Acquired corneal disease, including infectious ulcerative keratitis, stromal abscess, eosinophilic keratitis, and traumatic keratitis, is common in horses. A horse's environment may contribute to the development of corneal disease through exposure of the conjunctiva and cornea of each eye to a transitory population of bacteria and fungi. The composition of the microbial population affecting the eyes likely depends on season, geographic location, and ocular immune status. Because of the positions of the globes and corneas in the heads of horses, those structures are vulnerable to trauma or infection with microorganisms, which may also play a role in the development of corneal disease.

Keratitis in horses can be associated with epiphora, conjunctival irritation or chemosis, blepharospasm, uveitis, and other signs indicative of considerable discomfort. Ocular pain may be chronic because of the slow healing of the abnormal cornea. The superficial aspect of the cornea has dense sensory innervations; therefore, any defect in the corneal epithelium, regardless of size, will result in marked signs of ocular pain.

Ocular pain may be alleviated by systemic administration of drugs such as NSAIDs that also help control ocular inflammation. This class of drug, however, can have undesirable effects in horses, including renal and gastrointestinal tract toxicoses.

Other proposed methods of reducing the discomfort associated with ulcerative keratitis include topical administration of ophthalmic anesthetic and analgesic agents. Topical treatments with these preparations offer the benefit of minimal systemic adverse effects, especially in large animals in which the dose is insignificant, compared with body weight. Topical ophthalmic anesthetics such as 0.5% proparacaine hydrochloride solution are effective in providing short-term corneal anesthesia (determined via Cochet-Bonnet aesthesiometry) in dogs and cats, with a comparatively shorter duration of effect in cats. In horses, 0.2 mL (approx 4 drops) of 0.5% proparacaine induces corneal anesthesia, although not to the degree achieved by proparacaine treatment in dogs and cats. Topical ophthalmic anesthetics are appropriate for short-term use in diagnostic procedures.
such as tonometry and in therapeutic procedures such as corneal debridement. However, they are not a viable long-term treatment for ocular pain because they are known to delay healing and have corneal epitheliotoxic effects (eg, development of deep infiltrates, ulceration, and possible perforation) with chronic use.11–14

Topical administration of NSAIDs has been used to treat pain and inflammation associated with keratitis in humans and other species. However, toxic effects on corneal epithelial cells and prolonged increased healing time are associated with use of these drugs in humans and dogs.15–19 In a study17 of the effects of anti-inflammatory drugs on canine epithelial cells in vitro, suprofen had a concentration-dependent effect on both cell morphology and migration. In a study20 of corneal complications associated with topical ocular use of the NSAIDs ketorolac tromethamine and diclofenac sodium in 16 humans, 2 developed severe keratopathy, 3 developed ulceration, 6 developed corneal or scleral melts, and 5 developed perforations. Corneal melting was associated with topical application of diclofenac in 5 humans following ocular surgery in another study19; the authors of that report recommended cautious use of topical NSAID treatments following ocular surgery because of the potential for corneal melting.

To reduce pain associated with corneal ulceration and avoid the potential delayed wound healing and corneal melting associated with topical ocular NSAID use in dogs, topical treatment with opioid agonist-antagonists such as morphine sulfate and nalbuphine has been suggested. It is known that μ and δ opioid receptors are present in canine corneas.20 In 1 study,20 topical administration of 1% morphine sulfate solution was effective in providing analgesia to dogs with experimentally induced corneal ulcers and treatment with the topical solution did not interfere with corneal epithelial healing. Furthermore, although morphine can have adverse systemic effects in dogs,21 no such adverse effects developed in dogs treated with the 1% morphine sulfate ophthalmic solution, presumably because low doses were used in the eyes.20 The major drawback of topical treatments with morphine in a clinical setting is likely the tight control necessary for Schedule II drugs, of which morphine is one. As an alternative to morphine, 1% nalbuphine solution has been shown to decrease corneal sensitivity, as determined by assessment of CTT, 30 minutes after administration in healthy dogs’ eyes.22 The purpose of the study reported here was to test the effect of treatment with a topical ophthalmic preparation of 1% nalbuphine on corneal sensitivity in clinically normal horses.

Materials and Methods

Animals—Eight clinically normal adult Thoroughbreds from the University of Pennsylvania New Bolton Center research and teaching herds were used in the study. The study group was comprised of 7 geldings and 1 mare, which ranged in age from 4 to 9 years (mean age, 6.25 years). All horses were considered healthy on the basis of physical examination findings and free of corneal or adnexal disease bilaterally on the basis of results of slit-lamp biomicroscopy, assessment of corneal integrity (absence of corneal retention of fluorescein), and Schirmer tear testing (values > 10 mm/min23).

