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RADIOLABELED RED BLOOD CELLS: STATUS, PROBLEMS, AND PROSPECTS

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INTRODUCTION

Of the various cellular blood elements, red cells (RBC) are: (1) most abundant; (2) easy to separate and handle; (3) less susceptible to damage from physical or chemical manipulations; (4) not as dependent on energy and nutritional requirements in vitro, and (5) more amenable to labeling with radionuclides due to the availability of a variety of cellular transport mechanisms and of hemoglobin within, that is rich in active metal binding sites. Consequently, red cells have served as simple, convenient, and useful models for the study of among other things, cellular transport phenomena and membrane structure and function[1,2].

Red cells dispersed in plasma are not truly living cells. They are composed of water (65%), hemoglobin (32%), and other protein and lipid stroma (3%), and possess properties (circular, non-nucleated biconcave discs; marked elasticity, etc.) that are ideally suited for their functions to rapidly absorb oxygen in the lungs, to pass through smallest capillaries without damage, and to give up O_2 rapidly to the tissues. The average RBC count is about 5×10^9 per ml of blood and their surface area approximately 3×10^3 sq.m. (or 1500 times the body surface area). Normal red cells have a life span of 110-120 days; the usual rate of replacement is 0.8-1% per day. Hemoglobin, the most important component of the red cell, remains stable and does not undergo degradation or resynthesis during the life of the cell. The hemoglobin molecule contains four heme and globin molecules, and has an average molecular weight of 68000. The ferrous iron in heme is bound covalently to the four porphyrin nitrogen atoms. The fifth bond is to the imidazole nitrogen of the histidine of globin, and the sixth is in a reversible binding to oxygen.

DISCLAIMER

Radioactive labels that have been utilized for red cells mostly bind to hemoglobin but can also bind to other intracellular components as well as to surface proteins on the membrane. The desirable properties of an ideal radionuclide label, especially for diagnostic nuclear medicine applications, are as follows: (i) the radionuclide can be incorporated without altering the physical or biochemical properties of the cells and their in-vivo function; (ii) the radionuclide should have a gamma energy emission in high abundance suitable for imaging, a minimum of cell-damaging low-energy Auger and conversion electrons, and a physical half-life matched to the time frame of the study being performed; (iii) the label should be reasonably stable in vitro as well as in vivo, and once incorporated into the cell should not elute during the study, or get reutilized after cell destruction, and (iv) the radionuclide should have little or no particulate emission in order to minimize patient radiation dose.

Radionuclidic labels for red cells can be divided into two main categories - cohort or pulse labels, and random labels. The cohort labels bind to marrow precursors but not to cells already in circulation. Labeled cells of uniform age appear in the circulation after a few days and thus cohort labels are useful for the study of cell production rate and survival. The random labels are incorporated into circulating cells of all ages and the labeling process is usually carried out in vitro on a small sample of venous blood. Except for some earlier studies on ferrokinetics and red cell production, etc., using iron radionuclides, most of the red cell labels developed so far and those in predominant use at the present time involve random labeling and employ technetium-99m, chromium-51, indium-111, and gallium-68, roughly in that order. A listing of the various labels appears in Table 1. Also included is information on the compounds used and whether the labeling is carried out in vivo or in vitro. The various diagnostic applications of randomly used labels are shown in Table 2. The extent of usefulness depends, of course, on the properties of the label such as the half-life, decay mode, and in-vivo stability, etc. Labeled cells can be used for red cell survival measurements when the half-life of the radionuclide is sufficiently long. The major portion of this article will deal with random labels; only a brief discussion will be provided of the cohort labels.

COHORT LABELS

As mentioned above, the use of radionuclidic labels that get incorporated into bone marrow red cell precursors over a limited time period produces a labeled cohort of cells of approximately identical age. In an ideal situation, ~95% of the label would appear in the circulation within 4-5 days and the amount of the label in blood would remain constant until removed from the circulation due to aging of the cells, in a sigmoid fashion[3].

Such an ideal situation is not obtained with the use of either labeled glycine or radioiron. Nevertheless, these tracers have provided useful information on the normal red cell life span and hemoglobin synthesis[4]. The most important application of radioiron has been in ferrokinetic measurements[5,6]. Its use permits a complete functional analysis of the red cells at all stages, for example, quantification of erythropoeisis and life span, identification and evaluation of the sites of red-cell production and destruction, and quantitative determination of blood loss[7]. In combination with ^{51}Cr, radioiron can be used for measuring, simultaneously, both red-cell production and destruction[8]. Although it is not suitable for routine studies, the use of $DF^{32}P$ (diisopropyl fluorophosphate) has been advocated in special investigations, both as a cohort and a random label[9,10].

RANDOM LABELS

Random labeling of human erythrocytes is used more widely than cohort labeling, mainly because it involves procedures that are easier to perform in a clinical setting. In addition, and perhaps more importantly, radionuclides that possess favorable chemical and physical properties and that are available for research and clinical use, label red cells randomly.

1. Chromium-51. Chromium-51 labeling of red cells is the most popular technique at the present time for the measurement of red cell life span. Gray and Sterling in 1950 showed that ^{51}Cr in the form of chromate could be used for effectively tagging red cells of various species, including man[11]. Their observations led to the rapid development of ^{51}Cr-RBC for measuring red cell survival in human subjects[12,13]. Two problems are however, associated with this technique. The chromium label leaks from the red cells and this leakage, normally 1% per day, is often variable. Secondly, there appears to be an uneven labeling of cells depending on the age of the RBC and also on the nature of the hemoglobin itself. Appropriate corrections can be made to account for these problems and reliable results can be obtained.

   The chromium in the hexavalent form penetrates the red cell membrane and attaches to the globin part of the hemoglobin molecule. The predominant attachment is to the beta chain of globin[14]. The cationic trivalent form of chromium does not cross the membrane but binds to proteins in the plasma. Chromium-51 red cell survival curves can be erroneous in situations where cells contain abnormal hemoglobin and especially when greater or lesser amounts of the beta globin chain are present. The half-life of ^{51}Cr of 27.7 days is convenient for clinical studies although not ideal. Gamma rays of 0.32 MeV energy are emitted in 9.8% abundance and this allows for measurements using a sodium iodide well counter. In addition, this gamma emission is useful when double isotope studies are carried out employing the higher energy (1.10 and 1.29 MeV) iron-59, with appropriate crossover corrections[8].

The binding of ^{51}Cr to red cells in vitro is rapid and is >90% complete within 30 min at room temperature in ACD solution. The label is quite firm and resists repeated washing of the cells or dialysis. Factors that decrease efficiency of binding are: (i) prior contact of ^{51}Cr with ACD for periods exceeding an hour before addition of RBC; (ii) increasing the pH of ACD solution or autoclaving it; (iii) presence of calcium ions, and (iv) prolonged exposure of the ^{51}Cr-ACD mixtures to strong light. Erythrocytes can be damaged by excess metallic chromium (50 µg/ml RBC), and this was a limitation in the early work because of the low specific activity of ^{51}Cr available at that time. Alterations in the RBC are noticed even at 5 µg chromium per ml; however, such changes, mostly enzymatic, do not produce a significant effect on red cell survival. High specific activity ^{51}Cr, presently available, permits the use of less than 0.1 µg chromium per ml blood, thus allowing for the use of more activity with minimal damage to the cells.

The labeling procedure, briefly, is as follows. Approximately 16-20 ml of blood is withdrawn from the patient and added to a sterile multiinjection bottle containing 4-5 ml of ACD solution (commercially available, containing, per ml, 13.2 mg dextrose, 25 mg anhydrous sodium citrate and 8 mg anhydrous citric acid). Fifty to 100 µCi of ^{51}Cr as sodium chromate is then added and the mixture allowed to incubate for 30 min at room temperature with intermittent gentle swirling. Fifty mg ascorbic acid (0.2 ml of a 250 mg/ml solution) is added next to stop the tagging. The unlabeled ^{51}Cr can be removed by centrifuging and discarding the supernatant solution and injecting the saline-resuspended RBC, or the mixture injected as such and allowance made for non cell-bound ^{51}Cr in the calculations. The labeling efficiency should be determined on an aliquot of the well-mixed suspension in the latter case. Appropriate standards, properly diluted, are necessary for counting and comparing purposes.

The addition of ascorbic acid was proposed by Read and coworkers[13] in order to reduce the unbound chromate to trivalent chromium and thus prevent further labeling of the cells before or after injection (Cr^{3+} does not cross the cell membrane). This obviates the need for washing the cells prior to injection. By performing external body measurements for ^{51}Cr activity using a scintillation detector, the clinical usefulness of red cell survival studies can be enhanced further. For example, the potential sites of red cell sequestration in hemolytic states can thus be determined.

Damaging the cells (chemically[15,16] or by heat treatment[17,18]) following (or during) the ^{51}Cr labeling operation provides an agent suitable for the scanning of the spleen or for the study of splenic function. In early work, anti-D antibodies were also used to induce splenic trapping of the cells[18,19]. The procedure for heat damaging the

cells was developed later[17] and is almost exclusively used at the present time. The degree of heating (49° C, 15 min) however, has to be controlled very carefully since any excessive damage to the cells would result in hepatic as well as splenic sequestration of the activity.

2. Indium-and Gallium-Labeled RBC. Certain radionuclides of indium and gallium (e.g. ^{111}In, ^{113m}In, ^{67}Ga, ^{68}Ga) possess favorable properties for application in diagnostic nuclear medicine. Cell labeling with these nuclides is mainly carried out using a procedure developed by Thakur et al.[20,21] that employs the 8-hydroxyquinoline (oxine) chelates of these elements. Indium-oxine labeled leukocytes and platelets constitute an important class of radiopharmaceuticals and their value in clinical nuclear medicine is already well established[22,23]. Labeling of erythrocytes has also been carried out using the oxine chelates of indium and gallium[20,21,24-26]. The use of an acetylacetone (acac) complex of indium for labeling RBC and other cells has also been advocated[27-29]. It is claimed that the method of preparation of the acac complex is simple and that the labeling procedure may be less damaging to the cells.

Indium-oxine labeled red cells have been used in the detection of intermittent G.I. bleeding in animal models as well as in man[26,30,31]. The typical labeling procedure is as follows. Approximately 10 ml of venous blood is drawn into a syringe containing about 60 units of heparin, transferred to a round bottom tube, and centrifuged for 5 min at 1000-1500 G. The supernatant plasma and buffy coat are removed and the red cells washed twice with 5 ml of normal saline. The cells are then resuspended into 2 volumes of saline, incubated with 0.5-2 mCi of ^{111}In-oxine complex for 15 min, and washed twice with saline. The labeling efficiency generally is in the order of 90±5%. The cells are mixed with autologous plasma and injected intravenously. If the ^{111}In-oxine complex is not commercially available, it can be prepared in a typical case by the addition of 50 µg oxine to 1 mCi carrier-free indium-111 chloride adjusted to pH 5.5 with 0.3 M acetate buffer, extracting into an equal volume of chloroform, evaporating the chloroform layer to dryness, and finally dissolving the residue in 50 µl propylene glycol and diluting with 150 µl saline. It is often difficult to locate the source of intermittent G.I. bleeding by the usual procedures such as angiography, endoscopy, or scanning with ^{99m}Tc-labeled sulfur colloid or red cells. These methods show hemorrhage only at the time of bleeding. A tracer that would remain for a long enough period in the circulation to permit repeat imaging is preferable, and ^{111}In-RBC fulfills this requirement. The half-life of 67 hr and its abundant 173-and 247-keV gamma emissions make indium-111 ideal for this purpose. A major disadvantage is the relatively faster in-vivo elution of the label - approximately 7% per day in one reported case in man[30], and much higher in animals. In rabbits[26], 70% of the activity in blood had a $t_{\frac{1}{2}}$ of 3 hr and 30% a $t_{\frac{1}{2}}$ of 75 hr (Figure 1). Urine excretion reached a maximum of 2.5% per ml at 60-150 minutes; it was very slow thereafter up to 72 hr (Figure 2). It is claimed that despite the elution of the label, vascular imaging can be performed

for as long as 120 hr and bleeding in the G.I. tract detected up to 48 hr after injection[30]. A method to produce ^{111}In-RBC with higher in-vivo stability is highly desirable.

