such as surgery or needle biopsy. In particular, IPASs have been diagnosed correctly only after surgery (*3–5*).

The accessory spleen in this patient was also difficult to differentiate from hypervascular pancreatic tumors, such as islet cell tumors and acinar cell carcinomas, because the enhancement pattern of the mass was similar to that of those tumors, and the mass apparently was surrounded by normal pancreatic parenchyma. In this patient, however, we strongly suspected an IPAS by a careful examination of the CT and made a diagnosis using a spleen scintigraphy. Unnecessary invasive diagnostic methods were avoided.

Technetium-99mc-HDRBC scintigraphy is a highly sensitive and specific method for detection of splenic tissue (*6–9*), since up to 90% of the injected HDRBCs are trapped by splenic tissue (*9*). In our patient, the findings from dynamic CT, ^{99m}Tc-HDRBC scintigraphy and a careful review of previous reports made us believe that the mass should be diagnosed as an accessory spleen, although histopathological evidence was not obtained.

CONCLUSION

We noninvasively diagnosed accessory spleen in the tail of the pancreas using SPECT technetium-labeled heat-damaged RBCs. When a hypervascular mass is seen in the pancreas, IPAS should be in the list of differential diagnoses, and ^{99m}Tc-HDRBC SPECT should be considered.

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Evaluation of Solid-Phase Labels for Gastric Emptying Studies in Cats

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Development of appropriate radiolabeled diets for solid-phase gastric emptying studies in experimental animals is important for testing the effects of disease, drugs, surgical procedures and stress. This study evaluates the in vitro and in vivo stability of various radiolabels in commercially available dry, extruded and canned cat foods. **Methods:** Dry, extruded cat food was labeled with ^{99m}Tc-pertechnetate, ^{99m}Tc-sulfur colloid or ^{99m}Tc-disofenin. Canned cat food was labeled with ^{99m}Tc-Dowex resin beads, ^{99m}Tc-pertechnetate, ^{99m}Tc-sulfur colloid or ^{99m}Tc-disofenin. A sample of each labeled diet and ^{99m}Tc-sulfur colloid-labeled egg was digested in water, gastric juice, intestinal juice or gastric juice followed by intestinal juice. The samples were centrifuged and the activity in the samples counted before and after removal of the supernatant. Based on in vitro results, three labeled diets were fed to 10–12 cats for in vivo testing. **Results:** ^{99m}Tc-Dowex beads had the best labeling efficiency in vitro, but were not stable in vivo, resulting in unacceptable levels of circulating ^{99m}Tc. Technetium-99m-disofenin labeling resulted in in vitro percent solid-phase retention of 92.5% and 89.5% in water and gastric juice, respectively, for dry food and 86% and 94.9% in water and gastric juice, respectively, for canned food. **Conclusion:** Technetium-99m-disofenin is a suitable label for solid-phase gastric emptying studies using commercially available cat foods.

Key Words: technetium-99m-disofenin; radionuclide; gastric emptying; cat

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The use of physiologic meals and noninvasive methods, combined with the ease and accuracy of quantitation, has made scintigraphy the method of choice for gastric emptying studies (*1,2*). Scintigraphy is a valuable clinical and research technique that allows highly reproducible, quantitative characterization of different phases of gastric emptying (*3–8*).

Due to their size, ease of handling and similarities in physiologic and pharmacologic responses between monogastric mammals, dogs and cats are frequently used as experimental models in gastric emptying research (*5,9–28*). Unfortunately, with few exceptions (*11,13,14,21,23,26*), the diets used in these studies fail to meet the criteria of an appropriate test meal. Conclusions based on these models must be viewed with some skepticism.

The optimal solid-phase meal should: (a) be a diet of composition typical and physiologically appropriate for the subject; (b) have stable binding of the radiolabel; (c) experience no digestion, absorption or adsorption of the radiolabel in the stomach or small intestine; (d) be palatable; and (e) be readily available and easy to prepare (*11,29*). Labeled meals used for animal studies in the past have failed to meet many of these criteria. In many instances, the meal offered was not typical for the species and was not physiologically normal (*10,12,19,24,25*). Often, these meals were selected for palatability, ignoring issues of suitability for long-term maintenance or the period required to accommodate to these diets

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(*5,10,17,25,28*). In other studies, the issues of palatability and free-choice feeding were circumvented by force feeding or feeding through a gastrostomy tube (*9,12,24,25,27*). Both methods would be expected to affect gastric emptying. Few studies mention a period of accommodation to experimental diets. One must presume that, in order to meet institutional standards for animal care and use, the animals were maintained on a standard cat or dog food diet. It follows that, in many instances, experimental meals were novel to the research animals.

