The "Critical Colloid Dose" in Studies of Reticuloendothelial Function

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The [^{99m}Tc]sulfur colloid distribution in rat organs was investigated after the administration of different amounts of colloid particles. Saturation of the liver and spleen was not observed. Blood clearance was significantly reduced 15 min after injection above ~$3 \cdot 10^9$ particles per kg body weight. With an increasing number of injected particles, lung uptake increased and bone marrow uptake decreased. Microfiltration studies showed that the colloid is unaffected by dilution with saline but may be affected after incubation in normal rat plasma. We conclude that the distribution of [^{99m}Tc]sulfur colloid in organs varies with the number of injected particles and therefore, is not dependent upon the blood flow to the reticuloendothelial organs alone. The "critical colloid dose" may differ among the reticuloendothelial organs and cannot, therefore, be evaluated by blood clearance measurements alone. The considerable influence of the number of injected colloid particles on bone marrow uptake should also be recognized when carrying out dosimetric calculations.

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A classic approach to the estimation of the functional status of the reticuloendothelial system (RES) is to study the blood clearance of intravenously injected colloidal preparations. It has been shown by Dobson and Jones (*1*) and Biozzi et al. (*2*) that below a certain amount of colloid, called the "critical colloid dose," the blood clearance rate is merely a measure of the liver sinusoidal blood flow, since almost all colloid was cleared by the liver. Above this critical colloid dose, blood clearance was slower. Studies of the distribution of colloidal carbon in experimental animals showed a diminishing relative liver uptake with increasing amounts of colloid while the relative uptake in the lungs and the spleen increased (*2*). The necessity of administering colloids well above the critical dose has been stressed for reticuloendothelial (RE) function studies (*3*, *4*).

It has been pointed out that an optimal RE test colloid should not be taken up by cells other than macrophages (*3*). Smaller colloidal particles (~25 nm), such as colloidal carbon and thorium dioxide, are, however, also taken up by endothelial cells and hepatocytes, and the use of larger particles for RE function studies has, therefore, been advocated (*5*).

At our institution, a noninvasive method has been developed for investigation of the RE function in the rat, using a scintillation camera technique (*6*) and a highly standardized technetium-99m sulfur ($^{99m}Tc_2S_7$) colloid with a median particle size of ~300 nm (*7*). Using this method, a decreased hepatic uptake was demonstrated after the administration of gelatin, a well-known RE-blocking substance, and methyl palmitate, an RE-suppressing agent (*6*,*8*).

The present study was performed in order to investigate the colloid distribution in the RE organs after intravenous injection of different numbers of particles of $^{99m}Tc_2S_7$ colloid and, from the results, assess the critical colloid dose. It is well-known that particle size and plasma proteins can influence the distribution of intravenously administered colloids (*9*). Possible changes in the particle size due to dilution in isotonic saline and interaction with plasma proteins were, therefore, also evaluated at different particle concentrations.

MATERIALS AND METHODS

The gelatin-stabilized $^{99m}Tc_2S_7$ colloid was prepared from potassium perrhenate and sodium thiosulfate as described by Persson and Naversten (*10*). The number of particles in the colloid preparation has been calculated to be ~10^{10} per ml (*7*). The labeling efficiency of the preparations was checked with a gel chromatography column scanning technique (*11*), and was found to be 97–100%.

Fresh colloid preparations were diluted 1:10, 1:100, and

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1:1000 by volume with isotonic saline. Four different relative colloid particle concentrations were thus obtained, i.e., 100% (undiluted colloid), 10%, 1%, and 0.1%. One hour after preparation, the different particle concentrations were incubated 1:5 by volume with freshly obtained heparinized plasma from inbred Wistar rats (n = 10), in a water bath equipped with a sample stirrer, for 15 min at 37°C. In total, ten colloid preparations were made for dilution in saline and/or incubation in plasma. The particle size distribution of each colloid preparation was tested twice, and each plasma-incubated preparation three times.

The particle size distribution was obtained for the different particle concentrations and plasma incubations by microfiltration using Nuclepore polycarbonate membranes (25 mm diameter), held in Swinnex 25 (Millipore) filter holders (7). Samples were filtered through 2–3 filters of each pore size 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 μm (nominal sizes). The actual pore sizes according to the manufacturer were 0.09, 0.19, 0.40, 0.54, 0.71, and 0.86 μm, respectively. Filtration of small colloid aliquots was followed by passage of 2 ml distilled water. The fraction of activity passing each filter was then determined by measuring the activity retained on the filter and the activity passed through the filter. A radioisotope calibrator* or a NaI(Tl) detector was used. The mean fraction of activity passing each pore size was then calculated. Microfiltration will thus, in reality, give the activity-size distribution rather than the particle size distribution.

