Evaluation of gastrointestinal motility in awake rats: a learning exercise for undergraduate biomedical students

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Notwithstanding the current knowledge on gastric emptying and gastrointestinal transit, dyspepsia and dysmotility complaints are commonly misunderstood not only by patients but also by physicians (29). Nowadays, current medical curricula devote scarce time for students to perform “classical” human or whole animal experiments on physiology, which is no longer the research interest of most of their faculty members (3). In addition to students being unwilling to attend laboratories, another problem faced by many biomedical schools, especially in underdeveloped areas, is the shortage of facilities and personnel skilled in advanced laboratory techniques essential to accomplish learning objectives (4 and 4a).

Thus, we designed a simple and reliable hands-on activity for undergraduate students to assess gut motility in awake rats, which was followed by a small-group discussion. Our aims were 1) to provide students with a better understanding of digestive pathophysiology than what could be gleaned from typical medical curricula and 2) to motivate students to pursue additional education in gut physiology.

MATERIALS AND METHODS

General instruction. First, students (~30 individuals/term) were divided into three groups for a 2-h session. Each group was assigned to carry out one protocol. Each bench had animals as well as meals and basic instruments. Next, students were instructed to read the syllabus and perform the experiments under direct supervision of a postgraduate student and a lecturer. Finally, students were rejoined to discuss the results.

Animals. Male rats of the Wistar strain (weight: 180–220 g, n = 79) were obtained from the central housing station of our institution. All procedures were performed in accordance with the Guide of the Care and Use of Laboratory Animals (Brazilian College of Animal Experimentation). Protocols were approved by the local ethics committee. Animals were fasted for 24 h with free access to an oral rehydration solution up to 2 h before the experiments. This solution contained 75 meq/l Na⁺, 65 meq/l Cl⁻, 20 meq/l K⁺, 75 mmol/l glucose, and 10 mmol/l citrate.

Dye fractional retention experiments. Rats were randomly submitted to insulin (group I), control (group II), or hypertonic (group III) protocols. Each subset consisted of six to eight rats. Experiments were performed according to the technique described by Reynell and Spray (22) and previously adapted by us (10). Group I animals were pretreated 30 min earlier with an intraperitoneal injection of mono- component insulin (1 IU/kg). All animals were gavage fed with a liquid test meal (1.5 ml) consisting of 5% glucose solution (isotonic) except for group III animals, which received a 50% glucose solution (hypertonic). All test meals contained an equal dye (phenol red) concentration (0.5 mg/ml). After 10, 15, or 20 min of meal gavage, we obtained a blood sample (10 μl) from the rats’ tails and killed them by cervical dislocation. In groups I and III, blood was also drawn just before the insulin pretreatment and gavage.

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Teaching In The Laboratory

Using a laparotomy, the pylorus and cardia as well as the terminal ileum were quickly clamped. The gut was carefully removed, stretched along a meter stick on a plain tabletop, and divided into consecutive segments: stomach, proximal small intestine (~40%), middle small intestine (~30%), and distal small intestine (~30%). The volume of each segment was indirectly measured by liquid displacement after the segment was added into a graduate cylinder containing 100 ml of 0.1 N NaOH solution. Next, the segments were cut and homogenized by an electric mixer for 30 s. The suspension was allowed to sediment for 20 min, and 10 ml of the supernatant were centrifuged for 10 min (2,800 rpm). Proteins in 5 ml of the homogenate were precipitated with 0.5 ml of trichloroacetic acid [20% (wt/vol)], and the solution was centrifuged again for 20 min. Finally, 3 ml from the supernatant were added to 4 ml of 0.5 N NaOH. All samples were read by spectrophotometry at 560 nm and expressed as optic densities (ODs). In each experiment, a standard dilution curve was obtained by means of plotting the dye range of concentrations against the OD of 0.1 N NaOH solution (the blank). The linear coefficient (α) of the dilution curve set the solution concentration (C = OD) as well as the amount of phenol red (m) recovered from each segment (m = C × volume).

