in high quantities. Results of the AA incubation and its elimination experiments obtained by Hnatowich et al. appear only to be consistent with their findings.

In their dithionite experiment, not only did Hnatowich et al. use 5 times the recommended concentration, but, unlike our experimental conditions (pH 7.8–9) also used a low pH for incubation media which we measured to be 5.5–6. There is ample evidence in the literature (*8,9*) that dithionite quickly decomposes and forms a colloid that may be hard to detect at a low pH. Having no dithionite left to reduce protein disulfides, the reduced ^{99m}Tc will readily bind to the large quantity of colloid produced. This may not be separated from the protein, either by paper or gel chromatography and may be easily misunderstood as the radioactivity bound to the protein. The authors have neither performed HPLC nor have they confirmed that the radioactivity was bound to cysteine residues and not to other functional groups of the protein.

We realize that like the evidence provided by Mather et al. (*6*) and Pimm et al. (*7*) for 2-ME, and as discussed in various ways by Eckelman and Steigman (*10*) for other reducing agents, our testimony that AA reduces protein disulfides and not the dithionite is only indirect. However, additional independent support exists in the literature that AA-treated protein can be efficiently labeled with ^{99m}Tc by a transchelation technique in which no dithionite is involved (*11*).

Three separate clinical trials were performed in a series of patients in which two antibodies and one peptide labeled with ^{99m}Tc by the AA technique (*2,3*) were evaluated. These trials gave highly encouraging results (*12–14*) and the preparations appeared to be stable as indicated by in vivo distribution 3–4 hr postadministration.

Encouraged by these data, we performed additional experiments that are also indirect but more elaborate than just the paper and gel chromatography and, we believe, more convincing. We have shown that when dithionite-reduced ^{99m}Tc is added to 100–500 μg/ml of protein not treated with AA (final pH 7.8–9), only approximately 20% of the radioactivity binds to protein (*2*). When the Σ-amino groups on the unreduced protein are first blocked with fluorescein/isothiocyanate (FITC) and the protein is then incubated with dithionite-reduced ^{99m}Tc, this binding is significantly reduced. When protein was reduced with AA, amino groups were blocked with FITC and incubated with dithionite-reduced ^{99m}Tc, approximately 80% of the activity was bound to the protein. When protein was treated with AA and the sulfhydryls generated were subsequently blocked with iodoacetate (the excess of which was eliminated) and further treated with dithionite-reduced ^{99m}Tc, only about 15%–20% radioactivity was bound to protein. This is equivalent to nonspecific binding to Σ-amino groups. When protein is reduced with AA and none of the groups are blocked, labeling efficiency is 90%–95% (*15,16*).

These experiments indicate that when protein is not treated with AA but incubated with dithionite-reduced ^{99m}Tc (at pH 7.8–9) only approximately 20% of the activity is associated with the protein, most of which is bound to Σ-amino groups. These experiments, together with our spectrophotometric assay (*4*), show that the AA treatment of the protein generates cysteine residues to which approximately 80% of reduced ^{99m}Tc binds. We believe that these functional group blocking experiments provide additional evidence that it is AA that plays a major role in disulfide reduction and not dithionite. Our data further indicate that such nonspecific binding also occurs in some bifunctional chelating agent methods and that the nonspecific binding can be eliminated by FITC blocking (*15*). Thus, with our several years of experience with this method and with published and unpublished data generated by several such definitive experiments, we remain confident that under our experimental conditions, AA plays the major role in the generation of cysteine residues in protein molecules.

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REPLY: Regarding our recent article in the *Journal* (*1*), it may be worthwhile to stress again that we had no difficulty in radiolabeling two antibodies (Sandoglobulin IgG and B72.3 IgG) following the ascorbate/dithionite method as described (*2*), although it was necessary to increase the dithionite concentration by a factor of five. The issue is therefore not whether the labeling method works but rather the role that ascorbate plays in the process and, in particular, whether ascorbate is capable of reducing antibody disulfides. Furthermore, our conclusions were based on only two antibodies and should not be generalized to others.

