^{113m}In-LABELED COMPOUND FOR LIVER AND SPLEEN STUDIES

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Hepatic scintiscanning using radioisotopes has gained acceptance as a simple, accurate and safe procedure for evaluating liver diseases. Radioisotopes in a colloidal form, such as ^{198}Au, are commonly used because they are phagocytized by the reticuloendothelial system and provide an anatomic study of the organs containing RE cells. Since its introduction by Harper *et al* in 1964 (*1*) several different preparations of ^{99m}Tc have been used for various organ-scanning procedures including colloids for liver studies (*2*). The attractiveness of the short half-life of ^{99m}Tc made this isotope readily accepted. It lets one administer larger doses which, in turn, produces higher counting rates and more rapid statistically valid scans, allowing greater diagnostic accuracy. The radiation dose to the patient is considerably less than when isotopes with longer half-lives are used.

It is possible to prepare a colloid of ^{113m}In that is suitable for liver scanning (*3*). In our laboratory we have developed a technique of controlling the particle size of indium colloid to study the effect on organ distribution. It has several advantages over other colloids now being used; for example, it is simple to prepare, the radionuclide is readily available (because of the long half-life—118 days—of the ^{113}Sn-^{113m}In generator), the radiation dose is low and the cost per millicurie is lower.

As early as 1949, studies made on the distribution of particles in the reticuloendothelial system suggested that particle size played an important role in determining the site of localization (*4*). When colloidal particles are injected into the blood stream, they are trapped by the cells of the reticuloendothelial system, not only in the liver, but in the bone marrow and spleen as well. It was shown by Dobson *et al* that colloids had the common property of localizing in the liver and spleen of mice, rats and rabbits when the size of the particles were relatively large, with a marked Tyndall effect on illumination. When the size of the particles was smaller, with a less pronounced Tyndall effect, they had an increased tendency to localize in the bone marrow.

The spleen provides an exceptionally effective means for phagocytosis of unwanted debris in the blood. Guyton (*8*) has suggested that to remove large particles, such as old red cells, the capillaries of this organ are highly porous, thus allowing larger quantities of whole blood to pass out into the red pulp which is loaded with reticulum cells.

The removal mechanism of colloidal phosphates from the blood has been studied by others. Experimenting with dogs and rats, Gerst (*5,6*) found that calcium phosphate in the colloidal form is phagocytized by the liver and spleen to a much greater extent than the macrophages of bone marrow and

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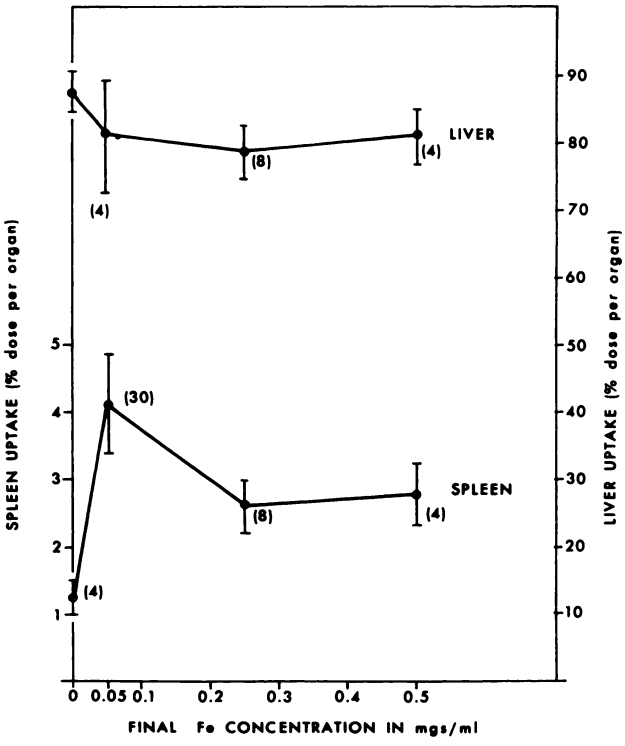

FIG. 1. Spleen and liver uptake using ^{113m}In-labeled particles with varying concentration of ferric chloride. Number of replicates in parenthesis ± standard deviations is also shown.

