Measurement of gastric emptying during and between meal intake in free-feeding Lewis rats

P. VAN DER VELDE,1 I. KOSLOWSKY,2 AND H. S. KOOPMANS1

1Department of Physiology and Biophysics, The University of Calgary, Health Science Centre; and
2Division of Nuclear Medicine, Foothills Hospital, Calgary, Alberta, Canada T2N 4N1

Van der Velde, P., I. Koslowsky, and H. S. Koopmans. Measurement of gastric emptying during and between meal intake in free-feeding Lewis rats. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R597–R605, 1999.—A new scintigraphic measurement technique is described that allows accurate assessment of gastric emptying in between as well as during a number of successive meals. Measurements were made every minute of food intake, gastric nutrient filling, and gastric emptying over a 6 h, 40 min period in conscious, free-feeding, loosely restrained rats. Before receiving access to the food, the animals had been deprived for a period of 31 h. Over the full duration of the experiment, an average rate of gastric emptying of 2.46 ± 0.18 (SE) kcal/h was established. During most meals, however, the gastric emptying rate was increased so that an average of 26.9 ± 2.7% of the ingested calories was emptied while the animals were feeding, with an average emptying rate of 0.15 ± 0.014 kcal/min or 8.88 ± 0.84 kcal/h. This transient increase in the rate of gastric emptying was followed by a subsequent slowing of gastric emptying after meal termination; in the 10-min postmeal interval, an average emptying rate of 0.96 ± 0.12 kcal/h was found. Despite these fluctuations during and immediately after meals, a relatively constant rate of caloric emptying is maintained over longer periods. There were no differences between the emptying rate during the first meal when the gastrointestinal tract was still empty, compared with later meals when the gastrointestinal tract had been filled with food. The emptying rate during the 10-min postmeal interval, however, was significantly reduced during later meals. The results suggest that gastric emptying is controlled by different mechanisms during and after the ingestion of food and that these mechanisms remain in effect at various degrees of gastrointestinal filling.

gamma scintigraphy; gastrointestinal motility; satiation; intestinal feedback inhibition

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
studies (5, 11, 12, 19) indicate that during a meal, the rate of gastric emptying may be temporarily increased. Failure to include this period in the measurement session will result in an underestimation of the total amount of food emptied from the stomach since meal onset and will change the observed rate of gastric emptying. An estimation of the total amount of calories emptied from the stomach over a 24-h period, based on data that do not include the feeding period, will greatly underestimate the actual daily caloric intake of the animal (5).

The aim of the present study was to investigate the stomach emptying pattern under natural, free-feeding circumstances by applying a new technique of gamma scintigraphic measurement that we modified for use in small animals such as the rat. A major advantage of scintigraphy is that it is a noninvasive technique that allows for normal GI function. The movements of GI contents, expressed in a two-dimensional plane, can be monitored continuously (in 1- or 2-min bins) without further handling of the animals, which makes this method well suited for measurement of the rate of gastric emptying. Traditionally this technique is used only to acquire a gastric retention curve after intake of a single meal; emptying patterns during the ingestion of food cannot be measured reliably if only the stomach is visualized. In contrast, our modifications allow continuous acquisition of information about variations in the pattern of gastric emptying during and between several consecutive meals, while simultaneously measuring cumulative food intake and the radioactively labeled nutrient content retained in the stomach.

MATERIAL AND METHODS

Subjects. The animal model used for this study was a parabiotic preparation made from male Lewis rats (Sprague Dawley, Indianapolis, IN) weighing 786 ± 13 g (SE) per pair at the time of the experiments. Because gamma camera scintigraphy requires a stable position of the subject with respect to the camera for the full duration of the measurement period, nonanesthetized animals have to be somewhat restrained in their freedom of movement during the measurement period. Single rats would easily twist and turn around in their cage unless severe restraint is applied. Even with severe restraint, rotation around the longitudinal axis can hardly be prevented. For that reason, parabiotic rats that were kept in specially designed restraining cages were used in our experiments. With this preparation, rats that had become accustomed to restraint could be held in place without extreme restriction of movement. The parabiotic union limited the animals’ freedom of forward movement while also preventing them from rotating around their longitudinal axis during the experiment. The animals appear healthy when maintained in these cages and eat the same amount of food as do single, free-moving rats of the same body weight.

