after injection is in a normally highly diffusible state but that it persists in blood for an abnormally long time allowing more time for the ^{64}Cu to diffuse out of blood. To clarify this, ^{64}Cu was injected intravenously by a continuous infusion pump to obtain a constant blood level. The rate at which the cranial content rose in the presence of this constant blood level was used as an indication of diffusibility. The rate of rise of the brain Cu in Wilson's disease was found to be the same as in the normal curve shown here suggesting the increased uptake of Figure 9 was due to the prolonged elevated Cu blood level after a single injection.

Figure 11. A diagram showing the somewhat dispersed bolus of radioactive tracer approaching the cranial blood pool and the same, more dispersed bolus as it would appear leaving this pool and the field seen by the detection system. The times of maximum rate of increase and decrease mark the entrance and exit of the most concentrated part of the bolus from the cranial blood pool. This differentiation is easily achieved by a simple circuit which will provide a useful output voltage which closely defines the rate of change or first derivative of the count rate meter output voltage. A capacitor C is placed in series with the rate meter output voltage and the right plate of the capacitor is shunted to ground by resistance R. Current flow through C and R will closely approximate the rate of change of the rate meter output provided the time constant C and R is short relative to rates of change in rate meter output voltage. For a varying signal such as found in the present work, a differentiating time constant of about 0.1 second is adequate. This might be obtained with a value for C of 1 mfd and R of 0.1 megohms. Ideally, the output impedance of the rate meter will be substantially lower than the value of resistance R. A reserve of display sensitivity must be available since this additional circuitry results in a considerable attenuation of the input voltage. Because of an enhancement of noise inherent in the differentiation process, additional high frequency filtering may be required.

Figure 12. Indicating the distribution of mode circulation times in a group of normals contrasted with a group with known cerebrovascular disease.

Figure 13. The precentage of gamma ray absorption by thin and thick regions of wet skull. Each point represents an average value for ten skulls. Broad beam collimation was used with a pulse-height threshold set at approximately 75% of photopeak energy of the isotope being used.

^{99m}Technetium Labeled Compounds*

P. Richards
Nuclear Engineering Department
H. L. Atkins
Medical Research Department
Brookhaven National Laboratory Upton, New York

Introduction

Advances in scintiphotographic instrumentation and the availability of an increasing number of radionuclides have been responsible for a rapid growth of nuclear medicine in recent years. The development and introduction of ^{99m}Tc has played an important role in this growth.

^{99m}Technetium was initially suggested for potential medical applications due to a com-

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bination of excellent physical characteristics.(1) The 6-hour half-life and absence of beta radiation make feasible the adminstration of millicurie amounts of the nuclide with relatively low radiation dosage to the patient. A gamma emission of 140 keV has satisfactory tissue penetration and yet is readily collimated making possible the use of collimators of high sensitivity. These factors result in greatly increased photon yields which when combined with advanced instrumentation result in statistically improved information and more rapid procedures. The development of the ^{99}Mo-^{99m}Tc generator at Brookhaven National Laboratory(1,2) has made this short-lived nuclide readily available in the user's facility.

^{99m}Technetium as the pertechnetate ion was first evaluated by HARPER, *et al*,(3,4) as a possible biological tracer and this work led to its present widespread use in brain tumor localization, as well as thyroid visualization. The development of a method for preparing a ^{99m}Tc-labeled colloid of sulfur by RICHARDS and clinical evaluation by HARPER(4,5) and ATKINS(6) has resulted in a radiopharmaceutical useful for liver, spleen and bone marrow scanning. The usefulness of this nuclide has been further enhanced by the preparation of ^{99m}Tc-labeled human serum albumin which is an important agent in several applications. There is little doubt the development of additional compounds of biological interest labeled with ^{99m}Tc will contribute to new or improved diagnostic procedures.

^{99m}-*Technitium HSA*

Human serum albumin (HSA) labeled with ^{99m}Tc is an excellent compound for nuclear medical applications due to the physical characteristics of the nuclide. The ability to administer large amounts of activity and the low radiation dosage to the patient allow the use of this material for placental localization by scanning, for cardiac visualization, and in the form of albumin aggregates for lung scanning.

A number of methods have been reported for preparing HSA labeled with. ^{99m}Tc(7,8,9). However, we have frequently had difficulty duplicating the results often obtaining inconsistent yeilds and generally low specific activities. It is usually desirable to use high specific activity HSA to reduce the risk of sensitivity reactions or impairment of respiratory reserve in the case of aggregates. Preliminary investigations indicated that the methods using iron and ascorbic acid as reductants for the technetium appeared to have the most promise. A systematic study of the chemical steps has been made with the following objectives: (1) to simplify the procedure, if possible, (2) to achieve consistent high yields, (3) to increase specific activity, and (4) to develop information useful in the preparation of other labeled compounds.

