PATHOLOGY AND CLINICAL MECHANISMS

OF

ELECTROCUTION INJURY

Raphael C. Lee,

Departments of Surgery, Medicine, and Organismal Biology (Biomechanics),

Pritzker School of Medicine, The University of Chicago,

Chicago, Illinois 60637, U.S.A.,

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Send Proofs to: Raphael C. Lee, M.D., Sc.D.
Department of Surgery, MC 6035
The University of Chicago
5841 South Maryland Avenue
Chicago, IL 60637
Phone: (773) 702-6302
Fax: (773) 702-0661
e-mail: rlee@surgery.bsd.uchicago.edu

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INTRODUCTION

The medical community is becoming increasing aware of the wide range of manifestations of electrocution injury. Almost everyone has once in his or her lifetime been shocked by electricity. The fear reflex generated by the bad experience of pain usually prevents us from further tampering with electricity. However, no matter how careful, accidents do and will happen, especially among electrical workers who have to handle commercial electrical power lines everyday.

Today, rates of electrocution injury among industrial workers range widely from one country to another. Within industrializing countries safety practices are often not the top priority, resulting in high rates of injury. In mature industrialized nations, electrocution shock rates continue to decline. In the United States, electrocution remains the fifth leading cause of fatal occupational injury with an estimated economic impact of more than 1 billion dollars annually (1). Although accurate statistics are difficult to find, it appears that the rates of injury may be highest among electrical workers. A study in Virginia suggested that public utilities have the highest rate of fatal electrical injuries among all industrial sectors. More than 90% of these injuries occur in men, mostly between the ages of 20 and 34, with 4 to 8 years of experience on the job (2). Another source (3) suggests that the average age of victims was 37.5 years and the average years of experience amounted to 11.3 years. For survivors, the injury pattern is very complex, with a high disability rate due to accompanying neurologic damages.

Away from the workplace, most injuries are due to either in-door household low-voltage (<1000 V) electrical contact or to out-door lightning strikes (4). Low-voltage (120 V or 220 V) household power-frequency electrocution shocks are common and usually results in minor

peripheral neurological symptoms or occasionally skin surface burns. However, more complex injuries may result depending on the current path, particularly following oral contact with household appliance cord disclosures or outlets in small children (5). Compared to a high-voltage shock that usually generates an arc and resulting explosive thermoacoustic blast, low-voltage shocks are more likely to produce a prolonged, "no-let-go" contact with the power source. This "no-let-go" phenomenon is caused by an involuntary, current-induced, muscle spasm (6). For 60 Hz electrical current the "no-let-go" threshold for axial current passage through the forearm is 16 mA for males and 11 mA for females (7, 8).

Injury often follows contact with higher frequency electrical power as well. There are approximately 200 deaths annually in the United States due to lightning injury. Yet, many more people survive it. The range of lightning injury extent is quite broad, depending upon the magnitude of exposure and the condition of the victim. According to many reports, if lightning strikes in the vicinity of several individuals, usually only one endures serious or fatal injury. Radiofrequency and microwave injuries are less common. Nonetheless, they represent an important medical problem to understand. At higher frequencies, when the wavelength is short enough to couple at the atomic level, the fields can be ionizing as well as cause molecular heating

In short, electrical trauma may produce a very complex pattern of injury because of the multiple modes of frequency-dependent tissue-current interactions, the variation in current density along the path through the body, as well as variations in body size, body position and use of protective gear. No two cases are the same. Today these variations create challenges in clinical diagnosis that delays effective medical management. Further advancement in medical care of electrocution

shock victims will follow the development of more accurate bioengineering models of injury. The objective here is to review the basic considerations for addition bioengineering research.

ELECTRICAL ENERGY TRANSPORT IN TISSUES

Broadly speaking, the human body is characteristically a compartmentalized (or lumped element) conducting dielectric. It consists of about 60% water by weight, in which 33% is intracellular and 27% extracellular (9). Body fluid in both the intracellular and extracellular compartments are highly electrolytic, and these two compartments are separated by a relatively impermeable, highly resistive plasma membrane. Current conduction within the body is carried by mobile ions in the body fluid. These mobile ions provide a conductivity of approximately 1.4 S m^{-1} in physiological saline. Since in metal wires the carrier for electrical current is an electron, when in contact with the human body the current carriers change from electrons to ions. This conversion occurs at the skin surface through electrochemical reactions (10).

At low frequencies (i.e., below microwave frequencies), it distributes so that the electric field strength is nearly uniform throughout any plane perpendicular to the current path (11, 12, 13). As a consequence, the electrical current density distribution depends on the relative electrical conductivity of various tissues and the frequency of the current. Experimental data supports this basic concept. Sances and co-workers (1981) measured the current distribution in the hind limb of anesthetized hogs (13). They found that major arteries and nerves experienced largest current density because of their higher conductivity. It was also observed that skeletal muscles carried the majority of the current due to its predominant volumetric proportions.

At a more microscopic scale, low frequency current distribution within tissue is determined by the density, orientation, shape and size of cells. Because the cell membrane functions an ionic

transport barrier, low frequency electrical current is mostly shielded from cytoplasmic fluid. In addition, the presence of cells diminishes the area available for ionic current and, in effect, makes tissues less conductive. As cell size increases, the membrane has less impact on a cell's electrical properties, because the volume fraction of the cell occupied by the membrane is proportionately decreased (14). Similarly, the resistivity of skeletal muscle that is measured parallel to the long axis of the muscle cells is less than what is measured perpendicular to the axis. Solid volume fraction is important, too. For example, the resistivity of cortical bone and epidermis is higher than other tissues because their free water content is lower.

The current distribution at higher frequencies, in RF and microwave ranges is dependent on different parameters. The cell membrane is no longer an effective barrier to current passage, capacitive coupling of power across the membrane readily permits current passage into the cytoplasm. Factors affecting the field distribution in tissues are frequency-dependent energy absorption and skin-depth effects. At the highest frequency ranges, including light and shorter wavelengths, other effects such as scattering and quantum absorption effects become important in governing tissue distribution. Table 1 provides a biological effect catagorization of the electrocution injury frequency regimes. Mechanisms of biological effects are different in each regime. A discussion of injury mechanisms must also be separated according to the frequency regime.