Study procedures—As an estimate of corneal sensitivity, CTT values were measured by use of a Cochet-Bonnet aesthesiometer; all assessments were conducted without the use of sedation or application of auriculopalpebral nerve blocks. Baseline CTT was measured for both eyes of each horse. Immediately thereafter, 0.2 mL of 1% nalbuphine solution2 was installed in 1 randomly selected eye of each horse and 0.2 mL of artificial tears solution1 was installed in the contralateral eye (control treatment). For all 8 horses, CTT of each eye was measured within 1 minute following nalbuphine or artificial tears administration and every 15 minutes thereafter for 60 minutes. For 5 of the 8 horses, CTT was also measured in both eyes at 120 minutes. On completion of CTT measurements, each eye was examined by use of a slit-lamp biomicroscope, stained with fluorescein, and observed with a cobalt blue filter to ensure that no corneal epithelial defects had been created by the Cochet-Bonnet aesthesiometer filament. The protocol used for this study was approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Measurement of corneal sensitivity—Values of CTT were measured by use of a Cochet-Bonnet aesthesiometer. The aesthesiometer had a 0.12-mm-diameter nylon filament that was adjustable in length from 5 to 60 mm. For each eye of each horse, the filament was directly applied to the central portion of the cornea to determine sensitivity. The CTT was defined as the length of the filament (in mm) that elicited a blink in 3 consecutive assessments. The filament was initially applied at maximum length (60 mm); if a consistent blink was not elicited, the filament length was decreased in 5-mm increments and retested until a consistent blink was elicited. For each eye at each time point, filament length (in mm) that elicited a blink in 3 consecutive assessments was recorded as the CTT value. Filament length was inversely related to the amount of pressure applied to the corneal surface (ie, the longer the filament, the less pressure exerted24,25) and was directly related to corneal sensitivity in that the longer the filament length required to elicit a blink, the more sensitive the cornea was. By defining CTT as the filament length (in mm) that elicits a blink, a decrease in CTT would be associated with a decrease in corneal sensitivity and an increase in CTT would be associated with an increase in corneal sensitivity. Thus, lower values of CTT are indicative of less sensitive corneas. Alternatively, aesthesiometer readings (in mm) can be converted to applied force measurements (g/mm² or mg/S, where S = 0.0113 mm² of sectional area of the filament) to derive the CTT; however, by use of that method, lower CTT values are indicative of more sensitive corneas. For the purposes of this study, the former method was used (without converting readings to applied force measurements) so that the relationship between CTT and corneal sensitivity would be more intuitive.

Measurement of conjunctival hyperemia and ocular tearing—At all time points including baseline, the extent of conjunctival hyperemia and ocular tearing were graded independently by a single observer (MEU) for each eye of each horse. The subjective assessments...
for each variable were made by use of a scale from 0 to 3, where 0 = none, 1 = mild, 2 = moderate, and 3 = severe.

**Treatments**—From a bulk supply of powdered nalbuphine, the 1% nalbuphine solution was prepared in an aqueous solution with a pH of 6.00 and a clear color. The commercially available formulation of nalbuphine is for administration via injection (1% [10 mg/mL] nalbuphine hydrochloride) and has a pH of 3.7; it is not appropriate for ocular use. Attempts to sufficiently alter the pH of the injectable formulation would have resulted in excessive dilution and were not therefore feasible. The prepared solution was given a 6-month shelf life based on standard FDA guidelines for compounded medications. The light-sensitive solution was protected from light and was known to turn yellow as pH increased, indicating that some of the drug was no longer in the solution. All nalbuphine solution was used within 1 month of delivery from the compounding pharmacy.

Each horse was randomly assigned to receive treatment of the left or right eye with 1% nalbuphine solution and treatment of the contralateral eye with artificial tears solution (control treatment). Following baseline CTT measurement in each eye, 0.2 mL of 1% nalbuphine solution was applied to the designated treated eye; by use of a 1-mL syringe with a hub of a 25-gauge needle attached, the solution was administered as a stream into the eye. The same volume of artificial tears solution was applied to the control eye in the same manner. For each eye in all 8 horses, a CTT value was obtained at 1, 15, 30, 45, and 60 minutes following nalbuphine or artificial tears administration. For each eye in 5 randomly selected horses, a CTT value was also obtained at 120 minutes following nalbuphine or artificial tears administration.

