A corticosteroid, usually MPA or TA, is commonly administered in the DIPJ to resolve lameness associated with navicular disease; resolution of lameness depends on diffusion of the corticosteroid from the DIPJ into the navicular bursa. Alternatively, the corticosteroid can be administered directly into the navicular bursa, but centesis of this bursa is technically more difficult. The speed at which MPA or TA diffuses between the navicular bursa and DIPJ and the maximum concentration of the corticosteroid capable of diffusing between these synovial structures are not known.

Determining the magnitude of diffusion of commonly used corticosteroids from the DIPJ to the navicular bursa as a treatment for horses that are lame because of disease of the navicular apparatus would be useful because centesis of the navicular bursa is technically more difficult than centesis of the DIPJ. To select the most appropriate site of injection of a corticosteroid to treat horses with disease involving both the navicular bursa and the DIPJ, clinicians should be aware of the volume and direction of diffusion of the corticosteroid between these 2 synovial structures. Studies undertaken to determine whether the DIPJ and navicular bursa communicate have revealed no consistent evidence of anatomic communication. No communication was found in a latex injection dissection study, and no communication was found in several contrast arthrography between the navicular bursa and DIPJ.

### Objective
To determine whether clinically effective concentrations of methylprednisolone or triamcinolone can be achieved in the navicular bursa after injection of methylprednisolone acetate (MPA) or triamcinolone acetonide (TA) into the distal interphalangeal joint (DIPJ) and whether clinically effective concentrations of these drugs can be achieved in the DIPJ after injecting the navicular bursa with the same doses of MPA or TA.

### Animals
32 healthy horses.

### Procedures
Horses in groups 1 through 4 received 40 mg of MPA in the DIPJ, 10 mg of TA in the DIPJ, 40 mg of MPA in the navicular bursa, and 10 mg of TA in the navicular bursa, respectively. Concentrations of corticosteroids that diffused into the adjacent synovial structure were determined.

### Results
For group 1, injection of MPA into the DIPJ yielded a mean ± SD concentration of 0.24 ± 0.072 µg of methylprednisolone/mL in the navicular bursa. For group 2, injection of TA into the DIPJ yielded 0.124 ± 0.075 µg of triamcinolone/mL in the navicular bursa. For group 3, injection of MPA into the navicular bursa yielded 0.05 ± 0.012 µg of methylprednisolone/mL in the DIPJ. For group 4, injection of TA into the navicular bursa yielded 0.091 ± 0.026 µg of triamcinolone/mL in the DIPJ.

### Conclusions and Clinical Relevance
A clinically effective concentration of methylprednisolone or triamcinolone diffused between the DIPJ and navicular bursa after intra-articular or intrabursal injection, which would justify injection of the DIPJ with MPA or TA to ameliorate inflammation of the navicular bursa. (Am J Vet Res 2008;69:611–616)

### Abbreviations
<table>
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<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>MPA</td>
<td>Methylprednisolone acetate</td>
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<tr>
<td>TA</td>
<td>Triamcinolone acetonide</td>
</tr>
<tr>
<td>DIPJ</td>
<td>Distal interphalangeal joint</td>
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<td>HPLC</td>
<td>High-performance liquid chromatography</td>
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In 1 study in equine cadavers, Evans blue dye injected into the DIPJ diffused into the navicular bursa in only 1 of 122 feet. In a study in which a combination of luxol fast blue dye and mepivacaine hydrochloride was injected into the DIPJ or navicular bursa, dye diffused from the DIPJ to the navicular bursa in 65% of the feet, but dye diffused from the navicular bursa to the DIPJ in only 12.5% of the feet. Mepivacaine concentrations were not measured in that study. A similar study in which dyes of molecular weight lower than that of luxol blue were used revealed time-dependent diffusion of dye from the DIPJ to the navicular bursa, but no direct anatomic communication was detected. An in vitro study in which diffusion of mepivacaine hydrochloride from the DIPJ to the navicular bursa and from the navicular bursa to the DIPJ was investigated revealed more diffusion of mepivacaine from the DIPJ to the navicular bursa than from the navicular bursa to the DIPJ. In an in vivo study, analogs of the DIPJ induced by injection of mepivacaine hydrochloride alleviated lameness associated with amphotericin B–induced navicular bursitis in all 6 horses within 5 minutes. Postmortem examination revealed that amphotericin B failed to diffuse from the navicular bursa into the DIPJ. These studies confirm that physical characteristics, including the molecular weight, of the drug injected and time play important roles in diffusion of compounds between the DIPJ and navicular bursa.

