1. Indium-NTA hydrolysis constant $(In(OH)L)(H^+)/(InL)$ is unknown but must be near 10^{-7}.
2. In(III) and Fe(III) probably form the complex (ML_2) with NTA.

It is stated that in analyzing the data the stability constant of the protein chelated conjugate is assumed to be the same as that of the iron chelate. It is impossible that the chelating agent conjugated to the protein by reactions of its anhydride would have the same metal ion affinity as the free unconjugated ligand, because at least one of its donor groups is lost in the conjugation process. This incorrect assumption is one reason why the results and conclusions will be incorrect since the relative magnitudes of the Fe(III) and In(III) stability constants with different ligands involve fairly uniform ratios (with $K_{Fe} > K_{In}$). Thus, this assumption carries over the finding that the free and bound In(III) will have about the same stability constants (i.e., the incorrect assumption leads directly to the incorrect conclusion).

It should also be noted that it is stated that the conjugates of EDTA, DTPA, and TTHA are chelates; this ignores the fact that In(III) citrate and Fe(III) citrate are chelates and that at various citrate concentrations these complexes will play important roles.

Several other problems exist in the manuscript. As has been discussed by two of us in a text on the determination and use of stability constants (*2*), accurate pH determination is important in the determination of true values of stability constants. Drs. Subramanian and Wolf state that the reaction does not involve hydrogen ions. Hydrolyzed forms (hydroxo complexes) of Fe(III) and In(III) chelates are probably present, whether conjugated or not, and hydrogen ions are most certainly involved. If one examines the curve shown in Figure 8, it is not obvious that equilibrium has in fact been reached. The curve shows a steady change even up to 95 hr. Confirmation of whether equilibrium had been achieved could have been accomplished by preparing the In(III) chelates and subsequent addition of Fe(III) to show that the same equilibrium was achieved no matter what the starting point.

In conclusion, the invalid assumptions and the ignoring of complex species actually present in solution causes one to have doubts on the validity of stability constants obtained by this new radiochemical method of determination of stability constants. It is important that the concerns listed above are taken into account before this method is applied by others.

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REPLY: We wish to thank Drs. Martell, Welch, and Motekaitis for their keen interest in our work (*1*) and for their concern for the readers of the *Journal*. We do, however, disagree with their analysis of our study and with their conclusions.

Our study was an attempt to develop a simple method that would allow the determination of the stability constants of bifunctional chelates of indium coupled to proteins under the very complex conditions present in biologic systems. We recognize that conditions in such systems may have some significant differences with conditions in pure solutions. In response to some of the specific issues raised by Martell et al.:

1. Under the conditions described in our work, e.g., in the presence of chelating agents, indium does not precipitate as $In(OH)_3$. This can be verified experimentally. Various chelating agents can be used to that effect (e.g., acetate, citrate, nitrilotriacetic acid)
2. We made no statement about Fe(III) existing as an aquo ion.
3. The blanket statement that all Fe(III) complexes are several orders of magnitude more stable than In(III) complexes does not agree with our experimental results. Indeed, the values of a prior publication by Martell (*2*) state differently and agreed with our data.
4. Equations 1 and 2 do not make any assumptions on protonated complexes.
5. We are puzzled by Martell et al.'s statement: " ... is unknown but must be near ...". What is the experimental basis for their assumption?
6. While it is true that we used the value of the stability constant of the free Fe-Che as that of HSA-Che-Fe, we did point out that "any differences in the values for the stability constant of the protein-bound and the free chelates may shed light on the modifications of the chelating ability caused by conjugation to protein." Thus, we recognized, and stated the limitations that Martell et al. suggested we had ignored. We had not.

Based on the work of Anderegg et al. (*3*) and Schwarzenbach et al. (*4*), who had studied the effect of the H^+ on metal chelates, specially of Fe(III), and used correction factors, it would appear that such correction factors would not affect the value of "K" as calculated in our work, under the conditions we described and used.

It is also of interest that steric factors appear to affect the pH at which hydrogen or hydroxylated chelates may be formed, as Bond et al. (*5*) had reported.

