LETTERS TO THE EDITOR

Re: Tc-99m Antibodies and Staphylococcal Endocarditis

I have read with much satisfaction the interesting article by Wong, Dhawan, Tanaka, Mishkin, and Reese (*1*), an experimental study on the imaging of infectious staphylococcal endocarditis in rabbits by means of Tc-99m complexes with antibodies, produced in a rabbit, against *Staphylococcus aureus*. The antibodies had not been purified by any separation based on an antibody-antigen reaction. Since a visualization of the area of the endocarditis was obtained, the authors propose that patients may serve as their own source for antibodies for radioimmunodetection, after in vitro chemical binding of radiotracer to the unpurified immunoglobulin fraction of their own blood.

As far as I can see, the authors have not taken into account the very strong affinity between the Fc portions of the IgG molecules and protein A, an antigen very abundant in the cell wall of most strains of *S. aureus* (*2*). Thus, I am not sure that the visualization of the area of the endocarditis is caused by an immunological antigen-antibody reaction with Tc-99m antibodies, since it may also be explained by a nonspecific chemical binding of the Fc portions of the Tc-99m antibodies to protein A.

The method described making Tc-99m antibodies an intermediate step where Tc-99m, reduced by $SnCl_2$, is neutralized to pH = 7.4 by sodium citrate and NaOH, before addition of antibodies. This may allow formation of Tc-99m(Sn)radiocolloids, and the observed complexing of Tc-99m to the antibodies may be in the form of Tc-99m-Sn aggregates rather than the "pure" reduced Tc-99m. It would be reassuring, therefore, if the authors could demonstrate a persisting immunological affinity of the Tc-99m antibodies, not merely of the bulk of untagged antibodies.

ERLING SUNDREHAGEN
The Central Hospital of Nordland
Norway

REFERENCES

1. WONG DW, DHAWAN VK, TANAKA T, et al: Imaging Endocarditis with Tc-99m-labeled antibody—An experimental study: Concise communication. *J Nucl Med* 23:229-234, 1982
2. GOUDSWAARD J, VAN DER DOUK JA, NOORDZIJ A, et al: Protein A reactivity of various mammalian immunoglobulins. *Scand J Immunol* 8:21-28, 1978

Reply

Dr. Sundrehagen is correct in pointing out that there is chemical binding of the Fc fragment of normal gamma globulin with *Staph. aureus* protein A. However, we strongly favor the hypothesis that visualization of the area of endocarditis in our experimental rabbit model was due to an immunologic reaction between Tc-99m-labeled anti-*Staph. aureus* antibody(Tc-99m AB) and the tissue, based on the following observations:

1. Imaging experiments with Tc-99m-labeled gamma globulin(Tc-99m GG) from unimmunized rabbit serum showed very little activity in the infected aortic valve. Based on percent injected dose/g tissue, the ratios of the infected valve plus vegetation to normal tricuspid valve, to the myocardium, and to the blood averaged less than 2:1—far below the observed ratios of 20-118:1 reported for Tc-99m AB.

2. No antibody titer was obtained for Tc-99m GG by tube agglutination assay. Instead, precipitate was observed in each sample up to 1:6400 dilution. The amount of precipitate present in each sample decreased with successive dilution. This was confirmed by assaying the radioactive precipitate following centrifugation and separation. In contrast, a definite antibody titer was obtained for Tc-99m AB. Precipitate was visible until the titer reached 1:800 and 1:1600, and none was seen thereafter. The antibody titer of Tc-99m AB was identical to that of unlabeled antibody.

For the following reasons we believe that the binding of Tc-99m to protein ligand presumably involves the reaction of a species of soluble Tc-99m(Sn)citrate complex with the protein molecule, rather than formation of a Tc-99m(SN)protein aggregate: 1. Radioprotein aggregates will not migrate upon electrophoresis; they remain at the origin of the electrophoretic plate. On the other hand, our labeled proteins such as HSA, fibrinogen, gamma globulin, etc. do migrate corresponding to the unlabeled protein fractions. 2. The final labeled product is clear and free of any colloidal particles under microscopic examination. 3. Ultrafiltration experiments with 0.22-μm Millipore filters on Tc-99m GG(Human) and Tc-99m HSA, labeled by our chemical method at pH 7.4, showed 17-20% of the radioprotein retained in the filter. This is comparable to the 15% retention rate for Tc-99m HSA labeled by a conventional kit method. In the same experiment, 99.4% of Tc-99m sulfur colloid activity remained on the filter. We conclude that, unlike NaOH, addition of sodium citrate/NaOH solution to a Sn-reduced Tc-99m solution will not cause formation of Tc-99m(Sn)colloids. This can be confirmed by visual observation of the solution after the addition of these reagents. When 0.1 *N* NaOH is added to acidic Sn-Tc-99m solution, a cloudy colloidal suspension is formed. This does not occur with addition of sodium citrate/NaOH solution.

We appreciate Dr. Sundrehagen's calling these interesting points to our attention.

DENNIS W. WONG
VINOD K. DHAWAN
Martin Luther King Hospital
Los Angeles, California

Potential Interfering Antibody Response In TSH Assays

In 1979 a patient was referred to us for thyroid evaluation. The free thyroxine index (FTI) was normal at 2.6 (normal range: 1.4-4.0) and the microsomal and thyroglobulin antibodies were negative; however, the serum thyroid-stimulating hormone (TSH) level* was elevated at 15.4 μIU/ml (normal < 8 μIU/ml). The