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*Radiolabeled Red Blood Cells:
Methods and Mechanisms*

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**Radiolabeled Red Blood Cells:
Methods and Mechanisms**

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RADIOLABELED RED BLOOD CELLS: METHODS AND MECHANISMS

STATEMENT OF OBJECTIVES

Upon completion of this course the recipient will be able to discuss the methods and mechanisms by which human red blood cells are radiolabeled with Tc-99m.

Specifically, the recipient should be able to:

1. List currently available methods by which human red blood cells are labeled with Tc-99m for clinical use.
2. Define the three general steps involved in any method of radiolabeling red blood cells with Tc-99m.
3. Compare and contrast how each of these general steps is accomplished using currently available methods.
4. State the relative advantages and disadvantages of currently available methods.
5. Describe the role of each component found in products used to radiolabel human red blood cells with Tc-99m.
6. Describe the pharmacokinetics of radiolabeled red blood cells.
7. Present the currently accepted mechanisms involved in the labeling process.
8. List several drug-drug interactions which interfere in the labeling process.
9. List clinical indications for Tc-99m red blood cells.

COURSE OUTLINE

RADIOLABELED RED BLOOD CELLS: METHODS AND MECHANISMS

- I. INTRODUCTION
- II. CLINICAL INDICATIONS
- III. REVIEW OF LABELING METHODS
- IV. Tc-99m LABELED RED BLOOD CELLS (RBCs)
- V. GENERAL STEPS IN LABELING RBCs WITH Tc-99m
 - A. Treatment with Stannous Ion
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 - C. Addition of Tc-99m pertechnetate
- VI. CURRENT Tc-99m RBC LABELING METHODS
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- X. RADIATION DOSIMETRY
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 - C. Volume of Whole Blood
 - D. Stannous Ion Dose
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By

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INTRODUCTION

Radiolabeled red blood cells have played an important role as diagnostic radiopharmaceuticals for many decades. Their current role as the drug of choice for cardiac blood pool imaging has resulted in an evolution in the methods of labeling and a better understanding of labeling mechanisms. In this article the use of radiolabeled red blood cells as a diagnostic radiopharmaceutical will be reviewed, current labeling methods will be presented and the current understanding of the mechanisms by which these cells are labeled will be discussed. Emphasis will be placed on technetium-99m red blood cells due to their importance in contemporary nuclear medicine practice.

CLINICAL INDICATIONS

The use of radiolabeled red blood cells includes five major areas:

1. Measurement of total red blood cell volume
2. Measurement of red blood cell survival time
3. Identification of sites of red blood cell distribution
4. Blood pool imaging studies including gated cardiac imaging and gastrointestinal bleeding
5. Selective spleen imaging with damaged red blood cells

The ideal properties of labeled red blood cells used for each of these indications are different. The physical properties of the radionuclide, in vivo stability, and ease of labeling all have different importance depending on the study to be performed. For example, determination of red blood cell survival time requires a radionuclide with a relatively long physical half-life and good in vivo stability whereas cardiac blood pool imaging studies are usually complete within 1 hour and require a short half-life radionuclide. However, since these

studies may be a high volume study in many nuclear medicine departments, the speed and ease of labeling becomes an important consideration.

REVIEW OF LABELING METHODS

The use of radioactive nuclides in the labeling of erythrocytes dates back to the work of Nobel laureate, George de Hevesy, when he introduced in 1942 the use of P-32 labeled erythrocytes for the determination of blood volume in patients. In this method, in vitro incubation of P-32 with red blood cells allows the erythrocyte hexoses and trioses to bind the P-32.

An improved labeling technique was described in 1950 by Sterling and Gray who observed that hexavalent Cr-51 in the form of sodium chromate provide a suitable label for red cells. Cr-51 was incubated in a manner similar to that of P-32. The Cr-51 method replaced the P-32 technique and is still a commonly used labeling method. However, the relatively low abundance (9%) of the 320 keV gamma ray of Cr-51 makes it unsuited for external imaging procedures. Cr-51 labeled red blood cells have been used for red blood cell survival studies by several early workers.

Radioisotopes of iron have been used extensively to study red blood cells. When radioactive iron is injected intravenously, it is cleared rapidly from the circulation (half-time 60 to 120 min) and about 80% of it is incorporated into the newly formed cells over the next 7 to 10 days. Unfortunately, the iron liberated from destroyed red cells is reutilized and rapidly reappears in newly formed cells. Radioactive iron, therefore, cannot be used clinically for autologous red cell survival studies, except in special circumstances, e.g., aplastic anemia when reutilization would be minimal. The principal isotopes of iron used in these studies are Fe-55 and Fe-59.

