Practical Solid and Liquid Phase Markers for Studying Gastric Emptying in Man

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This paper presents a method used to evaluate solid and liquid phase markers for radionuclide gastric emptying studies. We conducted in vitro and in vivo comparative experiments employing several radiolabeled markers. Among the solid phase markers tested, Tc-99m-sulfur colloid in vivo-labeled liver and I-131-fiber performed optimally. However, Tc-99m sulfur colloid in scrambled egg showed very acceptable performance and it is significantly easier to prepare. Among liquid phase markers, we found In-111-DTPA stabilized with 1% albumin to be a good agent and appropriate for dual isotope emptying studies.

Radionuclide gastric emptying studies are increasingly being utilized for the evaluation of a variety of clinical problems (*1–3*). They are also used as investigative tools to assess pharmacologic (*4–6*) and surgical interventions (*7–8*) on the stomach. The need for a specific marker of the liquid and solid phases of gastric emptying has prompted the use of a variety of radiopharmaceuticals (*2,3,5–7,9–14*). These radiopharmaceuticals differ greatly in the characteristics of the substrate. They include water-soluble chelates (DTPA), microspheres, digestible solids (liver), or indigestible solids (paper particles). Clearly, the emptying and fate of these radiopharmaceuticals depends on the nature of the substrate.

With regard to digestible solids, in vivo-labeled chicken liver (*14,15*) is one of the most elegant radiolabeled substrates, and is both physiologic and specific for solid phase emptying. Although there is very little leakage of the isotope from the solid, it is neither easy nor convenient to prepare. In our laboratory, we have investigated and used iodine-131-alpha cellulose as a marker of the solid phase which remains intact throughout its transit through the gastrointestinal tract (*16–18*), and is a very stable labeled compound. Unfortunately, the labeling procedure is time-consuming, and iodine-131 is unattractive as a radionuclide for external imaging in view of patient dosimetric considerations.

The purpose of this study was to find an appropriate marker for studying gastric emptying of liquids and solids that could be used in routine practice. The optimal criteria for such radiopharmaceuticals are: ease of preparation; incorporation into a standard meal of mixed composition; little or no crossover of activity between the liquid and solid phases of the meal; acceptable dosimetry; and gamma ray emissions that are appropriate for external imaging with a scintillation camera. Thus, we have compared the in vitro (in the laboratory) and in vivo (in the dog) performance of several radiolabeled substrates which have been employed for studies of gastric emptying in man.

MATERIALS AND METHODS

Liquid and solid phase markers were tested in an in vitro model and a subset of these markers were further evaluated in an in vivo dog model.

In Vitro Model

Procedure. The in vitro model simulated conditions prevailing physiologically in the stomach. It consisted of a 1-liter capacity beaker containing 500 ml of isotonic saline at 37 °C. An acid (0.2 N HCl) and pepsin (1,200 μg/ml) solution was infused into the beaker at 0.7 ml/min using a peristaltic pump. An overhead motor constantly agitated the solution at 120 rpm (Fig. 1).

Large fragments of the prepared solid markers listed below were added to the beaker, then agitated. Aliquots (10 ml) were removed at 30-min intervals for 3 hr. The aliquots were centri-

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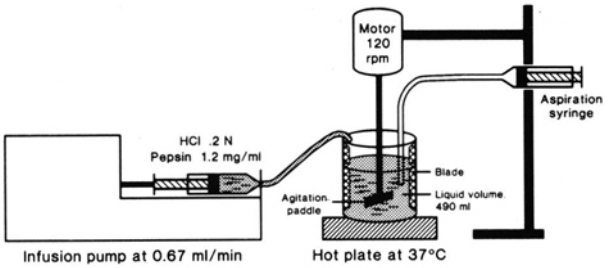

FIG. 1. In vitro model for evaluation of gamma emission labeled meal markers. See Materials and Methods for detailed description.

fuged at 640 G for 10 min, and a 1-ml sample of the supernatant was removed for scintillation counting.

For the liquid phase, the markers listed below were added to an unlabeled solid phase (pancakes prepared as indicated below), agitated, and 10-ml aliquots were removed at 30-min intervals for 3 hr. These aliquots were again centrifuged and a 1-ml sample of the supernatant was removed for radioscintillation counting.

