Mechanical Properties Of The Intervertebral Disc As An Estimator Of Postmortem Interval

Jennifer Noelle Jackson
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MECHANICAL PROPERTIES OF THE INTERVERTEBRAL DISC AS AN ESTIMATOR OF POSTMORTEM INTERVAL

by

JENNIFER NOELLE JACKSON
B.S. University of Central Florida, 2003

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Mechanical, Materials, and Aerospace Engineering in the College of Engineering and Computer Science at the University of Central Florida Orlando, Florida

Summer Term
2005
Currently, forensic scientists are only able to determine time since death (or postmortem interval) up to the first 60 hours. This is based largely on insect activity. Herein, it is proposed to use the degradation of the intervertebral disc (IVD) after death to determine a relationship between the mechanical properties of cadaveric tissue and time since death in order to extend the 60-hour window. To that end, 1 fresh human spine and 6 pig spines were each separated into sections (6 human and 48 pig), with each section having one intact disc. The sections were buried, unearthed, and cleaned, leaving only the disc and bone. To determine the mechanical properties, each disc underwent three different tests: cyclic conditioning, compression, and stress relaxation testing. The Schapery collocation method was used to create a theoretical curve from the data for the experimental curve. Observations were made involving the corresponding k values of the curve. Although there are trends in the data for k values that approximate the experimental stress relaxation curve, a correlation could not be determined.
With love and thanks to my close family and friends whose love and support will always be invaluable
ACKNOWLEDGMENTS

I would like to thank the following faculty/staff members and students for their assistance and support: Dr. Ted Conway, Shawn Franklin, Dave Carter, Lana Williams, Dr. Tosha Dupras, Mr. Jerry Bertetta, Dr. Richard Zarda, and Dr. David Nicholson. Also, a special thanks to Christian Feldt, Bernie Sam, and Corey Hopkins who sacrificed their time to help with trips to the FDA. I would also like to thank all of my friends and family for their support and encouragement.
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INTRODUCTION AND BACKGROUND

Introduction

Every person has a story to tell, both in life and in death. In death, a body can help determine important factors in a forensic case. The manner in which the person died may be identified, whether or not the body was murdered can be determined, and how long a body has been dead may be estimated [McKeown, 2003]. In forensic investigations of any kind of death, a precise determination of postmortem interval (PMI), or time since death, is one of the most important determinants. Comparing various data from autopsy reports (including the cause of death, the extent of corpse decomposition, and the location and stages of any insects present), the medical examiner attempts to estimate the time of death [Introna et al., 1998].

During the first 60 to 72 hours following death, things happen to the body that are very distinct (i.e. rigor mortis, livor mortis, bloating, etc.) and forensic investigators have reliable standards and methods used to indicate the timing of these processes and predict PMI. After that time frame has expired, there are currently no precise methods or techniques to indicate how long a person has been dead [McKeown, 2003]. Another method is needed in order to improve the estimation of the postmortem interval, beyond the initial 72 hours.
Most of the techniques that forensic investigators currently use are entomologically based and are only able to determine the PMI within the first 60 hours. So far there is no way of reliably determining time since death past the first two or three days. This small window is inadequate given that many corpses are found after the window has passed. One possibility for improvement is testing tissues of the body that seem to decay at slower rates. The intervertebral discs of the spine are a great example. These discs lie between vertebral bodies, or bones, and are protected by the dense proteins surrounding them. Because of these proteins, it takes longer for the intervertebral discs to decompose and they are left behind months longer than soft tissues of the body [McKeown, 2003]. This is why the spine, specifically the intervertebral discs, was chosen for study in order to determine whether the strength of the tissue changes in a mathematical or repeatable trend.

A protocol was designed to limit the variables in the experiment, which began with human spines. The first two were cut from cadavers that were bought by the anatomy lab at the University of Central Florida. Once the students were done dissecting the bodies, the spines were harvested for testing. Since the bodies had been embalmed and many of the discs had ossified because the spines were so old, the data obtained during such testing was not very useful. This may have also been attributed to the spines being frozen for a long period of time. Another human spine, which was younger than the previous two, was obtained from the Central Florida Tissue Bank, where fresh and younger spines could be attained. Although some of the data were questionable, it produced better results as compared to the previous two human spines. Unfortunately, human spines were not readily available from the tissue bank. Since there was a need for
younger spines that were in good condition and that could be ready when needed, the
dissection of six porcine spines obtained from the FDA in Bethesda, Maryland – two sets
of three spines – was performed. Each spine, both human and porcine, was dissected into
segments consisting of half a vertebral body, an intervertebral disc, and half a vertebral
body.

The first section of the first spine was never put in the ground, and it was tested
immediately upon reaching the lab. The rest of the sections were buried in a remote
location and one section was unearthed each day, alternating between three spines. The
spinous processes were cut away using a bone saw. Then the ligaments and tendons were
dissected away so only the vertebral bodies and the disc remained. Once it reached the
lab, the section was placed in clamps in a testing chamber filled with distilled water
heated to body temperature. The testing chamber was already securely fastened to the
Instron tensile/compression testing machine. The mechanical properties were determined
by performing three tests on each intact intervertebral disc. The tests consist of cyclic
conditioning, compression, and extensive stress relaxation testing.

Twenty minutes of cyclic conditioning prepared each specimen for the next two
tests and reduced any variability that may occur in the beginning of a test. Once this was
complete, the disc was compressed for thirty seconds. The ensuing data were used to
create a stress-strain curve for each specimen. Once the sample reached its compressed
height, it was held in compression for twelve hours. This stress relaxation test provided
the data for analysis.

Since the tissues of the spine are viscoelastic, the Schapery collocation method
was employed in order to determine a model that would match the experimental elastic
modulus equation closely. It is believed that any hope of a mathematical trend may lie in this model. Using the data obtained from the stress relaxation tests, a log-log plot of the time versus the relaxation modulus was used to choose decade values for the Schapery collocation method. From this, $k_e$, the long-time equilibrium (rubbery) modulus, was determined. The decade values were used to calculate the rest of the $k_j$ values, which were solved for using two methods. The first method assumes all coefficients less than 0.06 are zero so a simple backward substitution method is employed to calculate the $k_j$ values. The other more reliable technique uses the coefficients of the $k_j$ values as they are. This necessitates the use of circular references in EXCEL in order to calculate the $k_j$ values. On comparing the curves, the second method using circular references produced a closer curve to the theoretical curve. Then the $k_j$ values were examined to determine whether or not there is indeed a mathematical trend in the way the intervertebral discs degrade as a function of time. The graphs of the $k$ values were studied to determine any trends in the data between sets of spines that may lead to the estimation of postmortem interval, even if the estimation is for a range of time in days instead of hours.

**Spine Anatomy**

Before any experiments were conducted, general knowledge of anatomy of both pig and human spines was necessary. The skeletons of all mammals are very similar, as can be seen in Figure 1 of the human skeleton and Figure 2 of the pig skeleton. Their primary difference is that they experience different stresses resulting from their different means of support and locomotion, since the bones function mainly to resist compression stresses and to transmit muscle action by acting as levers [Walker, 1980].
Adapted from [Walker, 1980]

Figure 1 - Skeleton of a Human

Adapted from [Walker, 1980]

Figure 2 - Skeleton of a Pig
The Human Spine

The human spine consists of 7 cervical vertebrae, 12 thoracic vertebrae, 5 lumbar vertebrae, 5 fused vertebrae of the sacrum, and the coccyx. As shown below in Figure 3, the cervical vertebrae make up the neck. The ribs attach to the thoracic vertebrae that make up the chest region, while the lumbar vertebrae make up the lower back region.

Between each vertebra of the cervical, thoracic, and lumbar regions, there is an intervertebral disc. These discs are not vascular, in that they do not contain vessels that carry or circulate fluids. They depend on the endplates of the adjacent vertebral bodies to distribute needed nutrients. The endplates are composed of layers of cartilage that anchor the discs in place [Bridwell, 2001a]. These discs are actually joints that allow movement between the vertebrae. While resistant to compressive loads, each intervertebral disc allows small degrees of extension, flexion, lateral bending, and twisting. The combined small movements at each disc enable great mobility of the entire spine [Freemont et al.,]
The intervertebral discs are small cushions that serve as a shock absorbing system for the body, protecting the brain, vertebrae, and other structures [Bridwell, 2001a].

Each disc is composed of a nucleus pulposus and an annulus fibrosus, as shown above in Figure 4. Both the nucleus pulposus and the annulus fibrosus are composed of water, collagen, and proteoglycans, which are hydrophilic and cause the nucleus pulposus to swell. This swelling pressure is confined by the end-plates of the adjacent vertebrae and the annulus fibrosus surrounding it [Freemont et al., 2002]. The nucleus pulposus fills the space between the end-plates of the adjacent vertebrae and contains a hydrated gel-like material that resists compression. The annulus fibrosus is composed of concentric dense sheets of highly oriented collagen fibers, or lamellae. This strong ligament binds the end-plates of adjacent vertebrae together and encloses the nucleus pulposus [Bridwell, 2001a].

*The Porcine (Pig) Spine*

*Sus domesticus* (phylum: Chordata; class: Mammalia; order: Artiodactyla; family: Suidae), more commonly known as the modern domestic pig, has evolved over thousands of years through natural selection and more recently, the application of the Mendelian
genetic principles by animal breeders. Not only do we rely on these animals for food, but they are becoming increasingly important as animal models in biomedical research [Pond & Houpt, 1978; Swindle, 1998].

Thus far, pigs have rarely been used as models for musculoskeletal or orthopedic experiments because of the arrangement of their muscles and skeletal axis. However, farm and miniature pigs have similar proportions and distributions of muscles and bones that provide locomotion and support for the body, which enables their use as growth models of the system because of their rapid weight gain [Swindle, 1998].

The vertebral column, or backbone, of the pig is composed of 51-56 bones, while the human spine has only 33 [Bohensky, 1978; Odlaug, 1969]. The number of vertebrae that a pig has depends on the number of bones in the thoracic, lumbar, and caudal regions which show variation [Odlaug, 1969]. Differences in the average number of vertebrae are attributed to breed, but not sex, with additional ribs commonly associated with breeds of larger size [Pond & Houpt, 1978; Swine Basics, 2005]. A typical vertebral bone consists of the following: a body or centrum, a vertebral arch with spine, a pair of lateral and transverse processes, and the posterior articular facets at the place where the vertebrae meet [Bohensky, 1978; Odlaug, 1969; and Walker, 1980]. The size of the vertebral bodies and the spinous process length and inclination vary extensively along the vertebral column in association with the specific stresses to which each undergoes. There is a pad of cartilage, or intervertebral disc, located between adjacent vertebrae that forms a protective cushion [Bohensky, 1978].
Comparison between Human and Pig Spines

The vertebral column can be divided into five sections: cervical, thoracic, lumbar, sacral, and caudal. The cervical part of the vertebral column is located in the neck region. Nearly all mammals, including humans, pigs, and even tall giraffes, have 7 cervical vertebrae. The first two are referred to as the atlas and the axis, which allow free movement of the head [Bohensky, 1978; Odlaug, 1969; and Walker, 1980]. Note that the seventh cervical vertebra for the pig has an extremely long vertebral spine and that the articular facet for the first rib head is present on the centrum [Odlaug, 1969].

Moving down the spine, the next section of the vertebral column is the thoracic region. These vertebrae are characterized by their prominent vertebral spines and articular costal facets for rib attachments [Bohensky, 1978; Odlaug, 1969]. The vertebral spines are directed downward in humans, but are more or less erect in pigs [Odlaug, 1969]. Humans have 12, while pigs have 14-15 [Bohensky, 1978; Odlaug, 1969; and Walker, 1980].

The lumbar vertebrae can be distinguished by their massive centra, reduced vertebral spines, and long transverse processes [Bohensky, 1978; Odlaug, 1969].
has 5 lumbar vertebrae, while pigs have 6-7 [Bohensky, 1978; Odlaug, 1969; and Walker, 1980]. For a pig, as the number of ribs increases, the number of lumbar vertebrae decreases [Pond & Houpt, 1978; Swine Basics, 2005].

