Disease Processes of Synovial Membrane, Fibrous Capsule, Ligaments, and Articular Cartilage

C. Wayne McIlwraith, BVSc, PhD, FRCVS, Diplomate ACVS

1. Introduction
Noninfective arthritis is an extremely common problem in the horse. It is considered as nearly always traumatic in origin with a spectrum of injury ranging from traumatic synovitis and capsulitis through ligamentous injury, meniscal injury, osteochondral fracture, subchondral bone disease, to osteoarthritis (progressive degradation of articular cartilage). Knowledge of etiology, pathogenesis, diagnosis and treatment of each of the different equine joint conditions has advanced considerably even since publication of a comprehensive text on equine joint disease in 1996.

2. Relevant Anatomy and Physiology
The joint should be considered as an organ made up of critical tissues, all of which can be affected in the disease process of osteoarthritis: the joint capsule (with its components of fibrous joint capsule and synovial membrane), intra-articular and capsular ligaments, articular cartilage, and subchondral bone.

The fibrous portion of the joint capsule is composed of dense fibrous connective tissue that provides some mechanical stability to the joint. The collateral ligaments are associated with the joint capsule. Intra-articular ligaments normally have a synovial membranea cover. The fibrous portion of the joint capsule and the ligaments are principally composed of collagenous fibers and type I collagen predominates in these fibrous connective tissues of the joint. The insertions of the fibrous capsule and articular ligaments into the adjacent bones demonstrate a zonal organization. Parallel bundles of collagen first become invested with the fibrocartilaginous stroma and as they near the bone become calcified. The collagenous fibers enter the bone cortex in a manner analogous to Sharpey’s fibers. This gradual transition of joint capsule and ligaments to mineralized fibrocartilage and then to bone enhances the ability of the insertions to distribute forces evenly and decrease the likelihood of pullout failure.

Equine synovial membrane is a modified mesenchymal tissue consisting of two layers histologically: the intima, incomplete cellular lining layer that lies next to the joint cavity and overlies a deeper layer of connective tissue (fibrous areola or adipose), termed...
the subsynovial layer or subintima. The three principal functions of the synovial membrane include phagocytosis, regulation of protein and hyaluronan content of the synovial fluid, and regeneration. The synovial membrane acts as an important permeability barrier which, in turn, controls synovial fluid composition. Most small molecules cross the synovial membrane by a process of free diffusion that is limited by the intercellular spaces in the synovial membrane rather than by blood vessel fenestrations. The protein in synovial fluid is derived from plasma but the source of some 2% protein firmly bound to hyaluronan is unknown but probably it comes from the type B synoviocytes. In traumatic effusions, the changes in protein content and composition have been associated with both increased vascular permeability and increased protein synthesis by the synoviocytes. Hyaluronan is synthesized by the cells of the synovial membrane.

Articular cartilage is normally milky and opaque in the thicker regions but translucent with a slightly bluish tinge in the thinner regions. The articular cartilage of equine joints is generally of the hyaline type. However, fibrocartilage is also present in synovial joints at the junction of articular cartilage, synovial membrane and periosteum (called the transition zone), and also in menisci.

Histologically, adult articular cartilage has been divided into four layers and the chondrocytes have different appearances within these layers (Fig. 2):

1. The tangential or superficial layer, containing flattened or ovoid chondrocytes and tangentially oriented collagenous fibrils
2. The intermediate or transitional layer, containing larger chondrocytes that may be single or paired and randomly oriented collagenous fibrils
3. The radiate or deeper layer, containing chondrocytes arranged in vertical columns separated by collagenous fibrils that have an overall radial arrangement
4. The calcified cartilage layer, composed of mineralized cartilage and chondrocytes in various stages of degeneration

A basophilic-staining, undulating line of division between the radiate layer and the layer of calcified cartilage is termed the "tide mark" or "tide line." It delineates the elastic, nonmineralized layers of the articular cartilage from the layers of calcified cartilage that has little resilience.

The extracellular matrix of the articular cartilage is a complex of collagens, fibrils, amorphous proteoglycans, glycoproteins, and water.

Fig. 1. Diagram of a typical synovial joint. Courtesy of Bayer Animal Health.

