Formation of Sulfonamide Bonds Through Reaction of Dyes with Serum Proteins

TO THE EDITOR: I would like to comment on the recent article by Tsopelas and Sutton (*1*) regarding the identification of dyes that may be useful in visual identification of the sentinel lymph node. The goal of the study was to elucidate why particular dyes are absorbed by the lymphatic system. Indirect information on dye absorption was obtained through an investigation that used size-exclusion chromatography to determine the percentage of a particular dye that bound with rat plasma proteins. The authors were particularly interested in determining if binding was a function of molecular structure. Indeed, in the article the authors reported that the number of sulfonate groups, and the number of atoms between the sulfonate groups, on a dye correlate with its plasma protein binding. Although this finding is based on a model system in which rat plasma is used in place of lymph, the data may be useful in helping to identify dyes for use in sentinel node visualization.

Unfortunately, the authors' explanation of the dye–protein binding at a "molecular level" does not seem plausible. Although the authors use the term *binding affinity* throughout the article, they speculate that the binding of the dye with proteins occurs through covalent bond formation (i.e., sulfonamide formation). This explanation seems highly unlikely, as the sulfonamide-forming reaction of a protein amine with a sulfonic acid, as depicted in Figure 3 of the article, simply would not occur under physiologic conditions. Indeed, this reaction requires dehydration in an aqueous environment, which would be very difficult to perform. Some mechanism of activation of the sulfonate toward nucleophilic attack by the amine is required. For example, conjugation of dyes through sulfonamide bond formation with protein amines is a standard methodology, but sulfonyl chlorides, which are highly activated to nucleophilic attack, are used (*2*). Even conjugation with sulfonyl chlorides does not occur readily under physiologic conditions, as the protein amines are rendered nonnucleophilic by protonation at neutral pH. Further, sulfonates are fully ionized under physiologic conditions, such that the reaction in Figure 3 might best be depicted as below. When reaction components are depicted as ions, it is more apparent that the reaction to form the sulfonamide would not occur under physiologic conditions.

$$\text{Protein–}\overset{\oplus}{\text{N}}\text{H}_3 + \text{R–S}\overset{\ominus}{\text{O}}_3 \longrightarrow \text{Protein–}\overset{\oplus}{\text{N}}\text{H}_3\ \overset{\ominus}{\text{O}}_3\text{S–R (ion pair)}$$

Such a conversion as shown in Figure 3 of the article may be possible if specific enzymes are present in serum, which catalyzed the transformation. However, speculating that enzymatic coupling has occurred is not warranted unless substantial data are provided unequivocally proving that the dyes are bound to serum proteins through covalent bond formation. No data are provided that demonstrate that the sulfonamide is formed. Importantly, size-exclusion chromatography cannot delineate whether a peak observed for a protein–dye combination came about by a high-affinity interaction or from chemical bonding of the 2 species. Demonstration that a chemical species is bonded to a protein is relatively easy using mass spectral analyses. In addition, other analytic methods such as ion-exchange chromatography and isoelectric focusing electrophoresis can also be used to confirm that the dye is bonded and not simply associated with the plasma proteins.

An alternate, and perhaps more plausible, explanation for the observed dye–protein interaction than the sulfonamide bond formation proposed by the authors is that the dyes have a varying affinity for serum proteins brought about by ionic and lipophilic interactions based on each dye's unique structural features (e.g., lipophilic portions, 3-dimensional shape, and distance between sulfonates). As an example, Birkenmeier (*3*) reported an evaluation wherein blood proteins were separated using immobilized dyes. In that study, 2 dyes were evaluated as "affinity materials" for partitioning serum proteins. One dye, Remazol yellow GGL (DyStar) (*4*) has 2 sulfonate groups, one of which has 3 atoms between the sulfonate and an aromatic ring. The other dye, Cibacron blue F3G-A (Ciba-Geigy Corp.) (*4*) has 3 sulfonate groups directly attached to separate aromatic rings. These "affinity ligands" containing sulfonate groups displayed strong interaction with serum proteins but were not covalently bonded to the proteins.

In summary, the authors should further investigate the nature of the protein–dye binding and delineate whether it is brought about by covalent bonding or noncovalent interaction. With that information, they will be able to substantiate or refine the mechanism of molecular binding that is depicted in Figures 2 and 3 of their article.