Surgery. Briefly, the parabiosis was performed as follows: from two rats of similar size, an oval of skin of ~8-10 × 4-5 cm was cut out from the opposite flanks of the two animals, between the fore- and hind legs. Two parallel, longitudinal incisions of ~5 cm were made in the upper and lower portions of the exposed abdominal wall of both animals, leaving the blood vessels of the abdominal wall as intact as possible. The sides of the left and right partner were then attached by connecting the corresponding edges of the muscle incisions together with a continuous 2-0 cat gut suture. Thereafter, the skin was closed around the muscle connections with 3-0 silk suture and the animals were allowed 10 days to recover from the surgery. After removal of the silk sutures, the animals were given at least one more week to recover before they were adapted to the experimental conditions.

Apparatus. During experiments, the animals were kept in place in specially designed Plexiglas restraining cages, allowing the measurement of individual food intake while maintaining the rats in a stable position with respect to the gamma camera (Genesys; ADAC Laboratories, Milpitas, CA). The cages consisted of two connected compartments of 5.1 × 5.5 × 7.5 cm (length × width × height) that were separated by a longitudinal barrier and had additional perforated barriers 24 cm from the front of the cage, restricting the backward movement of the animal while allowing space for the tail to stick through (see Fig. 1). A padded semicircular opening (diameter 5 cm) in between the longitudinal barrier allowed the connective tissue of the parabiosis to pass through the longitudinal barrier. A perforated lid on top of the cage, not covering the head of the animals, allowed fresh air to circulate and body heat to dissipate.

Procedure. Eight pairs of male Lewis rats (~790 g per pair) were fed the liquid diet Ensure Plus (Ross Laboratories, St. Laurent, Quebec, Canada). The diet had a caloric density of...
1.5 kcal/ml and an energy distribution of 61% carbohydrate, 15% protein, and 24% fat. The animals were kept on a restricted feeding schedule, with food access between 1600 and 0900 and lights off between 1800 and 0600. Tap water was available ad libitum; the water contents of the diet, however, were sufficient for the needs of the rats, so that no significant additional water intake took place. The food deprivation occurred in a phase of the circadian cycle when the activity of Lewis rats is normally at a minimum (32). The rats were adapted for several weeks to the moderately restrained conditions that were used during the experiments. On nonexperimental days, they were kept free moving in larger home cages during the 7 h of food deprivation; this allowed time for grooming and undisturbed sleep during the light phase. In our experience, this schedule is sufficient to maintain apparent good health without signs of stress as indicated by secretions from the eyes or nose or by the occurrence of gastric ulcers. After a few days of the training period, the animals showed no obvious signs of stress and could be positioned readily in their restraining cages. Before the actual experiments, the animals had been deprived of food for an additional day, effectively subjecting them to a total deprivation period of 31 h.

Experimental design. The experiments began at 1600 (normal time for refueling of animals) and ran for 400 min (i.e., from 1600 until 2240), with lights on between 1600 and 1800. The rats were equipped with small radioactive markers on their shoulders to allow easier detection of movements during the study and were put in their restraining cages 1 h before the start of the study. They were positioned in the visual field of the gamma camera at least 15 min before the start of the experiment; no further handling of the animals occurred until after termination of the study. The radioactive, nondigestible marker 99mTc sulfur colloid was added to the liquid diet Ensure Plus (1.0 mCi/100 ml food), the well-mixed labeled diet was transferred to graded burettes, and the rats were allowed free access to the food at 1600. All experiments were performed using this single concentration of the standard diet. Lights were dimmed to a low level after 1800. During that period, the rats were further sheltered against the light via application of isolating material around the cages and gamma camera. Cumulative food intake during the experiment was measured at regular intervals for each animal individually.

Data acquisition. Continuous data collection took place in 1-min intervals using a dynamic planar protocol, with a resolution of the gamma camera of 128 × 128 × 16 pixels. After termination of the experiment, a first analysis of the data was performed on a Pegasys computer system (ADAC Laboratories, Milpitas, CA). In this process, a sequence of computer images was created showing the distribution of radioactivity within the GI tract of both animals over the full duration of the experiment. A summation of these images generated a composite picture that was used to draw regions of interest (ROI) around the desired GI structures. Total radioactivity in the different ROI was then obtained for each consecutive 1-min image and combined, resulting in 6 h, 40 min curves. Thereafter, this data set was corrected for radioactive decay (99mTc has a half-life of 6.02 h). Because no more radioactivity had been added to the food since the start of the experiment, this calculation applied equally to all labeled contents throughout the GI tract originating from various meals as well as to any eventually produced fecal material that contained radioactivity. The decay-corrected data set was finally analyzed using a standard spreadsheet (Quattro Pro 7, Corel) on an IBM-clone personal computer. No mathematical smoothing of the curves was performed, so as to avoid loss of accuracy of the data set.