Humans have 5 sacral vertebrae which are fused into one bone called the sacrum. The pig has only 4, which are not fused together [Bohensky, 1978; Odlaug, 1969; and Walker, 1980]. The larger number of sacral vertebrae in humans is attributed to the greater problems of support that are inherent in a bipedal gait [Walker, 1980].

Finally, humans have 3-5 caudal vertebrae that are fused, forming the coccyx. Pigs have 20-23 separate caudal vertebrae which extend out into the tail [Bohensky, 1978; Odlaug, 1969; and Walker, 1980]. A summary of the numbers of vertebrae for humans and pigs is shown below in Table 1.

<table>
<thead>
<tr>
<th>Region</th>
<th>Human</th>
<th>Pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Thoracic</td>
<td>12</td>
<td>14-15</td>
</tr>
<tr>
<td>Lumbar</td>
<td>5</td>
<td>6-7</td>
</tr>
<tr>
<td>Sacral</td>
<td>5 (sacrum)</td>
<td>4</td>
</tr>
<tr>
<td>Caudal</td>
<td>3-5 (coccyx)</td>
<td>20-23</td>
</tr>
</tbody>
</table>
It is well known that spine tissues are viscoelastic. Many studies have been conducted to directly or indirectly measure the time-dependent response of the spine to an applied external load. They may be described as being poroelastic when fluid flow is involved with the time-dependent properties. Holmes and Hukins [1996] studied how well simple models can be used to characterize the viscoelastic properties of a lumbar spine segment in compression. They used a simple linear model consisting of a spring and dashpot in parallel with another spring to analyze load-relaxation curves. They noted that in previous experiments, specimens consisting of half a vertebral body-intervertebral disc-half a vertebral body failed at the disc-vertebra interface. They assumed that the cutting in half of the vertebra in the transverse plane would not notably weaken the specimens. Tests were performed at constant temperature since relaxation times are temperature-dependent. The strain and cross-sectional area of each specimen was constant so it follows that the specimen’s stiffness behaves just like the load. The simplest model which could reproduce the experimental load-relaxation curve was equivalent to a mechanical system of two springs and two dashpots [Holmes and Hukins, 1996]. This study justified cutting the vertebrae in half in the present experiment since
the specimens would not be appreciably weakened. It also gives an idea of how the load-relaxation curves should behave.

One major concern in the planning of the present experiment was whether or not frozen spines could be used for testing, since it is more difficult to acquire fresh spines. According to Dhillon et al. [2001], freezing does not alter the elastic response of a disc. However, a recent study using porcine discs showed that frozen storage permanently altered its viscoelastic response to compression [Bass et al., 1997]. Dhillon and his colleagues [2001] compared values for certain variables in the equation for time-dependent axial strain of the disc. The results showed that the creep behavior of human intervertebral discs was not significantly altered by a typical freezing protocol. Also, the data show that cyclic, compressive preconditioning is an important pretesting regimen that can stabilize the behavior of the disc and reduce variability between specimens. Furthermore, the degree of pre-existing degeneration significantly affects disc response [Dhillon, 2001]. In the current experiment, fresh spines were used instead of cadavers or frozen tissues in order to get accurate data that will be as close to field conditions as possible. Also, each specimen underwent cyclic preconditioning testing in order to get the specimens ready for more rigorous tests.

A significant amount of research has already been done on the human spine. A study was conducted by Adams et al. [2000] that focused on how compressive damage to vertebral body endplates alters the distribution of matrix compressive stress in adjacent intervertebral discs. It focused on the change in disc height during compressive loading, and in many experiments the discs buckled. It was concluded that cadaver experiments can be used to simulate short-term mechanical changes in living discs because the effects
of frozen storage on motion segment mechanics and intradiscal pressure are relatively small [Adams et al., 2000]. This current study does not require failure or buckling. Of main interest is stress relaxation response.

Another consideration is that the intervertebral discs may already have damage from aging. Gruber and Hanley [2002] focused on the comparison between cellular and extracellular matrix (ECM) characteristics of the annulus fibrosus of intervertebral disc specimens. Their study shows that there were abnormal accumulations of ECM encircling the disc cells in the aging and degenerating annulus specimens. This contributes to decreased biomechanical function of the annulus. Gruber and Hanley [2002] discuss the contribution of the types of ECM changes to the compromise of biomechanical function and development of annular tears in a disc during aging and degeneration. They conclude that future work is needed for “a fuller understanding of the dynamic relation between disc cells and the surrounding ECM, which they continually produce and remodel,” [Gruber & Hanley, 2002, p. 804]. This paper may explain possible decreases in the function of the intervertebral discs during testing.

Martinez et al. [1997] studied the biomechanics of load-bearing of the intervertebral disc using a finite element model. The excretion of water from the nucleus pulposus through the peripheral annulus fibrosus layers occurs during the response of the disc to compressive loading. Saturated porous engineering materials undergoing mechanical consolidation have this characteristic ability to lose water during deformation. A miniature consolidometer was used to take pore pressure measurements of bovine intervertebral discs. After preparation, samples were stored in a solution for at least an hour to allow the disc to fully hydrate. Martinez and his colleagues [1997] were
also concerned about freeze/thaw cycles and bacterial growth during the testing period. Based on Biot’s theory of coupled solid-fluid interaction, the finite element program developed by Martinez et al. [1997] was an improvement over other finite element formulations because it enabled the disc to be modeled as a poroelastic material [Martinez et al., 1997]. In this present research, the sample was hydrated in distilled water during transport from the field to the lab. The expulsion of fluid and particles from the disc was observed during testing. A water filter was placed in the chamber to clean the water so that the discs could be observed through the sides of the testing chamber.

To better understand disc behavior during testing, a model can be created to simulate results. For example, Spilker [1980] constructed a simplified axisymmetric finite-element model of the vertebral body-intervertebral disc in order to simulate the mechanical behavior of an intervertebral disc under compressive loading. This publication shows how size and shape affect the mechanical behavior of this simplified model. Some of the component material properties also affect the mechanical behavior. While symmetric about a longitudinal axis, this model is only subjected to longitudinal loads in compression. In general, intradiscal pressure, disc bulge, and vertical deflection vary non-linearly with disc radius, $r_d$, disc half-height, $h_d$, nucleus to disc area ratio, and endplate to annulus modulus ratio, $K$. Many possible combinations of these four parameters can be used to match the model results with experimental results. Table 2 details the observations made in Spilker’s experiment. The results show that all parameters have a significant effect on predicted disc bulge and vertical deflection. Also, the effects of area ratio and $K$ are less significant on intradiscal pressure [Spilker, 1980].
Table 2 - Spilker’s Observations [1980]

<table>
<thead>
<tr>
<th></th>
<th>Nuclear Pressure</th>
<th>Disc Bulge</th>
<th>Vertical Deflection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increasing disc radius</td>
<td>decreases</td>
<td>decreases</td>
<td>decreases</td>
</tr>
<tr>
<td>Increasing disc half height</td>
<td>decreases</td>
<td>increases</td>
<td>increases</td>
</tr>
<tr>
<td>Increasing area ratios</td>
<td>decreases</td>
<td>increases</td>
<td>increases</td>
</tr>
<tr>
<td>Increasing modulus ratios</td>
<td>decreases</td>
<td>increases</td>
<td>increases</td>
</tr>
</tbody>
</table>

In other studies, Riches et al. [2002] used poroelastic theory to examine the mechanics of intervertebral discs associated with multiple cycles of compression and expansion phases. To simplify analysis, it was assumed that the lateral movement of solid and fluid within each disc occurs during the first ten seconds of deformation, and all subsequent displacement is vertical. This publication makes reference to and tests the idea that freezing does not significantly affect the elastic and time-dependent properties of human intervertebral discs. A non-linear diffusion equation was developed using numerous porosity and fluid pressure formulas, which aided in model comparison to experimental data. The results showed that dynamic equilibrium is reached during the fourth or fifth cycles. Riches et al. [2002] concluded that disc tissue expands faster than it compresses, which is consistent with the developed model [Riches et al., 2002]. This justifies extensive stress relaxation testing performed on each of the intervertebral disc specimens in the current experiment.

In addition to the problems already addressed, justification is needed to use porcine spines in addition to human spines for this research. Animal spines are frequently used in biomechanical testing because of the difficulty and expense of getting human cadavers, the fear of contracting diseases, and the lack of homogeneity in the
human spine. The results are said to be similar to those of humans, although none truly are like human bipedal locomotion. The L3-L5 vertebral bodies of pigs have been tested for their similarity to human lumbar vertebral bodies. Lin et al. [1997] showed that in porcine lumbar vertebrae, the direction of the main trabecular struts was parallel to the longitudinal axis of the spinal canal. This is similar to that of human vertebrae. Also, the load-deformation response was similar to the response of cancellous bone in human spines. These similarities could imply that the main resultant force on the vertebral body of a porcine spine is also similar, meaning perpendicular to the end-plate. Lin et al. [1997] found that the structural homogeneity in porcine vertebrae is better than those of humans for selecting an experimental specimen. They determined that it is suitable to use mature porcine lumbar vertebrae as an experimental model for axial loading of the spine [Lin et al., 1997]. This study validated the use of porcine spine segments in the present research.

In the current experiment, the intervertebral discs of both humans and pigs were tested in water heated to body temperature. Since discs are normally at that temperature in the body, in order to get accurate data, the samples were tested in water held at 98.6°F. Wang et al. [2004] studied the effect that heating has on the biomechanical properties of the intervertebral disc tissue in a porcine model. Vertebral body-disc-vertebral body segments were tested at various temperatures for 10 minutes. The optimum temperature was 70°F, with higher temperatures failing to demonstrate any noticeable effects. Uniformly heating intervertebral discs appeared to cause some contraction of collagen. However, the biomechanical effect in terms of stiffness or ultimate failure strength of the functional spinal unit was not noticeable. Based on these results, there was neither a
detrimental nor beneficial biomechanical effect from using heat treatments on intervertebral discs [Wang et al., 2004].

**Entomological Forensic Techniques**

As previously mentioned, many of the current forensic techniques for estimating postmortem interval are entomologically based. Forensic entomology is defined as “the investigation of insects and other arthropods recovered from crime scenes and corpses,” [Benecke, 2001, p. 2] and is based on “understanding the arthropod sarcosaprophagous community,” [Arnaldos et al., 2005, p.58]. Each group of arthropods, defined by the eating habits of its members, plays a specific role in the different phases of decomposition of organic material. There are five groups: necrophages arrive first and eat from the corpse; necrophiles feed on the necrophages inside the corpse by predation or parasitism; omnivores feed on the corpse and associated insects or animals; opportunists use the corpse as a refuge, heat source, etc.; and accidentals are present just by chance [Arnaldos et al., 2005].

The first techniques used to estimate postmortem interval relied on insects and their succession patterns. In 1855, a French doctor named Bergeret wrote the first modern forensic entomology case report that included an estimation of PMI [Benecke, 2001]. The estimate of PMI using forensic entomology provides an estimate of the age of the oldest larvae found on a corpse [Adams and Hall, 2003]. In 1900, Eduard Ritter von Niezabitowski made an important contribution to the field of forensic entomology in
providing experimental proof that human corpses share the same fauna with vertebrate and invertebrate animal corpses [Benecke, 2001].

Recently research has been conducted to estimate the postmortem interval during the later stages of decomposition. Kulshrestha and Satpathy [2001] used beetles infesting human remains to estimate the minimum postmortem interval (PMI). This study stated that little knowledge is currently available about the types of insects found on corpses in the later stages of decomposition, which includes the first 3-6 months. Beetles and their succession patterns are the main entomological evidence used to determine PMI in the forensic field during the later stages of decomposition. Forensic entomologists and medical examiners identify growth stages of insects on human remains to interpret the PMI. *Dermestes maculatus* (or skin beetle) first appears during Megnin third wave when body fats are rotten after 3-6 months [Kulshrestha and Satpathy, 2001]. Before this study, there was little data indicating that the PMI could be established beyond the first month.

