Electroporation of Cells and Tissues

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Invited Paper

***Abstract*—Electrical pulses that cause the transmembrane voltage of fluid lipid bilayer membranes to reach at least $U_m \approx 0.2$ V, usually 0.5–1 V, are hypothesized to create primary membrane "pores" with a minimum radius of ~1 nm. Transport of small ions such as Na^+ and Cl^- through a dynamic pore population discharges the membrane even while an external pulse tends to increase U_m, leading to dramatic electrical behavior. Molecular transport through primary pores and pores enlarged by secondary processes provides the basis for transporting molecules into and out of biological cells. Cell electroporation *in vitro* is used mainly for transfection by DNA introduction, but many other interventions are possible, including microbial killing. *Ex vivo* electroporation provides manipulation of cells that are reintroduced into the body to provide therapy. *In vivo* electroporation of tissues enhances molecular transport through tissues and into their constituative cells. Tissue electroporation, by longer, large pulses, is involved in electrocution injury. Tissue electroporation by shorter, smaller pulses is under investigation for biomedical engineering applications of medical therapy aimed at cancer treatment, gene therapy, and transdermal drug delivery. The latter involves a complex barrier containing both high electrical resistance, multilamellar lipid bilayer membranes and a tough, electrically invisible protein matrix.**

***Index Terms*—Bioelectric phenomena, biological effects, cell membrane, drug delivery, electroporation, high-voltage pulses, transmembrane voltage.**

I. General Background

ESSENTIAL features of electroporation include

1) application of short electrical pulses;
2) charging of lipid bilayer membranes;
3) rapid, localized structural rearrangements within the membrane;
4) transitions to water-filled membrane structures, which perforate the membrane ("aqueous pathways" or "pores");
5) tremendous increase in ionic and molecular transport.

Both applications and mechanistic understanding have received growing attention over the past two decades [1]–[10]. This is part of a persistent scientific interest regarding the interaction of electric and magnetic fields with biological systems [11]–[15], with short exposures to strong fields representing an important subset of the general problem.

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II. Weak Field Effects Comparison

There is a long-standing controversy regarding whether reported effects attributed to weak field exposures are real, capable of being understood in terms of established physics and chemistry in the context of biological systems. Briefly, the challenge is to understand how both physical and chemical signal-to-noise ratio (SNR) criteria can be satisfied for weak fields interacting with biological systems. Physical theories have considered competing thermal fluctuations, leading to "thermal noise" limits [16]–[18]. Significantly, more severe constraints arise if the alteration of biochemical processes by a weak field is estimated. For example, a fundamental limit due to "molecular shot noise" generates higher exposure thresholds than obtained from thermal noise alone [19], [20].

There is little doubt that animal sensory systems detect very weak steady (dc) fields. Anomalies that are a few percent of the earth's magnetic field ($B_{\rm earth} \approx 5 \times 10^{-5}$ T) can be detected [21], [22]. Similarly, sharks and rays sense extremely weak electric fields in sea water: their detection threshold is $\sim 5 \times 10^{-9}$ V cm^{-1}, accomplished in a ~1-s exposure. Biological sensing of weak magnetic fields is not as well understood. A magnetite-based mechanism is favored [23], [24], but a radical pair mechanism has also been proposed [25], [26]. The elasmobranch fish electric field sensory system is much better understood [27], satisfying both thermal noise [28] and molecular shot noise estimates [29]. Previous and ongoing theoretical efforts suggest that weak field sensory systems can be understood using known biophysical mechanisms for coupling fields to ongoing, metabolically driven biochemical processes.

In contrast, reports originating from *in vitro* studies of weak 50–60-Hz fields are difficult to understand. Most *in vitro* studies involve relatively unorganized cell systems compared to evolved sensory systems. Without involvement of a presently unknown, extremely strong biophysical mechanism coupling, it is difficult to understand how field-induced molecular changes can complete with other sources of change in the same biochemical pathway. Prolonged (> 100 s) exposures pose a particularly demanding challenge [30]. Briefly, unless the equivalent of a weak field-sensory system is involved, it is difficult to reconcile reports of effects due to weak field exposures with what is known about physics and chemistry in the context of a biological system.

Electroporation, however, involves orders of magnitude larger fields and readily passes conceptual tests based on SNR criteria, in spite of the short exposure time. Thermal noise is easily overcome [16], and even the delivery of a small number ($\overline{n} \approx 400$) of lethal bleoymcin molecules into individual cells [31] satisfies a molecular shot noise-based criterion: (S/N) $= \sqrt{\overline{n}} \approx \sqrt{400} = 20 \gg 1$. With no bleomycin initially present, the delivery of a small number of molecules can be regarded as a "signal" against zero background, just the opposite of weak field exposures that create small molecular changes (e.g., Ca^{++} transport) against a relatively large background. There is no doubt, therefore, that large, robust effects occur for exposures associated with electroporation.

III. Electroporation Motivation

Several classes of applications have been identified, with *in vitro* electroporation widely used to transfect suspended or anchored cells in laboratory apparatus by introducing DNA. Essentially, any size molecule can be introduced into the cell cytoplasm or transported across a vesicle membrane. For example, Ca^{++} ions can be gently introduced to mimic exposure to a hormone [32], antibody molecules (~150 000 $gmol^{-1}$) can be introduced to block specific biochemical pathways, and even micrometer-size particles can be introduced [33], [34]. In a very different application, biochemical reactions involving surface-immobilized vesicles can be rapidly initiated using microelectrodes [35].

