# The Efficacy of Radiolabeling the Albumin in Egg Whites with $^{99m}$Tc-Sulfur Colloid

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In 2009, the Society of Nuclear Medicine and Molecular Imaging published a standardized protocol guideline for gastric emptying scintigraphy that contains specific instructions on the exact meal and preparation procedure. Previous research has shown that the standardized meal and proper preparation of the meal for gastric emptying scintigraphy are not being adopted by some facilities. This research explores the differences of radiolabeling in the method of preparation of $^{99m}$Tc-sulfur colloid (SC)–radiolabeled eggs. **Methods:** Liquid egg whites were mixed with $^{99m}$Tc-SC before cooking in conjunction with the standardized protocol. A second sample set was prepared by adding the $^{99m}$Tc-SC to eggs after they were cooked. Each sample set was placed in a solution of HCl and pepsin to simulate gestation. Radiolabeling efficacy was tested on each sample set at 2 and 4 h after gestating in HCl and pepsin. **Results:** $^{99m}$Tc-SC added to the liquid egg whites before microwave cooking yielded radiolabeling efficacy of 70% $^{99m}$Tc-SC after 2 and 4 h of simulated gastric fluid gestation. In contrast, radiolabeling after cooking the egg whites yielded 50% radiolabeling after simulated gestation. **Conclusion:** The results from this experiment showed that the method of mixing the $^{99m}$Tc-SC with liquid egg whites before microwave cooking has higher binding efficacy than when adding $^{99m}$Tc-SC onto already cooked egg whites. These results highlight the importance of following the standardized protocol for the meal preparation of a gastric emptying study.

**Key Words:** gastrointestinal; radiopharmaceuticals; gastric emptying scintigraphy; radiolabeling; meal preparation; standardized meal

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Gastric emptying scintigraphy is the gold standard for evaluating gastric motility (*1*). The reliability of gastric emptying studies is dependent on careful adherence to the procedure guidelines, especially the standardized meal. However, previous literature has demonstrated that wide variations from the standard protocol exist (*2–4*). A common divergence from the standardized protocol is to add the $^{99m}$Tc-sulfur colloid ($^{99m}$Tc-SC) to the egg whites after they have been cooked rather than before (*2*,*5*). This research evaluates and contrasts the radiolabeling efficacy of adding $^{99m}$Tc-SC to egg whites before and after cooking.

A gastric emptying study is performed by radiolabeling the solid or liquid meal and measuring the radioactivity in the stomach for 1 min every hour up to 4 h (*1*,*5*). In 2009, the Society of Nuclear Medicine and Molecular Imaging (SNMMI) sought to reduce any variations that may occur in the preparation of a meal for a gastric emptying study by publishing a standardized procedure guideline for solid-meal gastric emptying (*1*,*3*). The SNMMI gastric emptying study protocol contains precise instructions for patient preparation, meal ingredients and preparation, radiopharmaceutical dose, camera acquisition, and image processing (*5*). According to the gastric emptying study protocol, the meal consists of 118 mL of liquid egg whites, 2 slices of toasted white bread, 30 g of jam or jelly, and 120 mL of water (*1*,*5*). The meal preparation consists of adding 18.5–37 MBq (0.5–1 mCi) of $^{99m}$Tc-SC to the eggs, which are then mixed well and cooked in a microwave oven or nonstick skillet. The eggs are stirred a couple of times during cooking until they are firm. It is essential to add the $^{99m}$Tc-SC to the eggs before cooking to increase binding efficiency between the $^{99m}$Tc-SC and the albumin in the eggs (*2*,*4*,*5*).

The standardized meal and meal preparation guidelines must be followed because normalized gastric emptying values depend on the contents of the meal (*4*,*5*). The emptying rate of the stomach depends on whether the meal is liquid or solid, whether it is high in fats or in carbohydrates and proteins, its digestibility and caloric density, and the amount consumed. For example, vegetables will stay in the stomach longer than foods that are more digestible (*3*), a liquid meal will empty from the stomach much more quickly than a solid meal, and a meal high in fat will stay in the stomach longer than a meal high in carbohydrates or proteins.

The standardized protocol includes instructions for preparation set by the SNMMI, but research has shown that the protocol is not always followed (*2*,*4*). Manipulating the method of preparation can result in variability, decrease comparison ability, and decrease the credibility of the gastric emptying results (*2*). In 2013–2015, the Intersocietal Accreditation Commission evaluated 129 accreditation-seeking facilities for compliance with the standardized protocol (*3*). Of those facilities, 69% were not compliant with meal preparation (*3*). Farrell et al. also noted that several nuclear medicine laboratories

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were following the standardized meal but not the preparation guidelines and were instead adding the $^{99m}$Tc-SC by squirting it onto cooked eggs (*2*). Although the most recent data show that adherence to the standardized protocol has improved, variations in meal and meal preparation are still widespread (*4*).

