DISTRIBUTION AND SCINTIPHOTOGRAPHY STUDIES OF ^{99m}Tc-IRON COLLOID IN THE RABBIT

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Technetium-99m has been found physically optimal for scintigraphy (*1,2*). Readily obtainable from a ^{99m}Tc generator as pertechnetate (TcO_4^-), it is valuable for scanning the brain (*2*), thyroid (*3*) and cardiac blood pool (*4*), for detecting extracranial neoplasms (*5*) and for making scintiphotographic dynamic studies of the heart and great vessels (*6,7*). The clinical application of ^{99m}Tc is further extended by a number of technetium-labeled compounds such as ^{99m}Tc in fat emulsion (*8*), ^{99m}Tc-Fe complex (*9*), ^{99m}Tc-albumin (*10,11*), ^{99m}Tc-macroaggregated albumin (*10,12*), ^{99m}Tc(V)-citrate complex (*13–15*) and ^{99m}Tc-S colloid. Currently six colloidal sulfur preparations are available and are used chiefly for photoscanning the liver and spleen (*16–22*). We have synthesized a new colloidal preparation, ^{99m}Tc-iron colloid with relatively small particles, a large fraction of which are localized in bone marrow. This report describes the preparation of the colloid and its distribution in rabbit tissue after intravenous injection.

MATERIAL AND METHODS

Preparation of ^{99m}Tc-Fe colloid. Using a modification of the method of Howard and Weber (*23*), ^{99m}Tc(VII)-pertechnetate is reduced with ascorbic acid in the presence of thiocyanate to ^{99m}Tc(V)-thiocyanate complex. The ^{99m}Tc-Fe colloid is formed by alkalinizing the reduced complex with NaOH in the presence of $FeCl_3$, Dextran and gelatin, then neutralizing the mixture with HCl and finally heating it to form a stable, clear, brown solution. The ^{99m}Tc-pertechnetate was "milked" from a ^{99m}Tc generator (donated by Nuclear Consultants Corp., St. Louis, Mo.) by elution with isotonic saline solution. One milliliter of 10 *N* HCl was added to 4.25 ml ^{99m}Tc eluate with constant stirring. To the eluate, 0.15 ml of 1% W/V $FeCl_3 \cdot 6H_2O$, an equal volume of 50% W/V ammonium thiocyanate and 1 ml of 5% W/V ascorbic acid were added in sequence and mixed well for 5 min. This mixture was the "reduced milk" used for subsequent labeling. To a flask containing 2 ml of 6% Dextran 70 (Baxter) were added slowly 0.5 ml of 1% W/V $FeCl_3 \cdot 6H_2O$, 1 ml of "reduced milk" and 0.5 ml of 20% W/V gelatin, U.S.P., while stirring continuously. The reaction mixture was titrated to pH 10.5–11.5 with 10 *N* and 1 *N* NaOH in sequence and then adjusted to pH 7.5 with 1 *N* HCl. The flask was heated in a boiling water bath for 5 min. After it was allowed to cool to room temperature, the solution was centrifuged at 20,000 G for 10 min. The clear, brown supernatant was the final preparation. The final product could be distinguished from the intermediate thiocyanate complex and the starting $^{99m}TcO_4^-$ by ascending paper radiochromatography (see section on paper chromatography and Fig. 1). The final solution prepared by this method usually had a total activity about 1/6th of that of the starting milk and contained approximately 98% ^{99m}Tc-Fe colloid and 2% ^{99m}Tc-pertechnetate. The colloid had a main molecular size intermediate between that of albumin (M.W. 69,000) and iron-Dextran complex (M.W. 156,000) (*24*) (see section on zone ultracentrifugation and Fig. 2). The dialyzable fraction of the preparation was 9% (see section on dialysis).

Laboratory animals. New Zealand male rabbits weighing an average of 3,350 gm were used. Animals were fed Purina chow and allowed water *ad libitum*.

Measurement of radioactivity. Radioactivity in the carcass and intestine was measured with a large ionization chamber and that in the other organs with a scintillation system adapted for large samples

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(Tobor, Nuclear-Chicago, Chicago, Ill.). Aliquots of samples of whole blood were counted in a well scintillation counter.

Paper chromatography. Ascending paper chromatography of urine samples and batches of the ^{99m}Tc-Fe colloid was performed on Whatman #3MM paper in 85% methanol for identification and quantitation of the colloid. Scanning the radioactivity on the paper chromatograms was carried out with an automatic windowless gas-flow counter and recorder (Scanogram II, Atomic Accessories, Valley Stream, N.Y.). The areas under the recorded peaks of the radioactivity on the chromatographed paper strips were measured with a planimeter. The percentage of the total radioactivity from an identified component was calculated from the ratio of the area under its respective peak to that of the total area of all radioactive components.

