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Thompson

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[54] RADIOACTIVE MACROAGGREGATES OF SERUM ALBUMIN

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[58] **Field of Search** 424/101, 1

[56] **References Cited**

OTHER PUBLICATIONS

Journal of Nuclear Medicine 5:259– 275(1964)–Applicant's Exhibit A

Colloidal Radioalbumin Aggregate for Organ Scanning pages 1– 8 Applicant's Exhibit B (Taplin et al. 1963)

Chemical Abstracts Vol. 60 1964, pp. 7056

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[57] **ABSTRACT**

Human serum albumin is made radioactive, denatured, and then agglutinated to form macroaggregates of a nearly uniform size. This latter is achieved by the use of weak organic acids. Their uniform size makes them more suitable for scintiscanning.

5 Claims, No Drawings

RADIOACTIVE MACROAGGREGATES OF SERUM ALBUMIN

This invention relates to radioactive macroaggregates of human serum albumin and particularly to a narrow size range useful for diagnostic scanning of organs of the human body.

It is well-known that radioactive macroaggregates of human serum albumin offer important advantages for diagnostic purposes involving scintiscanning techniques. Radioactive macroaggregates of from 5 to 100 microns have been injected into the antecubital vein so that the blood will carry them to the lung where they will be filtered out temporarily. In localized areas of the organ in which blood flow has been totally or partially blocked, there will be a corresponding absence of the radioactive macroaggregates. The degree of absence or presence of these macroaggregates is visualized with the automatic scintillation scanner.

The technique informs the physician about the presence of embolisms or other abnormal blood flow conditions in the organ being investigated. One article reporting on this diagnostic procedure is "Lung Scanning with Radioiodinated Macroaggregates of Human Serum Albumin" by Leonard Rosenthall, M.D. Journal de l'Association Canadiene des Radiologistes" Vol. XVI, Mar. 1965.

The human serum albumin used for organ scintiscanning in the past was made radioactive and then converted into macroaggregates by procedures which have been publicly described. One such disclosure was by G.V. Taplin et al. in connection with a Scientific Exhibit at the 11th Annual Meeting of the Nuclear Medicine Society in Berkeley, California in June 1964. At that time he released a manuscript entitled "-Human Lung Scanning with Macroradioalbumin-Aggregates," in which he stated that he started with a commercially available radioiodinated I^{131} human serum albumin (sold as Albumatope by E. R. Squibb and Sons) and adjusted it by the addition of NaOH to a pH of 10 ± 0.5; this required 0.8 ml. of a sterile 0.2 normal NaOH per 10 ml. of a 1 percent solution of the radioalbumin in physiological saline. This solution was then heated in a water bath at 79° C for 20 minutes during continuous mild agitation, and was then cooled below room temperature by immersion in ice water to arrest the denaturation process. Up to this stage no precipitation was observed.

Taplin et al., then treated this solution with HCl to convert the denatured albumin to macroaggregates. To do this, the pH was reduced to 5.0 by adding sterile 0.2 normal HCl (1.3 ml. per 10 ml. of the albumin solution). This suspension was then heated to 79° C for 3 to 5 minutes to produce macroaggregates of the radioalbumin.

A range of particle size of 5–25 microns was stated. However, careful tests of this procedure have shown it to be inconsistent and that some macroaggregates of much larger size are produced.

Macroaggregates which are larger than 30 microns can be used for lung scintiscanning without causing a serious embolism but they are not advisable for brain scintiscanning because of the possibility of permanent nerve injury due to prolonged oxygen depletion. For safe use in brain diagnosis the macroaggregates should be nearly 100 percent in the 5 to 30 micron range. Particles smaller than 5 microns flow too freely through the brain capillaries and do not become arrested sufficiently in a normal vascular bed to show up in the scanning apparatus. Particles larger than 30 microns involve the possible risk of an embolism and consequent brain damage.

In accordance with our invention, we have found that if the HCl of the Taplin et al process is replaced with a weak organic acid, such as acetic, citric, or lactic acid, the macroaggregates which are produced fall almost entirely within the desired 5 to 30 micron range. The cooled, denatured solution is adjusted to pH 5.1 ± 0.2 by the addition, during constant shaking over a 2 to 3 minute period, of sterile 0.2N acetic acid; this requires about 1.0 ml. of the acetic acid solution per 10 ml. of the albumin solution. This acidified mixture is heated for 240 ± 20 seconds at $79 \pm 2°$ C and the formation of macroaggregates is arrested by immersion of the flask in cold water preferably containing ice, while being occasionally shaken. Before sampling the contents it should be vigorously agitated for 30 to 60 seconds.

All solutions, including the initial radioactive albumin solution, preferably are in physiological NaCl so that the final product is physiologically acceptable for injection. The process can be carried out with sterile water but NaCl should be added to it to bring it up to a U.S.P. saline injection, before it is injected. The use of weak organic acids, allows for the addition of excess acid so that a buffer system is set up with the salt of the neutralized NaOH and the selected acid, and this may account for the consistently uniform size of the macroaggregate within the narrow range of 5 to 30 microns.