Labeling yields of >90% have been reported in the ^{68}Ga-oxine labeling of red cells[24]. The oxine complex is prepared by a method very similar to that used for preparing ^{111}In-oxine. If the ^{68}Ge/^{68}Ga generator provides ^{68}-EDTA, the complex has to be dissociated following the usual procedures[32], before preparing the oxine chelate. Gallium-68-RBC were shown to hold promise for imaging blood pools using positron tomography; the results were found to compare favorably with those obtained using ^{11}CO as a label[24].

A new approach was described recently for labeling the surface of red cells with ^{67}Ga[33]. The washed cells are first treated with tannic acid and then incubated with pH 7 ^{67}Ga chloride. The incorporation of gallium was reported to be >90% in 15 min and the in-vitro stability of the label was claimed to be good. Further work will be necessary to demonstrate the usefulness of this approach for in-vivo studies.

The use of ^{111}In-tropolone complex for labeling platelets was described in a recent report[34]. The possibility of labeling other cells with this complex was also suggested. Both oxine and tropolone form neutral chelates with indium and gallium with comparable stability constants and the cell labeling mechanisms of the two may indeed be very similar.

3. Miscellaneous Labels. Red cells have been labeled both in vitro and in vivo (cohort labeling) with various other compounds including ^{11}CO[35], ^{42}KCl[36,37], $Na_2H^{32}PO_4$[37], ^{125}I- or ^{131}I- P-iodobenzenesulfonamide[38], ^{131}I iodophenylhydroxylamine[39], ^{52}Fe[40] and ^{75}Se selenomethionine[41]. Any significant use of most of these has not resulted due to many obvious reasons.

Carbon monoxide which competes with oxygen to form carboxyhemoglobin was used (by inhalation) in one of the earlier methods for measuring blood volume with reasonable accuracy. Carbon-11, a positron emitter with a 20 min half-life has been used recently for blood volume measurements. The results are ~5-10% higher than with ^{51}Cr. Nevertheless, ^{11}CO could prove valuable for conditions where minimum radiation is desirable, and repeat blood volume studies are necessary. However, a cyclotron has to be available on-site and this imposes severe restrictions on the widespread use of ^{11}CO.

Based on various biochemical and kinetic considerations, radioiron is the best nuclide for localizing erythropoeisis. Imaging of ^{59}Fe in good resolution is extremely difficult because of the high gamma energies of 1.10 and 1.29 MeV. Iron-52, which is not routinely available, is a good candidate, although its short half-life of 8.3 hr and annihilation radiation of 511 keV make it less than ideal.

An interesting new method for labeling red cells using the enzyme-inhibitor approach was described recently[38], which may have some useful applications. In this study, radioiodinated p-iodobenzenesulfonamide, a lipophilic carbonic anhydrase inhibitor was employed to label red cells in whole blood rapidly and with high efficiency (95 ± 5%). In rats after i.v. administration, however, the activity was found to elute with a $t_{\frac{1}{2}}$ of about 30 hr.

4. Technetium-99m-Labeled Red Cells. Rapid and convenient kit procedures are presently available for labeling red cells with technetium-99m that provide essentially quantitative labeling yields. The current methodology has resulted from a slow progression of the various steps involved in the labeling process. This has closely paralleled the increase in our understanding of the chemistry of technetium-99m, which in turn has been slow to evolve[42].

(a) In-vitro methods. Soon after ^{99m}Tc was recognized as the "ideal" radiotracer for use in nuclear medicine imaging applications, efforts were begun to label red cells with this nuclide in-vitro. Several workers attempted to label RBC using the commonly available form of ^{99m}Tc which is the pertechnetate ion[43-45]. Pertechnetate moves in and out of the RBC rather freely and cannot be bound firmly to the cells in this chemical form. It was recognized early on that a reduced technetium species would be necessary to bind irreversibly with hemoglobin or other red cell components, and that reduction of pertechnetate within the cell would be a more effective way of achieving this goal. The use of stannous (Sn^{2+}) compounds to reduce pertechnetate for the purpose of labeling red cells was reported by several workers in quick succession[46-51], and stannous ion is still the most widely used reducing agent in the currently available procedures[42,52-54].

The labeling yields were initially limited to the 50-60% range and this made it necessary to separate the unbound ^{99m}Tc before injection. Several undesirable washing steps were required. The two earlier schemes involving "pretinning" and "post-tinning" of the cells are represented in Figures 3 and 4. These figures describe the various steps that were necessary to obtain good labeling yields and to remove unbound ^{99m}Tc before injection, when necessary.

Most current kit or non-kit procedures now use tinning of the cells first, using a suitable tin(II) compound such as pyrophosphate, glucoheptonate, DTPA, or citrate. The widely used BNL kit (approximately 20,000 kits are distributed annually to investigators worldwide) consists of the following: One Vacutainer reagent tube 100 x 15/16 mm-10 ml capacity, evacuated to draw up to 6 ml and containing a lyophilized preparation of: 2.0 µg tin, 3.67 mg sodium citrate, 5.50 mg dextrose, and 0.11 mg sodium chloride (maximum).

The labeling procedure using this kit is as follows:

Labeling Procedure. Use aseptic techniques throughout the procedure.

1. Add 1-3 ml of saline ^{99m}Tc pertechnetate to a sterile and pyrogen-free vial. Assay and store in lead shield.
2. Draw 4 ml of patient blood into a heparinized syringe and add to kit.
3. Mix immediately to dissolve the freeze-dried solids in the blood and gently rotate the tube for five minutes at room temperature.
4. Add 1 ml of a 4.4% EDTA solution. Draw an equal volume of air to avoid pressure buildup in the tube.
5. Mix by gently inverting about 5 times and centrifuge the tube upside down 5 minutes at approx. 1300 G.
6. Maintain the tube in the inverted position to avoid disturbing the packed RBC's. Using a standard 20 g sterile needle and a 2-3 ml sterile disposable syringe, withdraw 1.25 ml of RBC's and transfer to the premeasured technetium prepared in (1).
7. Incubate the technetium-RBC mixture for ten minutes at room temperature with gentle mixing.
8. Assay and dilute appropriately for injection. Cell separation and yield determination at this point consistently give 98+% yields.
9. The described procedure yields an excellent agent for blood pool imaging and red cell mass studies. Substitution of the following for Step 7 produces an ideal splenic agent: incubate the technetium-RBC mixture 15 minutes at 49° C with gentle mixing.

EDTA. To prepare the EDTA solution for use with this kit, take any commercially available disodium EDTA or calcium disodium EDTA solution for injection (for example: Endrate, Edetate disodium injection, USP, 15% solution in water, pH 7, Abbott Laboratories, North Chicago, IL, 60064, USA), and dilute with sterile water for injection to give a final concentration of 4.4%.

Alternately, an in-house EDTA preparation can be used if desired. To prepare this, weigh out 4.4 g disodium EDTA or calcium disodium EDTA (reagent grade) and dissolve in sterile water for injection or distilled water, under stirring and make up the volume to 100 ml. Sterilize this solution by autoclaving.

Aliquots of the sterile 4.4% EDTA solution (either in-house or commercial) can be dispensed into a number of individual sterile vials and stored in the refrigerator for subsequent use.

The various steps involved are shown schematically in Figure 5. The determination of labeling yield is carried out as follows:

Draw an aliquot (0.1 - 0.5 ml) of the well-mixed labeled red-blood-cell suspension into a syringe and add to a tube containing 2 ml saline. Mix briefly and centrifuge for 5 min at approx. 1300 x G. Withdraw supernatant solution and transfer to another tube. Make volumes in both tubes same with water. Count supernatant solution and RBC and calculate the yield as follows:

$$\text{Percent labeling yield} = \frac{\text{Activity in RBC's} \times 100}{\text{Activity in RBC's} + \text{Activity in supernatant solution}}$$

The ^{99m}Tc activity should be measured in a dose calibrator unless the sample has been allowed to cool down to <1 μCi in which case a NaI gamma counter can be used.

The procedure now uses 1 ml of 4.4% EDTA instead of 6 ml saline (as previously recommended[52]) after the tinning step prior to centrifugation. This improves the labeling yield and the in-vitro stability of the label as shown in Table 3. The blood clearance curves are shown in Figure 6. These were obtained in an asplenic dog and indicate the EDTA treatment producing a small but significant improvement in the in-vivo stability.

Red cell mass determinations using the BNL kit provide values that are in close agreement with those obtained using ^{51}Cr-RBC. The data are shown in Table 4[64]. Experiments with double labeled RBC (^{51}Cr labeling followed by labeling same cells with ^{99m}Tc) showed (Table 5) that it is the ^{99m}Tc label that elutes from the cells and leaves the circulation, rather than the intact labeled cells themselves[64]. These results suggest that the labeling operation does not significantly damage the RBC.

A study was recently completed to develop an in-vitro kit method for selectively labeling RBC with ^{99m}Tc in whole blood[54]. This new method eliminates the main drawbacks of the previous mentioned methods, namely the need for separating plasma, centrifugations, multiple transfers, etc. The overall effectiveness of such a procedure was thought to be greatly dependent on maximizing the availability of tin in the stannous form within the cells and at the same time effectively removing all the extracellular tin(II). Reduced technetium in most cases does not pass in or out of the cells and thus any premature extracellular reduction of pertechnetate results in considerably poor labeling yields. It was believed that the addition of an oxidizing agent which is not transported into the cells will render extracellular tin(II) ineffective by oxidizing it to tin(IV) (NaOCl was found most effective). The use of hypochlorite as an oxidant for tin (II) in plasma was suggested earlier by Narra and coworkers[55]. It was found[54] that hypochlorite alone did not completely oxidize all the tin(II) and that labeling yields greater than 92 ± 3% could not be obtained. Use of a chelating agent such as EDTA (in combination with NaOCl) was found to increase the labeling yields to consistently higher values (98 ± 2%) either by effectively sequestering the remaining tin(II) and thus making it more accessible to the hypochlorite, or by other possible mechanisms. Representative data are summarized in Tables 6 and 7.

Comparable labeling efficiencies at the tracer level are achieved regardless of whether glucoheptonate (GH), citrate (Cit), or other suitable ligands are used as complexing agents for tin in the kit. However, tin uptake into the RBC is higher with GH and Cit and the use of these ligands may thus be more advantageous especially in situations where excessive amounts of ^{99}Tc are present in ^{99m}Tc solutions (e.g., when using instant technetium, or the first generator milking following an overly long ingrowth period). The effect of carrier on labeling yields is shown in Table 8.

The data in Tables 6-8 were obtained using kits containing 15 µg stannous tin. Similar results are obtained using a later version of tin citrate kits that contains 50 µg tin.

The new kit for labeling RBC in whole blood consists of the following:

> One Vacutainer reagent tube 100 x 15/16 mm - 10-ml capacity, or a 10 ml multi-injection bottle, evacuated to draw up to 6 ml, and containing: 50.0 µg tin, 3.67 mg sodium citrate, 5.50 mg dextrose, and 1.40 mg sodium chloride (maximum).

The other reagents required during the labeling procedure (sodium hypochlorite, EDTA) can be prepared or obtained as follows. These may also become available as part of the kit in the future.

Sodium hypochlorite. To prepare the sodium hypochlorite (NaOCl) solution, dilute reagent grade NaOCl (for example, J.T. Baker, reagent NaOCl, 5.25%) with saline to give a 0.1% final concentration. This solution should be prepared fresh and used the same day.