Nonthermal destruction of bacteria and yeast has long been known [36]–[38]. Purposeful killing of microorganisms and other organisms is increasingly pursued, even though the basic mechanism of cell killing is not fully understood (discussed elsewhere in this issue).

Ex vivo applications typically involve blood cells, treated outside the body, with reintroduction to provide therapy. White cells are somewhat larger than red blood cells, and can be electroporated to load drugs into white cells in the presence of red cells [40]. Platelet cells can also be loaded [41]. The oxygen binding capability of red cells can be manipulated [42], and electroinsertion of appropriate proteins into the red cell membrane accomplished [43], all under sufficiently mild electroporation conditions that cells returned to the body circulate with long lifetimes.

In vivo applications include the local delivery of potent but relatively membrane impermeable anticancer drugs into solid tumors [44], [45]. Electrochemotherapy (ECT) has now reached clinical trials [46], [47]. Motivating *in vivo* experiments showed that orders of magnitude more bleomycin is delivered into cell interiors than by passive permeation. New anticancer drugs, which consisted of established drugs with added charge groups (to prevent spontaneous entry), would in principle be attractive but would face needing full regulatory approval as new drug entities.

Gene therapy is of great interest, predominantly approached by using virus to introduce foreign DNA into cells. Because viral methods face several problems, a physical method such as local tissue electroporation is of interest [49]–[55]. DNA is first introduced to a tissue (e.g., by needle injection), and then electrical pulses are used to electroporate the cells of a tissue within a treatment volume.

Both ECT and localized gene therapy can be regarded as examples of drug delivery, in which a therapeutic agent is introduced into desired regions or cells within the human body. Drug delivery is a significant research area [56], with particular interest in minimally invasive drug delivery such as transdermal drug delivery. Controlled delivery through the skin is potentially valuable, because the technology can be located outside the body and unobtrusive. One approach is based on the hypothesis that the ~100 multilamellar lipid bilayers of the skin's outermost layer can be electroporated [9], [57]. A brief description of skin electroporation is presented near the end of this paper.

IV. Early Investigations

The first observation of a new phenomenon may not lead to further study. To our knowledge, the first indication of electroporation was seen in electrical measurements on an excitable membrane, but apparently not pursued. However, the observation of electrically stimulated molecular transport through vesicle membranes [59] and inactive uptake of DNA into red blood cells [60] was followed by the important demonstration that DNA could be introduced to transfect cells [61], [62]. Although there is space to cite only a few of many additional papers, during these early years, there were also important experimental studies of reversible and irreversible electrical breakdown in artificial planar bilayer membranes and studies of breakdown and molecular uptake in vesicles and cells [63]–[70]. Since the early 1980's, there has been a large increase in the study and application of electroporation.

Initial theory of electroporation was tightly coupled to experiments on planar membranes, appearing as seven back-to-back papers (only the first is cited here [71]). Similar early theories invoked transient aqueous pores to estimate membrane rupture [72], [73]. Subsequent theory development has led to a reasonable quantitative description of several key features of electroporation [7].

V. Molecular Uptake

Most electroporation applications aim to transport extracellular ions or molecules into cells. A typical *in vitro* apparatus involves parallel plane electrodes contacting an aqueous electrolyte with suspended cells (Fig. 1). A typical experimental protocol involves suspending cells in an aqueous medium containing water soluble molecules of interest, applying one or more pulses with durations in the 10^{-6}—10^{-2} s range, waiting for cell membrane recovery ("resealing"), and then removing the cells for use or study. Some experiments use charged fluorescent molecules with a large extracellular concentration (Fig. 2). After an electroporating pulse and membrane recovery, molecules are present within cells interior (cytosol) at a concentration far below the equilibrium value (Figs. 8–10) expected for long-lasting membrane holes.

Biological systems contain large numbers of molecules that have the potential to interact and react. One source of biological control is spatial compartmentalization. Tissues with cell

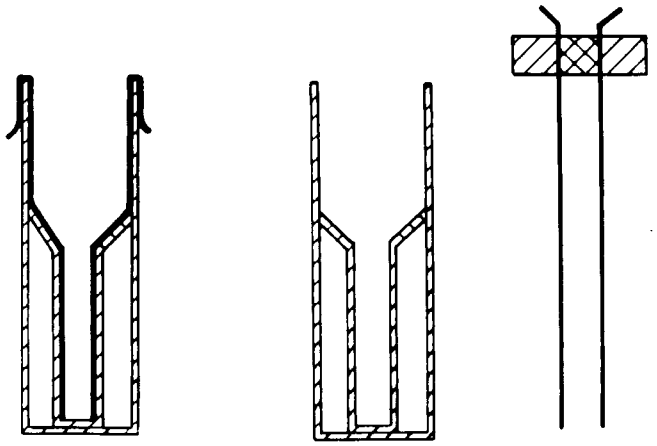


Fig. 1. Typical *in vitro* electroporation apparatus. Cross-sectional drawing of a cuvette with parallel plane metal electrodes for pulsing a cell suspension.

