85186

BLOOD CELLS RADIOLABELING ACHIEVEMENTS, CHALLENGES AND PROSPECTS.

JOLIE WEININGER AND JACOB TRUMPER

SOREQ NRC, 70600-YAVNE, ISRAEL

BUENOS AIRES
REPUBLICA ARGENTINA
1987

BLOOD CELLS RADIOLABELING: ACHIEVEMENTS, CHALLENGES AND PROSPECTS

Jolie Weininger and Jacob Trumper, Soreq NRC, 70600-Yavne, ISRAEL

1. INTRODUCTION

The potential value of the use of radiolabeled cellular blood elements for noninvasive medical diagnosis has long been recognized. A radionuclide is useful for diagnostic nuclear medicine if it is a high intensity photon emitter with suitable energy for imaging , if it emits no or minimal particulate radiation and if its physical half-life is of the order of magnitude of duration of the study being performed.

Ideally, for blood cell labeling, one would like to have available a number of radiopharmaceuticals, each of wich could be administered to the patient either in vivo or, if this were not possible, after being mixed with patient's blood in vitro in order to label a specific cell fraction. One would like a radionuclidic label wich assures reduced radiation dose to the cells and possesses physical properties favorable for imaging. The labeling of the blood cells must be stable both in vitro and in vivo. The physical and biochemical properties, as well as the in vivo function and behavior of the labeled cells, must be preserved. The label, once incorporated into the cells, should neither be released during the study, nor reused. All the above mentioned conditions must be achieved by simple, fast and yet very reliable techniques in order for the imaging agent to be accepted.

Plasma is used as the labeling medium in order to preserve cell viability. Since the labeling yields were only 50-60% at first, it was necessary to separate the plasma-bound activity before injection. Together with the separation procedures come

problems of maintaining the sterility and apyrogenicity of the preparation, and assuring the viability and normal physiologic function of the labeled cells.

During the last ten years many of these problems have either been overcome or reduced in magnitude. A variety of different cell fractions can now be labeled with reasonable reliability and are widely used for imaging in specific disease states. These examinations have proved their usefulness in the more accurate diagnosis of various specific pathologic conditions.

It is known that many pathologic processes tend to be characterized by infiltration or aggregation of one or another cell type, which is relatively specific to the process. The ability to label a particular cell type which will seek out a diseased area of the body should greatly expand the diagnostic capabilities of nuclear medicine by giving it specificity, the lack of which has generally been its major limitation.

2. RED BLOOD CELL LABELING.

Of all the nuclear medicine procedures that have come into use over the years, none has had as significant an impact as the widespread use of technetium-99m labeled red blood cells ------ (^{99m}Tc-RBCs) as an imaging agent. ^{99m}Tc-RBCs have revolutionized the field of cardiovascular nuclear medicine, in particular, by making possible non-invasive evaluation of various heart ----- parameters. ^{99m}Tc-RBCs are also widely used as a blood pool imaging agent for the visualization of different vascular ------ abnormalities: vascular malformations, hemangiomas and G. I. -- bleeding. The mass and life span of red blood cells may also be determined with ^{99m}Tc -RBCs. Intentionally demaged ^{99m}Tc-RBCs permit the evaluation of splenic function in an optimal ------- manner.

The labeling of RBCs, compared with that of other blood --- cells, is facilitated by several factors such as:

a) RBCs are the most abundant of all cellular blood elements
b) They are relatively easy to separate and manipulate in vitro and are not especially susceptible to physical or chemical damage.
c) In vitro they are less dependent on energy and nutritional requirements than the other blood cells.
d) RBCc are relatively easy to label due to the presence of a variety of cellular transport mechanisms and of hemoglobin with its active metal binding sites.

Radionuclidic labelingsof blood cells can be divided into two main categories: cohort (or pulse labels) and random labels. Cohort labels bind to bone marrow precursors that appear after a few days as labeled cells of uniform age in the circulation. These labels are useful for the study of cell production rates and survival. Most of the RBC labeling procedures developed so far and those in predominant use today involve random labeling (i.e. labeling of circulating cells of all ages) and use ^{99m}Tc, ^{51}Cr, ^{111}In or ^{68}Ga, as radiolabels.

