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Research Paper

Gastric pH and Gastric Residence Time in Fasted and Fed Conscious Cynomolgus Monkeys Using the Bravo® pH System

Emile P. Chen,[1,4] Kelly M. Mahar Doan,[1,3] Samm Portelli,[1] Robert Coatney,[2] Vernal Vaden,[2] and Wei Shi[1]

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Purpose. To measure fasted and fed gastric pH and gastric residence time (GRT) in Cynomolgus monkeys using Bravo® radiotelemetry capsules.
Methods. Continuous pH measurements were recorded with Bravo® capsules, which were either attached to the monkeys' stomach or administered as free capsules. Meals (either slurry or standard), were administered at designated times with monkeys chair-restrained during slurry meal ingestion.
Results. From the attached capsule studies, the fasted gastric pH (~1.9–2.2) was consistent among monkeys. Under fasted conditions, pH spikes were infrequently observed (once every 7.9 min to 3.6 h) with peaks reaching pH 9.4 and having short durations (<1 min). After feeding, the gastric pH rose quickly and remained alkaline for approximately 4.5–7.5 h before returning to baseline. Although significantly different ($p<0.05$), there was overlap between the fasted (153±87 min) and fed (436±265 (slurry) and 697±193 (standard) min) GRT due to considerable inter- and intra-subject variability.
Conclusions. Fasted gastric pH was similar between monkeys and literature human values. After a meal, the monkey gastric pH was elevated for a longer duration than that in human. The monkey GRT appears longer than that observed in human under both fasted and fed conditions, although this is likely dependent on the Bravo® capsule size.

KEY WORDS: Bravo® capsule; cynomolgus monkey; gastric pH; gastric residence time; radiotelemetry.

INTRODUCTION

It has long been recognized that a number of physiological factors of the gastrointestinal tract can affect oral drug absorption (1). Several studies have demonstrated that the bioavailability of drugs with pH-dependent solubility, dissolution or stability can be significantly affected by the gastric pH in both human and preclinical species (2–11). Prolongation of GRT may also change the extent of absorption of compounds and controlled-released dosage forms where dissolution is the rate limiting step (12,13). The rate of absorption of enteric-coated dosage forms, which are indigestible in the acidic environment of the stomach, is also dependent on the rate of gastric emptying as this affects the onset of drug absorption (14,15).

During early drug development, pre-clinical species such as Cynomolgus monkeys and Beagle dogs are frequently used to assess the performance of human oral formulations. As the gastrointestinal anatomy and physiology of the two species differ from humans in diverse aspects (16), neither species may best predict human oral bioavailability under all circumstances. When pre-clinical species are used as surrogates for human bioavailability/clinical formulation investigations, it is important to appreciate the gastric milieu used to extrapolate to the clinical relevance of the formulation. Therefore, the rate limiting physicochemical absorption properties of an investigational formulation along with the biological gastric conditions should guide the choice of an appropriate species during preclinical testing.

Many review articles have investigated the suitability of animals as appropriate models for predicting human oral drug absorption (16–20). Although there have been numerous studies to determine the gastrointestinal pH and transit time in human and other laboratory animals (21–36), limited information exists in Cynomolgus monkeys, a frequently used non-rodent species in the evaluation of clinical formulation prototypes and prediction of human oral bioavailability (37,38). Methods commonly used to monitor gastrointestinal function involve intubation and aspiration of gastrointestinal contents or employment of a trans-nasally delivered pH electrode. These methods [which were used to obtain the only

[1] Department of Preclinical Drug Metabolism and Pharmacokinetics, GlaxoSmithKline, 709 Swedeland Rd., King of Prussia, Pennsylvania 19406, USA.
[2] Department of Laboratory Animal Sciences, GlaxoSmithKline, King of Prussia, Pennsylvania, USA.
[3] *Present address*: Department of Clinical Pharmacokinetics Modeling and Simulation, Clinical Pharmacology and Discovery Medicine, GlaxoSmithKline, 709 Swedeland Rd., King of Prussia, Pennsylvania 19406, USA.
[4] To whom correspondence should be addressed. (e-mail: emile.p.chen@gsk.com)

ABBREVIATIONS: ANOVA, analysis of variance; FDA, Food and Drug Administration; GERD, gastroesophageal reflux disease; GRT, gastric residence time; IQR, interquartile range; MMC, migrating myoelectrical complex; Q_1, 25% quartile; Q_3, 75% quartile; RMSE, root mean squared error; SD, standard deviation.

reported Cynomolgus monkey gastric pH values (37,38)] may cause substantial discomfort and stress for the subjects (39). These methods may perturb gastrointestinal homeostasis, and therefore may affect the reliability of pH measurements. Furthermore, because catheter or tube-based techniques limit and interfere with the subject's movement, they can only be used over a short period of time, and therefore, can only be used to make a limited number of point measurements.

Monitoring gastrointestinal pH using radiotelemetry methods eliminates most of the discomfort and trauma of intubation, as well as mechanical stimulation effects, and allows continuous, unperturbed pH measurements. Radiotelemetry can also provide continuous pH measurements, which enables reliable assessment of pH fluctuation over long periods of time. One such technique, the Heidelberg capsule, has been successfully used to determine the gastrointestinal pH and gastric emptying in both human and dog (15,22,30,31,33–36,40,41).

The specific aims of these studies were to determine the gastric pH and residence time in Cynomolgus monkeys under both fasted and fed conditions using a new radiotelemetry pH monitoring technique, the Medtronic (Shoreview, MN) Bravo® pH monitoring system. This technique has recently been approved by the United States Food and Drug Administration (FDA) for human use, primarily for measuring esophageal acid exposure in gastroesophageal reflux disease (GERD) patients (42–47). Unlike the Heidelberg capsule, which was not designed to remain at a fixed location in the GI tract, the Bravo® capsule is a catheter-free system designed for attachment to the mucosal membrane of the gastrointestinal tract such that it can monitor pH at a fixed location over a prolonged period of time (up to 48 h) without interfering with the subjects' regular activities.

MATERIALS AND METHODS

pH Measurement System

Continuous (every six seconds) gastrointestinal pH measurements were made using the Bravo® pH System. The Bravo® pH Capsule with Delivery System is a miniature radiotelemetry catheter-free capsule consisting of a battery, pH electrode, and radio transmitter enclosed in a 6×5.5×25 mm plastic housing, similar in diameter to a 00 capsule and similar in height to a 000 capsule. Prior to each use, the pH capsule was calibrated in pH 1.07 and 7.01 reference buffers (Medtronic, Shoreview, MN). The Bravo® capsules were either attached to the mucosa in the gastrointestinal tract under endoscopic guidance or were administered orally and allowed to pass freely through the gastrointestinal tract. By sampling GI tract fluids and emitting a radiofrequency (433-MHz) to an external receiver in 6-second intervals over 48 h, the Bravo® capsule can measure pH between 0.5 to 9.0 with a precision of 0.01 U. After several days, the body naturally sloughs off the attached capsule, which passes unchanged through the subject's digestive tract.

