**Determination of abomasal emptying rate in suckling calves by use of nuclear scintigraphy and acetaminophen absorption**

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**Objective**—To develop nuclear scintigraphic and acetaminophen absorption methods for measuring abomasal emptying rate in suckling calves.

**Animals**—9 male Holstein-Friesian calves < 30 days old.

**Procedure**—Calves were fed 2 L of milk replacer, fresh cow's milk, or an isoosmotic (150mM) solution of NaCl, NaHCO₃, or sodium acetate containing technetium TC 99m-diethylenetriamine-pentaacetic acid (370 MBq) and acetaminophen (50 mg/kg). Right lateral scintigraphic images and venous blood samples were obtained periodically after feeding. Two power exponential equations were fitted to the scintigraphic data, and 3 pharmacokinetic models were fitted to the acetaminophen concentration-time data.

**Results**—Data from 32 feedings were analyzed, with half emptying time for scintigraphic evaluation ranging from 29 to 202 minutes. Siegel's modified power exponential equation provided a better description of the scintigraphic data than did Elashoff's power exponential equation. The first derivative of Siegel's modified power exponential formula provided the best pharmacokinetic model for the acetaminophen absorption data. Time to maximal acetaminophen concentration (Tmax) provided the most accurate index of abomasal emptying rate from the acetaminophen concentration-time data.

**Conclusions and Clinical Relevance**—Abomasal emptying rate is best determined in healthy suckling calves by use of Siegel's modified power exponential equation to model the scintigraphic data. Pharmacokinetic calculation of Tmax from the acetaminophen absorption curve provides an alternative method for determining abomasal emptying rate in healthy suckling calves that is accurate, inexpensive, practical, and safe. However, it is unclear whether diarrhea would alter the acetaminophen absorption curve in calves. (Am J Vet Res 2005;66:364–374)

A controversial aspect when scintigraphy is used to measure gastric emptying rate is the method used to describe the scintigraphic data. Various models and mathematical curves have been used for scintigraphic evaluation of gastric emptying. Ideally, the mathematical curve should be sufficiently flexible to describe an initial (lag) phase and a late (exponential) phase and...
should have as few parameters as possible, with each parameter having a clear graphic interpretation. Since the early 1980s, most scintigraphic emptying curves have been modeled by use of Elashoff’s power exponential curve or Siegel’s modified power exponential curve. It is currently unclear as to which model provides the best fit to the data; however, Siegel’s modified power exponential curve has the theoretic advantage of characterizing the lag phase in minutes instead of a nondimensional number (ie, \( \beta \)) reported in Elashoff’s power exponential curve. Accordingly, an objective of the study reported here was to determine which power exponential equation should be used to describe the scintigraphic data.

The rate of abomasal emptying is believed to play an important role in the etiopathogenesis of abomasal disorders in calves and is an important determinant of the rate of resuscitation in diarrheic dairy calves treated by administration of oral electrolyte solutions. The emptying rate also provides information regarding the rate of nutrient delivery to the small intestine during nutrition studies in suckling calves. A practical and inexpensive method for assessing abomasal emptying rate in calves would therefore be useful for clinical and research purposes. Although ultrasonography has been validated as a noninvasive method for assessing abomasal emptying rate, the suitability of other techniques should be investigated.

Acetaminophen is a widely used orally administered analgesic and antipyretic in humans, and acetaminophen absorption provides an accurate method of determining the emptying rate of liquid-phase meals in humans and horses but not the emptying rate of semisolid meals. We hypothesized that acetaminophen absorption would provide an accurate method of measuring abomasal emptying rate in suckling calves because milk replacer and fresh cow’s milk are liquid-phase meals. When administered orally, acetaminophen is absorbed in the small intestine, with the rate limiting step being the rate of gastric emptying in animals with physiologic small intestinal function. Acetaminophen absorption has been used to evaluate gastric emptying of liquid meals in horses, and a preliminary description exists for calves. Accordingly, the main objective of the study reported here was to develop methods to measure abomasal emptying rate by use of nuclear scintigraphy and acetaminophen absorption. A second objective was to evaluate the relationship between scintigraphic \( t_{1/2} \) and various indices derived from the actual acetaminophen absorption curve and from pharmacokinetic modeling of the acetaminophen absorption curve.

Materials and Methods

Animals—Nine healthy male Holstein-Friesian calves were obtained from a university dairy farm within the first week after birth. A cannula was surgically inserted in the abomasal body of each calf within 5 days after birth, as described elsewhere. After the calves recovered from surgery, they were housed unrestrained in separate stalls bedded with wood shavings. Calves were fed twice per day. Each meal consisted of a medicated milk replacer (minimum crude protein, 20%; minimum crude fat, 20%; maximum crude fiber, 0.15%; minimum calcium, 0.5%; maximum calcium, 1.0%; minimum phosphorus, 0.6%; and deconjugate, (50 mg/kg of milk replacer [equivalent to 0.5 mg of deconjugate/kg of body weight/d]). The milk replacer contained proteins derived only from milk and was fed twice daily at the rate of 60 mL/kg. Calves had access to fresh water at all times. The study was approved by an institutional animal care and use committee.

