LABELING OF TIN-SOAKED ALBUMIN MICROSPHERES WITH ^{68}Ga

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A method is described for the labeling of tin-soaked human serum albumin microspheres with ^{68}Ga. The radionuclide is a short-lived ($T_{1/2}$ = 68 min) generator product and a positron emitter; the labeled particles may be used for perfusion studies with a positron camera. The labeling procedure requires 40 min after elution of the ^{68}Ga generator and provides a labeling efficiency of 90 ± 5%. The in vivo stability of the particles was determined in a series of animal experiments which showed little washout of lung activity over a 2-hr period.

Radioactive albumin microspheres are popular for blood perfusion measurements partly because the particles are formed and sized prior to labeling. The possibility of introducing particles of incorrect size is virtually eliminated. Methods of labeling the pure albumin microspheres and those containing small amounts of iron have been developed for both ^{99m}Tc and ^{113m}In (*1,2*). A labeling kit containing pure human serum albumin microspheres designed for use with ^{99m}Tc has been available for several years (3M Co., St. Paul, Minn.). Our laboratory is investigating applications of the positron camera in clinical nuclear medicine; for this reason we have attempted to label microspheres with the positron-emitting radionuclide ^{68}Ga. This nuclide is short-lived ($T_{1/2}$ = 68 min) and is a generator product by decay of its parent ^{68}Ge ($T_{1/2}$ = 287 days).

Recently the 3M Company introduced for clinical use with ^{99m}Tc a labeling vial containing albumin microspheres coated with tin. The addition of tin to the particles simplifies the labeling procedure and improves the labeling efficiency. This report describes a method for the efficient (90 ± 5%) labeling of the tin-soaked albumin microspheres with ^{68}Ga, so that the product is suitable for human use.

MATERIALS AND METHODS

Cheletes of ^{68}Ga are prepared for human use in this laboratory according to a published procedure (*3*). A ^{68}Ge–^{68}Ga generator (New England Nuclear Corp., Boston, Mass.) is eluted with 0.005 *M* Na_2EDTA, adjusted to pH 7. The generator eluent is mixed with an equal volume of concentrated hydrochloric acid to convert gallium from the EDTA complex into the anionic chloride complex. In this chemical form, gallium is retained on an anion exchange column and is separated from all traces of EDTA by careful washing of the column with 6 *M* HCl. The column is rinsed with a small quantity (0.2 ml) of distilled water to remove most of the hydrochloric acid adhering to the resin; otherwise the eluent would be highly acidic and the final preparation, after neutralization, would be excessively saline. The column is now eluted with 1 ml of bacteriostatic water and the eluent, still acidic (pH 0), is neutralized with dilute sodium hydroxide using phenol red as an injectable acid–base indicator.

The optimum conditions for labeling were determined in a series of experiments in which each of the labeling variables was investigated separately. Since the 3M vials contain 5 mg of microspheres, each test was performed with 5 mg of particles. The labeling vial also contains 6 mg of the detergent Pluronic F-68; most tests were conducted with the same weight of this detergent.

The effect of pH on labeling efficiency was determined by dividing the anion exchange column eluent equally among several vials. Each vial was made up to 1 ml with bacteriostatic water; ½ ml of 0.10 *M* Na_2HPO_4 solution was added and the acidity was adjusted with dilute sodium hydroxide to a different value for each vial in the pH range 2.0–6.0. Half a milliliter of a solution containing 12 mg/ml of Pluronic F-68 was added, followed by 5 mg of the tin-soaked microspheres. The vials were ultrasonicated for 10 min. Following this, the content of each vial was transferred to a centrifuge tube and counted in an ion chamber with the particles still in suspension. The tube was then cen-

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trifuged briefly, the supernatant transferred into another tube, and the centrifuge tube counted again in the same geometry after resuspension of the particles in water. The activity in the supernatant was also determined as a check. The labeling efficiency was taken as the ratio of the activity in the tube containing only the suspended particles to that containing the suspended particles and labeling solution.

The effect of phosphate concentration was established in a similar manner except that the column eluent was first adjusted to pH 2.6–3.0 with dilute sodium hydroxide. The eluent was then separated equally among several vials and solutions containing different concentrations of Na_2HPO_4 (each adjusted to pH 3 with sodium hydroxide) were added to each vial. Bacteriostatic water was added so that each vial had the same total volume. The suspending agent and particles were then added and the vials were ultrasonicated for 10 min. The labeling efficiency was determined as described above.