TABLE 1. DISTRIBUTION OF PARTICLES FOR ORGANS IN MICE

Organs	% recovered dose per organ			
	^{113m}In-colloid			
	Carrier free	Fe carrier	^{99m}Tc-sulfur colloid (8)	^{198}Au-colloid
Blood	0.60 ± 0.3	0.16 ± 0.21	1.40 ± 0.40	0.09 ± 0.12
Lung	0.12 ± 0.2	0.45 ± 0.58	0.46 ± 0.11	0.10 ± 0.04
Liver	86.05 ± 2.50	80.71 ± 8.89	80.22 ± 14.7	78.86 ± 8.31
Spleen	1.25 ± 0.12	4.78 ± 0.7	1.47 ± 0.46	0.89 ± 0.34
Kidney	0.66 ± 0.07	0.36 ± 0.20	0.75 ± 0.34	0.22 ± 0.13
Total % recover	88.68	86.46	84.30	80.16
No. of replicates	4	30	12	8
	% recovered dose per gram of tissue			
Organs	^{113m}In-colloid		^{99m}Tc-sulfur colloid	^{198}Au-colloid
Liver	47.87 ± 8.60		53.66 ± 11.14	43.18 ± 3.78
Spleen	49.69 ± 10.93		11.18 ± 4.71	8.77 ± 3.5
No. of replicates	15		12	8

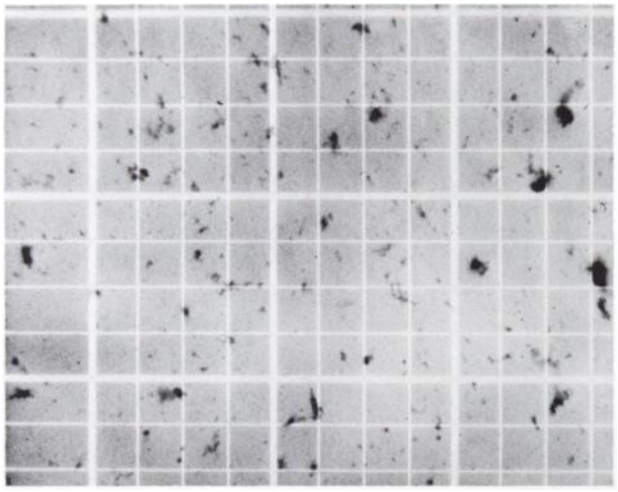

FIG. 2. Size of ^{113m}In-labeled particles prepared with iron carrier shown on a hemocytometer slide with light microscope. Majority of particles are in 1–5-micron range. Larger-appearing particles are aggregates of smaller-sized particles.

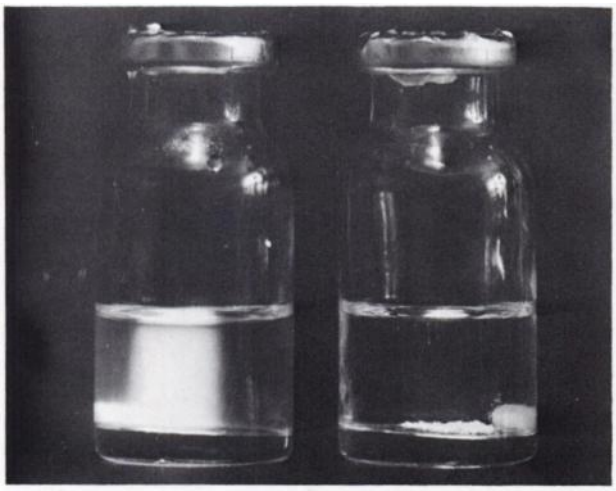

FIG. 3. Marked Tyndall effect is shown when 0.06–0.09 mg/ml of Fe carrier is added (left). Carrier-free colloid is clear (right).

lymph nodes. It seemed important therefore to develop a method of controlling ^{113m}In-colloidal particle size in an attempt to control the relative distribution in spleen, bone marrow and liver for clinical studies of these organs.

METHOD OF PREPARATION

Keeping the facts mentioned above in mind, we prepared ^{113m}In-labeled particles based on the use of phosphate buffers with and without iron as a carrier for liver and spleen studies.