Zone ultracentrifugation in a density gradient. Density gradient tubes were prepared according to the method described by Ricketts *et al* (*24*). The sample (0.2 ml) was carefully layered on top and the tubes were centrifuged at 20,000 rpm for 16 hr. Samples used included (1) a 1:20 dilution of the iron Dextran complex (Imferon, Lakeside Laboratories, Milwaukee, Wis.); (2) a 4% solution of albumin coupled with "Procion" red dye (Imperial Chemical Industries, Providence, R.I.); and (3) ^{99m}Tc-Fe colloid without dilution. After centrifugation, the displacement of the ^{99m}Tc-Fe colloid was compared with that of Imferon and albumin coupled with the dye.

Dialysis. One milliliter of ^{99m}Tc-Fe colloid was diluted to 500 ml with distilled water. A 20-μl aliquot was withdrawn and transferred to a test tube for counting. This sample represented the original fraction. Another milliliter of the colloid was placed in seamless cellulose dialyzing tubing (average pore diameter, 4.8 mμ) and dialyzed against 500 ml of distilled water under continuous agitation. At 10-min intervals from the starting time, a 20-μl aliquot of the dialysate was transferred to a clean test tube. This sampling procedure was continued for 150 min. The samples of the original fraction and dialysate were counted in a well scintillation counter, and the counting rates of the latter were plotted against time on graph paper. The percent dialyzable material was calculated directly from the ratio cpm/20 μl of dialysate at equilibrium to cpm/20 μl of original. After 150 min, dialysis was continued against a fresh supply of 500 ml of distilled water for another 2 hr, after which sampling and counting of the dialysate were repeated. In the second determination, the amount of diffusible material was negligible.

Scintiphotography. An Anger scintillation camera (Pho/Gamma, Nuclear-Chicago, Chicago, Ill.) with a 3-in.-thick multichannel collimator was used. Scintiphotos were taken of the anesthetized rabbits in the prone position. The instrument was calibrated to record the 140 keV photopeak of ^{99m}Tc within a 15% window. Usually 150,000 counts were collected per projection, the light intensity value was 760 and defocused imaging was used.

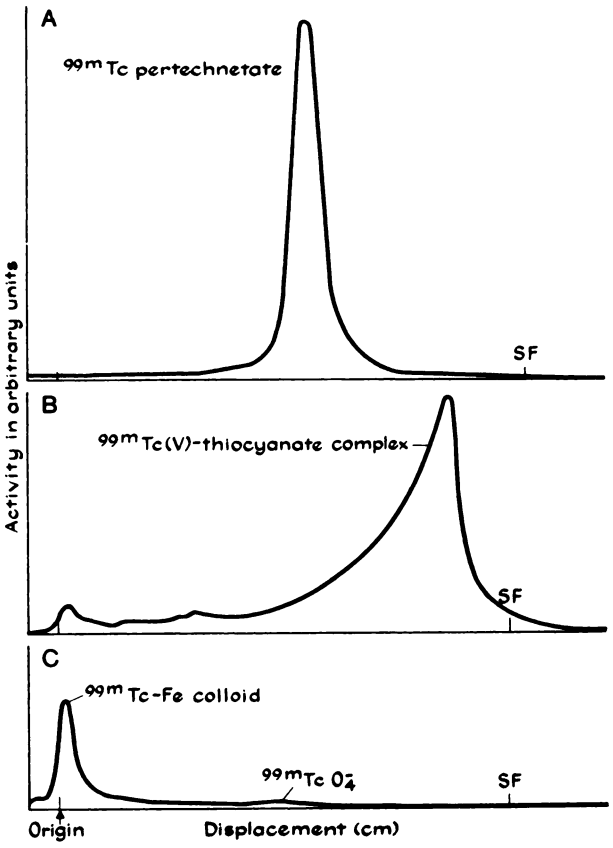

FIG. 1. Radiochromatograms of (A) starting material at pH 4, (B) intermediate product at pH 0.1 and (C) final product at pH 7.4 on Whatman No. 3MM paper in 85% methanol. Sample volume applied was 2–3 μl in each chromatograph.

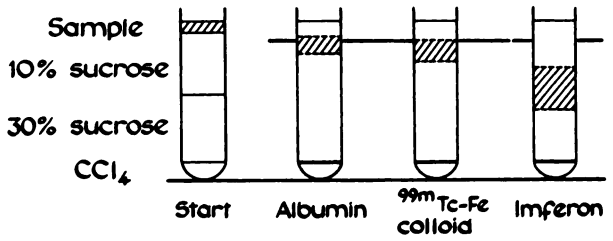

FIG. 2. Sucrose density gradient ultracentrifugation of ^{99m}Tc-Fe colloid, "Imferon," and serum albumin coupled with "Procion" red dye.

