# Biological Behavior of Erythrocytes Labeled In Vivo and In Vitro with Technetium-99m

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***The in vitro method of red blood cell labeling was compared to the commonly used in vivo technique to evaluate labeling efficiency, levels of radioactivity in cells, plasma, and urinary excretion. Comparative studies were performed on nine normal volunteers. In two subjects, in vivo labeling did not occur. In the other subjects, the maximum blood activity (5 min after administration for the in vitro method and 15–120 min for the in vivo method) was 18% higher with the in vitro method ($p < 0.005$). Whole body retention of radioactivity was higher (78.7 ± 7.9%) with the in vivo method. With the in vitro method it was 66.3 ± 9.0% ($p < 0.02$).***

***It is concluded that the in vitro method of red cell labeling provides significantly higher blood levels of radioactivity at the times required for data collection but reduced whole body retention over 24 hr.***

Labeled erythrocytes are used frequently in nuclear medicine laboratories for gated cardiac blood pool imaging as well as for a variety of other purposes such as gastrointestinal blood loss imaging, spleen imaging, venography, and hemangioma detection. The method most widely used for erythrocyte labeling is by an in vivo technique (*1*). An alternate method uses an in vitro labeling technique (*2*), to which several modifications (*3*) have been introduced. The in vivo technique has also been modified to improve labeling efficiency (*4*). This study compares the in vivo method with the in vitro method with respect to biodistribution and whole body retention parameters.

## MATERIALS AND METHODS

Nine hematologic normal male volunteers were studied twice, once with the in vitro method and a week later with the in vivo method. The in vitro method was used initially in order to avoid any effects of retained tin-pyrophosphate from the in vivo method.

In vitro labeling of erythrocytes with $^{99m}$Tc was accomplished with the use of a modification of the Brookhaven National Laboratory kit described by Smith and Richards (*3*). The contents of the lyophilized kit were 3.6 mg trisodium citrate dihydrate, 5.5 mg dextrose and 2 μg tin as stannous ion. Four ml of blood were drawn from a peripheral vein into a syringe pre-wetted with 1:1000 heparin solution. The blood was emptied into the kit and incubated for 5 min with mixing. Six milliliters of normal saline were then added to the kit and centrifuged for 5 min at 1,500 rpm with the rubber top down. Approximately 1.5–2.0 ml of packed red cells were then removed and added to an evacuated vial containing 1 mCi (37 MBq) [$^{99m}$Tc]pertechnetate. After 5–10 min incubation, the contents were divided into two aliquots, one for preparation of standards and the other for administration to the subject.

In vivo labeling was accomplished by the method of Pavel (*1*). The subject was administered 0.10 mg/kg of stannous (9.6–15 μg tin) pyrophosphate intravenously, followed in 30 min by 500 μCi (18.5 MBq) of [$^{99m}$Tc]sodium pertechnetate.

At the time of the in vitro labeling study, the subject also had a $^{51}$Cr red cell labeling study performed to measure the dilution volume of labeled erythrocytes. Sixteen milliliters of blood were drawn into a syringe containing 4 ml of modified acid-citrate-dextrose solution. The mixture was emptied into a siliconized vial to which was added 30 μCi (1.11 MBq) $^{51}$Cr-sodium chromate. Labeling was stopped at 30 min by addition of 50 mg ascorbic acid. After saving aliquots for standards and hematocrit, the $^{51}$Cr-labeled cells were administered intravenously at the same time as the in vitro labeled $^{99m}$Tc cells.

Blood samples were obtained at 5, 15, and 30 min, and 1, 2, 4, 8, 12 and 24 hr after administration of tracer. All urine was collected as separate samples over 24 hr. Radioactivity was assayed in a well counter for whole blood, plasma, and urine. Patient hematocrit and dilution volume were determined by standard methods. All samples were corrected for radioactive decay and background in keeping with standard analytical methods.