The fractional dye recovery value (in %) in each gut segment (X) was calculated according to the following equation:

\[
\text{Fractional dye recovery in segment } X = \frac{\text{Amount of phenol red recovered in segment } X}{\text{Total amount of phenol red recovered from all four segments}}
\]

Scintigraphic experiments. To verify if the dye retention values determined by the students were reliable, the authors performed scintigraphic experiments, which is considered the gold standard methodology for gastric emptying assessment. Therefore, separate groups of rats were submitted to the same protocols described above: i.e., insulin (group I), control (group II), or hypertonic (group III). Each subset consisted of six to eight rats. The test meal also consisted of a 5% glucose solution [insulin-pretreated animals (group I) or not (group II)] or a 50% glucose solution (group III). Instead of phenol red as a marker, the test meals now contained 10 MBq 99mTc coupled to phytye (Phytosid, Sydma Medical Reagents and Equipments, São Paolo, Brazil). After 15 min of meal gavage, rats were killed and submitted to gut exeresis. The stomach was numbered as segment 1. The small intestine was carefully removed, slightly stretched, and divided into consecutive segments: the proximal quarter, the intermediate small intestine, and the distal third small intestine, which were numbered as segments 2, 3, and 4, respectively.

The radioactivity of gut segments was determined by a γ-camera (Millenium MPR, General Electrics). Data were recorded by Solaris 2.0 computer system (Sun Microsystems) as the number of counts per minute after the background activity was discounted. To rule out eventual absorption of the marker, the carcass radioactivity was also recorded. Fractional marker retention was calculated for each gut segment as a ratio between the counts obtained in one segment and the sum of counts in all segments. Values obtained for each individual segment were multiplied by the number of the respective segment and summed up to calculate the geometric center of marker distribution throughout the gut (28).

Glycemia analysis. Under supervision of faculty members, the students performed the automated analysis of glycemia (Advantage glucometer 589, Boehringer Mannheim) from the blood samples obtained as described above.

Student assessment. At the end of the session, students were invited to share their results with their colleagues and tutors. Oral presentations were followed by a small-group discussion on gut motor behavior. They also evaluated the educational value of the present activity by answering the following questions: 1) Did the exercise motivate you to participate? 2) Did it help you to correct misconceptions? 3) Did it stimulate you to discuss the results? and 4) Did it stimulate you to pursue further studies? Answers were expressed on a 5-point scale (where 5 = strongly agree and 1 = strongly disagree). The eventual misconceptions of our students on gut motor behaviour were assessed by a comparison of the students’ answers (true, false, or do not know) to a questionnaire applied before and after the digestive system module. They answered the following questions:

1. Can the assessment of gastric emptying be done by measuring the remaining quantity of an insoluble marker added to a test meal in the stomach?
2. When studying intestinal transit, can we determine its function by measuring the quantity of the unabsorbed marker in different intestine segments?
3. Is gastric emptying of liquids characterized by a linear reduction of the gastric content volume, beginning from the end of ingestion period?
4. By adding a large quantity of glucose to a test meal, is gastric emptying accelerated?
5. Does hypoglycemia accelerate gastric emptying of a liquid test meal?
6. When gastric emptying is excessively rapid, does chyme rapidly reach the distal parts of the small intestine, which, in turn, does not affect transit in the colon?

Statistical analysis. Data are expressed as means ± SE or medians within an interquartile range. One-way ANOVA followed by the Student-Newman-Keuls test was used to compare the differences in fractional dye retention values. The Kruskall-Wallis test was used to compare the differences in the geometric center of the radioactive meal distribution. P values of <0.05 were considered statistically significant.

RESULTS

Figure 1 shows that hypertonic-fed animals exhibited greater (P < 0.05) gastric recovery values compared with isotonic-fed (control) animals (79.4 ± 3.1% vs. 44.1 ± 2.0%, 73.1 ± 5.9% vs. 38.0 ± 2.3%, and 76.3 ± 2.6% vs. 17.4 ± 2.5%) in all subsets at 10-, 15-, and 20-min postprandial intervals, respectively. On the other hand, insulin-pretreated rats exhibited lower (P < 0.05) values of gastric recovery than controls at 15- and 20-min postprandial intervals (15.4 ± 2.4% vs. 37.9 ± 2.3% and 8.1 ± 1.8% vs. 17.4 ± 2.5%). At the 10-min postprandial interval, the difference between values of insulin-hypoglycemia-fed and control groups did not attain statistical significance (38.0 ± 3.0% vs. 44.1 ± 2.0%).

