1. Introduction: State of the Art in 1975

Equine degenerative arthritis was reported as an entity in 1938 by Kelser and Callender\(^1\) based on a comparison of the pathological changes in humans and horses by the same authors.\(^2\) Equine osteoarthritis (OA; the common name now) was reviewed by Mackay-Smith in 1962,\(^3\) and it received its first clinical attention at AAEP in 1966 with a presentation on pathophysiology of equine degenerative joint disease and lameness by the clinical pioneers Raker et al.\(^4\) Until this stage, the studies had been postmortem studies, with little correlation to clinical signs or clinical significance, and there had been considerable extrapolation from human literature. Clinical problems in the fetlock joint were considered to correlate with articular cartilage lesions by Rooney,\(^5\) but in another study in Standardbred fetlocks, although there was good correlation between lameness and pathologic change in the synovial membrane and fibrous joint capsule, degenerative cartilage lesions were not well correlated with evidence of pain, and wear lines were commonly seen.\(^6,7\) Degenerative cartilage lesions were not considered to be painful if they did not involve subchondral bone.

Therefore, in 1975, articular cartilage lesions were considered the indispensable criteria of OA, but it was also recognized that they may not be the centrally important cause of clinical disease. Today, equine OA may be considered as a group of disorders characterized by a common end stage: progressive deterioration of the articular cartilage accompanied by changes in the bone and soft tissues of the joint. The deterioration of the articular cartilage is characterized by local splitting and fragmentation (fibrillation) of articular cartilage. Synovitis and joint effusion are often associated with the disease, and, clinically, the disease is characterized by pain and dysfunction of the affected joint.

In the early days, there was considerable extrapolation of equine OA to human OA, but the literature could be confusing, and, retrospectively, extrapolation was not appropriate. Human OA was initially classified conventionally into primary and secondary varieties.\(^9\) The term “primary” was used when the causes were unidentified and was typified by the insidiously developing disease of old people. The term “secondary” was used when an etiologic factor could be demonstrated. The term degenerative joint disease was used as a synonym for primary OA. However, as etiologic factors were identified, the distinction between primary and secondary lesions became difficult, and OA and degenerative joint disease are now used synonymously for
all forms of OA. By 1975, the terms thinning (synonymous with superficial erosions), partial-thickness of cartilage, ulcerations, and erosions (representing full-thickness loss of cartilage) were being used. Grooves or “wear lines” were common findings of articular cartilage in equine ginglymal joints and were recognized as being superficial or deep. Histologic definitions had developed in human work, including flaking or early fibrillation, and when the process extended to the radiate layer, it was described as “fibrillation.” As fibrillation extended through the radiate layer, it was recognized that vertical clefts form in full-thickness fragmentation and loss of articular cartilage can occur. This degree of change is represented grossly by full-thickness erosion. Wear lines are represented histologically by varying layers of fibrillation, with deep ones appearing similar to deep erosions.Variable amounts of necrosis among chondrocytes of fibrillated cartilage were recognized on histologic examination, and multicellular clusters or chondrones developed from other viable chondrocytes that were considered a reactive response. In addition to the above morphologic changes, histochemical staining indicated depletion of proteoglycans from the articular cartilage and an increase in the water content of the cartilage.

Although examinations of osteoarthritic joints were generally limited to morphologic observations in the mid-1970s, the concept of “use trauma” had become a central etiologic concept. These observations had been made in association with examination of highly mobile joints of the racehorse where changes were first seen in the joint margins, and a sequence of cartilage discoloration, fraying, erosion, and ulceration recognized. Wear lines secondary to chronic osteochondral fragmentation had been observed in the carpus and fetlock, as well as marginal osteophyte formation. These changes had been associated with concurrent traumatic damage to the attachment of the joint capsule and ligaments.

Because the information was never published in the referred literature, a 1942 MS thesis on equine degenerative arthritis from Cornell University generally went unnoticed. Sippel recognized the existence of synovial fossae that develop postnatally and are seen in all horses more than 3 yr of age. They are unassociated with lameness. In humans, it was recognized that fibrillation of articular cartilage could develop in the absence of clinical signs of OA. Two types of alterations in articular cartilage were described in the human hip in 1970. The first, or “non-progressive”, is limited to cartilage alteration and is related to age. The second type, a “progressive” lesion, is that of OA. It is still difficult to define whether non-progressive lesions have the potential to progress to clinical disease in humans, and the answer to this question in the horse awaits clarification.

Other than the use of radiography and clinical examination, little attention had been paid to diagnosis. The use of corticosteroids for therapy was introduced in 1955 by Wheat when he published the use of hydrocortisone to treat clinical muscular conditions in 94 horses and cattle. This paper was followed by a series of investigations by Van Pelt and co-workers, evaluating a number of corticosteroid preparations as treatments for a variety of clinical conditions. A number of clinical trials had also been reported. Mostly favorable results have been reported, but all studies were poorly controlled.

The first paper indicting corticosteroids as harmful in the horse was written by O’Connor in 1968. The report was based on some papers in the human literature and included the statement: “an endless destructive cycle is set into motion, which if continued will produce a steroid arthropathy which can render the horse useless”. An abstract written by an anonymous author was cited as a reference. Alarming statements in discussing corticosteroids were also published in an equine pharmacology textbook, but fortunately modern research has clarified and refuted many of these generalizations (presented later). In 1975, corticosteroids were the principal therapy for OA discussed, but, in 1970, there was a publication in which traumatic degenerative arthritis was treated with methylprednisolone acetate (MPA) or a hyaluronic acid/MPA combination (20 racing Thoroughbreds and Standardbreds).

The first course in arthroscopy of the human knee was held in the United States in 1973, and by 1975, the arthroscope began to achieve real clinical use in human orthopedics. Diagnostic arthroscopy initially met with considerable skepticism by human orthopedic specialists, until its value in the total evaluation of the knee was demonstrated. In the middle of the 1970s, arthroscopy moved into its second phase of its development with the realization of the potential to perform surgery under arthroscopic visualization. Large animal arthroscopy was first presented in the German literature in 1973, and diagnostic arthroscopy of equine carpal joints in three horses was reported in 1975.

2. Clarification of the Pathogenesis of Equine OA

Although it was recognized in 1966 that articular cartilage change in osteochondral fragmentation could be associated with concurrent traumatic damage to the attachments of the joint capsule and ligaments, little association had been made between primary disease in the synovial membrane and fibrous joint capsule and the development of osteoarthritic change in the articular cartilage. The significance of synovial membrane inflammation in the pathogenesis of equine OA was evaluated by the author in an experimental model based on the intraarticular (IA) injection of the polyeone antibiotic filipin. Filipin is a drug capable of disrupting lysosomes, and this experimental model produced morphologic and biochemical lesions of OA in the articular cartilage (Fig. 1). This research showed
that cartilage degradation could occur in the absence of instability or traumatic disruption of tissues, and loss of glycosaminoglycan (GAG) staining was associated with early morphologic breakdown at the surface of the cartilage. Recent research with cells and tissues of equine joints has shown that synovial cells are a good source of proteolytic enzymes, active against both collagen and proteoglycans and their component (GAG). Synoviocytes and chondrocytes isolated from healthy joint tissue exhibit no detectable enzyme production, but stimulation of the cells with an extract containing interleukin (IL)-1 produced high concentrations of stromelysin (also called proteoglycanase or metalloproteinase [MMP]-3). The mechanisms causing biochemical degradation have since been elucidated and will be discussed below.

At the time that it was recognized that synovitis caused loss of components from the articular matrix, the understanding of the structure of articular cartilage had evolved considerably from simply consisting of chondrocytes embedded within an amorphous matrix. Histologically adult articular cartilage was divided into four layers; the chondrocytes have different appearances within each layer (Fig. 2). However, it is in the make-up of the extra-articular matrix of articular cartilage that the secret of biological-based therapies lies.

The extra-articular matrix of articular cartilage is a complex of collagen fibrils, proteoglycans, hyaluronan (HA), glycoproteins, and water (Fig. 3). Type-II collagen comprises 90% to 95% of the collagen in articular cartilage and forms fibrils and fibers intertwined throughout the matrix. Equine Type-II collagen has been characterized biochemically by cyanogen bromide cleaved peptide profiles. The same authors also showed that chondrocytes harvested from juvenile horses exhibited synthetic activity in culture with high steady-state amounts of mRNA for Type-II procollagen. Type-II procollagen is expressed at low amounts in adult horses compared with younger horses, and this may have relevance to naturally occurring changes in cartilage in the joints of older horses. The authors also showed that IL-1β and tumor necrosis factor (TNF) α produced a dose-dependent decrease in the steady-state mRNA amounts for Type-II collagen. Small amounts of Type-VI, -IX, -XI, -XII, and -XIV collagen are also found in articular cartilage. These minor collagens help form and give stability to the Type-II fibrillar matrix of articular cartilage.
network. Aggregating proteoglycans (aggrecan) are contained within the Type-II collagen framework and also attach to it.8

The proteoglycans (previously called mucopolysaccharides) are the other major solid components of the articular cartilage matrix and occupy the spaces between the collagen fibers. They take several forms and consist of monomers formed by a protein core and GAG side chains (Fig. 3). Aggregating proteoglycans are the major proteoglycan constituent and are now called aggrecan. The major GAGs in adult articular cartilage are chondroitin-6 sulfate and keratan sulfate. The aggrecan molecules are contained only by the collagen network; hence, the proteoglycans impart compressive stiffness to the cartilage.

Non-collagenous, non-proteoglycan glycoproteins constitute a small but notable portion of articular cartilage and include link protein, chondronectin, fibronectin, cartilage oligomeric matrix protein, thrombospondin, and anchorin C-II.40

The articular cartilage is avascular, lacking both blood and lymph vessels. The deep layers of immature cartilage are penetrated extensively by vascular buds from the ossified portion of the epiphysis, and these appear to play an important role in nutrition of the cartilage from the subchondral region. Immature articular cartilage is an articular-epiphysial complex with deeper layers constituting a growth zone. In adults, the articular cartilage is separated from the subchondral vascular spaces by an end plate of bone (the subchondral plate), and nutrition of the articular cartilage occurs by diffusion from the synovial fluid. There are no nerves in articular cartilage, and the bearing surface of the joint depends on nerve endings in the joint capsule, ligaments, muscle, and subchondral bone for appreciation of pain and proprioception.

In summary, articular cartilage is a tissue consisting of aggrecan that is stiff in compression, collagen that is stiff and strong in tension, and a somewhat freely moving fluid carrying mobile ions (interstitial fluid). These components interact to provide the following mechanical and physical characteristics when young and healthy: a permeable matrix that is stiff in compression, a fibrous network capable of withstanding high tensile stresses, a fluid that flows under load or deformation and aids in dissipating high stresses in the tissue, and a high swelling pressure that results in a matrix swollen with water.

The chondrocytes synthesize all the components of the cartilage matrix. At each stage of growth, development, and maturation, the relative rates of matrix synthesis and degradation are adjusted to achieve net growth, remodeling, or equilibrium. A unique interaction exists between chondrocytes and the surrounding matrix. This may be facilitated by a cilium from each chondrocyte that extends into the matrix and acts as a “probe,” sensing changes in the matrix composition such as loss of proteoglycan or collagen or increase or decrease in HA concentration.41 This information is relayed to the cell. Interaction between the pericellular and territorial matrix in the chondrocyte cell membrane also may include transmission of mechanical signals by a change in matrix tension or compression. Other investigators have provided support to the idea that forces perceived by chondrocytes will dictate their shape and then stimulate alterations in cellular biochemistry and matrix metabolism.42

Dynamic load and the action of cytokines are considered to be involved in matrix turnover. Cytokines of principal interest at the moment are the ILs and TNFα. These factors act on chondrocyte receptors and influence the production and activation of MMPs. The activity of matrix MMP in turn is inhibited by tissue inhibitor of MMP (TIMP)-1 and -2,
Fig. 3. (A) Organization of Type-II collagen fibrils, containing Type-IX and -XI collagens and the multiple proteoglycan molecules that bind HA to form aggrecan in the extracellular matrix of articular cartilage. (B) Structure of proteoglycan molecule demonstrating the protein core and GAG side chains.
and it has been shown that there is a slight excess of TIMP over MMP concentration in typical articular cartilage. These cytokines will be discussed further in the section on pathobiology. Not all cytokines cause degradation. There are a number of growth factors such as insulin-like growth factor (IGF)-1, various members of the transforming growth factor (TGF) superfamily (including bone morphogenetic proteins (BMPs)), and growth/differentiation factors that are involved in articular cartilage synthesis.

3. How Can the Joint Tissues Be Injured or Insulted?

The joint is an organ, and there are a number of ways in which traumatic damage occurs, ultimately resulting in degradation of articular cartilage (Fig. 4). The possible pathways include abnormal forces on typical cartilage or typical forces on abnormal (diseased) cartilage. The reaction in various joint-associated tissues should not be considered in isolation. For example, in the carpus of a racehorse, considerable damage may be inflicted directly to the articular cartilage in regions of concussion as exemplified by the fractures that occur on the dorsal aspect of the joint. Intraarticular fractures of the carpus cause varying degrees of articular cartilage loss. Ulcerative lesions unassociated with fractures may develop as a consequence of direct concussion. However, cyclic fatigue damage to the collagen network could be an important step in the pathogenesis of an insidious osteoarthritic entity. Fatigue or damage in the collagen framework could expose chondrocytes to deleterious physical forces, causing injury and metabolic changes. Primary damage to the subchondral bone other than fracture may also occur on the proximal third carpal bone or the distal radial carpal bone and lead to secondary damage to the articular cartilage either from loss of support or from release of cytokines. Subchondral sclerosis may also lead to further physical damage to the articular cartilage because of decreased shock absorption. Acute synovitis and capsulitis is a common problem in these same joints and may also contribute to the degenerative process by the release of enzymes, inflammatory mediators, and cytokines.8

Fig. 4. Possible pathways for degradation of articular cartilage secondary to joint trauma. Courtesy of C.W. McIlwraith (redrawn from Fig. 3-1).8
When considering a traumatically injured joint, two basic pathobiologic processes should be considered: inflammation of the synovial membrane and fibrous joint capsule (synovitis and capsulitis) and physical or biochemical damage to the articular cartilage and bone. Acute synovitis and capsulitis can cause notable clinical disease and may also contribute to the degenerative process by the release of mediators and cytokines. These processes are outlined in Figures 4 and 5.