PHYSICS OF TISSUE INJURY

Low Frequency Electric Shocks

Until recently, low frequency electrocution injury was considered to be only a thermal burn injury, produced by Joule Heating (6). Over the past ten years, it has been shown that the pathophysiology of tissue electrocution injury is more complex, involving thermal, electroporation, and electrochemical interactions (15, 16, 17), and blunt mechanical trauma secondary to thermoacoustic blast from high-energy arc (18) (Table 2). While these forces can alter all tissue components, it is the thin plasma membrane of cells which has the greatest vulnerability. Thus, the cell's plasma membrane appears to be the most important structure in determining the rate of tissue injury accumulation.

The most important function of the cell membrane is to provide a diffusion barrier against free ion diffusion . The energy required for moving a solvated ion across a planar, pure phospholipid bilayer in an aqueous, physiological environment approaches ~68 kT (19), indicating the steep energy hurdle. Because most metabolic energy of mammalian cells is ultimately invested in maintaining the ionic difference across the cell membrane (20), the importance of the structural integrity of the lipid bilayer is apparent. If the membrane is permeabilized, the work required maintaining transmembrane concentration differences increases proportionately. The conductance of electropermeabilized membranes may increase by several orders of magnitude. ATP production and in turn, ATP-fueled protein ionic pumps, cannot keep pace which lead to metabolic energy exhaustion. If the membrane is not sealed, biochemical arrest and the permeabilized cell will become necrotic. Thus, in discussing tissue injury resulting from electrocution shock, the principal focus is directed at kinetics of cell membrane injury and the reversibility of that process.

DIRECT ELECTRIC FIELD EFFECTS. A cell within an applied DC or low-frequency electric field will experience electric forces which will act most forcefully across and along the surface of the cell membrane. The forces acting across the membrane can alter membrane protein conformation and disrupt the structural integrity of the lipid bilayer. The magnitude of the forces acting across the membrane is related to the induced V_m. V_m depends on a variety of factors, such as, the intra- and extracellular medium conductivity, cell shape and size, the external electric field strength E as well as how the electric field vector orients with respect to the point of interest on the cell membrane (21, 22, 23).

Given the most cells are either spheriodal in shape or cylindrical, the expressions which describe the relationship between the externally applied electric field and the induced transmembrane potential can be simplified to two simple forms. Considering physiologic conditions, the peak magnitude of induced transmembrane potential V^p_m at the electrode-facing poles of spherical cells can be expressed as

$$V^p_m = 1.5\, R_{cell} \cos(\phi) \cdot (1+(f / f_s)^2)^{-1/2} \cdot E_{peak}, \quad (1)$$

with R_{cell} being the radius of the cell, E_{peak} is the peak field strength in the tissue surrounding the cell, ϕ is the angle off axis from the field direction, f_s is the sub-β-dispersion frequency limit below which the cell charging time is short compared to rate of field change, and f is the field frequency. For cylindrical shaped cells, such as skeletal muscle and nerve cells, which are aligned in the direction of the field (herein assigned the z coordinate), the induced transmembrane potential takes a different form. Under these circumstances an electrical space

constant parameter becomes useful in describing the electrical properties of the cell. The induced transmembrane potential as a function of

$$V^{p}_{m}(z) \approx A\,\lambda_m \sinh(z/\lambda_m)\cdot(1+(f/f_s)^2)^{-1/2}\cdot E_{peak} \qquad (2)$$

when λ_m is the electrical space constant of the cell, *A* is a variable that depends on cell length, the position *z* = 0 corresponds to the mid-point of the cell (22). Figure 1 illustrates schematically the spatial variation of $V^{p}_{m}(z)$ on the cell size.

Equations (1) and (2) are valid as long as the electrical properties of the cell membrane remain constant. However, the major theme of this review article is that the transport properties of the cell membrane are altered by forces that are much greater than the natural physiologic forces. The natural transmembrane potential of mammalian cells, which has a magnitude of less than 100 millivolts, (24), originates from the difference in ionic strengths of the cell's intra- and extracellular fluids. When an imposed potential results in a transmembrane potential magnitude of greater that 200 mV, intra-membrane molecular alterations occur which may lead to membrane damage. The principal mechanisms of damage are electroporation of the lipid bilayer and electroconformational denaturation of the membrane proteins. Electroconformational damage to membrane proteins has been well documented for voltage-gated membrane protein channels. The processes occur quickly, on the order of milliseconds, after strong fields are applied.

Electroporation. Electroporation is the term ascribed to the biophysical process of electric field driven re-organization of lipids in the lipid bilayer by supraphysiologic electric fields (21, 25, 26). Current electroporation theory indicates that highly electrically polar water molecules are

pulled by Kelvin polarization stress into transient defects in the lipid packing order within bilayer leading to quasi-stable or stable pore formation. Although most commonly used to introduce foreign DNA into cells, electroporation of isolated cells has also been used to (1) introduce enzymes, antibodies, viruses, and other agents or particles for intracellular assays; (2) precipitate cell fusion; and (3) insert or embed macromolecules into the cell membrane. Recent reviews and books published have extensively treated this subject (21, 22, 23, 27, 28, 29, 30, 31). In this review we will briefly this phenomenon as it relates to the understanding of electrocution injury.

Electroporation can be either transient or stable depending on the magnitude of the imposed transmembrane potential, duration in which it is imposed, membrane composition and temperature. The time required for electroporation ranges from 10's of microseconds to milliseconds. The physical state of the lipid bilayer, whether liquid crystal or fluidic, which is strongly temperature dependent. After application of a brief electroporating field pulse, the transiently electroporated membrane will spontaneously seal. Sealing follows removal of water from the membrane defects. Sealing kinetics are often orders of magnitude slower than the field relaxation because the forces driving the molecular sealing events are not as strong as the electroporating electric field. Sealing of electropores requires reordering of membrane lipids and removal of water molecules from the pore: both time and energy consuming processes (32, 33, 34).

The threshold transmembrane potential for induction of membrane electroporation is remarkably similar across cell types. The threshold V_m for electroporation has been found to be in the range from 300-350 mV (32, 33, 34, 35). Several authors have developed models to explain the experimentally observed values of V_m required for electroporation and associated transmembrane aqueous dynamics (36, 37). Using empirical data as parameters in an asymptotic

approximation (38), the threshold V_m is predicted to be approximately 250 mV, which is quite consistent with reported experimental data.