Fig. 2. Histologic picture of articular cartilage.
Type II collagen comprises 90–95% of the collagen 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. Type II collagen is secreted as a procollagen and the complete sequence of equine type II procollagen messenger RNA has been reported. The same authors also showed that chondrocytes harvested from juvenile horses exhibited more synthetic activity in culture with high steady state levels of messenger RNA for type II procollagen. Type II procollagen is expressed at very low levels in adult horses compared to 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-1 beta and TNF alpha produced a dose-dependent decrease in the steady state messenger RNA levels for type II collagen. There are also small amounts of type VI, IX, XI, XII, and XIV. These minor collagens help form and give stability to the type II fibrillar network, aggregating proteoglycans (aggrecan) are contained within the type II collagen framework and also attached to it.

The proteoglycans (previously called mucopolysaccharides) are the other major solid component 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 glycosaminoglycan (GAG) side chains (Fig. 3). This aggregate is called aggregating proteoglycan or aggrecan. The major glycosaminoglycans in adult articular cartilage are chondroitin-6 sulfate and keratan sulfate. The aggrecan molecules are contained only by the collagen network and hence the proteoglycans impart compressive stiffness to the cartilage.

Noncollagenous, nonproteoglycan glycoproteins constitute a small but significant portion of articular cartilage and include link protein, chondronectin, fibronectin, cartilage oligomeric matrix protein (COMP), thrombospondin, and ancorin C-II.
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–epiphyseal 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: 1) a permeable matrix that is stiff in compression, 2) a fibrous network capable of withstanding high tensile stresses, 3) a fluid that flows under load or deformation and aids in dissipating high stresses in the tissue, and 4) a high swelling pressure that results in a matrix swollen with water. It has also been noted that an important function of aggrecan in cartilage is to retard the rate of strength and alignment when a tensile load is suddenly applied and that this mechanism may be useful in protecting the cartilage collagen network during nonphysiologic situations.

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 in the surrounding matrix. This may be facilitated by a cilium from each chondrocyte which 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 hyaluronan concentration. 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.

Dynamic load and the action of cytokines are considered to be involved in matrix turnover. Cytokines of principal interest at the moment are the interleukins and tumor necrosis factor alpha. These factors act on chondrocyte receptors and influence the production and activation of metalloproteinases. The activity of matrix metalloproteinase in turn is inhibited by tissue inhibitors of metalloproteinase (TIMP-1 and TIMP-2) and it has been shown that there is a slight excess of TIMP over metalloproteinase concentration in normal 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 TGF superfamily (including BMPs) and GDF that are possibly involved in articular cartilage synthesis.

The synovial joint requires two systems that require lubrication: a soft tissue system involving the sliding of synovial membrane on itself or other tissues and a cartilage on cartilage system. Lubrication of the synovial membrane is by boundary lubrication and hyaluronan is the principal component. Cartilage on cartilage lubrication uses two systems: boundary lubrication and hydrostatic lubrication. Boundary lubrication operates at low loads, the necessary component being primarily a glycoprotein lubricating fraction called lubricin. At higher loads, boundary lubrication fails and the joint is lubricated by hydrostatic or squeeze film lubrication. It has also been suggested that plugging of cartilage pores by hyaluronan after a squeeze film is present on the surface may facilitate hydrostatic lubrication (called boosted lubrication). It is now felt that cartilage on cartilage lubrication is not totally independent of hyaluronan.

Force attenuation studies have shown that bone and periarticular soft tissues are the principal shock absorbers in the joint and cartilage provides little shock absorption.

The subchondral plate and epiphyseal bone beneath it form an integral part of the joint structure providing structural support to the overlying articular cartilage. The subchondral plate consists of cortical bone that varies in thickness, depending on the joint. With exercise, remodeling occurs and the amount of dense cortical bone can increase, at least in the carpus and the fetlock, but there is marked variation between horses. These changes and the primary role of subchondral bone disease in many cases of joint disease will be detailed in the next article.

3. Pathobiology of Joints and Their Reaction to Insult and Injury

The reaction in the various joint-associated tissues should not be considered in isolation as evidenced by the example of 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. Intra-articular 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 net-
work could be an important step in the pathogenesis of a more 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 secondarily from release of cytokines. Subchondral sclerosis may also lead to further physical damage to the articular cartilage because of decreased shock absorption. Subchondral sclerosis can cause significant clinical compromise and may also contribute to the degenerative process by the release of enzymes, inflammatory 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 1) alleviate the immediate compromising effects of inflammation, including pain and reduced function; 2) 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 3) prevent or minimize the development of osteoarthritis (OA).
Synovitis and capsulitis as primary entities in athletic horses are presumed to be associated with repeated trauma. Severe injury to the fibrous joint capsule can also cause instability. 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. Some injuries may affect diffusion across the synovial membrane and others will have a primary effect on the metabolism of the chondrocyte. Mechanically damaged synoviocytes may release degradative enzymes and cytokines and these will alter the intra-articular environment and possibly affect articular cartilage. It has also been suggested that high intra-articular 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 lead to reperfusion injury. Flexion of a joint with sufficient synovial effusion could raise the intra-articular pressure to levels of impaired blood flow through the synovial capillaries. Ischemia and reperfusion could lead to the production of oxygen-derived free radicals.

