Novel approaches to evaluate osteoarthritis in the rabbit lateral meniscectomy model

by

Anthony Paul Pease, DVM

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Committee:

____________________
Hugo Veit
Committee Chairperson
D.V.M., Ph.D.

____________________
Spencer Johnston
VMD, A.C.V.S. Diplomate

____________________
John Robertson
V.M.D., Ph.D.

____________________
Hara Misra
B.V.Sc., M.S., Ph.D., A.C.F.E Diplomate

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NOVEL APPROACHES TO EVALUATING OSTEOARTHRITIS IN THE RABBIT LATERAL MENISCECTOMY MODEL
by Anthony Paul Pease, DVM
Hugo Veit, DVM, Ph.D. Committee Chairperson
Veterinary Medical Sciences, Biomedical Sciences and Pathobiology

(ABSTRACT)
A rabbit lateral meniscectomy model was used to induce osteoarthritis. Separate studies were conducted to evaluate the progression of osteoarthritis and to identify possible biological markers. First, 21 male, New Zealand White rabbits were divided into 3 groups (n = 7 / group). A randomly selected left or right stifle underwent a lateral meniscectomy. The 3 groups were: corticosteroid administration, forced exercise and surgical control. An open field maze was used to assess mobility weekly. The rabbits were euthanitized 47 days after surgery. Histopathologic examination found that the lateral meniscectomy induced more severe lesions than in the non-surgical contralateral stifle. It also showed a significant sparing effect on erosion of cartilage in the corticosteroid group. The corticosteroid group, but not the exercise group, caused a significant increase in mobility (p ≤ 0.008) compared to the surgical control.

Secondly, synovial fluid was harvested from the 12 rabbits on days 0, 6, 26, 40, and 57 with surgery occurring on day 12. Trypan blue was used in the lavage fluid to estimate the volume of harvested synovial fluid. There was a significant increase in the volume harvested on day 26 (p < 0.001). Superoxide dismutase concentration in synovial fluid increased after surgery, although not significantly.

These studies verify that the lateral meniscectomy model produce histopathologic lesions consistent with osteoarthritis. Furthermore, use of trypan blue appears to be a reliable concentration marker in a lavage sample to measure harvested synovial fluid.
Dedication

This study is dedicated to the faculty and staff of the Virginia-Maryland Regional College of Veterinary Medicine whose support was invaluable to the completion of this project. In addition, to my family and friends whose support throughout my veterinary as well as graduate training helped me to become a better researcher and veterinarian.
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Chapter 1

Introduction

One of the most limiting aspects of the study of osteoarthritis is the fact that few objective and reliable methods exist to evaluate the progression of the disease. The lack of objective methodology has slowed research for decades and coupled with the complexity of osteoarthritis has lead to only small advances in the understanding of this disease. Osteoarthritis, also known as osteoarthrosis or degenerative joint disease, has numerous causes of onset such as joint instability, trauma, or enzymatic degradation, leading to chondrocytes that are unable to compensate for or adequately repair degenerating cartilage. This leads to an increased rate of destruction of cartilage causing further severity of disease. Osteoarthritis is considered the second leading cause of disability in people over 50 years old in the United States next to cardiovascular disease. Because of the large number of humans effected by osteoarthritis, (approximately 80% of people over the age of 75 and 67% over the age of 35) a need to understand the mechanism of its onset and devise more accurate means of evaluating osteoarthritis is required. This disease is not just limited to humans. Rossdale et al. found that lameness is the most common cause for failure of race horses. Todhunter performed a retrospective study at the Large Animal Clinic of New York State College of Veterinary Medicine during a 4 year period, 7.9% of 5539 horses had lameness related to joint disease. In dogs, cranial cruciate rupture occurs both spontaneously as well as traumatically and this destabilization of the joint leads to the development of
osteoarthritis. Whitehair reported that of 591,548 dogs seen from July 1, 1967 to March 31, 1987 between the ages of 1 and 15 years old, 10,769 or approximately 1.8% diagnosed with cranial cruciate ligament rupture. Hip dysplasia is also a disease process in dogs that predisposes to the development of osteoarthritis. According to a study based on radiographs submitted to the Orthopedic Foundation for Animals between 1974 and 1984, 17.4% of the 141,754 dogs were found to have radiographic signs of hip dysplasia.

After the initial insult to articular cartilage with osteoarthritis, low-grade inflammation occurs due to the trauma and damage. This inflammation causes the release of superoxide radicals from activated phagocytes as well as other inflammatory catabolic enzymes such as collagenase and prostaglandins. These superoxide radicals have been found to degrade hyaluronic acid, collagen and proteoglycans, all of which are vital to the proper function of articular cartilage. Due to the presence of superoxide radicals, the activity of superoxide dismutase, which is a scavenger of superoxide radicals, increases in the inflamed area as a mechanism to maintain homeostasis and reduce the inflammation.

Some problems with the study of osteoarthritis in any species is the lack of uniformity inherent to clinical trials, lack of available tissues to analyze, and the fact that no sensitive and reliable biological marker exists for monitoring osteoarthritis at this time. For this reason, animal models have been used to explore different aspects of osteoarthritis. Analysis of synovial fluid, serum, urine and/or aspects of articular
cartilage are being used to help further define the etiology, pathogenesis and potential treatment modalities to prevent, slow or halt the progression of osteoarthritis.

**Research Objective**

The overall objectives of this study were to evaluate the effect of systemic corticosteroid administration and exercise on mobility and the development of osteoarthritis in the laterally meniscectomized rabbit model. In addition, the effect of a destabilized lateral meniscectomized joint on synovial fluid volume and superoxide dismutase activity was evaluated.

The specific objectives were to:

1. Evaluate the partial meniscectomy rabbit osteoarthritis model described by Colombo et al.\(^{19}\) for repeatability and appropriate representation of the pathogenesis of osteoarthritis.

2. Determine if the open field maze can be used to objectively assess pain after removal of the lateral collateral ligament and cranial aspect of the lateral meniscus.

3. Determine the effects of moderate forced exercise or systemic corticosteroids on the development of osteoarthritis in the surgical model.
4. Determine if trypan blue can be used as a concentration marker when performing lavaged arthrocentesis.

5. Determine if superoxide dismutase in synovial fluid can be used as a biological marker during the development of osteoarthritis.

**Overall Hypothesis**

We hypothesized that the lateral meniscectomy model combined with corticosteroids or exercise would produce osteoarthritis without altering the mobility of the rabbits. The use of superoxide dismutase and trypan blue as markers would be reliable methods to further evaluate development of osteoarthritis by this model.

**Hypotheses**

1. The lateral meniscectomy model will produce repeatable lesions that are kinetically comparable to lesions seen with clinical osteoarthritis.

2. The lateral meniscectomy will not cause increased pain in the rabbit model. However, if inflammation (including pain), limping, and decreased mobility is present, these changes will be noted by measuring voluntary movement compared with the movement prior to surgery.
3. Moderate exercise of the destabilized joint will cause an exacerbation of the osteoarthritis histologically, where as systemic corticosteroids will decrease the severity of osteoarthritis seen, based on mobility and histopathologic examination.

4. Trypan blue is a reliable concentration marker for arthrocentesis lavage procedures.

5. Superoxide dismutase levels in synovial fluid during this study will reflect the presence of this inflammatory product from the time of surgery to the end of the study, and will help ascertain the presence and degree of inflammation that may be associated with degeneration of articular cartilage.
Chapter 2

Review of the Literature

**Normal Joint Anatomy of the Stifle**

Synovial or diarthrodial (movable) joints consist of articulating surfaces of two bones that are covered by articular (hyaline) cartilage. These two bones are held in apposition by soft tissue structures that encase synovial fluid, called a joint capsule, and associated ligaments (Illustration 1). The actual joint capsule is under negative hydrostatic pressure and consists of two layers, a fibrous layer that is continuous with the periosteum and the synovial membrane. The latter is a thin vascular membrane with blood vessels and nerves forming a capillary bed plexus (or anastomosis). Fat may also be present more deeply in this membrane.

The synovial membrane serves three main functions: phagocytosis, regeneration of protein and hyaluronate of synovial fluid, and regeneration of joint capsule. Synovial membrane also removes wastes and filters nutrients out of and into the joint cavity, respectively. The synovial cells present in this membrane consist of two types: A and B. The majority of the cells are type A (or M cells) consisting of macrophages which are actively phagocytic. The other, type B (or F cells) resemble fibroblasts and secrete hyaluronic acid. The surface of the synovial membrane may also have finger-like projections that protrude into the joint cavity as folds or villi.

Three types of sensory nerve innervation are present within the synovial membrane, muscles, ligaments and bone marrow. These are low threshold nerve fibers.
associated with ordinary movement, proprioception and pain; high threshold myelinated fibers for noxious, excessive mechanical stimuli and small diameter; and unmyelinated fibers (C fiber) that are responsible for pain sensation due to severely abnormal stress on the joint capsule. The C fibers are the only fibers present in the synovial membrane and are in close association with the blood vessels. These C fibers are responsible for the release of Substance P and histamine if stimulated. In addition, once stimulated, the unmyelinated nerve fibers are sensitized and therefore more easily stimulated by later insults. If there is synovial membrane irritation and resultant inflammation, the type A cells and other leukocytes, plasma, and lymph migrate into the joint cavity causing inflammatory vasodilation along with stimulation of the C fibers.

This inflammation results in joint stiffness and pain due to increased synovial fluid production causing an increase in pressure inside the joint cavity. In addition, the inflammatory enzymes secreted accelerate degeneration of articular cartilage. Blood plasma, lymph, and leukocytes also enter the joint capsule and cause a change in the synovial fluid content which in turn could damage the articular cartilage by upsetting the nutrition or homeostasis of the chondrocytes or extracellular matrix.