In determining the effect of sodium chloride concentration on labeling efficiency, the eluent was again adjusted to pH 2.6–3.0 and divided equally among several vials. To each vial was added 0.5 ml of 0.10 *M* Na_2HPO_4 solution (adjusted to pH 3) and varying amounts of a sodium chloride solution so that the salt concentration was different in each. The volume was adjusted with bacteriostatic water to be the same in each vial. The detergent and particles were then added and the preparations were ultrasonicated and analyzed. The effect of $AlCl_3$ concentration was determined in the same manner.

The in vivo stability of the labeled particles was determined in dogs. The labeling was accomplished in 3M labeling vials so that the 5 mg of particles contained 1 mCi of ^{68}Ga. Approximately half of the suspension (500 μCi, 400,000 particles) was injected intravenously into the animal and the remainder used to determine the percent labeling.

The liver and lung regions of the animals were imaged with a positron camera every 30 min for 2 hr. The duration of each count was 1–5 min. Blood samples were taken at the same intervals. The images were processed by determining the number of counts about the liver and lungs in each image. The counts in each region, corrected for physical decay of the radionuclide, were used to establish the in vivo stability of the label.

RESULTS

The effect on labeling efficiency of pH, sodium chloride concentration, etc., is illustrated in Figs. 1–5. In Figs. 1 and 4 each datum point is the result of a single measurement, whereas in Figs. 2, 3, and 5 each point represents an average of up to five measurements.

Figure 1 illustrates the dependency of labeling on acidity, and indicates that the labeling should be performed at a pH between 2.6 and 3.0. As shown in Fig. 2, the optimum concentration of Na_2HPO_4 is about 0.025 *M*; the labeling procedure described below provides for this concentration. The high labeling efficiency obtained in the absence of Na_2HPO_4 indicates that $Ga(OH)_3$, like $GaPO_4$, is capable of labeling the particles, although somewhat less effectively.

The effect on labeling of the known contaminants in the preparation (NaCl and $AlCl_3$) was also determined. The sodium chloride is present following sodium hydroxide neutralization of the excess hydrochloric acid in the eluent. As shown in Fig. 3, salt does act to reduce labeling efficiency. Nevertheless, the 1–2% salt content of the labeling solution does not cause a reduction of more than several percent in efficiency. Furthermore, the labeling efficiency is not improved by evaporation of the eluent to remove virtually all hydrochloric acid prior to neutralization.

A much stronger dependency was found on Al^{3+} concentration. Aluminum is introduced into the preparation from alumina in the generator packing. The use of an anion exchange column reduces aluminum concentration over that present in the

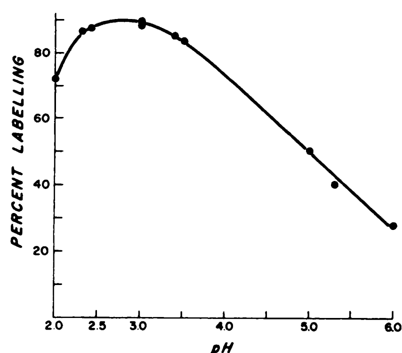

FIG. 1. Effect on labeling efficiency of pH.

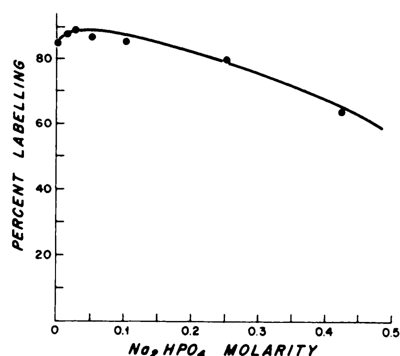

FIG. 2. Effect on labeling efficiency of Na_2HPO_4 molarity.

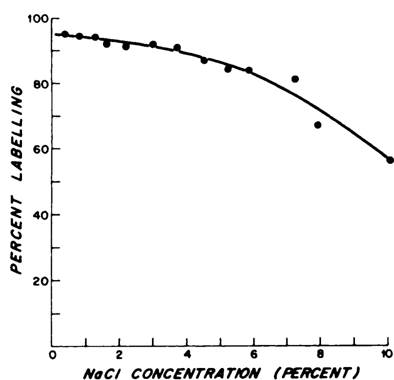

FIG. 3. Effect on labeling efficiency of sodium chloride concentration.