The ROI were drawn around the whole body of each separate animal (including eventual produced feces) and around the two rats as a pair (ROI 1), around the stomach of each animal in each pair (ROI 2), and around the area containing both the small and large intestines as well as the feces of each rat (ROI 3).

Artifacts due to movement of the animals as detected in the data analysis (via comparison of defined location of ROI with actual location of animal on successive 1-min frames) were corrected by hand by redefinition of the location of the ROI for the appropriate frames and subsequent recombinant of the curves that were generated via application of the modified ROI on the affected images. Occasionally, the rats made a significant movement in their cage during the data collection and stomach and intestines could not clearly be identified on the resulting 1-min image. In such cases, interpolated values between the previous and the subsequent image had to be calculated.

The use of small animals for scintigraphic studies has certain advantages over the use of conventional, large-bodied subjects; because the total body size of the rats did not exceed the measurement area of the gamma camera, it was possible to monitor the cumulative increase over time of radioactivity in the whole body (plus eventually produced feces) of the animal (ROI 1). This produced a cumulative food intake curve that could be used directly as an online measurement of both food size and timing.

Cumulative gastric emptying could be derived directly by measuring the total postgastric increase of radioactivity over time, i.e., in the small and large intestines, plus all activity in the feces that the rat releases (ROI 3). This allowed for more reliable measurement of gastric emptying than direct measurement of stomach contents alone (ROI 2), because data acquired during meals using the latter method would reflect a combination of food entering the stomach via intake and food leaving the stomach via the process of gastric emptying. Changes in emptying rate during meals will therefore be more accurately measured via analysis of ROI 3.

Meal criteria and data analysis. Although the general GI filling pattern for each animal can be graphically represented via the curves generated by the various ROI, a more detailed analysis was performed on the rate of emptying that occurred during and between meals. For these calculations, only meals that fulfilled the criteria of a minimum size of 1.0 kcal and a minimal postmeal interval of 10 min were included.

The total amount of nutrients emptied during the meal could be calculated via determination of the exact size and timing of the separate meals (ROI 1) and the change in radioactivity in the gastric emptying curve (ROI 3) during the concomitant period. A direct division of the two values gives the percentage of the meal that was emptied during feeding. These values could be compared with the average emptying rate for each animal over the entire experiment, the average emptying rate in the first 10 min of each postmeal interval, or the average emptying rate in between meals (calculated by dividing total number of calories emptied during periods without food intake by total time spent without feeding activity during entire experiment). The average rate of caloric intake was also calculated for each animal by dividing total food intake by total meal duration.

The data of one animal had to be discarded due to a spillage of labeled food into the rat’s restraining cage. Statistical analysis of the data was performed via ANOVA and t-tests (StatView 4.57, Abacus Concepts; and Q-Pro 7, Corel).
RESULTS

Typical curves of two single animals are shown in Fig. 2. The y-axis represents GI nutrient contents, expressed as kilocalories over time. All curves were originally obtained as decay-corrected cumulative radioactive counts per minute within the various ROI. This data set was transformed into GI caloric contents over time by comparing the radioactivity within the whole animal (ROI 1) with the actual food intake of the animals in milliliters, measured by taking regular readings from the graded burets. Meal size and timing were directly and accurately derived from the cumulative food intake data over the full duration of the experiment; meals are represented by an increase of radioactivity within the animal (plus radioactive feces). The intermeal intervals are periods without food intake and are expressed as a fixed plateau phase between rises (see Fig. 2, top curves). The heavy solid line represents cumulative gastric emptying as derived directly from ROI 3. The third curve (ROI 2) shows gastric nutrient filling over time, illustrating the amount of radioactively labeled food present in the stomach, which is increased by meals and followed by a steady decrease of gastric nutritive contents via the emptying process.

At the end of the 400-min period of the experiment, the average number of calories emptied from the stomach was $16.4 \pm 1.2$ (SE). Under the assumption that gastric emptying takes place at a relatively constant rate over the day (Van der Velde and Koopmans, unpublished observations), extrapolation of the average emptying rate of $2.46 \pm 0.17$ kcal/h that was observed in the present study would have resulted in a total amount of $59.1 \pm 4.2$ kcal being emptied over a 24-h period. This is not significantly different from the rat's average daily intake of $68.5 \pm 4.3$ kcal/day in the week preceding the experiment ($P > 0.05$, 2-tailed t-test). On average, the maximum amount of food found in the rats' stomachs during the course of the study was $15.1 \pm 2.0$ kcal, which equals $10.0 \pm 1.3$ ml of the diet. During the 6 h, 40 min measurement period, the rats ate an average of $29.6 \pm 1.9$ kcal. On the basis of our meal criteria, the animals took an average number of $6.0 \pm 0.37$ meals during the experiment, with an average meal size of $4.3 \pm 0.27$ kcal and an average duration of $7.4 \pm 0.44$ min.

