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In Vivo Electroporation: An Important Injury Mechanism in Electrical Shock Trauma

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27.1 Introduction

Most people have experienced some form of electrical shock considering our increasing exposure to electrical power. Public health records suggest that electrical injuries result in more than 3000 admissions to specialized burn units in the United States, accounting for 3%–4% of all burn-related injuries. In addition, it is estimated that there are far more accidental electrical shock accidents that do not reach medical attention than the ones that do (Tkachenko et al. 1999). Up to 40% of serious electrical injuries are fatal, resulting in an estimated 1000 deaths annually. There is a bimodal distribution of environmental electrical injuries with respect to age; work-related injuries affect mostly young adults and domestic accidents occur more in children (Spies and Trohman 2006). In the American workplace, it is the fifth most common occupational hazard.

For years, electrical injury was considered to be mediated only by Joule heating. However, it is now well established that electrical field exposure can affect living tissues through Joule heating, electropore formation in cellular membranes, electroconformational changes in membrane proteins, and mechanical trauma resulting from the stimulation of muscle contraction. All these effects are dependent on the magnitude of the tissue field strength and the duration of field exposure. Clinical pathology may manifest early or late and any organ can be affected.

In trained hands, by controlling the field strength, the frequency, and exposure duration, electrical shock trauma can serve as a very useful therapeutic tool. The cardiac defibrillator is the most commonly recognized example. But, in addition, electrical current can be applied to the brain to arrest intractable

seizures or to induce seizures to manage major psychiatric illnesses. These methods have been used and accepted for years. There are several other important examples. When used as therapy, the exposure is calibrated and controlled to limit the extent of injury to an amount that can be rapidly repaired by natural cellular healing mechanisms.

The subject of this chapter is the explanation of the clinical observed consequences of electrical shock injury, which exceeds natural repair and thus results in permanent tissue damage. Of course, it is the cell membrane that has the greatest vulnerability to damage by passage of electrical current through the body. Because the human anatomy is nonuniform and the tissues are anisotropic in their electrical properties, current passage through the body establishes tissue electrical fields that vary widely in magnitude from one anatomical location to another. The strongest electric fields are induced across the lipid bilayer of cell membranes. Therefore, the primary focus in understanding electrical shock pathogenesis focuses on the consequences of cell membrane alteration (Lee et al. 1988).

27.2 Injury Biophysics

Injury resulting from electrical shock may be mediated by effects of electric forces or elevated temperatures acting on the molecular scale (Lee and Astumian 1996). Indeed both mechanisms may ultimately lead to molecular conformational changes that alter protein function or permeabilize the cell membrane. It is this cell membrane damage, characterized by both breakdown of structural integrity and loss of function, that is the primary mechanism for the loss of tissue viability following electrical shock (Lee 2005). In this chapter, we will discuss the general concepts of what is known about the pathophysiological significance of electroporation in electrical injuries.

27.3 Electropore Formation

Electroporation is primarily mediated from the action of dielectric stresses acting on the interface between the high electrically polar water molecules and the transient defects in the lipid packing order within a bilayer (Lee et al. 1995, Ho and Mittal 1996, Weaver and Chizmadzhev 1996). The applied stress induces rearrangements of the ionic polar head-groups of bilayer lipids leading to localized hydrophilic pores (Böckmann et al. 2008). By the early 1980s, electroporation techniques were being used to introduce a variety of molecules including drugs and foreign DNA into cells and tissue (Chang et al. 1992, Aihara and Miyazaki 1998). Electroporation has been used on isolated cells to (a) introduce enzymes, antibodies, viruses, and other agents or particles for intracellular assays (Weaver 1993, Neumann et al. 1999, Teissié et al. 1999); (b) induce cell fusion (Neumann et al. 1989, Chang et al. 1992); and (c) insert or embed macromolecules into the cell membrane (Lynch and Davey 1996). Successful defibrillation of the heart also relies on electrically induced pores to reduce electrical nonuniformities and synchronize individual cells within the cardiac tissue. Electric shock is the only effective treatment for ventricular fibrillation and the success or failure of defibrillation has been attributed to one or both of these two mechanisms: (a) success in extinguishing ongoing fibrillatory activity (Zipes et al. 1975) and (b) failure to reinitiate a new arrhythmia (Frazier et al. 1989). Electroporation may produce both of these effects (Al-Khadra et al. 2000).