3. ^{99m}Tc-RED BLOOD CELL LABELING

A) In vitro Labeling Methods

After ^{99m}Tc was recognized as the "ideal" radioisotope and became the radioisotope of choice for nuclear medicine imaging, multiple efforts were begun to label RBCs with it.

The solution of technical problems involved in the improvement of labeling yields of ^{99m}Tc-RBCs was closely related to progress achieved in understanding Tc-chemistry and labeling mechanisms.

Very early it was recognized that ^{99m}Tc, as pertechnetate, moves in and out of the red cells almost freely and only reduced Tc species could bind irreversibly with the cell components.

Labeling methods which did not make use of reducing agents had, therefore, only minimal success. It was suggested that the pertechnetate could be reduced efficiently in the cell by pretreatment of the RBCs with a reducing agent This method gave

the first dependable results. Sn(II) compounds were among the first used to reduce pertechnetate and are still the most widely used reducing agents of all those available. Complexes like Sn-glucoheptonate (1), Sn-citrate (2), Sn-pyrophosphte (3), or Sn-DTPA were used to pretreat. or to "tin" the blood before labeling with ^{99m}TC - pertechnetate. Recently, by using --- radioactive stannous, it was shown that the plasma competes with the red cells for the available stannous-tin. The effect of the complexing agent on the percent distribution of Sn (II) in whole blood was reported (4). It was shown that, of the available stannous-tin, RBC-bound Sn (II) amounts there are about 50% for glucoheptonate, about 35% for citrate, about 4% for DTPA and about 1% for pyrophosphate. The rest, the extracellular Sn(II), is almost entirely plasma-bound.

The pretreatment of the whole blood with microgram amounts of stannous ion, as proposed by Smith and Richards (2) and confirmed later by Gutkowski et al. (1) and Weininger et al. (5) made possible the development of very simplified and reproducible high labeling yield procedures. Small samples of the patients blood are generally incubated for a few minutes with the -- Sn-complex. In different methods one or more consecutive washing steps follow. The blood is centrifuged and the plasma that contains tin not taken up by the RBC is removed before adding the ^{99m}Tc - pertechnetate, in order to assure constant high labeling efficiency.

Several kit and non-kit preparation procedures which consistently provide labeling yields of 95-98% have been published some 10 years ago (1,2,5).

The Brookhaven National Laboratory (BNL) procedure, later kit method, using Sn-citrate (2 µg tin) as reducing agent, is widely used clinically as well as in the study of mechanisms involved in in vitro labeling methods (2).

The removal of the plasma before the "tinning" step. proved - to be a very efficient way of improving the labeling ----- efficiency.

A Sn-glucoheptonate complex was used in two Soreq kit preparation procedures: a) using 3.12 μg Sn(II) for the pretreatment of 5 ml whole blood, and b) using 1,25μg Sn(II)for the pretreatment of previously separated packed red cells from 10 ml blood (5). Our experiments showed that of the various commonly used Sn(II) chelates, stannous glucoheptonate (Sn-GH) is transported more effectively into the cells and the uptake appears to be irreversible. This is the reason that in all our RBC in vitro labeling procedures Sn-glucoheptonate is always used as the reducing agent. Testing in humans showed that high intravascular activity and prolonged stability of the label in the blood pool were obtained.

Since only a small fraction of the tin in the kit is taken up by the RBCs and since extracellular Sn(II) in the plasma depresses the ^{99m}Tc labeling of the erythrocytes, the removal of non-cell-bound tin before the addition of pertechnetate improves the labeling. The labeling of preseparated red cells is more efficient, as only the remaining 3% plasma competes with the RBCs for the available Sn (II).

The Soreq procedure, by preseparated red cells are labeled, was chosen for clinical use in Israel, not only because of the easier preparation, but also because fo the significant reduction of the detrimental effects of Tc-carrier and other oxidizing agents possibly present in generator eluants (Table 1).