For the studies of colloid distribution in the organs, each colloid particle concentration was injected into groups of six male inbred Wistar rats. Experiments were performed on three separate days and three colloid preparations were made. The rats were anesthetized with ether. A fine plastic catheter was introduced into the right jugular vein and fixed with its tip in the superior caval vein. One milliliter of the different colloid concentrations was injected during 3–4 sec. After 15 min, a laparotomy was performed and the animal was killed. The liver, spleen, and the right lung were recovered and blood was collected from the abdominal cavity. Bone marrow was aspirated from both femurs. All specimens were weighed and put into plastic tubes. The activity content was measured with the radioisotope calibrator (for samples containing >3μCi>(0.1 MBq) or a well-type NaI(Tl) detector. The total organ uptake and the uptake per gram tissue as percent of injected activity were calculated.

Factors contributing to the total uncertainty when calculating activity content are the uncertainty in the measurement of injected activity (estimated to be ±2% with the radioisotope calibrator), statistical uncertainties in the number of registered photons (<±5% with the well-type NaI(Tl) detector), and the size and shape of the specimens, which will affect the counting efficiency of the detector (estimated to be ±5%). The total R.M.S. uncertainty will then be <±8%. The Kruskal-Wallis one-way analysis of variance by ranks was first used to test if there was any significant difference between the groups. When differences were found with this test, the groups were further analyzed with the Mann-Whitney U-test to establish the significance level. Values of $p < 0.05$ were considered significant.

RESULTS

Microfiltration of the ten colloid preparations showed that 84 ± 5% of the activity was found in the 0.2–0.8-μm range. The median activity size was in the 0.2–0.4-μm interval in five preparations and in the 0.4–0.6-μm interval in five preparations. The mean value of all median activity sizes was 395 ± 65 nm (±s.d.).

The effect of dilution with saline on the colloid particles was evaluated by comparing the median values of each activity-size distribution before and after dilution. Comparisons between colloid preparations and dilutions showed small differences in median activity sizes (< ±18%), with no marked tendency towards a size shift in either direction. The results for each colloid particle concentration have been combined in Figure 1, demonstrating the ranges of percentage of activity passing the different pore sizes.

Plasma incubation of the different colloid particle

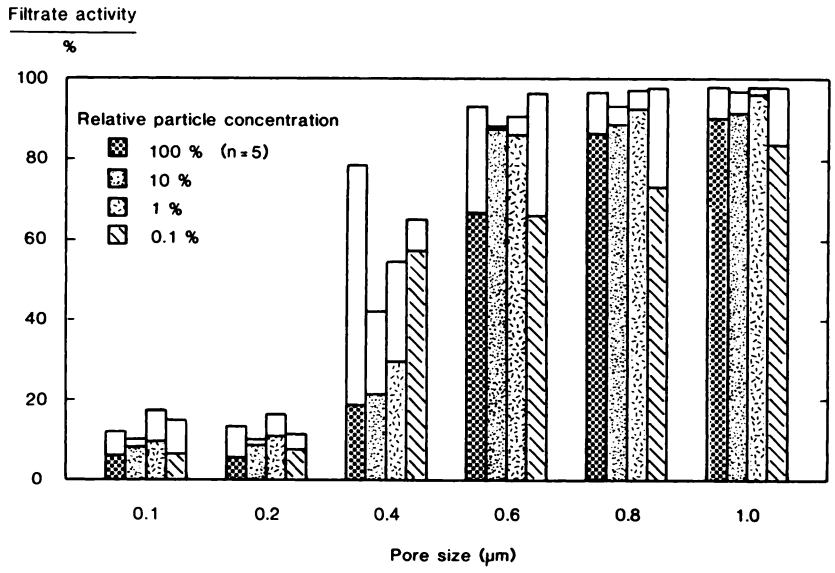

FIGURE 1
Microfiltration of the different $^{99m}Tc_2S_7$ colloid concentrations after incubation in saline. Columns indicate obtained ranges of percentage of activity passing the different filters.