Several other methods for labeling erythrocytes have been reported over the years. Because glycine is incorporated into protoporphyrin during heme synthesis, C-14 labeled glycine has been used as a label for red blood cells.

When Hg-197 or Hg-203 labeled bromomercurhydroxypropane (BMHP) is incubated at room temperature with whole blood, 90 to 98% of the label is rapidly bound to red blood cells. When a sufficient concentration of stable mercurhydroxypropane (MHP) is added to the cells, they are altered in such a manner that they are selectively removed from the circulation by the spleen. This damage can also be induced by heat and other chemical methods. Labeled red blood

cells damaged in this fashion are useful for selective spleen scanning.

In 1968, Rb-81 was described as a suitable red blood cell label. The main advantage of the Rb-81 is its short physical half-life (4.7 hrs) and suitable gamma-ray energy. It has also been reported useful for quantitative estimation of red cell uptake in the spleen.

A method for the measurement of red cell mass in the spleen by radionuclide scanning after the injection of red blood cells labeled with C-11 labeled carbon monoxide has been described. Because of its short physical half-life (20 min), large amounts of the radionuclide can be administered and the spleen visualized without damage to red blood cells. However, the major disadvantage of this method is the necessity of having a cyclotron nearby for the production of this short half-life positron-emitting radionuclide. The recent increase in the number of positron emission tomography (PET) facilities may increase the interest in this novel technique.

The introduction of lipid-soluble complexes of In-111 led to the use of this radionuclide to label platelets and white blood cells. The physical half-life of 2.8 days and suitable gamma emissions of 174 and 247 keV make it ideal for monitoring physiologic processes which are several days in duration. In-111 labeled red blood cells have been proposed for detection of gastrointestinal bleeding and red blood cell sequestration and survival studies.

Lipid-soluble complexes of Ga-67 and Ga-68 have also been reported as alternatives to more common methods for special applications such as the use of Ga-68 red blood cells in PET.

Tc-99m LABELED RED BLOOD CELLS (RBCs)

Most radionuclides previously mentioned lack physical properties which allow for their use in imaging procedures. These limitations restricted the use of red blood cells labeled with these nuclides to in vitro determinations or external probe counting techniques. The availability of a radiotracer with physical properties suited to imaging techniques and with chemical properties which would permit efficient labeling to red blood cells has greatly expanded the usefulness of labeled red blood cells as a diagnostic agent. The introduction of Tc-99m has singularly had the greatest impact on radionuclide procedures, including those with labeled red blood cells.

The use of Tc-99m labeled red blood cells as a blood pool imaging agent in cardiovascular nuclear medicine is well established. Clinical effectiveness of

this agent is based on its ability to distribute primarily within the intravascular pool of the body and to leave this compartment at a slow rate. Such behavior allows for the accumulation of high resolution images which can be obtained with the aid of a physiological gating device. Combined with the gamma scintillation camera, this procedure can yield diagnostic information about dynamic processes such as regional myocardial wall motion.

Tc-99m as the pertechnetate ion is not firmly bound to red blood cells and will diffuse into the extravascular fluid compartment, accumulating in organs such as the stomach, gut and thyroid gland. Such a distribution pattern results in lower blood-to-background activity ratios, poor detection of myocardial borders, interference with GI blood pool imaging and images which are difficult or impossible to interpret. It is, therefore, important that the Tc-99m be firmly and quantitatively bound to the cells and that this labeling persist in vivo during the observation period. In cardiovascular nuclear medicine this time period may be 1 hour, while in the evaluation of gastrointestinal bleeding the observation period may be as long as 24 hours.

Labeling of red blood cells with Tc-99m for spleen scanning was reported in 1967. However, efforts at that time to reproduce Tc-99m labeling of red blood cells had been unsuccessful. In these studies, Tc-99m was added as pertechnetate ion without the addition of any reducing agent. It is now well known that pertechnetate ion (with Tc-99m in the +7 oxidation state) is nonreactive, and binding to cellular components would not be expected under the reaction conditions employed by these authors. In 1971, a labeling method employing stannous chloride as a reducing agent for technetium was introduced with labeling efficiencies of 50 to 60% reported. The method involved the incubation of washed cells with pertechnetate followed by the addition of stannous chloride solution. It was observed that the presence of plasma greatly reduced the labeling efficiency by this method but that the labeled cells exhibited good in vivo and in vitro stability. All of the early methods involved erythrocyte separation from anticoagulated whole blood by centrifugation with subsequent incubation with stannous chloride followed by pertechnetate, or with pertechnetate followed by stannous chloride.