The effect on the labeling yields of the amounts of carrier Tc present in some generator eluants was studied and related to the reducing capacity of the very low amounts of available Sn(II). It was shown experimentally that labeling efficiencies of 95-98% can be obtained when the reducing agent contains about 1 μg Sn(II) and the ^{99m}Tc pertechnetate solution contains up to 10^{14} Tc atoms. We calculated that an eluate of 10 mCi ^{99m}Tc activity contains 10^{14} Tc atoms when the ingrowth period is about 50 hours (Fig. 1). In order not to exceed the reducing capacity of the available Sn (II), time controlled generator elutions must be performed.

In all the preparation methods several consecutive washings with saline solution and the removal of tin-plasma before labeling with pertechnetate, increases the standard labeling yield to almost 100%. Several centrifugations to obtain better separation between RBCs and plasma before incubation with the reducing agent, however do not significantly increase the subsequent labeling yield.

Srivastava and co-workers(6) recently developed a new in vitro technique, based on the BNL kit. This technique selectively labels RBCs in whole blood without outside handling of the blood sample and without centrifugations or washing steps. This method yields quantitative (>95%) labeling of red blood cells in 1 ml whole blood means of the following closed, one-vial system: To the vial containing the lyophilized stannous citrate mixture (50 µg tin) are added successively the heparinized blood and 5 min later 0.6 ml 1% sodium hypochlorite and 1 ml 4,4% disodium EDTA. Finally, the desired activity of ^{99m}Tc pertechnetate in 0,5 to 3 ml saline is added. After 15 min of incubation at room temperature a labeling yield of >95% is obtained. The addition of EDTA effectively reduces to a minimum the stannous tin content of plasma trapped between the packed red cells, thus assuring good labeling yields. It was reported that when samples of very low hematocrit blood were labeled without the use of EDTA, poor labeling yields were obtained due to the plasma effect. This new one-vial "whole-blood" method utilizes chemical inactivation (oxidation) of the extracellular, plasma-bound stannous tin, and thus obviates the need for cell separation prior to the addition of pertechnetate. Since the RBCs remain in their native plasma environment and there is almost no handling or manipulation, the RBCs do not suffer damage associated with the labeling procedure. Since there is no transfer involved, aseptic conditions are easy yo maintain.

It was shown experimentally, that when reducing agents containing 50 to 100 µg Sn (II) are used, the cellular tin uptake is measured in micrograms, i.e. about ten times higher than in

previous techniques. Thus, the drawback of limited stannous tin capacity for handling pertechnetate samples with high 99-Tc content, is virtually eliminated.

The Soreq optimized one-vial labeling method (7) for 2 ml whole blood uses as the reducing agent a lyophilized Sn-glucoheptonate complex with a formulated tin content of 50-65 μg Sn(II). This is the same formulation which has been widely used as a kidney and brain imaging agent and has been proved to have a shelf-life of at least one year. A very dilute sodium hypochlorite solution (0.26%) is added to oxidize the extracellular tin, thereby avoiding the plasma effect, without the use of EDTA. By adding to the RBCs the wanted ^{99m}Tc activity, in a volume not exceeding 0,5 ml, and incubating for 15 min, the red cells are labeled with an efficiency of 98-99%.

Studies performed on the RBCs labeled by this method showed that the labeling yield is almost unaffected by the presence of oxidizing agents in the generator eluate and also that the timing of the elutions does not have to be controlled. In cases when a bolus injection is needed, the whole activity may be obtained in about 1 ml volume by centrifugation of the vial in an inverted position and withdrawing the packed RBCs with a syringe.

Testing in humans showed that high intravascular activity and prolonged stability of the label in the blood pool were obtained. The experimental results are in good agreement with a biexponential decay, where 40% of the activity is cleared with a half-life of 3 hours and 60% with a half-life of 60 hours. As almost no free pertechnetate was observed in vivo during the first six hours after injection, this preparation was proved to be suitable for the diagnosis of patients with acute or intermittent upper gastrointestinal bleeding.

Mechanisms: Srivastava and co-workers (4) undertook exhaustive studies to define the mechanisms involved in the in vitro red cell labeling methods. They studied factors such as:

a) uptake and distribution of Sn (II) in blood components

b) role of oxidation and chelation in the chemical inactivation of the extracellular Sn(II).

c) uptake kinetics and distribution of ^{99m}Tc in tinned RBCs

d) effect of carrier ^{99m}Tc

e) effect of plasma and other suspending media

f) sites of binding of Sn(II) and ^{99m}Tc within RBCs

h) long-term retention of tinned RBCs following in vivo labeling.