Some insects, like the black soldier fly, have been used to estimate the PMI in the later stages of decomposition. Lord et al. [1994] observed and studied the black soldier fly, also known as *Hermetia illucens*, as a potential measure of postmortem interval. *H. illucens* is an omnipresent inhabitant of both surface and buried human remains throughout the Hawaiian Islands and the southern United States. In cases of advanced decomposition, life history data for these flies could possibly provide forensic investigators with valuable parameters for the estimation of PMIs. In order to do that, the data must be viewed within local environmental conditions and used in combination with data for other arthropod species that are cohabitants of the black soldier fly. Lord et al.
[1994] used five different cases to illustrate how the life data for the black soldier fly was used to determine the PMI for bodies that were in advanced stages of postmortem decomposition [Lord et al, 1994].

Rodriguez and Bass [1983] studied the relationship between insect activity and decomposition rates of human cadavers. During the winter when temperatures are cooler, there was a decrease in the number and types of insects, which slowed the degradation of the cadavers. The cadavers in this study were observed experiencing successive stages of decomposition. Rodriguez and Bass [1983] separated the process of decomposition into four stages: the fresh stage, the bloated stage, the decay stage, and the dry stage. The results show variation in the decomposition rates of each cadaver. This was caused by climatic differences during each season. This study shows that there is a direct relationship between the succession patterns of insects and the decay rates of human cadavers, thus indicating that insects are a major factor responsible for decomposition of the bodies [Rodriguez and Bass, 1983].

In a paper discussing the factors that affect decomposition, Campobasso et al. [2001] talks about the changes a body undergoes after death. This study states that there are four stages in the sequence of putrefactive changes (discoloration, bloating, liquefaction, and skeletonization) that follow a timeline. These stages are markers for identification purposes indicating the time since death. These putrefactive changes can be used to estimate PMI only if they are integrated with the environmental and circumstantial factors involving the corpse. Cadaver fauna, from insects to mammals, play a large role in the breakdown of a corpse. The insects mostly feed on decaying tissues of the corpses. These insects have highly specialized sensory organs that draw
them to organic putrefaction gases. Arthropod succession analysis involves the
association of each species of insect to one of the established stages which enables a good
approximation of PMI in cadavers with advanced decay. Campobasso et al. [2001]
conclude that a complete separation of PMI estimates from the examination of corpse
decomposition is not possible. Maximum and minimum limits can be estimated for PMI,
but a correlation must be made between the estimates in the putrefactive changes and the
collected insects to environmental conditions of the exposure site and to all other factors
affecting decay rates [Campobasso, 2001].

There are many variables that affect decomposition rates of corpses. Mann et al.
[1990] discuss the variables of decay and general observations of corpses from field
experience. One conclusion of this study was the significant effect that the ambient
temperature has on the decay rate of the human body. Furthermore, during the months
when the temperature fluctuates between hot and cold, it was very difficult to estimate
PMI. Similar to temperature, humidity/aridity can dry out a body where much of the skin
is retained for periods from two to six years after death. Most of the soft-tissue
destruction of bodies is due to feeding by insect larvae, although carnivores and rodents
will run off with parts of the body such as hands, feet, and bones. Clothing helps to
protect the body from sunlight, which maggots avoid, thus aiding in speeding up the
decay process [Mann et al., 1990].

In order to reliably apply PMI estimates to actual forensics cases, comparative
studies all over the United States need to be conducted. This may be difficult considering
that few human decay studies have been conducted because of negative public opinion,
difficulty in obtaining cadavers, and a lack of suitable areas for the placement and study of the bodies [Mann et al., 1990].

Marchenko [2001] also discusses how decay rates are affected by cadaver entomofauna to estimate PMI. He states that clothes on a corpse do not delay the invasion of insects although this can lengthen the decay period. This contradicts Mann’s conclusions. In addition, Marchenko [2001] discussed the delay of insect infestation if clothes are stained with combustible products, lubricants, and paint materials. Rain and humidity help to transfer these chemicals into the cadaveric tissues. This increases their repellent effect, which lengthens the decay period. This period varies because the time of death does not always coincide with the time when insects begin to infest and flourish on a cadaver, just like where a cadaver is found is not always the place of death. Insects serve as the basis for solving many problems including the season when the cadaver was placed, determination of PMI, whether or not the body was moved, and the initial location of the cadaver [Marchenko, 2001]. By only testing the spine in the current study, the main focus was on an accurate estimation of PMI. Other questions that need to be answered at a crime scene are not addressed in this experiment.

In studying insects, it is important to study factors affecting their development. Myskowiak and Doums [2002] studied the effects of refrigeration on the development of insects. When a rapid transport of insects from a crime scene is impossible, the insects are placed in a refrigerator for a varying amount of time. Low temperatures may induce PMI errors. The results showed that the total developmental time of the insects, from end of cooling to the beginning of adult stage of the insects, was significantly affected by the refrigeration treatment for all developmental stages of insects. If the forensic investigator
assumes an absence of development during refrigeration storage and normal development resuming after storage, errors in PMI estimation may occur [Myskowiak and Doums, 2002]. This shows that entomology techniques cannot always be reliable because of the temperature changes during transportation from the field to the lab.

Although refrigeration affects the development of entomofauna, freezing may have more damaging effects. A study was conducted to document the effects of freezing-thawing and mechanical injury on small animals. Micozzi [1986] showed that the rates of disarticulation were slower in fresh-killed animals than in frozen-thawed animals, although the sequences of disarticulating joints in the two were the same. “The mechanical disruption of the tissues caused by freezing also weakens the skin, connective tissue, and joints, thus facilitating aerobic decay and skeletal disarticulation, and making internal organs more susceptible to invasion by foreign organisms and insects,” [Micozzi, 1986, p.960]. This study showed that postmortem decomposition progresses from the “outside-in” in frozen-thawed animals; while it progresses from the “inside-out” in fresh-killed animals. An important conclusion shown in this publication was that previous studies using frozen-thawed animals to study various processes of postmortem change may not have given a correct account of the decomposition sequences that would occur in fresh-killed animals in order to determine the time since death [Micozzi, 1986]. Since crime scene conditions are desirable, Micozzi’s study justifies the use of fresh spines in this experiment.

Regarding entomology, traditional methods use morphological features to identify insects. However, a new diagnostic technique developed by Harvey et al. [2003] uses sequencing to study the mitochondrial DNA (mtDNA) of insects. This sequencing
focused on a section of cytochrome oxidase I encoding region of mtDNA. mtDNA can be isolated more easily than nuclear DNA, which is helpful in forensic studies where insect specimens may not be in the best condition. Since an accurate estimation of the PMI requires accurate identification of insects on the corpse, any misidentification could result in the application of incorrect developmental data. This method shows promise for an identification protocol. Further sequencing of more specimens is needed in order to develop a simpler and more reliable technique for insect identification [Harvey, 2003].

Schroeder et al. [2003] conducted another study involving mtDNA of blowflies (Family Calliphoridae), which are commonly found on dead bodies shortly after death. Species within this family have different developmental times so it is important to accurately identify every species for the correct estimation of PMI. The push for rapid determination of PMI introduces two problems: possible death before identification and lack of time for larval development. This study uses a new technique called PCR-RFLP to rapidly and accurately identify fly larvae. It combines the method of restriction fragment length polymorphism (RFLP) with the PCR technique. For this technique, regions of the mtDNA were amplified using special primers and digested with restriction enzymes. The results showed that the divergence in the DNA sequence of the three blowfly species examined is acceptable for their larvae identification [Schroeder et al., 2003]. This method can also be used to identify other species of insects.

As previously mentioned, pigs are frequently used for biomedical research, and forensic science is no exception. Turner and Wiltshire [1999] used pig carcasses as analogues for the human cadaver to study the decay process for a murder investigation. The original PMI was based on the finding of a final instar heleomyzid fly, but there is
little existing information on this species. Three freshly killed 4-month old pigs were buried within 5 hours of death, with one fitted with needle thermistors for temperature variation measurements of the corpse. Their investigation showed that the pig corpses were preserved longer than expected because of soil conditions and low seasonal temperatures. This study also showed the value of using pigs as models to gain a better understanding of the decay process [Turner and Wiltshire, 1999]. For obvious reasons, it is difficult to obtain human corpses for experimental studies. As a result, animal cadavers have been used as experimental models, but some are concerned that these models are not valid [Arnaldos et al., 2005]. For the purposes of the current study, the valid use of pig spines has been previously discussed.

Turchetto et al. [2001] concluded that the entomological approach to forensic investigations cannot always fully reconstruct the PMI. This approach becomes essential for determining the PMI when alterations of the body following death (livor mortis, rigor mortis, body temperature, bloating, etc.) are complete [Turchetto et al., 2001]. Since insects and their succession patterns cannot always be used to determine PMI, this opens the door for new research possibilities including the degradation of the spine as a tool for estimating PMI.

**Non-Entomological Forensic Techniques**

Early postmortem interval (PMI) can be determined through various physical methods, like body temperature or electrical stimulation of muscles, with significant accuracy. More recently, studies have been conducted using biochemical markers, like potassium or amino acids, in combination with other parameters to determine PMI.
Some of these non-entomologically based methods are briefly discussed in this section [Bauer et al., 2003].

Since there is a lack of knowledge on human decay rates, forensic scientists have begun to devise new methods for determining the PMI. Some forensic scientists study the degree of deterioration of associated materials, like clothing and paper, for example, exposed to the environment over vast periods of time [Morse and Stoutamire, 1980]. Another approach examines plants and related decomposition vectors of human skeletal remains [Warren, 1980].

Beyond the decay of corpses and other material, new techniques are being developed. Muñoz et al. [2001] developed a new formula for estimating the PMI based on vitreous potassium concentration ([K$^+$]). Based on linear regression and a correlation test, previous formulae use PMI as the independent variable and [K$^+$] as the dependent variable. However, in forensic investigations, it is crucial to use [K$^+$] as the independent variable. The new PMI dependence upon potassium concentration differs from the relationship in previous formulae. Applying both approaches to data showed that the new formula increases the amount of explainable variation and decreases the error in estimating PMI. This study shows that potentially serious errors in estimating PMI were introduced by the previous incorrect mathematical method that determined a regression line adjusted to [K$^+$] instead of to PMI [Muñoz et al., 2001].

While vitreous humor has been previously studied, Madea et al. [2001] performed a study to determine whether synovial fluid, found in the joint cavity of the knee, is an applicable fluid for determining PMI. Specifically, the data show potassium concentration development over time is almost the same in both synovial fluid and
vitreous humor. This is important if time since death is estimated by potassium concentration and traumatic injuries prevent the use of vitreous humor. The spread of values for all parameters was comparable for both fluids. The results indicate that synovial fluid can be used to estimate PMI, much like vitreous humor, especially in regard to the time dependent postmortem potassium concentration increase [Madea et al., 2001].

Temperature-based methods have also been used as a way of estimating PMI. They are considered to be the most reliable in practice, easily implemented, and likely to be of value, especially during the first 18-24 hours following death [Al-Alousi et al., 2002]. Nelson [2000] tests the notion that an averages-based method of short term PMI estimation is more accurate and holds true for longer periods of time when compared with other techniques. The temperature of a body increases soon after death and this can be used to estimate PMI. The averages-based method predicted the PMI significantly better than traditional methods and for periods over twice as long. This procedure requires contact with the body, which is necessary to pronounce death. At that time, a temperature probe can be placed in the body and a compact recording device can be set up without disturbing the crime scene or the body [Nelson, 2000].

Using new available technology, impedance has also been used to estimate the postmortem interval. Querido and Phillips [2001] have conducted several studies using abdominal impedance as a potential tool for estimation of PMI in rats. Electrical impedance of the abdomen was determined by measuring the voltage between electrodes on the anterior abdominal wall during the passage of current through the body. In terms of interpolating PMI, Querido states that it is impossible to use abdominal impedance
alone to distinguish between “numerically equal impedances on the rising and falling phases of curves showing impedance as a function of PMI,” [Querido and Phillips, 2001, p. 133]. They concluded that temperature-correction of postmortem abdominal impedance makes the relationship between impedance and PMI more useful. It has not been reported whether directionally-similar changes correlate with PMI. This technique has not yet been applied to human cadavers [Querido and Phillips, 2001].

