Use of Technetium-99m(V)Thiocyanate To Measure Gastric Emptying of Fat

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Technetium-99m(V)thiocyanate was evaluated as a radiopharmaceutical for measuring gastric emptying of fat. Olive oil was labeled with ⁹⁹ᵐTc(V)thiocyanate by direct extraction from acidic thiocyanate solution. After incubation with dilute HCl (pH 1.4) at 37°C for 3 hr, approximately 5% of the total radioactivity eluted into the aqueous phase. When incubated with human gastric juice (pH 1.8 and 2.2), approximately 8% of the activity was detected in the aqueous phase at 3 hr. Scintigraphic studies performed in two rabbits showed that olive oil labeled with ⁹⁹ᵐTc(V)thiocyanate emptied slowly from the stomach, with a gastric half-emptying time (T₅₀) of more than 3 hr. A low-nutrient soup labeled with ¹¹³ᵐIn-DTPA and mixed with ⁹⁹ᵐTc(V)thiocyanate labeled oil was consumed by six human volunteers. The oil emptied much more slowly (p < 0.02) (median T₅₀ = 198 min) than the aqueous component (median T₅₀ = 30 min). These observations indicate that ⁹⁹ᵐTc(V)thiocyanate is a suitable pharmaceutical to measure gastric emptying of extracellular fat.


Radioisotopic techniques are now widely used for clinical and research purposes to quantify the rate of gastric emptying, primarily because of the noninvasive nature and accuracy of these methods (1,2). Satisfactory radioisotope marker techniques are now available to quantify gastric emptying of digestible solid (3,4), non-digestible solid (5,6), and water-soluble liquid meals (4,7).

Dietary fat, although insoluble in water, is digested within the aqueous environment of the gastrointestinal tract. Knowledge of how the stomach processes fat is incomplete (8–12). Extracellular fat empties from the stomach more slowly than the aqueous phase of a meal (9–12). Recent studies (9,12) suggest that a small proportion of extracellular fat layers on top of the gastric contents, and that the majority is either stabilized in an aqueous emulsion or adheres to solid particles as it empties from the stomach. At present, there is no satisfactory radioisotopic or alternative technique for measuring gastric emptying of fat. Techniques utilizing intubation are technically complex and only applicable to research studies (9–11). Selenium-75-glycerol triether bound to butter has been used successfully as a lipid marker (12), but cannot be considered an ideal radiopharmaceutical because of the relatively high radiation doses associated with its use.

Technetium-99m is relatively inexpensive and widely available and, when incorporated in the relatively inert colloids of tin or sulphur, is routinely used to measure gastric emptying (1–4). In order to selectively label a substance, ⁹⁹ᵐTc may be bound to molecules that have specific chelating functions, or other physical properties of the labeled products are utilized. To develop a technetium-labeled pharmaceutical suitable for measuring gastric emptying of fat, we selected a technetium compound showing high lipid solubility, since lipid molecules do not contain chelating groups. Technetium-99m(V)thiocyanate (⁹⁹ᵐTc(V)(SCN)₅) was selected for further investigation. This compound is prepared in acid conditions, was expected to be more stable in air than stannous-reduced chelates and extracts readily into organic solvents. Moreover, an oily suspension containing this label was used intravenously for liver scanning (13,14), but it has been superseded by more convenient colloidal preparations.

MATERIALS AND METHODS
Preparation, Thiocyanate Content, and Flavor of Labeled Olive Oil