Radiographic contrast agents have a high molecular weight and high water solubility, and both of these factors reduce the rate of diffusion across a synovial membrane. Although the molecular weights of luxol fast blue dye and Evans blue dye are comparable to that of mepivacaine, mepivacaine diffuses more readily across the synovial membranes of the navicular bursa and DIPJ than do those 2 dyes, possibly because the hydrophilic and lipophilic ends of the mepivacaine molecule increase its ability to diffuse across synovial membranes. Mepivacaine is partially lipid soluble and has a molecular weight of 246.34 daltons. The similarly low molecular weights of triamcinolone (394.45 daltons) and methylprednisolone (374.46 daltons) may also enable those agents to diffuse across synovial membranes.

The degree to which a corticosteroid can diffuse from the navicular bursa into the DIPJ was examined in a clinical report in which the results of treating horses with lameness attributable to navicular disease by administration of MPA or TA into the navicular bursa were compared with the results of treating similarly affected horses by administration of the same drugs into the DIPJ. Of the horses that had resolution of lameness for 2 or more months, 60% had received the corticosteroid in the navicular bursa, whereas only 34% had received the corticosteroid in the DIPJ.

The duration of action of corticosteroids depends on many variables, including total dose and rate of hydrolysis. Whether the differences detected in degree and duration of clinical effect between sites of injection (ie, DIPJ or navicular bursa) are dose-related events or are caused by incomplete diffusion of the corticosteroid between the navicular bursa and DIPJ is not clear. We hypothesized that routinely used doses of MPA and TA instilled into the DIPJ would diffuse into the navicular bursa and achieve clinically effective minimum concentrations in the bursa and that these same doses of corticosteroids instilled into the navicular bursa would diffuse into the DIPJ and achieve clinically effective minimum concentrations in the joint.

Materials and Methods

Animals—Horses used in the study (n = 32) were adult mares from the University of Tennessee teaching herd. Most mares were American Quarter Horses or Tennessee Walking Horses. All horses were free from lameness in the distal portions of the forelimbs as determined by a lameness examination. The project was approved by the University of Tennessee Institutional Animal Care and Use Committee.

Procedures and collection of samples—Horses were allocated to 4 treatment groups on the basis of the drug injected and site of administration. Horses in group 1 received 40 mg of MPA in the DIPJ, horses in group 2 received 10 mg of TA in the DIPJ, horses in group 3 received 40 mg of MPA in the navicular bursa, and horses in group 4 received 10 mg of TA in the navicular bursa. One forelimb of each horse in each group was injected.

The trial was performed in 2 phases. Phase 1 was designed to establish the time of maximal diffusion of MPA and its active metabolite, methylprednisolone, or TA and its active metabolite, triamcinolone, from the DIPJ to the navicular bursa or from the navicular bursa to the DIPJ. Time of maximal diffusion for each treatment group was established by determining the concentration of the corticosteroid and its active metabolite in synovial fluid obtained from 6 pairs of horses (total, 12 horses/group) when samples of synovial fluid were collected 1, 2, 3, 6, 9, or 12 hours after injection. In phase 2, synovial fluid was obtained at the time of maximal diffusion from 6 additional horses for each treatment group so that, in total, 8 samples of synovial fluid/treatment group were obtained at the time of maximal diffusion. During both phases, when a synovial structure (DIPJ or navicular bursa) was injected with MPA or TA, only the synovial structure into which the drug diffused was sampled (navicular bursa or DIPJ, respectively). Because of the large number of synovial fluid samples, some horses were members of 2 treatment groups in phase 1. The contralateral limb was used in those horses, and at least 3 weeks were allowed to elapse before the contralateral forelimb was used. The allocation of the forelimb that was used first was randomized. Some horses from phase 1 were used again in phase 2. A period of 4 months elapsed prior to reuse of horses in phase 2, and the horses were allocated to a different treatment group.