It has been demonstrated that in the presence of synovial effusion in the hip, a position of extension and medial rotation causes an increase in intra-articular pressure which may compromise the blood supply to the capital epiphysis of the femur. It has also been demonstrated in inflammatory synovitis in the human knee that the rise in intra-articular pressure with isometric quadriceps contraction related to effusion volume and that the inflammatory process prevents reflex muscle inhibition. The latter is normally a locally protective mechanism that minimizes the potential for intermittent ischemia or oxidative injury.

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 intra-articular tissues is well recognized. The presence of cartilaginous wear particles increases the cellular production of prostaglandin E₂, cytokines, and the neutral metalloproteinases (collagenase, stromelysin and gelatinase). It has also been shown that the proteoglycans released into synovial fluid cause synovitis.

5. Injury or Insult to the Synovial Membrane

Injury or insult to the synovial membrane is important because of the potential to release metalloproteinases, prostaglandins, free radicals, and cytokines (IL-1 and TNF alpha). Four principal classes of proteinases are recognized: metalloproteinases, serine proteinases, cysteine proteinases, and aspartic proteinases. The synthesis of many of them have been shown to be altered by a variety of cytokines and hormones.
Metalloproteinases
These enzymes are considered to play a major role in the degradation of the extracellular matrix. Metalloproteinases are characterized by a requirement for Zn²⁺ in their active site. Calcium is also required for expression of full activity but does not reside in the active site. Neutral proteinase activity was identified by Sapolsky et al in 1974 and more specifically identified as a metalloproteinase (MMP) in 1976. Since that time the enzyme has been shown to be identical to stromelysin or MMP-3. Matrix metalloproteinases may be further subdivided into collagenases, gelatinases and stromelysins. Evidence linking MMP to matrix degradation includes their presence in increased concentrations in diseased cartilage, topographic relationship to OA lesions, synthesis by articular cells and activity at physiologic pH. Matrix metalloproteinases that have been incriminated in osteoarthritis include collagenase 1 (MMP-1), collagenase 2 (MMP-8), collagenase 3 (MMP-13), stromelysin 1 (MMP-3), and two gelatinases (MMP-2 and MMP-9). Evidence for varying roles of these MMPs in equine articular cartilage degradation is accumulating and will be defined below. The potential for MMPs to degrade collagen and proteoglycans will be discussed separately.

