at the right angles to the electric field [1]. In this connection, of great interest was the question of the distribution of electroinduced blebs over the cell surface.

The distribution was found to be non-uniform. We divided the membrane of the cells into four sections differently oriented with respect to the direction of the electric field. (Fig. 4) A calculation of the number of blebs in each section of all cells (by phase contrast microscopy) showed that sections A-A exposed to higher voltages contained on an average (72.2 ± 3.6)% of the total number of electroinduced blebs, whereas sections B-B contained (27.8 ± 3.6)%. We took into consideration electroinduced blebs belonging to different cells (for example, cell 1 has three blebs in A-A sections, cell 2 has two blebs in A-A sections and one in B-B, cell 3 has four blebs in A-A, two blebs in B-B and two blebs on the boundary of A-A and B-B sections. Then we

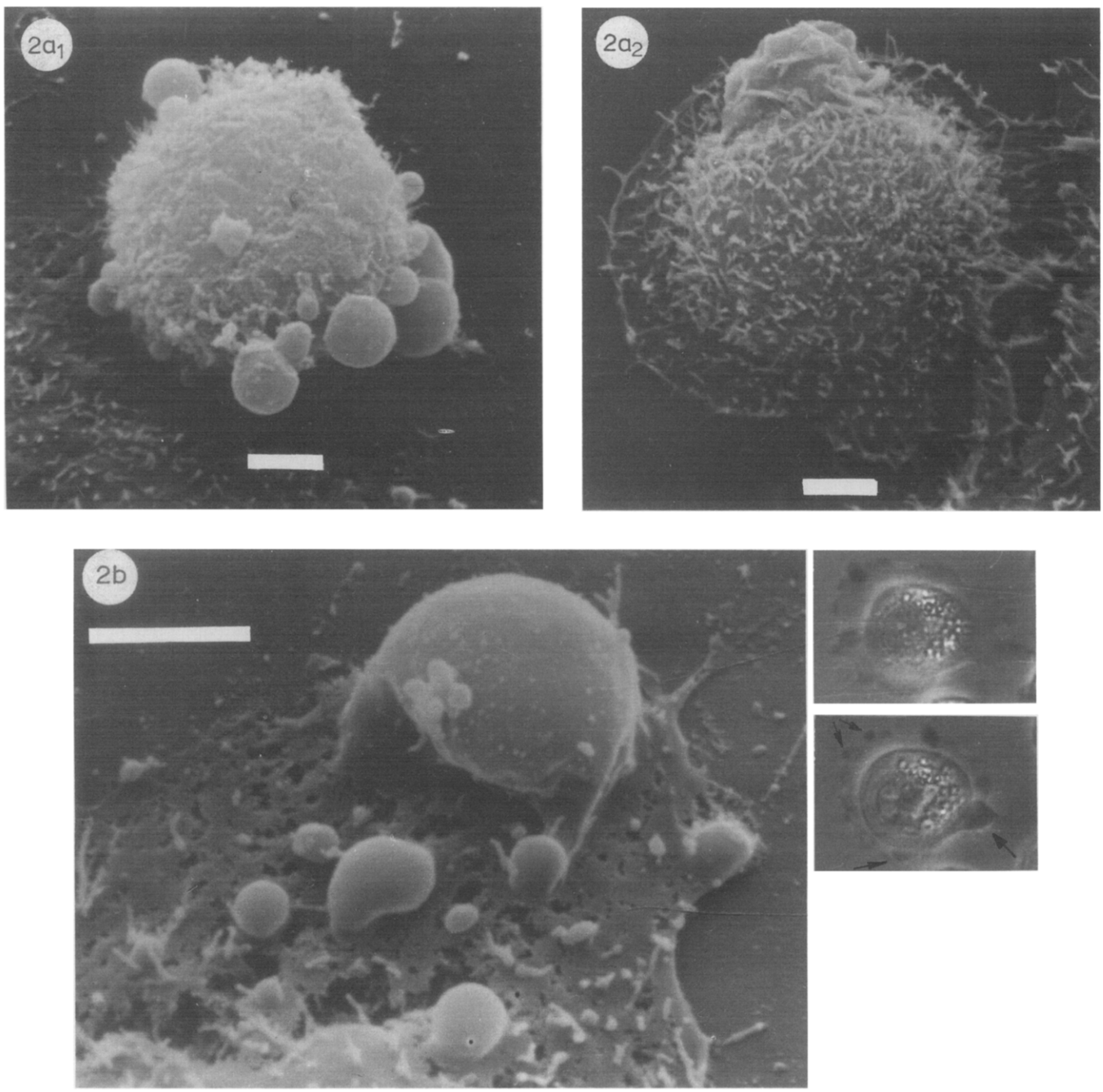


Fig. 2. Micrographs of electroinduced protuberances of the cell membrane on FBT cells after three electric pulses (1300 V/cm, 100 μs). (a) Hemispherical blebs on an unspread cell. (b) Electroinduced blebs shaped like bulges on the lamella of a spread cell. Right, the FBT cell before (top) and after (bottom) the pulses; the arrows point to the electroinduced bulges on the lamella (magnification 800×, phase contrast). Left, similar bulges as observed by scanning electron microscopy. (c) An electroinduced spherical bleb on the lamella. Such blebs were relatively rare. (a, b (left) and c). Scanning electron microscopy. The bar corresponds to 2 μm.

