

Licensed Medications, “Generic” Medications, Compounding, and Nutraceuticals—What Has Been Scientifically Validated, Where Do We Encounter Scientific Mistruth, and Where Are We Legally?

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1. Introduction

This report was encouraged by the American Association of Equine Practitioners' Ethics Committee, who perceived a need to clarify questions such as what are Food and Drug Administration (FDA)-licensed products and how they are scientifically validated compared with illegal medications (particularly some medications being erroneously represented as generics). This report will also address current knowledge of efficacy of various nutraceuticals and the legal definitions of an approved drug, a generic drug, a device, and a feed additive.

Getting a medication licensed is expensive and time consuming. The potential for disincentive to corporations in licensing a medication for veterinary use is real and prevalent. Legitimate manufacturers can be frustrated by the sale of illegal products claiming equivalency and by the sale of orally administered products that have little federal regulation and do not require proof of efficacy. The word “generic” has crept into the vocabulary of veterinarians

and clients, and it is usually an innocent error based on false information from a sales representative. Awareness of what is and what is not a generic drug is important for a veterinarian to practice legally.

This presentation focuses on medications used for lameness problems, in general, and for joint disease, in particular. This area provides a good example of the conflict between licensed and illegal drugs.

2. Evidence-Based Medicine

In the last 10 yr, evidence-based medicine (EBM) has become a common term in medical practices.¹⁻⁵ Although the concept is less advanced in veterinary circles compared with human medicine, it is important and was highlighted in a special issue of the *Equine Veterinary Journal* in 2003. EBM is the conscientious, explicit, and judicious use of the current best evidence in making decisions about the care of individual patients.² As such, it is hardly very specific. Although it is not a magical definition, it has been appropriately stated that the fun-

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damental underpinning of EBM is the application of appropriate experimental designs and statistics (the science that deals with the collection, analysis, and interpretation of data) to medical research and medical literature.⁴

There are various levels of experimental design and application of statistics. When it comes to testing a therapeutic modality in human medicine, the randomized controlled study is the gold standard. However, it has been pointed out that this kind of study is only one tool of many for investigating diseases, treatments, and clinical situations. For example, diagnosis of disease may be best addressed by a cohort study. It has been recommended that concerns regarding therapy and prevention of disease are best addressed by randomized, controlled, and blinded clinical trials, whereas questions concerning prognosis and harm are best addressed by cohort or case-control experimental designs.⁴ In this author's experience, randomized, controlled, and blinded clinical trials are a difficult challenge in the equine practice. On the other hand, observational, descriptive, and anecdotal studies do not provide proof of efficacy.

3. What Is Scientific Evidence?

The distinction of analytical science versus anecdotal evidence has been well described previously at the American Association of Equine Practitioners' annual convention.⁶ In the author's opinion, to prove efficacy of a treatment in joint disease, a controlled experimental study with a consistent level of disease (by a model) or an appropriately double-blinded controlled study with appropriate numbers are the only options. Critical concepts in these studies are sample size and power.⁴ Optimal sample size is the only way to provide a reliable statistical answer to the objective being investigated, and studies with low power have a poor chance of detecting a difference when it exists. A sample size smaller than the optimal number is ethically questionable, wasteful, and has little, if any, chance of being scientifically and more importantly, clinically significant. It is for this reason that power calculations are a necessary part of research applications and are necessary for peer review approval and funding as well as approval by Animal Care and Use Committees.

The literature on treatments of joint disease has a number of *in vitro* studies as well as bioavailability and absorption studies as indirect evidence of proof of effectiveness. Although such studies can be useful pilot studies, they do not answer the bottom line question of efficacy in treating or preventing the disease process in the living animal and, therefore, they need to be carefully examined in that light. Systemic absorption is a frequently asked question when studying the oral nutraceuticals. Although there have been some good studies done to document oral absorption of chondroitin sulfate in the horse,⁷ other manufacturers will cite indirect evidence of

absorption using serum levels of glycosaminoglycan (GAG) or hyaluronan (HA). Proponents of these are probably not knowingly misrepresenting their results, but they are likely unaware that both GAG levels and HA levels are markers of inflammatory joint disease.^{a8}

4. The FDA Licensing Process: What Is Involved and What Does It Mean?

First and foremost, an FDA-licensed medication is legal. It has been subjected to an approval process. More studies are required for a new chemical entity than a new formulation of an existing drug (generic).

If a company wants to license a drug that is a new and different type of medication, the company has to initially apply for an investigational new animal drug (INAD). There are a number of steps to the licensing process, including target animal safety studies, proof of efficacy (negative control, but positive controls are allowed in certain situations), and environmental toxicity. For drugs intended for use in food animals, evidence that the new drug imparts no harmful residues in the edible tissues is required. When the medication to be licensed is identical in active ingredient to an already licensed product, then a controlled study (positive control only) may be all that is required. A good example of this is the licensing of IV HA or Legend. The initial FDA approval of Legend was based on a positive-controlled study of traumatic arthritis in a group of horses compared with a group given intra-articular HA. Medications that were licensed for equine use but had no comparable product already licensed went through the INAD process. Historically, the two common models of joint disease used to evaluate these medications in a controlled fashion were an osteochondral fracture model (arthrotomy) and the Freund's adjuvant model (injecting Freund's adjuvant intra-articularly). The approaches that have been used to register products historically may not be valid today. Changes have been made as to what constitutes "substantial evidence of efficacy." For example, experimental models are not as acceptable today, and clinical studies are mandatory in most cases.^b

The previous examples provide an example of how the FDA licensing process is not necessarily the *sine qua non* of efficacy. Although the use of Freund's adjuvant model definitely documents anti-inflammatory activity, the model hardly simulates the clinical conditions commonly treated in the horse. Details on the degree of scientific proof for commonly used medications in equine joint disease will be discussed separately. Indeed, the level of proof does vary. In addition, the use of positive-controlled studies are flawed. If you have two groups that both improve (assuming that the tested drug actually works), it is possible that a negative control group (often excluded) would show the same improvement. Some examples of these

will also be presented as the individual medications are reviewed.

5. What Is a Generic Drug?

After the patent expires on an FDA-approved medication, a company can undertake to license a generic drug. However, a true generic drug must have the exact chemical formulation of the previously patented medication (pioneer drug). It is critical to recognize that a true generic drug is a licensed drug and has been through the FDA process. If it is not possible to have an exact copy, a bio-equivalency study is needed. Representing any medication as a generic that does not follow the guidelines described above is illegal. Two common such misrepresentations involve the products MAP-5 and Chondroprotec. There are numerous instances of MAP-5 being represented by distributors as generic Legend. In addition to it not being a generic drug, it is not licensed as a drug (it is only licensed as a device for extending semen). When a licensed medication is available, it is illegal for any veterinarian to administer MAP-5 intra-articularly or intravenously. Of greater concern are the quoted comments from certain representatives that MAP-5 is absolutely safe to be administered intravenously. Although the FDA licensing of IV Legend was relatively simple and based on a positive-controlled study, the development of the medication was rigorous, and it involved extraction of toxic elements and change in the molecular weight. MAP-5 is licensed as a semen extender. It is not licensed and is illegal for any musculoskeletal use.

