ELSEVIER

Burns Vol. 22, No. 7, pp. 509–519, 1996

Printed in Great Britain
0305-4179/96 $15.00 + 0.00

PII: S0305-4179(96)00051-4

The physicochemical basis for thermal and non-thermal 'burn' injuries

R. C. Lee[1] and R. D. Astumian[2]

[1]Electrical Trauma Research Program, Section of Plastic and Reconstructive Surgery, Department of Surgery, Department of Organismal Biology and Anatomy and [2]Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, Illinois, USA

One of the most basic problems of burn science may well be the confusing nomenclature we use. The word 'burn' is used to identify several different mechanisms of tissue injury. This article describes the problem of accurately characterizing and defining the various burn injuries on the basis of molecular events. The most important objective is to distinguish between the various physicochemical injuries on the basis of differences in their fundamental physicochemical mechanisms and physiological consequences. Also, pathophysiologically important biophysical processes such as the central importance of cell membrane permeabilization in acute cellular necrosis, which different types of burn injury have in common, are emphasized. The biophysics of membrane formation and permeabilization is presented to clarify the conditions for membrane damage as well as to discuss the potential for therapeutic intervention. Where feasible, plausible new strategies to reverse the molecular alterations caused by injury are hypothesized. Copyright © 1996 Elsevier Science Ltd for ISBI.

Burns, Vol. 22, No. 7, 509–519, 1996

Introduction

The prime objective of the trend toward molecular medicine is to devise ways to reverse or correct molecular pathophysiology caused by injury or disease processes. This dream, which may have seemed unattainable several years ago, is now on the verge of reality, and in several notable cases has been achieved. To apply this strategy to burn injury, it is necessary to know the molecular alterations resulting from physicochemical damage of lipids, proteins and genetic material. The capability to solve a problem nearly always requires having a clear definition. Thus, progress toward reversing or correcting damage due to burns is predicated on accurately discriminating and naming the molecular events involved. For the purposes of this discussion, the term physicochemical is used in reference to chemical events driven by physical forces acting at the molecular level. This would included heat-induced molecular changes such as macromolecule denaturation; electric force effects such as electroconformational coupling and membrane electroporation; frostbite-related ice crystallization leading to membrane disruption; and free radical-mediated effects such as that caused by toxic doses of ionizing irradiation.

Regrettably, the naming of the various types of burn injury has been somewhat ambiguous. Even today, injuries caused by electrical, chemical, radiation and thermal events are generally referred to as 'burns', despite the fact that the specific pathological molecular physics of these damaging processes are quite different. Thus, it is necessary to develop ways of communicating about these injuries that convey an understanding of the underlying pathophysiology and clinical manifestation. To begin, the origin of the problem must be addressed. The common macroscopic manifestations of physicochemical injuries, especially to the skin, caused by thermal insult, caustic chemical exposure (typically acids and bases, or strong reducing/oxidizing agents), exposure to ionizing radiation or electrical shock have considerable visual and physiological similarities. These injuries lead to inflammation and pain as well as changes in tissue appearance. These similarities in response to injury and approaches to therapy explain the application of the name 'burn' to these non-thermal injuries. As in other aspects of medicine, the naming of disease processes has required refinement accompanied with increased understanding.

In this review, an attempt will be made to describe the molecular basis and biological consequences of several types of injuries typically accumulated under the umbrella term 'burn' (*Table I*).

Thermal burn injury

Thermal burn is the term most often given to the damaging supraphysiological temperature effects

Table I. Physicochemical injuries called 'burns'

Acid	Flash
Alkali	Friction
Brush	Sun
Cement	Radiation
Electrical	Scald
Flame	Thermal

caused by heating tissue. It is generally appreciated that the effects are related to damage to proteins and that grossly visible changes in tissue follow. To understand the fundamental molecular basis of thermal burns, we must first recall that temperature, as defined by Boltzmann (*Figure 1*), is a measure of the kinetic energy of a moving object[1]. The relationship is defined by:

$$T = \frac{\text{kinetic energy}}{k_B} \quad (1)$$

where T is the absolute temperature (°K) of the object and k_B is Boltzmann's constant. Specifically, the time average speed v of a monoatomic molecule in free solution at temperature T is defined by the relationship:

$$k_B T \approx mv^2 \quad (2)$$

where m is the mass of the molecule with a speed v. As temperature rises both the molecular momentum transfer between colliding molecules and the frequency of intermolecular collisions increase. When sufficient energy is transmitted, alteration in molecular conformation can take place. As a consequence, at supraphysiological temperature the probability that proteins and other macromolecules will lose their native structure increases.

Figure 1. Photographic portrait of Boltzmann (circa 1990). Boltzmann was an important chemical physicist who provided a working physical understanding of temperature.

There are two conceptually different potential outcomes for the denatured protein which depend on the initial molecular structure and configuration. This can be best appreciated by examining the chemical reaction energy coordinate graph in *Figure 2*. The first possibility occurs when the native folded state of the protein, held by intramolecular bonds, is different from the most favoured configuration if no bonds were needed to maintain the native folded state. When this protein is heated, the intermolecular 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 established its initial folding after biosynthesis. The second possibility occurs when the native folded state of the protein is the same as the most preferred protein conformation in the absence of intramolecular crosslinks. Because the preferred configuration of a non-crosslinked protein is temperature dependent, the 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. The entropy S has to be multiplied by temperature to get the energy. Thus, the free energy is given by the relation[2] $G = H - TS$. In *Figure 2*, G is colour coded: orange is lowest energy and violet is highest energy. We have plotted G for a hypothetical protein that can change conformation between several gates of the protein. The conformational range of the first site is scaled on the vertical axis and the second site is scaled on the horizontal axis. Real proteins would have more dimensions, however this two-dimensional chart is useful to illustrate both consequences of heat denaturation. At normal body temperature T_1 the functional configuration of the protein is shown at position U (undamaged). When the temperature is raised to a higher level T_2, the landscape changes, the denatured form has the lower energy and the activation energy barrier is much lower. It is now energetically favourable for the protein to denature, reaching the most favoured damaged state at position D (damaged). When the temperature is lowered back to the normal body temperature the landscape changes again, effectively trapping the protein in the damaged state. If the lowest energy state U is the native state of the protein then the damage process is spontaneously reversible. The speed of spontaneous recovery is dependent on the height of the energy barriers along the most favoured recovery path (dotted line).