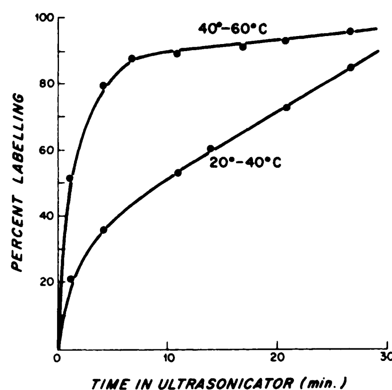

FIG. 5. Effect on labeling efficiency of ultrasonication time for two water temperatures.

generator eluent. An aluminum analysis showed that the generator used in this study on one occasion provided 490 μg of Al^{+3} in 10 ml of eluent. The amount of aluminum retained by the anion exchange column was 0.7 μg, with half of this removed when the column was washed with 0.20 ml of bacteriostatic water. Following this rinse, the 1 ml of eluent contained only 0.4 μg. Despite the strong effect of aluminum on labeling efficiency, shown in Fig. 4, this magnitude of aluminum produces negligible reduction in labeling efficiency.

Water temperature during sonication affects the labeling as shown in Fig. 5. The optimum temperature was found to be 40–60°C. At lower temperatures the labeling proceeds slowly, while higher temperatures affect the labeling efficiency adversely.

Figure 6 presents typical images obtained in dogs 5 min after administration of the radiopharmaceutical (Fig. 6A) and at 30-min intervals up to 2 hr after injection (Fig. 6B–E). Figure 6H shows the location of the regions. These were similar in each study and were adjusted so that each of two boxes would include, respectively, most of each lung, and a third would include a portion of the liver while avoiding the lungs as much as possible. In Fig. 6F and 6G, activity is displayed as apparent height (volumetric display) rather than brightness; in Fig. 6F the image is viewed laterally while in Fig. 6G it is viewed down the major axis.

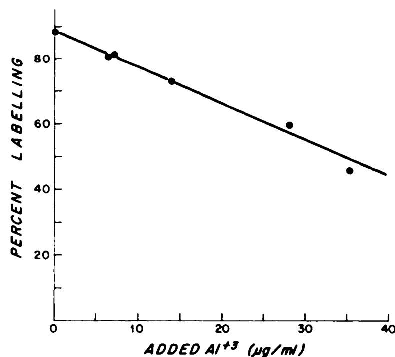

FIG. 4. Effect on labeling efficiency of added Al^{+3}.

Figure 7 summarizes the results obtained in the animal experiments. Six dogs were studied, with the counts obtained in each study being adjusted to the same administered activity (1 mCi). Each point is the average of the six measurements, plus and minus one standard deviation, and is corrected for physical decay. The data points for the lungs are the sums of counts in both lung regions. The blood samples were counted in a calibrated NaI(Tl) well counter so that the results could be expressed in percent of injected activity per milliliter of blood.

The optimal labeling procedure is as follows: The generator eluent, after mixing with an equal volume of concentrated hydrochloric acid, is added to the anion exchange column. The EDTA is washed off with 2 $\times$ 15 ml of 6 *M* HCl contained in glass syringes. The column is rinsed with 0.2 ml of bacteriostatic water to reduce the amount of residual acid introduced into the labeling solution. The column is then eluted with 1 ml of bacteriostatic water (Abbott Laboratories, Chicago, Ill.) and the eluent collected through a Teflon angiocath needle (Desert Pharmaceutical Co., Sandy, Utah) into a vented sterile vial. The acidity is reduced in the vial to pH 2.6–3.0 with a sterile solution 0.05 *M* in Na_2HPO_4, 1 *M* in NaOH, and containing 20 μg/ml of phenol red. The color change of the indicator (pink to yellow) is used to establish the pH. Approximately 0.7 ml is required. The final acidity may be checked conveniently by removing a drop of the solution with a 1-ml syringe and transferring it to a flat surface for measurement with a combination pH electrode. The content of the vial is then removed with a sterile syringe and filtered through a sterile 0.22-micron filter into the 3M labeling vial. The labeling vial is ultrasonicated (Ultrasonic Bath, 3M Co.) in water at 40–60°C for

