Journal of Zoo and Wildlife Medicine 34(1): 3–8, 2003
Copyright 2003 by American Association of Zoo Veterinarians

PHARMACOKINETICS OF FLORFENICOL IN LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*) AFTER SINGLE INTRAVENOUS AND INTRAMUSCULAR INJECTIONS

M. Andrew Stamper, D.V.M., Mark G. Papich, D.V.M., M.S., Gregory A. Lewbart, M.S., V.M.D., Stuart B. May, B.A., Delta D. Plummer, and Michael K. Stoskopf, D.V.M., Ph.D.

Abstract: The pharmocodynamics of single injections of florfenicol in yearling loggerhead sea turtles (*Caretta caretta*) were determined. Eight juvenile loggerhead sea turtles weighing 1.25 (±0.18) kg were divided into two groups. Four animals received 30 mg/kg of florfenicol i.v., and four received the same dose i.m. Plasma florfenicol concentrations were analyzed by reverse-phase high performance liquid chromatography. After the i.v. dose, there was a biphasic decline in plasma florfenicol concentration. The initial steep phase from 3 min to 1 hr had a half-life of 3 min, and there was a longer slow phase of elimination, with a half-life that ranged from 2 to 7.8 hr among turtles. The volume of distribution varied greatly and ranged from 10.46 to –60 L/kg. Clearance after the i.v. dose was 3.6–6.3 L/kg/hr. After the i.m. injection, there was a peak within 30 min of 1.4–5.6 μg/ml, and florfenicol was thereafter eliminated with a half-life of 3.2–4.3 hr. With either route, florfenicol plasma concentrations were below the minimum inhibitory concentrations for sensitive bacteria within 1 hr. Florfenicol does not appear to be a practical antibiotic in sea turtles when administered at these doses.

Key words: *Caretta caretta*, florfenicol, loggerhead sea turtle, pharmacokinetics, antimicrobials.

INTRODUCTION

Safe and effective antibacterial dosage regimens are poorly established for many reptiles, including sea turtles. Most antibiotic dosage regimens for these animals are empirical or extrapolated from other species. Cross-species extrapolation is risky because of differences in antibiotic disposition between reptiles and mammals and even between species of reptiles.

Although chloramphenicol may be valuable for treating some infections in companion and exotic animals, it may cause aplastic anemia in people. Because of the public health risk from residues of chloramphenicol in meat and milk, its use in food producing animals has been banned,[17] and its availability has decreased. Florfenicol, a structural analogue of chloramphenicol that is approved for cattle in the United States, lacks the *para*-nitro group of chloramphenicol, which has been associated with aplastic anemia.[10,18] Florfenicol also has greater activity than chloramphenicol against such gram-positive, anaerobic, and gram-negative bacteria as *Vibrio* sp. and *Aeromonas* spp. which are often associated with morbidity and mortality of salmonids and sea turtles.[1,4,7,9,11,20] Florfenicol use has replaced chloramphenicol use in food animals, but florfenicol's efficacy for treating infections in nondomestic animals other than fish has not been reported.

Pharmacokinetics vary among animal species with chloramphenicol, and probably with florfenicol also.[6] In cattle, the elimination half-life of florfenicol after i.v. administration is 2.65 hr, and 9–18 hr after i.m. injection, suggesting that the drug is released slowly from i.m. injection sites.[9] Prolonged elimination of a drug in reptiles might prevent the need for frequent injections. This study determined the pharmacokinetics of florfenicol after single i.v. and i.m. injections in juvenile loggerhead sea turtles (*Caretta caretta*), information that is necessary to estimate initial dosage regimens for clinical studies of florfenicol's safety and efficacy.

MATERIALS AND METHODS

Subjects and sample collection

Eight yearling juvenile loggerhead sea turtles, weighing 1.25 ± 0.18 kg, were studied. Each animal was first examined visually, and blood was collected for a complete blood count and for measurement of serum chemistries to assess health status using an eosinophil unopette (Unopette®, Becton Dickinson and Company, Rutherford, New Jersey 07070, USA). The turtles were confined to 200-L

From the Environmental Medicine Consortium (Stamper, Papich, Lewbart, Stoskopf) and the College of Veterinary Medicine Departments of Clinical Sciences (Stamper, Lewbart, Stoskopf) and Anatomy, Physiological Sciences, and Radiology (Papich, Plummer), North Carolina State University, 4700 Hillsborough Street, Raleigh, North Carolina 27606, USA; and North Carolina Aquarium, Pine Knoll Shores, P.O. Box 580, Atlantic Beach, North Carolina 28512, USA (May). Present address (Stamper): Walt Disney World Resort, Epcot, The Living Seas, EC Trl. W-251, P.O. Box 10000, Lake Buena Vista, Florida 32830-1000, USA. Correspondence should be directed to Andrew Stamper.