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, transition to the denatured state will occur. For a

large number of molecules at temperature T the fraction with a kinetic energy above the E is governed by the Maxwell–Boltzmann relation[3]:

$$\Gamma = \exp\left(\frac{-E}{k_B T}\right) \quad (3)$$

where k_B is Boltzmann's constant. Because the strength of bonds retaining the folded conformation of macromolecules is very dependent on the nature of the chemical bonds, the value of E 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 equations (2) and (3). For example, the accuracy of these equations in describing thermal damage to cell membranes has been reported[4–8]. Even the thermal injury to intact tissues such as human skin is reasonably described by this simple equation (3). It has been known for more than 50 years that the rate at which damage accumulates in heated skin can be estimated by convolving equation (3) with the temperature history.

The resulting expression is called the 'heat damage' equation[9]:

$$\frac{d\Omega}{dt} = A^*\Gamma \quad (4)$$

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 curve predicted by equation (4) is indeed the same as the human experimental skin temperature–time scald burn curve of *Figure 3* measured by Henriques and Moritz[9]. This temperature–time curve shape has also been obtained for heat damage to isolated cells[6].

Because the bilayer lipid component of the cell membranes is held together only by forces of hydration, the cell membrane is probably the most vulnerable structure to thermal trauma[1]. Even at temperatures of only 6°C above normal (i.e 43°C), the kinetic energy of the molecules in the cell membranes can exceed the hydration energy barrier which holds phospholipids in the membrane as a supramolecular assembly[7]. In effect, the warmed membrane goes into solution in the surrounding water, rendering the membrane freely permeable to small ions.

Electrical injury

Electrical burn is the term used to refer to injury caused by exposure to supraphysiological electrical

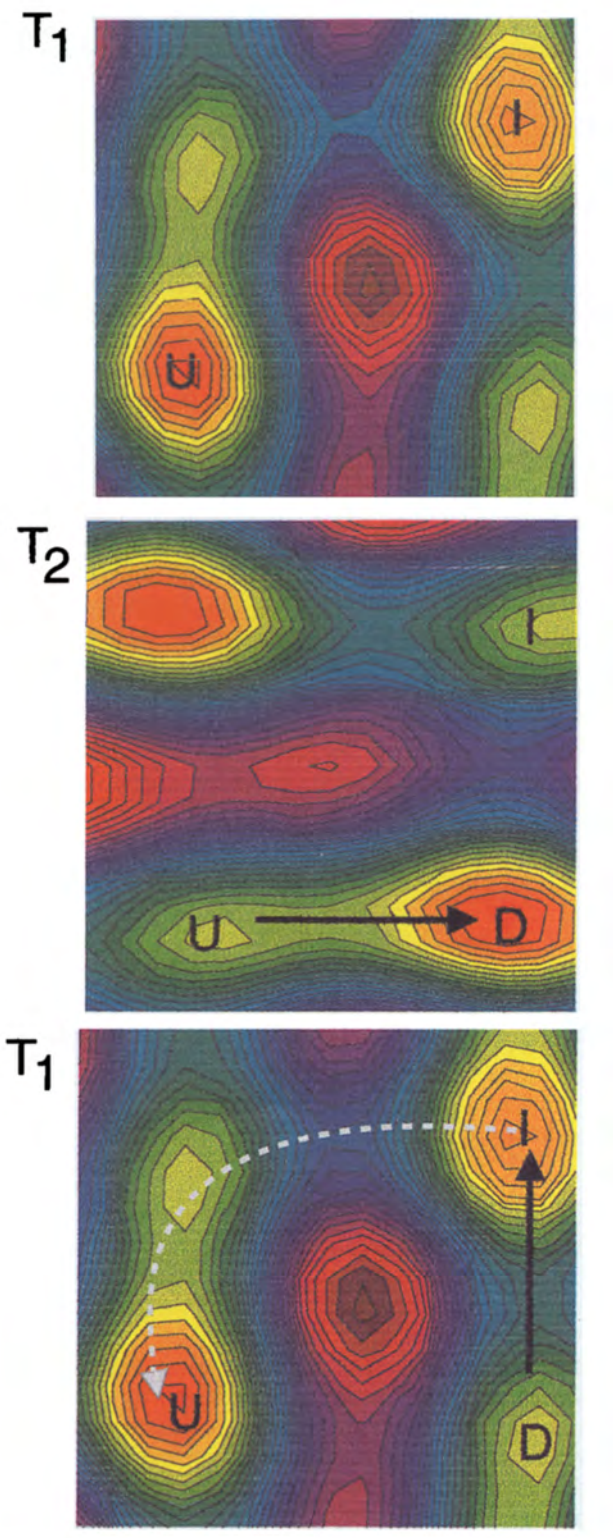


Figure 2. A common paradigm in chemical and biological physics is the representation of transitions between states of a system in terms of the diffusion of a hypothetical particle on a potential energy surface, such as shown in the figure for an arbitrary protein in solution. Orange represents the lowest energy, green the next lowest, blue follows, and red is the highest energy. As the temperature is raised from T1 to T2, it becomes energetically favourable to assume one of the denatured states along the path shown by the arrow. Upon cooling, the energy surface for the protein returns to its normal physiological topology. However, the protein is transiently trapped at state D since direct return to U is energetically improbable over the red hill. Eventually, the protein could return via the intermediate state I, but there are many potential denatured energy configurations and the probability of reaching its native undamaged site is small.

currents. By the inclusion of 'burn' in the term reference is also made to the longheld view that the mechanism of electrical shock injury is mediated by heat effects. Recently, it has become well known that the passage of electrical current through tissue can produce damaging effects by two fundamentally different physicochemical processes. One generally appreciated mechanism is heat exposure caused by the Joule heating effects of current passage. The other, more recently described mechanism[10] is due to the direct action of electrical forces on the electrically charged or electrically polarized components of tissue. Both mechanisms ultimately lead to alteration in molecular conformation and/or disruption of macromolecular structures such as proteins or the lipid bilayer of membranes. A fundamental distinction between heat and electric force mediated molecular effects is the mechanism of energy transmission to the molecule(s). In contrast to thermal forces which are random in direction and over time average to zero, electric forces produce effects on macromolecules by direct vectorial electrical coupling.