The stifle joints of rabbits and dogs have similar ligamentous attachments that help to maintain joint stability (Illustrations 2 & 3). The cranial and caudal cruciate ligaments, located in the intercondylar fossa of the femur, prevent the tibia from excessive motion cranially and caudally respectively. The lateral collateral and medial
collateral ligaments prevent lateral to medial movement and are present on each side of
the joint lateral to the joint capsule.

The meniscus, or semilunar fibrocartilage, is a C-shaped disk of fibrocartilage
present between both the lateral and medial condyles of the stifle and concave in the
central area to compensate for the incongruence that exists between the femur and the
tibia. According to Minns and Muckle, the meniscus has also been implicated in
providing stability as well as a mechanical barrier to the two articulating surfaces. It was
shown that the meniscus not only transfers load from the femur to the tibia, but also
prevents shearing forces from causing flaps and fissures in articular cartilage. After
meniscectomy, stress on the cartilage and subchondral bone of both the tibia and femur
has been found to increase 2 to 5 fold, and the instability caused a condition coined “post-
traumatic arthritis”. The conclusion was that the meniscus provided medio-lateral
stability in the flexed knee.

All connective tissues are derivatives of the mesenchymal stem cell. This one cell
type then differentiates into osteoblasts, chondroblasts, fibroblasts, mast cells, and others.
When the mesenchymal cells are in an area where cartilage is to develop, the cells
aggregate and secrete primarily type II collagenous fibrils and glycosaminoglycans as
well as other substances. The cells that produce these substances are called
chondroblasts. These secretions form a matrix of cartilage and in the process engulf the
chondroblast. As the cells develop, the matrix becomes more pronounced and the
chondroblasts begin to spread further apart. During this time, the chondroblasts reduce
cell divisions and mature, changing to chondrocytes.
Though mature and surrounded by matrix, chondrocytes are still metabolically active and produce extracellular matrix, including collagen and proteoglycans, in response to stimuli such as fibrillation of adjacent articular cartilage, or normal daily activity or inactivity. It has been shown that moderate exercise in the clinically normal joint causes no damage to cartilage but does seem to stimulate chondrocytes to increase metabolism. One reason expressed for this increased metabolism was that the chondrocytes had improved access to nutrients allowing increased metabolic activity.

Articular cartilage is grossly classified as having four zones. The most superficial tangential layer consists of collagen fibers and a few ovoid to flattened chondrocytes arranged in a meshwork pattern. The intermediate or transitional layer contains chondrocytes that are larger, randomly spaced with collagen fibers randomly oriented. In the radiate layer, chondrocytes are in vertical columns separated by collagenous fibrils. The deepest layer is called the calcified layer that contains mineralized cartilage and chondrocytes at various stages of degeneration. The most superficial portion of this calcified layer is called the tidemark (Illustration 4). The tidemark and calcified layers are the only parts of articular cartilage that are visible on radiographs which is why potential “joint space” is interpreted as a representation of the articular cartilage present. Articular cartilage by volume is made up of only 1 to 5% chondrocytes. Seventy to 80% of cartilage is water by total weight with the remaining 15 to 25% being mostly extracellular matrix and collagen. The dry weight of articular cartilage contains...
about 65% collagen, 25% proteoglycans and 10% glycoproteins, lipids and chondrocytes.

Hyaline cartilage is the connective tissue found covering articular surfaces. When fresh, the cartilage appears bluish-white and translucent. For this reason, the term hyaline was coined, derived from the Greek word *hyalos* meaning glass. It is avascular as well as aneural, meaning that the chondrocytes have no sensory innervation and no direct source of nutrients nor a method to remove waste. Instead, this is accomplished through compression and decompression of the articular cartilage. When compressed, the waste is expelled by diffusion into the interstitial space and out of the cartilage. During decompression, the absorption of nutrients from the synovial fluid is possible again by diffusion through the extracellular fluid. Compression and decompression of cartilage is possible through a complex network of proteoglycans intermeshed with collagen. Using hyaluronic acid, which is a non-sulfated glycosaminoglycan, as a root attached to the collagen fiber, link proteins attach in a bottlebrush three-dimensional configuration. This spacing is essential for binding of the interstitial fluid. Core proteins then attach to the link proteins giving the glycosaminoglycans a place to bind. The entire unit of core protein and glycosaminoglycan is what is called a proteoglycan aggregate (*Illustration 5*).

These aggregates stabilize the matrix, and provide the compressive properties to the cartilage due to their negative charge and ability to attract water. Proteoglycans are able to absorb solvent volumes of as much as 50 times their dry molecular weight. However, due to the bottlebrush organization, complete saturation of the proteoglycan
molecule is not attained, possibly due to stearic hindrance. Water in the proteoglycan matrix provides elasticity and a medium to transport materials. The intercellular fluid in the matrix allows the diffusion of nutrients into, and carbon dioxide and other waste materials out of, the chondrocytes. The chondrocyte expels waste during the compression that diffuses through the water and enters the interstitial space. Upon decompression, the complex returns to the original volume, causing the intake of nutrients present in the water of the extracellular matrix to enter by diffusion, while the waste products are carried away. The synovial membrane produces synovial fluid that enters the interstitial space and supplies these nutrients. Waste is removed from the joint capsule via the capillaries, or phagocytized by type A cells in the synovial membrane, and carried away via the lymphatics.

The compressive resilience of the cartilage, meaning the fact that cartilage does not collapse under load, is due to the proteoglycan matrix. The negative charge associated with proteoglycans cause them to repel one another, and this repulsion becomes stronger as the proteoglycans become closer. The collagen network provides the framework for articular cartilage and resists shear forces, as well as providing articular cartilage with elasticity.

Synovial fluid contains protein and nutrients derived from blood plasma and lymph, filtered through the synovial membrane, and secreted materials from type B cells. Hyaluronic acid is also added by type B synoviocytes and is thought to provide viscosity to the synovial fluid. This highly viscous synovial fluid, in conjunction with the
smooth surface of the tangential layer, aids in the low level of friction encountered in the joint. Fluid is also exuded from the articular cartilage under load, which is called weeping lubrication, and provides a surface that has a coefficient of friction less than ice sliding on ice.\(^{30,37-39}\)

It has also been proposed by Stachowiak et al. that a thin micelle layer is present on the surface of articular cartilage that traps synovial fluid to prevent direct cartilage on cartilage contact, further aiding this low coefficient of friction.\(^{38}\) Finally, Macirowski et al., as well as Bader et al., reported that fluid pressure in articular cartilage supports 90% of the joint’s mechanical load.\(^{36,37}\) It is clear that lubrication of a synovial joint is a complex balance of numerous mechanisms that must maintain homeostasis to provide a near frictionless environment for articular cartilage.

**The Degenerative Cascade Associated with Osteoarthritis**

Osteoarthritis, is the most common form of arthritis seen clinically. This disease is thought of as the “wear and tear” or primary degenerative form of arthritis. However, osteoarthritis is a complex and diverse set of events that leads to degeneration of articular cartilage (Scheme 1). Osteoarthritis is thought to involve not only mechanical damage but also a disturbance of homeostasis where catabolism outweighs anabolism and thus leads to degeneration.\(^{1-6}\) Buckwalter et al. believe that the process of osteoarthritis involves the disruption or alteration of cartilage matrix that results in a chondrocyte
attempting to repair the damage, but is unable to keep up with the catabolism and thus leads to a loss of tissue.

Though the scheme outlined by McIlwraith only addresses mechanical injury, immobilization serves as an important factor for osteoarthritis as well. Immobilization results in cartilage damage because without compression and decompression the chondrocytes have no means to absorb nutrients and remove wastes. Immobilization leads to a decrease in proteoglycan content, aggregation, and synthesis, as well as increased water content and increased extractability of the proteoglycans while the surface layer of the cartilage remains intact. Using a rabbit model, Jurvelin et al. have shown that the histologic changes caused by immobilization are observed after only 1 week, while it can take up to 8 weeks in the exercise model.

The characteristics of articular cartilage in the early stages of osteoarthritis include increased water content with loss of proteoglycans, constant collagen content with change in arrangement and size of fibers, and increased mitotic activity of chondrocytes. In intermediate to chronic stages, the presence of Type I collagen instead of Type II collagen is found in the repaired articular cartilage defect. As the degeneration progresses, loss of the tangential layer cartilage is noted as well as fibrillation, hypertrophy of chondrocytes, violation of tidemark with blood vessels and progressive loss of cartilage.

The tangential layer is believed to have lubricating properties, and its loss is a usual sequela to osteoarthritis. The belief is that in addition to the smooth surface of
the tangential layer, a thin film of micelles are present which aids the synovial fluid in preventing cartilage on cartilage contact. Researchers have found that once this tangential layer is removed, the underlying layers of articular cartilage do not have these protective mechanisms and there is increased friction and wear. In addition, decreased proteoglycan content of articular cartilage causes a decrease in fluid pressure that is responsible for 90% of the load bearing properties of articular cartilage.\textsuperscript{36,37}

Because of the above changes, an in vitro method of evaluating both wear (the amount of hydroxyproline collagen present in the synovial fluid is measured to relate to free collagen product), and friction under load, was created by the Mechanical Engineering Department on the Virginia Polytechnic Institute and State University campus. As a joint venture with the Virginia-Maryland Regional College of Veterinary Medicine, surgically induced osteoarthritic cartilage was compared to normal cartilage from the same rabbit on the in vitro apparatus. An increase in the friction coefficient in osteoarthritis cartilage is hypothesized to be due to the loss of the tangential layer, however, this increased friction does not directly relate to the amount of wear. The rationale for this apparent discrepancy of wear (since hydroxyproline levels are supposed to indicate the amount of wear) is that the loss of collagen in both normal and osteoarthritis cartilage occurs at a set rate due to physical forces and is poorly related to friction.\textsuperscript{3} The results of the study by Berrien, showed that the hydroxyproline levels present in normal and osteoarthritic cartilage were not statistically different within the
confines of the study, but the amount of friction was greatly increased in the osteoarthritis cartilage.