A major factor in determining the pore's kinetics is the physical state of the lipid bilayer, whether liquid crystal or fluidic, which is mostly governed by the temperature of the membrane. This is an important variable in tissue electroporation because tissue temperature varies considerably from one anatomic location to another. For example, skin temperature is typically lower than the temperature in cardiac muscle. Cell membranes are viscoelastic fluids. As such, the force required to deform the membrane under electrical stress is not only dependent on the amplitude of the applied voltage, but the duration of the application. The threshold transmembrane potential required for electroporation has been found to be in the range of 250–350 mV for field exposure greater than several milliseconds (Gowrishankar et al. 1998, Bier et al. 1999). Because of the viscous mechanical properties of the cell membrane, the threshold to create a pore is higher for shorter duration pulses.

Lifetimes of electropores may be transient or stable, depending on the magnitude of the induced transmembrane potential, its duration, membrane composition, and temperature. Transient pores have been observed to occur at lower applied voltages (340–480 mV) with sealing times averaged around 9 min whereas stable pores dominate at higher applied voltages (>540 mV) in rat skeletal muscle (Bier et al. 1999). In another study using liposomes, after an applied 1 V field was turned off, a stable micrometer-sized hole was seen to close within milliseconds (Chang et al. 1992). After application of a short electroporating field pulse, the sealing of transiently electroporated cells is spontaneous (Bier et al. 1999). However, stable defects require active modes of repair. Cells have evolved specialized proteins and molecular transport processes for sealing damaged membranes (McNeil and Steinhardt 2003, Togo 2004). The genetic expression of proteins involved in membrane sealing is increased to respond and adapt to repetitive membrane disruption. The sealing of electropores requires reordering of membrane lipids and removal of water molecules from the pore, which are both time- and energy-consuming processes (Gabriel and Teissié 1997, 1998). Sealing kinetics is often orders of magnitude slower than electropore formation. When the rate of molecular sealing events is overwhelmed by the rate of electroporation, as in the case of larger membrane defects, active transport of cytoplasmic transport vesicles is required to seal or patch the defect.

27.4 Current Path Parameters

The passage of current through the body exposes any cell or tissue in its path to electric fields. The risk of electroporation for any particular cell in the current path scales with the magnitude of the induced transmembrane potential (V_m), pulse duration, and membrane properties. The peak transmembrane potential induced by an externally applied electric field scales with the dimensions of the cell in the direction of the applied field. Generally, for media-suspended isolated cells with a characteristic diameter of 10–20 μm, the threshold field strength in the suspension media for electroporation is ~100 kV/m.

The fields required to electroporate adult skeletal muscle or peripheral nerve tissue are also much less due to their large lengths. As skeletal muscle cells in the extremities and peripheral nerve cells of humans and larger animals exceed 8 cm and 2 m, respectively, the electroporation thresholds begin to lower significantly (Lee et al. 2000). Electric fields as small as 0.2 kV/m may damage the membranes of these cells rendering them hundreds of fold more vulnerable to electroporation than small blood cells.

There is no set path in which electric current must pass through the body. Most frequently, the upper extremity is part of the current path found in major electrical shocks. The current passing along the long axis of the arm induces large muscle and nerve membrane potentials in cells with lengthwise orientation approximately parallel to the direction of the field lines (Danielson et al. 2000, Lee et al. 2000). The transmembrane potentials induced on the membranes of these cells are significantly larger than those experienced by skeletal muscle cells in any other orientation (Gaylor et al. 1988).

The cell membrane can be described as an "insulating shell" containing a conducting medium surrounded by a conducting buffer (Ramos and Teissié 2000). Normally, the intracellular and extracellular fluids have nearly equal ionic osmolarities, thus, their conductivities are similar. The lipid membrane conductivity, however, is typically 10^6–10^8 folds less than that of the surrounding fluid. Consequently, electric current established in the extracellular space by low-frequency fields are to a variable degree shielded from the cytoplasm by the electrically insulating cell membrane (Lee and Astumian 1996). This shielding leads to an induced transmembrane potential, which is sensitive to the geometry of the cell and its orientation in the field. A simple electrical cable model can provide quantitative insight into the importance of these parameters when considering the risk of electroporation injury.

