# PREPARATION OF $^{125}$I-LABELED MICRO-AGGREGATED HUMAN SERUM ALBUMIN FOR USE IN STUDIES OF RETICULOENDOTHELIAL FUNCTION IN MAN

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It is well documented that an estimate of the phagocytic capacity of the reticuloendothelial system can yield information that is quite useful in managing a variety of pathologic states (*1–3*). Indeed, it has been suggested that this determination may have clinical usefulness as an indicator of the extent or activity of disease in patients with Hodgkin's disease (*4*) as well as in certain infectious diseases such as pneumococcal pneumonia, typhoid fever, sandfly fever and tularemia (*5*).

However, the characteristics of an ideal test agent for this determination are quite specific. Not only is it necessary for the material to be safe from an immunologic and microbiologic standpoint, but also its plasma clearance in normal states must be both predictable and uniform. If it were otherwise, the annoying and often impractical assessment of clearance times in normal subjects would need to be completed each time a study in a diseased subject is required. The radiopharmaceutical product prepared in the manner described in this communication adequately fulfills these requirements. Several hundred clinical studies have been performed using this material without untoward effect.

## PREPARATION OF MICROAGGREGATES OF ALBUMIN

It was apparent at the outset that a batch of microaggregated albumin large enough to last at least a year should be prepared to determine long-term stability of the protein and to avoid needless evaluation of clearance rates in normal subjects. This same batch of microaggregated albumin would then be used as a diluent for the radioiodinated protein prepared from this batch. The procedure described by Iio and Wagner (*6*) was used to prepare 10 liters of aggregates. Thus 250.0 gm of Normal Human Serum Albumin U.S.P. was diluted to 10,000 ml with Sodium Chloride Injection (without preservative) U.S.P. The *p*H of the diluted albumin was then adjusted to 10.0 with 2*N* NaOH after which the albumin solution was subdivided into 50-ml aliquots in serum vials and placed in an oscillating water bath previously stabilized at 79°. The vials containing the albumin were immersed in the bath for 30 min while they were subjected to vigorous shaking at 100 oscillations/min.

At the end of this time the albumin was removed from the bath, cooled to 25° and pooled once again in a Pyrex flask. The *p*H was then adjusted to 7.5 with 2*N* HCl, after which clarifying serial filtrations were performed through 1.2, 0.8 and 0.45-micron membrane filters (*7*). Finally the batch was sterilized by filtration through a 0.22-micron membrane filter (*7*) and aseptically repackaged into 20-ml U.S.P. Type I glass serum vials (*8*). The microaggregated albumin must then be stored at 4° in a refrigerator.

**Control testing.** Pyrogen testing, according to U.S.P. methods (*9*), was performed at a test dose of 75 mg/kg. A U.S.P. sterility test was also made on a statistically significant number of vials of finished product. Total protein in the product was determined spectrophotometrically using the method of Weichselbaum (*10*).

## RADIOIODINATION OF MICROAGGREGATED ALBUMIN

At intervals determined by clinical needs, small aliquots of the microaggregated albumin were radioiodinated with $^{125}$I. This isotope of iodine was chosen because of its convenient physical half-life. Even more important, the absence of beta emission in the decay scheme of this radionuclide suggested that radiation-induced damage to the protein during periods of storage would be minimized. Our subsequent experience in the testing and use of this compound has confirmed this belief.

Stability studies using radiochromatographic methods indicated that it would be possible to use a