More recently, new members of the ADAM family, known as ADAMTSs, a disintegrin, and metalloproteinase with thrombospondin motifs (ADAMTS-4 and ADAMTS-11) are known as aggrecanase-1 and -2 because of their ability to cleave specific sites in aggrecan, a proteoglycan that maintains aggrecan. Three collagenases have been identified. The one that was recognized first and has been investigated the most is interstitial or tissue collagenase. Interstitial or tissue collagenase (also called matrix metalloproteinase [MMP]-1) is specific for collagen as a substrate and cleaves all three chains of the triple helix at one susceptible point between residues 775 and 776 (glutamic and glycine respectively) of the α1 (I) chain of collagen types I, II, and III. Collagenase also cleaves collagens type VII, VIII and X but does not cleave basement membrane type IV, V and VI or types IX and XI. Interstitial collagenase is produced by a wide variety of cells including macrophages, fibroblasts, synovial cells, osteoblasts, chondrocytes and endothelial cells. There is a second human collagenase called PMN collagenase (also called MMP-8). PMN collagenase is stored in specific granules of the PMN and secreted in response to appropriate stimuli but recent evidence supports a key role in human OA. Recently, evidence has accumulated in both human and equine studies that the primary collagenase involved in the degradation of type II collagen of articular cartilage may in fact be collagenase 3 (also known as MMP-13). Caron et al. found that MMP-13 is produced by equine chondrocytes and that MMP-13 expression was significantly stimulated by rhuIL-1. The equine MMP-13 cDNA had 93% homology with the human MMP-13 cDNA sequence. MMP-13 is expressed at low amounts in stationary equine chondrocyte cultures and rhuIL-1 beta significantly upregulates this expression. Human MMP-13 was initially cloned from a cDNA library obtained from a human mammary carcinoma. Subsequently MMP-13 was documented to be a product of human articular chondrocytes. Expression of MMP-13 by chondrocytes in human osteoarthritic cartilage has been demonstrated and it was shown that MMP-13 turned over type II collagen at least 10 times faster than MMP-1. Experiments with intact type II collagen demonstrated that MMP-13 cleaved type II collagen at the same bond as MMP-1 but this was then followed by a secondary cleavage that removed three amino acids from the quarter fragment amino terminus. Other researchers have demonstrated higher levels of expression for both MMP-1 and MMP-13 by OA chondrocytes compared to normal chondrocytes. In addition, they showed messenger RNA for MMP-8 was present in OA cartilage but not normal cartilage. Tumor necrosis factor alpha has also been shown to stimulate expression of all three collagenases. Freemont et al demonstrated the gene expression of MMPs 1, 3, 9 in articular chondrocytes during histologic development of the cartilage lesion of OA using 35S-labeled cDNA probes. In OA MMP gene expression was greatest in the superficial layer. In contrast, messenger RNAs for MMP 3 and 9 were expressed deeper in the cartilage; MMP 9 early in the disease and MMP 3 with a biphasic pattern in early and late stage disease. They concluded that the expression of genes for MMPs 3 and 9 is differentially regulated in human articular chondrocytes and, in individual cells, is related to the depth of the chondrocyte below the cartilage surface and the nature and extent of the cartilage lesion. Consideration of the breakdown of proteoglycan attention has focused on stromelysin (also called proteoglycanase or MMP-3) as well as an initially undefined enzyme called “aggrecanase” (now known as aggrecanase-1 and -2 or ADAMTS-4 and 11). Stromelysin was first purified by Galloway et al and was termed “proteoglycanase” because it degraded proteoglycan. Stromelysin has a wide variety of substrates including proteoglycans (aggrecan, decorin, fibromodulin, link protein), type IV, V, VII, IX and XI collagen (also cleaves type II collagen in nonhelicial sites). Stromelysin is also considered to have a significant role in activating pro-collagenase to collagenase. A second enzyme closely related to stromelysin and called stromelysin-2 (also MMP-10 and transin-2) has been cloned and characterized. At present its role in inflammatory diseases is unknown. In vitro studies of IL-1-induced cartilage degeneration have revealed evidence of collagen degradation that could be at-
tributable to stromelysin in addition to that attributable to collagenase. The molecular cloning and cartilage gene expression of equine stromelysin 1 (MMP-3) has been described recently by Balkman and Nixon.

Initial cleavage of the large aggregating proteoglycan, aggrecan, in situ occurs between the G1 and G2 domains (the E2 region), which causes release of the glycosaminoglycan bearing region of proteoglycan and the G1 domain remains attached to the hyaluronate (HA) backbone. Three related metalloproteinases examined (rabbit bone stromelysin, recombinant human stromelysin-I, and stromelysin-2) have all been shown to cleave cartilage proteoglycans at this location. The specific site of this cleavage is at the asparagine (341)-phenylalanine (342) bond. It may also be the primary site of cleavage during normal proteoglycan turnover in the cartilage matrix. Stromelysin also cleaves link protein in human neonatal cartilage aggrecan at the his-16-ile-17 bond. It has been shown that in addition to cleavage at the ASN341-PHE342 site (MMP site), cleavage between the G1 and G2 domains also occurs at the GLU373-ALA374 site and this has been attributed to aggrecanase. It has been demonstrated more recently that aggrecanase is the primary enzyme responsible for breakdown of proteoglycans in cartilage degradation whereas the principal role of stromelysin is in normal homeostasis and remodeling.

A 72-kD gelatin-degrading proteinase (gelatinase) (also called MMP-2, type IV collagenase, and matrixin) degrades denatured type II collagen, type IV collagen, and also has significant activity against fibronectin, elastin and collagen types V, VII, X and XI but not against collagens I and VI. A second gelatinase (92-kD) (also called type V collagenase, MMP-9 and invasin), is a major secreted product of stimulated PMN leukocytes and macrophages. Equine matrix metalloproteinases 2 and 9 have been characterized in the horse. It is known that the 1/4 and 3/4 fragments generated by cleavage of fibrin or collagens by collagenases can unwind and are then susceptible to further cleavage by MMP-2 and MMP-9. MMPs 2 and 9 are produced by a variety of equine cell types. These enzymes have been characterized in the horse at elevated levels in synovial fluids from horses with joint diseases. It is still not clear from current work what role these individual MMPs may play in equine cartilage degradation.