Generally, for most media-suspended, isolated cells with a typical diameter of 10 - 20 μm, the DC field strength threshold for electroporation is in the range of 1 kV cm^{-1}. By comparison the fields required to alter large cells is much less. Due to their relatively long lengths, skeletal muscle cells, up to 8 cm long in large animals, and nerve cells, up to 2 meters long, have much lower electroporation thresholds. Figure 2 illustrates the length scale of a large peripheral nerve such as the median nerve as an example. 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 as shown in Figure 1. Therefore, muscle and nerve cell membranes are likely to be damaged with electrical fields as small as 60 V cm^{-1}.

The distribution of electropore formation in a cell placed in an applied field was recently addressed by DeBruin & Krassowska (39, 40). Expanding from previous theoretical models, and including the fact that the membrane charging time of about 1 μs is very short compared with a 1-ms field duration, they conclude that supraphysiological V_m at the pole caps is large enough to create pores, and thereby effectively preventing a further increase in V_m in these areas.

This confirms early experimental findings which show a saturation of V_m that is independent of the field strength (for high-voltage shocks, 41, 42, 43). After the effect of ionic concentrations is included in the model, it is even able to confirm asymmetries in V_m observed in respect to the hyperpolarized (anode facing) and hypopolarized (cathode-facing) pole of a cell (33, 34, 44). Although the pore sealing time (time needed for pores to close) in the range of seconds predicted by the model is in agreement with some published experimental results (45), others have found

longer sealing times in the range of several minutes (32, 33, 46). This might be explained by the fact that: [1] this model is based on pure lipid bilayers instead of cell membranes embedded with proteins, and [2] it only considers primary pores formed by V_m (pores formed during shock) and not those formed after the external field pulse ends (secondary pores) that provide transport routes for macromolecules.

Tissue Electroporation. Investigation of tissue electroporation had been initially driven by the need for a better understanding of the pathophysiology of electrocution injury (47, 48). In the early 90's, it was studied in connection with cardiac defibrillation shocks (49, 50). More recently, tissue electroporation has begun to be envisioned as a potential therapeutic tool in the medical field. It has found use in (1) enhanced cancer tumor chemotherapy (electrochemotherapy, 51, 52), (2) localized gene therapy (53, 54), (3) transdermal drug delivery and body fluid sampling (55, 56, 57). Computational models of human high-voltage electrocution shock suggest that the induced tissue electric field strength in the extremities is high enough to electroporate skeletal muscle and peripheral nerve cell membranes (15, 58, 59, 60) and to possibly cause electroconformational denaturation of membrane proteins.

Bhatt and coworkers (15) measured electroporation damage accumulation using isolated, cooled *in vitro* rat *biceps femoris* muscles. After the initial impedance measurement, a electric field pulse was delivered to the muscle using current pulses that setup tissue field pulse amplitudes ranging between 30 - 120 V cm^{-1}, which was thought to be typical forearm field strengths in high-voltage electrocution shock. The duration of the DC pulses ranged from 0.5 - 10 ms. These short pulses reduce Joule heating to insignificant levels. Field pulses were separated by 10 seconds to allow thermal relaxation. The change in the normalized low frequency electrical impedance in the muscle tissue following the application of short-duration DC current pulses

indicated skeletal muscle membrane damage. A decrease in muscle impedance magnitude occurs following DC electric field pulses that exceed 60 V cm^{-1} magnitude and 1 ms duration. As seen in Figure 3 the field strength, pulse duration, and number of pulses were factors that determine the extent of electroporation damage.

Based on these results, Block et al (16) electrically shocked fully anesthetized female Sprague-Dawley rats through cuff-type electrodes wrapped around the base of the tail and one ankle (Fig. 4) using a current-regulated DC power supply. The objective was to determine whether electroporation of skeletal muscle tissue *in-situ* could lead to substantial necrosis. The study involved histopathological analysis and diagnostic imaging of an anesthetized animal hind limb. A series of 4 millisecond DC-current pulses, each separated by 10 seconds to allow complete thermal relaxation back to baseline temperature before the next field pulse, was applied. The electric field strength produced in the thigh muscle was estimated to range from 37 V cm^{-1} to 150 V cm^{-1}, corresponding to applied currents ranging from 0.5 – 2 A. These tissue fields were suggested to be on the same level as that experienced by many victims of high-voltage electrocution shock. Muscle biopsies were obtained from the injured as well as the collateral control legs six hours post shock and subjected to histopathological analysis. Sections of electrically shocked muscle revealed extensive vacuolization and hypercontraction-induced degeneration band patterns which were not found in non-shocked contralateral controls (Fig. 5). The fraction of hypercontracted muscle cells increased with the number of applied pulses. These results are consistent with the investigators hypothesis that non-thermal electrical effects alone can induce cellular necrosis. The pathologic appearance of the shocked muscle was similar to that seen in the disease malignant hyperthermia indicating that electroporation may lead to Ca^{2+}-influx into the sarcoplasm. Most recently, a similar muscle injury pattern has been

described in a human electrocution injury victim published by deBono in a clinical case report (61). These results suggested that direct electrocution injury of skeletal muscle *in-situ* can lead to the commonly seen pattern of injury in electrocution shock victims even in the absence of pathologically significant Joule heating.

Electrophysiological Responses to Electroporation. Compound muscle action potential amplitude (CMAP) records reflect the vectorial sum of fields produced by individual action potential (AP) conducting muscle cells. Only cells with intact membranes and with active ATP production are capable of generating action AP's. Thus, changes in CMAP amplitude can be used to quantify extent of tissue injury caused by events which damage cell membranes. In a recent report, CMAP recordings were used to estimate electroporation injury accumulation in the anesthetized rat hindlimb. CMAP's were produced by magnetic stimulation of the distal spinal cord. CMAP's were recorded via skin surface electrodes. Using this entirely non-invasive protocol, CMAP's changes in response to a series of applied 150 V cm^{-1} field pulses were recorded as a function of the number of field pulses applied. The data is shown in Figure 6. A saline injection was given intravenously 30 min. after the electric field application to simulate fluid resuscitation and as a sham-treatment for therapeutic investigations. The CMAP amplitudes decreased drastically after the electrocution shocks were applied, then they recover gradually with time. The larger the number of shocks, the larger the initial drop in CMAP, and the slower the recovery.

