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Electroporation: A General Phenomenon for Manipulating Cells and Tissues

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Abstract Electroporation is a fascinating cell membrane phenomenon with several existing biological applications and others likely. Although DNA introduction is the most common use, electroporation of isolated cells has also been used for (1) introduction of enzymes, antibodies, and other biochemical reagents for intracellular assays; (2) selective biochemical loading of one size cell in the presence of many smaller cells; (3) introduction of virus and other particles; (4) cell killing under nontoxic conditions; and (5) insertion of membrane macromolecules into the cell membrane. More recently, tissue electroporation has begun to be explored, with potential applications including (1) enhanced cancer tumor chemotherapy, (2) gene therapy, (3) transdermal drug delivery, and (4) noninvasive sampling for biochemical measurement. As presently understood, electroporation is an essentially universal membrane phenomenon that occurs in cell and artificial planar bilayer membranes. For short pulses (μs to ms), electroporation occurs if the transmembrane voltage, U(t), reaches 0.5–1.5 V. In the case of isolated cells, the pulse magnitude is 10^3–10^4 V/cm. These pulses cause reversible electrical breakdown (REB), accompanied by a tremendous increase molecular transport across the membrane. REB results in a rapid membrane discharge, with the elevated U(t) returning to low values within a few microseconds of the pulse. However, membrane recovery can be orders of magnitude slower. An associated cell stress commonly occurs, probably because of chemical influxes and effluxes leading to chemical imbalances, which also contribute to eventual survival or death. Basic phenomena, present understanding of mechanism, and the existing and potential applications are briefly reviewed.

Key words: electroporation, DNA introduction, reversible electrical breakdown, cell stress, molecular transport

The term *electroporation* is widely used to denote the dramatic phenomena that accompany large transmembrane voltages caused by electrical pulses [1–4]. However, other terms such as "electropermeabilization" and "electroinsertion " are also used, even though these suggest more a specific mechanism (viz. permeation) or a specific end result (viz. introduction of molecules into cells). Early studies using such pulses found both irreversible [5], and reversible [6] effects in cells, with some giving evidence for involvement of some type of pore [7]. Other studies used artificial planar bilayer membranes to show that irreversible effects generally occured in these simpler systems [8], but that for some planar membranes both irreversible and reversible effects occurred [9]. Since then many studies have extended these initial observations [1–4]. More recently, rapid optical measurements support the idea that some type of rapid membrane structural rearrangement occurs, coincident with membrane conductance changes, which is consistent with pore formation [10]. The occurrence of electrofusion is a companion phenomena which goes beyond permeability and insertion. Thus, an attractive hypothesis is that some type of aqueous pathways ("pores") appear because of the increased transmembrane voltage. Finally, physical models based on transient aqueous pores appear capable of explaining some essential features of the mechanical, electrical and molecular transport behavior. For all of these reasons, the term "electroporation" is increasingly used.

Common to all these terms is the idea that the natural barrier function of a membrane is overcome, so that ions and water soluble molecules can readily cross the membrane. Although the microscopic mechanism by which molecular transport occurs is not yet established, there has