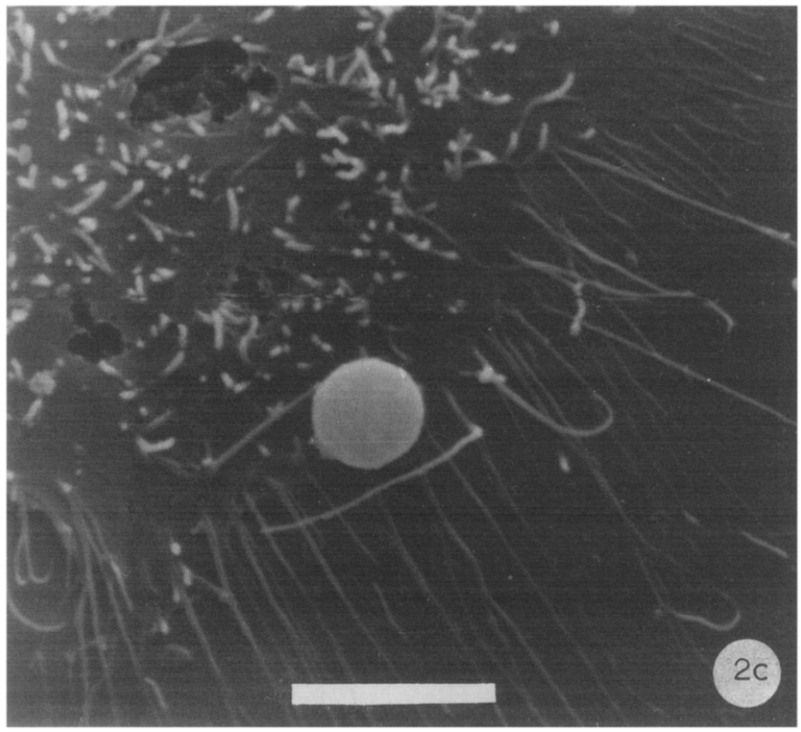


Fig. 2 (continued).

have, that in sections A-A there are ten and in sections B-B there are four blebs of the total number of electroinduced blebs).

Electroinduced blebbing as an osmotically-dependent process

It is reasonable to suppose that the formation and growth of electroinduced blebs may be accompanied by the change in the intracellular volume. To study the possible role of such processes in electroinduced blebbing, we raised the viscosity of the external medium by adding Ficoll (Pharmacia, $M_r = 400\,000$; 10% solution in PBS, pH 7.4) or increased the tonicity of the medium by adding inulin (Serva) at a concentration of 20 mM. Inulin does not penetrate into the cells during electroporation [12] and only slightly raises the viscosity of the solution.

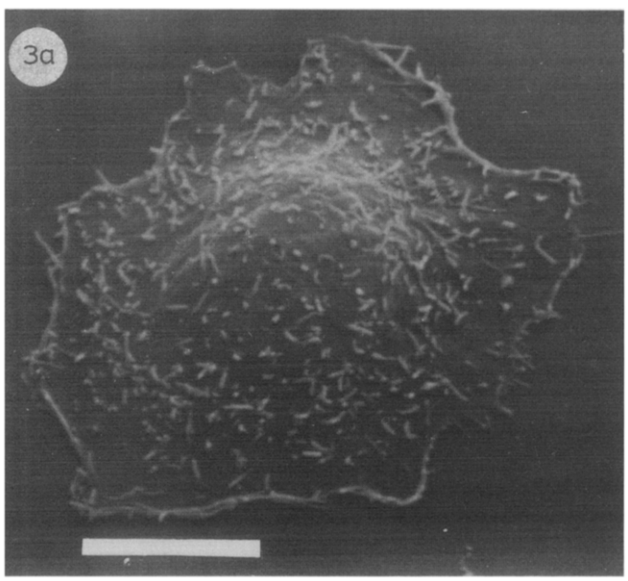
It turned out that both Ficoll and inulin considerably enhanced the cells' viability after a series of pulses (data not shown). The process of electroinduced blebbing was strongly affected by the presence of Ficoll or inulin in the medium. Thus, no blebs appeared on the cells in the medium with Ficoll within 10 min after the electrical treatment (phase contrast microscopy). When Trypan blue was added 3 min after the pulses in the presence of Ficoll (0.2% solution of the dye in PBS + 10% of Ficoll), the cells were not stained within 10 min even in the case of the lethal electrical treatment (8 to 10 pulses). However, when the medium with Ficoll was replaced by that without Ficoll (0.2% Trypan blue in PBS) 2 min after the pulses, blebs appeared and began to grow practically immediately, and dead cells stained with Trypan blue within 3 min.

