Normal Appearance and Reproducibility of Liver-Spleen Studies with Tc-99m
Sulfur Colloid and Tc-99m Microalbumin Colloid

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In order to determine the normal biodistribution and reproducibility of Tc-99m sulfur colloid and Tc-99m microalbumin colloid, each of ten normal subjects was studied twice with each of the two agents. In overexposed delayed analog images of the anterior chest, some thyroid radioactivity was demonstrated in all of 20 microalbumin colloid studies and in none of 20 sulfur colloid studies; lung radioactivity was seen in 19 of 20 (95%) sulfur colloid studies and in none of the microalbumin colloid studies (both p <0.001). Delayed digital images showed that the sulfur colloid gave a higher lung-to-heart ratio, a higher lung-to-liver ratio, and a lower bone marrow-to-liver ratio compared with microalbumin colloid (all p <0.05). In general, reproducibility was poor for both agents. We conclude that while there are some differences between these two radiocolloids, the differences do not indicate a superiority of the newer Tc-99m microalbumin colloid over the established Tc-99m sulfur colloid for liver-spleen imaging.


Tc-99m sulfur colloid was introduced for liver-spleen imaging in 1964 by Harper and associates (1). It quickly became the radiocolloid of choice for liver-spleen imaging, and currently this procedure is one of the more commonly performed in nuclear medicine. However, the normal biodistribution and reproducibility of Tc-99m sulfur colloid have never been evaluated quantitatively.

Recently, a Tc-99m microalbumin colloid was introduced as a possible replacement for Tc-99m sulfur colloid (2). In a direct clinical comparison in normal subjects, we have determined the biodistribution, normal range, and reproducibility of both radiocolloids.

METHODS AND MATERIALS

Normal subject selection and imaging protocol. Five male and five female normal subjects were recruited who had: (a) a normal complete blood count with differential, (b) a normal urinalysis, (c) a normal sedimentation rate, (d) no history of a viral or bacterial infection in the last month, and (e) no history of liver disease. Their age range was 22 to 44 yr (mean 33.2). All gave informed consent.

Each subject was studied four times, twice with Tc-99m sulfur colloid and twice with Tc-99m microalbumin colloid. The studies were paired so that the first two studies included one with each agent and the second pair included one with each agent. The order in which the agents were used in each pair was decided by chance. A minimum of three and a maximum of five days was allowed between studies.

For each study a gamma camera with 15-inch field of view was used and 1.5 mCi of the radiocolloid was injected intravenously in bolus fashion using a blood-pressure cuff. Digital images in the anterior projection were recorded for 30 min following injection (frames in 64 X 64 matrix were collected every second for 30 sec; then every 15 sec for 29.5 min). Beginning at 30 min, delayed analog and digital images were acquired simultaneously for 5 min in the anterior projection for the liver, spleen, and lower lungs, and in the posterior pro-
jection for liver and spleen, and for the lumbo-sacral spine. In addition, an overexposed analog image (exposed until cardiac blood-pool counts were faintly seen) was acquired in the anterior projection for the chest and neck.

**Image analysis.** The anterior analog images of the chest and neck were visually evaluated by one of us (WCK) for the presence or absence of lung uptake (lung density > cardiac density) and thyroid uptake (thyroid density > neck background).

In the digital images regions of interest (ROI) were visually placed over the lung, heart, and liver in the anterior images and over the liver, spleen, and lumbar spine in the posterior images. The liver and spleen ROIs were placed to correspond to the outline of the entire organ; the lung ROI was placed within the lower half of the right lung so as to occupy approximately one third of this lower half; the heart ROI was placed within the cardiac blood pool so as to occupy approximately one third of the cardiac area; and the lumbar ROI was placed over the fourth and fifth lumbar vertebrae. The lung and heart regions of interest were normalized to fixed numbers of pixels: 50 for the lung and 100 for the heart.

The delayed anterior digital images were used to measure the following ratios: (a) lung to liver, (b) heart to liver, and (c) lung to heart; and in the posterior projection: (d) spleen to liver, and (e) bone marrow to liver.

The digital images during the first 30 min were used to measure the following ratios in the anterior projection: (a) heart at maximum over heart at 5 min after peak, (b) heart at max. over heart at 25 min after peak, (c) lung to heart at 10 min, (d) heart to liver at 5 min, and (e) heart to liver at 10 min. In each study the shape of the heart time-activity curve was examined to confirm that a good bolus injection had been achieved.

The relative extraction efficiency during the first few minutes was determined as previously described (3). Briefly, the ratio of liver radioactivity at 2.5 to 3.5 min after injection over the integral of heart radioactivity from 0 to 3 min is calculated for each radiocolloid; then the ratio between the two colloid ratios is determined.

