Relationship Between the Phasic Period of Interdigestive Migrating Contraction and the Systemic Bioavailability of Acetaminophen in Dogs

Kazuyoshi Sagara,[1,2] Hiroaki Mizuta,[1] Minoru Ohshiko,[1] Masahiro Shibata,[1] and Keiichiro Haga[1]

Received September 5, 1994; accepted November 11, 1994

The relationships of the phasic period of interdigestive migrating contraction to gastrointestinal (GI) transit of drugs and their oral absorption were investigated in mongrel dogs by simultaneous oral dosing of acetaminophen (AAP) and salicylazosulfapyridine (SASP) at the starting points of the phase I and phase III periods of gastric contractions. Strain-gauge force transducers were surgically sutured onto the serosa of the GI tracts in the dogs to measure the interdigestive migrating contractions. The mean absorption time of AAP and the time for the first appearance of sulfapyridine (a bacterial metabolite of SASP in the colon) in plasma were used as the indices of gastric emptying time (GET) and small intestinal transit time (SITT), respectively. In individual dogs, the GET and the SITT at phase I showed a clear delay in comparison with those at phase III. For AAP used as a marker compound here, the systemic bioavailability after oral dosing to intact beagle dogs at doses of 3, 10, and 20 mg/kg was about 55, 63, and 79%, suggesting that AAP undergoes a non-linear hepatic clearance. At a dose of AAP 20 mg/kg, the systemic bioavailability of AAP was 100% in the case of dosing at phase III, but was reduced by half when dosing at phase I. These results indicate that, in oral dosing, the transit of drugs through the GI tract was clearly affected by the phases of gastric contractions. Phasic GI motility is thus concluded to be a cause for the inter- and intraindividual variations in the systemic bioavailability of drugs such as AAP that undergo a non-linear hepatic clearance, as a result of either gradual or rapid transport of drugs to enzymes on their first pass through the liver.

KEY WORDS: interdigestive migrating contraction; acetaminophen; salicylazosulfapyridine; gastrointestinal transit; systemic bioavailability; dogs.

INTRODUCTION

Marked intra- and interindividual variations in bioavailability are often observed for drugs that undergo high and non-linear hepatic clearance. For example, the peak plasma concentration of oral acetaminophen (AAP) and the systemic bioavailability of oral nifedipine is reduced in humans when the gastric emptying rate is slow (1,2). This study therefore focuses on motility in the GI tract.

Motility patterns in the GI tracts in fasted animals and humans follow complex cyclical processes and several phases of interdigestive migrating contraction (IMC). Phase I is a quiescent period. Phase II consists of intermittent and irregular contractions that gradually increase in strength, culminating in a period of intense contractions, namely phase III. The powerful contractions during phase III of the IMC are called the "housekeeper wave," these contractions play an important role in the emptying of drugs and preparations from the stomach. The emptying of the contents from the stomach has been said to be affected by the GI motility (3,4). However, the IMC phasic period has not yet been thoroughly studied from the aspect of its influence on GI transit and on the systemic bioavailability of oral drugs.

In the present study, the method chosen uses AAP and salicylazosulfapyridine (SASP) as respective marker compounds for the simultaneous determination of gastric emptying time (GET) and small intestinal transit time (SITT) of the drugs in beagle dogs (5). The mean absorption time of AAP and the time for the first appearance of sulfapyridine (SP: a bacterial metabolite of SASP in the colon) in plasma were used as the indices of the GET and the SITT, respectively. AAP was reported to undergo non-linear hepatic clearance (6). We have already reported on the oral bioavailability of several drugs and preparations in beagle dogs as indicated by tracing the GI transit of these marker compounds (7–9). Furthermore we proposed a method to regulate the GI physiology in beagle dogs by a combined-treatment of intramuscular pentagastrin and intravenous atropine sulfate (10–12). In this report, we discuss the influence of the phasic period on GI transit in dogs assessed by oral AAP and SASP at the dosing time to be timed with the appearance of the phase I and phase III contractions of the stomach. Moreover, we describe the relationship between the IMC phasic period in the dogs and the systemic bioavailability of oral AAP, as a model of drugs undergoing the non-linear hepatic clearance.

