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Radiopharmacology of a Simplified Technetium-99m-Colloid Preparation for Photoscanning[1]

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INTRODUCTION

Currently, the usefulness of colloidal preparations of technetium-99m is being evaluated for photoscanning the organs of the RES, i.e. the liver, spleen and bone marrow. The low radiation exposure resulting from internal administration of ^{99m}Tc colloid and the easily collimated 140 kev gamma emission, are the attractive features of this radiopharmaceutical.

Because of the six-hour half-life of ^{99m}Tc, the colloid must be prepared on the day it is used. Consequently, the method of preparation of the colloid must be relatively rapid and simple.

A satisfactory method for preparing colloidal ^{99m}technetium heptasulfide (Tc_2S_7) has been developed by Harper and associates (1). Although this technique is not complicated, it requires 30 to 60 minutes of preparation time and employs a reaction between pertechnetate ion (TcO_4^-) and hydrogen sulfide. The latter requires use of a fume hood and because of the potential toxicity of hydrogen sulfide, excess gas must be purged from the reaction mixture prior to administration of the final product.

A method for preparing ^{99m}Tc-sulfur colloid by reacting TcO_4^- with thiosulfate in sulfuric acid has been developed by Stern (2). This method employs carboxy methyl cellulose (CMC) as a colloidal stabilizer. This agent is relatively insoluble and is dissolved in pertechnetate solution only after heating for 15 minutes at 80°C. During preparation of CMC stabilized colloid, pH of reaction must be carefully controlled and the colloidal product sterilized by autoclaving. Garzon and associates (3) have prepared Tc-colloid by reacting colloidal antimony sulfide with TcO_4^- at 120°C for 30 minutes. This method has the advantage of providing a pre-formed colloid which may be labeled with ^{99m}Tc just prior to injection.

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A simple technique for the preparation of colloidal ^{99m}Tc, based on the reaction of thiosulfate ($S_2O_3^=$) with pertechnetate (TcO_4^-), has recently been investigated by Patton (4). By using perrhenate (ReO_4^-) as a carrier for TcO_4^- in the reaction sequence, Tc-sulfur colloid was rapidly obtained in high yield. Patton also demonstrated that this colloid produced good photoscanning visualization of the liver.

The observations of Patton have prompted us to further study the physical and chemical properties of this reaction in order to develop a more rapid and simple technique for preparing Tc-labeled colloid suitable for use in man. Our objectives were to optimize preparation of the colloid with respect to particle size and colloid yield, to make preparation time as short as possible and to evaluate the toxicity of the final product.

MATERIALS AND METHODS

REAGENTS

The following solutions of analytical grade reagents were used in these studies: sodium perrhenate ($NaReO_4$)[1] 5 mg/ml; sodium thiosulfate, ($Na_2S_2O_3 \cdot 5H_2O$) 8-16 mg/ml; gelatin, (U.S.P.) 4 mg/ml; 6% Dextran[2] in normal saline; 1N hydrochloric acid (HCl). Technetium-99m as pertechnetate ($^{99m}TcO_4^-$) was obtained by elution of ^{99}Mo generators of the BNL type with 0.9% sodium chloride.[3]

A phosphate buffer solution of pH 7.4 was prepared by dissolving 76.2 grams of dibasic sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$) and 4.6 grams of monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$) in 500 ml of pyrogen-free water. The final phosphate buffer mixture was made by adding two parts of the above phosphate buffer to one part of 1N NaOH.

All solutions were made using pyrogen free water (Abbott) and, when appropriate, were sterilized by millipore filtration using a syringe-adapted .45 micron filter.

PREPARATION OF THE COLLOID

The colloid was prepared by the following basic technique. Variable volumes of reactants were used during the study of the chemical and physical characteristics of the reaction, but the final total volume of the reaction mixture was kept at 14 ml. Sodium thiosulfate (1-2 ml), sodium perrhenate (.1 to 2 ml), 1 ml HCL, gelatin or Dextran (2 ml) and 6 ml of $^{99m}TcO_4^-$ in normal saline were

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[3]During the course of the these studies ^{99m}Tc-pertechnetate was used from ^{99}Mo generators which unlike BNL generators, do not use carrier free ^{99}Mo. In the former type of generator 4 to 5 times more alumina is required to retain the $^{98\text{-}99}Mo$ mixture. Although the saline-pertechnetate eluent obtained is clear and colorless, occasionally a flocculent precipitate has formed during the preparation of the colloid. This has been traced to a cationic contaminant in the eluent, possibly Al+++. This can be removed by dripping the saline-pertechnetate eluent through an additional column containing 4 ml. of Dowex 50 WX8 exchange resin (Na+ form) prior to millipore filtration.

added to a rubber-capped multidose vial (vented with a #25 needle) in a boiling water bath. After heating (1-24 min.), the vial was neutralized with three ml of the phosphate buffer mixture and cooled under tap water. A recommended final method for preparation of the colloid is presented in the results.