Chondroprotec is a patented product containing chondroitin sulfate. It is licensed as a topical wound treatment. It has been commonly represented as generic Adequan, but it is not. In the same fashion as MAP-5, being represented as "generic Legend," sales representatives selling and distributing Chondroprotec for Hymed Corporation have referred to it as "generic Adequan." This is both illegal and unethical. The fact that the company calls the product Chondroprotec (which implies cartilage protection) and packages it in a container-closure system that is identical to that used for an injectable product is an extreme example of misrepresentation.⁹ As pointed out in a written response to a letter from an indignant Vice President of the Hymed group,¹⁰ White⁹ suggested that the product should be called "Dermaprotec" or some other trade name more fitting to its intended use.

6. Nutraceuticals and Animal Supplements

The widespread use of nutraceuticals does not generally fall within legal impropriety. However, it does present a major area of advertising misrepresentation and "smoke and mirror" science. It is definitely inappropriate to tar all products with the same brush, but examples of the differing levels of science used will be discussed below.

With regard to regulation of these products, human and animal nutritional supplements are not regulated the same way. In 1994, the Dietary Supplement Health Education Act (DSHEA) amended the Federal Food, Drug, and Cosmetic Act to define a dietary supplement as a product intended to supplement the diet that contains at least one of the following substances: vitamin, mineral, herb or other botanical, amino acid, dietary substance for use to supplement the diet by increasing intake, and concentrate, metabolite, constituent, extract, or combination of any of the previously mentioned ingredients.¹² However, DSHEA does not cover animal products. An ingredient that is legal to sell for human use is not legal to sell for animal use.

The word nutraceutical has been borrowed from human counterparts. Many nutraceuticals or nutritional supplements marketed for the horse are illegal, because the manufacturer has not complied with FDA ingredient-recognition processes, not completed ingredient-definition applications as described by the Association of American Feed Control Officials (AAFCO), not followed state licensing requirements, and/or made false claims on the product label.¹¹ AAFCO is a not-for-profit organization of state and federal (including Canada) feed regulators, but it has no regulatory authority. Nick Hartog of Grand Meadows has been an advocate for addressing these issues facing the supplement industry. He is also a founder of the National Association of Equine Supplement Manufacturers. He feels that AAFCO must take some of the blame for the confusion and misrepresentation that occurs today with animal supplements because of the AAFCO's failure to take action when these products first became available (some as long as 10 yr ago). It is his feeling that lack of regulation has, in effect, "implied consent" to the production and marketing of these supplements.

There seem to be two principal issues with this lack of regulation. First, the issue is sufficiently low on the FDA radar screen that there is very little attention paid to it. Second, there is no incentive for a manufacturer of a glucosamine-chondroitin sulfate product to get licensed; such products are not proprietary, and other companies can simply grab on to the coattails of the company that has gone to the trouble of getting approval.

A product that is not a drug cannot make medical claims. A food product cannot say it can be used to remedy any illness or problem. Only a licensed drug can make claims for diagnostic, therapeutic, or preventative use. This creates a fine line between illegal and legal claims, which can be seen if one explores the advertisements for various nutraceuticals. Nebulous phrases, such as "promotes joint health," commonly appear in the text of advertisements. In other instances, nutraceutical advertisements clearly cross the line and strongly imply medical claims. For instance, in the recent issue of *Equestrian* magazine, one advertisement made sev-

eral thinly veiled medical claims, including “no other joint supplement works like Flex +,” “it takes away the pain of inflammation without harmful side effects,” “it is clinically proven to restore range of motion,” and “it supports maximum flexion in stress joints.”¹³

7. Drug Compounding

Drug compounding in veterinary medicine has greatly increased since the mid-1990s. Compounding estimates of \$50,000,000–\$60,000,000/yr have been cited,¹² but some feel that this estimate is low. It has also been reported that 13% of equine prescription drugs in the average equine practice were compounded, and in practices that were exclusively performance horse practices, it was up to ~30%. Compounding has always been part of veterinary medicine and remains of value. However, there has been a proliferation of compounding pharmacies and instances of unethical and possibly illegal practices including use of licensed drugs’ trade names. Compounded products are important to the equine practice in instances where medications that have been previously approved and developed for use in the horse are no longer available as a licensed medication. An example is intra-articular betamethasone esters, which are used to treat equine joint disease. Initially available as BetaVet Soluspan and then only available as Celestone Soluspan, the drug is currently unavailable as a licensed medication. Being one of the intra-articular corticosteroids that does not have deleterious side effects on the cartilage,¹³ its availability is desirable, and various compounders are producing it. However, at least one compounder (Wedgewood) lists compounded betamethasone esters in its catalogue as Celestone, which it is not. Many veterinarians report successful use without side effects, but there have been instances of serious reactive arthritis cases after injection. In one instance of which the author is aware, the insurance company did not allow the company to stand behind the clinicians involved. It is to be noted that adverse reactions with the licensed products of betamethasone have been experienced in isolated cases when those licensed medications were available. However, although a company manufacturing a FDA-licensed drug has to report all adverse reactions, there is no accountability with compounded medications. In the author’s experience, companies with licensed medications have stood behind the veterinarian when he/she encounters instances of adverse reactions. Equine practitioners need to be aware of such risks when using compounded drugs.

8. Assessment of Licensed Medications and the Pretenders

Commonly used medications and their level of scientific proof of efficacy will now be addressed.

HA (Sodium Hyaluronate)

HA is a linear polydisaccharide and polyionic non-sulfated GAG. The disaccharide units are linked by 1–4 glycosidic bonds to form a long, unbranched chain consisting of 10,000–12,000 disaccharide units.¹³ Studies in humans and animals have determined the molecular weight of synovial fluid HA to be 2–6 million daltons.¹⁴ HA is an integral component of both synovial fluid and articular cartilage in normal synovial joints. It is synthesized by the synoviocytes of the synovial membrane when it is in the synovial fluid, and it is synthesized locally by the chondrocyte when it is incorporated in the extracellular matrix of articular cartilage. HA confers the prosperity of viscoelasticity of synovial fluid and is responsible for boundary lubrication of the synovial membrane. Additionally, HA is a significant component of the lubrication system of articular cartilage.^{15,16} Since its use was first described in the horse,¹⁷ the mechanism for which HA has been hypothesized to benefit diseased joints has been varied and highly speculative, but the following discussion will be limited to proof of efficacy.

Most of the studies with intra-articular HA have been clinical studies. The first report was published in 1970; in this study, cases of traumatic, degenerative equine arthritis in 20 racing Thoroughbreds and Standardbreds were treated with methylprednisolone acetate versus a HA/methylprednisolone acetate combination. The investigators concluded that the combination of HA and methylprednisolone acetate resulted in a better and more lasting improvement than the corticosteroid alone.¹⁷ Unfortunately, methylprednisolone represents a positive control, and the lack of a negative control group was a flaw. In 1976, Asheim and Lindblad¹⁸ provided the first report of treatment of equine traumatic arthritis with intra-articular HA alone. In this study, 54 joints of 45 racehorses that had previously undergone conventional treatment and failed to improve were used.¹⁸ After a 1-yr observation period, 38 of 45 horses were free of lameness. Of the 38 horses free of lameness, 32 returned to the racetrack after treatment. The control group in this case was an historical control (failure to respond to previous treatment). It is preferable to having a positive control, but it does not account for all other factors causing improvement.

