Pharmacokinetics of long-acting nalbuphine decanoate after intramuscular administration to Hispaniolan Amazon parrots (*Amazona ventralis*)

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Objective—To evaluate the pharmacokinetics of nalbuphine decanoate after IM administration to Hispaniolan Amazon parrots (*Amazona ventralis*).

Animals—9 healthy adult Hispaniolan Amazon parrots of unknown sex.

Procedures—Nalbuphine decanoate (37.5 mg/kg) was administered IM to all birds. Plasma samples were obtained from blood collected before (time 0) and 0.25, 1, 2, 3, 6, 12, 24, 48, and 96 hours after drug administration. Plasma samples were used for measurement of nalbuphine concentrations via liquid chromatography–tandem mass spectrometry. Pharmacokinetic parameters were estimated with computer software.

Results—Plasma concentrations of nalbuphine increased rapidly after IM administration, with a mean concentration of 46.1 ng/mL at 0.25 hours after administration. Plasma concentrations of nalbuphine remained > 20 ng/mL for at least 24 hours in all birds. The maximum plasma concentration was 109.4 ng/mL at 2.15 hours. The mean terminal half-life was 20.4 hours.

Conclusions and Clinical Relevance—In Hispaniolan Amazon parrots, plasma concentrations of nalbuphine were prolonged after IM administration of nalbuphine decanoate, compared with previously reported results after administration of nalbuphine hydrochloride. Plasma concentrations that could be associated with antinociception were maintained for 24 hours after IM administration of 37.5 mg of nalbuphine decanoate/kg. Safety and analgesic efficacy of nalbuphine treatments in this species require further investigation to determine the potential for clinical use in pain management in psittacine species. (*Am J Vet Res* 2013;74:191–195)

Opioids are frequently used in veterinary medicine and are considered the most effective class of analgesic drugs for perioperative pain. Studies[1–7] have validated the clinical use of opioids, particularly those with κ-opioid receptor affinities, for birds. Butorphanol tartrate and nalbuphine hydrochloride, drugs that are κ-opioid receptor agonists and μ-opioid receptor antagonists, are currently considered the recommended opioid drugs for acute pain management in psittacine birds.[1–7] The accepted dose ranges for psittacine birds of 1 to 3 mg of butorphanol tartrate/kg and 12.5 mg of nalbuphine hydrochloride/kg result in a short half-life and require repeated parenteral administration every 2 to 3 hours to maintain effects in psittacine birds.[1,6,8,9] Nalbuphine hydrochloride appears to have good bioavailability after IM administration in Hispaniolan Amazon parrots (*Amazona ventralis*) and is rapidly cleared after IV and IM administration.[9]

Abbreviations

AUC	Area under the curve from 0 to infinity
$AUC_{extrapolated}$	Percentage of the area under the curve from 0 to infinity extrapolated from the last time point
AUMC	Area under the first moment curve from 0 to infinity
Cl/F	Plasma clearance per fraction of the dose absorbed
Cmax	Maximum plasma concentration
λz	Terminal rate constant
MRT	Mean residence time from 0 to infinity
$t_{1/2\lambda z}$	Terminal half-life
Tmax	Time to maximum plasma concentration
Vd_{area}/F	Volume of distribution (determined via the area method) per fraction of the dose absorbed

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Long-acting opioid drugs would address the need for frequent administration. This was determined in studies[2–4] in which liposomal-encapsulated butorphanol provided analgesia for up to 5 days in psittacine birds. Long-acting nalbuphine formulations, such as nalbuphine decanoate, may provide an alternative for long-term pain management in psittacine birds. The custom-synthetized long-acting ester nalbuphine decanoate resulted in analgesia for up to 60 hours in rats[10,11] and up to 48 hours in rabbits.[12] Additionally, nalbuphine is currently not on the Drug Enforcement Administration list of scheduled substances because of its low abuse potential, which would be advantageous in clinical settings. Clinicians would be more likely to dispense analgesic drugs that are not on the list of scheduled drugs than to dispense controlled drugs.

To our knowledge, no studies have been conducted on the use of long-acting nalbuphine esters in avian species. The purpose of the study reported here was to determine the pharmacokinetics of nalbuphine decanoate after IM administration to Hispaniolan Amazon parrots.