**Statistical analysis**—Values of CTT for nalbuphine-treated and control eyes in all 8 horses were recorded before and at 1, 15, 30, 45, and 60 minutes following nalbuphine or artificial tears administration. Measurements of CTT were obtained from the nalbuphine-treated and control eyes at the 120-minute time point. The CTT data were analyzed by use of mixed-model ANOVA for main effects of the nalbuphine-treated eye (right or left, which varied between horses) and measurement time point (ie, baseline, 1, 15, 30, 45, and 60 minutes, which varied within horses) and to assess differences between baseline CTT values and the CTT values measured at each subsequent time point. Analysis was performed by use of computer software with \( \alpha \) set at 0.05. A value of \( P < 0.05 \) was considered significant.

**Results**

Values of CTT were obtained for the 8 nalbuphine-treated eyes and 8 control eyes in the 8 study horses before and at 1, 15, 30, 45, and 60 minutes after solution administrations. Values of CTT were also obtained for 5 nalbuphine-treated eyes and 5 control eyes in 5 of the 8 study horses at the 120-minute time point. All values were collected successfully for all horses at all time points. Corneal touch threshold was not significantly different between nalbuphine-treated and control eyes at any time point. Mean ± SD of CTT for nalbuphine-treated eyes and control eyes was 38.8 ± 2.39 mm and 37.9 ± 3.14 mm, respectively (\( P > 0.10 \); Figure 1). There was a significant (\( P < 0.01 \)) main effect of measurement time point, but no significant interaction between treatment (nalbuphine or artificial tears solution) and measurement time point. Comparisons of baseline CTT and each subsequent measurement time point revealed no difference (\( P = 0.648 \)) between baseline readings and the reading taken 1 minute after treatment. Compared with baseline value, CTT was significantly (\( P = 0.04 \)) decreased at 15 minutes, marginally decreased (but not significantly [\( P = 0.07 \)]) at 30 minutes, and significantly decreased at 45, 60, and 120 minutes (\( P = 0.01, P = 0.01, \) and \( P = 0.04 \), respectively; Figure 2). Although readings were lower than baseline, and thus corneal sensitivity was presumably decreased
from that at baseline, at all time points except the 30-
minute time point, there was no treatment effect and no
interaction between treatment and time, suggesting this
was purely a result of repeated testing. Neither the main
effect of the treated eye (right or left) nor any interactions
involving the treated eye were significant ($P > 0.10$).

At baseline, there was no conjunctival hyperemia
and no ocular tearing evident in any of the horses’ eyes.
For all horses at all time points following administration
of nalbuphine or artificial tears solution, there was
no conjunctival hyperemia and no ocular tearing. All
scores for all eyes were 0, thereby precluding statistical
analysis of the data. None of the horses developed cor-
neal epithelial damage as a consequence of CTI testing,
as determined by results of fluorescein staining and slit-
lamp biomicroscopy.

**Discussion**

Options for the treatment of corneal pain in horses
are limited, as well as for treatment of other species in
which corneal pain and wound healing have been stud-
iied, including humans, dogs, and rabbits.21–23 Topical
administration of opioids has been suggested for use
in reducing signs of pain associated with corneal ul-
ceration. In dogs with experimentally induced corneal
epithelial and anterior stromal ulcerations, topical ad-
ministration of 1% morphine sulfate solution reduced
blepharospasm and decreased Cochet-Bonnet aesthe-
siometer readings (which corresponded to decreasing
corneal sensitivity).20 Furthermore, results of that study
also indicated that topical treatment with morphine solu-
tion did not delay corneal wound healing, consistent
with findings in humans with postsurgical corneal ulc-
ers26 and in rabbits26 and rats27 with experimentally
induced corneal ulcer.

Topical ocular application of nalbuphine has been used as an alternative to mor-
phine in dogs.26 In the present study, however, topical
administration of 1% nalbuphine solution had no ef-
fect on corneal sensitivity in eyes of clinically normal
horses at any time point. This finding contrasts with results of another study22 in clinically normal do-
s, in which corneal sensitivity decreased significantly for 30
minutes following topical treatment with 1 drop of 1%
nalbuphine solution.

To resolve these conflicting results, a more detailed
analysis of the analgesic effects of different opioids in
the components of the cornea in various species may
be helpful. The opioid receptors in a particular corneal
component in a given species and the action of spe-
cific drugs at each type of receptor need to be identified.
For example, although nalbuphine is a partial $\kappa$ opi-
oid receptor agonist and partial $\mu$ agonist-antagonist,28
morphine is thought to have agonist properties at the $\kappa$,$\mu$, and $\delta$ opioid receptors in the CNS of dogs.28 A
combined effect of actions at each distinct receptor type
may improve analgesia. In 1 study20 in dogs, $\delta$ opioid
receptors were detected in the corneal epithelium and stroma but $\mu$ opioid receptors were rarely detected in
the anterior and subepithelial portions of the stroma.

