Preparation and Evaluation of a ^{99m}Tc-SnF_2 Colloid Kit for Leukocyte Labeling

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Stannous fluoride colloid (SFC) kits for instant radiolabeling with ^{99m}Tc were prepared and evaluated for suitability as a leukocyte radiolabeling agent. Technetium-99m labeling for kits stored at −15°C for up to 3 mo was >95% as determined by instant thin layer chromatography while colloid particles of 1–3 μm were measured by electron microscope for these preparations. Canine leukocyte preparations labeled with [^{99m}Tc]SFC and characterized by triple density gradients of metrizamide in plasma demonstrated an 83% leukocyte association. Analysis of labeled cell preparation for up to 3 hr demonstrated label stability. Labeled leukocytes, when readministered in normal dogs, demonstrated bi-exponential blood clearance with uptake and subsequent clearance from lung. There was increasing uptake of labeled leukocytes by the liver until steady state was achieved. Furthermore, when whole blood samples were analyzed by the triple density gradient method, an increasing monocyte-to-granulocyte ratio was observed to occur with time. By 3 hr 95% of the whole blood activity was associated with the leukocyte fraction. Dogs in which a 24-hr sterile abscess was created demonstrated elevated blood-pool activity as compared to control with localization of the labeled cells at inflammatory sites within 3 hr following cell readministration.

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Despite the economic and dosimetric disadvantages of indium-111, labeled leukocytes remain the accepted nuclear medicine modality for detecting pyogenic abscesses. It is not surprising, therefore, that a decade of research has been devoted to the formulation of a suitable technetium-99m (^{99m}Tc) labeling procedure. Although several approaches to this problem have been studied, the use of radiocolloids has received the most attention.

Using a commercial ^{99m}Tc sulfur colloid (SC) kit and buffy coat from 50 ml human blood English and Andersen (*1*) demonstrated a 40% leukocyte labeling efficiency with good in vitro cell viability. Subsequent work (*2*) demonstrated labeled leukocyte localization in septic and sterile abscesses as well as acute inflammatory lesions in the dog model. Fisher et al. (*3*) and McAfee and Thakur (*4*) incubated ^{99m}Tc albumin microspheres with buffy coat from rabbit and human blood, respectively. Both experimentally induced sterile abscesses in rabbit and nonsterile abscesses in man were visualized. The use of a colloid from a different commercial source was later adapted to human whole blood by Marcus et al. (*5*).

In 1981 Scroth et al. (*6*) advocated the use of a commercial kit containing stannous fluoride colloid for labeling leukocytes in whole blood. Following administration of cells labeled by this method in man, sepsis was identified. Hanna et al. (*7*), using freshly prepared stannous fluoride colloid, reported cell viability and an improved leukocyte labeling efficiency. However, Mock and English (*8*) using a triple density gradient demonstrated much of the radiocolloid, prepared by these methods, to be associated with red blood cells. Furthermore, Rosenthall et al. (*9*) found the commercial kit source of the stannous fluoride colloid (SFC) to provide inconsistent leukocyte labeling in studies designed to identify the human septic abscess. They concluded that the study would be made more acceptable if the kit were to contain preformed colloids of appropriate size for leukocyte engulfment and thereby eliminate the

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required 1 hr incubation period following ^{99m}Tc inclusion.

Our aim in this study was to modify the stannous fluoride formulation to prepare an instant labeling preformed colloid for leukocyte labeling and to demonstrate the necessary in vivo characteristics of the labeled leukocytes required for clinical investigation.

MATERIALS AND METHODS

Preparation of ^{99m}Tc Tin Colloid

Stannous fluoride colloid was prepared employing a modification of the method of Mock and English (*8*). Using aseptic technique, aqueous solutions of stannous fluoride (0.250 mg/ml) and sodium fluoride (2.00 mg/ml) were prepared and sterilized by 0.22-μm membrane filtration. Equal volumes of solutions were combined and the pH adjusted to 6.1 with sterile sodium hydroxide. One-half milliliter aliquots of the resultant SFC were dispensed into sterile vials and stored at −15°C.