aquariums arranged in two groups of four, with each group connected by a recirculating system consisting of a 1/8 horsepower pump (Little Giant TE5MD-HC pump®, Aquanetics, San Diego, California 92110, USA), and a 60-W flow-through ultraviolet light sterilizer (Aquanetics). Two 300-W in-line heaters (Aquanetics) kept the water temperature at approximately 24°C.

The turtles were randomly assigned to i.v. and i.m. treatment groups (four in each group). Each animal received a single injection of florfenicol (Nuflor®, Schering-Plough Animal Health Corp., Kenilworth, New Jersey 07033, USA; 30 mg/kg, i.v. or i.m.) using a 25-ga needle attached to a 1-ml syringe. Intravenous doses were administered in the left cervical sinus. Intramuscular doses were injected into the left deltoid muscle. The plunger of the syringe was retracted prior to drug injection to verify that the drug was placed in the desired compartment.

Blood sampling alternated between right and left cervical sinuses at 0 (predose sample), 0.5, 1.5, 3, 6, 12, 24, and 48 hr after drug injection. For each blood collection, approximately 0.5 ml of blood was aspirated using a 1-ml tuberculin syringe with a 25-ga needle. The syringe and needle interiors were rinsed with 0.1 ml of 1,000 IU/ml Na heparin solution (Elkins-Sinn, Inc., Cherry Hill, New Jersey 08003, USA) as an anticoagulant. Excess heparin was shaken from the syringe. Blood was placed into polyethylene microcentrifuge tubes (Fisher Scientific Company, Pittsburgh, Pennsylvania 15219, USA) that were capped and immediately submerged in ice water. The blood was then centrifuged to harvest approximately 0.3 ml of plasma, which was placed in polyethylene microcentrifuge tubes via micropipet. The tubes were capped and stored at −70°C for 3 wk until high performance liquid chromatography (HPLC) analysis.

After analysis of concentrations from four animals in each group, we suspected that higher concentrations might have occurred earlier than the earliest collection time point of 0.5 hr. To more completely characterize the disposition of florfenicol, two additional turtles were administered i.v. florfenicol in the same manner as the initial animals, but from these turtles samples were collected at 0 (predose), 3, 5, 10, 20, and 30 min and 1 hr after drug administration. This study was conducted approximately 1 mo after the initial i.m. and i.v. study. To determine whether the drug was being excreted, aspirate flushes of 1 ml of saline were infused into the cloaca via polyethylene tomcat catheter (Sovereign®, The Kendall Company, Mansfield, Massachusetts 02048, USA) and aspirated at 0 and 30 min and 1 hr.

Florfenicol analysis

Florfenicol plasma concentrations were analyzed with reverse-phase HPLC. The HPLC apparatus consisted of a Waters Model 600 Pump (Millipore Corp., Milford, Massachusetts 01757, USA) and a Hewlett Packard Series 1050 Autosampler (Hewlett-Packard, Palo Alto, California 94304, USA), and data collection and analysis were performed on a Hewlett Packard HPLC^{2D} ChemStation running in Windows 3.1 on a Hewlett Packard Vectra 486/33N computer. The column was a Zorbax RX-C8, 4.6 mm × 15 cm (MAC-MOD Analytical Inc., Chadds Ford, Pennsylvania 19317, USA), with a Zorbax RX-C18 4 mm × 1.25 cm guard column (MAC-MOD Analytical Inc.).

Florfenicol was eluted with a mobile phase consisting of 73% distilled water and 27% (v/v) acetonitrile. No buffers or mobile phase modifiers were added. The mobile phase was filtered and degassed prior to use and was continuously sparged with helium during the analysis. The flow rate was 1.0 ml/min. Injection volume was 20 μl. Florfenicol was detected with UV detection at a wavelength of 223 nm. Retention time for florfenicol was approximately 5.5–6.5 min.

