THE MECHANISM OF ELECTROSURGICAL COAGULATION:
STEAM EVOLUTION VERSUS DIELECTRIC BREAKDOWN

by

STEPHEN F. BART

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Submitted to the Department of Electrical Engineering and Computer Science on May 21, 1982 in partial fulfillment of the requirements for the Degree of Bachelor of Science.

ABSTRACT

This work explores the nature of initial cell breakdown under the influence of electrosurgical current. Two possible hypotheses are introduced to explain this breakdown: (1) cell membranes are ruptured by steam evolution associated with intra-cellular fluid boiling caused by ohmic heating, (2) cell membranes are ruptured by a high voltage-induced dielectric breakdown phenomenon. To examine the role of steam evolution, bovine corneas were subjected to various durations of sinusoidal electrosurgical current with and without an elevated pressure. The elevated pressure allowed the intra-cellular boiling point to be increased, thereby allowing the possibility of altering the extent of cell damage for a given duration of current application. To study high voltage-induced breakdown, corneas were subjected to a high voltage pulse train waveform for short enough durations such that intra-cellular boiling could not occur.

Histological studies showed that the high voltage, pulse train waveform as well as the continuous sinusoidal waveform caused cell damage prior to intra-cellular boiling. We were unable to define with any certainty whether or not steam evolution plays a major role in initial cell disruption.

Thesis Supervisor: Alan J. Grodzinsky

Title: Associate Professor of Electrical and Bioengineering
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Finally there is Professor Alan J. Grodzinsky. His ideas and inspirations are intertwined with mine throughout this document. The time he has spent helping me with this project is vast. Yet gratitude is due for far more than this project. His intellect, energy and interest in the academic environment around him have been an inspiration that will always be with me; it was an honor to work with him.
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CHAPTER 1

INTRODUCTION

The use of electrical current as a surgical tool can be traced to the early 1900's. Today the use of electric currents to cut and coagulate tissues is a very common surgical technique. Modern electrosurgical generators supply current at frequencies between 100kHz and 3MHz; frequencies below 100kHz may stimulate nerve and muscle tissues, and at frequencies above 3MHz it becomes increasingly difficult to confine the current to wires. Output waveforms include pure sinusoids and bursts of sinusoids using various duty cycles that have been found empirically to produce desired tissue damage. The type of waveform modulation, waveform amplitude, and duration of current application are the parameters that govern power delivery and allow the output of the device to be tailored to specific surgical applications [1].

The electrosurgical device owes its widespread use to its ability to cut with little bleeding, seal "bleeders" and in general reduce operating time. There are also specific advantages to electrocoagulation in special surgery such as laparoscopic sterilization, neurosurgery, and retinal reattachment.
Despite the widespread use of electrosurgery, the literature shows a surprising lack of fundamental knowledge concerning the physical processes that occur at the cellular level under the influence of electrosurgical current. An extensive literature search [2] revealed numerous case studies expounding the problems associated with electrosurgical devices and techniques intended to overcome them. However, very few investigations have addressed the physical effects of electrosurgical current on the cellular level. In short, the surgeon becomes proficient in the instrument's use by trial and error. Little work has been done to try to understand the electrocoagulation process.

The most widely held hypothesis as to the mechanism of cellular destruction during electrocoagulation is what I term the "steam evolution" model [3]. Ohmic heating due to the applied current causes the cellular fluids to boil. At the boiling point, the phase change (with its accompanying large change in volume) causes the cell membranes to rupture. As a consequence of this belief, we see many papers which have developed theoretical temperature distributions using model tissue / electrode configurations [4,5,6,7,8,9,10].

While macroscopic tissue damage appears to correlate at
least qualitatively with total electrical power dissipation and hence tissue temperature [5,9,16], the mechanism responsible for initial cell membrane disruption may or may not be related to boiling of interstitial fluid. An understanding of this mechanism may substantially improve our ability to design new waveforms so as to achieve desired physiological effects while minimizing problems such as the sticking of tissue to electrosurgical electrodes.

Another possible model which is beginning to receive attention is a recent hypotheses by Belov [11] which I shall term the "breakdown" model. His idea is that short duration pulses with large peak-to-mean ratios (crest-factor) may cause cell membrane breakdown. Unfortunately local temperatures were not measured in Belov's experiments, and subsequent calculations show that there may have been significant heating of the tissue. Thus the hypothesis remains unsubstantiated.

In our work we are not directly interested in a detailed mechanism for breakdown of this type, but rather in the role that such a mechanism might play in electrosurgical coagulation. However in order to make plausible the existence of a high voltage-induced membrane breakdown phenomenon, we will describe a theoretical model (Crowley,
1973 [12]) that has been applied to the dielectric breakdown observed in artificial lipid bilayer membranes. The theory treats the cell membrane as an elastic, dielectric, non-conducting membrane surrounded on both sides by a highly conducting medium. By equating the mechanical and electrical stresses on the membrane, Crowley was able to show that an electromechanical instability results when the voltage across the membrane reaches a critical value. This critical voltage is a function of the membrane's electrical and mechanical parameters. In other words, above a certain critical voltage the membrane becomes unstable and ruptures. Crowley's calculations show that it is reasonable to believe that a breakdown mechanism of this type may play an important role in electrosurgical cell membrane rupture.

The objective of this research is to focus on the possible mechanisms responsible for the initial cell membrane damage that would precede full coagulation. Specifically we wish to explore the two general mechanisms of electrosurgical cell damage outlined above and try to clarify the roles each play in the electrocoagulation process. One problem that we face is the lack of a precise definition of electrosurgical coagulation at the cellular level. Vogler and Mylrea [13] equate tissue coagulation to
the fusing of membranes at approximately 80°C, although they present no direct histological evidence. Siegel and Dunn [14] studied the closure of incisions in arteries and veins and found that the most important factor for successful closure was the preservation of the arrangement of connective tissue collagen fibers. I refer the reader to Grodzinsky and Chen [15,16] for an overview of the changes in tissue characteristics associated with electrosurgical coagulation. However, since we are primarily interested in discriminating between two mechanisms for cell membrane damage, we can effectively side-step this issue by characterizing each mechanism empirically. In the end it is hoped that this work will increase the understanding of the electrosurgical process and help to solidify a definition of coagulation at the cellular level.
EXPERIMENTAL DESIGN AND RESULTS

A. Overview

The projects described within revolve around two basic experiments, each designed to clarify the role played by our two "membrane rupture models" in the electrocoagulation process. The first experiment examines the "steam evolution" model and hinges on the nature of thermodynamic phase equilibrium. The steam evolution model states that cell membrane breakdown results from the large volume change associated with the liquid-vapor phase transition in the intra-cellular fluid. Application of the Clapeyron equation [17] tells us that the liquid-vapor phase transition equilibrium line always has positive slope. Therefore if we increase the pressure on the fluid in a system, the boiling temperature will increase. The usefulness of all this is that we can subject a biological specimen to electrosurgical current and yet suppress the boiling of the intra-cellular fluid by increasing the applied pressure. In this way we can gauge the role of steam evolution in cell membrane breakdown.

The second basic experiment will be to subject a biological preparation to a high voltage, high crest-factor waveform without causing significant cellular heating (i.e. without letting the cellular fluid boil). This will allow us to judge the breakdown model's role in electrosurgical cell disruption.

The biological preparation we have chosen for our experiments
is Bovine cornea. This tissue consists of epithelial cell layers adjacent to a collagen/proteoglycan extracellular matrix. This central matrix, the corneal stroma, also contains cells (fibroblasts) which produce the collagen and proteoglycans. Because the cell layers are thin it is easy to see the full extent of changes brought about by the passage of current. Also, the preparation is easy to obtain and is cheap.

To accomplish these objectives, we have performed the following specific tasks:

Project 1) Theoretical calculation of electric field density and temperature change in the experimental apparatus due to a given applied voltage.

Project 2) Measurement of the change in temperature of the cornea versus time due to different settings on the Valleylab SSE3B electrosurgical generator.

Project 3) Histological characterization of normal Bovine cornea.

Project 4) Histological characterization of cornea which has been subjected to a high voltage, high crest-factor waveform.

Project 5) Histological characterization of cornea which has been
subjected to electrosurgical current of various durations.

Project 6) Histological characterization of cornea which has been subjected to electrosurgical current under an elevated pressure.

B. Experimental Procedures and Results

Project 1) Theoretical Calculation of Electric Field Density and Temperature Variation

All experiments were carried out in the same physical apparatus which I shall describe here. The apparatus consists of a solid brass pressure vessel, the design and dimensions of which are shown in Figure 1 (shown pictorially in Plate A). The vessel was equipped with high pressure pass-throughs to facilitate electrical connections. A plexiglass trough was constructed which contained a frame for holding a cornea and the current supplying electrodes (see Figure 1 and Plates A and B). The cornea is placed between two plexiglass blocks, which have holes that expose the center of the cornea (the blocks are also fitted with o-rings to hold the cornea securely). The blocks with the cornea slide into the trough as shown in Plate B. The trough can then be filled with a physiological saline, bringing the
cornea into electrical contact with the electrodes. The active electrode consisted of a stainless steel rod which slid into the hole in the center of the cornea holder allowing the distance between it and the cornea to be continuously adjustable. The common or return electrode was a piece of platinum foil glued to a square of plexiglass that could be slipped into the trough.