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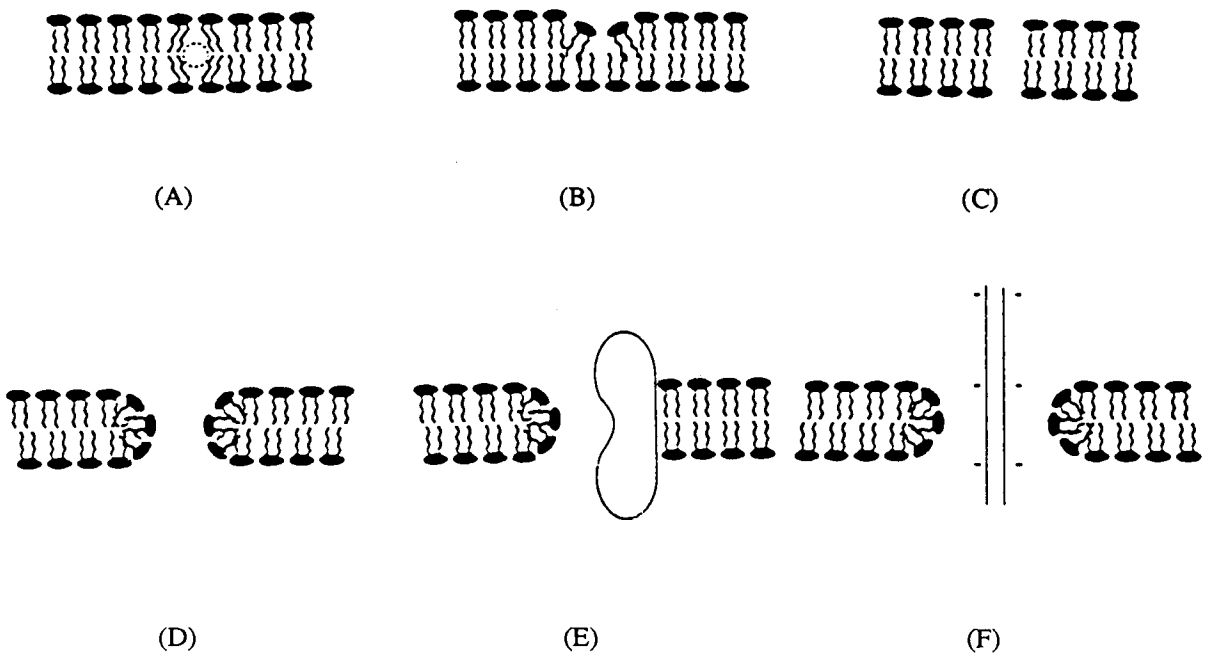

Fig. 1. Drawings of hypothetical structures for transient and metastable membrane conformations which are believed relevant to electroporation. **A:** Free volume fluctuation [52]. **B:** Aqueous protrusion or "dimple" [14,15]. **C:** Hydrophobic pore [8]. **D:** Hydrophilic pore [8,53,54], usually regarded as the "primary pores" through which ion and molecules pass. **E:** Composite pore with one or more proteins at the pore's inner edge [32]. **F:** Composite pore with "foot-in-the-door" charged macromolecule inserted into a hydrophilic pore [11]. The transient aqueous pore model assumes that transitions from A → B → C or D occur with increasing frequency as U is increased. Type F may form by entry of a tethered macromolecule, while the transmembrane voltage is significantly elevated, and then persist after U has decayed to a small value through pore conduction. It is emphasized that these hypothetical structures have not been directly observed, and that support for them derives from interpretation of a variety of experiments involving electrical, optical, mechanical, and molecular transport behavior.

been significant progress in understanding electrical behavior (voltage, conductance, and capacitance of the membrane), mechanical behavior (recovery or rupture of planar membranes), and some progress in understanding molecular transport (numbers of molecules which cross the membrane). However, there has been relatively little progress in understanding membrane recovery (restoration of the barrier as time progresses) and ultimate cell fate (survival or death).

NATURE AND ORIGIN OF PORES

Qualitatively, electroporation is though to involve the stochastic creation of microscopic pores through a joint contribution of "kT energy" (stochastic; associated with fundamental thermal fluctuations) and electrical energy (deterministic; associated with the elevated transmembrane voltage). These "primary" pores are thought to be transient (Fig. 1). As the pores evolve, they are believed to have fluctuating sizes, which leads to the expectation that many pores with a range of pore sizes (a "pore population") is transiently created. In the more complex cell membranes, interactions of transient aqueous pores with other molecules (e.g., protruding cytoplasmic macromolecules) and cellular structures (e.g., the cytoskeleton) may lead to long lifetime metastable pores which persist long after the membrane discharges (within μseconds of a pulse). Finally, consistent with the idea that electroporation is a physical process, electroporation is believed to be essentially universal. Differences in biochemical composition of the membrane appear to be overwhelmed by slightly different transmembrane voltages, so that (at least for short pulses) electroporation onset generally occurs if the transmembrane voltage reaches 0.5–1.5 V. Also supporting universality is the fact that electroporation-related phenomena have been widely observed in artificial planar bilayer membranes, vesicles, isolated cells of many types, and the cells of tissues.