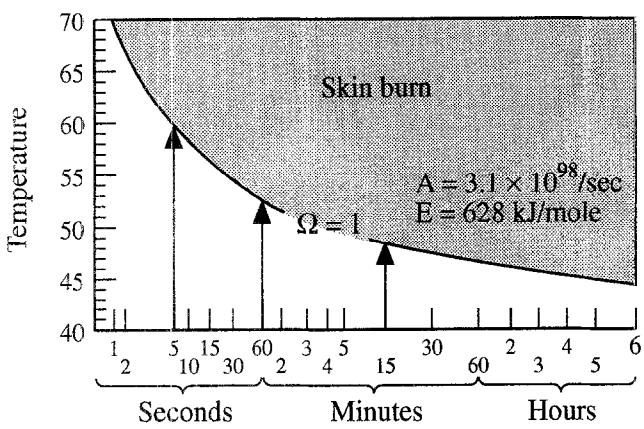


Figure 3. Kinetics of heat damage accumulation in tissue seems to follow closely the behaviour of a single chemical reaction despite the complexity of tissue molecular structure.

Another important distinction between electric force effects and heating effects is that tissue structures with dimensions much larger than that of a macromolecule are influential in electrical effects. For example, the vulnerability of a cell to electrical damage is rleated to its physical dimensions (*Figure 4*), whereas vulnerability to supraphysiological temperatures is not. In addition, because of the nature of the coupling between electrical energy and materials, electrical effects are frequently dependent. For human contacts, strong frequency dependence of damage mechanisms does not occur until the frequency is at or above the order of 10 000 cycles/s (i.e. 10 kHz) as summarized in *Table II*. However, because the vast majority of electrical injury contacts involve commercial electrical power which has operating frequencies between 0 and 60 Hz, the effects of frequency can be neglected.

Characteristically, the damage sustained by a cell injured by a pure electrical mechanism is quite different from that of a cell injured by pure supraphysiological temperatures. The pathophysiological significance of these differences is substantial. First, the distribution of injury through the cell is different. This is a consequence of the structure of cells, as illustrated in *Figure 5*. Because the cytoplasm and extracellular fluids have similar ionic strength, they are both good electrical conductors relative to pure water or oils. The electrical conductivity of the cell membrane, however, is characteristically 10^6–10^8 fold less than the surrounding media. From the simplest physical perspective, the cell can be described as an insulating shell the a highly conductive interior and exterior. Consequently, electrical current established in the extracellular space is to a large degree shielded from the cytoplasm by the electrically insulating cell membrane. This shielding limits the voltage drop

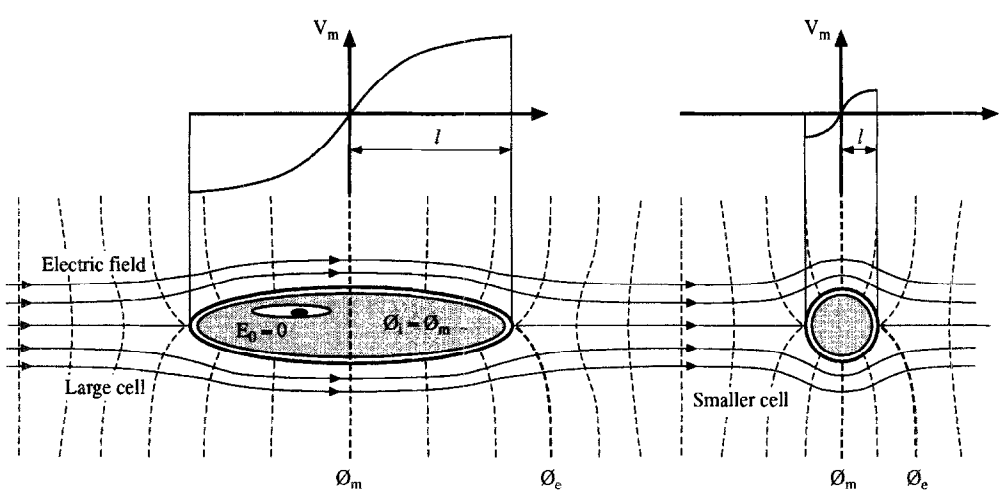


Figure 4. Distribution of current flow and electric force lines (solid) around cells exposed to the same applied uniform electric field. The current (and field lines) are solid. Lines of constant voltage (dashed) run perpendicular to the field lines. In effect, the cell membrane blocks flow of current into the cytoplasm, thus there are no solid or dashed lines in the cytoplasm, thus it is opotential throughout. The voltage difference across the membrane depends on the position on the membrane and the physical size of the cell, i.e. $\Delta V_m \propto E_0 l/2$. Large cells experience larger maximum induced transmembrane potentials because the voltage drop across the length of the cells scales with the length of the cell in the direction of the field lines.

Lee and Astumian: Thermal and non-thermal 'burn' injuries 513

Lee and Astumian: Thermal and non-thermal 'burn' injuries 513

Table II. Important frequency ranges of electrical injury

Frequency	*Description*	*Biological effect*
DC – 10 kHz	Commercial electrical power	Joule heating; destructive cell membrane potentials
10 kHz to 10 MHz	Electrothermy	Joule heating; dielectric heating of proteins
10 MHz to 10 GHz	Microwave	Dielectric heating of water
10^{21} Hz and higher	Ionizing irradiation (ultraviolet, X-ray, cosmic, alpha, electronic, gamma irradiation)	Generation of reactive oxygen intermediates