In all of our experiments the conducting media used to fill the cornea trough was 0.15 molar saline. This was chosen because it is approximately isotonic to biological tissues and is also a good conductor. The pressure vessel was pressurized with nitrogen gas.

Since it is imperative that we know the temperature versus time characteristics of our specimen given an applied voltage, it was necessary to calculate the electric field density at the specimen for the applied voltage. A simple electroquasistatic formulation (presented in detail in Appendix 1) yields:

\[ E_x = Kv(t) \quad (P1-1) \]

where \( E_x \) is the electric field at the specimen site and \( K \) is a constant that depends on the geometry of the electrode configuration. From this we can calculate the time average power dissipation density in the region surrounding the specimen:
\[ \langle PD \rangle_t = \sigma k^2 V_{rms}^2 \]  
where we have assumed that the bulk-average conductivity of the specimen and the surrounding saline solution are the same. This approximation is certainly correct to within an order of magnitude, since the increase in tissue conductance due to its fixed charge groups will compensate to some extent for the decrease in conductance associated with the specimen's fine structure, e.g. cell membranes. From this, thermodynamic arguments (see Appendix 1) yield:

\[ \frac{\Delta T}{\Delta t} = \frac{\sigma k^2 V_{rms}^2}{\rho c} \frac{^\circ C}{sec} \]  
where \( c \) is the specific heat and \( \rho \) is the mass density; both are assumed to be uniform throughout the fluid and specimen. After evaluating \( K \) for the specific geometry (see Appendix 1) we find:

\[ \frac{\Delta T}{\Delta t} = 5.6 \times 10^{-2} V_{rms}^2 \frac{^\circ C}{sec} \]  
This equation gives us our first approximation to the choice of applied voltages and application times for use in projects 4 and 5. The Valleylab SSE3B Cut #25 (\( V_{rms} = 64v \)) waveform (see Figure 2) was chosen because of its relatively low power (the number 25 means that the electrosurgical device would supply 25±7 watts (or 15%, which ever is larger [1]) to a 300Ω resistive load), thereby yielding an application time long enough to effectively differentiate between boiling and non-boiling states.
FIGURE 2
MONOPOLAR OUTPUT WAVEFORMS
As seen on an oscilloscope

ALL GRAPHS
Vertical Scale = 500 Volts/Centimeter
Horizontal Scale = 10 Microseconds/Centimeter
Generator Settings = "10"
Load on Generator = 300Ω
Project 2) Measurement of Cornea Temperature versus Time During Application of Current

The theoretical calculation carried out in project 1 assumes that the conductivity, specific heat, and density of the cornea are the same as the 0.15M saline bath. It also assumes that the electrosurgical generator will act as a ideal voltage source. Unfortunately it is very difficult to gauge how valid these assumptions are. In fact the output of the Valleylab SSE3B electrosurgical generator is not that of a perfect voltage source; it contains impedance matching circuitry that cause it to be non-linear for the first few moments of its operation. Thus the theoretical calculations in project 1 may be significantly in error. Since it is vital that we know the temperature versus time characteristics for any input that we may use, direct measurements were made.

Procedure
The temperature of the cornea was monitored using a thermocouple placed in direct contact with the cornea. The thermocouple was constructed from 3 mil constantin and chromel wires which were soldered into a thermal junction with stainless steel solder. The ends of the leads were soldered to copper wire and immersed in an ice/water bath as
a "zero degree" reference. All thermal junctions were insulated with epoxy. The leads were attached to a Hewlett Packard digital voltmeter from which the junction voltage was read. Note that we tried to record the thermocouple voltage directly using a strip chart, but were unable to isolate the amplifiers from the electrosurgical device's RF fields. The voltage to temperature transformation was accomplished using a published constantin-chromel junction voltage reference [18] (this calibration was double checked by placing the thermocouple in water at the melting point, room temperature, and the boiling point and checking the junction voltage with the table's value).

Results

Figure 3 shows the temperature versus time characteristic using the Valleylab SSE3B Pure Cut #25 waveform. (see Figure 2). Note that the slope in the linear region is about 2 °C/sec as compared to the 2.3 °C/sec value predicted in project 1 (this value is obtained by substituting $V_{rms} = 64v$ for the Cut #25 waveform into equ. (P1-4): see Appendix 1, equ. (A1-21)). The rolloff of the slope in Figure 3 results from the combination of two effects. As the temperature of the surrounding saline increases, the mobility of its conductive ions increases causing the system resistance to go down. As a consequence
of the Valleylab SSE3B's design, it tries to keep the input power constant, so it will reduce the applied voltage to compensate. This effect is small; a more important cause is due to water's \( \frac{540 \text{ cal}}{\text{gram}} \) heat of vaporization (the heat of vaporization represents the energy needed to change one gram of liquid into vapor with no change in temperature). As the slope flattens out, more and more of the energy being supplied by the electrosurgical current is going into satisfying the heat of vaporization instead of increasing the specimen's temperature. Thus as long as we remain on the slope of 2 portion of the graph no energy is being used to cause vaporization, hence no boiling is occurring even on a local level.

We now wish to compute the applied pressure necessary to suppress intra-cellular boiling in our specimen. Specifically, we will choose the pressure such that the 60 second point is still located on the slope of 2 segment of the graph so that no local boiling will occur before 60 seconds. Also, the time necessary to supply enough energy to satisfy the heat of vaporization will cause global boiling to be suppressed well past 110 seconds. In fact since the heat of vaporization is so large compared to the energy required to increase the tissue's temperature we can probably assume that no local boiling will occur at 110
seconds either (this assumption will not be critical to our results). If we extrapolate the slope of 2 section of the graph (figure 3) to intersect the 60 second point we see that the new boiling point must be about 175°C. This corresponds to an applied pressure of 120 psi [19]. Another way to view the effect of applying this pressure is to note that increasing the boiling point by 75°C simply shifts the graph 32.5 seconds to the right (as well as rescaling the temperature axis). Thus the 60 second samples will be in the same state relative to boiling as the 30 second samples at atmospheric pressure.

Project 3 Histological Characterization of Normal Cornea

The cornea is the anterior-most portion of the eye which is responsible for the major part of the refraction of incoming light. It is a slightly elliptical membrane of \(11\text{mm}\) mean diameter. Structurally, the cornea consists of 5 layers - (1) the corneal epithelium; (2) the anterior limiting lamina (Bowman's membrane); (3) the substantia propria or corneal stroma; (4) the posterior limiting lamina (Descemet's membrane); (5) the endothelium of the anterior chamber or mesothelium. Layers (1) through (3) are evident in plate (a) showing a normal bovine cornea.

The corneal epithelium covers the front of the cornea
and consists of three general layers of cells. The deepest cells are columnar; their basal surfaces are flat and their outer surfaces rounded, and they contain large round or oval nuclei. The cells of the second layer are polyhedral with oval nuclei. In the uppermost layer, the cells become progressively flattened with flattened nuclei (see Plate D).

Immediately beneath the corneal epithelium is the anterior limiting lamina or Bowman's membrane. It consists of fine closely interwoven collagen fibrils similar to those found in the stroma but contains no fibroblasts (described below).

The substantia propria or corneal stroma is composed of about 200-250 flattened, superimposed lamellae which are made up of bundles of modified connective tissue. The fibers of each lamella are arranged in a roughly parallel orientation. However, the orientations of adjacent lamella are staggered. Ground substance proteoglycans are interspersed between collagen fibrils; connective tissue cells called fibroblasts are found throughout the extracellular matrix of the stroma (see Plate C).

The posterior limiting lamina or Descemet's membrane covers the posterior surface of the substantia propria, and is a thin, homogeneous membrane. It serves as a basement membrane to the mesothelium, which consists of a single
layer of polygonal, flattened, nucleated cells [20].

Procedure

In the course of our study, 7 uneffected, control corneas were obtained and processed in the following manner. Bovine eyes were delivered on ice within 4 hours of slaughtering. The corneas were removed immediately and refrigerated in a bath consisting of their own aqueous humor. All experimental procedures were carried out within 24 hours. After the procedures were completed, the samples were chemically fixed in neutral buffered 10% formalin for a minimum of 24 hours.

In preparation for embedding, the center, treated section of the cornea was trimmed and placed into Tissue Tek II embedding cassettes. These were put through a series of ethyl alcohol baths to dehydrate the tissue and then cleared with xylene. The samples were then infiltrated with paraffin for 2 hours and mounted in paraffin blocks for microtomy.

The paraffin block containing the cornea was mounted and aligned on a microtome for sectioning. The block was iced and a 5 micrometer thick ribbon was cut. This ribbon was floated on a 42°C water bath (this procedure decompresses and flattens the ribbon). The sections were then placed onto slides and dried at 40°C before staining.

The staining procedure was an automated one which takes
the slides through xylene, a graded series of ethanol and finally stains the samples with routine hematoxylin and eosin (which stains nucleic acids purple and cytoplasm pink-red). The slides were coverslipped and dried overnight before handling [21,22].