27.5 Cable Model Theories

A distributed circuit model of a cell can be made with a series of parallel resistors and capacitors (Jack et al. 1975, Adrian 1983, Cooper et al. 1984) as illustrated in Figure 27.1a. This circuit model of the membrane is combined with the specific resistivities of the intracellular and extracellular media to

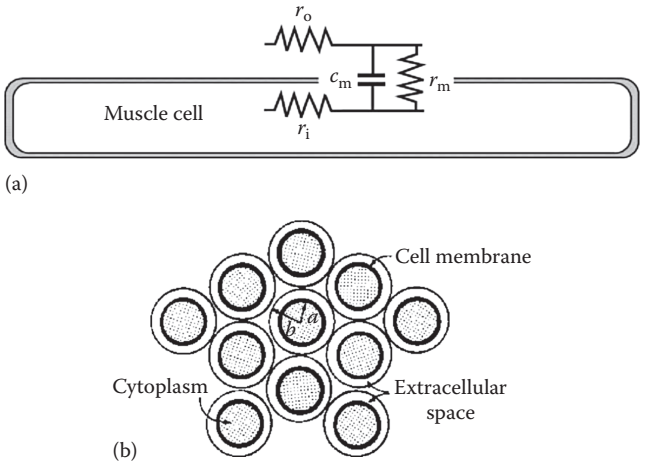


FIGURE 27.1 Circuit model of the cell. (a) Lumped element cable circuit model of a typical muscle cell. Cell length is equal to 2*L*. (b) A cross-sectional view of the arrangement of cells in a bundle which, in our model, is a hexagonal array of elongated cells with cell radius *a,* and extracellular fluid radius *b*.

yield the cable circuit representation. In the presence of an applied uniform field $E(t)$ to the cell's axis, a transmembrane potential will be superimposed on the natural resting potential of the membrane.

The induced transmembrane potential distribution can be estimated using an approach proposed by Gaylor et al. for both isolated muscle cells and for cells within intact tissue (Gaylor et al. 1988). Analyzing the circuit model leads to a differential equation for the induced transmembrane potential, $V_m(z, t)$, along the long axis of the cell, z:

$$\lambda_m^2 \frac{\partial^2 V_m(z,t)}{\partial z^2} = V_m(z,t) + \tau_m \frac{\partial V_m(z,t)}{\partial t} \tag{27.1}$$

The parameters λ_m and τ_m are the membrane electrical space constant and the membrane charging time constant, respectively. They can be defined as

$$\lambda_m = \sqrt{\frac{1}{(r_i + r_o)g_m}}, \quad \tau_m = \frac{c_m}{g_m} \tag{27.2}$$

where

r_i and r_o are the resistivities (Ω/cm) of the intracellular and extracellular fluids, respectively

c_m and g_m are the capacitance per unit length (F/cm) and the conductance per unit length (S/cm) of the membrane, respectively

For the case of a single cell in an infinitely large bath of extracellular fluid, r_o is negligible compared with r_i since extracellular space is much greater than the intracellular space. Thus, for a constant applied electric field E_o, an approximate solution arises for the induced transmembrane potential of the cell

$$V_m(z) = \frac{\lambda_m E_o}{\cosh(L/\lambda_m)} \frac{\sinh(z/\lambda_m)}{1 + \lambda_m r_i G_e \tanh(L/\lambda_m)} \tag{27.3}$$

where

G_e is the conductance of the membrane at the end-caps of the cylindrical cell

L is the cell half-length

Equation 27.3 suggests that the induced transmembrane potential will increase according to the length of the cell up to the point where the size of the cell exceeds the length of the electrical space constant. For the largest skeletal muscle cells, the electrical space constant λ_m reaches a maximum value of about 2 cm, which means that the induced electrical potential reaches a maximum value of $E_o\lambda_m$ even when the physical length of the cell greatly exceeds the electrical space constant. For human skeletal muscle cells typically 2–8 cm in length, Equation 27.3 gives a membrane potential at the ends of the cells of four to five orders of magnitude larger than the applied field (Gaylor et al. 1988).

There is a second effect due to cell crowding. For cells within intact tissue subjected to an electric field, the previous analysis can be used to include the effects of neighboring cells on the induced transmembrane potential. The cells are assumed to be ordered parallel to each other in a hexagonal array as illustrated in Figure 27.1b. To facilitate the comparison of induced transmembrane potential in tissue with the case of isolated cells, the quantity $V_c/2L$ is used as the "source" term, where V_c is the voltage drop across the full length of a cell. For the isolated cell case, $V_c/2L$ is equal to the applied field amplitude E_o. The boundaries are modeled as cylinders of radius a, and the cross-sectional area of the extracellular fluid surrounding each cell with radial thickness $b - a$ will determine the extracellular resistivity r_o. Generally, for cells that are not on the muscle surface, r_o is not negligible compared with r_i, which significantly affects the value of the space constant, λ_m, in Equation 27.3.