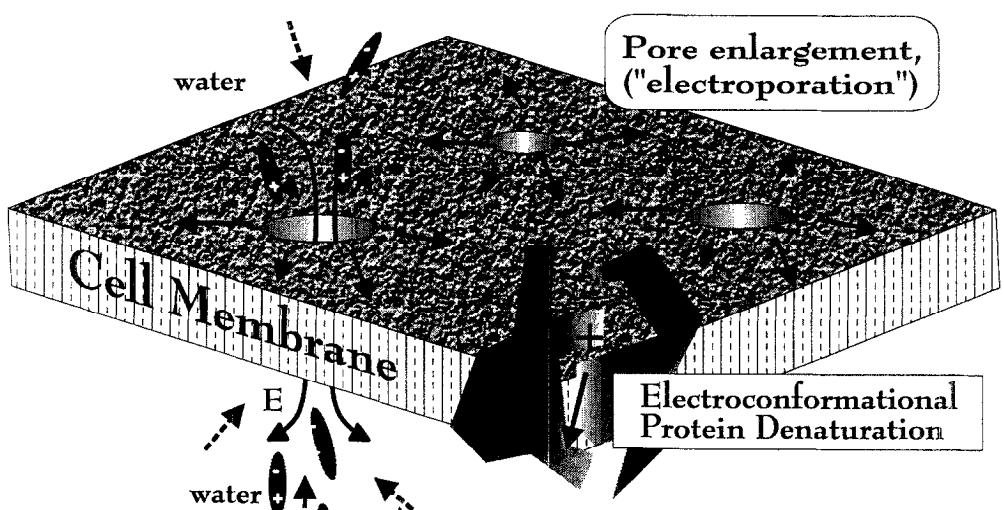
within the cytoplasm. If the membrane was a perfect insulator, the voltage in the cytoplasm would be uniform throughout. With current flowing around the cell, the voltage on the external surface of the cell changes and sets up an 'induced' transmembrane potential which varies according to position on the cell membrane. This induced transmembrane potential will range from zero at the axis of symmetry to a maximum at the extreme projections of the cell in the direction of the current passage. There are several reviews of this subject[11–13]. Conceptually, the maximum induced transmembrane potential will scale with the total voltage drop along the outer surface of the cell. However, the magnitude of the induced transmembrane potential depends on the size, geometry and orientation of the cell with respect to the field[14].

Because the cell membrane is very thin in comparison to the overall dimensions of the cell, the electric field strength established in the membrane is many fold greater than the field strength along the outer surface of the cell. For example, a 1-cm-long cell in an electric field of 150 V/cm will briefly experience a peak (because real membranes will not tolerate such potentials) induced transmembrane potential magnitude of approximately 75 V. Assuming a membrane thickness of 10 nm, the corresponding peak electric field magnitude within the plasma membrane is 7.5×10^7 V/cm which is of sufficient strength to denature proteins and permeabilize the membrane (electroporation).

Electroporation

Bilayer lipid membranes cannot maintain their structure when the transmembrane potential magnitude is too large. Structural defects or 'pores'[15] are formed in the membrane which effectively permeabilize the membrane to ions and molecules as large as DNA[16,17]. This electrically driven pore formation process, termed electroporation, typically occurs with submillisecond kinetics. The molecular physics responsible for electroporation is still debated, but in general involves the transport of water into molecular scale pores in the cell membrane (*Figure 6*) until the size of the pore exceeds a critical size[18] (beyond which it is energetically favourable for expansion rather than pore closure). Supraphysiological transmembrane potentials of greater than 600–800 mV are required to induce pores in the lipid bilayer. Unlike the rupture of a soap bubble following pin prick, the growth of pores in the bilayer lipid component of mammalian cell membrane is thought to be restricted by membrane proteins, which represent approximately 30 per cent of the total membrane mass. The kinetics of pore formation is transmembrane potential dependent within characteristic range of 10^{-2} to 10^{-1} ms. Electropores can be transient or stable.

When a 1-cm-long skeletal muscle cell is placed in a saline conducting medium with a 150 V/cm applied electric field, the membrane is rapidly electroporated resulting in a large increase in membrane electrical conductivity. As the conductivity of the membrane increases towards that of the cytoplasm, the



membrane electric field decreases and the cytoplasm field strength increases, both approaching that of the externally applied field[14]. The drop in the membrane field strength limits further membrane permeabilization. Although the intracellular field strength reaches that of the extracellular field, the intracellular membranes are not electroporated because of the relative small size of intracellular organelles. Unlike thermal injury in which all membranes and macromolecules are affected, damage resulting from electrical forces is typically restricted to the plasma membrane[17].

Electroconformational protein denaturation

Many membrane proteins span the entire thickness of the membrane. Generally, these proteins are composed of amino acids with acidic and basic side groups which can be acted upon directly by an intense intramembrane electric field. In addition to this mechanism, amino acids of these proteins are electrical dipoles which can align along the length of the transmembrane protein to create a large electric dipole that can also be acted upon by the field. Typically each amino acid unit contributes an electric dipole moment of about 3.5 Debye (D) to the entire protein[19]. In the α-helical structure of protein, many small peptide dipoles are aligned almost perfectly to form a larger dipole in the order of 120 D across the cell membrane. In general, a molecule under a strong electric field will tend to shift to a greater dipole moment in the direction of the field. This can be realized in several ways[20,21]:

1. Induced dissociation of ionizable side groups.
2. Induced separation of electrical charges along the macromolecule.
3. Alignment of weak dipoles in a cooperative fashion between subunits or monomers of a protein complex.
4. Reorientation of permanent dipoles on the protein within the direction of the field.
5. Induction of electrical dipoles by the applied field, but changing conformational state associated with a higher dipole moment or induction of dipoles as a result of polarizability of the protein. The magnitude of the side of alteration of the α-helical conformation on the protein's dipole strength can be appreciated by realizing that the random coil or β-helical structures have a relatively negligible dipole strength.

If the field strength become sufficiently intense, then the dielectric forces acting on the molecule can actually carry out destructive changes which are not spontaneously reversible. An example of this can be seen in the data from Chen and Lee[22], wherein the shape of the induced action potential following a 500-mV electric shock to a frog skeletal muscle cell membrane is altered. The alteration is consistent with loss of function of the potassium channel. In principle, these types of alterations can lead to functional neuromuscular and sensory types of