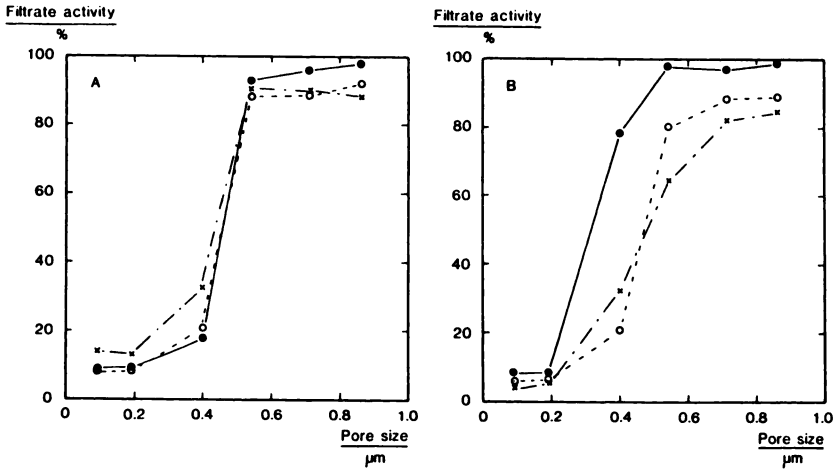

FIGURE 2
Examples of activity-size distributions after colloid incubation in rat plasma. A: (—●—) 100% relative colloid particle concentration, (–○–) 10% concentration, and (–×–) 10% concentration incubated in plasma. B: (—●—) 100% relative colloid particle concentration, (–○–) 100% concentration incubated in plasma, and (–×–) 10% concentration incubated in plasma.

concentrations resulted in more pronounced changes in median activity-sizes. The median activity-size increased in three cases by ~45% after contact with rat plasma, but was within ±15% in the other seven plasma samples. The considerable increase in median values for the three cases was obtained after incubation of three different colloid particle concentrations. The results of the microfiltration of colloid solutions incubated in plasma from two rats are given in Figures 2A and B, and show the observed variations in activity-size distribution. The results regarding activity-size distributions for colloid incubated in plasma are shown in Figure 3, and show the ranges of percentage of activity passing the different pore sizes. Results from the colloid preparations used in the plasma incubation are also included.

Total organ activity uptake in percent of injected activity after 15 min is shown in Table 1, and activity content per gram tissue (specific activity) in Table 2. One animal died before laparotomy and was therefore discarded. The results of the statistical evaluation of specific activities are given in Table 3.

The percentage liver uptake at various administered particle concentrations showed little variation. The values ranged from 80% to 92%, with no significant differences. Neither were any significant differences registered in liver specific activity. The splenic uptake varied greatly between individual animals, but no significant differences were found between the different concentrations.

The bone marrow specific activity was higher in animals receiving a small number of particles than in those receiving higher numbers. The difference between animals receiving the smallest and the highest number of injected particles was significant ($p = 0.005$). Regression analysis of the bone marrow specific activity for the different particle concentrations (Fig. 4) showed a high determination coefficient ($r^2 = 0.81$).

The pulmonary uptake increased with increasing particle number above 1% colloid particle concentration, and the mean uptake at the highest concentration was more than double that at the lowest ($p < 0.05$).

The specific blood activity was significantly higher in animals receiving the 10% and 100% colloid particle concentrations in comparison with the 0.1% and 1% concentrations. This, in turn, means a lower disappear-

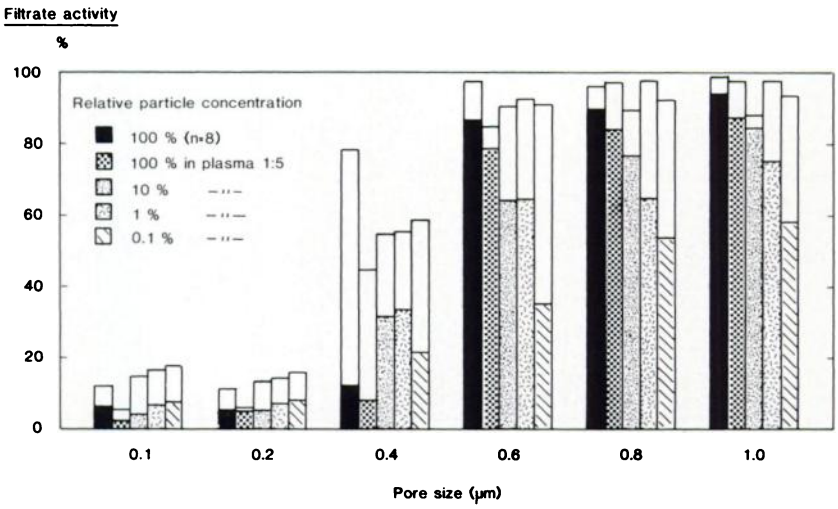

FIGURE 3
Microfiltration of the different $^{99m}Tc_2S_7$ colloid concentrations incubated in normal rat plasma for 15 min at 37°C. Columns indicate obtained ranges of percentage of activity passing the different filters.