Olive oil was labeled with ⁹⁹ᵐTc(V)(SCN)₅ by direct extraction from acidic thiocyanate solution according to the following method (13). Technetium-99m-pertechnetate (100–200 MBq) was added to a shielded 50-ml beaker containing 20 ml 0.9% saline. Concentrated hydrochloric acid (2.5 ml) and ferric chloride solution (0.5 ml containing 3.0 mg) were added, followed by ammonium thiocyanate (2.5 ml, 1.24 g) and ascorbic acid (0.5 ml, 25 mg). The contents of the beaker were stirred continuously while each solution was added, until the deep red ferric thiocyanate colour changed to pale pink or colorless over approximately 5 min. The ⁹⁹ᵐTc-thiocyanate complex was then extracted into olive oil (15 ml) by shaking with the reducing solution in a shielded 50-ml plastic tube for 5 min. Following centrifugation, the oil layer was removed and shaken with 10 ml phosphate buffer (sodium dihydrogen phosphate 2.2 M) for 2 min to remove any excess thiocyanate. Following a further centrifugation, the oil was washed with phosphate buffer a second time, immediately
before use. The yield of radioactivity in the labeled oil was 20%–30% of the added $^{99m}$Tc-pertechnetate. The amount of residual thiocyanate after the labeling procedure was quantified, utilizing spectrophotometric analysis of the intense color formed on the addition of ferric ions (15). The labeled oil was washed twice with phosphate buffer as in the labeling procedure and then finally with 0.01 M sodium hydroxide.

In order to ascertain the effect of the labeling conditions on the flavor of the oil, 20 ml oil was put through the labeling procedure in the absence of added radioactivity and tasted by two of the investigators.

In Vitro Studies

**Stability of Labeled Olive Oil in Dilute Hydrochloric Acid.** Labeled olive oil (50 g) was incubated with dilute hydrochloric acid (HCl) (300 ml, pH 1.4) in air at 37°C. The aqueous phase was stirred gently with a mechanical stirrer over a 3-hr period. Samples (200 μl) were removed from the oil and aqueous phases at 30-min intervals to determine the percentage of radioactivity eluting into the aqueous phase. This experiment was repeated once.

**Stability of Labeled Olive Oil in Human Gastric Juice.** The above experiment was repeated using 12 g oil and 60 ml pooled human gastric juice, pH 2.2 and 1.8 for duplicate experiments.

In Vivo Studies

**Animal Studies.** Two rabbits were each fed 3 ml oil containing approximately 20 MBq $^{99m}$Tc(V)thiocyanate and then imaged on a gamma camera for 3 hr. Images were collected at 30-min intervals on film and simultaneously stored in a computer to enable quantification of radioactivity in the stomach by selection of regions of interest over this organ.

**Human Studies.** Six healthy male subjects aged between 20 and 27 yr were studied. Following an overnight fast, each subject ingested at 1000 hr 290 ml of low-nutrient beef consomme soup (45°C) (Campbells Soups; Aust. Pty. Ltd.) blended with 60 g olive oil. In each case, consumption of the meal occurred within 1 min. The olive oil included 10 g of “active” oil containing 20 MBq of $^{99m}$Tc(V)thiocyanate and the aqueous component of the soup contained 15 MBq of $^{113m}$In-diethylenetriamine pentaacetic acid (DTPA). Each study was performed with the subject seated in front of a gamma camera. Data were corrected for radionuclide decay, Compton scatter, radionuclide gamma ray attenuation, and movement using previously described methods (16). The lag phase, before any isotope emptied from the stomach, and the 50% gastric emptying time was determined for both the oil and the aqueous phases of the meal (16). The experimental protocol was approved by the Human Ethics Committee of the Royal Adelaide Hospital.

**Statistical Analysis.** Gastric emptying data in the human studies were evaluated using the Wilcoxon rank sum test. A p value <0.05 was considered significant.

RESULTS

**Thiocyanate Content and Flavor of Labeled Oil**

Spectrophotometric analysis of the sodium hydroxide wash showed that following the two phosphate buffer washes less than 0.5 mg ammonium thiocyanate was present in the oil. The final product was palatable and it was concluded that the labeling procedure did not introduce any undesirable flavor characteristics into the oil.

**In Vitro Studies**

**Stability in Dilute Hydrochloric Acid.** The mean results for two experiments are shown in Figure 1, and demonstrate excellent stability of the label in acid and air conditions, with about 5% eluting from the oil into the aqueous phase over 3 hr.

**Stability in Human Gastric Juice.** Figure 2 shows the mean results for these two experiments and demonstrates excellent stability of the labeled oil over a 3-hr period, with approximately 8% of the total radioactivity eluting into the aqueous phase.