Experimental protocol—The study consisted of 2 experiments. Calves were used only in 1 of the 2 experiments.

Experiment 1—Five calves that were at least 5 days old, had a cannula inserted in the abomasal body at least 2 days before, and had been fed the preceding feeding of milk replacer at least 10 hours earlier were weighed and placed in a movable calf pen that allowed sternal recumbency and standing but prevented excessive lateral movement. The calf pens were modified in a manner similar to that described for use with sheep. Each of the 5 calves received 5 treatments in a Latin-square design; there was a washout period of at least 36 hours between successive treatments. We added technetium TC 99m-diethylamino-pentaacetic acid (\(^{99m}\)Tc-DTPA; 370 MBq) and acetaminophen (N-acetyl-L-aminophenol or paracetamol, 50 mg/kg, IM, every 6 hours) to 2 L of solution. The solution was mixed (at 18°C to 24°C) and placed in a bottle with a nipple. A calf was then allowed to suckle the mixture. The 5 treatments were milk replacer, milk replacer with atropine (0.01 mg/kg, IV; then 0.02 mg/kg, SC, q 30 min), isoosmotic NaCl (150mM), isoosmotic sodium bicarbonate (150mM), and isoosmotic sodium acetate (150mM). Atropine was administered because we were interested in examining a wide range of emptying rates and anticipated that atropine would delay abomasal emptying rate. The acetaminophen dose was determined on the basis of results of preliminary experiments and a pharmacokinetic study in crossbred male calves in which those authors recommended an initial dose of 50 mg/kg, IM, followed by subsequent doses of 30 mg/kg, IM, every 6 hours.

Experiment 2—Four calves that were at least 5 days old, had a cannula inserted in the abomasal body at least 2 days before, and had been fed the preceding feeding of milk replacer at least 10 hours earlier were weighed and placed in a movable calf pen. Each of the 4 calves received 3 treatments; there was a washout period of at least 36 hours between successive treatments. We added \(^{99m}\)Tc-DTPA (370 MBq) and acetaminophen (50 mg/kg) to 2 L of solution. The solution was mixed well (at 18°C to 24°C) and placed in a nipple bottle. A calf was then allowed to suckle the mixture. The 3 treatments were randomly assigned for each calf and consisted of milk replacer, fresh milk obtained from Holstein cows, or milk replacer again. Each calf received milk replacer twice to provide an estimate of the total variability in the measurement of abomasal emptying rate.

Scintigraphic measurements—Scintigraphic images were obtained for 30 seconds at 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, and 240 minutes after the end of suckling with the calf in a standing position. Except for the recording obtained immediately after the end of suckling, image collection started 15 seconds before the stated time to ensure that the time represented the middle of the counting interval. Time intervals were subsequently corrected on the basis of when a calf suckled and therefore represented the time since the start of suckling.

Scintigraphic images were obtained by use of a parallel-hole, low-energy, all-purpose gamma camera. The gamma camera was positioned for acquisition of dorsal and right lateral images. A dorsal image was obtained in a dynamic series during suckling to assist in identifying the anatomic location of the abomasum and to monitor the route of the sucked
fluid, thereby monitoring closure of the esophageal groove. The dorsal image used a 64 X 64 matrix, and data for the dynamic series were collected for 40 frames at a rate of 3 s/frame. The right lateral view also used a 64 X 64 matrix, but data were collected at a rate of 30 s/frame to enable us to monitor the rate of abomasal emptying after suckling.

After all images were acquired in experiments 1 and 2, calves remained confined in the moveable calf pens but were returned to their separate stalls. A radioactive tag was placed at the entrance to each stall; this tag was removed when activity had decreased to < 0 mR/h (usually within 24 hours), at which time calves were removed from the moveable calf pens and housed separately in stalls bedded with wood shavings. During the period when the radioactive tags were displayed, contact with the calves was kept to a minimum and investigators were required to wear disposable gloves.

Calves were euthanized at the end of the study by IV administration of an overdose of sodium pentobarbital (60 mg/kg). The abdominal incision, cannula site, and abomasal lumen were inspected for evidence of localized infection, peritonitis, or ulcers, and the carcasses were incinerated.

Analysis of scintigraphic data—A static right lateral image was used to define the abomasum as the region of interest (ROI), which was analyzed by use of a nuclear medicine software program. Intensity of the static image was adjusted to the hot body color scale of the software program, and the lower window was set at approximately –1,300. The upper window was then set to approximately 1,320 so that saturation was not evident in the first postnursing image. The ROI was drawn by use of the irregular option in the software program, thereby tracing the outline of the abomasum in an objective manner. The pyloric antrum was not included in the ROI because this region was not initially identifiable. Toward the end of the experiments, the pyloric antrum was sometimes identifiable but was usually superimposed on the small intestine. The total number of counts in the ROI recorded for each 30-second data collection period was determined, and decay was corrected by use of the following equation:

\[ A = A_0 \times e^{-\lambda t}, \]

where \( A \) is the corrected radioactive count at time \( t \), \( A_0 \) is the count at time 0, and \( \lambda \) is the decay constant. The value for \( \lambda \) was calculated as 0.693 \( /T_{1/2} \), with \( T_{1/2} \) representing the half-life of the isotope, which was 6.02 hours.