B) In Vivo Labeling

In vivo red blood cell labeling, as first proposed by Zimmer and Pavel (8), was crucial for the wide use of ^{99m}Tc-RBCs, especially in nuclear cardiology. Studies were performed which determined the optimum time lag between injection of the Sn (II) and the ^{99m}Tc-pertechnetate (30 min), the optimal stannous ion concentration (10-30 µg Sn(II)/kg body weight) and the optimal stannous preparation. Although no difference was found between pyrophosphate, diphosphonate and citrate in influencing the -- efficiency of red cell labeling is pyrophosphate has become the most widely used ligand.

Routinely performed examination have shown that about 70 - 90% of the injected dose is in the circulation during the first two hours after injection, and at least 95% of the activity in the blood is RBC-bound. The biologic half-life of RBCs labeled in vivo was found to be similar to that of RBCs labeled in vitro (5).

In vivo labeling is widely used, despite variable labeling efficiency and urinary excretion which were reported. Also, the eventual uptake by the gastric mucosa and thyroid of free pertechnetate, reduces the quality of the imaging. Attempts have been made to understand the source of extravascular activity and to reduce it. Especially high extravascular radioactivity was observed after bolus injection of the pertechnetate. One possible explanation of this undesirable effect could be that the amount of technetium injected exceeds the binding capacity of the Sn-RBCs, permitting the uptake of the excess b. the thyroid and

the gastric mucosa. The quality of the gated cardiac images was improved by injecting the pertechnetate slowly, thus increasing the number of Sn-RBCs that come in contact initially with the ^{99m}Tc pertechnetate. Improvement of the quality of the image was seen when 1-2 ml blood, containing the Sn-RBCs, was withdrawn --- for about 30 sec into the syringe containing the pertechnetate and the reinjected slowly. The image was also improved when the stannous content of the pyrophosphate kit used for pretreating the red cells is increased to exceed 1 mg Sn (11) (9).

C) In Vivo/In Vitro Labeling

A modified in vivo/ in vitro method for labeling erythrocytes was reported by Callahan et al. (10). Twenty minutes after the injection of Sn pyrophosphate (Sn-PYP), 3 ml of blood is withdrawn, incubated with 99m-Tc pertechnetate, and ---- reinjected after 10 minutes. The labeling efficiency of the method was shown to be around 95%, so that only 5% of the activity, injected as free pertechnetate, is available for extravascular distribution. This method seems to combine the superior binding efficiency of in vitro labeling with the convenience of the in vivo labeling method.

Effect of anticoagulants on RBC labeling efficiency. It was found that the use of ACD as an anticoagulant provides more efficient binding efficiency than heparin in all labeling methods, in vitro and in vivo/in vitro. The lower labeling efficiency obtained when heparin is used may be explained by the formation of ^{99m}Tc heparin complex in the presence of Sn (11). This complex undergoes renal clearance and raises the urinary bladder activity

D) Summary of Similarities and Differences between In Vitro and In vivo Labeling of RBCs with ^{99m}Tc Pertechnetate

Red cells are pretreated with stannous tin in both in vitro and in vivo labeling. The amounts of stannous ion that provide optimal Tc-labeling are almost the same in both cases: 1 µg Sn(II) for the in vitro labeling of 3 to 10 ml blood and 0,7-2,1 µg Sn (II) for in vivo labeling of the approximately 5 liters of circulating blood. Very special precautions must be taken in order to keep the stannous tin suitable for in vitro Tc-labeling even for very short periods. In contrast, for in vivo labeling, after the stannous is injected into the blood stream, it remains suitable for red blood cell labeling for a period of months.

In in vivo labeling only a fraction of the available tin is taken up by the RCBs: the remaining extracellular Sn(II) in the plasma competes with the red cells for the ^{99m}Tc, depressing the labelin yield. Therefore it becomes necessary either to remove physicaly or to oxidize the non-cell-bound tin before the addition of pertechnetate. On the other hand the non-cell-bound tin does not affect the in vivo labeling efficiency. Any time during the first 24h after Sn-PYP injection, in vivo labeling yields about 98% of the activity bound to the RBCs.