Technetium-99m stannous fluoride ([^{99m}Tc]SFC) was prepared by the addition of 15 mCi (555 MBq) ^{99m}Tc, obtained within 3 hr of eluting a ^{99}Mo/^{99m}Tc generator in 5 ml of sterile saline to the SFC kit. The preparation was allowed to incubate for 5 min at room temperature and evaluated for colloid size and radiochemical purity.

ANALYSIS OF [^{99m}Tc]SFC

Colloid Sizing

Aliquots of [^{99m}Tc]SFC were obtained and serially diluted with deionized water. Small drops of the diluted preparation were placed on electron microscope (EM) grids, previously coated with Formvar plasticizer, and allowed to dry at room temperature. The residue on each grid was examined by EM using a Phillips EM-400.

Radiochemical Purity

Radiochemical purity of the radiocolloid was determined by thin layer chromatography (TLC) using "mini" instant TLC (ITLC)-SG strips in acetone or saline. With each solvent, radiocolloid remains at the origin while free, unreacted ^{99m}Tc is found at the solvent front.

LEUKOCYTE LABELING

Leukocyte labeling was accomplished using the following procedure (Fig. 1). Fifty milliliters of heparinized venous blood (50 IU/10 ml) was collected to which hydroxyethyl starch, 6% (1–3 ml/10 ml blood) was added. The syringe was inverted several times to ensure adequate mixing. Erythrocyte sedimentation was allowed to proceed for 1 hr at room temperature in 2–50 ml plastic tubes. The supernate was pipetted into another 50-ml tube and centrifuged at 180 g × 8 min at room temperature to obtain a leukocyte pellet (LP) and platelet-rich plasma (PRP). Further centrifugation of the PRP at 1,000 g × 10 min at room temperature produced platelet-poor plasma (PPP) which was separated and retained at 37°C. The LP was resuspended in 5 ml PPP and incubated with previously prepared [^{99m}Tc]SFC for 1 hr at 37°C. The suspension was mixed every 15 min during the incubation period. The resultant ^{99m}Tc-labeled leukocytes were centrifuged at 180 g × 8 min and separated from the cell supernate. Activity associated with the cell pellet and supernate fractions were assayed in a dose calibrator and the percent activity in the cell pellet ("apparent" labeling efficiency) was obtained. Labeled leukocytes were washed in PPP, recentrifuged, resuspended in 5 ml PPP, and assayed.

ANALYSIS OF ^{99m}Tc LEUKOCYTES

Triple Density Analysis

The triple discontinuous metrizamide:plasma (MP)-density gradient as described by Mock and English (*8*) was used to determine the "true" leukocyte labeling efficiency of the labeled cell preparations (Fig. 2). Briefly, three MP-gradient solutions containing 40%, 50%, and 60% v/v of 35% w/v metrizamide in plasma were prepared, respectively. The discontinuous gradient was prepared by layering, in decreasing order of precent metrizamide concentration, 1.5 ml of each gradient solution in 7-ml tubes using the method of Madyastha (*10*). A 10-μl aliquot of the labeled cell preparation was mixed in 1 ml of PPP and loaded last on the gradient. The loaded gradient tubes were centrifuged at 2,000 g × 20 min which resulted in the separation of mononuclear leukocyte, polynuclear leukocyte, red blood cell (RBC), and free colloid fractions. Gradient tubes were subsequently imaged and an activity profile obtained (Fig. 3). The percent activity associated with each fraction was determined by a curve fitting program based on a fourth power polynomial fit.

Label Integrity

Labeled cell preparations were incubated at 37°C for up to 3 hr for the evaluation of label integrity. At 0, 30, 60, 120, and 180 min following cell labeling, 10-μl aliquot of the labeled cell preparations were loaded onto triple discontinuous MP-density gradients, centrifuged, and analyzed.

Cell Viability

The labeled leukocyte preparations were evaluated for cell viability by vital dye exclusion. Trypan blue, 1% (0.7 ml) was mixed with an aliquot of the cell preparation (0.3 ml) and examined by light microscopy.