Photomicrographs of the specimens were taken on a Zeiss photomicroscope using Kodachrome 25 color slide film. These photographic slides were then reproduced with a color Xerox machine to obtain the Plates in this document. The color Xerox reproductions unfortunately do not show the same clarity and detail as the original slides; however, the accompanying discussion should overcome this problem.

Results

In our work we shall be interested in the cells of the corneal epithelium and the state of the collagen fibers in the corneal stroma. The structures and characteristics described above can be seen in the control (normal) corneas shown in Plates C and D. Plate D shows the three general epithelial cell types. The parallel lamellae of the corneal stroma show up as a wavy texture in normal corneas (see Plates C, D and K). Note that the small spaces surrounding the nuclei are an artifact of the histological fixation process. Also as a consequence of microtoming, the lamellae often tear (see Plate C) along their parallel orientation.
Project 4  Histological Characterization of Cornea which has been subjected to a High Voltage, High Crest-factor Waveform

Here, we applied a high voltage, impulsive waveform to the specimen. In this way we could demonstrate whether or not cell damage could occur prior to steam evolution. This experiment therefore tests the possibility of a high voltage-induced breakdown mechanism.

Procedure

The corneas were placed in the frame with the epithelial cell layer facing the active electrode. The Valleylab Coag #100 setting (peak voltage = 1200 volts) was used to supply the impulsive waveform (see Figure 2) for various lengths of time. Specifically: for 1, 2, and 3 seconds, no boiling will occur; 5 seconds is probably too short for boiling to occur, but we can not be as sure; and 8 seconds was the time necessary for the experimenter to witness boiling (see Figure 3). Two specimens were tested for the one second experiment, and one specimen was tested at each of the other time durations. After the application of current the specimens were processed for histological observation as described in project 3.

Results
Plate E represents a specimen which has undergone 1 second of Coag #100 treatment. Although the damage is mild it is evident (more so on the original slide). Note the upper cell layer which has stained more darkly; this effect seems to be characteristic of moderately affected cells. In general, cell membranes remain intact here.

The specimen shown in Plate F was subjected to 2 seconds of Coag #100. Cell damage is much more extensive here. Note the ballooning effect and the large area of cells which have stained more darkly. Plate G is an enlargement of this area and makes it clear that the differentiation between cells has been destroyed in this region. The cells below were also quite disrupted. The stroma was unaffected.

At 3 seconds the cell damage is quite severe (Plate H). Many of the cell membranes have ruptured. The entire cell layer seems uniformly disrupted; it has become impossible to differentiate between the three cell types. The stroma shows the randomizing effect over half of its thickness. Keep in mind that no boiling has occurred in any of the specimens seen so far.

The damage accrued at 5 seconds (Plate I) does not seem vastly different from the 3 second case. In the upper cell layer the cytoplasm is gone. The stroma effect reaches through about two thirds of the stroma thickness.
Plate J shows the case that corresponds to observed boiling (8 seconds of Coag #100). The entire cell layer is gone over much of the cornea's surface. The cells that remain are completely disrupted. The collagen orientation stroma is randomized almost all of the way through.

Project 5) Histological Characterization of Cornea which has been Subjected to Pure Sinusoidal Electrosurgical Current of Various Durations

This project will allow us to develop an empirical understanding of the consequences of subjecting corneas to electrosurgical current. From this we will be able to gauge the extent of the damage imparted in the high pressure or high voltage experiments.

Procedure

Fresh corneas were placed in the experimental frame (see Plate B) with the epithelial cell layer facing the active electrode. They were then subjected to a 90 volt peak sinusoidal waveform (corresponding to Valleylab setting Pure Cut #25) for 10, 30, 60, or 110 seconds. These times were chosen to place the specimen in certain regions of the corresponding temperature versus time curve (see Figure 3). Specifically, 10 and 30 seconds were short enough to
preclude boiling; 60 seconds was the borderline for boiling; by 110 seconds, boiling was clearly visible in the fluid within the chamber. One specimen was tested at each of the above durations. Two additional specimens were tested, one at 30 and one at 60 seconds, with a thermocouple in contact with the cornea (see Figure 3). Also, two corneas each were subjected to a 50 volt (peak) 10kHz, sinusoidal voltage for 3, 5, and 10 minutes. Three minutes corresponded to pre-boiling, 5 and 10 minutes to boiling. These currents were supplied by a Kepco Bipolar Operational Power Supply. The effects of these lower power, longer duration currents could therefore be compared to the higher power, shorter duration currents supplied by the Valleylab electrosurgical generator.

Results

Plate K shows a cornea which has been subjected to 10 seconds of the Cut #25 waveform. It looks essentially normal except in the lower, columnar cell layer. Here we see a greater percentage of open space in the cells; the cytoplasm looks less homogeneous. This effect is the beginning of an effect called ballooning which is caused by increased osmotic activity due to the fracturing of large cytoplasmic proteins into smaller more osmotically active pieces. In general the cells are still discreet units, implying that
cell membranes have not ruptured. Note that the stroma is essentially normal (compare to Plate C).

Plate M shows a cornea which has been subjected to 30 seconds of Cut #25. Here the ballooning effect is much more pronounced and has effected all but the uppermost layer of cells. The differentiation between cells is beginning to break down.

Although it is difficult to see in Plate M, the stroma near the epithelial cell layer has undergone a change. It has lost its wavy parallel orientation and has taken on a much more random structure. This is believed to be a consequence of the denaturation of the collagen in the stroma which occurs at ~60°C. This collagen denaturation process is very important since it gives us an inherent temperature marker. Since this denaturation only extends about 1/6 of the way through the stroma, our belief that intra-cellular boiling is not yet occurring at 30 seconds is strengthened.

Plate P is an enlargement of the cell layer from a cornea which has undergone 60 seconds of Cut #25 (this image contains over 80% of the epithelium's cross section). Here all differentiation between cells is gone; the membranes are completely ruptured. The stromal lamellae have been changed to a randomly oriented state over half of the stroma's
thicknes (not shown in the magnification of Plate P).

Plate S shows a cornea which has been subjected to 110 seconds of Cut #25. In this case boiling was actually seen to take place in the saline surrounding the cornea. Again we see extensive cell damage; cell membranes have ruptured and much of the cytoplasm has run out to coagulate in a space between the cell layer and the stroma. Note that the stroma has taken on yet another appearance. No longer can any fine structure be detected; it has become very homogeneous. This effect reaches through the entire stroma.

Plate U shows a cornea which was subjected to a 50 volt (peak), 10kHz sinusoidal voltage for 5 minutes. The damage here is somewhat similar to the 10 second Cut #25 case (Plate K). The ballooning effect is more pronounced, but the stroma still appears normal.

Finally, Plate W shows a cornea which has undergone 10 minutes of the 10kHz waveform described above. Ballooning and membrane rupture is extreme. The stroma appears as if it has just barely begun to change to the randomized state. Note that no boiling was observed in the surrounding saline in this case.
Project 6  Histological Characterization of Cornea which has been Subjected to Electrosurgical Current under Elevated Pressure

In this project we subjected corneas to electrosurgical current for the same duration as in project 5. However, this time we applied an elevated pressure to stop any boiling of intra-cellular fluid which may have occurred in the previous experiment; specifically, in the 60 and 110 second cases.

Procedure

In this case as in the last, fresh corneas were placed in the experimental frame (see Plate B) with the epithelial cell layer facing the active electrode. The same 90 volt (peak) Cut #25 sinusoidal waveform was used for the identical durations as in Project 5. The difference was that the samples were sealed inside the pressure vessel, and a pressure of 120 psi was applied (nitrogen was used as the pressurizing agent). After the current was applied, the specimens were allowed to cool down and were then de-pressurized slowly. As in project 5, two samples were run for the 30 and 60 second durations; however, no thermocouple was used. Also, four control corneas were subjected to
elevated pressure without current application. A few samples were subjected to the 50 volt, 10kHz waveform described in project 5 along with a 100 psi. applied pressure. All samples were then processed for histological observation as described in project 3.

Results

The pressurized control corneas were indistinguishable from normal cornea as shown in Plates C and D. Plate K shows a cornea which was subjected to the Cut #25 waveform for 10 seconds. It is essentially indistinguishable from the control cornea (compare with Plate C). Comparison with the 10 second, 1 atmosphere sample (Plate K) shows slightly less disruption in the pressurized case.

The cornea shown of Plate N was subjected to Cut #25 for 30 seconds at 120 psi. Plate O is an enlargement of the same specimen. We see a considerable amount of cell damage. Cell membranes have broken down leaving a relatively homogeneous mixture of cytoplasm (see Plate O). The stroma remains unaffected. Comparison with the 30 second, 1 atmosphere sample (Plate M) shows that cellular damage looks essentially the same in both cases.

Plates O and R represent 60 seconds of treatment with elevated pressure. Plate Q seems to indicate less damage than the 30 second, 120 psi case described above, yet
examination of the magnified view (Plate R) shows considerable destruction. However, the integrity of many cell membranes is still evident. The upper layer cells are more disrupted than the 30 second case and the stroma shows the denaturation effect in the upper third of its thickness. Comparison with the 60 second, 1 atmosphere case (Plate P) indicates considerably less cell randomization and membrane rupture in the pressurized case.