Imaging of Electroporation. Technetium^{99m}-PYP (pyrophosphate) is widely used as a radiolabel tracer for various forms of soft tissue injury including electrical trauma. It is known to accumulate in damaged soft tissue, to clear moderately quickly from undamaged tissue, and to deposit over time in bone. The biggest drawback in using Tc^{99m}-PYP is that the mechanism of its

accumulation in damaged tissue is not yet well understood; it is believed to follow the calcium movement in cellular function (62). That increased tracer accumulation in the muscle tissue indicates loss of cell membrane integrity, tissue edema and predictive of tissue.

Using the *in vivo* rat hind limb electrocution injury model described by Block, Matthews et al (63) monitored the uptake of Tc^{99m}-PYP in the electrically shocked tissue as a function of the magnitude of the DC current. Either 0.5, 1.0 or 1.85 A of direct current was applied to the rat's hind limb. Intravenous saline infusion was used as sham-treatment. For each animal, a series of Tc^{99m}-PYP incorporation images (at 2 min. intervals) over the period of four hours was recorded. Their results supported earlier reports indicating that Tc^{99m}-PYP does accumulate in electroporated tissue. The plots of Tc^{99m}-PYP incorporation in Figure 7 suggests that the level of the tracer accumulation is positively correlated to the tissue field pulses applied. This indicates that quantitative imaging of Tc^{99m}-PYP uptake may be developed further as an indicator of the extent of electroporation or other membrane injury.

These experimental studies have shown that electroporation can lead to skeletal muscle tissue necrosis *in-vivo*. For several reasons, electroporation damage accumulation dynamics at the tissue level is different than for the case of isolated cells including the reduction of membrane lipid mobility caused by adhesion to large molecular weight biopolymers in the extracellular matrix of tissues. In addition, the distribution of electric fields, and in turn the induced transmembrane potential in tissue, is influenced by the packing density of the cells. Collectively, these results suggests that electroporation is likely to be an important mechanism of injury in electrocution shock victims.

THERMAL "BURN" INJURY. Passage of electrical current through Ohmic conduction leads to Joule Heating that can lead to severe burn injury in electrocution shock victims. Burn injury is used here to specifically refer to tissue injury by damaging supraphysiological temperatures. Burn affects are related to protein alteration or denaturation often followed by recognizabel changes in the optical properties of tissue. To understand the fundamental molecular basis of thermal burns, we must first recall that temperature, as defined by Boltzmann, is a measure of the kinetic energy of moving molecules of a medium. The relationship is defined by

$$k_B T \approx m v^2 \tag{3}$$

where k_B is Boltzmann's constant. Specifically, the time averaged speed v of a mono-atomic molecule in free solution at temperature T where m is the mass of the molecule. As temperature rises both the frequency of intermolecular collisions and the molecular momentum transfer between colliding molecules increase. When sufficient energy is transmitted to a macromolecule, an alteration in its molecular conformation can take place. As a consequence, at supraphysiologic temperature the probability that macromolecules such as proteins depart from their native structures increases.

There are two conceptually different potential outcomes for the denatured protein which dependent on the initial molecular structure and configuration. The first possibility occurs when the native folded conformational state of the protein, held by intra-molecular bonds, is different from the most favored conformation when no intramolecular crosslinks are needed to maintain the native folded state (thermodynamically lowest energy level). When this protein is heated, the intramolecular bonds are broken and it denatures to one of several preferred lower energy states

from which it will not spontaneously return to the native conformation. Conceivably, if the primary structure of the protein is undamaged it may be plausible to reconfigure the protein using similar chaperon-assisted mechanisms which establishes its initial folding after biosynthesis. The second possibility occurs when the native folded state of the protein is the same as the most preferred, energetically lowest protein conformation in the absence of intramolecular crosslinks.

Because the preferred configuration of a non-crosslinked protein is temperature dependent, protein will heat denature into conformations that are different from the preferred conformations at normal operating temperature. The free energy G, and hence relative stability, of any state of a protein is governed by competing tendencies: the tendency to form as many bonds as possible, measured in units of enthalpy H, and the tendency to be as disordered as possible, measured in units of entropy S. Because the entropy S is scaled according to temperature, S has to be multiplied by temperature to get the energy. Thus, G = H-TS.

When the temperature is raised to a higher level, the thermodynamic energy profile changes, the denatured form now has the lower energy and the activation energy barrier between different conformations is much lower. It is now energetically possible and favorable for the protein to denature. When the temperature is lowered back to the normal body temperature the landscape changes again, effectively trapping the protein in the damaged state due to the, now again, higher activation barriers.

The speed of the transition from natural to denatured states is governed by the Arrhenius rate equation which states that when the kinetic energy of the molecule exceeds a threshold magnitude E^i_a (for activation energy), the transition to the i^{th} state will occur (in this case from

natural to denatured state). For a large number of molecules at temperature T the fraction with a kinetic energy above E_a is governed by the Maxwell-Boltzmann relation (Γ) (64),

$$\Gamma^i = \exp(-E^i_a / k_B T) \tag{4}$$

where k_B is Boltzmann's constant. Because the strength of bonds retaining the folding conformation of macromolecules is very dependent on the nature of the chemical bond, the value of E^i_a is dependent on molecular structure. Despite this complexity the net rate of denaturation of cellular structures containing many different proteins is also often describable in terms of Eqs. 4. For example, the accuracy of these equations in describing thermal damage to cell membranes has been reported (65, 66, 67). Even the thermal injury to intact tissues like human skin is reasonably described by the simple Eq. 4. It has been known for more than 50 years that the rate at which damage accumulates in heated skin can be estimated by convolving Eq. 4 with the temperature history. The resulting expression is called the "heat damage" equation (68),

$$\mathrm{d}\Omega / \mathrm{d}t = A\,\Gamma \tag{5}$$

where Ω is a parameter reflective of the extent of damage, and A is a frequency factor that describes how often a configuration from which reaction is possible occurs which is also very dependent on molecular structure. The shape of the temperature-time curve predicted by Eq. 5 is indeed the same as the human skin temperature vs. time scald burn curve measured by Henriques and Moritz (69). This temperature-time curve shape has also been obtained for heat damage to isolated cells (67).