The same ligands are used for the Sn-preparations in both methods, and in both cases do not influence the labeling efficiency: the determining factor is the amount of available stannous-tin.

The choice of anticoagulant, the carrier Tc and other oxidants possibly present in the ^{99m}Tc pertechnetate seem to influence the labeling efficiency. The potential effect of patient medication on the quality of blood pool studies was recently reported. but further studies are necessary to pinpoint such interference more accurately.

Patient radiation dose. Average radiation doses to the whole body, as well as to various organs, have been calculated The whole body dose, 20 mrad/mCi, is lower than the radiation dose to many other organs (30-70 mrad/mCi). This difference is due to the lower average blood content of the whole body compared to the listed organs.

Table 2.

Patient radiation doses for normal and for heat damaged RBCs(11)

Organ	Dose (mrad/mCi)	
	Normal RBCs	Heat damaged RBCs
Whole body	20	20
Heart	78	
Spleen	50	2.870
Liver	70	11
Blood	55	27
Lungs	56	
Kidneys	54	
Red marrow	33	

4. LABELING OF PLATELETS AND LEUKOCYTES

Lipophilic chelates of ^{111}In with oxine, acetylacetone, tropolone and mercaptopyridine N-oxide (Merc) have been used successfully for labeling platelets and leukocytes. These methods require prior cell separation from relatively large samples of autologous blood. In vitro aggregation and in vivo viability of cells are better preserved when the labeling procedure is performed in autologous plasma. However, due to the chelation of In with plasma transferin and other lipoproteins, the labeling in plasma is generally inefficient. Efficient labeling can be achieved in nonplasma media, but only at the expense of cell viability.

Various investigators have modified nonplasma labeling techniques so as to allow efficient incorporation of radioactivity, while minimizing adverse effects on cell viability and making the procedure convenient for routine handling. Different salt balanced media and different proportions of various anticoagulants are used for cell separation with different centrifugal forces. All these factors affect cell viability.

Recently Thakur and colleagues (12,13) published kit procedures for the labeling of both platelets and leukocytes with ^{111}In with negligible alteration of in vitro aggregability, A water soluble sodium salt of Merc is used to chelate ^{111}In, permitting relatively high incorporation of the radioisotope in both platelets and leukocytes in plasma. The labeling yield is dependent on pH, Merc concentration and cell concentration. The blood cells were labeled using either preformed ^{111}In-Merc or first incubating the cells with dry Merc and then with ^{111}In. The latter method provided simple kit procedures that label both platelets and leukocytes with better efficiency than methods using either oxine or tropolone. In this procedure platelets were first incubated with 2.5 μg dry Merc and then labaled with ^{111}In. The same dry Merc concentration can be used for optimal labeling

of leukocytes when ACD is the anticoagulant. When instead of ACD, heparin is used, 20 μg Merc is necessary for optimal labeling. The reason for the need for this larger amount of Merc is not clearly understood. One explanation may be the formation of disulfides which cannot bind metal. Not more than 10 IU heparin/ml blood are used and generally a labeling efficiency of >75% is achieved.

The use of heparin as an anticoagulant for the labeling of leukocytes is important because it reduces the contamination of the preparation with radioactive platelets from about 30% to <3%. The radioactivity associated with erythrocytes remains <2%.

The use of dry Merc has several advantages: the need to prepare ^{111}In-Merc before proceeding to the cell labeling itself is eliminated thereby avoiding problems related to the quality control of this complex. The labeling method as described is very simple and fast. The use of Merc instead of either oxine or tropolone results in a much higher labeling efficiency in the ^{111}In labeling of both platelets and leukocytes (Fig. 2, Table. 3)

In dogs, platelets labeled with Merc in autologous PPP (platelet-poor-plasma) had a normal survival time (7,5 days). Twenty four hour old vascular thrombi were detected 40 min after injection of labeled platelets, the thrombi/blood ratio being about 60. Forty-eight hour old pulmonary emboli (PE) could be detected by scintigraphy 90 min after injection, the PE/blood ratio being about 46.