Moreover, the extracellular electric field amplitude between muscle cells is not constant in the z axis. The induced transmembrane potential for a cell embedded in other cells is approximately

$$V_m(z) = \frac{V_c}{2L}\frac{\lambda_m(r_o + r_i)/r_i}{\cosh(L/\lambda_m)}\frac{\sinh(z/\lambda_m)}{1+\lambda_m((r_i + r_o)G_e + r_o/(Lr_i))\tanh(L/\lambda_m)} \tag{27.4}$$

A comparison of the result predicted for cells within intact tissue (Equation 27.4) with the result predicted for isolated cells (Equation 27.3) suggests that cells surrounded by neighboring cells experience a higher induced maximum transmembrane potential than that of isolated cells. The decreased extracellular space increases resistance outside the cells and allows more current to flow through the ends of the cells producing the larger potential. Thus, the more closely packed the cells, the higher the induced potential.

The above analysis relates to the effects of an applied static electric field. Is this relevant to the contact with alternating electric currents that is used in standard commercial power supplies that operate near 60 Hz? The transmembrane potential response will depend on the charging time of the cell. Cooper gives a modal transient solution to the cable equation (Cooper 1986)

$$V_m(z,t) = \sum_{n=1}^{\infty} A_n \sin\left(\frac{\alpha_n z}{\lambda_m}\right) e^{(1+\alpha_n^2)t_n/\tau_m} \tag{27.5}$$

with

$$\alpha_n = \frac{n\pi\lambda_m}{2L} \tag{27.6}$$

Thus, the time-dependent terms decay with a time constant:

$$t_n = \frac{\tau_m}{1+\left(n\pi\dfrac{\lambda_m}{2L}\right)^2} \tag{27.7}$$

The time τ_l is the maximum time constant and represents the time required for the cell to attain the transmembrane potential distribution predicted by the cable model analysis. For long skeletal muscle and peripheral nerve cells, this time approaches τ_m (typically between 1 and 3 ms) but it decreases for cells of shorter length.

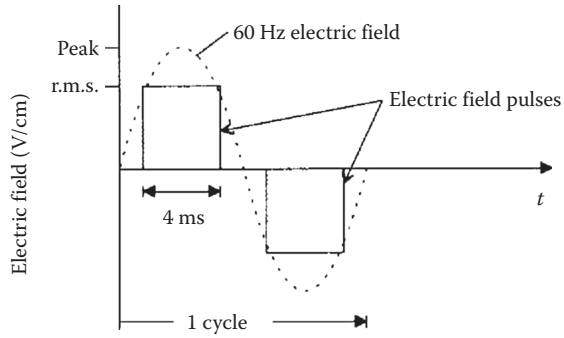


FIGURE 27.2 Approximation for a commercial sinusoidal electric current. Alternating 4 ms discontinuous square wave field pulses can roughly approximate a standard 60 Hz commercial sinusoidal electric current. The pulse magnitude will equal the average value of the field within the 4 ms timeframe.

For a 60 Hz applied field, large muscle and nerve cells should reach a steady-state level during each excursion of the sine wave because the root-mean-square duration of the 60 Hz sine wave is about 4 ms (Figure 27.2).

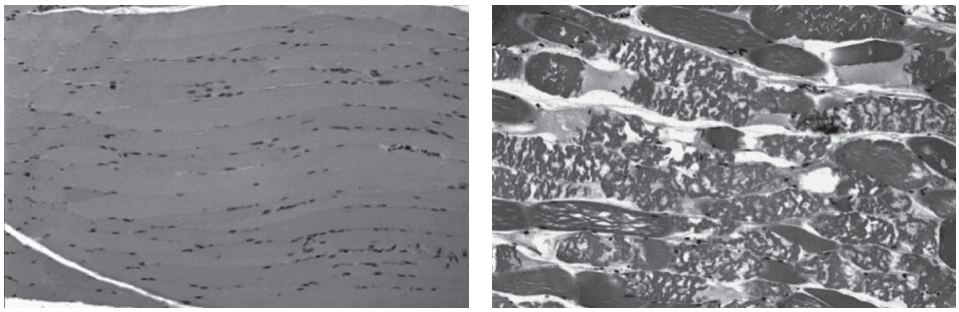
The model predicts a strong nonlinear dependence of induced V_m along the direction of the field. Moreover, cells that are surrounded by other cells can be expected to be more vulnerable to injury than cells on the muscle surface because they experience a larger imposed transmembrane potential. The increased potential for electroporation in electrical shock injuries of longer and more densely packed muscle and nerve cells in the extremities must be taken into account in assessing tissue damage. These crowding and size discrepancies must also be considered when predicting in vivo outcomes with in vitro studies.

