technical notes

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Albumin Macroaggregates Labelled with Tc^{99m}

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Introduction

SIX hours Tc^{99m} which can be easily "milked" as pertechnetate from an alumina column containing 67 hr Mo^{99}, has been used in the last few years as a tracer for medical diagnosis, especially for scintillation scanning.

Recently McAFEE and co-workers, in a preliminary note,[1] have reported the use of human serum albumin labelled with Tc^{99m} for scintillation scanning of the placenta. They have labelled the albumin by reducing the $Tc^{99m}O_4^-$ with a pH 4·5 solution containing ascorbic acid and ferric chloride, adding the reduced Tc^{99m} to the serum albumin and lowering to 2·5 the pH of the final solution. By this procedure they have obtained a labelling yield of about 90 per cent. The condition of the labelling reaction were carefully described by STERN *et al.* in a subsequent paper.[2] They stated that reduction or complexing of the pertechnetate by Fe^{3+} is necessary, in this case, in order to carry out the labelling of the albumin.

More recently HARPER and co-workers[3] have reported scintillation scannings of the lungs by means of albumin macroaggregates labelled with Tc^{99m}, but they have not described the labelling technique. Subsequently, during the preparation of the present note, LOKEN *et al.* published a paper[4] describing the use of some technetium compounds for the visualization of the body organs. They briefly reported the preparation of aggregates by the heating of the labelled albumin obtained using the procedure given by STERN and co-workers.

In the present note we describe the procedure to prepare Tc^{99m} labelled macroaggregates of denatured human serum albumin with a particle size suitable for scintillation scanning of the lungs. The technique used was the following: we reduced the $Tc^{99m}O_4^-$ with hydrochloric acid and ammonium thiocyanate. A protein precipitate, which retained more than 95 percent of the Tc^{99m}, was formed when the solution of reduced technetium was added to the human serum albumin. This precipitate was dissolved in saline solution, the pH was adjusted to 5–5·5 and the resulting solution was autoclaved at 120°C for 15 min to form the albumin aggregates. These aggregates were carefully washed; they retained about 60 per cent of the initial Tc^{99m} activity.

The size of the particles, as determined by microscopy, was of approximately 2 μ; the particles were grouped in clumps of different sizes from 5 to 80 μ dia.

These aggregates were localized mainly in the lungs, as shown by distribution tests carried out in rats and by scintillation scannings of rabbits and dogs after intravenous injection of the preparations.

Experimental

Preparation of the labelled aggregates

In several preliminary tests the labelling of the human serum albumin was tried using different agents to reduce the $Tc^{99m}O_4^-$. No significant yields were obtained. At the same time we tried the reduction in a thiocyanate-hydrochloric acid medium: the technetium compound so formed was easily extracted by ethyl ether, as was previously observed by CROUTHAMEL[5] for the reduction by a thiocyanate-sulphuric acid solution. Preliminary tests using this compound as a labelling agent were satisfactory. The procedure finally adopted was the following:

(1) Equal volumes of 10 per cent ammonium thiocyanate and 11 M hydrochloric acid were added to a saline solution containing $Tc^{99m}O_4^-$ from the "milker". After 5 min, 1 ml of 3 per cent human serum albumin was added, stirring carefully. We observed the formation of a bulky protein precipitate which retained 98 per cent of the Tc^{99m} activity. After 5 min the precipitate was separated by centrifugation and the solution was discarded.

(2) The precipitate was dissolved in 1 ml of saline solution, the pH was adjusted to 5–5·5 with drops of 10 percent sodium acetate buffer and, after adjusting the volume to 1·8 ml, the solution was autoclaved for 15 min at 120°C in a penicillin type vial. Subsequently the solution was stirred, forming a milky suspension of denatured albumin aggregates. The following steps were carried out under sterile conditions.

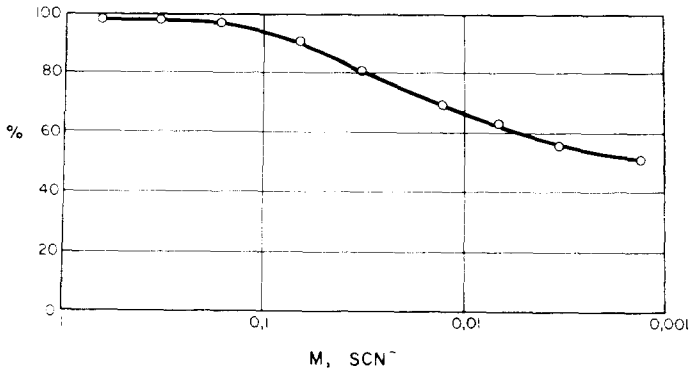
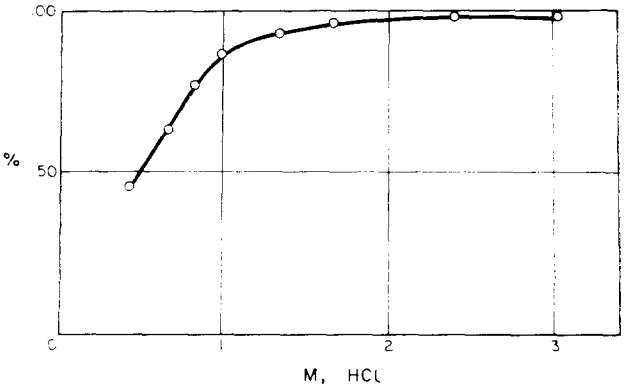