As for the medium with inulin, blebs were not formed within 10 min after electric pulses either; though, when the dye was introduced into the chamber 3 min after the pulses (0.2% Trypan blue in PBS + 20 mM inulin), the nuclei of 5–15% of the cells had been stained in 5 min. When the medium with inulin was replaced by that without inulin (0.2% Trypan blue in PBS) 2 min after the pulses, blebs appeared and started to grow on 15–30% of the cells virtually at once. Let us stress that the removing of Ficoll or inulin from the medium not later then 3–5 min after electrical treatment results in the development of typical electroinduced blebs not only on dead but also on viable cells.

In order to examine in more detail the morphology of the cell surface after the electrical treatment in the medium containing Ficoll or inulin, the cells were fixed directly in the chamber 5–6 min after the pulses by replacing the electrical-treatment medium by PBS with 10% of Ficoll, or, respectively, 20 mM of inulin, and 2.5% glutaraldehyde (pH 7.4).

Watching the cells directly through the phase contrast microscope during the fixation, we found in both cases that, in the first stage of fixation (within 0.5–1 min on addition of glutaraldehyde), the surface of most of the cells became covered with many small (0.5–3 μM) spherical protuberances (3 to 10 per cell) that ceased to grow quite soon (in 1 min). These spherical

formations were mainly localized at the edge of the lamella, but sometimes appeared in other parts of the cells. They did not look like the typical electroinduced blebs, as they were bright in phase contrast microscopy in comparison with the grey electrically-induced blebs, and were very easy to separate from the cells in the form of small bright vesicles. Except for single cases, we failed to detect these protuberances when examining the cells by scanning electron microscopy; virtually all of them were lost during the dehydration and drying. Note that the bright bulges like these arose only when the cells were fixed after the electrical treatment in the presence of Ficoll or inulin not later than 10–12 min after the pulses. When we placed the control cells in the medium with Ficoll or inulin for 10–15 min and then fixed in the presence of these substances without electrical treatment, we did not find any bright spherical protuberances. Neither did we observe any other bright



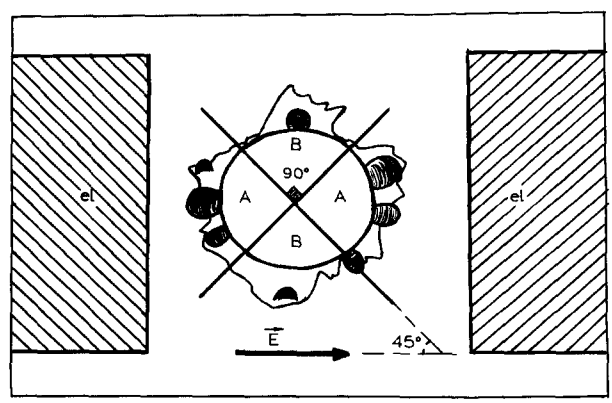


Fig. 4. Areas of the cell membrane differently oriented with respect to the electric field (arrow at the bottom) during the pulses (el-electrodes). Sections A-A of the membrane sustain the highest voltage during the pulses.