Plate T shows a cornea which has been subjected to 110 seconds of Cut #25. Cell damage is severe; note the long "cracks" within the epithelial layer which run parallel to the stroma-cell border. The upper cell layer is completely destroyed. The stroma fiber orientation has been randomized throughout the majority of its bulk. Note however that it does not show the amorphous structure of the specimen in Plate S, for which boiling was seen to occur. Cell disruption is roughly equivalent in these two cases.

Corneas which were subjected to 3 minutes of the 10kHz waveform (at 100 psi as described above) looked the same as those that received the treatment without pressure (i.e. they both showed damage similar to the 5 minute case shown in Plate U). Corneas that were subjected to 5 minutes of current also looked the same as their unpressurized counterparts (Plate U); however, there was some difference
in the uniformity of the cell damage. For example, some sections of the same cornea looked essentially normal, as shown in Plate V. The stroma was unaffected in all cases. A specimen which underwent 10 minutes of this treatment was virtually indistinguishable from the 10 minute case without pressure (see Plate W); again the stroma was unaffected.
In Chapter 2 the results of our experiments were described. Here our task is to interpret these results in terms of the possible mechanisms that could produce initial breakdown of cell membranes, including high voltage-induced dielectric breakdown and steam evolution due to boiling. Correlations will also be drawn between the cell damage caused by Coag #100, Cut #25 and the 10kHz sinusoidal waveforms used on this study. The dielectric breakdown model will be examined first.

A. High Voltage-Induced Dielectric Breakdown

The dielectric breakdown model rests on the assumption that there exists some phenomenon which will cause structures such as the cell membrane to rupture when they are subjected to high voltage. Our goal here is first to review a model mechanism in order to make such a breakdown phenomenon plausible and then to assess whether or not the conditions of our experiments' environment meet the criterion for such a breakdown mechanism. Finally we will review the results of project 4 (Coag #100 waveform) in light of this understanding and try to determine the role of such a breakdown mechanism in electrosurgical coagulation.
A model for dielectric cell membrane breakdown was proposed by Crowley in 1973 [12]. In this model the cell is treated as an elastic, non-conducting, dielectric surrounded by a highly conducting medium (see Figure 4). Since the membrane is modeled as a non-conducting dielectric, a voltage applied across it will cause a compressive electrical stress $T_{ex}$ in the x direction (perpendicular to the plane of the membrane):

$$T_{ex} = -\frac{\varepsilon V^2}{2l^2}$$

(3-1),

where $V$ is the applied voltage, $\frac{V}{l}$ is the electric field in the membrane and $\varepsilon$ is the dielectric permittivity of the membrane. The compressive deformations caused by the applied voltage are opposed by the elastic restoring stress $T_{mx}$ which is related to compressive strain as follows:

$$T_{mx} = E \ln \frac{l}{L}$$

(3-2),

where $E$ is Young's modulus, $L$ is the original thickness, and $l$ is the compressed thickness. The term $\ln \left( \frac{l}{L} \right)$ is a measure of membrane compressive strain for finite deformation, as opposed to the usual infinitesimal strain measure, $\frac{(L-l)}{L}$. Note that it is straight-forward to reformulate this one dimensional equation for more complex geometries.
FIGURE 4  Crowley's Membrane Model
By equating these stresses Crowley was able to show [12] that as voltage increases, the compressive deflection increases, approaching infinity at:

\[
\frac{\varepsilon V^2}{2EL^2} = 0.18 \quad (3-3).
\]

Thus, above some critical voltage which is a function of the membrane's electrical and mechanical parameters, an instability results which ruptures the membrane. This result can be reasoned qualitatively by noting that the electrical stress is inversely proportional to the compressed membrane thickness \( t \) (3-1) while the restoring stress is proportional to \( \ln (\frac{t}{L}) \) from (3-2). When the applied voltage produces a small enough \( t \), the electrical compressive stress dominates, tending to reduce \( t \) even further, and thereby initiating an instability which leads to membrane rupture.

This model was shown to predict the approximate threshold voltage for the breakdown of artificial lipid bilayer films. We must now decide whether the voltages we applied in our experiments were large enough to meet the critical voltage criterion of equation 3-3. From Crowley's paper [12] we can obtain the following average values for a lipid bilayer membrane:

\[
E = 10^5 \frac{N}{m^2}
\]

\[
C = \frac{\varepsilon}{L} = 0.005 \frac{F}{m^2}
\]
L = 100 \, \text{Å} = 10^{-8} \, \text{m.}

If we substitute these values into equation (3-3) we find:

\[ V = 270 \, \text{mV} \]  

(3-4)

In project 4 we applied a Valleylab Coag #100 waveform which has a peak voltage of about 1200 volts. A simple derivation (shown in detail in Appendix 2) shows that this waveform will cause a potential difference across the cell membranes of on the order of 1.2 volts. This tells us that the voltages applied in project 6 were in general high enough to cause dielectric breakdown by a mechanism such as Crowley's.

Recall from project 2 that 8 seconds of Coag #100 was necessary to produce observed boiling. There may be some question as to whether intra-cellular boiling is occurring at 5 seconds, but we may assume that no boiling is occurring at 3 seconds with a large margin for safety. If we review the results of the high voltage experiment (Plates: E, F, G, H, I, J) we see that for applications as short as one second duration, cellular changes have occurred. At 2 seconds (Plates: F, G) we see considerable cell damage and yet the stroma remains unchanged. This means that the stroma has not reached 60°C, which implies that the intra-cellular fluids did not boil, but damage did occur. By 3 seconds (Plate H)
serious cell disruption has occurred; membranes have broken down, and differentiation between cell types is gone.

From these results we draw the following preliminary conclusion: regardless of the role which steam evolution may play, when high voltage, high crest factor waveforms are applied to cellular tissues, considerable cell damage occurs well before the intra-cellular fluids begin to boil. It seems plausible that the mechanism involved in this cell disruption could be a high voltage-induced dielectric breakdown such as that proposed by Crowley [12].

B. Steam Evolution

The mechanism for membrane breakdown due to steam evolution is rather straightforward. As electrosurgical current passes through the tissues, ohmic heating results. When the intra-cellular fluid's boiling point is reached, steam is evolved. Inherent to this steam formation is a large change in volume which causes the cells to rupture. In projects 5 and 6 we have subjected 2 sets of corneas to equivalent input energies (recall that the temperature change is directly related to the energy input; see Appendix 1) and varied the pressure to see if cell damage could be suppressed.

Reviewing the unpressurized corneas (Plates: K, M, P and S) and comparing to their equivalent time, pressurized
counterparts (Plates: L, N, O, Q, R and T), we find that application of pressure did not suppress cell damage in a uniform and repeatable way. Plates K and L seem to indicate that damage was suppressed in the 10 second pressurized case. However the damage in both cases is very mild and subsequently hard to quantify. Careful inspection of the original histological specimens does not allow us to make any conclusive statement about suppression of damage in this case. The same situation is true for the 5 minute, 10kHz case (Plates U and V). The 60 second samples seem to show a definite reduction of damage in the pressurized case (compare Plate P to Plate R) and yet the 30 second samples showing the same type of damage do not (compare Plate M to Plates N and O).

C. Waveform Correlations

Given the findings above, it is natural to wonder how the cell damage caused by the different waveforms compares. In order to make such a correlation, we first need to relate the total electrical energy delivered as a function of time for each waveform. This turns out to be very difficult to quantify: (1) the resistance of the cornea is not known precisely, (2) the power delivered by the Coag #100 waveform has a tolerance of ±15%, and (3) we cannot be sure of the exact time of boiling due to the thermal inertia of the
thermocouple (±1 second) as well as inaccuracies associated with direct visual observation.

We have therefore compared the equivalent energy delivered by Cut #25 and Coag #100 waveforms (see Figure 3) by comparing the measured tissue temperature versus time characteristics for several specimens and then checking these results by comparing the extent of stroma denaturation for each time interval (similar denaturation implies a similar temperature which implies similar energy input). It was found that 3 seconds of Coag #100 is roughly equivalent to 30 seconds of Cut #25 (see Figure 2). Comparison of these two cases (compare Plate H to Plates N and O) shows that the damage is quite similar. In no case were we able to quantify any significant differences in the initial phases of cell damage caused by these two waveforms.

D. Conclusions

The results of our study clearly show that some type of cell membrane breakdown is initiated well before boiling can occur. This result was observed with Coag #100 and even with Cut #25 waveforms. As application of current is continued, cell breakdown continues. Significant cell damage has occurred and continues to occur at the onset of boiling; e.g., gross morphological changes in the stroma. Similar tissue damage effects after intra-cellular boiling have been
described recently for muscle [15,16]. In the present research, however, we confine ourselves to a description of the initial onset of damage to cells, which is the precursor of such macroscopic changes.

As to the mechanism for initial cell membrane breakdown, we conclude that it is very possible that a high voltage dielectric breakdown phenomena such as that proposed by Crowley [12] might be in operation in our situation, certainly for Coag #100 current. Due to the nature of the approximations used in Appendix 1, it is conceivable that Crowley's critical voltage was reached even in the Cut #25 case. It is possible, however, that a different mechanism for breakdown is at work.

