Comparison of various doses of carbon 13-labeled aminopyrine for a carbon 13-labeled aminopyrine demethylation blood test in healthy dogs

E. Michael Moeller, MS; Jörg M. Steiner, Dr med vet, PhD; David A. Williams, VetMB, PhD; Mark Tetrick, DVM; John Burr, DVM

Objective—To determine an optimal dose of carbon 13 (^{13}C)-labeled aminopyrine for use in a ^{13}C-aminopyrine demethylation blood test in healthy dogs.

Animals—9 adult dogs.

Procedures—Food was withheld from each dog for 12 hours. A 2-mL baseline blood sample was obtained from each dog and placed into an evacuated tube containing sodium heparin. Carbon 13-labeled aminopyrine was administered IV at doses of 1, 2, 5, or 10 mg/kg. Additional blood samples (2 mL) were obtained and placed into evacuated tubes containing sodium heparin 30, 45, 60, and 75 minutes after ^{13}C-aminopyrine administration. Hydrochloric acid was used to extract CO_2 from blood samples. The extracted gas was analyzed by fractional mass spectrometry to determine the percentage dose of ^{13}C administered as ^{13}C-aminopyrine and recovered in extracted gas (PCD).

Results—Gross evidence of clinical adverse effects was not detected in any dog after administration of ^{13}C-aminopyrine. The mean coefficient of variation (CV) for PCD was significantly lower than the mean CV for the summation of PCD values up to a given sampling time (CUMPCD). Mean PCD values among the 4 doses for each sample time were not significantly different. Administration of ^{13}C-aminopyrine at a dose of 2 mg/kg resulted in the lowest interindividual variability.

Conclusions and Clinical Relevance—The PCD is superior to CUMPCD for the quantification of ^{13}C-aminopyrine demethylation. Administration of ^{13}C-aminopyrine at a dose of 2 mg/kg is appropriate for use in the ^{13}C-aminopyrine demethylation blood test in healthy dogs. (*Am J Vet Res* 2006;67:1110–1114)

Abbreviations

PCD	Percentage dose of carbon 13 administered as carbon 13-aminopyrine and recovered in gas extracted from blood samples
CUMPCD	Cumulative PCD values up to a given sampling time
CV	Coefficient of variation

A number of tests are used to evaluate the liver; however, none are sensitive and specific for assessment of hepatic function. Abnormalities detected during abdominal palpation and radiography may indicate changes in liver size, but do not provide any indication of hepatic function.[1] Although ultrasonography of the liver may reveal morphologic changes in hepatic structure, it does not permit definitive assessment of hepatic function.[2]

Other modalities commonly used to evaluate the liver include the measurement of serum hepatic enzyme activities and serum bile acid concentrations. Alterations in serum hepatic enzyme activities may indicate hepatocellular damage, which may be associated with a decrease in hepatic function. However, some of these enzymes are released from other tissues during nonhepatic disorders and make interpretation of hepatic function difficult.[3] Presently, measurement of pre- and postprandial serum bile acid concentrations is the most clinically useful hepatic function test available; however, this test is neither sensitive for compromised hepatic function nor specific for identifying a particular hepatic disease.[4] Patients with mild liver disease may have serum bile acids concentrations within the reference range. Additionally, serum bile acids concentrations may be increased in patients with cholestasis in which hepatic function is initially normal.[4] In addition, serum bile acids concentrations do not correlate with the severity of hepatic disease.[4]

The indocyanine green, sulfobromophthalein, and ammonia tolerance tests have been developed to evaluate hepatic function. The indocyanine green and sulfobromophthalein tests evaluate hepatic perfusion and hepatobiliary function, but they use compounds with several disadvantages and are therefore rarely used.[5] The ammonia tolerance test measures the ability of the liver to extract and detoxify ammonia.[6] This test requires rapid processing of samples, and its clinical use is limited by its insensitivity. Therefore, a new test that is both sensitive and specific for assessment of hepatic function in dogs and cats is clearly needed.

In humans, the aminopyrine breath test has been developed to quantify hepatic microsomal enzyme

Received March 28, 2005.
Accepted December 30, 2005.
From the Gastrointestinal Laboratory (Moeller, Steiner, Williams), College of Veterinary Medicine, Texas A&M University, College Station, TX 77843; and Research and Development, The IAMS Company, Lewisburg, OH 45338 (Tetrick, Burr). Dr. Moeller's present address is Dallas Veterinary Surgical Center, 4444 Trinity Mills Rd, Ste 203, Dallas, TX 75287. Dr. Williams' present address is Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana, IL 61802.
Supported by a grant from The Iams Company.
Presented in part at the American College of Veterinary Internal Medicine Veterinary Medical Forum, Seattle, June 2000.
Address correspondence to Dr. Steiner.