In Vitro Evaluation of the Bravo® pH Measuring System

In order to test the integrity of the Bravo® radiotelemetry system, a series of *in vitro* tests using commercial buffers (VWR, West Chester, PA) were performed. These experiments were designed (1) to determine the amount of time it takes the capsules to recognize a new pH environment, going in either direction, by observing the transition time of the new pH to the receiver and (2) to assess the accuracy and consistency at each pH measured. Two series of buffer solutions were used for these studies.

The first and second series of buffers consisted of buffer solutions at pH 1, 3, 5 and 7 and pH 2, 4, 6 and 8, respectively. For each series, vials containing 30 ml of the buffer solutions were placed in a 25°C water bath and three to four calibrated capsules were each placed into a unique vial containing the highest pH buffer solution (i.e., pH 7 or 8). After 1 h, the capsules were removed from the highest buffer and transferred into unique vials containing the next highest pH buffer solution for an additional hour. This "stepping down" process was repeated for transitioning into the final two buffer solutions. The capsules remained in the lowest pH buffer solution (i.e., pH 1 or 2) for 48 h. The precise time of transferring each capsule into each buffer solution was recorded to the second. Aliquots of each pH buffer were taken throughout the experiment for a manual pH measurement with a Beckman (Fullerton, CA) Φ 360 pH meter. The above experiments were repeated at 37°C and were also performed, at each temperature, in the reverse direction, i.e., from pH 1 or 2 "stepping up" to pH 7 or 8.

In Vivo Evaluation of the Bravo® pH Measuring System

Animals

Fifteen male Cynomolgus monkeys (*Macaca fascicularis*) with body weights between 3–9 kg (4–9 years old) obtained from Primate Products, Inc., Miami, FL, were used for these studies. The monkeys were housed individually in stainless steel cages in a controlled environment (72°F±4°F; 50%±10% relative humidity) with a 12-h light/dark cycle. Filtered tap water (supplied and periodically analyzed by Philadelphia Suburban Water Company (Bryn Mawr, PA) was available ad libitum from an automatic watering system. Prior to the study day, the animals were fasted overnight.

To investigate meal size and its effect on gastric pH or GRT, the monkeys consumed two different sized portions in each study design. Test meals (prepared on the day of each study) consisted of either their standard meal of 60 g (eight) Monkey Diet biscuits (Lab Diet #5038 made by PMI Nutrition International, St. Louis, MO) and two quarters of fruit or approximately 15 g (~two) Monkey Diet biscuits homogenized with 50–60 ml of tap water. The nutrient composition for this biscuit diet contained: protein 15.5%, fat (ether extract) 5%, fat (acid hydrolysis) 5.9%, fiber (crude) 4%, ash <5%, nitrogen-free extract 60.7%, gross energy 4.03 kcal/g, physiological fuel value 3.50 kcal/g, metabolizable fuel value 3.22 kcal/g, minerals 4.8%. The pH of the homogenized test meal was 5.5–6.0. The monkeys were allowed 1 h to eat their standard meal and the slurry meal was administered via oral gavage with a 2 oz catheter tip syringe (BD (Franklin Lakes, NJ), # 309620) attached to a 16″, 18 French (6.0 mm) feeding tube/urethral catheter [Kendall (Mansfield, MA), # 8890-701819].

Before their initiation, all animal studies were reviewed and approved by the GlaxoSmithKline Institutional Animal Care and Use Committee, and were performed in accordance with the recommendations found in the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 1996) and

adhered to the "U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training" (NIH publication #85-23, revised in 1985).

Determination of Gastric pH Profiles Using Bravo® Capsules Attached to the Stomach Wall

A total of eleven monkeys were used for the gastric pH studies. The Bravo® capsules were attached to the gastric mucosal surface the day prior to the study. All animals had food withheld for 12 h prior to the procedure and water withheld for 2 h prior to the procedure in order to assure gastric emptying. Anesthesia was induced with ketamine (10 mg/kg intramuscular) and maintained with isoflurane (1–3% delivered in 100% oxygen) after endotracheal intubation. The animals were then placed in left lateral recumbency and a gastric endoscope advanced into the stomach for visualization. The stomach was mildly distended with air to allow identification of gastric structures, especially the greater curvature. The pH capsule delivery system was then advanced orally into the stomach along side the endoscope. The capsule was attached to the gastric mucosal surface by applying vacuum through the delivery system which forced a portion of the mucosa into the capsule opening. The capsule was stabilized following activation of its spring-loaded stainless steel pin being driven through the well containing the gastric mucosa (see reference 42 for more details of capsule attachment). Gastric pH readings were immediately recorded. Firm attachment was confirmed by endoscopic visualization. Air was evacuated from the stomach as the monkeys recovered. The function of the capsule and telemetry system was confirmed during recovery from anesthesia. After anesthesia recovery, the animals were returned to their home cages and maintained on light–dark cycles (from 6 A.M. to 6 P.M.).

On the second day of study, monkeys were transferred to chairs or remained in their cages, as described below. The designated slurry meal animals were moved to a study room and were chair-restrained (Plas Labs (Lansing, MI) Custom Rhesus Primate Chair #515-SASR/SP) in the same manner as a typical pharmacokinetic study. Each chair-restrained session lasted 6 h, during which fasted gastric pH was monitored in monkeys for the first 5 h before 50–60 ml of food slurry (0.25–0.3 g/ml, pH 5.5–6.0) was fed to the monkeys by oral gavage. One hour later, the animals were returned to their home cage while telemetric monitoring of gastric pH continued for a total monitoring time of 48 h. When the standard meal was offered, the monkeys remained in their home cages to allow for meal consumption over 1 h in a familiar environment.

Most capsules naturally detached from the gastric mucosa within 48–72 h of attachment. After the capsules detached they passed through the GI system and were eliminated in the feces.

Determination of GRT Using Freely Moving Bravo® Capsules

A total of twelve monkeys were used for the GRT studies and not all monkeys were involved in both a fed and fasted study design. All animals had food withheld for 12 h and water withheld for 2 h prior to the study day. On the day of study, animals were moved from their home cage to the study room and chair-restrained in the same manner as a typical pharmacokinetic study. Each chair-restrained session was 6 h.

To determine the GRT in fasted monkeys, a Bravo® Capsule was administered in the same manner as an oral solid dosage form would be administered in a pharmacokinetic study. The capsule was inserted into the tip of a size 16″, 18 French (6.0 mm) feeding tube/urethral catheter (Kendall (Mansfield, MA), # 8890-701819) attached to a syringe filled with water. The catheter was guided down the esophagus and the capsule delivered by expelling the water from the syringe. Six hours later, the monkeys were transferred to their home cages and telemetric monitoring continued for approximately 24 h. To determine the GRT in fed monkeys, the slurry meal was administered approximately 15 min after the monkeys were chair restrained. When the monkeys were offered the standard meal, they were allowed to remain in their cages for 1 h to consume their meal and were then chair restrained. Immediately following either meal, a Bravo® capsule was orally administered and the monkeys remained chair restrained for a total of 6 h. After the monkeys were returned to their home cages, telemetric monitoring continued for approximately 24 h.