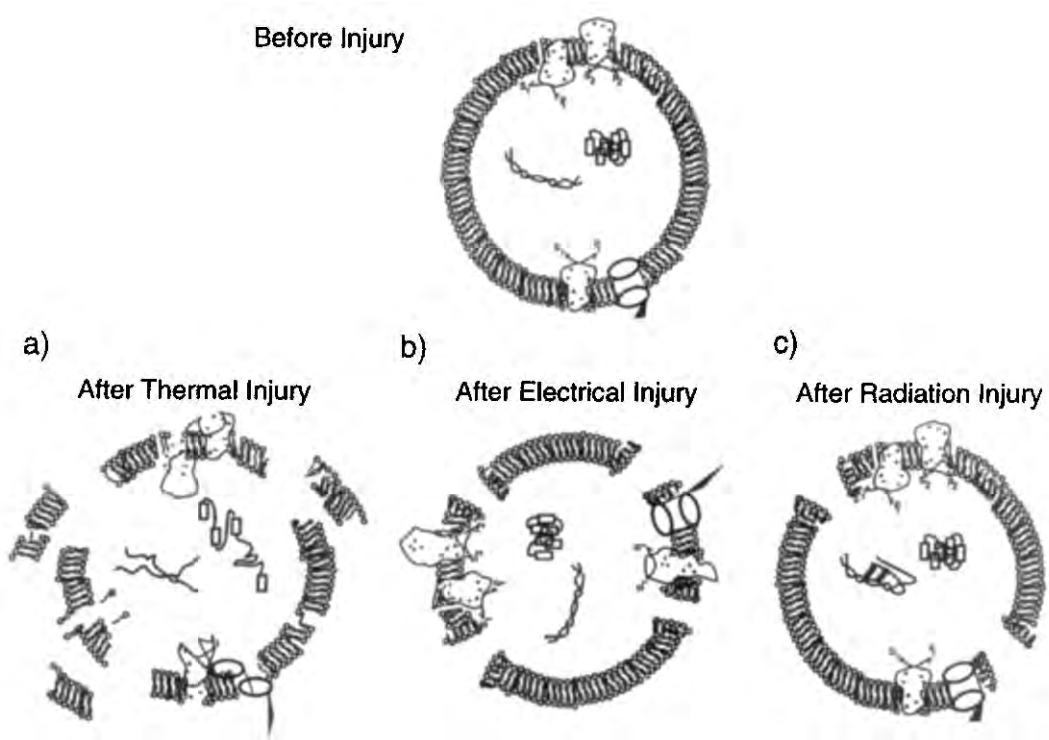


Figure 6. **a**, Diagrammatic representation of heat-induced damage to the cell. Membrane lysis and protein denaturation take place to an extent dependent on the thermal history. Membrane integrity is most heat labile and DNA is one of the most heat stable components. **b**, Diagrammatic representation of the cell damage caused by brief exposure to large electric fields typical of those occurring in high-voltage electrical shock. Here brief implies insufficient time to cause significant heating. The direct damage in the form of electroporation and electroconformational denaturation of proteins is limited to the membrane. Internal organelles and proteins are spared. **c**, Diagram of cell damage caused by ionizing irradiation. Proteins and lipids are altered by chemical reactions with oxygen derived free radicals. Lipid peroxidation leads to membrane lysis and nucleic acids are dimerized. Membrane lysis following very high doses (>50 Gy) leads rapidly to cell necrosis.

disorders. A therapeutic strategy to correct this problem must return the membrane protein to its native state. If the primary structure of the protein is intact, in theory it might be possible for the molecule to spontaneously return to its native stable state. The rate that this occurs can be influenced by increasing the membrane temperature as much as possible without heat damaging the lipid bilayer.

Microwave burns

In the presence of an alternating electric field molecules that are electrical dipoles will attempt to oscillate with the field. The oscillations will be constrained by viscous drag of interactions of neighbouring molecules and by structural bonding with adjacent structures. Smaller molecules like water, when not bound, are able to follow the field well into the megahertz frequency range. Causing water, which has a large dipole moment, to oscillate rapidly is an effective method of increasing the water's thermal energy. This is called 'microwave heating' which is one type of dielectric heating. If the amplitude of the field strength is constant, the heating rate increases with frequency until the gviscous drag on the rotating molecules prevents keeping up with the field. Kitchen microwave ovens are tuned to couple as efficiently as possible with the water.

Exposure to ambient microwave fields can cause thermal burns. The microwave penetration into physiological solutions has a characteristic depth of the order of 1 cm. If the microwave frequency current is passed by electrical wire directly to the body it would cause more uniform heating across the body. Tissue damage can occur that is essentially thermal in nature.

When the applied field is oscillating the effects on tissue are very frequency dependent. In the 10–100 mHz frequency range two types of heating occur, Joule heating as previously discussed and dielectric heating. Molecules that are electrical dipoles will attempt to oscillate with the field, but the oscillations are constrained by viscous drag of interactions of neighbouring molecules and by structural bonding with adjacent structures. Small molecules like water, when not bound, are able to follow the field up to the gigahertz (10^9 cycles/s) frequency range. Causing water, which has a large dipole moment, to rotate and tumble is equivalent to increasing its thermal energy. This is called 'microwave heating'. Given a constant field amplitude, microwave heating rate increases with frequency until the viscous drag prevents keeping up with the field. With frequencies above 1 GHz, the dielectric heating rate decreases with increasing frequency. Common microwave ovens are tuned to couple as efficiently as possible with water. Because mobile ions also respond to the field, there is Joule heating as well. However, microwave heating is more significant because tissue water content is much higher than dissociated salt. The therapeutic technique of RF hyperthermia operates according to the same mechanism.

Microwave electrical burns have different clinical manifestations than low frequency electrical shock. At low frequency the epidermis is a highly resistive barrier. In the microwave regime, the electrical power readily passed the epidermis in the form of 'capacitative' coupling with very little energy dissipation. Consequently, the epidermis is not burned. The microwave field penetration into tissue has a characteristic depth in the range of 1 cm, resulting in direct heating of subepidermal tissue water. It is possible to cause significant internal thermal damage without surface burn by changing the position of the extremity in the field.

Radiation injury

Radiation injuries are also called burns, although heating has no role in the tissue damage caused by ionizing irradiation. Radiation injury occurs following exposure to damaging levels of ionizing particle beam and electromagnetic irradiation, both of which alter atomic structure which mediates the damaging chemical reactions. The most common radiation burn is excessive ultraviolet light exposure during sun bathing which is often called a sunburn. Ionizing UV light can only penetrate the very thinnest layer of the epidermis. Higher frequency electromagnetic fields such as X-ray or gamma rays have sufficient energy to penetrate the entire body. Mechanistically, electromagnetic waves of frequency greater than UV light can energetically couple at the atomic level leading to the formation of unpaired electrons in the outer electron orbitals. Such high energy irradiation acting upon biological tissues produces protein, polysaccharide and lipid damage[23]. The reactive species from photo-ionization of water are primarily hydroxyl ions which are very reactive against biological macromolecules. The reaction with biological macromolecules leads to altered chemical bonding and molecular structure and ultimately blocks function. The hydrogen bonds of DNA and other proteins are particularly vulnerable. This vulnerability permits use of radiation for cancer therapy and for other conditions in which cell proliferation is to be blocked[24].