It has been shown that the response of the chondrocytes to an initial insult or some event that starts the degenerative cascade, cannot adequately repair the damage and reproduce its original integrity. Some studies have shown that reparative collagen is Type I in nature (fibrocartilage) rather than Type II (hyaline). However, it has also been shown that chondrocytes repair partial thickness defects using only Type II cartilage. One thought is that if the damage invades the tidemark, the calcified cartilage tends to produce Type I collagen, while Type II collagen is produced when more superficial defects are present. Nevertheless, it is clear that after the initial insult, cartilage does not fully return to its original state.

With the degeneration of cartilage in osteoarthritis, there is loss of proteoglycans through metalloproteinases and then prostaglandins decrease proteoglycan synthesis to create a net loss. In response to this loss, chondrocytes produce proteoglycans that are smaller and less likely to aggregate with hyaluronic acid, thereby decreasing the extracellular matrix. Osteoarthritis is also associated with an increase in the overall water content of the cartilage, as well as an increase in the strength by which this water is bound. Mankin et al. offered a review of three potential reasons for this increase.

1. That collagen is able to make an extracellular matrix gel with less proteoglycans that holds a greater amount of water than collagen – proteoglycan gel. The thought being that the loss of proteoglycans allows increased hydrogen bonding on the
surface of the collagen previously occupied by proteoglycans. An alternative theory is that collagen alone is a better gel former than proteoglycan and more collagen gel is produced, thus leading to greater water content.

2. That due to the rigid nature and negative charge of the proteoglycan structure and the bottlebrush formation, loss of some of the glycosaminoglycans or proteoglycans cause an increased available space due to “uncoiling” of the structure, allowing a greater amount of water to bind.

3. Changes in the proteoglycan constituents, such as the increase of chondroitin 4 sulfate, the decrease of keratin sulfate, or others present yet not identified, may provide an increase in water binding ability.

Regardless of the cause, the end result is swelling of the articular cartilage and loss of compressive stiffness due to lack of stearic hindrance to support the collagen network.

While trauma leading to the degeneration of cartilage with osteoarthritis is thought to be the initial and primary effect, synovial inflammation in the degenerative process of osteoarthritis is also important. Inflammation provides a source of enzymes such as collagenase and metalloproteinase, which are released by ruptured lysosomes in synoviocytes, as well as from invading neutrophils, macrophages, lymphocytes and even from damaged chondrocytes. Also, prostaglandins released by inflamed synovium, cause a decrease in proteoglycan content by suppressing glycosaminoglycan and proteoglycan synthesis. Vasodilation from histamine release as well as Substance P presence, will cause an increase in synovial fluid with more white blood cells and plasma protein, and
distension caused by this increase further irritates the synovium leading to pain and decreased blood flow.

Activated phagocytes are known to generate superoxide radicals, which in turn induce inflammation. During the inflammatory response, superoxide dismutase, a scavenger of superoxide radicals, is induced and appears at the inflammatory site. This enzyme is normally present at very low levels in the extracellular fluid and is regarded as a natural anti-inflammatory mechanism. It is thought that the superoxide radical is what causes the loss of viscosity present in synovial fluid via the degradation of hyaluronic acid. In addition, superoxide radicals have been found to degrade collagen and proteoglycans. This association between inflammation, superoxide free radicals, and superoxide dismutase, is a pivotal component in the natural homeostasis of the inflammation process.

Instability is believed to be the major cause of degenerative joint disease in the post-traumatic model. The lack of stabilization causes increased shear forces and fibrillation that outweighs and overwhelms the chondrocytes’ ability to repair the damage. The causes of this instability can be a torn or removed meniscus, and/or torn collateral or cruciate ligaments. To exercise this unstable joint should increase the amount of shear force present, and exacerbate articular cartilage damage.
Diagnostic Modalities

There are numerous methods employed to diagnose and assess the severity of osteoarthritis. Perhaps the most widely used in all species is pain. In most animals this is assessed by watching the animal at different gaits and assessing the amount the animal favors one leg over the others. Assessing pain in humans for quantitating the severity of osteoarthritis is done through a series of questions based on assessing the severity of pain during activity and at rest. Several indices have been developed to measure degree of pain, range of motion, physical function, walking distance and other parameters to determine severity of osteoarthritis. In animal models, pain is assessed by measuring voluntary motion. Deyo et al. described using an open field maze to assess free movement of rabbits as a quantitative assessment of pain.

Other methods, such as evaluating biological markers that are released over the course of osteoarthritis, have also been attempted. Rorvik and Grondahl believed that in order for a molecular marker to be reliable, it must not only relate to the nature of the disease, but also the stage of degradation either directly or in proportion to the degeneration. The supporters of biological markers claim that as degeneration occurs with osteoarthritis, biological components from the extracellular matrix, as well as inflammatory products can be measured in synovial fluid, blood or even urine to assess the severity of disease. Some examples include the use of serum or synovial fluid derived keratan sulfate, a glycosaminoglycan felt to be one of the first materials released during the early catabolic stages of osteoarthritis. Hyaluronic acid is
also found to increase during the course of degenerative joint disease, so assays have been used to measure levels in both serum and synovial fluid.\(^{63,74,78,79}\) Hydroxyproline is one of the most reliable markers to assess the severity of osteoarthritis, since it is used to measure the amount of wear based on the idea that collagen is approximately 12.5% hydroxyproline.\(^ {41,77,80-85}\) Tumor necrosis factor \(\beta\) (TNF \(\beta\)), interleukin 1 (IL-1), and Type II collagen, have also been used as markers due to the potential increase in concentration seen with inflammation (TNF \(\beta\), IL-1)\(^ {5,53,86-89}\) and decrease in concentration with destruction of cartilage (Type II collagen).\(^ {46,49}\) TNF \(\beta\) is believed to increase collagenase 3 secretion, IL-1 regulates the expression of Prostaglandin E\(_2\) and collagenase,\(^ {86}\) as well as decreasing the synthesis of matrix components, and chondrocyte production.\(^ {5}\)

A problem found with using these markers is that the elimination and therefore presence in synovial fluid and serum is highly variable.\(^ {78}\) This variability is thought to be due to the increased vasodilation of the inflamed synovial membrane and therefore the greater influx and efflux of materials into and out of the synovial fluid. The synovial inflammation causes increased removal of materials from the synovial fluid that is then is taken intravascularly to the kidneys and excreted. The belief is that the increased efflux of synovial fluid components by inflamed synovial membrane makes measuring breakdown products in synovial fluid highly variable and confounds the use of biological markers within such fluid.\(^ {22,78,90,91}\)
In smaller animals, synovial fluid analysis requires a lavage technique to gain an adequate sample from arthrocentesis. Lavages with sterile saline or Phosphate Buffered Saline (PBS) have been used at necropsy to increase the volume present, however, the exact amount of synovial fluid harvested becomes unknown. It was for this reason that Delecrin et al. added a fluorescent marker to the fluid to help quantitate the amount of synovial fluid aspirated from an opened joint at necropsy. This was accomplished by injecting a known volume of fluorescent dye, then determining the concentration after aspiration, the difference being the amount of synovial fluid present.

Measuring the degree of osteoarthritis through radiography is also possible. Joint space, presence of osteophytes, and joint effusion are usually assessed on a scale of 1 to 4. The main concern is that technique and position must be standardized to allow comparisons between patients to be valid. Another concern is that degree of damage caused by osteoarthritis is only appreciated radiographically when the disease is very advanced, and it is not possible to diagnose osteoarthritis early in the disease process.

Another method is histologic scoring which was first quantitated by Mankin et al. as a way to help standardize the reported degree of damage seen in articular cartilage, and to be able to compare the results between groups. Researchers have modified this method in the attempt to limit subjectivity and further standardize the method, but variables such as sectioning, staining and researcher subjectivity are still present. For example, Alcian Blue was found to be highly variable in staining quality based on different manufacturers. To overcome this limitation, all slides can be stained at the
same time, but comparisons between studies would be difficult. Another limitation of histology is the inaccessibility of samples since they are collected primarily post-mortem.

Staining an entire condyle with India Ink to assess surface morphology has also been used in conjunction with computer imaging to remove the variability of human assessment. India ink is a large molecule and is mostly absorbed when the tangential layer is disrupted therefore this method only assesses surface integrity.\textsuperscript{105,106} Scanning electron microscopy (SEM) has also been used to describe the surface contour of normal and osteoarthritic cartilage. Normal rabbit cartilage is smooth with only minor pits thought to be associated with lacunae (small empty areas surrounding the shrunken, fixed chondrocytes as an artifact of processing cartilage), and matrix variability due to processing. Osteoarthritic cartilage has disruption of the tangential layer as well as disorganization of the underlying chondrocytes and matrix (Illustration 6).\textsuperscript{44,107,108}

Arthroscopy and magnetic resonance imaging (MRI) have been suggested as new diagnostic methods. However, the technology is still new and their usefulness needs to be proven.

\textbf{Treatment Regimes}

Treatment validity of osteoarthritis is usually based on one, or a combination of several, of the diagnostic measurements described earlier. Since evaluation of the progression of the disease is difficult, the assessment of treatment is also very difficult. Some common treatments used for osteoarthritis include glucosamine sulfate which has been found to be absorbed orally and has some anti-inflammatory and chondroprotective
properties without the systemic effects of nonsteroidal or steroidal anti-inflammatory drugs. Chondroitin sulfate administered orally has too high of a molecular weight to be absorbed and is therefore only useful if given intravenously or intra-articularly.