CHROMATOGRAPHY

Separation of free pertechnetate ion from the colloid-bound ^{99m}Tc was accomplished using ascending paper chromatography[1] with 95% Methanol as the developing solvent. In this system, labeled colloid remains at the origin, while TcO_4^- migrates with an Rf of approximately .54.

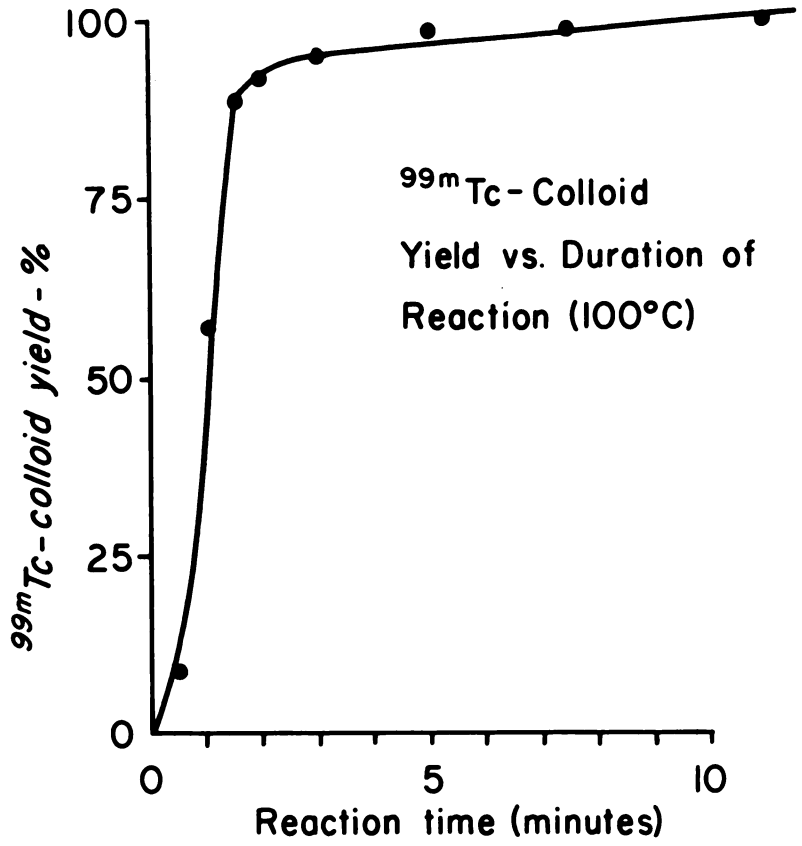

Fig. 1. Colloid yield (% $^{99m}TcO_4^-$ converted to ^{99m}Tc-colloid), as a function of reaction time.

Note the initial rapid and then gradual rise in % colloid yield. Concentration of reactants were: 1 ml. $Na_2S_2O_3 \cdot 5H_2O$ (8 mg/ml), .2 ml $NaReO_4$ (5 mg/ml), 2 ml 6% Dextran, 1 ml 1N HCl, and 6 ml. $^{99m}TcO_4^-$ saline eluent. After heating the mixture was neutralized with 3 ml. of the phosphate buffer.

[1]Whatman #1 Chromatography paper

DETERMINATION OF COLLOIDAL PARTICLE SIZE

The size of the ^{99m}Tc colloidal particles following various periods of heating was estimated by the methods of differential centrifugation (5, 6).

Three aliquots from each colloidal suspension were centrifuged at different pre-selected spin speeds. At the lowest spin speed, only particles greater than a calculated maximum diameter sedimented. At higher spin speeds, the sediment contained an additional fraction of particles whose size was smaller and at the highest spin speed, all particles except the smallest were sedimented. Thus, each batch of colloid was separated into four fractions of known particle size range from which the mean particle size was calculated. As the heating time of the reaction mixture was lengthened, the mean particle size increased, and the spin speeds were reduced accordingly.