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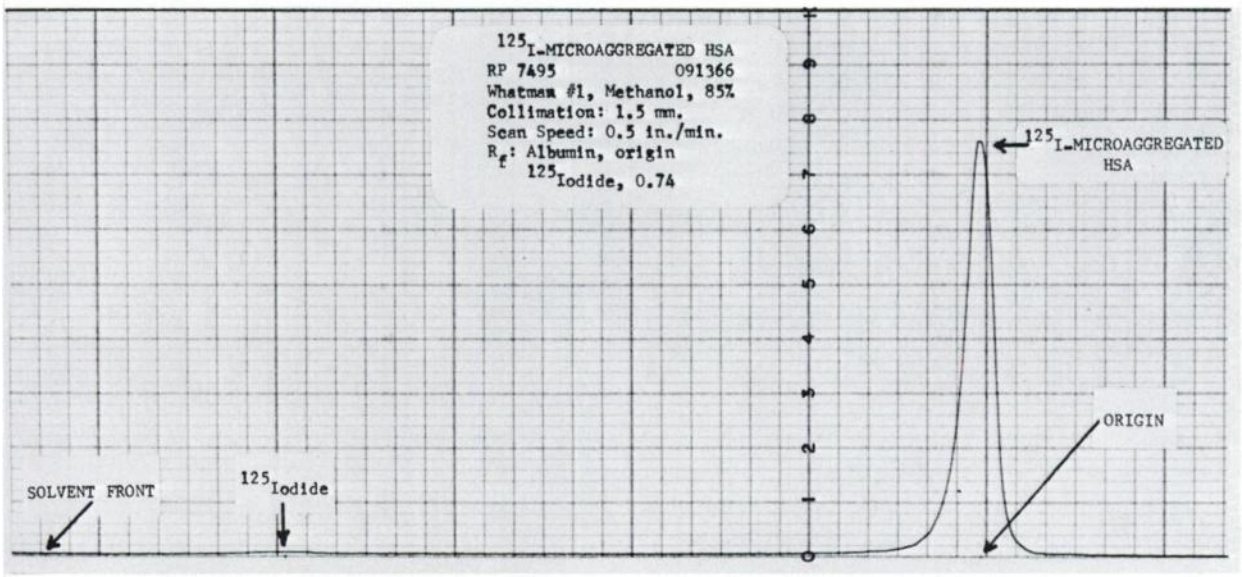


**FIG. 1.** Original radiochromatogram of $^{125}$I-microaggregated HSA (RP7495) on day of manufacture.

batch of labeled microaggregates for a period of 10–12 weeks without significant denaturation of the protein due to self-irradiation effects. Figure 1 shows a scan (*11*) of a paper chromatogram of radioiodinated microaggregated albumin performed on the day of preparation; the amount of nonorganically bound $^{125}$I present in the product is less than 1%. Figure 2 shows a scan of this same batch of the product performed some 14 weeks after its preparation. Little change is noted in the chromatographic behavior of the compound after more than 3 months of storage at 4°.

**Reagents and materials.** The iodination method we use is a modification of McFarlane's iodine monochloride system (*12*). In this procedure $^{127}$ICl is equilibrated in an exchange reaction with Na$^{125}$I and then reacted with the microaggregated albumin. The iodine monochloride reagent used in this reaction is prepared according to a method described by Bale (*13*) and results in a stock solution which is 0.02*M* in ICl, 2.0*M* in NaCl, 0.02*M* in KCl and 1.0*M* in HCl. It can be prepared by dissolving 0.5550 gm of KI, 0.3567 gm of $KIO_3$ and 29.23 gm of NaCl in approximately 150 ml of Water For Injection without preservative U.S.P. To this solution is then added 21.0 ml of concentrated HCl (specific gravity 1.18) and sufficient Water For Injection without preservative U.S.P. to make the final volume 250.0 ml. Free iodine which may be present can be removed by repeatedly shaking this solution with a few milliliters of $CCl_4$. Dissolved or suspended $CCl_4$ may then be removed by bubbling a current of air saturated with water vapor to volatilize the organic solvent. If carefully prepared, this stock solution contains 2.5 mg of iodine per ml and remains stable for at least 6 months when stored at 4° in a tightly closed container. If dilutions of the ICl are required for a procedure, a solution of 2.0*M* NaCl in Water For Injection without preservative U.S.P. should be used.

A borate buffer of *p*H 8.0, also necessary in the procedure, can be prepared by dissolving 4.676 gm of NaCl and 6.184 gm of $H_3BO_3$ in sufficient Water For Injection without preservative U.S.P. to make the final volume 250.0 ml. The *p*H is then adjusted to 8.0 with 1.60*M* NaOH. This results in a stock solution which is 0.32*M* in NaCl, 0.40*M* in $H_3BO_3$ and approximately 0.04*M* in NaOH.

The sodium radioiodide-$^{125}$I solution (*14*) to be used must not contain reducing agents or bacterial preservative because these substances seriously interfere with the iodination reaction. In addition, the concentration of the solution must be at least 50 mc $^{125}$I/ml if efficient labeling is to be carried out.