Preparation of calibration curve

A stock solution of florfenicol (1 mg/ml) was prepared by dissolving a pure reference standard (Schering-Plough Animal Health Corp., Union, New Jersey 07083, USA) in acetonitrile. The stock solution was kept refrigerated in a tightly sealed vial. The stock solution was then diluted serially with distilled water to make spiking solutions ranging in concentration from 1,000 to 1.95 μg/ml. Control (blank) plasma was obtained from untreated animals. Twenty microliters of the spiking solutions was added to 180 μl of blank plasma to produce 10 calibration standards ranging in concentration from 100 to 0.195 μg/ml. Thiamfenicol (Medichem China Group Company, Shenzhen, Guangdong, China) was added to each sample as an internal standard. A blank sample also was analyzed with each day's batch of samples.

A new calibration curve was prepared for each day's samples. Approximately 24 samples were analyzed each day. Calibration curves were linear, with an R^2 value of at least 0.99. Precision and accuracy had to be back-calculated to within 15% of the true value. Unknown concentrations were calculated by plotting the concentrations of florfenicol against the ratio of florfenicol:internal standard

peak height. Concentration of study samples was calculated from the peak height ratios.

Preparation of plasma

The samples from the study, as well as the prepared calibration plasma samples, were prepared by pipetting 100 μl into a clean screw-top glass tube. To this tube 100 μl of phosphate buffer and an internal standard were added. Phosphate buffer was prepared by adding 13.6 g of monobasic potassium phosphate (Sigma-Aldrich Corp., St. Louis, Missouri 63103, USA) to 1.0 L of distilled water. The pH of the buffer was adjusted to 7.0. After brief vortexing, 2 ml of ethyl acetate (Sigma-Aldrich) was added, and the admixture was gently rocked for 10 min at room temperature.

The tubes were centrifuged at 2,000 rpm for 10 min at −6°C. One milliliter of the supernatant was transferred to another clean glass tube and the contents evaporated under a flow of nitrogen (20 psi) at 45°C for 15 min. The residue in each tube was reconstituted with 200 μl of the mobile phase, briefly vortexed, and transferred to an HPLC vial for injection.

Pharmacokinetic analysis

Plasma concentrations of florfenicol were plotted on a semilogarithmic graph for analysis. The following parameters for disposition of florfenicol were calculated from plasma concentration vs. time curves: first order elimination rate constant (k_{el}), y-axis intercept (C_0), terminal half-life ($t_{½}$), area under the curve from time zero to infinity (AUC), volume of distribution area (Vd_{AREA}), and total systemic clearance (Cl_S).[8] Values for the elimination rate constant intercept of the curves and AUC were calculated with the use of a nonlinear curve fitting program (Parmsight Corp., Mountain View, California 94040, USA).

AUC was calculated using the trapezoidal method. Percent systemic availability from the i.m. injection (%F) was calculated from the ratio $AUC_{i.m.}$: $AUC_{i.v.}$. The values for the highest observed plasma concentration were designated as maximum plasma concentration (C_{MAX}), and time of maximum plasma concentration (T_{MAX}) was obtained from the observed values.

For some individuals, after the i.v. dose, it was possible to obtain a fit to a biexponential equation describing a two-compartment open model with the formula

$$Ct = Ae^{-\alpha t} + Be^{-\beta t},$$

where Ct is the plasma concentration at time = t, A is the intercept, and α is the rate constant for the initial steep phase (disposition) of the curve, B is the intercept, and β is the rate constant for the terminal (elimination) portion of the curve.

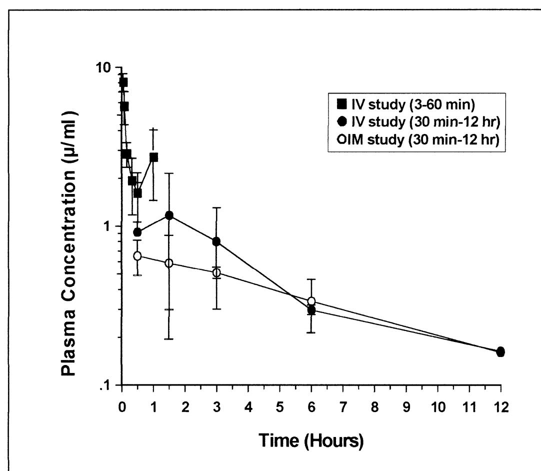

Figure 1. Mean florfenicol plasma concentrations with error bars (±SD) after i.v. and i.m. injections of 30 mg/kg (i.v. group [n = 4]; i.m. group [n = 2]) in juvenile loggerhead sea turtles (*Caretta caretta*).