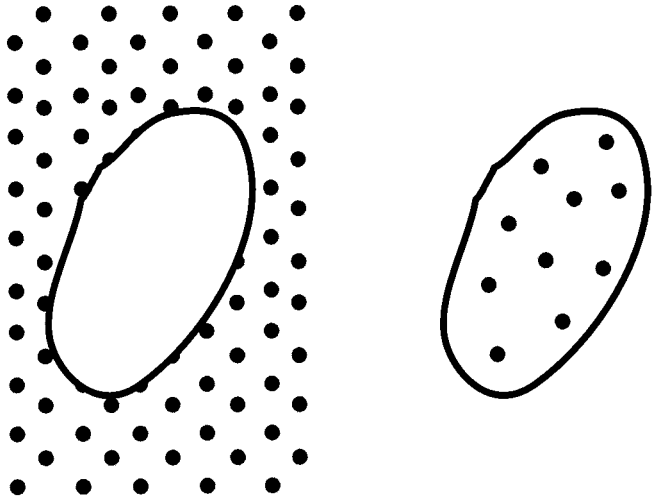


Fig. 2. Illustration of molecular uptake by electroporation. The irregular central object is a cell. (a) High extracellular concentration of a fluorescent molecule (solid ellipsoidal dots). (b) Nonequilibrium concentration of fluorescent molecules inside cell after a single electroporating pulse, membrane recovery, and removal of the extracellular supply solution [75], [76].

layers connected by tight junctions, cell membranes, and some subcellular structures all feature transport barriers based on a single lipid bilayer membrane, ~6 nm thick, containing macromolecules such as receptors, enzymes, and channels. Most biological molecules contain exposed charge groups. Importantly, a pure lipid bilayer membrane itself is a formidable barrier to transport of transport, preventing significant insertion (dissolution) of charge into the low dielectric constant ($K_{\rm m} \approx 2$) interior of a lipid bilayer [78]. For even a single charge, the free energy change is ~100 kT (Fig. 3), sufficient to prevent significant spontaneous entry.

VI. Membrane Structural Rearrangements

Fluid lipid bilayer membranes can be imagined to undergo various spontaneous rearrangements (Fig. 4), driven by thermal

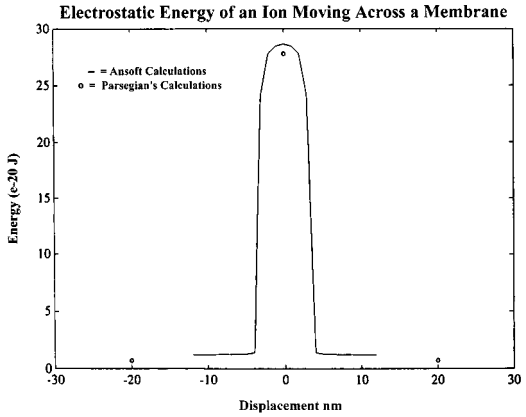


Fig. 3. Estimate of the lipid bilayer barrier [77]. Energy barrier for charge insertion based on the electrostatic energy change as a singly charged ion is moved from water into and across a bilayer membrane of thickness 6 nm.

fluctuations at a significant rate, if the associated free energy difference is small. This basic concept has been used to understand passive bilayer membrane permeation and to develop a theory of electroporation based on the emergence of "hydrophilic pores" as favored rearrangements for elevated transmembrane voltages.

VII. Primary Changes

Biological systems are electrically heterogeneous [15] [82]. Application of an electric field pulse results in rapid polarization changes that can deform mechanically unconstrained cell membranes (e.g., suspended vesicles and cells) followed by ionic charge redistribution governed by electrolyte conductivities and distributed capacitance. For most cells and tissues the latter charging times are of order $\tau_{\rm CHG} \approx 10^{-6}$ s. Thus, if $U_{\rm m}$ is to exceed 0.5–1 V, much larger pulses must be used if the pulse is significantly shorter than $\tau_{\rm CHG}$.

Electroporation is hypothesized to involve inhomogeneous nucleation of primary, hydrophilic pores [Fig. 4(d)] based on transitions from much more numerous hydrophobic pores [Fig. 4(c)]. The basic idea is that a circular region of membrane is replaced with a pore (Fig. 5).

As primary pores appear in the membrane, its resistance drops, and the voltages within the system redistribute on a time scale governed by the instantaneous values of the various conductivities and capacitance. Both experiment and theory show that the membrane capacitance change is small [89], [90], so that the main electrical result is drastically decreased barrier resistance. Overall, bilayer membrane electroporation results in dynamic, nonlinear changes as a heterogeneous pore population evolves rapidly in response to the local value of the transmembrane voltage $U_{\rm m,local}$ along the surface of a cell membrane. At the time of maximum membrane conductance, pores are nevertheless widely separated, occupying only about 0.1% of the electroporated membrane area [84], [90]. In this sense, electroporation is catalytic [91]. Not only is there the possibility of binding and lateral diffusion to the other side of the membrane as pores form and then vanish, the tremendous increases in rate (of transport) is due to small entities (pores) that occupy a small fraction of the membrane.