In those studies that used radiolabeled pet foods, in vitro (*11,14,21,26*) and in vivo (*13,14,21,26*) labeling stability has not been well documented. Established labels of human meals have been used in animal species without assessing in vivo stability (*9,10,17,18,20,25*). Unstable labels could result in erroneous gastric emptying rates due to: washout of the label in the liquid phase, retention of the label in stomach mucosa, gastric recycling of free pertechnetate or absorption of the label from the gastrointestinal tract.

It has been extensively documented that the stomach acts to selectively empty food based on form (solid compared with liquid phase), particle size and nutrient composition (*1,2,5,6,17,19,29–32*). Standard test meals (i.e., scrambled egg sandwich, liver in beef stew) used in man do not have a uniform distribution of radiolabel or nutrients. These test meals rely on chewing and trituration within the stomach to achieve a more uniform distribution. Since commercial cat and dog foods are generally ground and homogeneously blended during preparation, they have a relatively uniform distribution of nutrients before ingestion (*33*). The tendency of cats to gulp, rather than chew, both canned and dry food (provided the size of the dry food pieces is not too large) minimizes the variation in ingested particle size (*33*). Standardization of diets prepared in large quantities and stored either canned or in dry extruded form provides an advantage for long-term animal studies.

The goals of the study were to evaluate the in vitro and in vivo stability of several radiolabels in commercially available canned and dry, extruded cat foods.

MATERIALS AND METHODS

Diets

One canned (KSUGED1, Ralston Purina, Co., St. Louis, MO) and one dry extruded diet (KSUR1, Ralston Purina, Inc., St. Louis, MO) were selected for the study based on proximate analyses and composition that would be typical of premium or growth commercial cat foods. The diets had similar proximate analyses based on dry matter. The proximate analyses for each is given in the Appendix.

Stomach and Intestinal Juice

Immediately before each in vitro trial, 250 ml of simulated (USP) stomach and intestinal juice were made (*34*).

Animals

We used 22 normal, young adult (2- to 3-yr-old) cats from our gastrointestinal physiology/nutrition colony. All cats had normal complete blood counts, serum biochemistries and T4 levels. Cats were negative for feline leukemia and feline immunodeficiency virus. All cats had lived in the colony for at least 18 mo and had been accommodated to the nuclear medicine laboratory and restraint on the gamma camera during previous studies. Cats were fed the diets to be tested for at least 2 wk before the in vivo experiments. Cats were allowed to eat for 10 min twice daily to train them to meal feed. All animals were housed, and studies performed, in AALAC-approved facilities with the approval of the Kansas State University, Institutional Use and Care of Animals Committee.

Radiolabel Preparation

A $^{99}Mo/^{99m}Tc$ generator provided 1.48 GBq (40 mCi) [^{99m}Tc]pertechnetate ($^{99m}TcO_4^-$) in 10 ml of saline. Sulfur colloid (An-Sulfur Colloid, CIS-US, Inc., Bedford, MA) and disofenin (Hepatolite, DuPont Merck Pharmaceutical Co., Billerica, MA) kits were prepared according to manufacturer's directions. Quality control was performed on the kits per the manufacturer's recommendation before kit use.

One milliliter of $^{99m}TcO_4^-$ (148 MBq) was added to 2 ml of anion exchange resin beads (Dowex 2-X8, Bio-Rad Laboratories, Hercules, CA). Five minutes later, the beads were rinsed twice with 20 ml water. The water of the second rinse was placed in a well counter peaked for ^{99m}Tc and assayed for activity. In all trials, no activity was detected in the decanted rinse water.

Diet Labeling

Dry Food Labeling. Three 40-g samples of dry cat food were weighed out into plastic cups. Each was labeled by the addition of one of the following: 1 ml of the $^{99m}TcO_4$ eluate (TcO_4^--dry), 1 ml of the ^{99m}Tc-sulfur colloid preparation (SC-dry) or 1 ml of the ^{99m}Tc-disofenin preparation (Diso-dry). Each labeled dry food sample had 3 g of beef baby food (Gerber's Products Co., Fremont, MI) stirred in to coat the pieces.