The primary pores that participate in electrical behavior and molecular transport are thought

to be "hydrophilic pores," with a minimum radius of about 1 nm, and a statistical distribution of sizes up to several times this. It is likely that these primary pores cannot be visualized by any known form of microscopy, due to their small size, fluctuating and transient nature, and a lack of contrast between water and the membrane [11]. For this reason, understanding of the structure of primary pores is likely to be inferred from their ionic and molecular transport properties. Based on present knowledge, the very large pores observed by electron microscopy in erythrocyte membranes are believed to be secondary pores, perhaps caused by enlarging primary pores by pressure-driven flow [12], because they appear long after membranes are known to discharge.

Membranes are microscopic systems, and therefore experience fluctuations [13]. In the case of phospholipid bilayer, those with most relevance to electroporation are the membrane conformational fluctuations that involve entry of water and water soluble molecules into the membrane [14–16]. According to the transient aqueous pore hypothesis, the energy needed to form an aqueous pore is reduced as the transmembrane voltage is increased by application of an external electric field. The stochastic entry of, and departure from, water into a pore governs pore evolution [17,18]. This in turn ensures that a distribution of pore sizes will be present. Indeed, use of a transient aqueous pore theory that can quantitatively explain much of the electrical behavior for short pulses fundamentally and explicitly involves a heterogeneous pore population [17,19].

As shown in Figure 1, a variety of types of pores have been suggested. Hydrophobic pores are assumed to form first, with transitions to hydrophilic pores at larger r because the energy cost to make the pore circumference ("edge energy") is much larger than for hydrophilic pores [8]. This is easy to understand qualitatively: the interfacial energy for the hydrocarbon chains and water is much larger than that for the head groups and water. The hydrophilic pores are believed to be metastable over short time scales. Composite pores involving membrane proteins may also be possible, and may have smaller effective values of "edge energy" and longer metastable lifetimes. Entry of transported or of cell-attached intracellular molecules into a pore may prevent pores from shrinking, due to electrostatic repulsion, also leading to long lifetime pores. Finally, secondary very large pores may evolve from the primary transient aqueous pores because of pressure driven flows [12].

Vesicles and cells are separated from the external medium by a closed membrane, so that interfacial polarization plays an important role by causing large changes in the transmembrane voltage, $\Delta U(t)$, by external electric field changes, $\Delta E(t)$. The case of an isolated spherical membrane is well known to have ΔU described by [20]

$$\Delta U(t) = 1.5E(t)R_{cell}\cos\theta. \quad (1)$$

Here R_{cell} is the cell's radius, θ is the angle between the applied electric field, $E(t)$, and the site on the cell membrane at which U is measured. Generalizations of this equation to nonspherical shapes also predict a significant dependence on cell size. Note that unlike the exposure of biological systems to chemical challenges (usually concentration magnitudes, seldom concentration gradients), exposure to electric fields fundamentally involves the vectorial nature of the field (magnitude and direction). This leads to many more possible "exposure" conditions at the individual cell level for the same externally applied electric field [21].

It should also be emphasized that equation 1 is really valid only for small electric fields, viz. those which result in negligible electroporation. Once enough pores appear that the membrane conductivity changes, equation 1 is invalid. Equation 1 provides a general coupling mechanism, one with considerable amplification, i.e., a change in the external electric field results in a much larger change in the transmembrane electric field: $E_m = U/d$, where d is the thickness of the membrane. Because of the R_{cell} dependence, large cells generally require smaller electric field pulses than small cells. In the case of isolated cells, mammalian cells experience electroporation for electric fields of about $E \approx 1$ kV/cm for short pulses. Bacteria, which are significantly smaller, need a much larger E. In most work to date, the electric field exposure is brief: short pulses of about 1 μsec to long pulses of about 1 msec are common. The degree of electroporation at lower fields and longer times has received only slight attention [22].