Since these early reports, there have been numerous clinical reports that have been generally supportive of the use of HA. However, in many of them, the evaluations are subjective (with improvement noted but no consideration of the effects of rest, decreased exercise, and other treatments), and the definitions for criteria for successful treatment are absent. The duration of post-treatment observation periods are varied, and in some studies, they were of short duration. Most studies include response to intra-articular anesthesia as criteria for

case selection. This helps to localize the problem, but it provides little information about the specific diagnosis. A number of studies state or imply that the condition treated was osteoarthritis (OA), but the criteria for OA were not shown. In addition, horses in these studies are commonly injected with corticosteroid and HA combinations. In the author's experience, any condition beyond mild synovitis usually requires adjunctive corticosteroid therapy as well.¹⁹

Controlled experimental studies evaluating intra-articular HA have also been made. 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 weight bearing is suspect, because both treated and untreated limbs returned to pre-surgical weight-bearing values.²⁰ In a second study using amphotericin induced carpal lameness, the effectiveness of intra-articular hylan (a derivative of HA modified by cross linking) was evaluated in a double-blind study using gait analysis. The researchers concluded that the treatments with hylan did not significantly alter any of those variables compared with baseline or controlled values. Additionally, at least in this model of acute synovitis (amphotericin), there was no beneficial effect.²¹ The second study could be used as an example of the benefits of intra-articular HA. However, models will vary in their ability to prove effectiveness of a drug, even if the drug is useful. The amphotericin model was developed after previous work by this author with filipin.²² Filipin and amphotericin are both polyene antibiotics that cause lysis of lysosomes and promote quite severe synovitis. This synovitis can cause rapid degradation of HA itself. Our clinical experience supports usefulness of HA alone in mild synovitis but not in severe synovitis.

HA has also been assessed using a randomized double-blind, placebo-controlled clinical study in Standardbred trotters.²³ Seventy-seven trotters with moderate to severe lameness were grouped according to number of affected joints, and within each group, they were randomized for treatment with polysulfatic GAGs (PSGAG), sodium hyaluronate (HA), or placebo for 3 wk. The horses were inspected weekly with final examinations 2–4 wk 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 ($p < 0.01$). Additionally, the prevalence of sound horses increased significantly from 1–3 wk of treatment and into the last examination in all three groups, including the negative control group. Comparison of the two treatment groups with regard to the development of the lameness curve and time until soundness indicated a small, non-significant difference in favor of HA. No significant difference was detected between the two treatment groups and

the prevalence 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 ($p < 0.01$), for reduction of time until soundness for the treatment period and the total study ($p < 0.01$ and $p = 0.04$, respectively), and the prevalence of sound horses at the last examination ($p < 0.01$). All three treatments (including placebo) were useful in traumatic arthritis in horses, but HA and PSGAG gave better results than placebo. These results emphasize the importance of a placebo group.

More recently, 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, has been introduced and promoted.²⁴ Limitations were recognized with conventional HA preparations, and hylans were developed to improve the efficacy of viscosupplementation therapy or OA. Two combinations have been developed specifically as a device for viscosupplementation therapy in human OA, and there have been a number of positive reports on the use of intra-articular HA to relieve pain in an OA knee. A recent meta-analysis of 49 randomized controlled studies led to the conclusion that compared with placebo injections, the ability of intra-articular HA to relieve pain in an arthritic knee is small; even the small benefit is undermined by poor quality research in the presence of publication bias.^c The meta-analysis cites the contention that these preparations purportedly reduce OA pain by "viscosupplementation," restoring the viscoelasticity of the synovial fluid, and supplementing endogenous HA broken down by joint inflammation. Ultimately, the study concluded that there is very little data supporting this mechanism and that there is little evidence that one preparation or one dosing regimen is any better than another. Of the 49 randomized controlled trials reviewed, 19 met the inclusion criteria. Of these 19, at least 15 were industry-sponsored trials. To be included in the meta-analysis, a study had to have investigated knee OA in a single- or double-blinded randomized trial and compared a minimum of three weekly injections of HA to an intra-articular placebo. The trial had to report on at least one of five pain-outcome assessments. Additionally, a minimum follow-up time of 2 mo after treatment was required, and at least 50% of subjects had to have completed the trial for the study to be included. The trials ranged in size from 24 to >400 subjects, and drop-out rates in some of the larger studies were substantial. In those trials that were statistically significant, the overall effect size of HA in the meta-analysis was small (0.33)—comparable with that of non-steroidal anti-inflammatory drugs (NSAIDs). The "quality issues" that undermined this result included large drop-out rates, which can easily overlook the subjects in which intervention had no effect.

The authors of the report could also not come to a conclusion regarding high molecular weight HA having a greater effect than regular HA.

Another notable researcher in human OA concluded that there was little evidence to support the use of intra-articular HA.²⁵ Brandt et al.²⁵ concluded that even though HA was effective in many trials, it seemed no better than much less expensive analgesics or intra-articular saline. Brandt et al.²⁵ also concluded that it was not clear that improvement after HA injection was substantial because of either a placebo effect from the patient or a regression to the mean phenomena. In a third study, Felson and Anderson²⁶ reviewed large, randomized trials of >200 subjects that compared HA with an intra-articular placebo. They concluded that based on the best evidence from the large, randomized placebo-controlled studies, HA is no more efficacious than placebo injection in the treatment of OA of the knee. It would be interesting to see a similar meta-analysis of equine treatments. This author still supports the value of intra-articular HA for mild to moderate synovitis (in combination with corticosteroids for severe synovitis). The questionable results for intra-articular HA in human OA, as detected by meta-analysis, are hardly surprising to this author. When intra-articular HA was first available, we tried using it in OA with poor results. It was a surprise to see it licensed for human OA.

Intravenous HA

A formulation of HA for IV administration was developed in horses, and it has been licensed for several years now. Although the author was initially skeptical about the prospect of an IV HA product being effective, a controlled study in an equine model showed it to be quite efficacious.²⁷ The IV HA is given as a 40 mg (4 ml) IV injection. It goes under the trade name of Legend in the United States and Hyonate elsewhere. The evaluation of IV Legend was the first controlled investigation done using the osteochondral fragmentation model of equine OA developed at Colorado State University.²⁷ This model closely approximates a clinical condition of traumatic synovitis and OA in the horse, and a variety of outcome parameters and therapeutic routes can be investigated. Briefly, osteochondral fragments are created unilateral on the distal aspect of the radial carpal bone of 12 horses, and the horses are subjected to a controlled program of exercise using a high-speed treadmill (after recognizing the need to improve statistical power, we now use eight horses in each group for such studies). Six horses were treated with 40 mg of HA intravenously on days 13, 20, and 27. After osteochondral fragmentation, six 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 prostaglandin E₂ (PGE₂) within synovial fluid 72 days after surgery compared with placebo-control horses. Treatment with IV-administered HA had no significant effects on GAG synthetic rate or morphologic scores on articular cartilage (no deleterious effects occurred with HA treatment), and synovial fluid and HA fluid levels were not changed.

Intravenous HA has achieved widespread use clinically in the United States. It has been used not only as a direct therapeutic agent but also on a prophylactic basis. A prospective blinded study was done in 1996 to evaluate the effects of regular injections of IV HA at two weekly intervals.²⁸ 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 decrease the amount of other medication was not proven. A subsequent study in Thoroughbreds using synovial fluid and serum biomarkers at the same regimen as the controlled study previously cited failed to reveal any significant differences. Both these clinical studies show that there may be a benefit, but it is very difficult to show subtle benefits with clinical trials in the horse. Factors that complicate recognition of a subtle benefit include individual differences for the pain tolerance of horses as well as their athletic ability, the subjective nature in which we evaluate horses clinically, limited objective outcome parameters, and day-to-day variation in joint disease symptoms.