Scintigraphy model 1—An activity-versus-time curve was generated for each experiment, and Elashoff's power exponential equation was used to fit a modeled curve to the data. The equation was as follows:

\[ y(t) = 1 - (1 - e^{-\beta t})^{k}, \]

where \( y(t) \) is the proportion of maximal radioactivity remaining at time \( t \) (measured in minutes) from the start of suckling, \( k \) is the abomasal emptying rate, and \( \beta \) is a rate constant representing the extrapolated \( y \)-intercept for the terminal portion of the curve and is the time (measured in minutes) when the second derivative of the function is equal to zero. The highest value for \( y(t) \) represents the end of the lag phase and the start of abomasal emptying and therefore was set at 1.00; if radioactivity before the time of peak radioactivity was > 1 as follows:

\[ t_{lag} = \frac{\ln(1 - 2^{1/(k \beta)})}{\beta}. \]

The scintigraphic lag phase \( t_{lag} \), which provided an index of the time from the start of suckling to the start of abomasal emptying, was calculated for \( \beta > 1 \) as follows:

\[ t_{lag} = \frac{\ln(y)}{\beta}. \]

Scintigraphy model 2—An activity-versus-time curve was generated for each experiment, and Elashoff's power exponential equation was used to fit a modeled curve to the data. The equation was as follows:

\[ y(t) = 2^{e^{\lambda t^{1/2}}}, \]

where \( y(t) \) is the fraction of peak radioactivity remaining at time \( t \) from the start of suckling in minutes, \( 3^{-2} \) is the time required for the initial radioactivity to be reduced by half, and \( \beta \) is a constant that determines the shape of the curve.

Measurement of plasma concentration of acetaminophen—Venous blood samples were obtained for determination of plasma concentration of acetaminophen 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, and 240 minutes after the end of suckling isosmotic sodium acetate, sodium bicarbonate, and sodium chloride solutions and 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, and 720 minutes after the end of suckling milk replacer or cow's milk. Blood samples were collected via a catheter that had been aseptically inserted into a jugular vein of each calf before the study began. Blood samples were collected into 6-mL tubes containing sodium fluoride and potassium oxalate. Samples were centrifuged at 1,000 \( \times \) g for 15 minutes, and 3 mL of plasma was then harvested and stored at –20°C. All samples were analyzed for acetaminophen concentration within 3 months after collection.

Plasma was thawed at approximately 20°C and analyzed spectrophotometrically by use of a colorimetric nitration assay. Briefly, the assay was performed after removing interfering plasma proteins via deproteinization of a 0.1-mL plasma sample by the addition of 1 mL of 3% trichloroacetic acid followed by centrifugation for 6 minutes and harvest of the supernatant. The supernatant (0.8 mL) was mixed with 0.2 mL of 0.07M sodium nitrite solution, incubated at 37°C for 10 minutes, and alkalized by the addition of 0.1 mL of 8M sodium hydroxide. Absorption was measured at 430 nm against a plasma sample obtained at time 0 (before suckling), and the absorbance was proportional to the acetaminophen concentration in the plasma sample.

Mean ± SD recovery of acetaminophen in pooled calf plasma was 100.3 ± 4.9% (3 separate assays). The intra-assay and interassay coefficient of variation for pooled calf plasma was 3.1% and 5.3%, respectively (n = 10 assays). The assay was linear from 0 to 100\( \mu \)g/mL (R² = 0.9996). Limit of detection of the assay for calf plasma was 3 \( \mu \)g/mL. Therefore, the acetaminophen assay had acceptable recovery, precision, linearity, and sensitivity.

Analysis of acetaminophen absorption data—The maximum actual plasma concentration (Cmax) and time of Cmax (Tmax) were obtained from a plot of the plasma acetaminophen concentration-time data. Values for \( AUC_{\text{lag}} \) under the concentration curve (AUC) from 0 to 60 minutes (ie, \( AUC_{0-60} \)), 0 to 120 minutes (ie, \( AUC_{120} \)), and 0 to 240 minutes (ie, \( AUC_{240} \)) were calculated by use of the linear trapezoidal method. This method calculates the observed radioactivity dose had been emptied from the abomasum, was calculated as follows:

\[ \text{scintigraphic} \; \frac{1}{2} = \frac{(-1/\beta) \times (\ln(1 - 2^{1/(k \beta)}))}{\beta}. \]
Acetaminophen pharmacokinetic model 2—The overall absorption process for most orally administered drugs follows first-order kinetics, indicating that the rate of absorption is proportional to the amount of substance administered. A biexponential equation that assumes first-order kinetics for absorption and elimination from the central compartment and no lag time for absorption was applied as follows:

\[ C(t) = A \times (e^{-kt} - e^{-k2t}) \]

where A is a composite constant, kel is the apparent elimination rate constant from the central compartment, and ka is the apparent absorption rate constant. The value for A was calculated as follows:

\[ A = \frac{(F \times D \times ka) \times (V \times [ka - kel])}{D} \]

where V is the volume of distribution in the central compartment and both F and V are unknown. The t\text{\_4/5} for absorption was not discernible in our data and therefore was considered to equal 0. A hybrid constant (ie, ka) is dependent on the rates of all contributing processes but is determined primarily by the rate-limiting step, which is the abomasal emptying rate.29