Figure 1 also shows that there were no differences between dye recovery values of the proximal small intestine in insulin-pretreated and control groups (27.0 ± 3.1% vs. 26.1 ± 4.7%, 23.1 ± 2.5% vs. 34.7 ± 6.7%, and 17.9 ± 3.4% vs. 16.7 ± 4.5%) at 10-, 15-, or 20-min postprandial intervals, respectively. Dye recovery from the middle small intestine at the 15-min interval was greater (P < 0.05) in insulin-pretreated rats than in controls (57.4 ± 2.8% vs. 27.9 ± 5.8%), but no differences in these values were found at 10- and 20-min subsets (30.5 ± 4.1% vs. 24.4 ± 4.2% and 62.7 ± 4.8% vs. 54.0 ± 5.2%, respectively, for insulin-pretreated and control animals). Dye recovery from the distal small intestine of insulin-pretreated rats was similar (P > 0.05) to controls both at 10- and 20-min subsets (2.1 ± 0.4% vs. 3.1 ± 0.5% and 11.2 ± 4.1% vs. 11.8 ± 4.0%, respectively), whereas it was greater (P < 0.05) at the 15-min postprandial interval (5.6 ± 1.7% vs. 0.3 ± 0.2%) for insulin-pretreated and control rats.
hypertonic-fed rats showed lower ($P < 0.05$) dye recovery values in the proximal small intestine at the 10-min postprandial interval compared with controls ($7.0 \pm 1.8\%$ vs. $24.4 \pm 4.2\%$, $7.7 \pm 1.2\%$ vs. $27.0 \pm 5.8\%$, and $7.6 \pm 1.5\%$ vs. $54.0 \pm 5.2\%$ at 10, 15, and 20 min, respectively). In contrast, dye recovery from the distal small intestine of hypertonic-fed rats was not different ($P < 0.05$) from controls at either 10- or 15-min subsets ($3.8 \pm 0.8\%$ vs. $3.1 \pm 0.6\%$ and $0.2 \pm 0.2\%$ vs. $0.4 \pm 0.3\%$, respectively), but it was greater ($P < 0.05$) at the 20-min postprandial interval ($11.8 \pm 4.0\%$ vs. $5.4 \pm 4.1\%$ for hypertonic-fed and control rats, respectively).

The protocols induced remarkable changes in serum glucose levels. Compared with control rats, which maintained their glycemia values throughout the study ($64.3 \pm 3.0$ vs. $76.1 \pm 3.3$ mg/dl, $P > 0.05$), the insulin treatment dropped ($P < 0.05$) glycemia values ($57.5 \pm 3.6$ vs. $28.0 \pm 0.7$ mg/dl), which returned to basal levels 15 min after rats were fed ($55.3 \pm 3.7$ mg/dl, $P > 0.05$). On the other hand, hypertonic-fed rats became hyperglycemic ($57.7 \pm 2.9$ vs. $104.9 \pm 2.6$ mg/dl, $P < 0.05$).

Figure 2 shows the scintigraphic experiments of gastrointestinal motility. Compared with the fractional gastric retention values of control animals ($52.4 \pm 4.3\%$), hypertonic-fed animals showed increased values ($83.2 \pm 2.2\%, P < 0.05$), whereas insulin-induced hypoglycemic-fed rats exhibited lower ones ($27.4 \pm 3.6\%, P < 0.05$). As shown in Fig. 3, meal progression throughout the gastrointestinal tract was also affected. Compared with the transit index of the control group [median: 1.6 (interquartile range: 1.4–1.9)], the radioactive marker remained far behind ($P < 0.05$) in hypertonic-fed animals [median: 1.2 (interquartile range: 1.1–1.4)], whereas the center of mass advanced ($P < 0.05$) further in insulin-induced hypoglycemic-fed rats [median: 2.4 (interquartile range: 1.8–2.4)].