Oral HA

More recently, an oral HA product called Conquer has been on the market. An anecdotal case study as well as evaluation of blood levels of HA after oral administration has been cited as supportive evidence for efficacy of this product (on advertisements only). Although there are no publications yet in the referred literature, the suggestion that elevation of HA levels might imply therapeutic effectiveness is flawed and should be addressed.

Elevation of HA levels in the serum after oral administration of HA showing efficacy is intuitively logical. However, serum HA levels have been used for some time as a biochemical marker of rheumatoid arthritis and OA in humans. Increased levels of HA have been recognized in the serum of OA patients compared with healthy control subjects.^{28,29} In a more recent study, the prognostic value of different biochemical markers for morphologic progression of early knee OA was examined.³⁰ A total of 89 patients with knee OA were enrolled, and the follow-up period was 2 yr. Radiological OA progression was evaluated by measuring joint space, and pentosidine (using high performance liquid chromatography [HPLC]), cartilage oligomeric matrix protein (COMP), MMP-9, tissue-inhibitor of metalloproteinase (TIMP), and hyaluronic acid were evaluated prospectively. In a group of patients suf-

fering from knee OA, higher serum levels of pentosidine, TIMP, and COMP were detected compared with healthy control subjects. Using a correlation analysis method, it was found that the patients with higher basic serum levels of HA had a faster radiologic progression ($r = 0.56$, $p < 0.005$) and higher pentosidine levels ($r = 0.30$, $p < 0.05$).³¹ It was concluded that serum levels of HA and pentosidine had a predictive value for further development of knee OA and that additional joint space narrowing was detected in the patients with knee OA in the next 2 yr. This study not only shows the value of serum HA levels in predicting progression of OA, but it also highlights that serum HA levels cannot be used as an accurate indicator of increased HA absorption. Even if we did decide that HA was absorbed after oral administration, there is still a considerable distance between this finding and proof of therapeutic effectiveness.

To address this question, our laboratory has recently evaluated the use of IV Legend versus orally administered HA and placebo in our osteochondral fragment-exercise model. Results are forthcoming.

PSGAGs

PSGAGs belong to a group of polysulfated polysaccharides, and they include, in addition to Adequan, pentosan polysulfate (PPS) and GAG peptide complex (Rumalon). This group is often referred to as having chondroprotective properties, and because of this, PSGAG has been traditionally used where cartilage damage is said to be present rather than in the treatment of acute synovitis.³² Using the new alternative terminology for chondroprotective drugs, PSGAG would now be referred to as a disease-modifying osteoarthritic drug (DMOAD). Therapy with such drugs is meant to prevent, retard, or reverse the morphologic cartilaginous lesions of OA with the major criteria for inclusion being prevention of cartilage destruction.

Adequan is the commercially available PSGAG formulation in veterinary medicine, and Artepargon is the previously used human product. The chemical structure of the two products is identical, and only the concentration of the active ingredient varies.³³ The principal GAG present in PSGAG is chondroitin sulfate, and the product is made from an extract of bovine lung and trachea modified by sulfate esterification.

Both controlled *in vitro* and *in vivo* studies have been done with Adequan. *In vitro* studies have the convenience of low cost and not needing to sacrifice horses specifically for the research purposes. They can answer some simple questions, but extrapolation from *in vitro* results to *in vivo* effectiveness is dangerous, because they disregard dosage and absorption aspects. In one study on equine synovocytes stimulated to produce stromelysin (caseinase degradation assay), PSGAG was the only drug tested that inhibited stromelysin (other drugs tested were phenylbutazone, flunixin, betamethasone,

and HA).³⁴ There have been three other *in vitro* studies on the effects of PSGAG on equine cartilage explants, and the results are somewhat contradictory.³⁵⁻³⁷ Initially, it was reported that PSGAG caused increased collagen and GAG synthesis in both articular cartilage explants and cell cultures from normal and osteoarthritic equine articular cartilage.³⁵ The same author also reported that collagen and GAG degradation was inhibited by PSGAG in cell cultures 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 $\mu\text{g/ml}$ versus 25-50 mg/ml) and normal equine articular cartilage explants found dose-dependent inhibition of proteoglycan synthesis, little effect on proteoglycan degradation, and no effect on proteoglycan monomer size.³⁶ In a follow-up 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.³⁷

The first equine *in vivo* investigations on Adequan involved 250-mg injections of PSGAG administered intra-articularly two times per week for 3 wk and one time per week for the next 3 wk in clinical equine cases with joint swelling and lameness.³⁸ Significant improvement in synovial fluid protein concentrations and synovial fluid viscosity was reported as well as an overall impression of decreased clinical signs (lameness, swelling, effusion, and increased flexion). The first controlled experimental study was done using a Freund's adjuvant-induced model in the carpus of 30 horses.³⁹ The study concluded that the clinical signs of arthritis were reduced in treated animals. The same investigators, in a clinical trial of 109 horses, also felt that PSGAG improved clinical signs more frequently than horses not treated. It was on this basis that Adequan was approved, but further studies have been used to show the drugs effectiveness by the intra-articular route. PSGAG was also tested on chemically induced and physically induced lesions on the horse in our laboratory.⁴⁰ Treatment with intra-articular injection of 250 mg of PSGAG one time per week for 5 wk and carpal joints injection of sodium monoacetate revealed less articular cartilage fibrillation and erosion, less chondrocyte death, and markedly improved Safranin O staining. However, PSGAG did not have an effect on physically induced lesions (partial and full thickness), and thus, our conclusions from this study were that PSGAG could markedly decrease the development of OA, but it was of no benefit of healing cartilage lesions already present at the initiation of treatment.

There have been other controlled studies done that evaluate the effects of PSGAG with or without exercise in the repair of articular cartilage defects as

well as the development of OA in the carpus of ponies.^{41,42} A clear demonstration of effectiveness has been produced scientifically with intra-articular Adequan. However, there have been concerns regarding the potential for infection with PSGAG, initially based on anecdotal experience in a limited number of cases and then shown scientifically.⁴³ Possibly, there has been an overreaction to this experimental study. In a second study, the same authors showed that concomitant injection of 125 mg of amikacin would prevent infections from developing.⁴⁴ Unfortunately, scientific literature on efficacy of IM Adequan is not quite as impressive.

When IM PSGAG (500 mg every 4 days for seven treatments) was tested on the monoacetate model, relatively insignificant effects were shown.⁴⁵ Effects were limited to slightly improved Safranin O staining of GAG in induced joints when PSGAG was used. Because of the fears of the complications of intra-articular injection, most Adequan is used intramuscularly. More recent work confirms that the positive effects of IM Adequan in the Colorado State University OA model are less dramatic than with intra-articular Adequan.^d Circumstantial evidence of absorption has been shown after IM injection.⁴⁶ In this study, PSGAG was labeled with tritium, and scintillation was performed on synovial fluid and joint tissue. Levels of the drug 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. It needs to be recognized that this conclusion was based on the presence of radiolabeled tritium in the tissues and fluids and that anti-inflammatory activity in the equine joint after IM injection has not been defined.

Caron et al.⁴⁷ did a survey of 1522 equine practitioner members of the American Association of Equine Practitioners on information about Adequan use. Of practitioners responding, 90.5% reported use of PSGAG, and its use was significantly more common with 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), sub-acute 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 sub-acute OA and less effective for idiopathic joint effusion and acute synovitis. Obviously, this is anecdotal. Although the study provides valid information, it still does not clearly delineate the differences of efficacy of Adequan by intra-articular versus IM routes of administration.

