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Reprinted from

International Journal of Applied Radiation and Isotopes, 1969, Vol. 20, pp. 115–119. Pergamon Press. Printed in Northern Ireland

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1 1969

PERGAMON PRESS
OXFORD NEW YORK LONDON PARIS

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(*Received* 1 *June* 1968)

A technique is described for the preparation of human serum albumin macroaggregates, which can be supplied by the CNEA and labelled with Indium-113*m* by the users, to be applied in lung scintiscanning.

Particle sizes range from 10 to 150 μ.

The labelling technique is simple and insumes 10 min. The final preparation is sterilized by autoclaving.

Distribution studies in experimental animals have been made and showed a retention in the lungs of the radioactivity over 95%, 30 min after the intravenous injection.

AGGREGATS D'ALBUMINE HUMAINE MARQUES AVEC INDIUM-113*m* POUR SCINTIGRAPHIE PULMONAIRE

On décrit une technique de préparation d'aggregats d'albumine humaine (MAA) qui peuvent être fournis par la CNEA et marqués par les clients avec Indium-113*m* pour être utilisés en scintigraphie pulmonaire.

Il s'agit de MAA d'albumine humaine dont les particles mesurent entre 10 et 150 μ.

Le marquage s'effectue en 10 min au moyen d'une simple technique et la préparation est stérilisée en autoclave.

Des essais de distribution sur des animaux de laboratoire ont été réalisés et ont montré une retention d'activité dans les poumons supéricure à 95 pour cent, 30 min après l'injection intraveineuse.

МАКРОАГРЕГАТЫ АЛЬБУМИНА МЕЙЕННЫЕ ИНДИЕМ- 113м ДЛЯ СЦИНТИЛЛЯЦИОННОГО СКАНИРОВАНИЯ ЛЕГКИХ

Описывается способ приготовления макроагрегатов человеческого сывороточного альбумина, который может быть получен от CNEA и меченых индием- 113м экспериментаторами для применения в сцинтилляционном сканировании легких.

Размеры частиц лежат в диапазоне от 10 до 150 μ.

Техника мечения проста и занимает 10 минут. Конечный продукт стерилизуется в автоклаве.

Изучение распределения проделанное на экспериментальных животных показало сохранение в легких более 95% радиоактивности в течении 30 минут после внутривенной инъекции.

MIT INDIUM-113*m* MARKIERTE ALBUMIN-MAKROAGGREGATE ZUM SZINTILLATIONSUNTERSUCHEN VON LUNGEN

Es wird ein Verfahren beschrieben für die Zubereitung von Albumin-Makroaggregaten von menschlichem Serum, die von der CNEA geliefert und von den Verbrauchern mit Indium-113*m* markiert werden können, zur Verwendung bei Szintillationsuntersuchungen von Lungen.

Die Grösse der Teilchen schwankt von 10 bis zu 150 μ.

Das Markierverfahren ist einfach und dauert 10 Minuten. Das Endpräparat wird durch Autoklavenbehandlung sterilisiert.

Die Verteilungen wurden in Tierversuchen studiert und ergaben über 95% Rückstand der Radioaktivität in den Lungen 30 min nach der intravenösen Einspritzung.

INTRODUCTION

In 1966, Goodwin, Stern *et al.*[1,2] suggested the convenience of using Indium-113*m* for scintiscanning, due to its favorable desintegration characteristics. The energy of its gamma ray, 393 keV, is similar to the iodine-131 gamma emission, so the same collimator developed for this isotope can be used. Its short half-life ($T\frac{1}{2}$ 1.7 hr) and the absence of specificity for the thyroid, result in a low radiation dose for the patient.

In-113*m* is easily obtained from a long lived Sn-113 generator, from which it can be milked several times a day, thus rendering its application less expensive.

The good results obtained in lung scintiscanning with denatured albumin macroaggregates (MAA) labelled with I-131 by Taplin *et al.*[3] and Tc-99*m* by McAfee *et al.*[4] in the U.S.A. and by De Paoli *et al.*[5] and Testa *et al.*[6] in our laboratories, encouraged us to use this same agent for In-113*m*.

