

DISTRIBUTION AND SCINTIPHOTOGRAPHY OF A NEW LUNG-SCANNING AGENT, ^{99m}Tc -MACROAGGREGATED THIONIN: STUDIES IN THE RODENT

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Following the pioneer work of Taplin (1,2), Wagner (3,4), Quinn (5) and others in laboratory animals and in humans, lung scintiscanning with radioisotopically labeled macroaggregated human serum albumin (MAA) has become a useful and widely accepted procedure of considerable value for assessing regional pulmonary blood flow. As Taplin has indicated, the major disadvantage associated with using MAA tagged with ^{131}I or ^{51}Cr is the length of time needed for lung scanning with the conventional scintiscanner. This problem has been alleviated by using MAA labeled with ^{99m}Tc which has favorable physical characteristics for scintigraphy (6). Harper and his associates (6) first gave a brief description of the use of ^{99m}Tc -MAA for lung scanning. Subsequently, Stern *et al* (7) obtained ^{99m}Tc -MAA with low yield by tagging MAA with alkaline-dextrose-reduced pertechnetate; other workers (8-10) prepared the macroaggregates by pH adjustment and heat control of the ^{99m}Tc -labeled human serum albumin obtained by the procedure of Stern *et al* (11). In the meantime De Paoli *et al* (12) simplified the MAA labeling by using penta-valent ^{99m}Tc -thiocyanate complex as a tagging solution.

Following the observation of the preferential concentration of toluidine blue-0 in the parathyroid gland and pancreas (13,14), we succeeded in labeling this dye with ^{99m}Tc with hepatic localization (15,16). This led us to pursue further the related dyes. Consequently, we synthesized a new lung scanning agent, ^{99m}Tc -macroaggregated thionin (^{99m}Tc -MAT), the preparation of which is inexpensive as well as simple. This report describes the preparation of ^{99m}Tc -MAT and its distribution in the tissues of the rodent after intravenous injection.

MATERIAL AND METHODS

Preparation of ^{99m}Tc -macroaggregated thionin. ^{99m}Tc -macroaggregated thionin was synthesized by

the following steps: (1) reduction of ^{99m}Tc -pertechnetate (TcO_4^-) with ascorbic acid in the presence of thiocyanate to $^{99m}\text{Tc(V)}$ -thiocyanate complex (17); (2) binding of cationic thionin to anionic $^{99m}\text{Tc(V)}$ -thiocyanate complex and agar with aggregation (18-20); and (3) degrading the dye aggregates thermally.

^{99m}Tc -pertechnetate was "milked" from a ^{99m}Tc generator (from E. R. Squibb & Sons) by elution with isotonic saline solution. The ^{99m}Tc eluate was reduced in the manner we described previously (21) except that we used 0.5 ml of 10 N HCl instead of 1 ml of 5 N HCl. This "reduced milk" was used for subsequent labeling. To a flask containing 5 ml of a freshly prepared solution of 0.25% w/v thionin (from Allied Chemical Corp.; total dye content, 86%) in normal saline, 1 ml of the "reduced milk" and 1 ml of 0.2% agar-agar (U.S.P.) in normal saline were added slowly with continuous stirring. The reaction mixture was incubated for 5 min, adjusted to pH 4.4-4.5 with 10 N and 0.1 N NaOH in sequence and then heated in a boiling-water bath for 15 min. After the solution was allowed to cool to room temperature, it was centrifuged with a conventional horizontal centrifuge at 2,000 rpm for 3 min. The supernatant was drawn off with a syringe and the aggregate resuspended with 1 ml of physiological saline. Usually it took 75 min to prepare the aggregate if the reaction mixture was air-cooled. The time could be shortened to 40 min when the reaction mixture was cooled by tap water. The final product could be distinguished from the intermediate thiocyanate complex and the starting $^{99m}\text{TcO}_4^-$ by ascending radiochromatography on Whatman No. 3MM paper in 85% methanol (Fig. 1) as described

Received July 16, 1968; original accepted Sept. 19, 1968.