Markers. The following solid markers were evaluated in the in vitro model: a) labeled pancake (30 g liquid pancake mix, 10 g prepackaged scrambled egg, and 3 g flour) with [^{99m}Tc]-pertechnetate, Tc-99m sulfur colloid, Tc-99m-macroaggregated albumin, or I-131 fiber; and then cooked; b) labeled scrambled egg (2 large whole eggs) with Tc-99m sulfur colloid added and then cooked; c) in vivo-labeled (rabbit) liver with Tc-99m sulfur colloid, prepared as described previously (*14*).

The following liquid markers were evaluated in the in vitro model: DTPA labeled with: a) [^{99m}Tc]pertechnetate; b) [^{99m}Tc]pertechnetate with 0.5% bovine serum albumin (BSA); c) [^{99m}Tc]pertechnetate with 1% BSA; d) In-111; or e) In-111 with 1% BSA.

In Vivo Model

An in vivo dog model previously described (*19*) was used to evaluate the performance of several of the markers tested earlier in vitro. Four 15-kg mongrel dogs were placed under general anesthesia and two cannulae were surgically implanted, one in the stomach for aspiration of gastric contents and the other in the duodenum (Fig. 2). The duodenal cannula consisted of two polyvinyl chloride catheters. One catheter (5-French) had its tip positioned in the proximal duodenum for perfusion of C-14 PEG; the other catheter (10-French) was inserted at the level of the ligament of Treitz for continuous recovery of duodenal contents by siphonage. The purpose of the C-14 PEG was to quantify entry of meal markers into the duodenum.

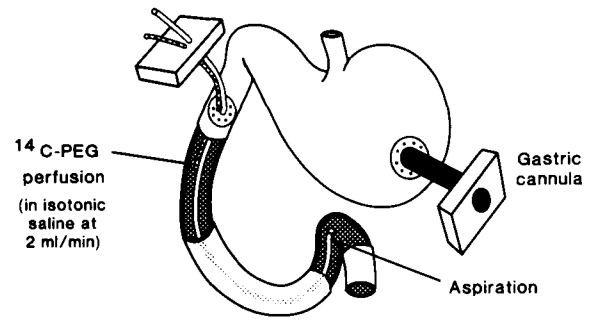

FIG. 2. In vivo dog model for evaluation of gamma emission labeled meal markers (*19*). See Materials and Methods for detailed description.

Three weeks after surgery, the dogs were put on a sling and studied. Duodenal perfusion and aspiration were carried out for at least 30 min to allow for equilibration. Then dogs ate the solid and liquid meal (detailed below) and test gastric sampling and duodenal perfusion were begun. Ten-milliliter gastric samples were taken every 10 min postprandially and the first duodenal sample was collected 5 min after the meal; all others were collected at 10-min intervals. This created a 5-min stagger in corresponding gastric and duodenal samples to allow for transit to the ligament of Treitz. Gastric and duodenal samples were also centrifuged and 1-ml samples of the supernatant were counted in gamma and beta scintillation counters to determine the percentage of the solid marker that had leaked off and the intraluminal dilution of markers. At the completion of the study (60 min after ingestion of the meal), the stomach was completely aspirated and lavaged with isotonic saline. Calculation of the gastric emptying of both liquid and solid radiolabeled markers was performed as described previously (*19*).

The following solid radiolabeled markers and meals were then evaluated in the in vivo model: a) labeled pancake (30 g liquid pancake mix, 10 g prepackaged scrambled egg, and 3 g flour), labeled with Tc-99m-macroaggregated albumin or Tc-99m sulfur colloid; b) labeled scrambled eggs (2 large whole eggs) labeled with Tc-99m sulfur colloid; c) in vivo-labeled (rabbit) liver 50 g labeled with Tc-99m sulfur colloid.

The liquid component of each of these meals was identical and consisted of 500 ml of water containing H-3 PEG. This latter marker was chosen for simplicity, since it can be counted in the presence of the C-14 PEG employed in the duodenal perfusion.

At the completion of the study, gastric contents were completely aspirated and analyzed to determine the stability of the radionuclide markers. The intragastric solid phase was defined as particles which sedimented after centrifugation at 640 G (*20*). Studies were done in duplicate for each marker.