TABLE 1. ^{99m}Tc DISTRIBUTION IN TISSUES OF THREE RABBITS AFTER INTRAVENOUS INJECTION OF Tc-Fe COLLOID*

Tissues	% dose per organ				% dose per gram			
	0.5 hr	1 hr	3 hr	5 hr	0.5 hr	1 hr	3 hr	5 hr
Kidneys	6.41 6.35–6.50	7.08 7.01–7.16	6.79 6.71–6.87	7.92 7.27–8.59	0.23 0.23–0.23	0.25 0.24–0.26	0.26 0.22–0.29	0.27 0.25–0.28
Bone marrow†	10.5 8.60–12.9	9.80 8.00–11.7	10.5 8.60–12.5	9.80 7.40–11.7	0.17 0.14–0.21	0.16 0.13–0.19	0.17 0.14–0.22	0.16 0.12–0.19
Spleen	0.22 0.16–0.29	0.26 0.12–0.39	0.13 0.12–0.14	0.13 0.08–0.18	0.16 0.14–0.19	0.11 0.11–0.11	0.06 0.05–0.06	0.07 0.05–0.09
Liver	10.7 10.4–11.0	10.4 10.4–10.4	10.3 8.69–11.6	10.3 8.68–12.2	0.07 0.07–0.07	0.07 0.07–0.07	0.07 0.05–0.08	0.06 0.05–0.07
Lungs	2.03 0.90–2.67	0.98 0.48–1.48	0.60 0.56–0.62	0.40 0.34–0.45	0.05 0.04–0.06	0.04 0.03–0.04	0.01 0.01–0.02	0.01 0.01–0.01
Intestine	4.92 4.15–5.32	4.28 4.23–4.33	5.87 5.03–6.41	5.87 5.82–5.96	0.01 0.01–0.01	0.01 0.01–0.01	0.01 0.01–0.01	0.02 0.01–0.03
Stomach	0.46 0.42–0.51	0.34 0.30–0.38	0.15 0.13–0.19	0.15 0.12–0.17	0.01 0.01–0.01	0.01 0.01–0.01	<0.01	<0.01
Testes	0.10 0.09–0.11	0.06 0.06–0.07	0.04 0.03–0.05	0.04 0.03–0.05	0.02 0.01–0.02	0.01 0.01–0.01	0.01 0.01–0.01	<0.01
Blood					0.11 0.10–0.11	0.06 0.06–0.06	0.01 0.01–0.01	0.01 0.01–0.01
Carcass‡	27.9 25.7–29.4	18.4 17.8–18.9	4.70 3.80–5.30	4.60 3.10–6.60				
Whole body	63.2 56.8–68.7	51.6 48.4–54.9	39.1 33.7–44.7	39.2 32.8–54.9				

* Mean values are centered above corresponding ranges. Radioactivity in organs includes that due to blood in each organ. Activity of stomach, small intestine and colon includes that of contents.

† Percent dose administered radioactivity in bone marrow is calculated using data from Ref. 41. Bone marrow of adult rabbits constitutes about 2.2% of total-body weight, and 85–90% of total marrow weight is active. Accordingly percent dose is estimated as follows: (Body wt in gm × 0.022) × 0.85 × % of dose/gm.

‡ For each time interval fraction of administered dose (%) taken up by bone marrow has been subtracted from that obtained from carcass including skeleton.

RESULTS

Tissue distribution studies. The distribution of radioactivity in various organs of the rabbit was studied at various intervals after intravenous injection of ^{99m}Tc-Fe colloid. Each of 12 rabbits received 1 ml (380 μCi) of ^{99m}Tc-Fe colloid through a marginal ear vein. At four different times after injection ranging from 0.5 to 5 hr, a group of three rabbits was killed by an intravenous injection of 300 mg of sodium pentobarbital (Diabutal, Diamond Labs, Des Moines, Iowa). Just before sacrifice, 2 ml of whole blood was drawn and heparinized. The following organs were taken *in toto* from each animal: intestine, liver, kidney, lung, spleen, stomach and testes. The gastric, intestinal and colonic contents were not removed. We also removed samples of bone marrow from the femurs. One rabbit was killed immediately after being injected with the same dose of the ^{99m}Tc-Fe colloid, but we did not dissect it. The radioactivity of this animal represented the standard injected dose from which the figures of the precent dose in the bulky carcass and intestine were derived. Suitable dilutions of the injected material were used as standards for smaller organs and blood samples. The weights and radioactivity of the various organs were measured. The radioactivity of each blood sample was measured. In one rabbit a urine sample was obtained from the excised urinary bladder 0.5 hr after administration of the dose. Ascending paper radiochromatography showed that all the radioactivity in the collected urine was in the form of the intact colloid.