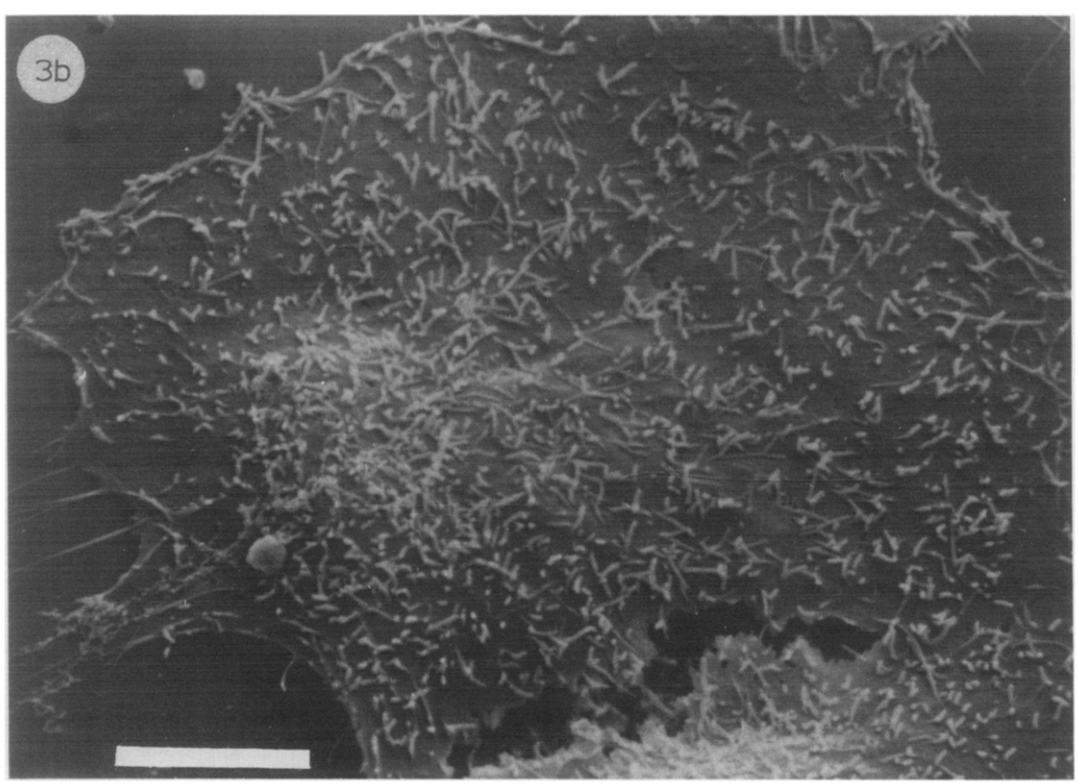
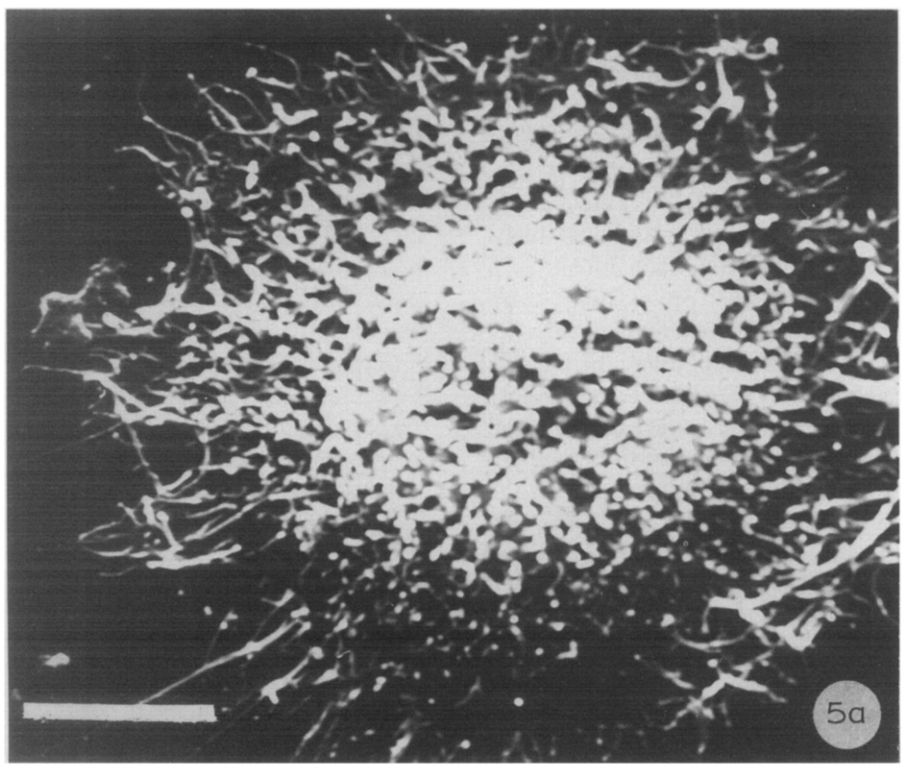


Fig. 3. Electron micrographs of the control FBT cells. (a) A typical FBT cell. (b) Some control cells (one of 20) exhibited single tiny protuberances on the cell membranes. Scanning electron microscopy. The bar corresponds to 4 μm.

spherical protuberances when we fixed the control cells or the cells treated with electric field in PBS and therefore having typical electroinduced blebs.

When examining the cells electrically treated in the presence of Ficoll or inulin with the scanning electron microscope, we detected no electroinduced blebs, either, although the cell surface had been considerably changed in both cases. The cells had a large number of wave-shaped hillocks (Figs. 5a,b), different from the microvilli typical for the control cells (cf. Fig. 3 and Fig. 5).



