Scintigraphic measurement of regional gastrointestinal transit in the dog

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Iwanaga, Yui, Jipu Wen, Mikael S. Thollander, Louis J. Kost, George M. Thomforde, Richard G. Allen, and Sidney F. Phillips. Scintigraphic measurement of regional gastrointestinal transit in the dog. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G904–G910, 1998.—Scintigraphic techniques can measure sequentially gastric emptying, small bowel transit, and colonic transit in humans, and comparable methods for experimental studies in animals would be useful. We developed such a method in dogs and examined the effects of prokinetic drugs on regional transit. Two isotopes were given to fasting dogs. Polystyrene pellets labeled with $^{99m}$Tc were mixed in a can of dog food and $^{111}$In-labeled pellets were given in a gelatin capsule coated with a pH-sensitive polymer, designed to dissolve in the distal bowel. Gamma camera images were obtained for up to 24 h. Prokinetic drugs were given by intravenous injection. Duplicate baseline studies showed good agreement in seven dogs. In a second group (n = 4), intra- and interanimal variabilities were established. Two novel prokinetic drugs (AU-116 and AU-130) accelerated small bowel and colonic transit. A simple noninvasive method for measuring whole gut transit in dogs was developed and validated. Two new prokinetics accelerated small bowel and colonic transit.

canine gut transit; colonic prokinetics; scintigraphy

ORDERLY SEQUENTIAL ABORAD movement of food, chyme, and feces is the ultimate goal of the enteric neuromusculature, and measurements of transit have been used often as practical indexes of gastrointestinal motility (17, 22). Gastrointestinal transit can be assessed noninvasively and most comprehensively by scintigraphy (17), which has been adapted for use throughout the gut in humans (4–6, 24). When two isotopes are used, gastric emptying, small bowel transit, and colonic transit can be quantified sequentially. Experimental animals, notably the dog, have been employed extensively for gastrointestinal myoelectrical recordings and manometry, but few comprehensive approaches to transit have been developed. Individual observations on transit have been reported (8, 12, 14, 15, 18, 20, 27). Our first aim was to validate our initial experiences in which we adapted the dog a system developed for whole gut transit (28). We proposed that such a model would be of value for the preclinical testing of prokinetics.

MATERIALS AND METHODS

Animals Studied

Two sets of healthy, female mongrel dogs, weighing 16–22 kg, were used. One group of seven dogs was used for protocol 1; the second group of four dogs was used for all subsequent studies (protocol 2). Before experiments were started, the dogs were dewormed routinely with fenbendazole (10 mg/kg body wt daily for 3 days). During the course of subsequent experiments canine giardiasis was diagnosed in the institutional kennels, and all animals were again treated empirically twice with fenbendazole by the same schedule. Two of the animals from protocol 2 whole gut transit was measured immediately before and after treatment; the drug did not alter transit significantly. Before the beginning of the experiments all dogs were trained to lay comfortably in a Pavlov sling resting on a gamma camera. The study was approved by the Institutional Animal Care and Use Committee of the Mayo Clinic. Experiments were performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (DHHS Publication No. (NIH) 85–23, revised 1985, Office of Science and Health Reports, Bethesda, MD 20892).

Experimental Procedure

Before each day of study, two $^{57}$Co external markers were placed at the level of the xiphoid to serve as a reference for the stomach. After a 24-h fast, a pH-sensitive capsule that disperses at a pH of 6.0–7.0 (4–6) was given with 50 ml of water. The capsule and water were placed in the mouth, and the jaws were held closed until the dog swallowed. The capsule contained 1 g of Amberlite IR-120 Plus resin pellets...
(1-mm diam; Sigma Chemical, St. Louis, MO) labeled with up to 0.25 mCi of $^{111}$InCl₃ (Amersham, Arlington Heights, IL). One hour was allowed for the capsule to empty from the stomach, during which three 1-min gamma images were acquired. The relationship of the capsule to the external $^{57}$Co markers indicated when the capsule left the stomach.

One hour after ingestion of the capsule a $^{99m}$Tc-labeled meal was given to the dog. The meal consisted of an egg cooked with 1-mm Amberlite 410 resin pellets labeled with $^{99m}$Tc (4–6) and one can of Alpo dog food (450 kcal; 9% protein, 6% fat, 1.5% fiber, and 78% moisture; Alpo Petfoods, Lehigh Valley, PA). The egg was cooked to a firm consistency to provide a solid medium with which to assess gastric emptying and small bowel transit.