The distribution of the ^{99m}Tc-Fe colloid in the organs at varying intervals after injection of the dose is shown in Table 1. The organs are listed in order of decreasing concentration of radioactivity. When the results were expressed as percent dose, it was found that (1) ^{99m}Tc activity was relatively high in the bone marrow, liver and kidney; (2) the uptake in bone marrow was equal to that of the liver (around 10%); (3) radioactivity tended to decline with time in all organs except the kidney, bone marrow, liver and intestine, the percent dose of which remained approximately constant throughout the whole period of observation; and (4) within 30 min after injection, about 40% of the ^{99m}Tc-Fe colloid

had been excreted in the urine; after 30 min, body radioactivity declined slowly with a biological half-time of approximately 30 hr. When the figures were expressed as percent dose/gm, the concentration of ^{99m}Tc activity was greatest in the kidneys and bone marrow, intermediate in the spleen and liver and lowest in the lungs, stomach, testes and blood. The concentration of ^{99m}Tc in bone marrow was about 2.5 times higher than that in liver. During the first hour following injection, the blood concentration of ^{99m}Tc declined rapidly as the colloid was deposited in the reticuloendothelial organs and excreted in the urine. Thereafter the blood concentration remained at a low but constant level. The concentration of ^{99m}Tc in the kidney, bone marrow and liver remained constant from 0.5 to 5 hr after injection.

Scintiphotographic studies. In another series of experiments, scintiphotographic studies were performed in five rabbits. Each animal received an intravenous injection of approximately 350 μCi/kg of ^{99m}Tc-Fe colloid. At varying intervals afterwards, a series of scintiphotos was exposed. The urinary bladder had been emptied before scintiphotography. The scintiphotographic findings confirmed those of the distribution studies described above. From 15 min to 5 hr after injection, there was clear delineation of the kidneys and bone marrow. Figure 3 is a representative scintiphoto of the abdomen and pelvis taken 1.5 hr after intravenous injection of the colloid. The image of the dorsolumbar region was partly obscured by that of the liver but was much less interfered with than when using ^{99m}Tc-S colloid (Fig. 4).

Chemical toxicity. The animals were carefully watched for any signs of untoward reactions after administration of the ^{99m}Tc-Fe colloid. In the preparation of the colloid the concentration of metallic iron was 0.25 mg/ml. Six rabbits were given an intravenous injection of 4 ml of this preparation (total dose of metallic Fe, 1 mg or 0.3 mg/kg) without provoking any immediate toxic reaction. They were observed for 100 days. At the end of this period they were killed, and gross postmortem examination performed on them revealed no abnormalities.

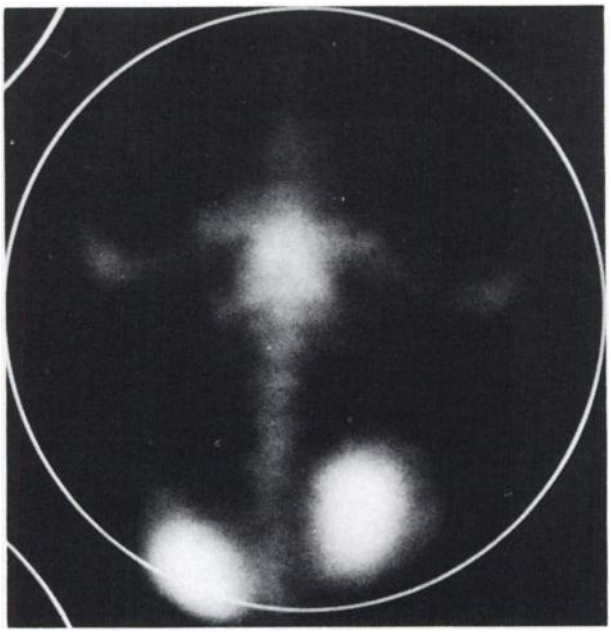

FIG. 3. Prone lumbopelvic scintiphoto of rabbit taken 1.5 hr after an intravenous injection of ^{99m}Tc-Fe colloid. Bone marrow and kidneys are clearly delineated.

