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H. Wayne Schultz, Ph.D.

An *in vitro* labeling procedure was developed based on optimal conditions of anticoagulant, stannous ion concentration, incubation time, and incubation temperature. This procedure was used to investigate the effects of various agents on Tc-99m RBC labeling yields. These agents were inorganic ions, therapeutic agents, biochemicals, chelating agents, oxidizing agents, an enzyme poison, and methemoglobinemia agents. The experiments for most of the agents consisted of both post- and pretinning procedures. For post-tinning, the selected agent was added to the blood sample before stannous ion. For pretinning, the agent was added to blood after the addition of stannous ion. In addition, a limited *in vivo* study in rats was conducted . For this study, the following agents were investigated for their effect on RBC labeling yield: sodium chromate, selenious acid, ferrous sulfate, digoxin and dimercaptosuccinic acid.

Agents that reduced the RBC labeling yields in post-tinning in vitro procedures were ferric, ferrous, cupric, and stannic ions. Agents that reduced the labeling yields in both post- and pretinning in vitro procedures were dimercaptosuccinic acid, dimercaprol, edetic acid, methylene blue, chromate amd selenite ions. Glucose and cysteine had no effect on the labeling yields of fresh or stored blood. Glutathione and inosine increased the labeling yields of stored blood. A high, but non-lethal concentration of cyanide ion did not alter the red blood cell labeling yield. When used at a ten fold increased concentration, it caused a reduction in the yields for both post- and pretinning procedures.

Factors Affecting Labeling Efficiency of Technetium-99m to Red Blood Cells

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Associate Professor of Medicinal Chemistry
in charge of major

Redacted for Privacy

Dean of Pharmacy School

Redacted for Privacy

Dean of Graduate School

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CHAPTER I

INTRODUCTION

RED BLOOD CELLS

Mature red blood cells are unique among cells in the body. These cells, also known as erythrocytes, essentially consist of a membrane surrounding a solution of protein and electrolytes. They differ mostly from other normal cells in that they lack a nucleus, mitochondria, and ribosomes. Thus, these cells are unable to synthesize protein and undergo mitosis (85).

Hemoglobin

More than 95% of the protein in red blood cells consists of hemoglobin. The remaining 5% includes enzymes used for energy production and for the maintenance of hemoglobin in its functional reduced form. Within one red blood cell, there are an estimated 200-300 million molecules of hemoglobin. Hemoglobin is a conjugated protein consisting of two pairs of polypeptide chains. Each polypeptide chain is attached to a highly

colored prosthetic group, heme. Heme is a 1:1 complex of iron and protoporphyrin. Therefore, each hemoglobin molecule contains four iron atoms. Hemoglobin reacts with oxygen and carbon dioxide to form oxyhemoglobin and carboxyhemoglobin, respectively. Both are physiological functional forms of hemoglobin.

Hemoglobin is comprised of various types of hemoglobins which differ in their polypeptide structure. The most frequently found form in adults is hemoglobin A. In this substance one pair of polypeptide chains is designated as alpha chains and the other pair as beta chains (85).

Red Blood Cell Membrane

The proposed membrane structure is a matrix formed from a double layer of phospholipids. The lipid molecules in the two layers are oriented such that their non-polar groups are directed toward one another and the hydrophilic polar groups are directed outwards to the aqueous enviroment on both the cytoplasmic and the plasma surfaces (85).

The membrane serves as a partial barrier to the penetration of all solutes. Nonpolar and polar solutes differ in their transport mechanism. Nonpolar solutes pass through the membrane at a rate proportional to their solubility in organic solvents, while polar solutes cross

the membrane at specific sites. Monosaccharides differ from most other polar solutes in that they easily cross the membrane barrier. The transport process depends on the steric structure of the monosaccharides; D-isomers are transported while L-isomers are not. The common monosaccharides require insulin for their penetration through the cellular membrane of most tissues. One exception to this general requirement is red blood cells. Glucose transport occurs with a concentration gradient and does not require energy. The mehanism appears to involve a passive transport process. It has been proposed that the movement of glucose into the cell is facilitated by its combination at the plasma surface with a membrane carrier protein (85).

The membrane permits the relatively free passive diffusion of such important polar substances as water and most anions, especially chloride and bicarbonate ions. The anions are thought to cross the membrane by one of two different pathways. The first pathway represents a rapid exchange reaction in which an internal anion is exchanged for an external anion. This pathway is most likely mediated by a carrier. There is no net loss or gain of the cellular anion content. The second pathway consists of a slower ionic diffusion resulting in a net loss or gain of anions (85).

The red blood cell membrane is only sightly permeable to sodium and potassium. Their movement is

largely dependent upon an energy transport mechanism. Within the human red blood cell, potassium is a relatively minor constituent. In plasma, this relationship is reversed. The gradients of these cations are preserved by a cation transport process (85).

The steady-state cation concentrations within the red blood cell are the results of an equilibrium between passive diffusion and active transport (pump). In the passive diffusion process, sodium diffuses into the red blood cell and potassium diffuses out. Active cation transport depends upon the membrane-associated enzyme, Na-K adenosine triphosphatase (ATPase) (14, 36). It involves an exchange of the inside sodium for potassium on the outside. The energy required for this process is supplied by adenosine triphosphate (ATP). For each molecule of ATP converted to adenosine diphosphate (ADP) three sodium ions are pumped out and potassium ions enter (22, 48).

RADIOLABELED RED BLOOD CELLS

Clinical Applications

Radiolabeled red blood cells may be classified into two types, normal and damaged. Except for labeling, normal radiolabeled red blood cells are unaltered. These cells behave as normal red blood cells in the circulation. Damaged radiolabeled red blood cells are

altered by heat or chemical treatment. Because of their alteration, they are removed from blood the by the spleen. These two types of radiolabeled red blood cells find various diagnostic applications. Normal radiolabeled red blood cells are used for 1) blood pool imaging, 2) detection of hemangiomas, 4) detection of gastrointestinal bleeding, 5) determination of red blood cell sequestration, and 6) determination of red blood cell mass (volume). The diagnostic applications of damaged radiolabeled red blood cells are 1) spleen imaging, 2) accessory spleen localization, and 3) detection of gastrointestinal bleeding. A discussion of the diagnostic applications of radiolabeled red blood cells follows.

Radiolabeled red blood cells provide a means for imaging the entire cardiac blood pool at various times during cardiac contraction. This procedure, known as a gated blood pool study, allows for the qualitative assessment of the relative size and configuration of cardiac structure and the quantitative determination of global and regional biventricular performance. It also allows for the detection of vascular wall abnormalities.

Hemangiomas are benign tumors composed of blood vessels that contain a large volume of blood. They are classified as either capillary or cavernous hemangiomas. In capillary hemangiomas, the majority of the vessels that make up the tumor have the size of normal

capillaries. Cavernous hemangiomas are distinguished by their large cavernous vascular spaces. Hemangiomas found on the skin or the membrane of the oral cavity have a characteristic appearance and do not pose a diagnostic problem. However, when they appear in internal organs e.g., liver, spleen, bone and skull, their diagnosis may be difficult. Radiolabeled red blood cells provide a useful and noninvasive procedure for the detection of both these types of hemangiomas.

Gastrointestinal (GI) bleeding is a common clinical problem. Major causes of upper GI bleeding are peptic ulcers, hemorrhagic gastric ulcers, neoplasms and drug-induced erosions. Lower GI bleeding may be caused by diverticular diseases, inflammatory lower diseases or neoplasms. Clinical detection of GI bleeding is usually relatively easy since there are normally only two exits of blood from the GI tract. However, localization of the site of bleeding presents a problem. Conventional barium studies often do not show actual bleeding sites. Angiography and endoscopy are more accurate, but require specialized equipment, trained personnel and are associated with discomfort and risk to the patient. Radiolabeled red blood cells have been found to be useful in detecting both massive and occult bleeding sites in the upper and lower GI tract. This is an easily performed procedure using ordinary nuclear medicine equipment and isotopes. It is possible with this

procedure to determine the extent of bleeding, as well as the site of bleeding.

Red blood cell sequestration may be determined from a scan by viewing the relative distribution of labeled red blood cells in the precardium, liver and scarum. Normally, the ratio of the radioactivity between spleen and liver is 1:1. In patients with active spleen sequestration the ratio varies between 2:1 and 4:1. Splenic sequestration accompanies hereditary splenocytosis, acquired hemolytic anemia, and hemolytic anemia secondary to lymphoma or leukemia. Information obtained from this procedure may be used in determining whether splenectomy is necessary in patients with splenic sequestration. Also, the procedure may be used to evaluate the degree of splenic sequestration. This information is useful in determining the possible efficacy of splenectomy in treatment of extramedullary hemolytic anemia.

The determination of red blood cell volume is clinically useful in the diagnosis of polycythemia and in pre- and postoperative management of surgical patients. Also, this procedure is used to follow the response of polycythemic patients to chemotherapy.

Splenic irregularities may be detected using damaged radiolabeled red blood cells. The procedure has been widely used since its introduction in 1960. Information provided by this procedure include 1) the localization of

space occupying lesions of the spleen, 2) demonstration of accessory splenic tissues, and 3) data on the size and position of the spleen.

Labeling Methods

Red blood cells may be labeled by either *in vivo* or *in vitro* procedures. The *in vivo* procedures are characterized by intravenous administration of a radioisotope to the patient. *In vitro* procedures are based upon removing a blood sample from the patient, treating it with a radioisotope label, and then injecting it back into the patient.

In vivo procedures may be further classified into cohort and random methods. Cohort labeling involves the incorporation of an isotopic radioisotope label into a cohort (i.e., a group) of red blood cells of the same age. Usually this is accomplished by administering a radioisotope which binds to red blood cell precursors to give labeled red blood cells of the same age. Cohort labeling procedures are useful for the study of red blood cell production rates and life span.

Random labeling occurs when the radioisotope is incorporated in red blood cells of varying ages. Most often, red blood cell labels are of the random type. Types of red blood cell labels produced by various radioisotopes are presented in Table 1.1.

Table 1.1 Red Blood Cell Labels (72).

Label	Compound used	In Vivo	In Vitro	Random	Cohort
Fe-52	Ferrous citrate	x			x
Fe-59	Ferric ammonium citrate	x			x
C-14	Glycine	x		x	x
C-11	Carbon monoxide	x		x	
P-32	Diisopropyl fluoro-phosphate	x		x	x
	Sodium phosphate	x		x	x
In-111	Oxine		x	x	
	Tropolone		x	x	
	Acetylacetone		x	x	
Ga-68	Oxine		x	x	
	Tropolone		x	x	
	Acetylacetone		x	x	
Cr-51	Chromate	x	x	x	
Tc-99m	Pertechnetate	x	x	x	

As another labeling variation, the radioisotopes may be incorporated as either an isotopic or nonisotopic label. Isotopic labeling involves a replacement of an atom in the molecule of interest by a radioactive isotope of the same element. This results in a labeled compound having a structure that is identical (except for a slight change in the mass of the substituted atom) to the unlabeled compound. Nonisotopic labeling involves the incorporation of a radioisotope into a molecule to give a product that is dissimilar in structure to the original substance. Most frequently, nonisotopic labeled red blood cells are obtained by complexing radioactive metal ions with red blood cells.

Red blood cells may be labeled with a variety of radioactive isotopes. The ideal radioisotope label should have the following properties: 1) high intensity photon emission, 2) energy of a sufficient level to provide suitable image, 3) minimal particulate radiation, 4) half-life sufficient for the duration of the study, and 5) does not alter the normal biological properties of the labeled red blood cells. Additional features for consideration of the radioisotope label are its availability and ease of labeling. Radiation properties of the various radioisotope labels that have been reported for labeling red blood cells are presented in Table 1.2. A brief discussion of each of these radioisotopes is presented below. Because of the

Table 1.2 Radiation Characteristic of Red Blood Cell Labels (68).

Radionuclide	Half-life[a]	Mode of Decay,[b] (%)	γ-Ray Energy, MeV
Fe-52	8.2 h	Positron (56) EC (44)	0.165 0.511
Fe-59	45 d	Negatron (100)	1.095 1.292
P-32	14.3 d	Negatron (100)	---
K-42	12.4 h	Negatron (100)	---
C-11	20.4 m	Positron (100)	0.511
C-14	5730 y	Negatron (100)	---
Se-75	120 d	EC (100)	0.121 0.136 0.265 0.280 0.401
In-111	2.8 d	IT (100)	0.173 0.247
Ga-68	68 m	Positron (88) EC (12)	0.511
Ga-67	78 h	EC (100)	0.093 0.184 0.296 0.388
Cr-51	27.8 d	EC (100)	0.320
Tc-99m	6 h	IT (100)	0.140

[a] y = year; d = days; h = hours; m = minutes

[b] EC = electron capture; IT = isomeric transition.

importance of technetium-99m to this research project, it is presented as an extended discussion.

Iron (Fe-52 and Fe-59)

Iron-52 decays by a positron emission (56%) and electron capture (44%). It has a half-life of 8.2 hours and emits gamma radiations having energies of 0.165 and 0.55 MeV. This isotope, which is produced by a cyclotron, is expensive.

Iron-59 decays by a negatron emission (100%) and has a half -life of 45 days. It emits 1.10 and 1.29 MeV gammas. The energies of these gammas are too high for imaging procedures.

The above radioisotopes are generally administered intravenously in the form of ferrous citrate or ferric ammmonium citrate. The iron ion is isotopically incorporated into hemoglobin during erythropoiesis (80). Red blood cells labeled with radioactive iron have been used in studies of red blood cell life span and blood loss (33, 54, 61).

Phosphorous (P-32)

Phosphorous-32 decays by a negatron emission (100%) and has a half-life of 14.3 days. It is generally used as sodium phosphate or diisopropyl fluorophosphate for labeling red blood cells. These agents label red blood cells by either in vivo (cohort) or in vitro procedures.

