

Current Treatments for Traumatic Synovitis, Capsulitis, and Osteoarthritis

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Pre-Treatment Considerations

In its broadest sense, the term *traumatic arthritis* includes a diverse collection of pathologic and clinical states that develop after single or repetitive episodes of trauma and may include one or all of the following: 1) synovitis (inflammation of the synovial membrane), 2) capsulitis (inflammation of the fibrous joint capsule), 3) sprain (injury of specific ligaments associated with the joint), 4) intra-articular fractures, and 5) meniscal tears (femorotibial joints).

Any of the above situations can potentially progress to osteoarthritis. To facilitate discussion of pathogenesis, diagnosis and treatment, it is convenient to divide articular trauma into three entities:

Type 1. Traumatic synovitis and capsulitis without disturbance of articular cartilage or disruption of major supporting structures. This includes acute synovitis and most sprains.

Type 2. Disruptive trauma with damage to the articular cartilage or complete rupture of major supporting structures. This includes severe sprains (A), meniscal tears (B), and intra-articular fractures (C).

Type 3. Posttraumatic osteoarthritis. This includes cases of disruptive trauma in which major residual damage is present. Patients may have deformity, limited motion, or instability of joints.

It must be recognized that there is considerable overlap in that cases of osteochondral fragmentation in the carpus or fetlock typically present as synovitis/capsulitis. The pathobiology associated with injury to each of the tissues of the joint have been detailed previously in these proceedings. There is obvious overlap between the entities of articular trauma and this needs to be recognized. However, each entity will be discussed separately as the specific treatments for each condition are most conveniently dealt with in this fashion. It should also be recognized that failure of a good response to treatment of traumatic synovitis and capsulitis commonly implies other damage within the joint. If one takes a problem-based approach and treats specifically for each problem present in the joint, the best results will be attained.

Radiographs are commonly made to eliminate the possibility of osteochondral damage. This is not always routine in sports medicine practices if the

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clinician feels comfortable that the problem is primarily localized in soft tissues. On the other hand, if there is failure to respond to therapy or the synovial fluid tap at the time of treatment reveals changes suggestive of more structural damage (and obviously if the lameness is of sufficient severity), radiographs need to be taken. Synovial fluid analysis (even gross inspection) is always useful. An evaluation of color and viscosity can be done while aspirating fluid or injecting the joint. With severe lameness associated with synovial effusion, synovial fluid analysis should always be performed to rule out infective arthritis. As implied above, diagnostic arthroscopy may be the only way to truly define the internal state of the joint and the degree of disease.

Principles of Treatment

There are a number of treatments for acute synovitis with or without accompanying capsulitis. The aim of these treatments is to return the joint to normal as quickly as possible. In addition to bringing relief to the patient and allowing it to return to normal work, suppression of synovitis and capsulitis is important to prevent the products of inflammation from compromising the articular cartilage and leading to osteoarthritis. These processes have been previously discussed in the first article. In addition to the potential deleterious effects of synovitis on articular cartilage, it is important to provide pain relief and minimize the potential microinstability associated with excessive synovial effusion. It has also been shown experimentally in the rabbit that joint inflammation weakens intra-articular ligaments in addition to affecting the cartilage.

In all traumatic entities in the joint, the goal—in addition to returning the joint to normal as quickly as possible—is to prevent the occurrence or decrease the severity of osteoarthritis. This in-depth seminar addresses medical treatments but it is important to note that timely removal of osteochondral chip fragments, timely and appropriate reduction and fixation of larger intra-articular fractures, accurate diagnosis of ligamentous and meniscal injuries with arthroscopy, and the appropriate treatment for osteochondritis dissecans entities are all critical treatments to preventing osteoarthritis. The remainder of this article details options available for treatment of the traumatic joint.

Rest and Immobilization

The usefulness of rest in cases of acute inflammation and capsular injury is obvious. The realities of racing or other athletic activities often prevent the proper application of this modality, which would allow a complete recovery in many cases. Bandage support may also assist healing of an acutely damaged joint. It has also been shown that a pressure bandage stimulates mechanoreceptors and this in turn can decrease pain sensation. Immobilization is important when there is any destabilizing injury

but is not ideal if the problem is limited to synovitis/capsulitis. However, prolonged immobilization may lead to muscle atrophy and adhesion formation within the joint as well as articular cartilage atrophy. Casting is only appropriate in cases of destabilizing injury. Passive flexion of limbs may help retain mobility and some hand-walking is recommended in most instances. We use passive flexion of the fetlock routinely after surgery to maintain capsular range of motion and minimize adhesions and fibrosis and, if it is considered appropriate (if the patient is willing), for primary capsular injury as well. Hand walking should always be continued even if training is stopped. If there is no destabilizing injury, hand walking will maintain motion on the joint capsule as well as prevent atrophic change in the articular cartilage.

Physical Therapy

Hydrotherapy may be useful immediately after a traumatic joint injury. Although the use of cold or hot water seems to be regularly debated, it is reasonable to assume that cold hydrotherapy is indicated in the acute stage of joint injury to retard the inflammatory processes of exudation and diapedesis and reduce edema.¹ The application of ice is extremely beneficial as a primary treatment for most acute joint injuries. After 48 hours, hot hydrotherapy may be indicated to relieve pain and reduce tension in inflamed tissues. The vasodilatory effect can aid in both fluid resorption as well as providing phagocytic cells.²

Swimming has also been used in the convalescent period with joint injury to maintain the horse's condition while relieving joint trauma. It is the closest treatment that can approximate nonweightbearing motion as practiced in human sports medicine. It is also possible that the massaging effect of the water on the limbs may help prevent fibrosis of the joint capsule. However, it should be recognized that swimming does not maintain joint tone, and a quick return of the horse to fast work is potentially dangerous.

There has been considerable use made in recent years of modalities such as electromagnetic therapy, electrostimulation, and low level laser for various musculoskeletal conditions including traumatic joint disease. There have been no controlled studies documenting their value but anecdotally symptomatic relief is considered to be achieved with these various modalities by people using them.

Any process that causes chronic fibrosis in the capsular tissues is contraindicated because it decreases joint motion and decreases the shock absorbing capabilities of the joint capsule. Diathermy and ultrasound have been used to produce deep heat in the tissues and to enhance vascularity and healing.¹ Repeated applications of ultrasound will cause bone resorption (osteoporosis). However, these techniques have not attained a prominent place in the treatment of joint conditions. Lini-

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ments are also commonly used.² The massaging effect of their application is probably as useful as their ability to produce heat.

Dimethyl Sulfoxide (DMSO [Domoso])

This polar chemical solvent has been used in the horse alone or mixed with corticosteroids to reduce soft tissue swelling and inflammation resulting from acute trauma.³ Its main value in this regard is considered to be the reduction of edema.⁴ More recently, DMSO has been shown to possess superoxide dismutase activity, whereby it can inactivate superoxide radicals. The drug has also been shown to enhance penetration of various agents through the skin and a 3-fold increase in the penetration of percutaneous steroid when mixed with DMSO has been reported.⁴ It has also been noted that when cortisone has been dissolved in DMSO the dilution of cortisone necessary to stabilize lysosomes was reduced from 1/10 to 1/1000 times. There has also been work to show an increased blood flow through experimental flaps and the presence of vascular dilation with DMSO application. This may also help with the resolution of soft tissue inflammation. The drug is bacteriostatic and produces collagen dissolution which may help in restoring pliability to fibrosed tissue.⁴

Such properties provide some rationale for its use in joint inflammation, and it has been demonstrated that the development of adjuvant polyarthritis in the rat is significantly inhibited by the local use of DMSO. The drug has a definite antiarthritic effect that seems independent of its ability to promote the absorption of corticosteroids.⁵ It was also shown that the local antiarthritic effect of hydrocortisone was increased 10-fold when DMSO was used as a carrier.⁵ Dimethyl sulfoxide has been used in the treatment against synovitis in horses.⁶ It is important that the medical grade DMSO (liquid or gel) be used. Gloves should be worn during its application.⁷

Joint Lavage

This technique was initially proposed to remove cartilaginous debris that caused synovitis.⁸ Production of synovitis with articular cartilage fragments and pu-

rified chondroitin sulfate has been demonstrated experimentally.⁹ In addition, synovitis of inflamed synovial membrane acts as a source of deleterious mediators (discussed in pathobiology) and this constitutes an additional reason for the use of joint lavage.

Lavage may be done under general anesthesia or standing. It is obviously possible to do more extensive lavage under general anesthesia. Clipping and aseptic preparation of the joint is performed after which two 12- to 14-gauge needles are inserted in the joint. The use of a fluid pump saves time for lavage. The clinical results from joint lavage are particularly gratifying in a patient with severe lameness associated with acute synovitis. Following the completion of lavage, therapeutic agents such as hyaluronan may then be administered. Joint lavage is used most commonly in association with arthroscopic surgery and is considered to be a significant part of the benefit achieved from surgery. It can, however, also be done standing and although the volume put in the joint is less than under general anesthesia, it still seems quite effective and there is no known data on minimum volume or flow requirements.

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Nonsteroidal Anti-Inflammatory Drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are substances other than steroids that suppress one or more components of the inflammatory response.¹ Such a broad definition would include both phenylbutazone-type drugs and the new intra-articular preparations such as HA, PSGAG, and pentosan sulfate. The term NSAID tends to be used more restrictively to describe anti-inflammatory agents

that inhibit some component of the enzyme system that converts arachidonic acid into prostaglandins and thromboxanes.² There are various nonsteroidal anti-inflammatory drugs available for and used in the treatment of joint disease in the horse. The most common one is phenylbutazone.

As defined above, all NSAIDs inhibit cyclooxygenase activity to some degree.³ With phenylbuta-

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zone, this effect is marked and one of the most important aspects of its therapeutic potential. However, other agents such as carprofen are relatively weak cyclooxygenase inhibitors leading to the conclusion that in addition to these agents' effects on prostaglandins, other mechanisms may contribute to their overall anti-inflammatory activity. Drugs such as ketoprofen inhibit 5-lipoxygenase *in vitro* in addition to cyclooxygenase but this property has not been demonstrated *in vivo*.⁴ Meclofenamate also inhibits human polymorphonuclear neutrophil (PMN) chemotaxis as well as degranulation and generation of superoxide free radicals. However, the significance of such findings to clinical anti-inflammatory therapy remains unclear. Although it is clear that in low doses aspirin and most of the newer NSAIDs inhibit the biosynthesis of prostaglandins (PGs) from arachidonic acid (and PGs have been shown to mediate fever, hyperalgesia, vasodilation [edema], and several interleukin-1 dependent responses), at higher doses these drugs inhibit non-PG-dependent processes such as the activity of a variety of enzymes, proteoglycan synthesis by chondrocytes, transmembrane ion fluxes and chemoattractant binding.⁵ The potential deleterious effect of suppressing prostaglandin E₂ (PGE₂) levels and inducing increased IL-1 secretion has recently been pointed out. It is well recognized that PGE₂ exerts a negative feedback on IL-1 release, an action which should lead to chondroprotection, so it is possible that NSAID-induced reduction of PGE₂ synthesis could adversely affect cartilage matrix by inhibition of this negative feedback pathway.⁶

Differential Activities of NSAIDs

A recent advance that should greatly increase our understanding of variations in the activity of different NSAIDs, as well as offering enhanced therapeutic usefulness, is the discovery of constitutive (COX-1) and inducible (COX-2) forms of cyclooxygenase.⁷⁻⁹ It is suggested that COX-1 is responsible for the production of PGs involved in normal physiologic functions, whereas COX-2, the production of which is produced by bacterial lipopolysaccharide and cytokines, would appear to have a role in inflammation. Many NSAIDs such as aspirin and indomethacin have been shown to be more potent inhibitors of COX-1 than COX-2, suggesting that they are likely to affect physiologic processes more than inflammatory ones.¹ It may be possible to reduce the toxicity of NSAIDs by choosing individual agents that are specifically active against the inflammation-associated isoenzyme COX-2 and leave the "physiologic" isoenzyme COX-1 unaffected to perform its homeostatic role.