MATERIALS AND METHODS

Materials

Drug compounds of AAP and SP were purchased from Sigma Chemical Co. (St. Louis, MO., USA). Salazopyrin^R tablets, containing 500 mg of SASP per tablet, were purchased from the Green Cross Co. (Osaka, Japan). In this study, 324 mg of crushed Salazopyrine^R tablets was equivalent to 250 mg SASP. All other reagents used were of analytical grade available from commercial suppliers.

Measurement of GI Motility

Five healthy male mongrel dogs weighing between 13 and 15 kg were used. Under pentobarbital anesthesia, a Silastic^R tube (Dow Corning, Midland, MI) was inserted into the superior vena cava through the right external jugular vein of each dog. This tube was used as the route for the sampling of plasma. The abdomen was opened by a midline incision, and a strain-gauge force transducer (F-12 IS-60, Star Medical, Tokyo, Japan) was sutured onto the serosa of the GI tract so as to allow measurement of circular muscle contraction according to the method reported (13). The transducer was placed on the gastric antrum 3 cm proximal to the py-

[1] Research Laboratories, Yoshitomi Pharmaceutical Industries, Ltd., Koiwai, Yoshitomi-cho, Chikujo-gun, Fukuoka 871, Japan.
[2] To whom correspondence should be addressed.

0724-8741/95/0400-0594$07.50/0

loric ring, on the mid-duodenum, on the jejunum 10 cm distal to the ligament of Treitz, on the ileum 20 cm proximal to the cecum, and on the colon 10 cm distal to the cecum. Actual absorption studies were started one month after the operation. GI motility was recorded on a polygraph (WR3001, Graphtech, Tokyo, Japan). The phases of the IMC in the stomach were characterized according to the method reported (14).

Measurement of GI Transit Time

In accordance with the method previously reported (5), the GET and the SITT were measured by using AAP and SASP as marker compounds. The mean absorption time of AAP (MAT) was used as the index of the GET. The MAT was calculated according to the following equation: MAT = $MRT_{p.o.} - MRT_{i.v.}$, where $MRT_{p.o.}$ and $MRT_{i.v.}$ represent the mean residence times of oral and intravenous dosing. The time for the first appearance of SP in plasma was used as the index of the SITT.

Absorption After Dosing at the Phase I and Phase III Periods of Gastric Contractions

Three kinds of experiments were carried out in the five dogs described above, at 1-week intervals: 1) the first experiment at the dosing time to be timed with the appearance of the phase I contraction of the stomach; 2) the second experiment at the dosing time to be timed with the appearance of the phase III contraction of the stomach; 3) the third experiment to determine the systemic bioavailability of AAP. In the first and second experiments, AAP (10 mg/ml) and SASP (25 mg/ml) were mixed and suspended in water, and were orally administered to the dogs at doses of 20 mg/kg and 25 mg/kg, respectively. In the third experiment, AAP was dissolved in a saline containing 50% (v/v) propylene glycol (50 mg/ml), and was intravenously administered to the five dogs at a dose of 10 mg/kg.

Each dog was fasted for 24 h prior to and until the end of the experiments, but was allowed free access to water. Blood samples were taken with heparinized syringes 0.08, 0.25, 0.42, 0.58, 0.75, 0.92, 1.1, 1.6, 2.1, 2.6, 3.1, 3.6, 4.1, and 5.1 h after the oral and intravenous dosing, and were centrifuged (1300 g for 15 min) to separate plasma. The plasma samples were kept frozen until the HPLC assay of AAP and SP. The amount of absorbed drug after oral administration was calculated by the point-area deconvolution method (15).

Relationship Between Doses of AAP and Bioavailability

Six healthy male beagle dogs weighing between 9 and 12 kg were used without the surgical operation described above. These intact dogs were fasted for 24 h prior and to the end of each experiment, but were allowed free access to water. In water, AAP was dissolved at concentrations of 3 and 10 mg/ml. Each solution was orally dosed to the six dogs at doses of 3 mg/ml/kg, 10 mg/ml/kg, and 20 mg/2 ml/kg, respectively. To determine the systemic bioavailability of AAP, AAP was intravenously dosed to the six dogs at a dose of 10 mg/kg. Blood samples were taken with heparinized syringes 0.25, 0.5, 1, 2, 3, 4, 5, and 6 h after the oral and intravenous dosing, and were centrifuged (1300 g for 15 min) to separate plasma. The plasma samples were kept frozen until the HPLC assay of AAP.