The various chemical steps were studied to determine whether any could be omitted and, if not, to find the optimum conditions. The steps examined included the following: (1) the pH of the initial solution, (2) the amounts of ferric iron and ascorbic acid, (3) pH adjustments prior to addition of HSA, (4) the amount of HSA, and (5) the adjustment required for labeling. For each set of conditions tried the percent labeling achieved at the end was determined by pasing the solution through a small Dowex 1 × 8 anion exchange column to remove uncomplexed pertechnetate; (complexed technetium is not retained by the resin). It is obvious that a considerable amount of back-tracking was required in these studies resulting from information derived from later experiments.

In all studies a volume of 5 ml of ^{99m}Tc saline solution as milked from a technetium generator was used. The acidity of the initial solution appears to be of importance to the procedure, as reported by others. However, in this study it has been found that the $FeCl_3$ and ascorbic acid lower the pH sufficiently to achieve high final yields, eliminating the need for the addition of acid at this point. The use of ferric iron is critical to the labeling procedure as is seen in A of Fig. 1. In this experiment the labeling yields were determined with varying amounts of $FeCl_3 \cdot 6H_2O$ and when 10 mg of ascorbic acid and 20 mg of albumin were used. It can be seen that with less than 3 mg of $FeCl_3$ the yield falls off rapidly. The role played by the ascorbic acid is not clear although it apparently reduces the ferric ion to the ferrous state which in turn reduces the technetium.

Ascorbic acid also forms a complex with the technetium and forrous iron when the pH is raised keeping the iron in solution, and in fact this complex has found application as a scanning agent.(8) When the pH is lowered the complex is apparently broken releasing the technetium in the desired state for binding with albumin. Ten mg of ascorbic acid is sufficient to complex 5 to 10 mg of $FeCl_3 \cdot 6H_2O$.

The pH manipulation after the addition of reductants also affects the final labeling yield as is shown in B of Fig. 1, with the yield increasing with increasing pH. Adjustment of the pH to 9.0—9.5 was selected as optimum as a result of these experiments. It has been determined that the addition of albumin may be made in the pH range of 9.5 to 4.8 with satisfactory final yields. The addition of HSA in a small volume (<0.5 ml) at pH of ~ 9 keeps the volume low and allows the later acidification to be made with a predetermined volume of HC1.

The optimum pH conditions for final labeling of HSA have been studied and the results are shown in C of Fig. 1. It can readily be seen that the binding efficiency increases with decreasing pH. A pH of 2.0—2.3 gives good labeling efficiency and minimum problems in later neutralization for intravenous administration. The duration of labeling was also found to be of importance in achieving high yields, particularly when small amounts of albumin are used. Fig. 2 shows the labeling efficiencies achieved for different amounts of HSA versus the time maintained at labeling pH. It is evident that relatively high yields may be obtained with small amounts of albumin with specific activity only limited by the amount of ^{99m}Tc available. After formation of the technetium-albumin complex the solution can be adjusted to a pH suitable for injection with sodium hydroxide or acetate without affecting the label.

As a result of these studies, a relatively simple procedure has been developed for preparing ^{99m}Tc-labeled HSA with consistent yields and of high specific activity. The suggested procedure for preparation is as follows:

To $\sim$5 ml of milked ^{99m}Tc-saline solution, while stirring:

1. Add 5 to 10 mg $FeCl_3 \cdot 6H_2O$ (dissolve).
2. Add 10 mg ascorbic acid (dissolve).
3. Adjust pH to 9.0—9.5 with *N* NaOH.
4. Add desired amount RSA (≤ 0.5 ml).
5. Adjust pH to 2.0—2.3 with *N* HCl (duration at pH determined by amount of HSA).
6. Adjust pH to 5.0—6.0 with NaOH or $NaC_2H_3O_2$.

Even though extremely high labeling efficiencies are achieved by this method, 90% or greater depend on the amount of HSA used, it may be desirable to remove unreacted pertechnetate. This is readily accomplished by passing the final solution through a small column (0.3 cm × 14 cm) of Dowex 1 × 8 anion resin that has been well washed with saline solution. The final solution is sterilized by filtering with a 0.22 micron membrane filter.

Confirmation of the formation of a ^{99m}Tc-albumin complex was made by means of gel filtration. Two ml samples of the product solutions were placed on a column of Sephadex G-25 (1 cm × 40 cm), eluted with saline, and the effluent collected in 2 ml fractions by means of an automatic fraction collector. The results of these studies are shown in Fig. 3. On bar graph A of the figure, where ^{131}I-labeled HSA was used as tracer, it can be seen that the albumin moves rapidly through the column and is collected in the early fractions. The location of albumin in these fractions was also confirmed by precipitation. Graph B shows a separation made with ^{99m}Tc-labeled HSA and indicates that the majority of the technetium activity appears in the same fractions as the albumin in A. Unreacted pertechnetate moves slowly through the column and a small percentage is seen in later fractions.