Anterior images were acquired at regular intervals for the first 8 h: each 5 min during the first 30 min after eating, each 15 min until 3 h, and then every 30 min for the next 5 h. Dogs were allowed to return to their cages, and further images were acquired at 12 and 24 h. A large field-of-view gamma camera with a medium-energy, parallel-hole collimator was used (General Electric Starcam, Milwaukee, WI). The mean downscatter of $^{111}$In into the $^{99m}$Tc window was calculated by subtracting $^{111}$In counts in the $^{99m}$Tc window from those in the $^{111}$In window. Data were stored on an on-line computer (General Electric Starcam) for later analysis. Two energy windows of acquisition were used, 140 keV ($\pm 20\%$ window) for $^{99m}$Tc counts (for gastric emptying and small bowel transit) and 245 keV ($\pm 20\%$ window) for $^{111}$In counts (for colonic transit). With use of variable regions of interest programs (5, 6), radioactivity was quantitated in the stomach and large bowel for $^{99m}$Tc and in three regions of interest in the colon for $^{111}$In (proximal, distal, and rectosigmoid colon). The external markers and the shape of the gastric image were used to construct regions of interest for the stomach and small bowel (Fig. 1). The midline plane was used to separate the proximal from the distal colon and a point in the left lower quadrant, opposite the ileocolonic junction, to separate the distal colon from rectosigmoid (Fig. 2). Stools were collected, fecal excretion of isotopes was quantified, and this was designated as colonic region 4. Counts in each region were corrected for the downscatter of $^{111}$In into the $^{99m}$Tc window using the mean value calculated during 1 h scanning before the ingestion of the meal.

Data Analysis

The time point zero was assigned to the beginning of the meal (Tc label) 1 h after ingestion of the $^{111}$In capsule. Gastric emptying was expressed as a lag phase (until 10% counts left the stomach), 50% emptying time, and the calculated slope of postlag emptying. For this calculation we used a linear regression model drawn through the points from the first data beyond the lag time until 90% of the radiolabel had left the stomach (4). An estimate of the goodness of fit of the linear regression model was obtained from the square of the correlation coefficient of the predicted line from the actual data points. Small bowel transit time was calculated by subtracting the time for 10% radiolabel to empty from the stomach from the time for 10% to enter the colon (5, 6). Colonic regional transit of the solid residue was assessed by the geometric center (GC), which is the weighted average of proportions of counts in each region (5, 6). In this way the midpoint of the total counts is expressed relative to the colonic region: 1, proximal; 2, distal; and 3, rectosigmoid colon. Counts in stool (beyond the colon) were 4. Higher values signify more distal passage of label. To correct for differences in the progress of the label before the injection of

Fig. 1. Scintiscan images of $^{99m}$Tc showing dog stomach and small bowel. Animal was oriented with head to right and with right side uppermost. Fundus and antrum were filled at 60 min, and gastric region of interest could be defined clearly. Very few counts are in small bowel at this time. Thereafter, the stomach emptied and the small bowel filled progressively with counts accumulating inferior to external markers (intestinal regions of interest).
drugs, changes in the GC were computed for the first and second postdrug hours; this was designated as change in GC. All scans were analyzed for the number of counts in the region of interest and expressed as a percentage of total counts in the field of view, thereby correcting automatically for decay. Intra- and interanimal variability was expressed as coefficients of variation (%CV) for each animal and for the group of four. Drug treatments were compared with vehicle alone by ANOVA.

Experimental Design

Protocol 1. Seven dogs were studied on two occasions (3–5 days apart), the aim being to establish the method and to obtain preliminary data of interanimal variability and intra-animal reproducibility.

Protocol 2. This protocol was developed from this background. Initial estimates of power led us to select a 4×4 Latin square design, featuring four animals and four treatment programs. For the individual indexes of gastric emptying, small bowel transit, and colonic transit, the design had 90% power to detect differences varying from 20 to 40%. Reproducibility was tested further in a block of experiments in which all four treatments were placebo.

Subsequently, three study blocks tested prokinetic drugs for possible effects on the large bowel. These were 1) placebo and three doses of AU-116 (0.1, 0.3, and 1.0 mg/kg iv); 2) placebo and three doses of AU-130 (0.03, 0.1, and 0.3 mg/kg iv); and 3) AU-116 (0.3 mg/kg iv), cisapride (1.0 mg/kg iv), and erythromycin (0.05 mg/kg iv).

These protocols featured the intravenous injection of drugs (or placebo) 2 h after the meal, i.e., 3 h after administration of the encapsulated markers. The timing was chosen to emphasize the effects of drugs on colonic transit.

