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In Vitro Studies of Leukocyte Labeling with Technetium-99m

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A method for labeling leukocytes in vitro with ^{99m}Tc is described. Separated leukocytes are incubated with ^{99m}Tc, followed by reduction with stannous chloride and washing with acid citrate dextrose solution. Maximum labeling occurs after at least 5 min incubation with pertechnetate, followed by at least 10 min incubation with stannous chloride. Labeling is similar at room temperature and at 37°C. The labeled leukocytes are viable, and reutilization of label does not occur in vitro. In acid conditions (pH 5.2), the elution of ^{99m}Tc from leukocytes is comparable with that of ^{32}P-diisopropylfluorophosphate, but ^{99m}Tc elution is greater at pH 7.2–7.4. Neutrophils label more heavily with ^{99m}Tc than do monocytes, lymphocytes, erythrocytes, or platelets.

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Phosphorus-32-diisopropylfluorophosphate (^{32}P-DFP) has proved to be an almost ideal label for the study of neutrophil kinetics since its introduction in 1960 (*1*). Extensive data concerning neutrophil production, intravascular distribution, survival, and turnover have been obtained with its use (*2*). Indeed, its only drawback is the impossibility of external counting and organ imaging because ^{32}P is a pure beta-emitter. Leukocytes labeled with ^{51}Cr have been used to measure organ uptake (*3–6*), but this nuclide suffers from several disadvantages, including significant labeling of lymphocytes (*4,5*), unreliable estimates of intravascular pool sizes (*5*), and elution (*7*).

Technetium-99m would be an ideal tracer for localizing the organ uptake of neutrophils if satisfactory cell labeling could be achieved. Our preliminary studies have shown that organ images can be obtained after the infusion of autologous ^{99m}Tc-labeled leukocytes (*8*). In this paper we report in vitro studies that define the conditions for optimal neutrophil labeling, the extent of tracer elution, and the relative uptakes by different types of blood cells. Our results suggest that labeling leukocytes with ^{99m}Tc is feasible and should provide a useful investigative tool when used in vivo.

MATERIALS AND METHODS

Leukocyte collection. White cell concentrates obtained from donations of normal blood were kindly provided by the New South Wales Blood Transfusion Service. The leukocytes were separated by dextran sedimentation, with hypotonic lysis of residual red cells (*9*). Thirty to fifty milliliters of white cell concentrates, obtained from 450 ml of venous blood anticoagulated with 75 ml of ACD-A solution,* were added to an equal volume of 3% dextran in isotonic saline and allowed to sediment for 45 min. The supernatant, containing leukocytes and platelets, was removed and centrifuged at 300 g and 4°C for 10 min, after which the cell button was resuspended in 20 ml of 0.6% saline containing 0.1% (W/V) saponin for 2 min in order to lyse any residual red cells. The leukocytes were then recovered by centrifugation (220 g for 3 min) and washed twice with

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* The ACD-A solution consists of 2.2 gm of trisodium citrate dihydrate, 0.8 gm of citric acid, and 2.5 gm of dextrose in 100 ml of distilled water.

ACD-A solution. The total duration of exposure of the leukocytes to hypotonic saponin, including centrifugation, was 5 min. Plastic syringes and siliconized glassware were used throughout.

Leukocyte labeling with ^{99m}Tc. Experience showed that leukocytes labeled more efficiently with ^{99m}Tc in the absence of plasma proteins (see Results). Accordingly, for optimal labeling, 5×10^7 washed leukocytes were suspended in 2 ml of ACD-A and incubated at room temperature with 0.1 ml of a solution of pertechnetate (0.1–0.2 mCi) in isotonic saline. Separate evaluation showed that maximum labeling occurred by 10 min and that subsequent reduction with stannous chloride was necessary (see Results). In practice, 15 min incubation, followed by reduction with 100 μg of stannous chloride (0.1 ml of a 0.1% W/V $SnCl_2 \cdot 2H_2O$ solution), was used in all studies unless otherwise indicated. The final measured pH of cell suspensions so prepared was 5.2. Labeled leukocytes were then washed twice with ACD-A solution and resuspended for study in ACD-A, citrate phosphate dextrose* (CPD), ACD-A plasma, heparinized plasma, or Hank's balanced salt solution.