Labelling was made on "preformed" macroaggregates in order to make the technique simple and fast. In this procedure the In-113*m* elution was adjusted to pH between 5 and 7, mixed with the MAA and the preparation was heated during 20 min at 1.5 kg/cm^2.

The sterile suspension obtained was washed once with sterile saline solution and resuspended in the desired amount of saline solution for injection. The activity retained by the protein was approximately 100 per cent.

The dia. of the particles, as determined by microscopy, was approximately 2 μ, grouped in clumps of different sizes, from 10 to 150 μ.

In the present note we report results of the percentage activity retained by the MAA precipitate as a function of pH and temperature, distribution studies in rats and anatomopathologic, histological and scintigraphic studies on dogs.

Some scintillation scannings of normal and pathological patients are shown.

EXPERIMENTAL

A 6 mCi Sn-In-113 generator from the New England Corporation was used; the human albumin employed was Behringwerke 20 per cent; measurements were made with a scintillation spectrometer, with a INa (Tl) crystal 2 × 2 in.

(a) *Preparation of the stock of MAA*

In preliminary tests the labelled MAA were produced by autoclave heating of the albumin solutions at different concentrations added to the isotope elution and adjusted to pH 6–7. After 20 min autoclaving the labelled MAA were formed but their sizes were variable and not reproducible. An attempt was made to get uniform MAA by changing the ClNa concentration, but it proved unsuccessful.

Later on, the labelling of preformed particles was tried and it was found that these particles retained almost the total activity. The procedure finally adopted was the following:

(1) Equal volumes of concentrated hydrochloric acid (10 ml), saline solution and 10% ammonium thiocyanate were mixed and 10 ml of 3% human albumin solution were added to this solution while stirring.

The bulky protein precipitate produced was separated by centrifugation and the supernatant was discarded.

(2) The precipitate was redissolved in 10 ml saline solution, a drop of 0·5% solution of Bromocresol green was added and the pH was adjusted to 4.5–5.0 with 20% sodium carbonate solution. The volume was made up to 20 ml with saline in order to get an albumin concentration of 15 mg/ml.

The preparation was separated in 5 ml portions in penicillin type vials hermetically closed and autoclaved during 20 min at 1.5 kg/cm^2. The albumin was denatured and showed a milky appearance when stirred. It was kept at 4°C.

and 10 mg of PO_4HNa_2 were added.

(b) *Labelling of the MAA*

(1) An aliquot of the elution from the Sn–In generator was taken (considering that during the labelling operation, approximately 45 min, its activity will decay to 75 per cent of the initial activity.) This aliquot was adjusted to pH 5–7 with 20 per cent sodium carbonate solution and the resulting solution was added to the MAA with vigorous agitation and heated in autoclave during 20 min at 1.5 kg/cm^2.

(2) The vials were cooled and centrifuged during 5 min at 2000 rev/min, discarding the supernatant by means of a sterile syringe. The precipitate was resuspended in 5 ml of sterile saline solution in order to eliminate the peptized protein. It was centrifuged again and the supernatant discarded as before.

(3) The precipitate was resuspended finally in 10 ml of sterile saline solution, obtaining a MAA suspension ready for injection, with a concentration of 7.5 mg of albumin per ml.

(c) *Labelled MAA yield as a function of pH and temperature*

We considered that the labelling mechanism was a case of adsorption of indium hydroxide on the MAA. As preliminary experiments showed that the yield of labelled MAA depended greatly on the pH of the medium, the following experiments were performed.

Definite volumes of In-113*m* eluate, adjusted to rising pH values, and equal volumes of MAA suspensions were added. After titrating the resulting suspension to the desired pH value, it was heated at 100°C during 10 min. The preparations were centrifuged for 15 min at 5000 rev/min. We measured the total activity of each preparation and the activity retained by the MAA precipitate after washing with solutions at the corresponding pH values.