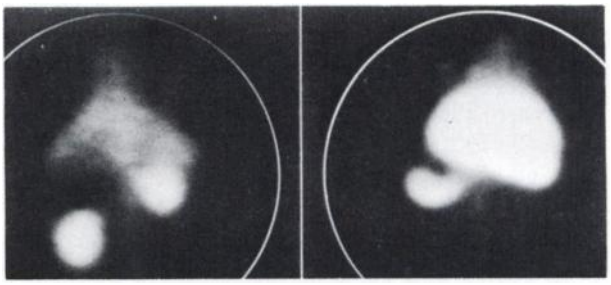

FIG. 4. Prone dorsolumbar scintiphoto of two rabbits after intravenous injection of same dose (350 μCi/kg of body wt) of ^{99m}Tc-Fe colloid (at left) and ^{99m}Tc-S colloid (at right). Pictures were taken 1 hr after injection with same accumulation of total counts (10,000) in each case. With ^{99m}Tc-Fe colloid there is good delineation of kidneys, while liver is poorly demonstrated; there is faint visualization of dorsolumbar marrow. In contrast, when using ^{99m}Tc-S colloid, there is clear visualization of liver and spleen and no demonstration of kidneys or dorsolumbar marrow.

DISCUSSION

With the procedure described above, ^{99m}Tc-Fe colloid is formed by the following sequence of mechanisms: (1) the positively charged colloidal ferric oxide is formed by adding NaOH to the reaction mixture containing $FeCl_3$ (*25*); (2) the anionic ^{99m}Tc(V)-thiocyanate complex is attached to the positively charged ferric oxide mainly by adsorption (*26,27*) and partly by complex formation (*28*); (3) the positive colloidal ferric oxide is converted to a negative colloid without coagulation taking place by preferential adsorption of hydroxyl ion in an excess of NaOH and in the presence of the protective substances, gelatin and Dextran (*25,27*); and (4) finally, the optimal growth of the particles is obtained by heating at a controlled temperature (*29*). It is important to stabilize the reaction mixture with gelatin before its titration with NaOH. Gelatin is a negative colloid at pH $>$ 4.7 and therefore has a strong protective action against the coagulation of ferric oxide during the latter's charge reversal. Dextran, a neutral hydrophilic colloid in

acidic, neutral or alkaline solution, is used to increase the labeling yield. This increase is small (around 2%), and the mechanism is not clear.

The result of dialysis of the preparation of ^{99m}Tc-Fe colloid, using seamless cellulose dialyzing tubing with average pore diameter of 4.8 mμ, indicates that the dialyzable fraction is 9%. According to Ostwald (*40*) the range of particle size of colloidal dispersions is from 1 to 500 mμ, so there is still a significant fraction of colloidal particles among 9% of the dialyzable fraction. The major fraction of the particles of ^{99m}Tc-Fe colloid ranges from 6 to 10 mμ on the basis of our results of zone ultracentrifugation and dialysis.

The results of the tissue-distribution studies indicate that there are high concentrations of ^{99m}Tc-Fe colloid in the kidney and bone marrow. Concomitantly there is a lower concentration in the liver than is observed with other ^{99m}Tc colloids. For example, the hepatic uptake of our colloid is only about 1/8th that of the ^{99m}Tc-S colloid prepared by Larson and Nelp (*21*). Consequently, the scintiphotographic image of bone marrow in the dorsolumbar spine is much less obscured by hepatic radioactivity when the iron colloid is used (Fig. 4). Furthermore, in the rabbit the thickest portion of the liver is on the sagittal plane relatively close to the spine (*30*). In contrast, the human dorsolumbar spine lies in relationship to the thin left hepatic lobe, which is more anteriorly located (*31*). Therefore it should be possible to visualize the dorsolumbar bone marrow clearly in humans.