The only other materials necessary for this procedure include a sterilizing membrane filter of 0.22-micron porosity (*15*), Amberlite IRA-400 anion exchange resin (chloride form, 20-50 mesh) (*16*), Sodium Chloride Injection U.S.P. without bacterial preservative (*17*), a sterile-disposable syringe of 12-ml capacity (*18*) and three glass test tubes.

The procedure is carried out in the following way: the anion exchange resin is prepared by hydrating it in Sodium Chloride Injection without preservative (*17*) for 1 hr. A volume of approximately 6-ml (wet) of this resin is then packed in the dispos-

able syringe with a two-way stopcock attached to the tip of the syringe. An additional volume of 50 ml of Sodium Chloride Injection without preservative is then passed through the column. To test tube #1 is added 30 mg of microaggregated human serum albumin and 1.5 ml of borate buffer. The required amount of $Na^{125}I$ and 1.5 ml of borate buffer are placed in test tube #2, and sufficient ICl (suitably diluted in 2*M* NaCl so the final volume is approximately 0.5–1.0 ml to carry out the reaction is placed in test tube #3. All tubes are then chilled at 4° for 15 min. Then in rapid sequence the contents of tube #3 are transferred into tube #2, and the combined contents of tube #2 are transferred to tube #1. The final mixture in tube #1 is then allowed to stand for 3 min at ambient temperature (approximately 25° in our laboratory). The contents of tube #1 are then passed through the ion exchange column in the syringe at a rate of 15-20 drops/min while the flow rate is regulated with the stopcock.

The amount of nonorganically bound $^{125}I$ in the solution is then determined by radiochromatographic means. Either paper-strip (*19*) or thin-layer (*20*) chromatography can be used; the solvent system used in both systems is 85% methanol. One microliter of the product is spotted at the origin of the paper strip or TLC medium and allowed to develop for a suitable period of time. Paper chromatography requires 2–3 hr for sufficient solvent flow while TLC requires only 15–20 min. The $R_f$ of the $^{125}I$-microaggregated albumin in both systems is at the origin; free $^{125}I$-iodide moves to an $R_f$ of 0.73–0.75 on the paper and remains with the solvent front on the TLC medium. The chromatogram is then scanned on a radio-chromatogram scanner (*11*). If the level of free iodide is 1% or less, the product is sterilized by filtration through a 0.22-micron membrane filter (*15*) into a sterile U.S.P. Type 1 glass vial (*8*). If the free iodide exceeds 1%, the iodinated albumin solution is subjected to dialysis against Sodium Chloride Injection without preservative U.S.P. for 1 hr before sterile filtration. Specific activities of batches prepared in this fashion have ranged from 100 to 200 μc/mg of albumin. Particle-size analysis by ultracentrifugation techniques show a major component (73%) of 80 millimicron and a minor component (27%) of 20 millimicron size.

**TABLE 1. HALF-TIMES OF $^{125}I$-MICROAGGREGATED HUMAN SERUM ALBUMIN PLASMA CLEARANCE IN 10 NORMAL CONTROLS**

| Dose (mg/kg body wt) | Half-time (min) |
|---|---|
| 0.03 | 5.8 ± 0.2* |
| 5.0 | 14.8 ± 0.2* |

* Mean standard error.

**Control testing.** The final product is subjected to pyrogen testing according to U.S.P. methods (*9*) at a dose level at least 10 times that which would be used in humans; it is also subjected to sterility testing using Fluid Thioglycollate and Fluid Sabouraud Media. A radionuclidic assay per unit volume of the finished product is also performed. The product is then stored at 4°.