Autopsy samples are now being used for DNA and RNA analysis in forensic science. This has drawn attention to advances in time-dependent degradation of nucleic acids in molecular biology. Bauer et al. [2003] studied postmortem degradation of RNA to determine its value in autopsy samples with longer PMIs. They show that FASN (fatty acid synthase-messenger) multiplex PCR (polymerase chain reaction) is an appropriate method to determine the extent of RNA fragmentation, which has a significant correlation to PMI [Bauer et al., 2003].

In summary, postmortem interval estimation can be improved. Furthermore, developing a degenerative model of the spine can be beneficial for future studies in biomechanics. Research has been conducted on the both human and pig spines and many models (both analytical and finite element) have been developed to simulate intervertebral disc behavior, but none of the models developed have examined a degenerative model of the intervertebral disc as a function of time. However, extensive research has been done to estimate the postmortem interval in forensic investigations. None of these techniques have involved the spine or the testing of the intervertebral discs, thereby missing the benefit of these protected tissues for study. Entomological techniques involving insects and their succession patterns dominate, while other
techniques, including the testing of synovial fluid and vitreous humor, temperature methods, abdominal impedance, and DNA and RNA studies are gaining recognition. In spite of the moderate success of these techniques, the field can certainly benefit from their combined use and from the introduction of new engineering techniques.
This chapter discusses the preparation and testing conducted to validate the hypothesis of this thesis, as well as explains how the results will be evaluated.

Protocol Development

Before testing commenced, a protocol was developed by testing frozen spines, as well as fresh spines that were in poor physiological condition. By running through the phases of the experiment before any real testing was done, unforeseen problems were solved to help maximize the usefulness of the results.

Some of the problems encountered, which will be discussed later, included the improper extraction of the spines at the tissue bank, the movement of the sample in the clamps during testing, and the length of time that the samples should be tested in order to get accurate and complete results.

Table 3 summarizes the types of spines tested in this study, as well as the number of samples tested from each spine and the time since death for each specimen. While waiting for a fresh human spine, frozen spines from human cadavers were tested and the results were poor. The human spine yielded acceptable data, but because of the problems discussed earlier with using human spines, pig spines were used for the remainder of this study.
Table 3 - Summary of Spines and Samples Used to Develop Protocol

<table>
<thead>
<tr>
<th>Type of Spine</th>
<th>Number of Samples</th>
<th>Time Since Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen Human Cadaver Spine</td>
<td>5</td>
<td>up to 1 year</td>
</tr>
<tr>
<td>Frozen Human Cadaver Spine</td>
<td>7</td>
<td>up to 1 year</td>
</tr>
<tr>
<td>Fresh Human Spine</td>
<td>6</td>
<td>days: 3, 6, 9, 12, 15, 18</td>
</tr>
<tr>
<td>Pig Spine 1</td>
<td>8</td>
<td>days: 1, 4, 7, 10, 13, 16, 19, 22</td>
</tr>
<tr>
<td>Pig Spine 2</td>
<td>8</td>
<td>days: 2, 5, 8, 11, 14, 17, 20, 23</td>
</tr>
<tr>
<td>Pig Spine 3</td>
<td>8</td>
<td>days: 3, 6, 9, 12, 15, 18, 21, 24</td>
</tr>
<tr>
<td>Pig Spine 4</td>
<td>8</td>
<td>days: 1, 4, 7, 10, 13, 16, 19, 22</td>
</tr>
<tr>
<td>Pig Spine 5</td>
<td>8</td>
<td>days: 2, 5, 8, 11, 14, 17, 20, 23</td>
</tr>
<tr>
<td>Pig Spine 6</td>
<td>8</td>
<td>days: 3, 6, 9, 12, 15, 18, 21, 24</td>
</tr>
</tbody>
</table>

The numbering of the specimens is straightforward. The first number indicates the spine number and the second number indicates the sample number. For example, sample 31 means sample 1 from spine 3. Each sample was numbered accordingly. Since there was only one human spine that provided acceptable data, each human sample began with a 1 indicating spine 1, the only human spine tested. After testing was complete, the bags that the samples were kept in were labeled H for a human sample and P for a pig sample.

**Specimen Preparation**

One fresh human spine (within hours of harvesting from a body) was sent from the Central Florida Tissue Bank, where the spine was isolated from the corpses with parts of the ribs still attached to the vertebral bodies. The spine was dissected into sections consisting of half a vertebral body, an intact intervertebral disc, and half of the other adjacent vertebral body. The vertebral bodies were later used to anchor the specimens during testing. The number of samples taken from a spine varied with the condition of the spine. If the spine was in good condition (i.e. some flexibility, little or no herniations), then more samples could be taken from it and tested.
Having the ribs still attached to the vertebral bodies is very important because otherwise, the vertebral bodies would be exposed to the environment, which would cause unwanted decomposition and degradation of the discs and bones. During protocol development, a spine was tested with some of the vertebral bodies exposed from the absence of attached ribs. This led to the decomposition of the bones and discs, which led to the corruption of data obtained from the testing of the specimens. This problem was remedied by specifying how the spine should be extracted from the body at the tissue bank. How the spines were prepared at the tissue bank was an unforeseen variable that was controlled through better communication with the tissue bank.

In order to combat the problems associated with aged human spines and their preparation from the Central Florida Tissue Bank, porcine (pig) spines were used later in the study. The research group was able to prepare the pig spines at the FDA and they were much cheaper to obtain than human spines from the tissue bank. Also, the pig spines were in much better condition since they were much younger and did not have ossified intervertebral discs so they provided better data for analysis to determine a correlation. Each human and pig spine was prepared the same way, as previously discussed.

All sections of a particular spine were buried on the same day in a discreet location in central Florida. Many different types of crime scenes could have been simulated, but a wooded area in a humid summer climate was chosen in this case because it is out of the way and easily accessible for the purposes of this study. At this location, the burial site (a human-made hole in the ground) was lined with chicken wire on all sides (forming a box that opened on the top) to keep out vultures and other animals that
may smell the tissue and try to dig the samples up and carry them away. The box was then filled with soil. A folding measuring stick was used to mark the locations where each specimen should be buried. Each sample was buried in the ground about 1 foot deep. The water table in this area is usually high so the depth of the site was only approximately a foot and a half deep because a deeper hole would allow water into the burial site, which could help preserve the tissue or alter its method of decay. Only three spines could be in the ground at any given time because there is only one machine to test the samples.

One section of a spine was unearthed every three days. If three spines were in the ground, then a section was dug up each day, alternating between the spines. The section of the spine, named for the vertebral bodies of the specimen, was recorded (i.e. T11-T12), as well as the moisture content of the soil, the amount of rainfall since the previous dig, and the amount of time that the section had been in the ground. A tensiometer was used to measure the moisture content of the soil, and a rain gage was used to measure the amount of rainfall that the area was subjected to during the previous three days.

The sample was cleaned once it was out of the ground. Cleaning involves several steps. First the sample was rinsed with distilled water to wash away much of the soil from its outer surface. Then, using a stryker saw, the articular and transverse processes were cut off of the specimen at the pedicle (refer to Figure 6).
Using a scalpel, the ribs were trimmed off, along with extra tissue and the longitudinal ligament than runs along the posterior surface of the vertebral bodies. Because the strength of the disc as it degrades with time since death is the main focus of this study, all other tissues, ligaments, and bones were removed from the sample. After cleaning was complete, only the disc and bone (or vertebral bodies) were left. The section was rinsed again with distilled water and placed in a sealed plastic container filled with distilled water for transportation to the lab.

Mechanical Testing

In the lab, the specimen was prepped for testing. It was placed between two circular stainless steel plates, specially designed for this experiment, and held in place by five screws on both the top and bottom plates. The specimen and clamps were then placed in a testing chamber on the Instron, which is a machine used to test the mechanical properties of different types of materials, from metals to biological tissues. Because of the mobility of the sample in the Instron using previous clamps, a new design was made
using a turn buckle to make the top and bottom plates parallel. Also, a screw was placed at the bottom which disables the bottom clamp from swaying back and forth during the cyclic tests.

After the sample was properly attached between the clamps, it was placed in the testing chamber filled with distilled water circulated by a filter and heated to 98.6°F to simulate normal body temperature conditions. This testing chamber was attached to the Instron and the top was covered with clear plastic wrap to minimize the amount of water loss from the chamber.

Three tests were performed on each specimen: 1) cyclic conditioning (composed of 90% compression and 10% tension); 2) compression to a specified displacement over a time span of 30 seconds; and 3) extensive stress relaxation testing.

Dhillon et al. [2001] showed that cyclic, compressive preconditioning is an important pretesting regimen that can stabilize the behavior of the intervertebral disc and reduce interspecimen variability. This justifies the cyclic conditioning performed on each sample in this experiment. Cyclic conditioning helps to prepare the specimens for rigorous stress relaxation testing. Since the human spine is normally in compression, the conditioning was composed of 90 percent compression and 10 percent tension. During protocol development, various percentages of compression and tension were tested. Ninety percent compression of the sample helped to better condition it for testing and to deliver the best results. The pig spines were tested the same way.

In order to develop a stress strain curve, each specimen was compressed to a specified displacement value over a time span of 30 seconds. The value of the displacement depends on the size and cross-sectional area of the sample. By looking at
the stress-strain curve, one can tell whether or not that particular sample will give good 
data. Usually, the stress-strain relationship is affected by the degree of pre-existing 
degeneration in the intervertebral disc specimen.

After the 30-second change from an initial value to a specified displacement, each 
specimen was then held in compression for 10 hours. Stress relaxation is described as the 
decrease in stress in a material subjected to prolonged constant strain at a constant 
temperature. During protocol development, each specimen was subjected to a varying 
amount of time (also the number of cycles of testing). After five hours (or 5 cycles of 
stress relaxation), the specimens were still not reaching an equilibrium value, although 
they were approaching it. This is why the number of hours of stress relaxation testing 
was doubled. Although 10 hours may be more than enough for some samples to reach 
equilibrium, the time required to reach equilibrium will vary from sample to sample. 
This long interval of testing will ensure that the stress of each specimen will decrease to a 
steady state value during the stress relaxation testing.

Riches et al. [2002] examined the mechanics of intervertebral discs associated 
with multiple cycles of compression and expansion phases. They concluded that disc 
tissue expands faster than it compresses. Their study justifies the extensive stress 
relaxation testing that was performed on each of the specimens in this experiment.

Data Analysis

In order to determine whether or not a relationship exists between cadaveric tissue 
and postmortem interval, the main focus in this experiment is on the data obtained from 
the stress relaxation tests. The data collected from the cyclic conditioning tests may also
be used to determine a relationship by plotting the maximum load values as functions of time.

The next chapter provides a detailed discussion of viscoelastic materials and the models used to analyze them. The process chosen for the analysis in this thesis is derived and an explanation is given on how it will be applied to the experimental data in order to determine a mathematical trend.
MATHEMATICAL MODELS FOR LINEAR VISCOELASTIC RESPONSE

Fundamentals of Tissue Mechanics

Consider a typical solid material, such as steel. Initially, a solid steel rod has a length, \( L_0 \). The rod elongates to length \( L \) after the application of a force \( F \). This change in length is denoted as \( \Delta L = L - L_0 \). Refer to Figure 7.

Figure 7 - A Solid Rod

The strain or deformation (\( \varepsilon \)) of the rod is defined as

\[
\varepsilon = \frac{\Delta L}{L_0},
\]

while the stress (\( \sigma \)) in the rod can be represented as

\[
\sigma = \frac{F}{A},
\]

where \( A \) is the cross-sectional area of the rod.
The stress-strain relationship can be determined empirically and shown on a stress-strain curve. A typical material will have a linear relationship between stress and strain, but biological materials do not follow the same principles.