Sodium phosphate P-32 labels various blood components such as red blood cell stroma, phospholipids, hexoses and trioses. Red blood cells labeled with sodium phosphate P-32 are useful for the determination of red blood cell mass (6). Diisopropyl fluorophosphate P-32 labels leukocytes and platelets along with red blood cells. Thus, red blood cells must be separated from whole blood before labeling wiht this agent. Red blood cells labeled with diisopropyl fluorophosphate have been used to determine red blood cell life span.

Phosphorous-32 finds limited diagnostic applications because it can not be monitored externally and further, it presents a certain radiation risk to the patient. This is because it emits a beta radiation and has a relatively long half-life.

Potassium (K-42)

Potassium-42 decays by beta emission (100%) and has a half-life of 12.4 hours. It is used in the form of potassium chloride K-42 to label red blood cells by *in vitro* and *in vivo* (cohort) labeling (6, 32). This radionuclide is rarely used for blood volume measurement because of its relatively short half-life and its undesired beta radiation.

Carbon (C-11 and C-14)

Carbon-11 decays by positron emission (100%) which

yields 0.511 MeV annihilation photons. It has a half-life of 20.4 minutes. Carbon-11 is used as carbon monoxide for labeling red blood cells by either *in vitro* or *in vivo* (cohort) labeling procedures (23). Because of its short half life, this radioisotope must be prepared at the time of use. Thus, a cyclotron is required to be available on site when this isotope is used in red blood cell studies.

Carbon-14 decays by a negatron emission (100%) and has a half-life of 5730 years. It has been used as glycine C-14 to label red blood cells for the measurement of red blood cell life span (5). Because of the long half-life of the isotope, this labeling procedure requires an extended time period for an accurate measurement. Further, its clinical use is limited as external monitoring can not be performed due to the lack of gamma emission.

Selenium (Se-75)

Selenium-75 decays by electron capture (100%) and has a half-life of 120 days. It emits gamma radiation having energies of 0.121, 0.136, 0.265, 0.285, 0.401, and 0.236 MeV. This radioisotope is used as selenomethionine Se-75 for labeling red blood cells. Using this compound, Se-75 is incorporated into the red blood cell at the erythroplast and reticulocyte stage of the red blood cell development (57). The major disadvantage of this

radioisotope is its very low incorporation (approximately 4 percent of the administered dose) into the circulating erythrocytes.

Indium (In-111)

Indium-111 decays by electron capture (100 percent) and has a half-life of 2.8 days. It emits gamma radiation having energies of 0.173 and 0.247 MeV. In general, these radiation properties are favorable for imaging. For red blood cell labeling procedures, this radioisotope is used in the form of various lipid soluble chelates e.g., oxine, acetylacetone, and tropolone (42, 46, 71, 74).

Oxine In-111 indiscriminately labels all types of blood cells and in addition, binds to plasma transferrin. Thus, the red blood cell labeling procedure requires separation of red blood cells from whole blood followed by resuspension in a transferrin-free medium. Oxine In-111 labeled red blood cells have been used for the detection of intermittent gastrointestinal bleeding. A major disadvantage of this isotope is that a significant (approximately 7 percent per day in humans) *in vivo* elution of the label occurs (21).

Gallium (Ga-68 and Ga-67)

Gallium-68 decays by positron emission (88 percent)

and electron capture (12 percent). It has a half-life of 68 minutes and emits 0.511 MeV annihilation photons. As with In-111, it is most often used in the form of lipid soluble chelates, being either oxine, acetylacetone, or tropolone. Oxine Ga-68 labels red blood cells and platelets. Thus, it is necessary to separate red blood cells by centrifugation before labeling with oxine Ga-68. Gallium-68 labeled red blood cells appear to have the greatest potential in blood pool imaging using positron tomography (83).

Gallium-67 decays by electron capture (100 percent) and has a half-life of 78 hours. It emits gamma rays with energies of 0.093, 0.184, 0.296, and 0.388 MeV. Gallium-67 is used in the form of gallium chloride to label red blood cells. Gallium chloride Ga-67 labels the surface of red blood cells and platelets (51). Thus, labeling red blood cells with Ga-67 requires separation of red blood cells from whole blood. Gallium-67 labeled red blood cells have been used for blood pool imaging.

Chromium (Cr-51)

Chromium-51 decays by electron capture (100 percent) and has a half -life of 27.8 days. It emits gamma radiation with an energy of 0.32 MeV. This radioisotope is most commonly used in the form of sodium chromate Cr-51 for labeling red blood cells (15, 24, 25, 63). The

chromate ion has the ability to penetrate the red blood cell membrane. It appears that following its entrance into red blood cells, the hexavalent chromium in the anion is reduced to the trivalent chromium cation. This cation becomes attached to the beta chain of hemoglobin (55, 56). The main disadvantages associated with procedures using Cr-51 labeled red blood cells are 1) reutilization of the chromium label upon destruction of Cr-51 labeled red blood cells, 2) red blood cells of varying ages are not uniformily labeled, 3) leakage (approximately 1 percent per day) of chromium label from the labeled red blood cells, and 4) reduced patient safety due to the radioisotope's long half-life.

Technetium (Tc-99m)

Technetium is an element that exists only in the form of artificial unstable isotopes. This element was discovered as a product upon treating molybdenum in a cyclotron (58, 59). The name technetium was derived from the Greek word "Texvntos" meaning artificial in recognition of its source.

The only technetium isotope of interest for nuclear medicine procedures is technetium-99m. The use of Tc-99m as a tracer in nuclear medicine was originally suggested because of its favorable radiation properties (65). These properties are 1) short half-life of 6 hours, 2) absence of beta radiation, and 3) gamma emission of 140

KeV photons.

The clinical use of technetium-99m is limited to brain and thyroid imaging (29). Pertechnetate ion may be reduced to technetium ion (e.g., Tc^{4+}) and these ions will undergo complexation to form Tc-99m labeled compounds. Because of the short half-life of Tc-99m, a generator has been introduced to facilitate its clinical applications. The usefulness further facilitated by the use of technetium-99m radiopharmaceutical kits. further facilitate the clinical applications of Tc-99m, products known as kits have been introduced.

^{99}Mo - ^{99m}Tc Generator

The technetium-99m generator was first developed at Brookhaven National Laboratory in 1957 (66, 67, 78, 79). The generator as presently used consists of a lead shielded chromatographic column of aluminum oxide with molybdate Mo-99 absorbed into it. The molybdate Mo-99 decays to pertechnetate Tc-99m which may be selectively removed from the column by elution with normal saline. The eluted solution consists of sodium pertechnetate ($NaTcO_4$).

Molybdenum-99 has a half-life of 67 hours. It decays by beta emission with 87 percent becoming Tc-99m (metastable state) and 13 percent becoming Tc-99 (ground state). Molybdenum-99 emits two photons of 740 and 780

KeV. Technetium-99m decays by isomeric transition to the ground state, Tc-99. Technetium-99 has a half-life of $2.1X10^{5}$ years and decays by negatron emission to stable Ru-99. The decay equations for the above isotopes are as follows:

$$^{99}_{Mo} \longrightarrow {}^{99m}_{Tc} + \beta^-$$

$$^{99m}_{Tc} \longrightarrow {}^{99}_{Tc} + \gamma$$

$$^{99}_{Tc} \longrightarrow {}^{99}_{Ru} + \beta^-$$

Technetium Radiopharmaceuiticals

Sodium pertechnetate which is obtained from a technetium-99m generator, finds little direct use as an imaging agent. The pertechnetate ion may be readily reduced to technetium ion. This ion complexes with a variety of compounds to form useful radiopharmaceutical imaging agents.

Various reducing agents have been used to reduce pertehnetate ion from a +7 oxidation state to a lower oxidation state such as 3+, 4+, or 5+. Reducing agents used include stannous chloride, ascorbic acid plus ferric chloride, concentrated hydrochloric acid, sodium borohydride, and ferrous sulfate. Among these, stannous chloride is the most commonly used reducing agent for the preparation of Tc-99m labeled compounds. The reduction of pertechnetate ion by stannous ion in acidic medium is

as follows:

$$2\,^{99m}TcO_4^- + 16\,H^+ + 3\,Sn^{2+} \longrightarrow 2\,^{99m}Tc^{4+} + 3\,Sn^{4+} + 8\,H_2O_2$$

Complexation occurs with agents containing electron donating groups, such -COOH, -OH, $-NH_2$, and SH. These groups donate electrons pairs to form a coordinate covalent bond with Tc-99m.

Technetium-99m Radiopharmaceutical Kits

Technetium-99m radiopharmaceutical kits consist of a sterile lyophilized mixture of reducing agent (usually a stannous compound) and the compound to be labeled. This mixture is contained in a sterile rubber stoppered vial. At the time desired for administration to a patient, the product is labeled by injecting into the vial an appropriate quantity of sodium pertechnetate. The reaction involving the reducing agent and the complexing agent occurs within a few minutes after addition of sodium pertechentate solution. Thus, the kit provides a stable and convenient form for preparaing Tc-99m radiopharmaceuticals.

Technetium-99m Clinical Applications

Technetium-99m labeled compounds constitute approximately 80 percent of all radiopharmaceuticals used in nuclear medicine. They have various clinical applications: Tc-99m sodium pertechnetate is used for

brain and thyroid imaging; Tc-99m diethylenetriaminepentaacetic acid and Tc-99m glucoheptonate for brain and kidney imaging; Tc-99m microaggregated albumin for lung perfusion imaging; Tc-99m dimercaptosuccinic acid for kidney imaging; Tc-99m phosphate complexes such as hydroxyethylene diphosphonate, pyrophosphate, methylene diphosphonate, and hydroxymethylene diphosphonate for bone imaging; and Tc-99m pyrophosphate for imaging myocardial infarcts. In addition to the above applications, Tc-99m labeled red blood cells have found wide range of diagnostic applications such as blood pool imaging, detection of GI bleeding, and spleen imaging.

TECHNETIUM-99m LABELED RED BLOOD CELLS

Technetium-99m RBC Labeling Procedures

Labeling of red blood cells with sodium pertechnetate Tc-99m was first reported by Fischer et al. (21) in 1967. In this procedure, red blood cells were incubated with sodium pertechnetate Tc-99m for 20 minutes. The labeled red blood cells were heat-damaged for use as a spleen imaging agent. This product, when administered to patients, was described as behaving like heat-damaged Cr-51 labeled red blood cells. The spleen image obtained was reported as excellent in quality. The labeling yield of Tc-99m labeled red blood cells was

not given in this report.

In 1968, Burdine and Legeay (8) attempted to increase the labeling yield by adding a sulfhydryl inhibitor, such as potassium permanganate. A 20 percent labeling yield was obtained with this agent, while only a 10 percent yield was obtained without the sulfhydryl inhibitor.

In 1969, Haubold et al., (30) reported that the labeling yield of Tc-99m RBCs by Fischer's procedure was 10 percent or less on the average. They found that higher labeling yields of Tc-99m labeled red blood cells were obtained when sodium pertechnetate Tc-99m was allow to stand for at least 30 minutes before its use. Their reason for this effect was that fresh pertechnetate solution contains reduced species that are unable to cross the red blood cell membrane and thus cause labeling of the hemoglobin. Also, in this study it was found that by allowing the packed RBCs to stand for 30 minutes before addition of pertechnetate the labeling yield increased from 10 percent to 81-89 percent. This effect of standing was associated with deoxygenation of the cells. Other investigators have reported difficulty in reproducing these labeling yields (12).

The inefficiency of the labeling technique described by Fischer et al., (21) was further confirmed by Wienstein and Smoak (80). They incubated red blood cells with sodium pertechnetate for 20 minutes at 37^{o} C.

After seven washings with saline, the labeling yield was only 1 to 2 percent. They reported that red blood cell labeling with Tc-99m was not irreversible since the radioactivity was removed by washing the labeled red blood cells.

Anghileri et al., (2) obtained a labeling yield of 0.2 percent using Fischer's procedure. They reported a 10 percent labeling yield using sodium chromate and ascorbic acid. They claimed that the use of sodium chromate as a carrier facilitated the entrance of pertechnetate ions into erythrocytes. These investigators stated that ascorbic acid reduced the chromate ion to the trivalent chromium cation which did not facilitate the exit of pertechnetate ion from the cells. Also, ascorbic acid was suggested to partially chelate ferrous ion in the heme and facilitate its complexation with pertechnetate ion.

Eckelman et al., (17) stated that the labeling of Tc-99m to red blood cells involved two steps 1) introducing pertechnetate ion into the red blood cells and 2) binding technetium within the red blood cells. They indicated that Fischer's procedure (21). depended upon the ability of venous blood to reduce pertechnetate ion to free technetium ions (e.g., 4+ oxidation state). They stated that the dependence on the cell's endogenous reducing ability was the source of the difficulty in Fischer's procedure. Thus, to improve

the yield of the labeling procedure a reducing agent must be used. Stannous ion because of its strong reducing property was selected for their study in labeling red blood cells with technetium-99m.

The addition of stannous ion to red blood cells before the addition of sodium pertechnetate is known as pretinning. Procedures of this type have been studied by various investigators (4, 47, 69). Berger and Johannsen (4) studied the Tc-99m RBC labeling yields as a function of stannous ion concentration. They obtained 85 to 95 percent labeling yields using 0.2 to 0.5 µg stannous ion per 3 ml packed red blood cells. Scharwtz and Kruger (69) reported a 98 percent labeling yield after the saline wash and 97 percent after the second saline wash using pertechnetate ion and stannous chloride. They also, reported a 43 percent labeling yield after the first wash and 17 percent after the second wash using pertechnetate ion alone. Nouel and Burnelle (47) used a large quantity of stannous ion and then removed excess extracellular stannous ion by washing the red blood cells with edetic acid, a strong chelating agent. The labeling yield was reported to be 97 percent after repeated saline washes.