The potential of topical administration of mor-
phine to provide corneal anesthesia without impairing
wound healing, as indicated by the results of the study
of morphine treatment of experimentally induced cor-
neal ulcers in dogs,29 needs to be reconciled with a body
of research suggesting that delayed corneal healing is
associated with a different opioid receptor agonist,
OGF.30–37 In an in vitro study,35 of rabbit corneas treated
with OGF (also known as [Met]$^3$-enkephalin), epide-
phelial healing was delayed. Opioid growth factor exerts its
effect on the $\zeta$ opioid receptors in basal epithelial cells
and inhibits DNA synthesis and cell migration, which
are necessary for corneal wound healing.31 The presence
of OGF and OGF receptors in the corneal epithelial cell
cytoplasm of dogs, cats, and horses has been reported.32
The effects of OGF are receptor mediated and can be in-
terrupted by the opioid receptor antagonist naltrexone.
Naltrexone is an antagonist primarily at $\mu$ and $\kappa$ opioid
receptors. Blocking of opioid receptors speeds healing,
which suggests that administration of opioids would
negatively affect corneal re-epithelialization.33–36 Opi-
oid growth factor is an endogenous opioid peptide and,
as such, may have effects (including slowing cell mi-
gration in corneas) that differ from those of exogenous
opioids such as morphine or nalbuphine, which do not
interfere with corneal epithelial cell migration or orga-
nization when administered topically. However, exog-
enuous OGF inhibits corneal epithelial cell healing.30,35,37
There is no evidence to suggest that exogenous opioids
such as morphine and nalbuphine interact at the $\zeta$ op-
oid receptor. It is known that the growth-retarding prop-
erties of OGF are $\zeta$ opioid receptor mediated; therefore,
it may not be unexpected that topical administration
of morphine does not inhibit corneal healing because
morphine acts primarily at $\mu$ and $\delta$ opioid receptors in
the cornea.31,27,33

In addition to differences in opioid receptor types
among tissues and species and differences among opio-
oids themselves in terms of their actions at different
receptors, another explanation for the present study's
failure to detect a decrease in corneal sensitivity fol-
lowing topical administration of nalbuphine solution
in eyes of clinically normal horses may be that periph-
ernally applied opiates, such as nalbuphine, are not ef-
fective in noninflamed tissue. This theory is supported
by findings of studies27,38 involving experimentally in-
duced corneal injuries in rats. Topical treatment with
morphine did not reduce pain sensitivity to less than
baseline levels in corneas of clinically normal rats but
did have that effect in rats with chemically cauter-
ized corneas.27,38 Expression of opioid receptors in the
cornea appears to be upregulated with inflammation,
which accounts for the effectiveness of exogenous opi-
oids in injured and inflamed corneas. It is possible that
the lack of effect of nalbuphine in our study was asso-
ciated with the use of noninflamed equine eyes. This,
however, is in contrast to findings of another study21 in
which nalbuphine significantly reduced corneal sensi-
tivity in normal canine eyes; the decrease in CTT was
significant 30 minutes following topical administration
of nalbuphine in that study. One explanation for the
nalbuphine-associated decrease in CTT in eyes of clini-
cally normal dogs but not in eyes of clinically normal
horses may be differences in the receptor types present
in noninflamed canine and equine corneas. As men-
tioned previously, the corneal epithelium of dogs has
primarily δ opioid receptors with few μ opioid receptors. Nalbuphine is primarily a κ opioid receptor agonist, and to our knowledge, no studies have specifically assessed whether κ opioid receptors are present in clinically normal or inflamed corneas of dogs or horses. The nalbuphine-associated decrease in corneal sensitivity detected in dogs but not in horses may indicate that the canine cornea has κ opioid receptors that are constitutive and that the equine cornea does not contain such receptors or that they are present but are inducible via inflammation. Anecdotally, we have used topical 1% nalbuphine solution in a small number of horses with chronic inflammation of the cornea attributable to various disease conditions, and our subjective impression was that horses had increased ocular comfort following treatment, although no experimental data exist to support this impression.