There are clinical impressions that some NSAIDs are most useful in the treatment of orthopedic problems, where others are better in the treatment of colic. This new data suggests a potential for targeting individual agents on inflammatory problems in specific areas.¹⁰ It has been suggested that

drugs such as aspirin and indomethacin, which are more effective inhibitors of COX-1 than COX-2 are more likely to produce mucosal damage and ulceration in the gastrointestinal tract than such agents as naproxen, carprofen, and meloxicam, which are relatively more effective against COX-2.^{11,12}

Other Activities of NSAIDs

NSAIDs are well known for their toxic effects and, in particular, gastric ulceration. However, provided the drugs are used at clinical dose rates, such problems seem relatively uncommon in the horse.^{1,2} Although it is recognized that several factors may predispose toward phenylbutazone toxicity in the horse—for instance, breed and age—high dosage is considered to be particularly important.¹ The greatest range of side effects have been reported in humans and include edema, renal papillary necrosis, tubular nephritis, hepatotoxicity, blood dyscrasias (bone marrow depression and aplastic anemia), and skin rashes. Marked individual human variation is seen between NSAIDs in terms of reported efficacy and toxicity and differential toxicities are now being recognized in the horse.

Another action of NSAIDs that should be considered in relation to joint disease is their effect on proteoglycan synthesis. It is now known that a number of NSAIDs affect cartilage anabolism in addition to modulating the inflammatory cascade. Most work has been done with sodium salicylate and aspirin in which it has been demonstrated there is inhibition of proteoglycan synthesis.^{13,14} Salicylate produces much more profound suppression of proteoglycan synthesis in osteoarthritic cartilage compared with nondiseased cartilage. Differences between drugs and the question of levels in the synovial fluid need resolution. Currently NSAIDs such as tiaprofenic acid, piroxicam, and sulindac do not appear to affect articular cartilage anabolism and phenylbutazone does not seem to have any effect on equine cartilage.^a It has also been suggested that some NSAIDs such as benoxaprofen stimulate proteoglycan synthesis² and it has been recently shown that carprofen increases proteoglycan synthesis by equine chondrocytes and explants *in vitro*.^b The other issue that always needs consideration is the beneficial anti-inflammatory effect of a NSAID versus potential deleterious effects in the cartilage. In a recently published study using cultured explants of equine carpal articular cartilage, it was shown that at all concentrations, the anticatabolic effects of both phenylbutazone and Depo-Medrol influenced the proteoglycan content of the explants far more than did the antianabolic or cytotoxic drug effect.¹⁵ The normal proteoglycan loss in culture was reduced by the presence of either phenylbutazone or Depo-Medrol and the effect was significant at clinically relevant concentrations of phenylbutazone (2–20 µg/ml) but not Depo-Medrol (20–200 µg/ml). Depo-Medrol caused a dose-dependent suppression of proteoglycan synthesis at

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all concentrations and chondrocyte viability was affected only at the 2000 $\mu\text{g/ml}$ dose. Phenylbutazone affected proteoglycan synthesis and cell viability only at 2000 $\mu\text{g/ml}$ concentration.¹⁵

Classification of NSAIDs

NSAIDs can be divided into two groups based on their chemical structure. Most NSAIDs are carboxylic acids but a few, most noticeably phenylbutazone, are enolic acids. To the clinician this division is probably academic. However, as knowledge of the cyclooxygenase isoenzyme system increases, it has been proposed that it will prove possible to identify the features of individual agents in each group that affect their relative affinities for COX-1, COX-2, and any other form of COX. For instance the acetic acid derivative sulindac has been shown to be 100 times more active against COX-1 than COX-2 whereas the related compound, diclofenac, is slightly more active against COX-2.² Carprofen is equipotent against COX-1 and COX-2 and naproxen is relatively more potent in its effect on COX-2. This knowledge should contribute to the development of the "ideal" anti-inflammatory acetic acid or propionic acid derivative.

Phenylbutazone

Phenylbutazone is the most widely used NSAID in the horse. Although the plasma elimination half-life ($T_{1/2}$) is the traditional way of assessing the duration of action of various drugs, including antibiotics and anti-inflammatory agents, many NSAIDs demonstrate different kinetics at the tissue level. It has been shown with phenylbutazone that although the $T_{1/2}$ is 4 to 8 h, the inflammatory exudate $T_{1/2}$ is 24 h.¹⁶ Similar results have been reported in the horse for flunixin, meloxicam, and carprofen.¹

It is felt that the drug is relatively nontoxic at repeated doses of 2.2 mg/kg twice a day or less.¹⁷ Because of the extended duration of action of phenylbutazone in inflammatory exudate, single daily dosing (4.4 mg/kg) seems to be sufficient in most cases. Although it is called an NSAID, like most cyclooxygenase inhibitors phenylbutazone inhibits prostaglandin synthesis at much lower doses than those required to suppress edema and leukocyte accumulation in inflammatory foci. It is therefore most likely to be useful where PGE₂ plays a major role. Increased PGE₂ production has been demonstrated a number of times in joint disease in the horse. PGE₂ also has the ability to amplify the pain-inducing properties of histamine and bradykinin.¹⁸ It is considered that phenylbutazone exerts its analgesic effect in part by inhibiting the production of PGE₂ peripherally at sites of inflammation. In addition, recent evidences highlighted a prostaglandin-dependent component of pain mediated at the spinal level and NSAIDs also exert some action at this level.² Because the major signs of inflammation such as swelling result from the accumulation of leukocytes and proteinaceous fluid at

the site of injury and infection, it is reasonable to understand that inhibition of a single mediator such as PGE₂ is hardly going to have an effect on leukocyte infiltration. Clinical experience suggests phenylbutazone can provide anti-inflammatory benefits such as reduction in edema associated with surgical wounds. However, such an effect, which has been seen at clinical doses of other NSAIDs like carprofen and ketoprofen, has been difficult to demonstrate experimentally for phenylbutazone. PGE₂ also plays a major role in pain production. Phenylbutazone definitely has a pronounced analgesic effect and although scientific demonstration of more marked anti-inflammatory effects have not been made, it is felt to work well clinically. It is commonly used as a first line of treatment with minor joint injuries.

The usual accepted dose for phenylbutazone is 4.4 mg/kg/d (administered once or twice daily). In severe musculoskeletal problems such as laminitis, the double of this dose has been used but this should only be for brief periods and augmentation with opioid-type analgesics would probably be preferred. High doses of phenylbutazone (15 or 30 mg/kg/d) cause anorexia and depression with death occurring in 4–7 d. A dose of 8.8 mg/kg is toxic if repeated on a daily basis. A leukopenia as well as a marked reduction in total serum protein level has been reported after 14 days at this dose rate.¹⁹ Postmortem examinations performed on horses, ponies, and foals dying of phenylbutazone toxicity have revealed gastrointestinal ulceration, renal papillary necrosis, and vascular thrombosis. Sites of ulcers included the glandular portion of the stomach and the small and large intestine. In addition, in animals given phenylbutazone orally, there were prominent ulcerations of the oral cavity. This appears to be a local effect as it was seen in two horses given phenylbutazone orally but not in two animals given the same dose intravenously. Renal papillary necrosis seems to be dose-dependent with mild lesions occurring in a horse given 8 mg/kg/d and more extensive necrosis involving the tubular epithelial cells in horses receiving 15 or 30 mg/d. It should also be remembered from a clinical point of view that the toxic effects of NSAIDs are additive since many of these result from their common ability to inhibit cyclooxygenase. Therefore, it is recommended that when the clinically recommended dose of an NSAID fails to give adequate analgesia, another type of analgesic such as an opiate or an alpha-2 agonist should be used. Phenylbutazone should be used with care in old and debilitated animals where it is less efficiently metabolized and eliminated.¹ It has been argued that phenylbutazone is relatively disappointing as an anti-inflammatory agent in terms of reduction of edema and leukocyte infiltration into inflammatory foci.²⁰ It may be that newer drugs will provide more effect at this level.

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Aspirin (Acetylsalicylic Acid)

Acetylsalicylate differs from other NSAIDs in its ability to acetylate and thereby irreversibly inhibit cyclooxygenase. This has a profound effect on platelet function. Aspirin is commonly administered at around 25 to 35 mg/kg PO. Aspirin has had limited clinical use in the horse.¹ However, its unique effect on platelets at low dose rates would suggest a rationale for its use in conditions such as navicular syndrome, chronic laminitis, and thromboembolic colic in which vasoactive agents are advocated and have been found of some value. Daily dosing or even administration of aspirin every two days should reduce clotting and thrombus formation in horses.

Meclofenamic Acid (Arquel)

Meclofenamic acid is used in the oral granule form at a dose of 2.2 mg/kg/d. Compared to other NSAIDs it appears it has an onset of action that is slow, requiring 36 to 48 h for full effect.²¹ Clinical experience suggests it is particularly useful in the treatment of chronic musculoskeletal problems.²² In clinical trials with 304 horses, it was found to improve 78% of the horses with navicular syndrome, 76% of those with laminitis and 61% of those with osteoarthritis.²¹ In a double-blind study comparing seven-day treatments of phenylbutazone (4.4 mg/kg) and meclufenamic acid (2.2 mg/kg), meclufenamic acid produced a favorable clinical response in 60% of the animals suffering from navicular syndrome or osteoarthritis, whereas phenylbutazone only produced improvement in 36% of such patients.¹ However, the drug has not achieved routine use because of the differential costs.

Excessive doses of meclufenamic acid produce signs of toxicity similar to those of phenylbutazone (the dose 13–18 mg/kg). Signs include anorexia, depression, weight loss, edema, diarrhea, oral ulceration, and reduced hematocrit.¹

Flunixin (Banamine, Finadyne)

Flunixin is used clinically in the horse at a dose of 1.1 mg/kg. Whether it is given orally or parenterally, the onset of action occurs after about 2 h and persists for as long as 30 h.² The maximal effect is obtained between 2 and 16 h. Because the drug has a short plasma half life, it is assumed that there is accumulation of the drug at inflammatory foci (the measured range is 1.6 to 2.5 h for $T_{1/2}$). Flunixin is rapidly absorbed after oral intramuscular administration with plasma levels occurring within 30 h. Like all NSAIDs, except salicylate, it is more than 90% protein-bound. In experimental systems for studying inflammation, a single intravenous dose of 1.1 mg/kg of flunixin suppressed PGE₂ production in inflammatory exudates for 12–24 h. This dose also inhibited the ex vivo production of thromboxane E₂ by equine platelets. Flunixin has been used most frequently for the treatment of colic but it is useful in the treatment of lameness in

horses. However, for economic reasons phenylbutazone is preferred when the latter drug is efficacious.

No adverse clinical or biochemical signs have been recorded in horses given three times the recommended dose of flunixin for 10 days or five times the recommended dose for 5 days. However, cases of toxicity have been reported in ponies and foals.

Naproxen (Equiproxen, Naprosyn)

Naproxen is given orally at a dose of 10 mg/kg. It is used much less than phenylbutazone for musculoskeletal conditions. It was originally marketed for primarily muscular conditions but experience in humans shows it to be very valuable in joint conditions. Naproxen has relatively close anti-inflammatory and analgesic doses and it would therefore be expected to have a greater anti-inflammatory effect than drugs such as phenylbutazone and aspirin. Work has demonstrated that naproxen does indeed seem to be more effective than phenylbutazone in an experimental model of equine myositis. In clinical cases of azoturia, naproxen produced a favorable response in 90% of horses with an average time for remission of five days.² Naproxen also seems to have a wide safety margin with no sign of toxicity in horses given the drug at three times the recommended dose for 42 days.