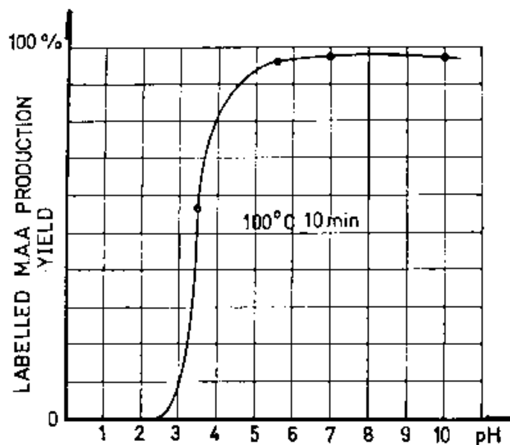

Fig. 1. Labelled MAA production yield at 100°C as a function of pH values.

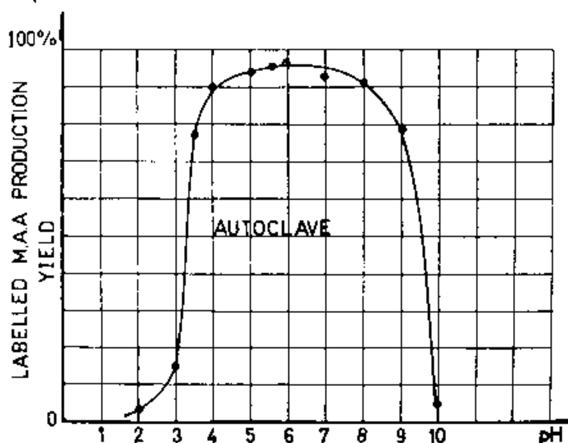

Fig. 2. Labelled MAA production yield in autoclave at 1.5 kg/cm^2 as a function of pH values.

These data are shown in Fig. 1 as labelled MAA yield as a function of pH. At pH 2 and 3, the protein is not labelled so the activity retained by the precipitate can be eliminated by washing.

From pH 3.5 onwards the activity is fixed to the protein completely, so no indium activity is recovered by washing.

The procedure adopted was the heating of the MAA suspension in the autoclave during 20 min at 1.5 kg/cm^2, to ensure the sterility of the preparation.

Solutions were prepared as in the previous experiment and were then autoclaved and centrifuged. The total activity of each preparation and the activity retained by the MAA precipitate were measured without previous washing.

The results are shown in Fig. 2, where it can be seen that at high pH, 9–10, the obtention yields of labelled MAA decrease. This fact seemed in disagreement with the mechanism accepted, but could be explained by the analysis

of the supernatants obtained at these pH values. A partial destruction of MAA was found, verified by observing the turbidity that appears when adding trichloro-acetic acid.

According to these findings, we finally decided to work at pH 5–7, for destruction of MAA during the sterilization process is minimum at these values.

(d) *Size of the particles*

The particle size was determined by microscopy in a hemocitometer. Figure 3 shows a microphotography of the macroaggregates.

(e) *Distribution tests*

In order to study the distribution of MAA in organs, 1 mg of labelled MAA suspended in 0.2 ml of saline solution was injected into the jugular vein of adult Wistar rats of approximately 400 g. The rats were sacrificed 30, 60 and 90 min after injection and the activity of the different organs was determined. After 30 min more than 95 per cent of the activity was found in lungs, while the sum of the activities retained in liver and spleen was only 3 per cent.

The animals sacrificed later showed a mobilization of the particles from the lungs to the liver.

In experiments with dogs[7], six mongrel dogs, weighing between 7 to 10 kg were injected through the femoral vein and catheterized in the pulmonary artery. ECG was determined in them simultaneously with the pulmonary arterial pressure at the time of injecting MAA doses, varying between 0.1–1.0 mg of MAA per kg of weight, with total activities ranging from 75 to 250 μCi. This study did not show any modification with respect to the normal pattern.

The animals were sacrificed and the organs were separated with a few days intervals in order to perform anatomic studies, macro and microscopic, on the trachea, lungs, heart and liver, which showed no modifications. No histological injuries were observed, which could be ascribed to the substance injected when the lung preparations were carefully studied, with special attention paid to blood and lymphatic vessels.

(f) *Scintiscanning*

In the experiments made in cooperation with the Centro de Medicina Nuclear, we used a Pho-Dot Nuclear Chicago scanner, with a INa (Tl) crystal of 3 × 2 in. and a 37 holes collimator, with a focal distance of 6·3 and a resolving power of 0.75 cm. The scanning speed was 45–60 cm/min. The doses of MAA given were of 0.1–0.5 mg per kg and total activities administered ranged from 0.3–0.5 mCi.