Chondroprotect

A number of chondroitin sulfate products have been used intramuscularly and orally, but it is Chondro-

protect that has achieved considerable clinical use. It is often misrepresented as "generic Adequan." Chondroprotect is a patented FDA clear-for-marketing wound care product for both human and animal use.¹¹ In a recent viewpoint reviewing a copy of U. S. Patent 5929050 for George Petito, the title of the patent is "Chondroitin sulfate composition and method for wound treatment."¹⁰ Despite the contention of the Vice President of the Hymed Group that their product is a PSGAG, this does not appear in the text of the patent. In fact, the patent states that the invention is "preferably chondroitin sulfate with a molecular weight range between 5000–50,000." Chondroitin sulfate contains one sulfate group at the four or six position on each of the disaccharide units that make up the molecule, and it would not fit the acceptable chemical identification of a PSGAG. An important point is that the substance in Chondroprotect is not the same as the drug substance PSGAG found in the FDA-approved drug Adequan. Adequan is the only drug that can legally call itself PSGAG. Adequan has a different molecular weight and a higher sulfate content than the chondroitin sulfate in Chondroprotect.

Chondroprotect is licensed as a device for wound care. A device is not a drug. Equally importantly, owners and veterinarians report that they have been told by sales representatives selling and distributing this product for Hymed that it is a "generic Adequan." It has also been documented in a letter that a veterinarian has been told that this product is "just like Adequan, but cheaper" and was to be given at a dose of 500 mg (5 ml) IM two times per week for 4 wk "just like Adequan."⁹ It has been suggested that only a small percentage of Chondroprotect has been used for its intended purpose of a wound-healing device.

The use of the name Chondroprotect also implies cartilage protection, and in the author's opinion, is a gross misrepresentation (albeit at a subtle level). In addition, Chondroprotect is packaged in a container identical to that used for an injectable product when its approved use is as a wound-healing device. Although there are provisions for the off-label use of drugs in veterinary medicine, there are no provisions in the regulations for the off-label use of a medical device as a drug. Furthermore, it is illegal for a manufacturer or distributor to promote such uses. A comparison of the efficacy of intramuscular PSGAG, Chondroprotect, and a compounded solution of ecetyl-D-glucosamine (Red Cross Drug, Blanchard, OK) was done using the Complete Freund's adjuvant carpalis model. All horses received the treatments by IM injection every 4 days for 4 wk, and all doses were 500 mg. On days 12, 19, 26, and 33, the primary outcome measures were taken for lameness score, carpal flexion, stride length, and carpal circumference. The study was blinded because the clinician evaluating the outcome measures was unaware of the treatment group assignment. PSGAG was significantly ($p < 0.05$)

more effective in the recovery of model-induced deficits in all parameters than were chondroitin and glucosamine injectable solutions, and there were no significant differences between the two test drugs. In this test system, these two compounds, often sold as "generic" versions of PSGAG were significantly less effective than PSGAG.⁴⁸

PPS

The use of this drug in the treatment of equine joint disease was reviewed in 1996 by Little and Ghosh.⁴⁹ PPS could also be considered a DMOAD, and it has been noted that, unlike NSAIDs, PPS does not possess analgesia activity. Therefore, 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. Little and Ghosh⁴⁹ reported that PPS fulfils these requirements. PPS is not derived from animal or bacterial sources; the "backbone" of PPS is isolated from beech wood hemicelluloses, which consists of repeating units of (1-4)-linked beta-D-xylanopyranoses. An anabolic effect on chondrocytes has been shown in a focal model of OA induced by unilateral meniscectomy in sheep. There is *in vitro* work showing that PPS stimulates proteoglycan synthesis by cultured chondrocytes in agarose culture and HA synthesis in cultured synoviocytes. *In vitro* studies have also shown that PPS will inhibit various processes that induce degeneration of the articular cartilage matrix.

There are no published placebo-controlled clinical studies for the application of PPS for equine joint disease, but the drug is approved for equine use 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.⁴⁹ It is also felt that the vascular effects of the drug could aid or decrease the rate of subchondral bone necrosis and sclerosis.

PPS has been recently evaluated in our osteochondral fragment-exercise model of OA.^e There were two groups. One group (n = 9) received PPS at a dose of 3 mg/kg one time per week for 4 wk. A control group (n = 9) received placebo saline at the same time. There was improvement in lameness, joint flexion, synovial fluid total protein levels, and levels of Type II collagen degradation markers and CS846 epitope (a synthetic marker of chondroitin sulfate). Treatment with PPS seemed to increase both serum and synovial fluid levels of 846, and this is considered a reparative response. It was also noted that CS846 increased in joints without a chip fragment as well as in joints with a chip fragment, potentially suggesting a systemic up-regulation of aggrecan synthesis rather than any response to pathologic change. PPS also resulted in significant improvement in reducing articular cartilage fibrillation and showed a strong trend (p = 0.063) for

overall improvement in cartilage histological appearance. We concluded that the dose administered (3 mg/kg one time per week for 4 wk) was a minimally effective dose; subsequent studies will focus on increasing the dose frequency to 3 mg/kg one time every 5 days for seven total injections.

NSAIDs

The term NSAIDs tends to be used restrictively to describe anti-inflammatory agents that inhibit some components of the enzyme system that convert arachidonic acid into prostaglandins and thromboxins.^{50,51} There are various NSAIDs available for use in the treatment of joint disease in the horse. The most commonly used NSAID is phenylbutazone. The discussion of NSAIDs is relatively non-controversial in the context of this presentation. However, there are some instances where both anecdotal experience and marketing and advertising have not been supported by controlled scientific evaluation.

The most significant advance in NSAID therapy in recent years has been the recognition of inducible COX (COX-2) being a distinct isoform encoded by a different gene from the constitutive enzyme (COX-1). Because COX-1 produces prostaglandins that serve protective physiological functions and COX-2 is responsible for production of prostaglandins that cause symptoms of inflammatory disease, NSAIDs that are selective at inhibiting COX-2 offer the hope that we can have more effective therapy without the usual gastro- and nephrotoxicity associated with this group of drugs. Inhibition of both COX-1 and COX-2 is marked with drugs such as phenylbutazone and is considered one of the most important aspects of phenylbutazone's therapeutic potential. However, other agents, such as carprofen, are relatively weak cyclooxygenase inhibitors, leading to the conclusion that in addition to effects on prostaglandin, other mechanisms may contribute to their overall anti-inflammatory activity. Anecdotally, the author has used carprofen (Rimadyl) on horses that showed signs of nephrotoxicity and diarrhea with phenylbutazone, and the secondary side effects were markedly reduced when carprofen was used.

An area where scientific study has been important is with ketoprofen. Ketoprofen was initially marketed as a dual inhibitor of cyclooxygenase and 5-lipoxygenase.⁵² Such activity would broaden the anti-inflammatory potential of the compound, in theory making it superior to other NSAIDs. However, such claims were based on early *in vitro* data.⁵² Ketoprofen had no effect on leukotriene B-4 (LTB-4) concentration in experimental models in rats, producing virtually 100% inhibition of PGE₂ and thromboxane-2 (TXB-2) production.^{53,54}

In a controlled *in vivo* study, synovitis was induced in the middle carpal joint of 12 horses by the injections of carrageenan. Although IV 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.^{55,56} The authors concluded that at clinical doses of 2.2 mg/kg/day, the drug should not be considered superior to other NSAIDs based on claims about its ability to inhibit 5-lipoxygenase. Also, in another study with experimentally induced synovitis, the analgesic and anti-inflammatory effects of ketoprofen and phenylbutazone were compared. All NSAID-treated horses had decreased PGE₂ compared with saline-treated horses. The effect lasted longer with phenylbutazone-treated horses than ketoprofen-treated horses.^{55,56} There were no treatment effects on LTB-4 (which supposedly happens 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.

