Iodinated E. Coli 70S Ribosomes as a Radiocolloid of Uniform Particle Size for Lymph-Node and Liver Scanning

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As an example of the use of biologic particles as carriers for radioactive tracers, E. coli 70S ribosomes were labeled with I-125 using chloramine-T. The labeled ribosomes, after treatment with glutaraldehyde, were injected into rabbits either subcutaneously (through the dorsum of the foot) or intravenously (through the ear). After subcutaneous injection, 40% of the activity accumulated in the lymph nodes during the first 5 hr, and the 70S ribosomal particles were shown to remain within the lymphatic system for at least 8 hr. After intravenous injection, 71% of the activity was detected in the liver within minutes by scintigraphic techniques. The effective half-time of the label in the liver from glutaraldehyde-treated I-125-tagged 70S ribosomal colloidal particles is 4–5 hr. No pyrogenic response was observed. Barring any deleterious side effects, the results indicate that biologic cell components of definite dimensions (in this case E. coli 70S ribosomes about 20 nm in diam) could be considered as radiocolloids for lymph-node and liver imaging.

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Various types of radiocolloids—e.g., Au-198, Tc-99m sulfur, Tc-99m Sb_2S_3, [^{197}Hg] S—have been used routinely in nuclear medicine for diagnostic investigations (*1–6*). The size distribution of these radiocolloids is heterogeneous, ranging from 4 to 100 nm (*7*). The results of Ege (*1*), who investigated the usefulness and application of radiocolloids in internal mammary lymphoscintigraphy, indicated that the Tc-99m Sb_2S_3 colloid gave far better results than the Tc-99m sulfur colloid. This variation was attributed to the difference in the size distributions of these two colloids. Other workers (*2–6*) have also indicated that particle size could very well influence the biologic behaviour of a colloid.

At present, one does not know whether a particular colloid is the optimum for a specific scintigraphic procedure. Refinements in techniques and changes in sizes or characteristics of the colloid would enhance diagnostic accuracy. The availability of naturally occurring particles of widely differing, but nevertheless uniform, colloidal size suggested the study of such particles for lymph-node and liver scintigraphy. This communication describes the initial investigation using the bacterial ribosome as a representative biologically occurring colloid having a size of 20 nm (*8*).

MATERIALS AND METHODS

A solution of [^{125}I] Na (c.f. of high specific activity ~15 Ci/mg) was used. All solutions were prepared with Sterile Water for Injection, U.S.P.

Using a variation of the technique of Schlamowitz et al. (*9*), 5 μl (7.5 μM) of highly purified *E. coli* 70S ribosomes was diluted with 100 μl of 0.5 M Tris-acetate buffer, pH 7, containing 5 mM $MgCl_2$ and 0.5 *M* NaCl. The solution was kept in an ice bath. Five μl of 200 m*M* aqueous chloramine-T were added. The reaction mixture was allowed to stand for about 5 min, after which an additional 8 μl of chlora-

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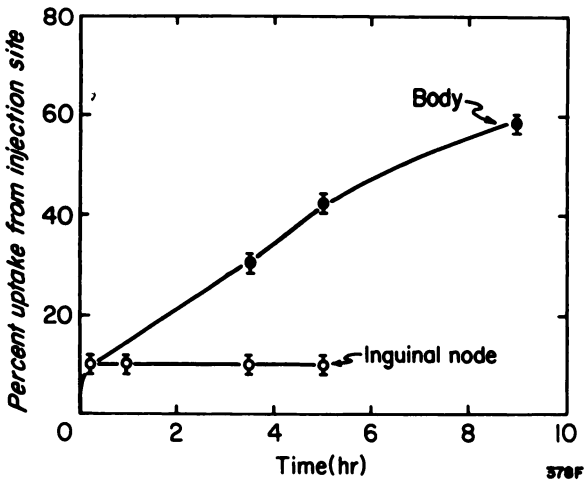

FIG. 1. Percent uptake of I-125-tagged *E. coli* 70S ribosomes from dorsum of right foot of a rabbit. Figures for "body" uptake exclude activity at injection site. Most of radioactivity was in lymphatic system.