For each injection, the horse was sedated with detomidine hydrochloride (5 to 10 µg/kg, IV), and a twitch was applied to the nose for restraint. The foot of 1 forelimb was desensitized by anesthetizing the palmar digital nerves at the level of the proximal sesamoid bones with mepivacaine hydrochloride; this was intended to prevent the horse from moving during centesis of the navicular...
The concentration of methylprednisolone or triamcinolone in synovial fluid was determined via HPLC. The HPLC solvent was 16% isopropanol-water solution and 1 mL of radiographic contrast solution was combined to achieve a total of 3 mL, and a lateromedial radiographic view of the foot obtained immediately after injection of the navicular bursa was examined to verify that only the navicular bursa had been injected. Detection of contrast solution in only the navicular bursa was interpreted as evidence of successful bursal injection. Inadvertent injection into the DIPJ caused the horse to be eliminated from the trial and replaced by another.

To obtain synovial fluid from the navicular bursa, 3 mL of physiologic saline solution was injected into the navicular bursa and immediately aspirated. After aspiration of the physiologic saline solution, 3 mL of a radiocontrast solution was administered through the needle, and a lateromedial radiographic projection of the foot obtained immediately after sample collection was examined to verify that the navicular bursa was the synovial structure from which the sample was collected. Detection of contrast solution in only the navicular bursa was interpreted as evidence of successful bursal centesis. Inadvertent centesis of the DIPJ, as determined by radiographic examination, caused the horse to be eliminated from the trial and replaced by another and that synovial fluid sample to be eliminated from analysis. To obtain synovial fluid from the DIPJ, 3 to 5 mL of physiologic saline solution was injected into the DIPJ and aspirated immediately after infusion through the same needle used to administer it. To inject the DIPJ, 2 mL of physiologic saline solution was combined with the MPA or TA to achieve a total of 3 mL. It was determined that needles were within the dorsal pouch of the DIPJ when synovial fluid was observed in the needle hub, low resistance to injection was confirmed, and back pressure in the syringe was detected. A blood sample was obtained from every horse for analysis of serum urea concentration at the time the physiologic saline solution was collected by synoviocentesis.

Each synovial sample was divided into 2 aliquots, which were stored at –80°C for later analysis. Serum obtained from the blood samples was also stored at –80°C.

**HPLC analysis**—Concentration of the parent corticosteroid (MPA or TA) and its active metabolite (methylprednisolone or triamcinolone) in synovial fluid was determined via HPLC. The column temperature was maintained at 45°C. The HPLC solvent was 16% isopropanol in HPLC-grade water, to which 0.1% trifluoroacetic acid solution had been added. Analytic separations were performed under isocratic conditions with the HPLC solvent flow rate fixed at 1 mL/min.

Solid-phase extraction cartridges were used to extract corticosteroids from synovial fluid samples. To extract a corticosteroid, cartridges were washed with 1 mL of methanol followed by washing with 1 mL of water. One milliliter of synovial fluid was applied and drained through the cartridge; the cartridge was again washed with 1 mL of 5% methanol in water, and corticosteroids were eluted with 1 mL of methanol. The methanol was evaporated in a heating block at 45°C by use of a stream of nitrogen gas. The extracted corticosteroids were redissolved in 0.1 mL of isopropanol-water solution (1:1 ratio) and placed in injection vials. Vials were placed in the autosampler and maintained at 4°C, and injections (20 µL) were performed automatically. Duration of each HPLC assay was 80 minutes, with this long duration ensuring that all analytes of interest were eluted from the column.

**Data analysis**—Dilution error, caused by injecting physiologic saline solution into the DIPJ or navicular bursa prior to aspiration, was corrected by use of a correction factor for urea–synovial fluid dilution. This factor was the ratio of the urea concentration in the serum to the urea concentration in the synovial fluid. The ratio of serum urea to synovial urea is a validated correction factor for dilution of synovial fluid caused by the sampling technique. Urea concentration of serum and synovial fluid was obtained with a urea ELISA. To determine urea concentration in the serum samples, 5 µL of serum was assayed in accordance with the manufacturer’s instructions, but for the diluted synovial fluid samples, 20 µL was assayed to ensure that a measurable amount of urea was detected. To correct for that increase in dose, the concentration of urea in diluted synovial fluid was divided by 4. Results of the HPLC analysis were multiplied by the dilution correction factor to obtain the true concentration of the corticosteroid in the synovial fluid.