EDTA. Directions for preparing and using the 4.4% EDTA solution are identical to those provided in an earlier section (vide supra).

The labeling protocol is as follows:

Use aseptic techniques throughout the procedure.

1. Draw 1 ml of patient blood (0.5 to 6 ml may be used) into a heparinized syringe and add to the kit tube.
2. Mix immediately to dissolve the freeze-dried solids in the blood and incubate for five minutes at room temperature.
3. Add 0.6 ml of 0.1% sodium hypochlorite solution. Mix by gently inverting the tube 3-4 times.
4. Add 1 ml of a 4.4% EDTA solution. Mix by gently inverting the tube 3-4 times.
5. Store the tube in a lead shield and add the desired quantity of technetium-99m pertechnetate in a volume of 0.5-3 ml.
6. Incubate the technetium-RBC mixture for fifteen minutes at room temperature with occasional gentle mixing.
7. The above procedure yields an excellent agent for blood pool imaging and red cell mass studies. Substitution of the following for Step 6 produces an ideal splenic agent: incubate the technetium-blood mixture 15 minutes at 49° C with gentle mixing.

In summary, the new kit method for selective labeling of RBC in whole blood: (i) eliminates the need for separating plasma, and thus cells remain in their native plasma environment, (ii) requires no centrifugation and thus greatly reduces handling of RBC, (iii) tolerates greater quantities of ^{99}Tc contamination in ^{99m}Tc eluates, (iv) involves one vessel operation and no transfers, and thus is more convenient to carry out in practice, and (v) provides consistently high labeling efficiencies (98 ± 2%). Preliminary studies in dogs have demonstrated that the in-vivo survival of the label in blood is equal to or somewhat superior than that using other in-vitro labeling techniques.

A typical procedure which is routinely used at BNL for preparing tin citrate kits containing 2 µg tin is described below.

Detailed Procedure for Preparing and Checking Red Blood Cell Kits

Reagents: (Sources given in parentheses; substitute sources may be used if reagent purity is comparable.)

Sterile water for injection, U.S.P., pyrogen free (Travenol Labs., Inc., Deerfield, IL)
Anhydrous dextrose, U.S.P. (J.T. Baker Chemical Co., Phillipsburg, NJ)
Trisodium citrate dihydrate, U.S.P. (Mallinckrodt Inc., Paris, KY)
Tin wire, 0.5 mm diameter (M5N-99.999%, Alfa/Ventron, Danvers, MA)
Concentrated hydrochloric acid, reagent grade
1 N sodium hydroxide, reagent grade

Apparatus:

Nitrogen glove box
Laminar flow hood
Hot plate-stirrer
Freeze drier
Magnetic stirrer
Balance
Repipet dispenser
Autoclave
Hot-air oven
Sample aluminum block
0.22 µm sterile Millipore filters

Glassware:

500-10 ml sterile Vacutainer tubes w/stoppers
1-1000 ml Erlenmeyer flask
Large (4") watch glass
2-disposable pipettes w/bulbs
1-10 ml volumetric
1-200 ml volumetric
1-250 ml volumetric
1-10.0 ml pipette (volumetric)
1-2.0 ml pipette (volumetric)
1-4.0 ml pipette (volumetric)
1-10 ml beaker
1-100 ml beaker
20-30 ml sterile multi-injection bottles

Procedure:

1. Prepare glassware and laminar flow hood according to standard USP procedures.
2. Thoroughly clean the nitrogen glove box by wiping inner walls and equipment with wet Kimwipes.
3. Boil ~700 ml sterile water for injection in sterile 1 liter Erlenmeyer flask covered with a sterile watch glass. Allow to cool overnight, covered with a watch glass and stored inside the laminar flow hood.
4. Wash 1 piece of tin wire 4.08 cm in length (0.5 mm diam.) weighing about 52.6 mg in beaker with acetone, dry with nitrogen, and transfer to a 10 ml volumetric inside nitrogen glove box. Cover the tin wire with 2 ml concentrated HCl. Heat gently to dissolve. After cooling, dilute to volume with boiled sterile water. This is solution "A", which contains ~5.3 mg/ml tin in 2.4 N HCl.
5. Prepare the citrate solution by dissolving 36.70 g trisodium citrate dihydrate in 80 ml boiled sterile water in a 100 ml beaker.
6. Add dropwise, under stirring, 4.0 ml solution "A" to the 100 ml beaker containing the above citrate solution while keeping the pH between 6 and 7.5 by adding 1 N NaOH dropwise as necessary. Final pH should be 7-7.5. Quantitatively transfer this solution to a 200 ml volumetric using water and dilute to volume with water. This is solution "B" containing ~105 µg/ml tin.
7. Aseptically, transfer 13 ml aliquots of solution "B" into sterile 30 ml multi-injection bottles and quick-freeze. Store the vials in a freezer, until ready to use.
8. Allow one 30 ml bottle containing 13 ml frozen stannous citrate to come to room temperature just before use. Transfer 10 ml of this solution into a 250 ml volumetric containing 2.75 g anhydrous dextrose dissolved in sterile water. Raise the volume to mark with sterile water. This is solutions "C" containing 4 µg/ml Sn(II). The above operations are carried out inside the nitrogen-filled glove box.
9. Remove solution "C" from the glove box after filtration through a sterile 0.22 µm Millipore filter into the sterile dispensing apparatus.
10. Dispense 0.5 ml filtered solution "C" into sterile Vacutainer tubes and immediately freeze by transferring to the aluminum block surrounded by dry ice. The dispensing assembly is kept under an atmosphere of sterile, filtered N_2. This operation is carried out inside the laminar flow hood with the uv light on.
11. Stopper the tubes making sure there is a big enough opening to allow for proper vapor escape.
12. Place the [illegible] inside freeze drier whose shelf has been precooled to <-40°C. Allow to pump ~24 hr without applying shelf heat and then 24 hr with shelf heated to 110°F. Fill the drier chamber with nitrogen to 16" Hg, and remotely stopper the tubes. Remove from the freeze-drier.
13. Immediately irradiate the stoppered tubes with a total of 2.5×10^6 rads using a ^{60}Co source.
14. Send out random samples to independent laboratories for sterility and pyrogen testing.
15. Check red cell labeling yields with representative sample tubes, in duplicate, using both no-carrier-added as well as carrier-added ^{99m}Tc solutions.

Typical images of the heart obtained following the injection of in-vitro kit labeled RBC are shown in Figure 7. A representative dyna[illegible] using gated data acquisition is shown in Figures 8 and 9.

(b) In-vivo method. Labeling of red cells with ^{99m}Tc can also be carried out in vivo. The method is based on the observation that prior administration of stannous compounds causes alterations in the subsequent in-vivo distribution of pertechnetate[56]. Pertechnetate alone does not bind strongly with red cells, but in vivo because of the prior introduction of tin(II) in the red cell compartment, the administered pertechnetate after finding entry into the cells gets reduced and firmly bound. Pavel and co-workers[53] introduced this method for RBC labeling in vivo and found stannous pyrophosphate to be most effective. Labeling efficiency (fraction of the total administered pertechnetate incorporated into the red cells), however, is variable, usually ranging anywhere between 60 and 90%. The exact role of stannous ion in the labeling process has not been completely elucidated. It has been proposed that tin(II) selectively activates the redox mechanisms in the choroid plexus and red cells thus causing in-situ reduction of the pertechnetate and its retention primarily at these two sites. Compared to the in-vitro method which routinely provides quantitative labeling of the cells (a necessary requirement for many applications), the in-vivo method results in generally poor and frequently irreproducible labeling efficiencies. Its usefulness, however, cannot be disputed, especially because of the convenience. The process requires only two injections and no outside handling of blood is involved. When higher labeling efficiencies are required, and for splenic studies, in-vitro labeling is the method of choice.

Various parameters such as the optimum quantity of the tin(II) preparation to be injected and the appropriate time delay between tin injection and the pertechnetate administration, etc. have been studied by various investigators[57-59]. Administration of 10 to 20 μg of stannous ion per kilogram of body weight is thought to be adequate for optimal labeling. A commercial stannous pyrophosphate kit containing 2 mg stannous ion is reconstituted with saline and a suitable aliquot (depending upon the patient's weight) is injected into the patient. After 30 min, the desired quantity of $^{99m}TcO_4^-$ (usually 10 to 30 mCi) is injected intravenously. The red cells get labeled almost immediately and the technetium activity incorporated into the cells has a clearance half-time of about 30 hr or more[60]. The stannous ion taken up by the cells appears to have a quite slow clearance. Thus, it appears that following pertechnetate injection up to several days after the patient has had a ^{99m}Tc-Sn-pyrophosphate bone study performed, significant in vivo cell labeling with ^{99m}Tc can occur.

The long-term retention of tin following in-vivo RBC labeling was the subject of a recent study[60]. The data from this study are summarized in Tables 9-10. It was found that: (i) There is a significant retention of tin in the RBC, even after a period of two months following a single Sn-PYP injection. (ii) Early blood samples (following tin administration) give high labeling yields (in vitro) with ^{99m}Tc; some labeling is achieved even with two-month samples. (iii) The kinetics of the ^{99m}Tc labeling of RBC (in vitro) slow down considerably

with the later samples. This may be due to the slow loss of Sn(II) from the cells or its oxidation to Sn(IV). Normal loss of RBC (and thus of tin) from the circulation may also be an important contributing factor. (iv) Significant in-vivo labeling of RBC results when $^{99m}TcO_4^-$ is injected up to 42 days after a single Sn-PYP administration. (v) Blood samples obtained 60 min after the injection of $^{99m}TcO_4^-$ showed that the activity in blood (% injected dose) was high for early periods after the Sn-PYP injection (30 min, 98.5) and dropped slowly with time (7 d, 41; 21 d, 27.5; 42 d, 25.4). The ratio of RBC to plasma activity also decreased with time (30 min, 19; 7 d, 1.75; 21 d, 0.39; 42 d, 0.20).

(c) Combined in-vivo/in-vitro ("In-vivitro") Methods. Some investigators claim to overcome the variable tagging achieved with the in-vivo procedure with the adaptation of the "in-vivitro" method[61-63]. The method basically is an in-vivo "timing" procedure followed by presentation of ^{99m}Tc-pertechmetate activity to a smaller number of RBC in vitro and reinjecting the labeled RBC in plasma into the patient. A typical procedure [61] is presented below in view of its use at various centers.

Modified in vivo labeling of red blood cells

1. Pretreatment with 0.5-1 mg stannous ion as stannous pyrophosphate (I.V. injection not through indwelling catheter)
2. Place 19 gauge butterfly needle into antecubital vein.
3. Attach 4-way stopcock to butterfly; place 10 ml syringe containing 4 ml heparin (10 units/ml) and 4 ml 0.9% NaCl on free port of stopcock.
4. Flush butterfly and tubing with heparin saline solution.
5. 20 min after injection of stannous reagent, attach a shielded 5 ml syringe containing 20 mCi of ^{99m}Tc pertechmetate to free port of stopcock.
6. Withdraw 3 ml of blood into ^{99m}Tc syringe.
7. Flush butterfly and tubing with heparin saline solution.
8. Invert the ^{99m}Tc-RBC syringe every 1 min for ten minutes; then inject labeled red cells via the indwelling butterfly needle.

(d) Mechanistic studies. The mechanism involved in the ^{99m}Tc-RBC labeling process is not completely understood. However, some evidence has been accumulated[64-67] to support the following conclusions: (1) stannous ion complexed with citrate or other suitable agents diffuses into the cell and becomes bound to a cellular component, (2) pertechmetate ion diffuses freely in and out of the cell, (3) pertechmetate, once inside the cell and if tin(II) is already present there, gets reduced and bound mainly to the globin part of hemoglobin, (4) the binding of technetium with globin is predominantly to the β-chain, (5) reduced forms of technetium cannot be transported across the cell membrane, and (6) any tin(II) remaining outside of the cells prematurely reduces the pertechmetate thus forbidding the entry of technetium into the cells and thereby causing low labeling yields. It thus becomes necessary to remove excess tin before adding pertechmetate. Data on the uptake and distribution of ^{99m}Tc and tin (^{117m}Sn or ^{113}Sn was used) in blood and within the red cell components[64] are described in Tables 11-14.