GENERAL STEPS IN LABELING RBCs WITH Tc-99m

Before presenting details of current labeling methods, it is worthwhile discussing the general

steps involved in labeling red blood cells with technetium since they are common to all methods. There are three general steps involved:

1. Treatment of RBCs with stannous ion
2. Removal of excess extracellular stannous ion
3. Addition of pertechnetate

Treatment of Red Blood Cells with Stannous Ion

Although it is technetium in the +7 (pertechnetate) oxidation state that crosses the intact erythrocyte membrane, only technetium that has been reduced to a lower oxidation state will firmly bind hemoglobin. Stannous ions are most commonly employed for reduction of technetium and the stannous chloride (as a stannous pyrophosphate complex) is preferred. At physiologic pH, stannous ions are subject to hydrolysis and precipitation that causes their rapid clearance from blood by the reticuloendothelial system. When complexed with pyrophosphate (or other soluble chelates), however, stannous ions are sufficiently soluble to be resistant to these effects, yet are not so strongly bound to pyrophosphate as to prevent their dissociation and attachment to red blood cells. In the in vivo and modified in vivo methods, treatment with stannous ion is accomplished by the direct intravenous administration of stannous pyrophosphate. Other chelates of stannous ions can also be used (such as pentetate, medronate, etc.) and would yield radiolabeled red blood cells in varying degrees of efficiencies. Pyrophosphate seems nearly ideal, however, because (a) it maintains the solubility of stannous ions in serum until they come into contact with the red blood cells and (b) most kits contain an optimal amount of stannous ion.

Reports on the quantity of stannous ion required for RBC labeling have been confusing because the quantity of tin to be given is stated in terms of either stannous ions, stannous chloride, or stannous pyrophosphate. For Tc-99m red blood cell labeling using either the in vivo or the modified in vivo technique, most clinicians utilize 10-20 micrograms Sn^{+2}/kg body weight. Depending upon the commercial formulation chosen, it may be necessary to inject one-third to one-half the contents of a vial of stannous pyrophosphate or an entire vial to provide this number of stannous ions. When the in vitro method of radiolabeling is employed, a much smaller number of stannous ions are employed, usually 1-15 micrograms total.

Removal of Extracellular Stannous Ions

The presence of stannous ion in the serum can

result in the undesirable reduction of Tc-99m pertechnetate prior to its entry into the red blood cell. Only the oxidized form of Tc-99m can be transported by the erythrocyte membrane.

In either the *in vivo* or the modified *in vivo* method, biological clearance of excess stannous pyrophosphate is the method by which extracellular stannous ions are reduced. The optimal time between the injection of stannous pyrophosphate and the administration of Tc-99m pertechnetate (*in vivo* method) or the incubation of the stannous ion pretreated cells with Tc-99m pertechnetate (modified *in vivo* method) is 20-30 minutes.

With the original *in vitro* labeling method, extracellular stannous ion could be removed by centrifugation, a step that physically separates stannous-treated cells from the noncellular stannous ions in serum. A modification of the *in vitro* labeling method has recently become commercially available. This product uses the non-penetrating oxidizing agent sodium hypochlorite to oxidize extracellular stannous ions, thus preventing the undesirable extracellular reduction of Tc-99m pertechnetate.

Addition of Tc-99m Pertechnetate

Actual red blood cell labeling with Tc-99m occurs whenever Tc-99m pertechnetate is brought into contact with RBCs that have been previously treated with stannous ions. This can be accomplished by either the *in vivo* or *in vitro* addition of Tc-99m pertechnetate to RBCs that have been pretreated with stannous ions.

CURRENT Tc-99m RBC LABELING METHODS

Nuclear medicine and nuclear pharmacy practitioners today have a choice of labeling methods from which to choose. With the recent approval of a commercially-produced *in vitro* kit, there are now three methods available, each of which has distinct advantages and disadvantages. These methods use different combinations of physical, chemical and biological means to accomplish the three general steps listed above. The following section will compare and contrast available methods.

In Vitro Kits

Although the stannous chloride method of labeling autologous red blood cells resulted in a clinically useful radiopharmaceutical, the procedure was long and required multiple washing steps as well as the extemporaneous compounding of a stannous chloride solution suitable for intravenous

(*i.v.*) injection. These disadvantages were partially eliminated with the introduction of simple kits for the preparation of Tc-99m red blood cells using stannous citrate and stannous glucoheptonate (gluceptate).