COUNTING

Radioactive samples were counted in a well-type sodium iodide scintillation crystal to less than a three per cent relative error according to the criteria of Loevinger & Berman (7). The distribution of the colloid in the various organs of the rabbit was measured by total body counting at time of sacrifice, thirty minutes after intravenous injection of two to four mC of the colloid. Animals were positioned 12 feet from a three-inch diameter sodium iodide crystal and repeated counts were made following removal of each organ. Each decrement in count rate with removal of an organ was expressed as a per cent of the initial total body count. The skeletal dose was determined by removing the total skeleton from the autoclaved carcass, dissolving it in 400 cc. of nitric acid and counting aliquots of the acid solution in a scintillation well counter.

TABLE I

RESULTANT COLLOIDAL PARTICLE SIZE AFTER VARIOUS REACTION TIMES

Time of Heating Minutes	*Mean Particle Size Micra*	*% of Particles with Diameter Greater Than Recorded Maximum Size*	
		%	Recorded Maximum Size-Micra
2	0.40	4.6	.97
2.5	0.46	7.3	.97
3.5	0.62	3.1	1.90
5	0.78	6.5	1.90
8	0.95	11.6	1.90
12	1.24	14.4	1.91
16	2.00	9.7	4.28
20	2.83	11.0	4.28
24	3.20	18.0	4.28

RESULTS

The yield of ^{99m}Tc colloid, (colloid binding), was determined under various conditions of final pH, time of heating and concentration of reactants.

All reactions were carried out at pH one. After conversion of ^{99m}Tc to colloidal form, the pH was adjusted to between two and ten. Over this range of pH, ^{99m}Tc activity was not dissociated from the colloid. In general, the final reaction mixtures were opalescent at low pH and became clear at pH seven to eight.

The effect of time of heating on colloid yield is shown in Figure 1, (concentration of reactants held constant). After three minutes of heating, 96% of the ^{99m}Tc was in the colloid fraction. By ten minutes, the binding approached 100 per cent.

The effect of varying concentrations of thiosulfate and perrhenate on the yield of ^{99m}Tc colloid were examined, using a heating time of three minutes. Figure 2 illustrates the effect of thiosulfate concentration on colloidal yield. At a thiosulfate concentration of .45 mg/ml of reaction mixture, 98% binding occurred. At concentrations of .45 to 1.0 mg/ml, binding was only slightly increased. The dependence of colloid yield on ReO_4^- concentration is seen in Figure 3. A consistent 99% yield of ^{99m}Tc colloid was noted when the concentration of per-