More recently, an intra-articular NSAID called buprenorphine has been used for equine joint disease. Its value as an intra-articular injection has been tested in an amphotericin B-induced aseptic arthritis in horses.⁵⁷ Buprenorphine or parabutoxyphenylacethydroxamic acid has been used as a suspension for intra-articular use in humans with success. In the controlled study cited above, aseptic arthritis was induced in the right intercarpal joint by intra-articular injection of amphotericin B (20 mg). One week later (day 0), horses were randomly assigned to four six-horse treatment groups and treated with intra-articular injection of 10, 20, or 40 mg of buprenorphine suspension (20 mg/ml) or 2.0 ml of sterile saline (control). The treatment was repeated again after 7 days. Intra-articular injection of amphotericin B consistently resulted in aseptic arthritis with a lameness index of 2.7 ± 0.17 on day 0. Intra-articular injections of 20 and 40 mg of buprenorphine significantly reduced the day 28 lameness index compared with control values. Both dosage levels also reduced the synovial fluid activity of betaglucuronidase.

Intra-Articular Corticosteroids

In 1955, Wheat⁵⁸ published the first report of intra-articular corticosteroid use, and their use is still widespread today. Various unsupported statements have been made regarding corticosteroids, and they provide a good example of non-scientific proclamations being used to cast a negative light on a treatment. More recently, various investigators have critically evaluated the effects of corticosteroids in equine joints in a scientific manner, and these results have helped identify a more definitive role for these agents in the management of joint disease.⁵⁹⁻⁶⁸ The consensus of these studies is that certain corticosteroids can indeed be harmful to the joint, but others are not. Despite this scientific

showing of the beneficial effects of betamethasone esters and triamcinolone acetonide, some scientific authors still do not seem to be able to differentiate these drugs from methylprednisolone acetate, which causes deleterious effect.⁶⁰ For this reason, the scientific literature on evaluation of three commonly used intra-articular corticosteroids will be briefly reviewed. All have been evaluated in controlled studies.

Methylprednisolone Acetate (Depo-Medrol)

A number of studies have evaluated the effect of methylprednisolone acetate (MPA) injected into normal joints.⁶³⁻⁶⁸ While some of these studies were done with higher doses of MPA compared with what is normally used in the field, the most recent study in the Colorado State University osteochondral-exercise model did confirm that MPA had deleterious effects on the cartilage. This was shown using the modified Mankin scoring system of articular cartilage degradation.⁶⁰

Betamethasone Esters

The lack of secondary articular cartilage degradation in cases of carpal osteochondral fragmentation treated with arthroscopic surgery was the initial prompt to investigate betamethasone esters. Additionally, the study questioned the validity of previous generalizations regarding the deleterious effects of corticosteroids.^{68,69} A number of joints had been injected repeatedly with betamethasone esters (Betavet Soluspan) without any changes seen at arthroscopy.⁷⁰ This study used the first generation of the osteochondral-fragment model (no burring back to 15 mm). The study was flawed, because the control limb was in the same horse with a fragment present; however, the lack of deleterious side effects of two intra-articular injections of corticosteroids were clearly shown.⁵⁸

Triamcinolone Acetonide

Triamcinolone acetonide (TA) has been studied in a controlled study using the osteochondral-fragment model.⁶¹ In this study, 18 horses were trained on a high-speed treadmill and had an osteochondral fragment created at the distal aspect of the radial carpal bone of one randomly chosen mid-carpal joint. Six horses were treated with intra-articular injection of polyionic fluid in both middle carpal joints (CNT group). Six horses were treated with 12 mg TA intra-articularly without an osteochondral fragment (the opposite mid-carpal joint was treated intra-articularly with a similar volume of polyionic fluid; TA CNT group). Six horses were treated with 12 mg of TA in the joint that contained the osteochondral fragment (with a similar volume of polyionic fluid in the opposite middle carpal joint; TA TX group). TA and placebo treatments were repeated at days 13 and 27 after surgery, and treadmill exercise proceeded for 5 days/wk from day 14 to day 72.

Horses that were treated intra-articularly in a joint containing a fragment (TA TX group) were less lame than horses in the CNT TA group. 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 into the hyperplasia and subintermal fibrosis. Analysis of articular cartilage and morphologic parameters evaluated using a standardized scoring system were significantly better in the TA CNT and TA TX groups, irrespective of which joint received TA. There was less staining with safranin-O-fast green (SOFG) in the TA CNT group compared with the TA TX and CNT group, although the GAG synthetic rate was elevated in the TA CNT group compared with the two other 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 compared with placebo treatments. Beneficial effects were recorded in both synovial membrane morphologic and biochemical articular parameters. Increased HA concentrations were observed in TA-treated joints, which also suggest a favorable corticosteroid effect on synoviocytes metabolism. This research supports a chondroprotective effect of corticosteroids in a controlled model of OA. It is in marked contrast to the detrimental effects of corticosteroids seen in the *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.⁶² Third carpal bones from joint with fragments showed significantly more vascularity, single labeled surface, and total labeled surface of mineralizing surface in subchondral and subjacent trabecular bone. No significant differences were seen in microdamage density or size between fragmented and non-fragmented joints. No significant effect of TA treatment was seen on any parameters examined. This information is important in view of the extrapolation from human clinical work done by some authors; they have suggested that intra-articular corticosteroids in horses may cause osteoporosis in the adjacent bone.

Fear of laminitis has caused a decrease in use of TA by some equine practitioners. There has been anecdotal associations made and maximum doses established based on an anecdotal report of no cases of laminitis in 1200 horses treated when a dose did not exceed 18 mg.⁷¹ Although some clinicians have reported no problems with higher total body doses than this, many clinicians are still wary of its use in general. A recent publication provides the first follow-up study with data on the potential for TA to produce laminitis. The conclusion of the study was

that there was no association between the occurrence of laminitis and the intra-articular use of TA.

In Vitro Assessment of Corticosteroids

Dose titration studies have been performed for corticosteroids using interleukin-1-conditioned equine cartilage explants.^{e73} In each situation, the optimal concentration *in vitro* to inhibit interleukin-1-induced matrix depletion extrapolates into a higher dose clinically than what many feel is necessary. It is for this reason that many clinicians feel that *in vitro* studies can be used to provide proof of principal answers. For example, our initial *in vitro* work showed that we could transfect synoviocytes with an adenoviral-IL-1ra vector.⁷⁴ On the other hand, it does not seem very useful to get exact dose regimens in that these need to be evaluated *in vivo*.

Compounding of Corticosteroids

The lack of availability of a licensed betamethasone product (both Betavet Soluspan and Celestone Soluspan are off the market) has led to a demand for compounded betamethasone esters. The author considers this legitimate use of compounding. However, the use of reputable compounders is critical. Compounders do not have to report adverse reactions like the manufactures of FDA-licensed products. Of more importance, in the case of an adverse reaction, many compounders will not stand behind the product. Recent experience with a well-known compounding company indicates that the veterinarian may be on his/her own in the event of an adverse reaction causing the horse's loss of ability or death. The situation represents quite a dilemma. Apprehension about using a compounded intra-articular drug has caused some veterinarians to use more intra-articular Depo-medrol. This has led to osteochondral chip fragment joints with more secondary OA change at the time of presentation for arthroscopic surgery. On the other hand, there are good corticosteroid products out there, and even with frequent use, no deleterious side effects have been seen.