Canned Food Labeling. Four 40-g samples of canned cat food were labeled by the addition of one each of the following: 2 ml of ^{99m}Tc-Dowex beads (Dowex-can), 1 ml of $^{99m}TcO_4^-$ (TcO_4^--can), 1 ml of ^{99m}Tc-sulfur colloid preparation (SC-can) or 1 ml of ^{99m}Tc-disofenin preparation (Diso-can).

Sulfur Colloid Egg Labeling. One milliliter of the ^{99m}Tc-sulfur colloid preparation was added to a raw egg that was cooked (scrambled) to a firm consistency (SC-egg). Total weight of the preparation was about 40 g.

In Vitro Digestion

For each labeled diet, four 10-g samples were transferred to conical centrifuge tubes. Each of the four samples had one of the following added to them: 10 ml of water, 10 ml of USP gastric juice, 10 ml of USP intestinal juice or 10 ml of USP gastric juice followed by 10 ml of USP intestinal juice 2 hr later. All tubes were placed on a shaker table in a 37°C water bath for 3 hr. Twenty-five milliliters of water were added to all of the dry extruded diet tubes 1 hr after being placed in the water bath. No water was added to the canned diets.

Three hours after the start of digestion, all tubes were centrifuged at 1320 × g for 20 min.

The activity in the tubes was counted in a dose calibrator. The supernatant was then removed and the solid pellet in the tube recounted. Percent solid-phase retention (%SPR) was calculated as follows: [activity in the pellet/total activity (supernatant and pellet)] × 100 = %SPR. To minimize the effects of day-to-day variation in technique, the in vitro experiments were performed five times over a 7-day period.

In Vivo Studies

Based on the in vitro results, trials were performed to qualitatively assess the in vivo stability of three of the diet labels: Dowex-can, Diso-dry and Diso-can. Water was available to all cats throughout the study.

Twelve cats were offered 100 g of canned food with 222 MBq (6 mCi) of ^{99m}Tc-Dowex beads added (Dowex-can). Cats were allowed to eat ad libitum for 10 min, at which time the remaining food was removed and weighed. Ingested meal size ranged from 25 to 73 g.

Ten cats were offered 80 g of canned food labeled with 111 MBq (3 mCi) of ^{99m}Tc-disofenin (Diso-can) and allowed to eat ad libitum for 10 min. Ingested meal size ranged from 30 to 77 g. Ten

TABLE 1
Stability of Dry Cat Food Radiolabels*

Label-diet	Method of digestion: Water	Gastric	Intestinal	Both
TcO_4^--dry	83.53[a]	85.31[e]	82.75[i]	81.44[m]
SC-dry	82.62[b]	76.77[f]	78.77[j]	82.21[n]
Diso-dry	92.50[c]	89.49[g]	85.53[k]	85.67[o]
SC-egg†	94.44[d]	93.28[h]	83.93[l]	88.30[q]

*Expressed as percent solid-phase retention; standard error of the least squares mean = 2.88%.

†For comparison; not a dry food label.

Selected statistical comparisons are:

a > b p = 0.823; a < c p = 0.029; a < d p = 0.008; a < e p = 0.663; a > i p = 0.849; a > m p = 0.608.

b < c p = 0.017; b < d p. = 0.004; b > f p = 0.153; b > j p = 0.347; b > n p = 0.919.

c < d p = 0.636; c > g p = 0.460; c > k p = 0.089; c > o p = 0.096.

d > h p = 0.777; d > l p = 0.011; d > q p = 0.134.

e > f p = 0.038; e < g p = 0.307; e < h p = 0.052.

f < g p = 0.002; f < h p = 0.001; g < h p = 0.353.

cats were offered 50 g of dry kibble labeled with 111 MBq (3 mCi) of ^{99m}Tc-disofenin (Diso-dry) and allowed to eat ad libitum. Ingested meal sizes ranged from 10 to 50 g.

Three hours after feeding the Dowex-can fed cats, and 3 and 6 hr after feeding the Diso-dry and Diso-can fed cats, right lateral whole-body and ventral cervical images were obtained. Images of 60 sec were acquired with an LFOV camera (Siemens 750 ZLC, Isleton, NJ) equipped with a low-energy, parallel-hole collimator using a 15% window centered at 140 KeV. Images were acquired to a computer (NucLear Mac, Scientific Imaging, Littleton, CO) in a 150 × 150 × 256 matrix and stored on removable hard disk.

Statistical Analysis

Comparisons of diet labels and methods of digestion were made by analysis of variance using the SAS™ general linear model (GLM) procedure (SAS Institute, Inc., Cary, NC). Follow-up tests for pairwise comparisons of interest were conducted based on protected least significant differences approach. In vivo results were analyzed by t-tests.