4. Synovitis and Capsulitis

Treatment of synovitis and capsulitis, particularly the acute form, is indicated to alleviate the immediate compromising effects of inflammation, including pain and reduced function; to prevent the development of permanent fibrosis in the joint capsule, which in turn will cause decreased motion and compromised shock absorption capabilities in that joint; and to prevent or minimize the development of OA.

Fig. 5. Factors involved in enzymatic degradation of articular cartilage matrix. FGF, fibroblast growth factor; PLA₂, phospholipase A₂; uPA, urokinase plasminogen activator; tPA, tissue plasminogen activator; PA, plasminogen activator. Dotted lines indicate factors that may inhibit degradation. Courtesy of C.W. McIlwraith (modified from Fig. 3-7).
Synovitis and capsulitis as primary entities in athletic horses are presumed to be associated with repeated trauma.\textsuperscript{36,45} Severe injuries to the fibrous joint capsule can also cause instability. The synovial membrane itself is mechanically weak and has no known biomechanical role, but it is recognized that synovial injury may have pathophysiologic consequences in the joint.\textsuperscript{46} Some injuries may affect diffusion across the synovial membrane, and others will have a primary effect on the metabolism of the chondrocyte.\textsuperscript{46} Mechanically damaged synoviocytes may release degradative enzymes and cytokines, and these will alter the IA environment and possibly affect articular cartilage. It has also been suggested that high IA pressures in injured joints associated with effusion could be sufficient to impair the flow of blood through the synovial capillaries. This would not only potentially lower the oxygen tension of the joint but could potentially also lead to reperfusion injury.\textsuperscript{47} Flexion of a joint with sufficient synovial effusion could raise the IA pressure to amounts of impaired blood flow through the synovial capillaries.

In addition to direct injury that may occur to the synovial membrane, the reaction of synovial membrane to articular cartilage damage or other mechanical destruction of intratissues is well recognized. The presence of cartilaginous wear and cartilage gene expression of equine stromelysin 1 (MMP-3) has been described by Balkman and Nixon.\textsuperscript{54} Stromelysin cleaves the protein backbone of cartilage proteoglycans at the asparagine341-phenylalanine342 bond.\textsuperscript{55} Cleavage because of aggrecanase, on the other hand, occurs at the GLU373-ALA373 site.\textsuperscript{56} Based on preliminary work in the horse using markers that differentiate these two cleavage sites, it appears that aggrecanase is the principal enzyme degrading proteoglycan in equine joint disease.\textsuperscript{4}

Equine matrix MMP-2 and -9 are two gelatinases that have been characterized in the horse.\textsuperscript{57,58} It is known that the one-fourth and three-fourth fragments generated by cleavage by fibrin or collagen by collagenases can unwind and are then susceptible to further cleavage by MMP-2 and MMP-9. MMP-2 and -9 are produced by a variety of equine cell types, and these enzymes have been demonstrated in elevated amounts in synovial fluids from horses with joint disease.\textsuperscript{57,58}

The MMPs are inhibited by TIMP-1 and TIMP-2.\textsuperscript{59,60} TIMP is found in many connective tissues and may be the common inhibitor found in articular cartilage. Currently, it is thought that the balance between MMPs and TIMP is important for the progression of articular cartilage degradation.

In summary, MMPs are considered to play a major role in articular cartilage degradation. They are secreted as latent proenzymes and activated extracellularly by serine proteinases. Plasmin may activate stromelysin, and stromelysin, in turn, is an important activator of collagenase. Up-regulation of these enzymes in synovial membrane and articular cartilage samples from traumatic equine joint disease has been demonstrated.\textsuperscript{41} It is also known that the production of MMPs is up-regulated by IL-1.

PGs

PGs (primarily E group) are produced in inflamed joints and can cause a decrease in the proteoglycan content of the cartilage matrix.\textsuperscript{62} The presence of
PGE$_2$ in synovial fluid from inflamed joints has been demonstrated in the horse$^{63,64}$, and in our laboratory at Colorado State University (CSU), we use PGE$_2$ measurements as an objective index of the amount of synovitis.$^{65,66}$ Actions of PGE$_2$ in joints include vasodilation, enhancement of pain perception, proteoglycan depletion from cartilage (by both degradation and inhibition of synthesis), bone demineralization, and promotion of plasminogen activator secretion. PGE$_2$ is released from chondrocytes on stimulation of these cells by IL-1 and TNF$\alpha$.

6. Oxygen-Derived Free Radicals

Oxygen-derived free radicals, including superoxide anion, hydroxyl radicals, and hydrogen peroxide, may be released from injured joint tissues. Studies have demonstrated cleavage of hyaluronic acid by free radicals.$^{67,68}$ There is also evidence that superoxide can degrade the alpha chains of collagen based on the finding that superoxide treatment inhibits gelatin.$^{59}$ Proteoglycans may also be cleaved by free radicals.$^{69}$ Increased free radicals in the synovial fluid of cases of equine joint disease has been demonstrated recently.$^{70}$

Nitric oxide (NO) has been recognized recently as an important physiologic mediator. It combines avidly with superoxide anion, and although this was originally thought to provide a protective function, it now seems that this reaction can generate further destructive species including peroxynitrite anion and hydroxyl radicals.$^{71}$ The role of NO in joint disease needs and is receiving further attention.

Cytokines and Articular Cartilage Degradation

Cytokines are defined as soluble peptides produced by one cell affecting the activity of other cell types. Studies of cytokines in joint tissues suggest that IL-1 and TNF$\alpha$ modulate the synthesis of MMPs by both chondrocytes and synovial cells$^{72-74}$ and are, therefore, important mediators in joint disease. Therefore, because IL-1 and TNF$\alpha$ may be produced by synovial cells,$^{72}$ they may be important in the deleterious effects of synovitis on articular cartilage. The typical turnover of the extracellular matrix of articular cartilage is considered to be regulated by the chondrocytes under the control and influence of cytokines and mechanical stimuli.$^{75}$ Articular cartilage degradation in association with disease represents an exacerbation of these typical processes. It is widely accepted that cytokines may induce proteoglycan depletion in articular cartilage by either increasing the rate of degradation or decreasing synthesis in association with the release of proteinases and PGs from chondrocytes. Recognition of the gene sequence for equine IL-1 by Howard et al. at CSU$^{76,77}$ has led to specific studies with equine tissues and equine IL-1. IL-1 containing extract was produced by May in 1990.$^{79}$

The notable role of equine IL-1 has been further established by determining the cDNA sequences for IL-1$\alpha$ and IL-1$\beta$.$^{76,77}$ After generation of these DNA sequences, the IL-1$\alpha$ and IL-1$\beta$ recombinant proteins were purified. Prior to this, only human recombinant IL-1$\alpha$ protein was available. Using equine articular cartilage explants, the effect of rEqIL-1$\alpha$ and rEqIL-1$\beta$ was studied. Similar treatment groups (500 ng) were created, and notable proteoglycan release was induced by both IL-1$\alpha$ and IL-1$\beta$ at concentrations greater or equal to 0.01 ng/ml with 38% to 76% and 88% to 90% of total GAG released by 4 and 6 d, respectively.$^{80}$ Notable inhibition of proteoglycan synthesis (42% to 64%) was observed at IL-1$\alpha$ concentrations greater than or equal to 0.01 ng/ml at 2 and 4 d. Increased PGE$_2$ concentrations were also observed at IL-1$\alpha$ concentrations greater than or equal to 1.0 ng/ml at 2 and 4 d. This work showed that much lower concentrations of equine IL-1 could cause these effects compared with previously reported studies using human recombinant IL-1.$^{80}$

The IL-1 system consists of the two agonist members, IL-1$\alpha$ and IL-1$\beta$, and these evoke signal production in response to binding only a few IL-1R transmembrane receptors to induce IL-1 effect (Fig. 6). The notable role of IL-1 in the pathogenesis of cartilage degradation in the horse was demonstrated by the work of Frisbie in our laboratory using gene therapy (discussed later).$^{81}$ This study

![Diagram of IL-1 activation of MMP, aggrecanase, and PGE$_2$ release acting through IL-1 receptors on the cell membrane.](image)
demonstrated that if IL-1 can be inhibited, cartilage degradation can essentially be stopped.

The role of TNFα in equine OA is less certain. Billinghurst et al. first demonstrated induction of IA TNF during acute inflammatory responses in equine arthritis. It appears that IL-1 is the principal cytokine responsible for articular cartilage degradation, and TNFα contributes to clinical morbidity and pain. IL-1 and TNFα have been demonstrated using reverse transcription-polymerase chain reaction in the synovial membrane of inflamed joints, and increased serum concentrations of soluble TNF receptors have been detected in human patients with rheumatoid arthritis and OA in comparison with healthy controls. Both receptors of TNF have been identified in synovial tissue, with greater numbers seen in joints affected by rheumatoid arthritis in comparison to OA.

7. Direct Morphologic and Biochemical Damage to Articular Cartilage with Trauma

Trauma can cause an immediate physical defect or initiate a degradative process. There can be direct damage to cells causing release of enzymes and cytokine-initiated release of MMPs and PGE2 from chondrocytes in response to IL-1. As outlined in Figure 4, any instability in a joint can lead to damage of typical cartilage. On the other hand, cartilage compromised by loss of GAGs or collagen is vulnerable to typical forces.

8. Primary Disease of Subchondral Bone

In addition to synovial mediated degradation of articular cartilage and direct mechanical damage, the subchondral bone can play a primary role in disease development (Fig. 4). Early subchondral bone sclerosis has been considered a possible pathway for mechanical destruction of articular cartilage in human OA because it causes a reduction in the joint’s shock absorbing ability and thereby places cartilage at risk of shear-induced tensile failure of cartilage cross-links, particularly under repetitive impulsive loading conditions. Work in our laboratory by Kawcak has demonstrated that microdamage in the subchondral bone can develop early when horses are subjected to athletic exercise on the treadmill. On postmortem examination of racehorse joints (euthanized for catastrophic injury in another limb), the range of microdamage includes not only microfractures but also primary osteocyte death. Not only is the mechanical support of the articular cartilage lost when subchondral bone microdamage progresses to macrodamage, cytokine release from the bone may also potentially influence that state of the articular cartilage. In Figure 7, a sample is shown from a horse euthanized because of catastrophic injury in the other limb. An incidental finding at postmortem was the presence of subchondral bone necrosis, with a peripheral area of sclerosis and intact cartilage in the distal palmar area of the metacarpus.

Figure 8 shows photographs of microdamage and osteocyte necrosis that can occur early in association with exercise.

9. Medical Treatments of Joint Disease: Recent Progress

A recent survey suggested that 60% of lameness problems are related to OA, stressing the importance of advancements of both medical and surgical treatment options. This section reviews medical options currently used for treating joint disease, emphasizing recent and/or future perspectives. The section after this will address these surgical options.

The aim of treatments for acute synovitis, with or without accompanying capsulitis, is to return the joint to typical as quickly as possible. In addition to bringing relief to the patient and allowing it to return to typical work, suppression of synovitis and capsulitis is important to prevent the products of inflammation from compromising the articular cartilage and leading to OA as previously described. It is important to provide pain relief and minimize the potential microinstability associated with excessive synovial effusion. Experiments in rabbits have shown joint inflammation weakens IA ligaments in addition to affecting the cartilage.

In all traumatic entities of the joint, the goal of treatment is 2-fold: return the joint to typical as quickly as possible and prevent the occurrence or reduce the severity of OA; in other words, to reduce pain and minimize progression of joint deterioration. Although this section addresses medical treatments, it is important to remember that timely removal of osteochondral chip fragments, timely and appropriate reduction on fixation of large IA fractures, accurate diagnosis of ligamentous and meniscal injuries with arthroscopy, and the appropriate...
The treatment of osteochondritis dissecans (OCD) entities are also critical treatments to prevent OA.

Physical Therapy and Shock Wave Therapy
Swimming and underwater treadmills are popular rehabilitation tools after arthroscopic surgery for joint injury and also, to a lesser degree, rehabilitation of non-surgical injuries. Underwater treadmills have become increasingly available and decrease weight bearing while potentially providing a massaging effect on the limbs and preventing fibrosis of the joint capsule. Controlled trials to establish evidence for the relative usefulness of these modalities would be an excellent contribution to our knowledge.

The only non-medical or non-surgical physical therapeutic tool that has been studied in a controlled fashion in the horse is extracorporeal shock wave therapy (ESWT). A controlled equine OA study has been done comparing ESWT to Adequan® and a sham treatment group.91 The study used our established short-term (70 d) OA model, where an osteochondral fragment is created at time 0 and treatments are initiated 14 d later.65 ESWT was administered on d 14 and 28 using the Versa Tron machine and a 12-mm probe, and a sham shock wave procedure was performed on the control horses on d 14 and 28.90 A positive control group involved IM Adequan® treatment every 4 d for 28 d. The shock wave energy was delivered mainly to the mid-

Fig. 8. Microdamage with (A) microcrack formation and (B and C) osteocyte necrosis in samples from subchondral bone from a horse exercised on pasture (B) and on a treadmill (C). Courtesy of C.E. Kawcak.
dle carpal joint capsular attachments, but some energy was delivered to the area of fragmentation. Notable improvement in clinical lameness, decreased synovial fluid TP (as a marker of synovitis), and less GAG amounts in the serum (a biomarker of early osteoarthritic change) was observed with ESWT compared to both control and Adequan®-treated horses. These results imply promise for this type of therapy in localized joint disease in horses, but clinical studies with sufficient numbers still need to be reported.

Non-steroidal Anti-inflammatory Drugs (NSAIDs)
The term NSAID is used to describe anti-inflammatory agents that inhibit some components of the enzyme system that converts arachidonic acid into PGs and thromboxains. The use of these agents in the horse was well reviewed in 1996. All NSAIDs inhibit cyclooxygenase (COX) activity to some degree. But recently, two different isoenzymes for COX, COX-1 and COX-2, were characterized. COX-1 has been associated with the “good” or “housekeeping” functions of the COX pathway. It is constitutively produced and has been shown to be important in the balance of typical physiologic function of the gastrointestinal and renal system, while having a lesser role in the inflammatory COX cascade. COX-2 has mainly been associated with inflammatory events, especially those driven by macrophages and synovial cells, and has been attributed with only minor roles in typical physiology, thus its “bad” or “inducible” role. Drugs that preferentially inhibit COX-2 enzyme have been developed. Although it appears logical that inhibition of COX-2 should minimize adverse effects of NSAIDs, there has been some suggestion that complete inhibition of COX-2 may not be optimal for the joint or the patient.