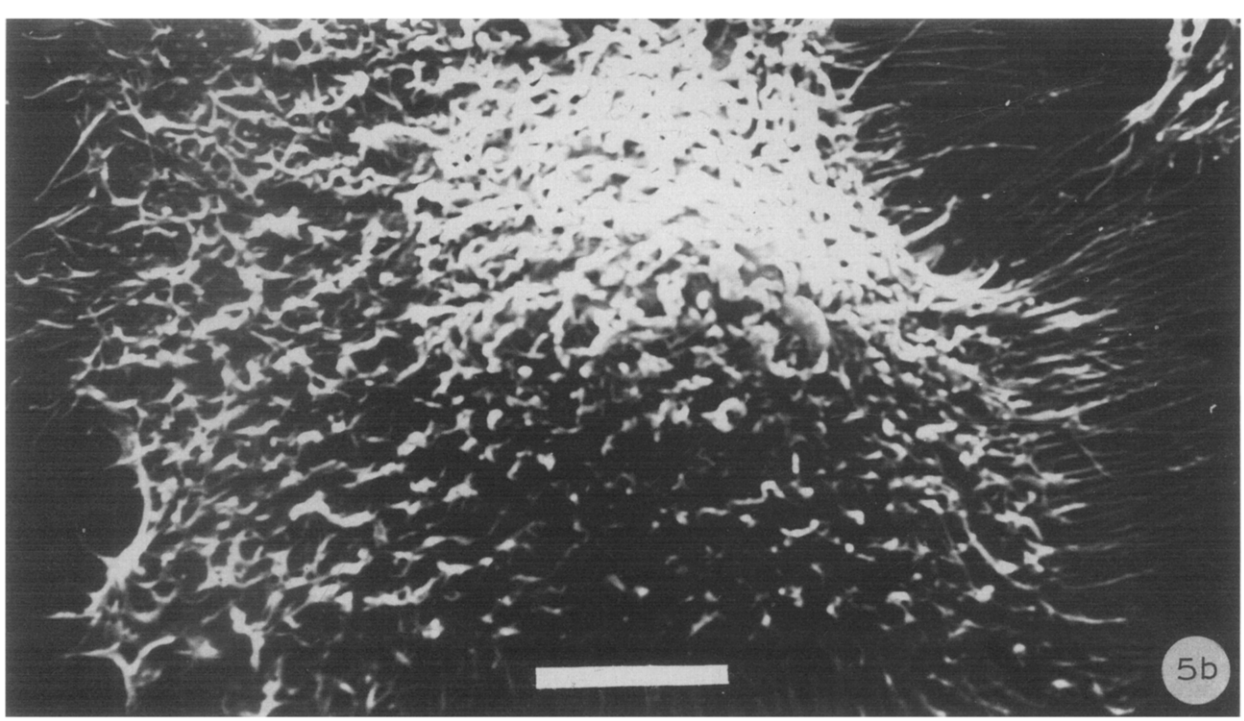


Fig. 5. Electron micrographs of FBT cells 6 min after the application of electric pulses in the presence of Ficoll (a) or inulin (b). Many wave-shaped protuberances can be seen on the cell surface, but electroinduced blebs are absent. Scanning electron microscopy. The bar corresponds to 4 μm.

However, on some cells electrically-treated in the presence of Ficoll (but not inulin), little bulges were observed resembling small electroinduced protuberances in the early stage of formation.

Electroinduced blebbing on the FBT cells treated with cytochalasin B

It is known, that treatment of cells with cytochalasin leads to the breakup (dissociation) of the actin cytoskeleton [13]. The treated cells are unable to keep their surface normal: they are deprived of microvilli, often from a lot of small blebs (Fig. 6a, see also Ref. 13). After the application of electric pulses to cytochalasin B-treated FBT cells, in 15–30 s electroinduced blebs appeared on their surface (Fig. 6b). Such blebs, as a rule, arose independently of the pre-existing cytochalasin B-induced blebs, that remained unchanged after the electrical treatment and did not increase in size. When a growing electroinduced bleb was forming close to pre-existing ones, it could capture these blebs' membrane, though, in general, the appearance of the electroinduced protuberances was random with respect to the cytochalasin blebs available.

Electroinduced blebbing on the other cells

Except the FBT cells, we have observed electroinduced blebbing on L-cells, primary culture mouse embryo fibroblasts (MEF) and rat fibroblasts (line RAT-1) after electrical treatment. We used no only the cells attached to the glass (as described above), but also those in suspension; on suspended cells electroinduced blebbing was also successful, but the percentage of dead cells was higher. However, we failed to obtain any electroinduced blebs on human erythrocytes. As the electric field intensity and the number of pulses increased, the erythrocytes were gradually turning into spherocytes and then into ghosts; no blebs were formed.