Assay for Plasma Concentrations of AAP and SP

To 100 μl of the plasma sample were added 1 μg of *p*-anisamide (internal standard), 1 ml of 0.5 M Na_2HPO_4-KH_2PO_4 buffer (pH 7.4), and 1 ml of ethyl acetate. After shaking for 10 min and centrifugation at 1300 g for 5 min, the supernatant (1 ml) was transferred to a glass tube and evaporated to dryness under reduced pressure. The residue was dissolved in 200 μl of the mobile phase for HPLC, and 100 μl of this solution was injected onto the HPLC column. The HPLC system consisted of a Shimadzu LC-6A pump, a Shimadzu SPD-6A UV detector (wave length 254 nm), and a Shimadzu C-R3A integrator. Separations were performed on a Nucleosil 7C18 column (15 cm × 4 mm i.d., M. Nagel) with a mobile phase of acetonitrile-1% AcOH (1 : 9, v/v) and a flow rate of 1.5 ml/min. The lower limits of the assay of AAP and SP in plasma were both 0.1 μg/ml.

Pharmacokinetic Analysis

The maximum plasma concentration (C_{max}) and the time to reach the C_{max} (t_{max}) were read from individual plasma concentration-time curves of AAP. The area under the plasma concentration-time curves (AUC) was calculated by the linear trapezoidal method, and the mean residence time (MRT) was computed by moment analysis (16).

Statistical Analysis

Differences in each bioavailability parameter and the GI transit time were statistically evaluated by the paired *t*-test.

RESULTS AND DISCUSSION

GI Motility

A typical record of the IMC is shown in Figure 1. Gastric contractions in each dog recurred regularly in cycles of 106.4 ± 16.9 (SD) minutes. In agreement with results reported (13), the onset of the gastric contraction was clear, and the phase II contractions seldom appeared after the phase I. The duration times of the phase I and phase III contractions in the stomach were 83.5 ± 14.9 and 21.6 ± 6.3 (SD) minutes, respectively. These values were similar to the results reported for mongrel dogs (17).

GI Transit Time

The gastric emptying in the five dogs studied was more rapid, with statistical significance, at phase III than at phase I period of gastric contractions (Table I). Similar results were observed in humans (4). Just as in the case of the GET, individual values of the SITT at phase I showed a delay up to 5 h or more in all five dogs, whereas those at phase III were 3–5 h. These observations indicated that the IMC played an important role not only in gastric emptying but also in transportation through the small intestine. In our previous report (5), marked inter- and intraindividual variations of the SITT (0.5-5 h) in beagle dogs were observed. In this study, the

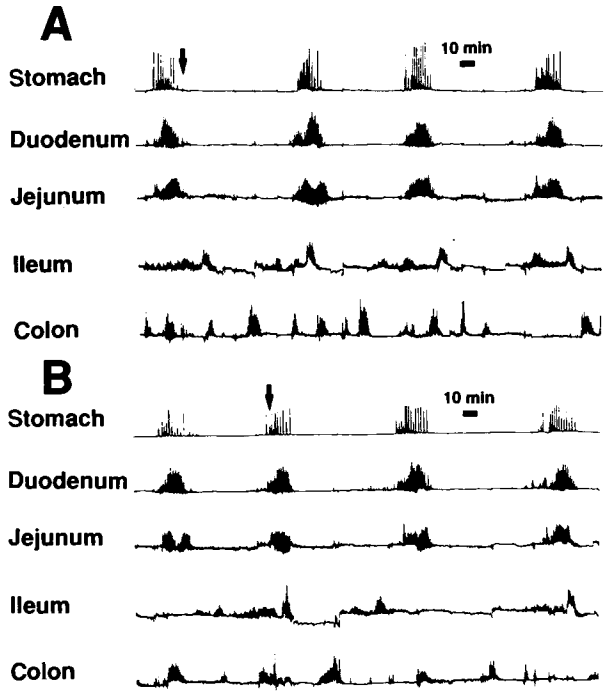

Fig. 1. Typical interdigestive migrating contractions recorded at five sites of the gastrointestinal tract for No.4 dog. The arrow indicates the dosing time of oral acetaminophen at 20 mg/kg and salycilazosulfapyridine at 25 mg/kg at the starting point of (A) phase I and (B) phase III periods of the gastric contractions.

drug administrations to the mongrel dogs were at the starting point of either phase I or phase III. The present results therefore suggest that the inter- and intraindividual variations in the SITT were caused by the phase difference of the phases at the dosing time. In fact, the values for the SITT in three out of five dogs could not to be determined until 5 h after the dosing. The extended values for the SITT at phase I appear to correspond to the time lag until the following phase III. These values of the SITT obtained in mongrel dogs at the two phase periods were found to be relatively larger than those in beagle dogs in our previous reports (5,7–10). Though the detailed cause was not clear, this discrepancy suggests the existence of a difference in the intensity of GI motility between the two dog strains.