Leukocyte Kinetics and Biodistribution

Normal Control

Cell kinetics. Fifty milliliters of canine venous blood was obtained and labeled leukocytes prepared as previously described. Following administration of the labeled cell preparation, 8-ml venous blood samples in heparin were withdrawn at 2, 5, 10, 15, 30, 60, 120, 180, 240, 300, and 360 min to obtain 1-ml samples of whole blood and plasma, respectively. A microhematocrit was obtained for each blood sample. Blood, plasma, and standard samples were counted in a NaI gamma well counter set with a lower level discriminator at 130 keV and a 20% window, for the determination of percent dose in circulating cells by the method of Goodwin et al. (*11*) (Appendix).

Isolation of radiolabeled cells. The percentage of ^{99m}Tc-

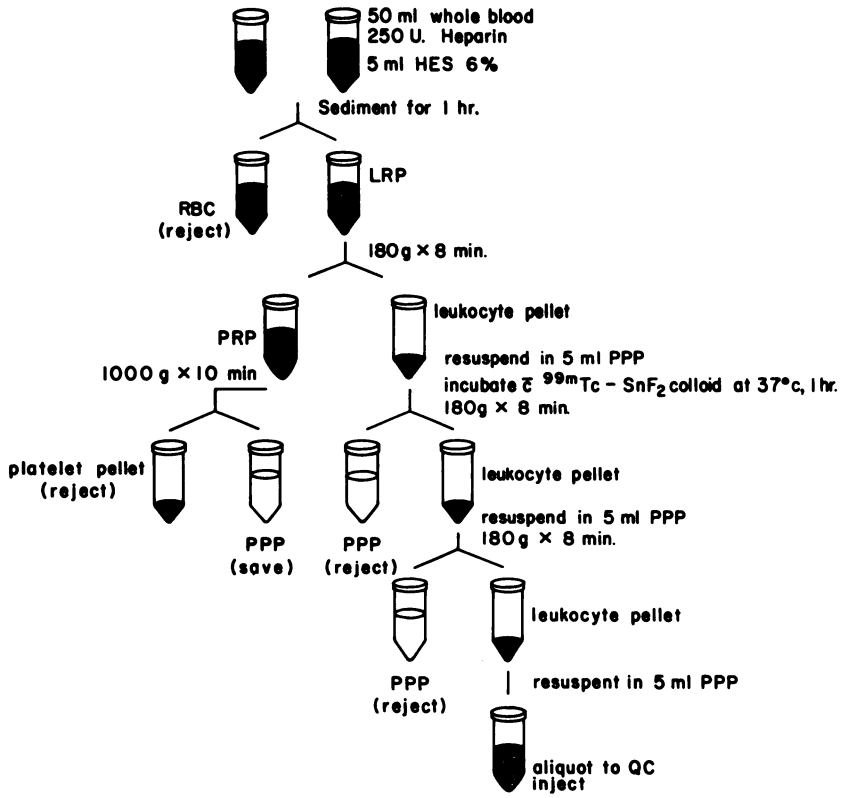

FIGURE 1
Schematic presentation of procedure for labeling leukocyte with [^{99m}Tc] SFC.

SFC associated with cell fractions from whole blood, collected over time, was determined in three control animals. A 25-μl aliquot of venous whole blood was obtained from samples collected at 5, 30, 60, 120 and 180 min following readministration of the labeled cell preparation and mixed with 1 ml of PPP. The whole blood suspensions were loaded onto triple discontinuous MP-density gradient tubes and centrifuged at 2,000 g × 20 min. Because the tubes contained nominal activity for imaging, layers from all columns were fractionated and delivered to separate tubes for counting. Activity associated with cell fractions and residual colloid were determined using a NaI scintillation well counter set with a lower level discriminator at 130 keV and a 20% window.

Biodistribution

Prior to labeled cell readministration, control dogs were anesthetized with pentobarbital (30 mg/kg) and positioned in the supine position. Rapid sequence imaging was performed in the anterior projection at 2 frames/sec for 1 min during bolus administration of labeled cells to obtain heart, lung, and liver regions of interest. Imaging was continued at 1 frame/60 sec for the next 6 hr to derive labeled leukocyte biodistribution over time among these organs.