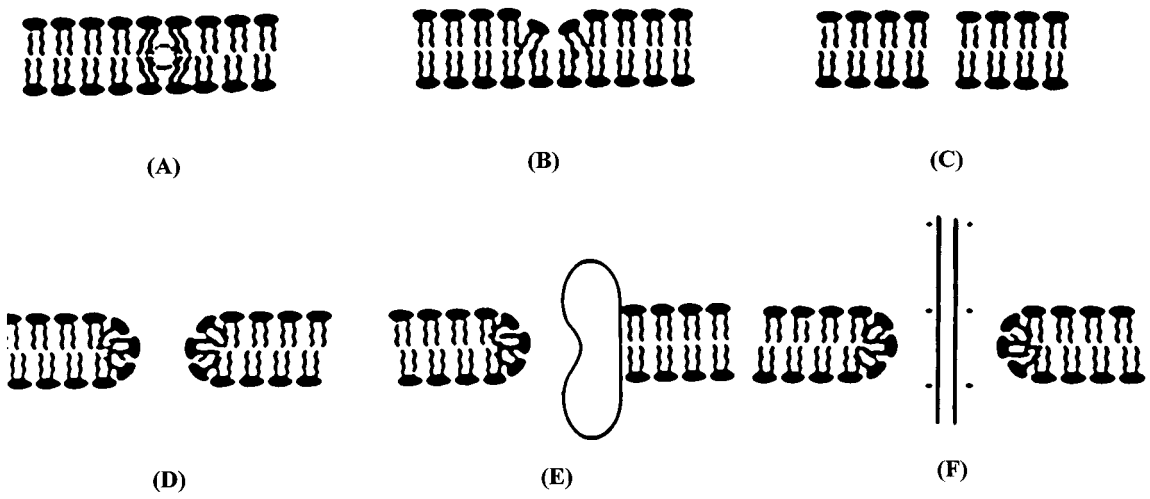


Fig. 4. Hypothetical bilayer membrane structural rearrangements [79]. (a) Free volume fluctuation allowing entry of an uncharged molecule [80] (dotted circle); (b) dimple (local membrane compression and thinning); (c) lateral fluctuation or "hydrophobic pore," envisioned to be a precursor to a hydrophilic pore [7], [71] and a possible route for water transport [81]; (d) hydrophilic pore believed to dominate electroporation onset [7] [71]; (e) composite pore involving a membrane protein [79]; and (f) "foot-in-the-door" interaction based on insertion of a long, charged molecule into a hydrophilic pore while U_{m} is large [79].

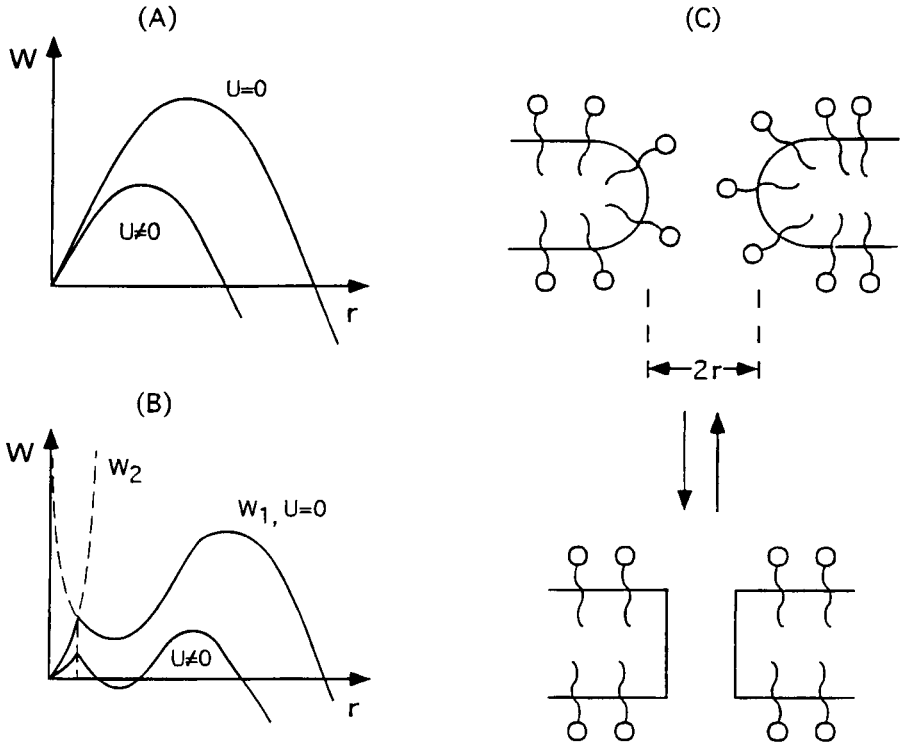


Fig. 5. Pore creation free energy change [7]. A transition from hydrophobic pores (lower right) to hydrophilic pores (upper right) is hypothesized, with a strong, nonlinear dependence on the local transmembrane voltage.

VIII. Secondary Heating Changes

Joule heating accompanies electrical currents passing through an aqueous electrolyte, so that electric field pulses increase the temperature of the bulk electrolyte surrounding cells. Typically,$(\Delta T)_{\mathrm{e}}$ is on the order of 1 °C–10 °C and is usually insignificant. However, pores concentrate the current crossing the membrane to the few sites occupied by pores, so there is the question of local heating. The estimate is $\Delta T \ll 1$ °C for cell membranes, but ΔT can approach 10 °C–100 °C within the dead stratum corneum of skin [9], [92].