Alterations in the binding of technetium-99m by red cells have been reported as a result of various patient medications[68,69] as well as from diseases or medications associated with RBC antibody formation[70]. The exact mechanisms responsible for these effects are not known.

(e) Comparison of ^{99m}Tc-RBC with ^{99m}Tc-HSA. The activity concentration of ^{99m}Tc in blood is significantly higher with ^{99m}Tc-RBC as compared to ^{99m}Tc-HSA (human serum albumin) for several hours after injection. For a number of reasons[71], ^{99m}Tc-HSA preparations with favorable blood clearance characteristics are not easily achievable. For blood pool imaging, especially of the cardiac chambers, labeled RBC have thus proven to be superior to labeled HSA in several recent studies[72,73] Some of the representative data are shown in Figures 6-8.

(f) Splenic studies. Heat-damaged technetium-99m RBC (^{99m}Tc-HDRBC) find application in the imaging of spleen which is useful in a numbr of clinical situations including trauma, investigation of left upper quadrant masses or pain, evaluation of spleen size, splenic infarcts and space occupying disease, and accessory splecus[74,75]. Specificity higher than ^{99m}Tc-sulfur colloid is obtained by using ^{99m}Tc-HDRBC. Heating at 49.5^{o} C is a very reliable technique for inducing splenic sequestration of ^{99m}Tc-RBC. The rapid blood clearance ($t_{\frac{1}{2}}$ 6 min) and a plateauing of the activity in the spleen by 30 minutes make it possible to accomplish imaging soon after the radionuclide administration[75]. High splenic uptake (~70%) allows the administration of small quantities of the radiotracer (~1 mCi is adequate for rapid imaging in multiple views) thus reducing the patient radiation dose considerably. Representative spleen images are shown in Figure 12, obtained using ^{99m}Tc-S colloid and ^{99m}Tc-HDRBC[52].

(g) Patient radiation dose. Average radiation doses to the whole body as well as to various other tissues and organs from the administration of normal and heat damaged ^{99m}Tc-RBC are shown in Table 15[75,76]. The whole body dose is generally lower than that to many other organs which is due to the lower average blood content of whole body.

CONCLUSION

Radiolabeling of red cells and their clinical and research application in nuclear medicine imaging and other areas, has been a field of intense interest during the last two decades. Significant advances have been made so that at the present time aufficiently stable labels are available for various applications. Technetium-99m labeled RBC have revolutionized the field of nuclear cardiology and it is now possible to evaluate various heart parameters externally without significant radiation dose or trauma to the patient.

The ideal radioisotopic label for the determination of red cell life span in normal and hemolytic states is yet to be developed. The tracers available so far especially ^{51}Cr-RBC have provided valuable information, but occasionally given misleading results as well. Caution has to be exercised when interpreting the results.

For various clinical procedures, the choice of label varies. Red cells labeled with long (~30 d) as well as intermediate (2-3 d) half-life nuclides with sufficient in-vivo stability are highly desirable. Future work will perhaps fulfill this need now that we have acquired a strong base of useful knowledge on radiolabeled red cells. Approaches such as using labeled antibodies to red cell antigens or receptors, or enzymes in the cell may provide encouraging results in terms of labeling RBC with the least damage.

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Table 1

Red Cell Labels

Nuclide	Compound used	In vivo	In vitro	Random	Cohort
^{55}Fe, ^{59}Fe	Ferric chloride Ferrous citrate	x			x
^{15}N, ^{3}H, ^{14}C	Glycine	x		x	x
^{15}N, ^{3}H, ^{14}C	DFP	x		x	x
^{32}P	DFP	x		x	x
^{51}Cr	Chromate	x	x	x	
^{111}In	Oxine, Acetylacetone, Tropolone		x	x	
^{68}Ga	-		x	x	
^{99m}Tc	Pertechnetate	x	x	x	

Table 2

Diagnostic Applications of Radiolabeled Red Cells

Normal

1. Nuclear cardiology
2. Blood pool imaging
3. Detection of vascular malformations
4. Detection of G.I. bleeding
5. Detection of hemangiomas
6. Red cell mass determination
7. Red cell life-span measurement

Heat-Damaged

1. Spleen imaging
2. Accessory spleen localization
3. Detection of G.I. bleeding

Table 3

Effect of EDTA Addition vs. Saline Addition on the Labeling Efficiency and Stability of ^{99m}Tc-RBC Using the BNL Kit[1]

	Saline Addition	EDTA Addition
Labeling yield, percent	97.1 ± 0.9	98.7 ± 0.6
^{99m}Tc washout[2]		
30 min	2.5 ± 0.3	1.6 ± 0.2
24 hr	10.7 ± 2.4	5.6 ± 1.4

[1]After incubating 4 ml blood with the kit for 5 minutes, and prior to centrifugation, either 6 ml saline or 1 ml 4.4% Na_2EDTa were added, n=5.

[2]Washed, labeled cells were incubated with saline at room temperature, and periodic aliquots removed to determine the loss of ^{99m}Tc from the cells.

Table 4

Technetium-99m/Chromium-51 Red Cell Mass Ratios

Patient No.	Sampling time after injection (min) 15	30	60	120
1	1.05	1.01	1.05	1.03
2	0.96	0.93	1.01	1.04
3	1.00	1.01	1.06	1.08
4	1.01	0.97	1.03	1.05
mean	1.01	0.98	1.04	1.05
s.d.	± 0.03	± 0.03	± 0.02	± 0.03

Table 5

$^{99m}Tc/^{51}Cr$ Ratios in Blood in Dog Using Double Labeled RBC

Time (hr) after injection	Ratio
0.25	1.00
0.5	0.99
2	0.96
4	0.89
21.5	0.59
25.5	0.54

Table 6

Effect of Sodium Hypochlorite (NaOCl), EDTA, and Plasma on the Tc-99m Labeling of Red Blood Cells Using BNL Tin Citrate (15 μg Sn^{2+}) Kits

Sample and treatment	Tc-99m Activity, %		
	RBC	Supernatant	2, 2 ml saline washes
Whole blood			
No NaOCl	1.8 ± 0.3	87.1 ± 1.6	11.3 ± 0.5
NaOCl (0.6 ml, 0.1 %) added	93.3 ± 0.4	5.2 ± 0.4	1.5 ± 0.2
NaOCl (as above) + EDTA (1 ml, 4.4%) added	98.0 ± 1.2	1.5 ± 0.3	0.5 ± 0.1
RBC (Plasma removed after tinning)			
NaOCl (as above) added	99.6 ± 0.3	0.4 ± 0.3	--

Table 7

Effect of Adding EDTA after NaOCl and Prior to Pertechnetate Addition on the Tc-99m Labeling of Red Blood Cells

Conditions	Tc-99m Activity, %	
	RBC	Supernatant (including 2, 2 ml saline washes)
Tin citrate kits, n=10 (15 μg Sn^{2+})		
No EDTA	93.3 ± 0.4	6.7 ± 0.4
1.0 ml EDTA, pH 4.6	97.7 ± 1.2	2.3 ± 1.2
1.0 ml EDTA, pH 7.2	98.0 ± 1.2	2.0 ± 1.2
Tin glucoheptonate kits, n=15 (15 μg Sn^{2+})		
No EDTA	95.2 ± 1.0	4.8 ± 1.0
1.0 ml EDTA, pH 4.6	98.0 ± 0.2	2.0 ± 0.2
1.0 ml EDTA, pH 7.2	97.6 ± 1.0	2.4 ± 1.0

Table 8

Effect of Carrier ^{99}Tc on RBC Labeling Yields (3 ml whole blood, n=4)

^{99}Tc added, equivalent to x mCi of ^{99}Mo decay[1], x =	% ^{99m}Tc Activity					
	Tin glucoheptonate kits (15 µg Sn^{2+})			Tin citrate kits (15 µg Sn^{2+})		
	0	500	600	0	500	600
RBC	97.3 ± 2.4	97.3 ± 0.8	72.7 ± 10.8	97.9 ± 1.8	96.8 ± 2.1	90.8 ± 7.3
Supernatant	2.7 ± 2.4	2.7 ± 0.8	27.3 ± 10.8	2.1 ± 1.8	3.2 ± 2.1	9.2 ± 7.3

[1]Approximately 1.27×10^{16} atoms or 2.09 µg of ^{99}Tc are produced upon the decay of 1 Ci ^{99}Mo.

Table 9

In-Vitro RBC Labeling with ^{99m}Tc of Blood Samples Obtained at Various Intervals Following Sn-PYP Injection in Normal Human Volunteers (n=4)

Time after Sn-PYP injection	Percent labeling yield		
	Time (min) of incubation, RBC's + $^{99}TcO_4^-$		
	15	60	300
30	98.5 ± 0.7	96.7 ± 1.2	—
24 hr	98.6 ± 0.3	98.4 ± 0.6	94.6 ± 2.3
7 d	55.7 ± 3.7	85.4 ± 5.8	96.5 ± 2.0
21 d	29.5 ± 4.3	47.6 ± 7.1	89.2 ± 5.2
42 d	21.4 ± 3.5	27.3 ± 6.8	61.7 ± 10.8
63 d	20.7 ± 13.7	31.7 ± 28.2	57.1 ± 30.1

Table 10
In-Vivo RBC Labeling with ^{99m}Tc in Normal Human Volunteers at Various Intervals Following a Single Sn-PYP Injection (n=3)

Time of $^{99m}TcO_4^-$ injection (post Sn-PYP)	Time (min) of blood sampling (post $^{99m}TcO_4^-$ injection)	^{99m}Tc in Blood (% of total injected, normalized)	Percent of ^{99m}Tc (total) RBC	Plasma (plus 2 ml saline wash)
30 min	30	100.0	94.5 ± 2.5	5.5 ± 2.5
	1440	71.6 ± 1.5	92.9 ± 1.0	7.1 ± 1.0
7 d	60	41.2 ± 9.5	63.6 ± 3.3	36.4 ± 3.3
	300	39.4	90.3	9.7
21 d	60	27.5 ± 2.8	27.9 ± 3.6	72.1 ± 3.6
	300	20.2 ± 1.2	43.8 ± 2.9	56.2 ± 2.9
42 d	60	25.4 ± 2.7	16.5 ± 0.8	83.5 ± 0.8
	300	16.6 ± 3.3	16.7 ± 2.5	83.3 ± 2.5

Table 11

Effect of Blood Volume and Amount of Tin(II) in Kit on RBC Tin Uptake (n=5)

Blood used (ml)	% Tin uptake into RBC	
	15 µg kit	50 µg kit
1	12.3 ± 1.2	9.5 ± 0.1
2	19.3 ± 1.2	12.5 ± 0.2
3	25.7 ± 0.3	15.9 ± 1.1
4	28.9 ± 3.7	20.9 ± 1.1

[1]Lyophilized kits labeled with ^{117m}Sn or ^{113}Sn were used. The kits contained the stated amount of tin(II), 3.67 mg trisodium citrate, and 5.5 mg dextrose. The kits were incubated for 5 min with the indicated volume of blood, and then the cells separated and washed (2x) to determine radioactivity uptake.

