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**Publication Date**
2023-09-06

# Use of Fe(II) or Sn(II) Alone for Technetium Labeling of Albumin

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## DISCLAIMER

Several methods are available for binding $^{99m}Tc$ to albumin (1-4). Many of these methods involve reducing systems which can convert heptavalent Tc(VII) to its lower oxidation states (5-6). It is possible that this reduced technetium is the form which directly or indirectly binds to human serum albumin (HSA). We have studied two reducing systems for $^{99m}Tc$ labeling of albumin and polypeptides. These two procedures involve the use of either Fe(II) or Sn(II) alone without ascorbate or other materials. One of these labeled polypeptides, $^{99m}Tc$-caseidin, prepared by the Fe(II) or the Sn(II) procedure exhibits a remarkable localization in the renal cortex, and these observations are the subject of a separate communication (7). In this paper, we describe optimization of parameters for $^{99m}Tc$ labeling of HSA in the Fe(II) and Sn(II) methods, compare these methods with others, and discuss the chemistry pertinent to technetium labeling of proteins.

## Materials and Methods

Fe(II) as $FeSO_4 \cdot 7H_2O$ was obtained from J. T. Baker Chemical Co. (Phillipsburg, N. J.), and Sn(II) as anhydrous $SnCl_2$ was obtained from Matheson Company, Inc. (Norwood, Ohio). Both were dissolved in suitable HCl solutions. HSA obtained in solid form from Pentex, Inc. (Kankakee, Ill.) was used in the study of Fe(II) procedure, and that obtained in solution from Cutter Laboratories, Inc. (Berkeley, Calif.) was used in the study of Sn(II) procedure and in all *in vivo* distribution studies. $^{99m}TcO_4^-$ was eluted in physiological saline from a generator (New England Nuclear Corp., Boston, Mass.). Labeling was carried out in a small beaker provided with a magnetic stirrer. pH was monitored with Beromatic II pH meter (Beckman Instruments, Inc., Fullerton, Calif.). When terminal elevation of pH of labeling

mixture was desired, the mixture was titrated with appropriate strengths of NaOH solutions to keep volume changes minimal. A disposable anion exchange column prefilled with AG1-X8($Cl^-$), 100-200 mesh, 0.7 x 4 cm (Bio-Rad Laboratories, Richmond, Calif.) was used to remove anionic $^{99m}Tc$. Flow rate through the column under gravity was about 0.4 ml/min. Adsorption of HSA to the AG1-X8 resin was evaluated by passage of prepared $^{99m}Tc$-HSA through a second column with and without prior saturation of the column with HSA. The adsorption was found to be negligible when the amount of $^{99m}Tc$-HSA applied was about 0.1 mg or more. Radioactivity of $^{99m}Tc$ in the generator eluate and in the preparation was assayed in an ionization chamber (Mediac, Nuclear-Chicago Corp., Des Plaines, Ill.). Ascending radiochromatographic analysis of the preparation was performed on Whatman No. 1 paper in methanol. After the development, the paper strip was cut into pieces and assayed for $^{99m}Tc$ activity in a well-type crystal scintillation counter. Where necessary, ionic strength of the reaction mixture was varied by adding solid NaCl. The ionic strength was calculated according to $1/2 \sum_i C_i Z_i^2$ where $C_i$ and $Z_i$ were concentration and valence, respectively, of the i th ionic species in the mixture (8). In the tissue distribution study, Sprague Dawley rats (Bioscience Lab., Berkeley, Calif.) weighing 220-284 gm were lightly anesthetized with sodium pentobarbital and given 0.2 ml of the preparation through a tail vein. Two ml cardiac blood, the entire liver, and kidneys were removed in the said order and assayed for $^{99m}Tc$ activity. Appropriate dilutions of the preparation were used as standards.

The steps in our preparation of $^{99m}Tc$-HSA using Fe(II) or Sn(II) were as follows: $FeSO_4$ or $SnCl_2$ solution and HSA solution

were added in that order to generator eluate. Unless stated otherwise, volume of the reaction mixture was 2 ml consisting of 1 ml generator eluate, 0.5 ml of the metallic salt solution, and 0.5 ml HSA solution. The mixture was then passed through a column of AG1-X8 resin either after terminal elevation of pH of the mixture by titration with NaOH solutions or directly without such prior titration. The reaction was sufficiently fast so that no delay was necessary between successive steps. Non-anionic $^{99m}Tc$ yield from the procedure was expressed as % initial $^{99m}Tc$ that was recovered in effluent from the column.

## Results

Fe(II) Method. When Fe(II) was used, optimal conversion of $^{99m}TcO_4^-$ to non-anionic forms of $^{99m}Tc$ never occurred unless terminal titration of an initially acidic mixture to a higher pH was performed regardless of the initial pH of the reaction mixture (Tables I and II). When the pH of the initially acidic reaction mixture was terminally elevated, the yield of non-anionic $^{99m}Tc$ increased with increasing terminal pH until precipitation occurred in the mixture with associated fall in recovery from the column (Table II). To evaluate whether non-HSA-bound $^{99m}Tc$ might be a major component of the non-anionic $^{99m}Tc$, reaction mixtures from which HSA was omitted were passed through the column. In such studies, either with or without terminal elevation of pH of the blank mixture, the percentage of $^{99m}Tc$ recovered in the column effluent was at most 0.15%. This suggested 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 and also, in the absence of precipitation in the mixture, that activity in the column effluent was a measure of the efficiency of labeling with $^{99m}$Tc in the procedure.