The aforementioned equation was reformulated to the following:

\[ C(t) = A \times (e^{\frac{1}{T2}} - e^{\frac{1}{T1}}) \]

where T1 is the time constant for absorption and T2 is the time constant for elimination. We used this equation because we were more interested in use of T1 to calculate acetaminophen t\text{\_1/2} for model 2, we expressed the preceding equation as follows:

\[ T_{max} = \frac{\ln(T2/T1)}{([1/T1] - [1/T2])} \]

The Cmax for model 2 was calculated by use of the following equation:

\[ C_{max} = A \times (e^{\frac{3\times T_{max}}{T1}} - e^{\frac{3\times T_{max}}{T2}}) \]

Because acetaminophen is incompletely absorbed and acetaminophen may have a multiple-compartment distribution, an accurate estimate of distribution volume and clearance cannot be obtained when acetaminophen is administered orally.30 However, an estimate for F for model 2 was calculated as follows:

\[ F = A \times V \times [([1/T1] - [1/T2])] / (D \times T1) \]

For this equation, we assumed that V = 0.64 L/kg (the calculated volume obtained in crossbred calves following IM injection of acetaminophen in 1 study31). A similar mean value for V (0.66 to 0.70 L/kg) was recently determined in another study32 of 24- to 29-day-old calves.

Acetaminophen pharmacokinetic model 3—Most drugs follow second-order kinetics or more complex pharmacokinetic models for elimination instead of the first-order kinetics assumed for pharmacokinetic model 2. In particular, pharmacokinetic studies33,34 in humans indicate that IV administration of acetaminophen is better described by a 2-compartment open model instead of a 1-compartment open model. Accordingly, we assumed first-order kinetics for absorption, no lag time for absorption, and a 2-compartment open model for elimination to create the following triexponential equation:

\[ C(t) = (A \times e^{\frac{t}{T1}} + B \times e^{\frac{t}{T2}} + C \times e^{\frac{t}{T3}}) \]

where A and B are the zero-time intercepts associated with the α and β elimination phases, respectively; α and β are

AUC from the start of suckling to time t (ie, AUC{\text{\_0-t}}) by use of the following equation:

\[ \text{AUC{\text{\_0-t}}} = \sum_{t=1}^{n} (C_i + C_{i+1}) / 2 \]

where t is the ith time and C_i is plasma concentration at t_i. The AUC{\text{\_0-t}} is believed to provide a good index of absorption kinetics and therefore abomasal emptying rate because plasma acetaminophen concentration in the first 60 minutes is dependent primarily on absorption, rather than metabolism or elimination.27

The aforementioned equation was reformulated to the following:

\[ C(t) = e^{\frac{t}{T2}} - e^{\frac{t}{T1}} \]

where T1 is the time constant for absorption and T2 is the time constant for elimination. We used this equation because we were more interested in use of T1 to calculate acetaminophen t\text{\_1/2} for model 2, we expressed the preceding equation as follows:

\[ T_{max} = \ln(T2/T1)/([1/T1] - [1/T2]) \]

The Cmax for model 2 was calculated by use of the following equation:

\[ C_{max} = A \times (e^{\frac{3\times T_{max}}{T1}} - e^{\frac{3\times T_{max}}{T2}}) \]

Because acetaminophen is incompletely absorbed and acetaminophen may have a multiple-compartment distribution, an accurate estimate of distribution volume and clearance cannot be obtained when acetaminophen is administered orally.30 However, an estimate for F for model 2 was calculated as follows:

\[ F = A \times V \times [([1/T1] - [1/T2])] / (D \times T1) \]

For this equation, we assumed that V = 0.64 L/kg (the calculated volume obtained in crossbred calves following IM injection of acetaminophen in 1 study31). A similar mean value for V (0.66 to 0.70 L/kg) was recently determined in another study32 of 24- to 29-day-old calves.
hybrid constants; and \( ka \) is the apparent rate constant for absorption.

Similar to before, we were more interested in determining \( T1 \) and its associated SE, \(^{39} \) rather than the associated \( ka \). Thus, the equation was reformulated as follows:

\[
C(t) = (A \times e^{t/T1} + B \times e^{t/T2}) - (A \times e^{t/T1}),
\]

where \( T2 \) and \( T3 \) are hybrid time constants. The \( C_{max} \) and \( T_{max} \) for model 3 were determined graphically after applying the estimated values for \( A \), \( B \), \( T1 \), \( T2 \), and \( T3 \) to the preceding equation.