Because there is no net electrical charge, free radicals have free access to the lipid bilayer[25]. Damage to the cell membrane leading to increased permeability results from heavy exposure. The precise molecular mechanics of membrane permeabilization are still under investigation. Some investigators suggest that it is related to desaturation of membrane lipids. The lipids in the membrane bilayer have protons stripped from their carbon backbone, leading to desaturation and bends in the structure[26]. Polyunsaturated fatty acids require greater molecular excluded volume and will self-aggregate by lateral diffusion in the lipid bilayer. Aggregation leads to bilayer instability and poration[27].

Radiation 'burns' also occasionally result as a complication of cancer radiotherapy. Skin wounds develop in heavily irradiated areas as a result of loss of epidermal and dermal cell proliferation potential. In addition, the effects of loss of vascular capillary function with resulting oedema and inflammation are manifesed in tissue breakdown. Loss of proliferation potential and the loss of expression of other proteins are a manifestation of cellular genetic damage from reactive oxygen intermediate-mediated DNA cleavage. At doses in excess of 80–100 Gray even

postmitotic cells are killed from bilayer membrane permeabilization[24]. Susceptibility to radiation injury is variable from one patient to the next. The injury mechanism is very different from thermal burns. The thermal energy absorbed by even lethal radiation doses is insufficient to raise the surrounding water temperature measurably. Molecular medicine strategies to correct the underlying problems would be very different when considering heat-mediated and ionizing irradiation-induced cell injury.

Chemical burns

While the specific molecular pathophysiology of burn injuries is generally not considered, chemical burns are the true exception. The immediate question raised regarding a chemical contact is what molecular alterations occur and what antidotes are known.

Hydrofluoric acid (HF) burn is an excellent example. HF is a weak acid that can diffuse great distances in tissue before it dissociates. However, HF is very reactive towards calcium and binds calcium from tissue, causing damage and severe pain. When HF diffuses to the bone, bone material can dissolve. The antidote is to provide intramuscular calcium as a barrier between the HF and bone. Chronic low level exposure results in leeching of calcium from bone. Direct contact with caustic chemicals can also damage tissue by two distinct processes. Direct chemical alteration of extracellular matrix and cellular molecules can occur. In addition, some chemical reactions are exothermic or endothermic, resulting in changes in tissue temperature. Of particular note in this regard is concentrated sulphuric acid (common industrially) which is very viscous. It is very important not to directly flush with H_2O, as the heat of neutralization would be extreme and cause severe thermal burn. Rather the affected area must first be wiped with a dry towel, following which cold water is appropriate.

Major differences and similarities in burn injury

Exposure to supraphysiological temperatures primarily leads to molecular changes ('damage') related to excessive intermolecular physical forces between neighbouring molecules. Vulnerability to thermal damage depends on molecular structure and strength of bonds but not on cell size or shape. Thus, the susceptibility to heat damage is quite similar across different tissue types. The damage which occurs is permeabilization of the cellular membranes, protein denaturaiton and other secondary consequences. Conceptually the changes in cell structure due to heat exposure *per se* are reflected in *Figure 6*. Macroscopically, changes in tissue optical properties result because the optical properties of tissue depend on the macromolecular form. Thus, the visible changes that occur in heat burn are useful in assessing the viability of injured tissue. This is not the case in other types of physicochemical injuries.

Electrical current passing through the body will produce a non-uniform injury pattern. In contrast to what is found for thermal injury, cell size and tissue structure are very important in determining vulnerability to tissue damage. The long axes of most skeletal muscle cells and nerve axons are oriented approximately parallel to the direction of the field lines. Human forearm skeletal muscle cells can approach 8 cm in length and nerves cells are much longer[28]. The transmembrane potentials experienced by these cells and peripheral nerve axons would be significantly larger than those epxerienced by non-parallel oriented skeletal muscle cells or experienced by smaller cell types such as fibroblasts or circulating blood cells. From this perspective, it is not surprising that skeletal muscle and nerve cells are the most frequently damaged in electrical injury. Vascular endothelial cells are connected together in such a way that in principal creates a large cell effect[29]. *Figure 6* indicates the damaged state of a pure electrical injured cell. The injury is primarily at the site of the cell membrane. Macroscopically the tissues' appearance does not resemble thermal injury because denaturation of proteins generally does not occur.

Ionizing radiation and chemical injuries have in common the ability to alter the primary structure of macromolecules without pathologically significant heating. Acute visible tissue changes result from triggering of tissue inflammation by the cell injury. The inflammation, particularly following a sunburn, is very similar in appearance to that produced by superficial thermal burns. In comparison to thermal burn and electrical injury, the major damaging effects on cells from irradiation injury are initiated at the protein primary structure level. This is illustrated in *Figure 7*. Renaturation is not possible, short of the type of damage excision and repair which is important for DNA protection[30]. In theory the therapy for radiation injury must involve free radical scavenging, DNA repair and sealing of permeabilized cell membranes.

While the major emphasis of this review has been the important differences that exist between the various types of burn injuries, it is important to recognize an important feature that these injuries have in common: loss of cell membrane integrity. Ionic compartmentalization, as permitted by the cell membrane, is essential for the chemical processes of

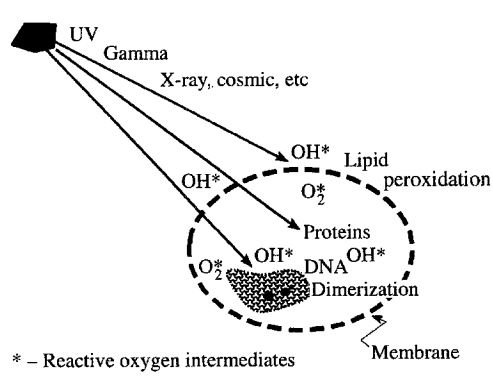


Figure 7. Although the molecular mechanisms are different the central role of membrane damage in the pathogenesis of cellular necrosis is well described for 'burn' injuries. Thus, an important first therapeutic step may be to induce membrane sealing.

life[31]. The most basic function of the cell membrane is to provide a barrier to restrict ionic transport and for this purpose the delicate bilayer lipid membrane is superb. The energy required to move solvated ions across a pure planar phospholipid bilayer is an aqueous environment approaches 100 k_BT, indicating the strong impediment to passive ion-diffusion across the lipid bilayer[25]. However, cell membranes are typically 30 per cent protein. Many membrane proteins permit and regulate membrane ion transport, including gated, ion-selective channels, ionic pumps or the establishment of other hydrophilic transport pathways. Roughly, these protein effects combine to make the mammalian cell membrane approximately 10^6 times more conductive to ions than the pure lipid bilayer[32]. Structural integrity of the lipid bilayer component of the cell membrane is essential for making possible the transmembrane physiological ionic concentration gradients at a metabolic energy cost that is affordable. Approximately, 95 per cent of the metabolic energy expended by cells is used to maintain the gradients.