Even though COX-1 is mainly responsible for the protective functioning of PGs, COX-2 also plays some accessory role or is, at least, more important than previously thought. The mainstream still believes that the beneficial effects of selective COX-2 inhibition in joint disease are ideal. Anecdotally, we have used carprofen (Rimadyl®) at the Orthopaedic Research Center at CSU in horses that have developed high creatinine concentrations and diarrhea in association with phenylbutazone use. The resolution of these adverse effects when the horse is placed on carprofen implies a protective effect with a drug that has more preferential COX-2-inhibiting activity than phenylbutazone.

Recently, a topical NSAID preparation, Surpass® (1% diclofenac sodium cream), was licensed. Research in humans had previously indicated that topical NSAID application was clinically beneficial, while reducing systemic adverse effects. Anti-inflammatory effects were also shown in experimentally induced SC inflammation. A clinical field trial of the topically applied diclofenac liposomal cream for the relief of joint inflammation in horses showed promising results.

A relatively recent paper has raised the issue of whether NSAIDs are deleterious to articular cartilage. The topic is not a new one, and in 1993, there was a suggestion that inhibition of the E group of PGs could have long-term unfavorable effects on cartilage metabolism. In vitro work in the horse had initially shown no evidence of deleterious effects on cartilage metabolism, but a recent paper based on administering phenylbutazone for 14 d to horses and then testing the serum on articular cartilage explants in vitro concluded there was decreased proteoglycan synthesis to a degree similar to that with rhIL-1β. Until in vivo deleterious effects are demonstrated, the author feels that in the absence of any clinical associations between the use of phenylbutazone and articular cartilage degeneration, continued appropriate use of NSAIDs is justified.

Intraarticular Corticosteroids
The use of IA corticosteroids for equine joint disease was extensively reviewed in 1996. Recent clarification of the benefits and deleterious adverse effects of IA corticosteroids in the horse represent a good example of clinical observation leading to scientific inquiry (Fig. 9). Based on the author’s observation of an apparent lack of correlation between the prior use of betamethasone esters (Betavet Soluspan®) and articular cartilage degradation during arthroscopic surgery for osteochondral chip removal, experimental studies were initiated of the three commonly used IA corticosteroids, namely MPA (Depo-Medrol®), triamcinolone (TA) acetonide (Vetalog®), and betamethasone esters (Betavet Soluspan®) were evaluated using the osteochondral fragment model. The first product studied was Betavet Soluspan® (later discontinued but then available as Celestone Soluspan®, which has since been discontinued). Osteochondral fragments were created arthroscopically on the distal aspect of both middle carpal joints in 12 horses, and one joint was treated with 2.5 ml of Betavet Soluspan® at 14 d after surgery and repeated in 35 d. The opposite joint was injected with saline as a control. No deleterious adverse effects to the articular cartilage were demonstrated. Exercise also did not have any harmful effects in the joints given corticosteroids.

The other studies of IA injection of corticosteroids (all studies with this model except betamethasone esters) were modified so that the opposite joint was not used as a control, and the chip fragment model was modified to effectively produce early osteoarthritic change.

MPA (Depo-Medrol®) and TA (Vetalog®) were tested using three groups and the experimental design is depicted in Figure 10. Eighteen horses were randomly assigned to each of three groups (six horses/group). Both middle carpal joints in the placebo control group (CNT) horses were injected IA with polyionic fluid. The corticosteroid control group horses (i.e. MPA CNT or TA CNT) were injected with corticosteroid in the middle carpal joint...
without an osteochondral fragment, and the opposite middle carpal joint was injected with a similar volume of polyionic fluid. The corticosteroid-treated group horses (MPA TX or TA TX) were treated with corticosteroid in the joint that contained the osteochondral fragment, and the opposite

Fig. 9. The relationship between clinical observation leading to scientific inquiry and, later, scientific inquiry leading back to useful clinical application.

Fig. 10. Design of experiments to assess the value of direct IA injection of a corticosteroid into an osteoarthritic joint (ST TX) and injection of IA corticosteroid in a remote joint (ST CNT) compared to a saline-injected control (CNT).
middle carpal joint was injected with a single volume of polyionic fluid. All horses were treated IA on d 14 and 28 after surgery and exercised on a high-speed treadmill for 6 wk, starting on d 15.

In joints containing an osteochondral fragment and treated with MPA, there was lower, although not notable, reduction in the degree of lameness; however, there were notably lower PGE\textsubscript{2} concentrations in the synovial fluid and lower scores for intimal hyperplasia and vascularity (no effect on cellular infiltration in the synovial membrane compared to placebo-treated joints). Of importance, modified Mankin scores (a score of histopathologic change in the articular cartilage) were notably increased in association with MPA, suggesting deleterious effects of IA administration of MPA. This is in contrast to the results with TA. Using the same experimental design, 12 mg of TA was used with each injection. Horses that were treated IA with TA in a joint containing a fragment (TA TX) were less lame than horses in the CNT and TA CNT groups. Horses treated with TA in either joint had lower protein and higher HA and GAG concentrations in synovial fluid. Synovial membrane from CNT and TA CNT had less inflammatory cell infiltration, intimal hyperplasia, and subintimal fibrosis. Analysis of articular cartilage morphologic parameters evaluated using a standardized scoring system were notably better from TA CNT and TA TX groups irrespective of which joint received TA. The results overall supported favorable effects of TA on degree of clinically detectable lameness and on synovial fluid, synovial membrane, and articular cartilage morphologic parameters, both with direct IA administration and remote site administration as compared to placebo injections.\textsuperscript{101} Repetitive IA administration of MPA to exercising horses has been shown to alter the mechanical integrity of articular cartilage\textsuperscript{103} but had no effect on subchondral or cancellous bone.\textsuperscript{104}

These in vivo studies, coupled with some in vitro work, have resulted in the recommendation that TA acetoneide be used, especially in high motion joints. There have been some options on low-dose MPA administration that have shown less negative effects based on in vitro titrations studies. However, the lower doses that are commonly used are unlikely to have the same beneficial effects. A greater concentration of corticosteroid is needed to inhibit the catabolic compared with the anabolic effects in articular cartilage.\textsuperscript{105} On the other hand, clinical improvement is more important to the clinician than in vivo data.

An area of concern is that no form of IA betamethasone esters are available as licensed medications in the United States. Although compounded product is available, there are concerns on using compounded drugs IA because of the recent FDA stance on bulk compounding but, importantly, risk of liability in case of a reaction and a malpractice suit. An unfortunate sequel to this has been a re-turn of some practitioners to use of Depo-Medrol®. The availability of a generic betamethasone ester preparation would help practitioners immensely; human rheumatologists are attempting to do this.

Fear of laminitis has inhibited the use of TA acetoneide by some equine practitioners, despite scientific studies demonstrating its effectiveness and its chondroprotective properties. There are anecdotal reports of laminitis after TA administration. A maximum dose has been established based on a report of no cases of laminitis in 1200 horses treated with TA when the dose did not exceed 18 mg.\textsuperscript{106} A recent publication provides the first follow-up study with data on the potential for TA acetoneide to produce laminitis; no association was found between the occurrence of laminitis and the IA use of TA acetoneide.\textsuperscript{107} Another traditional cliche is Depo-Medrol® should not be used in high-motion joints, but its use in low-motion joints (such as the distal tarsal joints) is appropriate. The implication is the state of the articular cartilage in low motion joints does not matter, and cartilage deterioration may promote ankylosis. Currently, no evidence exists that ankylosis is promoted in this fashion. The other side of this argument is that articular cartilage should be preserved whenever possible.

Intraarticular corticosteroids have commonly been combined with HA because of a perception that it might be protective against the effects of cortico-steroids. This perception has been based on tradi-tion rather than scientific proof but has become common thinking amongst equine practitioners.\textsuperscript{89}

Another study suggested that the effect of IA MPA on joint metabolism was different between inflamed and typical joints and also highlighted the potential for introducing in vitro culture artifacts (in addition to the effect of inflammation) when investigating the effective IA corticosteroids on chondrocyte function.

Using a model to induce synovitis by injection of lipopolysaccharide (LPS) into carpal joints, one study was demonstrated that acute synovitis prevented changes produced by IA MPA in control joints.\textsuperscript{108} However, in other studies with synovitis based on chip fragmentation, MPA was shown to be deleterious.

HA (Sodium Hyaluronate)

HA is non-sulfated GAG. The biological characteristics and therapeutic use of HA in an equine OA have been reviewed previously.\textsuperscript{109,110} HA has modest analgesic effects,\textsuperscript{111} but emphasis has been placed on its anti-inflammatory effects that may be physical (steric hindrance) or pharmacological (inhibition of inflammatory cells and mediators).\textsuperscript{89,110} Various in vivo and in vitro studies have shown protection against IL-1, driven PG synthesis, and inhibition of free radicals, but the ability of HA to inhibit the activity of MMPs is questionable.\textsuperscript{112,113} Several inflammatory mediators can augment the production of HA by synovial fibroblasts in vitro; thus, increased synthesis of HA in early OA may
constitute a protective response by the synovium to joint inflammation. Although this provides a rationale for exogenous administration of HA, it may also explain the increased concentration of HA in response to IA injection of a number of medications.

The author’s clinical impression is that HA alone is useful for mild to moderate synovitis, but for the treatment of most clinical cases adjunctive use of a corticosteroid is necessary. It has been claimed that HA preparations of molecular weight exceeding 1 × 106 daltons may provide better clinical resolution and chondroprotective events, but this is controversial. A recent survey of questionnaires sent to 20 members of AAEP (14 responses) found it was uncommon for the respondents to administer IA HA initially or alone, particularly in horses with established OA. Twelve of 14 supplemented HA injections with other forms of treatment (usually IA corticosteroids). Most clinicians reported being unimpressed by the efficacy of IV HA, particularly when used alone.

They considered effects on osteoarthritic signs to be moderate and the duration to be short. The prophylactic use of IV HA has been studied in both Quarter Horse and Thoroughbred race horses. One hundred forty horses were entered in the Quarter Horse study and received either IV saline or HA every 2 wk for the duration of the 9-mo study. Trends for HA-treated horses to race longer, require an IA injection of corticosteroid earlier, have a better speed index, higher average number of starts, and more money earned were observed when compared to placebo-treated horses. A similar study has been conducted in Thoroughbred racehorses using synovial fluid markers and starting with horses without musculoskeletal problems. No notable differences were found, but anecdotal reports from trainers and various equine disciplines have been positive regarding the prophylactic use of IV HA.

A randomized, double-blind, and placebo-controlled clinical study in 77 Standardbred trotters with moderate to severe lameness has been reported. Horses were randomized for treatment with HA, polysulfated GAG (PSGAG), or placebo for 3 wk. The mean and initial lameness score was notably reduced during treatment and at the last examination in all three groups (p < 0.01). Additionally, the prevalence of sound horses increased notably from 1 to 3 wk of treatment to the last examination in all three groups. Comparison of the two treatment groups in the development of the lameness curve and time until soundness indicated a small, non-significant difference in favor of HA. No notable difference was detected between the two treatment groups in the prevalence or accumulative incidences of soundness. The study detected a superior effect of the two drugs (250 mg of PSGAG IA 4 times or 20 mg of HA IA twice) compared to placebo for reduction of lameness score during the treatment period and the total study period, time until soundness, and the prevalence of sound horses at the last examination. All three treatments were effective in the treatment of clinical traumatic arthritis in horses, but HA and PSGAG gave better results than placebo. In a second paper, the same group compared IA saline to rest alone in 38 Standardbreds with traumatic arthritis. The mean lameness was notably lower when 2.0 ml of 0.9% NaCl solution was injected. The results ask the question whether this effect was because of withdrawing fluid and/or placing a needle in the joint.

The use of IV HA in the treatment of joint disease is now common. An experimental study of IV HA use documented a notable improvement in clinical lameness, decreased PGE2 and total protein concentrations in the synovial fluid, and decreased synovial membrane hyperemia and cellular infiltration. A recent survey of questionnaires sent to 20 members of AAEP (14 responses) found it was uncommon for the respondents to administer IA HA initially or alone, particularly in horses with established OA. Twelve of 14 supplemented HA injections with other forms of treatment (usually IA corticosteroids). Most clinicians reported being unimpressed by the efficacy of IV HA, particularly when used alone.

The use of Adequan® was reviewed extensively in 1996. At that time, there had been a number of in vitro studies, including one demonstrating that PSGAG was the only drug tested (others included phenylbutazone, flunixin, betamethasone, and HA) that inhibited stromelysin. There had been three other in vitro studies on the effect of PSGAG on equine cartilage that were contradictory. Initially, it was reported that PSGAG caused increased collagen and GAG synthesis in both articular cartilage explants and cell cultures from typical and osteoarthritic equine articular cartilage. However, other work had found a dose-dependent inhibition of proteoglycan synthesis, little effect on proteoglycan degradation, and no effect on proteoglycan monomer size. Various in vivo studies have supported the value of IA (250 mg) PSGAG in equine joint disease, including a clinical study, a study using a Freund’s adjuvant-induced model, a study of cartilage repair in exercised horses, and another equine carpal model using sodium moniodoacetate. In the latter study, there was notable reduction of articular cartilage fibrillation erosion, less chondrocyte death, and markedly improved Safranin O staining. At the same time,
PSGAG had no benefit in healing articular cartilage lesions that were already detected. A second study using IM PSGAG (500 mg q 4 d for 7 treatments) showed insignificant effects with treatment (limited to slightly improved Safranin O staining in sodium monooiodoacetate joints when PSGAG was used). In a recent unpublished experimental study where IM PSGAG was used as a positive control (administered every 4th d for 28 d starting 14 d postOA induction), there was some improvement in clinical lameness 56 d after initiation of treatment and decreased GAG amounts in the serum 14 d posttreatment (GAG is a marker of disease in this OA model). However, there was impressive improvement in the third test group (shock wave group therapy, discussed previously).