Experimental Abscess Model

Animals to serve as experimental sterile abscess models were handled in accordance with the Institutional Animal

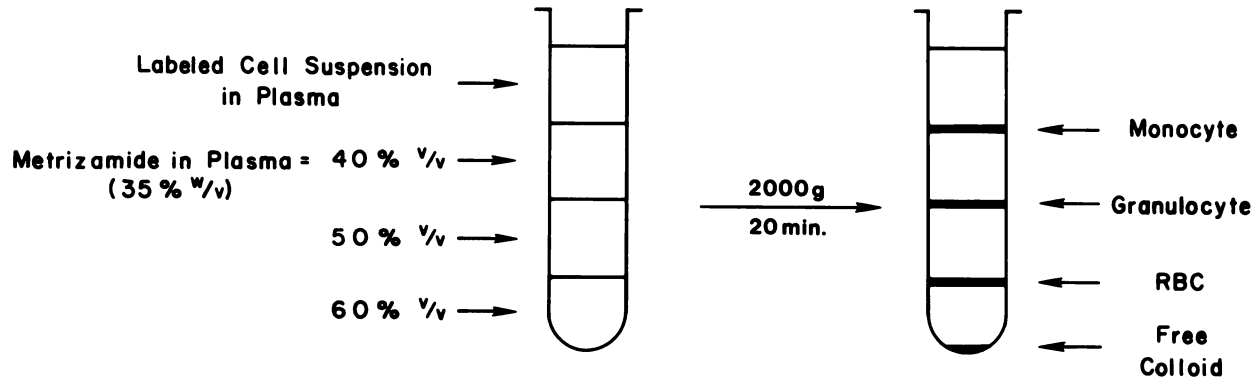

FIGURE 2
Separation of the labeled cell preparation by the triple discontinuous MP-density gradient method. Monocytes, granulocytes, RBCs, and free colloid form distinct bands during centrifugation.

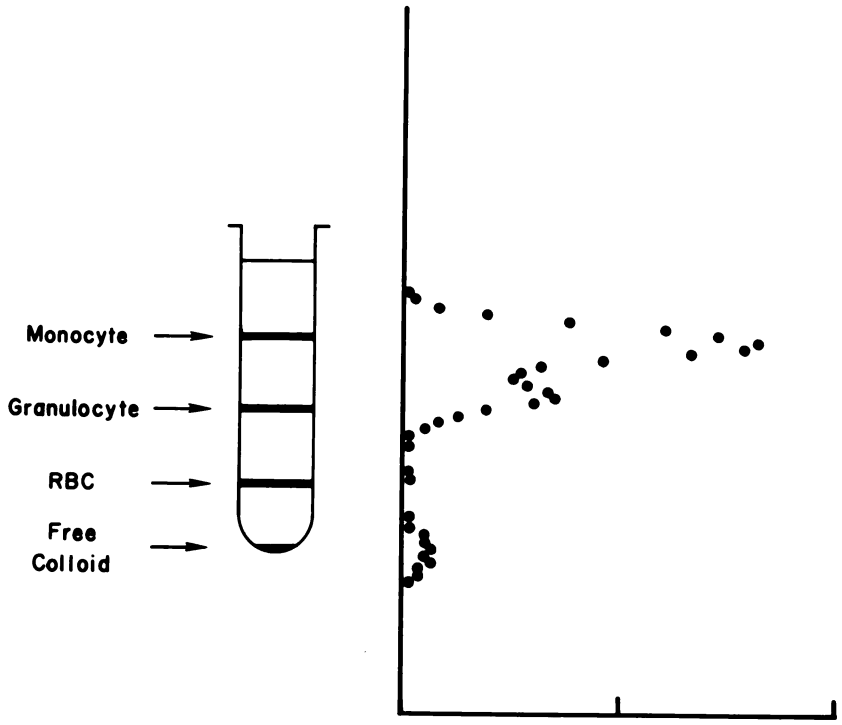

FIGURE 3
Representation of tube with separated labeled cell preparation on triple discontinuous MP-density gradient following centrifugation (L). The activity profile (R) was obtained by computer processing of the tube image.