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REPLY: We are grateful to D. Scott Wilbur for his précis regarding the nature of the dye–protein bond and accept that covalent bonding is not proven but proposed. The following response is offered nevertheless, as a rebuttal to his critique. Sulfonation or acylation reactions are chemical transformations that occur by an identical mechanism, that being the nucleophilic attack of nitrogen, oxygen, carbon, or sulfur atoms onto electrophilic sulfonyl or carbonyl centers, respectively. Such reactions are successfully performed in vitro under a variety of conditions, which usually include the use of activated substrates (sulfonyl- or acylchlorides, esters, anhydrides, etc.) in organic or even aqueous solvents. In a simple system in which a sulfonic acid–containing molecule is united with an amine such as lysine or arginine under neutral aqueous conditions, a water-solvated ion pair is expected to ensue (similar to the product depicted in the reaction sequence of the Wilbur letter). When the reaction medium is blood serum,

however, this complex milieu comprises molecules with the potential to promote alternative interactions.

Different enzymes have frequently been used for coupling organic molecules in vitro. In particular, good yields of oligopeptides can be synthesized from the reaction of (nonactivated) carboxylic acids and amines in organic-aqueous solvents with subtilisin or endopeptidase/chymopapain (*1*) and in aqueous solvents with thermolysin/α-chymotrypsin, papain, or penicillin acylase (*2*). Amide bonds formed in these enzyme-catalyzed acylation reactions result in an overall loss of water. Similarly, a phosphorylation reaction between 2 phosphate-containing molecules can be achieved in an aqueous buffer (*3*), where a phosphorus–oxygen bond is created at the expense of H_2O. The statement that a dehydration reaction would be difficult to perform in an aqueous environment has been otherwise shown in the literature.

In lymph, serum, or extracellular tissue, proteins are known to participate in covalent bond formation either with themselves or with other molecules. Examples of the "chemisorption" reaction include cross-linking of fibrin during blood clot formation, cross-linking of collagen to produce connective tissue, reaction with other proteins as a consequence of aging, disulfide bond formation of fibrillins during assembly of microfibrils via sulfhydryl oxidases, retinoylation of proteins, and substrate phosphorylation. Cytosolic enzymes/proteins are routinely involved in covalent bond-forming reactions with sulfotransferase in sulfonation/sulfation (*4*) or the ribosome 50S subunit, nonribosomal synthetases, or polyketide synthases in amino acid acylation (peptide synthesis).

Coupling reactions between biologic molecules and xenobiotic small molecules ex vivo have been shown to form covalent-bonded adducts at temperatures up to 37°C, including carboxylated dyes with horseradish peroxidase (*5*), amine- or sulfhydryl-specific dyes and cytochrome c (*6*), malachite green cation and chicken egg albumin (*7*), thiazole orange derivatives with oligonucleotides (*8*), 4-nitrobenyl-^{35}S-mercaptan S-sulfonic acid with rat cytosolic proteins, a leukotriene-tetraenoic acid with 15-lipooxygenase, and a ribozyme-catalyzed formation of dipeptides. In view of the evidence supporting the formation of amide and other covalent bonds between proteins and endogenous/xenobiotic molecules, one cannot preclude the possibility of an enzyme-mediated sulfonamide bond formation between proteins and sulfonic acid-dyes as reported earlier (*9*).

The literature also provides evidence of a "physisorption" interaction of protein hydrophobic groups and small aromatic water-soluble molecules, where for example, naphthalene sulfonic acid groups are particularly associated with arginine groups on the exterior of the protein. Preliminary observations in this laboratory thus far, after use of instant thin-layer chromatography (ITLC), suggest that a simple ion pair does not define the dye–protein interaction, nor does a hydrophobic interaction. At the time of publication (*9*), it was known that naphthol blue black (NBB) and Evans blue (EB), in separate experiments, migrated on ITLC-silica gel/glass paper in saline (0.9%) with migration coefficients of $R_f = 0.8$ in the presence or absence of excess arginine or of lysine, phenylalanine, or polylysine. Mixing any of these dyes with plasma for a brief time (~20 s) at room temperature, and then performing ITLC on the dye–protein mixture, resulted in a colored spot at $R_f = 0.8$, indicating no chemisorption or physisorption. This was confirmed by size-exclusion chromatography, a technique that serves to separate products from starting materials, where there was 100% recovery of the initial dye. However, altering the incubation conditions to 37°C for 10 min found, for NBB exclusively and EB predominantly, that the colored spots were visible at the baseline and near the baseline, respectively. Likewise for ^{99m}Tc-EB, the location of radioactivity on the ITLC strip correlated with blue color. These observations indicate that a strong affinity exists between dye and protein and that a simple ion pair association is absent. Furthermore, although Coomassie blue G (a sulfonic acid reported to bind to proteins by physisorption) has shown anomalous behavior in its interaction with polylysine (*10*), both Evans blue and NBB, with $R_f = 0.8$, have not. In an ongoing study, this laboratory is gathering additional evidence to elucidate the bond between serum proteins and naphthalene-sulfonic acid dyes.