Leukocytes were also labeled in parallel with ^{32}P-diisopropylfluorophosphate, using the technique of Mauer et al. (*1*).

Measurement of radioactivity. The ^{99m}Tc activity of the samples was measured in an automatic scintillation counter (Packard Instruments, Downers Grove, Ill.). Counts were corrected for physical decay and all results were expressed as counts per second (cps) at time zero (t_0). The measured counting efficiency for ^{99m}Tc in this system was 54.0 ± 1.7% (mean ± s.e.m.). For the ^{32}P-DFP, labeled cells were transferred to a vial containing 10 ml of scintillator (Insta-Gel, Packard) for counting in a two-channel Tricarb liquid-scintillation spectrometer (Packard), using a modified channels-ratio technique for quench correction. This system gave 89% counting efficiency for ^{32}P.

Cell counting. Leukocyte and erythrocyte concentrations were obtained using a Coulter Model-F cell counter (Coulter Electronics, Dunstable, U.K.).

Elution of ^{99m}Tc from labeled leukocytes. Two types of experiment were performed to determine the elution of label from the white cells. In the first, a suspension of labeled leukocytes was divided into ten aliquots, each of which was washed a different number of times, after which the residual radioactivity was measured. In the second series of experiments, a suspension of leukocytes that had been labeled and washed twice was divided into aliquots, each of which were incubated for different periods of time, after which the leukocytes and supernatant were separated and the radioactivity of each was expressed as a percentage of the activity of the whole suspension. The elution of ^{32}P-DFP from leukocytes was measured similarly for comparison.

Relative labeling of erythrocytes, neutrophils, lymphocytes, and monocytes. To determine the relative labeling of erythrocytes and leukocytes, whole blood was centrifuged and known volumes of white-cell-poor red cells (obtained from the lowermost one-eighth of the red cell column) and red-cell-poor white cells (obtained by sedimentation and red cell lysis) were mixed to form a final suspension containing as nearly as possible equal numbers of erythrocytes and leukocytes. This suspension was then labeled with ^{99m}Tc, after which white cells were separated by sedimentation and hypotonic lysis and red cells were obtained from the bottom of the centrifuged deposit. Because the red cells in these suspensions showed significant white cell contamination, the red cell activities were measured either as the activity of a hemolysate or by calculated correction for the activity of the white cells present.

In order to determine the relative labeling of neutrophils, mononuclear cells, and platelets, 10 ml of leukocyte-rich blood, obtained from the buffy coat, was incubated with 100–200 μCi of ^{99m}Tc and then reduced with $SnCl_2$. Labeled cell suspensions were heparinized, recalcified, and centrifuged through a Ficoll–Hypaque density-gradient column at 400 g and 20°C for 40 min (*10*).

The layer of mononuclear cells and platelets was collected and centrifuged at 220 g for 5 min. Platelets were recovered from the supernatant by centrifugation at 1,600 g for 15 min, followed by two washes. The mononuclear cells were washed four times with Eagle's minimum essential medium and incubated in plastic tissue-culture dishes for 2 hr at 37°C to allow attachment of monocytes (*11*). After removal of nonadherent lymphocytes, the adherent monocytes were recovered by vigorous pipetting with 1% ethylenediaminetetraacetate.

The layer of red cells and granulocytes was applied to 10 ml of 3% dextran saline solution and the granulocytes were separated by sedimentation. In some studies, the granulocytes were further purified by passage through nylon fiber columns at 37°C. The cell numbers and radioactivity of each fraction were measured after separation and washing.

Viability assays. Leukocyte viability was assayed by supravital staining with trypan blue (*12*), and neutrophil viability was determined by phagocytosis of heat-killed staphylococci (*13*).