Because the bilayer lipid component of the cell membranes are held together only by forces of hydration, the lipid bilayer of the most vulnerable to heat damage (70). Even at temperatures of only 6°C above normal (i.e. 43°C) the structural integrity of the lipid bilayer is lost (71). In effect, the warmed lipid bilayer goes into solution, rendering the membrane freely permeable to small ions. At slightly higher temperatures, published reports indicate that the contractile mechanism of muscle cells is destroyed immediately following exposure to 45°C and above (72). Experiments on fibroblasts demonstrated that heat-induced membrane permeabilization also begins to appear above 45°C (73).

Bischof and coworkers investigated the effect of supraphysiological temperatures on isolated rat muscle cells using a thermally controlled microperfusion stage (74). Cells were loaded with the membrane permeable fluorescent dye precursor calcein-AM. After entering the cell, the precursor is converted by nonspecific esterases into the membrane impermeable fluorescent Calcein. Using quantitative fluorescent microscopy Bischof et al measured time-resolved dye leakage from the muscle cells at several supraphysiological temperatures (Fig. 8). In addition, using Eqs. 5 and 6, the authors determined the activation energy necessary to thermally induce membrane permeabilization in the isolated muscle cells to 32.9 kcal mol^{-1} (74). Reported activation energy values for thermal damage in other cell types are in the range from 30 – 140 kcal mol^{-1} (67).

ELECTROCONFORMATIONAL DENATURATION OF TRANSMEMBRANE PROTEINS. In addition to membrane electroporation, which is mainly a lipid bilayer phenomenon, large supraphysiologic transmembrane potential differences can produce electroconformational changes of membrane proteins, ion channels and ion pumps. Approximately 30% of a cell's membrane consists of proteins, some of them embedded into the bilayer, others spanning across the entire membrane.

Many of them carry electric charges from amino acids with acidic or basic side groups that can be acted on directly by an intense V_m (charge separation or charge induction through dissociation). In addition, each amino acid has an electrical dipole moment of about 3.5 D giving the proteins an overall dipole moment that, in the case of an α-helical protein structure, can reach 120 D (75). In a strong external electric field those molecules will orientate themselves and thereby change their conformation to increase the effective dipole moment in the direction of that field.

If the field strength becomes sufficiently intense, those field-induced changes can cause irreversible damage to a membrane protein. In particular ion channels and pumps with their selective, voltage gated charge transport mechanisms (e.g., Ca^{2+} specific channel) are highly sensitive to differences in V_m. Chen and coworkers investigated the effects of large magnitude V_m pulses on voltage-gated Na^+ and K^+ channel behavior in frog skeletal muscle membrane using a modified double vaseline-gap voltage clamp. They found in both channel types, but more drastically in K^+ channels, reductions of channel conductance and ionic selectivity by the imposed V_m (76). In their most recent work, Chen et al. were able to demonstrate that these changes are not caused by the field-induced huge channel currents (Joule heating damage) but rather the magnitude and polarity of the imposed V_m (77). The consequences of this effect may underlie the transient nerve and muscle paralysis in electrocution injury victims.

RF and Microwave Burns

Although relatively less common, each year many cases of RF (radio frequency) or microwave field injuries occur. The victims are usually industrial workers. Above the low frequency regime (>10 kHz), tissue response strongly depends upon the field frequency. In the 10 - 100 MHz RF

range, two types of tissue heating occur, Joule and dielectric heating, with Joule heating outweighing dielectric heating. Small molecules like water, when not bound, are able to follow the field up to the gigahertz range (78). However, at microwave frequencies (100 MHz - 100 GHz), dielectric heating is more significant than Joule heating because both bound and free water are to be excited by microwave. A water molecule has a small size but a large dipole moment, hence water has a strong perceptivity to microwave which induces water molecules into rapid oscillative rotation, and in turn heats up the whole tissue. If the amplitude of the field strength is constant, the heating rate increases with field frequency until the viscous drag on the rotating molecule makes it lose pace with the field. Molecular dipoles of larger sizes oscillate more slowly, so that their most efficient induction frequency is in the radiofrequency range. For example, coupling of RF energy into proteins and DNA's is the basis of recent concerns about cellular phones. The therapeutic use of RF hyperthermia is based on the same mechanism.

Exposure to ambient microwave fields are known to cause burn trauma. Microwave burns have different clinical manifestations than low frequency electrocution shocks (79, 80, 81, 82). At low frequency the epidermis is a highly resistive barrier, whereas in the microwave regime, electrical power readily passes the epidermis in the form of "capacitive" coupling with very little energy dissipation. Consequently, the epidermis may not be burned unless it is very moist. The microwave field penetration into tissue has a characteristic depth in the range of 1 cm, resulting in direct heating of sub-epidermal tissue water. The rate of tissue heating is dependent not only on the amplitude of tissue electric field, but also on the density of dipoles. For example, microwave heating is much slower in fatty tissues (83).

Lightning Injury

Lightning arcs result from dielectric breakdown in air caused by build up of free electrical charges on the surface of clouds. The current through an arc can be enormous, but the duration is quite brief (1 - 10 ms). The primary current is confined to the surface of conducting objects connected by the arc. Peak lightning current range between 30,000 - 50,000 Amperes, which are able to generate temperatures near 30,000 K. This abrupt heating generates a high-pressure thermoacoustic blast wave known as thunder.

An individual directly struck by lightning will experience current for a brief period of time. Initially, the surface of the body is charged by the high electric field in the air. This can cause breakdown of the epidermis, and several hundred amperes to flow through the body for a 1 - 10 μs period, which is certainly long enough to induce electroporation. Following this, a much smaller current persists for several milliseconds, in which time the body is discharging into the ground. The duration of current flow is relatively short, so there is no substantial heating except a breakdown of the epidermis. However, disruption of cell membrane can wreak havoc on nerve and muscle tissues.