Post-tinning procedures have also received attention. In these procedures, stannous ion is added to red blood cells after the addition of sodium pertechnetate. The post-tinning technique was first reported by Eckelman et al., (17). In this procedure,

red blood cells were incubated with pertechnetate ion for 30 minutes at 37°C, and then incubated with stannous ion (62.55 µg/4 ml RBCs) for 15 minutes at room temperature. They reported labeling yields of 50 to 70 percent. Korubin et al., (38) and Atkins et al., (3) confirmed the efficiency of Eckelman labeling procedure and reported that the blood volume measured using Tc-99m labeled red blood cells was comparable to that measured using Cr-51 labeled red blood cells. McRae and Valk (44) found that a 5 minutes incubation time before and and after the addition of stannous ion gave maximum uptake of pertechnetate by the erythrocytes. Labeling yields of 50 to 70 percent was obtained.

Gutkowski and Dworkin (26) labeled red blood cells with technetium-99m by pretinning whole blood with stannous glucoheptonate. This reducing agent was obtained by using a kit that contained 200 mg sodium glucoheptonate and 0.1 mg stannous chloride. The treated red blood cells were incubated for 5 minutes at room temperature and then washed with edetic acid to remove excess extracellular stannous ion. They reported a labeling yield of 97 percent. Lower labeling yields were obtained when edetic acid was omitted in the procedure.

Smith and Richards (70) described a simple kit for the preparation of Tc-99m labeled red blood cells. This kit consisted of vacutainer tube containing 100 units of sodium heparin, 2.6 µg stannous citrate (1.0 µg stannous

ion), 2.5 mg sodium citrate, and 3.7 mg anhydrous stannous chloride. Labeled red blood cells were prepared by incubating heparinized whole blood with stannous ion (0.5 to 1.0 µg per 3 ml whole blood). A labeling yield of 97 percent was reported.

Another in vitro procedure was recently described by Mock and Wellman (45). They incubated heparinized whole blood with an acidic solution of stannous chloride (2 µg per 2 ml whole blood), then washed the pretinned red blood cells with edetic acid to remove excess extracellular stannous ion. A labeling yield of 98 percent was reported.

The first report of an in vivo labeling procedure was by Pavel et al., (53) in 1977. This was carried out by injecting intravenously a stannous pyrophosphate solution followed by a sodium pertechnetate Tc-99m solution. They found that the optimal time interval between injections of stannous pyrophosphate and sodium pertechnetate was 30 minutes. A labeling yield of 88 percent was obtained.

Hamilton et al., (27) studied the effect of various conditions on the labeling yield using an in vivo procedure. They obtained a maximum labeling yield of 88 percent with 10 µg stannous ion per Kg and 30 minutes lag time between the injection of stannous ion and pertechnetate ion solutions. In addition, they found no significant difference in the RBC labeling efficiency

with the use of either stannous pyrophosphate, stannous diphosphonate, or stannous tartarate .

Billinghurst et al., (7) studied the effect of stannous pyrophosphate concentration on in vivo red blood cells labeling with Tc-99m. They reported a maximum labeling yield of 86.3 percent with 4.7 μg stannous ion per Kg. They also reported that technetium becomes bound to other components in the plasma when a large amonut of stannous ion was injected.

Callahan et al., (9) reported a in vivo procedure for labeling red blood cells with Tc-99m. This was carried out by the intravenous injection of stannous pyrophosphate followed by withdrawing blood into a syringe containing sodium pertechnetate. After 10 minutes incubation, the labeled red blood cells were reinjected. They obtained labeling yields of approximately 95 percent.

Clinical Applications

Technetium-99m labeled red blood cells have found wide range of diagnostic applications. In particular, in vivo Tc-99m labeled red blood cells are used in multigated cardiac blood pool study. In this procedure, the maximum activity at end-diastole and the minimum activity at end-systole are used to calculate the ejection fraction of the left ventricle by the following equation (68):

$$EF\ \% = \frac{100\ (A_{ED} - A_{ES})}{A_{ED}}$$

where:

EF = The ejection fraction

A_{ED} = maximum end-diastole activity

A_{ES} = minimum end-systole activity

Another application of this study is the detection of any wall motion abnormalities by observing the difference between the end-diastolic and end-systolic images. These two images are concentric in normal heart. An abnormal motion due to ischemia or infarction can be detected by eccentric differences in these two images.

Among other areas of nuclear medicine procedures that use Tc-99m labeled red blood cells are blood pool imaging, red blood cell mass determination, and detection of GI bleeding and hemangiomas. Heat-damaged Tc-99m labeled red blood cells find application in spleen imaging, accessory spleen localization, and detection of GI bleeding.

Mechanism of Tc-99m Red Blood Cell Labeling

There has been increasing interest in the use of Tc-99m labeled red blood cells during the past decade (31, 52, 73, 77). However, the precise mechanism of labeling of Tc-99m to red blood cells is not known.

The first report of a mechanism was by Dewanjee (10) in 1974. This study attributed the perferential site of binding to the beta chain of hemoglobin. It was indicated that there is a similarity in the nature of binding of technetium and chromium ion to hemoglobin.

In 1980, Rehani and Sharma (64) studied the distribution of Tc-99m in various red blood cell components. The components isolated in this study were heme, beta and alpha chains of globin. They reported that the major part of technetium-99m was bound to the globin fraction. The preferential site of binding in the globin was the beta chain, where the radioactivity of Tc-99m was found to be 28 times that of the alpha chain. They indicated that this activity was somewhat similar to the factor of 30 reported with Cr-51 (16).

Problems

Major problems associated with the labeling of Tc-99m to red blood cells are 1) reduced Tc-99m red blood cell labeling yields due to interactions and 2) undesired in vivo Tc-99m RBC labeling. Prazosin and digoxin have been reported to cause a significant reduction in Tc-99m RBC labeling (41). It may be expected that other substances may also cause interference in labeling.

Undesired in vivo Tc-99m RBC labeling may occur in patients who are administered sodium pertechnetate after having previously received a stannous containing product.

Most frequently this occur with patients receiving technetium-99m kits containing stannous compounds. The first report of altered tissue distribution of Tc-99m attributable to prior stannous ion administration was reported by McRae et al., (43). Khentigon et al., (37) found similar results in a study using several stannous containing agents.

These two problems along with the limited information on the labeling mechanism provided the basis for this research. The goals were 1) to identify substances which may cause reduced Tc-99m RBC labeling yields, 2) to identify substances which may be useful to reduce or prevent undesired in vivo Tc-99m RBC labeling, and 3) to further the knowledge of the mechanism of Tc-99m red blood cell labeling.

EXPERIMENTAL DESIGN

The research plan consisted of first developing a general labeling procedure. Then, using this procedure, various agents would be investigated to determine their effect on Tc-99m RBC labeling yields.

Various labeling procedures were reviewed for their usefulness as a general procedure for this research. The procedure reported by Smith and Richards (70) was selected for use as a starting point for developing a general labeling procedure. This procedure offered the

advantage of being convenient and giving a high labeling yield. From the literature it was recognized that various conditions alter labeling yields. As a further step in establishing the general labeling procedure, the effect of various conditions on the labeling yield of the basic starting procedure was investigated.

Selected Conditions for the General Labeling Procedure

1. Anticoagulants. Three commonly used anticoagulant systems were examined. These were citrate phosphate dextrose (CPD), acid citrate dextrose (ACD), and heparin. Each system was prepared and added in the same quantity as that used for preservation of whole blood (85).

2. Stannous Ion Concentration. Seven stannous ion concentrations of 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 µg per ml were selected for investigation. This range of concentration was selected because of previous reports.

3. Stannous Ion Compound. Five different stannous ion compounds were selected for investigation. These were stannous chloride, stannous pyrophosphate, stannous glucoheptonate, stannous etidronate, and stannous dimercaptosuccinate. The first four were selected because they are used as reducing agents in preparaing Tc-99m products. The latter was selected because it is a stannous chelate. The chelate was of interest because it represents, as compared to the ionic salts, different

disssociation and biological characteristics. Sodium citrate was included in the preparation of solutions of these stannous compounds. This agent has been reported to increase the stability of stannous ion in aqueous solution (70). The concentration was selected on the basis of the found optimal stannous ion concentration.

4. Incubation Time. The effect of incubation time of whole blood with stannous ion was investigated over the range of 5 to 120 minutes. The selected incubation times were 5, 15, 30, 60, and 120 minutes. This range was used as these incubation times have been reported in the literature.

5. Incubation Temperature. Whole blood was incubated with stannous ion at room temperature (22 ^{o}C) and at 37 ^{o}C. These two temperatures were selected because of their use in previous reports.

6. Red Blood Cells Washed Before the Addition of Stannous Ion. The effect of separating and washing red blood cells before addition of stannous ion, as compared to incubation of whole blood, was investigated.

The incorporation of a washing step in the labeling procedure provides for the removal of plasma and/or excess extracellular stannous ion. The removal of plasma may be desirable because it contains components which could react with stannous ion to give lower labeling yields. The presence of extracellular stannous ion is undesired as it will reduce pertechnetate ion before it

crosses the red blood cell membrane. As a result, the Tc-99m concentration available for RBC labeling is reduced.

From the investigation of the effect of the above conditions, a set of optimal conditions was determined and incorporated into a general labeling procedure. This procedure was used to investigate the effect of the selected agents on labeling yields. Most of the selected agents were investigated using two sets of experiments. One involved the use of a post-tinning procedure which consisted of adding the agent of interest to blood before the addition of stannous ion. The other set of experiments used a pretinning procedure. This involved adding the agent to blood after the addition of stannous ion.

Selected Agents for Determining Effect on Labeling Yield

1. Inorganic Ions

Twenty inorganic ions were selected for investigation of their effect on labeling RBC with Tc-99m. These inorganic ions were selected because of their widespread use as drugs, diet supplements and foodstuffs. Also, they may be encountered as enviromental pollutants.

The concentration of each ion selected for addition to the red blood cell sample represented a high but not lethal value (13). This value did not take into account

any quantity of ions contained in the original blood sample. The normal blood concentration of some of these ions is presented in Table 1.3.

Such agents, in general, have the potential to cause reduced labeling labeling yields due to 1) oxidation-reduction reactions, 2) interference in the transport mechanism of stannous ion and/or pertechnetate ion through the red blood cell membrane, and 3) competition with stannous and pertechnetate ions for protein and enzyme binding sites in the red blood cells. Since labeling of red blood cells with Tc-99m depends upon an oxidation-reduction between stannous ion and pertechnetate ion, the presence of any additional substance with oxidizing or reducing property may interfere with the labeling process. Also, labeling depends upon transport of stannous ion and pertechnetate ion through the red blood cell membrane, followed by their binding to cellular components such as proteins and enzymes. Thus, any substance which interferes with the transport or competes for binding sites may interfere with the labeling process.

The effect of inorganic ions was studied in two sets of experiments. In the first set, designated as post-tinning, the inorganic ion was added to blood before stannous ion. In the second set, designated as pretinning, the inorganic ion was added to blood after the addition of stannous ion.

Table 1.3 Normal Values of Inorganic Ions in Blood (13).

Inorganic Ion	Normal Blood Concentration, µg/l
Al^{3+}	0 - 380
As^{5+}	60 - 200
Co^{2+}	0.35
Ca^{2+}	208 X 10
Cu^{2+}	220 - 1060
Fe^{2+}	440 - 560 X 10^{3}
Li^{+}	3 - 44
Mn^{2+}	9 - 16.5
Mo^{6+}	0 - 104
Mg^{2+}	126 X 10
Pb^{2+}	170 - 370
Se^{4+}	250 - 2750

2. Therapeutic Agents

Fifteen therapeutic agents were selected for investigation of their effect on labeling RBC with Tc-99m. Some of these agents and their therapeutic plasma concentration ranges are presented in Table 1.4. The concentration selected for each agent represented a high therapeutic plasma concentration. Most of the investigated agents were selected because of their wide use in treating cardiovascular patients. Such pateints frequently undergo procedures using Tc-99m labeled red blood cells. These agents consisted of beta-adrenergic blockers, calcium channel blockers, digoxin, quindine, lidocaine, prazosin and captopril. Other selected agents were ascorbic acid and tetracycline. Ascorbic acid is widely used as an essential vitamin and as a stabilizer (antioxidant) in various drugs and food products. Tetracycline is widely used as an antibiotic.

The potential interference in the labeling of red blood cells with Tc-99m might be expected with such agents because of their biochemical and chemical properties. Interference due to biochemical properties may occur because of alteration of the cell membrane transport system and/or alterationof the reactivity of cellular components with stannous and technetium ions. Interference due to chemical properties could be expected for ascorbic acid and tetracycline. Ascorbic acid is a

Table 1.4 Therapeutic Plasma Concentration of Selected Agents (1).

Agent	Therapeutic Plasma Concentration µg/ml
Propranolol	0.05 - 0.1
Atenolol	0.2 - 0.5
Metoprolol	0.05 - 0.1
Pindolol	0.05 - 0.1
Timolol	5 - 10
Verapamil	0.12 - 0.4
Diltiazem	0.05 - 0.2
Quinidine	2 - 8
Lidocaine	1 - 5
Digoxin	0.0005 - 0.002
Prazocin	0.01 - 0.075
Ascorbic Acid	10 - 20
Tetracycline	2 - 5

reducing agent and thus, may reduce pertechnetate ions before they cross the red blood membrane. Tetracycline has chelating properties, which in the labeling procedure, may result in the chelation of stannous ion to prevent its passage into the red blood cells.