Although a survey to assess the perceived efficacy of PSGAG in 1996 reported that PSGAG was considered more effective than HA for the treatment of subacute degenerative joint disease and less effective for idiopathic joint effusion and acute synovitis, and the author has used IM PSGAG routinely postsurgically, it now appears that there is little evidence for efficacy with IM administration. Articular cartilage concentrations of PSGAG after IM administration have been reported to inhibit some cartilage degrading enzymes, but the duration of administration have been reported to inhibit some evidence for efficacy with IM administration. Arportsurgically, it now appears that there is little PSGAG. This is a potential (but yet to be dem-

A principal reason for the persistent use of IM PSGAG in preference to IA PSGAG has been the work demonstrating a slightly increased risk of infection (compared to corticosteroids and HA). Apparently receiving less notice is a companion paper reporting all risks could be obviated with con-

PPS

The use of this PPS in the treatment of joint disease was reviewed in 1996. PPS could be considered as a disease-modifying osteoarthritic drug, and it was pointed out in the review article that PPS, unlike NSAIDs, does not possess analgesic activity. The conclusion was that to provide symptomatic relief and efficacy, a drug such as PPS must be capable of correcting the pathobiological imbalances that are detected within the OA joint, and the au-

Oral Joint Supplements

None of the oral supplements or oral nutraceuticals is licensed, and proof of efficacy is generally lacking. Most products include glucosamine and/or CS along with other added ingredients. Historically, the oral GAG products initially available for the horse included a CS product from bovine trachea (Flex- Free®) and a complex of GAGs and other nutrients from the sea mussel, Perna canaliculus (Syno-Flex®). Recently, a combination of glucosamine hydrochloride (GU), CS, manganese, and vitamin C has been marketed as a nutraceutical (Cosequin®), and a number of other products have simulated Cosequin®. Since that time, other products have attempted to compete on the basis of decreased cost (with no demonstration of comparable efficacy) or other added ingredients. Glucosamine sulfate is a precursor of the disaccharide subunits of cartilage proteoglycans. Although glucosamine salts have been reported as well absorbed after oral absorption in man, one study has reported an oral bioavailability of GU in horses to be 2.5%, with a large volume of distribution, which the authors interpreted as poor absorption from the intestinal tract but extensive tissue uptake. A second study in
dogs concluded that glucosamine absorbed orally, albeit a low percentage (12%), is likely because of extensive first pass metabolism in the gastrointestinal tract and/or liver prior to systemic availability.137

Recent work on the quantification of glucosamine in serum and synovial fluid after nasogastric or IV administration of GU to horses questions effective absorption of GU in the horse.138 Eight adult female horses with no evidence of joint disease were studied and were randomly assigned to two different groups (n = 4) for a crossover study. GU (20 mg/kg) was administered by nasogastric intubation or IV injection, and blood samples were collected. Glucosamine was assayed by fluorescence-assisted carbohydrate electrophoresis with glucosamine achieving a maximum concentration of 288 ± 53 μM after IV dose and 5.8 ± 1.7 μM after nasogastric dose. Synovial fluid reached a peak concentration at 250 μM postIV administration and 0.3 to 0.7 μM postnasogastric administration. The amounts of glucosamine obtained in synovial fluid after nasogastric administration with clinically recommended doses are lower than those that have been studied in vitro to elucidate glucosamine action on joint cells.

CS consists of alternating disaccharide subunits of glucuronic acid and sulfated N-acetylgalactosamine molecules and is a principal GAG of aggregating proteoglycan (aggrecan). CS is less sulfated but resembles PSGAG in structure and mechanism of action. Oral absorption of a CS has been tested in horses. A low-molecular weight CS (0.80 kDa) has been evaluated by quantifying the disaccharide content using a validated method that combined enzymatic digestion of plasma followed by fluorescence high-performance liquid chromatography.138 Low-molecular weight CS was absorbed to a greater degree compared to glucosamine; it was also demonstrated that the absorption may be influenced by the molecular weight of the polymer.139

In vitro studies can potentially help determine at what concentrations glucosamine or CS may inhibit the catabolic response in equine cartilage explants. One study done with cartilage discs incubated with LPS with varying concentrations of glucosamine, CS, or both revealed that glucosamine concentrations as low as 1 mg/ml decreased NO production relative to LPS-stimulated cartilage but that CS at either 0.25 or 0.50 mg/ml did not inhibit NO production. Glucosamine concentrations as low as 0.5 mg/ml decreased PGE2 production, whereas CS did not affect PGE2. The combination decreased MMP-9 activity but has no effect on MMP-2, and there was a trend for decreasing MMP-13 protein concentrations.140

In vitro dose titration studies of GU and CS alone and in combination have recently been reported based from our laboratory. There were no detrimental effects of GU, glucosamine sulfate, or GU plus glucosamine sulfate on typical cartilage metabolism. Higher doses of GU, CS, and GU plus CS appeared to limit total GAG release into the media, whereas intermediate doses enhanced GU, CS, and GU plus CS enhanced GAG synthesis and total cartilage content.141

The same dosages tested on IL-1-conditioned articular cartilage explants revealed no treatment effects for GU or CS alone but a protective effect of high dosages of GU plus CS for total GAG release into the media. The study suggested that GU plus CS might be beneficial to cartilage metabolism by preventing GAG degradation. However, the question of effective concentration of GU after oral administration is still an issue,138 and clear in vivo demonstration of reduction and degradation has yet to be demonstrated.

Other oral joint supplements used include Platinum Performance®, which is a combination of rare earth minerals and omega-3 fatty acids. This has been used postoperatively, but all information is anecdotal. Similarly, oral HA products are new to the market, and a recent controlled study in our laboratory did not demonstrate effectiveness in our equine OA model.14 Recently, another experimental study using the CSU equine OA model has demonstrated value for an oral supplement containing soy and avocado. This is the first well-controlled scientific study demonstrating a positive effect with an oral nutraceutical.

Summary

Conventional medications still form a large part of the equine veterinarians’ armamentarium. Increased attention is being paid to physical therapy regimens and positive results demonstrated with shock wave therapy and can perhaps decrease the use of medication for equine joint disease. COX-2 inhibitors are going to be useful to the veterinarian when the patient is not tolerating phenylbutazone well. Intraarticular corticosteroids continue to be the principal IA therapy. The use of MPA has decreased appropriately and the value of beta-methasone esters and TA acetoni are recognized. Continued availability of licensed medication is a challenge. Intraarticular HA continues to be used in conjunction with corticosteroids. Recent research challenges the degree of value gained from IM Adequan®, but all scientific research has been positive with IA use of the drug. It is predicted that PPS will become a licensed medication, and its value has been documented scientifically. Oral nutraceuticals continue to be somewhat of a “black box” as far as efficacy is concerned, but positive results in a controlled study with the product of Vétiquinol are exciting.

10. New Biologic Therapies

The knowledge gained from improved understanding of critical mediators in equine traumatic arthritis and OA has lead to the identification of new targets for therapy. Two obvious targets identified include MMPs and IL-1.
Inhibition of MMPs as a Therapeutic Approach

MMP inhibitors include peptide-based inhibitors (including hydroxamic acids), non-peptidyl inhibitors (this includes chemically modified tetracyclines such as doxycycline), and naturally occurring inhibitors (such as N-3 fatty acids, i.e. fish oils). Recent work has demonstrated that N-3 fatty acids, as found in fish oils, will inhibit MMPs and aggrecanase (which, as discussed before, is a key enzyme in the degradation of aggrecan).

In our laboratory, we studied the in vitro effect of the MMP inhibitor Bay-12-9566 using equine and canine articular cartilage explants in an IL-1 degradation model and using the COL2-3/4C_short immunoassay. A notable dose-dependent reduction in the catabolic effect of IL-1α on the release of proteoglycans and Type-II collagen from articular cartilage explants exposed to 10-fold increases in concentrations (1 nM:10 μM) was shown. No in vivo work has been done in the horse; however, it has been done in an experimental OA dog model. This study failed to demonstrate efficacy with an MMP inhibitor, and the prospect for these being a valuable biological therapy for horses seems low.

Novel Methods of Administering Therapeutic Proteins
(Including Gene Therapy)

The functional unit of DNA is the gene that is defined as the set of DNA sequences that produce a single polypeptide (protein). The gene sequence codes for a specific mRNA molecule that, in turn, carries the genetic information from the nucleus to the cytoplasm for translation into amino acid sequence (i.e. a protein). Although many recognized diseases relate to a lack of or a defect in or an imbalance of a particular protein(s), and because the gene is the basal unit ultimately responsible for protein production, it is also a logical therapeutic target. At the moment, most gene therapy protocols (at least the ones we have evaluated) are directed toward increasing amounts of selected therapeutic proteins in an attempt to alter specific disease dysfunction. Depending on the natural function of the protein, we might be able to enhance or repress certain direct effects on specific cellular processes.

The mechanism for in vivo gene therapy is demonstrated in Figure 11. The key component is the efficient transfer and expression of therapeutic genes by inserting the manipulated gene sequence into a vector. One example is IL-1 receptor antagonist (IL-1ra), which we have used in our laboratory. Previous work had shown IL-1ra inhibited the progression of OA when administered to laboratory animals with induced OA. After the gene sequence of the equine IL-1ra molecule was defined in our laboratory, the value of gene therapy with IL-1ra using an adenoviral vector in the treatment of equine OA was then investigated.

Proof of principal experiments demonstrating in vitro expression of an active equine IL-1ra protein after gene transfer of the equine IL-1ra gene sequence to cultured equine synoviocytes using an adenoviral vector were first performed. After confirmation that the adenoviral vector could infect equine synoviocytes and produce a biologically active IL-1ra protein, an in vivo dose titration study was done. Using the same adenoviral vector carrying the equine IL-1ra gene (AdeqIL-1ra), the optimal vector concentration to provide peak concentration and duration of IL-1ra protein expression was determined without notable adverse effects. Next, using our established experimental model of equine OA, this gene therapy treatment was tested and shown to notably reduce lameness and synovial effusion in the arthritic/fragmented joints. The horses receiving gene therapy also had notably less pathologic change seen on gross examination of the joints compared to placebo-treated arthritic/fragmented joints (Fig. 12). Microscopically, there was also notable improvement in the articular cartilage compared to the controls (Fig. 13).

Since our studies, gene therapy with IL-1ra, combined with IGF-1, has been tested for its capability of improving cartilage healing (discussed later). Gene therapy protocol using BMP-2 has been shown to aid healing in the presence of osteomyelitis in rabbits.

11. Diagnostic and Surgical Arthroscopy: Progress in the Last 15 Yr

By 1975, the arthroscope began to achieve common clinical use in human orthopedics and diagnostic arthroscopy of equine carpal joints in three horses. The acquisition of an arthroscope and development of expertise in its use began when the author recognized it as a possible way to monitor the development of synovitis in experimental studies. This is another example of the interrelationship between science and clinical applications (Fig. 9) because initial work in experimental equine arthritis resulted in exploration of arthroscopy. Experience with the limitations of what could be achieved with arthroscopic surgery has stimulated attempts to develop novel treatment techniques and recognized the need for early diagnosis and prevention of injury.

The application of arthroscopic techniques to the horse has revolutionized the treatment of traumatic joint injuries. The first detailed paper on diagnostic arthroscopy in the horse was published in 1978, and since then, arthroscopic surgery has been recognized as the diagnostic method of choice to evaluate articular cartilage and remains the gold standard for assessing pathologic changes in joints. As in human orthopedics, the common use of the arthroscope in horses in surgical practice grew as the technology and techniques of triangulation developed. These techniques were first detailed in a textbook in 1984. Diagnostic arthroscopy is especially valuable when response to medical treatment of a joint is suboptimal. In many instances,
Articular cartilage lesions are more extensive than what can be seen on radiographs, but these lesions can sometimes be better related to physical examination and the extent of clinical signs.

By 1990, arthroscopy in the horse had gone from being a diagnostic technique used by a few veterinarians to the accepted way of performing joint surgery. Prospective and retrospective data substantiated the value of the technique in the treatment of carpal chip fractures, fragmentation of the dorsal margin of the proximal phalanx, carpal slab fractures, OCD of the femoropatellar joint, OCD of the shoulder, and subchondral cystic lesions of the femur. (The results with tarsocrural OCD were published in 1991.) Advances and understanding of the pathogenesis of osteochondral disease and fragmentation in the carpus and fetlock have been reported, which naturally led to improvement in diagnosis and treatment. Parameters for the surgical treatment of joint injury have been carefully defined. Arthroscopic treatment of fractures in the previously considered inaccessible palmar aspect of the carpus have been described together with arthroscopy of the palmar aspect of the distal interphalangeal joint. Arthroscopy has also led to understanding of the contribution of soft-tissue lesions to joint disease. In the carpus, tearing of the medial palmar intercarpal ligament was first

Fig. 11. Schematic drawing representing in vivo gene transfer to the synovium. This is the fashion in which gene therapy with IL-1ra was done.
reported in 1992\textsuperscript{165} and its implications discussed by Phillips and Wright\textsuperscript{166} and Whitton et al.\textsuperscript{167–169}

In the fetlock joint, the success rate after arthroscopic removal of osteochondral fragments of the palmar/plantar aspect of the proximal phalanx has now been documented.\textsuperscript{170,171} Results for arthroscopic treatment of OCD for the distal/dorsal aspect of the third metacarpal/metatarsal bones\textsuperscript{172} and results of arthroscopic surgery to treat apical, abaxial, and basilar fragments of the sesamoid bones have also been reported.\textsuperscript{173,174}

The results of arthroscopic surgery for the treatment of OCD in the tarsocrural joint have been documented,\textsuperscript{158} and the arthroscopic approach and IA anatomy of the plantar pouch of this joint have also been described.\textsuperscript{175}

Considerable advances have been made in arthroscopic surgery of the stifle joint. Results of arthroscopic surgery for the treatment of OCD of the femoropatellar joint were reported in 1992\textsuperscript{176} and the syndrome of fragmentation of the distal apex of the patellar recognized and its treatment reported in the same year.\textsuperscript{177} The use of arthroscopic surgery for treating certain patellar fractures was discussed in 1990 and reported in the referenced literature in 2000.\textsuperscript{178}

In the femorotibial joints, the use of arthroscopic surgery to treat subchondral cystic lesions of the medial condyle of the femur\textsuperscript{179} and proximal tibia\textsuperscript{180} have been reported. Research has led to alternative methods of treating subchondral cystic lesions. After an initial demonstration that subchondral cystic lesions could develop on the medial condyle after 3-mm-deep, 5-mm-wide penetration of the subchondral bone plate,\textsuperscript{181} examination of the fibrous tissue of subchondral cystic lesions in horses demonstrated that it produced local mediators and neutral MMPs and caused bone resorption in vitro.\textsuperscript{182} Production of NO, PGE\textsubscript{2}, and MMPs in media of explant cultures of equine synovial membrane and articular cartilage has also been demonstrated in typical and osteoarthritic joints.\textsuperscript{183} Injection of corticosteroids into the lining membrane of subchondral cysts is now done in the clinical practice (Fig. 14).