All metalloproteinases are secreted as latent proenzymes that are activated extracellularly. Collagenase is probably normally activated by stromelysin, but collagenase, and possibly other metalloproteinases, can also be activated by plasmin (produced from plasminogen by the action of tissue or urokinase type plasminogen activators, kallikrein and cathepsin-B). Stromelysin is activated by plasmin and other proteinases that activate collagenase. Recent evidence suggests that latency is attributable to formation of an intramolecular complex between a single cysteine residue in the propeptide domain and the essential zinc atom in the catalytic domain (these are the only two domains common to all MMPs). Activation is associated with detachment of the cysteine residue from the complex and is referred to as the “cysteine-switch” mechanism of activation.

The metalloproteinases are inhibited by two tissue inhibitors of metalloproteinase known commonly as TIMP (TIMP-1 and TIMP-2). It inhibits all known MMPs by forming a 1:1 enzyme-inhibitor complex. TIMP is found in many connective tissues and may be the most important inhibitor found in articular cartilage. A deficiency of TIMP relative to levels of metalloproteinases has been demonstrated in osteoarthritic human cartilage. It is currently thought that the balance between MMPs and the TIMP is important for the progression of articular cartilage degradation.

In summary, metalloproteinases 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. Metalloproteinases are inhibited by TIMP and a relative deficiency of the latter may be important in cartilage degradation. With the development of techniques to study equine MMPs, more complete investigations are happening in the horse. Questions that it will be interesting to find answers to include: 1) do the aggrecan fragments released into equine synovial fluids reflect cleavage by aggrecanase or MMPs; 2) which collagenase plays the major role in type II collagen degradation, 3) when equine articular cartilage is cultured ex vivo with inflammatory cytokines, is there increased degradation of aggrecan type II collagen and are MMPs involved, and 4) does administration of MMP inhibitors to horses with joint disease lead to clinical improvement and does such improvement correlate with reduced levels of aggrecan and collagen degradation?

Serine Proteinases
Synovitis can also produce plasminogen activators (serine proteinases). Two types of plasminogen activator are recognized. Tissue plasminogen activator (tPA) and urokinase (uPA) both cleave plasminogen to active plasmin. This cascade plays a role in activating metalloproteinase. This system is also regulated by a series of plasminogen activator inhibitors. That IL-1 can stimulate production of tissue plasminogen activator has been established. Other serine proteinases include elastase and cathepsin-G. Neutrophils are the source of elastase and cathepsin-G.

Cysteine Proteinases
Cathepsin-B, H and L are lysosomal proteinases that belong to the class of cysteine proteinases of...
which cathepsin-B and cathepsin-L are best known. Both cathepsin-B and L cleave the internal peptides of collagen. Cathepsin-B also cleaves the hyaluronic acid binding region from cartilage proteoglycans and degrades the glycosaminoglycan attachment region to small fragments.\textsuperscript{90,16} It has been suggested that the proteolytic action of cathepsin-B, at least in human joints, appears to be related to cysteine protease inhibitors.\textsuperscript{65} The significance of their role in cartilage degradation is controversial.\textsuperscript{77}

Aspartic Proteinases

Cathepsin D, which is the most prominent lysosomal proteinase acting at acid pH, requires aspartic acid residues as part of the catalytic mechanism. Under inflammatory conditions and during periods of rapid extracellular matrix destruction, it is secreted extracellularly by macrophages in connective tissue cells, mostly as a proenzyme. Under the active metabolic conditions found in inflammatory joint disease, it is possible that CO\textsubscript{2} and lactic acid production could create an environment of sufficiently low pH in the pericellular space to permit the proteolytic activity of cathepsin D. However there has been no defined work in the horse.

Prostaglandins

Prostaglandins (primarily E group) are produced in inflamed joints and can cause a decrease in the proteoglycan content of the cartilage matrix.\textsuperscript{114,134} Prostaglandin E\textsubscript{2} can be released from synovial cells in response to IL-1.\textsuperscript{25} The presence of prostaglandin E\textsubscript{2} in synovial fluid from inflamed joints has been demonstrated in the horse\textsuperscript{75,132} and in our laboratory at Colorado State University we use PGE\textsubscript{2} measurements as an objective index of the level of synovitis.\textsuperscript{43,63} Actions of PGE\textsubscript{2} in joints include vasodilatation, enhancement of pain perception, proteoglycan depletion from cartilage (by both degradation or decreasing synthesis), bone demineralization and promotion of plasminogen activator secretion. PGE\textsubscript{2} is released from chondrocytes on stimulation of these cells by IL-1 and TNF\textsubscript{a}.