It is also essential that the meal preparation protocol be followed to ensure complete binding of the eggs (*4*). The $^{99m}$Tc-SC should be added before or during cooking of the eggs (*5*). When $^{99m}$Tc-SC is cooked together with liquid egg whites, the albumin protein in the egg whites denatures because of the heat and forms a stable bond with the $^{99m}$Tc-SC (*4*,*6*). However, when $^{99m}$Tc-SC is added to cooked eggs, it will not have a stable bond with the egg. The result is disintegration of the tagging between the albumin and the $^{99m}$Tc-SC during digestion, with at least a portion of the quantitative analysis being $^{99m}$Tc-SC not bound to eggs. If the $^{99m}$Tc-SC does not remain bound to the albumin, the quantitative analysis will reflect transit not of a solid meal but of a mixture of solid and liquid (*6*). It has become common practice to add $^{99m}$Tc-SC to eggs after they are cooked. This research examines the labeling efficacy of $^{99m}$Tc-SC added to liquid egg whites before and after cooking in a microwave oven.

## MATERIALS AND METHODS

The materials and methods for this research are similar to those of previous research examining the radiolabeling efficacy for gastric emptying studies (*7*,*8*). This research was performed in the Hillsborough Community College Nuclear Medicine Technology Program laboratory under supervision. The research methods were reviewed and approved by the Nuclear Medicine Technology Program, and it was determined that Institutional Review Board approval was not needed.

**TABLE 1**
Materials Used for This Research

| Material | Quantity |
|---|---|
| $^{99m}$Tc-SC | 5.92 MBq (160 μCi) |
| 1% bovine serum albumin | 0.5 mL |
| NaCl | 15 mL |
| HCl and pepsin | 3.5 mL |
| Distilled water | 50 mL |
| Sterile gauze pack | 1 |
| Test tubes | 24 |
| Test tube rack | 1 |
| 1,000-mL beakers | 2 |
| Hot plate with stirrer | 1 |
| Food scale | 1 |
| 3-mL syringes | 12 |
| Liquid egg whites | 118 mL |
| Paper cup | 2 |
| Square disposable plastic scale-tray | 2 |
| Metal laboratory spatula | 1 |
| Foam cup | 2 |
| Microwave | 1 |
| Dose calibrator | 1 |
| Well counter | 1 |
| Survey meter | 1 |

Several materials were used for this research (Table 1). The $^{99m}$Tc-SC was purchased from a licensed radiopharmacy following radiation safety guidelines and department protocol. A survey meter was used to measure the package before it was opened. A dose calibrator was used to measure the dose before it was added to the egg whites, which were transferred to foam cups for cooking in the microwave oven. A metal laboratory spatula was used to chop the egg whites and transfer them to the test tubes after they had been weighed. A square disposable plastic scale-tray was used to weigh the egg whites on the food scale. NaCl, HCl, pepsin, and distilled water were used to simulate the environment inside the human stomach (Fig. 1). A hot plate with a stirrer was used to warm distilled water inside a beaker, within which the test tubes were submerged. Bovine serum albumin, gauze, syringes, and NaCl were used to separate the simulated gastric fluids from the egg whites. A well counter was used to measure the radioactivity in each sample.

The method to determine radiolabeling efficacy used a simulated digestion process applied in previous research examining radiolabeling for gastric emptying studies (*7*,*8*). Two radiolabeling methods were used to tag the radiotracer to the egg whites. Egg whites for each sample were measured to 118 mL in a beaker and transferred to a foam cup for both preparation methods. For the precooking method, 2.59 MBq (70 μCi) of $^{99m}$Tc-SC were added to the egg whites and mixed well before they were cooked in the microwave. For the postcooking method, 3.1 MBq (84 μCi) of