In any case, steam evolution is not the sole mechanism for electrosurgical cell disruption. In fact, this study indicates that it may not even be an important factor. When interpreting this last statement it must be kept in mind that our experiments examined the initial effects of electrosurgical current on the cellular level. They do not necessarily apply to the macroscopic effects on gross tissue morphology. For example, it is understood that the Cut and Coagulate waveforms do indeed have very different effects on the macroscopic scale [1]; our cornea tissue model and specific experimental design were not designed to accentuate
these macroscopic differences.

In trying to model our tissue we ran into several problems: working with a biological system for which physical and electrical characteristics are not well documented, as well as trying to get statistically significant results with only a small number of specimens. Thus, while our main conclusion concerning membrane breakdown rests on firm ground, other correlations between different waveforms must still be considered tentative. There was also some difficulty using electronic equipment in the presence of the electrosurgical device's RF currents. We have attempted to be as objective as possible. To that end histological appraisals of researchers not involved in this project were obtained concerning the nature of the cellular damage that we have discussed.

E. Suggestions for Future Research

There are many directions which future work might take to try to develop a working understanding of the microscopic effects of electrosurgical current on biological tissues. A replication of this or similar studies might employ more sophisticated temperature probes for accurate, real-time measurement of specimen temperature using a non-electrical method (e.g. fiber optic probe sensors). In this way, more accurate monitoring of ohmic heating would be enabled,
without noise or artifacts associated with coupling of sensors and the applied field. Also more samples are needed to draw statistically significant conclusions with regard to waveform correlations.

In the study conducted by Chen and Grodzinsky [15,16], real time impedance measurements were made in beef muscle being subjected to electrosurgical current. They found that the admittance versus time plot exhibited a characteristic peak. Initially, the admittance increased because electrolytic mobilities increase with the increased temperature caused by ohmic heating. With time admittance decreased due to the loss of intra-cellular fluids that accompanied boiling. Figure 5 shows a plot of relative admittance of a saline bath versus time during the application of electrosurgical current. In this study we began a project to replicate this procedure using cornea. In a preliminary trial done with a slice of beef muscle placed in our cornea rack in saline soloution, we were not able to duplicate the characteristic admittance peak (see Figure 6; relative admittance versus time). This seemed to be due to the fact that our sample was immersed in a large bath of saline, which prevented it from drying out as intra-cellular boiling progressed (in the preceding study [15,16], there was no external bathing electrolyte). However, since the
corneal stroma is so drastically affected by electrosurgical current such a study of impedance versus time may still provide some new clues as to the mechanisms occurring during cellular disruption due to electrosurgical current. In Appendix 3 the Fortran and PDP-11 Macro programs used to take real-time voltage, current and temperature data from an analog to digital converter and then convert them to admittance versus time plots are given.

The other major avenue for future research is the further development and testing of a high voltage-induced dielectric breakdown model for cell membranes. This line of work is embryonic; further advances are essential.

As the microscopic effects of the electrocoagulation process come into focus they can be correlated with macroscopic effects to yield an overall understanding of the process. Such an understanding would allow the designers of these devices to tailor waveforms and probes to their specific physiological needs while minimizing the difficulties associated with use in a surgical setting. Ultimately, we would predict the use of microprocessors to monitor the "state" of the tissue in real time and to adjust the output characteristics of the electrosurgical generator accordingly, allowing it to indicate "good coagulation". In fact, some manufacturers have already made considerable strides towards realizing this goal.
REFERENCES


APPENDIX 1

We wish to derive the electric field at the specimen location given a voltage applied at the electrodes. Figure A1-1 shows the geometry of the cornea rack and the placement of the electrodes.

The quasi-static form of Faraday's law yields:

\[ v(t) = cE_{xc} + aE_{xa} + bE_{xb} \]  \hspace{1cm} (A1-1)

Since this is a one dimensional derivation we will drop the subscript \( x \). The letter subscript corresponds to the equivalently labeled section of Figure A1-1. Equation (A1-1) can be rewritten in terms of the respective resistances of the regions:

\[ v(t) = R_a \left( \frac{a}{\pi R^2} \right) \sigma_a E_a + R_b \left( \frac{b}{d w} \right) \sigma_b E_b + R_c \left( \frac{c}{\pi R^2} \right) \sigma_c E_c \]  \hspace{1cm} (A1-2),

where:

\[ R_a = \frac{a}{(\pi R^2) \sigma_a} \]  \hspace{1cm} (A1-2b)

\[ R_b = \frac{b}{d w \sigma_b} \]  \hspace{1cm} (A1-2c)

\[ R_c = \frac{c}{(\pi R^2) \sigma_c} \]  \hspace{1cm} (A1-2d).

Given the assumption that there is no bulk free charge in the system for frequencies of interest,

\[ \nabla \cdot \varepsilon E = 0 \]

in each region. We can use Gauss' Law with the surfaces
shown in Figure A1-1.

\[ \int_{S} \varepsilon E^\star \cdot \mathbf{n} \, da = \int_{V} \rho \, dv = 0 \]

\[- \varepsilon E_{\mathcal{D}} (dw) + \varepsilon E_{\mathcal{A}} (\pi R^2) = 0 \quad (A1-3)\]

\[- \varepsilon E_{\mathcal{A}} (\pi R^2) + \varepsilon E_{\mathcal{C}} (\pi r^2) = 0 \quad (A1-4)\]

Note that we have assumed that the permittivity is the same in all regions since the stroma is \( \sim 90\% \) water. Combining (A1-3) and (A1-4) we get:

\[ E_{\mathcal{C}} = \left( \frac{dw}{\pi r \tau} \right) E_{\mathcal{b}} = \left( \frac{R}{r} \right)^2 E_{\mathcal{a}} \quad (A1-5). \]

Substituting this into (A1-1):

\[ v(t) = [a(\frac{r}{R})^2 + b(\frac{\pi r^2}{wd}) + c] E_{\mathcal{C}} \quad (A1-6). \]

Now we define:

\[ \frac{1}{K} = [a(\frac{r}{R})^2 + b(\frac{\pi r^2}{wd}) + c] \quad (A1-7), \]

which allows us to write:

\[ E_{\mathcal{C}} = Kv(t) \quad (A1-8). \]

Defining the current density at the active electrode as \( J_{\mathcal{e}} \)

we can write a statement of conservation of charge:

\[ J_{\mathcal{e}} - \sigma_{\mathcal{C}} E_{\mathcal{C}} = 0 \quad (A1-9), \]
where: \[ J_e = \frac{i(t)}{\pi r^2} \] (Al-10).

These relations allow us to write a relation for the power dissipation density in terms of the electric field:

\[ PD(t) = \sigma E(t) \cdot E(t) \] (Al-11).

\[ PD_c(t) = \sigma_c K^2 v^2(t) \] (Al-12),

where we have used equation (Al-8). Taking the time average (assuming a sinusoidal input) yields:

\[ \langle PD_c \rangle = \sigma_c K^2 \frac{V_m^2}{2} = \sigma_c K^2 v_{\text{rms}}^2 \] (Al-13).

The power dissipated into a region \( P_d \) is equal to the power dissipation density times the volume of that region. Thus we can calculate the time average power dissipated in our sample:

\[ \langle P_d \rangle = \langle PD_c \rangle (\alpha \pi r^2) = \alpha \pi r^2 \sigma v_{\text{rms}}^2 \] (Al-14),

where \( \alpha \) is the thickness of the sample. Next we would like to derive a relation between the power dissipated in the sample and its temperature as a function of time. The increase in temperature of the sample per second can be related to the time average power dissipation via the specific heat and mass density.
\[
\left( \frac{\partial T}{\partial t} \right)_C = \frac{\langle PD \rangle}{c \rho_m} 
\]  \hspace{1cm} (Al-15),

where \( c \) is the specific heat and \( \rho_m \) is the mass density. Substituting equation (Al-13) we get the relation we are looking for:

\[
\left( \frac{\partial T}{\partial t} \right)_C = \gamma \left[ \frac{\sigma}{c \rho_m} \right] V_{rms}^2 
\]  \hspace{1cm} (Al-16),

where \( \gamma = 0.239 \frac{\text{cal}}{\text{J}} \) is the conversion factor between calories and joules.

For our apparatus' geometry (see Figure Al-1) we have the following dimensions:

- \( a = 0.6 \) cm
- \( b = 1.2 \) cm
- \( c = 1.9 \) cm
- \( d = 3.0 \) cm
- \( w = 3.8 \) cm
- \( r = 0.476 \) cm
- \( R = 1.0 \) cm
- \( a = 2.0 \) mm

Given these dimensions we can calculate: \( K = 0.485 \text{ cm}^{-1} \).