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.\textsuperscript{48,49} There is also evidence that superoxide can degrade the alpha chains of collagen based on the finding that superoxide treatment inhibits gelatin.\textsuperscript{150} Proteoglycans may also be cleaved by free radicals.\textsuperscript{25,122} Increased free radicals in the synovial fluid of cases of equine joint disease have been recently demonstrated.\textsuperscript{29}

Nitric oxide has recently been recognized 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.\textsuperscript{106} The role of nitric oxide in joint disease needs and is receiving further attention.

Cytokines and Articular Cartilage Degradation

Much of the destructive proteinases previously described are released by cytokines. 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 tumor necrosis factor (TNF\textsubscript{a}) modulate the synthesis of metalloproteinases by both chondrocytes\textsuperscript{25,122} and synovial cells\textsuperscript{24,151} and are important mediators in joint disease. IL-1 and TNF\textsubscript{a} may be produced by synovial cells\textsuperscript{25} and may therefore be of importance in the deleterious effects of synovitis on articular cartilage. It is considered that the normal turnover of the extracellular matrix of the articular cartilage is regulated by the chondrocytes under the control and influence of cytokines and mechanical stimuli.\textsuperscript{140} Articular cartilage degradation in association with disease represents an exacerbation of these normal processes. Accumulation of knowledge in this regard commenced with the initial studies by Fell and Jubb\textsuperscript{36,37} of cartilage-synovial interactions using in vitro systems. 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 prostaglandins from chondrocytes.\textsuperscript{136} Inhibited synthesis seems to be a more significant event than proteoglycan degradation in studies in the horse but these experiments have been done in vitro with human recombinant IL-1.\textsuperscript{100} Recognition of the gene sequence for equine interleukin-1 by Howard et al at CSU\textsuperscript{52,53} can lead to specific studies with equine tissues as well as equine IL-1. It is felt that IL-1 produces its effects by binding with an IL-1 receptor on the cell. The presence of IL-1 in equine osteoarthritic joints was first reported in 1990 by Morris et al.\textsuperscript{89} An equine IL-1 containing extract was produced by May in 1990.\textsuperscript{74} To think that interleukin-1 acts solely on its own in stimulating metalloproteinase release is probably naive but it does seem important. Todhunter showed in an in vitro experiment with canine articular cartilage explants that neither metalloproteinase activity nor proteoglycan degradation were inducible in canine cartilage explants treated with recombinant IL-1a. However, proteoglycan synthesis was significantly decreased by concentrations of 10 and 100 ng of rH IL-1a/ml. Metalloproteinase activity in the medium accompanied proteoglycan degradation of cartilage treated with lipopolysaccharide and monocyte conditioned medium. The metalloproteinase released into the medium was identified as prostromelysin by results of Western blotting.\textsuperscript{135}

In very recent work, the significant role of equine interleukin-1 has been further consolidated. As
mentioned above, expression constructs containing cDNA sequences and coding EqIL-1 alpha and EqIL-1 beta have been previously generated, prokaryotically expressed and the recombinant protein purified. Articular cartilage explants from two horses were separately randomized to rEqIL-1 alpha or rEqIL-1 beta treatment groups (0–500 nanograms). Significant proteoglycan release was induced by both at concentrations greater or equal to 0.01 ng/ml with 38–76% and 88–98% of total glycosaminoglycan released by four and six days respectively. Significant inhibition of proteoglycan synthesis (42–64%) was observed at IL-1 concentrations greater than or equal to 0.01 ng/ml at two and four days. Increased PGE2 concentrations were observed at IL-1 concentrations greater than or equal to 1.0 ng/ml at two and four days. Significant differences were not observed between rEqIL-1 alpha and rEqIL-1 beta groups for analyses. This work showed that much lower concentrations of equine IL-1 caused these effects compared with previously reported studies using human recombinant IL-1.

In addition to IL-1 being detected with in situ hybridization in osteoarthritic cartilage in humans, it has recently been shown that tumor necrosis factor alpha does indeed act on cartilage but only at specific sites where chondrocyte TNFα receptor (TNF-R) expression is high. It is therefore considered that focal loss of cartilage will occur at sites where chondrocyte P55 TNF-R expression is high as well as if sufficient TNFα is present. It has also been suggested that this may explain the focal nature of cartilage loss in some instances of osteoarthritis. There is evidence that while TNF alpha is important in joint swelling, a direct role in tissue destruction is unlikely. On the other hand, IL-1 is not a dominant cytokine in early joint swelling but has a pivotal role in evasive cartilage damage.