* Citrate phosphate dextrose consists of a solution of 3.0 gm of trisodium citrate dihydrate, 0.015 gm of sodium dehydrogen phosphate, and 0.2 gm of dextrose with distilled water to 100 ml (pH adjusted to 7.4).

TABLE 1. DEPENDENCE OF ^{99m}Tc UPTAKE BY LEUKOCYTES ON STANNOUS CHLORIDE CONCENTRATION AND INCUBATION TIME

Effect of concentration		Effect of incubation time	
μg/5 × 10^7 cells	cps/10^7 leukocytes* (mean)	Time (min)	cps/10^7 leukocytes* (mean)
0	1,140	0	6,810
0.1	7,060	5	21,660
1	15,980	10	27,670
10	32,080	15	28,580
100	30,480	30	31,970
		45	32,080
		60	30,140

5 × 10^7 leukocytes in 2 ml incubated with 100 μCi of ^{99m}Tc for 15 min and then either with $SnCl_2$ for 10 min using the concentrations shown or with 100 μg of $SnCl_2$ per 5 × 10^7 leukocytes for the times shown.

* Radioactivity of washed leukocytes; data submitted to analysis of variance (*14*). The standard error (s.e.m.) for the mean of each block of n samples (concentration or time) is: $\pm\sqrt{(\text{error variance})/n}$.

For each concentration mean, s.e.m. = ±1,120.

For each time mean, s.e.m. = ±640.

Variance ratios: for concentration, G between concentrations/within (4,5) = 151.61 ($p < 0.001$).

for incubation time, F between times/within (6,7) = 194.49 ($p < 0.01$).

least signicant differences: 2,160 for $p < 0.05$

3,190 for $p < 0.01$

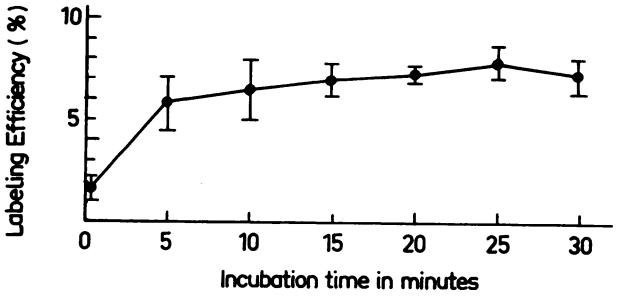

FIG. 1. Time course of ^{99m}Tc uptake by leukocytes suspended in ACD-A solution containing stannous chloride (100 μg per 2 ml; final pH 5.2). After incubation, cells were washed twice with ACD-A solution and resuspended in ACD-A prior to counting. Labeling efficiencies were calculated as

$$\frac{\text{cps/}10^7\text{ cells in washed cell suspension}}{\text{cps/}10^7\text{ cells in initial incubation mixture}} \times 100\%.$$

Each point represents mean of three experiments, and bars represent ±1 s.d. (individual observation).

Statistical methods. Conventional statistical techniques were used (*14*), including analyses of variance where appropriate.

RESULTS

Stannous chloride requirements. Leukocytes did not label in the absence of stannous chloride, and ascorbic acid was ineffective as an alternative reducing agent. With the addition of 100 μg of $SnCl_2 \cdot 2H_2O$ to 5 × 10^7 leukocytes, cell labeling approached a maximum at about 10 min and gained little thereafter (Table 1). Using an incubation of 15 min and the same quantity of leukocytes, the maximum cell labeling was found with 10 μg of $SnCl_2 \cdot 2H_2O$. Increasing the amount of $SnCl_2 \cdot 2H_2O$ to 100 μg caused no increase in labeling but had no adverse effect. In subsequent experiments, 100 μg of $SnCl_2 \cdot 2H_2O$ and 15 min incubation were used in order to allow for the possibility of higher cell numbers.