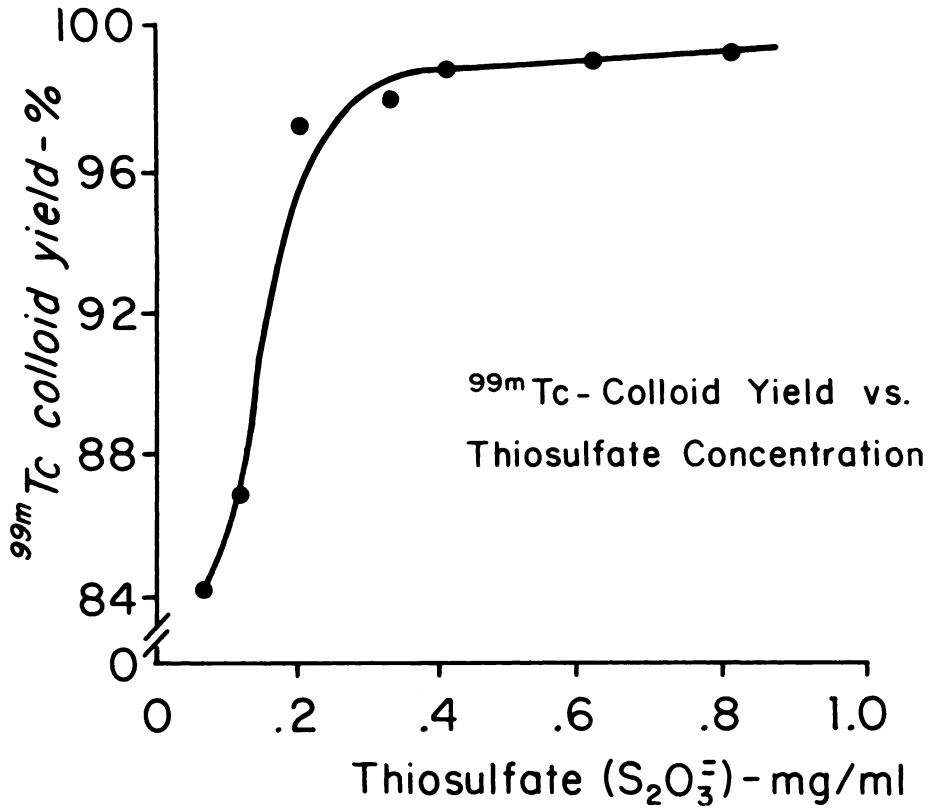

Fig. 2. % $^{99m}TcO_4^-$ converted to ^{99m}Tc-colloid, as a function of $S_2O_3^=$ concentration. Colloid was prepared as in figure #1, using a reaction time of 3 minutes.

rhenate was 0.1 mg/ml of reaction mixture. When no rhenium was present in the reaction mixture, binding still averaged 92 per cent. This is satisfactory for liver and spleen scanning, since 80 to 85% of the radioactivity is concentrated in these organs. The bone marrow, however, removes only about 10 to 15% of the labeled colloid. Therefore, if seven to ten per cent of the radioactivity injected for a marrow scan is free $^{99m}TcO_4^-$, a relatively high background of radioactivity occurs in the vascular and extravascular spaces which will impair the resolution of the marrow scan.

When the colloid was prepared under these various conditions, it was found to be stable for as long as 24 hours. Moreover, the ^{99m}Tc was not dissociated from the colloidal mixture after prolonged dialysis in 0.9% saline.

The effect of duration of heating on average particle size of the ^{99m}Tc-colloid was determined for heating times from two to 24 minutes. In these experiments the reaction mixture contained a concentration of .45 mg/ml of $S_2O_3^=$ and .2 mg/ml of ReO_4^-, a combination that gave 95% yield after two minutes of heating, and 98% yield or greater after three minutes of heating.

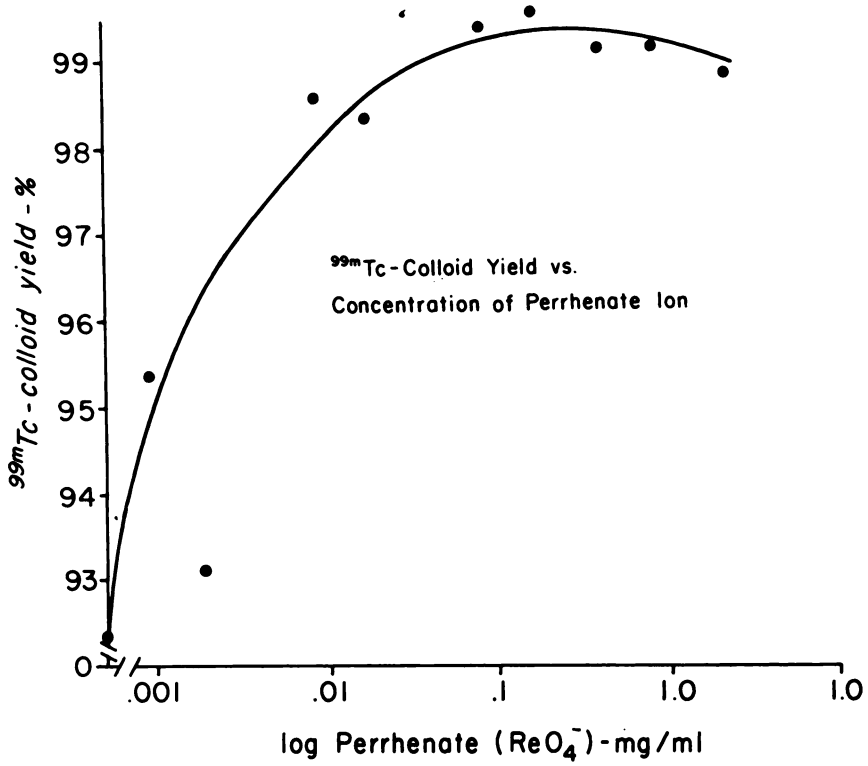

Fig. 3. $^{99m}TcO_4^-$ converted to ^{99m}Tc-colloid, as a function of ReO_4^- concentration. An apparent maximum yield is observed at .1 mg/ml ReO_4^-. Colloid was prepared as in figure #1, using a reaction time of 3 minutes.