The concentration of radioactivity in whole blood was

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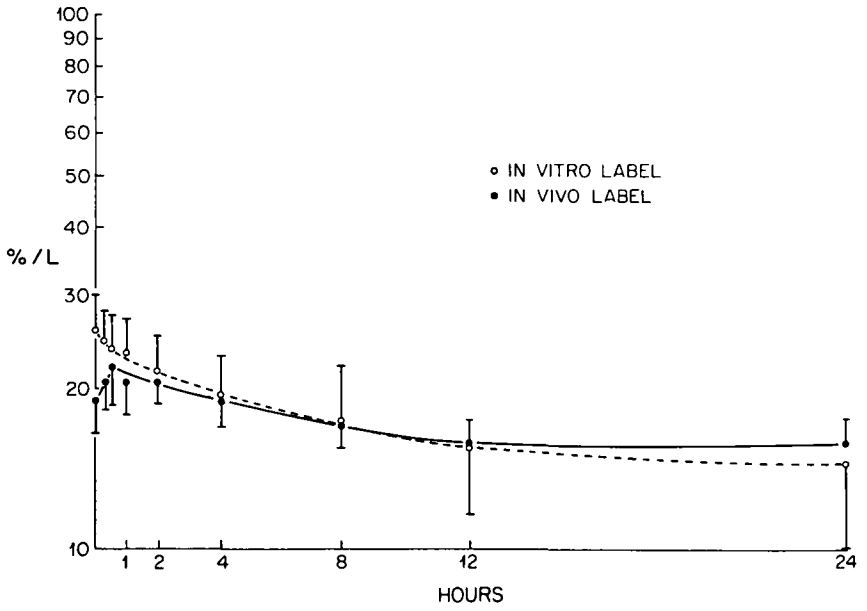


**FIG. 1.** Time-activity curve for $^{99m}$Tc labeled-red blood cells in whole blood normalized to 70 Kg whole body weight.

expressed as percent administered activity per liter and then normalized to a 70 kg body weight for comparison of subjects and to obtain mean values. The total amount of the administered activity in the blood pool of each subject was calculated by multiplying the percent administered activity per liter times the dilution volume in liters determined with the $^{51}$Cr-labeled erythrocytes. The fraction of $^{99m}$Tc in the red cells was determined by counting whole blood and plasma separately and subtracting plasma concentration times plasmacrit from the whole blood activity. Whole body activity was plotted on semi-logarithmic paper and the disappearance constants determined by curve stripping for each individual subject. Whole body activity is the reciprocal of urinary excretion.

## RESULTS

Whole blood activity normalized to 70 kg body weight is presented in Table 1. Maximum whole blood activity (percent administered activity/liter) was usually not reached until at least 30 min with the in vivo labeling method, whereas