IX. Secondary Membrane-Level Changes

Once the membrane resistance has dropped, a membrane tends to rapidly discharge, but pores are metastable, disappearing on a much longer time scale (often >1 s) than their creation time (~10^{-6} s). The strong electrical energy interaction at $U_{\mathrm{m}} \approx 1$ V is replaced by two or more orders of magnitude smaller electrical energy after discharge, and under these conditions the interaction of membrane constituents and inserted molecules (Fig. 4) can predominate. Membrane recovery (resealing) is not only slow; it is generally temperature dependent.

Some pores remain open for long times, during which ions and molecules can continue to cross the cell membrane by diffusion and electrically driven transport associated with diffusion potentials and metabolically driven membrane pumps [93].

Surfactants provided extracellularly can greatly accelerate membrane recovery by bridging a pore opening with a charged region of the surfactant molecule, thereby providing a new barrier to charged species transport at the site of a pore [94]. This provides an important approach to treatment for electrical injury, with cell damage now established to involve electroporation of long cells (e.g., skeletal muscle and nerve cells) rather than only tissue heating.

X. Secondary Cellular-Level Changes

Even if complete membrane recovery occurs, a cell may be sufficiently perturbed that it is killed. All that is needed is generation of sufficient cell stress, which can be accomplished by outward transport of essential ions and molecules, but is more easily accomplished by introducing extracellular molecules than are toxic. An extreme case is introduction of bleomycin during ECT, which can kill cells at very low extracellular concentrations ($\sim 10^{-9}$ M) if the cell is electroporated, but requiring orders of magnitude higher drug concentrations without electroporation.

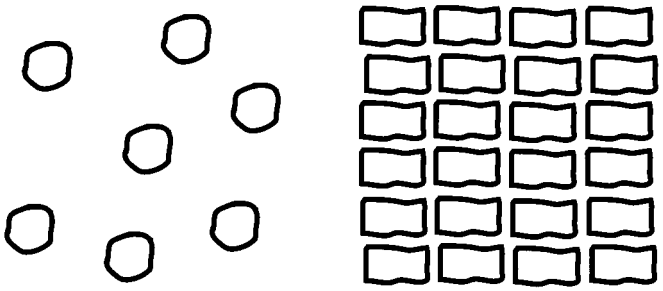
Until membrane recovery is completed, a cell is more susceptible to stress through loss of ions and molecules, which can be diluted into the extracellular fluid. As suggested by Fig. 6, this motivates the hypothesis that the ratio of intra- to extracellular volume

$$R_{\text{volume}} = \frac{V_{\text{extracellular}}}{V_{\text{intracellular}}} \tag{1}$$

is relevant [77]. Typical *in vitro* electroporation conditions involve a large value of R_{volume}. For example, a suspension of mammalian cells at 10^5 cells cm^{-3} has $R_{\text{volume}} \approx 10^4$ ($V_{1\ \text{cell}} \approx 10^{-9}$ cm^{-3}). In contrast, the extracellular volume of many "solid" tissues is ~0.1. Because of this 10^5-fold difference, *in vivo* tissue electroporation may involve minimal loss of essential ions and molecules into the extracellular fluid near electroporated cells. Solid tissue electroporation should therefore involve more favorable conditions for cell survival than typical *in vitro* conditions.

XI. Overall Cell Recovery

Several factors are thus believed to govern cell recovery. First, as noted above, pores within lipid bilayer membranes are generally metastable, with lifetimes at 25 °C of order ~1 s or more. Local molecular interactions reflecting the composition of the membrane are important once U_{m} has returned to a low value through membrane discharge. Further, membrane enzymes and channels may take a long time to recover from being driven into ordinarily improbable conformational states [96], [97]. Once membrane recovery is achieved, the long-term outcome for electroporated cells also depends on the recovery of their intracellular chemical pool, which is expected to depend on cell type and state, external medium composition, and R_{volume}. Thus, although cell membrane electroporation itself can be gentle, involving rapid nonthermal structural membrane rearrangements, biochemical imbalances can determine whether or not a cell 1) recovers and survives or 2) becomes irreversibly stressed, leading to death.



(a) (b)

Fig. 6. Schematic illustration of typical *in vitro* and *in vivo* extracellular volumes [77]. (a) Widely separated cells *in vitro*. (b) Closely packed cells in solid tissue. Cell survival may be optimal for *in vivo* tissue electroporation.

XII. Nonequilibruim Uptake

The transient aqueous pore theory of electroporation rests on the hypothesis that the primary pore creation rate is a nonlinear function of the local transmembrane voltage [7], [71], [90] $U_{\text{m, local}}$, with pores expanding or contracting rapidly. An unexpected prediction is appearance of a plateau in U_{m} for exponential pulses (Fig. 7).

If most ionic and transport occurs through pores by electrophoresis and electro-osmosis when U_{m} is large, then the transport driving force is approximately constant during most of the time when U_{m} is large. This is the basis of the qualitative prediction that charged molecule transport should exhibit a plateau in molecular uptake per cell as pulse magnitude is increased. Moreover, the transport per pulse can be a few percent of the equilibrium value (cell volume × extracellular concentration). As shown in Figs. 8–10, experiments are consistent with this prediction.