27.6 Thermal Injury

A discussion about electrical shock injury would be incomplete and misleading if thermal injury aspects were not mentioned. Current passage in a conducting media will increase the temperature through Joule heating, dielectric heating, or both. Because the components of cell membranes are held together only by forces of hydration, the lipid bilayer is the most vulnerable to thermal insult (Gershfeld and Murayama 1988, Lee et al. 2000). Alterations in the membrane molecular structure disturbs the membrane transport processes. Temperatures of only 6°C above normal (i.e., 43°C) may disrupt the structural integrity of the lipid bilayer (Moussa et al. 1979). As the melting temperature of the lipid composition is approached, the permeability of the cell membrane reaches a maximum. This may be due to increased area fluctuations causing local maximums in lateral compressibility and/or larger domain boundaries between solid and liquid phases where these pores are thought to occur (Heimburg 2007, Blicher et al. 2009). Experiments on fibroblasts have demonstrated that heat-induced membrane permeabilization also begins to appear at temperatures greater than 45°C (Merchant et al. 1998). Though not as susceptible as the bilayer components, there is also thermal denaturation of membrane-bound proteins, which may lead to irreversible unfolded conformations and aggregation at supraphysiological temperatures (Despa et al. 2005).

27.7 Current Path through the Body

The pathway that a current takes through the body during electrical shock determines the tissues at risk and the clinical manifestations. When the primary cause of tissue injury is electroporation and not heat, clinical diagnosis is challenging. Electroporated tissue appears to be edematous without other visible changes. A histological analysis of tissue biopsies has characteristic changes (Figure 27.3b). When electroporation damage exceeds cell membrane resealing capability, this injury will progress to necrosis.



(a) (b)

FIGURE 27.3 Human muscle cells as they appear in a normal and an electroporated patient. (a) Hematoxylin and Eosin stained skeletal muscle biopsy taken from a normal subject and seen under 40 times power magnification. Notice the regularly aligned sarcomeres as compared with figure (b) which shows edematous cells with loss of normal architecture and contraction band necrosis.

At frequencies less than 10 kHz, the skin is the primary resistive barrier to the flow of current into the body. Deeply calloused skin can have 20–70 times greater resistance (Childbert et al. 1985). This high resistance may result in a large fractional voltage drop across the interface and a significant amount of energy will be dissipated at the skin surface. If the body makes contact with a voltage drop greater than 200 V and the power source generates several amperes of current, the epidermis is destroyed almost instantaneously. Eliminating the epidermal barrier lowers total body resistance and leads to accelerated tissue damage (Püschel et al. 1985). Respiratory arrest may follow electrical shock from damage to the respiratory musculature or associated nervous system. This may be caused by tetanic contraction or paralysis of respiratory muscles in the lungs (Spies and Trohman 2006). Current passing through the heart or thorax can also cause cardiac dysrhythmias (Jensen et al. 1987) and/or direct myocardial damage (Ku et al. 1989, Walton et al. 1988). Electrical current passage directly through the brain will result in different outcomes than for current passage limited to one extremity. When the current path travels through brain tissue, it can lead to respiratory arrest, seizures, and paralysis (Hooshmand et al. 1989).

The current path through the body is a major determinant of the outcome, yet damage cannot always be predicted solely by pathway. Neurological manifestations of electrical shock can be especially perplexing because cognitive and emotional consequences often follow electrical shock (Cooper et al. 1992, Kelley et al. 1994, Pliskin et al. 1998, 2006, Chico et al. 1999). This is common even when no current passes directly through the brain. While the exact mechanism of this effect is not understood, it is generally recognized that the nervous system is tightly connected. Damage to peripheral nerves in the extremities can manifest in the brain.