Unauthenticated | Downloaded 03/26/23 06:24 AM UTC

function.[7-11] Results of numerous studies[9-12] indicate that this test is clinically useful and that test results correlate with disease severity as assessed by histopathologic evaluation of biopsy specimens obtained from patients with hepatic cirrhosis and chronic hepatitis.

In contrast to humans, breath tests are difficult to perform in dogs and cats, and thus a blood-based test would be preferable for use in these animals. The principles of the carbon 13 (^{13}C)-labeled aminopyrine demethylation blood test have previously been reported[13] and are similar to those of the breath test used in humans. Aminopyrine is administered orally or IV and is demethylated in the liver by microsomal enzymes. As a result, the methyl groups produced are then oxidized to CO_2. The CO_2 diffuses into the vascular space, is eventually carried to the pulmonary alveoli, and is released into the expiratory air.[8,14,15] By use of aminopyrine labeled with various carbon isotopes, either ^{13}C or carbon 14 (^{14}C), the amount of ^{13}C or ^{14}C derived from the labeled aminopyrine can be measured in the expired air (breath test) as either $^{13}CO_2$ or $^{14}CO_2$, respectively.[16] For the breath test, the amount of $^{13}CO_2$ or $^{14}CO_2$ is determined as a percentage of the oral or IV dose of ^{13}C-aminopyrine or ^{14}C-aminopyrine administered that is recovered in expiratory air.[16] Similarly, the blood test involves measuring the amount of $^{13}CO_2$ extracted from blood samples.[13]

Results of a preliminary study[13] indicated that a ^{13}C-aminopyrine demethylation blood test is technically feasible in dogs. In that study, ^{13}C-aminopyrine administered orally at a dose of 2 mg/kg to healthy dogs resulted in a detectible increase in the PCD in all dogs. Another study[17] was performed to evaluate the demethylation kinetics of ^{13}C-aminopyrine administered IV in healthy dogs and determine an appropriate parameter for quantification of aminopyrine demethylation. Results of that study indicated that IV administration of ^{13}C-aminopyrine, as previously detected for oral administration, does not result in any gross clinical adverse effects. In addition, PCD was found to be an appropriate parameter for the quantification of aminopyrine demethylation. Results of that study[17] also indicated that a single blood sample collected 45 minutes after IV administration of ^{13}C-aminopyrine is sufficient for assessment of hepatic demethylating capacity in dogs.

The purpose of the study reported here was to determine the optimal dose of ^{13}C-aminopyrine for use in a ^{13}C-aminopyrine demethylation blood test in healthy dogs. We used a dose of 2 mg/kg as an arbitrary starting dose because this dose is commonly used for the aminopyrine breath test in humans. The ideal dose would be one that gives the lowest variability of PCD values in healthy dogs and is cost-effective for use in a clinical setting.

Materials and Methods

Dogs—Nine young healthy adult dogs (4 males and 5 females) were enrolled in the study. Breeds of dogs included Labrador Retriever (n = 3), Brittany (2), Pointer (1), German Shepherd Dog (1), and Siberian Husky cross (2). Dogs were owned by and housed at an animal research facility[a] and remained in the care of this facility for the duration of the study. All dogs were determined to be healthy on the basis of results of physical examination, CBC, and serum biochemistry analyses. None of the dogs had a history of receiving drugs known to alter hepatic enzyme function. Dogs were closely monitored during and for several hours after each experimental period. The animal care staff monitored dogs for development of gross evidence of clinically adverse effects for several days after each experimental period. The study protocol was approved by the Animal Care and Use Committee at The IAMS Company (protocol No. 990022).

Procedures—The study was divided into 4 experimental periods in which ^{13}C-aminopyrine was administered at doses of 1, 2, 5, or 10 mg/kg. During the first experimental period, ^{13}C-aminopyrine administered at a dose of 2 mg/kg was evaluated, followed by evaluation of ^{13}C-aminopyrine administered at doses of 5, 1, and 10 mg/kg. Each dog was given the same dose of ^{13}C-aminopyrine during each experimental period. There was a 2-week resting period between each experimental period.