Hooke’s Law can be used to calculate Young’s Modulus, E, which is defined as stress divided by strain, or

$$ E = \frac{\sigma}{\varepsilon}. $$

Young’s Modulus defines the stiffness of the material. By increasing E, the material’s stiffness also increases. In general, the linear relationship between stress and strain does not apply to biological tissues. This means that they exhibit nonlinear stress-strain relationships. Regardless of the shape, stress-strain curves reflect the functions of the tissue [Lecture 7, 2003].

When a load is applied to a body, the inside of the material rearranges itself in response to the applied stimulation. A finite amount of time, however long or short, is required for any real material to rearrange itself. A purely viscous material is one in which these changes occur so quickly that the time is negligible in comparison to the time scale of the experiment; all of the energy needed to produce the deformation is dissipated as heat. Water is almost a purely viscous material. On the other hand, a purely elastic material is one in which the material rearrangements take virtually an infinite amount of time; the deformation of energy is stored and may be completely recovered when the forces acting on it are released. If only deformed to one or two percent, steel behaves almost completely elastically. However, all real materials are viscoelastic in principle. Under certain conditions, some energy may always be stored during material
deformation, and energy storage is always accompanied by some energy dissipation [Tschoegl, 1989].

The time it takes for a material to rearrange itself is comparable to the time scale of the experiment in a typically viscoelastic material. Consequently, material constants cannot be used to express stress-strain relations or strain rate like cases of purely elastic or purely viscous materials. Time dependent material functions are used to characterize the rheological behavior of viscoelastic materials [Tschoegl, 1989].

In general, it can be difficult to analytically represent the viscoelastic response of a material. However, it is possible when the assumption is made that the material behaves linearly, which is a valid assumption for relatively small strains [Conway, 1991]. This assumption will be used throughout this thesis.

**Basic Elements: Spring and Dashpot**

Viscoelastic materials under uni-axial stress behave similarly to that of models built from discrete elastic and viscous elements. The major elements of these models are springs (for elastic properties) and dashpots (for viscous properties).

![Figure 8 - (a) an elastic element and (b) a viscous element](image)
The elastic element is pictured as a helical spring, which allows total recovery over time, as shown in Figure 8(a). When a force P is applied to the spring, its length increases by a certain amount $u$, and the spring returns to its original length when the force is removed. This force acting on the elastic element simulates stress and is denoted by $\sigma$. The strain in the spring simulates the strain of the material and is designated as $\varepsilon$. If the material is linear-elastic, the relation is given by:

$$\sigma = G \cdot \varepsilon,$$

in which $G$ is the Shear Modulus or the Modulus of Torsional Rigidity [Tschoegl, 1989 and Ferry, 1970].

The viscous element describing permanent deformation is shown in Figure 8(b) as a dashpot, which is represented as a piston moving through a cylinder filled with a viscous liquid. The cylinder has a perforated bottom so that no air is trapped inside as the piston moves through the cylinder. There is a clearance between the piston and the cylinder walls. The viscous resistance of the piston is defined by the hydrostatic resistance of the liquid flowing in the clearance in response to the motion of the piston. The displacement of the piston relative to its initial position simulates deformation of a real body. A bar in tension of some materials shows a similar deformation: the bar is stretched when a load is applied. Force transmitted by the element simulates stress in the body, while resistance to motion simulates the viscosity factor. However, it is the time rate of change of the elongation that is proportional to the force, not the elongation itself. Therefore, the law relating stress-to-strain is given by:

$$\sigma = \eta \frac{d\varepsilon}{dt},$$

where $\eta$ is the linear viscosity coefficient [Flügge, 1967 and Junisbekov, 2003].
It should be expected that a combination of springs and dashpots represented by Equations 1 and 2 forms the constitutive equation describing linear viscoelastic behavior. Using springs and dashpots to represent viscoelastic behavior does not imply that these mechanical elements indicate molecular or supramolecular mechanisms in the material of the behavior they model. It is assumed that the spring and dashpot elements are massless, and thus have no inertia. In general, observed behavior can be represented by multiple models, which are all equivalent [Tschoegl, 1989].

For a homogeneous, isotropic material, the material displacements are separated into deviatoric ones (changes in shape) and dilatational ones (changes in volume) for convenience. Using tensor notation, the deviatoric stress, $S_{ij}$, is given by

$$S_{ij} = \sigma_{ij} - \delta_{ij}\sigma$$

(3)

where $\delta_{ij}$ is the Kronecker delta and $\sigma$ is the mean stress calculated by

$$\sigma = (1/3)\sigma_{ii}.$$  

(4)

Again using tensor notation, the deviatoric strain, $E_{ij}$, is given by

$$E_{ij} = \epsilon_{ij} - \delta_{ij}\epsilon$$

(5)

where $\epsilon$ is the mean strain calculated by

$$\epsilon = (1/3)\epsilon_{ii}.$$  

(6)

**The Generalized Kelvin Model**

The response of a viscoelastic solid can be represented by a parallel combination of a spring and a dashpot. This combination of elements is called the Kelvin-Voigt model (KVM) and is shown schematically in Figure 9.
In this parallel combination of elements, all of the elements undergo the same strain, and the stresses can be added together. The constitutive equation of the linear viscoelastic response using this combination is given by

\[ \sigma = G\varepsilon + \eta \frac{d \varepsilon}{dt} \]  

which is a generalization of the equation of classical elasticity theory.

For the KVM,

\[ S_{ij} = 2GE_{ij} + 2\eta \dot{E}_{ij} \]  

which results from the addition of the stresses imposed on the spring and dashpot. By setting \( \tau = \eta/G \), where \( \tau \) is the relaxation time and by dividing Eqn. (8) by the strain, the solution to Eqn. (8) becomes

\[ E_{ij}(t) = \exp\left(-\frac{t}{\tau}\right) \left\{ \exp\left(\frac{t}{\tau}\right) \frac{S_{ij}(t')dt'}{2\eta} + E_{ij}(0) \right\} \]  

If \( \tau \) and \( \eta \) are constants and \( E_{ij}(0) = 0 \), then Eqn (9) simplifies to

\[ E_{ij}(t) = \exp\left(-\frac{t}{\tau}\right) \left\{ \exp\left(\frac{t}{\tau}\right) \frac{S_{ij}(t')}{2\eta} dt \right\} \]  

The generalized Kelvin model (GKM) is a better representation for a viscoelastic fluid. This model uses several KVMs placed in series, as shown in Figure 10.
The total deviatoric strain is the sum of the strain in each KVM plus the strain in the dashpot and the spring,

\[ E_{ij}^{\text{total}}(t) = \sum_{n=0}^{N+1} E_{ij}^n(t). \]  
(11)

The deviatoric strains, \( E_{ij} \), for the spring and the dashpot, respectively, are,

\[ E_{ij}^0(t) = \frac{S_y(t)}{2G_0} \]  
(12)

and

\[ E_{ij}^{N+1}(t) = \frac{\int_0^t S_y(t') dt'}{2\eta_{N+1}} \]  
(13)

where \( \eta \) is a constant.

Combining Eqns. (10), (12), and (13) into Eqn. (11) yields the equation for the GKM,

\[ E_{ij}(t) = \frac{S_y(t)}{2G_0} + \frac{1}{2\eta_{N+1}} \int_0^t S_y(t') dt' + \sum_{n=0}^{N+1} \frac{\exp\left(-\frac{(t-t')}{\tau_n}\right)}{2\eta_n} \int_0^t \frac{S_y(t')}{\tau_n} dt'. \]  
(14)

It can be seen from Eqn. (14) that there is 2N+2 maximum number of parameters from a GKM. If the spring in series is removed (\( G_0 = \infty \)), then \( E_{ij}(0) = 0 \) because there is no instantaneous response at time \( t = 0 \). If the dashpot in series is removed (\( \eta_{N+1} = \infty \)), then no permanent deformation occurs in the material when the stress is removed [Conway, 1991].
The Generalized Maxwell Model

The response of a viscoelastic fluid can also be represented by a combination of a spring and a dashpot in series. This combination of elements is called the Maxwell model (MM) and is shown in Figure 11. In this series combination of elements, all the elements experience the same stress, and the strains can be added together.

\[ \sigma_{\text{spring}} = \sigma_{\text{dashpot}} = \sigma \]

and

\[ \varepsilon = \varepsilon_s = \varepsilon_d \rightarrow \dot{\varepsilon} = \dot{\varepsilon}_s = \dot{\varepsilon}_d \]

Therefore, the constitutive equation for the stress-strain relation is

\[ \dot{\varepsilon} = \frac{\dot{\sigma}}{k} + \frac{\sigma}{\eta} \]  

(17)

Since Eqn. (17) contains time derivatives, a simple proportionality constant does not exist between stress and strain. Similarly, the ratio of stress to strain, denoted by the modulus
E, must be expanded to account for this more complicated behavior. For example, in a stress relaxation test, the strain is constant so

$$\frac{1}{k} \frac{d\sigma}{dt} = -\frac{1}{\eta} \sigma$$  \hspace{1cm} (18)

Rearranging Eqn. (18) and taking the integral of both sides one has

$$\int^{\sigma}_{\sigma_0} \frac{d\sigma}{\sigma} = -\frac{k}{\eta} \int^{t}_{0} dt$$  \hspace{1cm} (19)

which after solving Eqn. (19) and rearranging yields

$$\sigma(t) = \sigma_0 \exp\left(-\frac{t}{\tau}\right)$$  \hspace{1cm} (20)

In Eqn. (20), $\tau = \eta/k$ is called the relaxation time as previously mentioned. The relaxation modulus $E_{\text{rel}}$ can be obtained directly from Eqn. (20) by noting that the initial stress is simply that needed to stretch the spring to a strain $\varepsilon_0$:

$$E_{\text{rel}}(t) = \frac{\sigma(t)}{\varepsilon_0} = \frac{\sigma_0}{\varepsilon_0} \exp\left(-\frac{t}{\tau}\right)$$  \hspace{1cm} (21)

$$E_{\text{rel}}(t) = k \exp\left(-\frac{t}{\tau}\right)$$  \hspace{1cm} (22)

As previously mentioned, a better representation for the response of a viscoelastic fluid is a generalized model, in this case a generalized Maxwell model (GMM), which is shown in Figure 12.
Since the MMs are in parallel, the same methodology can be applied in order to obtain the expression for the relaxation modulus:

$$\sigma(t) = [k_c + \sum k_j e^{-t/\tau_j}]\varepsilon_0$$  \hspace{1cm} (23)$$
and finally,

$$E_{rel}(t) = k_c + \sum k_j e^{-t/\tau_j}$$  \hspace{1cm} (24)$$

**The Viscoelastic Model Used to Analyze IV Disc Degeneration**

Since tissues in the spine are viscoelastic, a model should be chosen that describes its response to loading. A modified generalized Maxwell model, called the Wiechert model, was chosen for its convenience for describing stress relaxation of a material resulting from an imposed strain. As shown in Figure 13, the Wiechert model consists of an array of Maxwell units and a spring in parallel ensuring a viscoelastic-solid response.
It is easy to spot the difference between this model and the GMM. There is the absence of the dashpot in parallel with the Maxwell units allowing the model to immediately deform to an applied load. The element constants can then be computed from the experimental stress relaxation data for the given material.