1. In dogs:

We obtained scintiscannings with excellent concentration in both lungs, perfect cardiac silhouette and clear delimitation of both bases (Fig. 4).

2. In patients:

Figures 5 and 5a show the scintiscanning of a normal patient, compared with the corresponding chest radiograph.

Figures 6 and 6a show the scintiscanning and corresponding radiograph of a case of acute tuberculosis with infiltrations in the right lung and a cavern in the upper lobe.

DISCUSSION

Although several authors mention other compounds such as ferric hydroxide[2] and stannous hydroxide[8], which might coprecipitate indium, we believe that the procedure described in this paper offers the advantage of a preformed particle, of previously controlled size which can be rapidly labelled.

Moreover, the MAA have been routinely used and its physiological effects have been largely studied by Taplin *et al.*

Besides, even though the clinical incidence of interventricular communication is very low, it might be expected that in such a case the MAA could result less dangerous than ferric hydroxide as agents of brain embolism.

The radiation dose received by the patient in each scintillation scanning is low compared to the dose received when using ^{131}I labelled macroaggregates. When ^{99m}Tc is used as labelling isotope, the dose received is similar, but the greater durability of the Sn–In generator constitutes a great advantage over the Mo–Tc generator.

For all these reasons we consider very convenient the use of MAA labelled with In-113*m* in lung scintiscanning, in order to complete radiographic studies with valuable data related to lung circulation.

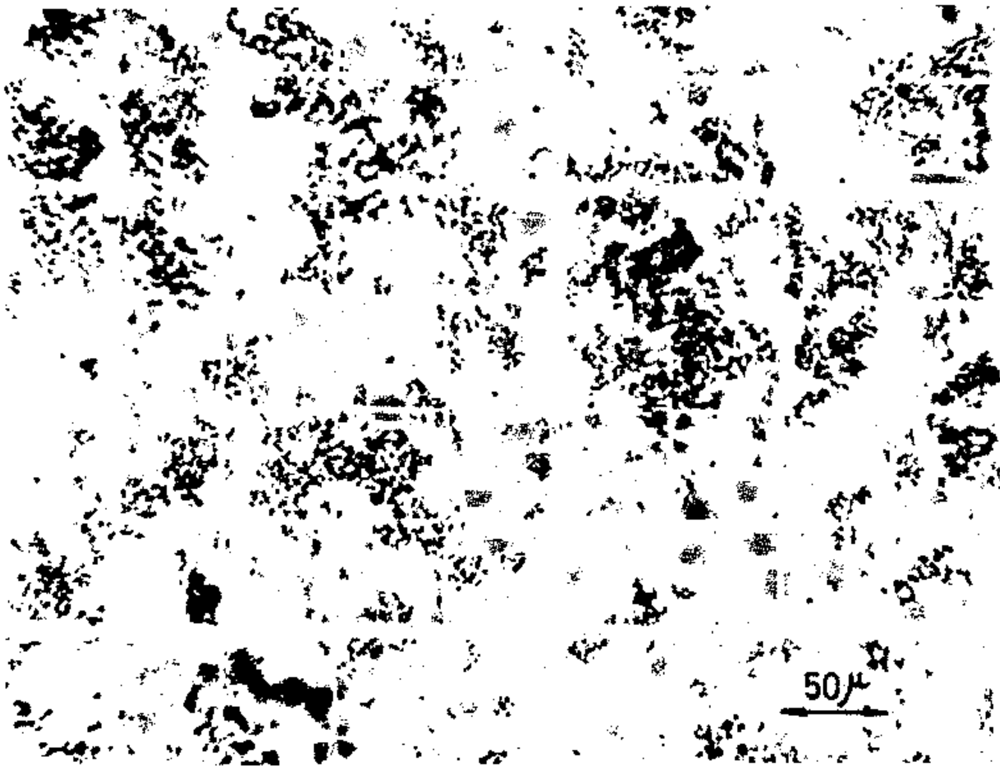

FIG. 3. Microphotography of the MAA.