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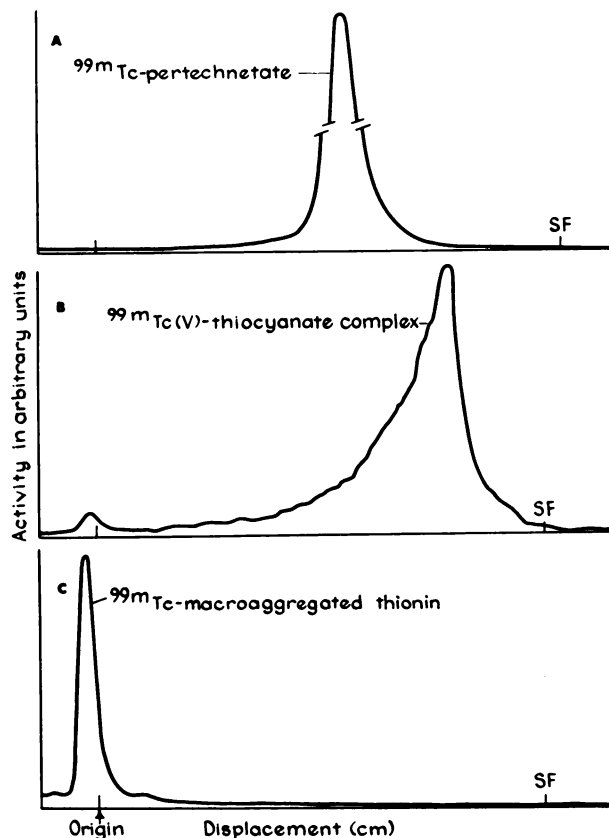


FIG. 1. Radiochromatograms of (A) starting material at pH 4, (B) intermediate product at pH 0.1 and (C) final product at pH 4.5 on Whatmann No. 3MM paper in 85% methanol. Sample volume applied was 2–3 μ l in each chromatograph.

previously (22). The final suspension prepared by this method usually had a specific activity about $\frac{1}{6}$ th of that of the starting milk and contained almost 100% ^{99m}Tc -MAT. When it was examined under the light microscope, the preparation appeared as conglomerates of aggregates assuming different shapes (Fig. 2). Particle sizes ranged from 5 to 100 micron. When the suspension was shaken, the larger aggregates broke into a rather uniform suspension of 20–30-micron particles.

Laboratory animals. Male Sprague-Dawley rats with an average weight of 200 gm and New Zealand male rabbits weighing approximately 3 kg were used. Animals were fed Purina chow and allowed water *ad libitum*.

Scintiphotography. An Anger scintillation camera (Nuclear-Chicago Pho/Gamma) with a 1.5-in.-thick multichannel collimator was used. Scintiphotos were taken of the rabbits strapped to a board in the prone position. The instrument was calibrated to record the 140-keV photopeak of ^{99m}Tc within a 20% window. One hundred thousand counts were collected per projection, the light intensity value was 516 and focused imaging was used. Scintiphotos

required an exposure time of 2 min to 1 hr, depending on the amount of radioactivity.

RESULTS

Tissue-distribution studies. The distribution of radioactivity in various organs of the rat was studied at varying intervals after intravenous injection of ^{99m}Tc -macroaggregated thionin. Each of 20 rats received 0.1 ml (60 μCi) of ^{99m}Tc -MAT through the right femoral vein after being anesthetized with 0.25 ml (15 mg) of Nembutal sodium given intraperitoneally. Each rat was put into an individual cage with an absorbing pad. At times after injection ranging from 5 min to 12 hr, a group of four rats was killed by an intravenous injection of 120 mg of Nembutal sodium. The distribution of the aggregate in the various organs of the rat was measured by total-body counting with the scintillation camera. Each animal was placed on the inverted cage in the supine position about 5 cm from the surface of the multichannel collimator, and repeated counts were made after the removal of each of the following organs: lungs, liver, spleen, stomach, intestine and kidneys. Each decrement in counting rate after removal of an organ was expressed as a percentage of the initial total-body count. The decrement in counting rate following the removal of the cage represented the radioactivity present in the urinary and fecal excretion.