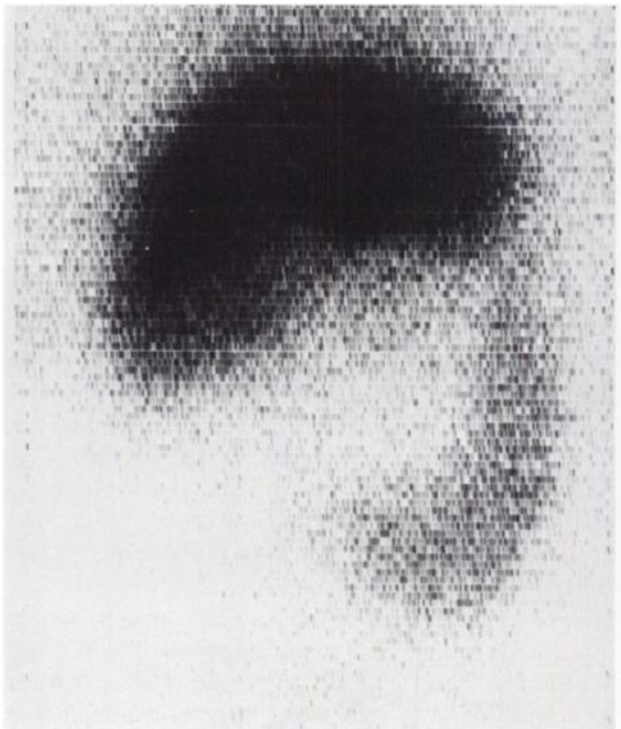

FIG. 4. ^{113m}In-labeled particles with iron carrier show excellent splenic uptake in dog, outlining long, narrow organ.