Table 12

Percent Distribution of Tin in Blood Components Following In-Vitro Labeling (Blood Volume 3 ml; n=10)

Tin content of kit, µg	Red cell bound		Plasma (including wash)
	Membrane	Non-membrane	
2	4.0 ± 2.0	17.5 ± 9.7	77.1 ± 12.1
15	5.7 ± 2.9	18.3 ± 5.1	77.1 ± 4.2

Table 13

Percent Distribution of ^{99m}Tc in Blood Components Following In-Vitro Labeling (n=10)

Tin content of kit, µg	Red cell bound		Plasma	Wash
	Membrane	Non-membrane		
2	1.7 ± 0.3	94.8 ± 2.0	2.7 ± 1.7	0.6 ± 0.3
15	1.3 ± 0.2	93.3 ± 3.2	6.1 ± 3.0	0.7 ± 0.1

Table 14

Percent Distribution of ^{99m}Tc and Tin in Hemoglobin (n=10)

Fraction	Technetium (^{99m}Tc)	Tin (^{113}Sn or ^{117m}Sn)
Heme	18.3 ± 9.8	90.1 ± 4.5[1]
Globin	80.5 ±10.1	12.9 ± 4.2

[1]Results not reliable due to high solubility of tin in acid. The method for separating heme and globin involved HCl/acetone treatment.

Table 15

Radiation Absorbed Dose from Technetium-99m-Labeled Red Blood Cells

Organ	Dose, rad/mCi	
	Normal RBC	Heat-damaged-RBC
Whole body	0.019	0.018
Heart	0.078	-
Spleen	0.050	2.87
Liver	0.070	0.011
Blood	-	0.027
Lungs	0.056	-
Kidneys	0.054	-
Red marrow	0.033	-

LEGENDS FOR FIGURES

Figure 1. Blood clearance of indium-111 labeled red cells in rabbit. Data from Reference 26, reproduced with permission.

Figure 2. Urine clearance of indium-111 labeled red cells in rabbit. Data from Reference 26, reproduced with permission.

Figure 3. An early scheme of labeling RBC with ^{99m}Tc using the "pretinning" method. Data from Reference 47, reproduced with permission.

Figure 4. An early BNL scheme of labeling RBC with ^{99m}Tc using the "post-tinning" method. Data from Reference 74, reproduced with permission.

Figure 5. Schematic representation of steps involved in the currently used BNL kit method.

Figure 6. Blood clearance curves of in-vitro labeled ^{99m}Tc-RBC in asplenic dog. Curve 1, saline procedure; curve 2, EDTA procedure; curve 3, 50 µg kit, whole blood procedure; curve 4, heat-damaged RBC, saline procedure.

Figure 7. Typical images (right anterior oblique, anterior, and left anterior oblique) of the heart obtained following the administration of in-vitro labeled ^{99m}Tc-RBC. Note the excellent visualization of the interventricular septum, space between the liver and heart, and the aorta and pulmonary artery. Data from Reference 51, reproduced with permission.

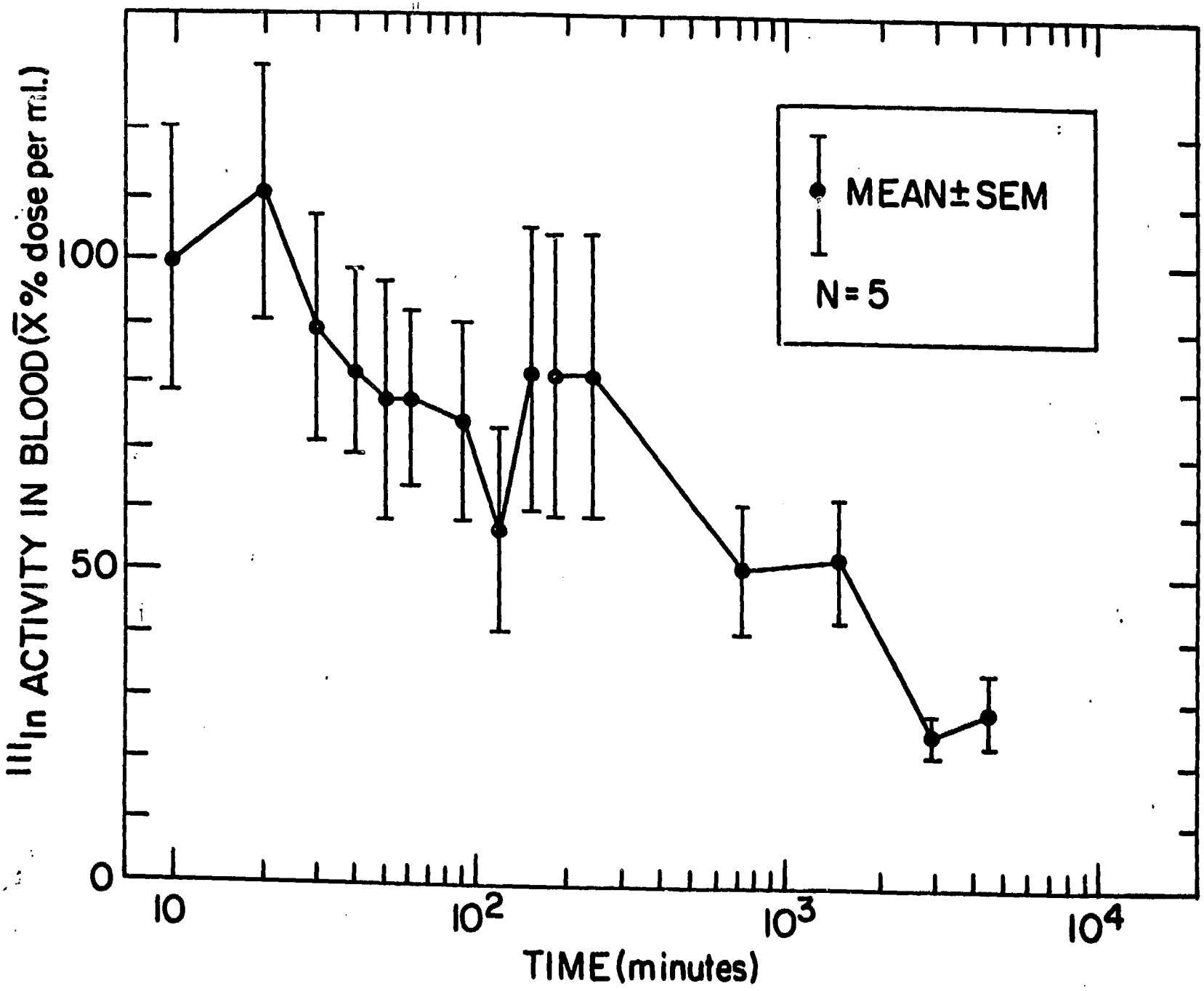
Figure 8. Dynamic views of the heart obtained using in-vitro labeled ^{99m}Tc-RBC. Note the emptying and the filling of the heart chambers.

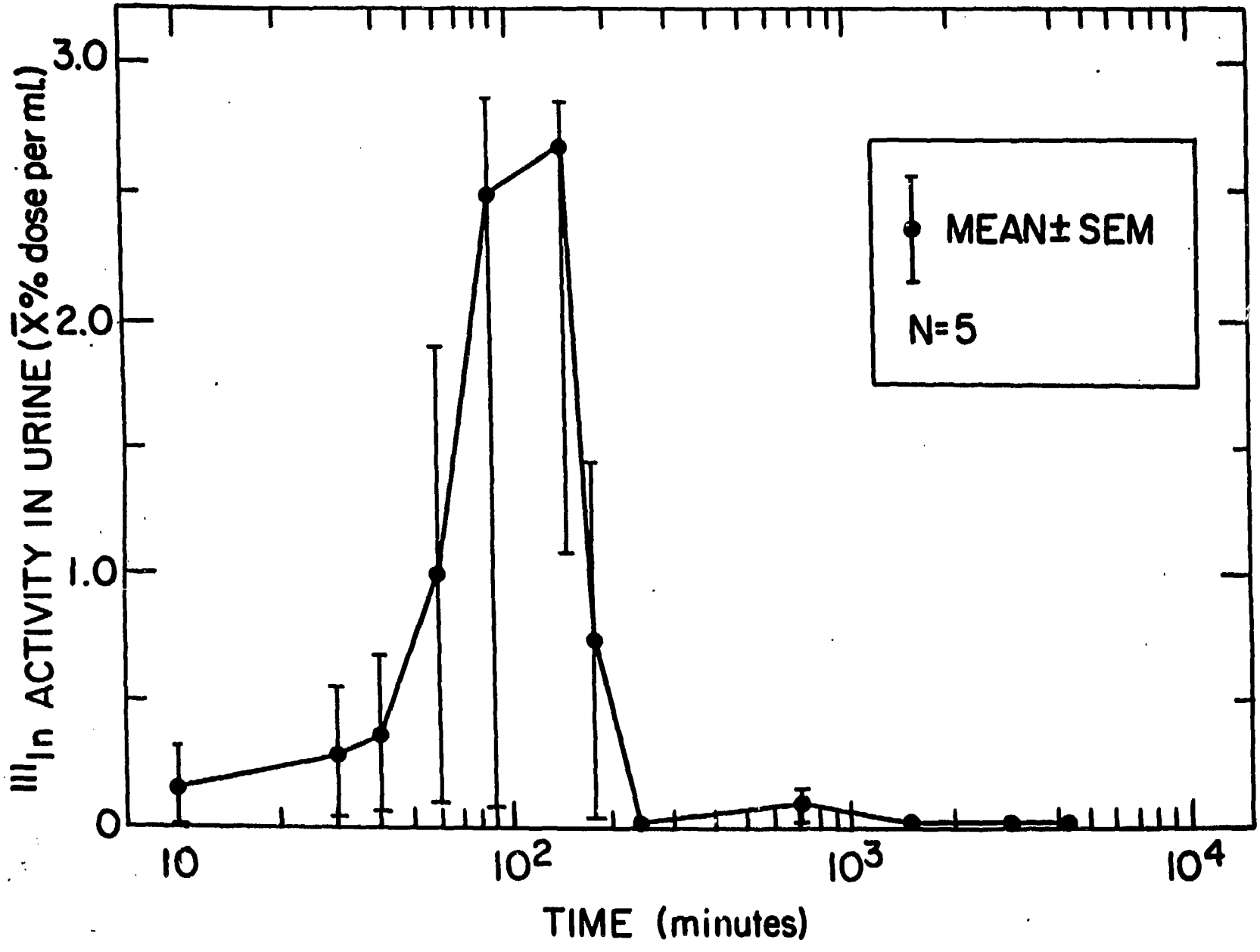
Figure 9. A typical gated stucy (MUGA) of the heart following administration of ^{99m}Tc-RBC.