RESULTS

In Vitro Studies

As shown in Table 1, the different radionuclide markers exhibited variable degrees of binding affinity to the solid and aqueous phases of simulated postcibal gastric contents. Among solid markers, Tc-99m sulfur colloid in vivo-labeled liver and I-131 fiber did not exhibit any appreciable leaching off of the radioisotope into the aqueous phase. Pancake labeled with Tc-99m-macroaggregated albumin and Tc-99m sulfur colloid scrambled eggs showed some leakage, but it was still within 2% at 3 hr.

With respect to the aqueous phase markers, In-111 DTPA stabilized with 1% BSA showed little binding to solid food

TABLE 1. Fate of Solid and Liquid Radionuclide Markers Exposed to Simulated Gastric Conditions In Vitro

Solid Radionuclide Marker	% Remaining on Solid Phase at: 1 hr	2 hr	3 hr
Pancake labeled with:			
Tc-99m pertechnetate	36.3	33.8	30.4
Tc-99m SC*	98.7	97.7	95.2
Tc-99m-macroaggregated albumin	99.6	99.1	98.5
Tc-99m SC scrambled eggs	98.9	98.6	98.6
I-131 fiber	100.0	100.0	100.0
Tc-99m SC in vivo-labeled liver	100.0	100.0	100.0

Liquid Radionuclide Marker	% Adhering to Solid Phase at: 1 hr	2 hr	3 hr
Tc-99m DTPA	11.5	15.2	20.0
Tc-99m DTPA (0.5 % BSA†)	6.5	10.0	12.7
Tc-99m DTPA (1% BSA)	3.7	7.5	7.5
In-111 DTPA	7.8	8.9	9.1
In-111 DTPA (1% BSA)	1.2	2.8	4.4

*SC = sulfur colloid.
†BSA = bovine serum albumin.

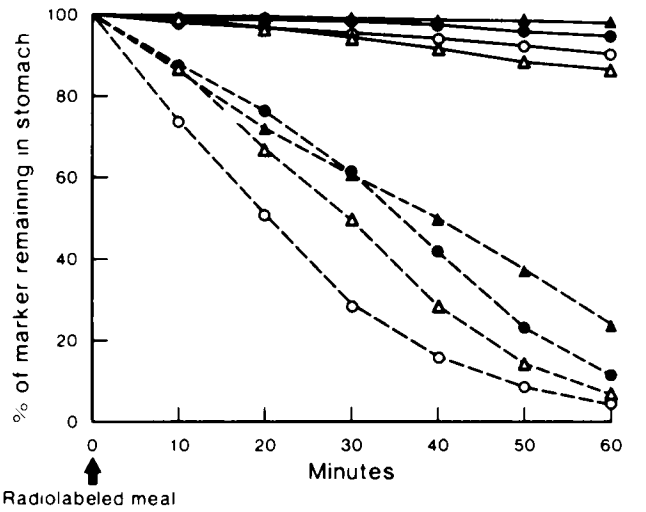

FIG. 3. Gastric emptying of labeled solid and aqueous markers quantified by marker dilution in a dog model. Note: All solid markers were selectively retained in the stomach relative to the aqueous marker. Solid marker (—) and aqueous marker (----) labeled with: ○, Tc-99m-macroaggregated albumin in pancake; ●, Tc-99m rabbit liver (sulfur colloid); ▲, Tc-99m-macroaggregated albumin in egg; △, Tc-99m sulfur colloid in pancake.

particles (< 5% at 3 hr), whereas all other markers tested had higher percent adherence to solids.

In Vivo Studies

The stability of radionuclide solid markers in vivo was inferior to that demonstrated in vitro. For example, even Tc-99m sulfur colloid in vivo-labeled liver showed a small degree of leakage into the aqueous phase at 60 min in the dog as opposed to none demonstrated in vitro. However, the performance of the pancake and egg markers (selected for this part of the study on the basis of their good performance in vitro) was somewhat close to that of the liver marker (Table 2). The reproducibility of these in vivo experiments, evaluated by duplicate tests, was good (Table 2).