Martell et al. have also assumed that the Fe(III)NTA complex would hydrolyze and dimerize. This is contrary to the findings of Bates et al. (*6*), who reported, in studies of Fe transfer to transferrin, that Fe-NTA is an active species that did not polymerize.

Martell et al. also suggest that at various citrate concentrations, this ligand will play important roles. They apparently failed to notice, on page 482, that the " ... mixture was dialyzed against water for 5 days at 4°C to remove any *unreacted* ^{114m}In-In-citrate." Thus, we have no citrate present in our studies.

In conclusion, we believe that our method, which is simple and can be readily performed in any laboratory, will provide reliable information that may be valuable in assessing the

degree of stability of indium complexes conjugated with various proteins. While we recognize that much more elaborate and complex methods can be used in pure solutions, our work describes a method applicable to the conditions normally encountered in nuclear medicine and radiopharmaceutical studies. We stand by our method, its simplicity, and by our results.

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Thymic Uptake of Gallium-67 Citrate: Adult Versus Pediatric Patients

TO THE EDITOR In the February 1990 issue of *The Journal of Nuclear Medicine*, Rossleigh et al. pointed out that thymic uptake of radiogallium post-therapy is most likely a normal finding in the pediatric age population (*1*). In that article, they commented that "we disagree with Tumeh et al., who stated that physiologic thymic regeneration does not accumulate gallium ... (*2*)." Our actual statement in that article was, "It seems, therefore, that although thymic hyperplasia could potentially give rise to a false-positive CT scan, this event is much less likely to occur on gallium scans, particularly in the adult population."

Indeed, to our knowledge, we have not encountered a single case of gallium-positive, chemotherapy-induced thymic hyperplasia in an adult. This includes patient populations at both the Brigham and Women's Hospital and the Dana-Farber Cancer Institute. We, therefore, concur with Rossleigh's observations if they are restricted to the pediatric population, but stand by our initial comments and underscore that in an adult population new post-therapy radiogallium uptake in the anterior mediastinum must be considered to be malignant in origin until proven otherwise.

Additionally, we would also ask the rationale behind performing 24-hr planar and 48-hr SPECT gallium imaging. Are the authors recommending these as the optimal time points for gallium data collection?

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REPLY: Thank you for the opportunity of replying to Drs. Tumeh and Kaplan's letter. The conclusion from our work that thymic uptake of radiogallium post chemotherapy is most likely a normal finding is based on our assessment of a pediatric population only. We are unable to make a comment on an adult population on the basis of our research. Peylan-Ramu et al. have confirmed our findings in a recent publication (*1*). They observed increased radiogallium uptake in the mediastinum after completion of chemotherapy in 10 of 62 patients with non-Hodgkin's lymphoma. All 10 patients were under 15 yr of age. All were asymptomatic, none were biopsied, and all remained well with a mean follow-up of 52.5 mo.

At the time when our study was undertaken, we would perform anterior and posterior whole-body studies at 24 hr and 48 hr postinjection. The 24-hr study provided us with a preview of probable abnormalities, which are better visualized at 48 hr, as Dr. Kaplan and others have also found to be the optimal time for gallium imaging for tumor. This preview did enable us to determine if a SPECT study was required at 48 hr and, thus, facilitate scheduling of the tomographic study.

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Inflammation Imaging

TO THE EDITOR: We would like to take issue with Dr. Rubin's editorial in the March 1990 issue of *The Journal of Nuclear Medicine (1)*. He describes six clinical settings where sepsis may be present. In all of them, significant derangements of the native anatomy and/or immunophysiology is present. In addition, the patients frequently are receiving broad spectrum coverage with antimicrobials. Several nuclear medicine approaches have successfully been used in these situations. Labeled white blood cells and gallium have widespread accepted use. As he correctly points out, the delay of 24–48 hr from injection to image is acceptable in these relatively indolent infections. He then offers the "hot" appendix as a paradigm for evaluation of nuclear medicine techniques where promptness of diagnosis is essential. His editorial refers to the Mountford et al. article in the same issue (*2*) as evidence that