In order to obtain a value for equation (Al-16) we must make certain approximations about the physical parameters of our tissue. We will approximate the tissue as having the same parameter values as water:

- \( \sigma_a = \sigma_b = \sigma_c = \sigma_d = \sigma = 1.0 \text{ mho/meter} = 0.01 \text{ mho/cm} \)
- \( c = 1.0 \text{ cal.} \) \( \frac{\text{g}}{\sigma \text{C}} \)
- \( \rho_m = 1.0 \text{ g/cm}^3 \)
Substituting these values into (Al-16) yields:

\[
\frac{\Delta T}{\Delta t} = 5.6 \times 10^{-4} V_{\text{rms}}^2
\]  

(A1-17),

also:

\[R_{\text{TOTAL}} = 284\Omega\]  

(A1-18)

\[R_c = 253\Omega\]  

(A1-19)

\[R_a = 25\Omega.\]  

(A1-20)

For the Valleylab Pure Cut #25 setting \(V_p = 90\text{v}\) or \(V_{\text{rms}} = 63\text{v}\); thus we get:

\[
\frac{\Delta T}{\Delta t} = 2.3 \frac{{}^\circ \text{C}}{\text{sec.}}
\]  

(A1-21).

When the Kepco supply was used the geometry was slightly different from that shown in Figure Al-1. A large electrode such as the right hand one in Figure Al-1 was placed symmetrically on the left side of the specimen. An equivalent computation for this geometry gives the results:

\[
\frac{\Delta T}{\Delta t} = 2.56 \times 10^{-4} V_{\text{rms}}^2
\]  

(A1-17K),

\[R_{\text{TOTAL}} = 428\Omega\]  

(A1-18K)

\[R_a = 25\Omega\]  

(A1-20K)

Here \(V_p = 50\text{v}\) or \(V_{\text{rms}} = 35\text{v}\), which yields:

\[
\frac{\Delta T}{\Delta t} = 0.32 \frac{{}^\circ \text{C}}{\text{sec.}}
\]  

(A1-21K)
FIGURE A1-1  Electrode/Cornea Geometry

FIGURE A2-1  Cell Membrane/Tissue Model
APPENDIX 2

Our purpose here is to derive the voltage that would develop across a cell membrane given an applied voltage to the tissue. In order to compute such a voltage we must construct an approximate model for our tissue system. The model we will use is shown in Figure A2-1. Since the corneal cells are closely packed and because the membrane is very thin compared to the radius of the cell, we will consider only current that flows normal to the membrane. Therefore, we will use a parallel, infinite extent model geometry. Because we neglect (tangential current lines that leak around the cell, this calculation overestimates the membrane voltage drop and thereby provides an upper bound estimate. However, other assumptions to be described below may actually result in an underestimate of transmembrane potential drop.

We start by assuming a uniform applied electric field, and write the appropriate boundary conditions. Assuming a uniform field in the x direction we can write:

\[ V = aE_a + bE_b + cE_c \]  \hspace{1cm} (A2-1),

where the time dependance and x direction notation have been suppressed. The letters a, b, and c refer to the respectively labeled regions in Figure A2-1 when used as subscripts, otherwise they refer to the corresponding dimensions. The
boundary between region a and b will be referred to as Boundary 1 and the boundary between region b and c: Boundary 2. Region a represents the intra-cellular space, and region c represents the extra-cellular space. These regions are assumed to be filled with the same type of fluid and thus are characterized by the same physical parameters, ε and σ (dielectric permittivity and conductivity). Region b is the membrane, which is characterized by the physical parameters ε_b and σ_b. The boundary conditions are as follows.

From Gauss' Law: \( \nabla \cdot \mathbf{E} = \rho \) we find:

\[
\nabla \cdot (\varepsilon_b \mathbf{E}_b - \varepsilon_a \mathbf{E}_a) = \sigma_{s_1},
\]

(A2-2a),

\[
\nabla \cdot (\varepsilon_c \mathbf{E}_c - \varepsilon_b \mathbf{E}_b) = \sigma_{s_2},
\]

(A2-2b),

where \( \sigma_{s_1} \) refers to the surface charge at boundary 1, etc.

From \( \nabla \times \mathbf{E} = 0 \) we have:

\[
\nabla \times (\mathbf{E}_b - \mathbf{E}_a) = 0
\]

(A2-3)

which is equivalent to saying:

\[
\phi_a = \phi_b
\]

(A2-4a).

at boundary 1. Similarly at boundary 2:

\[
\phi_b = \phi_c
\]

(A2-4b).

Conservation of charge yields:

\[
\nabla \cdot \mathbf{J} + \frac{\partial \rho}{\partial t} = 0
\]

(A2-5).

Combining this with the relation \( \mathbf{J} = \sigma \mathbf{E} \) yields two more
conditions:

\[ \hat{n}^*(\sigma_E E_b - \sigma E_a) = -\frac{\partial \sigma_1}{\partial t} \]  
(A2-6a),

\[ \hat{n}^*(\sigma_E c - \sigma E_b) = -\frac{\partial \sigma_2}{\partial t} \]  
(A2-6b).

Combining (A2-2) with (A2-6):

\[ (\sigma + j\omega \varepsilon)E_a = (\sigma E_b + j\omega \varepsilon)E_b \]  
(A2-7a)

\[ (\sigma E_b + j\omega \varepsilon)E_b = (\sigma + j\omega \varepsilon)E_c \]  
(A2-7b).

The charge relaxation in region a and c is given by:

\[ \tau = \frac{\varepsilon}{\sigma} = 7 \times 10^{-12} \]

From this we see that \( \tau >> T = 1.33 \times 10^{-6} \) sec, which tells us that \( \sigma >> j\omega \varepsilon \). This allows us to make approximations which transform (A2-7) into:

\[ \sigma E_a = j\omega \varepsilon E_b = \sigma E_c \]  
(A2-8).

This can be substituted into (A2-1) and solved for the electric field across the membrane:

\[ E_b(t) = \frac{V(t)}{\frac{j\omega \varepsilon}{(a+c)(\frac{\sigma}{\sigma_D}) + b}} \]  
(A2-9),

We can make further simplifications if we note that:

\[ \frac{j\omega \varepsilon}{\sigma} = \frac{j(4.7 \times 10^4) \kappa (8.85 \times 10^{-12})}{1} = j4.2 \times 10^{-5} \kappa \]
where \( \varepsilon_b = \kappa \varepsilon_s \). For a typical cell \( b = 100 \AA = 10^{-8} \text{m} \) and the diameter of the cell: \( a = 10^{-5} \text{m} \). Also we will assume that \( \kappa \) is 10 (such a value is typical of lipid dielectrics). These approximations allow us to eliminate \( a \) and \( c \) in (A2-9) to get:

\[
\frac{E_{bp}}{b} = \frac{V}{b} = \frac{V_{tp}}{bm} \quad \text{(A2-10),}
\]

where \( m \) is the effective number of membranes in the entire sample and \( V_{tp} \) is the total voltage drop across the specimen. The constant \( m \) can be further subdivided into \( m = N + n \) where \( n \) is the number of membranes in the tissue's cellular layer and \( N \) accounts for the underlying tissues. The resistance calculations in Appendix 1 (see equations (A1-18) and (A1-20)) indicate that about 10% of the applied voltage is dropped across the specimen. Thus for the Vallylab Coag #100 waveform, who's peak applied voltage is approximately 1200 volts, we expect that \( V_{tp} = 120 \text{v} \). The constant \( m \) is rather difficult to estimate; the corneal cell layer is about 10 cells thick, so we set \( n = 20 \). To be on the conservative side we will pick \( N \) large: \( N = 80 \). The result of these approximations is that:

\[
E_{bp} = 1.2 \times 10^8 \frac{\text{volt}}{m} \quad \text{(A2-11)}
\]

\[
V_{mp} = E_{bp} \times 10^{-8} \text{m} = 1.2 \text{v} \quad \text{(A2-12),}
\]
This derivation rests on many approximations, however it is certainly accurate to within an order of magnitude.
program cifssp

Version 4.
A sampling and storage program for molecular
diffusion and other transient experiments.
Sampling is done either synchronously at a
predetermined rate, or asynchronously at a
rate determined by variations in the measured
signal. Single samples can also be taken.

written: March 81 by PG
modified: February 82 by EF

3 channels user selectable

define storage

byte command
byte probnam(10)
byte outf1(12),prefix(6)
byte date(10),cmnt1(64),cmnt2(64),cmnt3(64)
byte home(2),erase(2),erlin(2)
byte gtime(2),line(2),line2(2),line3(2)
byte half(2),bottom(2)
integer minits,msecs,sig(3),chan,chnl

integer buff(2000)
integer buff1(2000)
integer buff2(2000)
integer buff(2000,3)
equivalence (buff,buff0)
integer time(2,2000)
common buff0,buff1,buff2,time

integer nbuff,icount,ncount,tcount,TH
integer RRrate,RRtime,TRNtin,endit
integer tstart,tstop,abflag
real sum
real rtime,nextim,rate,second

equivalence (prefix(1),outfil(1))

set constants

data probnam /'D','I','F','S','S','P','\..','V','O','4'/
data outf1 /'X','X','X','X','X','X','X','\..','D','I','F','\..','/'
data ncount /5/
data home="/033,"110/, erase="/033,"112/
data erlin="/033,"113/, gtline="/033,"131/
data line2="/041,"040/, line3="/042,"040/
data bottom="/067,"040/, half="/053,"040/

logical unit number for output file
data lunum /4/

size of buffers
data nbuff /2000/

initialize variables

icount = 0
abflag = 0
call scca(abflag)

get parameters from user & construct comments

type 80,home,erase
accept 90,prefix
type 81
accept 93,chan
type 84
accept 91,delta
type 82
accept 92,cmnt1
type 83
accept 93,TH

call getdat(date)
encode (64,85,cmnt2) prgnam,date,(outfil(x),k=1,10)
encode (64,86,cmnt3) delta,TH,chan