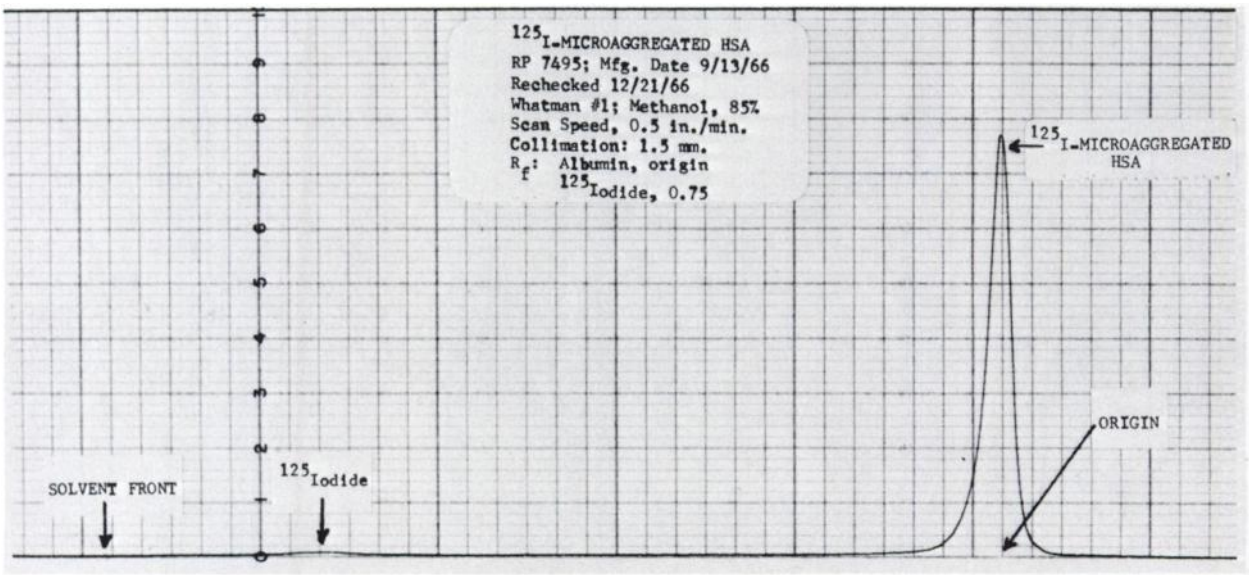


**FIG. 2.** Radiochromatogram of $^{125}I$-microaggregated HSA (RP7495) after 14 weeks storage at 4°.

### DETERMINATION OF PLASMA CLEARANCE

The normal plasma half-life for a batch of microaggregated human serum was obtained by performing clearance studies in 10 normal subjects (two women and eight men) ranging in age from 21 to 35 years. These studies were performed on individual groups of two or three volunteers over the 15-month period during which this batch was used. The results of these determinations, shown in Table 1, indicate no statistically significant shift of clearance time in these controls. Either further aggregation or deaggregation of the albumin after initial formulation would have resulted in progressive changes in the normal clearance rate (*4*).

Each subject received two doses of $^{125}$I-microaggregated albumin on each of two successive days: a 0.03-mg/kg dose to assess changes in perfusion of the RES and a 5.0-mg/kg dose to measure actual phagocytic capacity of the system (*5*). Each dose, whether 0.03 or 5.0 mg/kg, contained 10 $\mu$c of the $^{125}$I-labeled microaggregated albumin. Lugol's solution was administered to each subject before each dose to block thyroidal uptake of the $^{125}$I.

Three milliliters of heparinized blood were drawn 5, 6½, 8 and 10 min after the injection of the 0.03-mg/kg dose and 5, 9, 13 and 17 min after the 5.0-mg/kg dose. All blood samples were centrifuged to remove plasma, which was then passed through an Amberlite IRA-400 anion exchange resin (*16*) to remove nonorganically bound $^{125}$I. Plasma proteins were then precipitated with 10% sodium tungstate and 2/3*N* sulfuric acid. After centrifugation the spun sample was counted in an automatic gamma well counter.

Plots of plasma activity as a function of time were straight lines on semilogarithmic paper, thus permitting determination of the half-life of plasma clearance. Values for the 0.03-mg/kg and the 5.0-mg/kg doses on given days were averaged.

### DISCUSSION

The entire formulation procedure as well as subsequent dose preparations are carried out in a sterile glove box. All equipment and glassware used in the procedure are rendered pyrogen-free by some suitable means, and extreme care is used in the process to avoid introduction of pyrogenic contaminants. Unless otherwise specified, all reagents used are analytical reagent grade. Repeat paper or TLC determinations of free $^{125}$I are performed each 2 weeks on the stock $^{125}$I-microaggregated albumin to assure stability.

### SUMMARY

A simple method of preparing microaggregated human serum albumin labeled with $^{125}$I for use in RES function studies has been presented. Although an appreciation of proper radiopharmaceutical formulation techniques is required, no expensive or complex equipment is necessary to prepare this material. When properly formulated, the product has been shown to be safe and efficacious in more than 400 tests at this institution.

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