Acetaminophen \( t_{1/2} \) for model 3 was calculated by use of the following equation:

\[
\text{acetaminophen } t_{1/2} = T1.
\]

The AUC was calculated by use of the trapezoidal rule as follows:

\[
\text{AUC}_{\infty} = \sum_{i=0}^{n-1} (t_{i+1} - t_i) \times (C_i + C_{i+1})/[2 + (C_n \times T3)],
\]

where \( \text{AUC}_{\infty} \) is the total AUC and \( C_n \) is the last concentration. An estimate for \( F \) for model 3 was calculated as follows:

\[
F = \frac{\text{AUC}_{\infty}}{D}.
\]

Safety of the acetaminophen absorption test—Blood samples were obtained from the catheter inserted in the jugular vein of each calf at the start and end of experiments 1 and 2 to enable us to determine whether acetaminophen administration was safe. Hepatotoxicosis was evaluated by use of paired \( t \) tests to compare serum activities of \( \alpha \)-glutamyltransferase (GGT) and \( \gamma \)-glutamyltransferase (GGT) and serum concentrations of total bilirubin and cholesterol at the start and end of the experiments. Serum biochemical analyses were conducted by use of automated methods.\(^7\)

Statistical analysis—Data were fit by use of nonlinear regression,\(^1\) and the best scintigraphic and pharmacokinetic models were selected on the basis of the lowest value for Akaike's information criterion, visual examination of plots of observed-versus-predicted concentrations, and examination of residual plots.

Multivariable regression analysis\(^8\) was used to determine the linear association between acetaminophen absorption parameters and scintigraphic \( t_{1/2} \) by use of a dummy variable coding for each calf. This ANCOVA approach accounts for between-subject variability, thereby increasing the precision with which slope and intercept coefficients for the regression line can be estimated.\(^39\) The approach enforces a uniform slope but a different intercept value for each calf; this approach is reasonable whenever the slopes are similar,\(^32\) as was the situation for the study reported here. Dummy variables (\( C_i \) through \( C_{n} \)) were defined. The value for \( C_i \) was 1 for call i when \( i < n \) and –1 for call i when \( i = n \); otherwise \( C_i \) was 0.

The following regression equation was used to analyze the linear relationship between the dependent variable (\( y \)) and scintigraphic \( t_{1/2} \):

\[
y = b_0 + (\Sigma b_i C_i) + b_i,
\]

where \( b_0 \) is the intercept value, \( b_i \) is the coefficient value for the \( i \)th calf, and \( b_{s} \) is the coefficient value for scintigraphic \( t_{1/2} \). Dummy variables were entered into the model first to account for between-calf differences before analyzing the main factor of interest. Coefficients associated with \( C_i \) describe how much the intercept values for each calf vary from the mean, but this information was of minimal interest in the study reported here, and only the coefficients for \( b_{s} \) and \( b_i \) were reported. The adequacy of the final regression model was evaluated by examining residual plots and the normal probability plot of the standardized residuals. Outliers or influential observations were identified by calculating Cook's distance.

Clearance half-time was calculated by use of the estimated value for \( T2 \) in acetaminophen pharmacokinetic model 2 and the following equation:

\[
\text{Clearance half-time} = T2.
\]

Apparent \( F \) for models 1, 2, and 3 and clearance half-time were compared for the various test solutions by use of a repeated-measures ANOVA.\(^1\)

Results—Calves remained healthy for the duration of the study and readily suckled the 2 L of each test solution (mean suckling time, 2.4 minutes; range, 1.6 to 5.9 minutes). During necropsy, there was no evidence of infection of the abdominal incision or cannula site and abomasal ulcers were not observed in any of the calves.

Scintigraphy—A modified exponential formula provided an excellent fit to the scintigraphic data for 32 of 37 feedings; the exception was when atropine was administered in conjunction with milk replacer (Figure 1).
Atropine administration induced a biphasic rate of emptying that could not be accurately modeled as a single exponential equation; instead, the emptying process could be generally characterized as a rapid initial rate of emptying followed by a much slower rate of emptying. The acetaminophen absorption curve also reflected an initial rapid rate of absorption, followed by a sustained slower rate of absorption in calves administered atropine. Because scintigraphic $t_{1/2}$ could not be accurately determined in calves administered atropine, comparison of the 2 power exponential equations was performed on data from calves suckling milk replacer, sodium acetate, sodium bicarbonate, sodium chloride, and cow's milk, which provided a total of 32 feedings for the 9 calves.

Scintigraphic $t_{1/2}$ ranged from 29 to 202 minutes for the 32 feedings. Siegel's modified power exponential formula provided the best fit for the scintigraphic data, based on ease of model convergence, repeatability of model convergence from various starting estimates, and the lowest value for Akaike's information criterion in 26 of 32 feedings. Therefore, values for scintigraphic $t_{1/2}$ derived from Siegel's modified power exponential formula were used as the criterion-referenced index for abomasal emptying rate. Intrasubject variability (difference expressed as percentage of the mean) for scintigraphic $t_{1/2}$ was 21.9%, 1.4%, 2.0%, and 33.6% for the 2 separate feedings of milk replacer for the 4 calves of experiment 2, providing an overall mean estimate of intrasubject variability of 15 ± 16%.
Acetaminophen absorption—For the plasma acetaminophen concentration–time relationship, use of the actual values for Cmax (Figure 2), Tmax (Figure 3), or AUC (AUC\(_{60}\), AUC\(_{120}\), or AUC\(_{240}\)) revealed that Tmax had the strongest linear relationship with scintigraphic t\(_{1/2}\) (R\(^2\) = 0.87; Table 1). Inspection of the scatterplot and residual analysis revealed curvilinearity of the relationship between Cmax and scintigraphic t\(_{1/2}\) and between AUC\(_{60}\) and scintigraphic t\(_{1/2}\) (Figure 4).