TABLE 1
Activity Uptake in Rats at 15 min p.i. for Different $^{99m}Tc_2S_7$-Colloid Concentrations (Mean ± s.d.)

Relative concentration	n	Activity uptake (%) Liver	Spleen	Right lung
100%	6	86.0 ± 2.6	2.36 ± 0.62	0.76 ± 0.44
10%	5	86.7 ± 1.9	2.90 ± 1.06	0.59 ± 0.11
1%	6	88.3 ± 4.3	2.93 ± 1.45	0.29 ± 0.13
0.1%	6	84.1 ± 3.3	2.77 ± 0.75	0.34 ± 0.08

ance rate of the higher particle numbers during the time span of the examination (0–15 min).

DISCUSSION

Many factors which may control the rate of phagocytosis and colloid distribution in organs have been investigated during the last four decades. Some factors are determined by colloid properties, such as particle size, surface charge, antigenic properties, and the number of particles injected (*9*). Other factors are related to the state of the investigated subject, such as blood flow to the various RE organs, plasma levels of opsonins, the presence of specific antibodies, and the presence of RE depression or activation (*4,12*).

The general opinion is that the bone marrow has a preference for smaller colloid particles (*9,13*), and that smaller particles are cleared more slowly by the RES (*4,14*).

The extraction mechanism of [^{99m}Tc]sulfur colloid is still not fully understood, but may partly depend on the particle size. Autoradiographic studies with large-sized [^{99m}Tc]sulfur colloids (diameter >100 nm) performed by Chaudhuri et al. (*15*) and George et al. (*16*) indicate an exclusive uptake in the liver by the Kupffer cells. The latter study showed that extracted colloid particles were attached in groups to Kupffer cell membranes, that might explain why it is difficult to saturate the liver uptake with this colloid. In addition, the authors could not find morphological evidence of endocytosis of an osmium-stained [^{99m}Tc]sulfur colloid. For a smaller-sized [^{99m}Tc]sulfur colloid (~40 nm), Schell-Frederick et al. (*17*) demonstrated a markedly inhibited colloid uptake in isolated rat peritoneal macrophages in the presence of cytochalasin B at 0°C. This indicates an active ingestion of this colloid into the macrophages, i.e., endocytosis.

In the present study, no significant differences in particle size distribution between the different $^{99m}Tc_2S_7$ colloid concentrations were recorded, and the colloid particles thus seem not to be affected by dilution in saline.

Dornfest et al. (*18*) have observed the interaction between [^{99m}Tc]sulfur colloid and serum proteins. Our results indicate that an interaction between $^{99m}Tc_2S_7$ colloid and plasma may occur. A clear change in activity-size distribution was not found in all plasma samples tested, and no distinct pattern could be detected in our experiments. The colloid concentration does not seem to be a crucial factor. Changes in particle size may be explained by variations in plasma proteins among the different animals. A pattern may be correlated to specific plasma proteins and should be tested in further studies.

The concept of the critical colloid dose has recently been challenged by some authors (*19–21*). Bradfield (*20*) demonstrated that hepatic phagocytic depression was detected even with minute amounts of test particles (foreign red blood cells).

In the present study, the relative liver and spleen uptake was constant for varying numbers of injected particles between 10^7 and 10^{10}. Saturation of the liver and spleen was not reached with the particle amounts used. This finding is identical to those published by Haibach et al. (*22*), who investigated the liver uptake of [^{99m}Tc]sulfur colloid in a single-pass perfusion rat liver model, and Bradfield and Wagner (*23*), who studied [^{99m}Tc]sulfur colloid uptake after Kupffer cell blockage in mice. Atkins et al. (*13*), however, demonstrated hepatic saturation and increased bone marrow uptake with large amounts of particles of a small-sized [^{99m}Tc] sulfur colloid. Since colloidal preparations with larger particles usually contain fewer particles per unit volume than preparations with smaller particles (*7*), this may account for the differences in the ability to saturate the liver as reported by the above mentioned investigators. A previously unpublished study by us has shown, however, that hepatic saturation can indeed be produced with the $^{99m}Tc_2S_7$ colloid by repeated injections of large

TABLE 2
Specific Activity Content (%/g) in Rats at 15 min p.i. for Different $^{99m}Tc_2S_7$ Colloid Concentrations (Mean ± s.d.)