In a survey with 60 of 75 students about the practical, 82.9% of them considered that it motivated them to participate, 87.4% of them considered that it helped them to learn, 80.7% considered that it stimulated them to discuss the phenomena, and 75.0% of them considered that the session stimulated further study interest (Table 1).

From the results shown in Fig. 1, one can also see that hypertonic-fed rats showed lower ($P < 0.05$) dye recovery values in the proximal small intestine at the 10-min postprandial interval compared with controls ($9.7 \pm 1.8\%$ vs. $27.0 \pm 4.7\%$), whereas at 15 and 20 min, these differences did not attain statistical significance ($15.1 \pm 6.8\%$ vs. $34.7 \pm 6.7\%$ and $10.6 \pm 0.8\%$ vs. $16.7 \pm 4.5\%$, respectively). Dye recovery from the middle small intestine in all postprandial intervals was lower ($P < 0.05$) in hypertonic-fed rats compared with controls ($7.0 \pm 1.8\%$ vs. $24.4 \pm 4.2\%$, $7.7 \pm 1.2\%$ vs. $27.0 \pm 5.8\%$, and $7.6 \pm 1.5\%$ vs. $54.0 \pm 5.2\%$ at 10, 15, and 20 min, respectively). In contrast, dye recovery from the distal small intestine of hypertonic-fed rats was not different ($P < 0.05$) from controls at either 10- or 15-min subsets ($3.8 \pm 0.8\%$ vs. $3.1 \pm 0.6\%$ and $0.2 \pm 0.2\%$ vs. $0.4 \pm 0.3\%$, respectively), but it was greater ($P < 0.05$) at the 20-min postprandial interval ($11.8 \pm 4.0\%$ vs. $5.4 \pm 4.1\%$ for hypertonic-fed and control rats, respectively).
Table 2 shows additional results of the questionnaire from the students describing the major physiological principles learned by them. Note that most of the students did not have any idea that gastric emptying and gastrointestinal transit could be quantitatively assessed. In fact, most of the students began the digestive system module with the common idea that the gastric emptying rate is a linear process and that there is no relation between small intestine transit and gastric emptying. After this module, most of the students were able to change their earlier misconceptions, making inferences that the meal’s composition did affect gut motor behavior.

DISCUSSION

Here, we present a laboratory exercise for undergraduate students using simple and reliable methodology to show the phenomenon of gastrointestinal transit and its physiopathological relevance.

Evaluation of gastrointestinal motility. Since Cannon’s work, radiopaque meals have been used to show bowel movements. Later on, quantitative methods replaced qualitative methods. Gastric contents are aspirated at a preset interval, allowing a single measurement of gastric volume (13). Although providing useful data, this method presumes a linear process for gastric emptying of liquids as samples are taken once. More reliable data were later obtained by adding a poorly absorbed dye, usually phenol red, to the test meal (8). By measuring the changes in dye concentration, one can calculate the volume remaining in the stomach and its secretions. The fractional retention technique was later adapted to assess gut motility in rats (22). The double-sampling technique is judged as one of the best methods of assessing gastric emptying of liquids. It can be performed with the subject lying, sitting, standing, or moving, thus mimicking real-life situations.

The introduction, in the sixties, of radiolabeled oatmeal to measure its output from the stomach renewed research on gastric emptying (11). Laborious manual counts were later obviated by a γ-camera, which allowed more frequent scans. The development of markers like $^{99m}$Tc and $^{113}$In allowed simultaneous measurements of the outflow for liquid and solid phases (12). Plotting the counts against time allowed the calculation of gastric emptying half-time ($t_{1/2}$). The development of ultrasound enabled its use in gastric motility assessment with a probe over the epigastrium of a seated subject. The gastric emptying rate can be estimated from serial transverse sections (2). Recently, $^{13}$C-labeled octanoic acid breath testing was introduced to indirectly assess gastric emptying (9). Despite showing good correlation with scintigraphic data, it is not widely available and offers only a global assessment of the gastric function, an important drawback as abnormal distribution of food between the proximal and distal stomach of dyspeptic patients seems to better figure out dysmotility symptoms rather than gastric stasis (27). Despite being viewed as the gold standard for gastric emptying assessment in medical practice, scintigraphy is not yet worldwide available and implies radiation exposure. Our students performed only the dye dilution technique, whose safety, simplicity, and accuracy is well known (10) but could compare the data obtained by the dye dilution technique and scintigraphy.