As restraining Old World monkeys (macaque family) is known to affect gastrointestinal function and may cause inhibition of acid secretion and gastric emptying (48), the monkeys have been acclimated to the experimental setups with at least 10 h of chair restraining training prior to the pH studies.

Data Analysis

The pH data were uploaded from the external receivers to a computer *via* Bravo® Datalink (Medtronic, Shoreview, MN). Each pH data recording at each 6-second interval was extracted from the Polygram Net® (Medtronic, Shoreview, MN) software into a Microsoft® Excel spreadsheet for further evaluation and manipulation.

The means and standard deviations (SD) were determined for the pH recordings of the last 30 min of each *in vitro* pH solution, for the recordings from the second half of the fourth hour and for the last 30 min of the 48 h in the final buffer. To describe the overall error of the measurements, the root mean squared errors (RMSE) were determined for (1) the differences between the pH readings from the Beckman Φ 360 pH meter and mean pH values from the Bravo® pH monitoring system, (2) the differences between the mean pH from the second half of the fourth hour and the last 30 min of the 48 h in the final buffer and (3) for the SD for the pH recordings of the last 30 min of each pH solution.

Gastric pH data recorded from the slurry and standard meal study monkeys that underwent Bravo® capsule attachment were broken down into five and four intervals, respectively, for one-way analysis of variance (ANOVA) analysis and summarized by the mean, within monkey SD, between monkey SD as well as median values and 25 and 75% quartiles. The median, 25, and 75% quartile pH values over 30 min intervals from both meal type studies were also determined. Descriptive statistical analyses of "pH spikes", fasted pH fluctuations of pH values greater than 4, included calculations of the mean, median and range of the peak pH, the spike frequencies and the spike durations for the total fasted period, the overnight fasted (cage) and pre-meal fasted (chair) periods for the slurry meal monkeys and the overnight fasted

(cage) and pre-meal fasted (cage) periods for the standard meal monkeys. Additionally, the percent distributions of three pH spike duration periods, less than 1 min, 1 to 4 min and greater than 4 min, were also calculated.

The GRT from both the fasted and fed freely moving Bravo® capsule studies was assessed as the time elapsed from oral dosing that corresponded to a steep shift from an acidic pH to a basic pH≥7. The mean GRT and SD were determined for (1) the fasted monkeys, (2) the monkeys fed a slurry meal and (3) the monkeys fed a standard meal and statistical significance was determined by ANOVA with the post-hoc Tukey–Kramer multiple comparisons test.

RESULTS

In vitro Evaluation of the Bravo® pH Measuring System

The time profile in Fig. 1 is representative of a typical *in vitro* experiment performed over a 48 h period at 25°C. In order to evaluate the long-term stability of the pH reading, Bravo® capsules were transferred quickly from one pH buffer to the next at 1-h intervals for the first 4 h, and remained in the last pH buffer for the remainder of the 48 h. As seen in Fig. 1, the Bravo® capsule pH readings adjusted very rapidly each time they were moved to a new pH buffer. Each *in vitro* test was performed using three to four capsules and with most of the capsules, the pH reached the new steady-state within 6 s (one sampling interval) from the time the capsule entered a new buffer solution.

The pH fluctuation observation during each step was minimal. The overall SD (RMSE), calculated during the last 30 min of each pH step, were ±0.1 (25°C "stepping down"), ±0.3 (25°C "stepping up"), ±0.03 (37°C "stepping down") and ±0.04 (37°C "stepping up") pH units. There was no observable drift in the pH reading over the entire 48 h data collection interval. The mean pH reading from the second half of the fourth h was compared to the mean pH during the last 30 min of the 48th hour data collection period, and the difference of the means was <0.2 pH units in all cases. The pH readings from 19 of 28 capsules went up slightly at the end of the 48 h, while 8 of 28 capsules went down slightly. The RMSE were ±0.2 (25°C "stepping down"), ±0.05 (25°C "stepping up") and ±0.08 (37°C "stepping down" and 37°C "stepping up") pH units.

The insert in Fig. 1 illustrates the accuracy of the pH data generated by the Bravo® capsules in the pH range of 1–8 at both 25°C and 37°C *in vitro*. At both 25 and 37°C, the capsules slightly underestimate the pH of the buffer solution at all pH values tested, with RMSE of 0.3 (25°C "stepping down" and 25°C "stepping up") and 0.1 (37°C "stepping down" and 37°C "stepping up") pH units. This underestimation was lower than the variability (SD) of the fasted and fed gastric pH profiles (Table I).

Gastric pH Profiles Under Fasted and Fed Conditions

In Fig. 2, a typical slurry meal monkey gastric pH profile over a 36-h period is displayed. The measurements were divided into five time periods for analysis:

1. Gastric pH under fasted conditions while the monkeys were in their cages; beginning at 6 h after the Bravo® capsules were attached (to allow sufficient time for the

