Reply

Our paper (*1*) described a new method of labeling proteins with technetium-99m via a covalently linked bifunctional chelating agent, DTPA. Although it was not our intent to label free DTPA, we have tested the dithionite reduction method to label free DTPA with Tc-99m. When DTPA was present in the reaction mixture at the onset of reduction, 50% of the Tc-99m activity was associated with DTPA, as judged by TLC developed in saline. In an acetone/TLC system, 95% of the Tc-99m activity is at the origin. When DTPA was added to the reduced Tc-99m after 10 min of dithionite reduction, only 20% of the activity was associated with DTPA (at the solvent front in a TLC/saline system). In the acetone solvent, 95% of the Tc-99m activity was recovered at the origin. Vilcek et al. (*2*) also reported successful chelation of dithionite-reduced Tc-99m with DTPA. Jones et al. (*3*) reported that dithionite at high pH gave quantitative yields of the reduced Tc-99m that formed the required technetium complexes.

We have compared labeling efficiencies of DTPA-coupled proteins and unmodified proteins, although this was not reported in our paper (*1*). Technetium labeling of DTPA-protein complexes consistently yielded higher specific activities. Recently specific activities of 100 mCi/mg have been consistently obtained with DTPA-antimyosin Fab. Using unmodified proteins, by contrast, only 10–20% of the specific activities of Tc-99m-DTPA-proteins were obtained. Purified Tc-99m-DTPA-antimyosin Fab showed 15–20% of the injected dose still in the circulation at 24 hr after i.v. injection. However, we have not determined the biodistribution of directly labeled Tc-99m antimyosin Fab. We previously reported (*4*) that dual-labeled DTPA-antimyosin (I-125 and Tc-99m) injected into mice showed a percent Tc-99m liver activity greater than the percent I-125 liver activity. If all of I-125 and Tc-99m activities were labeled to antimyosin Fab, percent Tc-99m and percent I-125 activities should be the same, but the presence of greater Tc-99m liver activity in this study indicated that the radiolabeled antimyosin preparation contained an added Tc-99m contaminant. Table 1 shows the biodistribution of highly purified Tc-99m-DTPA-antimyosin Fab and I-125-DTPA-antimyosin Fab in mice at 1 hr after i.v. injection.

Concerning the in vivo stability of the Tc-99m-labeled fibrinogen, we now have both experimental and clinical data showing that the biological activity is retained over 24 hr. An average of 85% coagulability of Tc-99m-DTPA-fibrinogen was obtained from blood samples taken at various times up to 24 hr. No thyroid or gut activities were observed by whole-body scintigraphy. Similarly, Tc-99m-DTPA-antimyosin Fab has been used clinically, and myocardial infarct visualization was feasible as early as 10 min after intracoronary injection or as early as 6–7 hr after intravenous injection.

The above data suggest that the labeling of proteins with dithionite-reduced Tc-99m via the use of DTPA appears to provide a stable radiolabeled product.

TABLE 1. BIODISTRIBUTION OF Tc-99m-DTPA-ANTIMYOSIN-FAB AND I-125-DTPA-ANTIMYOSIN-FAB IN MICE AT 1 HR

Sample	Percent Tc-99m dose/organ Percent I-125 dose/organ* (n = 8)	(n = 6)
Blood	39.0 ± 6.8	30.4 ± 2.4
Heart	0.6 ± 0.1	0.5 ± 0.1
Spleen	0.4 ± 0.1	0.5 ± 0.04
Liver	12.5 ± 1.3	9.1 ± 1.9

* Means ± s.d.

The biodistribution of Tc-99m-DTPA-fibrinogen was provided in the paper.