Leukocytes labeled by this procedures clear rapidly from normal lungs and migrate to abscesses providing abscess/blood rations of about 75,24 hours after injection. The liver uptake in this case is much reduced as compared to cells labeled with ^{111}In-oxine, possibly due to better preservation of cell viability, i.e., Merc does not alter the phagocybility of neutrophiles.

The availability of kit procedures which allow efficient labeling of human platelets and leukocytes in plasma by a simple

and convenient method increases the potential value of radiolabeled platelets and leukocytes for noninvasive diagnosis.

5. SPECIFIC CELL LABELING USING MONOCLONAL ANTIBODIES

Labeling of a specific cell population in whole blood samples would eliminate the possible damage from cell separation procedures and keep the cells in their native plasma environment throughout the labeling procedure. A very promising approach is the labeling of cells with monoclonal antibodies directed against specific cell antigens. Various investigators have already started to search for cell specific antibodies (MAb) and to develop optimized methods for in vitro labeling with various radionuclides such as ^{123}I, ^{125}I, ^{131}I, ^{111}In and ^{99m}Tc.

Factors such as the nature and amount of oxidant, reaction time substitution levels and specific activity affect not only the chemical labeling yields, but also the specific binding of the labeled MAb to cells in the whole blood, and the blood clearance of the label. Radiolabeling frequently alters the biological behavior of MAb and compromises the specificity of binding to the in vivo antigens. Sensitivity of the labeling chemistry is quite variable for different antibodies. In order to achieve maximum efficiency in imaging and/or in therapeutic applications, individual antibodies may require careful specific optimization of the labeling procedure using different radionuclides.

Srivastava and co-workers (14,15) investigated Coller's 7E3 (16), an anti-platelet monoclonal antibody, which belongs to the IgG_1 subclass and is directed against the fibrinogen receptor on the platelet surface. This antibody inhibits ADP-induced platelet aggregation and the ADP-induced binding of fibrinogen to platelets. The antibody was chosen for a pre-clinical study in dogs because it cross reacts with dog platelets. Studies were carried out to check various factors affecting the binding to platelets of 7E3 following labeling with ^{123}I, ^{125}I and ^{131}I, as

well as after chelation labeling with ^{111}In and ^{99m}Tc. New modified methods for labeling and characterization of the Mab were recently reported and their usefulness for general antibody labeling was discussed (15). Both iodinated 7E3 and indium labeled 7E3 displayed greater binding specificity in fibrinogen coated bead assay.

The binding of radiolabeled 7E3 to dog platelets at various levels of saturation of the binding sites showed that when 0,1-1 μg was incubated per ml dog blood, 75^{+} 5% of the activity became bound to the cellular fraction, of which >95% was associated with platelets. Twenty to 30% of the label remained in the plasma with >90% of it being found to be associated with the IgG fraction. Experiments with human whole blood showed that >90% of the activity was platelet-bound. When blood was clotted by adding thrombin, 80-90% of the activity was incorporated in the clot despite repeated washing. Fifty percent of the maximum binding took place at 6 - 8 min: the maximum was observed at 45 - 60 min.

When blood was incubated in - vitro with ^{111}In-7E3 (100-150ml blood, 100 μg antibody) and the separated resuspended cells were reinjected, clots were visualized despite overlying herat blood pool activity. Three hours after injection the clot/blood ratio was about 15. Thus it was demostrated that the 7E3 antiplatelet monoclonal antibody binds highly selectively to human and to dog platelets in whole blood samples and that it can be radiolabeled with high specific activity without loss of binding efficiency to platelets. For this reason radiolabeled 7E3 is considered as a promising agent for the in vivo localization of thrombi and vascular lesions.

Several advantages of the antibody technique over conventional cell labeling are obvious: the antibody can be iodinated or labeled with metallic nuclides and imaging is possible quite sooon after injection (5-30 min for venous thrombi with ^{131}I-7E3-platelets as compared to 60 min for ^{111}In-oxine platelets) and at the same time the clot-to-blood ratios were approximately twice that from conventional labeling.