The introduction of these kits, although not widely available, greatly simplified the labeling procedure. One major advantage was that reagents could be prepared in advance and stored while quality control testing was undertaken.

The most widely used kit has been that of Smith and Richards and is referred to as the Brookhaven National Laboratory (BNL) kit. This kit is currently being distributed as an investigational drug (Cadema Medical Products, Inc., Middletown, NY). A modification of the *in vitro* kit has been introduced and is commercially available (Mallinckrodt Medical, St. Louis, MO). With this latter product, a small amount of sodium hypochlorite is added to whole blood that has been previously treated with stannous ion. Extracellular stannous ions are oxidized to the stannic form, and interference with labeling is minimized. Intracellular stannous ions are not affected by the addition of sodium hypochlorite. Unlike the centrifugation method, the chemical oxidation method does not require separation of red cells and can be performed in whole blood. Avoidance of centrifugation lessens the degree of cellular damage that occurs during radiolabeling.

As a result of experiments performed in the development of the BNL kit, important observations of problems with some Tc-99m solutions were made. The consequences of the chemical effects of technetium are not usually considered in the preparation of most routine Tc-99m radiopharmaceuticals. However, in this case the carrier Tc-99 in some generator eluates apparently exceeded the reductive capacity of the added stannous ion causing depressed labeling yields. It was pointed out that this problem may exist with other radiopharmaceuticals that use stannous ion, particularly when the quantity of Sn^{+2} used is very small or when poor formulation methods make the stannous ion unstable.

The chemical form of the stannous ion seems not to affect the labeling reaction since stannous ion has been combined with various anions including chloride, fluoride or citrate, in conjunction with other ligand molecules such as glucoheptonate, methylene diphosphonate, or pyrophosphate.

In Vivo Methods

In 1975, several groups reported altered distribution of Tc-99m pertechnetate in brain scans

of patients who had undergone previous Tc-99m pyrophosphate bone scans. In these patients, Tc-99m pertechnetate, which normally distributes throughout the extracellular fluid volume, was distributed primarily in the intravascular compartment. Further investigation showed that the majority of this intravascular radioactivity was associated with red blood cells. The occurrence of this phenomenon is affected by:

1. The amount of stannous ion administered in the bone scan dose
2. The interval between administration of pertechnetate and the brain scan
3. The interval of time between the bone scan and brain scan (no effect was observed when this interval exceeded 6 days)

The observation of this drug-drug interaction was soon followed by the development of a new method for the *in vivo* labeling of red blood cells using stannous pyrophosphate as the source of stannous ion.

In this method, labeling is accomplished with two consecutive *i.v.* injections, first of cold (nonradioactive) stannous pyrophosphate, followed in 20-30 minutes by Tc-99m pertechnetate. Reported results for average labeling efficiency using the *in vivo* method vary widely from 71-96%. The interval between pyrophosphate and pertechnetate injection also affects the composition of the plasma Tc-99m activity. With a short interval, the plasma activity is primarily Tc-99m pyrophosphate while as the interval increases to 30 minutes the technetium is equally divided between pertechnetate and pyrophosphate.

Modified In Vivo Methods

Currently, red blood cells can be labeled with Tc-99m by *in vivo* and *in vitro* techniques. Clinical comparisons have shown that the *in vitro* method results in a superior product. The need to remove a blood sample from the patient and the lack of a commercially-available kit until recently have prevented the method from gaining widespread acceptance. *In vivo* methods use readily-available components and do not require blood samples to be removed from the patient. However, the quality of images obtained with the standard *in vivo* method were often of poor quality.

In an attempt to optimize the biological behavior of Tc-99m red blood cells, modifications of existing labeling techniques have been developed. One such method reported by our laboratory is called the modified *in vivo* labeling method. This method

evolved from observations that the rate of incorporation of Tc-99m pertechnetate into human red blood cells *in vivo* proceeds at a measurable rate. During a time interval between *i.v.* injection of Tc-99m pertechnetate and firm binding to red blood cells, the Tc-99m is free to distribute to extracellular compartments. A standard *in vivo* technique was, therefore, modified so as to isolate pretinned red blood cells and Tc-99m pertechnetate from other body compartments. If sufficient time is allowed for the reaction to proceed to completion, approximately 90% of the total Tc-99m present will be firmly bound to the red blood cells at the time of *i.v.* injection. This results in increased intravascular retention and improved image quality.