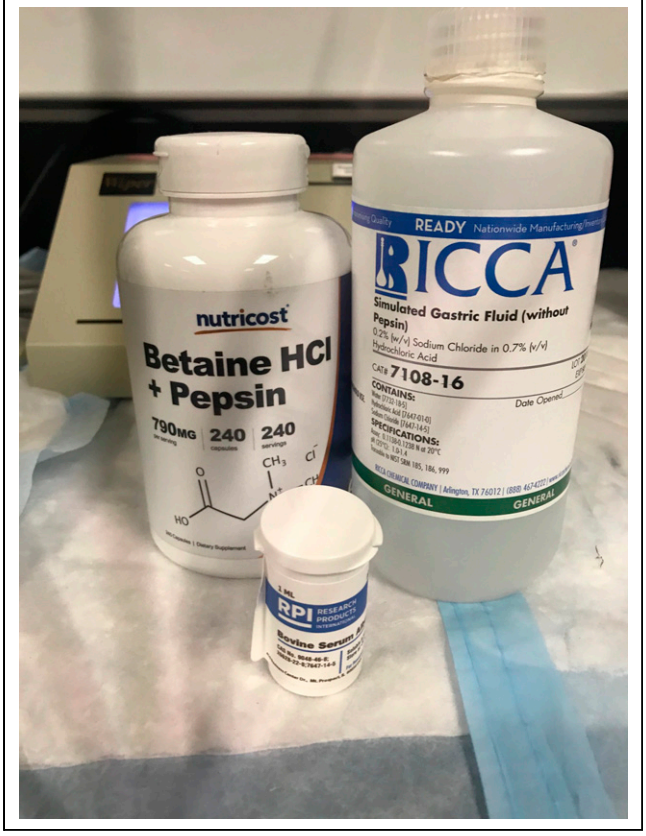


**FIGURE 1.** Materials used to simulate gastric environment and examine radiolabeling: simulated gastric fluid with HCl and pepsin. Bovine serum albumin was used to evaluate radiolabeling efficacy.

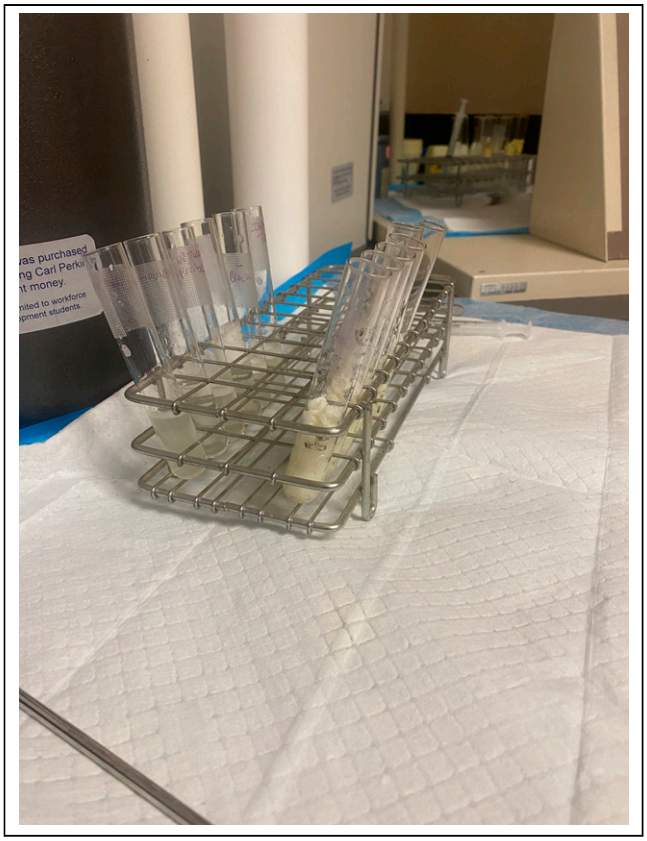

**FIGURE 2.** Twelve test tubes with radiolabeled cooked egg whites separated from simulated gastric fluid.

$^{99m}$Tc-SC were added to the egg whites after they were cooked in the microwave. For both methods, the egg whites were cooked to a firm consistency on high for 50 s, stirred for 5 s, and then cooked on high for another 15 s to ensure thorough cooking.

Two digestive processes inside the stomach had to be simulated: mechanical and chemical (*9*). After the egg whites had been cooked and allowed to cool for 10 min, they were chopped into small pieces with a metal laboratory spatula to mimic the mechanical process of chewing. Half of the 12 test tubes were labeled "precooked" and the other half "postcooked." Three test tubes in each set were labeled "2 h," and the other 3 were labeled "4 h." Both sets were then placed into square disposable plastic scale-trays, and 0.5-g samples were weighed out on a food scale and placed into each test tube.

During the chemical process, HCl and pepsin help break the food down to the macronutrients (*7*,*8*). To simulate the environment inside a human stomach, 3.5 mL of HCl and pepsin, 15 mL of NaCl, and 50 mL of distilled water were added to the egg whites in the test tubes, which were then placed in a water bath at 98.6°F to simulate the internal temperature of the human body. Stirrers at 125 rpm were used to simulate churning in the stomach. One timer was set for 2 h and another for 4 h, at which times the tubes were removed from the water bath.

Sterile gauze was moistened with 1% bovine serum albumin to prevent $^{99m}$Tc-SC from sticking to the gauze (*7*,*8*). The gauze was then folded and packed into the tubes so that the simulated gastric fluid rose above it. The simulated gastric fluid was then removed from the egg whites using a 3-mL syringe (Fig. 2) and transferred to another test tube, leaving just the egg whites tagged with the radiotracer. The egg whites were rinsed with 1 mL of NaCl, which was then filtered out and added to the simulated gastric fluid.