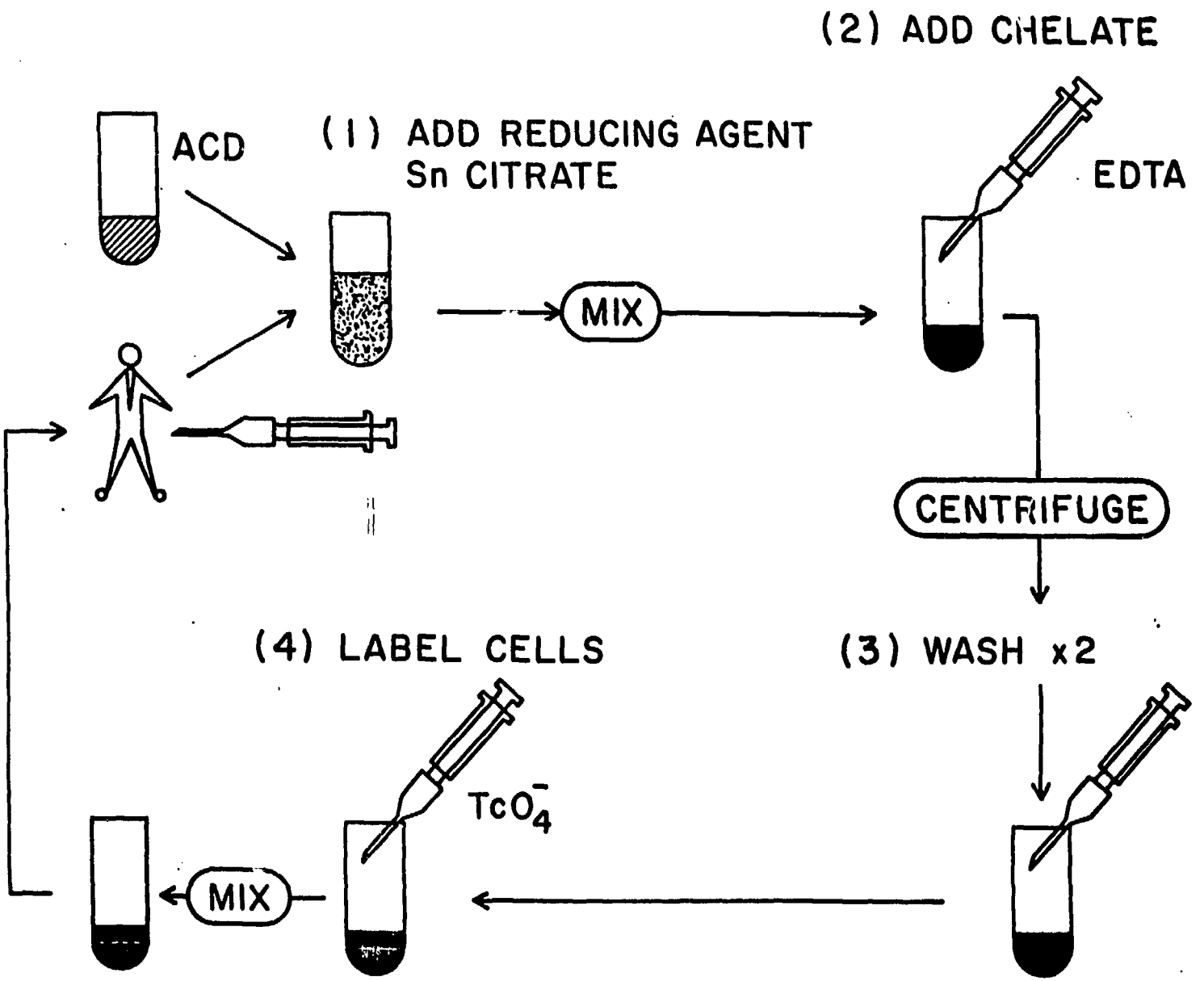
Figure 10. Change in blood concentration with time of ^{99m}Tc-RBC and ^{99m}Tc-HSA (average of 5 patients). The average blood clearance $t_{\frac{1}{2}}$ were 28.7 hr for ^{99m}Tc-RBC and 5.3 hr for ^{99m}Tc-HSA. Data from Reference 73, reproduced with permission.

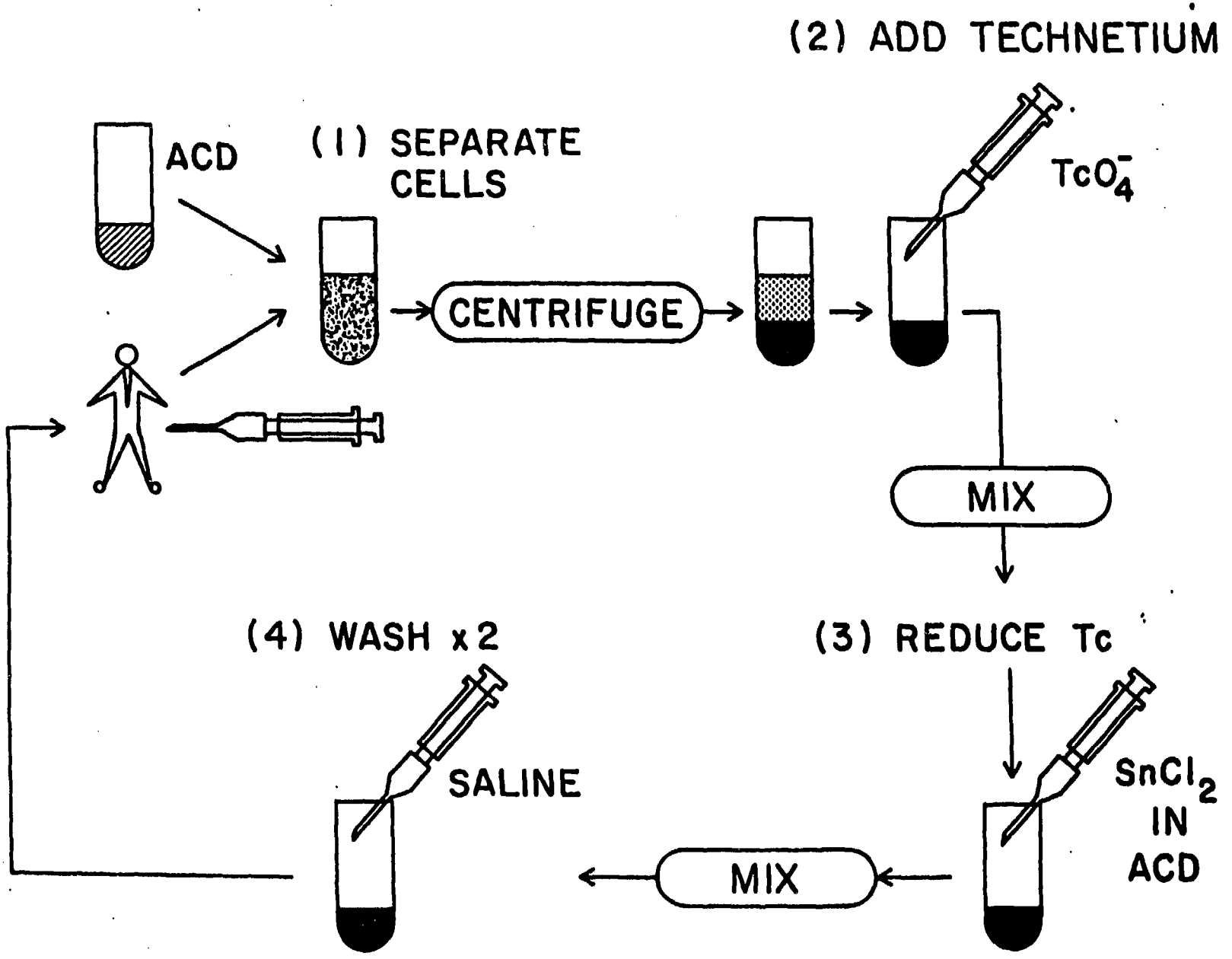
Figure 11. Comparison scintiphotos of ^{99m}Tc-HSA and ^{99m}Tc-RBC distribution in heart, lungs, and liver. Note the relative decrease in cardiac blood pool activity of ^{99m}Tc-HSA with time and the biliary excretion of ^{99m}Tc. In the ^{99m}Tc-RBC study, the relative cardiac blood pool radioactivity remains high compared to the liver. Data from Reference 73, reproduced with permission.

Figure 12. A comparison of spleen images obtained with ^{99m}Tc-sulfur colloid and BNL-kit-labeled ^{99m}Tc-heat damaged RBC. Note the absence of liver activity in the RBC image. Data from Reference 52, reproduced with permission.

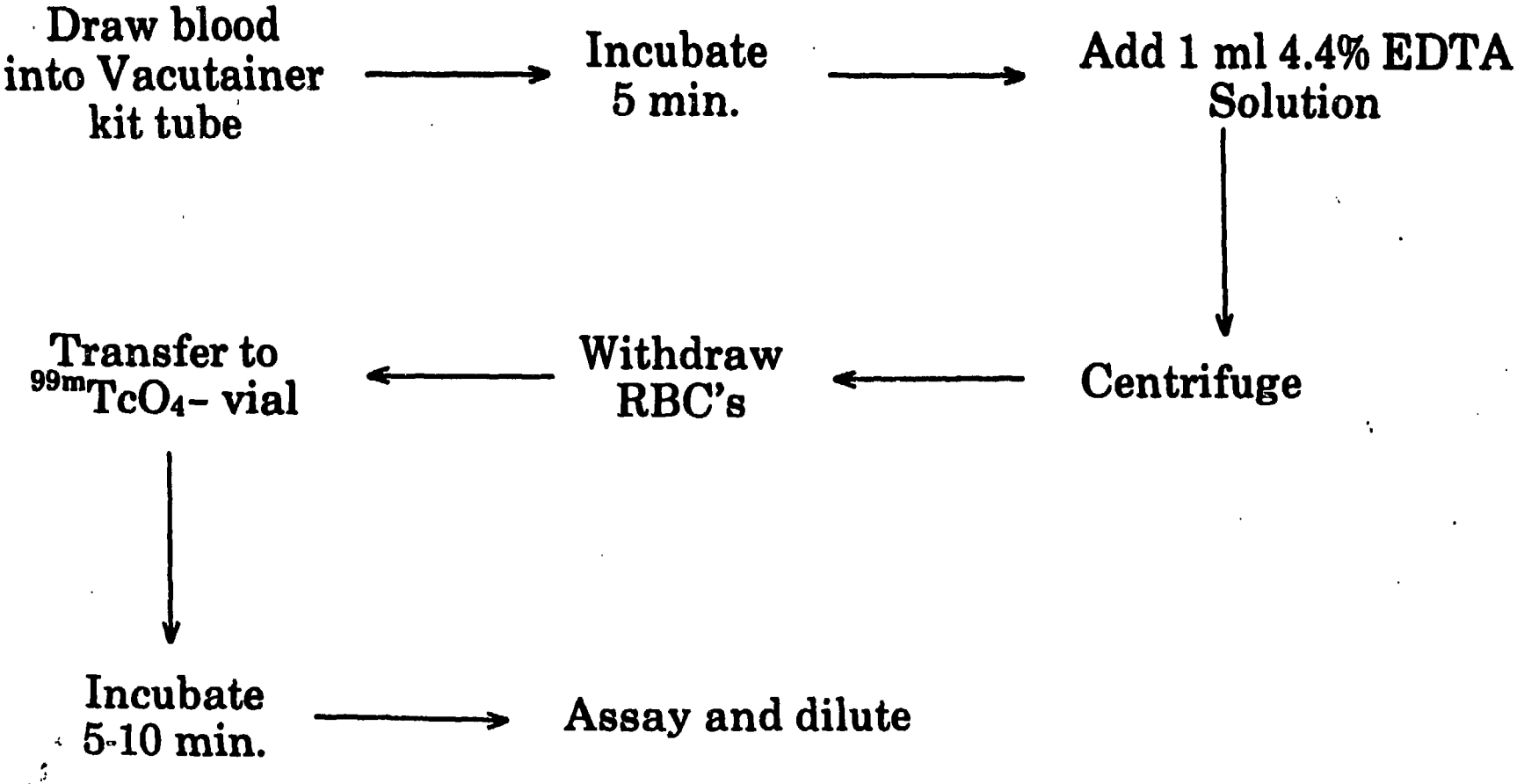

^{111}In ACTIVITY IN BLOOD ($\bar{X}$ % dose per ml.)
100
50
0
10
10^2
10^3
10^4
TIME (minutes)
MEAN ± SEM
N = 5