FIG. 1. Retention of Tc^{99m} by the first protein precipitate. (a) Retention vs. SCN^- concentration (HCl concentration 2·4 M).



(b) Retention vs. HCl concentration (SCN^- concentration 0·66 M).

(3) 5 ml of sterile saline solution were added, the preparation was centrifuged for 3 min at 2000 rev/min, and, with the help of a syringe, the supernatant solution was discarded. This step of the procedure was repeated once more and finally the precipitate was suspended in saline solution.

By this procedure the final preparation retained approximately 60 per cent of the initial Tc^{99m} activity.

The retention of the Tc^{99m} activity by the protein precipitate obtained in the first step of the procedure was studied by varying the amounts of ammonium thiocyanate and hydrochloric acid (Fig. 1). The results indicate that the retention is greatest for hydrochloric acid concentrations between 2 and 3 M and for thiocyanate concentrations between 0·2 and 0·7 M.

The active precipitate formed in the first step was dissolved in saline solution and studied by paper electrophoresis (6 hr; 100 V; 1 mA; barbital buffer pH 8·6). Simultaneously, samples of pure human serum albumin were analysed by electrophoresis under the same conditions. The colouring of the paper strips with amido-black showed that the protein of the precipitate and the pure albumin had the same electrophoretic mobility. The distribution of activity was determined in the paper strips after the electrophoresis. We found three active zones: the first one at the starting point, the second one with the albumin and the third one in the zone corresponding to the pertechnetate, as tested by simultaneous electrophoresis of a pure $Tc^{99m}O_4^-$ sample.

Since the small amounts of unbound Tc^{99m} were previously separated from the precipitate by centrifugation, the presence of free pertechnetate was

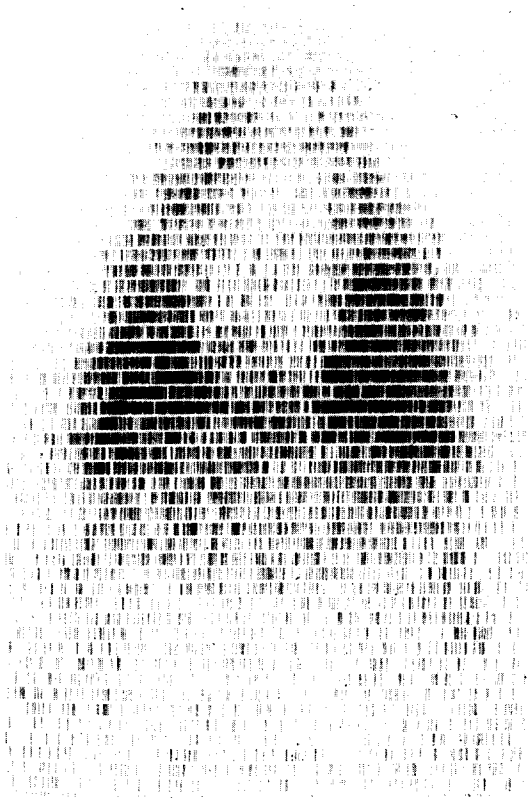


FIG. 2. Scintillation scanning of the lungs of a dog with Tc-labelled albumin aggregates (Courtesy of TESTA *et al.*).