Cartilage lesions of the medial femoral condyle have been described.\textsuperscript{184} Arthroscopy has allowed great advances in the recognition and treatment of meniscal tears and cruciate injuries.\textsuperscript{185–187} Successful treatment of Grades I and II meniscal tears
has been achieved and documented (Fig. 15) and lack of success recognized with lesions that are not completely accessible. Arthroscopy has also been used to remove fragments from the intercondylar eminence of the tibia, and internal fixation of one case has been reported. Techniques have also been developed for diagnostic and surgical arthroscopy of the caudal pouches of the femorotibial joints.

Diagnostic and surgical arthroscopy of the coxofemoral joint has been described, in which lesions have been identified and some surgical treatments performed. The use of the arthroscope is no longer confined to the limbs; the arthroscopic anatomy of the temporomandibular joint has been described recently.

The use of arthroscopy in assisting repair with internal fixation of articular fractures has become routine. This includes fractures of the metacarpal/metatarsal condyles and carpal slab fractures. These techniques can be used in both non-displaced and displaced fractures (Fig. 16). Techniques have been described for evaluation and treatment of problems in smaller joints such as the distal and proximal interphalangeal joints. In addition, joints in which lameness is less commonly encountered, such as the elbow, have been examined and treated arthroscopically.

Arthroscopic techniques for cartilage repair have been developed and will be described in the next section.

The use of the arthroscope for the evaluation and treatment of tendon sheath problems have been an other area of major advance. The arthroscope has been used to assess and treat tenosynovitis of the digital flexor sheath, and techniques for endoscopically assisted annular ligament release have been described. Intrathecal longitudinal tears of the digital flexor tendon have also been described and treated arthroscopically. The arthroscope has been used increasingly for carpal sheath conditions. Removal of radial osteochondromas and physeal remnants using arthroscopic visualization has produced excellent results, and superior check ligament desmotomy is now done arthroscopically, as well as release of the carpal canal. Techniques for tenoscopy of the tarsal sheath have been described. Arthroscopy of the synovial bursae

Fig. 14. Arthroscopic view of 18-gauge spinal needle being used to inject TA acetonide into the lining of a subchondral cystic lesion.

Fig. 15. A Grade-II tear of the medial meniscus prior to (A) and after removal and debridement of the torn portion (B).
has also been reported with the principal conditions being the treatment of contamination and infection.\textsuperscript{215–217}

The general advantages of arthroscopic surgery include:

1. An individual joint can be examined accurately through a small (stab) incision and with greater accuracy than was previously possible. The availability of such an atraumatic technique allows numerous lesions and “new” conditions that are not detected radiographically to be recognized.

2. All types of surgical manipulations can be performed through stab incisions under arthroscopic visualization. The use of this form of surgery is less traumatic, less painful, and provides immense cosmetic and functional advantages.

3. Surgical intervention is now possible in situations where it would not have been attempted previously. The decreased convalescence time, with earlier return to work and improved performance, is a notable advantage in the management of equine joint problems. The need for palliative therapies has decreased, as has the number of permanently compromised joints.

Although the technique appears uncomplicated and attractive to the inexperienced surgeon, some natural dexterity, good three-dimensional anatomical knowledge, and considerable practice are required for the technique to be performed optimally. Experience and good case selection are of paramount importance. The statement made in 1987 by a prominent human orthopedic surgeon is worth remembering: “Of those 9000 North American surgeons and the other surgeons of the world performing arthroscopy, many are ill-prepared and are therefore, not treating their patient fairly.” Overuse and abuse by a few is hurting the many surgeons that are contributing to orthopedic surgery by lowering morbidity of patients, decreasing the cost of healthcare, shortening the necessary time of patient’s returning to gainful employment, and adding to the development of a skill that has made profound change in the surgical care of the musculoskeletal system.\textsuperscript{147}

Back to positive connotations, arthroscopy remains the most sensitive and diagnostic modality for intrasynovial evaluation in the horse. This is different from human orthopedics where arthroscopy predominately is used for surgical interference, and much of its diagnostic function has or is being replaced by magnetic resonance imaging (MRI). Arthroscopy has continued to be of great benefit in the horse, with increased recognition of soft-tissue lesions in joints, tendons, sheaths, and bursa. However, as stated above, although there are many benefits gained from arthroscopy, it is technically demanding, and the need for training remains.

Last, but not least, with the development and successful treatment of many conditions with arthroscopy, the limitations that result from articular cartilage degradation and erosion have been recognized. In fact, the recognition of the limitations of arthroscopy because of residual OA and lack of articular cartilage is another example of clinical observation leading to scientific research, and this is discussed in the next section.

12. Recent Progress at Healing Articular Cartilage Lesions and Resurfacing Joints

The real challenge in OA treatment is the progressive loss of articular cartilage. The failure of osteochondral defects to heal is a major limiting factor in the prognosis after the treatment of articular fractures. Arthroscopic techniques for cartilage repair have been developed and reviewed.\textsuperscript{218,219} The aim of these techniques is to enhance both the quantity and quality of cartilage repair tissue while using the well-documented advantages of arthroscopic sur-

Fig. 16. Arthroscopic view of displaced fracture of lateral condyle of distal metacarpus before (A) and after (B) reduction under arthroscopic visualization and internal fixation.
The aims of all articular cartilage repair techniques are: to generate repair tissue with structural characteristics approximating typical articular cartilage and integration of the repair tissue with the adjacent tissue (both articular cartilage and subchondral bone). Still controversial is what “quality” of tissue is needed for it to remain in place and withstand exercise.

Overall, attempts at improving the repair of articular defects can be divided into two areas: stimulation of endogenous repair and articular grafting.

Stimulation of Endogenous Repair
Stimulating endogenous repair uses techniques to provide marrow elements access to the cartilage defect. The simplest example of this is debridement of the defect to subchondral bone which, as discussed above, provides a fibrocartilagenous repair with a high Type-II collagen but lower amounts of proteoglycan (GAG). In addition to defect debridement, other methods of endogenous repair that have been attempted surgically include: partial-thickness chondrectomy, spongialization, subchondral bone drilling, abrasion arthroplasty, and, recently, micropicking or microfracture.

Debridement and the Need for Removal of CCL
The healing achieved with debridement down to subchondral bone has been previously described. In a series of experiments in the author’s laboratory, however, we have recognized that it is easy to leave the CCL, and if CCL is left, healing is markedly restricted. In a recent study with arthroscopic examinations at 4 mo and postmortem examination at 12 mo, there was a notable increase in the amount of defect filling with repair tissue in defects where the CCL was removed.226 On histologic examination, the average percentage of repair tissue volume was notably greater in CCL removed (70.8 ± 5.1) than CCL retained (54.9 ± 5.0 group). There was also significant enhancement of repair tissue attachment when the CCL was removed compared to when the CCL was retained (Fig. 17).

Partial-Thickness Chondrectomy
Partial-thickness chondrectomy to relatively healthy chondral tissue (shaving) in cases of partial-thickness defects and fibrillation smoothes the cartilaginous area and may decrease further tissue exfoliation, producing (in conjunction with joint lavage) an early remission of synovitis. However, no controlled studies have been done. A study in rabbits, where articular cartilage was shaved on the underside of the patella, no evidence of repair was seen.227 Ultrastructural studies after arthroscopic cartilage debridement question whether any regeneration occurs and have suggested deleterious effects.228

Spongialization, Abrasion Arthroplasty, and Subchondral Bone Drilling
Spongialization is removal of sclerotic subchondral bone from the base of a full-thickness defect, and
this has been done in the horse. However, there is general evidence of subchondral cyst formation, and, subjectively, we believe that retention of an intact subchondral bone plate is essential for repair. Abrasion arthroplasty has also been called superficial intracordical debridement, as opposed to deep cancellous debridement, and has been used on sclerotic degenerative lesions in people.229 The concept is controversial and argues the necessity to expose cancellous bone to reach both blood supply and primitive mesenchymal stem cells. Criticisms have included difficulty with variables such as patient selection, arthroscopic debridement, joint irrigation, and variation in the degree of pathologic change in the joint.230 In vitro low oxygen tension is chondrotrrophic (the corollary of which is excessive oxygen tension is not conducive to cartilage formation), and this correlates with the low oxygen tension of cartilage in vivo and the general anaerobic metabolism of cartilage.231 Follow-up biopsies after abrasion arthroplasty have shown Type-I and -III collagen in most of the samples, although there is some focal amount of Type-II collagen.

The rationale for subchondral drilling is similar because it provides access to the cancellous bone plate, while still preserving some of the subchondral bone plate. In a study on full-thickness defects of the third carpal bone of horses, satisfactory functional healing was not achieved.232

Subchondral Micropicking (Microfracture)

Subchondral microfracture was a technique developed by Dr. Richard Steadman and has been extensively used in human clinical patients.233 Recently, it has been shown to provide equivalent repair to the commercially available autologous chondrocyte transplantation technique.234,235 Micropicking is a simple and atraumatic way to provide pluripotential cells (and growth factors) and has additional benefits of not having any heat associated with drilling. The rim of bone around the pick holes that subjectively seems to enhance repair tissue attachment. Studies in the horse have shown that microfracture notably increases the amount of repair tissue when cartilage lesions were debrided and microfractured compared to defects debrided alone.220 A short-term study also revealed a notable increase in Type-II collagen mRNA production 8 wk after microfracture.235 The aggrecan production was shown to gradually increase between 2 and 8 wk; however, this expression was not influenced by microfracture.235

Further Manipulation of Endogenous Healing Using Growth Factors (Protein Administration or Gene Therapy)

Recently studies have been designed to produce improved repair tissue matrix. In June 2002, James Richardson made the following statement: “There is great cleverness in each cell and learning to work with them, and to trust them to do the right thing in the right place is not new to surgery. We have always depended on the natural biology of tissue healing.”236 Several naturally occurring polypeptide growth factors play an important role in cartilage homeostasis.237,238 Endogenous growth factors not only promote synthesis of matrix components (anabolism) but also inhibit proteinases and inflammatory factors that can cause ongoing degradation after surgery (catabolism) (Fig. 18). The different effects in
matrix anabolic activity of IGF-1 and TGF-β, for instance, are considered important in counteracting the degradatory and catabolic activities of cytokines and MMPs. The effect of these and other growth factors have been studied in the horse.

Three-dimensional cultures of equine chondrocytes in fibrin gels were evaluated with either IGF-1 or TGF-β and cultured without serum supplements, and these two growth factors stimulated matrix component elaboration in a dose-dependent manner, with the most profound effects occurring at the highest concentration of IGF-1 and TGF-β. Other cartilage explant studies have used both typical and IL-1-depleted cartilage and revealed that IGF-1 had a positive effect on equine cartilage homeostasis. Because of these results, IGF-1 was selected as a candidate growth factor for in vivo evaluations in the horse. Elution studies showed that IGF-1 laden equine fibrin had maximal stimulatory concentrations of IGF-1 (greater than 50 ng/ml) remaining for a minimum of 3 wk after an initial loading dose of 20 mg. In vivo evaluation after placement of 25 μg of IGF-1 in fibrin into cartilage defects in the femoropatellar joint showed improved cell populations with more cartilage-like architecture after 6 mo. However, Type-II collagen concentrations only increased to 47% compared to 95% in typical articular cartilage with a Type-II collagen content of 39% in the control defects comparable to the amounts seen in empty full-thickness defects.

Other work in the same laboratory suggested IGF-1 had better application in combination with chondrocyte or mesenchymal stem cell grafts where there was more complete cartilage repair. At 8 mo after implantation of a mixture of chondrocytes and 25 mg of IGF-1 in femoropatellar defects in horses, there was improved joint surface, 58% Type-II collagen, and better neocartilage integration at the defect edges.

Resurfacing of articular defects using a regimen of autogenous fibrin laden with 50 mg of IGF-1 in 30 million chondrocytes/ml of fibrin has been used in clinical cases. The chondrocytes were mixed with fibrinogen and IGF-1 with activated thrombin to provide a two-component system for immediate injection. This polymerization process developed immediately on injection into the articular defect. This technique has been used in OCD defects and subchondral cystic lesions of fetlocks and stifles.

Gene therapy with transfected adenovirus (previously discussed with IL-1ra) using IGF1 has been used to transfect transplanted chondrocytes. Gene therapy transfecting transplanted chondrocytes with BMP-7 has also been reported.

In a recent collaborative effort between our laboratory, Cornell University, University of Pittsburgh,
and Harvard, we have evaluated the usefulness of a combined gene therapy protocol using the IL-1ra to decrease the effects of IL-1 on cartilage repair and IGF-1, which has been previously shown to enhance cartilage healing in an equine model and also reduce the deleterious effects of IL-1.\textsuperscript{249,250} Using an osteoarthritic IL-1 coculture (synovial membrane and articular cartilage), system gene transduction of IGF-1 and IL-1ra proteins was demonstrated using an adenoviral vector with protection of proteoglycan loss in the cartilage.\textsuperscript{249} There was also restoration of cartilage matrix without IL-1 detected using the same in vitro system.\textsuperscript{249} This combination gene therapy protocol was then evaluated using full-thickness articular chondral defects treated with microfracture in the horse.\textsuperscript{251} This protocol enhanced the quality of the repair tissue in full-thickness equine cartilage defects compared to microfracture alone because there was an increased concentration of Type-II collagen and aggregan content in the defects.