Despite differences in gastrointestinal tract morphology between rats and humans, their motility patterns are similar. To prevent a drive effect of the meal volume on gastric emptying rate, a fixed volume of meal was given to rats as they had similar body weights. Although meal gavage is aided by anesthesia, it was discarded as gut transit is faster in awake animals. Free access of rats to oral rehydration solution during the starvation interval allowed bowel clearance while assuring optimal hydration. For the sake of simplicity, glycemia was monitored using glucose oxidase strip method (16). An intraperitoneal injection of insulin was used to acutely decrease glycemia (7). In view of the counterregulatory hormones released by insulinic hypoglycemia, a 30-min interval was set for gut motility tests (30). Conversely, the stimulation of intestinal chemoreceptors induces neurohormonal reflexes that inhibit the gastric emptying rate (19). Thus, rats were fed with a hypertonic and caloric meal.

Further points for emphasis. In this work, hypertonic meals consistently delayed both the gastric emptying rate and gastrointestinal transit (both of phenol red and radiolabeled meals) compared with the respective indexes of isotonic-fed (control) rats (Figs. 1 and 2), confirming previous data (18). This phenomenon is dependent on meal caloric content, and it is likely that the stimulation of small intestine receptors by nutrients acts synergistically with hyperglycemia to retard gastric emptying. Normal volunteers and patients with insulin-
dependent diabetes mellitus also present a delay in the gastric emptying rate and gastrointestinal transit, indicating that such event does not involve insulin (25). A direct inhibitory effect on gut smooth muscle appears unlikely as acute hyperglycemia strengthens pyloric contractions in normal subjects (6). This delay of gastric emptying may be seen as a mechanism that restrains postprandial hyperglycaemia (20).

In contrast, insulin-induced hypoglycaemia accelerated both the gastric emptying rate and gastrointestinal transit (both of phenol red and radiolabeled meals) compared with the respective indexes in isotonic-fed (control) rats (Figs. 1 and 2), confirming previous data (17). This seems to be a result of hypoglycaemia and not the action of insulin, since it is preventable by glucose coadministration. Hypoglycaemia also accelerates the gastric emptying rate in humans (24). As hypoglycaemia represents a major threat, the increase in gastric outflow is viewed as a reflex that promotes recovery from eventual hypoglycaemia by facilitating meal absorption (1). Cholinergic stimulation by hypoglycaemia contributes to accelerate the gastric emptying, since such a phenomenon is abolished by vagotomy or atropine. In fact, the vagus nerve dorsal motor nucleus contains glucose-sensitive neurons (including those with projections to the gut), which act as a glucoprotective failsafe loop (32).

**Engaging student interest.** We are aware that the use of laboratory animals in a training activity for undergraduate students is a very sensitive topic, especially in the United States and Europe. Physiologists are nowadays supposed to balance in practical classes the participation of students in carrying out the experiments while restraining the number of laboratory animals used. If the access to rats is critical, one can assess the gastric fractional dye retention at a single postprandial time interval (e.g., 15 min), an option that allows only a static evaluation of the gastric motor function. To allow the participation of a greater number of students, one should add other postprandial time intervals (e.g., 10, 15, and 20 min, as we used in this work), which offer the advantage to the students of figuring out the dynamic process of gastric emptying. In addition, it is possible to induce less intense changes in serum glucose levels or in the test meal. Notwithstanding our commitment to the principles of refine, reduce, and replace, it is still hard to imagine that nonanimal methods will be able to completely replace in vivo teaching, because biological tissue properties are different from those of latex or other artificial materials. Also, real dynamics and intangible aspects of biological processes, such as peristalsis or pulsating bloodstream, are difficult to replicate in digital models (21). Although we have yet to have a student opt not to participate in a gut motility test, we have planned to accommodate those who seek an alternative pathway by asking them to write a short essay on gastric dysmotility.