The Wiechert model is most easily treated using operational calculus. Setting the time derivative operator $D = \partial / \partial t$, the stress transmitted by the $j^{th}$ arm of the model can be stated as

$$D\sigma_j(t) + \frac{1}{\tau_j} \sigma_j(t) = k_j D\epsilon(t)$$  \hspace{1cm} (25)

where $\tau_j$ is the relaxation time of the $j^{th}$ arm

$$\tau_j = \frac{\eta_j}{k_j}$$  \hspace{1cm} (26)

Also, it is known that

$$\epsilon(t) = \epsilon_0(t) = \epsilon_1(t) = \epsilon_2(t) = \ldots = \epsilon_N(t)$$  \hspace{1cm} (27)

so it follows that the total stress transmitted by the model is the sum of each $\sigma_j$ plus the stress in the equilibrium spring $k_\epsilon$:

$$\sigma(t) = k_\epsilon \epsilon(t) + \sum_{j=1}^{N} \sigma_j(t)$$  \hspace{1cm} (28)
Rearranging Eqn. (25) and substituting into Eqn. (28) yields

\[
\sigma(t) = \left( k_e + \sum_{j=1}^{N} \frac{k_j D}{D + \frac{1}{\tau_j}} \right) \varepsilon(t) \]  

(29)

Equation (29) is one form of the constitutive law for the Wiechert model, although it is somewhat inconvenient to use in this form. For any specified stress-time input function, in this case stress relaxation, the stress output must be obtained by first clearing the operational equation of fractions and then solving the time-dependent differential equation by separation of variables or another technique. Laplace transform methods are used in this thesis to reduce the complexity of the solution technique.

By using an overhead bar to represent the transform of a quantity and using the symbol “p” to denote the transform variable, Eqn. (29) can be written in the transform plane as

\[
\bar{\sigma}(p) = \left[ k_e + \sum_{j} \frac{k_j p}{p + \frac{1}{\tau_j}} \right] \bar{\varepsilon}(p) \]  

(30)

where \( \varepsilon(0) = 0 \).

The quantity in brackets may be considered as the viscoelastic analog of a modulus, \( \bar{E}(p) \). This expression is time-dependent and relates stress to strain in the Laplace transform plane.

For a given strain input function \( \varepsilon = \varepsilon(t) \), the resulting stress function can be obtained in three steps:

1. Obtain an expression for \( \bar{\varepsilon}(p) \), the transform of the strain function.
2. Form the algebraic product \( \bar{\sigma}(p) = \bar{\varepsilon}(p) \bar{\varepsilon}(p) \).
3. Obtain the inverse transform of the result to yield the stress function in the time plane.

The stress relaxation modulus can be calculated using the three steps above. To do this, the step function, \( \varepsilon(t) = \varepsilon_0 \), is used where \( \varepsilon_0 \) is an imposed constant strain at \( t = 0^+ \).

Then, the Laplace transform of a constant is:

\[
\varepsilon(t) = \varepsilon_0 \rightarrow \bar{\varepsilon}(p) = \frac{\varepsilon_0}{p}
\]  

(31)

Substituting Eqn. (31) into Eqn. (30) yields

\[
\bar{\sigma}(p) = 
\]

(32)

The inverse transform of Eqn. (32) is

\[
\sigma(t) = \left( k_e + \sum_{j=1}^{N} k_j \exp \left( \frac{-t}{\tau_j} \right) \right) \varepsilon_0
\]

(33)

By dividing both sides by \( \varepsilon_0 \), it is clear that the quantity in parentheses is just the time-dependent stress relaxation modulus for the Wiechert model.

The response of the Wiechert model can be correlated to the viscoelastic response of a real material by choosing the material constants, which appear in the model formulations (the \( k \)’s and \( \tau \)’s), from a reference to suitable experimental data. Model fitting is straightforward if each of the material’s major transitions is modeled as a single relaxation time process, as shown in Figure 14. The transition time for each step is given by \( \tau_j \). Increasing the number of \( \tau \) values allows the model to better approach the curve of the experimental data.
The value of $k_e$ is set equal to the long-time equilibrium (rubbery) modulus. Each successive $k_j$ value is chosen to raise the modulus to the next higher plateau. The $\tau_j$'s are chosen to position the relaxation properly along the time axis.

One can see from this type of analysis that a single Maxwell arm generally predicts too sharp a change in a given transition. The fit can be improved by assigning several Maxwell elements to each major relaxation. A convenient way of doing this is by using the Schapery collocation method (Tschoegl, 1989). In this method, one begins with an experimental plot of the relaxation modulus through a transition, as shown in Figure 15.
Using this hypothetical data as an example, one seeks to determine the value of the constants $k_e$, $k_j$, and $\tau_j$ in the model prediction for the relaxation modulus 

$$E_{\text{rel}}(t) = \frac{\sigma(t)}{e_0} = k_e + \sum_{j=1}^{N} k_j \cdot \exp \left( \frac{-t}{\tau_j} \right)$$  \hspace{1cm} (34)$$

so that the model prediction fits the experimental data. Schapery suggests arbitrarily locating the $\tau_j$’s at the decade values of log time, then solving for the associated $k_j$’s. In this example, $\tau_1 = 10^{-2}$, $\tau_2 = 10^{-1}$, $\tau_3 = 10^0$, …, $\tau_9 = 10^6$. By evaluating Eqn. (34) at these values of $\tau_n$, the following systems of equations are obtained:

$$k_1 e^{-(10^{-2}/10^{-2})} + k_2 e^{-(10^{-2}/10^{-1})} + ... = E_{\text{rel}}(10^{-2}) - k_e$$
$$k_1 e^{-(10^{-1}/10^{-2})} + k_2 e^{-(10^{-1}/10^{-1})} + ... = E_{\text{rel}}(10^{-1}) - k_e$$
$$...$$

(35)

Noting that terms like $e^{-(10^{-1}/10^{-2})} = e^{-10} \approx 0$ and terms like $e^{-(10^{-2}/10^{-1})} = e^{-1} \approx 1$, the system of equations can be rewritten as the triangular system:
On setting $k_e$ to the equilibrium rubbery modulus, this system can be easily solved by back substitution. Once the constants $k_e$, $k_j$’s, and $\tau_j$’s are calculated, the expression for the elastic relaxation modulus, $E_{rel}(t)$ can be found.

The relationship between cadaveric tissue and time since death will be determined by studying the plot of the $k_j$ values versus the amount of time (measured in days) that the sample was in the ground. These curves will be studied to determine a mathematical trend in the data. Obviously, the accuracy of the curves will increase as the number of samples tested increases.
This chapter discusses all of the data collected and analysis of the last human spine tested. A total of three human spines were tested before the protocol was revised for the testing of porcine spines. The first two human spines did not test well, which will be discussed shortly.

Originally the aim of thesis was to determine a correlation between time since death and the degradation of the human intervertebral (IV) disc. A protocol was developed for testing the human IV disc but did not take into account the various problems that occurred.

Human spines were ordered from the Central Florida Tissue Bank in Orlando, Florida. Since there is such a high demand for donated body parts for studies compared to the low donation rates of bodies, the research team was left waiting for a spine for months. In order to prepare for the eventual acquirement of a fresh human spine, two human spines were dissected from cadavers in the anatomy lab at the University of Central Florida. The cadavers had been embalmed and it is possible that they were subjected to freezing temperatures for up to a year. These factors could have affected the mechanical testing of their IV discs; however, those spines were simply used to develop a protocol for fresh human IV disc testing and analysis. Therefore, these data were not kept for analysis. Regardless, the data collected from the frozen spines was poor and
The discs moved in the clamps, which caused fluctuations in the graphs. Also most of the discs were ossified, meaning the disc had been replaced by bone, and the tissue was unable to completely relax during the stress relaxation tests. Despite the problems encountered for the frozen spines, testing was successful for the fresh human spine obtained from the tissue bank. The data analysis is discussed in the following section.

**Data Analysis**

During the stress relaxation tests, the load and displacement were recorded at each time interval of one second. The first sample was interrupted during testing at about 4.5 hours (16,384 sec), while the second sample was interrupted at about an hour (3,685 sec). The remaining four samples were tested for the full time span of approximately 5 hours (18,029 sec). Then the load was corrected for water loss over the time span of the testing by dividing the loss in load by the total time for testing. The corrected load was then calculated for each data point by multiplying that value by the elapsed time. The water loss was approximated for this data set by using the last 10,000 data points, and using all of the data points for sample two.

Before being placed in the testing chamber, the major and minor axis of each disc was measured with calipers to calculate the area of the disc. The height was also measured and recorded. With this data, the strain, which is held constant during the stress relaxation tests, was calculated by dividing the displacement of the disc during the first thirty seconds of compression by the initial displacement of the load cell of the Instron testing machine. Then the stress was determined by dividing the load by the area of that
specific disc. Using these strain and stress values, the elastic modulus at each data point was calculated to determine the experimental curve for the elastic modulus.

Now the Schapery collocation method was used to determine the theoretical curve for comparison with the experimental curve. The logarithm of time as well as the logarithm of the experimental elastic modulus values were calculated and plotted against each other to assess whether or not the sample reached a steady state value (i.e. relaxed) during the stress relaxation test. Also, using this data, decade values were chosen from the logarithm of time values in order to carry out the Schapery collocation method. For this set, it seemed reasonable to choose a final decade value of 4.2 since the largest value for the logarithm of time was 4.255. Eleven decade values were chosen for simplicity: the first decade value being zero and the others being multiples of 0.42. The elastic rubbery modulus, \( k_e \), was chosen as the final value of the logarithm of time, which was 4.255248. Then the \( E_{rel} \) values at each decade were calculated by taking 10 to the power of each decade value. Then \( k_e \) was subtracted from each of these \( E_{rel} \) values to determine the \( k_j \) values for the theoretical curve.

The \( k_j \) values were calculated using two methods in order to determine which one is more appropriate and accurate. The first method approximates all matrix coefficients less than 0.075 to zero, which creates a triangular matrix. This method uses backward substitution to calculate each \( k_j \) value. It is a simple “plug-and-chug” method. The second method takes advantage of circular references using Microsoft EXCEL to calculate each \( k_j \) value based on the other \( k_j \) values. This is an iterative process that continually refines the \( k_j \) values. The actual values of the matrix coefficients are used, eliminating the simple backward substitution technique. Both sets of values are listed for
each sample. By looking at the graphs, it is evident that the circular reference method creates a more accurate theoretical curve when compared to the experimental curve so these sets of $k_j$ values were used to plot each theoretical curve for all comparisons.

Finally, the theoretical and experimental curves were plotted on top of each other and compared. For the human IV discs, the two curves are almost identical for each sample, with the exception of a spike in the experimental curve in sample 12. With these good curves, the $k_j$ values were then evaluated to determine if a mathematical trend could be seen in the data.

Since the human spines were so hard and expensive to obtain and did not always provide good data, porcine spines were tested for the remainder of the study. Since there was only one human spine tested that had reasonable data, a comparison of $k$ values between multiple spines was unable to be completed. Figure 22 shows a general trend in the plots of the $k$ values of the samples. The negative $k$ values are artifacts of the model. They are not correct because physically they represent a spring pushing on itself. These negative values are a result from the finite transition times used in the analysis technique. The analysis is shown below to document the research that was completed.