When the lipid bilayer structure is damaged protein ion pumps cannot keep pace with the increased diffusion of ions across the membrane. Under these circumstances, the metabolic energy of the cell will be quickly exhausted leading to biochemical arrest and to necrosis (*Figure 8*). The types of burn injury discussed have in common the capacity to disrupt the transport barrier function of the cell membrane through structural alterations. Loss of cell membrane integrity occurs at supraphysiological temperatures, in frostbite injuries, in free-radical-mediated radiation injury, in barometric trauma and in electrical shock. Ischaemia-reperfusion injury, which is mediated by the effects of superoxide-free radicals on the plasma membrane[26], is probably the most common cause and is a substantial factor in many common medical illnesses. Under conditions of freezing, ice nucleation in the cytoplasm can lead to factors which are very destructive to the cell membrane, including the mechanical disruption of the membrane by the ice crystal growth and the damaging effects of increasing salt concentration as the ice spread and excludes ions[33]. Sudden changes in very strong barometric pressures can lead to explosion of the cell membrane. Electrical shock is the paradigm for necrosis primarily mediated by membrane permeabilization. Skeletal muscle and nerve tissue exposed to strong electrical fields (>50 V/cm) can experience membrane damage by at least three distinct physicochemical processes. These are lipid bilayer damage by electroporation, heat-mediated membrane poration and electroconformational membrane protein denaturation.

Considering structural stability, it is quite fortunate that cell membranes contain large proteins. In a pure surfactant membrane (i.e. a liposome), a defect sufficiently large to cause a conducting pore will expand until the entire structure ruptures[18]. Surface tension is responsible for causing the defect to expand. This is similar to a pinprick of a soap bubble. Because cell membranes also contain large proteins, some of which are anchored together in the intracellular and the extracellular space by other proteins, the defects in the membrane can become stable. By ultrastructural examination of electroporated cell membranes, Chang and Reese[34] and others have demonstrated that stable structural defects occur in cell membranes. Their studies demonstrated stable pore diameters may be in the range of 0.1 μm, which is large enough to pass nearly all biological macromolecules.

Drugs to seal cell membranes

This discussion immediately raises two questions: first, what are the strategies to seal biological cell membranes? Second, how soon must the sealing occur to prevent cell death? Both questions are unanswerable at this time. In an aqueous environment, at concentrations above the critical micelle concentration (CMC), surfactant compounds spontaneously self-assemble into supramolecular lamella called membranes. The most familiar example is the formation of bubbles when water-containing detergent is agitated. Biological lipid membranes are supermolecular assemblies of surfactants which spontaneously aggregate in an aqueous environment. Structures that orient the surfactant, such as the loop used to create bubbles, can trigger rapid formation of a supermolecular surfactant assembly such as the membrane forming across the loop. What is required to form closed membrane structures is a suitably high enough surfactant concentration in an aqueous environment, the correct range of temperature and time.

Sealing of permeabilized cell membranes is an important naturally occurring process in many cell types. Fusigenic proteins are macromolecules that induce sealing of porated cell membranes following exocytosis. They act to create a low energy pathway for flow of phospholipid across the defect or to induce fusion of transport vesicle to plasma membranes. The action of fusigenic proteins indicates that it is possible to seal damaged cell membranes using macromolecular templates. This has also been accomplished using synthetic surfactants, such as poloxamer 188 at a sub-CMC concentration of 0.1 mM (since our previous studies indicated that this sub-CMC concentration was quite effective) to seal cells against loss of calcein dye postelectroporation[35]. Others have reported similar observations[36].

Conclusion

Progress in science has been inexorably linked to finding the proper name for phenomena. The term 'burn' is ambiguous because it is used to describe a range of physicochemical injuries that are different and will require different therapeutic approaches. Advances in burn care have resulted from the development of strategies to interrupt processes that lead to secondary tissue damage and systemic illness. A logical extension of this effort is to focus on initial molecular consequences of physicochemical trauma with the goal of finding strategies which restore intact proteins to their native conformation, excisionally repair damaged nucleic acids and seal damaged cell membranes. This could, in effect, directly reverse the damage and mitigate the harmful effects of the physicochemical injury. Strategies to restore

denatured proteins and permeabilized cell membranes to their predamaged state seem to be the most appropriate first efforts[37].

Acknowledgements

The authors gratefully acknowledge Drs Mary Capelli-Schellpfeffer and Tina Desai for review of the manuscript and Bridget Scott for its preparation. Also, the following support has been vital to our research efforts: NIH Grant no. R29 ESO662 (Dr Astumian); NIH Grant no. R01 GM53113 (Dr Lee); Eastern Utilities Association; The Electric Power Research Institute, Empire State Electric Energy Research Corporation; Commonwealth Edison Company; New York State Electric and Gas Co.; Northeast Utilities Service Corporation; and The Public Service Company of Oklahoma.