Diffusion quantity and diffusion quantity standardized to a milligram-per-milligram basis of corticosteroid injected were analyzed via commercially available software with values of $P \leq 0.05$ considered significant. Within the linear model, both product (methylprednisolone vs triamcinolone) and site of injection (navicular bursa vs DIPJ) were treated as fixed factors.

**Results**

**Phase 1**—A peak diffusion time of 3 hours after injection for all groups was determined on the basis of visual assessment of diffusion curves (Figure 1).

**Phase 2**—The concentration of methylprednisolone and triamcinolone that had diffused from the DIPJ to the navicular bursa or from the navicular bursa to the DIPJ at 3 hours varied among horses. For group 1, injection of 40 mg of MPA into the DIPJ resulted in a mean ± SD concentration of $0.240 ± 0.072$ µg of methylprednisolone/mL in the navicular bursa (Figure 2). For group 2, injection of 10 mg of TA into the DIPJ resulted in $0.124 ± 0.075$ µg of triamcinolone/mL in the navicular bursa. For group 3, injection of 40 mg of MPA into the navicular bursa resulted in $0.050 ± 0.012$ µg of methylprednisolone/mL in the DIPJ. For group 4, injection of 10 mg of TA into the navicular bursa resulted in $0.091 ± 0.026$ µg of triamcinolone/mL in the DIPJ. Methylprednisolone acetate and TA did not diffuse readily and were excluded from further analysis. Prod-
uct injected (MPA vs TA) had no significant effect on mean rate of diffusion of the corticosteroid metabolite from one synovial structure to the other. However, a site effect was detected, with significantly (P = 0.05) greater diffusion of corticosteroid metabolite when the DIPJ was injected. Within the model, there was an interaction effect of site and product injected, which was primarily caused by the difference in diffusion of MPA from the DIPJ into the navicular bursa, compared with diffusion of MPA from the navicular bursa into the DIPJ, respectively (0.050 ± 0.012 µg/mL vs 0.246 ± 0.074 µg/mL; P = 0.017; Figure 2).

To compare approximate diffusion of methylprednisolone and triamcinolone on a milligram-per-milligram basis, results of methylprednisolone diffusion were standardized by dividing the raw methylprednisolone diffusion by 4 (Figure 3). When not taking the site of injection into consideration, triamcinolone diffusion values were greater than those for methylprednisolone on a milligram-per-milligram basis, although the difference was not significant (0.11 ± 0.039 µg/mL vs 0.037 ± 0.009 µg/mL; P = 0.09).

Comparison of the interaction between direction of diffusion and product injected revealed that the difference in diffusion of triamcinolone and methylprednisolone from the DIPJ was not significantly different, but that triamcinolone diffused 9 times as readily from the navicular bursa as did methylprednisolone on a milligram-per-milligram basis (mean ± SD concentrations, 0.0918 ± 0.026 µg/mL and 0.0124 ± 0.07 µg/mL for triamcinolone and methylprednisolone, respectively; P = 0.007; Figure 3).

Discussion

Measurable quantities of methylprednisolone and triamcinolone diffused from the DIPJ to the navicular bursa and from the navicular bursa to the DIPJ. On the basis of data indicating that clinically effective mini-

Figure 1—Time-mass diffusion graphs of MPA (black diamonds) and methylprednisolone (white diamonds) in the navicular bursa after injection of 40 mg of MPA into the DIPJ of 12 horses (A), TA (black squares) and triamcinolone (white squares) in the navicular bursa after injection of 10 mg of TA into the DIPJ of 12 horses (B), MPA (black diamonds) and methylprednisolone (white diamonds) in the DIPJ after injection of 40 mg of MPA into the navicular bursa of 12 horses (C), and TA (black squares) and triamcinolone (white squares) in the DIPJ after injection of 10 mg of TA into the navicular bursa of 12 horses (D). The MPA and TA were injected at time 0. Samples were collected from 2 horses at each sampling time (ie, 6 pairs of horses; 1 pair each for 1, 2, 3, 6, 9, and 12 hours after injection, respectively). Notice that the y-axis scale differs among the portions of the figure.