The total of 90 meals taken by the animals during the experiment was subjected to a more detailed regression analysis. A strong correlation was found between meal size and meal duration ($P < 0.0001$, $F = 53.2$). During intake of larger meals, a higher number of calories was emptied from the stomach ($P < 0.0001$, $F = 47.0$); however, the percentage of the meal that was emptied during feeding was less with larger meal size ($P < 0.025$, $F = 5.5$). Similarly, meals of longer duration caused a significantly higher number of calories to be emptied from the stomach ($P < 0.0001$, $F = 27.4$), but no effect on the percentage of the meal being emptied was found ($P > 0.38$). A higher rate of food intake during the meal caused a higher number of calories to be emptied from the stomach during the feeding bout ($P < 0.01$, $F = 8.0$) but had only a nonsignificant negative effect on the percentage of that meal that was emptied during feeding ($P > 0.14$). Meal size and intake rate were positively correlated ($P < 0.0001$, $F = 3.4$); the average intake rate tended to diminish during longer meals ($P > 0.06$).

Because all animals had been food deprived for $31$ h before the start of the experiment, it could be assumed that early in the experiment each animal would have a significantly lower amount of food in its intestines than in the later stages of data collection. Inhibitory feedback signals arising from the gut could therefore be expected to be on a lower level during the first meals, potentially leading to higher emptying rates in the early stages of the experiment. To explore this issue, four different meals were arbitrarily selected: the first, second, and last meal, and a meal taken halfway, i.e., more than $3.5$ h, into the experiment (see Table 1). No significant differences were found among the four meals in meal size ($P > 0.36$, $n = 15$), meal duration ($P > 0.28$), or rate of caloric intake ($P > 0.62$). Therefore, a
for first, second, and last voluntary meal

Table 1. Meal parameters and gastric emptying rates for first, second, and last voluntary meal and postmeal interval

<table>
<thead>
<tr>
<th>Time since start of experiment, min</th>
<th>First Meal</th>
<th>Second Meal</th>
<th>Meal &gt; 3.5 h</th>
<th>Last Meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meas. size, kcal</td>
<td>5.0 ± 0.9</td>
<td>3.4 ± 0.5</td>
<td>4.8 ± 0.5</td>
<td>4.6 ± 0.8</td>
</tr>
<tr>
<td>Meal duration, min</td>
<td>8.1 ± 1.4</td>
<td>5.7 ± 0.7</td>
<td>6.9 ± 0.6</td>
<td>8.1 ± 1.1</td>
</tr>
<tr>
<td>Amount emptied during meal, kcal</td>
<td>11 ± 0.3</td>
<td>0.8 ± 0.1</td>
<td>1.4 ± 0.3</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>%Emptied during meal</td>
<td>22.1 ± 2.8</td>
<td>25.6 ± 2.9</td>
<td>27.2 ± 3.8</td>
<td>26.4 ± 4.2</td>
</tr>
<tr>
<td>Rate of caloric intake, kcal/min</td>
<td>0.64 ± 0.07</td>
<td>0.61 ± 0.05</td>
<td>0.71 ± 0.07</td>
<td>0.59 ± 0.08</td>
</tr>
<tr>
<td>Emptied 10 min postmeal, kcal</td>
<td>0.19 ± 0.02</td>
<td>0.20 ± 0.05</td>
<td>0.08 ± 0.03</td>
<td>0.14 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE. No significant differences were found among the 4 conditions, except for emptying rate during 10 min after a meal (P < 0.0001, F = 43.7, n = 15).

direct comparison of the gastric emptying characteristics could be made among the four meals. There were no significant differences in the number of calories (P > 0.40) or percentage of the meal (P > 0.73) emptied during feeding. The emptying rate during the meal was more rapid compared with the average gastric emptying rate, the average emptying rate in between the meals, and the average emptying rate during the first 10 min of the postmeal interval, showing highly significant differences among the four conditions (Table 2, P < 0.0001, F = 43.7, n = 15). A direct comparison between the different conditions (t-test, 2-tailed, assuming unequal variance) showed a significantly faster emptying rate during meals compared with the average emptying rate (P < 0.0001). In the first 10 min after the meal, however, the gastric emptying rate was significantly diminished when compared to both the average emptying rate (P < 0.0001) and the average emptying rate in between meals (P < 0.001). To evaluate the accuracy of measurements based on a 10-min interval, we also measured the emptying rate during the last 10 min preceding each meal. Because measurements taken shortly after a preceding meal (when stomach emptying could be inhibited) could lead to an artificially diminished value, meals were excluded that were taken <30 min apart. No premeal information was available for the first meals, when the stomachs of the rats were still empty. For the remaining meals, no significant differences between the 10-min premeal interval and the average intermeal rate of gastric emptying could be established (P > 0.48, n = 50).