XIII. Skin Transport Barriers

Human skin provides a formidable barrier against dessication, mechanical injury, and entry of infectious microorganisms and toxic chemicals. It also stands in the way of transdermal drug delivery. The stratum corneum (SC) is the skin's outermost ~20-μm-thick layer and also the site of the main barrier function [99], [100], such that a "brick wall" [101] with multilamellar lipid bilayer ("mortar") surrounding keratin-filled corneocytes ("bricks") represents this tremendous barrier to ionic and molecular transport. We use a simplified version of the brick wall model (Fig. 11) to consider the skin electroporation hypothesis.

XIV. Skin Electroporation

The hypothesis of human skin electroporation is based on the recognition that one the order of 100 lipid bilayer membranes must be traversed in crossing the SC. If $U_{\text{m}} = 0.5$–1 V electroporates single bilayers, then pulses that cause the transdermal voltage to reach $U_{\text{skin}} = 50$–100 V should cause electroporation

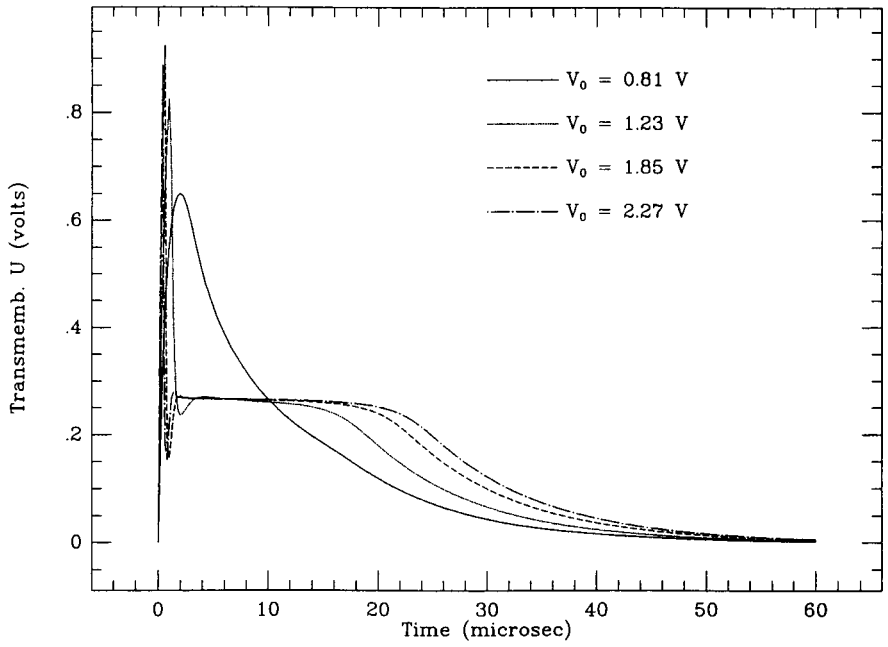


Fig. 7. Predicted behavior of $U(t)_{\mathrm{m}}$ showing a voltage plateau [90]. This simulation involved application of a single exponential pulse with $\tau_{\mathrm{pulse}} = 10\ \mu$s. A moderate pulse ($V_0 = 0.81$ V) creates relatively few pores, so U_{m} rises to about 0.65 V and then decays with the imposed pulse. The three progressively larger magnitude pulses ($V_0 = 1.23$, 1.85, and 2.27 V) all cause reversible electrical breakdown, seen as an abrupt voltage drop after reaching $U_{\mathrm{m}} \approx 0.8$ V, dropping to $U_{\mathrm{m}} \approx 0.28$ V for the interval 2 to about 20 μs, and then decaying. Our interpretation is that pores rapidly decrease their size, increasing the membrane resistance, so that a variable voltage divider effect creates the voltage plateau as long as sufficient pores are present.

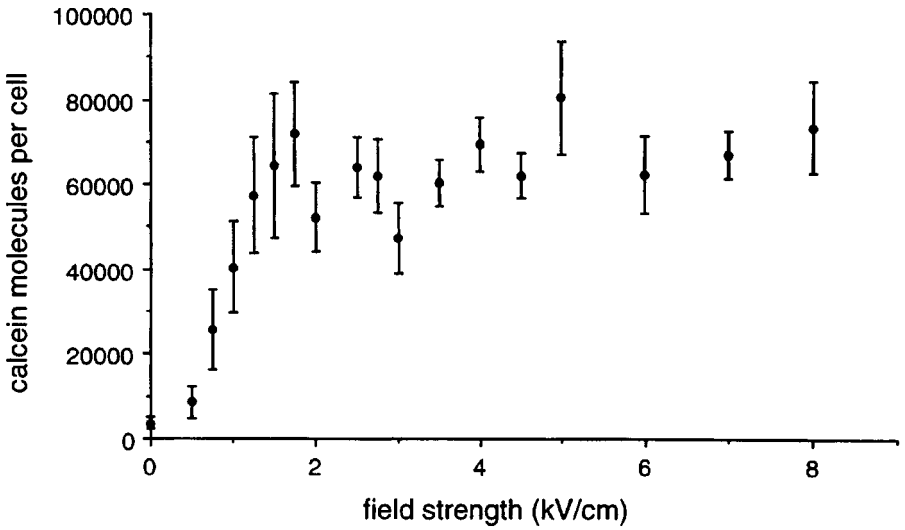


Fig. 8. Calcein uptake versus single pulse magnitude for red blood cell ghosts [98]. Calcein (623 g mol $^{-1}$; charge is –4) uptake per cell. Above ~1.5 kV/cm, the measured uptake is $\overline{n} = (7 \pm 1) \times 10^4$ molecules cell $^{-1}$, only about 0.7% of the equilibrium value.

