study, only four intact dogs were represented in the interval 0-1 in Fig. 3, whereas the other 12 intact dogs were depicted in the interval 2-4. In the interval 0-1, the predicted curve had a higher rate of change with fewer sample points. Without more sampling, it might be as easy to describe the data by a simple linear relation as by a multiexponential, compartmental model.

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REFERENCE

1. SAGAR VV, PICCONE JM, CHARKES ND: Studies of skeletal tracer kinetics. III. Tc-99m(Sn)methylenediphosphonate uptake in the canine tibia as a function of blood flow. *J Nucl Med* 20: 1257-1261, 1979

Reply

Dr. Allhands says he has seen better fits of data to predicted curves, and so have we. But as we clearly stated in the lead paragraph of our article, our purpose was only to see whether the experimental data were consistent with the theory at all, and it is obvious that they are. We were extremely careful to point out in the paper, and in other papers in this series, that the solutions are not unique and that others exist, even possibly better ones.

As we stated, the predicted curve of relative tibial uptake of Tc-99m MDP against relative femoral arterial blood flow was generated from studies done in four normal beagle dogs. We have since carried out this analysis in eight additional dogs, with extreme care to the details of blood collection. (The technique will be published in the next paper in this series (*3*)). Blood volume was estimated by taking the weighted average of all 17 published studies in 428 dogs collected in the FASEB handbook, *Blood and Other Body Fluids* (*1*). The curve of relative uptake against relative flow is shown in Fig. 1. In comparison with the curve published in our paper, it is evident that the data points now cluster even more closely about the predicted values than they did in the study with the first four dogs and, in fact, the squared deviations are less, i.e., the fit is better. Thus the experimental data support the theory even better than they originally did. The intercompartmental rate constants that we obtained in the normal dogs, in terms of fractional transfer per minute, were: k_{12} 0.400, k_{21} 0.117, k_{14} 0.284, k_{41} 0.590, k_{23} 0.033, k_{32} 0.840, k_{01} 0.014—based on the compartmental model that was used and referenced.

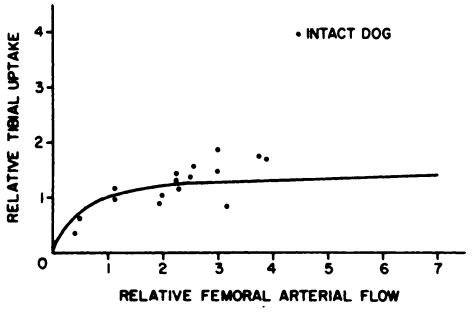

FIG. 1. Solid line is predicted curve of relative Tc-99m MDP uptake (experimental-to-control tibia) vs. relative femoral arterial flow, in dogs (see text). For comparison, solid circles are measured data points in 16 dogs. Flow was measured with electromagnetic flowmeter. There is a good fit of data to predicted curve.

Our approach in these matters is standard engineering practice, and it is therefore no wonder that we came up with highly relevant information. Our object in these studies has always been elucidation of knowledge, not curve fitting, and our methods have been explained in detail (*3*). They are the same as those used to put the astronauts on the moon. While there is still room for improvement in our data fit to the theoretical curve, we make no apologies for what we have done.

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REFERENCES

1. DITTMER DS, ed: *Blood and Other Body Fluids*. Bethesda, Federation American Societies of Experimental Biology, 1961, pp 3-4.
2. MAKLER PT, JR, CHARKES ND: Studies of skeletal tracer kinetics. IV. Optimum time delay for Tc-99m(Sn)methylenediphosphonate bone imaging. *J Nucl Med* 21: 641-645, 1980
3. CHARKES ND: Skeletal blood flow: Implications for bone scan interpretation. *J Nucl Med* 21: 91-98,1980

Re: Improved Protein Labeling by Stannous Tartrate Reduction of Pertechnetate

In the article by Pettit et al. (*1*), there is either a miscalculation or a typographical error in Table 1. The specific activity (column 4) is denoted in "mCi/μg," which is not possible. The higher specific activity attaintable, if the reaction was quantitative for Tc-99m human serum albumin (HSA) (line 1), is 2.3 mCi/100 μg to 0.023 mCi/μg. Assuming the unit for specific activity is a typographical error and should have been "μCi/μg," if one calculates the percent Tc-99m incorporated into HSA from the authors' statements and the data on lines 1, 2, 3, and 4 (using small amounts of HSA, 100 μg), the labeling efficiencies appear to be 13.7, 39, 14.4, and 18%, respectively. As an example, using the data from line 1 and the authors' statement that "This method of column preparation provided approximately 90% recovery of small amounts of protein," one calculates:

$$\frac{(0.9)\ (100\ \mu g\ HSA)\ (3.5\ \mu Ci/\mu g\ HSA)\ (100)}{(2300\ \mu Ci)} = 13.7\%$$

Thus, the statement, "Recoveries were typically 20-60% of the activity applied to the small column . . . " needs to be clarified, since the observed values in three of four experiments of recoverable labeled HSA average 25% *below* the typical lower limit (20%) stated in the article.

The authors state that ". . . aliquots of the labeled protein were analyzed by Sephacryl S-200 chromatography immediately following preparation and again at 4 hr and 20 hr later." Yet they show only one analytical elution curve for HSA (Fig. 1), and do not designate whether the data were obtained immediately after preparation, at 4 hr, or at 20 hr. It would have been helpful to review the three elution patterns along with the percent Tc-99m eluted in each case relative to the quantity of Tc-99m HSA applied to the column. The only statement made was that the elutions contained 80-100% recoveries of the protein fraction from the small column.