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Correlation of Tumor Radiation-Absorbed Dose with Response Is Easier to Find in Previously Untreated Patients

TO THE EDITOR: The quest for a correlation between tumor radiation-absorbed dose and response in radioimmunotherapy has been a difficult and, so far, mostly marginally productive effort. By the usual $P < 0.05$ requirement, Sgouros et al. (*1*) recently did not find statistically significant correlations for dose mean, maximum, minimum, and uniformity for tumors in 15 non-Hodgkin's lymphoma patients participating in a phase II study of therapy with a combination of unlabeled tositumomab plus ^{131}I-labeled tositumomab. For all patients, previous chemotherapy had failed. At the University of Michigan, we have studied patients undergoing the same treatment procedure. However, in some of our research,

including research with similar measurements of mean radiation-absorbed dose, the patients were all previously untreated (*2–5*).

In their discussion, Sgouros et al. (*1*) correctly commented that in one of our publications (*4*) we presented results for a restricted dataset, that is, not for all patients potentially available to us for evaluation and not for all time points at which they were scrutinized after therapy. The reasons for this practice were 3-fold: First, we eliminated axillary tumors from the study because we have found that they have a considerably lower radiation dose estimate than do abdominal and pelvic tumors (*2,5*). Second, we chose to include tumors from only those patients who went on to achieve a partial response, rather than patients who achieved a complete response, because we anticipated that the former would have a more widely distributed set of volume-reduction values at any time after therapy and might be a more homogeneous group. Third, we chose to look at our results only at 12 wk after therapy to reduce the work of the initial evaluation.

Sgouros et al. (*1*) also correctly commented that we determined several different dose–response relationships. In fact, we produced 4 probit-fit relationships (*4*). We used a time series of diagnostic conjugate views for 1, and we used those same data supplemented by a single intratherapy SPECT image for 3 others. Those 3 were for the dataset independent of the initial tumor mass, a data subset of tumors with an initial mass greater than 10 g, and a data subset of tumors with an initial mass less than or equal to 10 g.

Sgouros et al. (*1*) also wrote: "In no case was a statistically significant relationship observed" with our data. In fact, the *P* value was significant for 1 of our 4 probit-fit relationships. That is, for the SPECT-supplemented data subset consisting of tumors with an initial mass less than or equal to 10 g, a statistically significant *P* value of 0.029 was determined (*4*). This significance occurred for the best-fit sigmoidally shaped relationship between tumor volume reduction and radiation dose compared with no dose–response relationship, that is, compared with a constant volume reduction. (Note that we previously stated that the significance test was in comparison with a constant volume reduction of 50% (*4*). That statement was in error.) The data subset comprised 15 tumors in 6 patients. The curve was a slightly truncated version of the classic sigmoidal shape (*4*).

In addition to this result, since the time of our publication (*4*) one of the patients has been reclassified from PR to CR and so should be removed from the study as it was defined. Because the patient's 2 tumors were larger than 10 g, the removal has no effect on the data subset discussed above. However, the *P* value for the entire SPECT-supplemented dataset now has become statistically significant in the same sense as above ($P = 0.0496$). With the removal of the 2 tumors, this dataset now comprises 41 tumors in 9 patients. The curve is a considerably truncated version of the classic sigmoidal shape.

These observed correlations between radiation dose and response involve only a limited number of tumors and are only modestly robust. We expect that the modest robustness exists because the tumor mean radiation-absorbed dose, although quite important to response, is not likely to be the only determinant. For example, the uniformity of the radiation dose distribution may also be an important contributor. In addition, there are certainly measurement errors for both radiation dose and volume reduction.