These therapeutic agents were investigated for their effect on labeling yields when added to blood before the addition of stannous ion. This is representative of the situation that would be encountered with patients.

3. Biochemical Substances

Inosine glucose, cysteine, and glutathione were selected for investigation of their effect on RBC labeling with Tc-99m. The selection of these agents was based upon their use as nutrient, therapeutic and blood preserving agents.

Inosine has been reported to be a blood preservative. This agent offered the potential value of being useful for storing blood for later labeling. This could have practical value in a clinical setting where a large volume of patients is encountered. The selected concentration for inosine represented the concentration used as a blood preservative.

Glucose is major carbohydrate that provides a source of energy in living cells. It is widely used as a carbohydrate nutrient. It finds additional use in the treatment of insulin hypoglycemia (insulin shock) for

restoring blood glucose levels. Elevated blood glucose levels may be found in uncontrolled diabetic patients. Glucose is transported through the red blood cell membrane. It is a component that is essential for the life of the red blood cell. The concentration selected represented double its normal plasma concentration.

Cysteine is an amino acid required for the synthesis of glutathione. Glutathione occurs naturally in red blood cell where it acts as the principal reducing agent to protect red blood cells against oxidative damage. It is the essential co-factor for small number of enzymes such as glutathione peroxidase. The concentration selected represented double its normal plasma concentration.

The effect of these biochemical agents was investigated in two set of experiments. In one set, the agent was added to fresh blood. In the other set, the agent was added to two weeks stored blood.

4. Chelating Agents

Three chelating agents were selected for this investigation. These were dimercaptosuccinic acid (DMSA), dimercaprol (BAL) and edetic acid (EDTA). These agents were selected because of their ability to chelate heavy metals. Interference in labeling may possibly be expected due to chelation of stannous ion. This would prevent the ion from crossing the red blood cell

membrane. The concentration selected for each agent was that used as an antidote in heavy metal poisoning (1).

The effect of these agents was investigated in two sets of experiments. In one set, designated as post-tinning, the chelating agent was added to blood before the additon of stannous ion. In the other set, designated as pretinning, the chelating agent was added to blood after the addition of stannous ion.

5. Oxidizing Agents

Hydrogen peroxide and potassium permanganate were selected for this investigation. The selection of these two agents based on their widespread use as oxidizing agents in analytical procedures. Also, hydrogen peroxide has been previously used as an oxidizing agent in a study concerning Tc-99m RBC labeling (70). These agents were in addition to other oxidizing agents that were previously investigated in part 1, inorganic ions. Each agent was tested at a concentration that was equimolar to the stannous ion concentration.

The effect of these agents was investigated in three sets of experiments. First, designated as post-tinning, the oxidizing agent was added to blood before the addition of stannous ion. Second, designated as pretinning, it was added to blood after the additon of stannous ion. Third, the oxidizing agent was added to stannous ion before the addition of blood.

6. Enzyme Poison

Potassium cyanide was selected because of its toxic action on enzymes. Also, it has specific action on red blood cells. A list of enzymes inhibited by this agent is presented in Table 1.5. Two concentrations were selected. One concentration represented a high but not lethal concentration (19). The second concentration represented ten fold the first concentration.

Varying cyanide levels in the blood may be found in the general population. This may result from certain foodstuffs such as lima beans, sorghum, sweet potatoes, and kernels of fruits which contain moderate to high levels of cyanogenic glycosides. Non-dietary sources include tobacco smoke, drugs and enviromental pollutants. Somkers have higher levels of blood cyanide levels as compared to non-smokers (58, 73). Drugs such as sodium nitroprusside and succinonitrile increase cyanide levels in red blood cells and plasma.

The effect of potassium cyanide was investigated in two sets of experiments. In the first set, designated as post-tinning, potassium cyanide was added to blood before the addition of stannous ion. In the second set, designated as pretinning, potassium cyanide was added to blood after stannous ion.

Table 1.5 Enzymes Inhibited by Cyanide (84).

Enzyme	Minimum Inhibition Concentration, Moles/Liter
Catalase	5×10^{-6} [a]
Myoglobin (ox.)	8×10^{-7} [a]
Superoxide Dismutase	2×10^{-5} [b]
Nitrite Oxidase	5×10^{-6} [c]
Nitrite Reductase (ox.)	2×10^{-5} [c]
Nitrite Reductase (red.)	4×10^{-10} [a]
Nitrogenase	2×10^{-5} [c]
Lactoperoxidase	3×10^{-5} [a]

[a] Dissociation constant for enzyme-cyanide complex.

[b] Concentration of cyanide which gives 50% inhibition.

[c] Inhibition constant.

7. Methemoglobinemia Agents

Sodium nitrite and methylene blue were used in this investigation. The selection of sodium nitrite was based upon its oxidizing and reducing properties. Also, sodium nitrite enters the red blood cells and acts as direct oxidant with hemoglobin to produce methemoglobin. Interference in labeling may possibly be expected due to oxidation of stannous ion or reduction of pertechnetate ion by nitrite ion. The selected concentration used for this investigation represented a concentration reported to form methemoglobinemia (85).

Methylene blue is used to treat methemoglobinemia which is based upon its oxidizing property. This agent enters red blood cells and activates NADPH-dependent methemoglobin reductase enzyme. The NADPH-dependent enzyme reduces methylene blue, which rapidly reduces methemoglobin non-enzymatically to hemoglobin. Interference in labeling may possibly occur due to oxidation of stannous ion by methylene blue. The selected concentration used for this investigation represented a concentration reported to treat methemoglobinemia (85).

The effect of these agents was investigated in two sets of experiments. In the first set, designated as post-tinning, the agent was added to blood before the addition of stannous ion. In second set, designated as

pretinning, it was added to blood after stannous ion.

Statistical Calculations

Three determination were done for each experiment. Labeling yield was expressed as mean ± standard deviation (s.d.). A t test was performed to determine the significance of mean labeling yield of values which were not discernable different from their mean value.

CHAPTER II

EXPERIMENTAL

A. *IN VITRO* STUDIES

Materials

Chemicals having a high purity were used without further purification. Water purified by distillation was used for preparation of solutions.

Blood Samples

Samples of human venous blood were drawn from volunteers by a certified laboratory technologist into 10-ml vaccum blood collection tubes[1] containing an anticoagulant. The use of human volunteers for this project was approved by the Human Subject Committee.

Anticoagulants

Citrate Phosphate Dextrose Solution (CPD) - 3.2 g citric acid, 25.8 g sodium citrate, 2.18 g sodium dihydrogen phosphate monohydrate, and 25.0 g dextrose were dissolved in sufficient water to make one liter.

Acid Citrate Dextrose Solution (ACD) - 7.3 g citric acid, 22.0 g sodium citrate, and 24.5 g dextrose were dissolved in sufficient water to make one liter.

Heparin - A commercial solution[2] containing 75,000 USP units of heparin sodium in one liter of normal saline was used.

Stannous Chloride

Stock solution - 50 mg anhydrous stannous chloride was dissolved in 100 ml of a solution containing 0.75 percent sodium chloride and 0.5 percent sodium citrate in water.

Working solution - the stock solution was diluted with a solution containing 0.75 percent sodium chloride and 0.5 percent sodium citrate to give the following concentrations of stannous ion: 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 µg/ml.

Stannous Pyrophosphate

Stock solution - 50 mg anhydrous stannous chloride and 176.47 sodium pyrophosphate were dissolved in 100 ml of a solution containing 0.75 percent sodium chloride and 0.5 percent sodium citrate in water.

Working solution - the stock solution was diluted with a solution containing 0.75 percent sodium chloride and 0.5 percent sodium citrate to give 0.5 µg/ml of stannous ion.

Stannous Glucoheptonate

Stock solution - 50 mg anhydrous stannous chloride

and 100 g sodium glucoheptonate were dissolved in 100 ml of a solution containing 0.75 percent sodium chloride and 0.5 percent sodium citrate in water.

Working solution - the stock solution was diluted with a solution containing 0.75 percent sodium chloride and 0.5 percent sodium citrate to give 0.5 µg/ml of stannous ion.

Stannous Etidronate

Stock solution - 50 mg anhydrous stannous chloride and 215.5 mg sodium etidronate were dissolved in a 100 ml of a solution containing 0.75 percent sodium chloride and 0.5 percent sodium citrate in water.

Working solution - the stock solution was diluted with a solution containing 0.75 percent stannous chloride and 0.5 percent stannous citrate to give 0.5 µg/ml of stannous ion.

Stannous Dimercaptosuccinate

Stock solution - 50 mg anhydrous stannous chloride and 142.85 mg dimercaptosuccininc acid were dissolved in 100 ml of a solution containing 0.75 percent sodium chloride and 0.5 percent sodium citrate in water.

Working solution - the stock solution was diluted with a solution containing 0.75 percent sodium chloride and 0.5 percent sodium citrate to give 0.5 µg/ml of stannous ion.

Inorganic Ions

Each of the following solutions was prepared using normal saline as the solvent:

0.01% Aluminum chloride. This is equivalent to 1.14 µg aluminum ion per 0.1ml

0.001% Sodium arsenite. This is equivalent to 0.6µg arsenite ion per 0.1ml.

0.0015% Sodium arsenate. This is equivalent to 0.6 µg arsenate ion per 0.1ml.

0.0008% Cadmium chloride. This is equivalent to 0.5 µg cadmium ion per 0.1ml.

2.29% Calcium chloride. This is equivalent to 0.624 mg calcium ion per 0.1ml.

0.0014% Ceric sulfate tetrahydrate. This is equivalent to 0.5 µg ceric ion per 0.1ml.

0.0015% Chromic chloride. This is equivalent to 0.5 µg chromium ion per 0.1ml.

0.0023% Sodium chromate tetrahydrate. This is equivalent to 0.5 µg chromate ion per 0.1ml.

0.002% Cobaltous chloride hexahydrate. This is equivalent to 0.001 µg cobalt ion per 0.1ml.

0.0088% Cupric chloride dihydrate. This is equivalent to 3.18 µg cupric ion per 0.1ml.

8.36% Ferrous sulfate heptahydrate. This is equivalent to 1.68 mg ferrous ion per 0.1ml.

8.13% Ferric chloride hexahydrate. This is

equivalent to 1.68 mg ferric ion per 0.1ml.

0.002% Lead acetate trihydrate. This is equivalent to 1.11 µg lead ion per 0.1ml.

0.0008% Lithium chloride. This is equivalent to 0.132 µg lithium ion per 0.1ml.

26.52% Magnesium chloride hexahydrate. This is equivalent to 3.172 mg magnesium ion per 0.1ml.

0.002% Managanese chloride tetrahydrate. This is equivalent to 0.5 µg manganese ion per 0.1ml.

0.0008% Sodium molybdate dihydrate. This is equivalent to 0.31 µg molybdate ion per 0.1ml.

0.013% Selenious acid. This is equivalent to 8.25 µg selenite ion per 0.1ml.

0.0015% Stannic chloride pentahydrate. This is equivalent to 0.5 µg stannic ion per 0.1ml.

0.0002% Zinc sulfate heptahydrate. This is equivalent to 0.048 µg zinc ion per 0.1ml.

Therapeutic Agents

Each of the following therapeutic agents was prepared using normal saline as the vehicle:

0.003% Propranolol solution[3]

0.015% Atenolol solution[4]

0.003% Metoprolol solution[5]

0.003% Pindolol suspension[6]

0.030% Timolol solution[7]

0.012% Verapamil solution[8]

0.012% Nifedipine suspension[9]

0.006% Diltiazem solution[10]

0.000006% Digoxin solution[11]

0.000225% Prazosin suspension[12]

0.000225% Captopril solution[13]

0.024% Quinidine solution[14]

0.015% Lidocaine solution[15]

0.12% Ascorbic acid solution[16]

0.015% Tetracycline solution[17]

Biochemical Substances

Each of the following solutions was prepared using normal saline as the solvent:

4.86% Glucose solution

0.060% Cysteine solution

2.124% Glutathione solution

15.00% Inosine solution

Chelating Agents

Each of the following solutions was prepared using normal saline solution as the solvent:

0.182% Dimercaptosuccinic acid solution

0.124% Dimercaprol solution

0.380% Edetic acid solution

Oxidizing Agents

The following solutions were prepared using normal

saline as the solvent:

1.5% Hydrogen peroxide solution

0.0007% Potassium permanganate solution

Enzyme Poison

Each of the following solutions was prepared using using normal saline as the solvent:

0.0469% Potassium cyanide solution. This is equivalent to 18.75 µg cyanide ion per 0.1ml.

0.469% Potassium cyanide solution. This is equivalent to 187.5 µg cyanide ion per 0.1ml.

Methemoglobinemia Agents

Each of the following solutions was prepared using normal saline as the solvent:

0.50% Sodium nitrite solution. This is equivalent to 0.5 mg nitrite ion per 0.1ml.

0.035% Methylene blue solution

Methods

General Procedure

1. Collect 10 ml of human whole venous blood in a vaccum blood collection tube[1] containing 1.4 ml CPD solution.
2. Add 1 ml stannous chloride solution containing 0.5 µg

stannous ion to 3 ml whole blood, mix gently and allow to stand for 5 minutes at room temperature.

3. Add 2 ml normal saline to the above mixture, mix gently and centrifuge[18] for 5 minutes at 2,000 rpm.

4. Remove the supernatant by use of a syringe and discard this fraction.

5. Add 1.5 μCi sodium pertechnetate Tc-99m contained in 1 ml normal saline to the separated red blood cells (RBCs), mix gently and allow to stand for 10 minutes at room temperature.

6. Add 3 ml normal saline to the above mixture, mix gently and centrifuge[18] for 5 minutes at 2,000 rpm.

7. Remove the supernatant by use of a syringe and transfer to another tube.

8. Add sufficient water to each tube (i.e., one containing labeled RBCs and the other containing supernatant liquid) to give the same volume.