* formats for this section
80 format (x,2a,2a,'sample ID? (six characters)',$)
81 format (x,'number of channels to sample? (i)' ,$)
82 format (x,'brief description: ',$)
83 format (x,'TRANS threshold? (i)' ,$)
84 format (x,'thickness (microns) of sample? (r)' ,$)
85 format ('data sampled by: ',10a,' on: ',10a,
> ' file: ',10a)
86 format ('thickness (um): ',g8.3,
> ' TRANS threshold: ',i4,
> ' channels: ',i2)
90  format (6a)
91  format (g12.3)
92  format (64a)
93  format (i4)

open output file & write header

open (unit=lunum,name=outfil,type='NEW',form='UNFORMATTED')
write (lunum) prgnam
write (lunum) cmnt1,cmnt2,cmnt3

get command & go to appropriate section

type  280
accept 290,comand

210  abflag = 0
type  282,gtline,line2,erlin
accept 290,comand

215  if (comand .eq. 'S')  go to 300
if (comand .eq. 'T')  go to 400
if (comand .eq. 'R')  go to 500
if (comand .eq. 'E')  go to 700

280  format (/x,'commands are as follows:'
> /6x,'S - SINGLE SAMPLE'
> /6x,'T - TRANS mode'
> /6x,'R - REGULAR RATE mode'
> /6x,'E - exit program'
> /x,'enter command: ',$,)
282  format (x,2a,2a,2a,'enter comand (S,T,R,E): ',$,)
285  format (x,2a,2a,'elapsed time :','i6',' min','22x','DIFSSP.V04')
290  format (al)

SINGLE SAMPLE section

300  call getime(minits,ftime)
* call sampling macro
tcount = ncount
    call trnsam(-1,chan,buff0,buff1,buff2,time,
    >       tcount,minits+2,0)

* calculate sample
do 320  k = 1, chan
    sum = 0.
do 310  j = 1,tcount
    310  sum = sum + float(buff(j,k)) / 2.048
    320  sig(k) = ifix(sum / float(tcount))

* display & store sample
    write (lunum)  rtime,(sig(k),k=1,chan)
    icount = icount + 1
    type  380,gline,bottom,icount,rtime,(k,sig(k),k=1,chan)
    type  285,home,erlin,minits
    go to 210

* formats for this section
    format (x,2a,2a/x,'sample ','i6,10x,fl2.3,' seconds'
>      /,x(5x,'channel ','il,':',i6,' mv':;))

---------------------------------------------------------------
TRANS section
---------------------------------------------------------------

400 type  480,gline,line2,erlin
    accept 490,TRNtim
    if (TRNtim .lt. 0)  go to 400

call getime(minits,rtime)
    endtim = minits + TRNtim + 1
410 type  285,home,erlin,minits
    type  486,gline,line2,erlin,endtim,TH
    if (abflag .ne. 0)  go to 210

* call sampling macro
tcount = nbuff
    call trnsam(TH,chan,buff0,buff1,buff2,time,
    >       tcount,endtim,abflag)

c* calculate & store data
    tstart = icount + 1
    do 420  j = 1,tcount
        second = float(time(2,j)) * .001
        if (second .lt. 0.)  second = 65.536 + second
        rtime = second + float(time(1,j))*60.
do 415 k = 1, chan
   sig(k) = ifix(float(buff(j,k)) / 2.048)
   write (lunum) rtime, (sig(k), k=1, chan)
420   icount = icount + 1
   tstop = icount
   type 487, gtline, bottom, tstart, tstop,
      >         tstop, rtime, (k, sig(k), k=1, chan)
   c   * check time & repeat call to sampling macro if necessary
   c   call gettime(minits, rtime)
   if (minits .lt. endtime) go to 410
   c   * return to command section
   type 285, home, erlin, minits
   go to 210
   c   * formats for this section
   format (x, 2a, 2a, 2a, 'enter duration (min) for TRANS',
      >         ' node (i): ', $)
   format (x, 2a, 2a, 2a, 'TRANS mode ends at: ', i6,
      >         ' min', 10x, 'threshold: ', i5, )
   format (x, 2a, 2a, 'samples ', i5, ' through ', i5,
      >         ' were taken in TRANS mode'
      >         '/x, 'sample ', i6, 10x, 'f12.3, ' seconds'
      >         '/3(5x, 'channel ', i1, ': ', i6, ', mv', :, ) )
490   format (i5)

REGULAR RATE section

500   type 580, gtline, line2, erlin
      accept 590, RRrate
      if (RRrate .lt. 1) go to 500
505   type 581, gtline, line2, erlin
      accept 590, RRtime
      if (RRtime .lt. 0) go to 505
      rate = float(RRrate)
      c   call gettime(minits, rtime)
      nextim = rtime + rate
      endim = minits + RRtime + 1
      type 285, home, erlin, minits
      type 586, gtline, line2, erlin, endim, RRrate
      c   * sampling loop
      c   510   if (abflag .ne. 0) go to 210
      call gettime(minits, rtime)
if (rtime .lt. nextim)
   go to 570
else
   * take sample
tcount = ncount
call trnsam(-1,chan,buff0,buff1,buff2,time,
   tcount,minits+2,0)
   * calculate sample
do 530  k = 1, chan
   sum = 0.
do 520  j = 1,tcount
   sum = sum + float(buff(j,k)) / 2.048
   530   sig(k) = ifix(sum / float(tcount))
   * display & store sample
write (lunum)  rtime,(sig(k),k=1,chan)
icount = icount + 1
type 380,gtline,bottom,icount,rtime,(k,sig(k),k=1,chan)
type  285,home,erlin,minits
type  586,gtline,line2,erlin,endtim,RRrate
   * update next sample time
540   if (rtime .lt. nextim)  go to 570
      if (abflag .ne. 0)  go to 210
   nextim = nextim + rate
   go to 540

570 if (minits .lt. endtim)
   go to 510
else return to command section
   go to 210

* formats for this section
580 format (x,2a,2a,2a,'enter sampling rate in seconds (i): ','$')
581 format (x,2a,2a,2a,'enter duration (min) for REGULAR RATE',
   ' mode (i): ','$')
586 format (x,2a,2a,2a,'REGULAR RATE mode ends at: ',i6,
   ' min',10x,'rate: ',i5,' sec')
590 format (i5)

--------------------------------------------------------------------------------

exit section

--------------------------------------------------------------------------------

c * close file & stop
700 type  780,home,erase
call stopc
denefile lunum
close (unit=lunum)
type  781
stop 'End of Sampling Run'
subroutine getime(minit, rtime)

    Returns the elapsed time both in minutes (integer) and in seconds (real).

written: 30 January 82 by PG
modified: 30 January 82 by PG

integer minit, msecs
real rtime, second

call getc(minit, msecs)
second = float(msecs) * .001
if (msecs .lt. 0)  second = 65.536 + second
rtime = second + float(minit)*60.

creturn
d
.enabl lc
.title trnsam version 2
.globl trnsam,startc,stopc,getc

; Takes a/d converter sample every 16
; milliseconds, but only saves sample
; when it differs from previous sample
; by a minimum threshold.
;
; written: 13 October 81 by PG
; modified: 31 January 81 by PG
; modified: 2 February 82 by EF 3 channels user select

; register definitions

temp = r0
point = r1
chnl = r2
chnidx = r3
save = r4
list = r5

; device addresses

adccsr = 176770
adcdbr = 176772
adcvec = 000130
dac0 = 176760
dac1 = 176762
dac2 = 176764
rtccsr = 170420
rtcdbr = 170422
rtcvec = 000440

; device commands

rtcsta = 000200
go = 000001
khz = 000040
mode2 = 000004
model = 000002
mode0 = 000000
mntST2 = 001000
intov = 000100
ovflag = 000200

adcsta = 000200
extrig = 000002
intena = 000100
vmax1 = 000000
vmax10 = 000030
chan0 = 000000
chan1 = 000400
chan2 = 001000
com001 = go+intena+vmax10+chan0
com101 = go+intena+vmax10+chan1
com201 = go+intena+vmax10+chan2
com010 = go+intena+vmax1+chan0
com110 = go+intena+vmax1+chan1
com210 = go+intena+vmax1+chan2

; useful numbers
dacmin = 174000
dacmax = 003777

; argument list offsets
incrmt = 2
chan = 4
buff0 = 6
buff1 = 10
buff2 = 12
buff3 = 14
number = 16
tinlim = 20
abflag = 22

hitime = 2
lotime = 4

; start clock routine
startc: mov @adcdb,r1 ; dummy read to clear adc
mov #adcnt,#adcvec
mov #adcnta,#adcvec+2