For a given amount of HSA used, an optimum Fe(II)/HSA ratio existed for maximum labeling efficiencies. With relative excess of Fe(II) over HSA in the reaction mixture, co-precipitation of iron hydroxide and HSA occurred at a terminal pH of about 5. Under these circumstances, the resin column retained the precipitate, and the column effluent contained little of the HSA and $^{99m}$Tc used in the reaction. When the precipitate was redissolved by reacidifying the mixture prior to its application to the column, $^{99m}$Tc recovery in the column effluent was found to be good. When the reaction mixture contained 0.4 and 2 mg HSA, respectively, and 15 μmole Fe(II), the initial recovery was 0.05 and 0.13%, respectively, and subsequent to reacidification the respective recovery was 30 and 60%. These results indicated that poor $^{99m}$Tc-HSA yield in the case of relative Fe(II) excess resulted from poor recovery from the anion exchange column due to co-precipitation. Fig. 1 shows the plot of $^{99m}$Tc-HSA yield against Fe(II)/HSA ratio. The sharp diminution in yield after the maximum yield had been reached and as the Fe(II) concentration was increased coincided with appearance of precipitates in the solution. A sharp fall in the maximum yield at low concentrations of HSA (0.5-2 mg HSA) appeared to be a result of low concentrations of both HSA and Fe(II).

Results in Table III show that the yield of $^{99m}$Tc-HSA was greater the lower the initial pH of the reaction mixture. A lower initial pH is associated with a greater ionic strength as well as a higher hydrogen ion concentration. HSA (1mg) was found to salt out from a reaction

mixture at pH about 5 when its ionic strength exceeded about 0.8 M. Within this limit, as shown in Fig. 2, increasing ionic strength tended to enhance the yield of $^{99m}$Tc-HSA. Further, for a given ionic strength, a greater yield was associated with a higher initial hydrogen ion concentration.

Sn(II) Method. When Sn(II) was substituted for Fe(II) in the labeling procedure, high yield of non-anionic $^{99m}$Tc was possible without terminal titration of reaction mixtures. As shown in Fig. 3, when four different series of reaction mixtures at pH 1-4 containing varying amounts of Sn(II) and HSA were passed through the anion exchange column without prior terminal elevation of pH, maximum yields of 75-90% of activity in the column effluent was reached at pH about 2.5. At pH below 2 and above 3, the yield of activity in the column effluent tended to fall off rapidly. This suggested that $^{99m}$Tc-HSA could be readily made at pH about 2.5 using Sn(II). When HSA was omitted from the reaction mixture, the quantity of $^{99m}$Tc which was capable of passing through the anion exchange column plotted as a function of pH of the blank mixture resembled that found with mixtures containing HSA (bottom curve in Fig. 3). When the blank mixture was applied to a mixed anion (AG1-X8) and cation (AG50W-X2) exchange column (0.7 x 4 cm), at most 1% of the initial $^{99m}$Tc passed through the column. Superficially, it appeared that HSA labeling using Sn(II) entailed formation of certain cationic intermediate forms of $^{99m}$Tc. To further evaluate this possibility, generator eluate with added $H_2O_2$ was used to prepare reaction mixtures at pH 2-2.5 containing $H_2O_2$ (0.015%, v/v) in addition to Sn(II) with and without HSA in the mixture. When these mixtures were applied to anion exchange columns, less than 0.6% of the $^{99m}$Tc was recovered from the column regardless of whether the mixture contained HSA or not. Since formation of

cationic $^{99m}Tc$ in the blank mixture and, apparently, $^{99m}Tc$ labeling of HSA (vide infra) in the mixture containing HSA were both inhibited by the oxidant, it appeared that both the formation of the cationic $^{99m}Tc$ and the $^{99m}Tc$ labeling of HSA using Sn(II) involved formation of a cationic intermediate form of reduced $^{99m}Tc$.

The capability of this intermediate form of $^{99m}Tc$ to break through anion exchange columns raised the question whether such breakthrough of non-HSA-bound $^{99m}Tc$ might have accounted for a substantial portion of the $^{99m}Tc$ yield in HSA labeling. To evaluate this, reaction mixtures at pH 2.5 containing Sn(II) with and without HSA were passed through the anion exchange column, and the $^{99m}Tc$ recovered in the eluate was analyzed by paper chromatography. Results in Table IV show that about 94% of the applied $^{99m}Tc$ recovered from the mixture containing HSA remained at the first 2 cm, and about 4% reached the 5-7 cm region apparently as pertechnetate. In contrast, about 11% of the applied $^{99m}Tc$ recovered from the blank mixture (no HSA present) remained at the first 2 cm resembling labeled albumin, and about 82% reached the 5-7 cm region resembling pertechnetate. Since the blank $^{99m}Tc$ initially passed through the anion exchange column but subsequently a large fraction of this material behaved on the paper as pertechnetate, it is possible that the Sn(II) initially reduced the pertechnetate allowing it to pass through the column but this material was subsequently reoxidized, perhaps on the paper, to pertechnetate. The 11% of the $^{99m}Tc$ remaining close to the origin may have been associated with $Sn(IV)O_2$. 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.