Relative concentration	Specific activity content (%/g) Liver	Spleen	Right lung	Bone marrow	Blood
100%	8.90 ± 0.52	4.78 ± 0.84	0.82 ± 0.52	0.36 ± 0.09	0.162 ± 0.049
10%	9.76 ± 0.93	5.62 ± 1.46	0.56 ± 0.11	0.67 ± 0.14	0.144 ± 0.057
1%	9.28 ± 0.77	4.55 ± 1.49	0.30 ± 0.14	0.82 ± 0.12	0.039 ± 0.012
0.1%	8.96 ± 0.61	5.40 ± 1.64	0.37 ± 0.10	1.17 ± 0.23	0.060 ± 0.023

TABLE 3
Statistical Comparison of Specific Activity Contents After Administration of Different $^{99m}Tc_2S_7$ Colloid Concentrations

	p Values (<0.05) of compared particle concentrations					
Tissue	100%-10%	100%-1%	100%-0.1%	10%-1%	10%-0.1%	1%-0.1%
Liver						
Spleen						
Right lung		0.02		0.04	0.02	
Bone marrow	0.008	0.005	0.005		0.02	0.03
Blood		0.005	0.005	0.02		

amounts of particles. But since it also resulted in apparent hemodynamic changes in the animals, the use of such large volumes of colloid is not feasible for studies of RE function.

The blood elimination was significantly reduced 15 min after injection of the 10% and 100% colloid particle concentrations. A corresponding increase in lung uptake was also noted at these particle concentrations. Therefore, it is obvious that the distribution of this colloid varies with the number of particles injected, and is not solely dependent upon the blood flow to the RE organs. These results also indicate that the critical colloid dose was exceeded with the higher particle numbers despite the unchanged hepatic uptake. The injected 10% particle concentration corresponds to $\sim 3 \cdot 10^9$ particles per kg body weight. This can be compared with the number of particles injected in routine liver scintigraphy at our hospital, which is of the order of 10^8 particles per kg body weight.

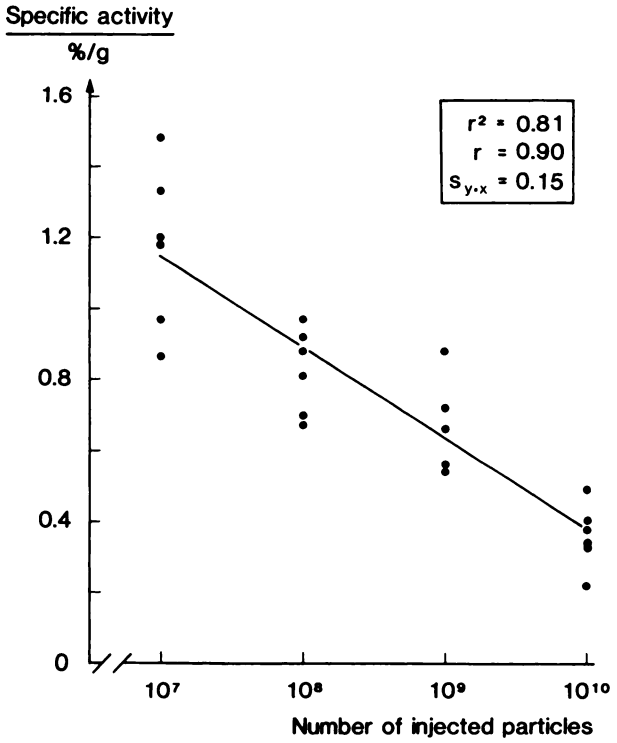

FIGURE 4
Correlation between specific activity uptake in bone marrow (%/g) and number of injected colloid particles.

In contrast to the findings of Atkins et al. (*13*), bone marrow uptake decreased significantly with increasing number of injected colloid particles in our study. Since bone marrow has a preference for smaller colloid particles (*9,13*), it is possible that bone marrow might be saturated by somewhat larger particles, like the $^{99m}Tc_2S_7$ colloid, in an amount not sufficient to produce a saturation of the liver Kupffer cells. Thus, the critical colloid dose may differ for the different RE organs and cannot be evaluated by blood clearance measurements alone.

From a dosimetric point of view, it is of interest to note the considerable influence of the number of injected particles on the uptake in bone marrow and lungs. In our study, the absorbed dose per unit activity will vary by a factor of ~3 in these organs. A concept often used for estimating the probability of inducing somatic or hereditary effects from irradiation of man is the effective dose equivalent (*24*). Red bone marrow and lungs have both been given a high weighting factor of 0.12. Variation in uptake in bone marrow and lungs due to varying numbers of injected colloid particles will thus highly affect the absorbed doses to the organs and affect the effective dose equivalent.

NOTE

* (Capintec CRC-4) Capintec, Ramsey, NJ.

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