Comparison between the rates of gastric emptying for each of the different markers, Fig. 3, showed excellent discrimination between emptying of the solids (slow) and H-3-PEG in water (rapid), which was the standardized liquid component of the meal. Thus, pancake and cooked egg markers behaved in a similar manner to liver. Mean percent variation in solid emptying was 1.0%, 0.7%, 5.9%, and 11.9% at 1 hr for the liver, egg, and the two pancake markers, respectively. Water left the stomach rapidly in all experiments, but the rate was a bit slower for the egg and liver meals than for the pancake meal (Fig. 3).

TABLE 2. Stability of Solid Radionuclide Markers In Vivo Shown in Two Separate Experiments

Solid Radionuclide Marker	% Leached off the Solid Phase into the Aqueous Phase* Experiments 1	2	Mean
Pancake labeled with:			
Tc-99m-macroaggregated albumin	5.9	8.6	7.3
Tc-99m SC	3.9	5.8	4.9
Tc-99m SC in vivo liver	1.2	1.1	1.2
Tc-99m SC scrambled eggs	4.0	4.7	4.4

*Complete gastric aspirates obtained 60 min after ingestion of the meal were tested.

DISCUSSION

Every laboratory performing radionuclide gastric emptying tests should select and validate appropriate markers and standardize the size, composition, and caloric content of the test meal (*21-23*). In addition, care should be taken to validate the different radionuclide markers employed in these techniques. In doing so, the most important factors to consider are, first, the stability of the marker in vivo (i.e., the percent of the isotope bound to the solid marker which remains attached to it without leaking into the aqueous phase of gastric contents). For aqueous markers, the reciprocal criteria apply (i.e., the percent of marker that does not adhere to solid particles and therefore truly reflects gastric emptying of liquids).

Second, it is important to evaluate the actual behavior of the marker in vivo. An acceptable solid marker should remain in the stomach in a particulate state long enough to test the ability of the normal stomach to selectively retain solids. Furthermore, the particulate state allows assessment of triturating capabilities of the antrum and the integrity of the antropyloric discriminatory mechanism (*15,24*). If a solid marker became semi-solid or liquid in the stomach, it would not be representative of the solid class of dietary components. The actual particle size is important in qualifying a solid marker as representative

of dietary solids. Meyer et al. (*24*) have shown that particles less than 1 mm in diameter are emptied by the stomach with liquids. For example, microspheres, although touted as a solid marker, in fact behave as an aqueous marker because of their microscopic size.

Our study describes in vitro and in vivo methods for evaluating the phase-specificity and stability of markers. Our results suggest that I-131 fiber and liver labeled in vivo with Tc-99m sulfur colloid are near optimal solid markers because they fulfill most of the criteria outlined previously. However, Tc-99m sulfur colloid in scrambled egg had excellent phase specificity and is an acceptable alternative in routine practice. Most importantly, the Tc-99m sulfur colloid in egg was easier to prepare than either the in vivo-labeled chicken liver or the I-131-fiber. Further, Tc-99m sulfur colloid in egg exhibits a gastric emptying rate in vivo quite similar to that of radiolabeled liver (Fig. 3). It is also selectively retained by the stomach, as expected for a solid marker.

The choice of a liquid phase marker is more limited. Technetium pertechnetate is both absorbed and secreted by the stomach. Technetium sulfur colloid in liquids will adhere to surfaces and not remain in the liquid phase. Water-soluble compounds, such as Tc-99m MDP undergo hydrolysis and may oxidize to free technetium pertechnetate to an unacceptable degree. The chelated compounds, Tc-99m DTPA and In-111 DTPA, have previously been shown to remain intact and to be unabsorbed by the gastrointestinal tract (*13*). In-111 DTPA exhibited good aqueous phase stability, especially when a small amount of albumin was added; it is easy to prepare using a standard DTPA kit and In-111-chloride, and could be used to perform dual isotope gastric emptying studies (*9,21*) with Tc-99m sulfur colloid in scrambled eggs.

In summary, Tc-99m sulfur colloid in eggs as a solid marker and In-111 DTPA stabilized with 1% albumin as a liquid phase marker are a combination that is quite adequate for practical purposes. It is also used by other laboratories (*4,25*). The more widespread use of standard validated radiopharmaceuticals and standardized meals would further facilitate comparisons between gastric emptying studies conducted at different centers.

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