^{99m}Tc-Fe colloid would have clinical value only if the desired dose contained quantities of iron which could be given safely. The positive colloidal iron hydroxide cannot be injected intravenously because the negatively charged serum precipitates it and causes the formation of emboli which lodge in the lungs and the glomeruli of the kidney (*32*). On the other hand, a negative sol containing an excess of both alkali and protective colloid does not precipitate serum (*33*). The negative colloidal iron preparation has been used in medicine since 1920 (*34*). The lethal dose of the negative colloidal ferric oxide for animals has been estimated as 30–60 mg of metallic iron per kg of body-weight (*35*). For mice, the LD_{50} for such colloid is 25 mg Fe/kg which is very close to the immediately lethal dose (ILD), 30 mg Fe/kg (*36*). When colloidal ferric oxide (total Fe = 50 mg, equivalent to 2 gm for a 70-kg adult) was given intravenously to a rabbit, no toxic reaction was provoked (*37*). According to Whipple *et al,* the negative sol of the iron has never given any untoward effects in dogs even when given intravenously in large amounts (50 mg/day for 2 weeks) (*38*). In our studies, the dose of iron administered to rabbits in 4 ml of the ^{99m}Tc-Fe colloid preparation (0.3 mg/kg) was not toxic. For man the lethal dose for the negative colloidal ferric oxide was assumed to be 28.6–57.2 mg Fe/kg, equivalent to 2–4 gm for an adult weighing 70 kg (*37*). Toxic symptoms, however, appear long before this dose is reached. Goetsch *et al* observed the effect of massive doses of iron given intravenously to patients (*39*). Of fourteen subjects, all but two had distressing reactions when the amount (0.608–1.32 gm) of elemental iron was given intravenously in one injection as negative colloidal sol. The maximal dose of *injectio ferri* B.P. is 6 mg. If one were to give humans 3 mc of ^{99m}Tc-Fe colloid intravenously for scintigraphy, and even assuming that one used a 5-day-old generator, the desired dose could be obtained by increasing the 1% $FeCl_3 \cdot 6H_2O$ to 0.6 ml (total dose of metallic iron, 1.2 mg) and using 3 ml of the tagging solution instead of 1 ml. This amount of iron is approximately 500–1,000 times less than the dose that would cause distressing reactions (*39*). We therefore believe that the colloid is without hazard from the standpoint of iron toxicity.

The radiation hazard from the ^{99m}Tc-Fe colloid is negligible. The limiting organ from the standpoint of radiation is the kidney. However, even with a relatively large dose of the ^{99m}Tc-Fe colloid (350 μCi/kg in the rabbit), the maximum absorbed radiation dose to the kidney was calculated to be only 280 mrads.

Harper and his associates have recently described a compound for renal scintigraphy, ^{99m}Tc-Fe complex, most of which is promptly excreted in the urine after intravenous injection without significant radioactivity appearing in the liver or intestine (*9*). The formation mechanism and nature of their complex remain unknown. The amount of $FeCl_3$ used in the preparation of ^{99m}Tc-Fe complex is relatively low (0.1 ml of 1% $FeCl_3 \cdot 6H_2O$), and there is still a fair amount (40–50%) of free $^{99m}TcO_4^-$ which must be removed by an anion-exchange resin column (*9*). In contrast, the ^{99m}Tc-Fe colloid prepared according to our method but without heat aggregation contained less than 5% free pertechnetate. If the titration of the reaction mixture is terminated at $pH < 7.8$, the amount of free TcO_4^- can be further reduced. We find it is not necessary to carry out further purification steps. The tissue distribution of such a nonaggregated preparation is similar to that of Harper's complex, as shown in Fig. 5, i.e., excretion mainly by the kidney.

The favorable physical characteristics of ^{99m}Tc and the results of the distribution of ^{99m}Tc-Fe colloid indicate a potential clinical usefulness of this

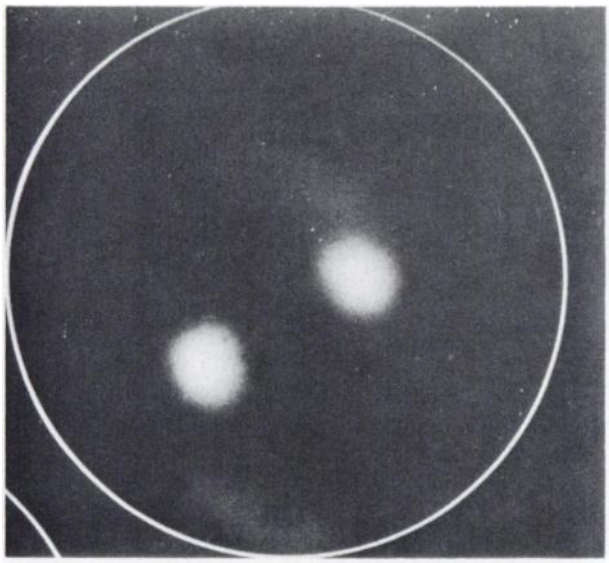

FIG. 5. Prone abdominal scintiphoto of rabbit taken 1.5 hr after intravenous injection of 300 μCi of ^{99m}Tc-Fe colloid without heat aggregation. Note clear delineation of kidneys and lack of visualization of bone marrow.

agent for bone-marrow visualization by scintigraphy. It might be especially suitable for evaluation of the bone-marrow involvement by tumors, such as Hodgkin's disease, metastatic carcinoma or leukemic infiltration. Such an evaluation is important to planning treatment. We would anticipate an advantage of this colloid over ^{99m}Tc-S colloid in bone-marrow scanning because of the relatively low obscurative localization of ^{99m}Tc-Fe colloid in the liver.