**TABLE 1. Concentration of $^{99m}$Tc-Labeled Erythrocytes in Blood (Normalized to 70 kg Body Weight)**

| | In Vitro Labeling % Administered Activity/Liter at: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Patient** | **5 min** | **15 min** | **30 min** | **1 hr** | **2 hr** | **4 hr** | **8 hr** | **12 hr** | **24 hr** |
| 1 | 29.09 | 25.44 | 24.06 | 23.21 | 22.59 | 20.19 | — | 15.66 | 14.48 |
| 2 | 21.27 | 19.56 | 18.72 | 18.57 | 16.60 | 15.45 | 12.08 | 11.43 | 10.21 |
| 3 | 23.92 | 22.87 | 22.03 | 20.45 | 18.69 | 16.42 | 14.56 | 12.34 | — |
| 4 | 32.23 | 30.67 | 30.76 | 30.13 | 27.30 | 25.41 | 20.68 | 18.90 | 16.56 |
| 5 | 22.68 | 24.09 | 20.74 | 21.68 | 18.45 | 18.10 | — | 13.90 | 12.09 |
| 6 | 21.67 | 19.94 | 20.10 | 19.87 | 18.60 | 17.48 | 14.52 | 12.90 | 11.68 |
| 7 | 29.40 | 26.43 | 26.37 | 24.57 | 23.32 | 20.64 | 17.01 | 15.61 | 13.01 |
| 8 | 28.36 | 25.63 | 25.45 | 25.31 | 22.92 | 20.00 | 17.73 | 15.07 | 13.32 |
| 9 | 27.48 | 26.86 | 25.95 | 25.51 | 22.75 | 20.12 | 16.69 | 14.54 | 13.20 |
| Mean | 26.23 | 24.61 | 23.80 | 23.26 | 21.25 | 19.31 | 16.18 | 14.48 | 13.07 |
| s.d. ± | 3.93 | 3.49 | 3.78 | 3.57 | 3.37 | 2.94 | 2.76 | 2.22 | 1.90 |
| | | | | In Vivo Labeling | | | | | |
| 1 | 16.94 | 18.39 | 19.81 | 19.55 | 18.28 | 17.22 | 15.80 | 14.46 | 14.17 |
| 2 | 17.87 | 17.72 | 18.09 | 17.99 | 17.20 | 16.01 | 14.44 | 14.06 | 13.56 |
| 3 | 21.34 | 22.86 | 22.40 | 18.66 | 22.34 | 20.69 | 18.88 | 17.85 | 17.26 |
| 5 | 16.49 | 19.68 | 20.53 | — | 21.15 | 19.58 | 17.74 | 16.85 | 17.14 |
| 6 | 17.01 | 18.30 | 18.63 | 18.76 | 19.98 | 17.69 | 15.93 | 12.30 | 14.06 |
| 8 | 23.16 | 24.15 | 22.95 | 22.14 | 23.02 | 20.32 | 18.37 | 16.12 | 16.47 |
| 9 | 20.22 | 22.82 | 23.73 | 23.53 | 22.83 | 21.56 | 19.21 | 17.56 | 17.97 |
| Mean | 19.00 | 20.56 | 20.88 | 20.11 | 20.69 | 19.00 | 17.20 | 15.60 | 15.80 |
| s.d. ± | 2.58 | 2.64 | 2.19 | 2.22 | 2.29 | 2.04 | 1.81 | 2.05 | 1.82 |

**TABLE 2. Maximum Percent Administered Activity in Whole Blood**

| Patient | In Vitro Label (5 min) | In Vivo Label (max) |
|---|---|---|
| 1 | 100.0 | 68.1** |
| 2 | 108.6 | 84.2** |
| 3 | 94.5 | 90.3* |
| 4 | 109.9 | — |
| 5 | 80.6 | 75.2† |
| 6 | 100.0 | 92.2† |
| 7 | 113.7 | — |
| 8 | 104.4 | 88.9† |
| 9 | 100.0 | 86.4** |
| mean ± s.d. | 101.3 ± 9.8 | 83.6 ± 8.8 |

*15 min.
**30 min.
†2 hr.

maximum activity was present at 5 min, the earliest sample, in subjects when the in vitro labeling method was used. Furthermore, when maximum levels of radioactivity were compared (Table 2), there was a rather consistent 18% higher level of activity in the blood with the in vitro method as compared to the in vivo method (101.3 ± 9.8% as opposed to 83.6 ± 8.8%, $p < 0.005$). These results are comparable to those obtained by Hegge, et al. (*5*).

Subjects 4 and 7 had no apparent labeling of red blood cells with the in vivo method, with the distribution being 26.2–26.7% in plasma and 23.6–27.9% in whole blood at 30 min, not significantly different than observed after administration of [$^{99m}$Tc]pertechnetate without pretreatment with stannous pyrophosphate (*6*). These data were omitted in the summary tables. Labeling efficiency for the in vitro method was consistently good (94.6 ± 3.3%, range 90.2–97.6%).

Distribution of blood radioactivity within the red cell and plasma fractions (Table 3) was essentially similar with both methods. The slight differences were not statistically significant.

Whole blood levels of radioactivity were consistently higher using the in vitro method until 8 hr, at which time the in vivo method resulted in higher blood levels (Fig. 1). This was confirmed by urinary excretion data (Table 4) which showed a somewhat higher whole body retention at 24 hr for the in vivo method (78.7%) as compared to the in vitro method (66.3%) where $p < 0.02$.