Our findings also indicate that the bioavailability of oral drugs in dogs sometimes fluctuates in response to the phasic period of the gastric contractions at the dosing time. When the dosing time is not at phase I but at phase III, oral drugs or preparations are rapidly transferred through the GI tract by the IMC. In such cases, the extent of absorption will frequently be affected by the reduction in the GI transit time.

Absorption of AAP

The relationship between the dose of AAP and its bioavailability was investigated in intact beagle dogs (Fig. 2). When AAP was administered to the intact dogs at a dose of 20 mg/kg, its systemic bioavailability was about 79%. However, the bioavailability was reduced to 63 and 55% at doses of 10 and 3 mg/kg, respectively. At the doses up to 450 mg in dogs (about 33–50 mg/kg), the extent of absorption was 80 to 98% for AAP and its conjugates, suggesting a completely absorbed drug (18). Thus, the diminished systemic bioavailability at lower doses of AAP seems to be due to a non-linear hepatic clearance of AAP. Such a biopharmaceutical property of AAP has been reported in humans (19) and rats (6,20). We will discuss the influence of the phasic period at the dosing time on the bioavailability of AAP.

After dosing at phase III in the surgically operated dogs, AAP was rapidly absorbed, and the systemic bioavailability reached 100% within 2 hours. On the contrary, at the phase I, the drug was slowly absorbed after dosing, and the systemic bioavailability was 56% after 5 hours. (Fig. 3 and Fig. 4). There were significant differences in the pharmacokinetic parameters in dosing at the two phases (Table II). Gastric emptying has been reported to be a rate-limiting factor for the absorption of AAP in humans (1,21). The absorption of AAP in humans has thus been slowed down by pethidine or diamorphine, both of which have reduced the rate of gastric emptying, though total urinary recovery of unchanged and conjugated AAP over a period of 24 hours has not decreased (22). We therefore concluded that the systemic bioavailabil-

Table I. Individual Gastric Emptying Time (GET) and Small Intestinal Transit Time (SITT) in the Different Phases of the Gastric Contractions in Dogs

	Phase I		Phase III	
No.	GET (h)	SITT (h)	GET (h)	SITT (h)
1	0.54	>5.1	0.16	3.6
2	0.86	5.1	0.28	3.1
3	0.54	>5.1	0.01	2.1
4	0.62	>5.1	0.05	5.1
5	1.01	4.1	0.15	4.1
mean ±SD	0.71 ±0.21	[b] –	0.13[a] ±0.11	3.6 ±1.1

[a] Statistically significant ($p < 0.01$) vs. at phase I.
[b] The mean was not able to be calculated.

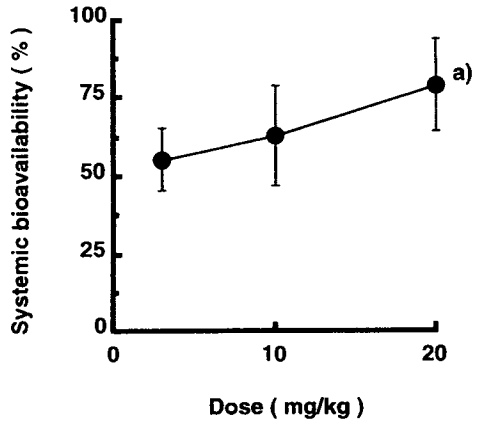

Fig. 2. Relationship between the oral dose of AAP and the bioavailability in dogs. a) Statistically significant ($p<0.05$) vs. 3 mg/kg. Each point represents the mean ±SD of 6 dogs.