Billinghurst et al first demonstrated induction of intra-articular TNF during acute inflammatory responses in equine arthritis. Prior to that time it had been demonstrated that recombinant human TNF α, like IL-1, causes cartilage degradation. It was presumed due to stimulating the chondrocyte to produce matrix degrading enzymes, however the role of TNF in individual clinical arthritides remains unclear. Based on inhibition studies, it would appear that IL-1 is the principal cytokine responsible for articular cartilage degradation and TNF-alpha contributes more to clinical morbidity and pain. IL-1 and TNF-alpha have been demonstrated using RT-PCR in the synovial membrane of inflamed equine joints. There are two types of TNF receptors (P55 and P75). Both receptors have been identified in synovial tissue and greater number of receptors are seen in joints affected by RA in comparison to OA. These receptors can be shed and act as soluble inhibitors of TNF (TNF binding proteins). Increased serum concentrations of soluble TNF receptors (sTNF-R) have been detected in patients with RA and OA in comparison to healthy controls.

Neuropeptides
It has been pointed out that in addition to its importance in the morbidity (pain sensation) of osteoarthritis, the nervous system has a potential role in the pathogenesis of the disease. The activation of articular nerves not only provides sensory perception of pain but also results in the release of neurotransmitters that have inflammatory potential. There is evidence that the peripherally released transmitters have deleterious effects on synovial and cartilage cell metabolism contributing to cartilage matrix depletion. Exposure of monocytes to substance P and other neuropeptides causes the release of the cytokines IL-1, IL-6, and TNF. Elevated synovial fluid concentrations of substance P and other peptides have been observed in humans with joint disease. Substance P levels are also elevated in horses with arthropathies. Substance P is not the only peptide found in nerves; others include peripheral peptides (VIP, MET-enkephalin, and somatostatin).

Fibronectin
It has been noted that the fibronectin content of osteoarthritic cartilage is considerably higher than that of normal cartilage but the significance of this or the role of fibronectin in the pathologic process has been controversial. It has been suggested that the appearance of fibronectin in diseased cartilage matrix may be a feature of the chondrocyte’s repair response to the loss of extracellular matrix and that fibronectin may interact with proteoglycans. It has also been recognized that fibronectin functions as a cellular adherence factor (it is a major cell surface glycoprotein) and is closely associated with the collagen of the extracellular matrix as well as proteoglycans.

Chondrocytes can be a source of some cartilage fibronectin, but plasma or synovial fluid may also be a source. Fibronectin in synovial fluid from osteoarthritic joints is derived from several sources, including synoviocytes, plasma, and increased production by degenerated articular cartilage. There has been more recent evidence that fibronectins may contribute to aggrecan degradation in osteoarthritis and inflammatory joint disease and that the fibronectins mediate their effects through catabolic cytokines.

6. The Role of Mechanical Factors and Subchondral Bone Change in the Pathogenesis of Articular Cartilage Degradation
The role of biomechanical factors has been frequently discussed in relationship to articular cartilage degeneration. It is well recognized that articular cartilage may be subjected to a harsh loading environment. Local intensity of loading is generally defined by mechanical stress (the force/
unit area acting on the tissue). The measurement of stress in physiologic situations is not yet possible and therefore much of the information in vivo is speculative. It has been suggested by some authors that the mechanical factors to which articular cartilage are normally exposed to in vivo are insufficient to destroy tissue directly but that when the chemical integrity of the matrix is compromised biochemically, direct mechanical damage becomes possible. However, other authors propose that subfracture impact loads can generate shear stresses that break collagenous crosslinks. This substructural damage, in turn, precedes chondrocyte enzyme production or entry of catabolic enzymes into the matrix. In an experiment of acute transarticular load in the canine patellofemoral joint, it was shown that fractures in the zone of calcified cartilage-bone interface with no visible abnormalities of the articular cartilage occurred. With India ink staining, there was superficial damage in the articular cartilage. The authors felt that their experimental model may also simulate the situation with severe ligamentous disruption in that unrecognized subchondral fractures or fractures in the calcified cartilage may be a common feature of musculoskeletal trauma and a major cause of subsequent osteoarthritis. In most situations, mechanical forces are considered more likely to destroy cartilage indirectly through insult to the subchondral bone, synovial membrane, or chondrocytes. It also needs to be recognized that while excessive forces may lead to articular cartilage loss, removal of all mechanical stimulation leads to atrophy. Normal cartilage structure and function is maintained by some intermediate level and frequency of loading.