Drugs

AU-116 \( \text{endo-4-[3-(4-amino-5-chloro-2-methoxybenzamido)-9-azabicyclo[3.3.1]non-9-yl] butyric acid} \) and AU-130 \([4-(4-amino-5-chloro-2-methoxybenzamidopiperidino)acetic acid hydrochloride\) were synthesized by Hokuriku Seiyaku (Katsuyama, Fukui, Japan). Cisapride was a gift from Janssen Research Foundation (Beerse, Belgium, courtesy of Dr. J. A. Schuurkes). AU-116, AU-130, and cisapride were dissolved in a solution containing 5% lactic acid and adjusted to pH 3–4. Erythromycin (Abbott Laboratories, Erythrocin Lactobionate-IV, Chicago, IL) was dissolved in distilled water and diluted with saline. The control observations featured 5% lactic acid solution alone. Drugs were used in the doses recommended by the manufacturers and based on earlier literature for erythromycin (10, 15).

RESULTS

Figures 1 and 2 are examples of scans of the stomach, foregut, ileum, and colon. Regions of interest could be defined adequately in all animals.

Protocol 1. In six of seven dogs \( ^{111} \text{In} \) capsules entered the small bowel within the first hour in both experiments. All capsules dispersed when they reached the distal small bowel. In one dog capsules remained in the stomach for 8 h in both experiments. Table 1 gives results for duplicate measurements of transit in this group of seven animals. When the two sets of experiments were compared, there were no significant differences between the means for all indexes of transit; more importantly, the interanimal reproducibility was consistently good.
Protocol 2. We evaluated inter- and intraindividual variability further by a block of four studies in each of four dogs, using the control vehicle only. In 13 of 16 experiments, $^{111}$In capsules were emptied into the small bowel from the stomach within the first hour. In two of four experiments in one dog, capsules remained in the stomach for 90 and 180 min, respectively, and in one of four experiments in another dog, the capsule remained in the stomach for 80 min.

The mean duration of the lag phase of gastric emptying in each dog was from 80 to 156 min; the group mean for four dogs was 105 min (%CV 33). The times for emptying of 50% of the $^{99m}$Tc (Fig. 3) showed less dispersion, individual means were 240–378 min, with a group mean of 285 min (%CV 22). Small bowel transit times (Fig. 3) were more variable, individual means were 41–120 min; the group mean was 83 min (%CV 48). Individual GC at 2 and 3 h after feeding were 1.0–1.4 and 1.2–1.7 (Fig. 3). The group means at 2 and 3 h were 1.1 (%CV 15) and 1.4 (%CV 16).

### Effects of AU-116 on Gastrointestinal Transit

#### Gastric emptying.
When AU-116 (0.1–1.0 mg/kg) was administered 2 h after the test meal, the time for half gastric emptying was unchanged (Table 2).

#### Small bowel transit.
AU-116 at a dose of 0.1 mg/kg did not accelerate small bowel transit, but at doses of 0.3 and 1.0 mg/kg, AU-116 shortened transit by approximately two-thirds (Table 2).

#### Colonic transit.
Treatment with the smallest dose alone resulted in no detectable change in the GC (Table 2). AU-116 at 0.3 and 1.0 mg/kg accelerated colonic transit significantly in the first hour after administration, but this effect was not noted in the second hour (Table 2).