The distribution of ^{99m}Tc -MAT in the organs at varying intervals after injection of the dose is given in Table 1. The organs are listed in order of decreasing radioactivity. It was found that (1) within 5 min after injection the extraction of ^{99m}Tc radio-

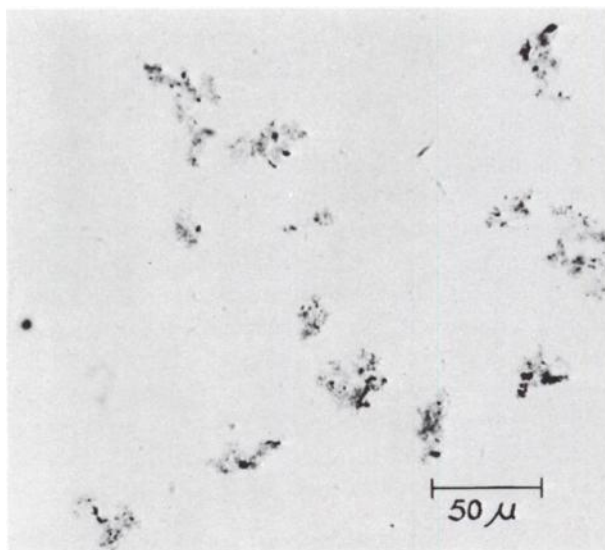


FIG. 2. ^{99m}Tc -macroaggregated thionin after standing ($\times 300$).

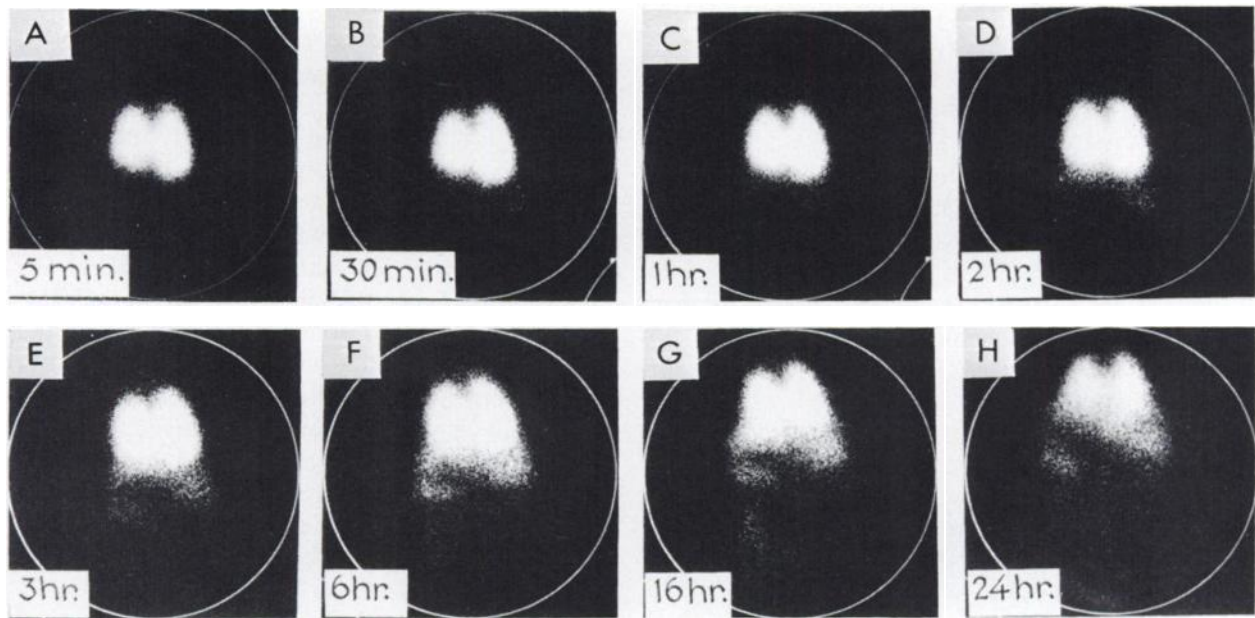


FIG. 3. Serial prone thoracolumbar scintiphotos of rabbit following intravenous injection of ^{99m}Tc-macroaggregated thionin. There is rapid pulmonary extraction of aggregate (A) with subsequent gradual clearance from the lungs (B-H) and transposition to liver (B-H), spleen (E-H) and bone marrow (G-H). Note faint delineation of kidneys at 16 and 24 hr (G-H). Head of rabbit is at top of illustration.