Although any source of stannous ion may be suitable for this procedure, we find that products containing the equivalent of 1 mg of stannous chloride dihydrate per vial are the most efficient and convenient. Products containing enough stannous ion for multiple patient doses may seem to be more economical. However, the possibility of oxidation of stannous ion in the unused portion of the vial may result in poor labeling of subsequent patients. Therefore, single dose preparations of stannous ion should be used in this method. It is important that sufficient time be allowed for distribution and clearance of extracellular stannous ion and pyrophosphate within the intravascular pool. For the modified *in vivo* method 15 to 20 minutes seems to be optimum. Anticoagulation of the reaction mixture is provided by the residual heparin solution in the infusion set. It is, therefore, important that the line be first flushed with the heparin-containing solution before red blood cells are withdrawn into the syringe containing pertechnetate. The source of pertechnetate should be a generator which has been previously eluted within 24 hours. This limits the amount of Tc-99 that may be present in the eluate, which has been shown to exert an adverse effect on labeling efficiency. A standard incubation time for this method is 10 minutes at room temperature. However, since factors such as temperature and hematocrit affect the rate of labeling, in certain instances it may be necessary to increase this incubation time.

Although this method may require a slightly longer labeling time than the standard *in vivo* method, the increased retention of intravascular Tc-99m results in shortened imaging time and, therefore, the total time necessary for the procedure is not lengthened over the standard *in vivo* labeling method.

PHARMACOKINETICS OF Tc-99m RBCs

The pharmacokinetics of technetium-99m red blood cells has been studied in patients and normal volunteers. After intravenous injection of pertechnetate during in vivo labeling, maximum whole blood activity was not reached until at least 30 minutes after injection. This suggests that pertechnetate freely diffuses into the extracellular fluid space, then reenters the intravascular pool as blood levels of pertechnetate fall.

Whole-body clearance was found to be biexponential for both in vivo and in vitro methods. The in vivo method of labeling resulted in a short $T_{1/2}$ component of 2.5 ± 0.7 hr ($10.9 \pm 6.1\%$) and a long $T_{1/2}$ component of 176.6 ± 163.6 hr ($90.5 \pm 5.0\%$), whereas the in vitro method resulted in whole body retention components of 2.7 ± 1.5 hr ($25.4 \pm 10.4\%$) and 75.6 ± 25.3 hr ($82.2 \pm 7.7\%$).

COMPARISON OF RADIOPHARMACEUTICALS FOR BLOOD POOL IMAGING

Several studies have compared two or more radiopharmaceuticals used for blood pool imaging, including Tc-99m human serum albumin (HSA) and Tc-99m red blood cells (prepared by either the in vitro, modified in vivo, or the in vivo method of radiolabeling). Based upon these studies, the following conclusions can be drawn:

1. Whenever Tc-99m HSA was compared to Tc-99m labeled red blood cells prepared by any method, labeled red blood cells were determined to be superior.
2. When in vivo and modified in vivo methods of labeling red blood cells were compared, the modified in vivo method was judged to be superior.
3. When in vitro labeled red blood cells were compared to in vivo and/or modified in vivo methods and judged on labeling efficiency and image quality, in vitro labeled cells were judged superior.
4. When availability and ease of labeling were considered in comparisons among all red blood cell labeling methods, the in vitro kit was found to be inferior because of the increased manipulation required and the potential for administration of cells to the wrong patient.

5. Comparisons of all methods of red blood cell labeling showed that the modified in vivo method gave image quality approaching that of in vitro methods but is far more easily performed with readily-available components.

For any given clinical situation, therefore, the selection of a blood pool agent will depend on the acceptable level of image quality, requirements for patient throughput, and the level of expertise of the technical staff.

DRUG INTERFERENCE

Drug interference with Tc-99m red blood cells for equilibrium blood pool imaging can be classified into two general categories: (1) agents that alter, by a direct pharmacological effect, cardiac function and have the potential to interfere with the interpretation of equilibrium blood pool images, or (2) agents that inhibit or diminish the radiolabeling of red blood cells by Tc-99m.

Agents that induce an alteration in cardiac function include (a) the beta adrenergic blockers, such as propranolol (b) calcium channel blockers, including verapamil and (c) the nitrates, notably, nitroglycerin. Studies performed in patients receiving these pharmaceuticals may not detect the presence of coronary artery disease or accurately reflect its severity.

It has been proposed that these interfering drugs be withdrawn from patients prior to exercise ventriculography. For beta blocking medications a 48-hour interval between withdrawal of the drug and the nuclear medicine study has been suggested, while for the calcium channel blockers the proposed interval is 48-72 hours, and 12 hours has been suggested for the nitrates.