References

1 Blackmore J. *Ludwig Boltzmann, His Later Life and Philosophy, 1900–1906 Book One: a documentary history.* Boston: Kluwer Academic Publishers, 1995.
2 Moore WJ. *Physical Chemistry*, 4th edn. New Jersey: Prentice-Hall, pp 90–96.
3 Tanford C. *Physical Chemistry of Macromolecules.* New York: John Wiley, 1961; p 635.
4 Moussa NA, McGrath JJ, Cravalho EG, Asimacopoulos PJ. Kinetics of thermal injury in cells. *J Biomed Eng* 1977; **99:** 155.
5 Rocchio CM. In: Lee RC (supervisor) *The Kinetics of Thermal Damage to an Isolated Skeletal Muscle Cell. S.B. Thesis.* Cambridge, MA: Massachusetts Institute of Technology, 1989.
6 Moussa NA, Tell NE, Cravalho EG. Time progression of hemolysis of erythrocyte populations exposed to supraphysiologic temperatures. *J Biomech Eng* 1979; **101:** 213–217.
7 Gershfeld NL, Murayama M. Thermal instability of red blood cell membrane bilayers: temperature dependence of hemolysis. *J Membrane Biol* 1968; **101:** 62–72.
8 Cravalho EG, Toner M, Gaylor D, Lee RC. Response of cells to supraphysiologic temperatures: experimental measurements and kinetic models. In: Lee RC, Cravalho EG, Burke JF (eds). *Electrical Trauma.* New York: Cambridge University Press, 1992.
9 Henriques FC, Moritz AR. Studies of thermal injury V: the predictability and the significance of thermally induced rate processes leading to irreversible epidermal injury. *Arch Pathol* 1947; **43:** 489–502.
10 Lee RC, Gaylor DC, Bhatt D, Israel DA. Role of cell membrane rupture in pathogenesis of electrical trauma. *J Surg Res* 1988; **44:** 709–719.
11 Lee RC, Kolodney MS. Electrical injury mechanisms; electrical breakdown orf cell membranes. *Plast Reconstr Surg* 1988; **80:** 672.
12 Lee RC. Tissue injury from exposure to power frequency electrical fields. In: JC Lin (ed) *Advances in Electromagnetic Fields in Living Systems*, vol 1. New York: Plenum Press.
13 Reilly P. Scales of reaction to electric shock: thresholds and biophysical mechanisms. In: Lee RC, Capelli-Schellpfeffer M, Kelley KM (eds) *Electrical Injury: a multidisciplinary approach to prevention, therapy & rehabilitation*, vol 720, New York: New York Academy of Sciences.
14 Gaylor DG, Prakah-Asante A, Lee RC. Significance of cell size and tissue structure in elelctrical trauma. *J Theor Biol* 1988; **133:** 223.
15 Litster JD. Stability of lipid bilayers and red blood cell membranes. *Phys Lett* 1975; **53(A):** 193.
16 Powell KT, Weaver JC. Transient aqueous pore in bilayer membranes: a statistical theory. *Bioelectrochem Bioenerg* 1986; **15:** 211.
17 Tsong TY. Electroporation of cell membranes. *Biophys J* 1991; **60:** 297–306.
18 Taylor GI, Michael DH. On making holes in a sheet of fluid. *J Fluid Mech* 19??; **58:** 625.
19 Tsong TY, Astumian RD. Electroconformational coupling and membrane protein function. *Prog Biophys Mol Biol* 1987; **50:** 1–45.
20 DeFelice LS. *Introduction to Membrane Noise.* New York: Plenum, 1981.
21 Tsong T, Gross C. Reversibility of thermally induced denaturation of cellular proteins. In: Lee RC, Capelli-Schellpfeffer M, Kelley KM (eds) *Electrical Injury: a multidiscplinary approach to prevention, therapy & rehabilitation*, vol 720. New York: New York Academy of Sciences.
22 Chen W, Lee RC. Altered ion channel conductance ionic selectivity induced by large imposed membrane potential pulse. *Biophys J* 1994; **67:** 603–612
23 Jagger J. *Solar – UV Actions on Living Cells, 1985*, New York: Praeger.
24 Pizzagrello DJ, Witcofski. *Basic Radiation Biology*, 1967; Philadelphia: Lea & Febiger.
25 Parsegian A. Energy of an ion crossing a low dielectric membrane: solutions to four relevant problems. *Nature* 1969; **221:** 844.
26 Halliwell B, Gutteridge J (eds). *Free Radicals in Biology and Medicine*, 2nd edn, 1991; Oxford: Clarendon Press.
27 Canaday D, Li P, Weichselbaum R, Astumian RD, Lee RC. Membrane permeability changes in gamma-irradiated muscle cells. In: Lee RC, Capelli-Schellpfeffer M, Kelley KM (eds) *Electrical Injury: a multidiscplinary approach to prevention, therapy & rehabilitation*, vol 720. New York: New York Academy of Sciences.
28 Lieber RL, Fazeli BM, Botte MJ. Architecture of selected wrist flexor and extensor muscle. *J Hand Surg (Am)* 1990; **15:** 244–250.
29 Cooper MS. Electrical cable theory, transmembrane ion fluxes, and the motile responses of tissue cells to external electrical fields. In: *Bioelectric Interactions, Symposium. IEEE/ Engineering in Medicine and Biology Society, 7th Annual Conference, Chicago*, 1986.
30 Darnell J, Lodish H, Baltimore D. *Molecular Cell Biology*, 2nd edn. New York: Scientific America Books, 1990.
31 Horgan J. In the beginning ... *Sci Am* 1991; **264:** 117.
32 Schanne OF, Ceretti ERP. *Impedance Measurements in Biological Cells.* New York: John Wiley, 1978.
33 Karisson J, Cravalho E, Rinkes I, Tompkins R, Yarrnush M, Toner M. Nucleation and growth of ice crystals inside cultured hepatocytes during freezing in the presence of dimethyl sulfoxide. *Biophys J* 1993; 2524–2536.
34 Chang DC, Reese JS. Changes in membrane structure induced by electroporation as revealed by rapid-freezing electron microscopy. *Biophys J* **58:** 1–12.
35 Lee RC, River P, Pan FS, Ji L, Wollmann RL. Surfactant-induced sealing of electropermeabilized skeletal muscle membranes in vivo. *Proc Natl Acad Sci USA*, 1992; **89:** 4524–4528.
36 Padanilam JT, Bischof JC, Le RC, Cravalho EG, Tompkins RG, Yarmush ML. Effectiveness of Poloxamer 188 in

arresting calcein leakage from thermally damaged isolated skeletal muscle cells. In: Lee RC, Capelli-Schellpfeffer M, Kelley KM (eds) *Electrical Injury: a multidisciplinary approach to prevention, therapy & rehabilitation,* vol 720. New York: New York Academy of Sciences.

37 Lee RC, Myerov A, Maloney C. Promising therapy for cell membrane damage. In: Lee RC, Capelli-Schellpfeffer M, Kelley KM (eds) *Electrical Injury: a multidisciplinary approach to prevention, therapy & rehabiliation,* vol 720. New York: New York Academy of Sciences.

Paper accepted 29 April 1996.

Correspondence should be addressed to: Raphael C. Lee, Burn/Electrical Trauma Research Center, MC6035, 5841 S. Maryland Ave., University of Chicago Hospitals, Chicago, IL 60637, USA.