**In Vivo Studies**

**Animal Studies.** The scintigraphic images demonstrated a slow passage of oil from the stomach into the small intestine with a gastric emptying half-time of more than 3 hr. A small proportion of the administered dose was excreted in the urine, suggesting that this activity has been absorbed either after enzymic digestion of the labeled oil or after elution from the oil phase. Computer analysis of the stored images are shown in Table 1. The two rabbits remained healthy without any sign of toxicity.

**Human Studies.** The meal was palatable and easily ingested by all subjects. In both experiments, the oil emptied much more slowly (p < 0.02) than the aqueous component of the meal. There was a significant lag phase (p < 0.02) before any of the oil emptied from the stomach, whereas emptying of the aqueous phase approximated a monoexponential pattern with minimal lag phase (Table 2). Emptying of the oil from the stomach commenced only after the majority of the aqueous phase had emptied (Figs. 3 and 4). A urine sample collected 3.50 hr after the meal in one of the subjects contained 3.5% of the dose administered in the oil phase.

The radiation exposures to the stomach wall, small, and large bowel were estimated at 1.5, 0.9, 3.5 mSv respectively. Total-body exposure was approximately 1.14 mSv.
DISCUSSION

Studies of the specific behavior of the lipid phase of a meal during gastric emptying are limited (8–12). Techniques utilizing intubation procedures have yielded discrepant results (9–11). It now appears that the majority of extracellular fat empties as an oil phase, while the majority of intracellular fat empties with the solid food phase (9). The present study indicates that $^{99m}$Tc(V) thiocyanate is a suitable pharmaceutical to measure gastric emptying of extracellular liquid fat. Approximately 95% of the label remains in the lipid phase after incubation with both hydrochloric acid and gastric juice for 3 hr. Using $^{99m}$Tc(V)thiocyanate as a marker of the lipid phase of a meal and $^{113}$In-DTPA as a marker of the aqueous phase, the emptying of aqueous and lipid phases were clearly defined and separated. The observation that the labeled oil emptied from the stomach much more slowly than the aqueous phase of a meal is consistent with previous studies (9–12). The presence of radioactivity in the urine in both the human and animal studies may reflect the absorption of some activity due to digestion of fat or elution of labeled tracer from the oil phase. Further studies are required to compare the rate of emptying of solid fats with solid meals from the stomach.

Thiocyanate is a relatively non-toxic substance, which is readily excreted in the urine (17) and naturally produced thiocyanate occurs at levels of $<$2 mg/100 ml blood. Thiocyanate is a detoxification end-product of the hypertensive agent sodium nitroprusside, administered intravenously in humans in doses of 0.5–1.5 mg$^{-1}$kg$^{-1}$min$^{-1}$ (18). Less than 0.5 mg free ammonium thiocyanate was detected in the oil. As a human dose (70-kg man), this quantity is equivalent to 0.0071 mg/kg. The LD50 of sodium thiocyanate is known to be 764 mg/kg orally in rats (19), which is equivalent to 717 mg/kg of ammonium thiocyanate. On a weight basis, therefore, the LD50 in the rat is approximately 10$^3$ times the dose given to the volunteers. The levels of thiocyanate likely to occur in the blood in our human subjects (approximately 0.1 mg/ml of blood) therefore compare favorably to the reported toxic levels of 50–100 mg/ml (19).

Other $^{99m}$Tc compounds require a reduction step for their preparation and may subsequently undergo reoxidation to $^{99m}$Tc pertechnetate, unless kept under atmosphere of nitrogen gas. Such compounds are unlikely to be suita-
Considerations for Accurately Measuring Gastric Emptying

In this issue, Cunningham et al. describe a radiopharmaceutical for measuring the gastric emptying of fat (1). The choice of radioactive markers is one of several important considerations in accurately measuring the rate of gastric emptying.

RADIOPHARMACEUTICAL

First, it is important that food be used as the marker of gastric emptying. Hunt and Stubbs have shown non-nutrient saline meals empty rapidly with exponential (first-order) kinetics, whereas nutrient meals show an initial emptying period that loads the duodenum, followed by a more linear (zero-order) emptying curve (2).

Second, solid-phase markers should be used. The mechanism of liquid


REFERENCES