TABLE 2
Stability of Canned Cat Food Labeled*

Label-diet	Method of digestion: Water	Gastric	Intestinal	Both
Dowex-can	99.32[a]	99.35[f]	99.59[k]	99.06[q]
TcO_4^--can	72.07[b]	80.33[g]	63.79[l]	75.51[r]
SC-can	71.04[c]	88.72[h]	70.92[m]	86.40[s]
Diso-can	86.01[d]	94.93[i]	77.80[n]	83.94[t]
SC-egg†	94.44[e]	93.28[j]	83.93[o]	88.30[u]

*Expressed as percent solid-phase retention; standard error of the least squares mean = 2.88%.

†For comparison; not a canned food label.

Selected statistical comparisons are:

a > b p = 0.0001; a > c p = 0.0001; a > d p = 0.001; a > e p = 0.232; a < f p = 0.995; a < k p = 0.946; a > q p = 0.949.

b > c p = 0.799; b < d p = 0.001; b < e p = 0.0001; b < g p = 0.045; b > l p = 0.044; b < r p = 0.399.

c < d p = 0.0003; c < e p = 0.0001; c < h p = 0.0001; c > m p = 0.976; c < s p = 0.0002.

d < e p = 0.041; d < i p = 0.03; d > n p = 0.046; d > t p = 0.612; e > j p = 0.777; e > o p = 0.011; e > u p = 0.1344.

f > g p = 0.0001; f > h p = 0.01; f > i p = 0.28; f > j p = 0.139; g < h p = 0.041; g < i p = 0.0005; g < j p = 0.002.

h < i p = 0.129; h < j p = 0.265; i > j p = 0.685.

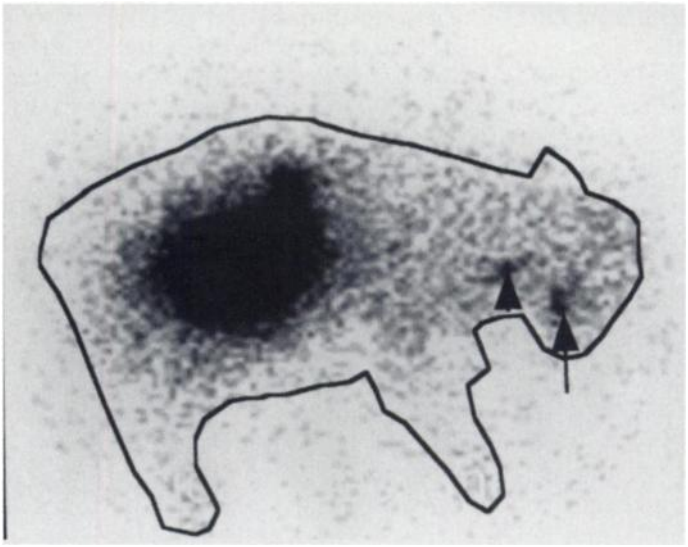

FIGURE 1. Right lateral, whole-body image of a cat 3 hr postingestion of a ^{99m}Tc-Dowex resin bead-labeled meal. Most counts originate from the gastrointestinal region. However, the cat's body can be clearly visualized and focal areas of increased radiopharmaceutical uptake are present in the regions of the thyroid (small arrow) and salivary glands (long arrow).

RESULTS

Chromatography of the sulfur colloid and disofenin kits demonstrated an average radiochemical purity of 97.9% (range 96.4%–99.9%) and 99.0% (range 97.8%–99.9%), respectively.

In Vitro Studies

Dry Extruded Cat Food. Disofenin-labeled dry cat food (Diso-dry) had the highest %SPR of the dry diet labels for each digestion tested. The average values for each dry diet label and digestive method are summarized in Table 1. Diso-dry had a significantly higher %SPR than SC-dry in water ($p = 0.017$) and gastric juice ($p = 0.002$). Technetium-pertechnetate-dry also had a higher %SPR in gastric juice than SC-dry ($p = 0.038$). There was no significant difference in %SPR between Diso-dry and sulfur chloride-egg digested in gastric juice ($p > 0.35$) or water ($p > 0.63$). While there was no