Referring to Table 1 and the authors' statement that "Recoveries . . . were inversely related to the amount of pertechnetate used

initially," it appears that either the data or the statement are contradictory. Compare the data, lines 2 and 3, which show that 3.0 mCi and 1.5 mCi Tc-99mO_4 were used in the reactions, respectively. Using 3.0 mCi, recovery was 39% Tc-99m HSA and using 1.5 mCi, the recovery was 14.4% (calculation method shown above). This certainly appears to be a direct relationship and not an inverse relationship relative to the quantity of Tc-99mO_4 used initially. Or perhaps I am interpreting the data incorrectly?

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REFERENCE

1. PETTIT WA, DELAND FH, BENNETT SJ, et al: Improved protein labeling by stannous tartrate reduction of pertechnetate. *J Nucl Med* 21:59-62, 1980

Reply

Dr. Stern is correct that Table 1 (*1*) contains a typographical error in the column headed "Specific activity" and should read "μCi/μg."

As Dr. Stern has also pointed out there are apparent discrepancies in the percent incorporation of starting activity (i.e., $^{99m}TcO_4^-$) into protein. The activity used represents the amount of activity drawn and calibrated by the clinical staff for our use. Preparation for and actual labeling often required 2-3 hr before a final reading of the activity associated with protein was obtained and the omission of a decay correction factor is an oversight on our part. In most cases percent incorporation was determined by counting the 0.5 ml fractions obtained from the small column as well as the column itself and summing these readings. The activity in fractions 7-13 (containing the labeled protein) was summed and divided by the total activity (total fractions plus column) to obtain percent incorporation. We would like to clarify that not all labeling results fell within the 20-60% range, but typically this could be expected.

The data presented in Table 1 were selected for potential clinical utility. Results for labeling were variable at all levels of activity. Unfortunately we have not been able to define or control these factors that produce the variability in the labeling results, especially when activity in the mCi range was used. A number of labelings were carried out using 100-500 μCi. The results of these studies, although not included in Table 1, prompted our statement regarding the inverse relationship between the amount of activity used for labeling and the amount incorporated. Again this relationship was not a precise mathematical one but rather a general trend.

The Sephacryl S-200 column assay used has been described elsewhere (*2*) and was routinely performed within 0.5 hr following the labeling procedure. Exceptions to this routine assay were related to binding and stability studies: (a) In order to detect transfer of the radionuclide from one protein to another, IgG was labeled and mixed with a large excess of HSA and allowed to stand for 1 hr before the assay. These results were given in Fig. 2. Figure 1 illustrated a routine assay of our labeled HSA. (b) The serial assays at 0, 4, and 20 hr were performed to estimate the rate of loss of the radionuclide from the protein. Although these results were not depicted by a figure in our paper, a copy of the results can be obtained by writing the senior author.

We thank Dr. Stern for his comments regarding our data and hope that this letter provides sufficient clarification of our results and their interpretation.

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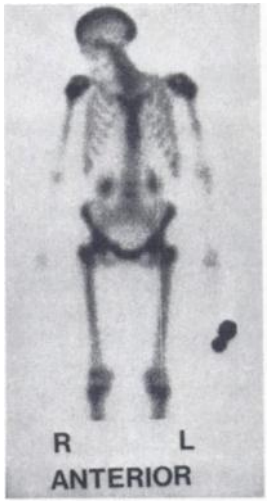
REFERENCE

1. PETTIT WA, DELAND FH, BENNETT SJ, et al: Improved protein labeling by stannous tartrate reduction of pertechnetate. *J Nucl Med* 21:59-62, 1980
2. PETTIT WA, DELAND FH, PEPPER GH, et al: Characterization of tin-technetium colloid in technetium-labeled aluminum preparations. *J Nucl Med* 19:387-392, 1978

Pitfalls of Absent or Faint Kidney Sign on Bone Scan

Detection of osseous abnormality by bone imaging depends upon the recognition of the areas of above-normal and/or asymmetrical tracer concentration. Diffuse symmetrical involvement of the axial skeleton may not be recognized unless one reviews a radiograph of the axial skeleton at the time the scan is interpreted (*1*). Sy et al. (*2*) observed faint or absent renal activity at the time of bone imaging when there was diffuse metastatic involvement of the axial



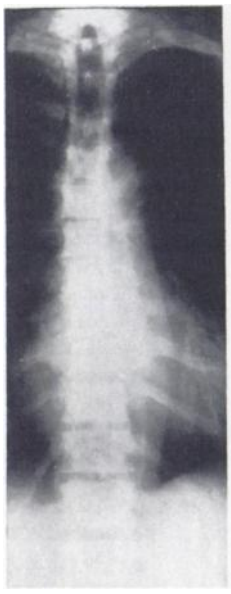


FIG. 1. (left) This 61-year-old white woman was admitted with palpable right breast mass and anemia. For the past 5 yr she had been treated intermittently with Leukeran for chronic lymphocytic leukemia. Breast biopsy revealed infiltrating duct carcinoma. Bone-marrow biopsy showed metastatic adenocarcinoma. Her skeletal survey was consistent with diffuse osteoblastic and lytic metastatic disease of axial skeleton. Technetium-99m MDP bone imaging, performed as part of metastatic workup, demonstrates diffuse increased uptake in axial skeleton, with no abnormal soft-tissue activity. Both kidneys are well visualized. Blood chemistry revealed BUN 15 mg/dl, creatinine 1.1 mg/dl, and alkaline phosphatase 304 U.

FIG. 2. (right) Radiograph of spine and pelvis shows diffuse osteoblastic and lytic lesions consistent with metastatic disease.