activity was about 90% in the lungs, 6% in the liver and 0.8% in the spleen; (2) the rate of clearance of the aggregate from the lungs was approximately exponential with a biological half-time about 7 hr; (3) radioactivity substantially increased with time in the liver, spleen and carcass (containing the bone marrow) indicating transposition of smaller particles of ^{99m}Tc-MAT to these organs after their formation in and clearance from the lungs; (4) ^{99m}Tc activity was low in the stomach and intestine but there was a slight increase in radioactivity up to 3 hr for the stomach and up to 6 hr for the intestine; and (5) kidney ^{99m}Tc activity was relatively low and tended to increase slowly with time. The urinary plus fecal excretion of ^{99m}Tc increased from 1.6% at 3 hr to 10% at 12 hr.

Scintiphotographic studies. In another series of experiments scintiphotographic studies were performed in four rabbits. The animals were strapped to a board in the prone position. Each animal received an intravenous injection of approximately 200 μ Ci/

kg of ^{99m}Tc-MAT in 1 ml. At varying intervals a series of the prone thoracoabdominal scintiphotos was made. The scintiphotographic findings confirmed and amplified those of the distribution studies described above.

Figure 3 is a series of scintiphotos obtained on a rabbit at intervals from 5 min to 24 hr after injection of ^{99m}Tc-MAT, clearly showing a rapid pulmonary retention of the aggregate with subsequent gradual clearance from the lungs and transposition mainly to the liver, spleen and bone marrow. Within 5 min the lungs were well delineated. Lung scintiphotos of good quality could be obtained up to 1 hr after injection; after that there was progressive obscuration of the lung bases as the result of a gradual

TABLE 1. ^{99m}Tc DISTRIBUTION IN TISSUES OF RATS AFTER INTRAVENOUS INJECTION OF ^{99m}Tc-MACROAGGREGATED THIONIN (% INJECTED DOSE)

Organs	Time after injection				
	5 min	1 hr	3 hr	6 hr	12 hr
Lung	89.0 \pm 2.7*	83.3 \pm 0.4	63.0 \pm 3.8	47.4 \pm 2.4	26.7 \pm 5.5
Liver	6.2 \pm 1.3	6.6 \pm 0.8	19.7 \pm 3.8	27.7 \pm 2.5	38.8 \pm 7.6
Spleen	0.8 \pm 0.6	0.5 \pm 0.5	1.9 \pm 0.5	2.1 \pm 0.3	4.7 \pm 1.7
Stomach	0.1 \pm 0.1	1.4 \pm 0.6	2.6 \pm 1.1	2.0 \pm 0.4	1.1 \pm 1.1
Intestine	0.4 \pm 0.3	1.3 \pm 0.9	1.1 \pm 0.7	2.4 \pm 0.6	1.6 \pm 1.0
Kidney	0	0	1.2 \pm 0.2	1.4 \pm 0.9	2.3 \pm 1.4
Excretion	0	0	1.6 \pm 0.6	4.5 \pm 1.6	9.8 \pm 0.9
Carcass	3.5 \pm 1.6	6.9 \pm 0.6	8.9 \pm 1.0	12.5 \pm 0.3	15.0 \pm 1.2

* Average of four rats \pm standard deviation.

accumulation of radioactivity in the liver. From 3 hr up to 24 hr, there was clear delineation of the spleen. At 16 and 24 hr the bone marrow and kidneys were faintly visualized.

Chemical toxicity and hypersensitivity. Observations were made in rats and rabbits to determine whether there were untoward reactions after the administration of ^{99m}Tc -MAT. In the preparation of the ^{99m}Tc -MAT used, the concentration of thionin was 10 mg/ml and that of agar was 2 mg/ml. In one series of experiments, four rabbits and ten rats were given an intravenous injection of 1 ml and 0.1 ml of this preparation, respectively, without provoking an immediate toxic reaction. They were observed for 30 days. At the end of this period they were killed, and gross postmortem examination revealed no abnormalities. Histological examination of the lungs was normal.