Carprofen (Zenecarp, Rimadyl)

Carprofen is a relatively recent addition to the NSAID armamentarium. It has a longer $T_{1/2}$ than the other NSAIDs. It is used in the horse at a dose of 0.7 mg/kg administered intravenously and can be given once daily. Carprofen has been tested in the horse for its anti-inflammatory and analgesic activity. A single dose of 0.7 mg/kg reduced the concentration of PGE₂ and inflammatory exudate for up to eight hours and the ex vivo generation of TXB₂ in blood for up to 15 hours.²³ It was, however, noted that the reduction in eicosanoid production by carprofen was modest compared with the reductions produced by therapeutic doses of phenylbutazone and flunixin.²⁴ However, carprofen demonstrated at 12, 24, 36, and 48 h an anti-inflammatory effect by way of reduced volume on a swollen area experimentally created in the necks of ponies.²³ Subsequent studies have demonstrated greater inhibition of PGE₂ and inflammatory exudate by carprofen at 4 mg/kg administered intravenously and this dose also causes moderate but significant inhibition of LTB₄ which indicates inhibition of 5-lipoxygenase by the high dose rate. Carprofen is more tolerated at the dose of 0.7 mg/kg given by the oral or intravenous route. However, intramuscular administration resulted in an increase in CPK levels, suggesting muscle damage.²⁵ In a randomized controlled study in osteoarthritis in dogs, carprofen was shown to be of significant benefit.²⁶

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Ketoprofen (Ketofen)

Ketoprofen (Ketofen) was originally marketed as a dual inhibitor of cyclooxygenase and 5-lipoxygenase.²⁷ Such activity would broaden the anti-inflammatory potential of the compound, in theory making it superior to other NSAIDs. However, such claims are based on early in vitro data⁴ and have been challenged with results from experimental models in rats. Ketoprofen had no effect on LTB-4 concentration in such models at doses that produced virtually 100% inhibition of PGE₂ and TXB-2 production.²⁸ In two studies involving ketoprofen on exudate eicosanoid concentrations in the horse, the drug significantly reduced PGE₂ but not LTB-4 levels. The dose rate used was 2.2 mg/kg once or twice daily (two doses). In another study, synovitis was induced in the mid carpal joint of 12 horses by the injection of carrageenan. Although intravenous administration of ketoprofen significantly reduced PGE₂ concentrations in synovial fluid at 6 and 9 h after administration, the LTB-4 levels were unaffected. Joint effusion was reduced at 3 h and lameness was reduced at 3 and 6 h after ketoprofen treatment.²⁹ At clinical doses of 2.2 mg/kg/d, the drug should not be considered as superior to other NSAIDs based on claims about its ability to inhibit 5-lipoxygenase.

In a recent study with experimentally induced synovitis in horses (sterile carrageenan) the analgesic and anti-inflammatory effects of ketoprofen (2.2 and 3.6 mg/kg) and phenylbutazone (4.4 mg/kg) were compared. All NSAID-treated horses had PGE₂ compared with saline treated horses. The effect lasted longer with phenylbutazone treated horses than ketoprofen treated horses.³⁰ There were no treatment effects on leukotriene B₄ (which would supposedly happen if ketoprofen was indeed inhibiting the lipoxygenase pathway). Only phenylbutazone treatment reduced lameness, joint temperature, and synovial fluid volume. The conclusion was that phenylbutazone was more effective than ketoprofen in reducing lameness, joint temperature, synovial fluid volume, and synovial fluid PGE₂. The results do not support lipoxygenase inhibition by either NSAID.

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Intra-Articular Corticosteroids

Although it has been implied by some that intra-articular corticosteroids have been replaced by HA and PSGAG, many clinicians have returned to or persisted in the use of corticosteroids.¹ The untoward effects of intra-articular corticosteroids in horses have been repeatedly cited in the veterinary literature and more recently in the lay press. The first report of intra-articular corticosteroid use was in 1955.² More recently, various investigators have attempted to critically evaluate the effects of corticosteroids in equine joints^{3–10} and these results are helping identify a more definitive role for these agents in the management of joint disease.

Historical Perspectives

Wheat first reported the use of hydrocortisone to treat clinical muscular conditions in 94 horses and cattle.² This report was followed by a series of investigations by Van Pelt and coworkers evaluating a number of corticosteroid preparations as treatments for a variety of clinical conditions.^{6,11–16} A few clinical trials have been reported since.^{17–21} Mostly favorable results have been reported but all studies were poorly controlled.

The first study indicating corticosteroids as harmful in the horse was written by O'Connor in 1968.²² The report was based on some studies in the human literature. The statement, "An endless destructive cycle is set into motion, which if continued will produce a steroid arthropathy which can render the horse useless" was referenced and the reference was an abstract written by an anonymous author.²³ Six other human-based references (four textbook chapters and two journal papers) were quoted in this paper and one of them alluded to corticosteroids producing Charcot-like arthropathy. Charcot's arthropathy is a neurogenic disease that results in the loss of sensation, loss of proprioceptive control, instability, and arthritis (most often seen as a sequel to syphilis). There has never been any scientific demonstration of a comparable response associated with corticosteroid use in horses.

A noted veterinary pharmacologist made some rather alarming statements in discussing corticosteroids in his textbook.²⁴ Examples include "A patient on corticosteroids can walk all the way to the autopsy room" and "A horse can wear a joint surface right down to the bone running on a glucocorticoid-injected joint." Photographs of a normal fetlock from an immature horse and a severely degenerative fetlock (that had been injected with corticosteroids) were also included. However, no substantiation was made for corticosteroids causing such gross damage. Instances of degenerative joint disease (DJD) caused by corticosteroids were persistently presented without proof of such pathogenesis.²⁴

More recently, the beneficial versus deleterious effects of corticosteroids have been revisited in humans^{25–29} and in horses.¹ Recent studies have looked at the morphologic and biochemical changes in equine articular cartilage under the influence of corticosteroids with or without the added effect of exercise^{3,4,8,10,30} as well as articular cartilage matrix metabolism and synovial membrane hyaluronan production under the influence of corticosteroids.^{9,30–32} Much new information has been gained and it is clear many previous generalizations are wrong and that there are many differences with regard to the type and dose of corticosteroid used, as well as the reaction of individual tissues.

Effect of Corticosteroids

These effects have been reviewed recently.³³ Corticosteroid effects are exerted through an interaction with steroid-specific receptors in the cellular cytoplasm of steroid-responsive tissues.^{28,34,35} The corticosteroid binds to the receptor, resulting in a change in the allosteric nature of the receptor-steroid complex. This then allows the complex reversibly bind to specific sites on the nuclear chromatin of glucocorticoid-responsive genes. Different corticosteroids interact differently with these receptors.³⁴ Due to this interaction, transcription of these genes is modulated and messenger RNA is

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produced that encodes for other proteins that produce the hormonal effect.^{28,34}

Corticosteroids are potent anti-inflammatory agents and inhibit inflammatory processes at virtually all levels. Traditionally, the primary anti-inflammatory effect of corticosteroids has been related to stabilization of lysosomal membranes with a concomitant decrease in the release of lysosomal enzymes. However, the anti-inflammatory effects are considerably more complex than this.³⁶ Glucocorticoid receptors have been demonstrated in neutrophils, lymphocytes, and eosinophils and it is possible that all glucocorticoid anti-inflammatory effects are exerted through receptor-mediated mechanisms.³⁶ A major effect of corticosteroids is their inhibition of movement of inflammatory cells (including neutrophils and monocyte-macrophages) into a site of inflammation.^{28,34-36} Corticosteroids also affect neutrophil function but to a lesser extent than movement and the effect on neutrophil function seems to be dose-dependent.^{28,34-36} It has been suggested that at physiologic (lower) doses of corticosteroids, the effects on neutrophilic phagocytosis and lysosomal membrane stabilization, inhibition of lysosomal enzyme release, and inhibition of neutrophilic chemotaxis may be less significant in how corticosteroids elicit their effect than previously believed.³⁶ There is also some evidence that the inhibitory effects of corticosteroids on prostaglandin production by leukocytes is more profound on monocytes-macrophages than it is on neutrophils.^{37,38} A poor correlation has been reported between neutrophil numbers and PGE₂ concentrations in synovial fluid after corticosteroid treatment of chronic inflammatory joint disease in man, suggesting either alternative sources of prostaglandin (macrophage) or differential effects of corticosteroids on cellular function.³⁹

Corticosteroids affect the humoral aspects of inflammation, predominantly by inhibition of prostaglandin production.^{36,39-41} There is much evidence to support inhibition of the generation of pro-inflammatory metabolites (prostaglandins) from arachidonic acid as the primary mechanism of anti-inflammatory action of corticosteroids (Fig. 7-22).³⁹⁻⁴¹ This action is considered to be largely due to the inhibition of phospholipase A₂ by the steroid-inducible group of proteins called lipocortins.⁴⁰⁻⁴⁴ NSAIDs exert their effect at an adjacent location along the pathway of eicosanoid production with corticosteroids being effective at inhibiting both the cyclooxygenase and lipoxygenase pathways and NSAIDs mainly acting by inhibiting the cyclooxygenase pathway, thereby limiting production of prostacyclin and thromboxane. It has been recently suggested that in addition to inhibition of the pro-inflammatory prostaglandin pathways, corticosteroids may have other anti-inflammatory effects at different levels and the finding that some prostaglandins have also been shown to exhibit anti-inflammatory effects

complicates the complete definition of the anti-inflammatory mode of action of corticosteroids.

The eicosanoids are important in both the induction and maintenance of inflammation and, once produced, they can interact with various cytokines. This further complicates accurate identification of modes of action. Many of the effects of IL-1 are associated with stimulation of prostaglandin production and inflammation and tumor necrosis factor (TNF) is known to induce production of PGE₂ in macrophages.⁴⁴ PGE₂ has been shown to suppress TNF activity and phospholipase A₂ synthesis is enhanced by IL-1, TNF, and lipopolysaccharide.

Data on specific activity of proteinases under the influence of corticosteroids varies. In one study in humans, messenger RNA expression for collagenase and tissue inhibitor of metalloproteinase (TIMP) as well as histologic inflammation scores were decreased after triamcinolone administration in arthritic joints.⁴⁵ However, corticosteroids did not suppress stromelysin activity by activated equine synovial cells under *in vitro* conditions.⁴⁶ In another study in humans, the presence of hydrocortisone caused decreased PGE₂ concentrations, increased TIMP concentrations, and decreased collagenase concentrations in normal osteoarthritic and rheumatoid synovial membrane.⁴⁷

Low doses of corticosteroids have also been associated with inhibition of plasminogen activator activity in human synovial fibroblasts.⁴⁸ Although hyaluronate synthesis has been observed to be decreased by corticosteroids in cultures of human skin fibroblasts as well as canine synovial membrane,^{49,50} synovial fluid concentrations of HA were increased after intra-articular corticosteroid injection in horses.³²

There were a number of early reports describing deleterious effects of corticosteroids on normal articular cartilage.^{49,51-55} Cortisone acetate administration in mice resulted in a decrease in chondrocyte size and impaired organelle development in association with single as well as repeated systemic corticosteroid injections.⁵⁴ In another study of intra-articular corticosteroids (once weekly for 2 to 12 weeks) in rabbits, progressive loss of endoplasmic reticulum, mitochondria, and Golgi apparatus was noted.⁵⁵ The same author also noted progressive loss of proteoglycans as well as an overall decrease in protein, collagen and proteoglycan synthesis and gross evidence of cartilage thinning fibrillation and fissuring. It is to be noted that in many of these studies, corticosteroids were administered daily for time periods of up to 12 weeks or very high dosages were used.

Equine Studies

Methylprednisolone Acetate (Depo-Medrol)

A number of studies have evaluated the effect of methylprednisolone acetate (MPA) injected into normal equine joints. The first was done by Marcoux

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in 1977.⁵⁶ Methylprednisolone (80 mg) was injected into equine carpal joints and compared the response to repeated injections of blood (simulating hemarthrosis). Marcoux injected 80 mg of MPA per joint in all four carpal joints of 6 horses for a total of five injections. The four joints of each horse were injected with either 80 mg of MPA, MPA plus blood, blood alone, or the vehicle associated with corticosteroid; two joints of each horse therefore received MPA. The author concluded that repeated injections did not have any direct toxic effects on the articular cartilage and that the injection of the vehicle did not alter the articular structures. However, evaluation methods were not well described. Levels of MPA were still elevated in the joint 47 days after a single injection. White color deposits were also noted in the synovial membrane of all MPA-injected joints.

In a second study, 8 mature horses with no prior signs of joint disease or history of intra-articular therapy were treated with eight weekly intra-articular injections of MPA.³ Treatments were given at a dose of 120 mg/joint into the antebrachio-carpal (radiocarpal) and middle carpal (intercarpal) joints with the left joints used as untreated controls. There were no gross differences but chondrocyte necrosis and hypocellularity were observed and the rates of proteoglycan and collagen synthesis were reduced in MPA-injected joints. After eight weekly injections, the proteoglycan content of the articular cartilage was reduced to 56.52% of the control values and the proteoglycan content decreased further at 4- and 8-week recovery periods to 40.77% and 35.17% of the control values respectively. The rate of proteoglycan synthesis as measured by ³⁵SO₄ uptake was reduced to 17.04% of the control values after the last injection. Four and eight weeks later, the rate of synthesis increased to 55.31% and 71.28% of the control values respectively, indicating a positive response. The authors asked whether cartilage that had lost 50% proteoglycan would be vulnerable to breakdown with exercise. The doses used in this study are high and with both joints injected, they become particularly high.