Initially, the colloid was examined by electron microscopy. The individual colloidal particles were spherical and very uniform in appearance with an average diameter of 20 millimicra. Many of these small particles however, were aggregated into clumps measuring 100 mμ or more in diameter. Subsequently, it become apparent that suspensions of the colloids when examined by differential centrifugation behaved like particles of much larger size, indicating that most of the individual particles within the suspension were aggregated.

Differential centrifugation of suspensions of the colloid after three minutes of heating indicated a mean particle size of 400-450 mμ. Results of the effect of heating from two to 24 minutes on resultant mean particle size are shown in Figure 4. There was an increase in particle size with increasing time of heating so that after 24 minutes, particles with an average diameter of 3.2 micra were observed.

Table I lists the observed mean particle size for each time of heating and also indicates the per cent of particles precipitated at the lowest spin speed used for each batch. (Column 3, Table I). This provides an estimate of the largest particles after any given time of heating. For example, after five minutes of heating, the average particle diameter was 0.78 micra and 6.5% of the particles were greater than 1.9 micra in diameter.

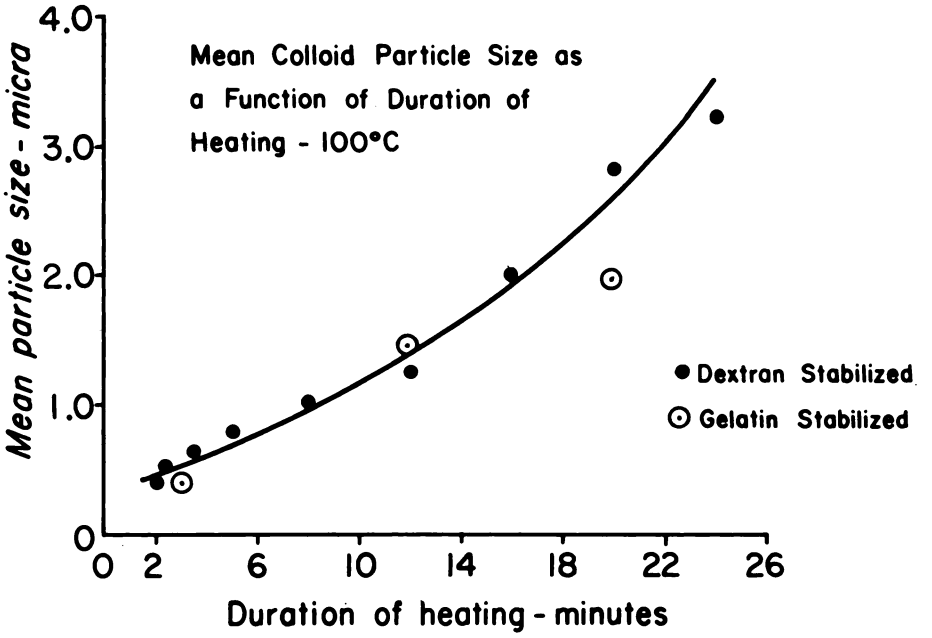

Fig. 4. Mean ^{99m}Tc-colloidal particle diameter, as a function of time of heating. As heating time was increased, there was a gradual increase in the mean size of the colloidal particles produced. Colloid for this experiment was prepared as in figure #1, except that the volumes of $Na_2S_2O_3$ and $NaReO_4$ solutions used in the procedure were increased to 1.5 ml and .5 ml respectively, a combination that gives 95% yield after 2 minutes of heating and 98% yield after 3 minutes of heating. See text for details.

Based on the above experiments, the following method has been adapted, as one which provides a rapid ^{99m}Tc-colloid preparation with an average particle size of 400 millimicra and 98% conversion of ^{99m}Tc to the colloidal form. Six ml. of $^{99m}TcO_4^-$ saline eluent, 1.5 ml. of sodium thiosulfate (8 mg/ml), 0.5 ml. of sodium perrhenate (5 mg/ml), 2 ml. of 6% Dextran and 1 ml. of 1N HCl are added to a 30 cc. rubber capped multidose vial. (Vented with a #25 needle). After heating this mixture in the vial for three minutes in a 100°C water bath, three ml. of the phosphate buffer mixture are added and the resultant colloidal suspension is cooled in tap water. In our experience, there is no difference in the colloid yield or particle size when Dextran or gelatin is used as the stabilizer. We prefer to use Dextran since it is commercially available in sterile form for intravenous administration.