Articular Grafting

Reattachment of Cartilage Flaps and Periosteal and Sternal Grafts

Direct repair of large OCD defects by replacing the flap and providing fixation with polydioxanone (PDS) pins has been described recently.\textsuperscript{252} Other than this, early attempts with direct grafting of periosteum or sternal cartilage met with disappointing results in the horse.\textsuperscript{224,225,253}

Implantation of Autologous Chondrocytes

Early attempts at grafts of cultured chondrocytes or cartilage regenerative cells in a matrix also were relatively unsuccessful.\textsuperscript{254–256} At present, there is one commercially available technique of autologous chondrocyte implantation into human knees, and it has been used principally for focal erosive defects\textsuperscript{267,268} and OCD.\textsuperscript{259} This is a two-stage procedure. After collection of cartilage, it is cultured, and 3 wk later, a piece of autogenous periosteum is sutured into the defect, and the cultured chondrocytes are injected beneath it. Despite publication implying excellent results, failures, particularly with detachment of the graft, can occur, and, recently, a clinical study with 2-yr follow-up showed no superiority over microfracture alone.\textsuperscript{254} Its use in an experimental study in the horse (10-mm-diameter defects on lateral trochlear ridge of talus) has been reported.\textsuperscript{260}

We have recently conducted experiments using a solid form of autologous chondrocyte cultures. Three hundred milligrams of cartilage was collected from the lateral trochlear ridge of the femur and chondrocytes cultured on a collagen membrane with implantation at 4 wk. The results were superior to empty defects and defects implanted with matrix alone and showed good promise. However, it is still a two-stage technique, which is a disadvantage of this technique.\textsuperscript{261}

Further work in our laboratory tested a one-stage technique in 15-mm defects on the trochlear ridge of the femur in the horse, and the success of this technique has resulted in its use in human clinical trials.\textsuperscript{262} Briefly, an articular cartilage biopsy is taken from the lateral trochlear ridge of the femur (follow-up at 12 mo postbiopsy reveals no apparent morbidity associated with the cartilage biopsy). The cartilage is morselized into approximately 1 mm\textsuperscript{3} and suspended in fibrin on a membrane (various membranes were tested, but the one that gave the best results was a PDS-reinforced foam). This morselized cartilage-fibrin-PDS membrane combination was then placed into the defect with the membrane uppermost and fixed with three specially developed polydioxanone-polyglycolic acid (PDS-PGA) staples (Figs. 19 and 20). The follow-up results at 12 mo were excellent (Fig. 20).

What About Stem Cells?
The use of mesenchymal-derived stem cells is a recent technique in the horse. Stem cells can be obtained from bone marrow or from fat. There is some evidence that stem cells are trophic for fibrillated cartilage. We are currently beginning on a project to evaluate the use of the IA injection of fat-derived cells in treating early OA in the horse. At the same time, the author has had some clinical experience of injecting stem cells IA into defects with early OA (Fig. 21).

Osteochondral Grafts

The use of osteochondral plug autografts to repair experimentally created defects in the medial condyle of the femur was initially investigated using autogenous sternal osteochondral allografts.\textsuperscript{263,264} Techniques of autogenous and allogeneic osteochondral plug grafting have been tested recently using the MosaicPlasty\textsuperscript{TM} technique.\textsuperscript{265–266}

Hydrogel (Salucartilage) Implant

The hydrogel implant obviates the problem of donor morbidity with osteochondral allografting and introduces a new concept for major defects causing a decreased joint space. The author has done three cases using a 15-mm implant, two in severe OA of the fetlock (Fig. 22) and one with severe OA in the medial femorotibial joint. This technique looks promising for joints with joint space collapse because of severe loss of articular cartilage.

13. Advances in Diagnosis of Joint Problems: Key to Early Detection

Introduction

Although osteochondral fragmentation, fractures, subchondral bone disease, and OA are common in the horse, diagnosis of these diseases usually occurs only after the disease has become established. The
detection of early or subtle disease in the past has been poor, but the situation is improving. Clinical examination and radiographic imaging are still the most commonly used techniques for diagnosis of osteochondral disease, yet osteochondral damage seen during arthroscopic surgery is usually more severe than that seen on radiographs. It is the author’s opinion that there is usually a good correlation between the severity of clinical signs (principally lameness and synovial effusion) and the amount of damage or disease found at arthroscopy of joints. However, in humans, although the most common complaint of a patient with OA is pain, only about one-half of the patients with radiographic OA have symptoms. The reason(s) that one-half the patients with radiographic OA have or do not have pain is not always clear because only some causes of pain have been studied. There is no “diagnostic test” for OA in man, but focus on MRI and biomarkers for diagnosis has occurred in recent years. Human clinical trials are now specific about the recording of outcomes measures. Outcome variables in OA clinical trials need to be selected on the basis of the therapeutic objective and are a critical part of assessing the results of medication. In a workshop of the World Health Organization and the American Academy for Orthopedic Surgeons, the methods to assess progression of OA of the hip and knee were reviewed. In addition, the European Group for the Respect of Ethics and Excellence in Science have made recommendations on methods for registration of drugs for OA. Equine veterinarians are equally in need of objective outcome parameters in assessing the results of various treatments for musculoskeletal disease in general and joint disease in particular in the horse.

The following measurements have been proposed to completely characterize joint disease in the horse: a measure of mechanical inputs into the joint; a measure of tissue architecture and geometry; a measure of tissue matrix properties, including measurement of material and biochemical matrix properties; and a measure of the amount of inflammation within the joint. The current state of diagnostic capabilities for horses will be presented here; some of these are already being used by the clinician, some are ready to be used, and some are futuristic.

Measurement of Mechanical Inputs

Clinical Examination

Assessment of joint effusion, range of motion, joint capsule thickening, and pain with flexion are currently used and subjectively graded by veterinarians doing lameness examinations. The lameness grading guidelines set up by AAEP are used often. Although flexion tests are commonly used, the reliability of these tests is controversial. Confounding...
Fig. 20. Construct of morselized cartilage before (A) and after (B) implantation and evaluation at 12 mo. Courtesy of Frisbie et al.\textsuperscript{262}

Fig. 21. Arthroscopic view of medial femorotibial joint treated with direct IA injection of fat-based stem cells (note focal erosion on medial femoral condyle).

Fig. 22. Surgical photograph after implantation of 15-mm-diameter hydrogel implant (Salucartilage\textsuperscript{®}) into severe degenerative lesion on medial condyle of distal metacarpus.
factors make this objective evaluation of a human for pain difficult. The principal factors are differences in observer scores and differences in a particular subject’s tolerance to pain. The same is true for a horse. Motion analysis, in which characteristics of limb movement and force can be determined, has been employed as a research tool. The abnormalities in these parameters can be characterized in patients with disease. For example, it has been found in humans that impulsive loading often leads to OA. Because data analysis involves sophisticated, expensive equipment and is often labor intensive, most gait analysis in veterinary medicine occurs in the research field.

Motion analysis systems that combine data from force plates, electromyogram analysis and muscle forces, and kinematics can provide sensitive information to an individual’s movement. These systems have been extensively studied in humans and are used clinically to evaluate an individual’s gait. Limb use and muscle forces play a large role in joint loading; recent work in our laboratory has shown this for the horse. For instance, in measuring contact forces across the carpus at the trot, the peak ground reaction force is 1350 pounds, whereas the peak muscle forces are 2700 pounds, leading to a total joint force of 4050 pounds. In other words, muscle forces are 2 times ground forces. Diagnostic techniques that describe kinematics and muscle forces potentially allow clinicians to identify those individuals with problems related to movement and, thus, allow for potential movement modification. At present, this approach is a research tool.

We have also evaluated the use of thin-film sensor systems to evaluate limb loading in horses. The system was attractive because it could be attached to the bottom of a horse’s hoof to measure force distribution throughout the sole surface, or it could be used like a force plate for jogging horses across. However, evaluation of the force plate-type system for accuracy and durability has shown it to be inadequate. Preliminary results indicate, however, that using an “in-shoe” system for the sensor film deploys results similar to the force plate.

Computer Models

Computer models of joint loading have been studied in both humans and animals. Modeling is the computer-based mathematical representation of the skeleton, ligaments, and muscles used to calculate forces in muscles and joints. The principle is to develop the model based on kinematic parameters and compare that model to those from imaging techniques such as computed tomography (CT) and MRI. Muscle, tendon, ligament, and ground reaction forces in tissue properties can be inserted into the model. Once developed, imaging based modeling can be performed so that subtle changes in joint geometry and loading can be detected. The ultimate goal is to develop long-term models in which data can be continually added. The clinical goal is to develop patient specific models in which abnormalities in loading and tissue response can be detected.

Collaborative research in the Equine Orthopaedic Research Laboratory at CSU, the Orthopaedic Research Laboratory at Columbia University, and the Steadman Hawkins Sports Medicine Foundation performed a kinematic and MRI study to develop a model of the equine carpus. This study determined the center of force of the joint surfaces in the carpus, and these data have been correlated with those obtained from MRI scans. At the moment, this is a research tool, but, ultimately, we hope the subtle irregularities in joint loading can be determined in clinical patients using MRI and CT.

Measurement of Tissue Architecture and Geometry

Radiography

Radiography is still the most widely used imaging technique for the diagnosis of osteochondral disease but is an insensitive method of diagnosis. Articular cartilage cannot be viewed radiographically except when there is extensive loss and decreased joint space, and 30% to 40% change in bone mineral density (BMD) is required before bone changes can be appreciated. In addition, multiple images are required for evaluation of a three-dimensional structure. Disease is often recognized after notable damage has occurred. This lack of sensitivity can prevent early and accurate diagnosis. Measuring joint space is fraught with error. The significance of osteophytes are often unrelated to IA pathological change, and considerable change in bone density is necessary to identify sclerosis and erosion. In a study correlating radiographic and histologic changes in the tarsi of horses, Laverty et al. found that radiographs were insensitive for detecting subchondral bone sclerosis and erosion when compared to histology. It has also been pointed out that superimposition of osteophytes may appear as sclerosis.

CT

CT has had increasing use in the horse, both as a research and clinical tool. Benefits of CT are visualization of the area of interest in three dimensions (which alleviates superimposition) and the ability to determine density patterns. Density patterns of bone can be determined by three-dimensional modeling of CT images (CT osteoabsorptiometry [CTO]) and allows three-dimensional evaluation of the joint in any plane. Hounsfield units, which are the CT measure of bone density, are determined and coordinated into ranges that are then represented by colors. This color map is then superimposed over a three-dimensional image of the joint surface to show a representation of the relative subchondral density (Fig. 23). The use of a density phantom has al-
lowed for objective measures of density to be determined. Because it has been shown that stress distribution within an osteochondral section is related to the density pattern, the subchondral density pattern represents the loading history of the joint.\textsuperscript{284} Considerable work in this area has been done in our laboratory by Kawcak. Initially, the subchondral density patterns of bones in equine carpal and metacarpophalangeal (MCP) joints were established. Subsequently, the effects of exercise has begun in foals 3 wk old compared to pasture-reared horses over time. In addition, we have evaluated the changes in bone density patterns with age.\textsuperscript{28}

In 1999, Riggs et al. identified substantial density gradients between the denser subchondral bone of the condyles and the subchondral bone of the sagittal groove in the distal MCIII and MTIII, which may help explain the etiology of distal condylar fractures. These density gradients were shown to equate to anatomical differences in loading intensity and locomotion; we hypothesized that difference in bone density results in stress concentration at the palmar/plantar aspect of the condylar groove, which may predispose to fracture.\textsuperscript{285} In a companion paper, linear defects in mineralized articular cartilage and subchondral bone were found in the palmar/plantar aspects of the condylar groove, adjacent to the sagittal ridge.\textsuperscript{286} These were closely related to the pattern of density of subchondral bone and were associated with intense focal remodeling of the immediate subjacent bone. Parasagittal fractures of the condyles originated in similar defects. This work and subsequent examination of CT’s in our laboratory has demonstrated a potential to diagnosis incipient condylar fractures in the racehorse (Fig. 24).

Fig. 23. CTO 3-dimensional evaluation of the surface of the third metacarpal condyles of a horse exercised on a treadmill (A) and a hand walked horse (B). Notice the increased density of subchondral bone (black areas) in the treadmill-exercised horse compared to the hand walked horse. Courtesy of Kawcak et al.\textsuperscript{89}

Fig. 24. CTO image demonstrating area of decreased bone density lateral to sagittal ridge in area where lateral condylar fractures occur (A) with color density gradient (B).
Results from human studies have shown that MRI is a sensitive and specific imaging tool for examination of hard and soft tissues in joints and that it is as good as, if not better than, arthroscopy for detecting subchondral lesions.\textsuperscript{287} MRI is the best measure of articular geometry; recently, an ability to quantify articular cartilage matrix properties using contrast enhancement has been demonstrated.\textsuperscript{288} Postmortem MRI, as well as other imaging modalities, including clinical examination, radiographs, nuclear scintigraphy, and arthroscopy, were used to evaluate an osteoarthritic MCP joint in a horse.\textsuperscript{289} Kawcak et al. (2001) have also used MRI to evaluate the effects of exercise on subchondral bone of horses and found that it could image osteochondral damage, including small fragments (Fig. 25).\textsuperscript{89}

Recently, there have been reports of the clinical use of MRI (high-field strength) in anesthetized as other imaging modalities, including clinical examination, radiographs, nuclear scintigraphy, and arthroscopy, were used to evaluate an osteoarthritic MCP joint in a horse.\textsuperscript{289} Kawcak et al. (2001) have also used MRI to evaluate the effects of exercise on subchondral bone of horses and found that it could image osteochondral damage, including small fragments (Fig. 25).\textsuperscript{89}

Recently, there have been reports of the clinical use of MRI (high-field strength) in anesthetized

Fig. 25. Imaging of an osteochondral fragment on the distal aspect of the radial carpal bone. (A) Gross photographic view, (B) CT osteoabsorptiometry image, and (C) MRI image of the distal aspect of the radial carpal bone showing the fragment. Courtesy of C.E. Kawcak.\textsuperscript{109}
Horses to diagnose specific changes in the distal limb and a paper on the use of a low-field strength standing MRI to image the distal limb. We have used a high-field strength MRI at CSU for 1 yr, and it is being used regularly on clinical patients to diagnose problems from the tarsus and carpus down effectively. Changes in the joint capsule and ligaments associated with joints can be diagnosed equally well to those in articular cartilage and bone (Fig. 26).

**Ultrasonographic Examination**

Ultrasonographic examination of joints was pioneered by Denoix. The technique can be used to evaluate soft tissues associated with the joint, including collateral ligaments, joint capsule, other associated ligaments, and menisci. The use of ultrasonography to image the medial palmar intercarpal ligament in the carpus also has been described recently.