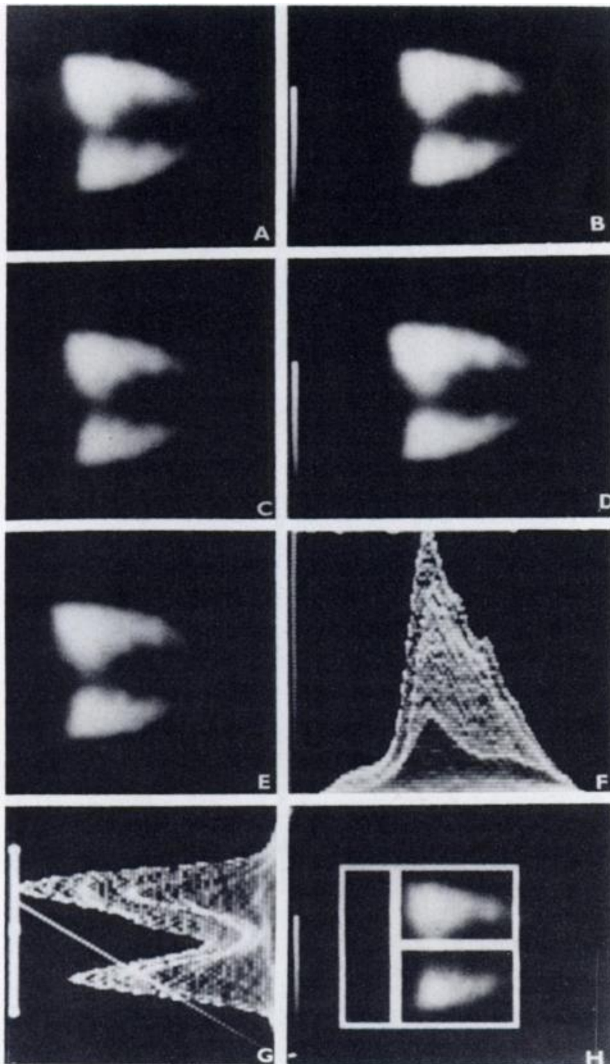

FIG. 6. (A–E) Lung images obtained at 30-min intervals from 5 to 125 min after injection. (F–G) Volumetric presentation of lung activity. (H) Lung image showing positions of regions.

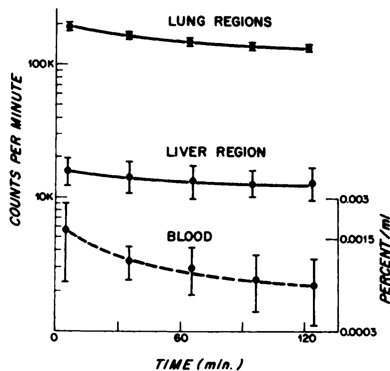

FIG. 7. Counts in lung and liver regions taken at 30-min intervals for 2 hr (left scale). Percent of injected activity per milliliter of blood (right scale). Averages of six measurements, with standard deviation, corrected for physical decay.

15 min, with regular shaking to complete the preparation.

The labeling efficiency is 90 ± 5%; approximately 5% of the activity remains on the filter and 10% remains in each of the two vials. The procedure requires 40 min after generator elution and the overall yield, with decay correction, is 65 ± 5%. The preparations have been found to be sterile and pyrogen-free. Microscopic examination of the labeled microspheres shows them to be unaffected by the labeling.

DISCUSSION

A method for the efficient binding of ^{68}Ga to tin-soaked human serum albumin microspheres has been described. The preparation is 1–2% in sodium chloride, has a pH of 3, and contains 10 ± 5% of free activity in solution. The in vivo stability of the label is high, at least up to 2 hr following injection—a time sufficiently long for perfusion studies. The free gallium activity did not materially affect the quality of the images obtained with this agent, provided the preparations contained less than about 15% free activity in solution.

Lung perfusion images using the positron camera may be obtained in patients with 0.5 mCi of ^{68}Ga microspheres. On the assumption of 90% labeling efficiency, the dose to the adult lungs is 1.3 rads, the dose to blood is 36 mrads, while that to the whole body is 12 mrads, based on values from the MIRD tables (*4*). Three-dimensional reconstruction of lung perfusion images with existing positron scintigraphic devices (*5*) may require twice this activity with a corresponding increase in radiation exposure.

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