Discussion

The application of high-intensity electric pulses to FBT cells in PBS leads to the formation and growth of spherical and hemispherical protuberances on the cell membrane. Electroinduced bleb formation is not associated with the cells' death and is reversible: cells remove electroinduced blebs and normally live. The electroinduced blebs are scattered over the cell surface in a non-uniform manner: blebs mainly form at those sites of the cells membrane which are subjected to the highest voltage during the electric pulses. Addition of inulin or Ficoll to the medium prevents (or strongly retards) the formation of electroinduced blebs and raises the viability of the cells after a series of pulses.

The formation of blebs after the application of high-intensity electric pulses was observed earlier on hepatocytes [14] and on Chinese hamster ovary cells [9]. In both cases, the formation of blebs was correlated with an increase in the permeability of the cell membrane for small molecules [14,9] and electroinduced blebbing was found to be reversible. Our data are in agreement with these results. It is important to note that the authors in Ref. 14 could observe very large protuberances only, since they used light microscopy (magnification up to 400 ×) and established only the very presence of blebs on hepatocytes after the electrical treatment. On the contrary, in Ref. 9 authors do not bring any light microscopic data on the formation and growth of electrically-induced blebs immediately after the pulses; the blebs were directly observed on fixed cells only. The authors did not therefore exclude the possibility that blebs observed by scanning electron microscopy could be the artefact of the fixation of the cells by glutaraldehyde. In addition, the authors of Ref. 9 performed the electrical treatment in a medium of low ionic strength (sucrose), which could greatly influence the cells' properties (e.g., that medium changes the cell membrane permeability [15], lowers the intracellular pH (Margolis and Gass, unpublished data), etc.).

Our data demonstrate that electroinduced blebbing is

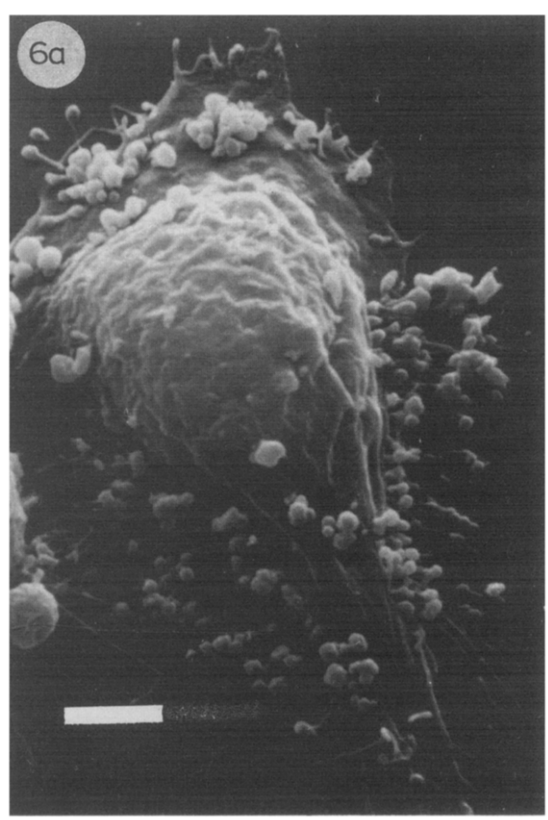
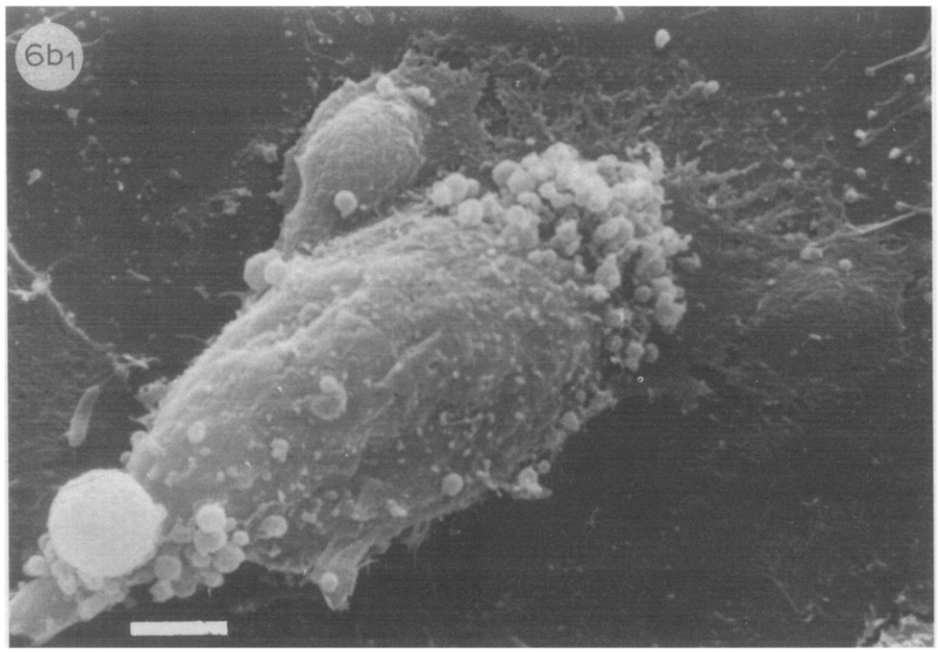
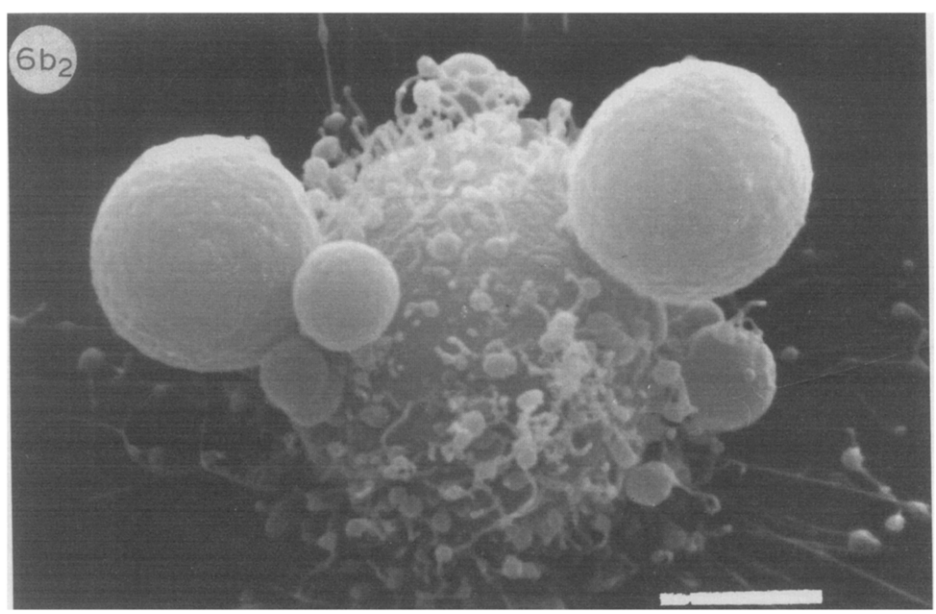