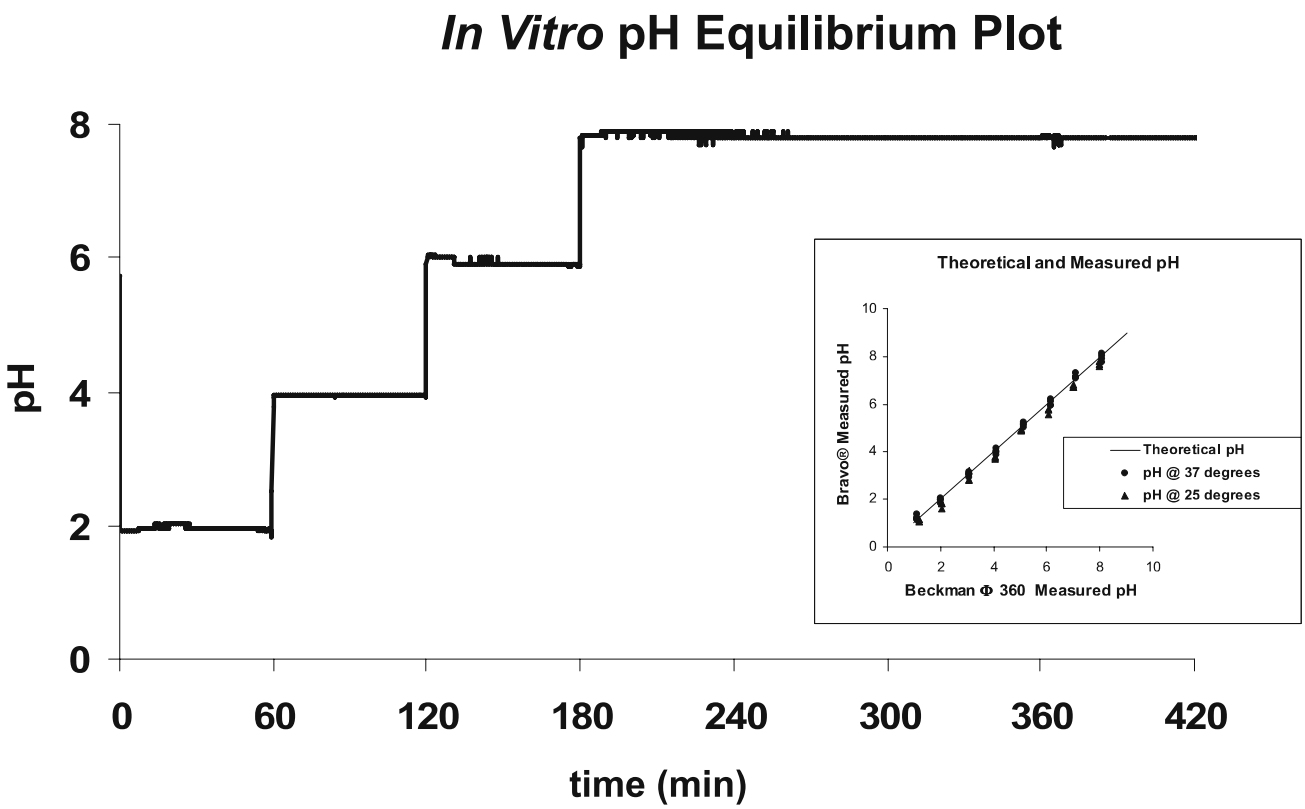

Fig. 1. Typical *in vitro* time course *vs* pH equilibrium plot initiating at pH 2 with step-wise increments to pH 8. *Inset* Beckman Φ 360 measured pH *vs* Bravo® measured pH at 25°C (*filled triangle*) and 37°C (*filled circle*).

Table I. Descriptive Statistics of Gastric pH in Monkeys with an Attached Bravo® Capsule Under Fasted and Fed Conditions

	Fasted		Fed		
	Cage	Chair	0–60 min	60–120 min	120–360 min
Slurry Meal Study (fasted[a], *n*=6; fed[a], *n*=5)					
Mean pH	1.97	1.91	4.79[b]	4.01[b]	3.82[b]
Within-monkey SD	1.40	1.10	0.89	0.84	1.08
Between-monkey SD	0.64	0.45	0.63	1.12	1.07
Median	1.54	1.62	5.14	4.40	3.47
25% quartile	1.06	1.17	4.21	2.84	2.55
75% quartile	2.03	2.25	5.62	4.96	5.18
Standard Meal Study (fasted, *n*=5); fed, *n*=5)					
Mean pH	2.21		4.86[c]	4.90[c]	4.27[c]
Within-monkey SD	1.28		0.92	0.58	0.87
Between-monkey SD	0.95		2.30	2.53	2.43
Median	1.69		5.10	5.59	4.62
25% quartile	1.21		3.02	2.84	2.29
75% quartile	3.03		5.85	5.85	5.81

[a] Six monkeys were included in the Fasted (slurry meal) pH analysis and five monkeys included in the Fed (slurry meal) pH analysis as the pH transmission from one monkey, 24504, ceased upon feeding

[b] ANOVA indicated that slurry meal fed gastric pH at all three time periods was significantly higher when compared to the fasted (cage and chair) gastric pH ($p<0.01$).

[c] ANOVA indicated that standard meal fed gastric pH at all three time periods was significantly higher when compared to the fasted gastric pH ($p<0.01$).

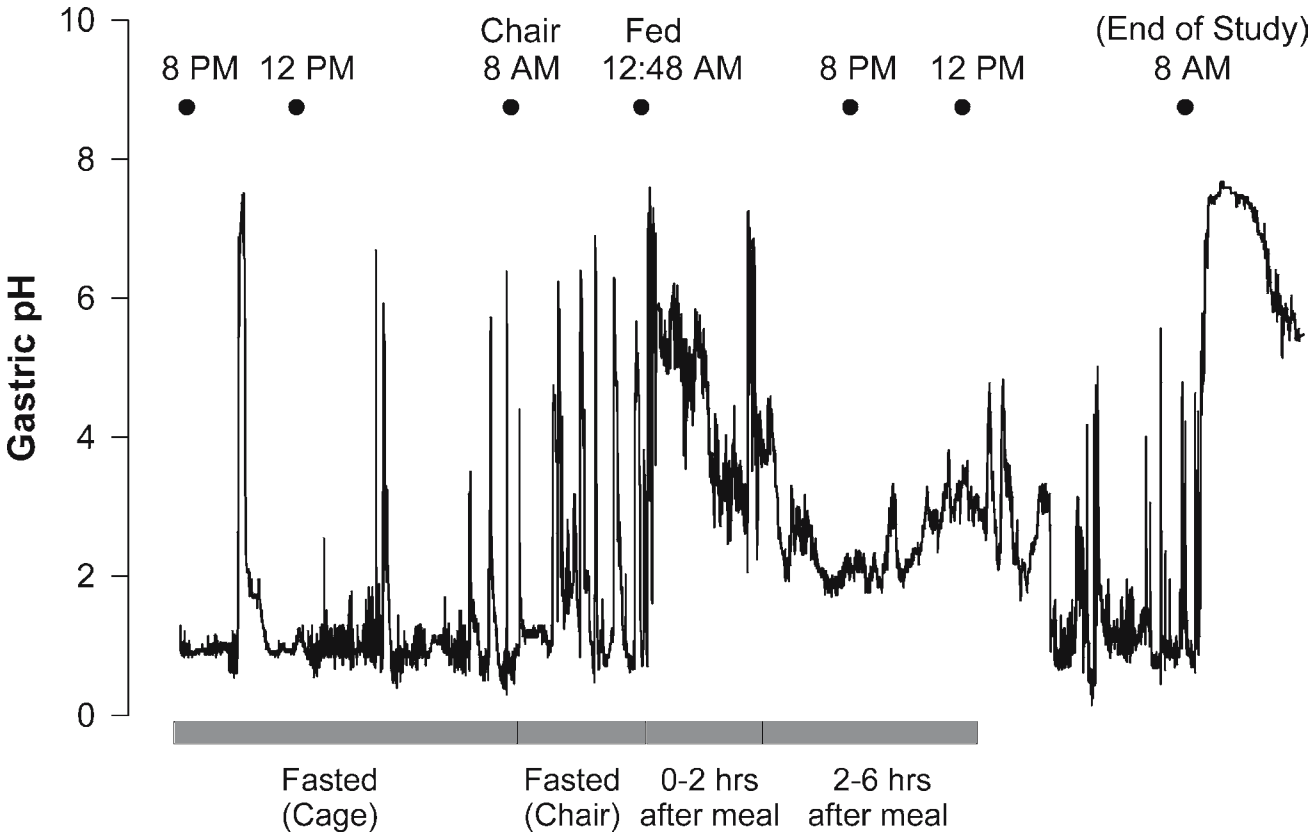

Fig. 2. Thirty-six hour time course of gastric pH using the attached Bravo® pH monitoring system. The Bravo® capsule was attached to the gastric mucosa of the anesthetized monkey at approximately 8 A.M. on day 1, allowed to recover over ~24 h, restrained in a typical PK study-chair at approximately 8 A.M. on day 2, fed the biscuit slurry meal at 12:48 P.M., removed from chair at ~2 P.M. and returned to home cage for remainder of study duration (end of study: 8 A.M., day 3). Occasional pH spikes at irregular intervals were observed in the pH profile under fasted conditions (before feeding at 12:48 P.M.). For this monkey, the spike frequencies were 1.1 and 4.3 spikes/h for the Fasted (cage) and Fasted (chair) periods, respectively.