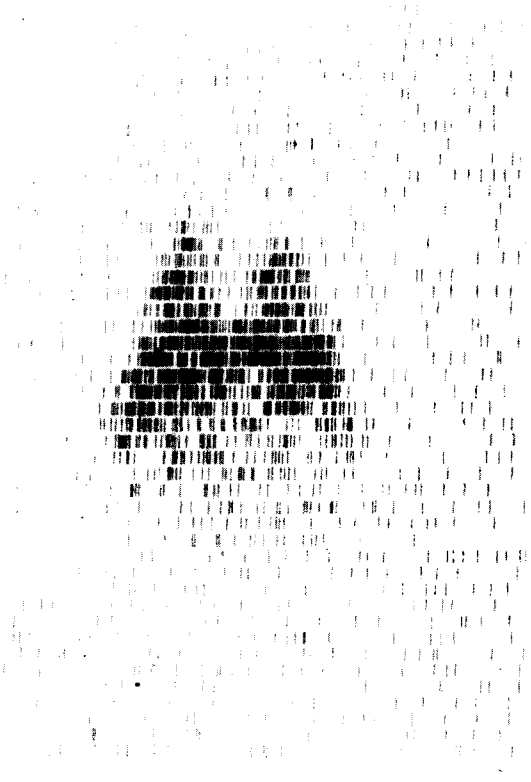


FIG. 3. Scintillation scanning of the lungs of a rabbit with ^{131}I-labelled albumin aggregates.

difficult to understand. Consequently, several tests were carried out in an attempt to explain these results.

The strongly acid solution of reduced Tc^{99m}, as obtained before adding the albumin in the first step of the procedure, was analyzed by electrophoresis: all of its activity was found at the starting point.* A second spot, which is due to pertechnetate, on the contrary, appeared when the pH of the solution was raised to 5 before the electrophoresis.

Moreover, when we studied the labelling yields in the second step of the procedure we determined that an important amount of Tc^{99m} had separated from the protein during the autoclaving. This Tc^{99m} fraction, which was subsequently separated by the washing in the third step, was found to consist of pertechnetate.

We could assume then, in order to explain these results, that the $Tc^{99m}O_4^-$ was reduced in the first step to a compound, probably a pentavalent thiocyanate complex,[5] which is stable in a strong acid medium and which can label the protein. When the pH of the solution was raised, the Tc (V) disproportionated into Tc (IV) and Tc (VII).[6,7] In this way the electrophoretic behaviour of the first protein precipitate could be explained: at a higher pH part of the technetium bound to the protein is transformed into pertechnetate and into a compound of Tc (IV), probably TcO_2, which in the paper electrophoresis remains at the starting point. To a similar mechanism might be ascribed the fact that during the heating to form the albumin macroaggregates part of the activity was lost by the protein as pertechnetate. The remaining activity could be partially bound to the protein and partially adsorbed on the albumin aggregates as TcO_2. In this case, in addition to the rise of the pH, the autoclaving could accelerate the transformation into Tc (IV) and Tc (VII).

This mechanism could also explain the results obtained in the following tests. A suspension of previously denatured albumin, was added, instead of the normal albumin, to the strongly acid solution of reduced technetium. We found that more than 90 per cent of the activity was retained by the aggregates. However, when these aggregates were injected, previously washed, into rats, we observed that only part of the activity was retained in the lungs. Moreover, when the labelled aggregates were suspended in saline solution and were heated at 120°C, about 30 per cent of the activity was found as free pertechnetate. If this activity was then removed by centrifugation and the remaining aggregates were injected, almost all the activity was found in the lungs after 30 min, even if the particle size had not been changed considerably during the treatment. In this case we could also assume that the reduced technetium has labelled the performed aggregates and, by the subsequent heating or pH change, it has disproportioned into Tc(IV) and Tc(VII), with the consequent partial separation from the protein.

Size of the aggregates

We have studied by microscopy the size of the albumin aggregates formed by the autoclaving of solutions containing different amounts of albumin. We have found that the best concentration was 17 mg/ml: with this concentration we have attained particles with a diameter of about 2 μ in aggregates of different sizes from 5 to 80 μ. Lower concentrations have yielded smaller aggregates which, when injected into rats and rabbits, were retained by the liver.

Distribution tests

0·8 mg of labelled aggregates, suspended in saline solution were injected into the jugular vein of Wistar rats of approximately 350 g, in order to study its distribution. The rats were sacrificed 30 min after the injection and the activity of different organs was determined. We found 90 per cent of the activity in the lungs and 3 per cent in the liver.

Moreover, TESTA and co-workers[8] and the authors have obtained perfectly defined scintillation scannings of the lungs after injection of the labelled aggregates into dogs and rabbits (Figs. 2 and 3).

In no case were toxicity reactions observed when albumin aggregates, in doses of approximately 2·5 mg/kg, were injected into rabbits and dogs. With doses of about 50 mg/kg, instead, we observed respiratory failure and various central nervous systems symptoms followed by the death of the animal. These results are in agreement with the toxicity experiments performed by TAPLIN[9] and co-workers with I^{131} labelled albumin macroaggregates.

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* This could be explained either by considering that the technetium forms a neutral complex or by assuming the strong adsorption or the combination of the reduced Tc^{99m} with the paper. We observed, in fact, that if paper pulp was added to the acid solution of technetium, almost all the activity was retained by the pulp, even after a careful washing with strong hydrochloric acid solutions.