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On Behalf of I-123 Fatty Acids for Myocardial Metabolic Imaging

In your issue for July, 1982 the teaching editorial on myocardial metabolic imaging (*1*) rightly recognizes the important role of positron emission tomography (PET) for in-vivo analysis of myocardial metabolism. The strength of this approach lies in the quantification of substrate utilization in small organ volumes. Nevertheless, rate constants for metabolic reactions are of comparable importance and at times describe a metabolic situation with greater relevance than substrate utilization. The measurement of rate constants is open to biplanar scintigraphy and single-photon tomography, and thus is of wide clinical practical interest. It particularly appeals to the many users who have no access to PET. Promising clinical studies have been done with 17-[^{123}I]heptadecanoic acid in the entire left-ventricular myocardium and in selected wall regions (*2–7*). Criticism has arisen about the validity of the technique, citing the possibility of altered kinetics relative to the natural substrate. This includes the concern about limiting diffusion and intracellular retention of free I-123, which then would dominate the washout curves (*8*).

Evidence obtained on this matter in small animals probably has little bearing on the data measured in man. The half-time of the first component of the tracer clearance in mice is in the range of about 1 min (*9*), whereas it is 10 to 25 min in man by various calculations (*2–7*); yet in all mammalian species intercapillary distance and the morphology of the muscle cells are very similar. Published data show that the terminally iodinated long-chain saturated fatty acids have kinetics very similar to those of the natural substrate, i.e., C-11-labeled palmitic acid (*2,6,9,10*). This is observed not only with the normal myocardium but also in variations due to disease. Moreover, metabolic intervention by glucose and insulin provokes similar responses with both C-11

palmitic acid and I-123 heptadecanoic acid (*6,10*). Recently we also observed a change in kinetics of I-123 heptadecanoic acid in normal man following acute ethanol administration. This signals a change of myocardial metabolism induced by ethanol. Moreover, chronic administration of ethanol in mice was found by us to cause changes of hepatic metabolism of I-123-labeled fatty acids similar to those occurring with C-14-labeled fatty acids.

We therefore emphasize that myocardial metabolism can be evaluated in terms of rate constants also by 17-[^{123}I]heptadecanoic acid—and possibly other fatty acids—as well as by PET. Since the process of beta-oxidation is rapid, the measured washout curves in man either with C-11 palmitic or 17-[^{123}I]heptadecanoic acid are taken to relate to the release of fatty acid from intracellular pools into the beta-oxidative pathway.

For the above reasons we believe that current developments in metabolic imaging with fatty acids should clearly include the new single-photon approach and measurement with I-123-labeled fatty acids.

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Reply

Certainly analogs of metabolic substrates labeled with single-photon emitters are of research interest, as indicated by Drs. Feinendegen and Shreeve. However, iodinated compounds can be used to study metabolism only in the same way that thallium-201 is useful for evaluating myocardial perfusion, i.e., as indirect indices of metabolic behavior rather than direct measurements. In addition, a major limitation of single-photon emitters is that these tracers provide only qualitative information because of image degradation due to overlapping anatomic structures, uncorrectable photon attenuation, background scatter, and lack of depth resolution, all of which prevent quantitative data from being obtained (*1*). It is of course possible to take regional data and process them to improve image quality visually, but such manipulations when applied to thallium scintigraphy, for example, make only modest improvements in the diagnostic capability of the test for coronary disease; they do not make the data suitable for quantitation of regional myocardial blood flow (ml/min-g) (*2,3*) or metabolism. Similarly, differences in the clearance rates of iodinated fatty acids can be obtained from normal and ischemic areas (*4*). Unfortunately, the physical limitations of the tracer preclude an accurate determination of the regional metabolic rate (mmole substrate/g-min) and provide only an index of comparison that may be affected by nonspecific conditions.

The intent of my editorial was not to slight the potential use of iodinated tracers. I chose to discuss positron emitters because these agents are better suited for direct quantitation of metabolic imaging. As noted in my editorial, carbon, oxygen, and nitrogen all have positron-emitting isotopes that can be incorporated into analogs that behave as physiologic substrates (*5*). The high energies and the use of electronic collimation allow measurement of a true regional metabolic rate. The major limitation of positron imaging is the relative scarcity of cyclotrons and tomographic cameras, as pointed out by Drs. Feinendegen and Shreeve. However, I believe it is necessary to understand these metabolic processes in vivo in man and to prove their applicability conceptually by optimal technology rather than to rely on the indirect assessment of metabolic function provided by iodinated tracers.

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