Tissue Distribution Studies. To evaluate the nature of the $^{99m}$Tc-HSA prepared by the Fe(II) and Sn(II) procedures described above, we studied $^{99m}$Tc distribution following intravenous administration of these preparations in rats. Since metallic salts were used in the procedure, attention was directed to possible inadvertent recovery of colloidal forms of $^{99m}$Tc in the $^{99m}$Tc-HSA preparation. For comparative purpose, similar studies were performed with $^{99m}$Tc-HSA prepared using ascorbate alone and ascorbate plus Fe(III) by a modification of the method of Harper et al (1) and of Persson and Liden (2), respectively. Table V shows $^{99m}$Tc distribution within the body of rats 15 min after administration of each of eight $^{99m}$Tc-HSA preparations. Five of the six scintiphotos of rats shown in Fig. 4 were taken 10 min after administration of Preparation #1, 2, 3, 4, and 7 of Table V. Using the Fe(II) method, for the same Fe(II)/HSA ratio of 0.5 μmole/mg (Table V, #4-6), a terminal pH of 5.6 resulted in a preparation with a good portion of its $^{99m}$Tc capable of rapid localization in liver while a lower terminal pH of 2.0 yielded a preparation similar to that obtained by the ascorbate plus Fe(III) procedure. Further, for a similar terminal pH of about 5.5 (Table V, #3 and 6), there was little yield of such liver-localizing forms of $^{99m}$Tc when Fe(II)/HSA ratio was reduced from 0.5 to 0.1 μmole/mg by increasing the albumin content of the reaction mixture to 50 mg.

With $^{99m}$Tc-HSA prepared at pH near 2.5 by the Sn(II) procedure, the $^{99m}$Tc distribution resembled that found with preparations obtained either by the ascorbate plus Fe(III) procedure or by an optimized Fe(II) procedure (Table V and Fig. 4, cf #2, 3, 4, and 7). When the Sn(II) procedure was carried out at near neutral pH, direct

injection of the reaction mixture resulted in localization of 84-89% of the administered $^{99m}$Tc in the liver and of 5.8-7.8% in the spleen 30 min after injection. These two ranges of measurement applied to results found with three different reaction mixtures at pH 5-6 consisting of 5 ml generator eluate, 0.5 ml Sn(II) (1 μmole), and 1 ml HSA (5, 10, and 15 mg). A rat scintiphoto taken at 10 min after the administration of such a mixture is shown in Fig. 5 (#9).

When Fe(II) and Sn(II) procedures were used in the preparation, renal localization of $^{99m}$Tc activity was less than that obtained when the HSA was labeled using ascorbate or ascorbate plus Fe(III) method (Table V). With the ascorbate procedure in particular, there was appreciable early urinary excretion of the administered $^{99m}$Tc possibly related to formation of $^{99m}$Tc ascorbate complexes (Fig. 4, #1). This appeared to account partly for its relatively poor retention in the blood (Table V, #1).

## Discussion

Ascorbate alone, ascorbate plus Fe(III), Fe(II) alone, and Sn(II) alone, have been used to achieve or to promote reduction of Tc(VII) apparently to Tc(V) in the presence of thiocyanate in a colorimetric determination of Tc which presumably depends on formation of a technetium(V)thiocyanate complex (5,6). It is often assumed that labeling of HSA with $^{99m}$Tc requires reduction of $^{99m}$Tc(VII) to its lower oxidation states (1,3). For this purpose, ascorbate without Fe(III) is a usable, albeit, an inefficient system. Addition of Fe(III) to the ascorbate results in a more efficient system. Use of Fe(III) alone does not result in labeling. In the present study, Fe(II) alone did result in labeling and did so as effectively as the ascorbate

plus Fe(III) system. We also found that Sn(II), which is a more powerful reducing agent than Fe(II) (9), was effective in causing $^{99m}$Tc binding to albumin at much lower concentration than Fe(II), but was totally inhibited by traces of oxidants. Drs. Richards and Lebowitz at the Brookhaven National Laboratories and others have used a similar Sn(II) system for labeling of proteins (10). The success of the Fe(II) and Sn(II) reducing systems is consistent with the hypothesis that reduction of Tc(VII) is involved in the labeling of proteins. It has been stated (11) that ascorbate reduces Fe(III) to Fe(II). Although ascorbate itself is a reducing agent, the relative ineffectiveness of ascorbate without Fe(III) could be related to a small probability of reaction between two similarly charged particles, such as ascorbate and pertechnetate.

Fe(II) in acid solution is relatively stable in air, and oxidation of Fe(II) to Fe(III) in acid solution (9) is slow. However, such oxidation occurs rapidly upon alkalinization of the solution (12). Thus, the finding of a low $^{99m}$Tc-HSA yield in acidic solution without terminal pH elevation and that of an enhanced yield with the terminal pH elevation when Fe(II) alone was used in the labeling reaction is consistent with the reduction thesis in that reduction of Tc(VII) to lower valency states should occur as a result of Fe(II) being oxidized to Fe(III).

The Fe(II) alone method does not seem to differ fundamentally from the ascorbate plus Fe(III) method. Both require an initially acidic pH and a subsequent pH elevation. In the ascorbate plus Fe(III) method, the ascorbate may generate Fe(II) from Fe(III) and keep them in solution even after pH elevation by forming iron ascorbate complexes.

In the Fe(II) method, since HSA is the only substance present to prevent iron precipitation at elevated pH, it is clear that both terminal pH and Fe(II)/HSA ratio are critical. When both terminal pH and the Fe(II)/HSA ratio are unduly high, $^{99m}$Tc-colloid may be produced through adsorption of labeled HSA to colloidal ferric oxide formed in the reaction mixture (13). Whatever the mechanism may be, such colloidal $^{99m}$Tc evidently was present in the preparation #5 and #6 of Table V.

Many proteins can chelate transition metals when such metals are in cationic forms (14,15). Technetium is a transition metal. It appears probable that $^{99m}$Tc labeling of proteins with $^{99m}TcO_4^-$ involves reduction of the anionic Tc(VII) to a cationic Tc which then complexes with electron-donating groups (ligands) of the protein. That Tc(VII) can be reduced to cationic Tc to form complexes with electron donors is supported by the following impressions: 1. As shown in the present work, the use of Sn(II) in inducing binding of $^{99m}$Tc to HSA appears to involve an intermediate cationic $^{99m}$Tc. 2. In the presence of thiocyanate, ascorbate reduces Tc(VII) to form a technetium(V)thiocyanate complex (6), and citrate can replace the thiocyanate in the complex to form a technetium(V)citrate complex (16). 3. Borohydride reduces Tc(VII) to a state that can form chelates with ethylenediaminetetraacetate and diethylenetriaminepentaacetate (17).