Sgouros et al. (*1*) gave a plot of volume reduction versus mean radiation dose for their data in Figure 4A of their article (*1*). This plot separately represented volume reduction at 3 different times after therapy, including 12 wk (designated 75 d because of a difference in time-zero definition). They reported that for these data no statistically significant correlation was observed. They did not give a *P* value for each relationship, but the range given for all their relationships was 0.25 to greater than 0.5. However, they did not examine their data for only nonaxillary tumors in only partial-response patients, and they did not fit a sigmoidally shaped probit function to the data. It appears that at 12 wk the probit fit would be statistically insignificant or exhibit a very weak dependence on mean dose. We assume it would also be so if the data were restricted to those for nonaxillary tumors in partial-response patients. Given that assumption, we are of the opinion that the crucial difference between this particular result of theirs and our result is that they examined patients who had disease relapse after chemotherapy, whereas we studied previously untreated patients.

Sgouros et al. (*1*) made a statement that can be used as a possible physiologic reason for the difference between their results and ours. That is, they said: "the effects of prior treatment . . . would be expected to differentially impact tumor radiosensitivity and, thereby, confound an absorbed dose–response relationship. . . " for patients who had disease relapse after chemotherapy. They also cited a reference for their statement (*6*). In that reference, Williams presages the result that is the subject of this letter by saying "Wisely, Koral et al. chose only untreated non-Hodgkin's lymphoma patients so as to minimize any analysis difficulty due to prior therapies." This statement was specifically made in regard to one of our publications (*3*), but it can equally well be applied to all (*2–5*), including that on which Sgouros et al. commented (*4*).

If the crucial difference between the 2 results cited is indeed pretreatment versus no pretreatment, it lends scientific weight to the supposition that dose–response relationships are easier to find for previously untreated patients than for patients with disease relapse. If this supposition is true, it implies that, while tumor dosimetry is being improved, researchers looking for dose–response relationships in radiopharmaceutical therapy should initially concentrate on previously untreated patients in the unusual situations in which both types of patient are available.

On the other hand, even in their patients with disease relapse, Sgouros et al. reported that "a trend toward increased response with increasing [dose] uniformity was observed ($r = 0.37$; $P = 0.06$). . ." (*1*). So, when uniformity of radiation dose can be assessed and a large number of tumors are available, it may be possible to find a statistically significant dose–response relationship, even for patients who have relapse of disease after previous chemotherapy.

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REPLY: Koral et al. have highlighted an important aspect related to tumor-absorbed dose versus response. We completely agree with them and thank them for emphasizing this point. It is encouraging that radioimmunotherapy has evolved to a stage at which it is being used as a first-line therapy. As Koral et al. suggest, the availability of such studies will remove an important confounding factor in establishing tumor (and normal organ) absorbed dose–response relationships.

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Tomographic Evaluation for a 3-Dimensional Organ

TO THE EDITOR: In his invited commentary in the May 2002 issue of *The Journal of Nuclear Medicine*, Dr. Meignan made a powerful argument for expanding the use of lung SPECT (*1*). The opportunities for quantification of alterations in region lung perfusion and ventilation in the diagnosis and understanding of pulmonary embolism are attractive and exciting—and dependent on tomographic imaging. As Dr. Meignan laments, it is unrealistic to hope for a PIOPED-type study of lung SPECT (although even PIOPED II might be criticized for bias against a competing modality using outmoded technology). However, much can be learned by careful clinical correlation as a means of evaluating diagnostic procedures. Outcome studies in patients on whom a diagnostic test central to their disease has been performed are essential to assess the accuracy and risk of new procedures and may be the only methodology available in many circumstances.

My group is persuaded that lung SPECT offers an ideal screening procedure for suspected pulmonary embolism: easily performed in any clinical setting with a rotating scintillation camera, available in most areas and at all times, accurate, and highly cost effective. We hope that others will heed Dr. Meignan's message and encourage the use of lung SPECT as the technique of the future.

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REPLY: In his letter, Dr. Corbus points out the essential role of outcome studies to assess the efficacy of new diagnostic procedures in patients with pulmonary embolism. Imaging techniques are continuously evolving, leading to different levels of technologic and clinical local expertise. This probably explains in part the broad variations in sensitivity (70%–100%) that have been reported for helical CT when a direct comparison was performed against other imaging modalities. Therefore, as claimed by Dr. Corbus, outcome studies relying on a 3-mo follow-up combined with validated diagnostic criteria to achieve a final diagnosis of pulmonary embolism must be encouraged. Musset et al. (*1*) have recently used this approach to assess the performance of single-array helical CT and have concluded that helical CT should not be used in isolation to exclude the diagnosis of pulmonary embolism.