Recently Som and co-workers (16) published a study about MAb 50H. 19, which reacts with human platelets. This MAb was converted to fragments, pretinned and made into kits for subsequent radiolabeling with ^{99m}Tc. The antibody, which cross-reacts with dog platelets, was used to evaluate in vitro binding to blood clots and in vivo experimental thrombi in dogs. The results of this study are summarized in Tables 4 and 5. Experimental thrombi in dogs could be visualized consistently within 2-3 hours postinjection in peripheral veins and arteries, pulmonary arteries and the rigth ventricle. The advantages of this method may be summarized as follows: short and simple pre-imaging preparation and rapid visualization of thrombi with no need for blood-pool subtraction or delayed imaging. The clinical effectiveness of this procedure still remains to be established.

REFERENCES

1. Gutkowski R.F., Dworking H.J., Porter W.C. et al., J Nucl. Med. 17 (1976), 1113.
2. Smith T.D. and Richards P. J. Nucl. Med. 17 (1976) 126-132
3. Stiegman J. and Richards P., Sem in Nucl. Med. 4 (1974) 269.
4. Srivastava S.C., Straub R.F., Meineken G.E. and Gil M.C., Current Applications in Radiopharmacology, Pergamon Press (1986) 166-183.
S. Weininger J., Trumper J., Lubin E. and Abrashkin S., Int. J. of Nucl. Med. Biol. 5 (1976) 190-195.
6. Srivastava S.C., Babich J.W. and Richards P., J Nucl. Med. 24 (1983) P 128.
7. Trumper J., Weininger j. and Lubin E., Proc. IV World Congress, Buenos Aires, 1986, 479-P.
8. Pavel, D.G., Zimmer, A.M. and Patterson V.N., J. Nucl. Med. 18 (1977) 305-308.
9. Lubin E. Unpublished results (1977).
10. Callahan R., Froelich J.M., McKusick K.A. et al., J. Nucl. Med. 23 (1982) 315-318.
11. Srivastava S.C. and Chervu L.R., Sem. in Nucl. Med. 14 (1984) 68-82.
12. Thakur M.L., McKenney S.L. and Park C.H., J. Nucl. Med. 26 (1986) 510-517.
13. Thakur M.L., McKenney S.L. and Park C.H., J. Nucl. Med. 26 (1986) 518-523.
14. Srivastava S.C., Straub R., Meineken G.E. et al., J. Nucl. Med. 25 (1986) P65.
15. Srivastava S.C., Meineken G.E., Scudder L.E. and Coller B.S. J. Nucl. Med., 26 (1985) P45.
16. Coller B.S., J. Clin. Invest., 76 (1985) 101-108.
17. Som P., Oster. H., Zamora P.O. et al., J. Nucl. Med. 27 (1986) 1315-1320.

Fig 1 The effect on the labeling yield of number of Tc atoms. On the upper abscissa, the number of Tc atoms is expressed in terms of ingrowth period calculated for 10 mCi activity (5).

Fig. 2 $\sim 9 \times 10^9$ Platelets suspended in 0,5 ml PPP were incubated either with Merc, oxine; or tropolone (dry). and labeled with ^{111}In. Results indicate that Merc was agent of choice (11).

Table 1 ^{99m}Tc-RBCs labeling yields as a function of oxidant quantities present in the pertechnetate solutions (5).

Table 3 Leukocytes labeling yields with ^{111}In using dry Merc, oxine, and tropolone (12).

Table 4 In vitro performances of ^{99m}Tc 50H. 19 (16).

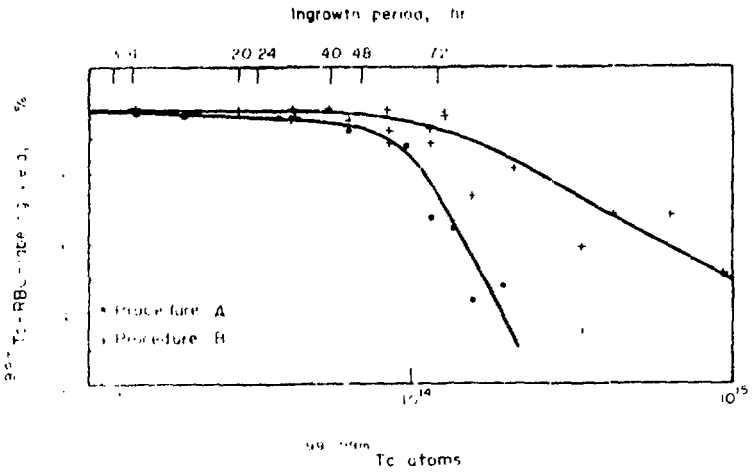

FIG. 1 The effect on the labeling yield of the number of Tc atoms. On the upper abscissa the number of Tc atoms is expressed in terms of ingrowth period calculated for 10 mCi activity