FIG. 4. Scintillation scanning of the lungs of a dog with In-labelled MAA. (Courtesy of Testa *et al.*)

Fig. 5

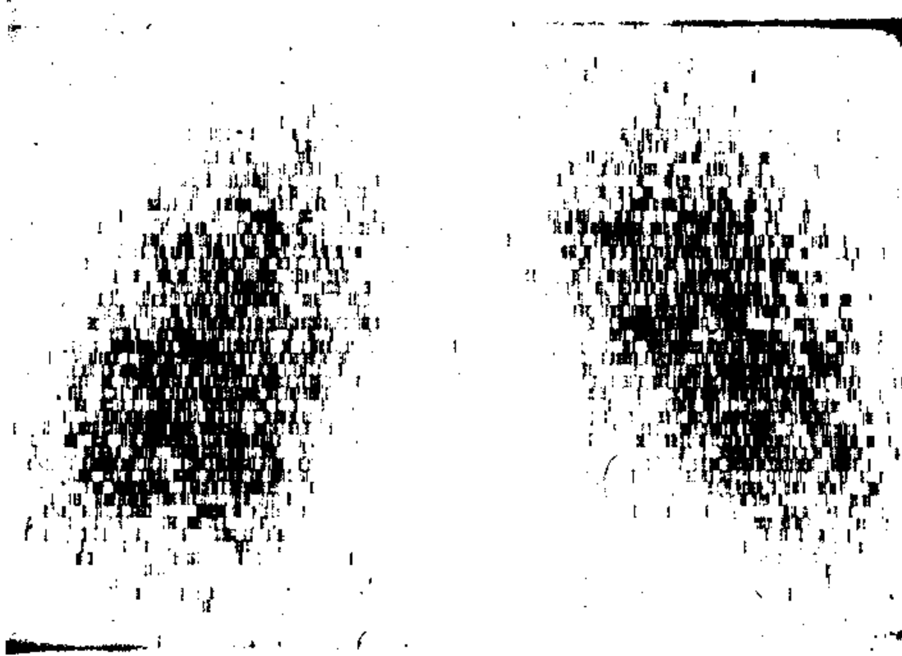

Fig. 5(a).

Fig. 5 and 5a. Chest radiograph and lung scintillation scanning of a normal patient.