### Table 1. Reproducibility of gastrointestinal transit: scintigraphic studies in seven dogs

<table>
<thead>
<tr>
<th></th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
<th>Dog 4</th>
<th>Dog 5</th>
<th>Dog 6</th>
<th>Dog 7</th>
<th>Means ± SE</th>
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<tr>
<td>50% Gastric emptying, min</td>
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<td></td>
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<td>Study 1</td>
<td>286</td>
<td>318</td>
<td>438</td>
<td>220</td>
<td>205</td>
<td>183</td>
<td>348</td>
<td>285 ± 34</td>
</tr>
<tr>
<td>Study 2</td>
<td>256</td>
<td>210</td>
<td>444</td>
<td>222</td>
<td>277</td>
<td>211</td>
<td>439</td>
<td>294 ± 39</td>
</tr>
<tr>
<td>Δ</td>
<td>-30</td>
<td>108</td>
<td>-6</td>
<td>-2</td>
<td>-72</td>
<td>-28</td>
<td>-91</td>
<td></td>
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<tr>
<td>Small bowel transit, min</td>
<td></td>
<td></td>
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<tr>
<td>Study 1</td>
<td>95</td>
<td>145</td>
<td>135</td>
<td>65</td>
<td>49</td>
<td>126</td>
<td>103 ± 16</td>
<td></td>
</tr>
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<td>114</td>
<td>60</td>
<td>25</td>
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<td>62</td>
<td>17</td>
<td>21</td>
<td>5</td>
<td>24</td>
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<td>Colon transit GC at 6 h</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Study 1</td>
<td>1.9</td>
<td>1.1</td>
<td>1.2</td>
<td>1.1</td>
<td>1.5</td>
<td>1.1</td>
<td>1.3 ± 0.1</td>
<td></td>
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<tr>
<td>Study 2</td>
<td>1.9</td>
<td>1.7</td>
<td>1.4</td>
<td>1.3</td>
<td>1.5</td>
<td>1.1</td>
<td>1.5 ± 0.1</td>
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</tr>
<tr>
<td>Δ</td>
<td>0</td>
<td>-0.6</td>
<td>-0.2</td>
<td>-0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
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<td>GC at 24 h</td>
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<td></td>
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<td>Study 1</td>
<td>2.7</td>
<td>2.8</td>
<td>4.0</td>
<td>2.8</td>
<td>3.0</td>
<td>2.7</td>
<td>3.0 ± 0.2</td>
<td></td>
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<tr>
<td>Study 2</td>
<td>4.0</td>
<td>2.8</td>
<td>4.0</td>
<td>2.7</td>
<td>2.8</td>
<td>2.8</td>
<td>3.2 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Δ</td>
<td>-1.3</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>0.2</td>
<td>-0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GC, geometric center. *$^{111}$In capsules did not leave stomach, and colonic transit was not evaluated.

Fig. 3. Individual data from 4 control studies in each of 4 dogs. Time for half emptying of $^{99m}$Tc from stomach and small bowel transit is shown in minutes. Colonic transit of $^{111}$In is expressed as geometric center (GC) of counts (see text for details).
Effects of AU-130 on Gastrointestinal Transit

No dose of AU-130 changed the half-time for gastric emptying (Table 3). However, AU-130 (0.1 and 0.3 mg/kg) accelerated small bowel and colonic transit; again, the effect on the colon was noted only for the first hour after injection. There was a small but statistically significant slowing of colonic transit after the 0.03 mg/kg dose in the second hour.

Comparison of AU-116, Cisapride, and Erythromycin

The smallest effective dose of AU-116 (0.3 mg/kg) was compared with cisapride (1 mg/kg) and erythromycin (0.05 mg/kg). When given intravenously 2 h after the meal, none of these agents accelerated gastric emptying (data not shown). AU-116 again accelerated small bowel (Fig. 4) and colonic transit (Fig. 5). Erythromycin did not change small bowel or colonic transit, and cisapride had similar quantitative effects to AU-116 (Figs. 4 and 5), but greater variability among animals did not allow the changes to reach a conventional level of significance.

DISCUSSION

Our first aim, to establish a simple and reproducible method by which transit throughout the gastrointestinal tract could be quantified in an experimental model, was achieved. We chose dogs because they are easily trained to lie still on the camera and because of the existence of extensive literature on motility in this species. The set of duplicate studies on seven dogs (28) yielded encouraging results, leading us to propose a practical study design, using a convenient 4 x 4 grid of animals and treatments. Reproducibility studies in protocol 2 confirmed the initial observations; intra-animal variability and interanimal differences were both quite small. Indeed, these levels of reproducibility are generally somewhat better than those obtained by duplicate studies in healthy humans (7). In the study by Degen and Phillips (7), group mean differences for duplicate studies were close to zero, but a few individuals had large differences between the two results. Thus the design we chose for the present studies had adequate power to detect differences likely to be important biologically. Preparation of the isotopic markers has been described in detail when the original methods were applied to humans (4). They are easily learned, simple and rapid, and can be scheduled readily to fit the institutional availability of the Tc and In radioisotopes.

Table 2. Effects of AU-116 on gastrointestinal transit in four dogs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Half-Time of Gastric Emptying, min</th>
<th>Small Bowel Transit Time, min</th>
<th>ΔGC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st h</td>
<td>2nd h</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>250 ± 63</td>
<td>141 ± 53</td>
<td>0.03 ± 0.03</td>
</tr>
<tr>
<td>AU-116 (0.1 mg/kg)</td>
<td>247 ± 63</td>
<td>122 ± 20</td>
<td>0.60 ± 0.18</td>
</tr>
<tr>
<td>AU-116 (0.3 mg/kg)</td>
<td>198 ± 43</td>
<td>36 ± 10*</td>
<td>0.86 ± 0.22*</td>
</tr>
<tr>
<td>AU-116 (1 mg/kg)</td>
<td>224 ± 29</td>
<td>44 ± 8*</td>
<td>1.09 ± 0.26*</td>
</tr>
</tbody>
</table>

Values are means ± SE and represent distal progression of GC for 1st and 2nd h after drug administration. *P < 0.05 vs. vehicle control.