Oral corticosteroid administration has been a standard treatment for pain associated with osteoarthritis in the past. By inhibiting phospholipase A₂, thus decreasing the production of inflammatory prostaglandins, corticosteroids are able to decrease inflammation thereby decreasing associated pain. In addition, corticosteroids were found by Colombo et al. to decrease the severity and frequency of osteophyte formation. The problem is that prolonged systemic administration may result in side effects, including iatrogenic hyperadrenocortism. Corticosteroids have also been used intra-articularly to prevent systemic side effects, however, this was found to degrade cartilage directly with cumulative use and to decrease matrix synthesis. Intra-articular hyaluronic acid is another treatment available providing both anti-inflammatory properties as well as theoretically increasing viscosity of the synovial fluid. Systemic nonsteroidal anti-inflammatory drugs (NSAIDs) are used to decrease the inflammatory cascade, similar to corticosteroids with less side effects since the NSAIDs work on cyclooxygenase rather than phospholipase A₂ in the pathway of proinflammatory mediators.
**Osteoarthritic Models**

The purpose of an animal model is to be able to study a disease process with as much control over extraneous variables as possible. The problem is the enormous variability of osteoarthritis. Numerous animal models have been used including rats, horses, dogs and rabbits all hoping to provide a model which is comparable to human osteoarthritis. Hulth et al. first used the rabbit model in 1970 in the attempt to make a slowly progressive model for osteoarthritis by removing the medial collateral ligament, the medial meniscus, as well as both cruciate ligaments. From this study, the first documented sighting of active mitosis of chondrocytes were made. The increased mitotic figures represents the chondrocytes’ attempt to repair ongoing degeneration caused by the instability and associated inflammation. This method was then refined to cause less joint instability by Colombo et al., who removed a portion of the lateral meniscus and lateral collateral ligament. This method seemed to cause little inflammation and only a mild amount of instability with reproducible histologic changes. The lateral meniscectomy model not only causes repeatable lesions in the rabbit model, but also generates lesions similar to those found in human degenerative joint disease. Another method is to transect the cranial cruciate ligament in the rabbit again causing instability in the joint.

Other models of instability include cranial cruciate transection in a dog, and an instability model in the horse involving transection of the lateral collateral ligament. The major problem with the dog and horse models is the availability of uniform
specimens. To obtain horses or dogs of the same sex, age and free of disease is difficult. The rabbit is easy to obtain and maintain, most colonies are pathogen free and all rabbits can be the same age, sex, relative weight and limited genetic variation. This helps to reduce any confounding variables that may exacerbate osteoarthritis, such as presence of joint disease prior to surgery, different weights, or even different sexes.

Several chemically induced osteoarthritic models have reported.\textsuperscript{23,73,126} However, these models represent more of an inflammatory arthritis rather than an osteoarthritis with a physical degenerative etiology. The physical degeneration of cartilage due to instability in the chosen rabbit model is felt to be more representative of the clinical osteoarthritis found in people and animals as a result of overuse or joint instability, and therefore more appropriate for the focus of this study.
Chapter 3

Materials and Methods

The Effect of Exercise or Corticosteroids on Articular Cartilage in the Rabbit Lateral Meniscectomy Model

Rabbits

Twenty-one New Zealand white, male rabbits weighing 2 to 3 kilograms were purchased from Hazelton Research Products (Denver, PA.) and housed in quarantine conditions for fourteen days to be monitored for normalcy and to allow time for acclimation to the new environment and handling. Normal activity and parameter assessment were defined as normal food and water consumption, normal stool formation, normal taxic behavior, no respiratory distress or nasal discharge, and a temperature between 38 and 40.6 degrees Celsius (°C). Rabbits were housed in a clean facility that required gowns and shoe covers as well as hand washing prior to handling of animals. In addition, the rabbits were placed individually into 4 square foot stainless steel cages and all housed in the same room with an ambient temperature of 21-22 °C. Rabbits were randomly assigned to 1 of 3 groups (n=7/group) after surgery was performed: exercise, systemic corticosteroid administration, and surgical control. A group combining exercise and systemic corticosteroids was also formed with the results being published in a future study. The surgical stifle (right or left) was assigned immediately prior to surgery. Both assignments were made using a random numbers table. The non-surgical stifle of the rabbit served as the non-surgical control.
Lateral Meniscectomy

After two weeks in quarantine, the rabbits were prepared for surgery. The lateral meniscectomy technique was followed as outlined by Colombo et al. For induction of anesthesia the following was used: atropine sulfate (Atropine Sulfate Injection, VEDCO, St. Joseph, MO.) at 0.1 milligram per kilogram (mg/kg), ketamine hydrochloride (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA.) at 20 mg/kg and xylazine (Rompun, Bayer Corporation, Shawnee Mission, KS.) at 5 mg/kg. All injections were given into the epaxial musculature. If necessary, one-half of the induction dose was given intramuscularly to maintain anesthesia during surgery. After the rabbit was anesthetized, the leg was prepared aseptically and the rabbit was moved into the operating room. The initial incision was made using a #10 scalpel blade on the lateral aspect of the stifle extending from approximately 2 centimeters (cm) proximal to the patella, to 2 cm distal. The subcutaneous tissue was dissected, as well as the underlying fascia to expose the lateral collateral ligament. This ligament was transected and a 3 to 4 millimeter (mm) portion was removed using a #64 Beaver blade (Becton-Dickinson, Franklin Lakes, NJ.). In addition, 3 to 4 mm of the sesamoid ligament was also removed. The lateral meniscus was identified, and the cranial one-third of the meniscus was removed using the same #64 Beaver blade. The joint capsule was closed using 4-0 Polydioxanone Suture (PDS, Johnson and Johnson, Somerville, NJ.) on a cutting needle in a simple continuous pattern. The subcutaneous tissue was also closed primarily using 4-0 PDS on a cutting needle in a simple continuous pattern, taking care to bury all knots. The skin was closed using Vet Bond Adhesive (3M, St. Paul, MN.) and then the rabbit
was moved from the operating room into the recovery room to be monitored until conscious and then returned to his cage. Buprenorphine hydrochloride (Buprenex, Reckitt and Colman Products, Richmond, VA.) at 0.01 mg/kg every twelve hours for three doses was given post-operatively for analgesia.

**Open Field Maze**

An open field maze was used to measure the amount of voluntary movement based on the method described by Deyo et al. Two wooden boards measuring 60 cm long and 10 cm high were varnished and hinged together at the end to make one half of a square perimeter. The corner of the room in which the rabbits were housed made up the other half of the square. The 4 square foot box was then divided into 16 one-foot squares using electrical tape placed on the floor (Illustration 7). These boxes were then numbered one to sixteen. Prior to surgery each rabbit was placed in this maze for 5 minutes and movement was recorded using the number of the box that all four feet entered. After 5 minutes the rabbit was placed back into his cage and the area was disinfected prior to the next rabbit entering. The observer always sat in the same place and no distracting movements were made. All rabbits were placed facing the same direction and in the same starting square once a week prior to surgery and then once a week until the end of the study (41 days after surgery). The movement results were analyzed using the Statistical Analysis System and compound symmetry analysis. The squares entered during the 5 minute period were summed and the square root was used to stabilize the variability among the groups.
Corticosteroid Administration

In the corticosteroid group (n = 7), triamcinolone acetonide (Vetalog, Fort Dodge, IA.) was given as a positive control at a dose of 0.05 mg/kg subcutaneously one time as a loading dose, then at 0.025 mg/kg subcutaneously every four days for the duration of the study.

Exercise

The exercise group (n = 7) was exercised on a treadmill twice a day. The schedule was modified from the study by Kamps et al. The rabbits were run at 0.3 miles per hour for 10 minutes each day, 5 days per week, starting 9 days after surgery, increasing to 15 minutes a day after the first week (Illustration 8).

Euthanasia and Necropsy

Rabbits were euthanitized 48 days after surgery using 1 to 3 mls of pentobarbital euthanasia solution (Beuthanasia-D, Sherig Plough, Atlanta, GA.) administered into the heart while anesthetized for joint lavage. A final synovial fluid sample was taken and then a standard necropsy was performed to examine all organ systems for gross lesions. The hind limbs were removed and both stifles were placed in 1:10 (tissue to formalin volume) ratio of 10 % phosphate buffered formalin fixative, until trimming for histopathologic staining.
Histopathologic Examination

Femoral and tibial sections were trimmed of excess soft tissue and cut using a fine bladed small band saw through the center of the osteoarthritic lesion present on the lateral condyle of the femur and tibia. For those sections without lesions, the location was approximated. The cut section was placed face down in the cassette, so it would be sectioned with the microtome (Illustration 9). Samples of joint capsule were also taken and placed individually into cassettes. All sections remained in 10 % phosphate buffered formalin until processing. The bone sections were placed into TBD-2 (Shandon, Pittsburgh, PA.) for decalcification for 24 hours. The next day, the cassettes were embedded in paraffin (EM400, Surgipath, Richmond, IL.) by placing the melted paraffin into a mold, adding the cassette, and cooling the mold until solid. The embedded tissue was sectioned using a microtome into 5 micron (µm) sections and placed onto a slide. The slide was stained with either Hematoxylin (Hematoxylin 7211, Richard-Allan Scientific, Kalamazoo, MI.) and Eosin (Eosin-Y with Phloxine, Richard-Allan Scientific, Kalamazoo, MI.), or Alcian Blue at pH 2.5 (Sigma Chemical Company, St. Louis, MO.) using the Leica Autostainer XL (Leica, Deerfield, IL), and then a coverslip was added. These stains were made as outlined in Carson’s text.129,130

The slides were scored by three blinded observers based on a slight modification of Kikuchi et al.’s method of histologic evaluation (Table 1) (Illustrations 10-12).103 Three researchers evaluated these slides, and then the median score was statistically analyzed using the One-Way Analysis of Variance for all groups, and within each group using Wilcoxon Sign Rank Test.
**Evaluation of Trypan Blue for Joint Lavage and Superoxide Dismutase in the Lateral Meniscectomy Rabbit Model**

**Rabbits**

Twelve male, New Zealand White rabbits weighing 2 to 3 kilograms were purchased from Covance (Denver, PA.) and housed in quarantine conditions for fourteen days to be monitored for normalcy and to allow time for acclimation to the new environment and handling. Normal activity and parameter assessment were defined as normal food and water consumption, normal stool formation, normal taxic behavior, no respiratory distress or nasal discharge, and a temperature between 38 and 40.56 degrees Celsius (°C). Rabbits were housed in a clean facility that required gowns and shoe covers as well as hand washing prior to handling of animals. In addition, the rabbits were placed individually into 4 square foot stainless steel cages and all housed in the same room with an ambient temperature of 21-22 °C. Groups were assigned randomly as before, with 8 rabbits in the surgical group and 4 serving as control rabbits that underwent no surgical procedure. The surgical stifle (right or left) was assigned immediately prior to surgery. Both assignments were made using a random numbers table. The non-surgical stifle of the rabbits undergoing surgery served as the non-surgical control limb.
**Time Line**

Day 0 = First day of the study, synovial fluid samples collected.