; start up real time clock
clr @rtccsr
mov #-1,@rtcdbr
clr rtcntH
clr rtcntL
clr tflag
mov #rtcint,#rtcvec
mov #rtcsta,#rtcvec+2

mov #khz,temp ; set rate to 1 kHz
bis #go,temp ; set go bit
bis #model,temp ; set to mode 1
bis #intov,temp ; allow interrup upon rtc overflow
mov temp,#rtccsr ; start clock
rts pc

; read clock routine
getc:    mov    rtcntH,@hitime(list)
mov    rtcntL,@lotime(list)
rts    pc

; stop clock routine

stopc:   clr    @#rtccsr
rts    pc

; sampling routine

trnsam:  mov    @timlim(list),limit    ;get parameters
mov    @number(list),count
mov    @incrmnt(list),inc
mov    @chan(list),chnls
asl    inc
asl    inc
asl    inc
asl    inc
mov    chnls,chnl
clr    chnidx

loopcl:  clr    @dachan(chnidx)      ;set DACs to zero
clr    indac(chnidx)
inc    chnidx
inc    chnidx
sob    chnl,loopcl

mov    buff0(list),ch0      ;set up buffers
mov    buff1(list),ch1
mov    buff2(list),ch2
mov    buff3(list),time

; take samples

sample:  mov    chnls,chnl
clr    chnidx

loopc:   mov    #1,adflag
mov    adcors(chnidx),@#adccsr ;coarse sample - channel chnidx

wai tc:  tst    adflag     ;wait for adc to sample
bne    waitc
add    adcout,indac(chnidx)

cmp    indac(chnidx),#dacmin ;zero out offset with DAC
bge    okmin
mov    #dacmin,indac(chnidx)

okmin:   cmp    indac,#dacmax
ble    okmax
mov    #dacmax,indac(chnidx)
okmax:  mov  indac(chnidx),@dachan(chnidx)
inc  chnidx
inc  chnidx
sob  chnl,loope

;initialize variables
mov  chnls,chnl
cir  chnidx
loopi:  cir  newav(chnidx)
cir  oldav(chnidx)
inc  chnidx
inc  chnidx
sob  chnl,loopi

mov  #36,point
jgp  nowait

loop16:  dec  point  ;take initial samples
dec  point
first:  tst  tflag  ;has it been 1 msec?
beq  first
cir  tflag

nowait:  mov  chnls,chnl
cir  chnidx
mov  point,save

loof:  mov  #1,adflag
mov  adcfin(chnidx),@adccsr ;fine sample - channel 0
waitf:  tst  adflag  ;wait for adc to sample
bne  waitf
add  adcout,oldav(chnidx)
add  adcout,newav(chnidx)
mov  adcout,circ(point)
add  #40,point
inc  chnidx
inc  chnidx
sob  chnl,loof

mov  save,point
tst  point
bne  loop16
mov  rtcntH,@time  ;record data & time
inc  time
inc  time
mov  rtcntL,@time
inc  time
inc  time
mov  chnl, chnl
clr  chnidx

loopmv:
  mov  indac(chnidx), temp
  mov  temp, save
  asl  temp
  asl  temp
  add  save, temp
  asl  temp
  mov  oldav(chnidx), save
  asr  save
  asr  save
  asr  save
  add  save, temp
  mov  temp, @ch(chnidx)
  inc  ch(chnidx)
  inc  ch(chnidx)
  inc  chnidx
  inc  chnidx
  sob  chnl, loopmv

dec  count
beq  done ; stop if buffers are full

; wait for signals to change

change:
  cmp  rtcntH, limit
  bpl  done ; reached time limit?

  tst  @abflag(list)
  bne  done ; abort request?

  dec  point
  dec  point
  bic  #177740, point

next:
  tst  tflag
  beq  next ; has it been 1 msec?
  clr  tflag

  mov  chnl, chnl
  clr  chnidx
  mov  point, save

loopav:
  mov  #1, adflag
  mov  adcfin(chnidx), @adcsrc ; fine sample - channel 0

waitav:
  tst  adflag
  bne  waitav ; wait for adc to sample
sub  circ(point),newav(chnidx)
add  adcout,newav(chnidx)
mov  adcout,circ(point)
add  #40,point
inc  chnidx
inc  chnidx
sob  chnl,loopck
mov  save,point
mov  chnls,chnl
clr  chnidx

loopck: mov  newav(chnidx),temp          ;has signal 0 changed by more
sub  oldav(chnidx),temp              ;than inc?
bpl  check	neg  temp
check: cmp  inc,temp
bpl  try
jmp  sample                            ;if so, take another sample
try:  inc  chnidx
inc  chnidx
sob  chnl,loopck
jmp  change                             ;if so, take another sample
done: mov  @number(list),temp          ;send # of samples back
sub  count,temp
mov  temp,@number(list)                ;to calling program
rts  pc

; adc interrupt service routine

adcint: bis  #mntST2,@rtccsr            ;allow count of rtc to be latched
mov  rl,-(sp)                          ;push old value of rl
mov  @adcoibr,rl                       ;move data to work register
bit  #4000,rl                          ;check for negative result
beq  ret
bis  #170000,rl
ret:  mov  rl,adcout
mov  (sp)+,rl                           ;pop old value of rl
clr  adflag
rti

; rtc interrupt service routine

rtcint: bic  #ovflag,rtccsr            ;clear overflow flag in csr
mov  #1,tflag                           ;set millisec. flag
inc  rtcntL                             ;incr low order clock count
cmp  rtcntL,#-5536.                     ;time to incr high count?
beq  incnth
rti
in cntH: clr rt cntL
inc rt cntH ; reset low order counter
rti ; incr high counter on rtc overflow

; storage

rt cntH: .word 0
rt cntL: .word 0
adc out: .word 0
adelag: .word 0
chnls: .word 0
inc: .word 0
ch:
ch0: .word 0
ch1: .word 0
ch2: .word 0
time: .word 0
count: .word 0
limit: .word 0
tflag: .word 0
adc ors: .word com001,com101,com201
adc fin: .word com010,com110,com210
da chan: .word dac0,dac1,dac2
old ay: .word 0,0,0
ind ac: .word 0,0,0
new av: .word 0,0,0
circ: .blkw 20
.blkw 20
.blkw 20
.end
ADMIT1.FOR

Written by STEPHEN BART
Modified by SB March 25, 1982

This program takes an output file generated by DIFSSP.FOR (version 4),
calculates the admittance, and prepares the new file for OUT.FOR.

define storage

integer lunum, output, y, otemp, adm, jy(3), nchan, chan
byte prgnam(10), file(10), infil(12), cmnt1(64),
c cmnt2(64), cmnt4(64)
real ovolt, oamp, time, volt, amps, temp, a, b, c
data output/1/, lunum/4/
5 type 10
10 format (' Enter output file name (aaaaaa.ADM): ',$)
accept 20, file
20 format (10a1)
   if (file(1) .eq. ' ') go to 999
25 open (unit=1, name= file, type = 'NEW',
   c form = 'UNFORMATTED', err=1030)

Input scaling parameters

type 30
30 format (' Enter volts/volt: ',$)
accept 31, a
31 format (f5.2)
   if (a .ne. 0.0) volt=a
type 40
40 format (' Enter volts/amp: ',$)
accept 41, b
41 format (f5.2)
   if (b .ne. 0.0) amps=b
type 50
50 format (' Enter volts/degC: ',$)
accept 51, c
51 format (f5.2)
   if (c .ne. 0.0) temp=c

Open DIFSSP output file

60 type 65
65 format (/x,'input file? (aaaaaa.DIF): ',$)
accept 70, infil
70 format (12a)
   if (infil(1) .eq. ' ') go to 999
   open (unit=lunum, name=infil, type = 'OLD',
   c form = 'UNFORMATTED')

c read data from DIFSSP file
c
read (lunum) prgnam
80 read (lunum) cmnt1,cmnt2,cmnt4
   decode (64,82,cmnt4) chan
82 format (62x,i2)
   nchan = chan-1
   if (nchan .eq. 0) go to 1050
   type *,'nchan is', nchan
   encode (64,82,cmnt4) nchan
   encode (64,84,cmnt1) volt,amps,temp
84 format ('STEPHEN BART ',' volts/volt:','f5.2,
   volts/amp:','f5.2,' volts/degC:','f5.2)
c
  write output file
  write (output) prgnam
  write (output) cmnt1,cmnt2,cmnt4
  read, modify, and write the data
  100 read (lunum, end=110) time,(jy(i),i=1,chan)
     ovolt = volt*float(jy(1))*0.001
     if (ovolt .eq. 0) go to 101
     oamp = amps*float(jy(2))*0.001
     otemp = temp*float(jy(3))*0.001
     adm = ((oamp/ovolt)*1000.0)+0.5
     go to 102
100 adm = 0
102 if (chan .eq. 3) go to 104
  write(output) time,adm
  go to 100
104 write(output) time,adm,otemp
105 go to 100
110 close (unit=output)
   close (unit=lunum)

c
   Repeat sequence

c
999 type 1000
1000 format (' Do you wish to run again ? (Yes=1):',$)
   accept 1010,y
1010 format (i1)
   if (y .eq. 1) go to 5
1020 stop
1030 type 1040
1040 format (' open Error')
1045 stop
1050 type 1055
1055 format (' ERROR More than one channel of
c data is necessary!')
   stop
end