Dr. Corbus also underlines the potential incremental value of lung SPECT, coupled with a quantification of regional ventilation and perfusion (*2*), thus focusing on the functional role of pulmonary nuclear imaging. One has to remember the paramount role of nuclear imaging techniques in the knowledge of lung physiology and pathophysiology. With this respect, ventilation imaging by means of radioactive gases is invaluable, and it is regrettable that ^{81m}Kr is no longer produced in some countries.

For these reasons, like Dr. Corbus, we truly hope that quantified lung SPECT will soon be extensively used and evaluated by outcome studies (*1*). Lung SPECT would also certainly benefit from a cost-effectiveness analysis of diagnostic strategies including this modality (*3*).

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Measurement of Gallbladder Ejection Fraction

TO THE EDITOR: We would like to comment on a flaw we observed in the methods of determining gallbladder ejection fraction using fatty-meal stimulation proposed by Krishnamurthy and Brown (*1*) and Jacobs and Peterson (*2*) in recent communications.

Both articles recognize the variable latent period between ingestion of a fatty meal and the onset of gallbladder contraction (B − A in Fig. 2 of Krishnamurthy and Brown (*1*)) but then propose that the ejection period used for ejection fraction calculations be determined at the trough of activity in the gallbladder or at the end of 60 min of imaging relative to peak activity (C − B in the same figure). In Figure 2 of Krishnamurthy and Brown (*1*), all 4 curves—w, x, y, and z—have variable ejection periods and have very different ejection fractions of approximately 20%, 40%, 60%, and 80%, respectively. However, the slope of gallbladder emptying is nearly identical for all 4 curves during the ejection phase, and therefore, gallbladder contraction is also nearly identical for all 4 curves even with the wide range of proposed ejection fractions (20%–80%).

A better estimate of gallbladder function would be the parameter that we use: Gallbladder ejection fraction is calculated using the ejection period between the onset of gallbladder contraction and 30 min after the onset of gallbladder contraction. The onset of gallbladder contraction is a marker of endogenous cholecystokinin secretion similar to exogenous Kinevac (sincalide for injection; Bracco Diagnostics, Inc.). A consistent 30 min of the ejection period is used for ejection fraction calculations instead of the variable-length ejection periods of the other methods. This simulates the 30-min-after-Kinevac gallbladder ejection fractions used for comparison with the normal values that clinicians use; that is, gallbladder ejection fraction is normal if it is >35%–40% at 30 min after injection of Kinevac.

In the past year, our clinicians have told us that our method produces gallbladder ejection fractions that help them discriminate between normal and abnormal gallbladder function. They continue to send patients to us for gallbladder ejection fraction determination to identify those with biliary dyskinesia and abdominal pain who would benefit from cholecystectomy or sphincterotomy. They also report that whether the patient's usual symptoms were reproduced with fatty-meal ingestion is a clinically useful finding and should be included with the study results. We are now reviewing our results from 653 studies performed in 2002.

In order to use our proposed method of gallbladder ejection fraction determination, dynamic 1-min images for 60 min after ingestion of the fatty meal must be obtained to identify the onset of gallbladder contraction and determine the gallbladder ejection fraction. The protocol of Jacobs and Peterson (*2*) obtains images immediately after ingestion of the fatty meal and 15, 60, and 75 min after ingestion. This protocol would not have the temporal resolution necessary to accurately determine the onset of gallbladder contraction. Dynamic images are also necessary to differentiate between gallbladder contraction and filling patterns that represent cystic duct syndrome and those that represent dysfunction of the sphincter of Oddi (*3*).

Other differences in methods are the fatty meal used. Krishnamurthy and Brown (*1*) used 240 mL (8 oz) of half-and-half per 70 kg of body weight (24 g of fat and 1,339 kJ [320 kcal]). Jacobs and Peterson (*2*) used 90 mL (3 oz) of whipping cream with a teaspoon of sugar regardless of body weight (30 g of fat). We originally used Lipomul (Lee Pharmaceuticals) in the amount applied for gallbladder contraction studies with ultrasound protocols but had difficulties obtaining an adequate supply. We then switched to a cheaper alternative, Microlipid (Mead Johnson & Co.), that was readily obtainable through our hospital pharmacy. Microlipid has the same concentration of fat per milliliter as does Lipomul, so we used the same volume as Lipomul (volume in milliliters equals 0.68 × body weight in pounds or 1.5 × body weight in kilograms) for a standard fatty meal (105 mL per 70 kg of body weight equals 52.5 g of fat or 1,977 kJ [472.5 kcal]).