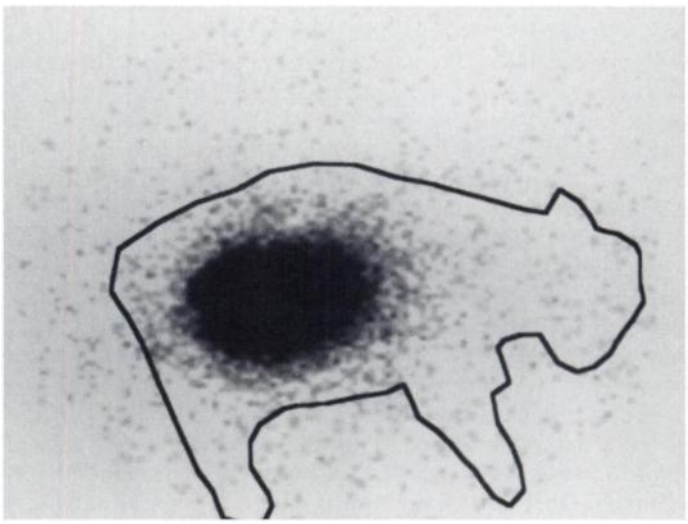

FIGURE 2. Right lateral, whole-body image of a cat obtained 3 hr postingestion of a ^{99m}Tc-Disofenin-labeled meal. Only the gastrointestinal region can be clearly visualized, confirming that the radiopharmaceutical is not absorbed from the gastrointestinal tract.

significant difference between Diso-dry and TcO_4^--dry in gastric juice ($p > 0.30$), Diso-dry had higher %SPR in water ($p = 0.029$). No statistical difference between water and the different methods of digestion was observed for any given dry diet label ($p > 0.05$).

Canned Cat Food

Results of %SPR for canned diet labels and SC-egg are summarized in Table 2. Dowex-can had higher %SPR in water, intestinal juice and gastric plus intestinal juice than TcO_4^--can, SC-can or Diso-can ($p < 0.01$). Dowex-can's %SPR was not statistically different than Diso-can ($p = 0.28$) or SC-egg ($p > 0.14$) in gastric juice, but was higher than TcO_4^--can ($p = 0.0001$) and SC-can ($p < 0.04$). Diso-can had higher %SPR in water compared with TcO_4^--can ($p = 0.0008$) and SC-can ($p = 0.0003$) but was lower than SC-egg ($p = 0.04$).

The method of digestion resulted in no difference in the in vitro stability of Dowex-can labeling ($p > 0.94$). In all instances Dowex-can %SPR exceeded 99%. For TcO_4^--can, SC-can or Diso-can digested in gastric juice, %SPR was significantly higher than when incubated in water ($p < 0.05$). No difference was identified between SC-egg in gastric juice compared with water ($p > 0.77$).

The %SPR was significantly lower after digestion with intestinal juice compared with water for TcO_4^--can ($p < 0.045$), SC-egg ($p = 0.011$) and Diso-can ($p < 0.045$) but not SC-can ($p > 0.97$). The addition of intestinal juice (to the gastric plus intestinal juice group) resulted in a decrease in %SPR for Diso-can ($p = 0.008$) but not other canned diet labels ($p > 0.22$).

In Vivo Studies

No activity was detected outside the gastrointestinal tract for the Diso-dry and Diso-can groups during in vivo studies. The Dowex-can group had activity outside the gastrointestinal tract including readily apparent thyroid and salivary uptake. Figures 1 and 2 represent typical examples of whole-body right lateral images obtained 3 hr after cats were offered Dowex- and disofenin-labeled meals, respectively.

DISCUSSION

The composition of the diets used in these labeling studies would be typical of high-quality commercially available cat foods. The two diets selected for study had similar dry-matter proximate analyses to minimize the effects of differing levels of fat, protein and carbohydrate. In general, as in these two diets, canned cat foods contain a larger percentage of meat to provide protein and fat, and dry foods rely more on plant sources (*33*). Carbohydrate sources vary as well. As with these two diets, canned cat foods usually contain 68%–78% water and dry extruded cat foods less than 10% (*33*).

Water was added to the dry food diets at 1 hr to simulate the natural situation where cats drink after ingestion of a dry meal. From pilot studies with these diets, our colony of cats usually drank 2–2.5 times as much water (by weight) as dry food consumed. The addition of the water resulted in a nutrient density similar to that of the canned diets.

The in vitro percent solid-phase retention (%SPR) of the ^{99m}Tc-disofenin label in USP gastric juice for dry and canned food exceeded 89% and 94%, respectively. Technetium-99m-disofenin label also demonstrated excellent stability in water. The combination of good label stability in water and gastric juice suggests it to be an excellent choice for solid-phase gastric emptying studies, with only a small amount of activity moving with the liquid phase despite the animal being allowed to drink, or the amount of gastric secretions (factors beyond the researchers control in a normal physiologic state).