monkey to recover from the anesthesia and the stress that may have resulted from the procedure) and ending when the monkey was moved to the study room and placed in a chair. This encompassed a 12 h period between 8 P.M. to 8 A.M.
2. Gastric pH under fasted conditions while the monkeys were in the study chairs. This period began when the monkeys were moved from their cages to the study chairs and ended when the monkeys were fed approximately 5 h later (normally between 8 AM and 1 PM).
3. Gastric pH during the 60 min period immediately after feeding.
4. Gastric pH between 1 to 2 h after feeding.
5. Gastric pH between 2 to 6 h after feeding.

For the monkeys that received a standard meal, the first two fasted condition intervals were combined, as these monkeys remained in their cages until meal time. The last three intervals remained the same for pH analysis.

Gastric pH Under Fasted Conditions

The gastric pH under fasted conditions, regardless of whether the monkeys were in their cages or restrained in chairs, was characterized by relatively steady acidic pH punctuated by occasional pH spikes at irregular intervals (discussed below). Descriptive statistics of gastric pH during fasted conditions are summarized in Table I.

As reported in Table I, the mean, fasted gastric pH of the slurry meal monkeys in their cages or while chair-restrained was 1.97 and 1.91, respectively, and was similar to the mean fasted pH of the standard meal monkeys (2,21). For the monkeys in the slurry meal group, there was no statistical difference in the fasted pH values whether the monkeys were in their cages or chair restrained. The between monkey SD were lower than the within monkey SD during the fasted periods.

Gastric pH Spikes Under Fasted Conditions

For the purpose of this analysis, a pH spike was defined as a fasted pH fluctuation rising above a pH value of 4, as it is the nearest integer pH level above the mean plus one within-monkey SD of the fasted pH. The duration of a pH spike was defined as the lapse between the time pH rose above and fell below pH 4. It is worth noting that such pH spikes were not observed in the *in vitro* experiments.

Typical examples of pH spikes are shown in Fig. 3. When analyzing the total fasted period (cage and chair together) from all monkeys, the mean peak pH value was 5.22 (range: 4.03 to 9.44). The frequency of the pH spikes varied significantly among the eleven monkeys studied, ranging from 0.28 spikes/h (one spike every 3.6 h) in monkey W167 to 7.56 spikes/h in monkey A12608. The median frequency was 2.67 spikes/h or one spike every 22.5 min. The duration of the spikes (quantified at pH of 4) also varied widely, ranging from 0.1 min (limit of sampling resolution) to 72.1 min.

However, the spikes occurred less frequently but with longer duration during the overnight period as compared to a 5 h period prior to the meal. The median and mean frequencies

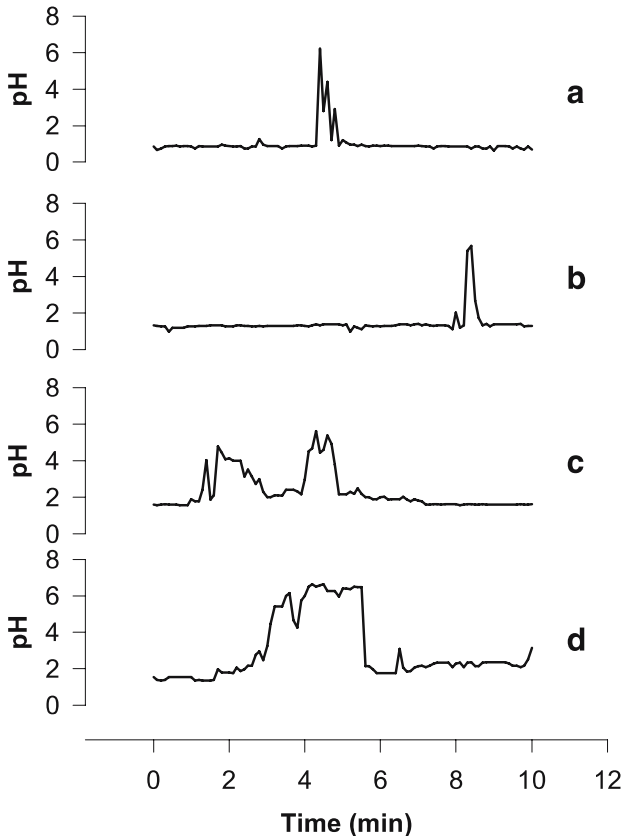

Fig. 3. Four examples of pH spikes. The pH spike examples in **a** and **b** display spikes with durations lasting less than one minute, and were more frequently encountered. They accounted for 75.9 and 85.4% of the spikes observed during the overnight and the morning pre-meal periods, respectively. The spikes in **c** and **d** have durations between 1 and 4 min and greater than 4 min, respectively and were less frequently observed. Spike durations between one and four minutes accounted for approximately 11.6 and 10.0% of the spikes observed during the overnight and the morning pre-meal periods, respectively and spikes with durations greater than four minutes accounted for approximately 12.5 and 4.6% of the spikes during the same respective periods.

were 1.50 and 1.85 spikes/h overnight, and 4.41 and 6.02 spikes/h during the morning pre-meal period. This increase in spike frequency occurred in nine out of eleven monkeys, whether they had spent their morning hours in the chair (monkeys fed slurry meal) or in their home cage (monkeys fed standard meal). In fact, the median fold-increase in spike frequency is higher in the monkeys that stayed in their home cage in the morning than those that were moved to the study chairs (4.7-fold *versus* 2.0-fold). Spikes with longer duration were also more frequently encountered during the overnight period than the morning pre-meal period. Overnight, 75.9% of spikes had durations of less than 1 min, while 12.5% of spikes had durations greater than 4 min. In the morning pre-meal period, 85.4% of spikes had durations less than 1 min, and only 4.6% of spikes had durations greater than 4 min.