A regression analysis was performed on the total of all 90 meals taken over the time course of the experiment, exploring the effects of time of day and of different levels of GI filling. The timing of the meals had no significant negative effects on meal size (P > 0.74), meal duration (P > 0.83), or intake rate (P > 0.65), nor did it have effects on the amount (P > 0.80) or percentage (P > 0.26) of kilocalories emptied during feeding. The amount of calories emptied during the 10-min postmeal interval, however, significantly decreased over the time course of the experiment (P < 0.025, F = 5.4).

**DISCUSSION**

To our knowledge, this study presents, for the first time, data of food intake, gastric caloric filling, and gastric emptying measured simultaneously over several hours in conscious animals with free access to a standard liquid diet. Gamma scintigraphy is currently considered the gold standard for gastric emptying studies, but for practical reasons, it is still mainly used in human studies for the determination of a gastric retention curve after a single meal. A main limitation of the standard method is that this approach is unsuitable to accurately measure changes in GI contents during the ingestion of food: gastric contents during this period are the result of food moving into and out of the stomach simultaneously. If only a gastric retention curve is measured, changes in the rate of gastric emptying during this period may remain largely undetected by this method. Our adaptation of this noninvasive technique, measuring the cumulative amount of radioactive label in the intestines and feces, allowed us to measure gastric emptying both during and between several meals taken voluntarily over 6–7 h. The rats could be left relatively undisturbed throughout the experiment, and data could be collected over extended time periods without repeated handling of the animals. This procedure minimizes disruption of the feeding pattern and stress-related inhibition of gastric emptying.

The most striking result of the present study was the finding that the rate of gastric emptying, which is fairly constant when measured over longer time periods, appears to vary considerably in periods when feeding occurs. Three different phases could be identified: during food intake there was an elevated rate of gastric emptying, which was followed by an attenuation of the emptying rate directly after meal termination. Therefore, stomach emptying continued at a fairly constant intermediate rate. The major part of the incoming food, in our study on average 73.1 ± 2.7% of total meal size, remained stored in the stomach during the meal. Pregastric and gastric reflex mechanisms that inhibit gastric motility and decrease intragastric pressure are thought to be in-

Table 2. Average gastric emptying rates during and between meals and postmeal intervals

<table>
<thead>
<tr>
<th>Emptying Rate vs. Average Emptying Rate</th>
<th>P Values</th>
<th>Emptying Rate vs. Emptying in Between Meals</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average emptying rate, kcal/h</td>
<td>2.46 ± 0.18</td>
<td>NA</td>
<td>&lt; 0.025</td>
</tr>
<tr>
<td>Emptying rate during meals</td>
<td>8.88 ± 0.84</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>In between meals</td>
<td>1.68 ± 0.12</td>
<td>&lt; 0.025</td>
<td>NA</td>
</tr>
<tr>
<td>During first 10 min postmeal</td>
<td>0.96 ± 0.12</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>During last 10 min premeal</td>
<td>1.71 ± 0.09</td>
<td>&lt; 0.025</td>
<td>&gt; 0.48</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 15). Values for last 10 min premeal calculated for meals >30 min apart. NA, not applicable.
volved in this process; swallowing food induces a vagal reflex that induces relaxation of the proximal stomach (1, 6, 7, 10, 17) that counteracts the increase of intragastric pressure that would normally result from gastric distension due to the arrival of new food in the stomach. An increase of intragastric pressure in itself activates another reflex mechanism (2, 10, 33), resulting in a further relaxation of the gastric fundus. Postgastric feedback mechanisms arising from intestinal osmo-, chemo-, and mechanoreceptors are also thought to be involved in the inhibition of gastric motility and may induce gastric relaxation, lowering the rate of gastric emptying. However, these inhibitory mechanisms appear to be less effective in controlling gastric emptying during meals; our data confirm and extend recent findings of Kaplan et al. (12) and McHugh and Moran (19) showing that in the rat and rhesus monkey, the emptying rate of the stomach is increased during food intake. Kaplan et al. (12) have already demonstrated that this elevated emptying rate is maintained for the full duration of a meal, and our data show that this still holds true for multiple meals ingested at various levels of GI filling. Similar to previous results of Kaplan et al. (12), the present study found a direct correlation between ingestion rate and the number of calories emptied during meals. However, the percentage of the meal that was emptied during feeding was not affected by the ingestion rate. In accordance with the “volume differential hypothesis” proposed by Kaplan et al. (12), the arrival of fresh food in the stomach and the resulting dynamic changes in intragastric pressure appear to be the major driving force behind the described phase of rapid gastric emptying.