In a third study, we injected 100 mg MPA three times at two weekly intervals into the middle carpal joints of four normal horses and tissues were collected two weeks after the last injection.¹⁰ Horses remained clinically normal during the study and significant radiographic changes were not observed. However, safranin O matrix staining intensity and uronic acid content were significantly lower in the treated joints. Articular cartilage fibrillation was not evident in any joints. The latter two studies did show, however, that some regressive changes occurred to normal equine articular cartilage when 6 α -methyl-prednisolone acetate was used.

MPA has also been evaluated using equine osteochondral fracture models.^{4-6,57} Synovitis is a common feature of all of these. Meagher created large osteochondral fractures off the distal aspect of the

radial carpal bone bilaterally in 5 horses using arthrotomy; one other horse was used as a nonoperated control. Fragments were 1-cm wide and 2.5 cm in length, which is more like a slab fracture. Three weeks later these horses were galloped for 4.5 to 5 miles by chasing them around a pasture in a pickup truck. One middle carpal joint received methylprednisolone acetate (120 mg), the other one was an untreated control. The first injection was given three weeks after surgery and injections were repeated every two weeks for four injections. Horses were galloped from the 22nd day until the 78th day. Changes occurred including cartilage erosion and periarticular proliferation in the non-treated joints (probably related to instability or arthrotomy) with change being more severe in the joints injected with MPA. This study was considered to confirm previous statements that adequate rest is required after injection of intra-articular corticosteroids.

Most recently we have re-evaluated the effects of intra-articularly administered MPA in exercised horses with our arthroscopic carpal osteochondral fragmentation model. Eighteen horses were randomly assigned to each of three groups (6 horses in each group). An osteochondral chip fragment was created in one randomly chosen middle carpal joint of each horse. Both middle carpal joints in the placebo control group (CNT) horses were injected intra-articularly (IA) with a polyionic fluid. The MPA control group horses (MPA CNT) were injected with 100 mg MPA IA 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 MPA treated group horses (MPA TX) were treated with 100 mg MPA IA in the joint that contained the osteochondral fragment and the opposite middle carpal joint was injected with a single volume of polyionic fluid. All horses were treated IA on days 14 and 28 after surgery and exercised on a high speed treadmill for six weeks starting on day 15 after surgery.

The results of this experiment were that there was no significant clinical improvement in the degree of lameness associated with MPA administration. The lack of a significant reduction of joint pain (manifested by lameness) was in contrast to our previous work with triamcinolone acetonide (TA) and also with anecdotal reports of decreased lameness associated with the clinical use of MPA. Joints that contained an osteochondral fragment and were treated with MPA had lower PGE₂ concentrations in the synovial fluid and lower scores for intimal hyperplasia and vascularity (there was no effect on cellular infiltration) in synovial membrane compared to placebo-treated joints. However other parameters observed at post mortem and evaluated in the articular cartilage (histologic and histochemical evaluation of articular cartilage glycosaminoglycan (GAG) content and rate of GAG synthesis) suggested possible deleterious effects of intra-artic-

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ular MPA administration when compared to the controls.

It was noted that there was lower synovial fluid volume in 10 of 12 MPA-treated joints independent of fragmentation. These findings are compatible with anecdotal clinical reports of "red, dry joints" in association with MPA administration and are in contrast to results with intra-articular administration of TA where similar color or volume changes were not seen. There was also a higher color score attributable to MPA plus fragmentation. Synovial fluid protein concentration was higher in fragmented joints but at day 72 after surgery all joints from horses receiving MPA treatment had significantly more total protein in the synovial fluid compared to nonfragmented joints in the control group horses. Fragmented joints from MPA control group horses had higher total protein concentrations in the synovial fluid as compared to fragmented joints or control group horses, suggesting that MPA administration in a nonfragmented joint increased protein concentration compared to saline administration and this also is in contrast to the decreased protein levels in synovial fluid after TA administration. Significantly higher GAG concentration was seen in the synovial fluid from joints injected directly with MPA and this was felt to be a direct result of MPA administration. GAG synthesis in the articular cartilage was decreased under the influence of MPA so that it was felt that increased fluid GAG levels were most likely resulting from increased degradation of GAG in the articular cartilage. Increased HA levels were also associated with MPA administration which has also been reported after administration of other corticosteroids.⁵⁸ In addition, all joints receiving MPA treatment had significantly inferior modified Mankin scores on histologic evaluation, illustrating the deleterious effect with intra-articular administration of MPA on articular cartilage. This is again in contrast to the lack of deleterious effects with betamethasone⁴ and the improvement in Mankin scores associated with TA administration.⁵ Although a significant loss of safranin O fast green staining is observed in nonfragmented joints treated with MPA compared to the contralateral joints, no differences between joints were observed in the MPA treated or control groups and there was poor correlation between safranin O staining and biochemical analysis of the GAG content of the articular cartilage. Recent studies question the accuracy of Safranin O–Fast Green (SOFG) staining in assessing the GAG content in the articular cartilage⁵⁸ and we feel the results of SOFG staining need to be interpreted with caution. Biochemical analysis of the total articular cartilage GAG content did not reveal a detrimental effect with MPA treatment. However, cartilage from joints opposite those receiving MPA had significantly higher GAG content compared to both contralateral joints and joints from the control group horses. This suggests that there may be a beneficial remote effect on

GAG content of cartilage associated with MPA administration. Similar remote effects were seen with TA. Although total articular cartilage GAG content was not adversely affected by MPA administration, GAG synthesis on day 72 after surgery was lower in MPA treated joints as compared to joints from control horses, suggesting a direct negative effect on articular cartilage GAG synthesis associated with MPA treatment. This is in contrast to previous data following TA administration where there were no negative effects on the rate of GAG synthesis but is consistent with previous *in vitro* and *in vivo* studies.

In conclusion, there was no significant clinical improvement in lameness associated with MPA although there was a decrease in PGE₂ levels in the synovial fluid and lower synovial membrane vascularity and intimal hyperplasia scores. On the other hand, there were deleterious effects on articular cartilage with direct administration of MPA with possible deleterious effects associated with MPA in the contralateral joint. These findings are in contrast to the positive effects seen when TA was assessed using the same model but are consistent with previous studies where MPA has been administered intra-articularly in normal and abnormal joints. Our studies further confirm the potential detrimental effects of MPA in articular cartilage in horses. More recently the effect of intra-articular MPA on the biomechanical properties of articular cartilage has been evaluated.⁵⁹ Eight two-year-old horses had MPA or 2.5 ml of pH-adjusted polyethylene glycol, sodium chloride, and Myrastyl-gamma-picolinium chloride. They were injected at 14-day intervals for a total of four treatments per horse (100 mg MPA each time). Horses underwent a standard treadmill exercise protocol until euthanasia at day 70. There were significant differences demonstrated between intrinsic material properties and thickness of the cartilage between MPA and treated joints. Diluent-treated cartilage had 97% increase in compressive stiffness modulus, was 121% more permeable and had an 88% increase in shear modulus compared to MPA-treated articular cartilage. Diluent-treated cartilage was also 24% thicker than MPA-treated cartilage. These findings indicated that repetitive intra-articular administration of MPA to exercising horses alters the mechanical integrity of articular cartilage, which could lead to early cartilage degeneration.

In another study, the effect of MPA was tested in joints that also had lipopolysaccharide-induced synovitis. Intra-articular MPA alone was associated with decreased proteoglycan synthesis and increased protein and collagen synthesis in the articular cartilage. Total protein synthesis by synovial membrane was also increased by MPA alone. In contrast, no differences in protein or proteoglycan synthesis were observed in explants from the joints with synovitis with or without intra-articular MPA. The results suggested that the effect of in-

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tra-articular MPA on joint metabolism was different between inflamed and normal joints. The authors also suggested caution in interpretation of in vitro culture results when investigating the effect of intra-articular corticosteroids on chondrocyte function.⁶⁰ Caron et al showed that MPA inhibited the stimulation of MMP-13 expression by rhIL-1 β .

We have recently done an in vitro study with cartilage explants to try to determine a minimally effective dose for MPA. A traditional dose in a carpal joint, for instance, has been 100 mg. Our hypothesis was that we could perhaps inject considerably less MPA and still have the same effect. Clinical reports from equine veterinarians confirm that they indeed do get clinical responses with lower doses. However, in our in vitro study using human recombinant IL-1 with equine cartilage explants, we needed a dose equivalent to 100 mg to achieve effective suppression of IL-1 mediated degradation in the cartilage. We plan to do this work again with our newly acquired equine interleukin-1.

In the meantime, we need to be cautious with the use of MPA. We should try to use as low a dose as possible and be well aware of the deleterious side effects. We prefer using betamethasone or triamcinolone acetonide (described in the following sections).

Betamethasone

Using an osteochondral fragment exercise model that we developed, we evaluated betamethasone esters. Osteochondral fragments were created arthroscopically on the distal aspect of both antebrachiocarpal bones in 12 horses to evaluate the effects of intra-articular betamethasone with and without exercise.^{4,6} One middle carpal joint of each horse was injected with 2.5 ml betamethasone at 14 days after surgery and the procedure was repeated at 35 days. The opposite joint was injected with 2.5 ml saline as a control. Six of the horses were maintained in box stalls throughout the study as nonexercised controls and six were exercised five days per week on a high speed treadmill with a regimen of 2 min trot, 2 min gallop, 2 min trot. Three weeks after the second injection, horses were clinically examined for lameness and synovial effusion, radiographs were taken and the horses euthanized. Mild lameness was seen in all horses in the exercised group at the end of the study. Four of these were lame in the saline-injected limb, one in the corticosteroid treated limb and one had bilateral lameness. Of the five nonexercised horses evaluated for lameness (one horse was removed from the study), two were lame in the saline-injected joint, two in the steroid-treated limb and one was sound. No differences were noted on radiographs or on palpation of the steroid-treated limbs vs control limbs in either exercise group. Firm reattachment of the osteochondral fragment was seen in all but three joints. Gross articular cartilage damage subjectively seemed worse in the exercised horses

but was not different between steroid and saline-treated joints in the same horse. The results of histologic examination did not show any consistent detrimental effects of betamethasone with or without exercise. Histochemical staining showed a decrease in glycosaminoglycans in the steroid-treated limbs of rested horses, although the decrease was not significant at $p < 0.05$. The exercised horses had similar levels of glycosaminoglycans in treated vs control joints. Chemical assays showed no significant difference in water content or uronic acid concentration (a measure of GAG content) of the treated vs control joints. The use of betamethasone in this carpal chip model did not show any consistent detrimental effects in either rested or exercised horses. This was our first evaluation of an intra-articular corticosteroid using our arthroscopic fragmentation-exercise model. At that stage our laboratory was not doing good biochemical analysis of GAG content or GAG synthetic rate. In subsequent experiments, we have modified our fragmentation (to increase the degree of synovitis and decrease the tendency for healing of the fragment) and also are evaluating the articular cartilage with more sophisticated means.