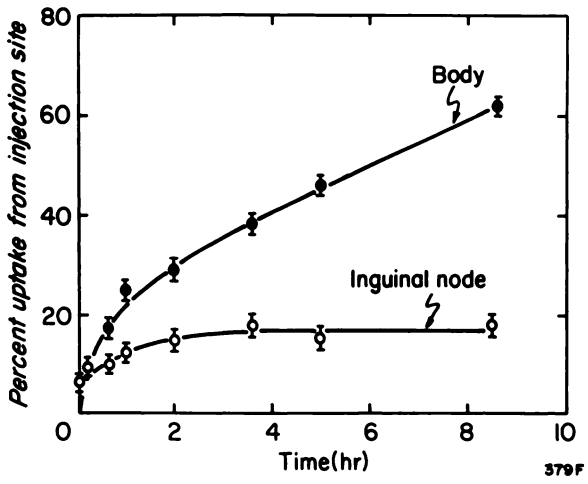

FIG. 2. Percent uptake of Tc-99m antimony sulfide colloid from dorsum of right foot of a rabbit. Figures for "body" uptake exclude activity at injection site. Most of radioactivity was in lymphatic system.

mine-T were added, followed by rapid addition of 250 μCi of $Na^{125}I$ ($\sim$20 μl). The reaction was allowed to proceed for 30 min, with occasional stirring; then 25 μl of 5% aqueous glutaraldehyde were added. After 2 hr, 10 μl of 1.3 M $NaHSO_3$ (prepared in the same Tris-acetate buffer) were added to neutralize excess chloramine-T and glutaraldehyde, and the reaction mixture was chromatographed on a 70- by 4.5-mm Sephadex G-25 column that was previously conditioned with 100 μl 25% HSA solution. Depending on the source of radioactive iodine, 55–82% incorporation of I-125 was achieved. The observed fluctuations in labeling efficiency were due to differing contents of iodate in the labeling material (*10*). The purified, iodine-tagged *E. coli* 70S ribosomes were injected into rabbits either subcutaneously (through the dorsum of the foot) or intravenously (through the ear), and scintigrams of the lymph nodes or the liver were obtained with a gamma camera. For comparison purposes, similar experiments were carried out using Tc-99m antimony colloid (*7*).

Flow of radiocolloid through the lymph nodes and organs was followed on the persistence scope directly with a 35-mm camera. Scintiphotos were made every half-hour for the first 2 hr, and then hourly for the next 6 hr. Still others were taken at longer intervals for several subsequent days. In this way, results could be obtained quickly and experiments interspersed between the normal flow of diagnostic procedures for patients.

RESULTS AND DISCUSSION

Lymph-node imaging. Half an hour after subcutaneous injection in the dorsum of the foot, about 8% of the total activity was detected in the inguinal node. The activity level of the inguinal node remained constant for about 5 hr. No crossover drainage of the radiocolloid to the contralateral inguinal node could be detected. During this time, 40% of the radioactivity was absorbed into the lymphatic system (Fig. 1), and activity could be detected in the popliteal, lumbar, and mesenteric nodes. Injection with Tc-99m antimony colloid gave similar results (Fig. 2). Figure 3 shows the uptake of 70S ribosomal radiocolloid by popliteal, inguinal, lumbar and mesenteric nodes of a rabbit 3 hr after subcutaneous injection in the dorsum of the right foot.