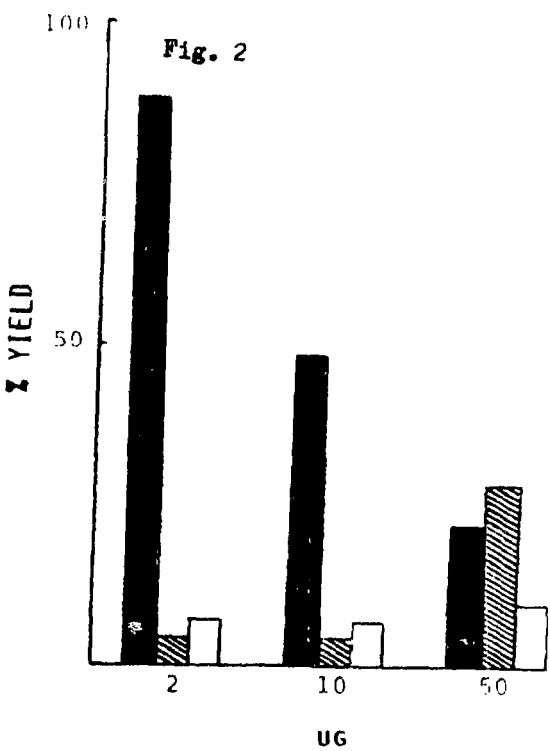

Fig. 2

~9 × 10^9 Platelets suspended in 0.5 ml PPP A were incubated either with (■) Merc; (▨) oxine; or (□) tropolone (div), and labeled with ^{111}In by following Method II. Results indicate that Merc was agent of choice

Table I. [illegible] RBC's Cr labeling yields as a function of oxidant quantities present in the pertechnetate solutions

Oxidizing agent	Quantity * (μg)	Procedure A (%)	Procedure B (%)
MoO_4	800	98.3	97.6
H_2O_2	0.09	97.0	
	9.00	93.0	97.0
$Cr_2O_7^{-2}$	0.13	98.5	98.5
	0.39	89.5	93.6
	0.66	47.0	73.9

* One microgram of Sn^{+2} is equivalent to 2.5 μg MoO_4 (assuming a valency change of Mo^{+6} to Mo^{+3}), 0.288 μg H_2O_2, 1.12 μg $Cr_2O_7^{-2}$

TABLE: 3

Leukocytes Labeling Yields with ^{111}In Using Dry Merc, Oxine, and Tropolone

μg	Merc	Oxine	Tropolone
10	46 ± 4.5	6.9 ± 2.3	8.7 ± 4.2
25	96 ± 2.3	10.4 ± 2.2	7.8 ± 3.2
50	55 ± 5.7	5.6 ± 2.1	8.2 ± 3.9

* 60 million leukocytes in 0.5 ml plasma were first incubated with the agent and then with dry ^{111}In

TABLE: 4

Technetium-99m 50H 19 Binding to Cellular Blood Fraction

	In vitro		In vivo	
	Cells (%)	Plasma (%)	Cells (%)	Plasma (%)
Dogs*	57.6 ± 2.9	42.5 ± 2.9	56.2 ± 5.4	43.8 ± 5.4
Humans†	61.2 ± 2.1	38.8 ± 2.1	-	-

* n = 4
† n = 8
Each point represents mean ± s.d.

TABLE: 5

Technetium-99m 50H.19—Clot Binding [illegible]

	In vitro	In vivo
	Percent binding to clot	Percent binding to clot
Dogs*	57.2 ± 1.9	69.9 ± 2.7
Humans†	74.6 ± 5.8	-

* n = 4.
† n = 8
Each point represents mean ± s.d.