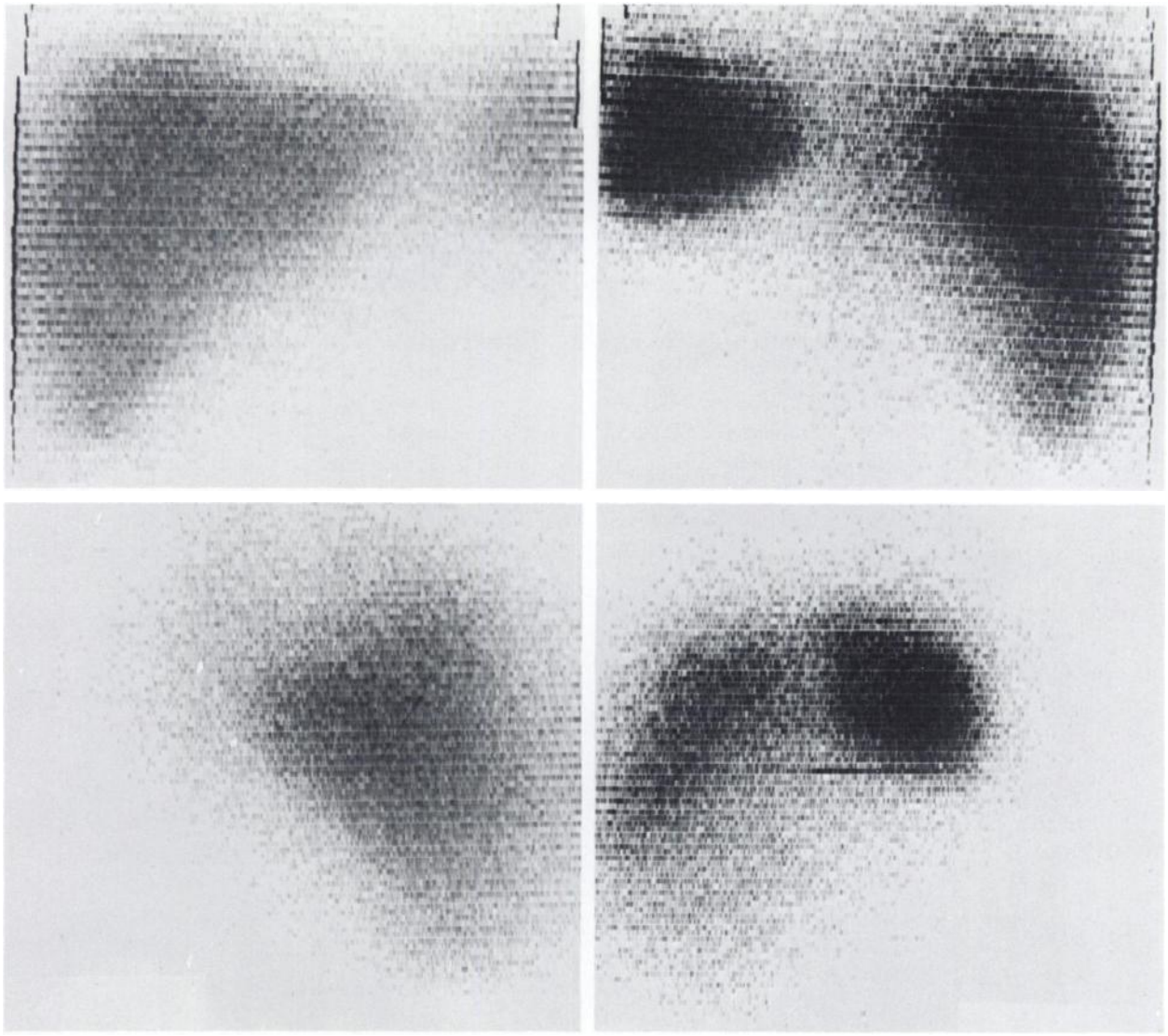

FIG. 5. Normal scan with anterior (top left), posterior (top right), R. lateral (bottom left), L. lateral (bottom right) shows excellent splenic visualization. Scan was made with 5-in. dual-crystal scanner and 85-hole lead collimator.

The particles are prepared by simple mixing of the components. Autoclaving for 20 min at 15 psi and 250°F is required if the generator is not sterile. Using a sterile generator, the rest of the components can be autoclaved in small vials at any time before the doses are prepared. Because of the short half-life of ^{113m}In, the generator must be eluted just before the doses are prepared.

The following stock reagents are made up monthly with pyrogen-free water, placed in small serum vials and sterilized by autoclaving for 20 min at 15 psi and 250°F: eluent—HCl solution 0.05 *M* (pH 1.4–1.6); $FeCl_3$, reagent grade, solution—1 mg of Fe^{3+} per ml; USP gelatin—2% solution. Phosphate buffer is prepared by adding 40.87 gm reagent-grade monosodium phosphate and 64.87 gm reagent-grade disodium phosphate per liter with a final pH of 7.3. The particles are prepared as follows:

In a 20-ml vial containing a small Teflon-coated magnet bar the compounds are added in the following order during mixing on the magnetic stirrer:

1. eluent from generator.
2. ferric chloride solution.
3. gelatine solution.
4. phosphate buffer solution.

The iron concentration was varied from 0.05 to 0.5 mg/ml to study the effect on the organ distribution of the particles. The carrier-free material was prepared in the same manner, eliminating Step 2.

The experimental compound with maximum splenic uptake contained between 0.06 and 0.09

mg/ml of Fe^{3+}. This compound was prepared as follows:

1. 4 ml eluent.
2. 0.5 ml ferric chloride solution.
3. 0.25 ml gelatine solution.
4. 1 ml phosphate buffer.

The preparation time for the particles is approximately 1–5 min.

RESULTS

Bioassays were done in white mice and rabbits, and scintiscans were performed in dogs. All animals were injected intravenously with the experimental solutions.

Table 1 shows the distribution of the particles for various organs in white mice, sacrificed 15 min after intravenous injection, as they compare with assays done using ^{99m}Tc-sulfur colloid (*9*) and commercially available ^{198}Au colloid (Squibb "Aureotope" colloidal suspension). A significant increase in uptake by the spleen was noted when iron was added to the solution. As is shown in Fig. 1 the uptake by liver is the same with or without iron carrier while the optimum iron concentration for maximum splenic uptake was 0.06–0.09 mg/ml.

The distribution of these particles agrees with the findings of Dobson *et al* (*4*). Commercially available ^{198}Au has a particle size of 3–35 mμ while ^{99m}Tc-sulfur colloid has particles in the range of 20–100 mμ (*7*). The size of the labeled particles shown in Fig. 2 would explain the higher uptake by the spleen using this compound rather than smaller sized colloids. Carrier-free ^{113m}In-colloid was not visible using the light microscope and had no Tyndall effect on illumination (Fig. 3).

The increased uptake of the Fe-^{113m}In particles by the spleen makes this material very useful in combined spleen and liver studies. Uptake by the spleen using the Fe-^{113m}In preparation was equal to liver per gram of tissue. It was also significantly higher than the splenic uptake using ^{99m}Tc-sulfur colloid and ^{198}Au colloid (Table 1).

Three dogs were injected intravenously with 1 mCi of the indium compound, and scintiscans were made. As is shown in Fig. 4, the liver and spleen are readily visualized.

Toxicity studies were conducted with white mice using doses over 1,000 times that of the clinical dose for humans with no visible side effects. Monosodium-disodium phosphate buffers are common buffering systems for biological use, and the concentration of ferric ion used is so minute that no toxicity would be expected, especially in the unionized state (*10, 11*). The pH of the final solution was in the range of 6.6–7.0. Toxicity data on the other components of the colloid have been presented elsewhere (*11*).