As one can see from the results of the questionnaire shown in Table 2, our students began the digestive system module with the common idea that gastric emptying of a test meal is a linear process, independent of physicochemical properties of the meal. In addition, they had scarce knowledge about the neurohumoral regulation of gut motor behavior and the functional motility interactions between the stomach and small intestine. Therefore, this exercise allows our students to realize a more complex view of gut motor function and to preview the effects of variations in a meal’s properties (e.g., osmolarity and volume). It also helps our students to figure out the role of the autonomic nervous system on gastric emptying rate and gastrointestinal transit of a test meal as well as the role of duodenal feedback on gastric retention and gastric emptying of a test meal, key concepts for a better understanding of dyspepsia and dysmotility by future health professionals.

The present session contemplates all the conceptual, motivational, and technical goals described for active learning, such as 1) enthusiastic faculty members and fellow tutors; 2) a hypothesis-driven exercise that actually produces results not found in textbooks, is reliable, and relatively simple to do; and 3) motivates students (3, 4). In this properly supervised setting, students develop cognitive skills of experimental design and observational and technical skills to make precise measurements plus the ability to solve problems. Moreover, they are introduced to the principles of ethical care of animals. Students also make links between physiological concepts presented in the introductory lecture on gastrointestinal motility and the laboratory data. Oral presentations by students to their peers and tutors followed by a small-group discussion on gut motor behavior at the end of the session may also be useful for student assessment. Student feedback about the session has been encouraging. Toward the end of the academic term, they are required to preregister for an elective discipline. At this time, 68% of them expressed an interest in our Honors Digestive Physiology course. When asked what got them interested

### Table 2. Student evaluations of the educational value of the practical exercise

<table>
<thead>
<tr>
<th>Question</th>
<th>Before the Digestive System Module</th>
<th>After the Digestive System Module</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Could the assessment of gastric emptying be done by measuring the remaining quantity of an insoluble marker added to a meal in the stomach?</td>
<td>True 26* False 4 Without Response 45</td>
<td>True 55* False 11 Without Response 6</td>
</tr>
<tr>
<td>2. When studying intestinal transit, can we determine its function by measuring the quantity of the unabsorbed marker in different intestinal segments?</td>
<td>True 37* False 3 Without Response 35</td>
<td>True 60* False 3 Without Response 9</td>
</tr>
<tr>
<td>3. Is gastric emptying of liquids characterized by a linear reduction of the gastric content volume, beginning from the end of the ingestion period?</td>
<td>True 7 False 18* Without Response 50</td>
<td>True 20 False 38* Without Response 14</td>
</tr>
<tr>
<td>4. By adding a large quantity of glucose to a test meal, is gastric emptying accelerated?</td>
<td>True 15 False 14* Without Response 45</td>
<td>True 19 False 38* Without Response 15</td>
</tr>
<tr>
<td>5. Does hypoglycemia accelerate gastric emptying of a liquid test meal?</td>
<td>True 20 False 15 Without Response 40</td>
<td>True 43 False 16 Without Response 13</td>
</tr>
<tr>
<td>6. When gastric emptying is excessively rapid, does chyme rapidly reach the distal parts of the small intestine, which, in turn, does not affect transit in the colon?</td>
<td>True 8 False 13* Without Response 54</td>
<td>True 16 False 31* Without Response 25</td>
</tr>
</tbody>
</table>

Values are numbers of student responses; 75 students total responded anonymously to the questionnaire. *Expected correct answers for the students.
in the physiology program, they mentioned “the lab experience.”

Conclusions: In summary, a hypercaloric meal delays the gastric emptying of liquids in awake rats, whereas acute hypoglycemia speeds it up. The hands-on exercise uses a safe, inexpensive, and reliable methodology that favors student engagement. Evaluating major factors involved in gastrointestinal transit physiology enables students to grasp the nature of bowel movements, its relationship to dietary factors, and its link to physiopathological phenomena.

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