Application of the 3 pharmacokinetic models to data derived from the 32 feedings indicated that Maes’ first derivative of Siegel’s modified power exponential formula (pharmacokinetic model 1) provided the best fit for the data (Figures 5 and 6; Table 1). The strongest relationship between any of the studied variables and scintigraphic t\(_{1/2}\) was provided by Tmax from pharmacokinetic model 1 (R\(^2\), 0.91). Intrasubject variability (difference expressed as percentage of the mean) for Tmax was 19.6%, 10.0%, 10.3%, and 13.1% for the 2 separate feedings of milk replacer for the 4 calves of experiment 2, providing an overall mean intrasubject variability of 14 ± 5%.

The apparent F calculated by use of pharmacokinetic model 1 indicated no significant (P = 0.27) main effect of treatment, with least-squares mean ± SE estimates ranging from 0.38 ± 0.09 (150mM NaCl) to 0.58 ± 0.06 (milk replacer). The apparent F calculated by use of pharmacokinetic model 2 also indicated no significant (P = 0.50) effect of treatment, with apparent F ranging from 0.45 ± 0.08 (cow’s milk) to 0.62 ± 0.07 (150mM sodium acetate). The apparent F calculated by use of pharmacokinetic model 3 also indicated no significant (P = 0.08) effect of treatment, with apparent F ranging from 0.47 ± 0.10 (150mM NaCl) to 0.83 ± 0.10 (150mM sodium acetate).

Mean half-times for clearance could be calculated from the estimated values for pharmacokinetic models 2 and 3. These values were similar for all test solutions (pharmacokinetic model 2, P = 0.30; pharmacokinetic model 3, P = 0.53), ranging from 229 (150mM NaCl) to 388 (150mM sodium acetate) minutes with a median value of approximately 300 minutes.

### Table 1—Summary of linear regression equations (dependent variable regressed against scintigraphic t\(_{1/2}\)) for acetaminophen absorption as an indicator of abomasal emptying rate for suckling calves.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Coefficient</th>
<th>Intercept</th>
<th>R(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (µg/mL)</td>
<td>-0.101 (0.026)</td>
<td>39.4 (2.6)</td>
<td>0.811</td>
</tr>
<tr>
<td>Tmax (min)</td>
<td>1.55 (0.21)</td>
<td>7.4 (24.4)</td>
<td>0.866</td>
</tr>
<tr>
<td>AUC(_{60}) (µg X min/mL)</td>
<td>-0.77 (0.16)</td>
<td>188.0 (18.4)</td>
<td>0.808</td>
</tr>
<tr>
<td>AUC(_{120}) (µg X min/mL)</td>
<td>-1.52 (0.29)</td>
<td>399.5 (34.1)</td>
<td>0.826</td>
</tr>
<tr>
<td>AUC(_{240}) (µg X min/mL)</td>
<td>-1.68 (0.46)</td>
<td>669.1 (52.9)</td>
<td>0.797</td>
</tr>
<tr>
<td>Pharmacokinetic model 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(_{1/2}) (min)</td>
<td>2.39 (0.25)</td>
<td>18.3 (28.7)</td>
<td>0.876</td>
</tr>
<tr>
<td>Cmax (µg/mL)</td>
<td>-0.110 (0.023)</td>
<td>39.4 (2.6)</td>
<td>0.844</td>
</tr>
<tr>
<td>Tmax (min)</td>
<td>1.26 (0.12)</td>
<td>22.2 (13.5)</td>
<td>0.909</td>
</tr>
<tr>
<td>Pharmacokinetic model 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(_{1/2}) (min)</td>
<td>1.26 (0.18)</td>
<td>-25.6 (20.7)</td>
<td>0.847</td>
</tr>
<tr>
<td>Cmax (µg/mL)</td>
<td>-0.103 (0.026)</td>
<td>37.2 (3.0)</td>
<td>0.794</td>
</tr>
<tr>
<td>Tmax (min)</td>
<td>1.32 (0.14)</td>
<td>12.0 (19.9)</td>
<td>0.897</td>
</tr>
<tr>
<td>Pharmacokinetic model 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(_{1/2}) (min)</td>
<td>0.79 (0.14)</td>
<td>-17.5 (16.8)</td>
<td>0.737</td>
</tr>
<tr>
<td>Cmax (µg/mL)</td>
<td>-0.112 (0.023)</td>
<td>39.1 (2.7)</td>
<td>0.837</td>
</tr>
<tr>
<td>Tmax (min)</td>
<td>1.15 (0.20)</td>
<td>18.6 (22.8)</td>
<td>0.776</td>
</tr>
</tbody>
</table>

Data were obtained for 9 calves that suckled 2 L of milk replacer, cow’s milk, a 150mM solution of sodium acetate, a 150mM solution of sodium bicarbonate, and a 150mM solution of sodium chloride; each solution contained acetaminophen (50 mg/kg). Values in parentheses are the SE of the estimates.