Although a 1 percent radioactive albumin solution is preferred, it may range from 0.1 to 1.5 percent correspondingly less or more of the NaOH solution and weak acid will be required; the pH and time and temperature remaining unchanged. Although the acid is preferably a 0.2 normal solution, it can be of a higher or lower concentration, and correspondingly less or more of it would be added to attain the pH range.

The macroaggregates of this invention serve to produce the necessary transient blockage to blood flow through an organ such as the lung or the brain so that the resulting degree of presence or absence of the radioalbumin will show up in the scintiscanning apparatus. As the macroaggregates are of small size they are broken down in that organ to smaller sizes which pass through the vascular bed before any damage occurs due to an embolytic effect. The ultimate small sizes are removed by phagocytosis through the reticuloendothelial system, principally in the liver and spleen and are thus eliminated from the system.

The diluent employed in forming the pharmaceutical preparation to contain the radioactive macroaggregates may be such materials as sodium chloride injection U.S.P. or distilled water. A suitable pharmaceutical preparation would contain, for a single injection, about 100–300 microcuries in a volume of 10 ml. and this will require about 1–10 mg. of the radioactive albumin.

The following examples illustrate preparations of this invention. The examples are illustrative only, and the invention should not be construed as being limited thereto.

EXAMPLE 1

Macroaggregates of iodinated I^{131} Serum Albumin Particle Size 5-30u.

10 Ml. of a sterile 1 percent solution of iodinated I^{131} serum albumin U.S.P. is adjusted to pH 10 ± 0.5 by the addition of sterile 0.2N NaOH (0.6 ml. of NaOH solution per 10 ml. of albumin solution). The mixture is heated 20 minutes at 79° C. with continuous shaking then cooled for 10–15 minutes in an ice-water bath with occasional shaking. The pH is adjusted to $5.1 \pm .2$ by the slow addition (2–3 minutes) of sterile 0.2N acetic acid (1.0 ml. of the acetic acid solution per 10 ml. of the albumin solution). The mixture is reheated 4 minutes at 79° C and cooled as above. The preparation is shaken vigorously for 30–60 seconds and then sampled by sterile technique and the particle size, particle count, sterility and lack of free I^{131} assayed. The assay showed 11.6 ml. of macroaggregated iodinated I^{131} serum albumin mixture of nearly 100 percent particle size 5–30 μ. To prepare it for injection, sufficient NaCl should be added to constitute a physiological saline solution which will not be irritating.

EXAMPLE 2

Under sterile conditions place in a sterile vial 6.0 ml. of sterile 1 percent iodinated I^{131} serum albumin U.S.P., 24.0 ml. of steride 1 percent serum albumin in physiological saline. Add to it during mild agitation 1.8 ml. of sterile 0.2N NaOH in physiological saline to adjust to pH $10 \pm .5$. Stopper with a sterile closure and cap. Place inside appropriate shielding.

Heat the mixture at 79° C (water-bath) for 20 minutes with continuous shaking. Cool in an ice bath for 10–15 minutes with occasional swirling.

Adjust the pH to 5.1 ± 0.2 by the addition over 2 to 3 minutes of 3.0 ml. of sterile 0.2N acetic acid U.S.P. in physiological saline with continuous shaking.

Heat the suspension for 4 minutes at 79° C with continuous shaking. Cool in an ice bath at 5° C. Shake 30–60 seconds vigorously and sample. Provides 34.8 ml. of a pharmaceutical preparation of macroaggregated I^{131} serum albumin which is nearly 100 percent in the 5–30 μ range.

EXAMPLE 3

Following the procedure of Example 2, 0.2N citric acid U.S.P. in sodium chloride injection U.S.P. is used in place of acetic acid and it gives a suitable stable radioactive preparation of the desired particle size in a yield of 34.8 ml.

EXAMPLE 4

Instead of the acetic acid of Example 2, 0.2N lactic acid in physiological saline is used.

The invention has been described with particular reference to macroaggregates of the serum albumin which have been made radioactive by the use of iodine 131. The invention can be practiced as well by using other known radioactive elements such as iodine 125, chromium 51 and technetium 99^{m}. Such radioactive serum albumin can be substituted in the above examples.

What is claimed is:

1. The method of producing macroaggregates of previously NaOH denatured radioactive human serum albumin which comprises the slow addition over a 2 to 3 minute period of a weak organic acid selected from the group consisting of acetic, citric and lactic acids to a pH 5.1 ± 0.2, heating the mixture for 240 ± 20 seconds at 79 ± 2° C and cooling in ice water.

2. The method of claim 1 wherein the acid is acetic acid.

3. The method of claim 1 wherein the acid is citric acid.

4. The method of claim 1 wherein the acid is lactic acid.

5. An injectable pharmaceutical preparation comprising a suspension in physiological saline solution of radioactive macroaggregates of human serum albumin, which in size are substantially 100 percent in the 5–30 μ range and containing 100–300 microcuries per 10 ml., said macroaggregates being prepared by the process of claim 1.

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