The amount and the percentage of ingested food that emptied from the stomach during each meal did not significantly differ during the first, second, middle, and last meal of the 6.7-h experimental period (see Table 1). This result is quite interesting because there are two major changes that occur in the GI tract, especially during the early phase of the experimental period that started after a food deprivation period of 31 h. The stomach becomes more and more distended with food; the food that is emptied from the stomach moves down the gut and is digested and absorbed along the way and may gradually spread over a larger and larger surface area. Apparently, these changes had no major effect on the amount or percentage of ingested food that emptied from the stomach during each meal. In particular, the increase in stomach emptying that normally occurs during a meal was not affected by the level of intragastric meal volume, because our data show that the intragastric food contents were quite different during each of these meals yet similar amounts and percentages of ingested food were released during all the meals. In addition, the amount emptied during a meal did not seem to be controlled by the amount or the spread of food that was present in or absorbed from the small intestine. Again, similar amounts and percentages of ingested food left the stomach during each of these meals, but, especially during the first or two meals after the deprivation period, one may assume that the amount of food that was already present in the small intestine (and quite probably the length of small intestine that was in contact with and stimulated by food) was smaller than during the later meals. The most likely explanation for the consistent pattern of emptying during the meals is that the increase in gastric distension and intragastric pressure during the meal allows a certain percentage of the gastric contents to pass on immediately into the digestive tract.

The percentage of the meal that was emptied during feeding (27% in our study) is quite comparable to the values that can be derived from the study of Kaplan et al. (12) (~35%), thus validating results that were acquired via two different techniques. In their carefully designed study, glucose solutions were infused directly into the stomach at rates similar to normal ingestion rates for the rat, whereas in our experiment, the rats were allowed normal oral intake of the meals, thus allowing the activation of the receptive relaxation reflex. It should be kept in mind that all our experiments were carried out using one single concentration of the diet. Different concentrations would not necessarily have induced exactly the same percentage of the meals to be emptied from the stomach.

Another basic result of this study was the decrease in gastric emptying rate that occurred during the 10 min after a meal compared with the average emptying rate during the intermeal intervals. The average emptying rate between meals was 1.68 kcal/h, whereas the emptying rate in the 10 min after a meal was 0.96 kcal/h or 57% of the average intermeal rate. These data show that emptying slows down just after the meal-related delivery of food to the upper small intestine. This effect is relatively short lived in nature, as illustrated by the fact that the emptying rate during the 10-min premeal intervals was not different from the average emptying rate in between meals when calculated for meals >30 min apart. An important conclusion that can be drawn from the emptying rate during the 10-min premeal interval is that meal initiation apparently is not caused by lack of nutrient availability on the level of the GI tract.

The results suggest that when stomach contents are at a stable level, the presence of food in the small intestine inhibits stomach emptying, as has been hypothesized for many years (4, 5, 9, 14, 20). Our data also show that this inhibition of gastric emptying just after a meal is greater in the later meals than in the earlier meals (P < 0.025, see Table 1). The decrease in gastric emptying rate immediately after a meal is most likely to be caused by increased stimulation of the small intestine. It could be hypothesized that, after the initial fast and during the further course of the experiment, more and more food had been released from the stomach and had spread over a larger surface area of the small intestine, supposedly until a balance had been reached between the rate of gastric emptying and the absorptive capacity of the gut. A faster rate of gastric emptying, as occurs during a meal, would then lead to an increase in nutrient availability in the intestines. Thus the signals that were generated by the presence of
Food in the small intestine could intensify and lead to a greater reduction in the rate of gastric emptying in the 10-min postmeal interval (14). The greater inhibition of postmeal gastric emptying cannot be explained by signals originating in the mouth, throat, and esophagus, because these signals remain the same when meals of the same size have been eaten.