ELECTROPORATION IS A NONTHERMAL BIOCHEMICALLY MILD PHENOMENON

Electroporation can be regarded as a nonthermal phenomenon because the dramatic membrane rearrangement of pore formation begins to occur extremely rapidly (estimated to be of order 10^{-8} sec or less), before any significant temperature rise occurs. Essentially all the heating takes place in the extracellular medium, for which the rate of temperature rise is $dT/dt \approx \sigma_e E^2/c_e\rho_w$. Typical electric field pulses are in the range 10^3–10^4 V/cm^{-1}, and for physiological media the electrical conductivity is $\sigma_e \approx 1.4$ S, c_e is the electrolyte specific heat, and ρ_w the mass density of water. As a result, $dT/dt \approx 10^3$–10^5°C sec^{-1}, but because of the short exposure time, the temperature rise often is only about 1°C per pulse. Electroporation has also been demonstrated in special, low conductivity media for which the extracellular heating is significantly smaller, but this may also change the chemical stress of cells as molecules from the external medium enter the cell through pores.

Electroporation usually occurs under biochemically mild conditions. For the above electric field pulses the transmembrane voltage reaches values about five to fifteen larger than the physiologic value (around 0.1 V). However, the resulting transmembrane voltage of 0.5–1.5 V corresponds to a potential energy too small to disrupt most molecules. Moreover, within an aqueous pore the local electric field is significant reduced because of the "focusing fields" (spreading resistance fields [17,23]) near the entrances to a pore, and these local fields accelerate a charged molecule to only a fraction of the energy needed to chemically alter most molecules. At the larger electric fields sometimes used to electroporate small microorganisms is there evidence that macromolecules (DNA) in solution can be directly altered, but this does not occur for the smaller electric field pulses used with the larger eukaryotes.[24]

Finally, some biological systems experiencing electroporation have clearly experienced negligible damage. For example, electro-insertion of proteins into red blood cell membranes [25], and electroporation loading of platelets [26] both showed circulating survival times close to controls, and in vivo transdermal delivery of a small fluorescent molecule into hairless rats showed no damage by histology [27]. Such experiments demonstrate that conditions causing significant molecular transport do not necessarily result in damage.

PROMPT MEMBRANE DESTRUCTION (RUPTURE) DUE TO ELECTROPORATION

Many experiments show that planar and cell membranes can be significantly damaged by some pulses. Early non-pore theories [28] could not explain the critical transmembrane voltage or the stochastic nature of rupture in planar membranes, but a transient aqueous pore model did [8,29,30]. Figure 2 shows the predicted pore energy as a function of pore size and transmembrane voltage. Transient aqueous pore models correctly predict the magnitude and stochastic nature of the destruction of planar bilayer membranes. In this phenomenon, escape of one or more large "critical" pores over the pore energy barrier for planar membranes (Fig. 2) is believed to account for the prompt rupture of these membranes.

In the case of cells, it is not yet known whether a portion of the membrane, e.g., a region bounded by cytoskeletal elements, behaves like a small planar membrane and can therefore exhibit prompt rupture. There are fundamental reasons for expecting that an unconstrained closed membrane (e.g., a vesicle) cannot exhibit prompt rupture, as there is no boundary at which membrane phospholipids can accumulate upon expansion of a large pore. For this reason, the pore energy curve of Figure 2 is believed appropriate only for a planar membrane [8,19]. In the case of closed membrane (e.g., vesicle), there is no such boundary, and for this reason no opportunity for the membrane to vanish by evolving a large pore which then expands to that boundary. Instead, larger pores are favored for larger transmembrane voltages, but as the membrane discharges through the pores (REB), the vesicular membrane returns to its initial state [31]. This inability to promptly rupture via critical pores probably applies generally to cell membranes. However, if the more complex cell membrane has bounded portions which behave like small planar membranes, then these regions may experience rupture. Moreover, cell membranes may allow a variety of long lifetime metastable pores (e.g., Fig. 1), which may allow small ions and molecules to cross the membrane long after the transmembrane voltage has decayed to a small value. Then even very small voltages (< 1 mV)