When lightning reaches the ground, it spreads out radially from the contact point. A substantial shock current can be experienced by a person walking nearby, if their feet are widely separated. For example, with an average lightning current of 20,000 A, a step length of 50 cm, and an individual located 10 m away from strike point, the voltage drops between the legs can reach 1500 V. This can induce a 2 - 3 A current flow through the body between the legs for a 10 μs period.

The immense current pulses in a lightning induces large magnetic field pulses in its surrounding. The magnetic field pulses set up secondary electrical currents inside. The secondary currents, forming closed loops around the penetrating magnetic field lines, are in theory large enough to cause cardiac arrest, seizures and other harmful effects. Also, electrical currents will be induced through any electrical circuits penetrated by the magnetic field. Consider the scenario of a person standing on wet ground and leaning on a metal golf club. The golf club links the upper body to the soil, which, in effect, forms a closed loop consisting of golf club, body and wet soil. A large magnetic pulse can drive large currents through this circuit.

Ionizing Radiation

Radiation injury occurs following exposure to damaging levels of ionizing particle beam or electromagnetic irradiation, both of which alter the atomic structure and lead to damaging chemical reactions. Radiation injuries are often called burns although heating is totally insignificant. The most common radiation injury happens after excessive ultraviolet (UV) light exposure often referred to as sunburn.

Mechanistically, electromagnetic waves of frequencies greater than ultraviolet light can excite electrons in an atom leading to the formation of unpaired electrons in the outer electron orbitals. In biological tissues this will result in damages to proteins, polysaccharides, and lipids (84). The reactive species from photo-ionization of water are primarily reactive hydroxyl radicals that can attack biological macromolecules, leading to altered chemical bonding, altered molecular structure, and ultimately, blocked biological functioning of them. The hydrogen bonds in DNAs and proteins are particularly vulnerable. This vulnerability is the rationale behind the use of radiation in cancer therapy and its use in other procedures to block cell proliferation (85).

Because some free radicals have no net electrical charge, they have relatively free access to the lipid bilayer (86). Although, the precise molecular mechanics of membrane permeabilization is still under investigation, some researchers suggest that it is mainly caused by membrane lipid peroxidation: protons get stripped off from their carbon backbone in the membrane lipids, leading to desaturation in their structure (86). Polyunsaturated fatty acids are bulkier and tend to self-aggregate by lateral diffusion in the lipid bilayer, which leads to bilayer instability and poration (87).

Clinical manifestations start to occur when the rate of free radical generation exceeds the cell's ability to scavenge them. DNA is particularly susceptible to free radical mediated abnormal crosslinking. This forms the basis for cancer radiotherapy where a carefully calibrated dose of radiation is delivered to a tumor to damage only its DNA with tolerable adverse effect to other macromolecules. If the dosage is too high in relative to the patient's scavenging ability for free radicals, tissue injury occurs. As a consequence, visible tissue changes would appear as the cell injury triggers the tissue inflammation cascade. This radiation induced inflammation, particularly that due to sunburn is very similar in appearance to that produced by superficial thermal burns.

ANATOMIC PATTERNS OF INJURY

The location and extent of tissue damage depends on the field strength, frequency, duration of contact, and tissue properties. The pattern of injury in a shock victim also depends on the current path through the body, protection from clothing, health status, and presence of a thermoacoustic blast, fractures due to muscle spasm, seizures, etc. The type and pattern of injury is of central importance to the clinical management of the victim.

Commercial Power Frequency Injuries

ESTIMATING THERMAL INJURY PATTERNS. The first task toward understanding clinical patterns of electrocution injury is to estimate the tissue field strength along the current path. This was first reported by Tropea and Lee was used to estimate the field distribution in a sample human upper extremity (58). Because of the variation in anatomic contact, use of protective equipment and power source parameters, they assumed a simplified "worst-case" scenario of perfect mechanical and electrical contact. The worst-case was considered to be a perfect mechanical contact with bare, wetted hands and a hand-to-hand current path. They numerically solved Laplace equations for the electric field of the human upper limb in a fully three-dimensional finite-element computational model based on an average male adult body such as the size, shape, and the position of skin, fat, muscle and bone transferred from a standard anatomical cross-sectional atlas (88). From this, the Joule heating dynamics in tissues during and following high-voltage electrocution shock was derived (58). These simulations were based on a hand-to-hand potential drop ranging from 1 to 20 kV. The resistance for a single upper extremity was calculated to be 384 Ohm and the total hand-to-hand resistance to be 1268 Ohm, which agreed well with Freiberger's measurements (89) made more than 60 years ago. The calculated electric field strength in the upper extremity for a worst-case contact with a 10 kV hand-to-hand 60 Hz source was found highest in the distal forearm due to its small cross-section, where it ranged from 65 to 140 V/cm (Fig. 9). The field strength maxima at joints reflected the high percentage of bone and skin in the anatomic cross-sections at these locations. Appropriate thermal and electrical properties were assigned to each tissue, and the temperature rise due to Joule heating was determined using the Pennes Bioheat equation (90) with an added energy source term for Joule heating. This equation describes the balance between heat dissipation due to conduction

heat transfer and cooling through blood perfusion, and heat generation due to electric current. The result was the temperature profile for each anatomic cross-section as a function of duration of current flow (i.e. contact) and the contact voltage. They found that when the tissues were electrically connected in parallel, skeletal muscle sustained the largest temperature rise and then heated adjacent tissue. In addition, the tissue perfusion was found to be the most important parameter in determining the kinetics of tissue cooling. Estimates of Lethal Contact Times (LT) required to produce substantial heat damage at any point in the model was derived by a convolving Eq. 5 with the tissue temperature history for a number of adjacent points in the center of muscle in selected anatomic cross-sections. The LT were defined by solving for the time for $\Omega=1$ in Eq. 6. The LT were determined for points selected in the center of the muscle mass for various cross sections is shown in Figure 10. The four curves displayed represent the LT of contact as a function of contact voltage at different positions along the axis of the upper extremity. These values would be different if the size of the upper extremity changed, if the shape changed as would occur by a change in position, if the electrical contact was mediated by an arc, or if other deterministic parameters changed.