CLINICAL RESULTS

Over 400 patients have been injected with this Fe-^{113m}In compound to date. Using doses of 1–1.5

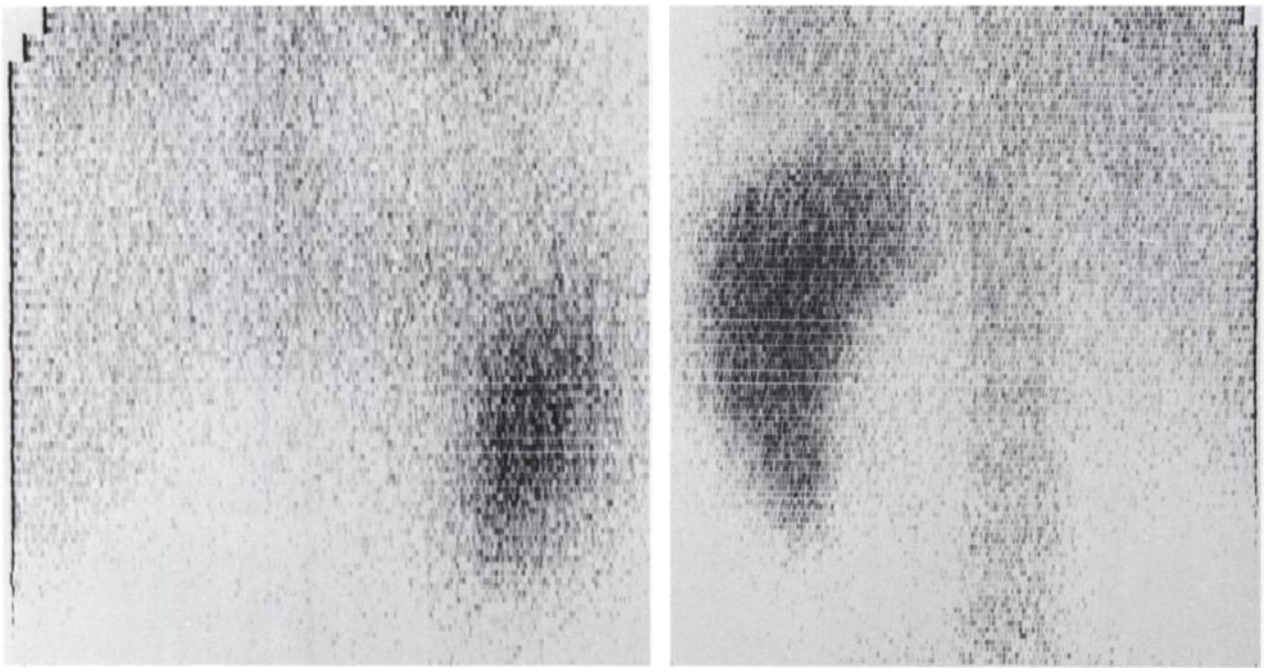

FIG. 6. Scan on patient with cirrhosis: anterior (left), posterior (right). Views show increased uptake in bone marrow and greatly enlarged spleen. Scan was made with 5-in. dual-crystal scanner and 85-hole lead collimator.