9. Count the activity in each of the two tubes using a NaI(Tl) well-type scintillation counter[19].

10. Calculate the percentage of labeled red blood cells using the following equation:

$$\% \text{ Labeled RBCs} = \frac{100 \quad A_{RBC}}{A_{RBC} + A_S}$$

where:

A_{RBC} = RBC Activity

A_S = Supernatant Activity

Effect of Anticoagulant

CPD - The general procedure was conducted.

ACD - The general procedure was conducted with the exception that 1.5 ml ACD solution was used in step 1 in place of 1.4 ml CPD.

Heparin - The general procedure was conducted with the exception that 0.6 ml (45 USP units) heparin sodium solution was used in step 1 in place of 1.4 ml CPD.

Effect of Stannous Ion Concentration

Stannous chloride solutions containing 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 µg/ml of stannous ion were used in separate labeling experiments. Labeling was conducted using a modification of the general procedure. The modification involved changing step 2 to the following:

Add 1 ml stannous chloride solution containing the appropriate quantity of stannous ion to 3 ml whole blood, mix gently and allow to stand for 5 minutes at room temperature.

Effect of Stannous Compound

Solutions of stannous pyrophosphate, stannous glucoheptonate, stannous etidronate and stannous dimercaptosuccinate were used in separate labeling experiments. Labeling was conducted using a modification of the general labeling procedure. The modification involved changing step 1 to the following:

Add 1 ml of the appropriate stannous compound containing 0.5 µg stannous ion to 3 ml whole blood, mix gently and allow to stand for 5 minutes at room temperature.

Effect of Incubation Time

Incubation times of 5, 15, 30, 60, 120 minutes were used in separate labeling experiments. Labeling was conducted using a modification of the general procedure. The modification involved changing step 2 to the following:

Add stannous chloride solution containing 0.5 µg stannous ion to 3 ml whole blood, mix gently and allow to stand for the appropriate incubation time at room temperature.

Effect of Incubation Temperature

Room temperature (22° C) and 37°C were used in separate labeling experiments. Labeling was conducted

using a modification of the general procedure. The modification involved changing step 2 to the following:

Add 1 ml stannous chloride solution containing 0.5 µg stannous ion to 3 ml whole blood, mix gently and allow to stand for 5 minutes at the appropriate temperature.

Washed Red Blood Cells

Labeling was conducted using a modification of the general procedure. The modification involved changing step 2 to the following:

Add 3 ml normal saline to 3 ml whole blood, mix gently and centrifuge for 5 minutes at 2,000 rpm. Remove the supernatant by use of a syringe and discard this fraction. Add 1 ml stannous chloride solution containing 0.5 µg stannous ion to the separated red blood cells, mix gently and allow to stand for minutes at room temperature.

Addition of Inorganic Ion Before Stannous Ion

The inorganic ions listed in the materials section were used in separate labeling experiments. Labeling was conducted using a modification of the general procedure. The modification involved changing step 2 to the following:

Add 0.1 ml of the appropriate inorganic ion to 3 ml whole blood, mix gently and allow to stand for 5 minutes

at room temperature. Add 1 ml stannous chloride solution containing 0.5 µg stannous ion to the mixture, mix gently and allow to stand for 5 minutes at room temperature.

Addition of Inorganic Ion After Stannous Ion

The inorganic ions listed in the materials section were used in separate labeling experiments. Labeling was conducted using a modification of the general procedure. The modification involved changing step 2 to the following:

Add 1 ml stannous chloride solution containing 0.5 µg stannous ion to 3 ml whole blood, mix gently and allow to stand for 5 minutes at room temperature. Add 0.1 ml of the appropriate inorganic ion to the mixture, mix gently and allow to stand for 5 minutes at room temperature.

Addition of Therapeutic Agent Before Stannous Ion

The therapeutic agents listed in the materials section were used in separate labeling experiments. Labeling was conducted using a modification of the general procedure. The modification involved changing step 2 to the following:

Add 0.1 ml of the appropriate therapeutic agent to 3 ml whole blood, mix gently and allow to stand for 5 minutes at room temperature. Add 1 ml stannous chloride solution containing 0.5 µg stannous ion to the mixture,

mix gently and allow to stand for 5 minutes at room temperature.

Addition of Biochemical to Fresh Blood

The biochemicals listed in the materials section were used in separate labeling experiments. Labeling was conducted using a modification of the general procedure. The modification involved changing step 2 to the following:

Add 0.1 ml of the appropriate biochemical to 3 ml whole blood, mix gently and allow to stand for 5 minutes at room temperature. Add 1 ml stannous chloride solution containing 0.5 µg stannous ion to the mixture, mix gently and allow to stand for 5 minutes at room temperature.

Addition of Biochemical to Stored Blood

The biochemicals listed in the materials section were used in separate labeling experiments. Labeling was conducted using a modification of the general procedure. The modification involved changing step 2 to the following:

Allow whole blood to stand for two weeks at $0^{\circ}C$. To 3 ml of the stored blood, add 0.1 ml of the appropriate biochemical, mix gently and allow to stand for 5 minutes at room temperature. Add 1 ml stannous chloride solution containing 0.5 µg stannous ion to the mixture, mix gently and allow to stand for 5 minutes at room temperature.

Addition of Inosine to Whole Blood Before Storage

Labeling was conducted using a modification of the general procedure. The modification involved changing step 2 to the following:

Add 0.1 ml inosine solution to 3 ml whole blood, mix gently and store for two weeks at 0°C. Add 1 ml stannous chloride solution containing 0.5 µg stannous ion to 3 ml stored blood, mix gently and allow to stand for 5 minutes at room temperature.

Addition of Chelating Agent Before Stannous Ion

The chelating agents listed in the materials section were used in separate labeling experiments. Labeling was conducted using a modification of the general procedure. The modification involved changing step 2 to the following:

Add 0.1 ml of the appropriate chelating agent to 3 ml whole blood, mix gently and allow to stand for 5 minutes at room temperature. Add 1 ml stannous chloride solution containing 0.5 µg stannous ion to the mixture, mix gently and allow to stand for 5 minutes at room temperature.

Addition of Chelating Agents After Stannous Ion

The chelating agents listed in the materials section were used in separate labeling experiments. Labeling was

conducted using a modification of the general procedure. The modification involved changing step 2 to the following:

Add 1 ml stannous chloride solution containing 0.5 µg stannous ion to 3 ml whole blood, mix gently and allow to stand for 5 minutes at room temperature. Add 0.1 ml of the appropriate chelating agent to the mixture, mix gently and allow to stand for 5 minutes at room temperature.

Addition of Oxidizing Agent to Stannous Ion Before Blood

The oxidizing agents listed in the materials section were used in separate labeling experiments. Labeling was conducted using a modification of the general procedure. The modification involved changing step 2 to the following :

Add 0.1 ml of the appropriate oxidizing agent to 1 ml stannous chloride solution containing 0.5 µg stannous ion, mix gently and allow to stand for 5 minutes at room temperature. Add 3 ml whole blood obtained in step 1 to the mixture, mix gently and allow to stand for 5 minutes at room temperature.

Addition of Oxidizing Agent to Blood Before Stannous Ion

The oxidizing agents listed in the materials section were used in separate labeling experiments. Labeling was conducted using a modification of the general procedure.

The modificaton involved changing step 2 to the following:

Add 0.1 ml of the appropriate oxidizing agent to 3 ml whole blood, mix gently and allow to stand for 5 minutes at room temperature. Add 1 ml stannous chloride solution containing 0.5 µg stannous ion to the mixture, mix gently and allow to stand for 5 minutes at room temperatue.

Addition of Oxidizing Agent to Blood After Stannous Ion

The oxidizing agents listed in the materials section were used in separate labeling experiments. Labeling was conducted using a modification of the general procedure. The modification involved changing step 2 to the following:

Add 1 ml stannous chloride solution containing 0.5 µg stannous ion to 3 ml whole blood, mix gently and allow to stand for 5 minutes at room temperature. Add 0.1 ml of the appropriate oxidizing agent to the mixture, mix gently and allow to stand for 5 minutes at room temperature.

Addition of Enzyme Poison to Blood Before Stannous Ion

Labeling was conducted using a modification of the general procedure. The modification involved changing step 2 to the following:

Add 0.1 ml of the appropriate potassium cyanide solution to 3ml whole blood, mix gently and allow to stand for 5 minutes at room temperature. Add 1 ml stannous chloride solution containing 0.5 µg stannous ion to the mixture, mix gently and allow to stand for 5 minutes at room temperature.

Addition of Enzyme Poison to Blood After Stannous Ion

Labeling was conducted using a modification of the general procedure. The modification involved changing step 2 to the following:

Add 1 ml stannous chloride solution containing 0.5 µg stannous ion to 3 ml whole blood, mix gently and allow to stand for 5 minutes at room temperature. Add 0.1 ml of the appropriate potassium cyanide solution to the mixture, mix gently and allow to stand for 5 minutes at room temperature.

Addition of Methemoglobinemia Agent to Blood Before Stannous Ion

The methemoglobinemia agents listed in the materials section were used in separate labeling experiments. Labeling was conducted using a modification of the general procedure. The modification involved changing step 2 to the following:

Add 0.1 ml of the appropriate methemoglobinemia agent to 3 ml blood, mix gently and allow to stand for

minutes at room temperature. Add 1 ml stannous chloride solution containing 0.5 µg stannous ion to the mixture, mix gently and allow to stand for 5 minutes at room temperature.

Addition of Methemoglobinemia Agent to Blood After Stannous Ion

The methemoglobinemia agents listed in the materials section were used in separate labeling experiments. Labeling was conducted using a modification of the general procedure. The modification involved changing step 2 to the following:

Add 1 ml stannous chloride solution containing 0.5 µg stannous ion to 3 ml whole blood, mix gently and allow to stand for 5 minutes at room temperature. Add 0.1 ml of the appropriate methemoglobinemia agent to the mixture, mix gently and allow to stand for 5 minutes at room temperature.

B. IN VIVO STUDIES

Materials

Animals

Twenty-seven male one month old Sprague-Dawley rats ranging in weight from 180 to 220 g were used. The use

of animals for this project was approved by the Animal Care Committee.

Stannous Chloride

Stock solution - 50 mg anhydrous stannous chloride was dissolved in 100 ml of a solution containing 0.75 percent sodium chloride and 0.5 percent sodium citrate in water.

Working solution - the stock solution was diluted with a solution containing 0.75 percent sodium chloride and 0.5 percent sodium citrate to give a stannous concentration of 2 µg/0.1 ml.

Test Solutions

Normal saline- 0.9% sodium chloride solution in water.

Each of the following solutions was prepared using normal saline as the solvent:

0.0123% Sodium chromate solution. This is equivalent to 2.67 µg chromate ion per 0.1ml.

0.069% Selenious acid solution. This is equivalent to 44 µg selenite ion per 0.1ml.

44.36% Ferrous sulfate solution. This is equivalent to 8.96 mg ferrous ion per 0.1ml.

0.024% Digoxin solution[11]

0.1% Dimercaptosuccinic acid solution

Methods

General Procedure

1. Inject 0.1 ml stannous chloride solution containing 2 µg stannous ion into the tail vein.
2. After 15 minutes withdraw approximately 15 ml blood by cardiac puncture (animals were anesthesized with sodium phenobarbital) using two vaccum blood collection tubes[1] containing CPD solution as an anticoagulant.
3. Centrifuge[18] 3 ml of the above blood sample for 5 minutes at 2,000 rpm.
4. Remove the supernatant by use of a syringe and discard this fraction.
5. Add 1.5 µCi sodium pertechnetate Tc-99m contained in 1 ml normal saline to the separated red blood cells, mix gently and allow to stand for 10 minutes at room temperature.
6. Add 3 ml normal saline to the above mixture, mix gently and centrifuge[18] for 5 minutes at 2,000 rpm.
7. Remove the supernatant by use of a syringe and transfer to another tube.
8. Add sufficient water to each tube (i.e., one containing labeled red blood cells and the other containing supernatant liquid) to give the same volume.
9. Count the activity in each of the two tubes using a NaI(Tl) well-type scintillation counter.[19]
10. Calculate the percentage of labeled red blood cells

using the following equation:

$$\% \text{ Labeled RBCs} = \frac{100\ A_{RBC}}{A_{RBC} + A_S}$$

where:

A_{RBC} = RBC Activity

A_S = Supernatant Activity

Injection of Inorganic Ion Before Stannous Ion

Solutions of sodium chromate, selenious acid, ferrous sulfate and sodium chloride were used in separate labeling experiments. The sodiuim chloride solution served as the control. Labeling was conducted using a modification of the general procedure. The modification involved changing step 1 to the following:

Inject 0.1 ml of the appropriate test solution into the tail vein. After 15 minutes inject into the tail vein 0.1 ml stannous chloride solution containing 2 µg stannous ion.

Injection of Inorganic Ion After Stannous Ion

Solutions of sodium chromate, selenious acid, and sodium chloride were used in separate experiments. The sodium chloride solution served as the control. Labeling was conducted using a modification of the general

procedure. The modification involved changing step 1 to the following:

Inject 0.1 ml stannous chloride solution containing 2 µg stannous ion into the tail vein. After 15 minutes inject into the tail vein 0.1 ml of the appropriate test solution.

Injection of Digoxin Before Stannous Ion

The experiment was conducted using a modification of the general procedure. The modification involves changing step 1 to the following:

Inject 0.1 ml digoxin solution into the tail vein. After 15 minutes inject into the tail vein 0.1 ml stannous chloride solution containing 2 µg stannous ion.