of the multilamellar bilayers within the SC [9], [98]. This should create primary aqueous pathways similar to single bilayer membrane transient aqueous pores. For $U_{\mathrm{skin}} < 5$ V, iontophoresis through preexisting pathways occurs; and for $5 \leq U_{\mathrm{skin}} < 50$ V, electroporation of the cell linings of sweat ducts and hair follicles should occur [110]. However, most of the skin's area is occupied by the dead SC, which should electroporate for $U_{\mathrm{skin}} > 50$ V. Many electrical and molecular transport measurements confirm this expectation. *In vivo* protocols using transdermal pulsing show that skin irritation and damage is minimal [45]–[47], [111], [112]. However, the electrically invisible keratin matrix within electroporated corneocytes now becomes important as larger molecules may become trapped. [103].

XV. Microconduit Creation

To overcome the keratin matrix barrier, the skin electroporation hypothesis has been extended to include introduction of keratolytic molecules into corneocytes [113], [114]. Previous

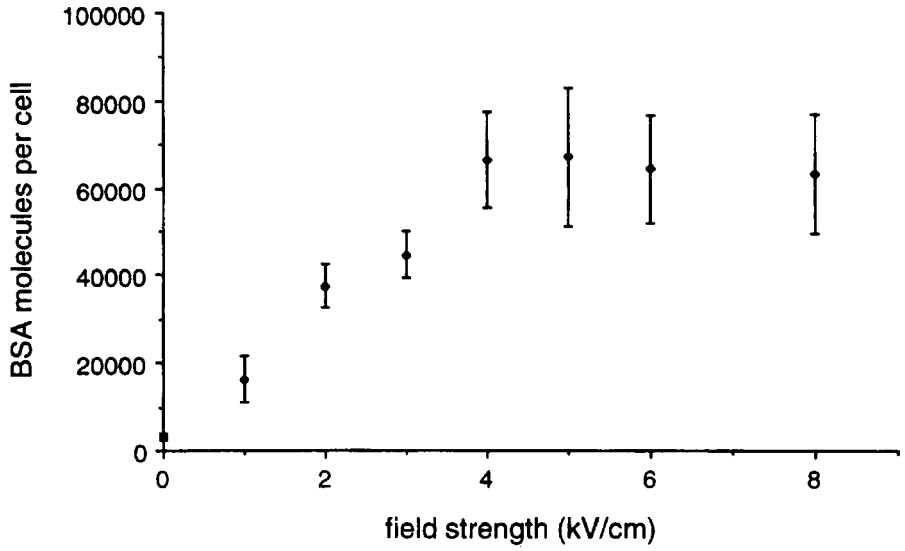


Fig. 9. BSA uptake versus single pulse magnitude for red blood cell ghosts [98]. Bovine serum albumin (~65 000 g mol $^{-1}$; charge ≈ -25) uptake per cell. Above ~4 kV/cm, the measured uptake is $\overline{n} = (6.5 \pm 1) \times 10^4$ molecules cell $^{-1}$, about 7% of the equilibrium value. BSA requires larger pores, which may involve field-induced primary pore enlargement as the larger. Charged molecule is pushed by pore-focused fields toward the pore, and this may be the basis of the plateau beginning at a larger field value than for calcein (Fig. 8).

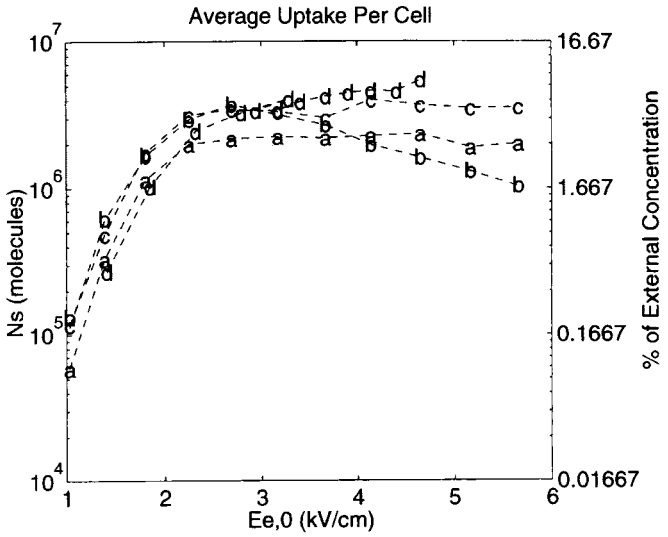


Fig. 10. Calcein uptake versus single pulse magnitude for yeast cells [82]. In spite of a very heterogeneous response, above ~2.5 kV/cm the measured uptake is $\overline{n} = (1.5 \pm 1) \times 10^6$ molecules cell $^{-1}$, about 2.5% of the equilibrium value.