Benjamin (4) has shown that anodic dissolution of zirconium by electrolysis followed by addition of $^{99m}TcO_4^-$ and HSA could result in excellent $^{99m}$Tc binding to HSA. Direct electrolytic reduction of Tc(VII) was shown to be unnecessary. As an explanation for these findings, it was proposed that binding of $^{99m}$Tc to HSA occurred

through formation of certain Zr(IV) species in the electrolyzed solution and subsequent co-ordination of $^{99m}TcO_4^-$ and HSA as ligands to the Zr(IV). However, he reported that such $^{99m}Tc$ binding to HSA was almost totally inhibited by traces of contaminating oxidants in the generator eluate (18,19). It seems likely, therefore, that traces of reducing agent, perhaps hydrogen gas or Zr(II), an extremely powerful reducing agent (20), might have been formed in the electrolyzed solution and served to reduce the $^{99m}Tc$(VII). This is analogous to the finding in the present study using Sn(II) in that addition of traces of $H_2O_2$ to the generator eluate totally abolished $^{99m}Tc$ binding to HSA. Benjamin also reported (4) that platinum could not replace zirconium as the anodic crucible for a successful labeling but that iron presumably could. When a platinum crucible was used, even addition of Fe(III)$Cl_3$ or Zr(IV)$Cl_4$ did not seem to increase the labeling efficiency beyond 10%. Possibly, when the iron crucible was used, Fe(II) formed in the solution was responsible for the labeling. While all of these results are readily explained using the thesis that Tc(VII) reduction causes direct Tc complexing to HSA, the Tc(VII)-metal-HSA indirect complexation thesis needs to explain the following difficulties: 1. If Tc(VII)-Zr(IV)-HSA complexes are formed with certain Zr(IV) ions in the electrolyzed solution, why are they not formed with Zr(IV) ions of $ZrCl_4$ origin? 2. Since both Fe(II) and Fe(III) are known to form complexes and indeed many Fe(III) complexes are more stable than analogous Fe(II) complexes (21,22), why is Fe(II) effective in the labeling but Fe(III) is not? 3. It has been stated (6) that Sn(II) reduces Tc(VII) to reduction states lower than Tc(V). If so, it is difficult to imagine that

Sn(II) could co-ordinate Tc(VII) as a ligand without causing its reduction to a lower valency state. This argument is even more applicable to use of Zr(II) in producing Tc binding to HSA, because Zr(II) is an even stronger reducing agent than Sn(II). 4. While ascorbate alone is a poor agent for inducing $^{99m}$Tc labeling of HSA, it nonetheless is effective in so doing. Recently, penicillamine and borohydride have been shown to be effective in inducing binding of $^{99m}$Tc to various materials (17,23). None of these agents are transition metals, but all are reducing agents. All systems which have been used to induce $^{99m}$Tc binding to proteins are reducing systems (or could contain reducing agents); not all such systems are capable of forming Tc(VII)-metal-HSA complexes. We conclude that formation of Tc(VII)-metal-HSA complexes is not a necessary mechanism for binding technetium to albumin and that if such a mechanism is operative in binding technetium to albumin under some circumstances it is at present unclear what these circumstances are.

The Fe(II) and particularly Sn(II) methods discussed in the present study are simple methods for $^{99m}$Tc labeling of HSA. Compared to the use of ascorbate plus Fe(III) (2,3,24), the Fe(II) procedure involves mixing generator eluate, Fe(II) solution, and HSA solution in one step, a single pH adjustment step, and a separation step on a small disposable anion exchange column. The labeling is further simplified in the Sn(II) procedure through elimination of the pH adjustment step. This elimination saves laboratory time. It may also improve reproducibility in routine service work since a given pH end point is not readily reproducible by titration. In the Fe(II) procedure, if a significant yield of colloidal forms of

$^{99m}$Tc in the prepared $^{99m}$Tc-HSA is to be avoided, one should employ a low terminal pH and/or a low Fe(II)/HSA ratio at some sacrifice of $^{99m}$Tc-HSA yield. In the Sn(II) procedure, there is no such tendency for the quantity and the quality of the product to work against each other.

We do not intend to prescribe a rigid protocol for the Fe(II) and Sn(II) procedures, since a given protocol can not be optimal for all purposes. However, the parameters affecting the labeling are presented as a basis for individual determination of labeling protocols.

The preparation can be sterilized using a Millipore filter. Toxicity of $SnCl_2$ has been studied (26). The amount of $SnCl_2$ required in the present Sn(II) procedure appears to be negligible in this regard.

$SnCl_2$ has been used to prepare $^{99m}$Tc- and $^{113m}$In-colloid for imaging of reticuloendothelial organs (25,26). Published methods (25,26) of preparation of the labeled colloid involve titration of an initially acidic reaction mixture to pH 5-7 and the use of gelatin as a stabilizer. In the present study, mixing of generator eluate, Sn(II) solution, and HSA solution at pH 5-6 appeared to constitute a simple method for making the $^{99m}$Tc-colloid without titration and with non-toxic non-allergenic HSA as a stabilizer (27).