Fig. 6. Electron micrographs of FBT cells treated with cytochalasin B before (a) and after (b) the application of pulses. (a) The cells are devoid of microvilli and form many protuberances on the cell membrane – blebs. (b) Electroinduced blebs formed on the cell surface after electric pulses. The formation of electorinduced blebs is, generally, independent of the pre-existing (cytochalasin) protuberances. Scanning electron microscopy. The bar corresponds to 2 μm.





not a consequence of the fixation of cells by glutaraldehyde, because we have directly observed the formation and growth of such blebs by light microscopy before the fixation. It is interesting to note that in some cases, when glutaraldehyde fixation may really cause the formation of spherical protuberances (after electrical treatment in the presence of Ficoll or inulin), these protuberances differed very much from the electroinduced blebs and, as a rule, were completely vanished during preparation of samples for scanning electron microscopy.

The ability to form blebs in apparently a universal property of cells and may be caused by various reasons. Blebs are often observed on dead cells, independently of the cause of their death (lethal dose of radiation (Dvoryankina et al., unpublished data), hypoxia [14] and treatment with toxins [16], mechanical stresses, for example during isolation of hepatocytes [14], etc.). Living cells can form blebs as well (after a nonlethal electrical treatment (Refs. 9,14, and this paper), after incubation of the cells with cytochalasin [13], just after isolation of hepatocytes [14], after a short incubation under the conditions of hypoxya [14,16], etc.). Thus, blebbing is probably a consequence of some damages in

the cell membrane or in some other cell structures (e.g., cytoskeleton); they can be reversible (as in living cells) or irreversible (the cells die).

Electroinduced blebbing has been observed on hepatocytes [14], on Chinese hamster ovary cells [9], on FBT, MEF, RAT-1 and L-cells (this work). In all cases, electroinduced blebs were reversible. As for the human erythrocytes, free of cytoskeleton (only membrane spectrin-actin skeleton), no blebs appeared up to the hemolytic voltages. It seems to be possible that the formation of blebs after electrical treatment is typical only for the cells having cytoskeleton.