When considering possible pathways for mechanical destruction of articular cartilage, an early concept was that early subchondral bone sclerosis causes a reduction in the joint’s shock absorbing capability and thereby places cartilage at risk of shear-induced tensile failure of cartilage crosslinks particularly under repetitive impulsive loading conditions. Recent work in our laboratory has demonstrated that when horses are subjected to athletic exercise on the treadmill microdamage in the subchondral bone can develop. In looking retrospectively at racehorses, the range of microdamage includes not only microfractures but also primary osteocyte death. It is felt that not only is mechanical support of the articular cartilage lost when subchondral bone microdamage progresses to macродamage but also potentially cytokine release from the bone can influence the state of the articular cartilage. We feel that subchondral bone is very important in the development of a number of articular lesions but it is a lot more complicated relationship than simply being secondary to subchondral stiffness and details are furnished in the next article.

7. Morphologic and Biochemical Reaction of Articular Cartilage to Insult
As discussed previously, trauma can cause an immediate physical defect or initiate a degenerative or degradative process. Degradation of articular cartilage is manifested in a sequence of morphologic changes including chondromalacia or softening, fibrillation (superficial or deep), and erosion (see also the section on osteoarthritis later in this article). It has now been well recognized (and the processes reviewed previously) that major biochemical changes take place and may indeed precede morphologic defects. Some of these processes are outlined in Figures 4 and 5. To varying degrees in different situations, synovial inflammation (synovitis), release of degradative mediators within the articular cartilage, and/or mechanical and cytokine-related factors from the subchondral bone can contribute to this degradation.

8. Formation of Osteophytes and Enthesophytes
In addition to the central process of articular cartilage breakdown in osteoarthritis, a second pathologic process characterized by proliferation of new cartilage and bone at the periphery of joints (osteophytosis) occurs. These spurs are usually called osteophytes but the term osteochondrophytes may be more accurate. Osteophyte formation is commonly considered to be a characteristic component, and a secondary consequence of osteoarthritis but this concept is to be questioned.

Using the canine cranial cruciate desmotomy model, Marshall demonstrated that osteophyte formation started early after surgery and could not be related to any major articular cartilage damage or subchondral bone change. He also documented that the first sign of an osteophyte was the appearance of fibrous tissue outside the bone and that this tissue then underwent chondroid metamorphosis and endochondral ossification. Using a filipin-induced equine model, this author demonstrated osteophyte formation unrelated to significant cartilage damage and formation followed the same sequence of fibrous tissue, then cartilage and bone. This connective tissue response at the junction of the synovial membrane, cartilage perichondrium and periosteum is characteristic of a marginal osteophyte (there are also central osteophytes in the interior of the joint). Osteophytes need to be distinguished from enthesophytes, which are bone proliferations at ligament, tendon or joint capsule insertions into bone. In situations where osteophytes are consistently associated with structural articular cartilage change and parallel them, it would seem appropriate to consider osteophytes a significant lesion of osteoarthritis.

Etiologies proposed for osteophytes include aging, mechanical instability, proliferative responses secondary to synovitis, and tissue responses to stretching of the synovial membrane at its insertion or forces of any soft tissue attachments.

Proceedings of the Annual Convention of the AAEP 2001
in the area of the transition zone.91 The concept that osteophytes develop as a secondary response to stabilize an osteoarthritic knee or increase available joint surface area is an old one. It is only relatively recently that it was shown marginal osteophytes stabilize the varus-valgus motion of the knee in humans with knee osteoarthritis.105 The authors of this study felt that the osteophytes might increase stability by pressing directly against slackened collateral ligaments, thus reducing ligamentous pseudolaxity. They also felt that surgical removal of such osteophytes should not be done to prevent even greater instability.

Work with an equine model demonstrates that osteophytes and enthesophytes can result from chemical synovitis and capsulitis and in the absence of instability.83 It is also recognized that osteophytes and enthesophytes are also seen in the horse without articular cartilage damage (defined arthroscopically) and also in the absence (in some instances) of clinical significance. Their removal is certainly inappropriate.

Enthesophytes have been recognized as a consequence of aging in humans.10 They are also seen in association with instability or without instability in horses. Remodeling and smooth margins to enthesophytes generally indicate the enthesophyte to be a sign of previous insult but of doubtful clinical significance.

References


146. von Rechenberg.