Cmax = Maximal plasma concentration of acetaminophen. Tmax = Time to Cmax. AUC\(_{60}\) = Area under the curve of the plasma acetaminophen concentration-versus-time relationship for the first 60 minutes after the start of sucking. AUC\(_{120}\) = Area under the curve of the plasma acetaminophen concentration-versus-time relationship for the first 120 minutes after the start of sucking. AUC\(_{240}\) = Area under the curve of the plasma acetaminophen concentration-versus-time relationship for the first 240 minutes after the start of sucking.
Safety of the acetaminophen absorption test—We did not detect significant changes in serum activity of AST (P = 0.39) or ALP (P = 0.32) or serum concentration of total bilirubin (P = 0.40) or cholesterol (P = 0.20) between the start and end of the experiments (data not shown). However, serum activity of GGT decreased significantly (P < 0.001) from the start (353 ± 166 U/L) to the end (107 ± 70 U/L) of the experiments.

Discussion

The major findings of the study reported here were that Siegel’s modified power exponential equation provided the best model for describing the scintigraphic data, the first derivative of Siegel’s modified power exponential equation provided the best pharmacokinetic model for describing the acetaminophen absorption data, pharmacokinetically derived values for Tmax did not measure radioactivity at 2 sites because only 1 view was impractical, and studies in human infants,39,40 cats,41 sheep,42 goats,42 and cows42 have used only 1 site. We elected not to acquire lateral and ventral scintigraphic images of the abomasum with the calf restrained in lateral and dorsal recumbency because this method of restraint may influence the rate or pattern of abomasal emptying and may result in abnormal positioning of the small intestine over the ROI (ie, the abomasum for the study reported here). Variations in gastric emptying in humans have been observed with changes in posture and gravity.36,38 Therefore, we allowed the calves to stand with minimal restraint while obtaining scintigraphic images and permitted the calves to become stenally recumbent or stand whenever images were not being acquired.

Scintigraphic determination of gastric emptying rate is strongly influenced by the ability to accurately define the ROI. Defining the ROI on a static scintigraphic image is subjective unless a standardized method based on image intensity is applied, as was the case in this study. It was difficult to differentiate the abomasal body from the forestomach and pyloric antrum from the duodenum immediately after sucking, and the subsequent passage of 99mTc to the small intestine overlying the abomasum also increased the difficulty in accurately identifying the abomasal body. Therefore, we acquired a dynamic dorsal image while each calf was sucking to ensure closure of the esophageal groove and passage of the test solution into the abomasum. Groove closure was observed in all calves, including those administered atropine.

Atropine was administered to delay abomasal emptying, thereby providing a wide range of emptying times to test the robustness of acetaminophen absorption as a measure of abomasal emptying rate. Surprisingly, we found that atropine administration caused a biphasic emptying pattern characterized by an initial rapid rate of emptying followed by a slower emptying rate (Figure 1). This phenomenon does not appear to have been described in suckling calves, and we are uncertain of the mechanism, although a similar biphasic emptying pattern has been observed in humans.39 Atropine may interfere with gastric accommodation by facilitating passage of ingesta to the small intestine as a result of increased abomasal luminal pressure34,44, once the pressure gradient between the abomasum and duodenum equilibrates, the rate of emptying is slowed as a result of vagal inhibition of abomasal and small intestinal motility.46

Plasma or serum concentrations of acetaminophen have been measured by use of various analytic methods, such as gas-liquid chromatography,45,46 high-performance liquid chromatography,47,48 fluorescence polarization immunoassay,12 colorimetry, spectrophotometry,49,50 and an enzymatic method.32 The fluorescence polarization immunoassay is widely used in the United States but is expensive.52 We used the colorimetric nitration method because it was simple, was inexpensive, could be rapidly conducted,39 and measured acetaminophen concentration and not the concentration of sulfate or glucuronide conjugates.34 Although the colorimetric nitration method measures salicylate as well as acetaminophen, the method does not measure acetylsalicylic acid44; thus, the test can be performed on samples obtained from cattle that have been administered aspirin.

The plasma concentration of acetaminophen after oral administration depends on the rates of absorption, metabolism, distribution, and clearance.30 The absorp-
tion of a drug from the gastrointestinal tract depends on a number of factors, including gastric emptying rate, luminal pH, binding materials in the lumen, gastrointestinal surface area, local blood flow, metabolism of the drug by luminal bacteria or gastrointestinal epithelial cells, and the influence of diseases. Acetaminophen is a low–molecular-weight compound (formula weight, 151 g) and a lipophilic weak acid (pKα, 9.3). Small lipophilic compounds have high membrane solubility and are rapidly absorbed from the small intestine; this means that the rate of acetaminophen absorption is primarily influenced by the rate of abomasal emptying. In the study reported here in which calves suckled various test solutions, acetaminophen absorption may also have been influenced by luminal pH and binding materials in the intestinal lumen. However, the apparent F of acetaminophen was similar for all solutions, suggesting that pH and physical characteristics of the test solutions did not affect acetaminophen absorption. The apparent F (approx 50%) was lower than that reported for humans (> 85%) and dogs (79%); following oral ingestion of acetaminophen at rates of 30 and 20 mg/kg, respectively. This result may reflect high first-pass metabolism in calves.