## Summary

Simple methods for the preparation of $^{99m}$Tc-human serum albumin using Fe(II) and Sn(II) as a reducing agent in the labeling reaction are presented. The Fe(II) method consisted of achieving the labeling

by alkalinizing an initially acidic reaction mixture containing $^{99m}TcO_4^-$, Fe(II), and albumin, and subsequent removal of unbound $^{99m}Tc$ by passage through a small anion exchange column. The Sn(II) method consisted of achieving the labeling in a reaction mixture at pH about 2.5 containing $^{99m}TcO_4^-$, Sn(II), and the albumin and direct passage of the mixture through the anion exchange column to remove free $^{99m}TcO_4^-$ without any pH titration.

In the Fe(II) procedure, labeling was favored by a high initial acidity and ionic strength, a near neutral terminal pH, and an optimum relationship between the amounts of Fe(II) and albumin present. In general, for any concentration of albumin, labeling increased with increasing Fe(II) concentration until the point of Fe(II) excess where co-precipitation of albumin and iron hydroxide from the alkalinized mixture occurred.

In the Sn(II) procedure, optimum labeling and recovery occurred at pH about 2.5. $^{99m}Tc$-albumin prepared at pH near 2.5 was administered intravenously to rats. Fifteen min after the administration, the $^{99m}Tc$ remaining in the blood and that found in the liver were similar to corresponding values found with $^{99m}Tc$-albumin prepared by an ascorbate plus Fe(III) procedure or by an optimized Fe(II) procedure.

Data are presented which support the notion that reduction of Tc(VII) to lower valency states is the key prerequisite for Tc binding to albumin.

Acknowledgement

The author, M.S.L., a James Picker Foundation Fellow, wishes to thank the Foundation for its support. This work was supported in part under AEC contract #W-7405-ENG-48.

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## Figure Legends

Figure 1. Non-anionic $^{99m}Tc$ yield as a function of relative amounts of Fe(II) and HSA in the preparation of $^{99m}Tc$-HSA using the Fe(II) method. Reaction mixtures had initial pH 0.6 and contained $^{99m}TcO_4^-$ and the indicated amounts of Fe(II) and HSA. Ordinate represents the yield on a linear scale in % of initial $^{99m}Tc$ recovered from AG1-X8 columns. Abscissa represents Fe(II)/HSA ratio on a logarithmic scale in μmole/mg. Four sets of data were shown for four different amounts of HSA used in the preparation: 50 mg (closed circles), 10 mg (boxes), 2 mg (open circles), and 0.5 mg (triangles).

Figure 2. Non-anionic $^{99m}Tc$ yield as a function of ionic strength and initial pH of reaction mixtures in the preparation of $^{99m}Tc$-HSA using Fe(II). The reaction mixture contained $^{99m}TcO_4^-$, 2.5 μmole Fe(II), 1 mg HSA, and varying amount of NaCl added in solid form prior to pH adjustment to 5.5. Two sets of data were shown for initial pH 1.2 (open circles) and initial pH 4.1 (closed circles). The ordinate represents yield on a linear scale in % initial $^{99m}Tc$ recovered from AG1-X8 columns. The abscissa represents the logarithm of calculated molar ionic strength of the mixture at pH 5.5.

Figure 3. Non-anionic $^{99m}Tc$ yield as a function of concentrations of Sn(II) and HSA and pH of reaction mixtures in the preparation of $^{99m}Tc$-HSA using Sn(II). Results shown are from five separate experiments, including one performed without addition of HSA to the reaction mixture. Accompanying inset identifies the five sets of reaction mixtures. These mixtures were directly passed through Ag1-X8 columns without any pH adjustment. The yield is shown as % initial $^{99m}Tc$ recovered from the column. The abscissa represents pH of the mixture.

Figure 4. Scintiphotos of rats 10 min after intravenous administration of $^{99m}$Tc-HSA preparation #1, 2, 3, 4, and 7 of Table V, and a $^{99m}$Tc-Sn-colloid preparation (#9) described in text. Scintiphotos were obtained using a 3/16 inch pinhole collimator and an Anger camera.

Table I. Yield of Non-anionic $^{99m}Tc$ as a Function of pH of Untitrated Reaction Mixtures in the Preparation of $^{99m}Tc$-HSA Using Fe(II)*(10 mgm HSA)

| pH | Non-anionic $^{99m}Tc$(%) |
|---|---|
| 0.7 | 10.5 |
| 1.5 | 4.4 |
| 2.1 | 2.4 |
| 2.8 | 0.9 |
| 4.6 | 0.4 |
| 6.0 | 0.2 |

*A series of reaction mixtures at varying pH containing $^{99m}TcO_4^-$, 5 μmole Fe(II), and 10 mg HSA were directly passed through AG1-X8 columns without terminal pH elevation prior to the passage. The yield is given as % initial $^{99m}Tc$ recovered in column effluent.

Table II. Yield of Non-anionic $^{99m}Tc$ as a Function of Terminal pH of Titrated Reaction Mixtures in the Preparation of $^{99m}Tc$-HSA Using Fe(II)*(10 mgm HSA)

| Terminal pH | Non-anionic $^{99m}Tc$(%) |
|---|---|
| 2.5 | 67 |
| 3.5 | 73 |
| 5.0 | 80 |
| 6.6 | 89 |
| 9.0 | 36** |

*A set of reaction mixtures at initial pH 0.75 containing $^{99m}TcO_4^-$, 6 μmole Fe(II), and 10 mg HSA were titrated to varying final pH and then passed through AG1-X8 columns. The yield is expressed as % initial $^{99m}Tc$ recovered in column effluent.