Injection of Dimercaptosuccinic Acid After Stannous Ion

Solution of dimercaptosuccinic acid and sodium chloride were used in separate experiments. The sodium chloride solution served as the control. Labeling was conducted using a modification of the general procedure. The modification involved changing step 1 and 2 to the following:

1. Inject 0.1 ml stannous chloride solution containing 2 µg stannous ion into the tail vein. After 2 hours inject 0.5 ml of the appropriate test solution into the tail vein.

2. After 24 hours withdraw approximately 15 ml blood by cardiac puncture using two 10-ml vacutainer tubes containing CPD solution as an anticoagulant.

ENDNOTES

1 . Vaccum blood collection tubes, Terumo Medical Corporation, Elkton, MD.

2. Heparin sodium injection USP, Ricker Laboratories In.

3. Propranolol hydrochloride, 1mg/ml injection, Ayerst Laboratories, New York, NY.

4. Atenolol, 50mg tablet, Stuart Pharmaceuticals, Wilmington, DE.

5. Metoprolol tatarate, 1mg/ml injection, Geigy Pharmaceuticals, Ardsley, NY.

6. Pindolol, 5 mg tablet, Sandoz Inc., East Hanover, NJ.

7. Timolol maleate, 5mg tablet, Merck Sharp & Dohme, West Point, PA.

8. Verapamil hydrochloride, 2.5mg/ml injection, Knoll Pharmaceutical Co., Whippany, NJ.

9. Nifedipine, 10mg capsule, Pfizer Laboratories, New York, NY.

10. Diltiazem hydrochloride, 30mg tablet, Marion Laboratories Inc., Kansas city, MO.

11. Digoxin, 250 g/ml injection, Burroughs Wellcome Co., Research Triangle Park, NC.

12. Prazosin hydrochloride, 1mg capsule, Pfizer Laboratories, New York, NY.

13. Captopril, 25mg tablet, Squibb, Princeton, NJ.

14. Quinidine gluconate, 80mg injection, Eli Lilly, Indianapolis, IN.

15. Lidocaine hydrochloride, 10mg/ml injection, Astra Pharmaceuticals, Westboro, MA.

16. Ascorbic acid, 50mg/ml injection, Eli Lilly, Indianapolis, IN.

17. Tetracycline hydrochloride, powder, Squibb, Princeton, NJ.

18. Model TJ-6 Centrifuge, Model TJ-R Refrigeration unit, Beckman Instruments, Palo Alto, CA.

19. NaI(Tl) well-type scintillation counter, modular components were used. Harshaw NaI(Tl) detector. The Harshaw Chemical Co. Division of Kewanee Oil Co.

CHAPTER III

RESULTS AND DISCUSSION

Anticoagulant

The effect of the selected anticoagulant systems on Tc-99m RBC labeling yields is presented in Table 3.1. Citrate phosphate dextrose (CPD) resulted in the highest labeling yield followed by acid citrate dextrose (ACD) and then by heparin. No attempt was made to vary the concentration of either ACD or heparin in order to achieve higher labeling yields. Based on the results of this experiment, CPD was used as the anticoagulant for further labeling experiments.

The effect of different anticoagulants on RBC labeling with Tc-99m has received limited attention in the literature. Only ACD and heparin have been used as anticoagulants in labeling procedures (2, 3, 26, 70). A controversy appears to exist as to which agent is most desired for RBC labeling procedures. Weinstein (81) reported that heparin gave consistently higher labeling yields than obtained with ACD. The labeling yields were not given in this report. Porter et al., (62) compared ACD to heparin in the preparation of *in vivo*/*in vitro* Tc-99m labeled red blood cells. They found that the average binding efficiency was 93.47 percent with ACD and

Table 3.1 Effect of Anticoagulant on Tc-99m RBC Labeling Yields.

Anticoagulant	Labeling Yield, %[a]
Citrate Phosphate Dextrose	99.25 ± 0.65
Acid Citrate Dextrose	94.38 ± 1.50
Heparin	88.36 ± 2.10

[a] Mean ± s.d. for three determinations.

87.23 percent with heparin.

The labeling procedure used in the present research is very similar to that reported by Smith and Richard (70). They used 50 units of sodium heparin and 5 mCi of sodium pertechnetate Tc-99m per 3 ml of whole blood. A 97 percent labeling yield was obtained. The present research used 45 units of sodium heparin and 1.5 μCi of sodium pertechnetate Tc-99m. The labeling yield was 88 percent. The high labeling yield of 97 percent may be due to the use of a greater quantity of Tc-99m. It has been reported that heparin forms a complex with technetium when it is reacted with sodium pertechnetate in the presence of stannous ion (40). Thus, for a given quantity of heparin, the larger the quantity of Tc-99m used the smaller the percent of loss of activity due to interaction with heparin.

Stannous Ion Concentration

The effect of various stannous ion concentrations on Tc-99m RBC labeling yields is presented in Table 3.2 and Figure 3.1. Using stannous ion concentrations that ranged from 0.125 to 8.0 μg per 3 ml of whole blood, it was found that a maximum labeling yield of a 99.25 percent was obtained using 0.5 μg Sn(II) per 3 ml of whole blood. Labeling yields obtained using 0.25, 0.5 and 1.0 μg Sn (II) per 3 ml whole blood were not significantly different as determined by a t test.

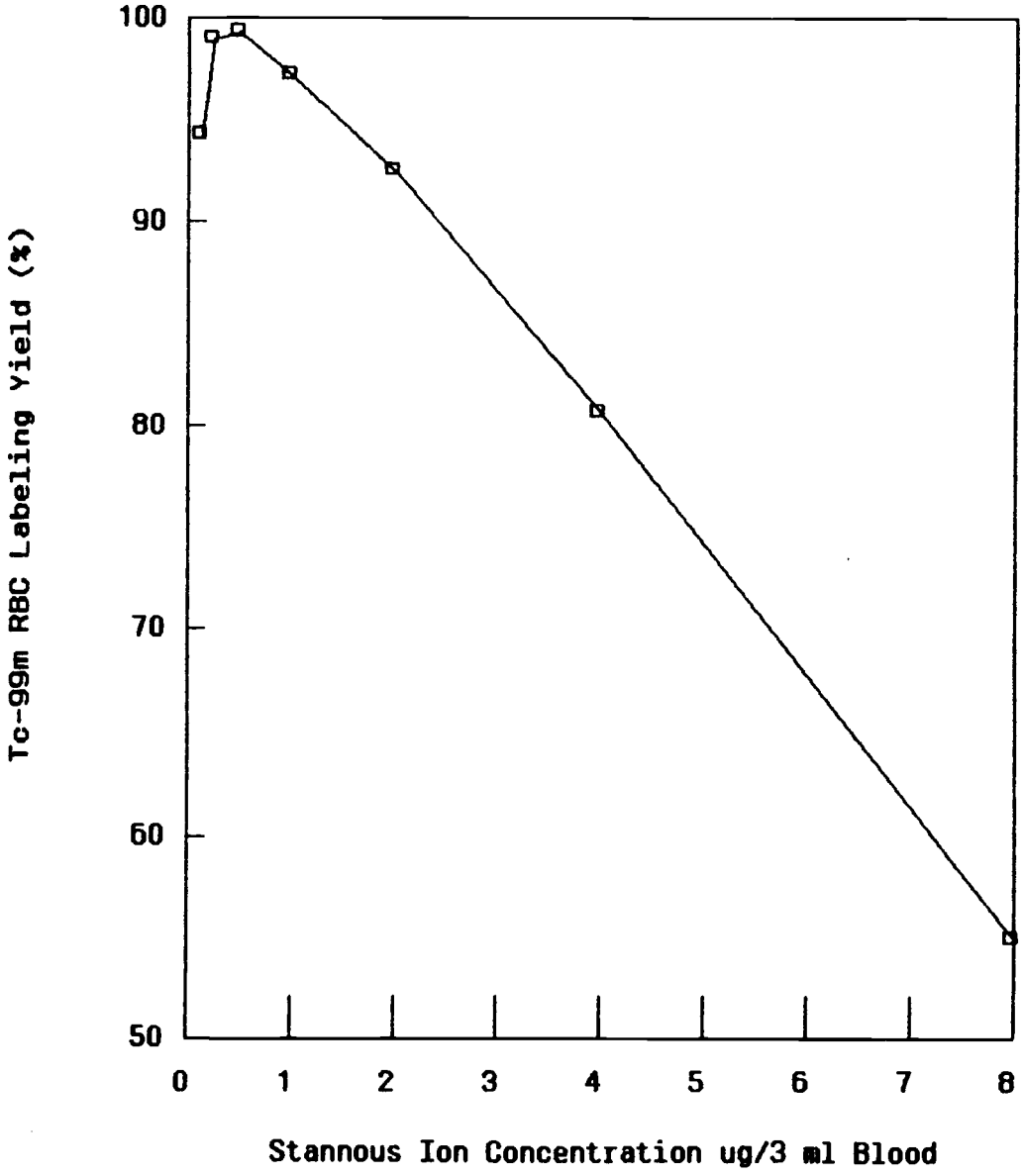
Table 3.2 Effect of Stannous Ion Concentration on Tc-99m RBC Labeling Yields.

Stannous Ion Concentration, µg/3 ml Blood	Labeling Yield,[b] %	t Value[a]
0.125	94.32 ± 1.48	
0.250	99.00 ± 0.78	0.603
0.500	99.25 ± 0.65	2.200
1.000	97.15 ± 0.93	
2.000	92.48 ± 1.5	
4.000	80.53 ± 1.25	
8.000	54.88 ± 1.6	

[a] $t\ (\alpha/2,\ n_1 + n_2 - 2) = t_{0.05,4} = 4.604$

[b] Mean ± s.d. for three determinations.

Figure 3. 1 Effect of Stannous Ion Concentration on Tc-99m RBC Labeling Yields.



Based on these results, the middle value of 0.5 µg Sn (II) per 3 ml of whole blood which represents was used in all further labeling experiments

Berger and Johannsen (4) studied the labeling yields of Tc-99m labeled red blood cells as a function of stannous ion concentration. They reported maximum labeling yields of 85-95 percent at 0.5 µg stannous ion per 4 ml of whole blood. These results were confirmed by Schwartz and Kruger (69).

Smith and Richards (70) obtained a labeling yield of 97 percent using 0.5 to 1.0 µg stannous ion per 3 ml whole blood. Mock and Wellman (45) reported that the optimal stannous ion concentration was 2 µg per 2 ml whole blood which gave 98 percent labeling yield. They removed excess extracellular stannous ion by washing the red blood cells with a 5 percent edetic acid solution before incubation with Tc-99m. Nouel and Brunelle (47) also used a large amount of stannous ion and then removed excess extracellular stannous ion with edetic acid. They reported a maximum labeling yield of 97 percent. The washing step with edetic acid may explain why a large quantity of stannous ion gives high labeling yields.

The curve given in Figure 3.1 for stannous ion concentration versus labeling yields of RBC with Tc-99m is similar to that obtained by Smith and Richards (70) and Billinghurst et al. (7). Several workers (26, 35, 69, 70) have shown that red blood cells have limited

capacity to take up stannous ion. Smith and Richards (70) stated that stannous ion remaining in the plasma will reduce pertechnetate before it can cross the red blood cell membrane. Reduced technetium can not cross the red blood cell membrane and in turn will reduce labeling yields.

Stannous Ion Compound

The effect of various stannous ion compounds on Tc-99m RBC labeling yields is presented in Table 3.3. Four of these stannous ion compounds gave similar high labeling yields. However, stannous dimercaptosuccinate gave a low labeling yield of 38.32 percent. These results suggest that the stannous compounds of pyrophosphate, glucoheptonate, and etidronate are dissociated to the same quantity of free stannous ion as stannous chloride. It may be expected that stannous dimercaptosuccinate would give a lower quantity of free stannous ion than the other stannous compounds because of the strong chelating property of dimercaptosuccinic acid. Stannous chloride was selected for use in all further labeling experiments. This was based on the above results, as well as its popular use in labeling red blood cells with Tc-99m.

In vitro labeling procedures have commonly involved the use of stannous chloride (17, 18, 38, 69). Other stannous compounds reported to be useful include stannous

Table 3.3 Effect of Stannous Compounds on Tc-99m RBC Labeling Yields.

Stannous Compund	Labeling Yield, %[a]
Stannous Chloride	99.25 ± 0.65
Stannous Pyrophosphate	98.73 ± 0.60
Stannous Glucoheptonate	98.13 ± 0.78
Stannous Etidronate	98.01 ± 0.55
Stannous Dimercaptosuccinate	38.32 ± 2.3

[a] Mean ± s.d. for three determinations.

citrate (66), stannous glucoheptonate (26), and stannous pyrophophate (7). None of these agents have been studied on a comparative basis for their effect on in vitro labeling efficiency. Some attention has been directed to the effect of various stannous compounds on in vivo labeling of red blood cells. For example, Hamilton et al. (27) studied the effect of stannous pyrophosphate, stannous diphosphonate, and stannous tartarate. They reported that these stannous compounds gave no significant difference in RBC labeling efficiency.

Incubation Time

Whole blood was incubated with stannous ion over the time period ranging from 5 to 120 minutes. The effect of the incubation time period on Tc-99m RBC labeling yields is presented in Table 3.4. No discernable difference in in the labeling yields were found for the studied incubation range. As 5 minutes incubation time is more convenient than using longer time peroids, all further labeling experiments involved incubating whole blood with stannous ion for 5 minutes.

Early workers (17, 69, 7) used incubation times varying from 15 to 30 minutes. They did not investigate the effect on labeling yields with various incubation times. Simth and Richards (70) reported that labeling yields of 97 percent occured with an incubation time of 5 minutes. Mock and Wellman (45) studied the effect of

Table 3.4 Effect of Incubation Time on Tc-99m on RBC Labeling Yields.