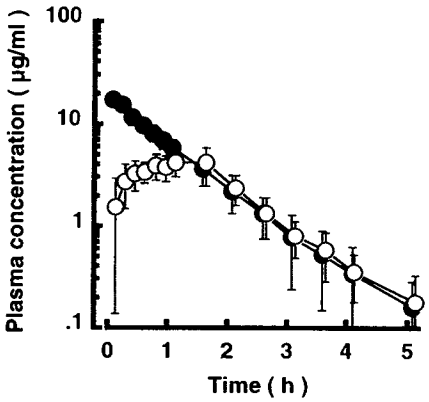

Fig. 3. Plasma concentration-time curves of acetaminophen after oral dosing at the different phases of the gastric contractions in dogs ○: phase I, ●: phase III. Each point represents the mean ±SD of 5 dogs.

ity of AAP in the dogs decreased dependent upon the dosing at phase I because a slow gastric emptying increased the cumulative extent of hepatic first-pass metabolism, as described above. For drugs with the characteristic of non-linear hepatic clearance, variations in bioavailability would result from combined factors, as follows: 1) a drug input rate that determines the extent of hepatic clearance of the drugs before entering the systemic circulation; 2) the individual capacity for hepatic clearance.

We thereby indicated the relationship between the IMC and the GI transit of drugs, and pointed out a probable cause for the inter- and intraindividual variations in the systemic bioavailability of drugs such as AAP that undergo non-linear hepatic clearance. Moreover, when a certain sustained-release preparation of nifedipine has been administered to humans, the systemic bioavailability of nifedipine has been reported to be reduced with a delay in the gastric emptying of the preparation, suggesting the possibility that drug is metabolized excessively in the hepatic first-pass (2). Our findings would be useful in interpreting an individual variation in the drug absorption for oral drug delivery systems affected by a varied GI motility, such as floating systems or single-unit sustained-release systems.

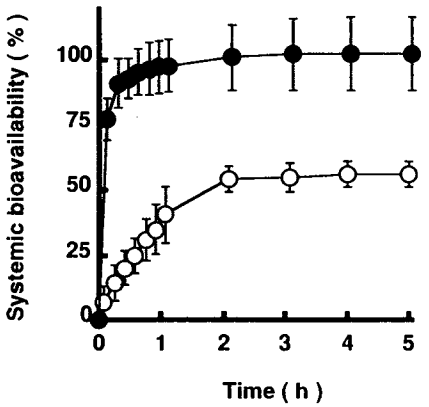

Fig. 4. Absorption profiles of acetaminophen after oral dosing at the different phases of the gastric contractions in dogs. ○: phase I, ●: phase III. Each point represents the mean ±SD of 5 dogs.

Table II. Pharmacokinetic Parameters After Oral Dosing of Acetaminophen at the Different Phases of the Gastric Contractions in Dogs

Motility	C_{max} (μg/ml)	t_{max} (h)	$AUC_{0-5\,h}$ (μg · h/ml)	MRT (h)
phase I	5.5 ± 0.8	1.1 ± 0.5	9.6 ± 1.0	1.5 ± 0.2
phase III	17.7 ± 2.3[a]	0.1 ± 0.1[b]	17.2 ± 3.9[b]	1.0 ± 0.2[a]

Results are expressed as the mean ± SD of 5 dogs.
[a] Statistically significant ($p < 0.01$).
[b] Statistically significant ($p < 0.05$) vs. at phase I.