**Measurement of Tissue Matrix Properties, Including Measurement of Material and Biochemical Matrix Properties**

**Nuclear Scintigraphy**

Nuclear scintigraphy has been found to be extremely helpful in detecting cortical bone disease and, in particular, stress fractures in horses. Its notable use has been in detecting stress fractures of the pelvis, tibia, femur, and humerus before they become complete fractures. A nuclear scintigraphic image shows the physiologic distribution of radioisotope throughout the bone and therefore is more sensitive than radiographs in detecting early OA in human knees. In humans, nuclear scintigraphy has been the best early predictor of joint space narrowing in knees. In some cases, it has been more sensitive than arthroscopy and MRI for detecting early and subtle subchondral bone pathology. However, nuclear scintigraphy cannot distinguish stress response because of subchondral bone adaptation from osteochondral damage. Osteochondral fragments show up as discreet, focal areas of increased radioisotope uptake, but any remodeling change because of stress will also show increased uptake of radioisotope. Because of this, mild to moderate increases in uptake of radioisotope in the joints of horses, especially young, exercising horses, can lead to confusion. Scintigraphy can be used as a sensitive screening tool but cannot demonstrate a specific anatomical problem.

More objective means of assessment have been used to eliminate some of the subjectivity with nuclear scintigraphy. Using computer programs, areas of particular interest can be highlighted, the counts/pixel determined for that area, and normalized to the counts/pixel for a reference area within the same limb. This is of particular benefit because the distribution of radioisotope within an area varies between animals and between different regions within the same animal. If we outline an area of interest such as the distal condyles of the
third metacarpus, normalize the count to a reference area such as the cortical area of the first phalanx, it is possible to eliminate the influence of individual horse uptake in assessing this area (Fig. 27). Careful selection of the reference area to ensure it is typical and has no increased uptake compared to the surrounding bone is necessary. This technique takes into account the regional limb response to exercise and, therefore, potentially reduces the effect of exercise induced increases.

Material properties of subchondral tissues can be inferred from the CT examination. The densities of subchondral and cortical bone have been shown to be proportional to their strengths; therefore, a measure of bone density can give the clinical an impression of bone strength. This is commonly used in an outpatient setting for humans in which peripheral quantitative CT is used for diagnosis and to monitor therapy for osteoporosis. However, unlike cortical bone, there is a maximum density at which subchondral bone can be damaging to articular cartilage. Thus, there exists an appropriate density range at which subchondral bone must be maintained to avoid joint damage, a range that we have not yet determined.

Histologic properties of osteochondral tissues can be assessed using optical coherence tomography, referred to by Kawcak as an in vivo form of biopsy. In humans, optical coherence tomography has shown a fairly good correlation between images and histologic change.

**Synovial and Serum Biomarkers**

Conventional synovial fluid analysis will not provide a specific diagnosis, but it will give an indication of degree of synovitis and metabolic derangement within the joint. It will not define the degree of articular cartilage damage but merely the degree of synovitis. Previous attempts to develop techniques such as synovial sediment analysis have also not solved this problem. Over the past 10 to 15 yr, researchers have found biochemical and immunologic biomarkers to quantitate breakdown products of the articular cartilage. Reviews of this in the horse have been published.

The terms biomarker, biochemical marker, and molecular marker have all been used to describe either direct or indirect indicators of musculoskeletal turnover. These markers are often molecules that are typical products and by-products of the metabolic process occurring within the musculoskeletal system. Disease alterations occur between the anabolic and catabolic processes within the skeletal tissues; consequently, concentration of biomarkers may increase or decrease. In joint disease, these molecules can be released into the synovial fluid when the source is articular cartilage, menisci, ligament, or synovial membrane. If the underlying subchondral bone is involved, molecules from osseous tissue will usually be delivered into the bloodstream. Biomarkers can potentially be used to clarify pathobiological processes in the joint, differentiate diagnostically between affected and non-affected joints and distinguish the degree of degradation in articular cartilage, and monitor the response to therapy.

Direct biomarkers originate principally from cartilaginous structures and provide specific information about alterations in cartilage matrix, anabolism, or catabolism. On the other hand, indirect biomarkers are not derived principally from cartilage but have the potential to influence the metabolism of chondrocytes or the integrity of the matrix and include proteolytic enzymes and their inhibitors, growth factors, proinflammatory cytokines, and other molecules from non-cartilaginous sources including MMPs, aggrecanase, TIMP, IGF-1, IL-1, IL-6, TNF-α, HA, and C-reactive protein. Indirect markers used in the horse have been reviewed recently.

Individual Direct Biomarkers of Cartilage Metabolism

**Biomarkers of Anabolic Processes**

The carboxypropeptide of Type-II collagen (CPII) is a useful measure of Type-II collagen synthesis (Fig. 28). Although CPII concentrations were not notably higher in synovial fluid samples of joints with osteochondral fragmentation, their concentrations were notably higher in the serum of horses with

Fig. 27. Nuclear scintigraphic view of an MCP joint of a healthy horse. PSB, proximal sesamoid bone; McIII, third metacarpal condyle; P1?, presumed proximal phalanx. Courtesy of Kawcak et al.
osteochondral fragmentation. \(^{309}\) In the same study with horses, another synthetic marker, CS-846, was notably higher in the synovial fluid of joints with osteochondral fragmentation compared to control joints and serum amounts were also notably higher. \(^{309}\) CS-846 and CPII concentrations were not linearly related to greater fragmentation but were notably higher with Grades I and II. Discriminate analysis using a combination of serum CS-846 and CPII concentrations allowed for 79% of horses to be correctly classified as having osteochondral damage.

**Biomarkers of Catabolic Processes**

Measuring the degradation of Type-II collagen with biomarkers has proven to be a benefit in monitoring OA and OCD in the horse. Antibodies have been developed to identify Type-II collagen fragments that have been cleaved and/or denatured, exposing previously inaccessible regions (neoepitopes) of the molecule (Fig. 29). Using these antibodies, notable increases in concentrations of degraded Type-II collagen have been demonstrated in synovial fluid and serum samples from horses, dogs, and rabbits with experimental OA. \(^{310}\) Initially, the Col-2–3/4\(_{\text{short}}\) immunoassay for detecting collagenase-cleaved collagen fragments (detect both Type-I and -II collagen degradation) was developed. This assay had been used in the author’s laboratory for monitoring collagenase-induced collagen degradation and to measure the inhibitory effect of a synthetic MMP inhibitor on IL-1-induced degradation of equine articular cartilage explants. \(^{143}\) Recently, a collagen degradation immunoassay that is specific for Type-II collagen degradation and is equine specific was developed. \(^{311}\) The antibody in this assay is designated as 234CEQ.

In a recent study of skeletal markers in osteochondrosis (OC) in foals, \(^{312}\) a combination of notably higher serum concentrations of CPII, higher concentrations of Col-2–3/4\(_{\text{short}}\), and lower concentrations of 234CEQ correlated with high OC scores (radiographically). This study suggests that there is increased collagen turnover in OC, and by measuring the serum amounts of specific biomarkers of collagen metabolism, it is possible to identify horses with OC. \(^{312}\) An earlier study in cases of OC found that there were notably higher concentrations of CPII and lower concentrations of CS-846 and keratan sulfate (KS) epitopes in synovial fluids of affected compared to typical joints. \(^{313}\)

Other biomarkers that have been measured in the horse include KS and cartilage oligomeric matrix protein, but until now, these have proven less useful. On the other hand, the development of monoclonal antibodies that distinguish the two different
sites of aggrecan degradation can help identify which is the most responsible for aggrecan degradation in the horse.\textsuperscript{314} At present, it appears that aggrecanase is more important than stromelysin in this degradation process.

**Individual Direct Biomarkers of Bone Metabolism**

**Biomarkers of Anabolic Processes**

During typical Type-I collagen synthesis, as with Type-II collagen, cleavage of carboxy- and amino-terminal propeptides (PICP and PINP, respectively) of the procollagen molecule occurs, and these cleaved propeptide fragments can be exploited as markers reflective of bone formation. In a preliminary study, PICP was shown to have potential value as a molecular marker for monitoring changes in matrix turnover after tendon injury,\textsuperscript{315} and increases in PICP with age and exercise have been demonstrated.\textsuperscript{316,317}

Osteocalcin is a small, non-collagenous protein associated with bone assembly and turnover. Concentrations in the horse appear to vary with age, administration of corticosteroids, and general anesthesia. In a study in our laboratory where various serum markers were used to differentiate changes with exercise from pathologic change in joints, concentrations of osteocalcin and CS-846 provided the best correlation to the modified Mankin score ($r^2 = 0.72$) and clinical degree of pain ($r^2 = 0.70$) using multivariate linear regression (step-wise model selection).\textsuperscript{318}

Bone-specific alkaline phosphatase (BAP) is expressed at high concentrations on the cell surface in bone forming osteoblasts. In a study with treadmill exercise in young horses, serum BAP amounts were not different between exercise and control groups, although previously there had been a suggestion that there was a correlation between amounts of BAP and the amount of arthroscopically defined joint damage.\textsuperscript{319}

**Biomarkers of Catabolic Processes**

The release of a fragment of the Type-I collagen non-helical telopeptide (ICTP), which includes the collagen cross-linking region, has been evaluated as a marker of bone resorption in humans.\textsuperscript{320,321} Concentrations of ICTP in the horse have not been shown of value in detecting pathological processes.\textsuperscript{315,316}

A relatively new set of antibodies recognizing Type-I collagen C-telopeptides (CTXs) has proven useful as markers of specific bone resorption based on clinical data from cases of human joint disease.\textsuperscript{322} In the same study from our laboratory the ability of serum markers to differentiate exercise from pathology and correlate biomarkers to clinical parameters of pain in an osteoarthritic model, CTX
was less useful than CS-846, CPII, and GAG biomarker concentrations in predicting whether a horse was from a control, exercised, or an osteoarthritic horse.\textsuperscript{311} Other work in our laboratory has identified CPII and CTX-1 as potential serum indicators of the exercise effects on the developing skeletal system in young horses. Higher serum concentrations of CTX-1 and lower concentrations of CPII were found in trained foals compared to other groups, but these differences later disappeared during an additional 6 mo of identical exercise.\textsuperscript{323}

One of the principal aims of biomarker research is to diagnose early subchondral bone disease and, thereby, potentially predict fracture. This was the basis of a study funded by the Grayson-Jockey Club Foundation and carried out with racing Thoroughbreds in southern California reported elsewhere in these proceedings by Dr. Frisbie.

Gene Chip Microarray

Gene chip microarray is the latest advance in biomarkers and represents a molecular approach to defining a disease process. The principal is to have an array of a large number of gene sequences (cDNAs) on a computer chip. The entire human genome is currently available on a computer chip (Affymetrix), and the same company, in corroboration with an Australian company Genetraks\textregistered, has produced an equine gene chip containing more than 3000 sequences.\textsuperscript{33a} The production of this chip facilitates the simultaneous relative quantitation of multiple mRNAs and allows for comprehensive assessment of expression amounts.

In recent work from our laboratory\textsuperscript{bb} (Frisbie et al., unpublished data), the potential usefulness of gene chip microarray as a diagnostic tool in OA has been explored. Blood samples were taken during the development of experimental OA (using the carpal chip fragment exercise model) in the horse, and we were able to identify notable upregulation of 18 different genes in the OA group compared to the controls. This change in gene expression started early in the development of the osteoarthritic disease process. It is envisioned that this ability will be combined with conventional immunologic biomarkers (previously discussed) to provide a diagnostic platform for OA and other diseases.

Summary

Our ability to diagnose cartilage and bone disease in an individual with a single sample using biomarkers is progressing. We are not there yet, but we can monitor disease process with regular sampling.

14. Prevention of Equine Joint Injuries: What Are We Doing?

Lameness has been repeatedly shown to be a notable cause of wastage in young horses in race training.\textsuperscript{324,325} Joint-related diseases are the most common cause of lameness in all athletic horses. The forelimb is the most commonly affected by musculo-skeletal injury, and 85% of forelimb injuries are located between the carpus and MCP joint.\textsuperscript{320} As discussed previously, OA is associated with repetitive cyclic trauma of the joint and most commonly is Type-I (associated with capsulitis and synovitis).\textsuperscript{45} It is now recognized that the joint is an organ; consequently, all tissues in the joint can be the primary site of injury or disease. Although there have been many advances in treatment, there has been less work on prevention. Firth recently stated signs of joint injury are likely to occur in equine athletes, and we tolerate and treat the problem after it has occurred.\textsuperscript{327} He also stated that when the injury is severe, we spend much time and expense on long duration and costly treatments and that this is not a particularly rational approach. He expressed surprise at all the attention created for detecting slight abnormality in the form, range of motion, flexion, function, appearance, and radiograph features of the joint before sale, that we are less assiduous in taking an active role in either the individual clinical case or in equine athletes in general after sale, but await clinical signs before intervening.

As discussed previously, the diagnostic modalities are able to establish the presence of end-stage disease but are less effective for earlier stages of OA. Debate continues on the primary site of injury that leads to OA associated with cyclic trauma (be it articular cartilage, subchondral bone, or joint capsule), and this author feels that all three are involved individually or together in different situations. Thus, it is important to consider the joint as an organ in assessing early changes associated with exercise. A number of studies characterizing early changes have now been done, with the most attention given to articular cartilage and subchondral bone.

Response of the Joint to Change in Exercise

Within limits, tissue strength or other capability to function is increased by exposing it to an appropriate stimulus.\textsuperscript{327} This is physiological load and physiological response of the tissue to the new demand but not necessarily implying damage. If the stimulus is beyond physiologic loading, damage to the tissue might occur, at which point the load and stimulus could be considered pathological. Differentiation of this threshold is key to training without injury. However, complicating factors include individual variability between horses, training surface, training method, and last but not least, our ability to easily detect the earliest of these injury processes. A critical question is at what point will gradually increasing activity lead to damage of a particular tissue.

The concept of microdamage, particularly in bone and tendon, has been developed in recent years. In subchondral bone, this microdamage can include diffuse microdamage, microcracks, cell death, or failure of remodeling. There is evidence that change can occur early in exercise,\textsuperscript{47} and disease
occurs when the rate of microdamage has exceeded the processes that might repair it.

Wild Horses and OA

Man is commonly implicated as the cause of injury in the equine athlete. However, horses in athletic competition are not the only ones that sustain joint injury. A study of wild horses culled to sustain equine health and the environment in New Zealand provided interesting results.328 On examination of left and right MCP and carpal joints, MCP OA (recognized macroscopically with Indian Ink staining and confirmed histologically) occurred and was worse in older than younger horses. In the carpi, distal radial carpal bone chip fragments and medial palmar intercarpal ligament ruptures were observed in few and many cases, respectively. The lesions were chronic (in other words, not associated with the recent herding, transport, or slaughter). The important point from these studies is that lesions commonly attributed to the high speed and fatigue associated with intensive athletic training or competition can be found in animals that have not experienced training.