Rate of ^{99m}Tc uptake by leukocytes. In order to determine the optimal incubation time, leukocyte suspensions were incubated with ^{99m}Tc for 30 min at room temperature (Fig. 1). Duplicate aliquots were removed every 5 min, reduced for 15 min with

TABLE 2. RELATIONSHIP OF TEMPERATURE TO ^{99m}Tc UPTAKE BY LEUKOCYTES

Experiment No.	Leukocyte radioactivity (% of radioactivity at 37°C)		
	4°C	20°C	37°C
1	61.0 ± 2.0	95.6 ± 11.4	100.0 ± 9.0
2	—	91.7 ± 6.4	100.0 ± 5.7
3	43.0 ± 3.5	83.6 ± 5.7	100.0 ± 4.0

Cells incubated with ^{99m}Tc (100 μCi per 5 × 10^7 leukocytes) for 15 min at temperatures shown, followed by $SnCl_2$ reduction and washing at 20°C.

Each figure represents the mean (± s.e.m.) of 3–4 samples, expressed as a percentage of the labeling at 37°C.

Uptake at 4°C was significantly ($t = 4.11$, $p < 0.001$) less than at 20°C, while the uptakes at 20°C and at 37°C were not significantly different ($t = 1.52$, $0.2 > p > 0.1$) (method of mean paired differences).

TABLE 3. ELUTION OF ^{99m}Tc FROM REPEATEDLY WASHED LEUKOCYTES

No. of washings	Leukocyte activity (percent of initial mean)	No. of washings	Leukocyte activity (percent of initial mean)
0	100.0	5	15.4
1	29.3	6	15.3
2	17.8	7	14.1
3	15.8	8	13.5
4	15.4	9	12.7

Leukocytes labeled with ^{99m}Tc (1 mCi per 50 × 10^7 cells), treated with $SnCl_2$, and washed with ACD-A for the number of times shown. Aliquots removed for measurement of residual leukocyte activity.

Data submitted to analysis of variance (*14*). The standard error (s.e.m.) of the mean of each block of n samples is $\pm\sqrt{(\text{error variance})/n} = \pm 4.7$

Variance ratios: F between washings/within (9,10) = 32.69 ($p < 0.01$).