Acetaminophen metabolism is a species-, age-, and dose-dependent phenomenon. Absorbed acetaminophen may undergo extensive first-pass elimination by the liver in ruminants, whereby acetaminophen undergoes extensive hepatic biotransformation and is detoxified to sulfate and glucuronide conjugates. When there is first-pass metabolism following oral administration, metabolism of absorbed acetaminophen may be inducible and dose-dependent, meaning that the Cmax for acetaminophen could vary with F and the frequency of administration as well as the abomasal emptying rate. For these reasons, Tmax would appear to provide a theoretically more appropriate index of abomasal emptying rate than Cmax in calves; this supposition was supported by the results of our study that indicated actual and pharmacokinetically derived values for Tmax were linearly related to scintigraphic t1/2. Similar results have been reported for humans, but with a lower R² value for Tmax (0.58) than that obtained in the study reported here. This result indicates that acetaminophen is a useful liquid-phase marker for abomasal emptying rate in suckling calves. Because the highest R² values were obtained for Tmax, we recommend that Tmax be used as the primary factor of interest when acetaminophen absorption is used to assess abomasal emptying rate.

Maximal plasma acetaminophen concentrations ranged from 15 to 53 µg/mL after administration of acetaminophen at a rate of 50 mg/kg in 2 L of test solution (Figure 2). Maximal concentrations were lower or similar to those obtained in ponies or horses administered a lower dose of acetaminophen (administration at a rate of 20 mg/kg in 350 to 1,000 mL of water resulting in Cmax values of 26, 49, 48, and 48 µg/mL in 4 studies1,21,49-51). In those 4 studies, plasma acetaminophen concentrations were assayed by use of the same colorimetric nitration assay used in our study. In contrast, when serum acetaminophen concentrations were analyzed by use of fluorescence polarization immunoassay in samples obtained from horses administered acetaminophen at a rate of 20 mg/kg, a lower value was obtained for Cmax (16.7 µg/mL). The Cmax value was 14 µg/mL in humans administered acetaminophen (14 mg/kg) when acetaminophen was assayed by use of high-performance liquid chromatography. It is not clear whether the range of values for Cmax reflects differences in dose, analytic method, nature of test solutions, or emptying rate from the stomach.

We found that Cmax was not as accurate a predictor of abomasal emptying rate as was Tmax. Variability in Cmax after oral administration of acetaminophen may reflect differences in F, gastric emptying rate, or acetaminophen clearance. The absorption of acetaminophen can be influenced by luminal pH; abomasal and small intestinal pH are affected by the stimulatory effects of test solutions on gastric secretions as well as by the pH of the test solutions. In this study, we did not standardize pH of the test solutions and variability in

acetate, N-acetyl-p-benzoquinoneimine, that binds covalently to hepatic macromolecules (unless it is biotransformed), resulting in dose-dependent hepatic necrosis. Therefore, we investigated whether there was serum biochemical evidence of hepatotoxicosis in the calves of the study reported here. We did not detect changes in serum AST and ALP activities and serum concentrations of total bilirubin and cholesterol. Serum GGT activity decreased severely in the calves during the study, but this is a typical response in colostrum-fed calves. Finally, the Cmax values for plasma acetaminophen concentration in the calves in our study (< 50 µg/mL) were considerably lower than the concentration (> 120 µg/mL) needed to induce hepatic damage in humans, although the plasma concentration required to induce toxic effects in calves is unknown. Therefore, we concluded that acetaminophen can be safely administered (50 mg/kg, PO) to suckling calves.
pH may theoretically have influenced \( F \) and therefore \( C_{\text{max}} \).

We believe that the calves in this study were physiologically normal, although there was variability in their ages (5 to 30 days of age) when used in the study. Other studies have been conducted within a few days after surgical placement of an abomasal cannula. We do not believe that the abomasal cannula altered emptying rate because placement of a percutaneous endoscopic gastroscope tube did not slow gastric emptying in cats and a much more invasive duodenal reentrant cannula did not alter emptying rate in milk-fed calves, compared with the rate for calves without a reentrant duodenal cannula.

We concluded that the acetaminophen absorption test provides a practical, inexpensive, and accurate method for measuring abomasal emptying rate in healthy suckling calves. It is unclear whether diarrhea or other enteric or systemic disease will affect the pattern of acetaminophen absorption because our study was performed in healthy calves. Future studies need to be completed in calves with naturally acquired or experimentally induced diarrhea to determine whether acetaminophen absorption is an accurate test in calves with enteric disease.

References