Materials and Methods

Animals—Nine adult (range, 6 to 22 years old; median, 8.3 years old) Hispaniolan Amazon parrots of unknown sex were used in the study. Mean ± SD body weight of the parrots was 289.2 ± 18.7 g. All parrots were considered healthy before and during the study as determined via physical examination. For the duration of the study, the birds were housed in stainless steel cages (0.6 × 0.6 × 0.6 m), provided toys and perches, and maintained on a light cycle of 12 hours of light and 12 hours of darkness. A commercial pelleted diet[a] formulated for psittacine birds was fed to all parrots, and fresh water was provided ad libitum. The Institutional Animal Care and Use Committee at the University of Wisconsin School of Veterinary Medicine approved the experimental protocol.

Experimental design—Parrots were manually restrained for drug administration and blood collection. Parrots received a single dose of nalbuphine decanoate (37.5 mg/mL, IM) in a pectoral muscle. Blood samples (0.2 to 0.3 mL/sample) were collected from the jugular veins or brachial veins before (time 0) and 0.25, 1, 2, 3, 6, 12, 24, 48, and 96 hours after drug administration. Blood samples were placed in lithium heparin tubes and centrifuged at 1,030 × *g* for 7 minutes within 1 hour after collection. Plasma samples were harvested and stored at –70°C until analysis. All birds were monitored subjectively during the study for signs of adverse effects, including sedation, excitation, vomiting, and diarrhea.

Synthesis of nalbuphine decanoate—Nalbuphine decanoate was synthesized by modification of a method described elsewhere.[13] Nalbuphine hydrochloride[b] (0.5315 g) was added to a round-bottom flask containing a stir bar and 16 mL of methylene chloride.[c] Triethylamine[d] (0.7 mL) was added, and the solution was cooled to 0° to 5°C in an ice bath. Decanoyl chloride[d] (0.35 mL) was added in a drop-wise manner with vigorous stirring under argon gas at 0° to 5°C. The solution was removed from the ice bath, warmed to 23°C, and stirred for 3 hours. The desired product, nalbuphine decanoate, and a small amount of nalbuphine hydrochloride were separated via thin-layer chromatography on a silica gel[e] by use of a solution of ethyl acetate and hexane (3:1) as the solvent. The reaction mixture was washed twice with 10% sodium carbonate and water. The crude product was collected in a preweighed round-bottom flask, evaporated, and lyophilized. Weight of the crude product obtained was 0.5518 g. This material was dissolved in methylene chloride and purified with a 3.0 × 17.5-cm column[d] containing silica gel, 70 to 230 mesh, at 6 nm and eluted with a solution of hexane and acetone (70:30). All fractions that contained product were combined and evaluated via thin-layer chromatography in a solution of ethyl acetate and hexane (3:1). Fractions that contained product were combined in a preweighed round-bottom flask, evaporated, and lyophilized. Weight of the pure product was 0.446 g. The actual yield of product was 60% of the theoretical value. The nalbuphine decanoate product was then dissolved in 100% ethanol. After a short isocratic separation, the product was analyzed for purity via atmospheric pressure chemical ionization on a liquid chromatography–mass spectrometry mass spectrometer. A molecular ion at 512.4 m/z was detected that corresponded to the singly charged mass of nalbuphine decanoate. Prior to injection, nalbuphine decanoate was dispensed into a sterile 20-mm screw-capped test tube, dried via flash evaporation, and dissolved in sterile sesame oil at a concentration of 18.75 or 37.5 mg/mL. The interval from dissolution in oil to injection into the birds was < 24 hours. Prior to injection, the drug was stored at room temperature (approx 21°C) and protected from light.