Care and Use Committee of the Virginia Commonwealth University. Dogs were anesthetized with pentobarbital and a 2-ml osmotic pump containing 60 mg/ml morphine sulfate was surgically implanted into the neck muscle. Turpentine (0.2 ml) was injected into the thigh while an equivalent volume of sterile saline was injected into the contralateral site. Blood for leukocyte labeling was withdrawn 24 hr post-turpentine administration. Following labeling, 5 mCi (185 MBq) of labeled cells were readministered. Samples for whole blood and plasma counting and for the determination of hematocrit were obtained. Animals were imaged continuously for 3 hr in the anterior projection with the collimator positioned over both thighs.

RESULTS

In Vitro

Following SFC labeling with ^{99m}Tc, > 95% of the activity was associated with the colloid as determined by ITLC. Kits stored at −15°C for up to 3 mo demonstrated radiochemical purity similar to this initial value. Colloid size for [^{99m}Tc]SFC as determined by EM ranged from 1–3 μm (Fig. 4).

The "apparent" labeling efficiency of the leukocyte pellet following a 1-hr incubation period with [^{99m}Tc] SFC was 66.011 ± 16.60%. When the cell pellet was subsequently washed with plasma, labeling efficiency improved to 89.52% ± 4.26% (n = 8). The triple discontinuous MP-density gradient results (Table 1) demonstrated that a mean of 83% of activity can be ascribed to the leukocyte fraction while 4% is fixed to residual red cells and 13% remains as free colloid.

Labeled cell preparations incubated at 37°C for up to 3 hr in plasma and separated by the triple discontinuous MP-density gradient technique demonstrated label stability with a monocyte-to-granulocyte ratio of 2:1 throughout this study period. Trypan blue studies per-

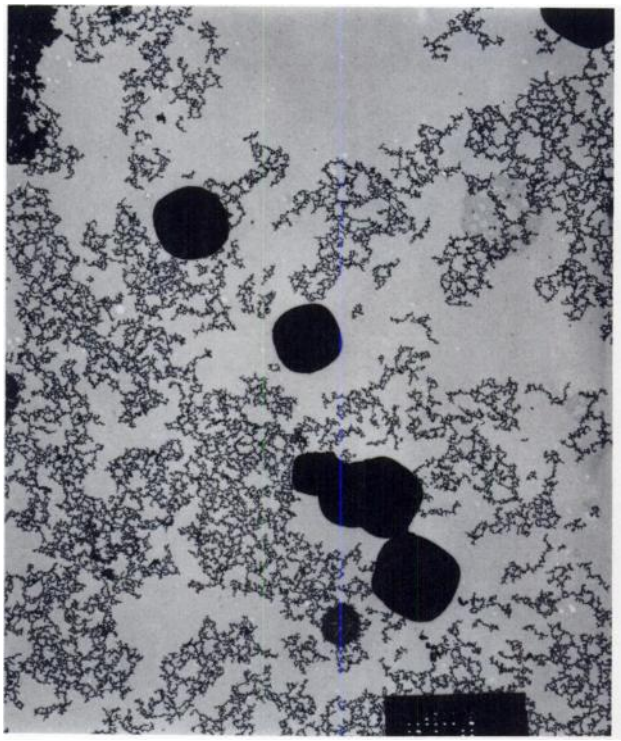

FIGURE 4
Electron micrograph of SFC (7,000×). Colloids are 1–3 μm in size.

TABLE 1
Association of [^{99m}Tc]SFC with Metrizamide:Plasma Density Fractions in Labeled Leukocyte Preparations*

Gradient fraction	% Association (mean ± s.d.)
Monocyte	63.69 ± 19.83
Granulocyte	19.64 ± 14.39
RBC	4.18 ± 4.12
Free colloid	12.54 ± 9.02

*n = 5.

formed on these cells revealed > 95% retained the ability to exclude vital dye.