**Precipitation occurred in the reaction mixture at pH 9.

Table III. Yield of Non-anionic $^{99m}Tc$ as a Function of Initial pH of Reaction Mixtures in the Preparation of $^{99m}Tc$-HSA Using Fe(II)*(1 mgm HSA)

| Initial pH | Non-anionic $^{99m}Tc$(%) |
|---|---|
| 0.6 | 32 |
| 1.4 | 18 |
| 2.5 | 7.4 |
| 4.3 | 5.4 |
| 4.6 | 4.3 |

*A set of reaction mixtures at varying initial pH containing $^{99m}TcO_4^-$, 2.5 μmole Fe(II), and 1 mg HSA were titrated to pH 5.5 and then passed through AG1-X8 columns. The yield is given as % initial $^{99m}Tc$ recovered in column effluent.

Table IV. Paper Radiochromatogram of (A) $^{99m}$Tc-HSA Prepared at pH 2.5 Using Sn(II), (B) Corresponding Blank Preparation (no HSA present), and (C) $^{99m}TcO_4^-$ (generator eluate)*

| cm from origin | A $^{99m}$Tc-HSA | B Blank | C $^{99m}TcO_4^-$ |
|---|---|---|---|
| 0-2 | 93.67** | 11.32 | 0.07 |
| 2-3 | 0.96 | 0.82 | 0.02 |
| 3-4 | 0.54 | 0.79 | 0.10 |
| 4-5 | 0.40 | 1.51 | 0.69 |
| 5-6 | 1.85 | 30.95 | 26.61 |
| 6-7 | 2.02 | 50.58 | 71.19 |
| 7-8 | 0.39 | 3.11 | 1.31 |
| 8-9 | 0.14 | 0.44 | 0.01 |
| 9-10 | 0.03 | 0.48 | 0.00 |

*$^{99m}$Tc-HSA was prepared by mixing 1 ml generator eluate, 0.5 μmole Sn(II) in 0.5 ml 0.2 N HCl, and 20 mg HSA in 0.5 ml saline and subsequent passage of the mixture (pH 2.5) through an AG1-X8 column. The corresponding blank was prepared by mixing 1 ml generator eluate, 0.5 μmole Sn(II) in 0.5 ml 0.1 N HCl, and 0.5 ml saline and similar passage of the blank mixture (pH 2.5) through the column.

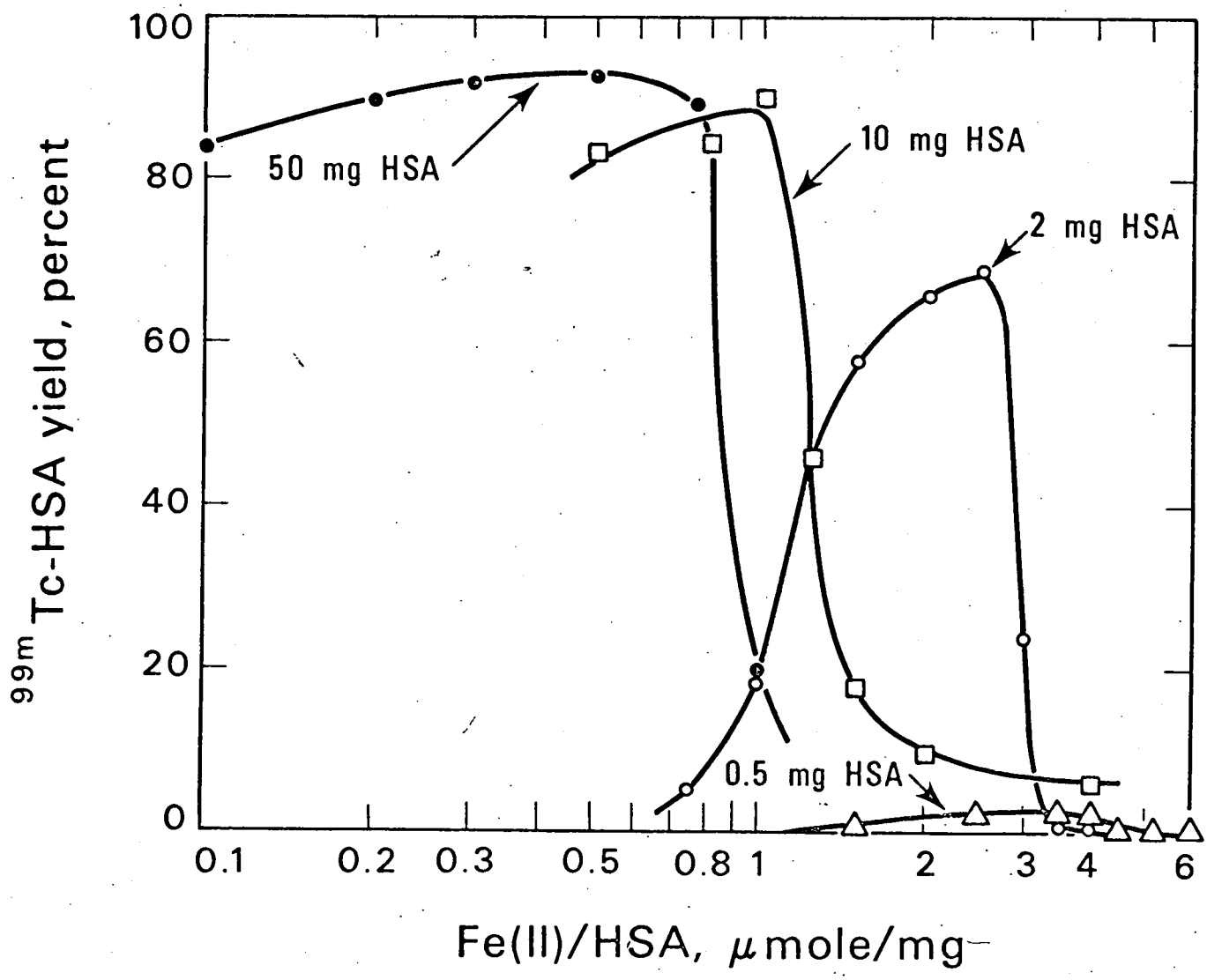
**% applied $^{99m}$Tc found at the indicated position on the paper is shown. Solvent front was at 10 cm.

