TECHNETIUM LABELING OF ALBUMIN

In their recent article Lin, Winchell, and Shepley (*1*) state that "the ^{99m}Tc activity which passed through the anion exchange column when HSA was present in the reaction mixture represented ^{99m}Tc bound to HSA" in the preparation with Fe(II) reducing agent. Furthermore, in the case of Sn(II), they state that "when HSA was present in the reaction mixture, most of the activity behaved chromatographically as though it were bound to HSA consistent with the assumption that reduced technetium efficiently bound to HSA when it was present."

We feel that their data in Table 5 are not completely consistent with these statements. It seems that the eluate of the anion exchange column can or cannot give liver localization; that is, the Tc-HSA is not the overwhelming constituent of the anion exchange eluate in all preparations. In the case of Preparations 4, 5, and 6 in Table 5 the eluate of 4 gives little liver uptake, but the eluate of 5 and 6 gives substantial liver uptake. A similar situation is described by the authors for Sn(II) preparations done at low and high pH. The use of anion exchange causes the yield of Tc-HSA to appear erroneously high in some cases because the liver component cannot be separated from the Tc-HSA on the anion exchange column. This makes predictions concerning in vivo distribution and stability difficult.

In recent work in this laboratory (*2*) we have shown that anion exchange chromatography does not distinguish between Tc-HSA and hydrolyzed Tc(IV). Gel chromatography (Sephadex G25) can, on the other hand, separate these components and give a better indication of the subsequent in vivo distribution. Table 1 contains data for Tc-HSA preparations at 2 pH values analyzed by gel chromatography. The ^{99m}Tc-HSA was prepared by a stannous chloride method (*3*). One aliquot was put directly on gel chromatography. A separate aliquot was put across a Dowex anion exchange column, and the eluate was then put across a gel chromatography column. The yields are reported as percent ^{99m}Tc present as Tc-HSA and percent present as hydrolyzed reduced technetium.

Control experiments were performed which indicate that ^{99m}Tc-HSA is not destroyed by the Dowex anion exchange column at least at low pH. All determinations across Dowex at pH 6.1 seemed to be more variable than the low pH determinations.

TABLE 1. YIELD OF ^{99m}Tc-HSA ON GEL CHROMATOGRAPHY

	Before anion exchange		After anion exchange	
Final pH	Tc-HSA (%)	Hydrolyzed Tc (%)	Tc-HSA (%)	Hydrolyzed Tc (%)
A. 2.5	86	14	84	16
6.1	77	23	77	23
B. 2.5	94	6	94	6
6.1	94	6	81	19
6.1	94	6	94	6

Mix 1 ml $^{99m}TcO_4^-$, 25 mg HSA and 250 μg $SnCl_2 2H_2O$ at pH 2.5 for 1 and 30 min for Groups A and B, respectively. The pH was raised with 0.15 ml 0.8 M NaH_2PO_4. A 0.5-ml aliquot was used for analysis.

The elution of the Dowex eluate across Sephadex indeed indicates that hydrolyzed technetium crosses the column. This hydrolyzed, reduced technetium must be responsible for the liver localization. With the use of anion exchange as a single analytical and preparative tool, no indication of the hydrolyzed reduced technetium fraction can be obtained, and thus no indication of the in vivo distribution can be obtained.

In addition, we found a number of minor points inconsistent with our results. Chromatography in 85% methanol of a Sn(II) reaction mix with no HSA present gave quantitative yields of origin material if performed in a nitrogen atmosphere. We found that both albumin and the hydrolyzed technetium remain at the origin and therefore this system would also not necessarily indicate the purity of the Tc-HSA. We have further discovered (*3*) that the reactions are not "sufficiently fast" as stated by the authors with 25 and 50 mg HSA. The use of 250 mg HSA requires only 1 min to achieve yields of 95% Tc-HSA. Use of 25 and 50 mg HSA requires 30 min mixing at low pH to achieve 95% yield of Tc-HSA. This fact might explain why their low pH solutions seemed to give higher yields of HSA by their bioassay method.

We feel the use of analytical techniques that can accurately identify various components in the products is becoming increasingly important. With the large number of preparations being formulated for

nuclear medicine use, failure to identify and quantify components will lead to misuse of the compounds, claims of in vivo instability which are not accurate, and incorrect internal radiation dose calculations.

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AUTHORS' REPLY

Drs. Eckelman and Richards have enriched the armamentarium of investigators interested in technetium radiopharmaceutical development by their demonstration of the utility of gel chromatography in separating technetium-labeled constituents in various mixtures. Perhaps the single most useful aspect of this technology involves the separation of technetium-labeled materials which remain at the origin in methanol paper chromatography. Certainly gel chromatography could have been usefully employed in analyzing the Fe(II) and Sn(II) labeling procedures described in our paper. However, we doubt that such analysis would significantly alter our results or our interpretation of these results.

Most investigators in this field are aware of the fact that in the technetium labeling of albumin by any method, technetium activity not bound to albumin may be found at the origin in methanol paper chromatography. Whether such technetium represents hydrolyzed technetium (IV) or other as yet unidentified forms of technetium remains a moot point. As described in our paper, we found that much of the activity in an acidic pertechnetate-tin(II) mixture could pass an anion exchange column, but that virtually none of the activity could pass a mixed anion and cation exchange column. We interpreted this as an indication of the presence of a cationic form of the technetium in the mixture. When a small amount of albumin is added to such a mixture, the cationic form of the technetium may not quantitatively bind to the albumin. It is our feeling (without experimental verification) that some of the activity found at the origin in the paper chromatography may represent the unbound cationic technetium.

In our work, we also found that when a pertechnetate-tin(II)-albumin mixture at near neutral pH was passed through an anion exchange column, the activity recovered was almost entirely in a colloidal form. Subsequently, we found that the activity in the near neutral mixture itself already was quantitatively in a colloidal form. Indeed, these observations have led us to the development of a technetium-tin(II) colloid (*J Nucl Med* 13: 58–65, 1972). Our awareness of the presence of variable quantities of colloidal technetium in our preparations of the technetium-labeled albumin was the basis for performing the in vivo distribution studies shown in Table 5 of our paper.

Drs. Eckelman and Richards stated that the reaction was not "sufficiently fast" as we had indicated. The reaction time depends on the concentration of the tin(II) and the albumin and also on the desired labeling yield. Since we decided to incorporate a radiochemical separation step into our procedure, we were not interested in prolonging the reaction time for a technetium recovery from the column exceeding 90%. Therefore in this frame of reference, the reaction was "sufficiently fast".

Lastly, we would like to thank Drs. Eckelman and Richards for their interest and critical review of our paper. Only through such critical analysis of data can procedures emerge from the hands of investigators which can be reliably used by practitioners of nuclear medicine.

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IMPORTANCE OF PROPER BOWEL CLEANSING BEFORE ^{131}I WHOLE-BODY SCAN OR RETENTION STUDY

The importance of proper bowel cleansing before whole-body scanning with ^{85}Sr or ^{67}Ga is well established (*1,2*). Because 10–15% of the administered dose of these radionuclides is excreted from the body by the gastrointestinal tract, the accumulation of the tracer within the bowel may be mistaken for a lesion. In a similar manner, false-positive studies may result from the use of radioiodine scanning in the detection