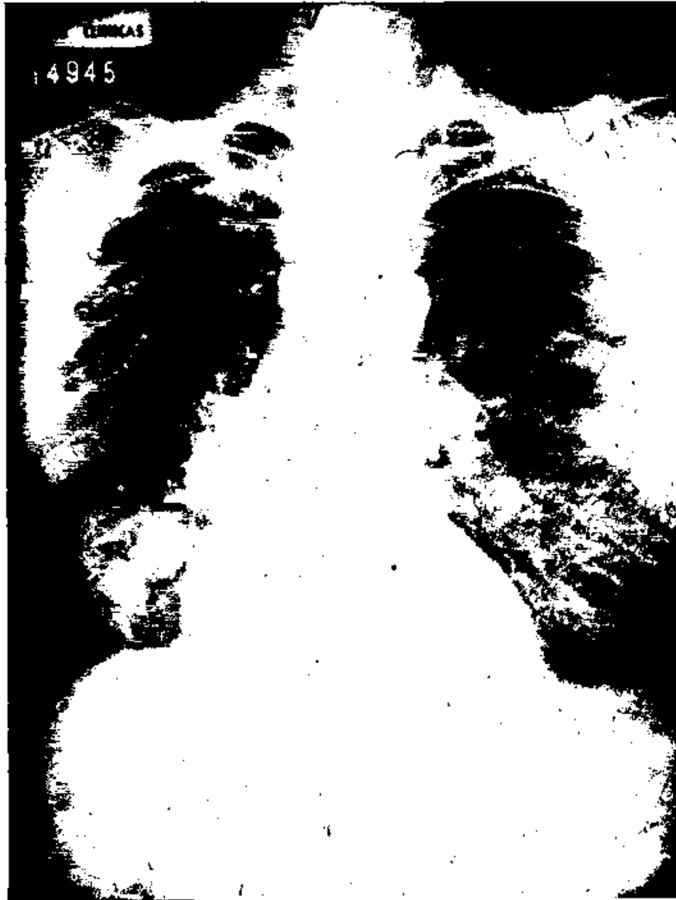

Fig. 6

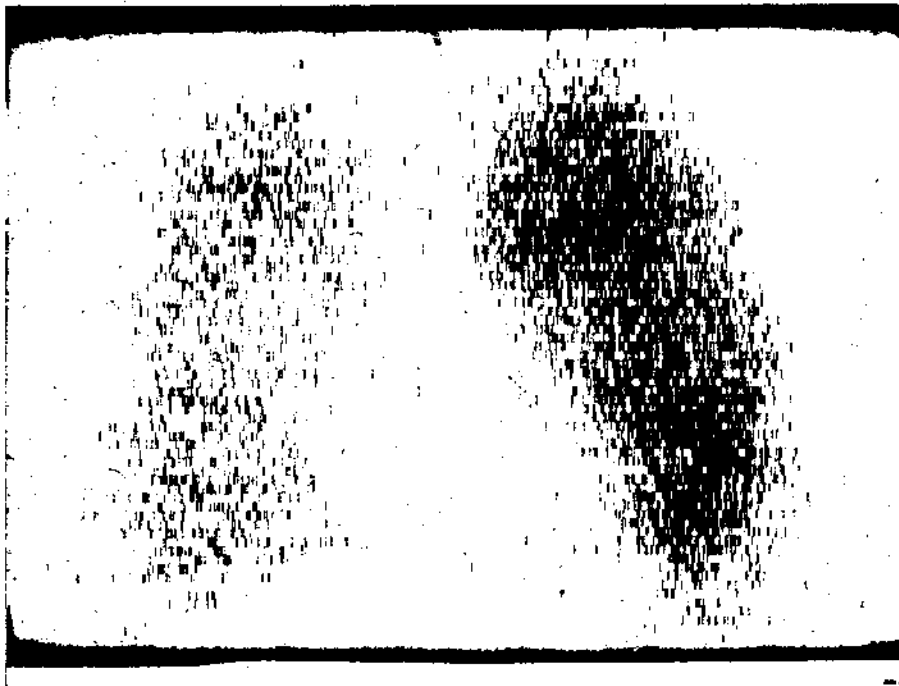

Fig. 6(a).

Fig. 6 and 6a. Chest radiograph and lung scintillation scanning of a patient with acute tuberculosis.

Acknowledgements—We are grateful to Drs. H. J. Testa, A. J. Olivari and V. Pecorini of the Centro de Medicina Nuclear, Hospital San Martin, for their highly important collaboration in the biological experiences performed with the In-113*m* labelled MAA.

REFERENCES

1. Stern H. S., Goodwin D. A., Scheffel U., Wagner H. N. Jr. and Kramer H. H. *Nucleonics* **24,** 57 (1966).
2. Goodwin D. A., Stern H. S., Wagner H. N. Jr. and Kramer H. H. *Nucleonics* **24,** 65 (1966).
3. Taplin G. B., Johnson D. E., Dore E. K. and Kaplan H. S. *Hlth Phys.* **10,** 1219 (1964).
4. McAffee J. G., Stern H. S., Fueger G. F., Baggish M. S., Holzman G. B. and Zolle I. *J. nucl. Med.* **5,** 936 (1964).
5. De Paoli T., Hager A., Nicolini J. O. and Radicella R. CNEA-193, Bs.As. (1967); and *Int. J. appl. Radiat. Isotopes* **17,** 551 (1966).
6. Testa H. J., Chwojnik A., Olivari A. and Pecorini V. *Medicina* **26,** 311 (1966).
7. Testa H. J., Olivari A. J. and Pecorini V. XLII P.A.M.A. Congress, Bs. Aires, Nov. 1967. Personal communication.
8. Maass R., Alvarez J. and Arriaga C. *Int. J. appl. Radiat. Isotopes* **18,** 653 (1967); and personal communication.
9. Peterson C. C. and Bonte F. J. *Int. J. appl. Radiat. Isotopes* **18,** 193 (1967).