This study also addressed the question of whether exercise after corticosteroid injection causes significant deleterious effects on articular cartilage, at least in the short term. It showed that exercise did not harm articular cartilage exposed to betamethasone. It also implies that there may be considerable differences in metabolic responses of articular cartilage to the various corticosteroids used routinely and also pointed out that no evaluation of therapeutic dose (in the way of dose titration studies) has ever been done with any of the intra-articular corticosteroids used in the horse.¹

Triamcinolone Acetonide

Our work with triamcinolone acetonide (TA) suggests that it may indeed be chondroprotective in the horse.⁶ In this study 18 horses were trained on a high speed treadmill and then had an osteochondral fragment created at the distal aspect of the radial carpal bone of one randomly chosen midcarpal joint. Six horses were treated with intra-articular injection of polyionic fluid in both middle carpal joints (CNT), six horses were treated with 12 mg TA intra-articularly in the middle carpal joint without an osteochondral fragment (the opposite midcarpal joint was treated IA with a similar volume of polyionic fluid) (TA CNT), six horses were treated with 12 mg TA in the joint that contained the osteochondral fragment (the opposite middle carpal joint was treated IA with a similar volume of polyionic fluid) (TA TX). Triamcinolone and placebo treatments were repeated at days 13 and day 27 after surgery and treadmill exercise proceeded five days per week, beginning on days 14 and ending on day 72. Clinical exams were performed at the beginning and end of the study. Synovial fluid samples were obtained

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from the joints on days 0, 14, 21, 28, 35, and 72, and analyzed for total protein, nucleated cell counts, hyaluronan levels (HA), glycosaminoglycan (GAG) and prostaglandin E₂ (PGE₂). Euthanasia was done at day 72 and synovial membrane samples were taken and assessed histologically. Articular cartilage samples were aseptically collected to determine GAG content of the cartilage as well as GAG synthetic rate. A split plot with repeated measures design was used as the statistical model and a multivariate analysis of variance was performed to determine statistical significance of both main and interaction effects of independent variables.

Horses that were treated intra-articularly 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 groups had less inflammatory cell infiltration, intimal hyperplasia and subintimal fibrosis. Analysis of articular cartilage morphologic parameters evaluated using a standardized scoring system were significantly better from TA CNT and TA TX groups, irrespective of which joint received TA. There was less staining with SOFG in the TA CNT group compared with the TA TX group and the CNT group, although the GAG synthetic rate was elevated in the TA CNT group as compared with the other two groups.

In conclusion, the results from this study supported favorable effects of TA on degree of clinically detectable lameness and on synovial fluid, synovial membrane and articular cartilage morphological parameters, both with direct intra-articular administration and remote site administration as compared to placebo treatments. The beneficial effects were recorded in both synovial membrane morphologic biochemical articular cartilage parameters. Increased HA concentrations were observed in TA treated joints which also suggests a favorable corticosteroid effect on synoviocyte metabolism. This research supports a chondroprotective effect of corticosteroids in a controlled model of osteoarthritis and is in marked contrast to the detrimental effects of corticosteroids seen in in vivo osteochondral fragment models where methylprednisolone was used.

In the same study, the effect of TA on dynamics of bone remodeling and fragility was assessed.⁷ Third carpal bones from joints with fragments showed significantly more vascularity, single-labeled surface, and total-labeled surface of mineralizing surface in subchondral and subjacent trabecular bone. Trends were also seen toward high vascular canal volume and osteochondral junction remodeling sites in third carpal bones from fragmented joints. No significant differences were seen in microdamage density or size between fragmented and nonfragmented joints. No significant influence of TA treatment was seen on any parameter measured. The results from this study show

that osteochondral fragmentation induces significant changes in remodeling of opposing bone and that administration of corticosteroids into joints with fragmentation does not significantly alter bone remodeling or fragility. This information is particularly useful in view of the extrapolation from human clinical work that has suggested that intra-articular corticosteroids in horses may cause osteoporosis in the adjacent bone.

In summary, the critical evaluation of intra-articular TA administration in this study resulted in no substantial detrimental effects and some chondroprotective effects on joint tissues.

Clinical Impressions

There have also been some clinical reports questioning the extent of the deleterious effects of corticosteroids. McKay and Milne looked at Thoroughbreds that received intra-articular corticosteroids on the racetrack.¹⁹ Conclusive evidence of corticosteroid arthropathy in racehorses was not seen where there was no prior radiographic evidence of osseous changes in the joint. In another review of case records by Owen in which the intra-articular injection of a corticosteroid had been considered to result in arthropathy, all cases had evidence of prior osseous changes in the joint, including three cases of carpal chip fractures, two of osselets, one of a proximal first phalanx chip fracture and one of a fractured tuber scapulae in the shoulder.⁶² This author pointed out in his paper how the term Charcot's arthropathy in man was sometimes used incorrectly to describe corticosteroid-induced arthropathy. Unfortunately, the lay public has been told that corticosteroids purely inhibit pain and therefore permit horses to continue to run and to degenerate their joints. It would seem that the beneficial effects of corticosteroids go far beyond being painkillers. Some of the beneficial effects in clinical practice have been outlined by Genovese.⁶³

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Sodium Hyaluronate (Hyaluronan)

Hyaluronic acid (hyaluronan) is a linear polydisaccharide and polyionic nonsulfated glycosaminoglycan. The disaccharide units are linked by 1–4 glycosidic bonds to form a long unbranched chain consisting of 10,000 to 12,000 disaccharide units¹ forming particles of widely varying size. Under physiologic conditions, hyaluronic acid is anionic and associated with monovalent cations. It has been suggested that when the cation of a polysaccharide is undetermined, the compound is properly referred to as hyaluronan (HA).¹ Estimates of molecular weight vary and depend on the source of the compound, the method of isolation and the method used in determination of molecular weight. Studies in humans and animals have determined the molecular weight of synovial fluid hyaluronate to be 2–6 million daltons.² Concentration of synovial fluid HA varies between species and between joints of an individual with the smaller joints generally exhibiting a higher concentration.^{3,4} The various methods employed in the determination of equine synovial fluid HA concentration have resulted in a range of normal values. The values for normal equine synovial fluid have fallen into the range of 0.33 to 1.5 mg/ml, depending on the investigator and technique employed. The wide range of values reported indicates a comparison of absolute values between studies is impossible.

Synthesis and Function of Endogenous Hyaluronate

Hyaluronan is an integral component of both synovial fluid and articular cartilage in normal synovial joints. Synovial fluid HA is synthesized by the synoviocyte of the synovial membrane. Hyaluronan

that is incorporated in the extracellular matrix of articular cartilage is synthesized locally by the chondrocyte. Hyaluronan is removed from the joint via the lymphatic system. Once in the peripheral circulation, HA is rapidly taken up primarily by the liver and degraded by endothelial cells of the hepatic sinusoids.^{1,5} It has been shown recently that in addition to the liver, the articular tissues are capable of local degradation of HA; however, no degradation is apparent within the joint cavity.⁶

Hyaluronan confers the property of viscoelasticity to synovial fluid and this viscoelasticity is proportional to and dependent upon the concentration and degree of polymerization of HA in the fluid.^{7,8} It has been demonstrated that HA is responsible for boundary lubrication of the synovial membrane and, more recently, is a significant factor in the lubrication of articular cartilage.^{9,10} Hyaluronan may also influence the composition of synovial fluid through steric hindrance of active plasma components and leukocytes from the joint cavity.¹¹ Solutions containing HA have the ability to exclude solutes and particles from the solution in proportion to the size of the particle, concentration, and molecular weight of the hyaluronate in solution.^{11,12} Solutions containing HA may also modulate the chemotactic response within the extracellular fluid of connective tissues through reduction of cell migration¹³ and reduced rates of perfusion and flow of solutes.¹⁴

A molecule of HA is the nucleus of the proteoglycan aggregates (aggrecan) in the extracellular matrix of articular cartilage. It is believed that the compressive stiffness in articular cartilage is dependent on the integrity of the matrical proteoglycans.¹⁵

Possible Mechanism of Action of Exogenous Sodium Hyaluronate

Beneficial effects after intra-articular administration of HA have been reported in a number of equine studies¹⁶⁻²⁵ as well as other animals. The mechanism through which beneficial effects have been achieved remains controversial. The therapeutic effect(s) of exogenously administered HA may result from the supplementation of the actions of depleted or depolymerized endogenous HA or, alternatively, result from other properties that have been ascribed to HA based on experimental work, including modulation of increased synthesis of endogenous HA. The mechanisms through which HA has been hypothesized to benefit diseased joints has been varied and highly speculative.

Alterations in synovial fluid HA concentration and molecular weight in various pathologic states have been described but the results are somewhat conflicting. Generally, there is a reduction in synovial HA concentration and molecular weight with equine joint disease. The concentration has been reported as lower in horses with traumatic arthritis.²⁶ On the other hand, in another study synovial fluid from equine joints with acute traumatic synovitis was not significantly different in HA concentration than from normal joints.²⁷ In a third study, there was no significant difference in the concentration of synovial fluid HA between normal equine joints and those with acute or chronic arthritis; however, joints with septic arthritis and those with radiographic evidence of osteoarthritis had reduced concentrations compared to controls.²⁸ The molecular weight of synovial fluid HA was not significantly different when fluid from nonclinical equine joints were compared with those of acute or chronic arthritis.

The mechanisms through which HA has been hypothesized to benefit diseased joints has been varied and highly speculative. It has not been determined what concentration or degree of polymerization of HA is necessary for effective intra-articular soft tissue lubrication. In one study utilizing a synovial membrane assay to evaluate the ability of various solutions containing HA to lubricate soft tissues, synovial fluid from human rheumatoid arthritis patients had similar lubricating properties to normal bovine synovial fluid.¹⁰ The half-life of exogenous intra-articular HA injected into normal equine joints has been estimated to be 96 h.²⁹ The half-life of exogenously administered HA is reduced in diseased joints. In a sheep experimental model the half-life was reduced from 20.8 h in normal joints to 11.5 h in arthritic joints.³⁰ It has, however, been shown that although most exogenously administered hyaluronate is rapidly cleared from the joint, a proportion remains associated with synovial tissues.³¹ It has been suggested that some of the exogenous HA and its breakdown products localize in the intercellular space surrounding the synovio-

cytes, influencing the metabolic activity of these cells.³² The mechanism by which exogenous HA produces clinical benefit beyond its presence in the joint is of great interest.

Other effects of exogenous HA have been identified experimentally. Anti-inflammatory effects have been demonstrated in a number of in vitro studies and include an inhibition of chemotaxis of granulocytes, macrophages, and migration of lymphocytes, as well as reduction of phagocytosis by granulocytes and macrophages.³²⁻⁴¹ It has been suggested that the anti-inflammatory effect of HA is the result of reduced interaction of enzymes, antigens, or cytokines with target cells through steric hindrance.^{5,13,14,40} Recent evidence suggests that reduced chemotaxis and phagocytosis of activated neutrophils are mediated through the interaction of HA with the CD44 cell receptors of neutrophils.⁴² The HA-inhibited neutrophil-mediated degradation in a concentration and molecular weight-dependent fashion and it has been shown effective in reducing the production of prostaglandin E₂ by interleukin-1-stimulated rabbit chondrocytes.^{42,43} In a controlled clinical trial in human arthritic patients, hyaluronate treatment reduced synovial fluid levels of PGE₂ and elevated levels of cyclic AMP.⁴⁴ These studies suggest that the anti-inflammatory properties of HA may be attributable in part to its ability to reduce production of soluble inflammatory mediators and to augment signal transduction pathways. The proliferation of rabbit synovial cells in culture was inhibited by the addition of HA to the culture medium. It was found that this effect was markedly dependent on the molecular weight and concentration of HA. At the molecular weight and concentration of HA present in normal synovial fluid, proliferation was inhibited. At lower molecular weights or concentrations as found in rheumatoid synovial fluid, HA was significantly less inhibitory. It is therefore felt that changes in synovial fluid HA associated with arthropathies may contribute to a favorable environment for rheumatoid pannus expansion.⁴⁵

It has also been recently demonstrated that a commercial preparation of 800-kDa HA^a was tested in an in vitro cartilage chondrolytic system. It was found that the HA was effective in blocking the ability of a fibronectin fragment to cause cartilage degradation and release of half of the total cartilage PG from cartilage and this was associated with a decreased concentration of fibronectin fragment on the superficial cartilage surface and decreased penetration into the cultured cartilage tissue. It was concluded that the blocking activity appeared to be associated with the ability of HA to block penetration of the fibronectin fragment rather than direct effects on cartilage tissue.⁴⁶ In a study evaluating the value of hyaluronic acid in a canine stifle immobilization model, it was found that the immunolocalization of TNF α was absent or greatly reduced in articular cartilage of the injected stifle along with

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increased retention of proteoglycan histochemical staining. Immunoreactivity of TNF receptors were similar to that of TNF α . In this study the pattern of distribution of stromelysin in regions where proteoglycans were degraded supported the role of stromelysin in the destruction of proteoglycans in atrophic articular cartilage.⁴⁷

Effect of Molecular Weight

It has been repeatedly stated that the injection of HA into a pathologic joint results in increased synthesis of high molecular weight endogenous hyaluronate by the synoviocytes.^{18,21,23,48} Many of these authors reached such conclusion based on a hypothesis by Asheim and Lindblad in 1976⁴⁹ and opinions expressed by Balazs.⁵⁰ Direct effect on HA synthesis was not clearly demonstrated, however. In a later in vitro study it was demonstrated that hyaluronate and molecular weight greater than 5×10^5 daltons stimulated the synthesis of hyaluronate in a concentration-dependent manner. However, HA preparations of molecular weight less than 5×10^5 daltons had little or no effect except at high concentrations where HA synthesis was depressed.⁵¹ In a more recent study on the influence of exogenous HA on the synthesis of HA and collagenase by equine synoviocytes (monolayer culture), it was found that exogenous HA influenced neither the rate of synthesis nor the hydrodynamic size of the newly produced HA by control or principal cell cultures. The authors concluded that the principal mechanism of action of HA did not appear to be stimulation of synthesis of HA, of augmented molecular weight, or marked inhibition of collagenase synthesis.⁵² Exposure of synoviocytes from normal and diseased joints to a number of commercial HA preparations failed to significantly influence endogenous HA biosynthetic activity and in higher concentrations significantly stimulated collagenase synthesis. This study provided some objective evidence that HA in the extracellular environment may modulate the synthesis of HA via synoviocytes. Whether these in vitro effects occur in vivo have not been clearly demonstrated. It is also possible that normalization of synovial fluid HA concentration and molecular weight may occur secondarily as a result of other benefits derived from the exogenous sodium hyaluronate therapy rather than through direct pharmacologic effects.