Carbon-14-labeled ascorbic acid and ^{59}Fe have been used as tracers in the process to determine the behavior of these reagents. Graphs C and D of Fig. 3 show the results of these studies. The major portions of the iron and ascorbic acid are not associated with the labeled albumin and the iron is probably complexed by the ascorbic acid. It is evident from these experiments that gel filtration can be used to concentrate the ^{99m}Tc-HSA and to separate it from the iron and ascorbic acid, as well at the uncomplexed pertechnetate. This may be useful in the preparation of labeled-alubumin aggregates, as it has been reported that the iron may interfere with the formation of aggregates.(10)

^{99m}-*Technetium DTPA*

The technetium-albumin studies have suggested the possibility of the formation of a ^{99m}Tc-labeled chelate by the reaction of pertechnetate with ferrous iron in the presence of a chelating agent. Experiments of this type have resulted in the development of a new compound, ^{99m}Tc-labeled DTPA (diethylenetriaminepentaacetic acid). Preliminary studies with mice indicate that the chelate when administered intravenously is rapidly excreted via the urinary tract, ~90% in 2 hours. Due to its rapid clearance the chelated form of technetium may be a useful agent for renal studies, brain scanning, or for dynamic vascular studies.

A simple method has been developed for preparation of the technetium chelate using the trisodium salt of Ca-DTPA. The following procedure requires ~15 minutes to carry

To ~4 ml of ^{99m}Tc-saline solution, while stirring:

1. Add 10 mg $FeSO_4 \cdot 7H_2O$.
2. Add 1 ml of conc. HCl.
3. Add 10 mg of Ca-DTPA.
4. Adjust pH to 3.0 ± 0.1 with NaOH and stir for ~1 minute.
5. Adjust pH to 5.5—6.5 with NaOH.

Labeling efficiency for the procedure is 85-90%. The complexed pertechnetate may be removed and the solution sterilized by the same method as in the albumin procedure. Other chelating agents (e.g. EDTA) may also be labeled by this general method.

Labeled chelate prepared by this process was separated by means of gel filtration to confirm the association of the ^{99m}Tc activity with the DTPA. Fig. 4 shows the results of these studies. Plot A indicates the location of the technetium activity by counting and B the location of the DTPA-iron complex determined spectrophotometrically. It is evident that the ^{99m}Tc and the DTPA appear in the same fractions proving the chelate formation. Graph C is a run in which the iron-DTPA complex was formed in the normal manner and the technetium was added *after* the complex formation. It can be seen that the ^{99m}Tc is not now associated with the DTPA but is located in the fractions where unreacted pertechnetate normally appears.

Summary

The radionuclide ^{99m}Tc has played an important role in the rapid growth of nuclear medciine due to its combination of excellent physical characteristics and its ready availability from the ^{99}Mo-^{99m}Tc generator. The development of compounds labeled with ^{99m}Tc having different biological distributions has greatly increased its usefulness and made it the nuclide of choice for several applications. A detailed study of the chemistry invovled in labeling human serum albumin with technetium has resulted in the development of a reproducible method for labeling with high efficiency and the production of a product of high specific activity. As a result of these studies a new compound, ^{99m}Tc-DTPA, has been prepared which may further enhance the usefulness of the nuclide in nuclear medicine.

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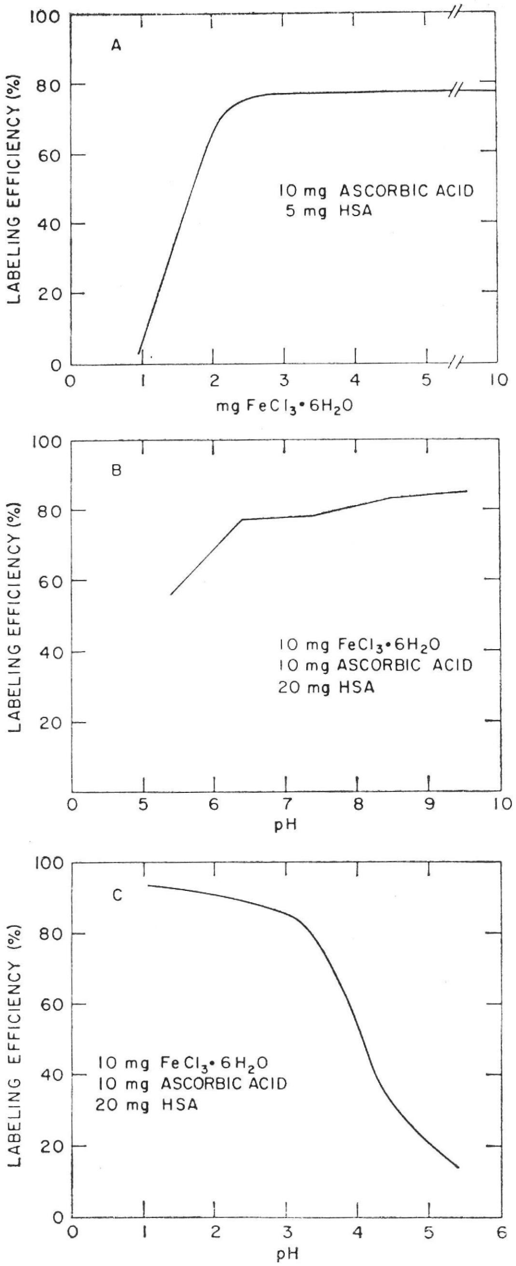