Food was withheld from each dog for 12 hours prior to the initiation of each experimental period. A 2-mL baseline blood sample was collected from each dog and placed into an evacuated glass tube[b] containing sodium heparin. The ^{13}C-aminopyrine[c] was dissolved in deionized water, and the solution was sterilized by passage through a 0.1-µm pore-size syringe filter[d] and stored in an amber glass bottle at 4°C until administered. The ^{13}C-aminopyrine was administered IV at the dose that had been predetermined for each experimental period. Additional blood samples (2 mL each) were collected from each dog 30, 45, 60, and 75 minutes after ^{13}C-aminopyrine administration and stored in evacuated tubes containing sodium heparin. Samples were stored at 22°C and shipped overnight to the Gastrointestinal Laboratory at Texas A&M University for analysis.

The CO_2 was extracted from each blood sample by addition of 1 mL of 6N hydrochloric acid.[e] Immediately after addition of the acid, samples were vortexed to prevent acid coagulation and to maximize CO_2 release. Gas samples were then analyzed by use of fractional mass spectrometry using an automated breath-carbon analyzer[f] to measure the fraction of $^{13}CO_2$ in the CO_2 extracted from blood samples.

Data analysis—The PCD and CUMPCD values were calculated as previously described.[13,18] The PCD values for each sample time in each experimental period were compared with the baseline sample for that experimental period by use of repeated-measures ANOVA. This was performed to determine whether there was a significant difference between PCD values for each sample time and the baseline value. The Dunnett multiple comparison test was used as a posttest to compare values for each sample time with the baseline value.

Mean ± SD and CV values were calculated for PCD and CUMPCD for each dose. Mean CV values for PCD and CUMPCD for all doses and sample times were compared by use of a *t* test. The PCD values for the various doses were compared by use of repeated-measures ANOVA and Bonferroni's multiple comparison tests. A statistical analysis package[g] was used for data analysis; values of $P < 0.05$ were considered significant.

Results

Gross evidence of clinically adverse effects was not observed in dogs during, or for a period of several days after, each experimental period. The PCD values increased initially and then began to decrease with time for all dogs and all doses. Consequently, the mean PCD values for each dose also increased with time and

Unauthenticated | Downloaded 03/26/23 06:24 AM UTC

peaked 45 minutes after administration of ^{13}C-aminopyrine at doses of 1, 2, and 10 mg/kg and 30 minutes after administration of ^{13}C-aminopyrine at a dose of 5 mg/kg (**Figure 1**). The CUMPCD values for all dogs and doses increased with time, and the mean CUMPCD values for each dose also increased with time (**Figure 2**).

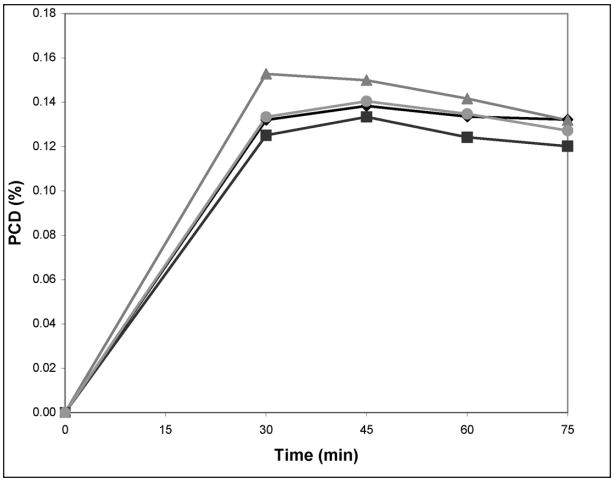

Figure 1—Mean PCD values after IV administration of ^{13}C-labeled aminopyrine at doses of 1 (diamond), 2 (square), 5 (triangle), and 10 (circle) mg/kg in 9 dogs. Time 0 = Baseline sample before administration of ^{13}C-aminopyrine.