**Statistical analysis.** In determining the mean, standard deviation, and range of results for each radiopharmaceutical, the first study with each agent in each patient was used. These results were also used in comparing the two agents, but this comparison is not as powerful statistically as the following method.

The results of the two studies with each agent in each patient were averaged. Then a ratio was calculated using the average value for each agent in each patient. These ratios were used in determining the mean and standard deviation of differences between the two radiopharmaceuticals for each parameter.

In evaluating reproducibility, the fractional change from the first to second study with each radiopharma-

![Tc-99m-Sulfur Colloid](image1)

![Tc-99m-Microalbumin Colloid](image2)

**FIG. 1.** Posterior views for the two normal subjects who showed greatest change in spleen-to-liver ratio; one was studied with Tc-99m-sulfur colloid and one with Tc-99m-microalbumin. The first sulfur colloid study gave a spleen-to-liver ratio of 0.59, the second gave 1.22. The first microalbumin study gave a spleen-to-liver ratio of 0.78, the second gave 1.22.

ceutical in each patient was calculated and used to determine the mean and standard deviation.

Differences between the two agents in biodistribution and reproducibility were tested by the Student's t-test or the sign test as appropriate.

**Radiopharmaceuticals.** The sulfur colloid kits and the microalbumin colloid kits were obtained commercially. The technetium-99m was obtained from a commercial generator. Eighty six percent of the Tc-99m sulfur particles ranged from 0.1 to 1.0 μ in size (4); the Tc-99m microalbumin particles ranged from 0.2 to 3.0 μ in size (manufacturer's package insert).

Most individual preparations of both radiocolloids were evaluated for labeling efficiency by development of silica gel strips with saline.

**RESULTS**

**Analog images.** In the overexposed anterior chest images, diffuse lung uptake was seen in 19 of 20 (95%) Tc-99m sulfur colloid studies and none of 20 microalbumin colloid studies (p < 0.01). A mild degree of thyroid uptake was seen in all of 20 microalbumin studies and none of 20 sulfur colloid studies (p < 0.01). In properly exposed anterior images of the liver, spleen, and lower lungs, lung uptake of a mild degree could be seen in 6 of 20 (30%) sulfur colloid studies, but in none of the 20 microalbumin colloid studies (p < 0.05). In properly exposed posterior images, the bone marrow was never seen with either radiocolloid.

Figure 1 shows the analog images from the patients showing the least quantitative reproducibility in spleen-to-liver ratio in the posterior projection for Tc-
colloid showed an initial blood clearance ratio (heart at max. over heart 5 min later) that was 80% of the same for microalbumin colloid, a hepatic uptake (liver over heart at 5 or 10 min) that was 76 to 77% of the ratio for microalbumin colloid, and a lung uptake (lung over liver at 10 min) that was 196% of that for microalbumin colloid (all p <0.05). In the delayed images the sulfur colloid showed a bone-to-liver ratio 63% of that for microalbumin colloid, a lung-to-liver ratio 150% of that for microalbumin, and a lung-to-heart ratio 152% of the microalbumin figure. In hepatic extraction efficiency the two agents showed no significant difference.

The results for reproducibility for the first 30 min and for the delayed images are shown in Table 4. There were no significant differences between the two radiocolloids in any parameter and, in general, reproducibility was poor.

**Radiopharmaceutical quality control.** Chromatographic evaluation of the labeling efficiency of sulfur colloid with Tc-99m was performed in 16 of 20 (80%) preparations at 10 to 90 min after labeling; the average efficiency was 99.6% with a range of 99.0 to 99.9%. The labeling efficiency for microalbumin colloid with Tc-99m was determined in 19 of 20 (95%) preparations at 15 to 120 min after labeling; it averaged 99.4% with a range of 98.8 to 99.9%.

**DISCUSSION**

Our results indicate that Tc-99m microalbumin colloid is cleared from the blood and into the liver faster than Tc-99m sulfur colloid during the first 10 to 15 min after injection. By 30 min, however, the levels in the blood are about the same. The bone-marrow uptake of the microalbumin colloid is higher than that of the sulfur colloid, whereas in lung uptake the reverse is true. The reproducibility of all of these measurements is poor for both radioagents. There is consistently some thyroid