9. Oral Nutraceuticals

As mentioned previously, it is important to recognize that none of the oral supplements or oral nutraceuticals are licensed, and proof of efficacy is generally lacking. Most products include glucosamine and/or chondroitin sulfate along with other ingredients.

Historically, the oral GAG products initially available for the horse included a chondroitin sulfate product from bovine trachea (Flex-Free) and a complex of GAGs and other nutrients from the sea mussel *Perna canaliculus* (Syno-Flex). More recently, a combination of glucosamine hydrochloride, chondroitin sulfate, manganese, and vitamin C has been marketed as a "nutraceutical" (Cosequin). There have been a number of scientific studies done with Cosequin that will be discussed below. Most other

products have simulated Cosequin and attempted to compete on the basis of decreased cost (with no proof of comparable efficacy) or other added ingredients. Scanning horse magazines, one can see a plethora of products now available. There are a number of ways that efficacy has supposedly been shown with glucosamine and chondroitin sulfate products, and these methods will be examined.

Bioavailability

Proof of the absorption of the oral dose attaining therapeutic blood and tissue levels provides indirect evidence that these products may work. It has also been suggested that glucosamine and chondroitin sulfate exhibit a high degree of tropism for articular cartilage.⁷⁵ A study in dogs showed that glucosamine and chondroitin sulfate (measured as total disaccharides) are bioavailable after oral dosing. The mean bioavailability of glucosamine after single dosing was ~12%. The mean bioavailability of chondroitin sulfate after single dosing was 5%.^{76,77} This paper was particularly interesting in that it suggested that the low molecular weight chondroitin sulfate used in this study displayed significant accumulation on multiple dosing. This is the low molecular weight product used in Cosequin. Evidence of the oral absorption of chondroitin sulfate has been shown in the horse. The oral bioavailability of 8.0-kDa chondroitin sulfate was 32% compared with 22% for 16.9-kDa chondroitin sulfate.⁷ The oral bioavailability of glucosamine hydrochloride in horses was found to be 2.5% with a large volume of distribution, which the clinicians interpreted as poor absorption from the intestinal tract and extensive tissue uptake.⁷³ A more recent publication from the same laboratory confirmed that after oral dosing, the mean C_{max} for glucosamine was 10.6 $\mu\text{g/ml}$, and the mean bioavailability was 2.5%. This was interpreted as providing evidence that glucosamine is absorbed orally, albeit it low, and that it is most likely due to extensive first pass metabolism in the gastrointestinal tract and/or liver before systemic availability. These authors also pointed out that these data in horses was in contrast to the bioavailability of glucosamine in the dog (12%).⁷⁷ The absorption of low molecular weight chondroitin sulfate was also evaluated in this study by quantifying the disaccharide content using a validated method that combined enzymatic digestion of plasma followed by fluorescence HPLC.⁷⁸ Low molecular weight chondroitin sulfate was absorbed to a higher extent compared with glucosamine, and it was also shown that its absorption may be influenced by the molecular weight of the polymer.⁷⁸

More recent work on the quantification of glucosamine in serum and synovial fluid after nasogastric or IV administration of glucosamine hydrochloride to horses questions effective absorption of glucosamine hydrochloride in the horse.⁴ Eight adult female horses with no evidence of radial carpal joint disease were studied and were randomly assigned to

two different groups ($n = 4$) for a cross-over study. Glucosamine hydrochloride (20 mg/kg) was administered by either a nasogastric intubation or IV injection. Blood samples collected before dosing and at 5, 15, 30, 60, 120, 180, 240, 360, 480, and 720 minutes post-dosing. Synovial fluids were collected 48 h before dosing in both radiocarpal joint, at 1 h after dosing in the left joint and 12 h after dosing in the right joint. This was repeated, crossing over treatment, at 7 days. Glucosamine was assayed in serum and synovial fluids by fluorescence-assisted carbohydrate electrophoresis (FACE). Analysis was performed in triplicate. The mean maximum concentration of glucosamine in the serum was $288 \pm 53 \mu\text{M}$ following IV dose and $5.8 \pm 1.7 \mu\text{M}$ following nasogastric dosing. Synovial fluid reached a peak concentration of 250 μM after IV dosing and 0.3–0.7 μM after nasogastric dosing, and glucosamine was still detectable (0.1–7 μM) in most horses in the synovial fluid at 12 h after dosing. It was concluded that the levels of glucosamine obtained in synovial fluid after nasogastric administration with clinically recommended doses are lower than those that have been studied in vitro to elucidate glucosamine action on joint cells. Further work is needed to determine its mechanism of action at these pharmacological concentrations.

In Vitro Studies

The above studies on bioavailability have been extrapolated into in vitro studies. In vitro studies do not clarify the mode of action, but help determine at what concentration glucosamine or chondroitin sulfate might inhibit the catabolic response in equine articular cartilage explants. One study was done with cartilage disks incubated with lipopolysaccharide in the presence of varying concentrations of glucosamine, chondroitin sulfate, or both.⁷⁹ Media was analyzed for nitric oxide (NO), prostaglandin E_2 , PGE_2 , and keratan sulfate. Cartilage was extracted for analysis of metalloproteinases. In all experiments, glucosamine concentrations as low as 1 mg/ml decreased NO production relative to LPS-stimulated cartilage, without GLN over a 4-day period. In general, chondroitin sulfate at either 0.25 or 0.50 mg/ml did not inhibit NO production. The addition of chondroitin sulfate to glucosamine containing media did not further inhibit NO production. On the other hand, glucosamine at concentrations as low as 0.5 mg/ml decreased PGE_2 production, whereas chondroitin sulfate (CS) did not affect PGE_2 . The combination of glucosamine and chondroitin sulfate decreased MMP-9 activity, but had no effect on MMP-2 activity. The combination tended to decrease MMP-13 protein concentrations, and decrease keratan sulfate.⁸⁰ Two other in vitro studies have been done looking at glucosamine with equine articular cartilage. In one study with articular cartilage obtained from antebrachiocarpal and middle carpal joints of horses, explant discs were treated with lipopolysaccharide or recombinant hu-

man interleukin-1 to induce cartilage degradation. Three concentrations of glucosamine (0.25, 2.5, or 25 mg/ml) were tested. The results showed that maximal NO production, proteoglycan release, and MMP activity were detected 1 day after the addition of LPS or recombinant IL-1 β to the media. The addition of 25 mg/ml glucosamine prevented the increase in NO production, proteoglycan release, and MMP activity induced by LPS, or rhIL-1.⁸⁰ In another study, the potential mechanisms of action of glucosamine inhibition of MMP expression and activity in LPS-stimulated equine chondrocytes were evaluated.⁸¹ Glucosamine had no effect on activated MMP activity, but inhibited MMP protein expression as determined by Azocollo digestion (glucosamine, 3–50 mM) and MMP-13 ELISA (glucosamine, 1.5–50 mM). Resting mRNA concentrations for MMP-1, MMP-3, and MMP-13 mRNA were significantly lower in cultures exposed to glucosamine concentrations of 50 and 20 mM than those of positive controls. It was concluded that glucosamine appeared capable of pretranslation, and possible translational, regulation of MMP expression, which suggests a potential mechanism of action for the chondroprotective effects of glucosamine.⁸¹

Dosage titrations of glucosamine hydrochloride (GU) and CS alone and in combination have been tested in vitro in our laboratory, both on normal equine cartilage explant matrix metabolism and IL-1-conditioned equine articular cartilage explant matrix metabolism.^e In normal equine articular cartilage, the explants are collected from the stifle and the treatment groups included control (no GU or CS), 12.5, 25, 125, and 250 μ g/ml of GU; 12.5, 25, 125, and 250 μ g/ml of CS, and 12.5, 25, 125, and 250 μ g/ml each of GU plus CS. Explants were labeled with ³⁵S sulfate to assess glycosaminoglycan degradation and synthesis. Total GAG content in the explants and media was also analyzed. There were no detrimental effects of GU, CS, or GU plus CS on cartilage metabolism. Higher doses of GU, CS, and GU plus CS seem to limit total GAG release into the media, whereas intermediate doses of GU, CS, and GU plus CS enhanced GAG synthesis and total cartilage GAG content.