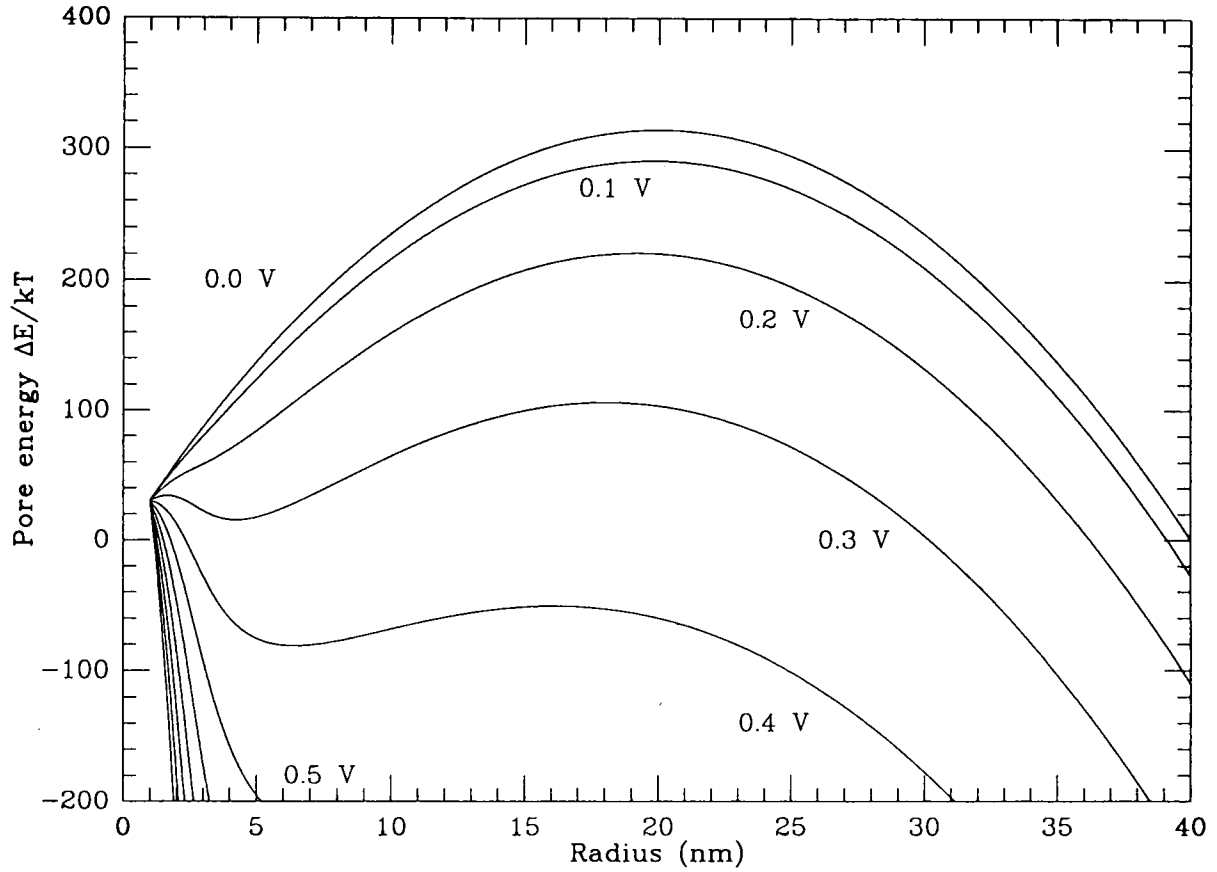

Fig. 2. Computed plot of the hydrophilic pore formation energy, ΔE(r,U), vs. pore radius, r, for a planar membrane [19,23]. In order to make the comparison to the average thermal fluctuation energy easier, the pore energy, ΔE, which is a function of both pore radius, r, and transmembrane voltage, U, is expressed as a ratio to kT, the mean thermal energy. Such "primary pores" (Fig. 1B) are believed to occur in the bilayer membrane portion of cell membranes. For a planar membrane, pores which expand to radii larger that the barrier peak location (a critical radius, r_c) can expand to the boundary of the supporting structure for the membrane (the aperture in a planar membrane experiment; possibly a cytoskeletal element in a cell). This is rupture. Vesicles and cell membranes without a pore/cell structure interaction are not expected to rupture [31]. Instead, cell lysis for large pulses probably involves a secondary response due to chemical imbalances associated with the presence of many pores.

may transport significant numbers of charged molecules [32].

ELECTRICAL BEHAVIOR FOR A SHORT PULSE

The electrical conditions which have been observed to cause electroporation are simple to state in approximate form: elevation of U(t) to 0.5–1.5V for durations of microseconds to milliseconds. A great many observations together suggest that this is essentially universal, i.e., that the onset of electroporation is predominantly a physical phenomenon, with the chemical properties of different membranes overwhelmed by only slight changes in U(t). The electrical and mechanical behavior of some artificial planar bilayer membranes has been determined, with mechanical destruction (rupture) typically occurring for moderate values of U (e.g., 300–500 mV). Paradoxically, for short pulses much larger U can be reached for some types of membranes, but rupture does not occur. Instead the membrane achieves a high conductance state, and rapidly discharges via "reversible electrical breakdown" (REB) [9,33]. Related behavior for longer pulses has also been observed [34,35].

A brief description of the electrical behavior is worthwhile, primarily because electroporation is caused by electrical stimuli, but also because the extent and progression of electroporation can be followed by electrical measurements. A "signature" of electroporation is the tremendous increase in electrical conduction which can be measured, and which is believed due to ionic