The lack of nongastrointestinal activity encountered during the in vivo studies suggests minimal digestion or breakdown of the radiopharmaceutical and little absorption of the radiopharmaceutical or its breakdown products from the gastrointestinal tract. Three-hour postfeeding images were selected for in vivo assessment based on reported gastric emptying times (*26*). Six-hour images were made for disofenin-labeled diets to assure there was no absorption of radiopharmaceutical from the colon. No biliary or urinary activity was identified during any of the 20 disofenin-labeled studies presented here.

Based on in vitro results, it is anticipated that after a disofenin-labeled meal, 5%–14% of the activity emptying from the stomach actually represents liquid phase (either water or gastric juice). Two factors suggest that %SPR in in vivo studies may be higher than estimated from in vitro work. First, exposure to gastric juice resulted in increased %SPR for disofenin-, sulfur colloid- and pertechnetate-labeled diets. The mechanism for this was not investigated but likely involves pH since exposure to intestinal juice had the opposite effect. Second, centrifugation results in a more complete separation of solid and liquid phases than is expected by the stomach's normal sieving action. In the natural state, some water (and, therefore, liquid-phase counts) is trapped in the solid food matrix. No attempt to quantitate the water content of the solid pellet was made.

Disofenin was easy to prepare and relatively inexpensive. Radiopharmaceutical purity of the prepared kits was high when prepared according to manufacturer's directions. Unlike sulfur colloid, disofenin label preparation does not require heating.

A previous report suggested using ^{99m}Tc-sulfur colloid for labeling dry extruded cat food (*26*). No attempt to assess the stability of the label was presented in that report. Our results suggest that sulfur colloid is an inferior label with more than twice the amount of unbound activity as disofenin in both water and gastric juice. It is possible that the relatively high fat content of the diets used in this study favored the lipophilic disofenin label.

Of interest were the minimal (and statistically insignificant) differences between disofenin-labeled diets and SC-labeled egg. Sulfur colloid-labeled egg and in vivo sulfur colloid-labeled chicken liver serve as the standards for human gastric emptying studies (*1,2,30*). Our results with disofenin labeling are similar to those previously reported for both ^{99m}Tc-sulfur colloid egg or in vivo ^{99m}Tc-sulfur colloid-labeled chicken liver (*2,30*). Reduction of the food particle size with the SC-egg and chicken liver labels results in decreased label retention (*2,30*). Perhaps if the SC-egg in this study had been ground to the consistency of the canned cat food, the disofenin label would have proven superior.

The decrease in %SPR observed with disofenin-labeled diets and SC-labeled egg exposed to intestinal juices raises the question of accuracy of these solid-phase labels for intestinal transit studies. While it appears that the label is retained in the luminal contents of the small intestine, a significant amount is likely associated with the liquid, rather than solid, components of intestinal chyme. The clinical significance of this is unknown.

Since 1982, reports promoting the use of ^{99m}Tc-Dowex 2-X8 resin beads for gastric emptying in humans and dogs have appeared in the literature (*21,23,35*). These reports found high in vitro stability similar to that achieved in this study but do not contain references to in vivo stability. Despite in vitro stability exceeding that of all other labels, ^{99m}Tc-Dowex beads resulted

in obvious nongastrointestinal free pertechnetate in in vivo studies in cats. Uptake by gastric mucosa would result in an erroneously decreased gastric emptying rate. Disparity between in vitro and in vivo results have been found with other labels (*10*). This underscores the need for in vivo testing of radiolabels.

CONCLUSION

Technetium-99m-disofenin is suitable for solid-phase labeling of cat food for gastric emptying studies. Little justification exists for using a meal other than cat food in gastric emptying studies in cats. Cat food provides researchers with a more normal meal that cats can be maintained on long term and yet is easily labeled. Issues with nutritional completeness, effect of a novel meal, accommodation to test meals and palatability can be eliminated by the use of cat food.

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APPENDIX
Diet Proximate Analyses

	As fed (%)	Dry matter (%)
Dry extruded diet		
Moisture	5.65	0
Fat	26.0	27.6
Protein	40.2	42.4
Ash	6.5	6.9
Crude fiber	1.19	1.26
Canned diet		
Moisture	75	0
Fat	7.3	29.2
Protein	11.5	46
Ash	1.6	6.4
Crude fiber	0.26	1.04

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