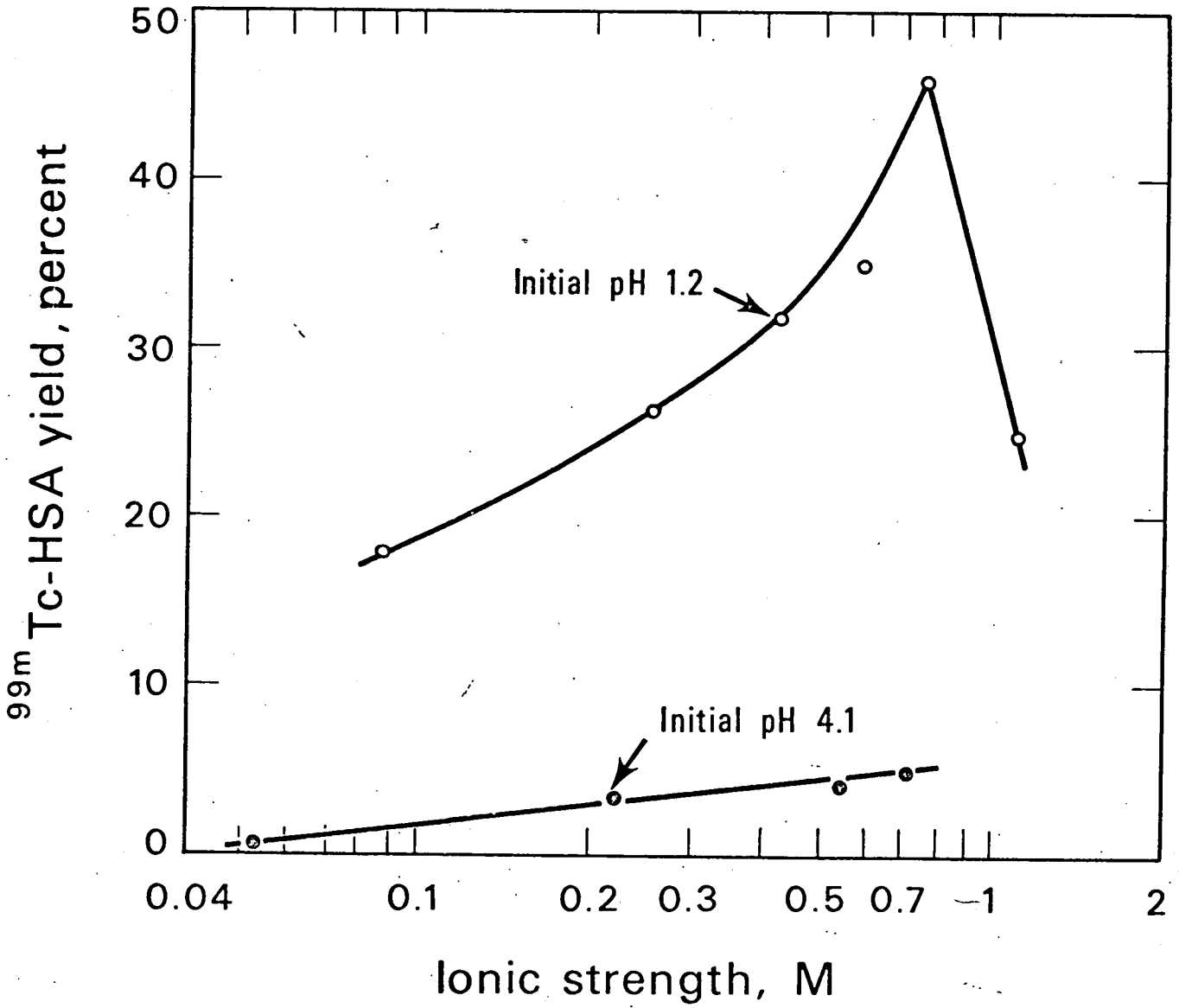
Table V. $^{99m}$Tc Distribution in Tissues of Rats 15 min after Intravenous Administration of $^{99m}$Tc-HSA Prepared Using Ascorbate alone (A), Ascorbate plus Fe(III) [A+Fe(III)], Fe(II) alone [Fe(II)] and Sn(II) alone [Sn(II)]*