**TABLE 2**
Radiolabeling Efficacy Results for Precooked Sample

| Time | Simulated gastric fluid | Precooked egg whites | Radiolabeling efficacy |
|---|---|---|---|
| 2 h | 5,784 | 12,656 | (12,656/18,440) × 100 = 69% |
| | 5,676 | 12,689 | (12,689/18,365) × 100 = 69% |
| | 5,974 | 12,679 | (12,679/18,653) × 100 = 68% |
| 4 h | 4,542 | 12,628 | (12,628/17,170) × 100 = 74% |
| | 5,200 | 12,630 | (12,630/17,830) × 100 = 71% |
| | 5,467 | 12,659 | (12,659/18,126) × 100 = 70% |

Mean of precooked sample set is 70%. All measurements are in counts per minute.

## RESULTS

The activity of the egg white samples in each test tube was counted using the well counter. The simulated gastric fluid with the rinse activity was also counted using the well counter. Each was recorded in counts per minute. To determine the radiolabeling efficacy percentage, the total counts of the egg whites and the gauze was divided by the total counts of the egg whites, gauze, simulated gastric fluid, and rinse and then multiplied by 100.

The mean percentage for each method was calculated by adding the radiolabeling efficacy percentages and then dividing by 6 (the number of samples in each set). At both time points for the precooking method, most activity was still tagged to the egg whites in all 6 samples. At both time points, the precooking method had a higher radiolabeling efficacy, at an average of 70% (Table 2), versus 50% for the postcooking method (Table 3).

## DISCUSSION

This study examined the labeling efficacy of $^{99m}$Tc-SC added to egg whites before and after cooking in a microwave oven. Some limitations to the study include the lack of actual

**TABLE 3**
Radiolabeling Efficacy Results for Postcooked Sample

| Time | Simulated gastric fluid | Postcooked egg whites | Radiolabeling efficacy |
|---|---|---|---|
| 2 h | 12,548 | 12,176 | (12,176/24,724) × 100 = 49% |
| | 11,666 | 10,954 | (10,954/22,620) × 100 = 48% |
| | 11,165 | 11,358 | (11,358/23,023) × 100 = 49% |
| 4 h | 10,903 | 11,478 | (11,478/22,381) × 100 = 51% |
| | 11,729 | 12,572 | (12,572/24,301) × 100 = 52% |
| | 12,196 | 11,973 | (11,973/24,169) × 100 = 50% |

Mean of postcooked sample set is 50%. All measurements are in counts per minute.

human gastric fluid, the measuring of samples only at 2 and 4 h instead of the full protocol time length, and the limited sample size. Evaluation of labeling efficiency before digestion would also be useful. Despite these limitations, this research found that the radiolabeling efficacy of $^{99m}$Tc-SC was much higher when added to the egg whites before they were cooked.

Our findings support previous findings in similar research on meal preparation methods. Knight et al. determined binding efficacy to be much better when $^{99m}$Tc-SC was added to whole eggs before they were cooked (*7*). Similarly, McKee et al., who used a simulated digestion method to examine the radiolabeling of whole eggs before and after microwave cooking, found that labeling before cooking yielded 73% binding whereas labeling after cooking yielded 43% binding (*8*). Our current study yielded slightly higher binding for egg whites using similar methods but supported the same outcome that adding $^{99m}$Tc-SC before cooking yields a much higher binding efficacy. Tafti et al. reported that variations in gastric emptying study protocols are still widespread but are lessening (*4*). Future research should be done with larger sample sizes and other meal preparation methods.

## CONCLUSION

Gastric emptying scintigraphy remains the gold standard for evaluating gastric motility. The reliability of the examination depends strongly on adherence to the standardized meal and meal preparation. However, there is a lack of consistency across facilities, with a common variation being to add the $^{99m}$Tc-SC to cooked scrambled egg whites. This study documented the efficacy of $^{99m}$Tc-SC radiolabeling of egg whites according to the SNMMI protocol guidelines (i.e., before cooking) versus after cooking. The precooking method had higher efficacy than the postcooking method. These results highlight the importance of following the standardized protocol in preparing the meal for a gastric emptying study.

## DISCLOSURE

No potential conflict of interest relevant to this article was reported.

## KEY POINTS

**QUESTION:** Is radiolabeling of egg whites with $^{99m}$Tc-SC as effective after cooking as before cooking?

**PERTINENT FINDINGS:** The bond between $^{99m}$Tc-SC and the albumin in egg whites is not as strong when the $^{99m}$Tc-SC is added after the egg whites are cooked and is more likely to disintegrate during digestion.

**IMPLICATIONS FOR PATIENT CARE:** Gastric emptying scintigraphy results may be less accurate when the $^{99m}$Tc-SC is added to the egg whites after they are cooked.

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