Taken together, our data suggest that, during and after meals, changes do occur in the level and expression of inhibitory signals on gastric emptying. During a meal, a larger amount of nutrients is emptied into the intestines (12) compared with the average rate of emptying over the full day. This initial phase of rapid emptying, which, to a large extent, escapes intestinal feedback regulation and is possibly driven by increases in intragastric pressure, would then generate a higher level of receptor stimulation in the gut, inducing stronger inhibitory feedback signals on gastric emptying via relaxation of the gastric fundus and increased resistance in the pyloric-duodenal region (4, 14, 17, 20, 21). In the later phase of the meal, some of the intestinal contents will also be absorbed into the bloodstream (28), possibly activating postabsorptive inhibitory mechanisms (16, 27). Thus in this phase, the stomach may already receive a higher level of inhibitory signals, but the arrival of fresh food would still induce changes in intragastric pressure, causing relatively rapid emptying. After meal termination, however, with the level of stomach filling stabilized, a more precisely regulated intestinal phase of gastric emptying would take place: a higher level of stimulation of intestinal receptors and/or stimulation of a longer stretch of intestine would evoke stronger inhibitory signals on gastric emptying. A phase of slower stomach emptying would then be established until the excess of food was fully absorbed from the gut. Thereafter, stomach emptying would continue at a standard intermediate rate.

The stomach is often considered a main source for the generation of satiety signals (12, 19, 29). Our data, showing faster gastric emptying during feeding, do not exclude the possibility that under natural feeding conditions both gastric and postgastric factors may be involved in meal termination; not only is there an increased distension of the stomach, but a substantial part of the meal is also already leaving the stomach during the meal, thus providing a possible intestinal or postabsorptive signal for the inhibition of food intake.

Another important conclusion is that the absolute level of gastric distension by itself does not control the termination of food intake. The first meal is terminated at a level of intragastric meal volume that is insufficient to terminate the second or third meal (see Fig. 2). Successive meals are started and terminated at increased levels of gastric filling, showing that the feeding control system must either adapt to circadian changes in the degree of gastric distension or use distension along with other cues to terminate a meal. Five hours into the study, the two individual animals depicted in Fig. 2 held 3 and 14 ml of food, respectively, in their stomachs. It should be kept in mind that the measurements of gastric nutrient contents were based on the intake of radioactively labeled food. Any (unlabeled) secretion of gastric juices into the lumen of the stomach would add to the total gastric volume but would remain undetected by the gamma camera.

An interesting result from the present study is that food is emptied from the stomach at a rate that is fairly constant throughout the 6.7-h observation period. Although there is an increase in the rate of stomach emptying during each meal and a slowing of gastric emptying just after the meal is complete, a look at the cumulative curves in Fig. 2 shows that gastric emptying continues at a fairly steady rate between meals throughout the study period. This is somewhat different from many scintigraphic studies showing an exponentially declining emptying rate, which may be explained by the fact that most of these studies measure gastric emptying only after one single meal, sometimes including at least part of the feeding period itself. Because gastric emptying is faster during the meal, whereas the last traces of the radioactive marker will not empty easily from an almost-empty stomach, the best-fitting curve would suggest an emptying rate with an exponential decay. The present experiment avoids these problems by allowing the rats to eat several meals, thus measuring gastric emptying from a generally well-filled stomach.

Food intake patterns and GI transit can be altered under the influence of stress (31). An attempt was made to minimize these effects in our study: our rats were used to the restraint of parabiosis and they had undergone several weeks of adaptation to the experimental conditions. Although restraint is commonly described as a potent stressor, these effects are thought to be stronger during a single exposure and appear to decrease during repeated restraint (23). Furthermore, the animals were kept in relatively loose restraint compared with most restraining studies. On a behavioral level, no clear aversion to the conditions was found; the animals could be readily positioned in their restraining cages after a few days of training and did not display obvious visual signs of discomfort during the experiments. Ideally, the total number of calories emptied from the stomach over a 24-h period should be similar to the average daily food intake of the animals. The fact that in the present experiment the estimated gastric emptying rate was not significantly different from the average daily food intake of the rats suggests that the data that were generated in this study are in a normal physiological range. These data underscore the importance of inclusion of the ingestion period of a meal in the gastric emptying measurement (5, 12).

During the 6 h, 40 min measurement period, the animals ate an average of 29.6 ± 1.9 kcal, leaving 3.6 ml of food intake that were not accounted for after application of our meal criteria. This discrepancy results from small meals of <1.0 kcal in size or from occasional drops falling from the feeding spouts that are readily licked up by the rats without inducing further feeding behavior. The total intake covered almost 50% of the animal's daily needs over a time period that included <5 h of the 12-h dark period.
Normally, ~85% of the daily intake of the rat, a nocturnal animal, takes place in the dark phase. Thus the rats ate an acceptable amount of food during the study compared with their normal intake in this phase of the circadian cycle. Because other meal parameters, such as the relationship between meal size and meal duration, were also in good accordance with behavioral studies, the data suggest that the animals were behaviorally well adjusted to the experimental conditions and that, at least at a behavioral level, the measurements were not affected in a major way by external stress factors.