Figure 2—Mean ± SE concentrations of methylprednisolone (MP) and triamcinolone (TAC) in synovial fluid of the DIPJ or navicular bursa (NB) 3 hours after injection of 40 mg of MPA or 10 mg of TA into the DIPJ or NB in 8 horses. a,bValues with different letters differ significantly (P ≤ 0.05).
that the cytoplasm of target cells largely determine the duration of action of a corticosteroid. However, the rate of diffusion into the navicular bursa or DIPJ, respectively, may vary because of variability in the volume of synovial fluid of the DIPJ or NB (corrected on the basis of the quantity of MPA or TA injected). The rates of hydrolysis by synovial tissue enzymes of MPA and TA are hydrolyzed into an active ester form. The rate of hydrolysis and onset of action depend on the site of administration. Methylprednisolone acetate, which is slowly hydrolyzed into an ester when administered IM, is rapidly hydrolyzed in synovial fluid. The inconsistent diffusion of MPA and TA may be attributable to interindividual differences in hydrolysis.

In 1 study, administration of 40 mg of MPA into the tarsocural joint of horses resulted in an undetectable concentration of methylprednisolone in the joint by 4 to 39 days. In a similar study, administration of 6 mg of TA into clinically normal tarsocural and intercarpal joints of horses resulted in a high concentration of triamcinolone in the joints for 4 days, but the concentration was low by 14 days. The high variability of concentration of the metabolite of MPA or TA in the tarsocural and intercarpal joints of horses in those studies is consistent with the high variability of the concentration of these metabolites in the navicular bursa and DIPJs of horses in the study reported here.

The sampling times used in phase 1 were chosen on the basis of the number of horses in another study in which investigators measured methylprednisolone concentration in the centrodistal joint after injecting 80 mg of MPA into the tarsometatarsal joint of 8 horses. The sample size of 8 horses/sample group was chosen on the basis of use of that number of horses in another study in which investigators examined diffusion of methylprednisolone from the tarsometatarsal joint to the centrodistal joint in horses. The highest concentration of methylprednisolone in the centrodistal joint was reached 6 hours after 80 mg of MPA was administered into the tarsometatarsal joint (sampling times were 0.5, 1, 3, 6, 9, and 12 hours after injection). We chose to sample 6 pairs of horses at various time intervals once each, rather than repeated sampling of 2 horses 6 times. Variation caused by the sampling method during collection of multiple samples from a single joint would have substantially distorted the results. Centesis of the navicular bursa may not be
innocuous, and collection of multiple samples of this synovial structure was inevitable from an animal welfare perspective. However, this precluded statistical comparison of the sampling times, and the choice of 3 hours was selected on the basis of visual analysis of the diffusion curves.

In a study\(^7\) in which 1 intercarpal joint received MPA, MPA and methylprednisolone were not found in the synovial fluid at any time in the contralateral (noninjected) joint, although low (< 10 ng/mL) concentrations of MPA and methylprednisolone were found in plasma. Those results led our group to believe that use of the DIPJ and navicular bursa of both forelimbs of the same horse in our protocol was justified. Allowing a period of at least 3 weeks to elapse before use of the contralateral joint or bursa ensured that administration of a corticosteroid into a synovial structure on 1 limb would not affect the concentration of a corticosteroid administered into the contralateral synovial structure. We cannot explain the reason that there was a 9-fold difference in magnitude of diffusion between triamcinolone and methylprednisolone from the navicular bursa but no difference in the diffusion of these 2 products from the DIPJ.

Results of the study reported here suggested that administration of MPA or TA into the DIPJ for treatment of horses with inflammation of the navicular apparatus can be effective. Results also suggested that the clinical experience of longer-acting effects of intrabursal injection, compared with the duration of effects after DIPJ injection, even of TA, may be more attributable to the high concentration of a corticosteroid administered into the navicular bursa than a difference in direction of diffusion.

References


b. Breeze high-performance liquid chromatography system with column heater, 717 Plus autosampler, and 2487 UV and visible absorbance detector set at 234 nm, which was interfaced with Waters’ Satin Bus and Breeze Analytical Software, Waters Corp, Milford, Mass.

c. Zorbax SB-C18 StableBond column, 4.6 X 150 mm, (5 µm) Agilent Technologies Inc, Santa Clara, Calif.

d. Oasis HLB extraction cartridges, 1 mL, Waters Corp, Milford, Mass.

e. DIUR-01K quinichrom urea assay kit, BioAssay Systems, Hayward, Calif.

f. SAS Institute Inc, Cary, NC.