RESULTS

HPLC analysis

The HPLC analysis of florfenicol was rapid, with a high degree of reproducibility. The precision was ±11.88% at the low concentration and ±5.4% at the high concentration. The accuracy, defined as the percent deviation from the true value, was 17.96 and 1.95% at the low and high concentrations, respectively. The limit of quantitation was defined as the lowest calibration point that had an accuracy within 20% of the true value, which was 0.195 μg/ml. Concentrations below this value were not used for pharmacokinetic analysis.

Although samples were collected for 72 hr after dosing, there were no measurable concentrations of florfenicol after 12 hr in any animals with either route. In three of the animals (one i.v. and two i.m.) there were no detectable concentrations in any samples.

Figure 1 presents the data for i.v. and i.m. doses on a semilogarithmic graph. After the i.v. dose, there is a biphasic decline in florfenicol plasma concentration. The initial steep phase from 3 min to 1 hr has a half-life of 0.05 hr (3 min), and there is a longer slow-phase of elimination with a half-life that ranges from 2 to 7.8 hr among one out of six turtles. One turtle had levels below the limit of quantitation of the assay when sampled at 30 min.

Table 1. Pharmacokinetic values for i.v. and i.m. florfenicol in juvenile loggerhead sea turtles (*Caretta caretta*) after a 30 mg/kg dose. Note: One turtle in the i.v. study and two from the i.m. study had levels below the limit of quantitation of the assay. They have been omitted from the analysis.[a]

	i.v. dose					±SD i.v. study	i.m. dose	
	3–60 min		30–72 hr					
Turtle no.	CB-1	T-2	CB-14	CB-4	CB-15		CB-16	CB-17
Clearance	n.a.	n.a.	3.58	6.33	3.6	n.a.	n.a.	n.a.
AUC (μ hr/ml)	44.95	11.21	8.38	4.74	8.32	16.61	6.17	3.48
Terminal half-life (hr)	25.0	3.1	7.8	6.6	2.0	9.31	4.3	3.2
Volume of distribution (L/kg)	24.09	12.16	40.11	59.9	10.46	20.79	33.56	56.54
C_{max} (μ/ml)	7.34	8.79	1.27	0.38	2.36	3.79	0.79	0.53

This last phase was highly variable among turtles, and the ability to discern this phase was limited by the few data points. The volume of distribution varied greatly from 10.46 to 60 L/kg. Clearance after the i.v. dose was 3.6–6.3 L/kg/hr (Table 1).

Florfenicol appeared to be absorbed quickly from the i.m. injection, with the greatest concentration observed at the first sampling time, 30 min. The elimination half-life was 3.2–4.3 hr after i.m. administration. There does not appear to be an absorption-limited decrease in plasma concentrations from the i.m. injection, as there is in cattle.[9] The average systemic availability, on the basis of AUC, after an i.m. dose was 67.4%. Cloacal flushes performed on the two turtles during the 1-hr study demonstrated florfenicol concentrations at 30 min (3.95 μg/ml, $n = 1$) and 1 hr (26.27–54.66 μg/ml, $n = 2$).

DISCUSSION

A rapid and accurate HPLC analysis for detection of florfenicol in plasma of sea turtles using a single sample volume of 100 μl was developed. In three animals there was no detectable florfenicol in any samples. These zero values for AUC, terminal half-life, volume of distribution, and maximum plasma concentration were not included in calculating the average shown in the tables and graphs. It may be possible these samples had levels below detectable limits at the time of sampling.

Florfenicol appears to be eliminated in sea turtles with an initial steep decline in plasma concentrations after an i.v. dose followed by a longer elimination period (Fig. 1). Because plasma was not collected before 30 min for the i.m. study, or for most of the i.v. study (except for two animals), a complete description of the pharmacokinetic profile in all animals is not possible for this study.

Conclusions from the i.v. study should be regarded cautiously because the i.v. study was performed as two separate experiments. Although the i.v. pharmacokinetics are difficult to discern because only two animals were sampled earlier than 30 min, our data suggest that there is an early, steep decline in florfenicol plasma concentration that was missed in most of the animals because of the sampling schedule. This may explain the low concentrations detected in the other eight animals that received florfenicol i.m. or i.v. but had low plasma concentrations by 1 hr. The terminal half-life in the 0–60 min i.v. turtles is highly variable because of the low number of samples and the inconsistency of the levels. As with the original i.v. study turtles, the determination of the terminal half-life for the i.v. study was done using nonlinear least-squares regression analysis, which is highly dependent on the data points used. In this case, only a couple of data points were available to define the terminal half-life, and consequently the results were highly variable from turtle to turtle and not reliable since they were taken from only a couple of points. If measurable concentrations of the drug were sampled and detectable at a longer period of time postinjection on turtle T-2, a longer $t_{½}$ would mostly likely have been determined. One of the original i.v. turtles and two of the i.m. turtles had levels below the limit of quantitation of the assay when sampled at 30 min. It is undetermined whether these discrepancies were on account of physiological parameters, an error in delivery of the drug, sampling error, or analysis of the data. Retrospective analysis of the experiment did not detect these problems in the experimental design and execution.