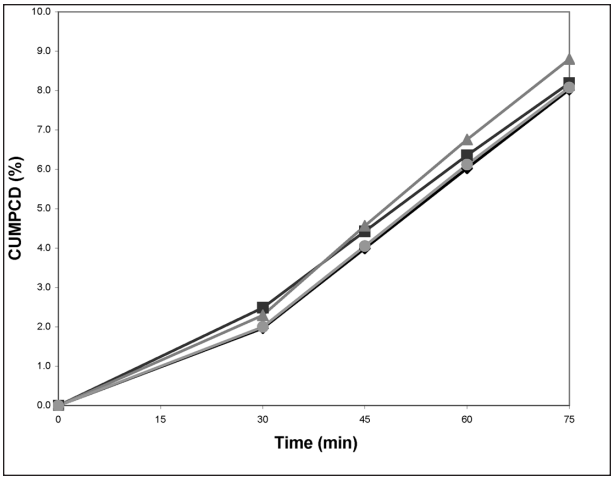

Figure 2—Mean CUMPCD values after IV administration of ^{13}C-aminopyrine at doses of 1 (diamond), 2 (square), 5 (triangle), and 10 (circle) mg/kg in 9 dogs. Time 0 = Baseline sample before administration of ^{13}C-aminopyirne.

As determined by repeated-measures ANOVA, the mean PCD values for all sample times differed significantly ($P < 0.001$ for all doses) with time for all 4 doses. When compared separately by use of the Dunnett multiple comparison test, mean PCD values after ^{13}C-aminopyrine administration differed significantly ($P < 0.01$ for all 4 doses) from the baseline sample.

The mean ± SD CV (21.5 ± 4.4%) for the PCD value from all doses was significantly ($P < 0.01$) lower than the mean ± SD CV (23.8 ± 4.6%) for the CUMPCD value for all the doses and sample times (**Table 1**). Therefore, the PCD value was used as an estimate of hepatic demethylation of ^{13}C-aminopyrine for the remainder of the study.

No significant ($P = 0.41$, 0.73, 0.58, and 0.70) differences were detected in mean PCD values among the 4 doses (1, 2, 5, and 10 mg/kg, respectively) when compared by use of repeated-measures ANOVA. No dose was significantly ($P > 0.05$ for all comparisons) different from any other dose.

To compare interindividual variabilities, CV values were calculated for each dose. No significant ($P = 0.07$) differences in the mean CV values for PCD between doses were detected. There were also no significant (all values of $P > 0.05$) differences when the mean CVs for PCD for individual doses were compared.

Discussion

In the study reported here, gross evidence of clinically adverse effects was not observed in any dog during any experimental period. Although gross evidence of clinically adverse effects was not seen, subclinical adverse effects, such as subclinical organ damage, could not be definitively excluded. The safety of ^{13}C-aminopyrine needs to be further evaluated in dogs with altered hepatic function.

A 2-week resting period was provided to dogs between experimental periods to eliminate any possible induction of hepatic demethylating enzymes caused by ^{13}C-aminopyrine administration. We assumed that any microsomal enzyme induction that may have occurred after administration of a single dose of ^{13}C-aminopyrine would have returned to baseline values during this 2-week resting period. To prove or

Table 1—Mean ± SD (CV) values for PCD and CUMPCD before (time 0; baseline) and after IV administration of ^{13}C-aminopyrine at doses of 1, 2, 5, and 10 mg/kg in 9 dogs.

	PCD				CUMPCD			
Time (min)	1	2	5	10	1	2	5	10
0	0 (NA)	0 (NA)	0 (NA)	0 (NA)	0 (NA)	0 (NA)	0 (NA)	0 (NA)
30	0.1321 ± 0.0310 (23.6)	0.1251 ± 0.0223 (17.8)	0.1528 ± 0.0419 (27.4)	0.1334 ± 0.0437 (32.7)	1.9678 ± 0.4639 (23.6)	2.4878 ± 0.5391 (21.7)	2.2933 ± 0.6281 (27.4)	2.0011 ± 0.6557 (32.8)
45	0.1383 ± 0.0311 (22.5)	0.1334 ± 0.0239 (17.9)	0.1500 ± 0.0339 (22.6)	0.1404 ± 0.0363 (25.8)	3.9889 ± 0.8888 (22.3)	4.4267 ± 0.8629 (19.5)	4.5644 ± 1.1921 (26.1)	4.0544 ± 1.2512 (30.9)
60	0.1336 ± 0.0237 (17.8)	0.1242 ± 0.0227 (18.2)	0.1416 ± 0.0268 (18.9)	0.1347 ± 0.0317 (23.6)	6.0267 ± 1.2269 (20.4)	6.3600 ± 1.1551 (18.2)	6.7522 ± 1.6291 (24.1)	6.1178 ± 1.7469 (28.6)
75	0.1322 ± 0.0239 (18.1)	0.1201 ± 0.0230 (19.1)	0.1319 ± 0.0211 (16.0)	0.1272 ± 0.0280 (22.0)	8.0222 ± 1.4936 (18.6)	8.1922 ± 1.4237 (17.4)	8.8022 ± 1.9658 (22.3)	8.0811 ± 2.1647 (26.8)

NA = Not applicable.