In Vivo

In normal control dogs (n = 4) the time course of percent recovery of labeled cells in blood, corrected for plasma activity and decay, was biexponential with ~30% and 5% of the dose recovered at 2 min and 3 hr, respectively. The t½ of the second component was calculated to be 11.75 hr (Fig. 5).

Whole blood obtained 5 min after the readministration of the labeled cell preparation and fractionated by the triple discontinuous MP-density gradient method showed 82% of the activity associated with leukocytes, 8% with RBC and 10% with colloid. By 3 hr the RBC associated activity and free colloid cleared the blood pool leaving 95% of the activity associated with the leukocytes. The initial monocyte-to-granulocyte ratio of 4:1 increased to 12:1 at 3 hr. Dynamic imaging studies displayed an initial uptake of labeled cells in lungs followed by clearance (t½ = 52 min) and was a major component to the pattern of blood clearance. There was also a progressive increase in activity in the liver with time (Fig. 6).

In the canine abscess model (n = 3), the percentage of labeled cells recovered from blood, corrected for plasma and decay, was higher than controls. In this abscess group the percent dose in blood was ~20% for each of the 2–4 hr postadministration periods (Fig. 7). Multiple abscesses could be visualized in the scintiphoto of the left thigh of this model at 1 hr postlabeled cell readministration. These sites were better defined at 3 hr while no uptake was seen at the saline control during the study period (Fig. 8).

DISCUSSION

While the advantages of an efficient, specific, and stable ^{99m}Tc labeling procedure for leukocytes are obvious, previous attempts have been laborious, nonspecific, and frequently unstable. Radiocolloid methods have obvious appeal in that a specific leukocyte function can be exploited for specific labeling. However, the effectiveness of colloid labeling is highly dependent upon colloid size, and in many cases this critical property has not been fully controlled. Failure to control this property has led to nonreproducible results as well as nonspecific labeling of other species such as red blood cells. Our data demonstrates colloid sizes within the 0.264-μ to 3.04-μ range are required for association with leukocytes (*12*).

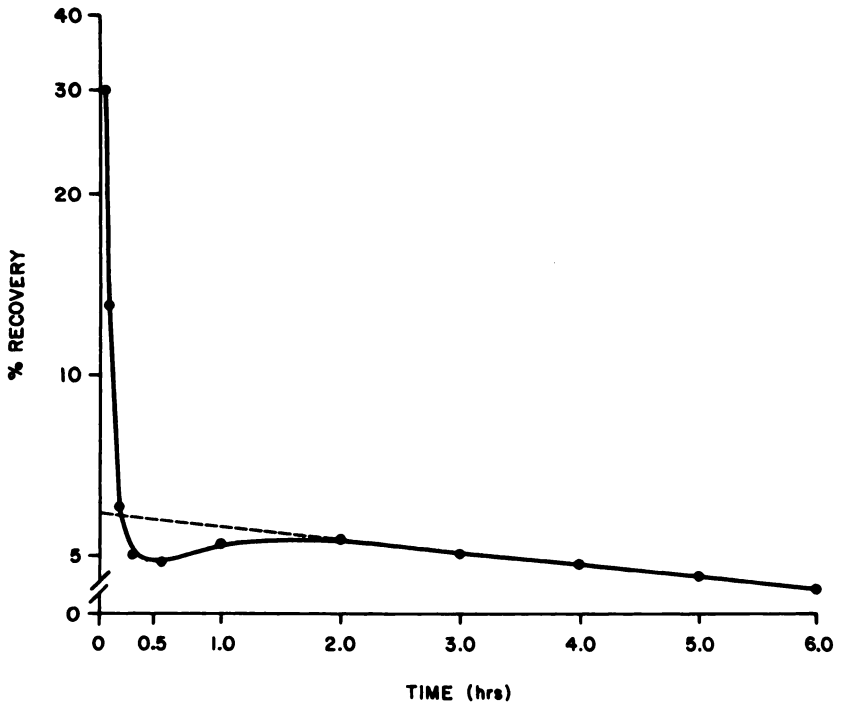

FIGURE 5
Recovery of administered activity in circulating cells (whole blood minus plasma activity) in control dogs.