27.8 Challenges in Clinical Diagnosis

To illustrate the clinical consequences of the most common modes of electrical shock injury, the following clinical cases of an electrician on the job and a young boy at home are presented. Mr. V is a patient who was recently evaluated by the physicians of the Chicago Electrical Trauma Research Institute. He is a 40-year-old patient suffering from multiple post-electric injury complaints. The injury occurred while performing his job as an electrician in May 2000. His right (dominant) hand came in electrical contact with what he thought was a "de-energized" 12,500 V transformer circuit breaker. The contact was mediated by the ignition of an arc between his hand and the high-voltage side of the transformer. A snapping loud arc was heard, and then the transformer faulted, which generated a high-energy acoustic shock wave that knocked the patient to the ground in front of the transformer housing. On contact, Mr. V experienced what he described as a "burning" sensation and severe pain in the back of his head as if being shot. This was followed by a brief

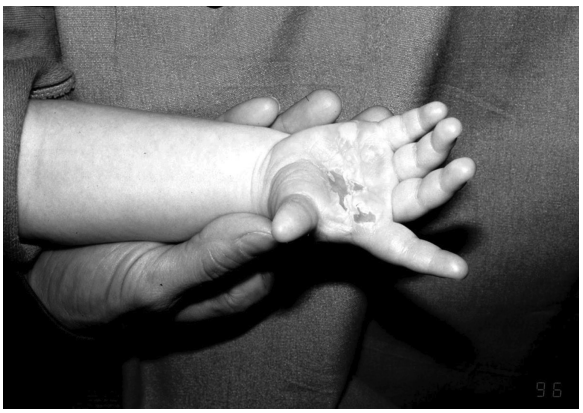


FIGURE 27.4 Right hand of a 1-year-old child after brief contact with an open fluorescent bulb power supply. Besides the superficial burn, this child presented with temporary muscle paralysis of the lumbrical muscle of his right index finger and the inability to flex his index following a brief contact with an open fluorescent bulb supply. Patient fully recovered within a couple weeks.

period of loss of consciousness after which he woke up confused on the way to the hospital with a burning sensation in his hands and feet.

In the emergency ward, Mr. V was complaining of chest pain, headache, dizziness, numbness of his hands and feet, and diffuse upper body myalgias. No major skin burns were noted except for a small arc wound. Mr. V's electrocardiogram was normal. Neuro-imaging results revealed no evidence of abnormalities. No evidence of skull fractures was found at that time. He was discharged the following day.

Mr. V followed up immediately with his primary care physician and was referred to a neurologist because of "black-out" episodes with subsequent disorientation. A brain MRI was completed but did not show any abnormalities. Mr. V tried several anticonvulsants, which did not help these episodes. A 24 h cardiac rhythm evaluation revealed episodic supra ventricular tachycardias.

Over the past 5 years, Mr. V has undergone multiple evaluations from neurologists and neuropsychologists. Various diagnostic imaging techniques have failed to show a clear pathology yet neuropsychological studies show considerable disabilities. He has been receiving treatment for post-traumatic stress disorder and depression since 2001. Mr. V returned to light-duty work 9 months following the injury but finds simple tasks such as using as screw driver or walking down a flight a stairs to require his full concentration. He remains unable to return to the dangerous power line work he was engaged in pre-trauma.

Another example is the case of the young boy in Figure 27.4 who got electrocuted while briefly holding on to an open fluorescent bulb supply with his bare wet hand. He presented with a superficial burn to his hand and temporary loss of function of the intrinsic muscles of his hand. His symptoms resolved completely after a couple of weeks and the wound healed without grafting.

A multidisciplinary medical team approach is often needed to manage electrical shock patients due to the various symptoms that it may present (Capelli-Schellpfeffer et al. 1995, Chico et al. 1999). There is considerable need to increase the research efforts to understand the relationship between electrical contact parameters such as voltage, current capacity, and duration of current passage, frequency, anatomical current path, and pre-existing medical conditions. These many variables increase the complexity of diagnosing and treating electrical shock.

27.9 Summary

The significance of nonthermal modes of tissue damage such as electroporation in the pathogenesis of electrical shock injury has become increasingly recognized over the past 20 years. Peripheral neurons and skeletal muscle cells are particularly vulnerable to electroporation injury because excitable tissue

must detect and respond to action potentials, which in the context of this discussion have relatively small associated electric fields. When the electrical shock is very short, there is often very little visible burn injury. However, clinical manifestations of such injury can be severe. Understanding underlying mechanisms of injury and developing an effective therapy to treat electroporated membranes is a high priority.

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