Measurement of plasma concentrations of nalbuphine—Plasma concentrations of nalbuphine were determined via high-performance liquid chromatography–tandem mass spectrometry by use of electrospray ionization in a modification of a method described elsewhere.[14] The mobile phase consisted of 0.1% formic acid in water and 0.1% formic acid in methanol with a flow rate of 250 µL/min. Separation was achieved via a column maintained at room temperature (approx 21°C). Sample preparation consisted of adding 300 µL of acetonitrile that contained 250 ng of levorphanol/mL (internal standard) to 100 µL of parrot plasma. The solution was mixed with a vortexer, which was followed by centrifugation. Supernatant was removed, placed in a sterile tube, and dried under nitrogen gas. After the material was dried, 75 µL of 0.1% formic acid in water and 75 µL of 0.1% formic acid in methanol were added to each tube, and the solution was filtered through a 0.45-µm syringe filter into a high-performance liquid chromatography vial. The injection volume was 15 µL. Standard curves were prepared by the addition of nalbuphine hydrochloride to untreated parrot plasma at concentrations that ranged from 1 to 5,000 ng/mL. The estimated limit of quantification was 1 ng/mL, and the limit of detection was 0.5 ng/mL. The assay was linear ($R^2 > 0.99$) for the concentrations examined (ie, 1 to 5,000 ng/mL). The recovery of nalbuphine was from 71% to 89% over this range of concentrations. Interassay and intra-assay variability were each < 15%.

Table 1— Noncompartmental pharmacokinetics after IM administration of 37.5 mg of nalbuphine decanoate/kg (equivalent to 26.9 mg of nalbuphine/kg or 26.19 mg of nalbuphine base/kg) to 9 Hispaniolan Amazon parrots (*Amazona ventralis*).

Parameter	Bird 1	Bird 2	Bird 3	Bird 4	Bird 5	Bird 6	Bird 7	Bird 8	Bird 9
$AUC_{extrapolated}$ (%)	3.4	25.0	3.9	4.4	3.3	9.8	7.5	17.6	10.5
AUC (h•ng/mL)	3,954.8	2,158.7	3,168.2	3,038.0	3,228.1	1,721.4	3,410.4	4,874.9	4,365.0
AUMC (h•h•ng/mL)	53,605.6	109,624.6	62,316.0	79,863.6	69,428.9	33,693.8	109,693.5	261,484.9	176,698.4
Cl/F (mL/min/kg)	110.4	202.2	137.8	143.7	135.2	253.6	128.0	89.5	100.0
Cmax (ng/mL)	278.5	39.0	147.5	108.2	108.8	83.2	118.2	93.6	129.7
$t_{1/2\lambda z}$ (h)	10.6	34.7	14.6	19.6	14.1	13.9	27.0	36.7	30.3
λz (1/h)	0.0656	0.0200	0.0473	0.0353	0.0490	0.0499	0.0257	0.0189	0.0229
MRT (h)	13.6	50.8	19.7	26.3	21.5	19.6	32.2	53.6	40.5
Tmax (h)	1.0	3.0	1.0	3.0	3.0	1.0	6.0	1.0	6.0
Vd_{area}/F (L/kg)	100.9	606.5	174.7	244.2	165.5	305.2	299.4	284.2	262.3

Pharmacokinetic analysis—Pharmacokinetic analyses were performed with computer software.[f] The calculated noncompartmental parameters included AUC calculated via the linear trapezoidal method, $AUC_{extrapolated}$, AUMC, Cl/F, $t_{1/2\lambda z}$, λz, MRT, Vd_{area}/F, and volume of distribution at steady state. Values for Cmax and Tmax were determined directly from the plasma concentrations. Values for the extrapolated concentration at time 0 were determined by log-linear regression of the first 2 time points.

Results

Calculated pharmacokinetic values after IM administration of nalbuphine decanoate to Hispaniolan Amazon were summarized (**Tables 1 and 2**). The Cmax was 109.4 ng/mL at 2.15 hours. The mean $t_{1/2\lambda z}$ was 20.4 hours. Plasma concentrations rapidly increased after IM administration, with a mean concentration of 46.1 ng/mL at 0.25 hours after administration. Plasma concentrations of nalbuphine remained > 20 ng/mL for at least 24 hours in all birds (**Figure 1**). None of the parrots had abnormal behavior throughout the study, and there were no adverse effects detected subjectively.

Table 2—Noncompartmental pharmacokinetic values after IM administration of 37.5 mg of nalbuphine decanoate/kg (equivalent to 26.9 mg of nalbuphine/kg or 26.19 mg of nalbuphine base/kg) to 9 Hispaniolan Amazon parrots.