We were aware that Ellman's reagent is slowly reduced by ascorbate and, therefore, unless precautions are taken, an erroneously *high* reading for antibody thiols may result. In our inves-

tigation, we were unable to detect thiols using Ellman's reagent on a Sandoglobulin IgG purified from ascorbate after treatment. Although the measurement was performed immediately after purification, a possibility exists that the absence of detectable thiols was due to the reformation of antibody disulfides once ascorbate was removed. Therefore, we also measured thiol concentrations without the ascorbate purification by measuring the absorbance at a fixed time after the addition of ascorbate to the antibody and to the cysteine standards. Once again, we were unable to detect thiols. Furthermore, we also used dithiodipyridine as a thiol indicator in place of Ellman's since the former is less sensitive to interference by ascorbate. Again, we were unable to detect thiols in samples of Sandoglobulin IgG treated with ascorbate and even without ascorbate purification. By contrast, we did observe a definite thiol signal in each study in which dithionite was present. Although these are difficult measurements and prone to experimental error, our results have the plausibility of consistency: in each case, a thiol signal was detected with dithionite but not ascorbate. We concluded that treatment of this antibody with ascorbate at a tenfold higher molar ratio to that recommended could not have generated more than about 1%–2% of the possible thiols (the upper limit on the assays), whereas dithionite treatment at fivefold higher concentrations did generate about 1%–5% of the possible thiols. On this basis, we feel that dithionite played a more important role in antibody reduction than did ascorbate.

Our experimental protocols were reproduced as faithfully as possible to those reported and when it was necessary to make changes, they were carefully described. For example, we were unable to achieve good labeling efficiencies unless the dithionite concentration was increased by a factor of five. We went up to a 35,000:1 ascorbate-to-antibody molar ratio (i.e., an increase by a factor of ten) for the thiol measurements to increase the concentration of thiols generated. We prepared ascorbate solution at pH 6 by adding ascorbic acid to solutions of ascorbate rather than the reverse and the dithionite was freshly prepared in nitrogen-purged 0.05 *M* carbonate buffer at pH 11.5 rather than bicarbonate buffer at pH 11.

The pH at a reduction of 5.5–6 measured by Thakur for our conditions contrasts with the pH value of 7.8–9 used in his work and was suggested as possibly generating artifact in our radiolabelings (*3*). Presumably this low pH was a result of adding a tenfold excess of ascorbate. However, we added the excess ascorbate primarily for the thiol measurements; most of the radiolabeling was performed with the lower ascorbate-to-antibody molar ratios. More importantly, elimination of the ascorbate entirely would have maintained the acidity and we observed no difference in labeling efficiency with or without ascorbate.

The yet unpublished studies with FITC and iodoacetate treatment of antibodies are interesting and may shed some light on the mechanism of the ascorbate/dithioinite labeling method (*3*). However, the issue of whether ascorbate is capable of disulfide reduction of antibodies can only be addressed by assaying for thiols after antibody treatment with ascorbate. These studies should be expanded. Meanwhile, useful information on the role of ascorbate could result simply from multiple labeling studies on a variety of different antibodies with and without ascorbate. It is possible that under certain circumstances the role of ascorbate is to stabilize reduced ^{99m}Tc and prevent colloid formation, as suggested by Thakur (*3*).

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May *Randoms* Evolve into *Scatter*?

TO THE EDITOR: We read with great interest the recent editorial "Something Borrowed, Something Blue" in the January issue of the *Journal* (*1*). However, we were somewhat bewildered when we learned about the intention that "*Randoms* will evolve into *Scatter* to denote a more generic and fundamental phenomenon . . . ." In our opinion, there must be a profound misunderstanding.

We fully agree that scatter is a basic physical phenomenon and "tends to degrade information unless dealt with appropriately." Compton scattering is the predominant interaction mechanism of gamma-quanta from radionuclides with body tissues in nuclear medicine. Most of the scattered photons can be suppressed by using an appropriate energy window, although in practice this may not be sufficient due to the limited energy resolution of the radiation detection devices. Thus, additional techniques to "correct" the measured data for scattered events are mandatory for accurate quantitation of local radiotracer concentrations.

Randoms, or more explicitly, random *coincidences*, on the other hand, are intimately connected to coincidence detection techniques as commonly used in PET. The terms random, chance or accidental coincidences simply denote the fact that two (or more) spatially and temporally independent events have been registered within the resolution time of the coincidence unit. These random coincidences increase rapidly with counting rate, as there is an increasing probability that unrelated events will accidentally produce overlapping timing signals. In principle, random coincidences cannot be distinguished from truly coincident events and will occur even in the absence of true coincidences. The contribution of the randoms to the recorded total coincidence rate, however, can be taken into account on a statistical basis either directly by an additional measurement with an appropriate time delay inserted in either branch of the coincidence unit or, in the case of a twofold coincidence system, by estimating the random coincidence rate from the uncorrelated *singles* input rates and the resolving time of the coincidence device (*2*).

In conclusion, both scatter and randoms do degrade information in PET. In any quantitative study, each of them must carefully be taken into account. The origin of both phenomena, however, is different (fundamental physical process versus coincidence counting technique) and should not be mixed up.