Direct Effects on Cartilage Healing

It is doubtful that exogenous HA has any direct effect on articular cartilage. It is well recognized that there is decreased proteoglycan aggregation in articular cartilage with osteoarthritis (and proteoglycan aggregation is mediated by a link protein to an HA backbone); various investigators have demonstrated in vitro that addition of HA to a medium of disaggregated proteoglycan subunits results in aggregation.⁵³ In view of these findings, some authors have theorized that one of the benefits of

intra-articular HA lies in its ability to increase proteoglycan aggregation in articular cartilage. However, there are no convincing data to support proteoglycan-aggregating effects of exogenous HA in hyaline cartilage in vivo. If one considers the molecular size of pharmaceutical HA, it would seem unlikely that exogenous HA would gain access to the cell membrane of the chondrocyte. Exogenously administered HA has been shown to interact with proteoglycans and the chondrocyte cell surface via the HA binding domain of the proteoglycan molecule.

There have been no demonstrated direct effects on intact articular cartilage. However, in vitro studies have demonstrated that high concentrations of HA suppress IL-1 α and TNF α induced release of ³⁵SO₄ proteoglycans from chondrocytes in culture.^{54,55} The influence of intra-articularly injected high molecular weight HA on the healing of superficial and deep lesions of the articular cartilage in an experimental animal model has been investigated.⁵⁶ The HA injections appeared to have no effect, either positive or negative, on the healing of intracartilaginous and osteochondral joint lesions. However, the positive effects of high molecular weight HA on experimentally induced cartilage degeneration have been recognized. In one study using a partial meniscectomy of OA in the rabbit knee, high molecular weight HA injected intra-articularly twice a week starting immediately after surgery inhibited cartilage degeneration in both the femoral condyle and tibial plateau. High molecular weight HA offered better protection than a lower molecular weight product and therefore showed that at least in the rabbit model, intra-articular high molecular weight HA was more effective than lower molecular weight HA in inhibiting cartilage degeneration and early osteoarthritis.⁵⁷

Clinical Use of Hyaluronan

The first report of the clinical use of HA for intra-articular treatment of equine joint disease was published in 1970,²³ in which cases of traumatic degenerative equine arthritis were treated with methylprednisolone acetate versus HA/methylprednisolone acetate combination in 20 racing Thoroughbreds and Standardbreds. The investigators concluded the combination of HA and methylprednisolone acetate resulted in a better and more lasting improvement than the corticosteroid alone. In 1976, Asheim and Lindblad provided the first report of treatment of equine traumatic arthritis with intra-articular HA alone in 54 joints of 45 racehorses previously treated unsuccessfully by conventional means. Through a one-year observation period, 38 of 45 horses were free of lameness and 32 returned to the racetrack after treatment.⁴⁹ Since these early reports, numerous clinical and experimental studies have been conducted to evaluate the efficacy of HA in the treatment of equine joint disease.^{17-19,21,24,25,29-31,35,36,58,59} The clinical reports

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have generally supported the use of HA but in many of them the evaluations are subjective and the definitions of criteria for successful treatment are absent. The duration of post-treatment observation periods are varied and some studies were of short duration. Most studies include response to intra-articular anesthesia as a criterion for case selection, which helps in localizing the problem but provides little information about the specific diagnosis. A number of studies state or imply that the condition treated was osteoarthritis but the criteria for osteoarthritis were not demonstrated. It would seem that many of the cases were synovitis or capsulitis rather than osteoarthritis.

Attempts have been made to assess the clinical response to sodium hyaluronate therapy in the horse more objectively.^{6,58} In one model using bilateral osteochondral fractures created by arthrotomy, the authors concluded that HA had a protective effect on the articular cartilage and resulted in reduced lameness. However, the conclusion that HA treatment was responsible for the return to normal weightbearing is suspect since both treated and non-treated limbs returned to presurgical weightbearing values. The effectiveness of intra-articular Hylan, a derivative of HA modified by cross-linking, was evaluated in a double blind study that employed the use of gait analysis.⁵⁹ In this study, treatment with Hylan did not significantly alter any of those variables compared to baseline or control values and the conclusion was, at least in this model of acute synovitis (amphotericin), that there was no beneficial effect.

A chondroprotective effect for HA was reported based on a study involving experimentally induced arthritis in dogs.^{60,61} However, treatment with HA has been reported to result in exacerbation of histologic, biochemical, and gross morphologic changes associated with osteoarthritis experimentally induced in sheep by medial meniscectomy.^{62,63} Treatment with HA improved weightbearing and resulted in a lower gross pathologic score of osteoarthritis but resulted in a higher score for osteophytosis and higher histologic score for osteoarthritis as well as reduced proteoglycan content. One of the arguments espoused by proponents of the use of HA has been its actions of physiologic therapeutic modality in the treatment of joint disease allowing rapid return to athletic function without the risk of deleterious effects that have been associated with some other treatments. This notion of safety has been challenged and the sheep meniscectomy demonstrates that rapid return to function may not be an appropriate goal in every case.

Controversy exists concerning the relationship between molecular weight of exogenous HA and the clinical efficacy of treatment in equine joint disease. Certain advantages have been claimed in promotional material for products of higher molecular weight.⁶⁴ Although many of the *in vitro* effects of

HA have been shown to be enhanced with higher molecular weight hyaluronate (including inhibition of fibroblast proliferation, inhibition of phagocytosis, enhanced synthesis of hyaluronate by cultured synoviocytes, and inhibition of PGE₂ production by IL-1 stimulated chondrocytes),^{2,13,34,37,39,51} the correlation between molecular weight and clinical effect are less clear. In a comparative study of five sodium hyaluronate products in the treatment of traumatic arthritis in horses, horses treated with hyaluronate of molecular weight greater than 2×10^6 daltons exhibited significantly longer duration of soundness than those treated with hyaluronate less than 2×10^6 daltons.⁶⁵ In another blinded study, the clinical effect of sodium hyaluronate with a molecular weight of 0.13×10^6 vs 2.88×10^6 daltons were compared in the treatment of 69 racing Thoroughbreds with carpalis. There were no clinically significant differences in the response to the two drugs, questioning whether the molecular weight of administered HA had any effect on therapeutic response.⁶⁶ The post-treatment observation period in this study was two weeks, therefore the duration of effect was not evaluated.

Recently, a randomized double-blind and placebo-controlled clinical study was carried out in Standardbred trotters. Seventy-seven trotters with moderate to severe lameness were grouped according to number of affected joints and within each group were randomized for treatment with polysulfated glycosaminoglycan (PSGAG), sodium hyaluronate (HA), or placebo for three weeks. The horses were inspected weekly with final examination two to four weeks after the end of treatment. The mean and initial lameness score was significantly reduced during treatment and at the last examination in all three groups ($p < 0.01$).⁶⁷ Additionally, the prevalence of sound horses increased significantly from one to three weeks of treatment and to the last examination in all three groups ($p < 0.03$). Comparison of the two treatment groups with regard to the development of the lameness curve and time until soundness indicated a small nonsignificant difference in favor of HA. No significant difference was detected between the two treatment groups and the prevalences or cumulative incidence of soundness. The study detected a superior effect of the two drugs compared with a placebo for reduction of lameness score during the treatment period ($p = 0.03$) and the total study period ($p < 0.01$), time until soundness ($p = 0.04$), and the prevalence of sound horses at the last examination ($p < 0.01$). All three treatments affected traumatic arthritis in horses but HA and PSGAG gave better results than placebo.

Viscosupplementation

It has been known for many years that synovial fluid from osteoarthritic joints is lower in elasticity and viscosity than synovial fluid from normal joints and

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that the decrease in rheologic properties of synovial fluid results from reduction in molecular size and concentration of HA in the synovial fluid.^{2,68} This phenomenon led Balazs to introduce viscosupplementation therapy, which is the injection of HA or its derivative in an attempt to return the elasticity and viscosity of the synovial fluid to normal or higher levels.⁶⁹ Viscosupplementation with HA has been used as a specific therapeutic technique in osteoarthritis (OA), especially in Italy and Japan. However, 6 to 10 injections are often required to achieve efficiency and suggested reasons for this have included that the elastoviscous properties of current HA preparations are inadequate to restore sufficiently the elasticity and viscosity of the synovial fluid in the arthritic knee, or that the injected HA has eliminated too quickly from the joint to be effective.⁶⁸ Because of this limitation in viscosupplementation with conventional HA preparations, hylans (chemically cross-linked hyaluronans) were developed to improve the efficacy of viscosupplementation therapy of OA. Cross-linked HA improves its utility for viscosupplementation in several ways: 1) the rheologic properties are increased, 2) it has a longer retention time in the synovial space, and 3) because of the cross-links, it becomes more resistant to free radical production. One particular combination of hylan, G-F20 (Synvisc^R)^b has been developed specifically as a device for viscosupplementation therapy in OA of the knee. In a Canadian multicenter trial of human osteoarthritis, it was shown that patients treated with Synvisc had an equal response to nonsteroidal anti-inflammatory drugs (without the consequent side effects).⁶⁸ The results of four clinical trials in Germany have also validated the efficacy and safety of Synvisc.⁷⁰ In another more recent study in Canada, 1537 injections were performed in 336 patients involving 458 knees. The overall response and the change of activity level were judged better or much better for 77% and 76% of the treated knees after the first course of treatment (three weekly injections) and 87% and 84% after a second course. The mean time elapsing between the first and second course (8.2 ± 0.5 mo) is an evaluation of the duration of benefits. Local adverse events were observed in 28 patients (32 knees) with an overall rate of 2.7% adverse events per injection. The adverse events were characterized by pain and/or transient swelling in the injected joint, mostly mild or moderate in intensity. The conclusion was that Synvisc provided good clinical benefits and an acceptable safety profile in current clinical practice. The occurrence of adverse events after an intra-articular injection is infrequent and unpredictable. Hylan G-F20 is a cross-linked HA preparation of average molecular weight over 6 million. It is to be noted that purified human umbilical hyaluronate and a commercial preparation of HA^d intended for intra-articular viscosupplementation did not demonstrate the same

degree of boundary lubricating ability as bovine synovial fluid or purified lubricin. The data did show that HA possesses some boundary lubricating ability in excess of that produced by physiologic saline alone but could not replicate the boundary lubrication provided by synovial mucin. This study also supported earlier observations of an interaction between lubricin and hyaluronate, however.⁷¹

As mentioned previously, a double blind study with amphotericin-induced synovitis has been done in the middle carpal joint of horses. The response to treatment with Hylan was compared with that in untreated horses using 3-D motion analysis, synovial fluid analysis, and synovial histologic exam. Treatment with hylan did not significantly alter any of these variables compared with baseline control values. When one considers where Hylan has been used in humans compared to an acute synovitis model in the horse, it may be that viscosupplementation is more appropriate for osteoarthritis.