Parameter	Geometric mean	Minimum	Median	Maximum
$AUC_{extrapolated}$ (%)	7.4	3.3	7.5	25.0
AUC (h•ng/mL)	3,178.8	1,721.4	3,228.1	4,874.9
AUMC (h•h•ng/mL)	88,897.8	33,693.8	79,863.6	261,484.9
Cl/F (mL/min/kg)	137.3	89.5	135.2	253.6
Cmax (ng/mL)	109.4	39.0	108.8	278.5
$t_{1/2\lambda z}$ (h)	20.4	10.6	19.6	36.7
λz (1/h)	0.0339	0.0189	0.0353	0.0656
MRT (h)	28.0	13.6	26.3	53.6
Tmax (h)	2.1	1.0	3.0	6.0
Vd_{area}/F (L/kg)	242.9	100.9	262.3	606.5

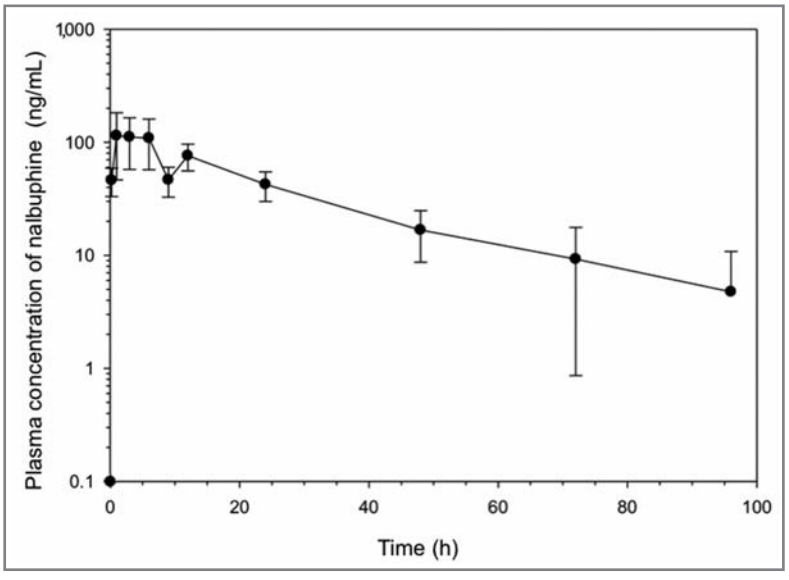

Figure 1—Mean ± SD plasma concentrations of nalbuphine before (time 0) and after IM administration of 37.5 mg of nalbuphine decanoate/kg (equivalent to 26.9 mg of nalbuphine/kg) to 9 Hispaniolan Amazon parrots (*Amazona ventralis*).

Discussion

To our knowledge, the study reported here is the first in which the pharmacokinetics of a long-acting ester of nalbuphine in an avian species has been described. Nalbuphine decanoate administered IM to Hispaniolan Amazon parrots rapidly resulted in a mean Cmax of 46.1 ng/mL at 0.25 minutes after administration and maintained plasma concentrations > 20 ng/mL for at least 24 hours in all birds. The mean $t_{1/2\lambda z}$ was 20.4 hours. The prolonged period for plasma concentrations and the prolonged $t_{1/2\lambda z}$, compared with results of previous studies of nalbuphine hydrochloride, were suggestive of the extended-release characteristics of the preparation and consistent with data from mammalian species in which nalbuphine long-acting esters result in a prolonged effect.[10–12]

A depot formulation is an established method for increasing the duration of a short-acting drug. It is produced by esterifying a drug to form a bioconvertible prodrug-type ester and then formulating it in an injectable oil.[15] In the present study, the prodrug ester was nalbuphine decanoate, and the oily formulation was

sesame oil. This formulation results in a drug reservoir at the site of injection. The rate of drug absorption is controlled by the interfacial partitioning of drug esters from the reservoir to the tissue fluid and the rate of bioconversion of drug esters to regenerate active drug molecules.[15]