conduction through transient aqueous pores. The behavior of the transmembrane voltage, U(t), during membrane charging, and the subsequent appearance and evolution of a pore population, is intimately connected with the number and size of the pores. The success of a transient aqueous pore model in providing a quantitative description of U(t) under these conditions provides confidence that electroporation is a valid concept.

What happens as pulse size is increased? Very small and short pulses merely charge up membrane, with a time constant (e.g., 1 μs) due to the membrane's capacitance and the resistance of the external charging pathway. Somewhat larger pulses bring the membrane to several hundred millivolts and lead to rupture of a planar membrane. Progressively larger pulses lead to a larger membrane conductance, G(t), and increasingly faster decay of U(t). This is the origin of reversible behavior, as the membrane discharges before a single large pore can evolve and then lead to rupture. In theoretical models this is found to accompany the non-linear appearance of pores of many sizes, such that the membrane conductance becomes progressively larger for larger pulses. This phenomenon is "reversible electrical breakdown" (REB), which occurs when U(t) reaches 0.5 to 1.5 V [1–3]. According to both experimental evidence,[9] and theoretical models [36], less than about 0.1% of the membrane area becomes aqueous during REB.

MOLECULAR TRANSPORT DUE TO ELECTROPORATION

For essentially the same conditions that cause REB, a large increase in molecular transport across cell membranes is found. In spite of the importance of this enhanced transport, only a few studies have made quantitative determinations of molecular uptake or release [37–39], especially in terms of the "number of molecules per cell" [40,41]. In the largest number of electroporation applications to date, DNA is introduced into cells, and the degree of transformation scored, not the number of transported DNA molecules. Large numbers of smaller molecules can also be introduced into cells, which suggests widespread potential applications (Table II). The detailed mechanism by which molecular transport occurs during electroporation is not understood, but includes as candidates electrical drift (electrophoresis), electro-osmosis (electrically driven flow), diffusion [32], and endocytosis [32].