Effect of Food on Gastric pH

In Fig. 4, the effect of food on the gastric pH of four representative monkeys is displayed; two monkeys were fed a slurry meal made from two biscuits (Fig. 4a, b) while the other

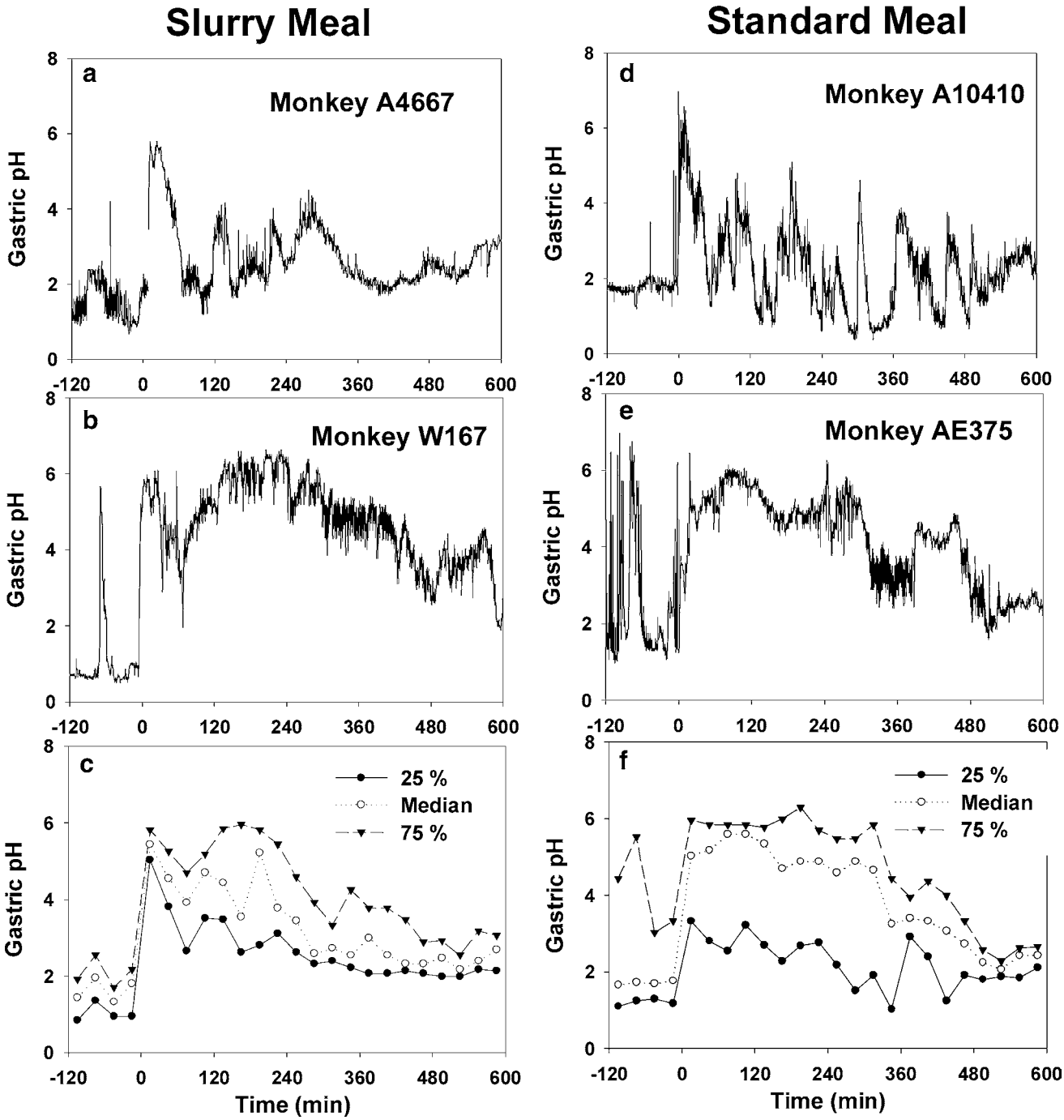

Fig. 4. Food-effect time course of gastric pH from four monkeys. The example in **a** displays a rapid change of gastric pH following a slurry meal followed by a rapid decline in pH back to acidic conditions (monkey A4667). In **b**, the gastric pH from monkey W167 remains alkaline following a slurry meal. These same two scenarios are also displayed in **d** (monkey A10410, rapid pH change) and **e** (monkey AE375, prolonged alkaline pH) following a standard meal. Subpanels **c** (slurry meal) and **f** (standard meal) represent the median, 25, and 75% quartile plots at each 30-min interval following both meal types from all monkeys (n=5/meal type). Meal time is defined as time=0 in all panels.

two monkeys were fed a typical meal consisting of eight biscuits and two quarters of fruit (Fig. 4d, e). In all monkeys (five monkeys per meal type), gastric pH rose sharply to reach the peak value approximately 30 min following the meal consumption (represented by time 0). The mean peak pH from all monkeys was 6.5, and the median time to reach the peak was 27.9 min (2.0 to 60.5 min).

Considerable between-monkey variability was observed for the duration of gastric pH elevation subsequent to the meal. In some monkeys, the pH declined rapidly following the peak and returned to the pre-meal baseline within 2 h, while the pH in other monkeys remained relatively alkaline for several hours before returning toward baseline. The duration of pH elevation did not correlate with the types of ingested meal

(slurry or standard), as prolonged and short durations were observed in both slurry and standard meal groups.

The median, 25% quartile and 75% quartile gastric pH, were calculated in 30 min intervals for monkeys in both slurry (Fig. 4c) and standard (Fig. 4f) meal groups, respectively. The overall pattern of gastric pH time course following a meal were similar regardless of the type of meal consumed, although the standard meal median time course was prolonged. The median pH stayed above pH 4 for approximately 240 min (slurry meal) or 360 min (standard meal) before returning toward baseline pH, attaining median pH values ≤3 at 270 min (slurry meal) or 450 min (standard meal) after meal consumption. Regardless of the type of meal, the median gastric pH had mostly returned to the pre-meal baseline by 480 min.

Descriptive statistics of gastric pH during various time periods of the slurry and standard meal studies is summarized in Table I. The mean fed gastric pH values ranged between 3.82 and 4.79 for slurry meal monkeys and 4.27 and 4.90 for standard meal monkeys among the three time periods analyzed. ANOVA indicated that the tabulated fed gastric pH at all three time periods (from both meal types) was significantly higher than the fasted gastric pH while the monkeys were in their cages or restrained in chairs ($p<0.01$). In general, the between monkey SD were similar to or larger than within monkey SD during the fed periods.

GRT under Fasted and Fed Conditions

A representative pH profile obtained from a typical monkey using an unattached (free) Bravo® capsule under fasted and fed (slurry meal) conditions can be observed in Fig. 5. Under the fasted condition (Fig. 5a), the pH dropped quickly to below 2.0 after the Bravo® capsule was administered. Approximately 68 min later, the pH rose quickly to >7.0 and was maintained at this elevated level for the remaining period of the study. This was interpreted as the transit of the capsule from the stomach into the intestine. The GRT for this fasted monkey was estimated to be approximately 68 min.

The pH profile under the fed (slurry) condition was studied in the same monkey (Fig. 5b). The monkey was administered the Bravo® capsule immediately following an oral gavage of the biscuit slurry meal. Initially, the pH declined gradually from approximately 5–6 to <2.0. This was consistent with the observation from the pH time course following feeding from monkeys with attached Bravo® capsules. The pH remained acidic until it rose rapidly at ~303 min to a pH>7.0. The GRT for this monkey, calculated as the total duration between dosing of the Bravo® capsule to the time pH rose sharply to an intestinal pH was 303 min. Similar pH profiles were observed in monkeys offered the standard meal.