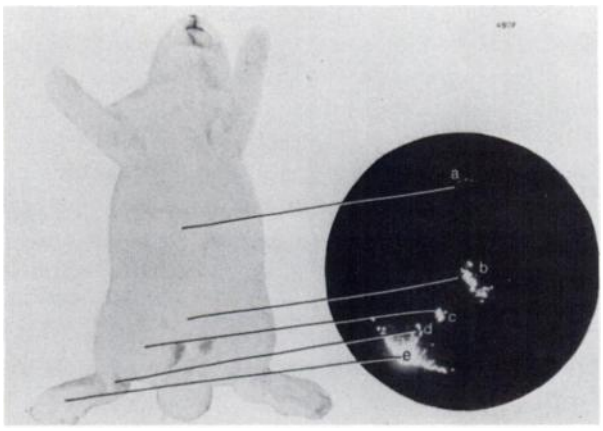

FIG. 3. Scintigram (circular inset) of lower portion of rabbit taken directly from persistence scope of gamma camera 3 hr after subcutaneous injection of I-125-tagged 70S ribosomes in dorsum of right foot of a rabbit. (This procedure sacrificed some resolution but, being extremely rapid, interfered little with the normal flow of patient procedures.) (a) = Some of the mesenteric nodes shown at edge of scope; (b) = Lumbar nodes; (c) = Right inguinal node; (d) = Popliteal node; (e) = Injection site.

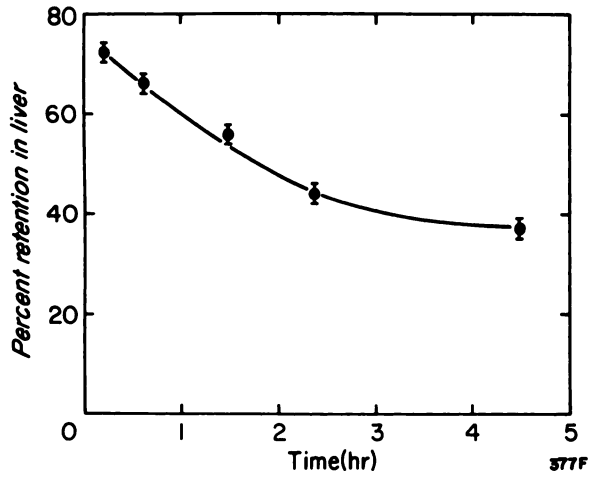

FIG. 4. Percent retention of I-125-tagged *E. coli* 70S ribosomal colloid in the liver of a rabbit.

Liver imaging. Ten minutes after injection of I-125-tagged 70S ribosomes, 71% of the injected radioactivity was found in the liver. Gradually the rabbit's extrahepatic background began to increase, showing breakdown of ribosomal radiocolloid. After 1.5 hr, some activity was detected in the thyroid and bladder regions due to release of I-125. Figure 4 indicates an effective hepatic half-time of 4 to 5 hr for I-125 in glutaraldehyde-treated, iodinated 70S ribosomal colloidal particles. Since I-125 has a physical half-life of 60 days, the measured half-time is an indication of the biologic degradation of the ribosomes and consequent release of radioactivity from the liver. Release of label from liver using Tc-99m antimony colloid (*11*) was observed to be similar at comparable times.

The results indicate that ribosomal particles behave as radiocolloids in vivo, and are potentially useful scintigraphic agents. The ribosomes are readily purified and are stable during the labeling procedure. The final product is a colloid with a particle size of about 20 nm, as determined by electron microscopy. The iodination and fixation had not altered the size from that of untreated ribosomes (*8*). The use of I-125 or I-131 as a label would, however, result in several times the radiation dose required for the currently used Tc-99m antimony sulfide. The objection could be less important as I-123 becomes increasingly available.

Finally, while possible immunologic effects will require further investigation, no pyrogenic responses were observed with the glutaraldehyde-fixed ribosomes. The success of this experiment suggests a new approach by which a series of uniformly-sized radiocolloids could be prepared. Using these readily available biologic particles of various but uniform sizes, the optimum colloid size for a particular application could be determined, and applied to clinical use.

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