Least significant differences: for $p < 0.05$ = 14.7%

$p < 0.01$ = 21.0%

$p < 0.001$ = 30.4%

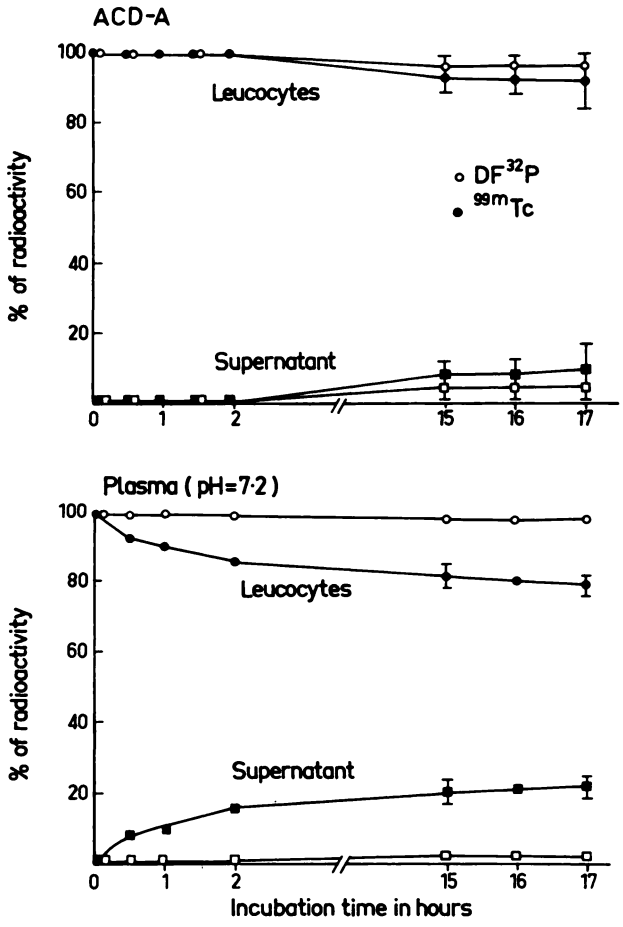

FIG. 2. (Top) Elution of ^{99m}Tc from leukocytes suspended in ACD-A was negligible over first 2 hr, although by 17 hr approximately 9% of total radioactivity (mean of five experiments) had been lost, compared with approximately 5% loss for ^{32}P-DFP labeling. (Bottom) Elution of ^{99m}Tc was much more marked in the presence of plasma (15% in 2 hr and 22% in 17 hr); that of ^{32}P-DFP in plasma was only about 3% by 17 hr. Bars represent ±1 s.d. of three experiments.

$SnCl_2$, and washed twice. The labeling efficiency, defined as

$$\frac{\text{cps}/10^7 \text{ washed cells}}{\text{cps}/10^7 \text{ cells in initial incubation mixture}},$$

measured after 10 min incubation in 30 separate experiments, was 8.4 ± 0.6% (mean ± s.e.m.). With the use of about 100 μCi of ^{99m}Tc for 5×10^7 cells, this efficiency corresponds to about 168,000 cps for 5×10^7 cells at t_0 and 10,500 cps for 5×10^7 cells at 24 hr.

Effect of temperature. The ^{99m}Tc uptake by leukocytes was similar at room temperature (20–25°C) and at 37°C (Table 2). In subsequent experiments all labeling was done at room temperature.

Effect of suspending medium. The labeling yields of leukocytes suspended in ACD-A (pH 5.2), CPD (pH 7.4), or plasma were compared. Cell labeling was found to be most effective in ACD-A: taking the labeling in ACD-A as 100%, that in CPD was 58 ± 4% ($p < 0.01$), that in plasma at pH 7.2 was 76 ± 6% ($p < 0.05$), and that in plasma at pH 5.2 was 62 ± 3% ($p < 0.01$).

Label elution. Two washings were sufficient to remove all loosely bound technetium. Up to nine further washings made no important difference (Table 3). For the elution studies, cell suspensions in ACD-A or ACD-A plasma were used at room temperature. At predetermined intervals, aliquots were removed and centrifuged. Elution of ^{32}P-DFP from labeled leukocytes was studied similarly for comparison. As shown in Fig. 2, elution of ^{99m}Tc from leukocytes suspended in ACD-A was negligible over the first 2 hr. However, by 17 hr a 9% loss had occurred, compared with a loss of 5% for ^{32}P-DFP.

TABLE 4. EFFECT OF THE SUSPENDING MEDIUM ON ^{99m}Tc ELUTION

Duration of incubation (hr)	Radioactivity of leukocytes remaining after incubation (%)						
	(1) ACD-A pH = 5.2	(2) Saline pH = 5.2	(3) Acidified plasma pH = 5.2	(4) CPD pH = 7.4	(5) Hank's BSS pH = 7.4	(6) ACD–plasma pH = 7.2	(7) Heparin–plasma pH = 7.4
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	97.7 ± 1.0	99.6	95.2	93.1	95.9 ± 2.2	88.7 ± 1.4	98.5
3	97.0 ± 1.7	99.7	94.1	90.6	94.3 ± 3.3	86.8 ± 0.5	89.9
5	96.1 ± 2.1	99.0	94.1	86.8	92.2 ± 2.1	84.1 ± 1.7	83.2
15	92.8 ± 1.9	91.9	93.0	72.6	71.9 ± 5.0	81.2 ± 2.0	77.7
17	91.0 ± 2.7	91.9	92.0	71.9	71.5 ± 4.7	77.7 ± 1.5	76.0
15–17	91.8 ± 1.6	91.9 ± 0	92.5 ± 0.5	72.2 ± 0.4	71.7 ± 2.8	79.4 ± 1.4	76.9 ± 0.9

Leukocytes labeled with ^{99m}Tc in ACD-A solution followed by $SnCl_2$ reduction and ACD-A washing were resuspended in the solutions shown. Residual cell activity was measured in cells from aliquots removed at the times shown.