Day 6 = Synovial fluid samples collected.

Day 12 = Day of surgery.

Day 26 = Synovial fluid samples collected.

Day 40 = Synovial fluid samples collected.

Day 57 = Synovial fluid samples collected and rabbits euthanitized.

**Lateral Meniscectomy**

The surgical procedure was followed as previously described and performed on 8 rabbits.

**Trypan Blue with Synovial Fluid**

Trypan blue (Sigma Chemical Company, St. Louis, MO.) was made using 100 mg of a 40% pure powder diluted in 10 ml of distilled water. This 0.4% suspension was then filtered using #4 filter paper and diluted further by adding 10 ml of the 0.04% suspension to 90 ml of distilled water. A sample of the solution was read in a spectrophotometer using a 1 cm wide cuvette at a wavelength of 600 nanometers (nm). Concentrations of 10, 20, 30, 40 and 50 microliters (μl) were placed in a cuvette containing an appropriate amount of distilled water to bring the total volume to 1 ml. These samples were run in triplicate and analyzed to make a standard curve.
To determine the effect of synovial fluid absorbance, bovine synovial fluid was obtained from grossly normal appearing stifle joints at necropsy and was added to the standard dilution. Thus, 950 ml of distilled water and the trypan blue was added at the concentrations above and synovial fluid was added at 50, 40, 30, 20, 10, and 0 µl respectively. The 0.04% trypan blue was further diluted by adding 1 ml of the stock solution to 9 ml of distilled water to produce a 0.004% suspension. A standard curve was obtained as before and synovial fluid was added to the standard dilution as described earlier. The mean values of the absorbance were then statistically compared using a regression analysis.

**Synovial Fluid Collection**

Synovial fluid was collected from each rabbit twice prior to surgery and then once a week until the end of the study. The rabbits were anesthetized as outlined previously. Both stifles were shaved, aseptically prepared, and the femoropatellar joints were lavaged with sterile saline. A 22 gauge, 1 inch needle was inserted adjacent to the lateral aspect of the patella into the femoropatellar joint. A 3 ml syringe was used to inject 0.6 or 1 ml of sterile saline that was immediately aspirated. The lavage fluid used contained 0.004% trypan blue and the sample obtained from the 0.6 ml or 1 ml lavage was divided into two 1.2 ml conical vials (Cryule Vial, Wheaton, Millville, NJ.), one for spectrophotometry and one for superoxide dismutase analysis. All samples were placed in a -4° C freezer for 24 hours and then placed into a -70° C freezer for long term storage.
The extinction coefficient for trypan blue was found to be $5.17 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ using the equation:

$$A = a \times b \times c$$

**Where:**

$A$ = absorbance, $a$ = extinction coefficient, $b$ = light path (1 cm), $c$ = trypan blue molar concentration

The molar concentration of trypan blue ($c$) was calculated post injection, by using the equation above solving for $c$ and multiplying the result by a dilution factor of 20 and a molecular weight of 960.8. This provided the concentration of trypan blue in grams/liter.

Then the equation below was used:

$$\frac{c_{\text{post}}}{c_{\text{pre}}} \times 100 = \text{percent trypan blue present after lavage}$$

**Where:**

$c_{\text{pre}}$ = pre-lavage molar concentration of trypan blue

$c_{\text{post}}$ = post-lavage molar concentration of trypan blue

The percentage results were then statistically analyzed using the Tukey-Kramer method.

*Euthanasia, Necropsy*

These were performed as described previously.
**Superoxide Dismutase Assay**

This assay was performed using the Bioxytech® SOD-525™ Spectrophotometric Assay kit purchased from Oxis Health Products, Inc. (Oxis International, Inc. Portland, OR.). The procedure was modified to allow the use of a 96 well tissue culture plate.

Three hundred microliters (µl) of buffer (2-amino-2methyl-1, 3-propanediol) warmed to 37 degree Celsius (° C) was placed into each well. Thirteen microliters of deionized water was added to 4 wells as a blank and 13 µl of sample was added to all other wells. Each time period was run in triplicate on the same plate to account for variability. Next, 10 µl of reagent R2 (1, 4, 6-trimethyl-2-vinylpyridinium trifluoromethanesulfonate) was added to all the wells. The plate was incubated for 1 minute at 37 ° C. Next, 10 µl of reagent R1 (5, 6, 6a, 11b-tetrahydro-3, 9 ,10-trihydroxybenzo[c]fluorene) was added to all the wells. The plate was then vortexed for 3-4 seconds and then placed in the SpectraMax 250 Microplate Reader (Molecular Devices, Sunnyvale, CA.). The assay was run for 5 minutes recording every 11 seconds at a wavelength of 525 nm. The superoxide dismutase activity was recorded as optical density per minute for each sample. The estimated volume of synovial fluid recorded using the trypan blue assay was divided by the optical density to give activity of superoxide dismutase per milliliter of synovial fluid. The results of this assay were analyzed using a repeated measure analysis of variance.
Chapter 4

Results

Open Field Maze

During all observations, only one rabbit appeared to favor the surgical leg immediately after surgery but returned to normal use on the next two observations. In addition, one rabbit consistently did not move when placed in the open field maze prior to surgery and after surgery. There was no statistical difference of voluntary movement between the groups prior to surgery (Figure 1). In addition, no significant decrease in mobility was observed after surgery was performed. There was a significant increase (p \leq 0.008) in mobility in the group of rabbits that received systemic corticosteroid injections compared to the surgical control group.

Histopathologic Examination

Analysis of the severity of histopathologic lesions between groups revealed a trend showing the administration of corticosteroids systemically resulted in a lower average score compared to the exercise and surgical control groups (Table 2). The histopathologic criteria that had a lower score included the loss of superficial layer, erosion of cartilage, fibrillation and/or fissures, and loss of proteoglycan, though the individual scores were not statistically significant (p \leq 0.631, 0.335, 0.211, and 0.528, respectively) (Figure 2). When analyzing the difference between surgical and non-surgical contralateral stifle histopathologic lesions between all groups, systemic
administration of corticosteroid seemed to have a significant sparing effect on the erosion of cartilage severity scores ($p \leq 0.042$) (Figure 3).

When each group was compared individually between surgical and contralateral non-surgical stifles, the histopathologic score of the exercise, corticosteroid, and surgical groups showed significantly more severe lesions in surgical sections versus non-surgical sections. For all criteria $p \leq 0.03$, except for cluster formation in the surgical group ($p \geq 0.059$) (Figure 4).

**Trypan blue Lavage**

The standard curve of trypan at 0.04% and 0.004% showed a significantly different slope when synovial fluid was added ($p \leq 0.041$ and $p \leq 0.001$ respectively). However, the variance of the samples were very small ($R^2 = 99.3\%$ and 98.3 % respectively) and the difference between slopes was between $-0.0006557$ and $-0.00010667$ (Figure 5).

There was no significant difference between the volume of synovial fluid aspirated on day 0 compared to day 6. A significant increase was found in the calculated volume of synovial fluid after surgery (day 12) on days 26, 40 ($p \leq 0.0001$) and 51 ($p \leq 0.02$) when compared to day 0 and day 6. There was also a significant decrease in the volume of synovial fluid aspirated post surgery on day 40 ($p \leq 0.0019$) and day 57 ($p \leq 0.0001$) compared to day 26 (Figure 6).

When comparing surgical to non-surgical legs over time, days 0, 6, 40 and 57 showed no statistical difference ($p \leq 0.94, 0.61, 0.38$, and 0.06, respectively). However,
day 26 showed a statistically significant increase in the volume of synovial fluid from the surgical leg versus the non-surgical leg ($p \leq 0.001$) (Figure 7). Also, when all time periods were combined, the surgical leg had a significantly greater volume of synovial fluid harvested compared to the non-surgical leg ($p \leq 0.005$) (Figure 8).

In addition, the volume of synovial fluid aspirated from the surgical joint did significantly increase from day 6 to day 26 ($p \leq 0.0007$) with surgery occurring on day 12. The non-surgical leg also showed a significant increase in synovial fluid volume harvested from day 6 to day 26 ($p \leq 0.001$). Comparing day 26 to day 40 showed a significant decrease in synovial fluid volume harvested from the surgical leg ($p=0.002$), but not in the non-surgical leg ($p \leq 0.28$) (Figure 7).

**Superoxide Dismutase Assay**

The superoxide dismutase activity was not statistically different across time or between any two time periods when compared between groups (Figure 9). When adjusted using the estimated volume of synovial fluid based on the trypan blue assay, no significant difference across time or between any two time periods was noted between groups (Figure 10).
Chapter 5

Discussion

The lateral meniscectomy model created greater histologic osteoarthritic lesions in all surgical rabbits compared to non-surgical stifles. These lesions were comparable to lesions described by Mankin observed in human osteoarthritis and readily repeatable. This repeatability was based on the fact that all stifles that underwent surgery had a higher histologic severity score compared to the non-surgical stifle.