Table 3. Effects of AU-130 on gastrointestinal transit in four dogs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Half-Time of Gastric Emptying, min</th>
<th>Small Bowel Transit Time, min</th>
<th>ΔGC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st h</td>
<td>2nd h</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>268 ± 35</td>
<td>94 ± 36</td>
<td>0.20 ± 0.13</td>
</tr>
<tr>
<td>AU-130 (0.03 mg/kg)</td>
<td>305 ± 69</td>
<td>69 ± 23</td>
<td>0.45 ± 0.09</td>
</tr>
<tr>
<td>AU-130 (0.1 mg/kg)</td>
<td>330 ± 54</td>
<td>31 ± 6</td>
<td>0.58 ± 0.14*</td>
</tr>
<tr>
<td>AU-130 (0.3 mg/kg)</td>
<td>292 ± 63</td>
<td>44 ± 11</td>
<td>0.79 ± 0.10*</td>
</tr>
</tbody>
</table>

Values are means ± SE and represent distal progression of GC for 1st and 2nd h after drug administration. *P < 0.05 vs. vehicle control.
Our second goal was to test our system for its responsiveness to pharmacological manipulations predicted to alter transit. Our initial attention was directed to the large bowel because region-specific colonic prokinetics are not yet available clinically, despite constipation being a common clinical problem which usually warrants drug therapy. Constipation can result from the inability of motor forces to propel feces from the proximal colon to the rectosigmoid region in a timely fashion (5, 22); one pathophysiological mechanism that has been proposed is that colonic muscle is unable to develop strong propulsive forces, i.e., high-amplitude propagating contractions (HAPCs), for the mass movement of feces (22). In this regard, some of the proposed colonic prokinetics have an attractive rationale. When given orally to dogs, R-093877 induced HAPCs during the first hour after treatment (2); intravenous cisapride and AU-130 also stimulated HAPCs in dogs (25a).

Substituted benzamides are derivatives of para-aminobenzoic acid and, although structurally related to procainamide, have none of its antiarrhythmic or local anesthetic properties. AU-116 and AU-130 are newly synthesized benzamide derivatives which have stimulatory effects on gastrointestinal motor activity in conscious dogs (25a). In this study, we demonstrated the ability of these compounds to accelerate small bowel and colonic transit. Their stimulatory effects on colonic transit were dose dependent and were only apparent for the first hour after intravenous injection at doses of 0.3 and 1.0 mg/kg. AU-116 and AU-130 have half-lives after intravenous injection of 1.3 and 0.8 h, respectively (Iwanaga, unpublished observations). They are thought to act by stimulating 5-HT₄ receptors, although the affinity of AU-130 for these receptors was less than that of cisapride (25a). Cisapride did not yield changes in transit that were statistically significant, although the drug had very similar quantitative effects to those of AU-116 and AU-130. It should be pointed out that cisapride has more specific regional effects on the upper gut, and 100–300 μg/kg produced phase III-like gastric complexes (9). The nonbenzamide 5-HT₄ agonist, SDZ-HTF-919, also stimulated colonic transit in dogs for the first hour after intravenous injection (20).

We also measured gastric emptying, and no agent had a significant effect on the half-time of the emptying of solids or on other indexes of gastric function, lag time, or postlag emptying slope. The dose of erythromycin was small and probably too low for an effect on gastric emptying in the fed state. However, as little as 30 (9) and 100 μg/kg (10) stimulated gastric phase III complexes in fasting dogs. Cisapride was given at an effective dose (9), but it must be appreciated that we administered all drugs 2 h after feeding by intravenous injection. Moreover, the lag phase of gastric emptying for our high calorie meal was ~100 min, and the emptying halftime was 285 min. Clearly, our experimental aims and design, which were directed mainly to small bowel and colonic transit, would have minimized the effects of any of the drugs on gastric emptying. A different study plan will be necessary to evaluate these.

In conclusion, scintigraphy using dual isotopes (⁹⁹mTc and ¹¹¹In) is a simple and practical technique for the study of gastrointestinal transit in the dog. Transit in the small bowel and colon were sensitive to prokinetic drugs but different experimental approaches will be needed if the stomach is to be evaluated fully. AU-116 and AU-130 accelerated small bowel and colonic transit, showing that they have potential as anticonstipation drugs. The method validated here should be useful for the predenic study of motility agents.

This study was supported in part by the National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-32121 and by Hokuriku Seiyaku, Katsuyama, Japan.

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Received 4 May 1998; accepted in final form 1 July 1998.

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