In the tables that follow, the circular reference column shows the $k_j$ values calculated using the circular reference technique, while the diagonal matrix column shows the $k_j$ values calculated using the backward substitution method. The theoretical curves in the following figures represent a best fit curve using the $k_j$ values to the experimental curve. Both curves are graphed in the following figures, but they are very similar and lie almost on top of each other.
Human Sample 11

Table 4 - Schapery Collocation Data for Human Sample 11

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Table 4 - Schapery Collocation Data for Human Sample 11

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<td>109.1288 200.9153</td>
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<td>293.6544 213.9409</td>
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<tr>
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<td>52.0863 91.2375</td>
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<td>94.6882 158.3795</td>
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<tr>
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<td>407.5521 277.7292</td>
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<td>-73.1610 6.6691</td>
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Figure 16 - Stress Relaxation Curves for Human Sample 11
Human Sample 12

Table 5 - Schapery Collocation Data for Human Sample 12

<table>
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<tr>
<th>Ke</th>
<th>3378.726</th>
<th>E0-Ke</th>
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<th>K0</th>
<th>1509.5575</th>
<th>1002.9504</th>
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<td>2389.776</td>
<td>K4</td>
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<td>781.1553</td>
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Experimental vs. Theoretical Stress Relaxation Curves

Figure 17 - Stress Relaxation Curves for Human Sample 12
Human Sample 13

Table 6 - Schapery Collocation Data for Human Sample 13

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<td>E9</td>
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Experimental vs. Theoretical Stress Relaxation Curves

Figure 18 - Stress Relaxation Curves for Human Sample 13
### Table 7 - Schapery Collocation Data for Human Sample 14

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<tr>
<td>E4</td>
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<td>E4-Ke</td>
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#### Experimental vs. Theoretical Stress Relaxation Curves

![Stress Relaxation Curves](image)

**Figure 19 - Stress Relaxation Curves for Human Sample 14**
**Table 8 - Schapery Collocation Data for Human Sample 15**

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<td>48057.1721</td>
<td>diagonal matrix</td>
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<tr>
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<td>E1-Ke</td>
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<tr>
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**Figure 20 - Stress Relaxation Curves for Human Sample 15**
Table 9 - Schapery Collocation Data for Human Sample 16

<table>
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<th>diagonal matrix</th>
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Experimental vs. Theoretical Stress Relaxation Curves

Figure 21 - Stress Relaxation Curves for Human Sample 16
### Diagonal Matrix:

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Figure 22 - k Values of All Human Samples
PORCINE INTERVERTEBRAL DISC DATA AND ANALYSIS

The last chapter discussed the problems encountered using human spines for this study. Since the spine is fresh from the body, the researcher had to be ready to test when a spine was available in order to prevent freezing of the spine. Once a spine was available, it was in poor condition from age and many of the discs had ossified. It was uncommon to receive a spine less than 60 years old. The surgeons at the tissue bank were not careful to leave the ribs and other bones attached to the spine to prevent exposure of the discs to outside elements. Also, these spines are not cheap and it was hard justifying the money spent on the spines when the data did not turn out as well as it would with a younger, healthier spine. Because of these reasons, porcine spines were used for the rest of the study.

The FDA (Food and Drug Administration) in Bethesda, Maryland, was doing some experiments on pigs during the same time that this study took place. They were varying the diets of common pigs that they were raising and then sacrificing the pigs for their hearts and discarding the rest of the body. The research team made two trips to the FDA and dissected six spines (three per trip) out of the bodies before they were incinerated. This controlled two variables: 1) the spines were young and the discs were still elastic; and 2) the spine was dissected according to the developed protocol so the discs were not exposed to outside elements. Once the spine was dissected from the body,
it was cut into half vertebral body-intervertebral disc-half vertebral body segments and eight of these were used from each spine. The samples were then placed in clean bags and grouped by spine. Once back to the lab in Florida, the first sample was immediately tested (since a day had elapsed since dissection) and the rest were buried in the ground. The data analysis is similar to that previously conducted for the human spines but is summarized again below.

**Data Analysis**

During the stress relaxation tests, the load and displacement were recorded at each time interval of one second. A duration of five hours was not quite long enough for the tissue to relax in the previous human IV disc stress relaxation tests, so each pig IV disc was tested for ten hours to ensure that the tissue was able to relax. Each sample was tested for 43,229 seconds, with the exception of sample 23, which ended early at 23,846 seconds. Then the load was corrected for water loss over the time span of the testing and recalculated for each data point. The water loss was approximated for this data set by using the last 11,000 data points.

Before being placed in the testing chamber, the major and minor axis of each disc was measured with calipers to calculate the area of the disc. The height was also measured and recorded. The strain, which is held constant during the stress relaxation tests, was calculated by dividing the displacement of the disc during the first thirty seconds of compression by the initial displacement of the load cell of the Instron testing machine. Then the stress was determined by dividing the load by the area of that specific sample.

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1 The size of the data files were too large to include in this thesis. All raw data and calculations for the human and pig samples are on cd, which can be requested by contacting Dr. Ted Conway.
disc. Using these strain and stress values, the elastic modulus at each data point was calculated to determine the experimental curve for the elastic modulus.

Now the Schapery collocation method was used to determine the theoretical curve for the elastic modulus. The logarithm of time as well as the logarithm of the experimental elastic modulus values were calculated and plotted against each other to assess whether or not the sample reached a steady state value (i.e. relaxed) during the stress relaxation test. Also, using these data, decade values were chosen from the logarithm of time values in order to carry out the Schapery collocation method. For this set, it seemed reasonable to choose a final decade value of 4.568 since the largest value for the logarithm of time was 4.635474. Many attempts were made to choose a value closer to 4.635474, but more negative k values were calculated as the final decade value approached 4.635474. Eleven decade values were chosen for simplicity: the first decade value being zero and the others being multiples of 0.4568. The elastic rubbery modulus, $k_e$, was chosen as the final value of the logarithm of time, which was 4.635474. Then the $E_{rel}$ values at each decade were calculated by taking 10 to the power of each decade value. Finally, $k_e$ was subtracted from each of these $E_{rel}$ values to determine the $k_j$ values for the theoretical curve.

The $k_j$ values were calculated using two methods in order to determine which one creates a more accurate curve. The first method approximates all matrix coefficients less than 0.06 to zero, which creates a triangular matrix. This method uses backward substitution to calculate each $k_j$ value. It is a simple “plug-and-chug” method. The second method takes advantage of circular references using Microsoft EXCEL to calculate each $k_j$ value based on the other $k_j$ values. This is an iterative process that
refines the $k_j$ values. The actual values of the matrix coefficients are used, eliminating the simple backward substitution technique. Both sets of values are listed for each sample. By looking at the graphs and by knowing that less rounding error occurs, it is obvious that the circular reference method creates a more accurate theoretical curve when compared to the experimental curve so these sets of $k_j$ values were used to plot each theoretical curve for all comparisons.

Finally, the theoretical and experimental curves were plotted on top of each other and compared. For the porcine IV discs, there were problems with some of the data and they could not be used for analysis. The next section lists the samples not used for analysis and discusses the reasons why.

**Samples with Poor Data**

Unfortunately, one cannot predict when a sample will provide poor data or when the Instron tensile/compression testing machine will have a power failure. There were 48 total pig samples tested, and 13 of these could not be used. For set one, which includes spines 1, 2, and 3, samples 12, 21, 22, 31, and 32 were not able to be used because their corrected load plots were irregular, indicating the movement of samples in the clamps during testing. A common corrected load vs. time plot is shown below in Figure 23 for sample 14. Notice that the load reaches a steady state value.
The other corrected load graphs are shown below to validate the data being excluded from the analysis. The problems with the corrected load graphs indicated that the samples were moving in the clamps, in which case the k values are of no importance.
Figure 25 - Corrected Load Graph for Sample 21

Figure 26 - Corrected Load Graph for Sample 22
Also, in set one of the porcine spines, sample 27 could not be used because the theoretical stress relaxation curve was not smooth. The data collected was choppy in the beginning and the theoretical curve took this into account and therefore, cannot be used. One can see this bend early on in the curve shown below in Figure 29. Note that EXCEL can only graph up to 31,000 data points so the repeated graphs in the legend are merely the rest of the data points.
Although there was nothing wrong with sample one of spine one, it was tested to make sure that the protocol was also sufficient to test porcine spine samples. The corrected load curve is shown below in Figure 30. Since it was merely used to validate procedure, the disc measurements were not recorded and therefore, the data is not admissible.
Set two of the porcine spines includes spines 4, 5, and 6. This set had just as many samples that did not test well. The IV discs in samples 58 and 67 had degenerated (to the point that the end plates of the disc fell off at the touch) by the time they were dug up from the ground so they could not be tested at all. Samples 41, 42, 47, 48, 57, 65, and 68 could not be used because again, the stress relaxation curves are not smooth or do not reach a steady state value. A typical stress relaxation graph is shown below in Figure 31 for sample 13, which is followed by the curves of the samples that could not be used for analysis. The disc measurements can be found in Appendix A, while the stress relaxation curves for each sample analyzed can be found in Appendix B.
Figure 31 - Typical Stress Relaxation Curves

Figure 32 - Stress Relaxation Curves for Sample 41
Figure 33 - Stress Relaxation Curves for Sample 42

Figure 34 - Stress Relaxation Curves for Sample 47
Experimental vs. Theoretical Stress Relaxation Curves

Figure 35 - Stress Relaxation Curves for Sample 48

Figure 36 - Stress Relaxation Curves for Sample 57
Figure 37 - Stress Relaxation Curves for Sample 65

Figure 38 - Stress Relaxation Curves for Sample 68
Analysis of k Values

Once the k values for the samples with poor data were excluded, the k values for
the remaining samples were compared to see if a trend could be seen in any of the data.

In order to compare like samples with like samples, the k values were compared between
the sets because the samples for spine 1 were on the same time scale as spine 4, while the
same theory applies for spines 2 and 5 and spines 3 and 6. See the following figures for k
value plots. The k values calculated using circular references are shown below in Table
11.

<table>
<thead>
<tr>
<th>Pig Spines 1 - 3:</th>
<th></th>
<th></th>
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<tr>
<td>K1</td>
<td>4.974</td>
<td>-2.138</td>
<td>2.259</td>
<td>4.325</td>
<td>-61.709</td>
<td>-0.047</td>
<td>-5.351</td>
<td>-5.016</td>
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<tr>
<td>K6</td>
<td>72.559</td>
<td>60.605</td>
<td>41.039</td>
<td>1.82</td>
<td>20.472</td>
<td>262.811</td>
<td>-0.687</td>
<td>-3.484</td>
<td>50.757</td>
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<tr>
<td>K7</td>
<td>72.090</td>
<td>12.913</td>
<td>8.408</td>
<td>74.466</td>
<td>110.506</td>
<td>110.506</td>
<td>110.506</td>
<td>0.133</td>
<td>13.294</td>
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<tr>
<td>K9</td>
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<td>1.847</td>
<td>0.218</td>
<td>-0.377</td>
<td>-0.077</td>
<td>2.471</td>
<td>1.248</td>
<td>0.646</td>
<td>-2.547</td>
<td>-3.789</td>
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<tr>
<td>K10</td>
<td>-0.350</td>
<td>#DIV/0!</td>
<td>0.510</td>
<td>1.072</td>
<td>0.184</td>
<td>-0.407</td>
<td>-0.590</td>
<td>-0.039</td>
<td>-0.371</td>
<td>0.770</td>
<td>0.595</td>
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</table>

<table>
<thead>
<tr>
<th>Pig Spines 4 - 6:</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<td>K1</td>
<td>11.073</td>
<td>1.080</td>
<td>6.125</td>
<td>3.315</td>
<td>0.836</td>
<td>0.866</td>
<td>6.569</td>
<td>1.050</td>
<td>1.699</td>
<td>6.731</td>
<td>-2.238</td>
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<tr>
<td>K7</td>
<td>4.889</td>
<td>-0.145</td>
<td>-0.847</td>
<td>-2.392</td>
<td>-0.339</td>
<td>2.591</td>
<td>5.136</td>
<td>3.015</td>
<td>-8.052</td>
<td>-3.267</td>
<td>1.061</td>
</tr>
<tr>
<td>K9</td>
<td>9.686</td>
<td>0.834</td>
<td>-3.600</td>
<td>0.522</td>
<td>-0.098</td>
<td>-0.221</td>
<td>4.745</td>
<td>8.195</td>
<td>-0.388</td>
<td>3.596</td>
<td>0.952</td>
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<tr>
<td>K10</td>
<td>-1.893</td>
<td>0.338</td>
<td>2.262</td>
<td>0.409</td>
<td>0.509</td>
<td>0.295</td>
<td>-0.437</td>
<td>-0.873</td>
<td>0.874</td>
<td>-0.272</td>
<td>-0.627</td>
</tr>
</tbody>
</table>
Figure 39 - $k$ Values vs. $k$ Values for Porcine Spines 1, 2, and 3

Figure 40 - $k$ Values vs. $k$ Values for Porcine Spines 4, 5, and 6
Graphs of the 11 k values for set 1 were plotted against set 2. These graphs were studied to observe trends in the data. If trends can be determined from the graphs, it may be possible to narrow down time since death to a range of time in days based on the behavior of the stress relaxation curves of a sample. These graphs can be found in Appendix C.

In order to rule out the range of logarithm of time values as a reason why there is not a trend in the data, the analysis was repeated for a span of 4.5 and 4.6 both having 11 k values. All three plots were very similar with 4.658 being very close to that of 4.6. This was the same for all k values for both sets of spines. The graph for k0 values for set 1 is shown below.