The results of the open field maze indicate that rabbits treated with systemic corticosteroid injections without exercise increased mobility compared to surgical controls. Through the reduction of phospholipase A2, corticosteroids decrease inflammation and therefore reduced pain. Other than decreased inflammation, another theory for the increased mobility in non-exercise rabbits compared to surgical controls, is the ability of corticosteroids to increase gluconeogenesis and inhibits insulin from acting on insulin receptors. This increase may have caused a hyperglycemia in the rabbits making them more hyperactive. Though the glucose level in the blood was not measured, this theory may explain the increased mobility seen in the open field maze with the systemic corticosteroid administration group. In addition, no significant decrease in mobility was noted throughout the study, regardless of the group. The open field maze itself provides a method to evaluate pain with voluntary movement. Therefore, the lack of a decrease in mobility after surgery implies that the lateral meniscectomy model does not cause enough pain to limit the rabbit’s desire to move. Corticosteroid administration
actually increased motility compared to the surgical control group, most likely due to a combination of effects seen with systemic corticosteroid administration.

The open field maze study was conducted in the same room as the rabbits were housed and subtle distractions were present including the observer. However, the observation that no lameness was noted after 9 days after surgery, and that there was not significantly decreased mobility throughout the study, (Figure 1) suggests that the development of osteoarthritis in this model does not limit a rabbit’s desire to ambulate. The study also shows that the open field maze is an easy and objective method to assess voluntary movement in the New Zealand White rabbit.

The criteria (Tables 1&2) for the histologic evaluation of the cartilage sections were selected based on a scoring system developed by Kikuchi et al. The criteria were used as a representation of early changes in osteoarthritis. Transferred forces through the subchondral bone were hypothesized to increase after surgery due to destabilization of the joint and partial loss of the meniscus. Histopathologic examination of the epiphyseal growth plate was included to see if any changes could be seen in the chondrocyte organization but none were seen. However, the epiphyseal growth plate was not uniformly present, or the section was not oriented parallel to the vertical plane of chondrocytes, thereby making an accurate evaluation nearly impossible in some sections, so the results of the score was not included in this study.

Corticosteroid administration every 4 days had a significant sparing effect on the erosion of cartilage caused by surgery. In addition, corticosteroid administration had a slight effect on reducing cartilage damage overall, except for proteoglycan loss. This
result could be due to a decrease in the inflammatory component of osteoarthritis caused by the administration of corticosteroids. Though not considered the primary etiology in this osteoarthritis model, the corticosteroid administration would cause a decrease in the catabolic enzymes such as collagenase and prostaglandins present due to the inflammatory cascade. The loss of proteoglycans observed could be due to the adverse effect of corticosteroids on matrix formation. \(^{119}\) This result supports the theory that corticosteroids decrease the production of extracellular matrix, even when administered systemically once every four days for prolonged periods of time. By administering corticosteroids every 4 days, it is believed that iatrogenic hyperadrenocortism is avoided by allowing enough time between doses to avoid additive effects. However, it is possible that hyperglycemia was caused by the corticosteroid administration. Therefore, the use of systemic corticosteroids would help decrease pain associated with inflammation, which combined with a possible hyperglycemia, would explain the increased mobility seen in the open field maze.

Moderate exercise of a destabilized joint did not exacerbate the histologic lesions (Figures 2-4). One explanation for this is that the supporting musculature of the stifle helped stabilize the joint, and the relative low speed of exercise did not produce enough traumatic force to severely damage the cartilage. Though no evidence of limping was observed, the rabbits may have favored the destabilized joint by placing less weight on the surgical leg, again decreasing trauma to the articular cartilage. A second explanation is that the moderate exercise increased proteoglycan synthesis and the metabolism of the chondrocytes, allowing for the articular cartilage to compensate for the destabilization.\(^{28}\)
A third explanation is that the exercise was not severe enough to be different from the exercise that can be obtained with normal cage movement.

Overall sources of error with the histologic grading include variability of the histologic section and staining consistency. Though all sections were taken as close as possible to the same point, the 5 µm sections could still be skewed from one sample to the next. Also, although the slides were stained at the same time, the stain uptake appeared variable between sections. The individual bias of the observers was one other source for error.

Trypan blue is an inert dye that stains non-viable tissue and is readily measured at a wavelength 600 nm. Two volumes were used for lavage, 1 and 0.6 ml. This was done because 1 ml of lavage solution distended the femoropatellar joint too severely causing increased blood contamination. Therefore after the first sampling, the lavage volume was decreased to 0.6 ml. Statistical analysis showed that increasing concentrations of synovial fluid in the trypan blue standard caused a significant difference in the standard curve (Figure 5). The small variance results in even small differences to be statistically significant. However, from a practical standpoint, synovial fluid is not considered to interfere with the absorbance of trypan blue. Based on this data, trypan blue makes an excellent marker for synovial fluid lavage, since the synovial fluid itself does not confound it and since trypan blue is relatively inert.

This study showed that the volume of harvested synovial fluid in the rabbit stifle increased significantly after surgery (day 12). Surgery causes an acute synovitis due to the incision through the synovial membrane resulting in vasodilation and increased
extravasation of fluid into the stifle joint. This explains the larger volume of synovial fluid immediately after surgery that then, though still significantly higher then prior to surgery, decreased over time. Though the study ended, two explanations for the decreasing synovial fluid volume after surgery can be offered. One is that the acute inflammatory event (surgery) had passed and the joint was slowly returning to homeostasis. The second, and more likely, is that the acute inflammation from surgery was over, but due to the destabilization that occurred, a low grade inflammatory process persisted causing an increased volume of synovial fluid in the joint from baseline, but not as high as that caused by the surgery. This inflammatory event was perpetuated by degradation of the cartilage releasing enzymes and debris that would, along with joint instability, continue to cause inflammation of the synovium and synovial effusion.

The overall significant increase in synovial fluid volume harvested in the surgical stifle compared to the non-surgical stifle, suggests that response was a direct result of surgery and not a systemic response. However, when the surgical and non-surgical legs are compared over time, an overall increase of synovial fluid harvested was present in both stifle joints. This increase was uniform prior to surgery suggesting that a mild synovitis was present due to the lavage procedure. The lavage on day 26 was significantly different between the surgical and non-surgical legs, but the increase when compared to the synovial fluid harvested prior to surgery in both the surgical and non-surgical legs, is even more pronounced. Two theories can be offered for this increase in synovial fluid harvested. One is that surgery may have a systemic response causing a bilateral synovitis. After surgery on days 40 and 57, the volume of synovial fluid
harvested nearly equilibrates in both surgical and non-surgical legs. This shows that though the initial trauma of surgery causes an acute synovitis in the surgical leg, the lateral meniscectomy produced an increase in synovial fluid production both in the surgical and non-surgical leg. This increase in the non-surgical leg could be due to the lavage procedure itself, it could be an indication of a systemic response to the osteoarthritis, or it could be due to increased weight bearing on the non-surgical contralateral limb. The systemic response would be due to the presence of inflammatory products in the blood causing a generalized systemic inflammatory response not just localized to the affected leg. A second theory is that the rabbits favored the surgical leg, though not severely enough to be noted in the open field maze, inducing a mild synovitis with associated effusion in the non-surgical contralateral limb. The rabbits that had no surgical procedure were not included in the statistical analysis because the variability of individual rabbits could not be compensated for and therefore a fair comparison could not be accomplished.

The main limiting factor of the trypan blue analysis in this study is that the total amount of synovial fluid present from in the joint is not harvested. Therefore, the volume of the synovial fluid must be estimated based on the amount harvested. The short duration that the trypan blue was in the joint and its relative inert properties makes these preliminary findings worth investigating further.

The superoxide dismutase activity based on the Oxis Biotech® Assay showed no statistical significance or trends when the data points were analyzed. The appearance of the data points was suggestive of pathological processes and further investigation is
warranted. Superoxide dismutase activity appeared to increase from day 0 to day 6, illustrating that the injection and lavage of the stifle joint caused a slight inflammatory response. When superoxide dismutase activity was adjusted to show activity per milliliter of synovial fluid using the results attained from the trypan blue assay, the increased activity was very similar throughout the three groups (the surgical leg, the non-surgical leg and the control rabbits) at day 0 and day 6. This would be expected with the introduction of a needle through the synovial membrane as well as distending the joint with fluid, causing a mild synovitis that would increase free radical production and therefore increase superoxide dismutase concentration.

The data points at day 26 (14 days after surgery) show that the superoxide dismutase activity in the control rabbits was at the same concentration as the surgical legs with a different volume of synovial fluid. In addition, the contralateral normal leg of the rabbits that underwent surgery had a higher concentration of superoxide dismutase activity than the two other groups. This suggests that the contralateral non-surgical stifles of the surgical rabbits have more concentrated amount of superoxide dismutase (more superoxide dismutase present in less volume of synovial fluid) than the control rabbits or the surgical legs. This finding was deduced by the previous results reported from the trypan blue study, that found surgical legs had a larger volume of synovial fluid than the contralateral leg. An increase in superoxide dismutase activity in both stifles after surgery is attributable to a systemic inflammatory event, or possibly inflammation in the surgical stifle causing the rabbit to place more weight and thereby place abnormal stress on the non-surgical contralateral limb. As for the difference between the concentration of
superoxide dismutase between the surgical and the contralateral normal legs, Myers et al. showed that in the dogs that underwent anterior cruciate ligament transection increased protein clearance 3 fold when compared to the opposite non-surgical limb. This rapid turnover could remove excess superoxide dismutase present in the surgically destabilized limb, however the non-surgical leg might show an increased concentration since it could have a slower clearance rate. This increased concentration may also be partially explained by increased weight shifting on the contralateral non-surgical leg, or it could be an indication of a systemic response to osteoarthritis.