Advantages of the fatty meal are that it is much cheaper than a dose of Kinevac, avoids spasm of the sphincter of Oddi that can occur when Kinevac is injected too quickly, and truly mimics a patient's meals. Disadvantages include less literature support for the use of fatty meals in nuclear medicine studies and a requirement for more gamma camera imaging time. In addition, some patients are nauseous and refuse to ingest the fatty meal or immediately regurgitate the ingested meal. Given our current excellent results with fatty-meal stimulation, we are debating whether to go back to Kinevac even now that it is available again. We may limit Kinevac use to only patients who cannot tolerate a fatty meal.

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REPLY: We appreciate the comments of Drs. Chen and Campbell on our article. As we tried to point out in our article, the value of gallbladder ejection fraction depends on various factors including fatty-meal content, total calories, and duration of postmeal data collection (*1*). Authors can choose to measure gallbladder ejection fraction during 30 min of ejection period as long as normal values are established for that fixed duration. Figure 2 of our article is only a schematic representation of various types of emptying curves one may encounter with half-and-half as the fatty meal at a dose rate of 240 mL (8 oz)/70 kg of body weight. Individual values are shown in Table 1. For patients, the highest gallbladder counts are normalized to 100% by the custom-designed hepatobiliary software we used (KHB-Quant). The slope of the curve may change for patients.

The responsibility for establishing normal values for a particular technique rests with the physician conducting and interpreting the study and not with the clinician who depends on the test result. Clinicians rely on the reference values cited next to the patient result. The physician who conducts the test and interprets the hepatobiliary study should validate the local technique, especially when it deviates from the published technique. Because it is expensive to perform studies on healthy subjects, and often diffi-

cult to justify ethically, patient outcome results are frequently used to establish normal limits (*2*).

While using the results of a published study, it is necessary to follow the borrowed technique in its entirety, including the content and calories of the total meal, and the dose rate and infusion duration of the octapeptide of exogenous cholecystokinin (CCK-8). Any deviation from the borrowed validated technique would call for revalidation. Gallbladder ejection fraction measured with the fatty meal ultimately depends on the total quantity of endogenous cholecystokinin released into the circulation. Disadvantages of the fatty meal are its longer test duration (2 h) and greater variability in ejection fraction (*1*). Because gallbladder emptying does not follow a gaussian distribution, one cannot rely on the traditional method of using the mean ± 2 SDs to set the normal range. An arbitrary lower limit needs to be chosen from the results of outcome studies (*2*). Gallbladder ejection fraction can be controlled to any desired level simply by varying the dose rate and duration of infusion of cholecystokinin (*1–3*). We use 35% as the lower limit of normal with a 3-min infusion of 10 ng CCK-8 per kilogram, and 50% as the lower limit with 3 ng/kg/min for 10 min (*2*,*3*). We hope that Drs. Chen and Campbell soon publish the results on their 653 patients to enable others to adopt their technique. Now that CCK-8 is again available for clinical use in the United States, the need for fatty-meal stimulation has lessened.

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REPLY: With the paucity of literature investigating the utility of fatty-meal gallbladder stimulation studies, we were pleased to read about Drs. Chen and Campbell's experience and apparent success with their technique. Our fatty meal (90 mL [3 oz] of heavy whipping cream) has been well tolerated by our patients and was chosen because of our inability to obtain other standard meals previously described (such as Lipomul [Lee Pharmaceuticals] and Calogen [Scientific Hospital Supplies]) and the ability to deliver a high fat content in a small volume. Lactose intolerance has not been an issue, and for those unfortunate few with a "milk allergy," we have given 30 mL (1 oz) of cooking oil with success.

Our technique for data acquisition has evolved such that only 2 static images are acquired: immediately before meal administration (after the initial dynamic 60-min acquisition) and 75 min later. Patient tolerability and the efficiency of gamma camera use were both improved by our not requiring the patient to lie motionless under the camera for more than 2 h. Like Drs. Chen and Campbell, we found that our referring physicians were well pleased with the results.

With the return of Kinevac (sincalide for injection; Bracco Diagnostics, Inc.), however, we have resumed doing our gallbladder stimulation studies using a 30-min infusion of Kinevac (0.02 μg/kg) and a 40-min dynamic acquisition. There continues to be more literature support for the use of Kinevac than for fatty-meal stimulation, and since many of our studies are done after hours, the overall time required for completion of the study is lessened. Perhaps the next time Kinevac is no longer available, our whipping cream may be served on a scoop of premium ice cream.

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