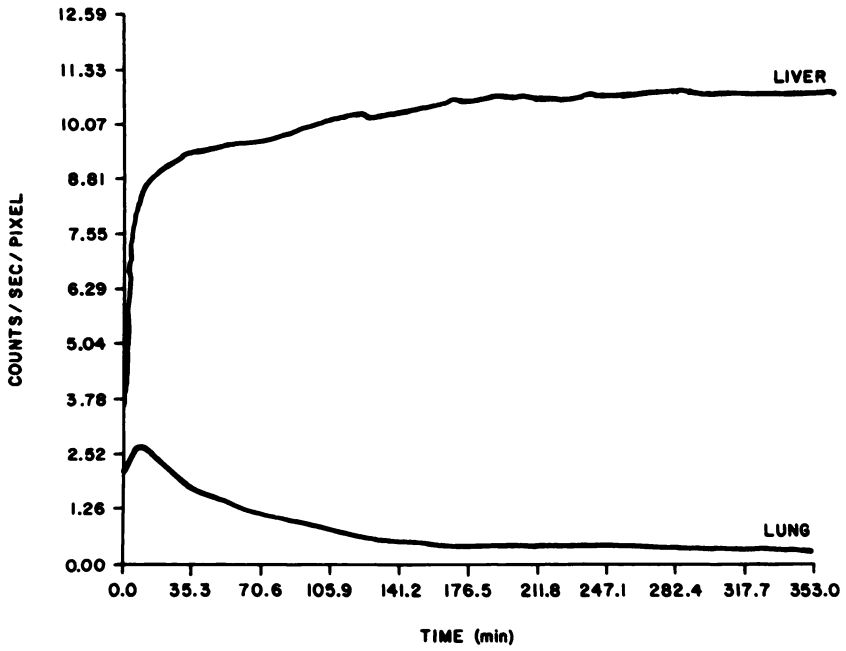

FIGURE 6
Graphic representation of dynamics of labeled leukocytes in dog. Continuous imaging of the chest and abdomen was obtained in the anterior view. Splenic activity could not be quantitated in this view.

In the present study, an efficient, stable, and specific technique utilizing a kit of preformed colloidal stannous fluoride has been developed. Utilizing this kit, a mean leukocyte labeling efficiency of 83% with only minimal red cell labeling was achieved. The leukocyte label was stable over a 3-hr incubation period and no change in the labeled monocyte-to-granulocyte ratio was observed. Additionally, the kit when maintained at −15°C appeared stable for at least 3 mo without loss of labeling effectiveness.

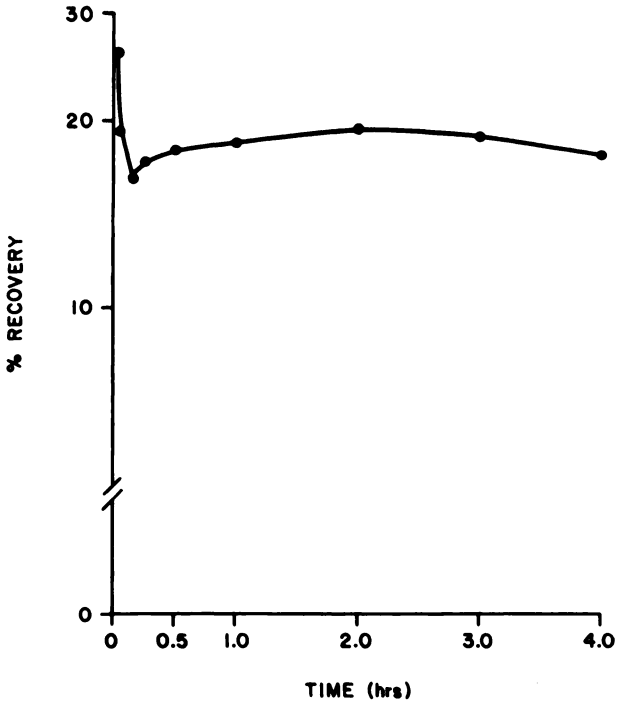

FIGURE 7
Recovery of administered activity in circulating cells (whole blood minus plasma activity) in the abscess dog model.