Unauthenticated | Downloaded 03/26/23 06:24 AM UTC

disprove this contention, measurement of microsomal enzyme activities in hepatic biopsy specimens would have been required. However, obtaining hepatic biopsy specimens in dogs used in our study was not possible because of animal welfare guidelines at the facility in which dogs were housed. Additionally, assays for hepatic microsomal enzyme function in dogs were not available.

Although the mean PCD value for ^{13}C-aminopyrine administered at a dose of 5 mg/kg peaked earlier than that for ^{13}C-aminopyrine administered at a dose of 2 mg/kg (as well as at the other 2 doses), we do not believe that this was caused by induction of hepatic microsomal enzyme function. If this were the case, we would have expected the mean PCD value for ^{13}C-aminpyrine administered at a dose of 1 mg/kg to peak earlier than that for ^{13}C-aminopyrine administered at a dose of 2 mg/kg and perhaps at a dose of 5 mg/kg. Because mean PCD values for ^{13}C-aminopyrine administered at doses of 1 and 10 mg/kg did not peak earlier than or at the same time as that for ^{13}C-aminopyrine administered at a dose of 2 mg/kg, we are confident in our assumption that hepatic microsomal induction did not lead to the delayed peak time for the PCD value detected after administration of ^{13}C-aminopyrine at a dose of 5 mg/kg.

Data for ^{13}C-aminopyrine administered at a dose of 2 mg/kg were collected during a kinetic study performed previously.[17] The remaining 3 doses of ^{13}C-aminopyrine were evaluated during various experimental periods so that doses were actually evaluated in the following order: 2, 5, 1, and 10 mg/kg. All dogs received the same dose during each experimental period to enhance the possibility of identifying potential adverse effects of repeated ^{13}C-aminopyrine administration.

Intravenous administration of ^{13}C-aminopyrine resulted in an increase in the PCD value of gas extracted from blood samples in all 9 dogs and for all experimental periods. For all 4 doses, the mean PCD value at each sample time after ^{13}C-aminopyrine administration was significantly greater than the mean PCD value at baseline.

One of the parameters used to assess the potential clinical usefulness of a new diagnostic test is interindividual variability of that test in a group of healthy animals. This is based on the assumption that the lower the interindividual variability in healthy dogs, the easier it would be to differentiate between clinically healthy dogs and dogs with disease. Interindividual variability can be assessed by calculating CV. In the study reported here, the CV values for PCD and CUMPCD were calculated and compared. Results of our study indicated that the mean CV value for PCD was significantly ($P < 0.01$) lower than the mean CV value for CUMPCD. Taking into consideration the relative ease of collecting a single blood sample after administration of ^{13}C-aminopyrine for determination of the PCD value, compared with collection of multiple samples as necessary for determination of CUMPCD, this finding indicates that determination of PCD is preferable to CUMPCD for assessment of hepatic ^{13}C-aminopyrine demethylation. These findings are consistent with that of another study.[17] However, whether determination of CUMPCD would be clinically more useful than determination of PCD in dogs with altered hepatic function cannot be definitively determined on the basis of results of the study reported here.

Significant differences in CV values among the 4 doses were not detected. However, ^{13}C-aminopyrine administered at a dose of 2 mg/kg had the lowest CV value, compared with the other doses. As previously mentioned, a low interindividual variability in healthy dogs is desirable. Use of ^{13}C-aminopyrine at a dose of 2 mg/kg would also be more cost-effective than use of ^{13}C-aminopyrine at doses of 5 and 10 mg/kg because less ^{13}C-aminopyrine is needed for the test. Thus, we concluded that administration of ^{13}C-aminopyrine at a dose of 2 mg/kg is appropriate for use in the ^{13}C-aminopyrine demethylation blood test in healthy dogs. Unfortunately, in the study reported here, dogs with hepatic dysfunction were not evaluated. Demethylation kinetics may be severely altered in dogs with hepatic dysfunction; therefore, administration of ^{13}C-aminopyrine at a dose of 2 mg/kg may not be optimal for use in dogs with hepatic dysfunction. Additional studies are required to fully evaluate the clinical usefulness of a ^{13}C-amionpyrine demethylation blood test in dogs with hepatic disease. An initial clinical study[19] has been reported since the completion of the study reported here, but further studies are required.

a. Nutritional Research Center, The IAMS Co, Lewisburg, Ohio.
b. BD vacutainer, Preanalytical Solutions, Franklin Lakes, NJ.
c. 4-dimethyl-$^{13}C_2$-aminoantipyrine, Isotech Inc, Miamisburg, Ohio.
d. Gelman Sciences, Supor Acrodisc, 0.1 µm, sterile, VWR Scientific Products Corp, West Chester, Pa.
e. Hydrochloric acid, Sigma Chemical Co, St Louis, Mo.
f. Automated breath carbon analyzer, Europa House, Crewe, UK.
g. GraphPad Prism, version 3.0, GraphPad Software Inc, San Diego, Calif.