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**TABLE 1. NORMAL RADIOCOLLOID PARAMETERS: FIRST 30 MIN**

<table>
<thead>
<tr>
<th></th>
<th>Peak heart</th>
<th>Peak heart</th>
<th>Liver Heart</th>
<th>Liver Heart</th>
<th>Lung Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart 5 min after peak</td>
<td>Heart 25 min after peak</td>
<td>(5 min)</td>
<td>(10 min)</td>
<td>(10 min)</td>
</tr>
<tr>
<td><strong>Tc-99m sulfur colloid</strong></td>
<td>138*</td>
<td>221</td>
<td>31.8*</td>
<td>42.7*</td>
<td>0.126*</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>56</td>
<td>86</td>
<td>9.5</td>
<td>13.3</td>
<td>0.052</td>
</tr>
<tr>
<td><strong>Standard deviation</strong></td>
<td>51–223</td>
<td>103–360</td>
<td>14.2–46.5</td>
<td>17.8–62.8</td>
<td>0.057–0.212</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>169*</td>
<td>232</td>
<td>43.3*</td>
<td>59.1*</td>
<td>0.069*</td>
</tr>
<tr>
<td><strong>Tc-99m microalbumin colloid</strong></td>
<td>43</td>
<td>58</td>
<td>16.6</td>
<td>24.2</td>
<td>0.027</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>78–231</td>
<td>103–318</td>
<td>21.0–73.9</td>
<td>30.6–114</td>
<td>0.026–0.122</td>
</tr>
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* Difference between radiocolloids significant at p <0.05 level.
uptake of Tc-99m from the microalbumin colloid whereas none was seen with Tc-99m sulfur colloid. Since there is no significant difference between the two radiopharmaceuticals in labeling efficiency, some in vivo dissociation of the radiolabel must occur with Tc-99m microalbumin colloid.

A recent comparison of these two tracers in patients demonstrated a significantly higher spleen-to-liver ratio, liver-to-background ratio, and spleen-to-background ratio for Tc-99m sulfur colloid (2). In addition, a slower blood clearance for the microalbumin colloid was postulated because of its smaller particle size, but clearance was not directly measured (2). The difference between the previous and present studies with respect to the spleen-to-liver ratio remains unexplained; the belief that Tc-99m microalbumin colloid is cleared more slowly from the blood is found incorrect when blood clearance is directly measured.

Although Tc-99m sulfur colloid has been widely used in clinical practice for over 15 yr, the appearance of the normal sulfur colloid liver-spleen study has never been carefully defined. Our data indicate that lung radioactivity is occasionally seen on properly exposed anterior images, and bone-marrow radioactivity is rarely seen on properly exposed posterior images. The spleen normally may have up to 50% more radioactivity per pixel than the liver in the posterior view, and has a considerable range of normal size.

One previous study stated that normal splenic uptake in the posterior view was less than that of the liver, but no group of normal subjects was studied (5). Another paper arbitrarily defined a spleen-to-liver ratio near unity (comparable amounts) as normal (6), but it is clear from the present data that a spleen with considerably more radioactivity than the liver, when regions of maximum radioactivity are compared in the posterior view, is within the normal range. A number of reports have correlated increased splenic uptake with disease states. The most
common association in patients without liver disease has been with melanoma (6–9); none of these studies quantitated the spleen-to-liver ratio or used normal controls.

Other reports have dealt with increased lung uptake of Tc-99m sulfur colloid (10–12); again none of these quantitated the ratio between lung and liver. Since normal subjects routinely have a small amount of lung uptake of Tc-99m sulfur colloid—which is always visible in overexposed images—future studies of increased lung uptake should quantitate the lung uptake and compare the results with appropriate controls. The fact that no lung uptake of Tc-99m microalbmin colloid was ever seen in overexposed images supports the hypothesis that the normal lung uptake of Tc-99m sulfur colloid is not related to phagocytosis; phagocytic cells are not considered to reside normally in the pulmonary capillary bed (13). However, the exact mechanism of lung uptake of Tc-99m sulfur colloid remains unknown.

The lack of reproducibility in all parameters for both agents is of interest and unexplained. Variations either in the subject’s intravascular phagocytic system or in the radiocolloids are possible causes. However, it seems unlikely that a normal subject’s phagocytic system would change significantly between closely spaced studies. Detailed evaluation of the individual radiopharmaceutical preparations was not done.

We conclude that the absence of lung uptake and the increased bone-marrow uptake of Tc-99m microalbmin colloid are of interest, but that the higher background radioactivity relative to the spleen and liver (2), and the thyroid uptake, suggest that it should not replace Tc-99m sulfur colloid. Knowledge of the normal range of various parameters in the Tc-99m sulfur colloid liver-spleen study and the degree of reproducibility that can be expected in these measurements should permit improvement in future studies of the biodistribution of Tc-99m sulfur colloid in disease states.

### Footnote
* Gelman ITLC System, Ann Arbor, Michigan.

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### References


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