When the ^{99m}Tc-colloid is injected intravenously into rabbits, it is rapidly cleared from the plasma ($T_{1/2}$ of 1-3 minutes) and on sacrifice, 95% or more is recovered from the organs containing the RES. (Table II). In man, the colloid is cleared from the plasma with a similar half-time and photoscanning shows good visualization of the liver, spleen and bone marrow.

In three patients, total urine and stool radioactivity was measured for 48 hours following intravenous injection of the colloid. Table III indicates that 3.9% or less of the radioactivity was excreted after 48 hours. By contrast, 39% of intravenously injected $^{99m}TcO_4^-$ is excreted in the same time period (8).

COMMENT

The preparation of ^{99m}Tc sulfur colloid using sodium thiosulfate and rhenium as a carrier for technetium is relatively simple and very rapid. The reaction of thiosulfate with hydrogen ion (H^+), is a classic method for preparing colloidal solutions of sulfur. Small amounts of other products, such as polymers of thiosulfate, free sulfur or sulfurous acid may also be formed in this reaction (9). When $^{99m}TcO_4^-$ is incorporated into this reaction sequence, technetium heptasulfide (Tc_2S_7) is probably formed (10), which is also colloidal in behavior.

Since the combined solutions of sodium thiosulfate, sodium perrhenate and Dextran do not react in the absence of H^+ ion, a sterile mixture of these solutions may be prepared and 4 ml. aliquots can be stored in 30 cc. multidose vials. These pre-mixed aliquots are stable under refrigeration for as long as three months.

For routine preparation of the colloid in our laboratory, a colloid kit is assembled consisting of three units, a 30 cc. multidose vial containing a four ml. "pre-mix" aliquot of rhenium, thiosulfate and Dextran; a sterile disposable syringe with one ml. of 1N HCl, and a disposable syringe with three ml. of sterile phosphate buffer mixture. A calibrated aliquot of ^{99m}Tc-saline eluent is obtained from the ^{99}Mo generator and added to the "pre-mix", acidified, and heated for three minutes, after which it is neutralized in the phosphate buffer and cooled. Because the colloid is stable for at least 24 hours, it can be prepared each morning with adequate ^{99m}Tc activity to last throughout the day.

To be useful in clinical studies, a radiopharmaceutical must be sterile, nonpyrogenic and pharmacologically safe. The reactants used in the preparation of this Tc-colloid are easily sterilized by millipore filtration, and pyrogen tests

of the final produce in rabbits have been negative. Moreover, the reactants used in this preparation have previously been administered to man in large amounts without apparent toxicity. Thiosulfate, for example, can be taken orally in doses up to 12 gms without effect except mild catharsis (11). Intravenous colloidal sulfur was used extensively during the 1930's for the treatment of arthritis, (12) and repeated single injections of up to 60 mg. were well tolerated. Studies in rats on the toxicity of rhenium as sodium perrhenate indicated that intraperitoneal injections of 600 mg/Kg body weight were without effect. A LD_{50} in rats of 900 mg/Kg was reported (13). In man, intravenous sodium perrhenate was explored as a method of cancer therapy in the 1930's. Repeated intravenous or intramuscular injections of 100 mg/day produced no ill effects (14). Large amounts of intravenous phosphate may lower the ionized serum calcium concentration temporarily. However, 50 times the amount of phosphate contained in three ml. of the phosphate buffer has been given by rapid intravenous infusion with complete safety (15).

SUMMARY

Using a modification of a method suggested by Patton, ^{99m}Tc-sulfur colloid can be rapidly produced by the reaction of thiosulfate with pertechnetate and perrhenate (ReO_4^-) carrier. The factors influencing the rate of reaction, ^{99m}Tc-colloid yield and resultant colloidal particle size are presented.

The colloid can be stabilized with Dextran and, for daily use, aliquots of the reactants can be stored in a pre-mixed sterile form. Using such a "colloid kit" ^{99m}Tc-colloid of 400 mu size can be routinely prepared in less than five minutes with 98% yield. The preparation is very suitable for photoscanning organs of the RES in man.

TABLE II

DISTRIBUTION OF ^{99m}Tc COLLOID WITHIN THE RES OF THE RABBIT[1]

Organ	*% Total Dose*
Liver	77%
Lungs	3%
Spleen	2%
Skeleton	14%
Carcass	2%

[1]Average of 5 rabbits

TABLE III

EXCRETION OF RADIOACTIVITY AFTER I.V. ^{99m}Tc COLLOID

	% Dose—48 Hours	
Patient	*Urine*	*Stool*
1.	3.6	0.3
2.	2.7	0.1
3.	2.3	1.0

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