Intravenous Hyaluronan

A formulation of HA for intravenous administration has been developed for use in horses and has been licensed for several years now. It is given as a 40 mg (4 ml) intravenous injection and goes under the trade name of Legend in the United States and Hyonate everywhere else.^e Anecdotal information from personal communication with veterinarians and personal experience suggests efficacy^f; however data from a controlled study has only recently become available. A controlled investigation of intravenously administered HA was done using an osteochondral fragmentation model of equine arthritis.⁷² Osteochondral fragments were created unilaterally on the distal aspect of the radial carpal bone of 12 horses and the horses were subjected to a controlled program of exercise using a high speed treadmill. Six horses were treated with 40 mg sodium hyaluronate intravenously on day 13, 20, and 27 after osteochondral fragmentation and 6 control horses were similarly treated with physiologic saline. Horses treated with HA intravenously had lower lameness scores (were less lame), significantly better synovial membrane histologic scores (cellular infiltration and vascularity), and significantly lower concentrations of total protein and PGE₂ within synovial fluid 72 days after surgery compared with placebo-treated horses. Treatment with IV administered HA had no significant effects on glycosaminoglycan synthetic rate or morphologic scores in articular cartilage (no deleterious effects occurred with HA treatment) and synovial fluid HA levels were not changed.

The mechanism by which intravenously administered hyaluronate achieves therapeutic levels intra-articularly is uncertain. Assuming the plasma clearance of HA in the horse is similar to that identified in rabbits,⁸ it must be assumed that the beneficial effects seen in the experimental study are

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associated with localization of HA (or part of the molecule) at the synovial membrane level. It is well recognized that synovial membrane of the horse is highly vascularized and it is perhaps possible an intravenous administration provides the synovio-cyte with more exposure to exogenous HA than intra-articular administration. Hyaluronan receptors are not confined to connective tissue cells. There are three main groups of HA cell receptors identified to date: CD 44, RHAMM, and ICAM-1. Some have yet to be classified and the first and third of these were already known as cell adhesion molecules with other recognized ligands before their HA binding was discovered.⁷³ CD44 is a multipurpose receptor. It is widely distributed in the body and recent studies in our laboratory have shown expression of this receptor on equine synoviocytes, neutrophils, and lymphocytes. Although there is low expression of CD44 receptors on chondrocytes in normal cartilage, we have shown increased expression in osteoarthritic equine chondrocytes.

Intravenous HA has achieved widespread use clinically in the U.S. It has been used as a direct therapeutic agent as well as on a prophylactic basis. A prospective blinded study was done in 1996 to evaluate the effects of regular injections of intravenous HA at two weekly intervals.⁷⁴ Seventy horses were treated from May 1 to December 1 and 70 horses received placebo (racing Quarter Horses). Positive trends were noted but the hypothesis that prophylactic use of HA would cut down the amount of other medication was not proven.

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^aArtz Seikagaku, Inc., Japan.

^bSynvisc, Biomatrix, Inc., 65 Railroad Ave., Ridgefield, NJ 07657.

^cHylartin[®] V, Pharmacia, Piscataway, NJ 08854.

^dLegend, Bayer, Inc., Shawnee Mission, KS.

^eMcIlwraith, CW, unpublished data.

Polysulfated Glycosaminoglycan

Polysulfated glycosaminoglycan (PSGAG) belongs to a group of polysulfated polysaccharides and includes, in addition to Adequan,^a pentosan polysulfate (Cartrophen^R)^b and glycosaminoglycan (peptide complex (Rumalon^R)).^c This group has often been referred to as having chondroprotective properties (previously discussed), and, because of this, PSGAG has been traditionally used where cartilage damage is considered to be present rather than in the treatment of acute synovitis.¹ Using the new alternative terminology for chondroprotective drugs, PSGAG would now be referred to as a disease modifying osteoarthritis drug (DMOAD). Therapy with such drugs is meant to either prevent, retard, or reverse the morphologic cartilaginous lesions of osteoarthritis with the major criterion for inclusion being prevention of cartilage destruction. Based on some work in the horse, this classification would seem to be valid.

Adequan^R is the commercially available PSGAG formulation in veterinary medicine and ArteparonRd is the previously used human product. The chemical structure of the two products is identical and only the concentration of the active ingredient varies.² The principal GAG present in PSGAG is chondroitin sulfate (Fig. 7–25). PSGAG is made from an extract of bovine lung and trachea modified by sulfate esterification.

Mechanism of Action

There have been numerous *in vitro* and *in vivo* studies of PSGAG. PSGAG is a heparinoid. There have been varying opinions as to binding of PSGAG in cartilage but affinity for proteoglycans, collagen, and noncollagenous protein have all been proposed.^{3,46} PSGAG has been shown to inhibit the effects of various enzymes associated with cartilage degradation, including neutral metalloproteinases (both collagenase and stromelysin),^{3,5–7} serine proteinases,^{8,9} as well as lysosomal elastase,^{10,11} and cathepsin G.¹⁰ PSGAG has also been shown to have a direct inhibitory effect on PGE₂ synthesis.¹² Some other work in which PSGAG reduced proteoglycan breakdown associated with conditioned synovial membrane suggests an anti-interleukin-1 effect.^{13,14} In addition to anti-degradative effects, PSGAG has been shown to stimulate the synthesis of sodium hyaluronate both *in vitro*^{15,16} in the horse.¹⁷ It should be noted, however, that increased HA content in synovial fluid has been seen in association with other intra-articular medications by our research group and the significance is to be questioned.

In addition, enhanced glycosaminoglycan synthesis has been demonstrated *in vitro* in association with PSGAG. In radioactive labeling studies,^{18–20} it was demonstrated that both glucosamine (proteoglycan) and proline (collagen) had increased labeling after treatment of human osteoarthritic cartilage with PSGAG (this effect was less marked with normal human articular cartilage).¹⁸

In Vitro Equine Studies

In a study on equine synoviocytes stimulated to produce stromelysin (measured by the caseinase degradation assay), PSGAG was the only drug tested (others tested were phenylbutazone, flunixin, beta-methasone, and sodium hyaluronate) that inhibited stromelysin.⁷

There have been three *in vitro* studies on the effects of PSGAG on equine cartilage and the results are somewhat contradictory. Initially, it was reported that PSGAG caused increased collagen and glycosaminoglycan synthesis in both articular cartilage explants and cell cultures from normal and osteoarthritic equine articular cartilage.²¹ The same author also reported that collagen and glycosaminoglycan degradation was inhibited by the PSGAG in cell culture studies and also that osteoarthritic tissues were more sensitive to PSGAG than normal tissues. However, another investigator, using smaller doses of PSGAG (50 and 200 $\mu\text{g}/\text{ml}$ vs 25 to 50 mg/ml) and normal equine articular cartilage explants, found a dose-dependent inhibition of proteoglycan synthesis, little effect on proteoglycan degradation, and no effect on proteoglycan monomer size.²² In another subsequent study using osteoarthritic equine articular cartilage explants and small (0.025 mg/ml) and large (25 mg/ml) doses of PSGAG, the same investigator found a decrease in proteoglycan synthesis, little effect on proteoglycan degradation, no change in the size of the proteoglycan monomer, and no change in the aggregability of the monomer.²³ Three non-equine *in vitro* studies and one equine study have shown decreased degradative effects of certain enzymes on articular cartilage in the presence of PSGAG.^{6,7,24,25} However, the precise mechanisms of action of PSGAG are uncertain and the interaction of PSGAG with cytokines involved in joint disease has not been well investigated.

In Vivo Studies

The earliest animal studies were not done in horses. Using a canine lateral meniscectomy model, Ueno

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demonstrated dramatic morphologic differences between articular cartilage from control and PSGAG-treated joints. PSGAG was given intramuscularly at 25 mg/kg for 13 treatments.²⁶ Later work with a canine meniscectomy model also showed a protective effect when PSGAG was administered subcutaneously.¹⁹ These authors suggested that the PSGAG likely acts by inhibiting matrix degrading enzymes. Favorable effects (lower active neutral metalloproteinase activity, increased chondrocyte counts, and maintenance proteoglycan content) have also been reported using intra-articularly administered PSGAG in a meniscectomy model of osteoarthritis in rabbits.^{6,25} PSGAG has also been tested on the canine anterior cruciate ligament transection model and is reported to have both a prophylactic and therapeutic effect.^{24,27} PSGAG has also been tested using the rat air pouch model of inflammation and improved proteoglycan content extractability and aggregation as well as reduction of leukocyte infiltration into the pouch were noted.²⁸ It was felt that reduced leukocyte infiltration would reduce cartilage exposure to leukocyte derived proteinases and other mediators of cartilage damage. PSGAG was also tested in clinical cases of osteoarthritis in boars (intramuscularly at 5.2 mg/kg for 6 treatments and saline was put into control joints). The degree of lameness was significantly decreased and there was also increased hyaluronic acid in the synovial fluid.²⁹ The drug has also been used in the treatment of canine hip dysplasia.³⁰

The first equine investigation involved 250-mg injections of PSGAG intra-articularly twice weekly for 3 weeks and then once weekly for the next 3 weeks in clinical equine cases with joint swelling and lameness.³¹ A significant improvement in synovial fluid protein concentration and synovial fluid viscosity was reported, as well as an overall impression of decreased clinical signs (lameness, swelling and effusion, and increased flexion). Intra-articular PSGAG was then tested using a Freund's adjuvant-induced model in the carpus of 30 horses. This study concluded that the clinical signs of arthritis were reduced in treated animals.²⁴ The latter investigators, in a clinical trial in 109 horses, also felt that PSGAG improved clinical signs more frequently than horses not treated.

PSGAG was then tested on chemically-induced, as well as physically-induced lesions in the horse in our laboratory.^{32,33} Treatment with intra-articular injections of 250 mg PSGAG once weekly for 5 weeks in carpal joints injected with sodium monoiodoacetate revealed less articular cartilage fibrillation and erosion, less chondrocyte death, and markedly improved safranin O staining. PSGAG, however, did not have any effect on physically induced lesions (partial and full thickness). Our conclusions from this study were that PSGAG could markedly decrease the development of osteoarthritis but was of no benefit in healing cartilage lesions already

present at the initiation of treatment. A second study using intramuscular PSGAG (500 mg q 4 d for 7 treatments) showed relatively insignificant effects with treatment. The effects were limited to slightly improved safranin O staining in sodium monoiodoacetate joints when PSGAG was used.

More recent studies have evaluated the effects of PSGAG with or without exercise on the repair of articular cartilage defects as well as the development of osteoarthritis in the carpus of ponies.^{34,35} The authors concluded that PSGAG was beneficial in ameliorating the clinical, radiographic, and scintigraphic signs of joint disease. In another study, the effects of both hyaluronan and PSGAG were evaluated in the repair of equine articular cartilage defects in ponies.³⁶ Neither drug showed any beneficial effects. However, the project was terminated 11 weeks after defect induction.

Potential Complications of Intra-Articular Use

Intra-articular infection after intra-articular injection is always a potential risk. However, research has demonstrated that PSGAG may have greater potential in this regard. Potentiation of a subinfective dose of *Staphylococcus aureus* in the middle carpal joint of horses has been demonstrated in our laboratory.^{37,38} Using a subinfective dose, infection occurred in 8 out of 8 PSGAG injected joints, whereas it only occurred in 3 horses that received intra-articular sodium hyaluronate and 4 that received intra-articular methylprednisolone acetate. This infection could be prevented by 125 mg amikacin but was not abolished by prior filtration of Adequan. In another study, PSGAG was shown to inhibit equine complement activity.³⁹ The classical and ultimate complement pathways have bactericidal activity and both were shown to be inhibited by PSGAG in vitro. This inhibition could be a factor in the ability of bacteria to induce septic arthritis.