Following readministration of the labeled cell preparation in control animals, a rapid clearance of the blood-pool activity occurs. Leukocyte associated activity in density fractions obtained from whole blood and their corresponding incubated labeled cell preparation were in agreement when the colloid content in the whole blood sample was adjusted to the colloid content found in the cell preparations. This difference in colloid content is presumably a result of clearance by the liver and spleen as supported by the kinetic data. Furthermore, the increase over time of the monocyte-to-granulocyte ratio in whole blood samples is consistent with previous findings that while granulocytes marginate in repository sites, monocytes are transitory in the lungs and re-enter the circulation with time (*8*).

In animals with abscesses, there is a marked alteration of the blood-pool clearance with apparent mobilization of labeled cells from repositories allowing early visualization of the abscess when the stannous fluoride colloid label is employed. The high specificity of labeling, the minimal labeled red cell contamination, and the blood-pool clearance characteristics achieves a good target-to-background ratio upon early imaging which intensifies throughout the 4 hr examined. The favorable labeling properties, the blood-pool characteristics, and proven success in early detection of animal aseptic abscesses, warrant clinical evaluation of the [^{99m}Tc]SFC kit.

APPENDIX

$$\text{Net cpm/ml WB in cells} = \left[\frac{\text{Net cpm in}}{\text{1 ml WB}}\right]$$

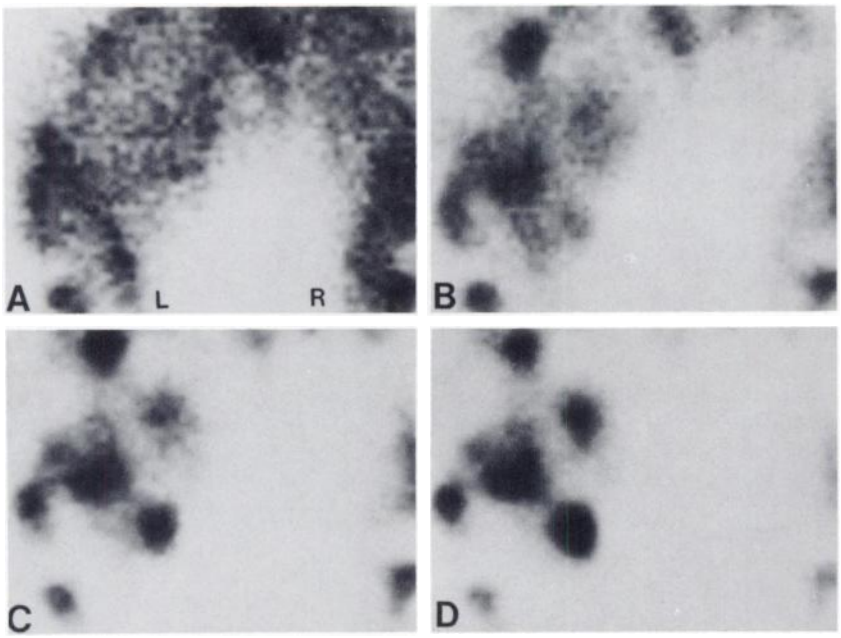

FIGURE 8
Scintiphotos of dog thighs obtained at 0 hr (A), 1 hr (B), 2 hr (C), and 3 hr (D) following labeled cell readministration in the abscess dog model. Turpentine (L) and saline (R) were administered 24 hr prior to the labeled cell injection.

$$- [1 - (\text{Hct} \times 0.97 \times 0.91) \times \text{Net cpm in 1 ml Pl}]$$

$$\% \text{ Dose in Circulating cells} = \frac{\text{Net cpm/ml WB in cells} \times \text{TBC (ml)} \times 100}{\text{Net cpm injected}}$$

0.97 = Correction factor for plasma trapping in microhematocrit tube;
0.91 = Correction factor for total body Hct/peripheral Hct ratio;
TCB = Total blood volume;
WB = Whole blood;
Hct = Hematocrit;
PL = Plasma.

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