Doxorubicin causes a dose-related cardiomyopathy that may interfere with the diagnosis of abnormal cardiac function. However, the radionuclide ventriculogram is often performed to monitor doxorubicin-induced cardiotoxicity.

Poor radiolabeling of red blood cells with Tc-99m or early dissociation of Tc-99m from the labeled red blood cell brought about by concomitant drug therapy can adversely affect image quality. Table 1 lists several of the drugs known to interfere with Tc-99m red blood cell labeling or that may be responsible for deterioration of the labeled cell.

The anticoagulant used in red blood cell labeling techniques has been shown to affect the results. Decreased labeling and increased urinary excretion of Tc-99m has been reported when stannous pyrophosphate and Tc-99m pertechnetate were

injected through a venous catheter containing heparin. The fact that heparin is used successfully as an anticoagulant in the *in vitro* and modified *in vivo* method suggest that the effect of heparin may be dose related or temporally related to the addition of the various components of the reaction.

Heparin and acid-citrate-dextrose (ACD) solution have been used as the anticoagulant in the modified *in vivo* method in patients and normal volunteers. Some groups report higher labeling efficiency and improved image quality with minimal renal and urinary bladder activity with ACD. Others have shown labeling efficiency to be independent of the selection of ACD or heparin as the anticoagulant.

RADIATION DOSIMETRY

To calculate accurate dosimetry data, detailed knowledge of the pharmacokinetics of each agent must be known and applied. For example, it is known that the initial distribution and rate of elimination of Tc-99m labeled red blood cells is a function of the method of labeling. To date, these factors have not been carefully applied to dosimetry calculations.

One factor that affects the dosimetry of blood pool imaging agents is the blood volume of the individual organs. This factor has been applied to dosimetry calculations. In these calculations, the effective half-life is assumed to be equal to the physical half-life, and distribution to the organs is assumed to be solely a function of blood volume. The dosimetry calculated by this method results in higher values than those given from other sources. These data are summarized in Table 2.

MECHANISMS OF LABELING

It has been shown that in red blood cells labeled with Tc-99m, the majority of radioactivity is associated with hemoglobin. Further investigation has shown that 87% of the activity is associated with the globin portion of the molecule and 10% with the heme. It was therefore concluded that pertechnetate ion in the lower valence state (probably technetium +4) binds irreversibly with globin, with the highest specific activity found in the beta-chain, most probably by coordinate covalent bond formation.

Various studies have concluded that the process of pertechnetate binding to the red blood cell essentially involves passive diffusion of pertechnetate into the cell. More recently it has been shown that the pertechnetate ion is transported across the red blood cell membrane by the band-3 anion transport

system. This system is responsible for maintaining the transmembrane concentrations of chloride and bicarbonate. Since there is no mechanism inside the cell to reduce pertechnetate in the absence of a reducing agent, pertechnetate is readily transported out of the cell by this system when the red blood cells are suspended in a vehicle containing chloride or bicarbonate as exchangers. The role of intracellular reduction of pertechnetate, which results in binding of the Tc-99m to hemoglobin, has been well documented.

FACTORS AFFECTING LABELING EFFICIENCY

Using a method to stop the labeling reaction between Tc-99m and red blood cells at the time of sampling, it has been shown that Tc-99m is incorporated into red blood cells *in vivo* at a measurable rate, reaching a value of 91.4% at 10 minutes following injection. This suggests that significant amounts of non-red blood cell-bound Tc-99m, probably as pertechnetate, is available for distribution to the extravascular compartments.

The incorporation of Tc-99m pertechnetate into pretinned red blood cells in a system isolated from other body compartments was shown to be affected significantly by the temperature and hematocrit of the reaction mixture, the dose of stannous ion administered and the presence of plasma. The volume of whole blood, activity of Tc-99m, and patient population have no significant effect on the rate or extent of Tc-99m labeling.

Temperature

There is a direct relationship between the temperature of the reaction mixture and rate and extent of labeling. The temperature data suggest that increased labeling and shorter incubation times could be obtained if, in the modified *in vivo* method, the syringes were maintained at 37°C rather than allowed to slowly cool during the incubation period. Elevation of the syringe temperature to 49 to 50°C for 35 minutes has been shown to sufficiently damage the red blood cells so as to be able to do selective spleen imaging.