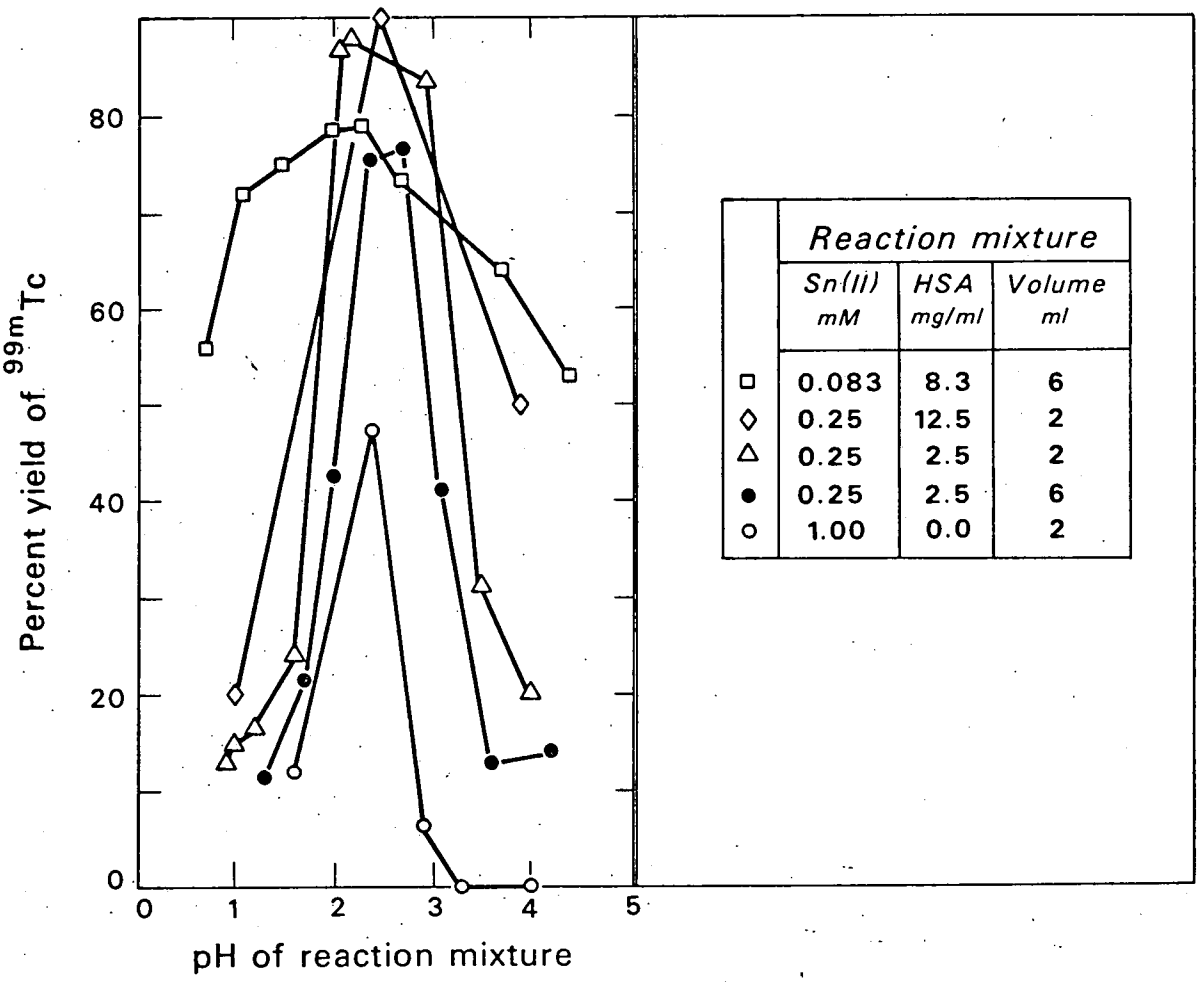
| Prepa-ration (#) | Method | Reaction mixture: HSA (mg) | Reaction mixture: Fe(II) or Sn(II) (μmole) | Reaction mixture: pH initial →final | Yield (%) | % dose per gram: blood | % dose per gram: liver | % dose per gram: kidney | % dose per organ: blood** | % dose per organ: liver | % dose per organ: kidney |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | 50 | | 0.4→5.5 | 15 | 4.4 | 0.9 | 2.4 | 66 | 6.9 | 3.8 |
| 2 | A+Fe(III) | 50 | | 2.7→6.8→2.3 | 81 | 6.0 | 1.0 | 2.4 | 94 | 7.9 | 3.8 |
| 3 | Fe(II) | 50 | 5 | 1.1→5.5 | 94 | 5.8 | 1.1 | 1.8 | 90 | 9.7 | 2.9 |
| 4 | Fe(II) | 10 | 5 | 0.8→2.0 | 48 | 5.5 | 1.0 | 1.6 | 99 | 8.8 | 2.9 |
| 5 | Fe(II) | 10 | 5 | 0.8→3.0 | 60 | 4.5 | 2.6 | 1.6 | 76 | 23 | 2.8 |
| 6 | Fe(II) | 10 | 5 | 0.8→5.6 | 72 | 3.1 | 4.5 | 1.4 | 50 | 38 | 2.5 |
| 7 | Sn(II) | 50 | 0.5 | 2.6 | 89 | 6.5 | 0.9 | 1.5 | 93 | 6.8 | 2.5 |
| 8 | Sn(II) | 50 | 0.1 | 2.7 | 87 | 6.7 | 0.8 | 1.5 | 96 | 6.1 | 2.3 |

*Left half of the table describes the labeling procedure; the right half presents the corresponding measured distribution. Initial values and end points of pH of reaction mixtures are shown for all procedures. Sn(II) procedures involved no pH adjustment. $^{99m}$Tc yield in the preparation is given as % initial $^{99m}$Tc recovered from AG1-X8 columns. The preparation was administered to groups of three rats each. Mean values are shown for the measured distribution. Liver and kidney activities include that in blood contained in these organs.

**Calculations based on the assumption that blood volume was 6.4% of body weight.


100
80
60
40
20
0
$^{99m}$Tc-HSA yield, percent
50 mg HSA
10 mg HSA
2 mg HSA
0.5 mg HSA
0.1
0.2
0.3
0.5
0.8
1
2
3
4
6
Fe(II)/HSA, μmole/mg


50
40
30
20
10
0
$^{99m}$Tc-HSA yield, percent
Initial pH 1.2
Initial pH 4.1
0.04
0.1
0.2
0.3
0.5
0.7
1
2
Ionic strength, M




DBL 7010-5955

#1 A

#2 A + Fe (III)

#3 Fe (II)

#4 Fe (II)

#7 Sn (II)

#9 $^{99m}$Tc-Sn colloid