The whole body retention curve derived from urinary excretion could usually be described by a biexponential curve although in one subject both the in vivo and the in vitro study were best described by a monoexponential curve and in another subject the in vivo study appeared to be monoexponential. The in vivo method of labeling resulted in a short $T_{1/2}$ component of 2.5 ± 0.7 hr (10.9 ± 6.1%) and a long $T_{1/2}$ component of 176.6 ± 163.6 hr (90.5 ± 5.0%), whereas the in vitro method resulted in whole body retention components of 2.7 ± 1.5 hr (25.4 ± 10.4%) and 75.6 ± 25.3 hr (82.2 ± 7.7%).

## DISCUSSION

Despite its several drawbacks, the in vivo method of erythrocyte labeling with $^{99m}$Tc is widely used mainly because of the following convenience factors: a) an IND application is not required and investigative consent need not be obtained; b) less manipulation of materials and technician time is required; and c) there are no problems with mixing of blood samples when many patients are examined at the same time.

Major disadvantages of the in vivo method are poorer labeling efficiency and lower blood levels of radioactivity as well as occasional nonlabeling. In addition, as Hegge, et al. (*5*) have shown, background counts tend to be much higher.

In vitro labeling of erythrocytes results in higher blood levels of activity and considerably reduced background, enabling the use of a smaller amount of administered activity or of shorter acquisition times. Furthermore, the long term (24 hr)

**TABLE 3. Whole Blood, Plasma, and Red Cell Concentrations of $^{99m}$Tc Label (% Administered Activity/Liter)**

| | | | In Vitro | | | In Vivo | | |
|---|---|---|---|---|---|---|---|---|
| Patient | Dilution* Volume (ml) | Hematocrit | Whole Blood at 5 min | Plasma at 5 min | % in Red Blood Cells | Max % Whole Blood | Plasma at 30 min | % in Red Blood Cells |
| 1 | 3,521 | 0.455 | 28.40 | 2.58 | 95.0 | 19.34 | 1.42 | 96.0 |
| 2 | 5,683 | 0.409 | 19.11 | 2.89 | 91.1 | 14.81 | 1.05 | 95.8 |
| 3 | 4,103 | 0.439 | 23.02 | 6.08 | 85.2 | 22.00 | 1.76 | 95.4 |
| 4 | 3,761 | 0.415 | 29.22 | 2.74 | 94.5 | — | — | — |
| 5 | 3,599 | 0.442 | 22.39 | 3.72 | 90.7 | 20.88 | 1.30 | 96.4 |
| 6 | 4,616 | 0.415 | 29.22 | 1.20 | 96.8 | 19.98 | 1.09 | 96.7 |
| 7 | 5,233 | 0.459 | 21.72 | 2.74 | 93.6 | — | — | — |
| 8 | 4,313 | 0.419 | 24.20 | 3.80 | 90.9 | 20.61 | 1.49 | 95.6 |
| 9 | 3,405 | 0.387 | 29.37 | 1.87 | 96.1 | 25.36 | 1.83 | 95.6 |
| Mean | — | — | — | — | 92.6 | — | — | 95.9 |
| s.d. ± | — | — | — | — | 3.6 | — | — | 0.5 |

*Determined by $^{51}$Cr.

**TABLE 4. Retention of $^{99m}$Tc at 24 Hr (% Administered Activity)**

| Patient | In Vitro Label | In Vivo Label |
|---|---|---|
| 1 | 70.1 | 78.0 |
| 2 | 85.2 | 82.3 |
| 3 | 56.5 | 91.4 |
| 4 | 72.2 | — |
| 5 | 59.8 | 68.1 |
| 6 | 68.9 | 83.0 |
| 7 | 58.6 | — |
| 8 | 60.6 | 70.8 |
| 9 | 64.5 | 77.2 |
| Mean ± s.d. | 66.3 ± 9.0 | 78.7 ± 7.9 |

retention of radioactivity appears to be less which would result in a lower radiation absorbed dose to the patient.

The Brookhaven National Laboratory in vitro labeling kit is presently undergoing Phase III trials by a commercial sponsor* prior to a new drug application to the United States Food and Drug Administration. If approved in the near future, as expected, the greatest impediment to the use of this more reliable method on a widespread scale would be removed.

## FOOTNOTE

*Cadema Medical Products, Middletown, NY.

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