REFERENCES

1. R. C. Heading, J. Nimmo, L. F. Prescott, and P. Tothill. The dependence of paracetamol absorption on the rate of gastric emptying. *Br. J. Pharmac.* 47, 415-421 (1973).
2. I. R. Wilding, R. A. Sparrow, S. S. Davis, and R. J. Horton. The role of gastric emptying in the absorption and metabolism of nifedipine given in a modified release pellet formulation. *Int. J. Pharm.* 84, 59-67 (1992).
3. N. E. Schindlbeck, C. Heinrich, and S. A. Muller-Lissner. Relation between fasting antroduodenal motility and transpyloric fluid movements. *Am. J. Physiol.* 257, G198-G201 (1989).
4. R. L. Oberle, Tzyy-Show Chen, C. Lloyd, J. L. Barnett, C. Owyang, J. Meyer, and G. L. Amidon. The influence of the interdigestive migrating myoelectric complex on the gastric emptying of liquids. *Gastroenterology* 99, 1275-1282 (1990).
5. H. Mizuta, Y. Kawazoe, K. Haga, K. Ogawa, and T. Yokobe. Determination of small intestinal transit time in beagle dogs using salicylazosulfapyridine. *Yakugaku Zasshi* 109, 760-765 (1989).
6. G. M. Cohen, O. M. Bakke, and D. S. Davies. First pass metabolism of paracetamol in rat liver. *J. Pharm. Pharmac.* 26, 348-351 (1974).
7. H. Mizuta, Y. Kawazoe, and K. Ogawa. Gastrointestinal absorption of chlorothiazide: evaluation of a method using salicylazosulfapyridine and acetaminophen as the marker compounds for determination of the gastrointestinal transit time in the dog. *Chem. Pharm. Bull.* 38, 2810-2813 (1990).
8. H. Mizuta, Y. Kawazoe, and K. Ogawa. Effect of small intestinal transit time on gastrointestinal absorption of 2-[3-(3,5-di-tert-butyl-4-hydroxyphenyl)-1H-pyrazolo[3,4-b]pyridin-1-yl]ethyl acetate, a new non-steroidal anti-inflammatory agent. *Chem. Pharm. Bull.* 38, 2825-2828 (1990).
9. H. Mizuta, Y. Kawazoe, Y. Ikeda, and K. Ogawa. Gastrointestinal absorption of nitrofurantoin: Evaluation of a newly developed method for determination of the gastrointestinal transit time in dogs. *Yakugaku Zasshi* 111, 794-799 (1991).
10. K. Sagara, Y. Nagamatsu, I. Yamada, M. Kawata, H. Mizuta, and K. Ogawa. Bioavailability study of commercial sustained-release preparations of diclofenac sodium in gastrointestinal physiology regulated-dogs. *Chem. Pharm. Bull.* 40, 3303-3306 (1992).
11. K. Sagara, I. Yamada, Y. Kawazoe, H. Mizuta, and M. Shibata. Gastrointestinal physiology-regulated dogs: utilization of a new thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]-diazepine, an antagonist of platelet-activating factor, and its preparations. *Biol. Pharm. Bull.* 17, 117-120 (1994).
12. K. Sagara, M. Kawata, H. Mizuta, and M. Shibata. Utility of gastrointestinal physiology regulated-dogs: Bioavailability study of a commercial sustained-release dosage form of theophylline. *Biol. Pharm. Bull.* 17, 931-934 (1994).
13. Z. Itoh, S. Takeuchi, I. Aizawa, and R. Takayanagi. Characteristic motor activity of the gastrointestinal tract in fasted conscious dogs measured by implanted force transducers. *Am. J. Dig. Dis.* 23, 229-238 (1978).
14. G. M. Carlson, B. S. Bedi, and C. F. Code. Mechanism of propagation of intestinal interdigestive myoelectric complex. *Am. J. Physiol.* 222, 1027-1030 (1972).

15. K. Iga, Y. Ogawa, T. Yashiki, and T. Shimamoto. Estimation of drug absorption rates using a deconvolution method with non-equal sampling times. *J. Pharmacokinet. Biopharm.* 14, 213-225 (1986).
16. K. Yamaoka, T. Tanigawara, T. Nakagawa, and T. Uno. A pharmacokinetic analysis program (MULTI) for microcomputer. *J. Pharmacobio-Dyn.* 4, 879-885 (1981).
17. Z. Itoh, and I. Takahashi. Periodic contractions of the canine gallbladder during the interdigestive state. *Am. J. Physiol.* 240, G183-189 (1981).
18. R. G. Sample, G. V. Rossi, and E. W. Packman. Thirty-vella dog as a biologic model for evaluation of drug absorption from the intestinal mucosa. *J. Pharm. Sci.* 57, 795-798 (1968).
19. R. Ameer, M. Divoll, D. R. Abernethy, D. J. Greenblatt, and L. Shargel. Absolute and relative bioavailability of oral acetaminophen preparations. *J. Pharm. Sci.* 72, 955-958 (1983).
20. Y. Tone, K. Kawamata, T. Murakami, Y. Higashi, and N. Yata. Dose-dependent pharmacokinetics and first-pass metabolism of acetaminophen. *J. Pharmacobio-Dyn.* 13, 327-335 (1990).
21. J. A. Clements, R. C. Heading, W. S. Nimmo, and L. F. Prescott. Kinetics of acetaminophen absorption and gastric emptying in man. *Clin. Pharmacol. Ther.* 24, 420-431 (1978).
22. W. S. Nimmo, R. C. Heading, J. Wilson, P. Tothill, and L. F. Prescott. Inhibition of gastric emptying and drug absorption by narcotic analgesics. *Br. J. Clin. Pharmac.* 2, 509-513 (1975).