Study 1 represents the mean ± s.e.m. of five experiments, and Studies 5 and 6 the mean ± s.e.m. of two experiments each.

Data from 15 and 17 hr were submitted to analysis of variance;

F between experiments/within $(6,7) = 121.2$ ($p < 0.01$).

Elution from cells incubated at pH 7.2–7.4 (Studies 4–7) significantly greater ($p < 0.001$) than from cells incubated at pH 5.2 (Studies 1–3).

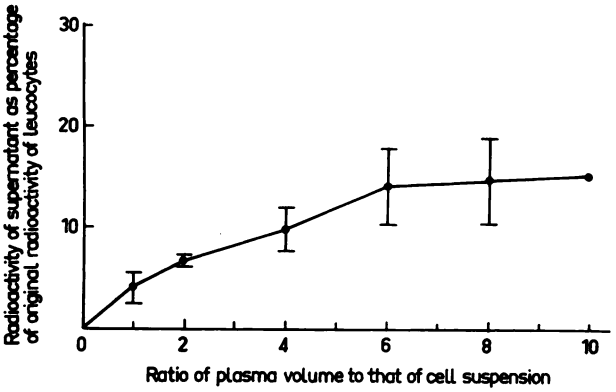

FIG. 3. Effect of increased plasma volume on elution. Elution of ^{99m}Tc by incubation of cells for 2 hr in presence of excess plasma was maximal when cell suspension was mixed with six times its volume of plasma. Bars represent mean ± 1 s.d. of duplicate experiments.

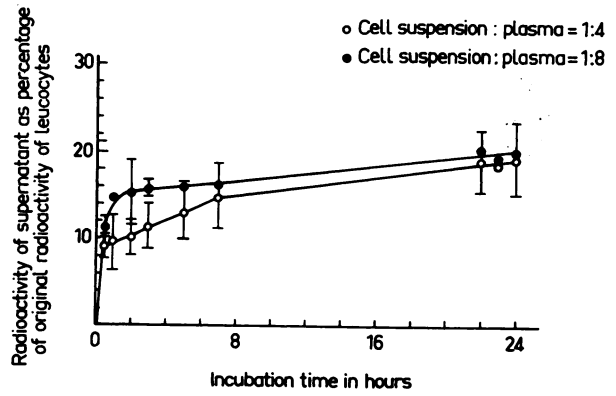

FIG. 4. Time course of ^{99m}Tc elution from labeled leukocytes suspended in four volumes of plasma (○) or in eight volumes of plasma (●). Points represent mean ± 1 s.d. of seven experiments (1:4) or two experiments (1:8).

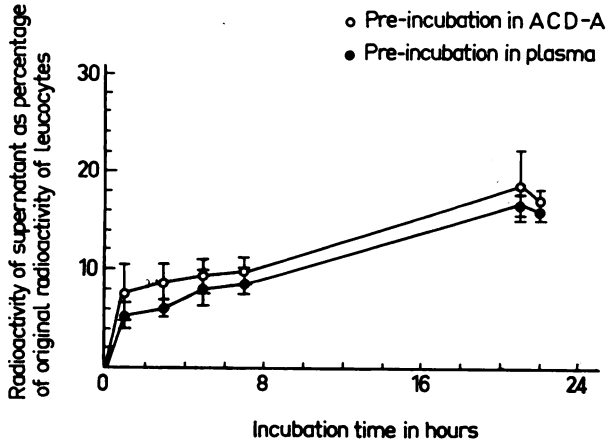

FIG. 5. Effect of preincubation of labeled cells with plasma. Leukocytes labeled with ^{99m}Tc were incubated in plasma and ACD-A for 2 hr, followed by centrifugation and resuspension with plasma. Preincubation of labeled cells with plasma for 2 hr did not alter subsequent loss of label, compared with cells preincubated with ACD-A. Mean ± 1 s.d. of duplicate experiments shown.