Hemodilution with abnormal health state or hemodilution with lymph contamination could have occurred. Hemodilution caused by abnormal health status was unlikely because all the animals were in good health prior to the study. Lymph contamination was unlikely because samples were collected from the external jugular vein, which is large and easy to access. Blood was collected easily, and Hct and serum quality were checked subsequently. No

abnormalities were noted. Dilution of the blood by heparin was unlikely, as syringes were shaken to remove excess heparin and needles were changed to preclude the presence of residual heparin.

Florfenicol concentrations in the cloaca were well above plasma levels within 30 min. Most drugs studied in turtles to date are eliminated more slowly than in mammals, but these drugs have been renally excreted.[2,19] In the rat, florfenicol is 60–70% excreted by the kidney and 30–40% via hepatic metabolism and biliary excretion.[12,13,16] In mammals, chloramphenicol is metabolized in the liver to glucuronides. The parent compound is excreted by glomerular filtration, and the metabolites are excreted mainly by tubular secretion.[14] Renal clearance of chloramphenicol in green sea turtles (*Chelonia mydas*) is approximately 0.24 ml/min/kg, much lower than the renal clearance rate in dogs.[3,15] In this study, the overall clearance rate of 60–105 ml/min/kg after i.v. florfenicol administration in loggerhead sea turtles was much faster than the green sea turtles renal clearance rates and the 3.8 ml/min/kg clearance rate of florfenicol in cattle.[9] Although loggerhead sea turtles may have faster overall elimination rates than green sea turtles, renal clearance may not be the only source of elimination of florfenicol in loggerhead sea turtles. In rats, florfenicol concentrates in the bile.[16] We detected florfenicol in the cloaca before the predicted 26–28 hr required for ingesta to travel from the small to the large intestine in sea turtles kept at 25°C, so biliary excretion could not explain the swift appearance of florfenicol in the cloaca.[5] Breakdown of florfenicol into metabolites could explain low florfenicol plasma levels, but no other peaks were detected chromatographically.

Peak plasma concentrations of florfenicol in cattle after i.m. doses of 20 mg/kg are 1.4–5.6 μg/ml.[9] Plasma concentrations in loggerhead sea turtles after i.m. injections were lower (mean 0.65 μg/ml) than the breakpoint for minimum inhibitory concentration (MIC) for susceptible bacteria used for quality control of susceptibility testing (2–8 μg/ml for *Staphylococcus aureus* and 4–8 μg/ml for *Escherichia coli*).[1,11] The MICs for *Pseudomonas aeruginosa* and *Aeromonas veronii* biovar *sobria* isolated from a sick loggerhead sea turtle housed at the veterinary school were 64 and 1 μg/ml, respectively.

The plasma concentration of a bacteriostatic drug like florfenicol should be maintained, presumably, above the MIC throughout most of the dosing interval for successful therapy. This is unlikely to be achieved with i.m. administration of florfenicol unless much higher doses are administered, and the drug's safety in turtles at higher doses is not known.

Florfenicol given at 30 mg/ml i.m. or i.v. has impractical plasma half-lives to be useful against most bacteria, including *Pseudomonas* and *Aeromonas* spp. This study illustrates that drug elimination rates in reptiles are not always slower than in mammals, substantiating the need for pharmacokinetic studies of other drugs in reptiles.

Acknowledgments: We thank Kari Snook, Jennifer Owen, and Charlene Stamper for their sample collection assistance, as well as the staff of the North Carolina Aquarium at Pine Knoll Shores for their support. We also thank Robert Bakal and Airess Eatmon for performing florfenicol MIC analysis on bacteria isolated from sea turtles. We thank the Schering-Plough Corporation for supplying the reference standard used in the HPLC assay.

LITERATURE CITED

1. Aguirre, A. A., G. H. Balazs, B. Zimmerman, and T. R. Spraker. 1994. Evaluation of Hawaiian green turtles (*Chelonia mydas*). J. Wildl. Dis. 30: 8–15.