Studies with Induced and Controlled Exercise Programs

Previous studies have involved treadmill exercise,329–334 exercise on grass,335–343 and in controlled studies, testing joint disease treatments.65,66,102,344

Treadmill Studies

Several publications have been produced based on a study where 12 untrained horses (18–21 mo) were paired commonly and randomly assigned to an exercise group (Group 1) that underwent 19 wk of progressive high-intensity training on a high-speed treadmill. Group 2 underwent walking exercise only.329–339,345 Comparisons were made between the dorsal aspect and palmar aspect of the middle carpal joints. Dorsal cartilage had higher collagen content, DNA content, and chondrocyte numerical density, but lower GAG content than palmar cartilage.345 Cartilage from horses undergoing high-intensity training had a notably higher GAG content than cartilage from horses undergoing low-intensity training, with maximal differences being observed in cartilage from dorsal radial and dorsal intermediate carpal articular surfaces. Overall, no effect of exercise on collagen was observed, but at sites predisposed to clinical lesions, cartilage from horses undergoing high-intensity training contained notably less collagen than from horses undergoing low-intensity exercise. There was superficial loss of toluidine blue staining, primarily at dorsal sites and in the high-intensity exercise group. Overall, the changes were maximal at sites predisposed to clinical lesions and indicate that load variations within the joint may risk exceeding the physiologic threshold at high-load sites that are predisposed to clinical injury.345

In examination of the subchondral bone, horses undergoing high intensity had thicker subchondral bone thickness, with greater osteoid perimeter and, at individual sites, had a smaller osteoid seam, width, and eroded cavity. Exercise-related differences were most marked at dorsal locations. Again, the conclusion was that the combined effect of exercise and local load variations within a joint may lead to maximal adaptive responses and overload at sites predisposed to injury. Mechanical studies of the cartilage were done with an automated creep indentation apparatus in the dorsal cartilage was less permeable, thinner, and had a loss of chondrocyte alignment compared to palmar cartilage.345 Dorsal radial cartilage and third carpal cartilage of strenuously trained horses were notably less stiff than that of gently exercised horses. The former had reduced superficial toluidine blue staining compared to that of the gently exercised group. The CCL layer was also examined and was shown to be greater in the high-intensity exercise group. The CCL area was thick without alteration in hyaline non-CCL thickness, and the response was maximal at sites that withstand high intermittent loads (dorsal locations). It was suggested that increased CCL thickness with exercise may maintain the articular surface thickness gradient in the face of alterations in hyaline cartilage and/or subchondral bone stiffness.345 Another interesting finding was that fibronectin was found to be localized to sites of cartilage degeneration and released into the matrix in the local area (dorsal radial carpal cartilage had increased superficial staining and fibrillated cartilage showed increased intracellular and local matrix staining in the superficial zone).331

In another treadmill study, Kawcak et al. showed the development of microdamage, including microracks, diffuse microdamage, cellular death, and increased remodeling in horses exercised on a high-speed treadmill for 6 mo compared to horses that were walked.87,89 An important finding in this study was marked variation between horses, with one horse having osteochondral fragmentation in both the carpal and MCP joints, whereas another horse had no gross articular damage.

Massey University Grass Exercise Study

This study was performed in 2-yr-old Thoroughbred racehorses on grass and sand race tracks in New Zealand.338–345 Fourteen Thoroughbred fillies were used, four chosen from one group of yearlings, that had been born and raised on one farm, reared on pasture, and had received no grain. Seven horses were placed in race training, and seven were controls that were kept at pasture in enclosures approximately 100 × 25 feet. Horses were exercised 6 d a wk, with the regimen consisting of 4 wk of slow cantering, 4 wk of fast cantering, followed by 4 wk of fast cantering with fast gallop superimposed twice weekly on Wednesday and Saturday. The
horses were worked in pairs, whereas the seventh horse was worked with a horse from an adjacent stable. The duration of the phases of exercise were timed, and all distances and times were recorded. The training period was for 13 wk. Volumetric BMD, measured with CT, was marked by higher in the epiphysis and slightly higher in the diaphysis in trained compared with untrained horses. However, greater bone size in the trained horses had the most effect on an index of bone strength. Histologic examinations with a calcin label showed that there were fewer active osteones (Haversian system containing calcin label), which were a smaller diameter, and had a greater bone apposition rate in trained compared with untrained horses. The conclusion was that bone responded rapidly to early training.

Examination of bone and articular cartilage in the carpus showed macroscopic lesions in the cartilage were few and mild and not notably different between groups. High BMD was confined in the dorsal area of the subchondral bone and was notably higher in trained than in untrained horses. Adaptive increase in density was associated with thickening of the trabecular junctions that were oriented proximodistally. Hyaline cartilage was notably thicker in the concavity of the radial articular facet compared to dorso-lateral to the sagittal ridge showed signs of early degeneration, but the scores with Indian Ink staining were relatively low (indentation stiffness and collagen content) and relatively high water content. Effects of exercise on side (left versus right) were not detected for any measure. Overall, indentation stiffness correlated positively with reflectance score.

Global Equine Research Alliance (GERA) Study
A recent study by GERA tested the hypothesis that early exercise of young horses may modulate the musculoskeletal to better withstand training and racing. The study was prompted by earlier work in Dutch Warmbloods that suggested lesions of OC may be reduced in exercising foals. Thirty-six foals born in August 2000 in New Zealand were randomly divided into two groups. The conditioned group lived in pasture and took part in a conditioning program of increasing exercise amount from approximately 10 d after birth until breaking at 18 mo. The other group exercised spontaneously at pasture only. The results of examination of six foals from each group at 18 mo are currently being evaluated. However, preliminary results are available in articular cartilage and bone.

Articular cartilage samples were taken from the palmar and dorsal regions of the left and right distal third metacarpal bones and the palmar region of the left hind third metatarsal bone in the fetlock joints, incubated with fluorescence stains and examined under confocal laser scanning microscopy to assess chondrocyte viability within 24 h of collections. The number of viable and dead chondrocytes was determined based on the fluorescent staining characteristics. The subchondral epiphyseal BMD adjacent to the articular cartilage sample site was measured using CT data from regions of interest, 2 mm proximal to the interface of CCL and subchondral bone. The mean percentage of viable chondrocytes (pooled sites) was 14% greater in the conditioned than in the non-conditioned group, and the mean percentage of viable chondrocytes was 34% greater in dorsal sites in the conditioned than in the non-conditioned group. Variability and the percentage of viable chondrocytes was much less in the conditioned group (both sites) and in palmar sites in both the conditioned and non-conditioned groups. Irrespective of treatment group, the mean percentage of viable chondrocytes was 16% greater in palmar than dorsal sites, and also the percentage of viable chondrocytes was 6% greater in the medial compared with the lateral sites, with no notable interaction with treatment group or region. Mean BMD and mean percentage area of high-density bone in the region of interest were not notably different between conditioned and non-conditioned groups.

Combined with previous work from the Massey University grass study, it appears that articular cartilage responds by both increasing in thickness in certain locations and also increasing the number of viable chondrocytes with exercise. These data are also interesting in light of a recently reported in vitro study where intermittent compressive strain on chondrocytes suspended in agarose suggested that the compressive strain could protect cartilage by suppressing the expression of aggrecanase-1 and aggrecanase-2 (as previously discussed considered to be the major matrix degrading enzyme responsible for cleaving aggrecan). In the same fetlocks, cartilage structure and biochemical and biomechanical properties varied markedly with site in the joint. Sites just medial and lateral to the sagittal ridge showed signs of early degeneration, but the scores with Indian Ink staining were relatively low (indentation stiffness and collagen content) and relatively high water content.
match that of a racehorse. The soil loading conditions for horse racing are extreme with 9-kN force applied at a rate of 10 m/s. A system has been developed that replicates the strain rate and loads applied to the hoof to the soil. By replicating the strain rate and load in the system, it is possible to measure the tangent modulus of the track at the high strain rates encountered in horse racing, and this system has now been tested to provide quantitative baseline data from tracks around the country. In tangent with these studies at the racetrack, impact hoof landing velocities in treadmill trotting and galloping on a treadmill were also measured. Horizontal hoof velocity and impact were notably greater in the lead limb relative to the trailing limb at gallop and notably greater than during the trot. These results provided a starting point for both computer and mechanical models simulating hoof strike with the goal of reducing equine injuries.

In addition to these evaluations of the racetrack surface, attention has also been paid to the base where concerns over slope and irregularity have long been expressed. Using ground-penetrating Doppler radar, it is now possible to evaluate the base of the racetrack non-invasively, and we have been able to develop data that show irregularities in the base as well as corresponding problems in the cushion that could predispose to injury and can be corrected by the track superintendent. Newer synthetic tracks such as Polytrak have also been evaluated. Overall, the work so far shows that variability within a racetrack is probably more of an issue than variability between racetracks. The overall objective of these studies is to validate the racetrack and eliminate and minimize track role in injury.

16. Trying to Put Objective Data Behind the Conformation Questions

Conformation is another factor that is sometimes blamed for musculoskeletal injury. There have been no reports on the relationship of overall body conformation to clinical findings in the racing Thoroughbred, and two studies were done developing an objective method for recording specific body measurements to investigate the role of conformation in musculoskeletal problems in the racing Thoroughbred and look at these changes with growth and their relationship to musculoskeletal problems. In the initial study, annual photographs were taken more than 4 yr and conformation measurements made from photographs using specific reference points. Correlation analysis revealed highly significant moderate to strong relationships between long bone lengths, and wither heights for all ages and all long bone lengths showed moderate to strong relationships with each other for all ages. Wither height, croup height, and length of neck top line, neck bottom line, scapular, humerus, radius, and femur increased notably from age 0 to 1 yr and age 1 to 2 yr. Hoof length (medial and lateral, right
and left) grew notably between the ages of 0 and 1 and 1 and 2 yr but decreased in length between age 2 and 3 yr. The ankle of the scapula, shoulder, and radiometacarpus notably increased between all age groups (became more upright) and the angle of the dorsal surface of the hoof decreased notably from ages 0 to 1 and 1 to 2 yr. It was concluded that longitudinal bone growth in the distal limb increased 5% to 7% from weanling to age 3 yr and was presumably completed prior to the yearling year. Increase in shoulder angle contributed to increase in height. Back in the knee, conformation would improve from weanling to 3 yr of age.

In a second study, conformation was measured, and clinical observations were recorded for each horse on a regular basis. Clinical outcomes significantly \( (p < 0.05) \) associated with conformational variables included effusion of the front fetlock, effusion of the carpus, effusion of the hind fetlock, fracture of the left or right carpus, right front fetlock problems, and hind fetlock problems.

Offset knees (offset ratio) contributed to fetlock problems. Long pasterns increased the odds of a fracture in the front limb. An increase in the carpal angle as viewed from the front (carpal valgus) could serve as a protective mechanism as the odds for a carpal fracture, and carpal effusion decreased with an increase in the carpal angle. This study demonstrated relationships between conformation and musculoskeletal disease in the racehorse that could be measured objectively. The immediate information could be useful in selection and management of the racing Thoroughbred but, more importantly, will lead to more data being accumulated in this area because it will represent part of the solution to decreasing the injury rate.

17. Radiographic Screening of Thoroughbred Yearlings: Can it Help Reduce Injury?

Radiographic changes are often detected in young horses prior to training or athletic competition. These changes can represent typical variation in the appearance or development in bone structures or be the result of acquired, congenital, or developmental abnormalities. The prevalence of selected radiographic changes has been described for several breeds, including Warmblood horses in Sweden and clinically typical Standardbred horses. Howard et al. described the result of survey radiography of 582 Thoroughbred sale yearlings and paid particular attention to the presence of OCD and sesamoiditis.

A thorough understanding of the prevalence of specific radiographic changes at important times in the life of a performance horse is a prerequisite for veterinarians advising clients at yearling sales and researchers trying to identify radiographic problems that warrant concern or further investigation. A large study done to evaluate the prevalence of radiographic changes in Thoroughbred yearlings and the association with racing performance was done in collaboration between the equine sale companies, Equine Medical Associates (Lexington, KY) and CSU.

Radiographs and routine pre- and postsale examinations of 1062 yearlings were used to identify individual radiographic changes in sale yearlings. Overall, 946 (81%) yearlings started at least one race during ages 2 or 3 yr. Fourteen of 24 (58%) yearlings with moderate or extreme palmar supracondylar lysis of the third metacarpus, eight of 14 (57%) with enthesophyte formation on the proximal sesamoid bones, and 19 of 30 (63%) of those with dorsal medial intercarpal joint disease started a race. The odds of starting a race when age 2 or 3 yr were 3 times lower for yearlings with these changes \( (p < 0.01) \) compared with yearlings that did not have these changes. Twenty-five of 36 (69%) yearlings with proximal dorsal fragmentation of the first phalanx in the hind fetlock started a race, and these yearlings were also less likely (odds ratio = 0.51, \( p = 0.07 \)) to start a race. Yearlings from enthesophyte formation on hind proximal sesamoid bones placed in a smaller percentage of starts (16%, \( p=0.01 \), earned less money, and had lower earnings/start compared with starters without this change.

Many of the changes observed on radiographs of sale yearlings did not appear to influence future racing performance, but some are associated with reduced performance. The results of this study need to be applied in parallel with the clinical impression of veterinarians experienced in examining radiographs of sale yearlings. Some of the findings supported those established in the literature as incidental, and others suggested new areas of concern because they had not previously been reported in Thoroughbred sale yearlings. Further studies such as these are to be encouraged in the interest of identifying changes that could lead to problems when a horse goes into an athletic career; on the other hand, it is equally important to eliminate findings that have no influence on later soundness. This study has lead to an interest in evaluating changes in other disciplines such as cutting.

18. Summary

A number of methods including early exercise, early diagnosis of problems, and thorough evaluation of horses before they go into training have been discussed for their potential for decreasing the amount of day-to-day and catastrophic injury. Much of this research is costly and difficult to do but is worthwhile on both the welfare and economic point of view, and we must continue to strive to find ways of prevention of injury in our athletes. Early manipulation of the musculoskeletal system and early diagnosis of the various joint conditions are key to the future for optimal joint health.
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Milne Lecture: From Arthroscopy to Gene Therapy


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MILNE LECTURE: FROM ARTHROSCOPY TO GENE THERAPY


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