Hematocrit

Whole blood hematocrit has a major effect on the rate and extent of red blood cell labeling. Normal values for the hematocrit vary with an individual's age and sex. The normal hematocrit value for adults is 36 to 46% for women and 42 to 52% for men. There is a slight decrease in the hematocrit level after 50 years of age. However, in

Table 1

Drugs Suspected of Interfering with Labeling of Red Blood Cells with Tc-99m	
Drug	Possible Mechanism
Heparin	Formation of Tc-99m labeled heparin
Methyldopa, hydralazine	Oxidation of Sn ⁺²
Digoxin, prazocin, propranolol	Unknown
Iodinated contrast media	Competition between iodide and pertechnetate for transport by the band-3 anion transport system

Table 2.

Radiation Absorbed Dose Estimates for Tc-99m RBC (rads/mCi)		
Organ	Estimates Using Cell Kinetics Data	Estimates Using Organ Blood Volume Data
Total body	.016	.018-.019
Spleen	.018	.039-.062
Bladder Wall	.12	-
Testes	.012	-
Ovaries	.02	-
Blood	.052	-
Red Marrow	.022	-
Liver	-	.040-.098
Kidneys	-	.043-.066
Lungs	-	.048-.064
Heart	-	.075-.081

patients with anemia and in patients with significant blood loss, hematocrit values as low as 12 to 15% can be seen. Thus, these individuals would be expected to show decreased labeling efficiency with resultant increase in extravascular concentration of Tc-99m activity. This effect may be partially overcome by increasing incubation time when patients with known low hematocrit values are studied with the modified in vivo method.

Volume of Whole Blood

Increasing the volume of whole blood from 1.5 to 4.5 ml in the modified in vivo method did not significantly alter the labeling parameters. The normal range of red blood cell count in men is 4.5 to 6.5 million/ul and in women it is 3.9 to 5.6 million/ul. Therefore, the number of cells in the whole blood reaction mixture can be varied from approximately 7.5×10^9 to 2.2×10^{10} without effects on labeling.

It has been shown that the presence of plasma exerts a competing effect on labeling. The effect of diluting red blood cells with saline has less of an effect on relative labeling than when dilutions are done with plasma. This suggests that the effects of hematocrit on labeling efficiency is due partially to the concentration of red blood cells and partially to the presence of plasma.

Stannous Ion Dose

Changes in blood disappearance of Tc-99m pertechnetate at stannous ion doses as low as 1 ug/kg have been reported. A plateau of labeling efficiency at 10 ug/kg has been reported in several studies which has also been shown to be the minimum dose of stannous ion that resulted in satisfactory red blood cell labeling. Decreases in labeling efficiency have been reported at doses in the 35 to 40 ug/kg range.

SUMMARY AND CONCLUSIONS

Radiolabeled red blood cells represent a unique radiopharmaceutical dosage form which allows for the determination of cellular volume and kinetics and the visualization of the intravascular blood pool of organs. While a variety of radiolabels have been used in the past, Tc-99m has gained widespread use for imaging and in vitro measurements.

The selection of the method of labeling red blood cells with Tc-99m depends upon personal preference and the clinical indication being addressed. The modified in vivo method employs some aspects of the in vivo and in vitro methods and results in reproducibly high labeling efficiency without the

added efforts of in vitro processing of cells.

The efficiency of labeling red blood cells is affected by temperature, hematocrit, stannous ion dose, mass of technetium, and choice of anticoagulant. Awareness of these factors and control of them, when possible, will result in a highly effective radiopharmaceutical for a variety of nuclear medicine procedures.

Radiolabeled red blood cells provide the opportunity to study transport mechanisms associated with cell labeling. In addition, the use of selective pharmacological agents can increase understanding of the mechanism of labeling. Continued research into these labeling methods will further expand the clinical applications of this radiopharmaceutical.

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3. The mechanism of localization of Tc-99m red blood cells for cardiac blood pool imaging is best described as:
 - A. active transport
 - B. capillary blockade
 - C. compartmentalization
 - D. extravasation
4. The correct dosage for tin used in the in vivo labeling of red blood cells is:
 - A. 10-20 micrograms stannous ion per kg body weight
 - B. the entire contents of any stannous pyrophosphate kit
 - C. 5 mg stannous pyrophosphate
 - D. 10 mg stannous gluceptate
5. The role of stannous ion in the labeling of red blood cells with Tc-99m is the:
 - A. extracellular reduction of pertechnetate
 - B. intracellular reduction of hemoglobin
 - C. intracellular reduction of pertechnetate
 - D. facilitation of Tc-99m transport into the cell
6. When using the "Brookhaven Kit" to label red blood cells with Tc-99m, the mole fraction of the metastable isomer in a generator eluate has been shown to have:
 - A. no effect on labeling efficiency
 - B. a direct relationship to labeling efficiency
 - C. an inverse relationship to labeling efficiency
 - D. an inverse relationship to the rate of labeling