Figure 6 summarizes the observed GRT values under fasted and fed conditions from 28 experiments in a box-and-whisker plot. Under fasted conditions, the median GRT was 171 min, with 25 and 75% quartiles of 68 and 224 min, respectively. Under slurry meal fed conditions, the median GRT was 313 min, with 25 and 75% quartiles of 263 and 510 min, respectively. The median GRT for standard meal fed conditions was 673 min, with 25 and 75% quartiles of 620 and 842 min, respectively. There was a statistically significant difference between the fasted GRT and both the slurry ($p<0.05$) and standard ($p<0.001$) meal GRT. Although statistically significant, there was overlap between the fasted and slurry meal groups. The range for fasted GRT, fed (slurry meal) GRT, and fed (standard meal) GRT was 31–294, 192–950 and 406–932 min, respectively. There was no statistical difference between the GRT following consumption of the slurry *vs* standard meal.

DISCUSSION

Fasted Gastric pH

Table II compares the descriptive statistics for gastric pH measurements from the monkey and human under fasted and fed conditions. In most cases, the fasted gastric pH between monkey (37,38) and human (30,31,40) are very similar, with

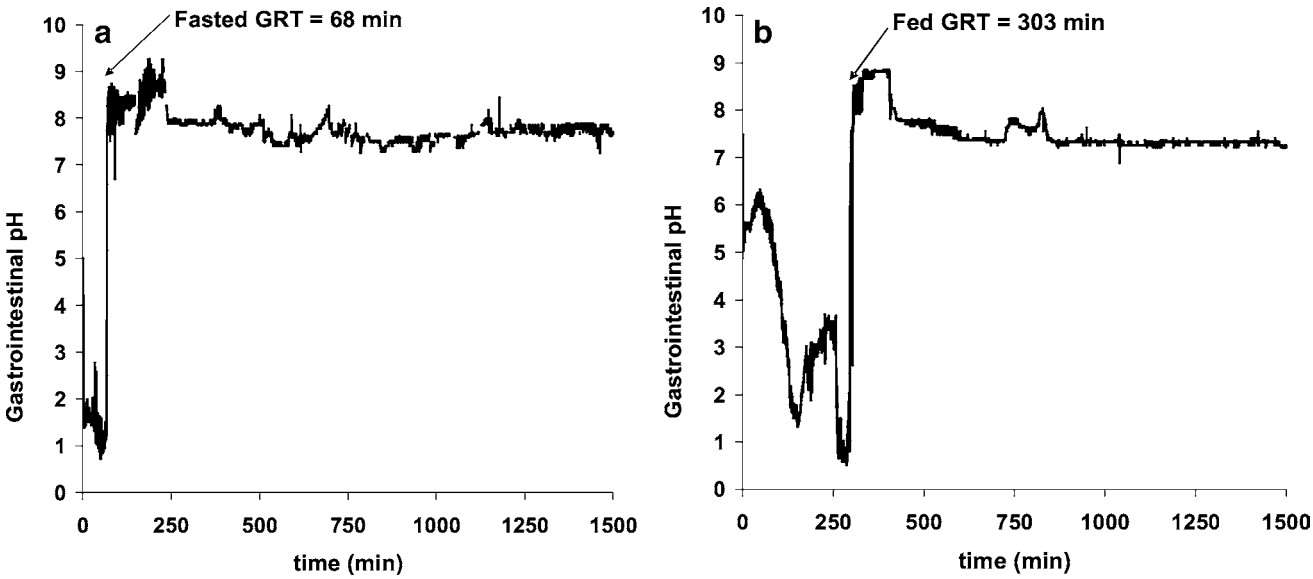

Fig. 5. Fasted and fed GRT profiles. pH *vs* time profiles obtained by continuous monitoring of the Bravo® capsule in monkey A2614 after an overnight fast or immediately following a slurry meal. Oral administration of the capsule occurred at time=0. Gastric emptying time coincided with a sharp increase in pH (*arrow*) for fasted (**a**) and fed (**b**) conditions.

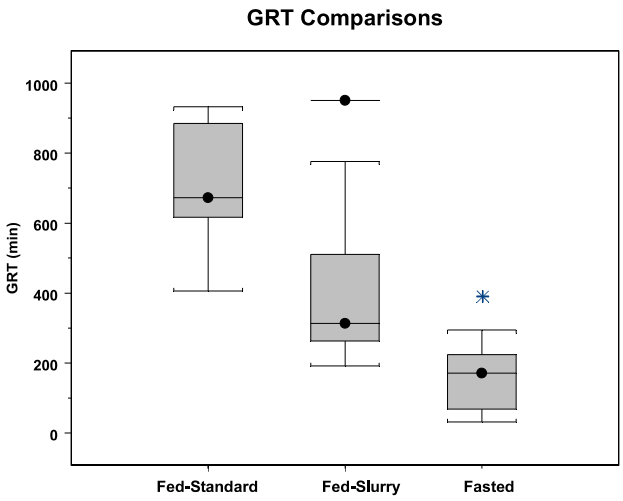

Fig. 6. GRT box-and-whisker plots. Median GRT values of fasted and fed (slurry and standard meals) monkeys are depicted as —●— in each box (n=6–13 individual studies using 1 of 12 monkeys). The 25% (Q_1) and 75% quartiles (Q_3) are represented by the *ends of the boxes* and the *ends of the* "*whiskers*" represent ±1.5 • IQR($IQR = Q_3 - Q_1$). The —●— in the Fed–Slurry plot at 950 min is an outlier GRT value that falls beyond 1.5•IQR. *p<0.05 (Fasted compared to Fed–Slurry) and p<0.001 (Fasted compared to Fed–Standard) by one-way ANOVA and post-hoc Tukey–Kramer multiple comparisons test.

typical pH ranges between 1 and 3. This is consistent with the pH findings from the present study.

Equally important to drug absorption is the extent and duration of pH fluctuation. For drugs where solubility or dissolution is pH dependent, the bioavailability may be significantly altered if there were significant and prolonged elevation in pH. Two previous studies reported considerable variation in fasted gastric pH in dogs (25) and Cynomolgus monkeys (37). In contrast, from the present study, the monkey gastric pH under fasted conditions showed little fluctuation over time, except for the occasional pH spikes of very short durations. Even though the pH spikes occurred more frequently during the morning period when a pharmacokinetic study is more likely to take place, there was also a lower incidence of spikes with long durations. Typically, the pH spikes with durations of 4 min or longer was calculated to only happen every 3.6 h in the morning pre-meal period.