SUMMARY

A method for synthesizing ^{99m}Tc-Fe colloid has been described. The preparation consists of relatively small particles. Distribution and scintiphotography studies in rabbits indicate that about 40% of the intravenously injected colloid is rapidly excreted in the urine. The remainder is deposited chiefly in the bone marrow, kidneys and liver. Because of the relatively low uptake in the liver, the results suggest that the colloid is potentially useful in clinical scintigraphy of bone marrow. Hazards from iron toxicity or radiation are considered to be negligible.

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REFERENCES

1. RICHARDS, P.: A survey of the production at Brookhaven National Laboratory of radioisotopes for medical research. In *V Congresso nucleare,* Comitato nazionale recerche nucleari, Rome, vol. 2, 1960, 223.

2. HARPER, P. V., LATHROP, K. A., JIMINEZ, F., FINK, R. AND GOTTSCHALK, A.: Technetium 99m as scanning agent. *Radiology* **85**:101, 1965.

3. ANDROS, G., HARPER, P. V., LATHROP, K. A. AND MCCARDLE, R. J.: Pertechnetate-99m localization in man with applications to thyroid scanning and the study of thyroid physiology. *J. Clin. Endocrinol.* **25**:1,067, 1965.

4. WITCOFSKI, R. L. AND BOLLINGER, T. T.: The use of ^{99m}Tc pertechnetate in cardiac scanning. *J. Nucl. Med.* **6**:555, 1965.

5. BOLLINGER, T. T., WITCOFSKI, R. L., WHITLEY, J. E. AND MAYNARD, C. D.: The demonstration of extracranial neoplasms with ^{99m}Tc pertechnetate. *J. Nucl. Med.* **6**:687, 1965.

6. ROSENTHALL, L.: Applications of the gamma-ray scintillation camera to dynamic studies in man. *Radiology* **86**: 634, 1966.

7. KRISS, J. P.: Diagnosis of pericardial effusion by radioisotopic angiocardiography. *J. Nucl. Med.* In press.

8. HARPER, P. V., LATHROP, K. A. AND MCCARDLE, R. J.: Improved liver scanning with 6-hour Tc^{99m} in fat emulsion. *J. Nucl. Med.* **4**:189, 1963.

9. HARPER, P. V., LATHROP, K. A., HINN, G. M., GOTTSCHALK, A., JIMINEZ, F. AND PALOYAN, D.: Technetium-99m-iron complex. In *Radioactive Pharmaceuticals,* ed. G. A. Andrews, R. M. Kniseley and H. N. Wagner, Jr., AEC Conf.-65111, 1966, p. 347.

10. HARPER, P. V., LATHROP, K. A. AND GOTTSCHALK, A.: Pharmacodynamics of some technetium-99m preparations. In *Radioactive Pharmaceuticals,* ed. G. A. Andrews, R. M. Kniseley and H. N. Wagner, Jr., AEC Conf.-65111, 1966, p. 335.

11. STERN, H. S., ZOLLE, I. AND MCAFEE, J. G.: Preparation of Technetium (Tc^{99m}) labeled serum albumin (human). *Intern. J. Appl. Radiation Isotopes* **16**:283, 1965.

12. STERN, H. S., MCAFEE, J. G. AND ZOLLE, I.: Technetium-99m-albumin. In *Radioactive Pharmaceuticals,* ed. G. A. Andrews, R. M. Kniseley and H. N. Wagner, Jr., AEC Conf.-65111, 1966, p. 359.

13. YEH, S. H. AND KRISS, J. P.: Distribution and scintiphotography studies of a new compound: ^{99m}Tc(V)-citrate complex. *J. Nucl. Med.* **7**:364, 1966.

14. YEH, S. H. AND KRISS, J. P.: Distribution and scintiphotography of a new complex, pentavalent technetium-99m citrate: Studies in the rodent. *J. Nucl. Med.* **8**:666, 1967.

15. KOUNTZ, S. L., YEH, S. H., WOOD, J., COHN, R. AND KRISS, J. P.: Technetium-99m(V)-citrate complex for estimation of glomerular filtration rate. *Nature* **215**:1,397, 1967.

16. HARPER, P. V. LATHROP, K. A. AND RICHARDS, P.: ^{99m}Tc as a radiocolloid. *J. Nucl. Med.* **5**:382, 1964.

17. ATKINS, H. L., RICHARDS, P. AND SCHIFFER, L.: Scanning of liver, spleen and bone marrow with colloidal 99m-technetium. *Nuclear Appl.* **2**:27, 1966.