TABLE I. Sources of Heterogeneous Electroporation Within Cell Populations

Source	Significance
Cell size	Generalization of equation 1
Cell shape	Generalization of equation 1
Cell orientation	Generalization of equation 1
Field nonuniformity	Electric field varies within chamber
Cell–cell separation	Perturbation of local field by nearby cells
Tissue heterogeneity	Perturbation of local field by tissue
Membrane composition	Composition variation within cell population[a]
Pore statistical behavior	Electroporation is fundamentally stochastic (kT fluctuations)

[a]The observed universality of electroporation onset at about U ≈ 1 V argues against significant membrane composition effects, but minor contributions to heterogeneity may be due to membrane properties. Much greater variation is expected after the pulse, i.e., during the membrane recovery phase, when transmembrane voltage effects are greatly reduced.

The first three mechanisms can clearly lead to increased molecular transport for water soluble molecules if pores are involved, but it is not clear whether endocytosis is a primary field-stimulated membrane process, or a secondary cell-stimulated process that occurs for chemically unbalanced cells.

In order to better understand the basic nature of electroporation, and also for many applications, it will be important to know the order of magnitude of molecular transport. The molecular size, shape and charge can all be expected to be of interest, as direct interaction of molecules with pores may involve all three of these properties. Simple exclusion by geometric size ("sieving") can be considered, but distortions of the pore by a nearby or entering molecule may also occur. Similarly, the shape of a molecule, particularly if a molecule is long, should be important (e.g., Fig. 1E). Electrostatic exclusion because of a Born energy repulsion associated with the lower dielectric constant of the membrane interior regions, and interaction with charged head groups within hydrophilic pores and any composite pores, can also be significant. As noted above, only a few quantitative molecular transport studies have yet been carried out, but more can be expected and should be of great value.

INSERTION OF MACROMOLECULES INTO THE MEMBRANE

In contrast to transmembrane transport, it has also been reported that membrane proteins can be stably inserted into the membrane by electroporation [25]. As suggested in Figure 1E, if a molecule with a hydrophobic region separated by two hydrophilic regions enters a large pore caused by electroporation, then the pore can be expected to shrink down to the nonrepulsive hydrophobic region of the molecule. As is well known qualitatively, and understandable quantitatively by considering the electrostatic energy of the system [42], a molecule within a collapsed pore is stable. The biochemical mildness of electroporation is supported by the observation that red blood cells with electroinserted molecules have long circulating half-lifes in laboratory animals, as significant change in the red blood cell membrane would result in rapid clearance of the cells.

ELECTROPORATION ONSET APPEARS TO BE UNIVERSAL

Almost all experiments using short (1–100 μs) and longer (1–10 ms) pulses with many different types of cells have observed electroporation [1–3]. There is a common observation that short pulses cause major effects at U = 0.5–1.5 V. Moreover, a transient aqueous pore theory can explain much of the short term (within 100 microseconds) behavior by a purely physical mechanism. Thus, even though there are differences in membrane composition, it appears that a slight difference in electric field energy within the membrane overwhelms details of membrane composition. In this sense electroporation appears to be universal; it occurs in essentially all artificial planar bilayer and cell membranes.

HETEROGENEITY OF ELECTROPORATION WITHIN A CELL POPULATION

Universality does not mean, however, that all cells within a population of cells exhibit the same behavior. In fact, there are fundamental reasons for expecting the individual cells within a population to have a distribution of electroporation behavior, mainly because the transmembrane voltage changes due to a uniform externally applied field are expected to be different for different cells [32] (Table I). Specifically, equation 1 (and its generalizations to nonspherical cells) indicates that variations in cell size, shape and orientation are important in determining the magnitude of the transmembrane voltage change, $\Delta U(t)$, at different sites on a cell membrane. Cell populations are also well known to exhibit cell-to-cell variations in composition and function ("biological variability"), which may be very important for post-pulse phenomena, e.g., membrane recovery. It is therefore not surprising that electroporative phenomena (e.g., molecular uptake, survival) are found to vary for the individual cells within an electrically pulsed population [43,44].