The LT curves are helpful to estimate the likelihood of significant thermal damage following a certain electrocution shock exposure. Unfortunately, the contact time of an actual electrocution shock injury is almost never known. It seems from the work of Jones and others (91), that for accidents involving high-power electrical sources, contact times are very likely to be on the scale of fractions of a second. This is because the acoustic blast resulting from arcing is likely to push the victim away. Whereas longer contacts are more likely with lower voltages. Thus, the usefulness of the LT analysis rests in its suggestion that the duration of contact is the most

significant parameter in determining the extent of thermal injury to subcutaneous tissues in high-voltage, high-current electrocution shock.

IMAGING ELECTROPORATION DAMAGE PATTERNS. In most electrocution injury cases, the treating physician does not know the voltage, current and the contact time experienced in a particular high-voltage accident. Since the muscle damage sustained lies invisible underneath the skin, non-invasive imaging methods such as magnetic resonance imaging (MRI) have rbeen explored as important tools in early diagnosis. MRI allows the detection of the edema (T_2-weighted imaging sequences) and evaluation of permeability changes (contrast enhanced T_1-weighted sequences) in electrically induced muscle injury with high spatial resolution.

Using the previously described rat hind limb electrocution injury model, Jang et al acquired multi-slice T_2-weighted and contrast agent (Gd-DTPA) enhanced T_1-weighted images to obtain information about edema localization and contrast agent distribution volume, respectively (92). Images of relative contrast agent distribution volume in the electrically shocked hind limb were obtained by subtracting the pre-injection T_1-weighted image sets from the respective post-bolus injection T_1-weighted image sets (93). MRI scans of non-shocked rat hind limbs served as a control for image interpretation. Significant differences in both the T_2 - and contrast enhanced T_1 -weighted images were observed between control (non-shocked) and electrically shocked animals (Fig. 11). The investigators found a strong regional correlation between edematous tissue (T_2) and areas of increased contrast agent distribution volume (T_1) confirming the cell membrane permeabilizing (electroporating) nature of the muscle injury imaged.

Because muscle bundles are encapsulated in layers of perimysium and epimysium, severe edema will increase the local interstitial hydrostatic pressure so much that it obstructs the local blood

flow (compartment syndrome). The prolonged ischemia alone will also cause muscle necrosis. This effect is especially pronounced in deep muscles injured by electric shock(5). As discussed in Lee, 1997 (5), if edema is present in a muscle group, damage should be expected. The MRI images of electrically shocked hind limbs shown in Figure 11 demonstrate also that electrocution injury is not uniformly distributed in the bulk muscle tissue: some muscle flaps are spared and others endure more severe damage. The inhomogeneous injury pattern of muscle electrocution injury is probably a combination of the following factors: [1] Non-uniform distribution of electrical current intensity in different muscle flaps; [2] Non-uniform electrical resistance distribution of muscle itself and the break-in barrier resistance of muscle sheaths; [3] Differences in relative orientation of different muscle cell bundles and muscle flaps with respect to the electrical current entrance and exit points;. Although at this time the cause for the inhomogeneous injury pattern can only be speculated on, the early differentiation of injured vs non-injured muscle flaps via MRI will greatly help treating physicians on surgery planning and management.

COMBINED THERMAL AND ELECTROCUTION INJURY MECHANISMS. The preceding discussion indicates that there are thermal and direct electrical mechanisms of tissue injury in victims of electrocution shock. These mechanisms produce different cellular injuries, suggesting that therapeutic strategies will be different. Thus it is important to discuss this important matter further.

Because of the universal vulnerability to supraphysiologic temperature exposure of all tissue types, prolonged contact causes direct thermal damage to all tissues in the current path. For tissue field strengths larger than 30 volts/cm, generation of heat is so much faster than the heat convection due to blood perfusion that the heating process is considered quasi-adiabatic. Clearly,

as boiling and vaporization begin (which dissipate heat much more quickly), this approximation no longer holds.

Significant membrane permeabilization in human skeletal muscle cells with average length can occur in field applications above 25 V cm^{-1} for any contact time more than 20 – 40 milliseconds. However, the extent of the Joule heating mediated damage depends on the duration of the contact. Figure 12 shows that if the field strength is less than 25 V cm^{-1}, it will take forever to heat up a tissue with normal blood circulation. On the other hand all field exposure levels shown lead to membrane permeabilization. At higher field strengths, the field strength and exposure time combination to reach certain tissue temperatures are plotted as separate curves. At higher field strength, a lower exposure time is needed to reach the same temperature by way of Joule heating.

The importance of the duration of contact is again illustrated in Figure 9. For a fixed current (10 A) contact, the electric field strength in the tissue as a function of the position along the arm is plotted as derived from the 3-D model described above. The shading underneath the curve reflects the variation in predicted contact time as a function of position needed to raise skeletal muscle tissue temperature from 37°C to 45°C. As mentioned earlier, heat-induced cellular membrane damage accues in a temperature dependent fashion above 42.5°C (74, 74). Obviously the tissue sections experiencing higher field strengths require less exposure time to produce temperature rise above 45°C.

The predicted combined electroporation and thermal injury distribution in the human upper extremity model suggested in Figure 9 following electric shock provides some insight into the variation in injury pattern when contact duration and strength are known. Clearly, brief duration

contacts result in membrane breakdown without significant burn injury. With high field or long duration contacts, on the other hand, thermal damage predominates, and may overshadow the electroportion effect on clinical outcome. Additional simulations of this nature with morphable models would be useful for further insight. Simulations that include the changes in tissue properties as a function of applied field strength, hydration state and temperature (which changes its chemical nature) would also be quite important to complete. In a recent report by DeBono, 1999 (61), a 100 kV injured human arm (current path from palm to shoulder) is amputated and dissected. He found that on the forearm, the damage (to both the muscle and the nerve tissue) is much more severe on the radial (thumb) side than that on the ulnar side, which would not be predicted assuming constant and uniform tissue properties.

Even if the bulk tissue has a uniform field strength, different muscle cells will be injured differently due to differences in cell size, orientation and anatomical site, as demonstrated by the theoretical model of Gaylor et al 1988 (14). For example, a long muscle aligned in parallel with the field gradient will experience a much larger transmembrane potential than one aligned in perpendicular with it. They suggested that under certain assumptions, skeletal muscle cells in the interior of a bundle may experience a higher level of transmembrane potential than those near the exterior fascia and cells next to a bone will experience a higher transmembrane potential than those away from it. These predictions seem to agree with the general injury pattern reported (94, 95, 96) as well as experimental observations (92).