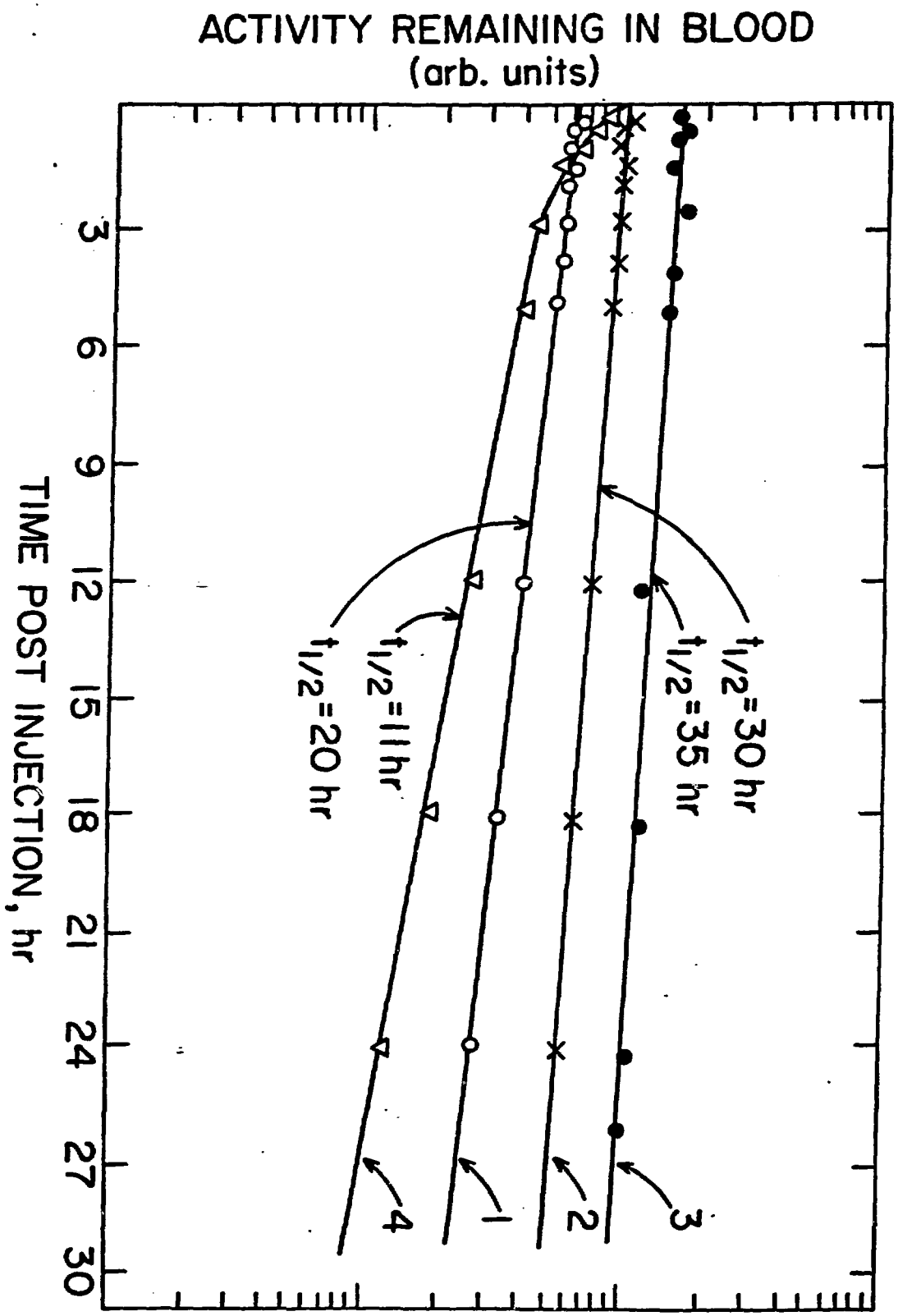

3.0
2.0
1.0
0
^{111}In ACTIVITY IN URINE ($\bar{X}$% dose per mL)
MEAN± SEM
N=5
10
10^2
10^3
10^4
TIME (minutes)

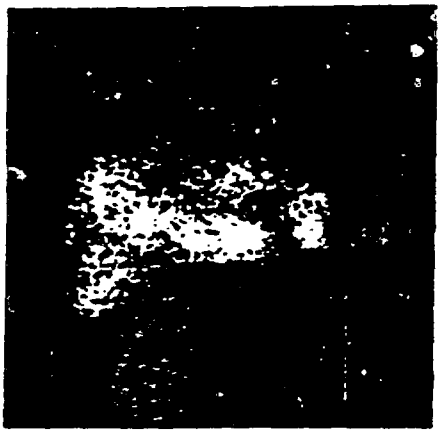

(2) ADD CHELATE
ACD
(1) ADD REDUCING AGENT
Sn CITRATE
EDTA
MIX
CENTRIFUGE
(4) LABEL CELLS
(3) WASH x2
TcO_4^-
MIX


(2) ADD TECHNETIUM
ACD
(1) SEPARATE CELLS
TcO_4^-
CENTRIFUGE
MIX
(4) WASH x2
(3) REDUCE Tc
SALINE
$SnCl_2$ IN ACD
MIX

BNL Kit Procedure for Preparation of ^{99m}Tc Labeled RBC's

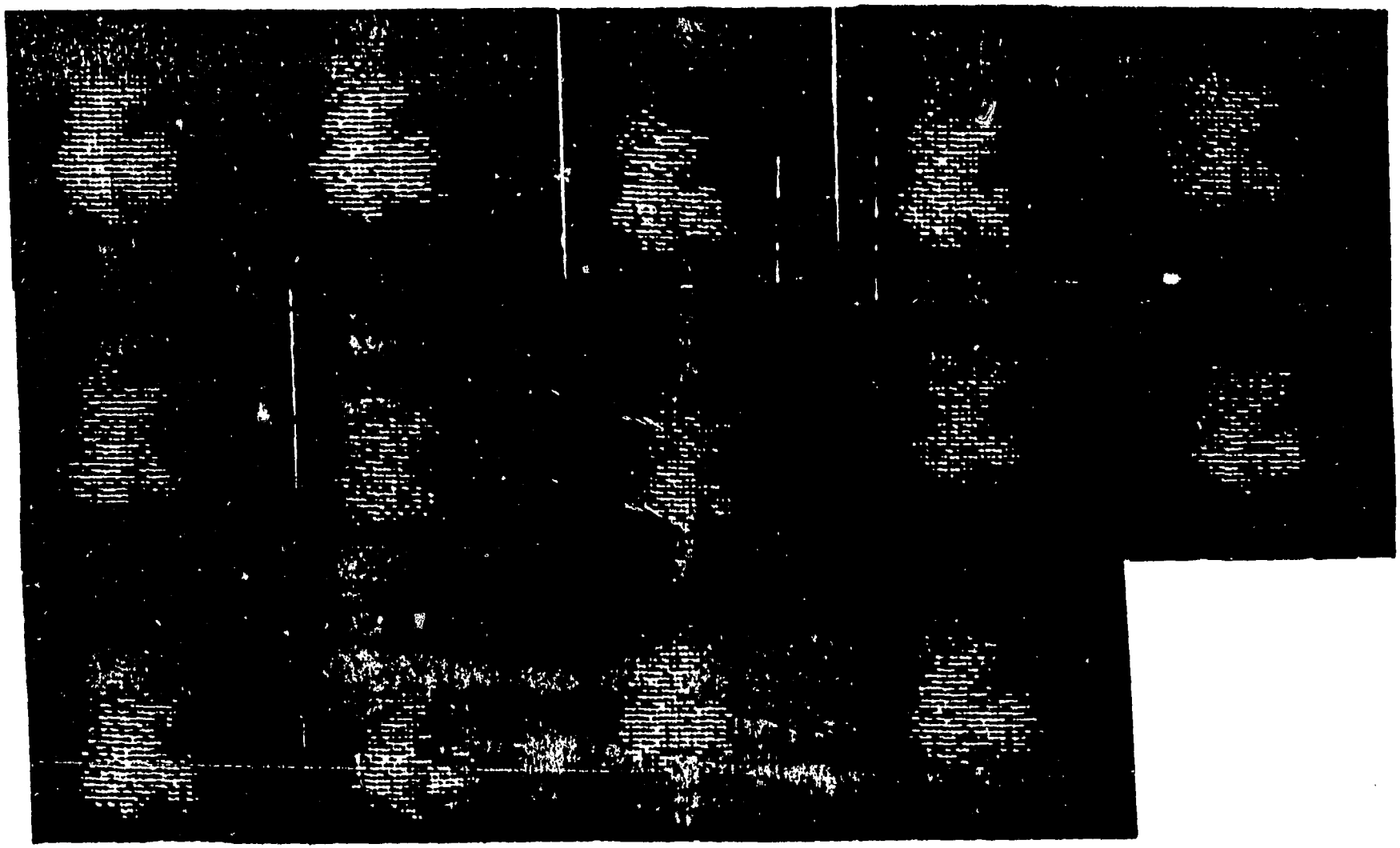


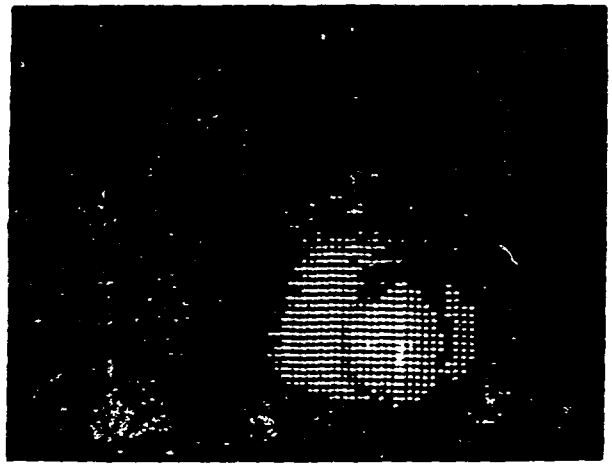

ACTIVITY REMAINING IN BLOOD
(arb. units)
TIME POST INJECTION, hr
3
6
9
12
15
18
21
24
27
30
$t_{1/2}$=20 hr
$t_{1/2}$=11 hr
$t_{1/2}$=35 hr
$t_{1/2}$=30 hr
4
1
2
3