2. Beck, K., M. Loomis, G. Lewbart, L. Spelman, and M. Papich. 1995. Preliminary comparison of plasma concentrations of gentamycin injected into the cranial and caudal limb musculature of the eastern box turtle (*Terrapene carolina carolina*). J. Zoo Wildl. Med. 26: 265–268.

3. Bovée, K. C., and T. Joyce. 1979. Clinical evaluation of glomerular function: 24-hr creatinine clearance in dogs. J. Am. Vet. Med. Assoc. 174: 488–491.

4. Campbell, T. W. 1996. Sea turtle rehabilitation. *In:* Mader, D. R. (ed.). Reptile Medicine and Surgery. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 427–435.

5. Davenport, J., S. Antipas, and E. Blake. 1989. Observations of gut function in young green turtles *Chelonia mydas*. J. Herpetol. 1: 336–342.

6. Davis, L., C. A. Neff, J. D. Baggot, and T. E. Powers. 1972. Pharmacokinetics of chloramphenicol in domestic animals. Am. J. Vet. Res. 33: 2259–2266.

7. Fukui, H., Y. Fujihara, and T. Kano. 1987. In vitro and in vivo antibacterial activities of florfenicol, a new fluorinated analog of thiamphenicol against fish pathogens. Fish Pathol. 22: 201–207.

8. Gibaldi, M., and D. Perrier. 1982. Pharmacokinetics, 2nd ed. Marcel Dekker, New York, New York.

9. Lobell, R. D., K. J. Varma, J. C. Jounson, R. A. Sams, D. F. Gerken, and S. M. Ashcraft. 1994. Pharmacokinetics of florfenicol following intravenous and intramuscular doses to cattle. J. Vet. Pharmacol. Ther. 17: 253–258.

10. Manyan, D. R., G. K. Arimura, and A. A. Yunis. 1975. Comparative metabolic effects of chloramphenicol analogues. Mol. Pharmacol. 11: 520–527.

11. Marshal, S. A., R. N. Jones, A. Wanger, J. A. Washington, F. V. Doern, A. L. Leber, and T. H. Haugen. 1996. Proposed MIC quality control guidelines for National Committee for Clinical Laboratory Standards susceptibil-

ity tests using seven veterinary antimicrobial agents: ceftiofur, enrofloxacin, florfenicol, penicillin, G-Novobiocin, pirlimycin, premafloxacin, and spectinomycin. J. Clin. Microbiol. 34: 2027–2029.

12. Matsui, H., M. Komiya, C. Ikeda, and A. Tachibana. 1984. Comparative pharmacokinetics of YM-13115, ceftriaxone, and florfenicol in rats, dogs, and rhesus monkeys. Antimicrob. Agents Chemother. 26: 204–207.

13. Anonymous. Nuflor® Technical Monograph. Schering-Plough Animal Health, Kenilworth, New Jersey.

14. Sande, M. A., and G. L. Mandell. 1993. Antimicrobial agents. *In:* Gilman, A. Goodman, T. W. Rall, A. S. Nies, and P. Taylor (eds.). Goodman & Gilman's The Pharmacological Basis of Therapeutics, 8th ed. McGraw-Hill, New York, New York. Pp. 1117–1145.

15. Schmidt-Nielsen, B., and L. E. Davies. 1968. Fluid transport and tubular intercellular spaces in reptilian kidneys. Science 159: 1105–1108.

16. Scott, G., and J. Stuart. 1996. Freedom of Information Act. NADA #141-063. Food and Drug Administration, Rockville, Maryland. 19: 39–43.

17. Settepani, J. 1984. The hazard of using chloramphenicol in food animals. J. Am. Vet. Med. Assoc. 184: 930–931.

18. Skolimowski, I. M., R. C. Knight, and D. I. Edwards. 1983. Molecular basis of chloramphenicol and thiamphenicol toxicity to DNA in vitro. J. Antimicrob. Agents Chemother. 12: 535–542.

19. Stamper, M. A., M. G. Papich, G. A. Lewbart, S. B. May, D. D. Plummer, and M. K. Stoskopf. 1999. Pharmacokinetics of ceftazidime in loggerhead sea turtles (*Caretta caretta*) after a single intravenous and intramuscular injection. J. Zoo Wildl. Med. 30: 32–35.

20. Syriopoulou, V. P., A. L. Harding, D. A. Goldman, and A. L. Smith. 1981. In vitro antibacterial activity of fluorinated analogs of chloramphenicol and thiamphenicol. J. Antimicrob. Agents Chemother. 19: 294–297.

Received for publication 12 March 2002