reduction method of Mu-9-IgG, an antibody against a colorectal cancer mucin antigen (*10*), and subjected it to cysteine challenge as described by the authors. At 64 m*M* cysteine, 75% of ^{99m}Tc was removed from the antibody after 1 hr. At 0.64 m*M* cysteine, the amount of ^{99m}Tc remaining bound to antibody was 88%, 84%, 81% and 80% at 1, 2, 3 and 4 hr postincubation, respectively. At the 0.64 m*M* level, ^{99m}Tc-Mu-9 is exhibiting similar resistance to cysteine challenge as the indirectly labeled C110 Mab. The chosen level of cysteine used in experiments was based on the high level of cysteine in tissues (*1*). However, inspection of papers cited by Hnatowich et al. (*1*) shows that plasma levels of cysteine and glutathione are in fact much lower than the authors used in challenge experiments. Plasma level of free cysteine is about 0.01 m*M* (*11*) and glutathione is a little higher, at 0.025 m*M*, while intracellular levels are much higher (*12*). Therefore, relevance of the 0.64 and 64 m*M* cysteine challenge experiments to plasma conditions is questionable.

Also, in the results section of the paper it impressed us that for SDS-PAGE only indirectly labeled material was run under "non-reducing" conditions, which makes results somewhat ambiguous. With gel results it would be interesting to know how many free thiol groups were present on directly labeled antibody, in order to gauge the extent of reduction during pretinning. Indirectly labeled Mabs show extensive aggregation according to SDS-PAGE, both by autoradiography and optical density, which is not shown by HPLC. The HPLC system in use engendered collection of 0.35-ml samples and gave a separation of four samples (1.4 ml) between species of MW 150,000 and species >300,000 dalton. For indirectly labeled injectate, the presence of significant levels of aggregate masked by the HPLC system's limited resolving power but indicated by SDS-PAGE, cannot be ruled out. Further suspicion of aggregation is suggested by high 24-hr liver uptake of both indirectly labeled antibodies and by serum, liver and kidney samples run on HPLC at 2.5 hr postinjection, in which both antibodies show distinctly higher MW shoulder. The indirectly labeled B72.3 Mab at 2.5 hr showed 27% ID/g in liver compared to 10% ID/g for corresponding C110 Mab, which may raise particular suspicion about the B72.3 radiolabeled antibody's integrity. Both indirectly labeled antibodies seemingly exhibit much higher liver uptake than ^{111}In-C110 conjugate. It is unfortunate that uptake of radioactivity in the gastrointestinal tract was not reported. If aggregates are present in indirectly labeled Mabs, they compromise the whole-body clearance study since a significant portion of injectate from indirectly labeled IgGs will quickly localize in the liver.

Taken together, HPLC traces for two B72.3 species (direct and indirect labels) are somewhat more different than those for C110 agents. Indeed, for C110 the serum, urine, liver and kidney traces are quite similar, notwithstanding the very different preparation methods and inherent inconsistencies of animal data. Further, both B72.3-labeled agents targeted to LS174T xenografts despite poor immunoreactivity retention, although tumor uptake of the direct-labeled antibodies was far below our results with the same tumor xenograft (*6*). The 24-hr blood clearance of direct-labeled B72.3 in normal animals is faster than seen with our antibodies (*6*) and faster than direct-labeled C110 antibody, raising concerns about this conjugate's utility in the tumor-localization experiment.

Finally, one must try to consider all possible aspects in designing a clinically useful Mab imaging agent. Replacement of nonspecifically localized activity from kidney to liver when using this chelate in lieu of the direct-labeled agent, will be a disadvantage when imaging the abdomen, particularly in liver metastases. Any such agent will have utility problems similar to ^{111}In-labeled antibodies and is therefore less desirable. We believe that a univalent (Fab') fragment is optimal to minimize HAMA and that the fragment should be labeled to the highest possible specific activity so that a clinical dose of 20–30 mCi of ^{99m}Tc will only require milligram to submilligram doses of Mab. At present, state-of-the-art direct labeling of antibody fragments with ^{99m}Tc has achieved a 5-min quantitative yield requiring no postlabeling purification, is capable of 150 mCi/mg specific activities, and produces virtually no HAMA (*13*). If indirect-labeling technology described in this paper can improve upon this, it will be a laudable achievement.

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Gary L. Griffiths
Hans J. Hansen
Immunomedics Inc.
Morris Plains, New Jersey

David M. Goldenberg
Garden State Cancer Center
at the Center for Molecular Medicine and Immunology
Newark, New Jersey

REPLY: We wish to thank Drs. Griffith, Hansen and Goldenberg for their thoughtful comments regarding our recent contribution to the *Journal* (*1*) and for the opportunity to further discuss this interesting subject. Many issues have been raised that will be addressed in their order of appearance.

Drs. Griffith et al. view pretinning as inferior to their thiol-reduction direct method. Would that they had provided experimental data in support of this view. No conclusions on this matter are possible from our article since we did not compare direct-labeling methods. As such, any properties of pretinning which we

observed, such as decreased immunoreactive fraction, are by themselves useless for purposes of this comparison.