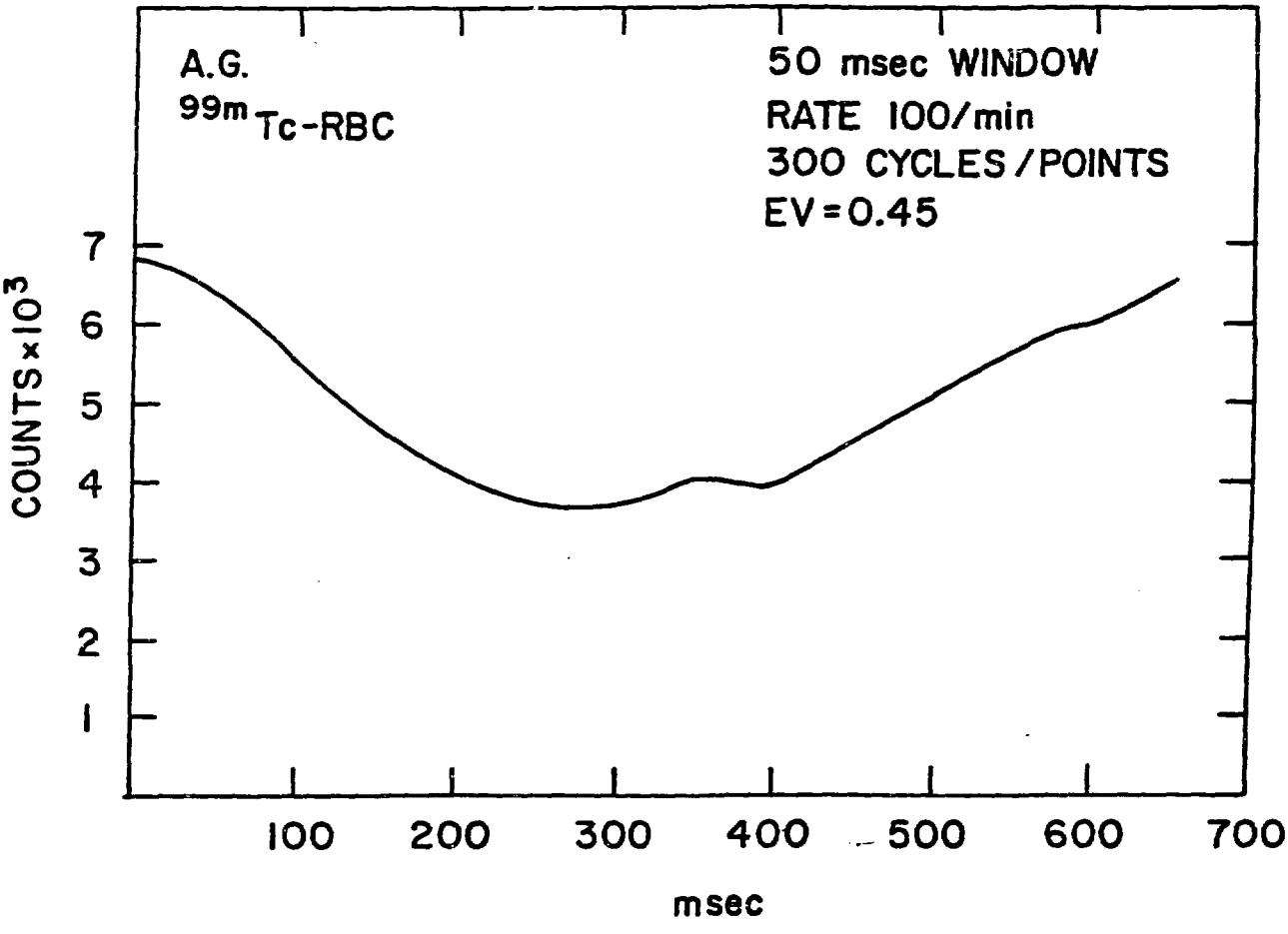


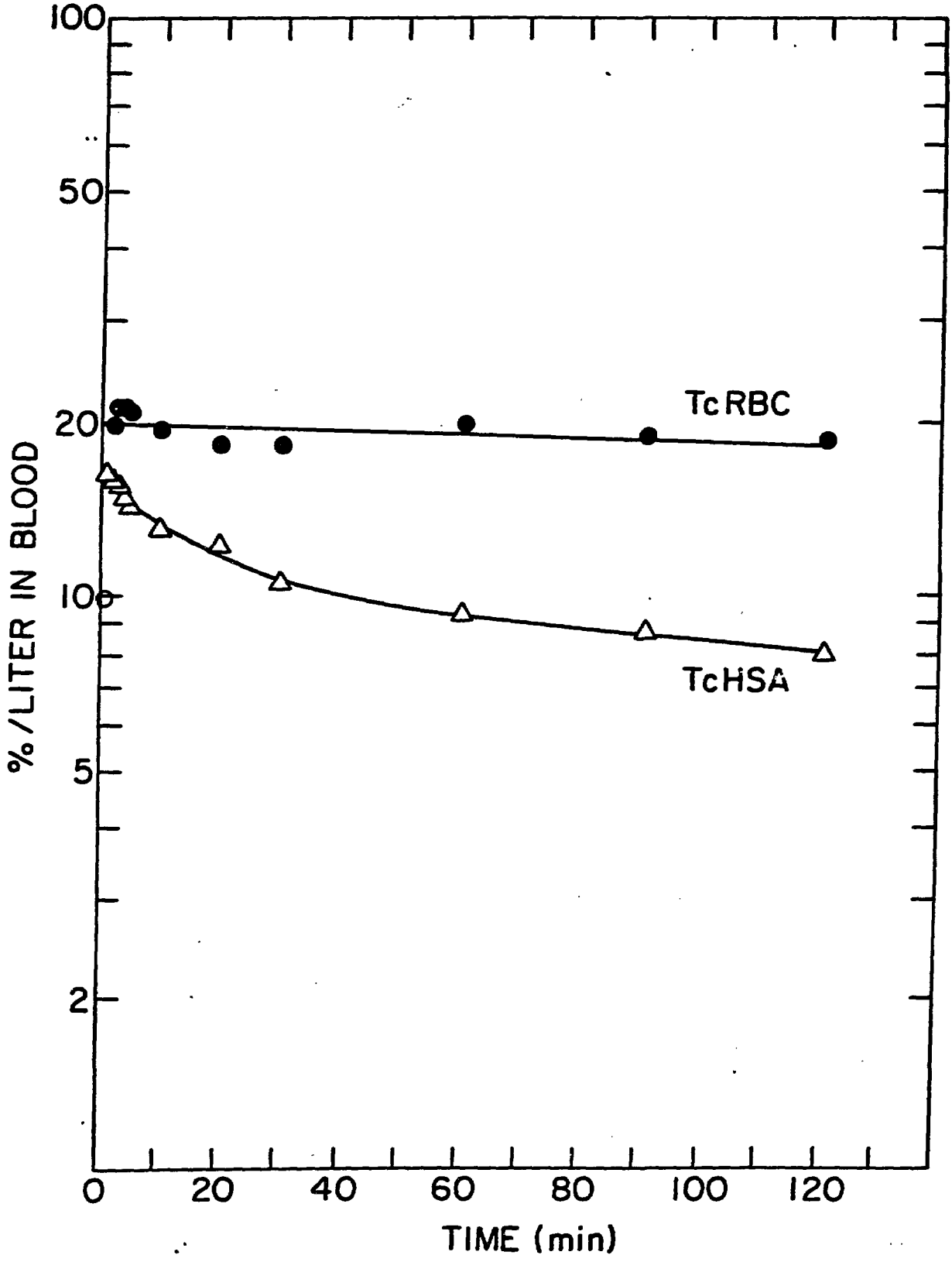
AG

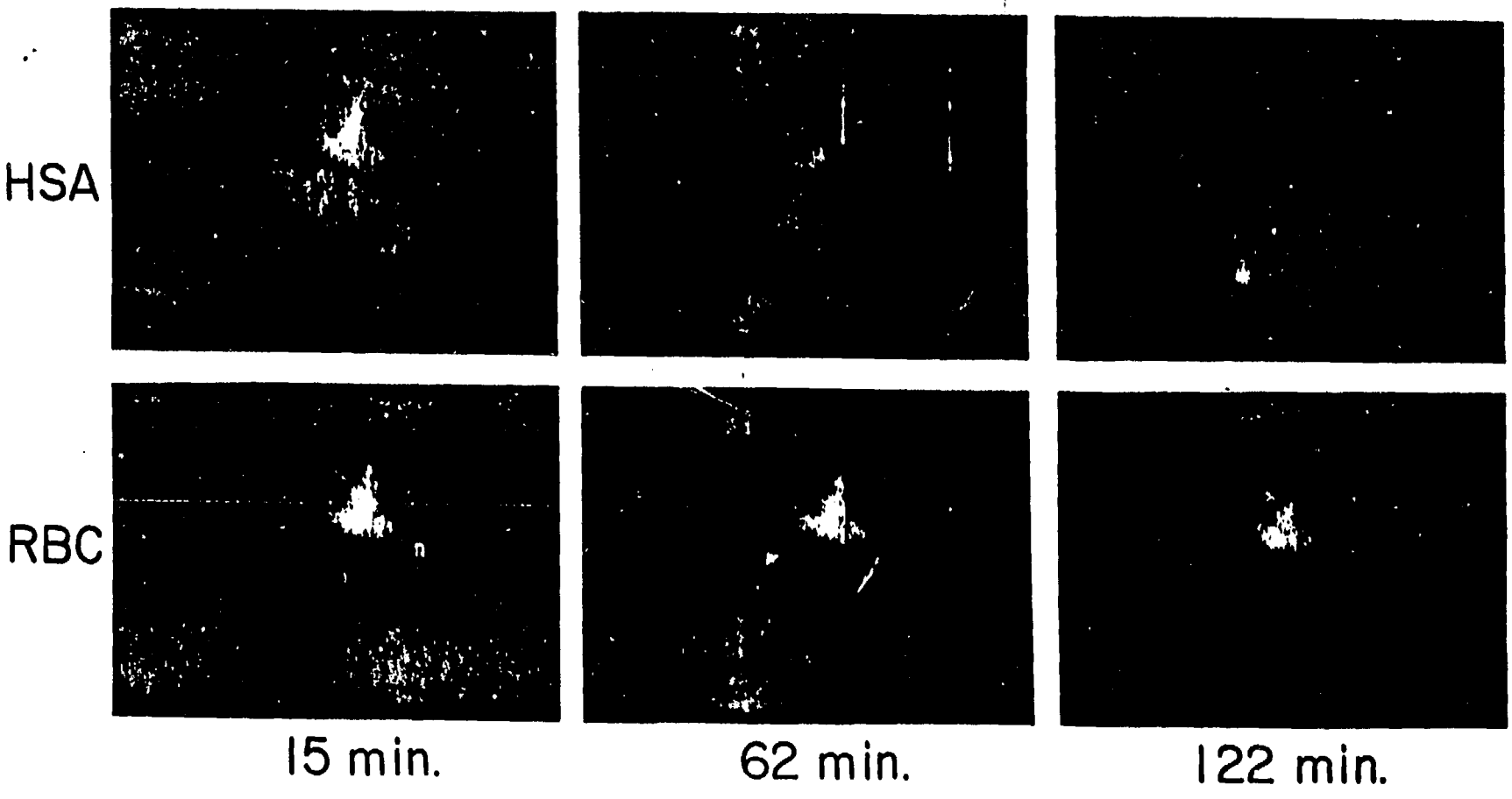
^{99m}Tc RBC






A.G.
99m Tc-RBC
50 msec WINDOW
RATE 100/min
300 CYCLES/POINTS
EV=0.45
COUNTS×10³
7
6
5
4
3
2
1
100
200
300
400
500
600
700
msec

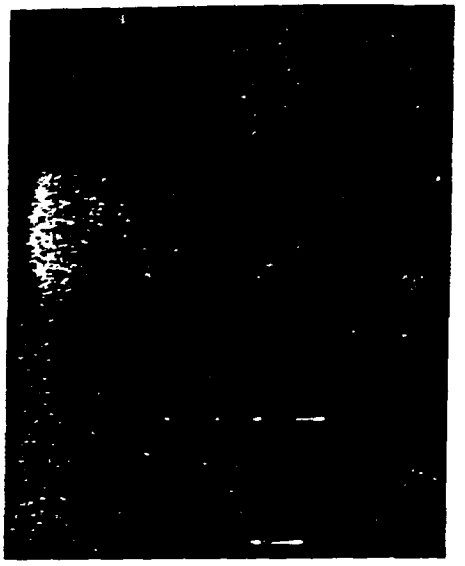

100
50
20
10
5
2
% / LITER IN BLOOD
Tc RBC
Tc HSA
0
20
40
60
80
100
120
TIME (min)

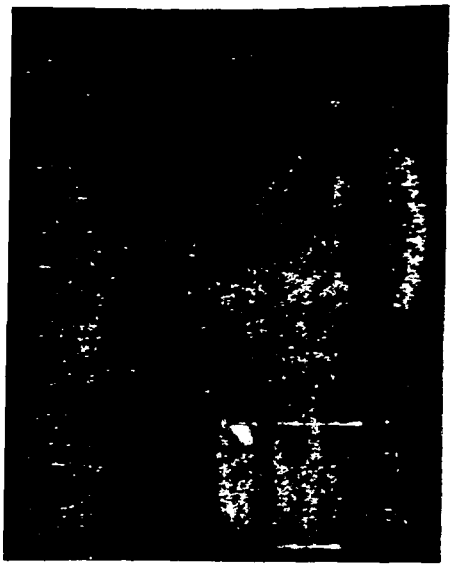

HSA
RBC
15 min.
62 min.
122 min.

R.C.

HEPATITIS, RECOVERED

^{99m}TcS COLLOID





ANTERIOR

POSTERIOR

^{99m}Tc RBC