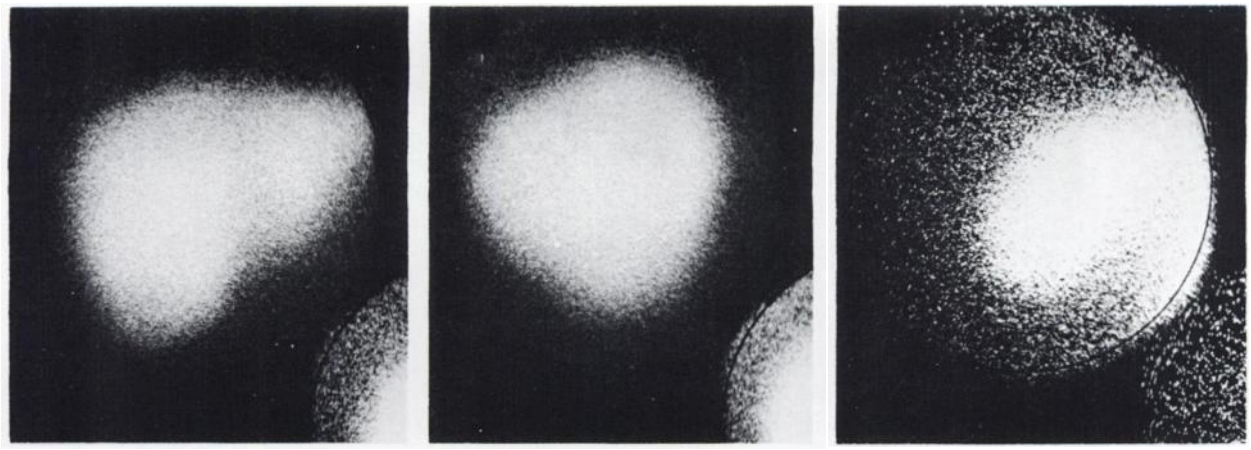

FIG. 7. Normal scintiphoto using Fe-^{113m}In colloid: from left to right, anterior view, R. lateral and posterior oblique of spleen.

mCi excellent scintiscans and scintiphotos were obtained of the liver and spleen. Scintiscans were done using an Ohio Nuclear scanner with dual 5-in. crystals and an 85-hole lead collimator (Figs. 5 and 6). The counting rate from a dose of 1 mCi (in the range of 70,000 and 80,000 cpm) was proved sufficient to run scans at a speed of about 350 cm/min with 250 counts/cm, which provides good statistics.

Scintiphotos were obtained using a Nuclear-Chicago Anger scintillation camera with an 11-in. crystal (Fig. 7). Excellent quality scintiphotos with about 300,000 dots/picture were made in about 1 min with 1.5 mCi.

Scans and scintiphotos can be started immediately after administration of the dose to the patient because, as is shown in Fig. 8, the blood clearance of Fe-^{113m}In particles is extremely rapid, with more than 99% being trapped by the reticuloendothelial system in less than 10 min.

CONCLUSIONS

A method of preparation of ^{113m}In-labeled particles for combined liver and spleen studies is described.

We have been able to increase the splenic uptake of ^{113m}In-colloid by increasing the particle size by adding iron as carrier.

The method used to prepare these Fe-^{113m}In particles for liver and spleen studies has proved to be simple, rapid and clinically useful. Excellent quality scintiphotographs and scintiscans have been obtained. It was possible to visualize the spleen in all patients studied with the exception of two who had undergone splenectomy. Because of the frequent association of hepatic and splenic diseases, it is an advantage to examine both organs at the same time.

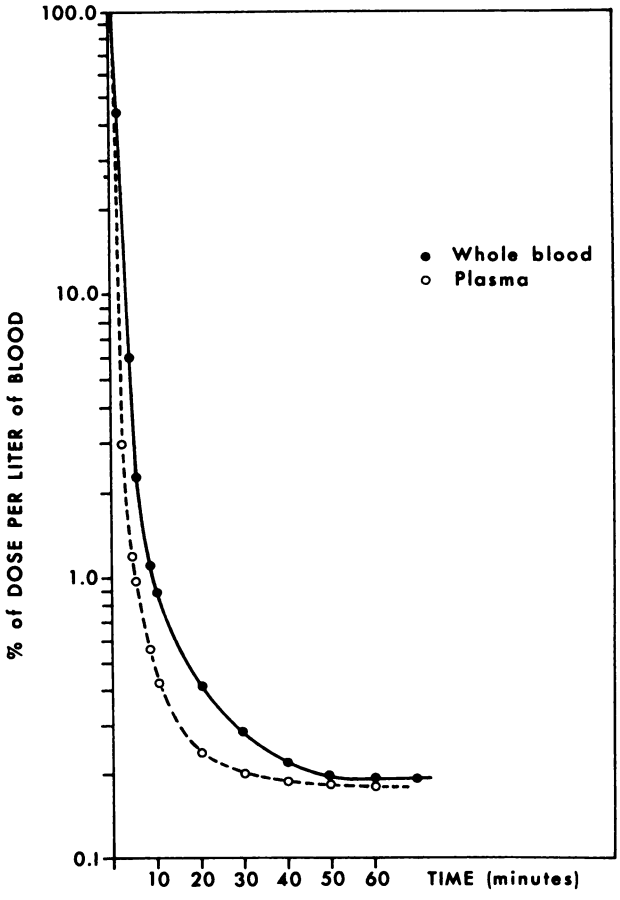

FIG. 8. Clearance of Fe-^{113m}In-labeled particles in humans comparing whole blood with plasma. Slight difference in clearance rate of whole blood and plasma is possible due to adhesion of colloid to red cells.

Because of the physical properties of ^{113m}In, it is possible to administer millicurie doses to the patients, which result in larger counting rates, more rapid scans and greater diagnostic accuracy.

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