By using the standard liquid diet Ensure Plus both during the training period as well as during the study itself, a relatively normal feeding pattern was obtained. The chosen diet is quite comparable in composition with the standard North American diet, allowing all the macronutrients to stimulate the gut in a normal way. The use of a less natural or less complex food source, such as a single macronutrient like glucose or a nonnutritive substance, may generate a different emptying pattern (12). In in vitro pilot studies in which enough acid was added to the diet to reach pH 2–3, it was shown that our diet, compared with other commercially available test diets, had a low tendency to separate into distinct solid and liquid components.

Instead, it assumes a semisolid structure, which in scintigraphic studies is an advantage because it forms a reasonable compromise between the different emptying characteristics of liquid and solid meals (13). Also under these circumstances, the radioactive marker that was used in the experiments, 99mTc sulfur colloid, remained well attached to solid particles and evenly distributed throughout the diet after a 24-h test period. 99mTc colloid is widely in use as an accurate solid phase marker that shows little dissociation from a solid test meal when mixed with gastric acid or when subsequently mixed with bile and pancreatic juice and has minimal levels of absorption from the GI tract (22). It has the additional advantage of being a relatively safe isotope with a low radiotoxicity and a short half-life of only 6.02 h.

The quality and resolution of the images that were acquired via the gamma camera were sufficient to accurately discriminate and outline the desired GI structures throughout the study. Contrary to human studies, the small size of rats did not allow for detailed measurement of movements of food within the stomach. However, their small body mass also had the advantage that the effects of tissue attenuation of the gamma radiation were less severe, so that all measurements could be made with a single, anteriorly placed gamma camera.

In summary, we describe a new technique for the measurement of the movement of radiolabeled food through the GI tract while an animal is free feeding over 6–7 h. Scintigraphy is a noninvasive technique that avoids the use of tubes and direct handling for the infusion or withdrawal of stomach or gut contents but has confirmed many earlier results that used these techniques. By defining three ROI within the gamma camera image, we have been able to measure continuously and concurrently the time and rate of spontaneous food intake, the changing nutrient content within the stomach, and the minute-by-minute rate of gastric emptying. The major change from current scintigraphic technique is the measurement of radioactivity in the intestines and feces to obtain a more accurate measurement of the rate of gastric emptying.

Our data show that fluctuations in gastric emptying rate, caused mainly by food intake, occur over the 6 h, 40 min study period, which included the early dark phase of the circadian cycle. During meals, gastric emptying is fast and appears to be influenced by the delivery of new food to the stomach, whereas between meals, gastric emptying seems to be under more accurate feedback regulation by the intestines or other postabsorptive signals. The higher gastric emptying rate during feeding is followed by an inhibition of emptying directly after the meal, which ensures that over longer periods of time, a fairly constant emptying rate can be maintained. The present data suggest that this immediate postmeal inhibition of gastric emptying is affected by the length of intestine stimulated by food.

**Perspectives**

The advantages of our modification of gamma camera scintigraphy are the ability to use it over several meals, to concurrently measure food entering and leaving the stomach during a meal, and to continuously measure gastric emptying at critical times, such as just before, during, or just after voluntary meals. Thus the technique is helpful in further defining the relationship between spontaneous meal intake and stimulation of the upper GI tract. The availability of a wide range of scintigraphic markers should allow for the acquisition of detailed information about gastric contents and emptying rate in free-feeding animals for various food components under a variety of conditions and treatments. The present results argue against fixed set points for stomach distension as major signals for meal initiation or termination. The continuous availability of nutrients in the intestines and the different levels of stomach filling at the start and end of different meals suggest that meal patterns are not likely to be regulated by signals arising from the GI tract alone.

Special thanks are due to Dr. Damini Dey for expert assistance in adapting computer programs for ROI analysis.

This research was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-4527 and the Medical Research Council, Canada, Grant MA-13718.

Address for reprint requests: P. Van der Velde, Dept. of Physiology and Biophysics, The Univ. of Calgary, Health Science Centre, Calgary, Alberta, Canada T2N 4N1.

Received 9 March 1998; accepted in final form 21 October 1998.

**REFERENCES**