Time, minutes	Labeling Yield, %[a]
5	99.25 ± 0.65
15	99.23 ± 0.78
30	99.20 ± 0.57
60	99.18 ± 0.68
120	99.10 ± 1.04

[a] Mean ± s.d. for three determinations.

incubation time on RBC labeling yields over the range of 1 to 10 minutes. From their results, they recommended a 5 minutes incubation time for obtaining maximum labeling yields of 98 percent. The results obtained in these two investigations are similar to those obtained in this study.

Incubation Temperature

Whole blood was incubated with stannous ion at 22° C (room temperature) and 37° C. The effect of these incubation temperatures on Tc-99m RBC labeling yields is presented in Table 3.5. No discernable differences in the RBC labeling yields were obtained for these two different temperatures. As room temperature is more convenient than using an elevated temperature, all further labeling experiments used room temperature.

Early labeling methods (7, 17, 35, 38) used an incubation temperature of 37°C. No basis was given for the selection of this temperature. Later, other investigators reported that incubation at room temperature gave optimal labeling yields, while incubation at 37°C did not improve the labeling yields (45, 69, 70).

Red Blood Cells Washed Before Incubation with Stannous Ion

The effect of washing red blood cells before

Table 3.5 Effect of Incubation Temperature on Tc-99m RBC Labeling Yields.

Temperature	Labeling Yield, %[a]
22 °C	99.25 ± 0.65
37 °C	99.15 ± 0.89

[a] Mean ± s.d. for three determinations.

incubation with stannous ion is presented in Table 3.6. It may be seen that the labeling yield was not affected by separating and washing the red blood cells. All further labeling experiments were conducted using whole blood without a washing step.

General Labeling Procedure

A general labeling procedure was developed by selecting the optimal conditions as found in the above results. This procedure consistently gave a 99 percent labeling yield. The optimal conditions selected were: 1) the anticoagulant was CPD, 2) the stannous ion concentration was 0.5 µg per 3 ml whole blood, 3) the incubation time with stannous ion was 5 minutes, and 4) the incubation was conducted at room temperature. The general labeling procedure was used in all further labeling experiments.

Inorganic Ions

The effect of various inorganic ions on Tc-99m RBC labeing yields is presented in Table 3.7. Little or no effect on labeling yields, whether added to blood either before (post-tinning) or after the addition of stannous ion (pretinning), was obtained with the following: Al^{2+}, AsO_4^{3-}, Ca^{2+}, Cd^{2+}, Ce^{4+}, Co^{2+}, Cr^{3+}, Li^{+}, Mn^{2+}, AsO_2^{-}, MoO_4^{2-}, Mg^{2+}, Pb^{2+}, and Zn^{2+}. These results suggest that the yields of Tc-99m labeled red blood cells would

Table 3.6 Effect of Washing RBCs Before Incubation with Stannous Ion on Tc-99m RBC Labeling Yields.

Procedure	Labeling Yield, %[a]
Washed RBCs	99.12 ± 0.73
Non-washed RBCs	99.25 ± 0.65

[a] Mean ± s.d for three determinations.

Table 3.7 Effect of Inorganic Ions on Tc-99m RBC Labeling Yields.

Inorganic Ion	Concentration, µg/3 ml blood	Labeling Yield, %[a] Post-tinning	Pretinning
Al^{3+}	1.14	98.23 ± 1.3	97.42 ± 1.5
AsO_2^-	0.60	97.31 ± 1.2	97.60 ± 1.6
AsO_4^{3-}	0.60	97.65 ± 1.5	98.23 ± 1.4
Ca^{2+}	624	97.01 ± 1.5	97.37 ± 1.8
Cd^{2+}	0.50	97.26 ± 2.5	97.63 ± 2.1
Ce^{4+}	0.50	98.34 ± 0.9	98.65 ± 0.7
Co^{2+}	0.001	99.23 ± 0.42	99.18 ± 0.5
Cr^{3+}	0.5	98.55 ± 1.1	98.18 ± 1.4
Li^{+}	0.132	97.59 ± 2.1	98.60 ± 1.2
Mn^{2+}	0.05	99.24 ± 0.52	99.08 ± 0.6
Mg^{2+}	3172	97.92 ± 1.8	97.82 ± 1.6
MoO_4^{2-}	0.31	96.60 ± 1.3	96.39 ± 1.7
Pb^{2+}	1.11	96.68 ± 2.1	98.89 ± 1.5
Zn^{2+}	0.048	96.84 ± 3.0	97.66 ± 2.8
Fe^{3+}	1.68×10^3	65.73 ± 3.1	98.14 ± 1.7
Fe^{2+}	1.68×10^3	62.62 ± 2.6	98.24 ± 1.6
Cu^{2+}	3.18	48.11 ± 2.6	97.37 ± 1.8
Sn^{4+}	0.5	93.49 ± 1.2	96.48 ± 1.4
CrO_4^{2-}	0.5	40.85 ± 2.1	42.19 ± 1.8
SeO_3^{2-}	8.25	45.98 ± 2.2	46.08 ± 1.9
Control	0	99.12 ± 0.4	99.12 ± 0.4

[a] Mean ± s.d. for three determinations.

not be reduced because of high blood levels of these inorganic ions in patients. Also, these results suggest that these inorganic ions are not involved in the labeling mechanism.

The addition of ferric, ferrous, cupric and stannic ions to blood before the addition of stannous ion (post-tinning) resulted in reduced labeling yields. These inorganic ions had no effect when they were added to blood containing stannous ion (pretinning). In vivo results with ferrous ion were similar to the in vitro results (Table 3.8). Discussion of the individual results for these ions follows.

The addition of ferric ion to blood before the addition of stannous ion (post-tinning) decreased the RBC labeling yield to 65.73 percent. This reduced labeling yield may be due to an oxidation-reduction reaction occuring between ferric ion and stannous ion and/or interference with the transport of stannous ion through the red blood cell membrane. The oxidation-reduction reaction is most likely to occur because the oxidation potential of ferric/ferrous is greater than that of stannic/stannous. Respectively, these values are 0.77 V and 0.07 V (28). The addition of ferric ion to blood after stannous ion (pretinning) had no effect on RBC labeling yield. In this situation the stannous ion has entered the red blood cell before any interfering action may occur due to the presence of ferric ion. These

Table 3.8 Effect of Inorganic Ions on In Vivo Tc-99m RBC Labeling Yields.

Agent	Dose Inorganic Ion Conc.	Labeling Yield, %[a] Post-tinning	Pretinning
CrO_4^{2-}	2.67 µg	42.91 ± 3.1	45.36 ± 2.22
SeO_3^{2-}	44 µg	54.73 ± 4.2	52.28 ± 2.10
Fe^{2+}	8.96 mg	62.80 ± 6.07	-----[b]
Control	0	96.49 ± 1.5	96.49 ± 1.5

[a] Mean ± s.d. for three determinations.

[b] This procedure was not carried out.

results suggest that ferric ion interferes with the labeling mechanism due to an extracellular interaction.

The addition of ferrous ion to blood before stannous ion (post-tinning) reduced the labeling yield to 62.62 percent. Possible reasons for reduced labeling yield include interference of stannous ion uptake by red blood cells and/or an oxidation-reduction reaction occuring between ferrous ion and pertechnetate ion. Ferrous ion with an oxidation potential of 0.44 V (28), has been used as a reducing agent in the preparation of technetium-99m labeled compounds. Thus, in the present research, ferrous ions may reduce pertechnetate ions before they have the opportunity to enter the red blood cell. The addition of ferrous ion to blood after stannous ion (pretinning) had no effect on the labeling yield. Such results suggest that the interference of ferrous ion with the labeling mechanism is probably due to an extracellular interaction.

The addition of cupric ion to blood before the addition of stannous ion (post-tinning) decreased Tc-99m RBC labeling yield to 48.11 percent. Possible reasons for this reduced labeling yield include an oxidation-reaction between cupric and stannous ions and/or an interference with the transport of stannous ion through the red blood cell membrane. The oxidation-reduction reaction is most likely to occur because the oxidation potential of cupric/cuprous is greater than that of

stannic/stannous. Respectively, these values are 0.34 V and 0.07 V (28). The addition of cupric ion to blood after the addition of stannous ion (pretinning) had no effect on RBC labeling yields. This is probably because stannous ion passes through the red blood cell before any possible interference due to the presence of cupric ion. These results suggest that cupric ion interferes extracellularly with the labeling mechanism.

The above results with ferric, ferrous and cupric ions may be indicative of a potential reduced labeling yields of technetium-99m labeled red blood cells in patients having high blood levels of such ions.

The addition of a relatively large quantity of stannic ion to blood before stannous ion (post-tinning) reduced the Tc-99m RBC labeling yield from 99.25 percent to 93.49 percent. Thus, it appears that stannic ion has a minor role in the labeling mechanism. Further, it appears that the presence of stannic ion as an impurity in stannous products will not have a significant effect on Tc-99m RBC labeling yields. The stannic ion is a common impurity in stannous compounds.

The addition of chromate and selenite ions to blood either before (post-tinning) or after (pretinning) addition of stannous ion significantly decreased the Tc-99m RBC labeling yields. The *in vivo* results with chromate and selenite ions were similar to those obtained *in vitro* (Table 3.8). Individual results with these two

ions are discussed below.

The reduced labeling yields with chromate ions may possibly occur due to 1) an oxidation-reduction reaction between chromate and stannous ions, 2) an interference in the transport of stannous ion and/or pertechnetate ion through the red blood cell membrane, and 3) a competition of chromate ion with stannous ion and/or pertechentate ion for red blood cell binding sites. The oxidation potentials of chromate ion (0.12 V) and stannous ion (0.07 V) (28) suggest that an oxidation-reduction reaction is unlikely to occur between these two ions. Holt et al., (34) reported that the presence of stannous pyrophosphate in blood inhibited Cr-51 RBC labeling. They found that washing red blood cells after the addition of stannous pyrophosphate did not inhibit RBC labeling. This suggested that extracellular stannous pyrophosphate interacted with sodium chromate. They indicated that this interaction probably involved complexation between chromium and stannous pyrophosphate.

Since chromate ion reduced the labeling yields in the post- and pretinning procedures, the interference in the labeling is most likely due to an intracellular effect. It is known that this ion crosses the red blood cell membrane (24). The intracellular effect may be related to the inhibition of glutathione reductase enzyme as chromate ion has been reported to inhibit

glutathione reductase in erythrocytes (39).

The reduced labeling yields with selenite ion in post-and pretinning procedures may be due to 1) an interference with the transport of stannous ion and/or pertechnetate ion, 2) an interference with the reduction of pertechnetate ion with stannous ion within red blood cells, and 3) a competition of selenite ion with stannous ion and/or pertecchnetate ion for binding sites in the red blood cells. The reduced labeling yields with selenite ion in both post-and pretinning procedures suggest that selenite ion crosses the red blood cell membrane and interferes with the RBC labeling mechanism through an intracellular interaction. It has been reported that a correlation exists between increased selenium concentration and increased glutathione peroxidase activity in whole blood (76). Thus, the labeling mechanism may involve glutathione peroxidase enzyme.

Therapeutic Agents

The effect of various therapeutic agents on Tc-99m RBC labeling yields is presented in Table 3.9. Little or no effect on labeling yields was obtained with any of these agents. The *in vivo* results with digoxin were similar to the *in vitro* results (Table 3.10).

The results obtained with digoxin and prazosin in this investigation are in contrast with an *in vivo* study

Table 3.9 Effect of Therapeutic Agents on Tc-99m RBC Labeling Yields.

Agent	Concentration, μg/3 ml Blood	Labeling Yield, %[a]
Propranolol	0.3	98.68 ± 1.2
Atenolol	1.5	98.51 ± 1.9
Metoprolol	0.3	98.77 ± 0.9
Pindolol	0.3	98.68 ± 1.3
Timolol	30	98.70 ± 0.89
Verapamil	1.2	98.30 ± 1.5
Nifedipine	1.2	98.25 ± 1.8
Diltiazem	0.6	98.67 ± 1.3
Quinidine	24	97.98 ± 2.0
Lidocaine	15	98.67 ± 1.6
Digoxin	0.006	98.48 ± 1.45
Prazosin	0.225	97.73 ± 1.6
Captopril	0.225	98.63 ± 2.1
Ascorbic Acid	60	98.29 ± 0.9
Tetracycline	15	98.51 ± 1.3
Control	0	99.20 ± 0.5

[a] Mean ± s.d. for three determinations.

Table 3.10 Effect of Digoxin on In Vivo Tc-99m RBC Labeling Yields.

Agent	Dose	labeling Yield, %[a]
Digoxin	2.4 μg	96.97 ± 4.2
Control	0	96.49 ± 1.5

[a] Mean ± s.d. for three determinations.

reported by Lee et al., (41). They found that both digoxin and prazosin in rats caused a significant reduction in the labeling of RBC with Tc-99m. Digoxin was administered in a relatively high dose of 150 - 600 µg/Kg to give a 79.7 percent labeling yield. This dose of digoxin is about 3 to 15 times greater than the normal high dose of digitalization. In the present research, digoxin was administered 12 µg/Kg intravenously. Prazosin in the above reported study when administered orally in 0.2 - 0.6 mg dose to 180-200 g rats gave a labeling yield of 83.8 percent. In the present investigation, prazosin was not studied in vivo. However, when this agent was used in the in vitro labeling procedure at 0.225 µg per 3 ml whole blood it appeared to have little effect on the labeling yield.