In another series of experiments a hypersensitivity study was performed in rats and rabbits. Ten rats receiving an intravenous injection of 0.1 ml of ^{99m}Tc -MAT were given the same dose intravenously after 4 weeks had elapsed. The same study was performed in four rabbits using doses of 1 ml of ^{99m}Tc -MAT per animal. None of the rats or rabbits developed any signs of hypersensitivity.

DISCUSSION

The results indicate that ^{99m}Tc -MAT suits the parameters set up by Stern *et al* for developing a new lung-scanning agent (23). The particle size of the aggregates is optimal for lung-scintigraphy. ^{99m}Tc -MAT gives an initial high uptake in the lungs, a suitable pulmonary clearance half-time and scans of good quality comparable with the published results of ^{99m}Tc -MAA. The preparation of the former is simpler than that of the latter.

The radiation hazard from ^{99m}Tc -MAT is low. The limiting organ from the standpoint of radiation is the lung. However, even with a large dose of ^{99m}Tc -MAT (200 $\mu\text{Ci}/\text{kg}$ in rabbits), the maximum absorbed dose to the lung was estimated to be 5 rads.

^{99m}Tc -MAT would have clinical value only if the desired dose contained quantities of thionin and agar that could be given safely. The LD_{50} of thionin in terms of its absolute active ingredient is 7.40 mg/kg in rats (24), and the fatal dose is 8.75 mg/kg in rabbits (25). The signs of acute toxicity are increased rate and depth of respiration, tonic convulsions and death due to cardiac and respiratory failure (24). Small intravenous doses of thionin (1/10th of the LD_{50}/kg in rabbits) do not cause any detectable signs of pathological changes even when administered over a long period of time (24).

When agar is injected intravenously in the form of agar sol-gel, the lowest fatal dose is 16.5 mg and 27.0 mg of agar per kilo of the rabbit and rat, respectively (26). The fatal outcome is due to the occurrence of pulmonary emboli and thrombi with agar which result in passive bronchoconstriction and pulmonary inflation with the attendant symptoms of asphyxia (27). In our studies the dose used in the rat was 5 mg/kg of thionin and 1 mg/kg of agar; that in the rabbit was 3 mg/kg of thionin and 0.6 mg/kg of agar. Neither acute nor chronic toxicity was observed in these animals. If one were to give 1.5–1.75 mCi of ^{99m}Tc -MAT intravenously to a 70-kg man for lung scintigraphy (28), even assuming that one used a 5-day-old generator, the desired dose could be obtained by using 2 ml of the tagging solution instead of 1 ml. The amounts of thionin and agar would be 0.14 mg/kg and 0.03 mg/kg, respectively. The latter is approximately 1/500th the fatal dose for the rabbit and 1/900th that for the rat. Toxicity due to the above dose of thionin is unlikely because the usual total human therapeutic dose of toluidine blue, a related dye of thionin, is from 1.4 to 4.3 mg/kg (24). The LD_{50} of toluidine blue in the rabbit is 13.44 mg/kg (24). Consequently, we believe that ^{99m}Tc -MAT can be safely administered to humans.

^{99m}Tc -MAT can be prepared sufficiently sterile for clinical purposes. Except for thionine, the reagents may be prepared in bulk ahead of time in autoclavable, multiple-dose, rubber-capped bottles; single doses of thionine are kept in the stoppered test tubes for the convenience of centrifugation. The reagent bottles and thionin tubes are autoclaved and stored. Amounts of the reagents necessary for the reaction are precalibrated in the sterile syringes.

SUMMARY

A method for synthesizing ^{99m}Tc -macroaggregated thionin has been described. Distribution and scintigraphy studies in the rat and rabbit indicate that intravenously injected ^{99m}Tc -MAT has an initial pulmonary uptake of 90% with subsequent gradual clearance from the lungs and transposition mainly to the liver, spleen and bone marrow. Hazard from radiation is considered to be low. Neither chemical toxicity nor hypersensitivity was observed in the rat and rabbit in the experiments designed. The amount which may be injected safely in humans has been discussed. The results suggest a potential usefulness of the aggregate in clinical lung scintigraphy. In view of its simple preparation and the physical characteristics of ^{99m}Tc , it should be a useful addition to the present lung-scanning agents.

ACKNOWLEDGMENT

This work was supported by a grant from the Milheim Foundation for Cancer Research.

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