The elution of ^{99m}Tc was much more marked in plasma, with a 15% loss at 2 hr and 22% at 17 hr. The elution of ^{32}P-DFP into plasma was only about 3% in the same time.

The effect of the suspending medium on ^{99m}Tc elution was studied further (Table 4). Elution was significantly greater ($p < 0.01$) in neutral solutions (CPD, Hank's balanced salt solution, or plasma) than at pH 5.2 (ACD-A, buffered saline, or acidified plasma) (*15*). In these studies the volume of the suspending medium was four times the volume of the cell suspension. Increasing the plasma volume led to increased elution of the label: elution was maximal when the cell suspension was mixed with six times its volume of plasma (Fig. 3). Most of the plasma-associated elution had occurred by 2 hr, with not much more at 24 hr (Fig. 4). Although greater elution occurred into plasma, preincubation of labeled cells with plasma for 2 hr did not alter the subsequent loss of label, compared with cells preincubated with ACD-A only (Fig. 5).

Relative labeling of erythrocytes, granulocytes, lymphocytes, and monocytes. In cell suspensions containing roughly equal numbers of leukocytes and erythrocytes, the mean ^{99m}Tc uptake by leukocytes was 5.5 times that by red cells, and the specific activity of the leukocytes was similar to that observed in pure leukocyte suspensions (Table 5). The presence of excess numbers of red cells decreased the individual uptakes of both leukocytes and erythrocytes, although the ratio of leukocyte to erythrocyte activity was significantly increased.

The relative labeling of granulocytes, lymphocytes, and platelets is shown in Table 6. The activity of

TABLE 5. RELATIVE LABELING OF LEUKOCYTES AND ERYTHROCYTES BY ^{99m}Tc

Ratio of leukocytes to erythrocytes	Percent of activity in pure leukocyte suspension		
	Leukocytes (L)	Erythrocytes (E)	Ratio L/E
1:1	92.3 ± 3.1	16.7 ± 1.7	5.5 ± 0.5
	$p < 0.01$	$p < 0.05$	$p < 0.01$
1:10	63.9 ± 0.9	6.0 ± 0.8	10.7 ± 1.6
	$p < 0.001$	$p < 0.01$	
1:100	23.9 ± 2.1	2.4 ± 0.1	10.0 ± 0.6
Erythrocytes only		4.6 ± 1.0	

Mixtures of leukocytes and erythrocytes in the proportions shown were incubated with ^{99m}Tc, reduced with $SnCl_2$, and washed twice. Activity (cps per 10^7 cells) of isolated leukocytes or erythrocytes (see text) expressed as percentage of activity in control suspensions of pure leukocytes labeled with ^{99m}Tc at the same time.

Significant differences between adjacent means (two-tailed t test) as shown.

granulocytes isolated by centrifugation through a Ficoll–Hypaque gradient was similar to the activity of granulocytes further purified by passage through nylon fiber columns. The mononuclear cell fraction, obtained by Ficoll–Hypaque centrifugation, was further separated by allowing the monocytes to adhere to plastic tissue-culture plates in tissue-culture medium, leaving the nonadherent lymphocytes in suspension. Assuming a specific activity for granulocytes of 100%, the relative labeling for lymphocytes was 26.6%, for monocytes 27.9%, and for platelets 6.2%.

The practicality of isolating labeled granulocytes directly from whole-blood or buffy-coat preparations labeled with ^{99m}Tc was investigated further. The activity trapped on nylon fiber columns through which labeled whole buffy-coat suspensions or unwashed granulocyte-rich fractions from a Ficoll–Hypaque gradient had been passed was greater than that found when washed gradient-separated granulocytes were used. When either plasma alone or red cells alone were labeled with ^{99m}Tc and passed through nylon fiber, the uptake of activity was negligible (0.6% and 2.0%, respectively). In the case of whole buffy coat or unwashed granulocytes, the increased adherent activity was shown to be due to the adherence of labeled platelets.