Biochemical Substances

The effect of selected biochemical substances on RBC labeling with Tc-99m is presented in Tables 3.11 and 3.12. Labeling yields decreased from 99.25 to 77.86 percent in blood that was stored for two weeks at refrigerated temperature. Cysteine and glucose had no discernable effect on Tc-99m RBC labeling yields of either fresh or two weeks old blood. This result suggests that labeling interference due to high glucose blood levels in patients would probably not occur. Also, these results suggest that glucose and cysteine are not

Table 3.11 Effect of Biochemical Substances on Tc-99m RBC Labeling Yields.

Agent	Concentration, mg/3 ml Blood	Labeling Yield, %[a] Fresh Blood	2 Weeks Stored Blood
Glucose	4.86	98.54 ± 0.7	77.35 ± 1.0
Cysteine	0.06	98.49 ± 1.2	78.34 ± 2.4
Glutathione	2.124	98.37 ± 0.8	93.18 ± 1.4
Control	0	99.25 ± 0.6	77.86 ± 2.3

[a] Mean ± s.d. for three determinations.

Table 3.12 Effect of Inosine on Tc-99m RBC Labeling Yields After Two Weeks Storage.

Agent	Concentration, mg/3 ml Blood	Labeling Yield, %[a] Fresh Blood	Stored Blood
Inosine	15	86.20 ± 2.3	89.76 ± 2.1
Control	0	77.86 ± 2.3	77.86 ± 2.3

[a] Mean ± s.d. for three determinations.

useful as blood preservatives for labeling red blood cells of stored blood with Tc-99m. Further, it appears that these agents are not involved in the labeling mechanism.

Glutathione was found to have no effect on Tc-99m RBC labeling yields of fresh blood but increased the labeling yield of two weeks old blood. Also, inosine significantly increased red blood cell labeling yields when it was added either before or after storage.

The above results with glutathione and inosine suggest that reduced labeling yields of stored blood may be due to an alteration of enzymes and/or the red blood cell membrane. From these results it appears that glutathione and inosine may be useful as preservatives in storing blood for labeling at later time.

Chelating Agents

The effect of various chelating agents on Tc-99m RBC labeling yield is presented in Table 3.13. The addition of chelating agents to blood either before or after the addition of stannous ion significantly decreased RBC labeling yields.

The reduced labeling yield in the post-tinning procedures may be due to chelation of stannous ions by the chelating agent. This would reduce the amount of free stannous ion available for intracellular reduction

Table 3.13 Effect of Chelating Agents on Tc-99m RBC Yields.

Agent	Concentration, mg/3 ml Blood	Labeling Yield, %[a] Post-tinning	Pretinning
DMSA	0.187	28.6 ± 2.3	51.3 ± 1.8
BAL	0.187	38.3 ± 4.1	38.4 ± 3.7
Edetic Acid	1.875	47.9 ± 2.6	89.4 ± 1.7
Control	0	99.2 ± 0.6	99.2 ± 0.6

[a] Mean ± s.d. for three determinations.

The reduced labeling yields in pretinning may be due to an alteration of red blood cell membrane by the chelating agent. Such alteration may result in reduced uptake of technetium ions. It has been reported that edetic acid causes a marked change in red blood cell shape and the elasticity of the membrane (49, 50). Another basis for this reduction in labeling yields may be that these complexing agents alter the intracellular stannous ion concentration by competing in an equilibrium reaction.

The *in vivo* results obtained with DMSA were different from the *in vitro* results (Table 3.14). It was found that DMSA increased the RBC labeling yields as compared to the control. The increased yield was possibly due to DMSA reacting with stannous ion in the blood to form a chelate. Such chelate formation could result in a higher blood level of stannous ion after 24 hours than might otherwise occur. It has been reported by Dewanjee and Wahner (11) that stannous as Sn-DMSA has a slower blood clearance than the free ion after 24 hours.

Oxidizing Agents

From the results presented in Table 3.15, it may be seen that neither hydrogen peroxide or permanganate, whether added before (post-tinning), or after the addition of stannous ion (pretinning), affected the

Table 3.14 Effect of DMSA on In Vivo Tc-99m RBC Labeling Yields.

Agent	Dose	Labeling Yield, %[a]
DMSA	0.5 mg	90.13 ± 1.2
Control	0	76.38 ± 1.5

[a] Mean ± s.d. for three determinations.

Table 3.15 Effect of Oxidizing Agents on Tc-99m RBC Labeling Yields.

Agent	Conentration, µg/3 ml Blood	Labeling Yield, %[a] Post-tinning	Pretinning	Pre-mixed with Sn^{2+}
H_2O_2	3	98.1 ± 0.7	98.9 ± 0.8	35.9 ± 1.4
MnO_4^-	0.5	98.6 ± 0.6	97.1 ± 0.8	37.8 ± 2.0
Control	0	99.2 ± 0.6	99.2 ± 0.6	99.2 ± 0.6

[a] Mean ± s.d. for three determinations.

labeling yields. The result obtained by the addition of hydrogen peroxide to blood after stannous ion was similar to that obtained by Smith and Richards (70).

The effect of these oxidizing agents is probably limited to reactions that occur extracellularly. When they were added to the blood before addition of stannous ion (post-tinning), they undoubtedly interacted with the various components, such as proteins and enzymes, that are present in whole blood. Thus, no further reaction would occur on addition of stannous ions. The results of the pretinning procedure suggest that all of the stannous ion has entered the red blood cell. This is desirable, as extracellular stannous ion will react with pertechnetate ions to cause extracellular labeling. The Tc-99m in this type of label is very loosely held to the red blood cell and when injected into patients it becomes separated from the red blood cell.

Enzyme Poison

The effect of potassium cyanide on Tc-99m RBC labeling yield is presented in Table 3.16. From the results, it may be seen that potassium cyanide at a high but not lethal concentration, had no effect on labeling yields for either post- or pretinning procedures. When cyanide ion was added at a 10 fold increased concentration, the labeling yield was greatly decreased for both types of procedures. This suggest that an

Table 3.16 Effect of Cyanide on Tc-99m RBC Labeling Yields.

Agent	Concentration, µg/3 ml Blood[b]	Labeling Yield, %[a] Post-tinning	Pretinning
KCN	18.75	98.70 ± 0.8	98.61 ± 1.1
KCN	187.5	22.26 ± 3.6	22.84 ± 2.7
Control	0	99.30 ± 0.7	99.30 ± 0.7

[a] Mean ± s.d. for three determinations.

[b] Cyanide concentration.

enzyme system may be involved in the labeling mechanism.

Methemoglobinemia Agents

The effect of these agents on Tc-99m RBC labeling yield is presented in Table 3.17. It may be noted that the addition of sodium nitrite to blood either before (post-tinning) or after (pretinning) stannous ion had no significant effect on Tc-99m RBC labeling yields. The nitrite ion has both oxidizing and reducing properties, depending on the interacting materials. Apparently it does not cause any significant oxidation-reduction reactions with either stannous or pertechnetate ions. The oxidation potential of nitrite ion is 0.46 V (28). It may preferentially oxidizes other cellular components.

The addition of methylene blue to blood either before or after stannous ion significantly reduced the labeling yields. This agent has the ability to cross the red blood cells membrane. Methylene blue based on its electron acceptor property and ability to cross the red blood cell membrane finds in the treatment of methemoglobinemia. The results of this experiment suggest that methylene blue interferes in the labeling mechanism due to an intracellular reaction. The possible interfering reaction for methylene blue include 1) oxidation of stannous ion and 2) oxidation of an enzyme system involved in reducing pertechnetate ions to technetium ions. The first effect is most likely to

Table 3.17 Effect of Methemoglobinemia Agents on Tc-99m RBC Labeling Yields.

Agent	Concentration, µg/3 ml Blood	Labeling Yield, %[a] Post-tinning	Pretinning
Sodium Nitrite	500[b]	98.49 ± 1.2	98.96 ± 0.7
Methylene Blue	35.3	40.25 ± 2.3	65.60 ± 1.4
Control	0	99.35 ± 0.6	99.35 ± 0.6

[a] Mean ± s.d. for three determinations.

[b] Nitrite concentration.

occur because the oxidation potential of methylene blue is greater than that of stannous. Respectively, these values are 0.5 V and 0.07 V (28). Should the latter effect occur, the role of stannous ion in the labeling procedure would be to form reduced enzyme. From these results it appears that methylene blue may be useful to reduce or prevent undesired *in vivo* Tc-99m RBC labeling.

CHAPTER IV

SUMMARY AND CONCLUSION

Technetium-99m labeled red blood cells (RBCs) are widely used in blood pool imaging, detection of vascular malformation, red blood cell mass determination, and detection of gastrointestinal bleeding.

Technetium-99m labeled red blood cells was first reported in 1967. This procedure did not utilize a reducing agent and gave labeling yields of less than 10 percent. Others found that high labeling yields were obtained by using a strong reducing agent. Labeling yield was further improved through the optimization of stannous ion concentration.

The mechanism involved in the labeling of Tc-99m to red blood cells has received limited attention. It is postulated that pertechnetate ion reversibly crosses the red blood cell membrane and in the presence of stannous ion an oxidation-reduction reaction occurs. The reduced Tc-99m becomes bound to the beta-chain of hemoglobin.

Two major problems are associated with the labeling of technetium-99m to red blood cells. These are 1) reduced labeling yields due to interactions, and 2) undesired *in vivo* Tc-99m RBC labeling. Abnorml Tc-99m RBC labeling may occur after administration of sodium

pertechnetate Tc-99m to patients who have previously received a radiopharmaceutical containing stannous ion.

The goals of this research were to 1) identify interfering substances which may reduce the Tc-99m RBC labeling yields, 2) identify substances which may be useful to reduce or prevent undesired *in vivo* labeling, and 3) further the knowledge of the labeling mechanism.

An *in vitro* labeling procedure was developed based on optimal conditions of anticoagulant, stannous ion concentration, incubation time, and incubation temperature. This procedure was used to investigate the effects of various agents on Tc-99m RBC labeling yields. These agents consisted of inorganic ions, therapeutic agents, biochemicals, chelating agents, oxidizing agents, an enzyme poison and methemoglobinemia agents. The experiments for most of the agents involved both post- and pretinning procedures. For post-tinning, the selected agent was added to the blood sample before stannous ion. For pretinning, the agent was added to blood after the addition of stannous ion. In addition, a limited *in vivo* study in rats was conducted. For this study the following agents were investigated for their effect on RBC labeling yield: sodium chromate, selenious acid, ferrous sulfate, digoxin, and dimercaptosuccinic acid.

Twenty inorganic ions were investigated. It was found that the following ions did not alter labeling

yields in either post-tinning or pretinning procedures: aluminum, arsenite, arsenate, calcium, cadmium (II), cobalt (II), ceric, chromium (III), lithium, manganese (II), magnesium, molybdate, lead (II), and zinc. Reduction in labeling yields was found in post-tinning with ferric, ferrous, cupric, and stannic ions. These ions probably interfere with the labeling through an extracellular interaction. Chromate and selenite ions were found to reduce labeling yields in post- and pretinning procedures. These ions probably interfere with the labeling process due to an intracellular interaction. In vivo results with ferrous, chromate, and selenite ions were similar to those obtained in vitro.

Fifteen therapeutic agents were investigated. Included were beta-adrenergic blockers, calcium channel blockers, antihypertensive agents, quinidine, lidocaine, digoxin, ascorbic acid, and tetracycline. Little or no effect on Tc-99m RBC labeling yields was found with these agents. In vivo results with digoxin were similar to those obtained in vitro.

Labeling yield decreased from 99.25 to 77.86 percent for blood that was stored two weeks at refrigerated temperature. Neither glucose or cysteine altered the labeling yields of fresh or two weeks old blood. Both glutathione and inosine were found to increase labeling yields of stored blood. Because of this action, these

agents may be useful as preservatives for storing blood to be labeled at a later time.

Dimercaptosuccinic acid, dimercaprol, and edetic acid reduced labeling yields in both post- and pretinning procedures. DMSA increased the in vivo RBC labeling yields.

The oxidizing agents, hydrogen peroxide and potassium permanganate, were found to have no effect on labeling either in post- or pretinning procedures.

Cyanide ion did not alter the red blood cell labeling yield at a high non lethal value. When it was used at a ten fold increased concentration, reduction in yields ocuured for both post- and pretinning procedures.

Two methemoglobinemia agents were investigated. These were sodium nitrite and methylene blue. Sodium nitrite a methemoglobinemia forming agent, was found to have no effect on labeling yields in either post- or pretinning procedures. Methylene blue a therapeutic agent for reducing methemoglobin to hemoglobin, decreased the labeling yields in both post- and pretinning procedures.

It has been established in the literature that labeling of red blood cells with Tc-99m requires the entrance of pertechnetate ions into the red blood cells followed by its reduction to technetium ions. High labeling yields are obtained by using stannous ion, a strong reducing agent. This ion crosses the red blood

cell membrane and causes an intracellular reduction of pertechentate ions. It has not been determined whether stannous ions directly react with pertechentate ions to give the reduced product of technetium ions. This interaction could possibly involve the reduction of pertechentate ions by an enzyme system that has been activated by stannous ions.

Based on the results of this research, the reduction process of pertechnetate ions may possibly involve an enzyme system. Reduced labeling yields were obtained with chromate, selenite, methylene blue, and high concentration of cyanide ion. All of these agents appeared to interfere intracellularly in the labeling mechanism. Further, these agents have been reported to affect the reactivities of various enzyme systems. The following interactions may be noted: 1) chromate ion inhibits glutathione reductase enzyme, 2) selenite ion activates glutathione peroxidase enzyme, 3) methylene blue activates NADPH-dependent methemoglobin reductase enzyme, and 4) cyanide acts as an unselective enzyme poison.

Further support for an enzyme system being involved in the reduction of pertechnetate ions is that the labeling of aged red blood cells using inosine as a blood preservative resulted in increased labeling yield as compared to a control sample. It is possible that aging impairs an enzyme system that is involved in the labeling.

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