MEMBRANE RECOVERY

The contraction and disappearance of pores can also vary during the recovery phase, after the transmembrane voltage has returned to small values. Under these conditions, the biological and biochemical nature of the membrane should be relatively more important. Moreover, the consequence of having many pores may vary from one membrane to another. For artificial planar bilayer membranes some membranes may rupture more readily than others, due to differences in surface and "edge" energies. For cells, post-pulse behavior can vary significantly. For example, some primary pores may expand into very large pores by secondary processes which are particular to the cell type and the experimental conditions. As another example, some experiments show a long term (seconds to minutes) persistence of molecular transport in a subpopulation of cells within a large population of cells. Finally, for the same conditions, one cell type may mostly survive while another may be mostly killed. The processes that occur after U(t) has decayed to low levels because of ionic conduction through pores is poorly understood.

CELL STRESS AND DEATH DUE TO ELECTROPORATION

A general trend is found in the use of electroporation to introduce molecules into cells. For a given pulse shape, small magnitude pulses cause no effect, but at about 1 kV/cm (mammalian cells; short pulses) some cells experience molecular uptake. As larger electric fields are used, the percentage of participating cells increases, but the percentage of surviving cells simultaneously decreases. Eventually, for very large fields essentially no cells survive. Why does this occur? There are at least two hypotheses: (1) a prompt membrane rupture occurs in some portions of the cell membrane, leading to a large

TABLE II. Existing and Likely Electroporation Applications

DNA introduction
Loading drugs into cells
In situ enzymology (load reagents)
Insertion of proteins into membranes
Tumor tissue drug delivery
Localized gene therapy
Isolated cell fusion[a]
Low-energy cell killing
Loading dyes and tracers into cells
Intracellular immunoassays
Release of intracellular compounds
Transdermal drug delivery
Noninvasive tissue sampling
Cell/tissue fusion[a]

[a]The many possible applications of cell fusion by electric fields are worthy of an entirely separate discussion.

hole in the membrane; and (2) chemical imbalances occur, due to the influx and efflux through both transient and metastable pores. Although important to almost all applications, a good understanding of cell stress and resultant cell death does not yet exist.

Very early studies demonstrated nonthermal killing of microorganisms by electric field pulses which are now associated with electroporation [5]. Much more recently, compelling evidence has been gathered that electroporation plays an important role in cell death and the associated tissue damage of electrocution injury [45], and that membrane recovery can be significant improved by providing a nonionic surfactant [46]. Thus, like essentially all natural phenomena from which technologies are crafted, there are both desired and undesired outcomes. In order to obtain optimal outcomes, considerable additional understanding of electroporation will be needed.

GENERALLY ATTRACTIVE FEATURES OF APPLICATIONS

The study of electroporation is compelling for two general reasons: (1) fundamental aspects of membrane structure and behavior are involved, and (2) significant applications in biological research, biotechnology and medicine are likely. As noted in Table II, significant applications are likely. It is worth noting that electroporation achieves a chemical and biological result by a physical means. This means that unlike chemical manipulations of biological systems, there is no chemical residue. Furthermore, electroporation onset appears to be universal at the membrane level. Finally, the fact that electrical phenomena are involved has the important technical consequence that electronic systems can be used to both cause electroporation and to then measure its electrical consequences. For example, in tissue electroporation, rapid electrical measurements allow the occurrence and after-effects of electroporation to be monitored [47]. Although the potential applications of tissue electroporation are large, only a few studies have been reported. Some have emphasized the ability to make electrical measurements [47], and combined molecular transport and electrical measurements [27], while others have focused on important biological consequences of molecular transport [48–50]. Finally, electrofusion of cells to tissue is being investigated [51], and many other aspects of electrofusion (not discussed here) also appear to have significant applications [1–4]. Much more can be expected in the future.

SUMMARY

Electroporation investigation and application are still at an early stage. Although several basic phenomena have been discovered, and electroporation appears to be a universal, electroporation mechanism remains incompletely understood. In spite of this, it is compelling that electrical stimuli can be used to universally alter the natural barrier function of cell membranes, and that electrical measurements can often be used as a partial indicator of barrier restoration. For this reason, the number of existing and future applications in biological research, biotechnology and medicine is large.

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