Day 57 (45 days after surgery) showed that the surgical rabbits overall had the same level of superoxide dismutase activity in the stifle joints of the surgical and contralateral normal limbs. This is supported by the trypan blue results, that suggested the stifles were returning to homeostasis. However, since less synovial fluid was present in the normal leg, a higher concentration of activity per milliliter of synovial fluid was present. Again, these findings suggest an increased superoxide dismutase activity that may be due to a decreased clearance in the contralateral normal leg. The increased activity in the control rabbits infers an additive effect of multiple lavages, causing an increased inflammatory response, and also could be due to the lysis of red blood cells, which contain a large quantity of superoxide dismutase, during the lavage procedure.

This hypothesis of a systemic inflammatory response arising from the instability of one joint leading to the involvement of multiple joints in osteoarthritis warrants further investigation due to its potential biologic and medical implications. However, the suggestion that osteoarthritis of a single joint can cause increased superoxide radicals and
synovial fluid production in other joints should guide further studies to address the affected, as well as the non-affected joints.

The benefit of combining the results of the superoxide dismutase assay and the trypan blue analysis, is that a more precise comparison of the samples over time can be performed. The variability of harvested synovial fluid present in each sample can be taken into account, eliminating the inaccuracy of normal lavage techniques. Also, the use of the Bioxytech® SOD-525™ Spectrophotometric Assay kit for superoxide dismutase analysis avoids the need of batch sampling, since only a small amount of synovial fluid is needed for analysis.

The main source for error in this assay is that the activity level obtained is not a true quantitation of superoxide dismutase. No other marker was coupled with the result (such as protein), and so the concentration that is referred to above is inferred based on the activity present that is read as optical density per minute. Other sources of error with the superoxide dismutase assay include slight interference of trypan blue at the 525 nm, and the presence of lysed red blood cells due to the collection procedure. The first variable is accounted for as there is trypan blue in every sample, and the interference is minimal since the absorbance of trypan blue is 600 nm. The second variable of lysed red blood cells can only be offset by a larger sample size. The problem arises since red blood cells contain superoxide dismutase and could therefore skew the results if lysed. In this study, approximately 24 samples per time period were analyzed, but this number varied due to inadequate sample volume or severe blood contamination (only 12 samples were analyzed for day 0; 23 on days 6 and 26; 22 samples on day 57).
The lack of statistical significance of the superoxide dismutase activity is explained due to the high variability of each sample and the small sample size. The variability could be because of incomplete mixing of the synovial fluid and the sterile saline used for lavage, interference from the trypan blue, the low relative activity and therefore amount of superoxide dismutase in each sample, or individual variation between rabbits. In future work, more samples will be needed to account for these variables.
This study found that:

1. The lateral meniscectomy model caused reproducible histologic lesions in 6 weeks that are comparable to lesions seen with human osteoarthritis.

2. The lateral meniscectomy did not cause a significant increase in pain as measured by voluntary movement, limping, or observation of generalized discomfort as compared with pre-surgery movement in any treatment. Systemic corticosteroid administration increased voluntary movement.

3. Moderate exercise of the destabilized joint did not cause significant histological exacerbation of osteoarthritis. Systemic corticosteroids had a significant sparing effect on the erosion of cartilage.

4. Trypan blue provided a reliable marker for calculation of concentration in arthrocentesis lavage procedures.

5. Superoxide dismutase activity in synovial fluid during this study showed an increased production, possibly due to the lavage techniques as well as surgery, though no statistical significance can be attributed to these findings. The superoxide dismutase activity appears to support the findings of Myers et al., of increased synovial fluid turnover in an osteoarthritic joint. Our data suggests that there may be a systemic response or a compensation by the contralateral limb too subtle to be seen
with observation of movement. In the control rabbits, superoxide
dismutase activity suggested that multiple lavages of a normal joint cause
a mild, yet progressively heightened, inflammatory response.

Further studies related to this project could include use of the trypan blue lavage
so that substances such as hyaluronic acid, keratin sulfate, Type II collagen or
hydroxyproline could be more accurately measured in a harvested synovial fluid centesis.
In addition, further exploration of the systemic effects of osteoarthritis, both with
synovial fluid volume and superoxide dismutase, should be re-evaluated using a larger
sample size to help reduce individual variability. The hypothesis of a systemic response
could be address by evaluating other diarthrodial joints, such as carpal or gleno-humeral,
that are not directly contralateral to the surgically induced osteoarthritic joint. However,
due to the limited size of the rabbit, this might be better evaluated in the canine or equine.
Also, nuclear scintigraphy may aid in identifying areas of inflammation by highlighting
areas of increased concentration of the radiolabeled marker during the soft tissue phase.
It would also be beneficial to continue the experiment past 45 days after surgery, to see if
the regression of synovial fluid volume continues to more normal levels, or remains
elevated. The use of more uniform histologic stains, and perhaps the aid of computer
imaging, might allow for a more objective method of evaluating the severity of histologic
lesions. Also further exploration of the effects of corticosteroids and exercise together
could be evaluated as could more specific methods to assess pain in this rabbit model
such as joint manipulation.
Literature Cited


Appendix

Illustration 1

*Joint Anatomy of Diarthrodial Joint*

Illustration 2

*Ligaments of the Stifle*

Illustration 3

Menisci and ligaments, proximal end of the left tibia

Illustration 4

*Normal Layers of Cartilage*
Illustration 5

Association of Collagen, Hyaluronic Acid, and Proteoglycans

Illustration 6

Scanning electron microscopy of surgically induced osteoarthritis lesion surrounded by normal cartilage.

Taken from femur of rabbit from surgical group

Magnification of 20 X
Illustration 7

Open field maze

Four foot square divided into sixteen boxes measuring one square foot each
Partition used to exercise two rabbits at the same time. Rabbits were exercised at 0.3 miles per hour for 10-15 minutes, twice a day, 5 days per week.
Illustration 9

Photo of tibial and femoral condyles

Line represents the band saw cut made through the lateral condyles of the femur and tibia (from left to right) and thus the beginning of the section for histology.
Illustration 10

Sections of articular cartilage for surgical group stained with Alcian Blue viewed at 40 X magnification

Surgical Femur – Surgical Group

Non Surgical Femur – Surgical Group

The surgical femur has slightly noticeable irregularities in the surface and decreased proteoglycan staining. The non-surgical femur also has some slight surface irregularities.
Illustration 11

Sections of articular cartilage from exercise group stained with Alcian Blue viewed at 40 X magnification.

Surgical Femur – Exercise Group

Non Surgical Femur – Exercise Group

The surgical femur has noticeable irregularities in the surface and decrease proteoglycan content, determined by decreased stain intensity compared to the non-surgical femur.
Illustration 12

Sections of articular cartilage from corticosteroid group stained with Alcian Blue viewed at 40 X magnification

The surgical femur has noticeable irregularities in the surface, clusters of chondrocytes and decreased proteoglycan staining. In contrast, the non-surgical femur has a smooth surface and few chondrocyte clusters.
Scheme 1

Factors effecting degeneration of articular cartilage

This figure illustrates the square root of the mean number of squares moved in each group over all time periods. The square root was taken to help decrease variability.

Note: different letters indicate statistically significant difference.
No values are statistically different. However, the corticosteroid group does appear to have less severe lesions than the other two groups. This table illustrates the variability of the scoring system. The error bars represent one standard deviation.

Note: All rabbits in this experiment underwent a lateral meniscectomy in one stifle.
This table represents the histologic severity score when the mean surgical stifle severity score of the 3 observations is subtracted from the non-surgical stifle severity score. This table illustrates the variability of the scoring system and the fact that the corticosteroid group has consistently less severe than the other two groups. The error bars represent one standard deviation.

* The erosion of cartilage was found to be statistically the same between the surgical and non-surgical stifles (p \leq 0.042)
Figure 4

Histologic severity scores of the surgical versus non-surgical stifles

This table illustrates the median of the Wilcoxon Sign Rank Test and shows that each individual group has a significant increased score compared to the non-surgical contralateral joint with the exception of the surgical control group with regards to cluster formation (marked with an *) where $p = 0.059$
This table uses a standard concentration of trypan blue mixed with an increased amount of bovine synovial fluid. For Sample 1 and 2, the concentration of synovial fluid (up to 50 microliters) is inversely proportional to the concentration of trypan blue. The results show that synovial fluid does not interfere with the absorbance of trypan blue. The error bars represent one standard deviation.
Figure 6

*Calculated volume of synovial fluid using the trypan blue assay*

This table illustrates a statistically significant increase in synovial fluid volume after surgery (day 12) followed by significant decreases in volume until the conclusion of the study. The error bars represent the standard error.

Note: Means with the same letters are statistically the same.
Figure 7

Synovial fluid volume harvested using the trypan blue assay comparing surgical to non-surgical legs.

The results show a significant bilateral increase in synovial fluid content after surgery on day 12 that equalizes but remains elevated throughout the duration of the study. The error bars represent the standard error.
Combining all time periods, there was a significant increase in synovial fluid volume in the surgical legs compared to the non-surgical legs ($p \leq 0.005$).
This figure illustrates that the data points, though not significant, have a steady increase in superoxide dismutase activity over time, with surgery on day 12 (black line), which then slowly resolves in the surgical rabbits. The error bars represent the standard error.

Note: no values are statistically different
Figure 10

Superoxide dismutase activity per milliliter of synovial fluid

This figure shows that superoxide dismutase activity per milliliter of synovial fluid is higher in the non-surgical stifle and the repeated joint lavage causes a steady increase in activity over time. Surgery occurred on day 12 (black line).