References

1. Ticer JW. Roentgen signs of endocrine disease. *Vet Clin North Am Small Anim Pract* 1977;7:465–486.
2. Cartee RE. Diagnostic real time ultrasonography of the liver of the dog and cat. *J Am Anim Hosp Assoc* 1981;17:731–737.
3. Center SA, ManWarren T, Slater MR, et al. Evaluation of twelve-hour preprandial and two-hour postprandial serum bile acids concentrations for diagnosis of hepatobiliary disease in dogs. *J Am Vet Med Assoc* 1991;199:217–226.
4. Center SA. Serum bile acids in companion animal medicine. *Vet Clin North Am Small Anim Pract* 1993;23:625–657.
5. Center SA, Bunch SE, Baldwin BH, et al. Comparison of sulfobromophthalein and indocyanine green clearances in the cat. *Am J Vet Res* 1983;44:727–730.
6. Stahl J. Studies of the blood ammonia in liver disease: its diagnostic, prognostic, and therapeutic significance. *Ann Intern Med* 1963;58:1–24.
7. Gikalov I, Bircher J. Dose dependence of the 14C-aminopyrine breath test. Intrasubject comparison of tracer and pharmacological doses. *Eur J Clin Pharmacol* 1977;12:229–233.
8. Henry DA, Kitchingman G, Langman MJ. [14C]Aminopyrine breath analysis and conventional biochemical tests as predictors of survival in cirrhosis. *Dig Dis Sci* 1985;30:813–818.
9. Baker AL, Krager PS, Kotake AN, et al. The aminopyrine breath test does not correlate with histologic disease severity in patients with cholestasis. *Hepatology* 1987;7:464–467.

Unauthenticated | Downloaded 03/26/23 06:24 AM UTC

10. Miotti T, Bircher J, Preisig R. The 30-minute aminopyrine breath test: optimization of sampling times after intravenous administration of 14C-aminopyrine. *Digestion* 1988;39:241–250.

11. Beyeler C, Reichen J, Thomann SR, et al. Quantitative liver function in patients with rheumatoid arthritis treated with low-dose methotrexate: a longitudinal study. *Br J Rheumatol* 1997;36:338–344.

12. Schneider JF, Baker AL, Haines NW, et al. Aminopyrine N-demethylation: a prognostic test of liver function in patients with alcoholic liver disease. *Gastroenterology* 1980;79:1145–1150.

13. Moeller EM, Steiner JM, Williams DA, et al. Preliminary studies of a canine 13C-aminopyrine demethylation blood test. *Can J Vet Res* 2001;65:45–49.

14. Schoeller DA, Kotake AN, Lambert GH, et al. Comparison of the phenacetin and aminopyrine breath tests: effect of liver disease, inducers and cobaltous chloride. *Hepatology* 1985;5:276–281.

15. Villeneuve JP, Infante-Rivard C, Ampelas M, et al. Prognostic value of the aminopyrine breath test in cirrhotic patients. *Hepatology* 1986;6:928–931.

16. Hofmann AF, Lauterburg BH. Breath test with isotopes of carbon: progress and potential. *J Lab Clin Med* 1977; 90:405–411.

17. Moeller EM, Steiner JM, Williams DA, et al. Kinetic analysis of demethylation of 13C-aminopyrine in healthy dogs. *Am J Vet Res* 2004;65:159–162.

18. Boutton T. Tracer studies with 13C-enriched substrates: humans and large animals. In: Coleman A, Fry B, eds. *Carbon isotope techniques*. New York: Academic Press, 1991;219–242.

19. Chiaramonte D, Steiner JM, Broussard JD, et al. Use of a ^{13}C-aminopyrine blood test: first clinical impressions. *Can J Vet Res* 2003; 67:183–188.

Unauthenticated | Downloaded 03/26/23 06:24 AM UTC