The physiological basis of the pH spikes observed in monkeys from this study is not known. Interestingly, Bueno *et al.* (49) reported alkalinization of the dog gastric antrum of ~5 pH units lasting 15–30 min which occurred regularly at 90–120 min intervals at the cessation of periods of the antral spiking activity phase of the migrating myoelectric complex (MMC). As bile salts were detected in the gastric samples taken at the beginning of periods of antrum motor quiescence, the authors associated the observed alkalinization with regurgitation of intestinal fluid into the stomach. In the present monkey study, bile colored fluid was observed in the stomach of some monkeys during capsule attachment with gastroscopy and the fluid was flowing retrograde through the pylorus; however, there is no direct evidence of its composition or origination. Unfortunately, pH measurements were not taken during the time of these observations, and therefore, a correlation between pH spikes and bile regurgitation cannot be established.

Fed Gastric pH

Similar to the observations made in human (31), the gastric pH in monkey climbed quickly following a meal to a median pH>4, before declining back toward the fasted state pH. However, the return of gastric pH towards baseline in monkey was slower than that observed in human. The human gastric pH returned to the fasted value within 2 h after a meal. Although the gastric pH in some monkeys returned toward baseline within 1–2 h after a meal, the mean monkey gastric pH remained above pH 3 for 4.5 to 7.5 h before declining and had mostly returned to the fasted pH baseline by 8 h after meal consumption.

It should be noted that neither the type nor amount of the meal appeared to have an overt effect on the extent and duration of pH elevation. In both meal type groups, there were monkeys that had either gastric pH that quickly declined or gastric pH that remained alkaline for several hours before returning to the fasted pH.

The results of the present study were in contrast to those by Kondo *et al.* (37), which reported that monkey gastric pH remain between pH 5 and pH 7 for 9 h after a standard meal

Table II. Gastric pH Species Comparison

Species	Source	Method	Number of Subjects	Fasted pH	Peak pH After Meal	Fed pH (60 min)
Cynomolgus monkey	Present study (slurry meal)	Bravo® capsule	6[a]	1.1–2.1[b]	6.1–6.2[b]	4.2–5.6[b]
	Present study (standard meal)	Bravo® capsule	5	1.2–3.0[b]	6.1–6.6[b]	3.0–5.9[b]
	(37)	pH electrode	10	1–3[c]	–	5–7[c]
	(38)	gastric fluid aspirates	16	1.2–4.3[d]	–	–
Human	(40)	Heidelberg capsule	10	0.4–4.0[d]	–	–
Human (young)	(31)	Heidelberg capsule	24	1.4–2.1[b]	6.4–7.0[b]	~2–4.5[e]
Human (elderly)	(30)	Heidelberg capsule	79	1.1–1.6[b]	3.9–5.5[b]	~2–6[f]

[a] Six monkeys were included in the Fasted (slurry meal) pH analysis and five monkeys included in the Fed (slurry meal) pH analysis as the pH transmission from one monkey, 24504, ceased upon feeding
[b] Interquartile range (IQR), fed pH represents data between 0 and 60 min post meal
[c] Median range
[d] Range
[e] IQR estimated from Fig. 4 (31)
[f] IQR estimated from Fig. 4 (30)

of biscuits and fruits. The difference between the two sets of findings is not clear, although the experimental design of the two studies, including amount and composition of food, the size and age of monkeys were similar. One difference was the method of pH measurement. In the study implemented by Kondo and colleagues, an electrode was inserted through the nostril and removed for each measurement for a total of eight measurements. The disparity of the two measurement approaches may have contributed to the present study's unique findings.

GRT

Previous studies using Heidelberg and Bravo® capsules in conjunction with dual gamma scintigraphy (35,50) demonstrated that a steep shift in pH correlates with the movement of either capsule from the stomach into the duodenum. Therefore, these references provide the foundation for using the pH shift as the determinant for GRT. Additionally, human GRT values observed with the Bravo® capsule (Table III) were on the order of that observed by Lui *et al.* (40) and Mojaverian *et al.* (33) using Heidelberg capsules, suggesting that the Bravo® capsule is as reliable a tool for GRT determinations.

It was observed that the GRT of the Bravo® capsule in the Cynomolgus monkey was variable both across monkeys and within the same monkey when studied on multiple occasions (data not shown). This may be due to the size and shape of the capsule. The capsule was rectangular in shape with an end on width of 5–6 mm and a length of 25 mm. Numerous studies in humans and animals indicate an inverse relationship between particle size and rate of gastric emptying (51–53). The large size of the Bravo® capsule relative to the pyloric sphincter of the Cynomolgus monkey may contribute to the longer and more variable retention times. Further, Stotzer *et al.* (52) reported that cylindrical markers, similar to the Bravo® capsule, have more variable emptying times. One potential cause of this increased variability of emptying times of a cylindrical marker is that the orientation of the marker, relative to the pylorus may play a role in passage into the small intestine, with an end on orientation passing more readily through the pylorus.

Because of the high variability of the GRT in the monkey with the Bravo® capsule, this species may not be the ideal candidate for certain pharmacokinetic investigations, namely, enteric coated formulation studies with similar sized tablets/capsules. High variability in gastric emptying time in the Cynomolgus monkey would be expected with formulations of comparable sizes. It would be rather difficult to design the pharmacokinetic blood sampling scheme following dosing when a uniform GRT is not anticipated. At present, the GRT of smaller formulations in the Cynomolgus monkey is unknown and further work is necessary in this area to truly determine the extrapolation to humans.

CONCLUSION

The Medtronic Bravo® pH monitoring system was investigated as a technique to ascertain gastric pH and GRT in the Cynomolgus monkey. The Bravo® pH monitoring system proved to be a useful system for obtaining these measurements and the catheter-free feature has advantages over experiments involving a tethered capsule. The experimental results indicate that fasted gastric pH was similar between Cynomolgus monkeys and literature human values. However, following a meal, the monkey gastric pH was elevated for a longer duration than that in human. The monkey GRT was longer than that

Table III. GRT Species Comparison

Species	Source	Method	Number of Subjects	Fasted GRT (min)	Fed GRT (min)
Monkey	Present study	Bravo® capsule	12 males: 6 (fasted and fed standard meal), 4 (fed slurry meal)	153±87[a]	436±265[b,c] 697±193[d,e]
Human	(54)	Bravo® capsule	8 males	52.4±25.4	143±131[f]
	(40)	Heidelberg capsule	5 males, 5 females	59.7±14.8	–
	(36)	Heidelberg capsule	4 males	114±66	–
	(33)	Heidelberg capsule	13 males, 3 females: 6 (liquid and standard meals) 10 (fasted)	72±48	156±54[g] 288±90[h]
	(34)	Heidelberg capsule	12 young males, 12 young females, 12 elderly males	–	204±36[h] (male) 276±72[h] (female) 270±66[h] (elderly)
	(35)	Heidelberg capsule	6 males	–	216±48[g]

Data represented as mean±SD
[a] n=13 individual experiments
[b] slurry meal
[c] n=9 individual experiments
[d] Standard biscuit meal
[e] n=6 individual experiments
[f] Meal type not defined
[g] Liquid fatty meal
[h] Standardized solid meal

observed in human under both fasted and fed conditions, although this is likely dependent on the Bravo® capsule size. The GRT obtained during fasted conditions, was statistically different when compared to the GRT obtained following a standard or slurry meal.

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