QUESTIONS

1. Which of the following radionuclides has NOT been used to label red blood cells?
 - A. Rb-81
 - B. C-11
 - C. K-43
 - D. Ga-68
2. The first described use of radiolabeled red blood cells was for:
 - A. equilibrium gated cardiac blood pool imaging
 - B. first pass angiocardiology
 - C. detection of gastrointestinal hemorrhage
 - D. measurement of total blood volume
7. Which of the following methods has NOT been used to remove extra-cellular stannous ion in labeling red blood cells with Tc-99m?
 - A. centrifugation
 - B. biological clearance
 - C. in vivo reduction
 - D. oxidation

8. The only commercially available kit for the labeling of red blood cells with Tc-99m is based on the:
- modified in vivo method
 - in vitro labeling using chemical oxidation
 - in vitro labeling using centrifugation
 - in vivo method
9. The pertechnetate ion reaches the intracellular space of the red blood cell via which of the following mechanisms?
- Na⁺/K⁺ ATPase pump
 - calcium channels
 - band-3 anion transport system
 - passive diffusion
10. When using the modified in-vivo method for labeling red blood cells with Tc-99m, classify the following factors as having a direct (D), inverse (I) or no (0) effect on labeling efficiency.
- hematocrit _____
 - temperature _____
 - volume of blood _____
 - quantity of Tc-99m _____
11. In radiolabeled red blood cells, intracellular Tc-99m is found in the highest specific activity in:
- heme
 - alpha chain of globin
 - beta chain of globin
 - mitochondria
12. The kinetics of labeling red blood cells with Tc-99m suggests that the optimum time (minutes) for incubation is:
- 1
 - 10
 - 30
 - 60
13. The temperature (degrees C) needed to damage red blood cells for selective spleen imaging is:
- 4
 - 37
 - 49
 - 64
14. Increasing the dose of stannous ion from 10 micrograms/kg to 40 micrograms/kg has been reported to results in:
- chemical damage to red blood cells
 - increase in labeling efficiency
 - decrease in labeling efficiency
 - hemolysis
15. Which statement best describes the effect of injecting both stannous pyrophosphate and Tc-99m pertechnetate through a heparinized catheter?
- decreased labeling efficiency
 - increased urinary excretion
 - labeling of catheter
 - all of the above
16. When all methods of labeling red blood cells with Tc-99m are compared, the method most often found to have the highest labeling efficiency is
- in vivo
 - in vitro kit
 - modified in vivo
 - none of the above
17. When in vivo and modified in vivo methods are compared, the method most often found to give superior images is
- in vivo
 - modified in vivo
 - both of the above
 - none of the above
18. When the in vitro method is compared to in vivo methods the best image quality is most often found with
- in vivo
 - modified in vivo
 - in vitro kit
 - none of the above

19. When ease of labeling and minimizing risk of misadministration are taken into consideration the most inferior of all methods is
- in vivo
 - in vitro kit
 - modified in vivo
 - none of the above
20. A drug which decreases the efficiency of labeling red blood cells with Tc-99m by interfering with transport of pertechnetate by the cell membrane is:
- digoxin
 - heparin
 - prazocin
 - iodinated contrast media
21. A drug which will form a complex with reduced Tc-99m and thus compete for red blood cell labeling is:
- doxorubicin
 - propranolol
 - heparin
 - prazocin
22. When using the modified in vivo labeling method, the 20 minute interval between stannous ion injection and mixing blood with Tc-99m is required in order to allow time for:
- uptake of tin by the RBC
 - transport of pertechnetate by RBC membrane
 - clearance of extracellular stannous ion
 - equilibration of stannous pyrophosphate with blood
23. The statement which best describes the whole body clearance of Tc-99m red blood cells is:
- single exponential with a half-life of 120 hours
 - single exponential with a half life of 24 hours
 - bi-exponential with half lives of 2.5 and 75-175 hours
 - bi-exponential with half lives of 12 and 210 hours
24. Using the in vivo method, following the intravenous injection of pertechnetate, maximum whole blood activity is reached in _____ minute(s).
- 1
 - 10
 - 30
 - 60
25. When using in vitro labeled cells, the most important consideration in patient safety is:
- minimizing cell lysis when withdrawing blood
 - minimizing the chance for misadministration of blood products to the wrong patient
 - limiting the quantity of tin injected
 - encouraging frequent voiding to minimize radiation absorbed dose to the bladder