The dose-corrected AUC (ie, absolute bioavailability) for nalbuphine decanoate after IM administration was 121.4 h•ng/mL, whereas the dose-corrected AUC for nalbuphine hydrochloride is 245.1 h•ng/mL.[9] Although a direct comparison of these values cannot be made, this may be suggestive of bioavailability < 100% (approx 50%). The Cl/F for nalbuphine decanoate was 137.3 mL/min/kg, whereas the Cl/F for nalbuphine hydrochloride after IM administration is only 68.0 mL/min/kg.[9] The apparent lower bioavailability of nalbuphine decanoate would explain the reason that Cl/F appeared to be twice as high, which would result in an apparent increase in clearance. The mean $t_{1/2\lambda z}$ for nalbuphine decanoate was 20.4 hours, whereas the mean $t_{1/2\lambda z}$ for nalbuphine hydrochloride is only 0.35 hours.[9] The $t_{1/2\lambda z}$ calculated in the present study may have been overestimated because of an absorption rate effect. The Cmax for nalbuphine hydrochloride is 3,685 ng/mL after IM administration of a dose of 12.5 mg/kg,[9] whereas the Cmax for nalbuphine decanoate was 109.4 ng/mL after IM administration of a dose of 37.5 mg/kg. The lower Cmax after nalbuphine decanoate, in addition to the prolonged half-life, is suggestive of prolonged absorption that resulted in a flip-flop phenomenon. In a flip-flip phenomenon, the absorption rate of the drug is much slower than the elimination rate, which results in the absorption rate being the rate-limiting step for the plasma profile. The lack of a complete crossover study that included IV administration precluded further conclusions regarding the clearance and a possible flip-flop effect in the present study. Therefore, the terminal portion of the plasma profile and mean $t_{1/2\lambda z}$ was prolonged and the Cmax was less, compared with results after administration of nalbuphine hydrochloride.

The Vd_{area}/F after IM administration of nalbuphine decanoate was 242.9 L/kg, whereas the Vd_{area}/F for nalbuphine hydrochloride is 2.08 L/kg in Hispaniolan Amazon parrots.[9] This difference, as well as the lower plasma concentrations achieved when higher doses of nalbuphine decanoate were administered, is typical for a sustained-release formulation. Lower bioavailability may also contribute to a larger Vd_{area}/F, but the relative difference is much greater (100-fold as great) than can be expected from any bioavailability differences, given the plasma profiles. The lack of a complete crossover study that included IV administration precluded further conclusions regarding the volume of distribution in the present study.

The therapeutic plasma concentration of an analgesic drug varies with species and with the method used to provide a painful stimulus, so caution should be used when plasma concentration alone is used to predict the analgesic concentration.[16] The antinociceptive effects are likely determined by the concentration at the receptor, which typically lags behind the plasma concentration (ie, hysteresis).[16] The antinociceptive effect of nalbuphine hydrochloride administered IM at a dose of 12.5 mg/kg lasted for 3 hours, compared with the thermal threshold at baseline. Mean ± SD plasma concentration at 3 hours in another study[9] in Hispaniolan Amazon parrots in which nalbuphine was administered at 12.5 mg/kg was 27.76 ± 20.38 ng/mL. Furthermore, 4 of 8 parrots in that study[9] had plasma concentrations < 1 ng/mL at 3 hours after administration. The affinity of the drug for the receptor may account for a longer duration of action than is predicted by the half-life or plasma concentrations. Additional studies that involve the use of integrated pharmacokinetic-pharmacodynamic models for nalbuphine hydrochloride and nalbuphine decanoate may make it possible to discern any hysteresis after administration of nalbuphine in parrots.

The dose of 37.5 mg/kg selected for the study reported here was determined on the basis of results of a preliminary study of 2 birds. This dose is much higher than that used in rats and rabbits. The primary adverse effect of nalbuphine hydrochloride administration in humans is sedation.[17] No adverse effects were detected in the parrots. Although sedation was not quantified, the parrots subjectively did not appear to be sedated after nalbuphine administration. These findings were consistent with those in studies[8,9] on the pharmacokinetics and antinociceptive effects of nalbuphine hydrochloride in Hispaniolan Amazon parrots.

In Hispaniolan Amazon parrots, nalbuphine decanoate administered IM at a dose of 37.5 mg/kg appeared to have a prolonged $t_{1/2\lambda z}$, compared with results after administration of nalbuphine hydrochloride, and maintained for 24 hours plasma concentrations that could be associated with antinociception. Safety and analgesic efficacy of various nalbuphine treatments are needed to determine the potential for pain management in psittacine birds.

a. Exact, Kaytee Products Inc, Chilton, Wis.
b. Spectrum Laboratories Inc, Gardena, Calif.
c. Fluka, St Louis, Mo.
d. Sigma-Aldrich Corp, St Louis, Mo.
e. EMD Chemicals, Gibbstown, NJ.
f. WinNonlin, version 5.2, Pharsight Corp, St Louis, Mo.