The same dosages were then tested on IL-1-conditioned articular cartilage explants. Explants were again labeled with ³⁵S sulfate to assess glycosaminoglycan degradation and synthesis and the total GAG content in the explants and media was analyzed. There were no treatment effects for GU or CS alone, but a protective effect of high dosages of GU plus CS was found for total GAG release into the media. The study suggested the GU plus CS might be beneficial to cartilage metabolism by preventing GAG degradation. However, the question of effective concentration of GU after oral administration is still an issue.^f

Other studies done on bovine cartilage evaluating the response of cartilage to simulated conditions of in vivo stress show variable response.⁸² The met-

abolic response of cartilage from aged animals under simulated conditions of in vivo stress was greater than that seen in non-stressed or young tissue. It was suggested that by enhancing the “protective” metabolic response of chondrocytes to stress, glucosamine and chondroitin sulfate might improve its ability for repair and regeneration.

In Vivo Studies

In vivo studies have been done in a number of species, including humans. While a number of studies have reported positive results in humans, a recent meta-analysis published in *JAMA* showed that studies in knee OA are generally of low to mediocre quality, are sponsored largely by manufacturers, and show strong evidence of publication bias.⁸³ Glucosamine trials in the hip have been virtually non-existent during the past 30 years. The first large scale, independent, randomized trial of glucosamine for knee OA—a multi-center study of 1000 subjects, sponsored by the NIH, commenced in 2001. A new randomized, double-blind study suggested that symptoms of knee OA in patients who take glucosamine sulfate can actually worsen over time. In this trial, persistent worsening of symptoms was just as common in patients receiving glucosamine as in their counterparts who received placebo.

Two clinical trials evaluating glucosamine hydrochloride and chondroitin sulfate have been conducted in horses: one with degenerative joint disease and the other with navicular disease.^{84,85} The study on degenerative joint disease involved 25 cases, but was not blinded or controlled, and improvement was seen within 2 wk, and DJD was confirmed by physical examination, diagnostic intra-articular anesthesia, and radiographs or fluoroscopy of the distal interphalangeal, metacarpophalangeal, tarsal-metatarsal, or carpal joints (quite a variety). Horses weighing <545 kg were given 9 g of the glucosamine-chondroitin sulfate compound twice daily for 6 wk. Horses weighing >540 kg were given 12 g twice daily for 6 wk. Each 3 g included 1800 mg of glucosamine hydrochloride, 600 mg purified chondroitin sulfate, 16 mg of manganese, and 104 mg of ascorbate. Within 2 wk of the start of administration of the compound, the lameness grade, flexion test, and stride length were significantly improved. There was a further significant improvement in lameness at 4 wk, whereas flexion score and stride length did not show further improvement.⁸⁴

In the navicular study, 14 horses with a progressive forelimb lameness of 3- to 12-mo duration diagnosed as navicular syndrome were selected. Horses were randomly allocated to treatment with the nutraceuticals or placebo. There were eight in the test group and six in the control group. Lameness was assessed by an algofunctional lameness index, comprising a combined sum score of standing posture, hoof tester examination, and lameness

scores at various levels of work. Overall clinical efficacy was rated on a visual analog scale. Owners assessed lameness through a pre-assigned questionnaire incorporating an algofunctional lameness index and overall clinical condition at weekly intervals. The median algofunctional lameness index and overall clinical condition scores were improved for horses treated with the nutraceuticals compared with placebo in horses. The degree of improvement in algofunctional lameness index assigned by owners after 8 wk was also improved between the treatment groups. Radiographic scores were not sufficiently different.⁸⁵

In Vivo Experimental Studies

Two studies have been formed in horses using complete Freund's adjuvant to induce arthritis.^{86,87} The first study found that Cosequin did not result in any clinically detectable benefits. Treatment began 10 days before model induction and continued until 26 days after model induction. The parameters of efficacy included lameness score, stride length, carpal circumference, maximum carpal flexion, and synovial fluid protein. In this study, there were two groups of six horses, which limited statistical power, a short evaluation period, lack of blinded observers, and a model that is controversial. All of these factors weaken the strength of the conclusions. In the second study, PO and IM chondroitin sulfate was compared, and both had a significant treatment effect. Here there were no negative controls and similar caveats to the previous study.⁸⁷

Glucosamine hydrochloride has been evaluated using young horses in training programs to assess its effect on serum markers of bone and joint metabolism. While both studies used the labeled dose for Cosequin for 8 wk in Quarter horses and 4–8 wk in Standardbreds, neither found the treatment significantly altered serum concentration of markers.^{88,89} However, it should be pointed out that other studies with FDA-licensed medication using markers have not shown an ability for biomarkers to differentiate treatment effects.⁹

A double-blinded study on the effects of another oral supplement with tarsal degenerative joint disease has been reported.⁹⁰ The study was done in eight riding horses. All horses were mildly lame (grade I or II) in one or more limbs, and all were diagnosed as having OA of the distal intertarsal and/or tarsal-metatarsal joints in one or both hind limbs on the basis of physical examination, diagnostic anesthesia, and radiography. Horses were admitted into the study in pairs. Horses received the first treatment orally for 2 wk, followed by 2 wk without treatment, and then the alternative treatment was administered orally for 2 wk. During each treatment period, the horses received a loading dose of 60 ml/day for 5 days, followed by a maintenance dose of 30 ml/day for 9 days. Gait analysis was performed. It was concluded that compared

with the placebo treatment with the active solution resulted in significant increases in left-right symmetry of peak vertical ground reaction force, vertical impulse, tarsal joint range of motion, and tarsal joint energy generation during stance. The product used was Corta-Flex (Nature's Own Inc., Aiken, SC 29801). Although the investigators of this study are very experienced in gait analysis, the technique has still not achieved general acceptance in lameness assessment; of concern is that, because of the low numbers, the strength of statistical power in this study is questionable.

Summary

This paper reviewed what has been scientifically shown with licensed medications, while hopefully also pointing out how to understand what a true generic medication is. The equine practitioner is potentially compromised legally in using certain medications that have been purported to be generics, and there are definite scientific mistruths with the way some of these products are represented. Truth in advertising is definitely compromised in many instances of nutraceuticals marketing, and there are numerous examples of insinuations clearly intended to mislead, but staying on the side of legality. It is to be emphasized that when equine veterinarians use licensed medications, the patient gets the best care in that an accurate diagnosis is made. It is an unfortunate reality that many instances of lameness and joint disease are presented after client-prescribed periods of oral nutraceuticals have failed to yield results.

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