Similarly, the in vitro stability to cysteine challenge of pretinned antibodies was not compared with any other direct-labeling method. However, here Dr. Griffith et al. have repeated our challenge experiment on their Mu-9 IgG antibody labeled with ^{99m}Tc by their thiol-reduction method. Although instability to cysteine was comparable at high cysteine concentration (i.e., 64 m*M*) for their labeled antibody relative to that we described for the pretined antibody, they report that at low concentration (i.e., 0.64 m*M*), their antibody seems to show greater cysteine stability. But as always comparisons are more reliable when performed entirely in one laboratory. Accordingly, we have now compared both the B72.3 and C110 IgG antibodies as to stability of the ^{99m}Tc label to cysteine challenge when attached via antibody reduction by one thiol (mercaptoethanol) and pretinning. We are in agreement with Dr. Griffith et al. in that the thiol-reduced antibodies show greater stability.

Drs. Griffith et al. are mistaken that our conditions of SDS PAGE were different for the directly and indirectly labeled antibodies. Both were run under nonreducing conditions. They are correct that we did not quantitate the extent of −SH generation following antibody reduction. With regard to the high molecular weight species observed in the indirectly labeled antibodies, we did not investigate the causes or effects of these species. We agree that liver levels may have been elevated in the case of animals receiving these indirectly labeled antibodies due to the presence of what may well have been labeled protein aggregates. A comparison of HPLC radiochromatograms of indirectly versus directly labeled antibodies (our Fig. 6) does show in serum a hint of these high molecular weight species. It is also possible that whole-body clearance may have been retarded by RES clearance of these aggregates. However, in our view it is equally possible that clearance may have been accelerated if transchelation to cysteine occurs rapidly within the Kupffer cells.

Nevertheless, the tendency toward aggregate formation in the indirect case is less a disadvantage than it may seen. As stated in our report, since postconjugation labeling was performed, the antibody could have been purified from these high molecular weight species prior to labeling.

We are also able to agree with Dr. Griffith et al. that HPLC traces for the B72.3 antibody do appear to be quantitatively different (direct versus indirect) than tracer for the C110 antibody, although the significance of this observation is unclear. It is possible that lower tumor uptake and more rapid clearance we have observed relative to that reported by our colleagues from CMMI, with the same antibody and tumor model, may simply be due to interlaboratory variations.

Finally Dr. Griffith et al. list the advantages of direct labeling in their hands, such as high specific activities and ease of labeling. We have no comment on these issues as the goals of our study did not include comparing directly and indirectly labeled antibodies in these terms. They have also mentioned higher liver activity levels that may be expected with the more cysteine-stable indirectly labeled antibodies, presumably because of decreased transchelation to cysteine in the liver. Indeed, in our opinion this last point is perhaps the most important, in that it offers another opportunity to caution that increased instability to cysteine is not necessarily disadvantageous as illustrated by the lower levels of ^{99m}Tc in the blood, liver and all other organs (with the exception of kidneys) that may result. As we attempted to emphasis in our article, whether increased instability is a net advantage or disadvantage will depend on whether clearance through this mechanism occurs more extensively in tumor relative to normal tissues. Head-to-head comparison studies in patients will offer the best opportunity to resolve this critical issue.

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D.J. Hnatowich
G. Mardirossian
M. Rusckowski
M. Fogarasi
F. Virzi
P. Winnard, Jr.
University of Massachusetts Medical Center
Worcester, Massachusetts

Gallium-67 Citrate in Liquid Gastric Emptying Scintigraphy

TO THE EDITOR: In a recent study, Bellen and co-workers (*1*) concluded that ^{67}Ga citrate when administered orally was found to have a very high fecal excretion (97.2% of injected dose) and a very low urine excretion. Because of its lack of gastrointestinal absorption, ^{67}Ga citrate may, for convenience, be the agent of choice as a radioactive colonic transit marker.

Scintigraphic solid phase gastric emptying measurements in combination with liquid phase measurements are useful in investigating abnormal gastric-emptying rates associated with many disease states. Technetium-99m stannous colloid mixed with oatmeal or cooked with scrambled eggs provides a simple but effective method for determining solid-phase gastric-emptying rates. For concurrent liquid phase measurements to be performed, it is necessary to use a radiopharmaceutical labeled with a different isotope. Indium-III-DTPA and ^{113m}In-DTPA have previously been used with successful results. However, both radiopharmaceuticals present with problems. First, ^{111}In-DTPA is expensive with limited availability. Second, since October 1992 ^{113m}In generators are no longer available worldwide.

On the basis of results presented by Bellen et al., we investigated use of ^{67}Ga citrate as an alternative radiopharmaceutical for concurrent liquid and solid-phase gastric-emptying studies. The primary concerns in this approach were (1) the possibility of ^{67}Ga citrate binding to the solid phase (in this case radiolabeled scrambled eggs) and (2) the effects of downscatter into the ^{99m}Tc window from the higher-energy ^{67}Ga isotope.

Gallium-67 citrate (10 MBq) was added to 2 beakers of 100 ml 0.14 *M* PBS buffer (pH1.5) and a "spoonful" of scrambled eggs. The mixture was mixed on a magnetic stirrer for 2 hr and 30 min. The two phases where then separated and activity in each phase was counted using a dose calibrator. The experiment was repeated three times. At each time point, 90% of activity was recovered in the liquid phase (mean ± s.e.; 92% ± 0.7).

The downscatter correction ratio was calculated from a control study in which dual acquisition was performed on a patient who had been injected with ^{67}Ga citrate only and scanned over the abdomen. The correction ratio was derived from the number of counts collected in the ^{99m}Tc window (20%, centered on 140 keV)