References

1. Sanchez-Migallon Guzman D, Flammer K, Paul-Murphy J, et al. Pharmacokinetics of butorphanol after oral, intravenous and intramuscular administration in Hispaniolan Amazon parrots (*Amazona ventralis*). *J Avian Med Surg* 2011;25:185–191.
2. Paul-Murphy JR, Sladky KK, Krugner-Higby LA, et al. Analgesic effects of carprofen and liposome-encapsulated butorphanol tartrate in Hispaniolan parrots (*Amazona ventralis*) with experimentally induced arthritis. *Am J Vet Res* 2009;70:1201–1210.
3. Paul-Murphy JR, Krugner-Higby LA, Tourdot RL, et al. Evaluation of liposome-encapsulated butorphanol tartrate for alleviation of experimentally induced arthritic pain in green-cheeked conures (*Pyrrhura molinae*). *Am J Vet Res* 2009;70:1211–1219.
4. Sladky KK, Krugner-Higby L, Meek-Walker E, et al. Serum concentrations and analgesic effects of liposome-encapsulated and standard butorphanol tartrate in parrots. *Am J Vet Res* 2006;67:775–781.
5. Buchwalder T, Huber-Eicher B. Effect of the analgesic butorphanol on activity behaviour in turkeys (*Meleagris gallopavo*). *Res Vet Sci* 2005;79:239–244.
6. Paul-Murphy JR, Brunson DB, Miletic V. Analgesic effects of butorphanol and buprenorphine in conscious African grey parrots

(*Psittacus erithacus erithacus* and *Psittacus erithacus timneh*). *Am J Vet Res* 1999;60:1218–1221.
7. Curro TG, Brunson DB, Paul-Murphy J. Determination of the ED50 of isoflurane and evaluation of the analgesic properties of butorphanol in cockatoos (*Cacatua* spp.). *Vet Surg* 1994;23:429–433.
8. Sanchez-Migallon Guzman D, KuKanich B, Keuler NS, et al. Antinociceptive effects of nalbuphine hydrochloride in Hispaniolan Amazon parrots (*Amazona ventralis*). *Am J Vet Res* 2011;72:736–740.
9. Keller DL, Sanchez-Migallon Guzman D, Klauer JM, et al. Pharmacokinetics of nalbuphine hydrochloride after intravenous and intramuscular administration to Hispaniolan Amazon parrots (*Amazona ventralis*). *Am J Vet Res* 2011;72:741–745.
10. Liu KS, Hu OY, Ho ST, et al. Antinociceptive effect of a novel long-acting nalbuphine preparation. *Br J Anaesth* 2004;92:712–715.
11. Chu KS, Wang JJ, Hu OY, et al. The antinociceptive effect of nalbuphine and its long-acting esters in rats. *Anesth Analg* 2003;97:806–809.
12. Ho ST, Wang JJ, Hu OY, et al. The antinociceptive effect of a long-acting nalbuphine preparation in rabbits. *Acta Anaesthesiol Sin* 2003;41:99–103.
13. Aungst BJ, Myers MJ, Shefter E, et al. Prodrugs for improved oral nalbuphine bioavailability: inter-species differences in the disposition of nalbuphine and its acetylsalicylate and anthranilate esters. *Int J Pharm* 1987;38:199–209.
14. Leung GN, Leung DK, Wan TS, et al. High throughput screening of sub-ppb levels of basic drugs in equine plasma by liquid chromatography-tandem mass spectrometry. *J Chromatogr A* 2007;1156:271–279.
15. Ansel HC, Allen LV Jr, Popovich NG. Parenterals. In: *Pharmaceutical dosage forms and drug delivery systems*. 7th ed. Philadelphia: Lippincott Williams & Wilkins, 1999;397–449.
16. Bailey PL, Egan TD, Stanley THJ. Intravenous opioid anesthetics. In: Miller RD, ed. *Anesthesia*. Philadelphia: Churchill Livingstone, 2000;273–376.
17. Schmidt WK, Tam SW, Shotzberger GS, et al. Nalbuphine. *Drug Alcohol Depend* 1985;14:339–362.