Viability of labeled leukocytes. Supravital staining with trypan blue (*12*) following ^{99m}Tc labeling of leukocytes showed fewer than 5% dead cells. The phagocytic indices of neutrophils (*13*) before and after ^{99m}Tc labeling did not differ significantly from each other or from the mean normal value found in our laboratory (Table 7).

TABLE 6. RELATIVE LABELING OF GRANULOCYTES, MONONUCLEAR CELLS, AND PLATELETS BY ^{99m}Tc

Lymphocytes (%)	Monocytes (%)	Platelets (%)
26.6 ± 1.5	27.9 ±1.5	6.2 ± 0.8

Leukocyte- and platelet-rich cell suspensions were labeled with ^{99m}Tc, with $SnCl_2$ reduction and washing, and the activities (in each case expressed as percent of granulocyte labeling) of isolated washed lymphocytes, monocytes, and platelets were measured. Each value is the mean ± s.e.m. of 3–5 experiments.

TABLE 7. PHAGOCYTIC ACTIVITY OF ^{99m}Tc-LABELED GRANULOCYTES

Separated white cells	Phagocytic index (mean ± s.e.m.)
Before labeling	28.7 ± 1.4
After labeling	30.4 ± 0.4

The phagocytic activity of granulocytes suspended in ACD-A plasma was measured, before and after labeling with ^{99m}Tc, using a heat-killed suspension of staphylococci (13). In each sample, the phagocytic index was determined as the mean number of organisms ingested per cell in a count of 100 neutrophils. Each value represents the mean of six samples. The difference between the means was not significant ($0.3 > p > 0.2$).

DISCUSSION

The data presented here show that neutrophils can be labeled in vitro with ^{99m}Tc. Labeling is complete when the cells are incubated for a minimum of 5 min with the tracer and then for a minimum of 10 min with stannous chloride, the latter step being essential. Labeling is equal at room temperature and at 37°C and is greater in the absence of plasma and in acid (pH 5.2) rather than in neutral solutions (pH 7.2–7.4). Elution is increased in neutral rather than acid (pH 5.2) solutions and in the presence of excess plasma. In acid plasma, the elution of ^{99m}Tc and ^{32}P-DFP are both similarly low, but in neutral plasma the elution of ^{99m}Tc over 15 hr is greater than that for ^{32}P-DFP.

Neutrophils label better with ^{99m}Tc than the other blood cells, the order of affinity being neutrophils, monocytes and lymphocytes, erythrocytes, and platelets. This differs from the relative affinity of blood cells for ^{51}Cr, which labels large lymphocytes and monocytes more heavily than small lymphocytes, neutrophils, erythrocytes, and platelets in that order (*16*). The mechanism by which ^{99m}Tc is incorporated into leukocytes is unknown. In sarcoma I cells in mice, ^{99m}Tc has been shown to label nuclear mitochondrial and microsomal fractions (*17*). In the case of red cells (*18,19*), ^{99m}Tc from pertechnetate, like the chromate ion, binds to the beta chain of hemoglobin, the binding being made irreversible by reduction (*20*).

Technetium-99m has been used as a cell label in studies of cell-mediated immunity in vitro (*17,21*). Its greater usefulness, however, is likely to be in the study of cell migration and organ uptake using external scanning techniques. Its use in the study of neoplastic cell migration (*22*), platelet sequestration and destruction (*23*), and the organ distribution of leukocytes (*8*) has already been reported. As a neutrophil label in man, it could prove of great value, particularly in investigating the role of the spleen in neutropenia. Although the leukokinetic studies in neutropenic patients reported by Bishop et al. (*24*), Vincent et al. (*25*), and Uchida and Kariyone (*26*) all indicate that margination is important in cases of neutropenia associated with splenomegaly, the inability to scan cells labeled with ^{32}P-DFP has made

it difficult to prove that the spleen is the site where neutrophils are marginated. Studies following the reinfusion of autologous ^{99m}Tc-labeled cells could overcome this difficulty.

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