18. GARZON, O. L., PALCOS, M. C. AND RADICELLA, R.: A technetium-99m labeled colloid. *Intern. J. Appl. Radiation Isotopes* **16**:613, 1965.

19. STERN, H. S., MCAFEE, J. G. AND SUBRAMANIAN, G.:

Preparation, distribution and utilization of technetium-99m-sulfur colloid. *J. Nucl. Med.* **7**:665, 1966.

20. PATTON, D. D., GARCIA, E. N. AND WEBBER, M. M.: Simplified preparation of technetium 99m sulfide for liver scanning. *Am. J. Roentgenol. Radium Therapy Nucl. Med.* **97**:880, 1966.

21. LARSON, S. M. AND NELP, W. B.: Radiopharmacology of a simplified technetium-99m-colloid preparation for photoscanning. *J. Nucl. Med.* **7**:817, 1966.

22. BLUM, A.: Technetium-99m sulfide colloid preparation. *J. Nucl. Med.* **8**:692, 1967.

23. HOWARD, O. H. AND WEBER, C. W.: Rapid spectrophotometric determination of technetium in uranium materials. *Anal. Chem.* **34**:530, 1962.

24. RICKETTS, C. R., COX, J. S. G., FITZMAURICE, C. AND MOSS, G. F.: The iron Dextran complex. *Nature* **208**: 237, 1965.

25. POWIS, F.: Negative colloidal ferric hydroxide. *Chem. Soc. J.* **107**:818, 1915.

26. SEN, K. C., GANGULY, P. B. AND DHAR, N. R.: Studies on adsorption. V—Coagulation of negatively and positively charged ferric hydroxide sol and of negatively charged antimony sulphide sol by electrolytes. *J. Phys. Chem.* **28**: 313, 1924.

27. DHAR, N. R., SEN, K. C. AND GHOSH, S.: Studies in adsorption. VI—A new interpretation of the Schulze-Hardy law and the importance of adsorption in the charge reversal of colloids. *J. Phys. Chem.* **28**:457, 1924.

28. HARRAR, N. J. AND GERMANN, F. E. E.: A study of organic-acid iron solutions. III—Complex-colloid equilibrium. *J. Phys. Chem.* **36**:688, 1932.

29. THORNE, P. C. L. AND ROBERTS, E. R.: *Inorganic Chemistry,* 6th Ed., Oliver and Boyd, Edinburgh and London, 1954, p. 479.

30. LRAIGIE, E. H.: *Practical Anatomy of the Rabbit—An Elementary Laboratory Text-Book in Mammalian Anatomy,* 8th Ed., Univ. of Toronto Press, Toronto, 1948, p. 138.

31. JONES, T.: *Anatomical Studies for Physicians and Surgeons,* 9th Ed., S. H. Camp & Co., Jackson, Michigan, 1939, p. 24.

32. POLSON, C. J.: The fate of colloidal iron administered intravenously. *J. Pathol. Bacteriol.* **31**:445, 1928.

33. FISCHER, H. W. AND KUZNITZKY, E.: Das negative Eisenhydroxyd. III. Mitteilung. Eisen und Arsen. *Biochem. Z.* **27**:311, 1910.

34. DOZZI, L.: Ricerche cliniche sull'ossido idrato ferrico colloidale. *Gazz. Ospedali Clin.* **41**:182, 1920.

35. BEST, C. H. AND TAYLOR, N. B.: *The Physiological Basis of Therapeutics,* 3rd Ed., Williams & Wilkin, Baltimore, 1943, p. 98.

36. NISSIM, J. A.: Physico-chemical properties and toxicities of different iron preparations. *Brit. J. Pharmacol.* **8**:197, 1953.

37. NISSIM, J. A.: Intravenous administration of iron. *Lancet* **II**:49, 1947.

38. WHIPPLE, G. H. AND ROBSCHIET-ROBBINS, F. S.: I. Iron and its utilization in experimental anemia. *Am. J. Med. Sci.* **191**:11, 1936.

39. GOETSCH, A. T., MORE, C. V. AND MINNICH, V.: Observations on the effect of massive doses of iron given intravenously to patients with hypochromic anemia. *Blood* **1**:129, 1946.

40. WEISER, H. B.: *A Textbook of Colloid Chemistry,* 2nd Ed., John Wiley & Sons, New York, 1949, p. 2.

41. DIETZ, A. A.: Distribution of bone marrow, bone, and bone-ash in rabbits. *Proc. Soc. Exp. Biol. Med.* **57**:60, 1944.