Fig. 1. Studies on the labeling of HSA with ^{99m}Tc.
(A) Effect of varying the amount of Fe^{+3} on labeling efficiency.
(B) Manipulation of pH prior to addition of HSA.
(C) Optimum pH for labeling.

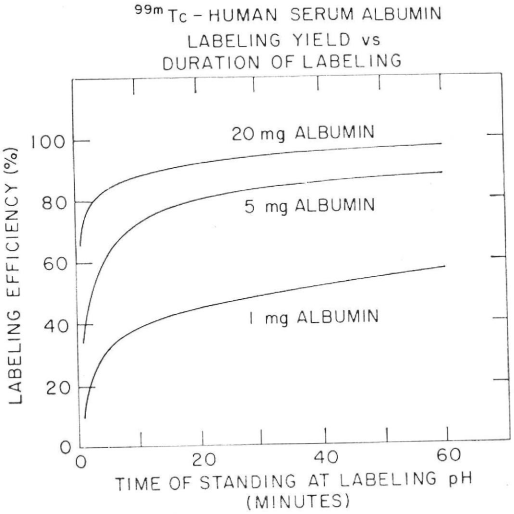

Fig. 2. Labeling efficiencies for different amounts of HSA versus duration at labeling pH.

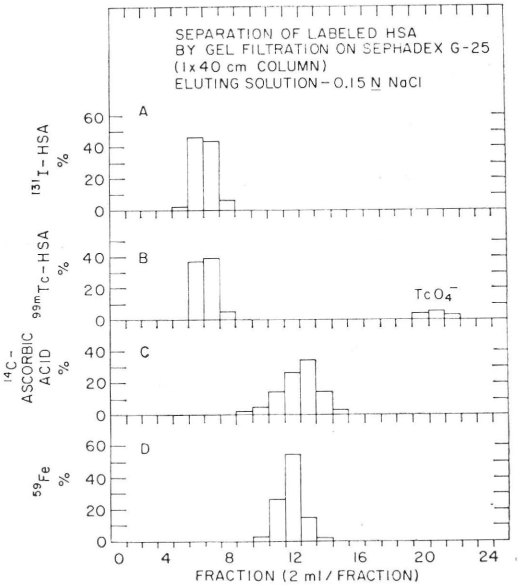

Fig. 3. Separation of ^{99m}Tc-HSA by gel filtration to confirm albumin complex formation and study of the behavior of iron and ascorbic acid by the use of radioactive tracers.
(A) The location of ^{131}I labeled HSA.
(B) The location of ^{99m}Tc labeled

HSA.
(C) The location of ^{14}C-ascorbic acid.
(D) The location of ^{59}Fe-iron.

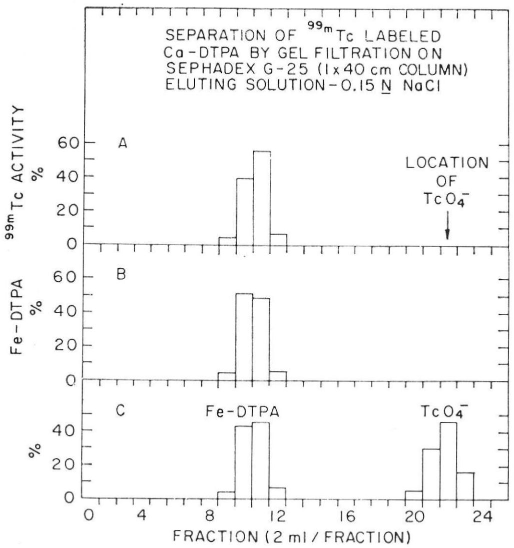

Fig. 4. Separation of ^{99m}Tc-DTPA by gel filtration to confirm formation of technetium chelate.
(A) The location of ^{99m}Tc activity.
(B) The location of the iron-DTPA chelate.
(C) The location of unchelated pertechnetate.

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