![k0s for Pig Set 1](image)

Figure 41 - k0 Values for Porcine Spines 1, 2, and 3
Since changing the range did not seem to make a difference, the number of k values was changed from 11 to 17 to see how that affected the curves. Increasing the number of k values made the curves less similar as can be seen in Figure 42.

To validate the choice of using 11 decade values, a range of decade values from 5 to 11 was studied for one sample. Using only 5 or 6 decade values produced a curve that did not fit the experimental curve very well around the transition area between 3000 and 5000 seconds. Although there was little difference between the curves approximated using 7, 8, 9, 10, or 11 decade values, it was determined by the author that 11 decade values accurately modeled the experimental stress relaxation curve.
DISCUSSION OF RESULTS

As previously mentioned, the data collected from the human spines, with the exception of one, were not helpful in determining a correlation between time since death and the degradation of the spine. Focusing on the data from the human spine used for study and the six porcine spines, the values for the elastic moduli in the stress relaxation curves in most cases are much higher for the human spines than for the pig spines. This could be attributed to the forces on the spine and its position in the body. A human spine is vertical and experiences loads due to gravity along the axis of the IV discs (discs are in compression). However, a pig spine is horizontal and experiences gravitational loads perpendicular to the axis of the IV discs (discs are in shear and bending). When the pig IV discs are tested in compression they will be weaker than the human IV discs. Human IV discs tend to be more elastic to cushion the spine during everyday movement.

Since there was only one set of acceptable data for a human spine, a comparison between the k values could not be done. The data was still analyzed and observations were made. The k values: k0, k1, k2, k3, and k5, all have increasing slopes for all samples excluding sample 16, which can be seen in Figure 22. The number of negative k values increased the closer the values were to the rubbery modulus.

The bulk of the analysis was conducted on the porcine spines, since there were two different sets and a total of six spines. It should be noted that the decade values were
the same for both sets of spines so they could be compared to each other. Examining the k values for both methods, using backward substitution and circular references, led to the observation that the trends in the k values are different for each method. The author was not concerned with the k values calculated using backward substitution because a curve created using this method was less accurate compared to a curve created using circular references. There was not a significant trend in those k values.

The first set of pig spines includes spines 1, 2, and 3. General trends were observed between spines 1 and 3 for the following: k1 for samples 3 through 8; k3 for samples 3 through 7; k7 for samples 4 through 8; and k9 for samples 5 through 8. These graphs can be found in Appendix C.

Another significant observation is that of sample 25. The Schapery collocation method produced a curve that was basically identical to the experimental curve. This may explain why the k values for this sample are so extreme compared to the other samples. Since other experimental curves do not match the theoretical curves perfectly, the k values are lower because the Schapery collocation model is undercompensating for the errors, meaning the values are not as high or low as they should be. If the data were perfect for each sample, the k values would be different and could possibly have a more detectable trend.

The second set of spines includes spines 4, 5, and 6. Spine 6 had a generally decreasing slope for most of the k values, those up through k6, which can be seen by inspection of values in Table 11.

Each set followed the same time line. Spines 1 and 4, spines 2 and 5, and spines 3 and 6 were tested on the same days during the relative time line of day 1, day 2, etc. from
the start of the cycle. Since the data were bad for some of the samples and there is a need
to compare like samples with like samples, only samples 3 through 6 were compared for
each spine, except for k10 where only 4 through 6 were compared. There is a general
trend for k3, k9, and k10 for spines 1 and 4. k7 and k10 followed the same general trend
for spines 2 and 5. k4 and k6 also show similar trends for spines 3 and 6. These graphs
can be examined in Appendix C.

In general, the first set of pig spines had many more negative k values than the
second set. Obviously these negative values affect the trends in the k values, which in
turn affect the theoretical curves of the relaxation modulus.

In addition to varying the number of decade values as discussed in the analysis,
the dominant terms in the curves were studied for two samples – one with a smooth curve
and the other with fluctuations in the curve. All negative k values or those less than 2
were removed from the equation that calculated the $E_{rel}(t)$ values for the sample. Pig
sample 14 did not have any negative k values, but it did have k9 and k10 values less than
2. These were excluded from the equation, and in doing this, the curve did not
significantly change. Then pig sample 24 had negative k2, k4, k6, and k9 values in
addition to a k10 value less than 2. When all of these were excluded from the equation,
the curve was actually better at approximating a smooth curve from a shaky curve, which
can be seen by comparing graphs in Appendix B. Excluding negative or small k values
may help in analyzing imperfect stress relaxation curves.

There are many variables that may have affected the data, preventing a correlation
from being found. First of all, the process of decomposition could not be controlled.
Although the amount of rainfall in the area and the moisture content in the water were
observed, these variables did affect the rate of decomposition. It was noted that when there were periods of heavy rainfall, the samples did not decompose as quickly because the water tended to preserve the tissue.

Secondly, anatomy also plays a role in the analysis of the data. The number of vertebrae that a pig has varies, so it was difficult to get the same discs (i.e. L1, L2, etc.) from each spine and test them at the same times during the study (i.e. day 4, day 10, etc.). Also, even though the pigs were relatively young, they were on high fat, high cholesterol diets that may have promoted abnormal tissue growth or facilitated the herniation or deterioration of the IV discs.

In addition to those variables affecting the data, the technique used to evaluate the data may not have been the ideal method for this study. The Schapery collocation technique is not unique. This means that different sets of elastic moduli and relaxation times can be obtained from a given set of experimental data. This was proven when the range was adjusted from 4.568 to 4.5 and 4.6 and again when the number of k values was changed from 11 to 17. It is necessary to use the collocated parameters to reconstruct the original curve to determine whether the reconstruction produces an accurate curve, in this case to examine a trend. It happened in this case, that some of the calculated k values became negative. This results from the fact that the matrix inversion used to calculate the $k_j$ values is mathematically an ill-posed problem. Small variations on one side of the matrix may produce large changes on the other side [Tschoegl, 1989].

It is possible that a correlation may still exist if some of the above mentioned variables are controlled or if another technique is chosen for analysis. It is concluded by the author that the Schapery collocation technique is insufficient for determining a
correlation between time since death and the degradation of the human spine. Although many positive observations were made, a correlation could not be statistically proven.

This study developed a protocol, as well as a testing chamber and clamps, for testing human and porcine intervertebral discs. This protocol can now be applied to a similar study on a larger scale of 50 to 100 spines. Only then will more definite trends be established in the data. With these trends, postmortem interval could possibly be measured in a range of time that spans days, which is a significant improvement for the techniques currently available to estimate postmortem interval past the first 60 hours. Even if the data is only able to determine PMI for a range of days, this will be a significant help to forensic investigators.
FUTURE WORK

The Schapery collocation technique was used in this thesis to determine whether or not there was mathematical trend in the data that correlated time since death of a body to the degradation of the spine. The number of $k_j$ values was varied as well as the range of decade values in order to get a more complete set of data for the theoretical curves. Neither of these attempts seemed to make a difference in determining a correlation, although using too many $k_j$ values can make the curve much less accurate. Many attempts were made to find a range that produced all positive $k$ values, but with so many data points, it was very difficult to find one, so one was chosen that minimized the number of negative $k$ values. If one wanted to keep playing with that range, then it is possible that a correlation could be found that way. A program could be written to continually change that range and recalculate the $k$ values in order to determine a trend.

However, there are other techniques that can be used for model fitting that use the Wiechert or Kelvin models to describe observed behavior. One could use Procedure X discussed by Tschoegl [1989]. This method first writes the relaxation modulus $E_{rel}(t)$ in logarithmic form and separates out the term with the longest relaxation time. The $E$ and $\tau$ values can be determined from the intercept and slope of the resulting asymptotic equation. This method generally does not allow more than a few relaxation times to be
determined because there is not enough sufficient space between them. Although this method is not unique either, it could possibly determine a trend in the data.

Another method described by Tschoegl [1989] is called the multidata method which uses all of the data, not just a subset like the Schapery collocation method. This method reduces the error in determining $E_j$'s or $k_j$'s. The $\tau_j$ values are predetermined so the error needs to be minimized with respect to the $E_j$ values. Introducing an equation to minimize the error creates square matrices that can be solved to produce the same equation as that of the Schapery collocation method, but the results will have less error.

In addition to changing the analysis technique, the protocol could be modified. The testing could be done with specimens consisting of only the intervertebral discs themselves instead of half a vertebral body - IV disc - half a vertebral body segments that were used in this study. This may reduce any error in the stress relaxation data for the discs, but new clamps would need to be made for testing the discs only. Also, more samples could be taken from the spine and tested. In this study, only 8 samples were taken from pig spines and 6 from the human spine, but more could have been used from the pig spines.

Besides the intervertebral discs of the spine, other parts of the body could be tested to estimate postmortem interval. Maybe certain bones could be tested for brittleness after death as a function of time since death.

Certainly either of the two methods described in this section could produce better confidence in the results. The objectives of this research require a new direction to better correlate the degradation of the spine with time since death. It is hoped that future work may find a better correlation in the data.
APPENDIX A: DISC MEASUREMENTS
### Table 12 - Human Disc Measurements

<table>
<thead>
<tr>
<th>Sample</th>
<th>Major Axis</th>
<th>Minor Axis</th>
<th>Disc Height</th>
<th>Area</th>
<th>Test Date</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>2.71</td>
<td>1.96</td>
<td>0.31</td>
<td>4.17</td>
<td>3/15/2003</td>
<td>L4-L5, good</td>
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<tr>
<td>12</td>
<td>2.5</td>
<td>1.9</td>
<td>0.28</td>
<td>3.73</td>
<td>3/18/2003</td>
<td>L2-L3, poor</td>
</tr>
<tr>
<td>13</td>
<td>2.21</td>
<td>1.67</td>
<td>0.27</td>
<td>2.9</td>
<td>3/21/2003</td>
<td>T12-L1, poor</td>
</tr>
<tr>
<td>14</td>
<td>1.8</td>
<td>1.56</td>
<td>0.2</td>
<td>2.21</td>
<td>3/24/2003</td>
<td>T10-T11, poor</td>
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<tr>
<td>15</td>
<td>1.46</td>
<td>1.44</td>
<td>0.205</td>
<td>1.65</td>
<td>3/27/2003</td>
<td>T8-T9, poor</td>
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<td>16</td>
<td>1.42</td>
<td>1.37</td>
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<td>1.465</td>
<td>1.11</td>
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<td>1.28</td>
<td>4/2/2003</td>
<td>T4-T5, sample too small to test</td>
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</tbody>
</table>

### Table 13 - Porcine Disc Measurements for Spines 1, 2, and 3

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<tr>
<th>Sample</th>
<th>Major Axis</th>
<th>Minor Axis</th>
<th>Disc Height</th>
<th>Area</th>
<th>Test Date</th>
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<td>1.535</td>
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<td>0.295</td>
<td>1.11516722</td>
<td>5/13/2003</td>
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<td>1.45683505</td>
<td>5/14/2003</td>
<td>L2-L3, mostly broken</td>
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<td>1.12311937</td>
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<td>0.21</td>
<td>1.37161935</td>
<td>5/17/2003</td>
<td>T12-L1, first good one</td>
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<td>sp2sa3</td>
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<td>0.18</td>
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<td>sp3sa4</td>
<td>1.39</td>
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<td>0.2</td>
<td>1.13537159</td>
<td>5/22/2003</td>
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</tr>
<tr>
<td>sp1sa5</td>
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<td>1.11</td>
<td>0.2</td>
<td>1.18563707</td>
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</tr>
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Table 14 - Porcine Disc Measurements for Spines 4, 5, and 6

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Figure 44 - Stress Relaxation Curves for Sample 13
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Figure 50 - Stress Relaxation Curves for Sample 23
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Experimental vs. Theoretical Stress Relaxation Curves

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Experimental vs. Theoretical Stress Relaxation Curves

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Figure 96 - k10 Comparison for Spines 1 and 4
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REFERENCES