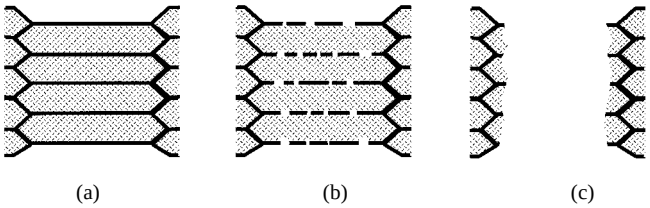


Fig. 11. Simplified brick wall model [102]. Illustration of skin site before electroporation, after electroporation alone, and creation of a microconduit by introducing keratolytic agents by electroporation. The dark, solid lines represent five to six lipid bilayer membranes between corneocytes, with aqueous pathways (light regions in center and right parts) created by electrical pulses (electroporation). Hatched regions indicate keratin matrix within corneocytes. A single local transport region [103] is depicted, showing only 1/3 of the 15–16 corneocyte layers of the SC. LTR's can be forced to occur at predetermined sites by exposing a small skin area to electroporating pulses [107]. For simplicity, ordered stacks (columns) of corneocytes similar behavior is expected with disordered (offset) columns. (a) Prior to aqueous pathway creation. (b) After electrical pulses. Early experiments used viable frog skin [108], with a much thinner barrier. (c) Minimum size microconduit (~50 μm diameter), caused by removal of most of a single stack of corneocytes after keratin matrix disruption. Recent experiments [109] used an electrically insulating mask with a 200-μm diameter hole to create a single microconduit of about the same size.

studies have shown that electroporation spontaneously concentrates at localized transport regions (LTR's) located almost randomly over the skin [103]–[106]. Still other experiments have shown that an electrically resistant mask with an array of microholes (40–100-μm diameter) can restrict electroporation to predetermined sites [107]. This provides an approach to creating single microconduits at predetermined locations [109]. This process is analogous to semiconductor microfabrication: a spatially localized physical perturbation (here electroporation) is followed by a chemical disruption (here of the keratin matrix) that results in localized removal of material (here components of the SC). The result is *in situ* creation of microconduits, which are SC-spanning openings that can transport essentially any size molecule.

XVI. Breakdown and Electropermeabilization

Breakdown often means "dielectric breakdown," either irreversible or reversible, and usually requires $U_{\rm m} \gg 1$ V. Even though the electric field within the membrane is very large [$E_{\rm m} \approx 2 \times 10^{-8}$ V m $^{-1}$ (2×10^{-6} V cm $^{-1}$), for $U_{\rm m} \approx 1$ V], there is not enough energy ($zeU_{\rm m}$) to ionize most molecules. Instead, charged, mobile molecules are transported. Given the partial success of the transient aqueous pore theories, it seems better to interpret the membrane's high conductance as due to membrane-penetrating aqueous pathways, not dielectric breakdown.

Permeability refers to a phenomenological parameter $P_{\rm m}$ for molecular transport. An example is diffusive permeation. The steady-state rate is $\dot{n} = A_{\rm m} P_{\rm m} \Delta c_{\rm s}$, with $A_{\rm m}$ the membrane

area and $\Delta c_{\rm s}$ the slowly changing concentration difference across the membrane for molecular or ionic species "s." Transport takes place by dissolution (partitioning) into the membrane, diffusion across the membrane, and then dissolution into the medium on the other side of the membrane. The associated diffusive membrane permeability is $P_{\rm m} = g_{\rm s} D_{\rm s}/d_{\rm m}$, where $g_{\rm s}$ is the partition coefficient (solubility in the membrane relative to the bathing medium for the case that both sides of the membrane contact the same medium), $D_{\rm s}$ is the diffusion constant of "s" within the membrane, and $d_{\rm m}$ is the membrane thickness.

Although "electropermeabilization" is used to describe molecular transport across electroporated cell membranes, transport appears to involve more than a permeability increase. Not only are contributions from diffusion involved; local electrophoresis and electro-osmosis appear to dominate during a pulse, and then the local electric field within the aqueous pathway plays an important role. For example, the far-from-equilibrium results of Figs. 8–10 are consistent with transport through a dynamically changing pore population, not a long-lifetime membrane opening.

The view that electroporation involves more than electropermeabilization is further supported by electroinsertion of proteins into cell membranes [43], [115]–[117]. This is much more than a permeability change. Further, electroporation theory [7] predictions include:

1) either rupture or reversible electrical breakdown for an artificial planar bilayer membrane, depending on the applied pulse characteristics;
2) the approximate magnitude of the transmembrane voltage associated with rupture;
3) the magnitude of maximum transmembrane voltage ($U_{\rm m} \approx 1$ V for short pulses) during reversible electroporation;
4) the stochastic nature of rupture;
5) the maximum fractional aqueous area ($\sim 0.1\%$) during reversible electrical breakdown;
6) the order of magnitude of the number of transported small charged molecules for a single pulse, including the existence of an approximate plateau as a function of pulse magnitude.

In short, the electroporation hypothesis provides testable predictions (to date mostly observed experimentally) that involve more than electropermeabilization.

XVII. Summary

Most transport barriers protecting active compartments of biological systems are based on one or more lipid bilayer membranes. Applications of short pulses that drive the transmembrane voltage to 0.5–1 V appear to create primary aqueous pathways ("pores") with radii $r_{\rm p} \approx 1$ nm. A dynamic interaction involving both membrane charging and discharging governs a rapidly evolving pore population, which in turn controls electrical behavior and molecular transport. Many applications of electroporation exist, and more are likely, ranging from *in vitro* to *ex vivo* to *in vivo* manipulations that provide controlled transport of molecules in and out of cells and tissues.

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James C. Weaver, photograph and biography not available at time of publication.