Note: no values are statistically different. The synovial fluid volume is calculated from the trypan blue assay results. The error bars represent the standard error.
<table>
<thead>
<tr>
<th>Loss of superficial layer</th>
<th>+0</th>
<th>+1</th>
<th>+2</th>
<th>+3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slight</td>
<td>Moderate</td>
<td>Focally severe</td>
<td>Extensively severe</td>
<td></td>
</tr>
<tr>
<td>Erosion of cartilage</td>
<td>Not Detectable</td>
<td>Moderate</td>
<td>Focally severe</td>
<td>Extensively severe</td>
</tr>
<tr>
<td>Not Noticeable (1 very small)</td>
<td>Moderate (1 small)</td>
<td>Marked (2 small or 1 medium)</td>
<td>Extensively severe (3 small, 2 medium or 1 large)</td>
<td></td>
</tr>
<tr>
<td>Loss of proteoglycan</td>
<td>Stain similar throughout section</td>
<td>Moderate loss of Alcian stain</td>
<td>Marked loss of Alcian stain</td>
<td>Total loss of Alcian stain</td>
</tr>
<tr>
<td>Disorganization of chondrocytes</td>
<td>Mildly noticeable irregular distribution of cells</td>
<td>Moderate irregular distribution of cells</td>
<td>Marked irregular distribution of cells</td>
<td>Very extensive irregular distribution of cells</td>
</tr>
<tr>
<td>Cluster formation</td>
<td>3-4 small or 1-2 medium</td>
<td>5-6 small, 3-4 medium or 1-2 large</td>
<td>7 or more medium or 5-6 large</td>
<td>7 or more small, 5-6 medium or 3-4 large</td>
</tr>
<tr>
<td>Disorganization of growth plate</td>
<td>Chondrocytes in vertical columns</td>
<td>&lt;2 rows of chondrocytes skewed</td>
<td>Focal area of chondrocytes skewed</td>
<td>Diffuse area of chondrocytes skewed</td>
</tr>
</tbody>
</table>
## Table 2

*Mean histopathologic severity score*

<table>
<thead>
<tr>
<th>Observer</th>
<th>Treatment</th>
<th>Loss of Superficial Layer</th>
<th>Erosion of Cartilage</th>
<th>Fibrillation &amp; Fissures</th>
<th>Loss of Proteoglycans</th>
<th>Disorganization of Chondrocytes</th>
<th>Cluster Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise</td>
<td>1</td>
<td>1.62 +/- 0.96</td>
<td>1.54 +/- 1.05</td>
<td>1.08 +/- 0.95</td>
<td>1.46 +/- 0.88</td>
<td>1.15 +/- 0.80</td>
<td>1.15 +/- 1.07</td>
</tr>
<tr>
<td>Steroid</td>
<td>1</td>
<td>1.46 +/- 1.05</td>
<td>1.46 +/- 1.05</td>
<td>0.92 +/- 1.04</td>
<td>1.31 +/- 0.63</td>
<td>1.46 +/- 0.88</td>
<td>1.00 +/- 0.91</td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>1.38 +/- 1.12</td>
<td>1.31 +/- 1.11</td>
<td>0.77 +/- 1.01</td>
<td>1.15 +/- 0.99</td>
<td>1.23 +/- 1.01</td>
<td>0.85 +/- 0.80</td>
</tr>
<tr>
<td>Exercise</td>
<td>2</td>
<td>1.00 +/- 1.15</td>
<td>1.08 +/- 1.19</td>
<td>0.92 +/- 1.12</td>
<td>1.62 +/- 1.12</td>
<td>1.15 +/- 0.90</td>
<td>0.46 +/- 0.52</td>
</tr>
<tr>
<td>Steroid</td>
<td>2</td>
<td>0.92 +/- 1.04</td>
<td>0.69 +/- 1.11</td>
<td>0.62 +/- 0.87</td>
<td>1.62 +/- 0.96</td>
<td>1.08 +/- 0.86</td>
<td>0.77 +/- 0.60</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>0.92 +/- 1.04</td>
<td>0.92 +/- 1.12</td>
<td>0.85 +/- 1.21</td>
<td>1.08 +/- 1.19</td>
<td>0.92 +/- 1.04</td>
<td>0.85 +/- 1.07</td>
</tr>
<tr>
<td>Exercise</td>
<td>3</td>
<td>1.08 +/- 1.19</td>
<td>1.08 +/- 1.19</td>
<td>1.00 +/- 1.22</td>
<td>1.46 +/- 1.20</td>
<td>1.15 +/- 1.28</td>
<td>1.15 +/- 1.28</td>
</tr>
<tr>
<td>Steroid</td>
<td>3</td>
<td>1.00 +/- 1.22</td>
<td>1.00 +/- 1.22</td>
<td>1.08 +/- 1.12</td>
<td>1.38 +/- 1.26</td>
<td>1.08 +/- 1.19</td>
<td>1.31 +/- 1.18</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>1.08 +/- 1.26</td>
<td>1.15 +/- 1.21</td>
<td>1.08 +/- 1.32</td>
<td>1.08 +/- 1.32</td>
<td>0.92 +/- 1.26</td>
<td>0.85 +/- 1.21</td>
</tr>
</tbody>
</table>

This table shows the average histologic severity score per criteria by observer +/- one standard deviation. The scores from the three observers were combined to arrive at the mean and standard deviation represented in the results.
Curriculum Vitae

Anthony Paul Pease
12806 Cambleton Drive
Upper Marlboro, MD 20774
Phone: 301-249-7993
Email: apease@vt.edu

Present Position: Intern in Surgery and Medicine at the Marion duPont Scott Equine Medical Center
Born: March 28, 1973; Fairfax, VA.
Martial Status: Single

Education

Bachelor of Science
University of Maryland College Park
May 1995

Doctor of Veterinary Medicine
Virginia-Maryland Regional College of Veterinary Medicine
May 1999

Master of Science Degree in Veterinary Medical Science

Novel approaches to evaluate osteoarthritis in the rabbit lateral meniscectomy model

This two-part project was part of a parallel program where the research was conducted concurrently with the Doctorate in Veterinary Medicine training. Responsibilities included devising and implementing an original research project, supervision of up to 28 rabbits in the research population, participation in surgery under the supervision of Dr. Spencer Johnston, Diplomate ACVS in Small Animal Surgery. In addition, I performed necropsy and histopathologic evaluation of all rabbits under the supervision of Dr. Hugo Veit, Associate Professor in Pathology.

Defense date June 5, 2000
Work Experience

Present Position

Intern in Medicine and Surgery
Marion duPont Scott Equine Medical Center
Responsibilities include care for surgical and medical cases on a rotating basis, case presentations, participation in daily rounds and weekly grand rounds, and emergency on-call every third night. The internship involves evaluation and problem solving skills as well as assisting the resident, clinical instructor and faculty. In addition, I assist on all surgeries performed by my service and all emergency surgeries when on-call. Finally, interns are responsible for monitoring and assisting the resident with post-operative care. My supervisors include Drs. Martin Furr, Michael Murray, Ken Sullins and Nat White.

Previous Experience

Clinical Veterinarian Externship in Research and Development
The Iams Company, Lewisburg, OH
Responsibilities include daily observation of 600 dogs and cats in a closed colony including health checks, physical exams, screening tests, blood collection, and urinalysis. Also assist with radiographs and surgery as needed. In addition, aid in nutritional research studies and diagnosis, treat and report on any ailments affecting the dog colony.

Remote Area Medical Volunteer
Associated with Tennessee University
Free spay and neuter clinic offered on weekends in rural areas. Responsibilities included pre-surgery physical exams, vaccinations, anesthesia, and primary surgeon.

Laboratory Technician
United States Department of Agriculture, Beltsville MD
Coccidia research involving White Leghorn Chickens. Responsibilities include fecal cytology, fluorescent antibody staining, cervical dislocation, necropsy, statistical analysis and assistance with manuscript preparation.

Emergency Small Animal Assistant
Emergency Animal Hospital, Glenn Dale, MD
This hospital served for after hour small animal emergencies. Responsibility included assisting the veterinarian with triage, radiographs and surgery. Also, with the day to day care of the animals in the hospital and assisting the technicians as needed.
Awards and Honors
WARDs Humane Treatment of Animals Essay Scholarship 1998-1999
Winslow Scholarship 1998-1999
H.M & M.M Raulet Scholarship 1996-1997
The United Professional Horsemans Association Award 1996-1997
One of two people selected out of a class of 81 to participate in the parallel program 1995-1999

Presentations
Arytenoid chondritis treated with a partial arytenoidectomy. – Marion duPont Scott Equine Medical Center, Case Presentation for faculty, clinical instructors and residents. (September 1999)

Dysphasia due to a laryngeal mass in an elderly pony - Marion duPont Scott Equine Medical Center, Case Presentation for faculty, clinical instructors and residents. (December 1999)

Effects of exercise or corticosteroids in the rabbit lateral meniscectomy model - Marion duPont Scott Equine Medical Center, Case Presentation for faculty, clinical instructors and residents. (March 2000)

End-to-end small intestinal resection and anastomosis due to epiploic foramen entrapment - Marion duPont Scott Equine Medical Center, Case Presentation for faculty, clinical instructors and residents. (April 2000)

Publications


Associations and Licenses
Member of the American Veterinary Medical Association
State licensed in Maryland and Florida
References

Peter K Shires BVSc, MS, Diplomate ACVS
Professor, Small Animal Surgery
Director Veterinary Educational Technologies
Virginia-Maryland Regional College of Veterinary Medicine
Virginia Tech
Blacksburg, VA 24061-0442
Phone: 540-231-5891 Fax: 540-231-7367

Martin Furr, DVM, Diplomate ACVIM
Associate Professor of Medicine
Marion duPont Scott Equine Medical Center
P.O. Box 1938
Leesburg, VA 20176
Phone: 703-771-6800 Fax: 703-771-6810

Maurice Docton, BS, DVM
Clinical Veterinarian
Animal Care Center, Research and Development
6571 St. Right. 503 North
P.O. Box 189
Lewisburg, OH 45338
Phone: 937-415-8988 Fax: 937-415-8923

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Anthony Paul Pease, DVM