Because PSGAG is classified as a heparinoid, some effect on the hemostatic mechanisms is expected.^{40,41} Local hematomas were sometimes described as transient complications in early clinical trials in humans and heparin-associated thrombocytopenia is known to occur with heparin use in people.⁴¹ However, the drug has been used extensively in humans in earlier reports with a low complication rate. However, it has been suggested that the risk of hemarthrosis in humans is high.⁴⁰ It is therefore interesting that hemarthrosis has not been seen in the horse, despite extensive use. In addition, one of the author's primary uses of Adequan is following arthroscopic surgery where there is considerable articular cartilage loss and subchondral bone exposure. These joints typically have persistent effusion, which could be classified as hemarthrosis and the use of intra-articular PSGAG seems to treat this condition quite effectively.

Intramuscular Use of PSGAG

Most Adequan is used intramuscularly. As discussed previously, the positive effects using the monoiodoacetate (MIA) model that had been seen with intra-articular PSGAG could not be emulated by the intramuscular route. However, defects in this MIA model have since been recognized. There is minimal objective data supporting effectiveness for intramuscular Adequan but the drug is widely used and anecdotal reports support its value. The issue of absorption after intramuscular injection was addressed by Burba et al.¹⁷ In this study, PSGAG was labeled with tritium and scintillation done on synovial fluid as well as joint tissues. It was felt that levels of drug consistent with that seen in other non-equine studies were obtained and it was concluded that therapy every 4 days was effective in maintaining anti-inflammatory levels in the joint.

Clinical Use of PSGAG

We primarily use Adequan following surgery when there is significant loss of articular cartilage (grade III or grade IV damage). Typically these horses will have persistent bloody synovial effusion. The use of intra-articular Adequan has marked beneficial effects in these instances. We like to give one injection intra-articularly (using 0.5 ml amikacin concurrently) and then follow up with intramuscular therapy at weekly intervals and a dose of 500 mg. The drug is also used to considerable extent on a prophylactic basis. Caron et al.⁴² did a survey of 1522 equine practitioner members of AAEP seeking information on Adequan use. Of practitioners responding, 90.5% of practitioners reported use of PSGAG. Use of PSGAG was significantly more common by practitioners involved predominantly with racehorses or show horses. Standardbred racehorse practitioners had a significantly higher level of intra-articular use of Adequan. Overall, PSGAG was reported to be perceived as moderately effective for all four categories of joint disease: idiopathic synovitis, acute synovitis (with lameness), subacute OA (mild radiographic changes), and chronic OA (moderate to severe radiographic changes). Use of PSGAG was considered more effective than HA for the treatment of subacute OA and less effective for idiopathic joint effusion and acute synovitis.

Pentosan Polysulfate

The use of this drug in the treatment of equine joint disease has been recently extensively reviewed.⁴³ Although pentosan polysulfate (PPS) as the sodium salt has been used in Europe for over 30 years as an antithrombotic-antilipidemic agent, its potential as a disease-modifying antiarthritic agent has only been realized in recent years. Also, a new calcium derivative of PPS (CAPPS) has recently been developed that is more effectively absorbed after oral administration than sodium pentosan

polysulfate (NaPPS) and this offers hope for wider use of this drug. PPS could also be considered as a disease-modifying OA drug. It has been pointed out by Little and Ghosh that PPS, unlike NSAIDs, does not possess analgesic activity.⁴³ Therefore, in order to provide symptomatic relief and efficacy, a drug such as PPS must be capable of correcting the pathobiologic imbalances that are present within the OA joint, and these authors feel that PPS fulfills these requirements.

PPS is not derived from animal or bacterial sources but, rather, the "backbone" of PPS is isolated from beechwood hemicellulose, which consists of repeating units of (1-4)-linked beta-D-xylano-pyranoses. An anabolic effect on chondrocytes has been demonstrated in a focal model of OA induced by unilateral meniscectomy in sheep.⁴⁴ Studies on chondrocytes in agarose culture showed that PPS stimulated proteoglycan synthesis.⁴⁵ Also, in an experimental model of joint disease in rabbits, oral administration of CAPPS (10 mg/kg q 7 d) maintained the normal articular cartilage ratio of aggrecan to dermatan sulfate (interpreted by the authors as chondrocyte phenotype).⁴⁶ PPS also stimulates HA synthesis by cultured synoviocytes obtained by both rheumatoid and osteoarthritic joints.⁴⁷ These *in vitro* effects of PPS in HA synthesis were confirmed in a rat air pouch model of inflammation and in this model increased synthesis of HA was not stimulated by PSGAG.²⁸

A number of *in vitro* and *in vivo* studies have shown that PPS will inhibit various processes that induce degeneration of the articular cartilage matrix. For example, PPS has been shown to inhibit MMP3.⁴⁸ There is a suggestion that PPS may modulate receptor-mediated binding of cytokines.⁴³ In an ovine model of OA (medial meniscectomy), weekly intra-articular injections of PPS for four weeks improved joint function and reduced mean radiographic scores and Mankin histologic scores of articular cartilage damage in the femoral condyle.⁴⁹

There are no published studies describing the application of PPS for equine joint disease but the drug has been used in Australia. Anecdotally it is considered that treatment improves the clinical parameters of synovial effusion and lameness in most horses when used in clinical cases. Marked alleviation of lameness after racing was the most noted change with this drug.⁴³ It is also felt that because of the vascular effects of the drug that it could aid or decrease the rate of subchondral bone necrosis and sclerosis.

An interesting study involved the simultaneous administration of sodium pentosan polysulfate and IGF-1 early in the pathogenesis of iatrogenic canine OA. The combination of drugs significantly reduced the severity of lesions, whereas IGF-1 alone had little effect.⁵⁰ The presence of PPS appeared to decrease the amount of total and active metalloproteinases in the cartilage. The authors suggested that the PPS reduce the enzymatic breakdown of

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IGF-1, binding protein or receptor, thus allowing IGF-1 to exert its influence.

Oral Glycosaminoglycans

Oral glycosaminoglycan products available for horses include a purified chondroitin sulfate product from bovine trachea (Flex-Free^R)^e and a complex of glycosaminoglycans and other nutrients from the sea mussel *Perna canaliculus* (Syno-Flex^R)^f. More recently, a combination of glucosamine hydrochloride, chondroitin sulfate, manganese, and vitamin C has been marketed as a "nutraceutical" (Cosequin^R)^g. There have been some positive anecdotal reports for such supplements.⁵¹⁻⁵⁵ Cosequin has been evaluated using the Freund's adjuvant model of inflammatory joint disease in horses.⁵⁶ The oral supplement was used at the recommended dose beginning 10 days prior to arthritis induction and continuing for a further 26 days. No benefit was demonstrated based on clinical (lameness, stride length, carpal circumference, carpal flexion) and synovial fluid (protein) parameters. However, it is questionable whether the Freund's adjuvant model simulates any clinical equine joint entity and one must question the value of studies using this model.

The oral administration of glucosamine sulfate has been associated with decreased pain and improved range of motion compared to placebo in a controlled clinical trial in humans.⁵⁵ In another controlled study, glucosamine sulfate was as effective as ibuprofen at relieving symptoms of osteoarthritis in people.⁵⁴ In vitro studies using glucosamine sulfate have demonstrated increased glycosaminoglycan and proteoglycan synthesis and in vivo studies have demonstrated anti-inflammatory activity through inhibition of lysosomal enzyme activity and free radical production.

There is conflicting evidence regarding the enteral absorption of orally administered glycosaminoglycans.⁵¹⁻⁵³ The initial focus with oral glycosaminoglycans was on chondroitin sulfate and there has been some supportive evidence presented for absorption of active molecules.^{30,52} In interpretation of such studies, however, one has to be careful whether radiolabeled macromolecular sulfate, chondroitin sulfate, or labeled but inactive monomer or other degradation products are being absorbed. It is not valid to extrapolate between antienzymic data (involving intact chondroitin sulfate molecules) and the detection of tritium label in tissues. Some earlier studies that failed to show absorption have been since criticized for the lack of specificity of the methods used. However, favorable absorption of chondroitin sulfate and dermatan sulfate from the gastrointestinal tract with reduced N-acetylglucosaminidase and granulocyte elastase activity, as well as increased HA concentration in treated patients, has been reported.⁵² For instance, in another study when chondroitin sulfate was administered to healthy human volunteers and serum concentrations of GAGs using the dimethyl methylene

blue assay done, it was reported that neither intact nor depolymerized chondroitin sulfate was effectively absorbed.⁵¹ This study was criticized for low dosage combined with a low sensitivity with the DMMB assay used.⁵³

As mentioned in the previous section, absorption of calcium pentosan polysulfate after oral administration in rats has been reported as effective and what has been reported to be of sufficient level to maintain cartilage proteoglycan concentration and biosynthesis.⁴⁶ There was also some evidence for oral bioavailability of glucosamine sulfate and tropism for articular cartilage after oral administration.⁵⁴ The pharmacokinetics, organ distribution, metabolism, and excretion of glucosamine were studied in the dog using uniformly labeled (14-C)-glucosamine (sulfate) intravenously or orally in single doses. In humans, unlabeled glucosamine sulfate was given intravenously and orally and glucosamine was measured in plasma and urine with a glucosamine-specific ion-exchange chromatographic method. The result showed that the bioavailability, pharmacokinetics, and excretion pattern of glucosamine was consistent with those found in the dog with radiolabeled glucosamine and with those reported in a previous study in the rat.⁵⁷ In the dog study, radiolabeled glucosamine was given intravenously and orally and the distribution of radioactivity orally was the same as intravenously.

In one human study, cartilage biopsies were taken before and after 4 weeks of glucosamine sulfate oral supplementation in a few treated subjects as well as assessing the overall results clinically. There were reductions in joint pain, analgesic use and the improvement of joint function with glucosamine sulfate administration. Electron microscopy showed a typical picture of established osteoarthritis. In those given glucosamine sulfate, it was considered that they "showed a picture more similar to healthy cartilage."⁵⁸ Exogenous glucosamine increases matrix production and seems likely to alter the natural history of OA. Glucosamine also has a mild anti-inflammatory activity that is probably via a free radical scavenging effect.⁵⁹ Numerous in vitro studies have demonstrated that glucosamine stimulates the synthesis of proteoglycan and collagen by chondrocytes.⁴⁷

An additional mechanism by which chondroitin sulfate may benefit joint tissues is by the prevention of fibrin thrombi in synovial or subchondral microvasculature.⁶⁰ The anti-synovitis effect may be significant. It is interesting that in a study with extract of *Perna canaliculus* in humans, at the end of six months 19 of the 28 rheumatoid patients (67.9%) and 15 of the 38 osteoarthritic patients (39.5%) felt they had benefitted from the treatment.⁶¹ However, other studies have questioned both the GAG content and the therapeutic value of this extract.

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There is better evidence for glucosamine absorption than with chondroitin sulfate. Glucosamine is an amino monosaccharide that is a basic constituent of the disaccharide units of glycosaminoglycans of articular cartilage.⁴⁵ Glucosamine is the hexosamine present in keratan sulfate and the precursor of D-galactosamine (the hexosamine in chondroitin sulfate).² Exogenous glucosamine has been suggested as the preferred substrate for GAG synthesis because a higher energy expenditure is required with endogenous glucose.⁴⁵ There is good evidence for effective absorption of glucosamine sulfate (up to 87%) after oral administration in humans. In vitro studies have also documented enhanced chondrocyte synthesis of GAGs and collagen by glucosamine. It has been pointed out that although research mainly documents the effects of glucosamine as the sulfate salt, the veterinary product contains glucosamine hydrochloride.

New oral glycosaminoglycan/glucosamine products continue to be developed. There is quite an explosive market in both humans and horses despite very little scientific validation. A control study is needed to address the effectiveness of these oral glycosaminoglycan-type products and also to evaluate their efficacy relative to other systemically administered products, such as Adequan.

A clinical trial was conducted in 25 horses over a 6-week period. In this study, Cosequin was associated with decreased lameness, improved lameness, and improved lameness scores, but there were no controls.⁶²

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^aAdequan[®], Luitpold Pharmaceuticals, Inc., Shirley, NY.

^bCartrophen[®], not available in US.

^cRumalon[®], not available in US.

^dArteparon[®], Luitpold Pharmaceuticals, no longer manufactured.

^eFlex-Free[®], Vita-Flex Nutrition Co., Staten Island, NY.

^fSyno-Flex[®], Vetri-Science Laboratories, Essex Junction, VT.

^gCosequin[®], Nutramax Laboratories, Edgewood, MD.