It is reasonable to suppose that formation and growth of electroinduced blebs may be accompanied by a certain change in the intracellular volume. Depending on the conditions (medium composition, treatment parameters, registration time, etc.), electrical treatment may in principle evoke both an increase and a decrease in the volume of the cells. Thus, the formation in the membrane of pores permeable to ions but impermeable to large osmotically-active molecules may lead to the colloid-osmotic swelling and even lysis of the cells [1,12,17]. On the other hand, electric field can deform spherical cells, leading to squeezing out the intracellular medium [18,19]. In this connection, it seems interesting that the presence of inulin or Ficoll in the external medium during and, for a while, after the electrical treatment prevents (or strongly retards) the formation of blebs. In the case of Ficoll, it is likely to be associated with the significant increase in the viscosity of the medium (e.g., the water entrance into the cell is slowed down; or the viscous medium mechanically inhibits growth of blebs from outside; or, perhaps, both processes contribute). Let us note that Ficoll only slightly raises the osmotic pressure outside (10% Ficoll correspondence to 0.25 mM). The effect of inulin, which increase the viscosity only insignificantly, seems to be associated with its osmotic activity. These results indicate that the formation of electroinduced blebs is assisted by some osmotically-dependent processes which lead to the movement of water into the cell, but not out of it as it was suggested in Ref. 9. The presence of inulin in the external medium does not prevent water from running out of the cells, but hinders water running in, simultaneously blocking the formation of blebs. The penetration of other substances (as Trypsin blue) into the cells remains unaffected.

However, the mechanism of electroinduced blebbing apparently cannot be explained only by osmotically-dependent processes that come into effect as the cell membrane permeability goes up. Thus, the correlation between the voltage on different sites of the cell membrane during the pulse and the probability of the formation of electroinduced blebs (see above) cannot be explained only by the disturbance of osmotic equilibrium between the cell and the outside medium. Of interest is also the fact, that formation of electroinduced blebs in cytochalansin-B-treated FBT cells occurred independently from the pre-existing ones. In principle, the colloid-osmotic swelling of the cells after electroporation could have resulted in growth of these cytochalasin blebs.

In this work as elsewhere [9,14], the parameters of the electrical treatment inducing blebbing (electric-field intensity and pulse duration) are close to those used for electroporation, electrofusion and electrically-stimulated introduction of DNA into the cells [1,9,20]. In the majority of relevant works the role of electric field in those processes is explained by the induction of local permeable structures in the membrane – pores of a characteristic size of 5–7 nm [4,6,9,17]. However, data have been accumulated recently which make it doubtful whether the mechanism of electrofusion and electrotransfection can be explained by the formation or pores only [7–10]. Of special importance is the discovery of the electroinduced long-lived fusogenic state [7,21]. It is pointed out in these papers that the areas of the highest competence to fusion are localized on those sites of the cell membrane where maximum voltage drops during the pulse. That is the localization which is typical to the herein-studied large-scale changes in the properties of cell membranes after electrical treatment that manifest themselves in the formation of electroinduced blebs.

There seems to be no evidence to suggest that blebs take part directly in electrofusion and electrically-stimulated introducion of DNA into cells, since both processes take place efficiently in the presence of Ficoll (Ref. 22; Agarkova, unpublished data), which inhibits blebbing in our experiments. Nevertheless, the occurrence of such electrically-induced osmotically-dependent structures (blebs) reflects those rather long-lived (about 3–5 min) changes that take place in certain areas of the cell membrane (and possibly in other cell structures) under the influence of high-intensity electric pulses. The mechanism of such changes still remains to be clarified. It is possible, that a local rupture in the cell's cytoskeleton induced by electric field underlies the electroinduced blebbing. It is unknown now, whether this local rupture can be caused by electric field simultaneously with electroporation, or it is the secondary effect following the electroporation and disturbance of osmotic equilibrium. A theoretical analysis of the properties of the membrane skeleton of an erythrocyte has shown [23] that a breakup of the chemical bond but in one unit of the skeleton network can, as a result of relaxation of the skeleton, initiate the formation of relatively large areas in the cell membrane which would be free of membrane skeleton and poor in integral proteins. Some similar processes may come into effect under certain conditions in other types of cells. The formation of such areas after electrical treatment – and in the first place, in those areas of the cell membrane

where maximum voltages drop during the pulse – may reveal itself in the subsequent formation of blebs (Refs. 9,14, and this paper), in the appearance of a singular long-lived fusogenic state, described in Refs. 7 and 21, and in the alteration of the state of a considerable portion of membrane lipids following electrical stimulation, discovered in Ref. 8. It goes without saying, that the above concepts require a direct experimental verification.

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