The failure to identify a decrease in corneal sensitivity following topical administration of nalbuphine in eyes of clinically normal horses in the present study may be related to the time course of action of this drug in this tissue. The analgesia associated with topical application of morphine in people with postsurgical corneal abrasions is time dependent. In those patients, the analgesic effect of morphine at 20 minutes after administration was greater than that at 10 minutes. This is similar to the effect of ocular administration of nalbuphine in clinically normal dogs; no significant decrease in CTT was detected at 15 minutes after treatment, but a significant decrease was evident at 30 minutes. For this reason, CTT readings were obtained in the present study by use of the Cochet-Bonnet aesthesiometer until 60 minutes after treatments were applied and again at the 120-minute time point in 5 of the 8 horses. Even so, topical application of 1% nalbuphine solution had no effect on corneal sensitivity in eyes of clinically normal horses at any time point.

Dose dependency should be considered as an explanation for the lack of effect of nalbuphine on corneal sensitivity in the present study, as well as for the lack of effect of the drug on wound healing in other studies. It may be that the studies in which morphine was administered topically to wounded dog and rabbit corneas did not reveal a delay in healing because of the dose-dependent nature of adverse effects associated with opioids, particularly morphine. Amounts of morphine solution used topically in eyes are extremely low, and adverse effects locally (eg, miosis) or systemically (eg, respiratory depression) have not been detected with in vivo usage. Because nalbuphine's analgesic effects may be dose dependent, it is possible that the amount of drug used in the present study (0.2 mL) was not sufficient to achieve analgesia in the relatively large surface area of the equine cornea. This is unlikely, however, because the volume of nalbuphine solution used in the eyes of the horses in the present study was proportionally equivalent to that used in the eyes of dogs (ie, 1 drop or 0.05 mL) and humans (ie, 2 drops or 0.1 mL) on the basis of corneal surface area and lacrimal lake volume.

Finally, it is possible that the difference in CTT between eyes treated with nalbuphine solution and eyes treated with artificial tears solution did not differ significantly with time because of the small sample size. However, given that the difference in CTT between the 2 groups was clinically insignificant (ie, less than the 5-mm interval by which the Cochet-Bonnet filament was adjusted to measure CTT [< 1-mm difference at baseline and at 1, 15, 60, and 120 minutes after treatment, and only 2.5 mm and 3.75 mm greater in nalbuphine-treated eyes than in artificial tears–treated eyes after 30 and 45 minutes, respectively]), this is unlikely.

It is important to identify alternatives to control corneal pain in horses. The potential complications associated with topical use of anesthetics and NSAIDs along with the short duration of action of currently available topical anesthetic preparations reduces the usefulness of these classes of drugs as treatment options for corneal pain. Systemic administration of NSAIDs commonly used in horses has many risks, including development of nephrotoxicosis and gastric and colonic ulceration. Both flunixin meglumine and phenylbutazone, the 2 most commonly used NSAIDs in horses, are potent nonspecific COX inhibitors that do not discriminate between the constitutive COX-1 necessary for several normal cellular functions and the inducible COX-2 associated with inflammation. The inhibition of the COX-1 isoform is generally responsible for adverse effects on renal perfusion and development of gastric and colonic irritation and ulceration associated with NSAID use. Renal and gastrointestinal tract abnormalities may develop in a horse treated with an appropriate dose and dosing frequency of NSAIDs as a result of the individual animal’s idiosyncratic responses. In horses, systemic and topical administrations of corticosteroids to provide analgesia would generally be contraindicated in the face of corneal ulceration because of drug-associated prolongation of healing time.

Limitations of the present study included the fact that apparently normal, noninflamed corneas were evaluated and a small number of horses were used. Even with a small number of horses, it was clear that topical ophthalmic 1% nalbuphine solution was nonirritating to the eyes of clinically normal horses. Future studies to assess the corneal healing times in horses following treatment with nalbuphine are indicated, as are studies of corneal sensitivity in horses with experimentally induced corneal wounds treated topically with nalbuphine. In addition, controlled clinical trials of the effectiveness of topical administration of nalbuphine as an ocular analgesic in horses with painful corneal conditions such as band keratopathy, nonhealing ulceration, eosinophilic keratitis, and bacterial or fungal keratitis should be pursued. Further considerations would also include determining the presence of opioid receptors (including κ opioid receptors) in both normal and inflamed corneas of horses to determine the analgesic effectiveness of not only nalbuphine but also other classes of topical ophthalmic opioid preparations in this species.

a. Cochet-Bonnet aesthesiometer, Model L12 No. 9018, Luneau Ophthalmologies, Chartres Cedex, France.

b. Prescription Center, Fayetteville, NC.
c. Artificial tears solution, Akorn Inc, Lake Forest, Ill.
d. GLM procedure, SAS, version 9.1, SAS Institute Inc, Cary, NC.