Microwave and RF Burns

Of a few reports on microwave injuries in the literature, a large portion result from use of radiofrequency electrocautery devices during surgery where microwave is delivered to heat

denature proteins in order to control bleeding or to rapidly vaporizing tissue water in order to cut (97). Use of these devices will occasionally result in unintentional tissue injury. Most of the injury occurs below the epidermis, with the fat tissue largely spared. Fat doesn't attenuate the field well either. Skeletal muscle has a high level of hydration, hence it carries a heavy load of thermal input under microwave. Clinical experience indicates that a characteristic "layered burn" occurs: burned skin and muscle with fat spared in between.

Microwave burns demonstrate similar patterns as the higher frequency RF burns. However, there is more Joule heating component in RF heating, hence fat is not as well spared as in microwave heating. Another more common complications of RF hyperthermia procedures are nerve injuries (78). Therapeutic guidelines on RF hyperthermia require a maintenance of tissue temperature at 42ºC to 43ºC. Exposure to this temperature for 0.5 – 1 h will generate substantial heat-mediated membrane permeabilization. Patients with RF burns usually have deep 4th degree burns penetrating all tissues. Most of these injuries occur as a side effect of either a tumor-treating therapy or a healing-enhancement physical therapy.

Lightning Injury

Victims of lighnting injury usually manifest fern-like patterns of burn injury that is mostly confined to the outer layers of the skin along with neuromuscular dysfunction (3, 98, 99, 100). The seriousness of a lightning strike should not be judged by the superficial surface burn occurred on the skin. The duration of the primary lightning current pulse and its induced secondary electric currents are so brief that heating is typically not found in subcutaneous tissue., rather the injury appears to be a pure electrical phenomenon. As previously described, there are some exceptions to this, such as when lightning flashes over to charge up a car or truck to which

a victim is in contact, thus the electrical discharge of the car or truck through the victim takes long enough for heating and burning to occur in addition to the damage from electrical effects.

Transient nerve dysfunction can have a variable course of recovery depending upon the severity of field exposure. Nerves can regain function in hours or require months. The mechanism of recovery is unknown. Permanent sequelae have been correlated with demonstrable anatomical lesions (101). Dr. Cooper (3) has categorized lightning strike victims in three groups of severity. The first group is "mild injury" in which the effects are transient. The victim is "stunned" by shock, may have fallen, has been fully alert, slightly disoriented and amnesic for the event. Memory of the event is impaired for hours to days. There may also be temporary blindness and deafness. The patient may be hypertensive or hypotensive as a result of a loss of autonomic control. Recovery is gradual but usually complete. The second "moderately injured" group of victims is disoriented, combative and may manifest seizure activity. They have keraunoparalysis that will persist for several hours. Vascular spasms lead to cold, mottled, cyanotic skin over the affected distal extremities. Characteristically, they have superficial thermal injury to the skin, but occasionally the burns are deeper. Myocardial infarction may have occurred or occur in response to the stress (102). These patients generally survive (103, 104) with permanent neurophysiologic disturbances such as paresthesias, loss of cutaneous sensation, sleep disorders, etc. The third "severely injured" group of victims has lesions in central nervous system (CNS) (100) and/or myocardial infarction (98, 99). Prolonged circulatory arrest will add hypoxia / reperfusion injury to the brain. Although, survival is possible, rehabilitation potential is small.

Radiation Injury

Ionizing UV light can only penetrate the very thinnest layer of skin. Soft X-rays with wavelengths below 10 nm (124 eV – 12.4 eV) have a very shallow penetration into the tissue. Radiation with energies between 12.4 keV and 124 keV (0.1 – 0.01 nm) is widely used for diagnostic purposes. It penetrates through the body but energy deposition is so low that it can only be used for superficial therapies. Both of these X-ray classes can be produced by X-ray tubes. Deep therapy X-rays used in cancer treatments have energies of more than 124 keV and are typically produced in linear accelerators. These radiation beams penetrate easily the entire body and produce damage along their travel pathway. To optimize cancer treatment effects with a minimum of damage in non-cancerous tissue, cancer therapies therefore use multiple beams intersecting at the center of the tumor.

Immediately after an exposure to intense irradiation, no visible changes are manifest. In irradiated areas, skin wounds will develop as cells lose their ability to proliferate or produce extracellular matrix. All irradiated cells are affected. Tissue breakdown ensues as widespread edema and inflammation occur as a result of the loss of capillary function. At radiation doses in excess of 80 - 100 Gy, even post-mitotic cells are killed via extensive lipid peroxidation and resultant bilayer membrane permeabilization (87). In nerve cells this leads to CNS arrest, which in turn leads to death within hours of exposure. The clinical syndrome resulting from this massive exposure is called the "Neurologic Syndrome" (105).

SUMMARY AND CONCLUSIONS

Given the importance of electrical power to human culture, the problem of electrocution injury is one that will continue to exist for the foreseeable future. Electrocution injury has been poorly

understood and perhaps less than optimally managed in the past. Improvement requires a better understanding of injury mechanisms, anatomical patterns of injury and therapy. A prompt, accurate clinical diagnosis of electrocution injury is one of the most difficult tasks in the medical field (5) because it usually calls upon an understanding of the interaction between electric current and human tissue. Specifically speaking, the difficulty involves the following:

1. The exact tissue damage mechanism and damage level depend on a host of parameters: the characteristics of the power source (DC or AC current, voltage, frequency, etc.), path and duration of closed circuit, area and impedance of contact spot, etc. Correspondingly, there is a whole spectrum of damage characteristics depending on the values of these parameters. The physician needs to do a 4-dimensional (spatial plus temporal) detective work in order to arrive at a correct diagnosis.

2. Electrical damage to the tissues is not easily detectable by visual inspection or physical examination. And often times its sequelae will not manifest themselves after a certain period of time: electrically injured tissue may initially appear viable, only to become visibly necrotic at a later point (in a number of days) (94, 106, 107).

The molecular structure of biological systems can be severely altered by the effects of high-energy commercial frequency electrical power. The mechanisms of damage include cell membrane electroporation, Joule heating, electroconformational protein denaturation, and others.

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