Tc-99m-Labeled Red Blood Cells for the Measurement of Red Cell Mass in Newborn Infants: Concise Communication

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In vitro and in vivo investigations were performed to examine the binding of Tc-99m to neonatal red blood cells (RBC). Labeling efficiency was about 90%, and unbound Tc-99m less than 3% after one washing, in premature and full-term newborns and in children. Thus presence of high percentages of fetal hemoglobin (Hb F) did not influence the labeling of RBCs with Tc-99m. RBCs of 11 newborns were hemolysed and the distribution of Tc-99m on RBC components was analyzed. Although Hb F percentage averaged (60.0 ± 8.1)% (s.d.), only (11.9 ± 3.7)% of Tc-99m was bound by Hb F, whereas (45.0 ± 6.1)% was associated with Hb A. RBC membranes bound (13.7 ± 4.3)% and (29.3 ± 4.0)% were found unbound in hemolysates. These results indicate that Tc-99m preferentially binds to beta chains. In vivo equilibration of Tc-99m RBCs and of albumin labeled with Evans blue was investigated in five newborn infants. Tc-99m RBCs were stable in each case during the first hour after injection. Elution of Tc-99m from RBCs was (3.4 ± 1.5)% per hour. Body-to-venous hematocrit ratio averaged 0.86 ± 0.03.

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Low values of red-cell mass at birth are associated with high morbidity and a high mortality rate (*1,2*). Therefore, an accurate method for red-cell-mass determination that is not harmful to the patient and gives rapidly available results is desirable for newborns at risk. Measurements of RBC mass in newborn infants have been performed using P-32 (*3*), Cr-51 (*4,5*), and nonradioactive Cr-50 (*1*). The high radiation dose from Cr-51 and P-32 and the requirement of equipment for neutron activation of Cr-50 explain why reports on RBC mass determinations in newborn infants are extremely rare. The utility of Tc-99m for the labeling of red blood cells has been clearly established by several investigators (*6–13*). The usefulness of Tc-99m RBCs in newborns cannot be derived from studies in adults, since Tc-99m binds preferentially with the beta chains in adult hemoglobin (Hb A_1) (*14*). In fetal hemoglobin (Hb F) the beta chains are replaced by gamma chains. The present study attempted to examine the binding of Tc-99m with neonatal RBCs and Hb F, and to study the stability of the label in vivo.

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METHODS

We used a commercial freeze-dried reagent kit,* containing 0.02 mg of $SnCl_2$ and 40 mg of human serum albumin in a vial. Sodium pertechnetate was eluted daily from a Tc-99m generator. Activities were measured in a well scintillation counter. All Tc-99m activities were corrected for decay by reference to a standard.

RBC labeling. Four ml of blood were drawn in a 5-ml sterile syringe containing 0.8 ml of ACD solution. The kit was dissolved by adding of 5 ml of preservative-free 0.9% NaCl solution and careful mixing. One milliliter each of this solution and the blood were transferred to a sterile tube and gently mixed for 5 min at room temperature. The mixture was centrifuged for 10 min at 1,000 *g* and 10°C. The eluate of Tc-99m was diluted with 0.9% NaCl so that 1 ml contained 0.5 μCi of Tc-99m per kg of body weight of the patient. Using a 20-

TABLE 1. LABELING EFFICIENCY AND UNBOUND Tc-99m AFTER ONE WASHING (IN PERCENT ± 1 s.d.)

	Newborns (gestational age in wk)			
	26–30	31–36	37–40	Children
n	7	7	15	11
Labeling efficiency	90.9 ± 2.7	88.7 ± 3.1	90.3 ± 3.5	89.5 ± 4.6
Unbound Tc-99m	1.8 ± 1.1	2.1 ± 0.7	2.2 ± 0.8	2.2 ± 0.7

gauge (1.2 × 75 mm) needle, 1 ml of the packed RBCs was withdrawn into a 2-ml syringe containing 1 ml of the diluted Tc-99m. The preparation was incubated for 15 min at room temperature with repeated gentle mixing. The mixture was transferred to a sterile tube, diluted with 2 ml of 0.9% NaCl, and centrifuged at 10°C for 10 min at 1000 *g*. The supernate was removed.

Radioactivity of the supernate and the labeled RBCs was determined. Percent labeling efficiency was expressed as 100 times the ratio of radioactivity in the RBCs to the sum of radioactivity in RBCs and supernate. The labeled RBCs were resuspended in 1 ml of 0.9% NaCl solution. One milliliter of this preparation, suitable for i.v. injection, contained about 0.25 μCi of Tc-99m per kg of body weight of the patient. Percentage of unbound Tc-99m in this mixture was determined by diluting 0.5 ml in 2 ml of 0.9% NaCl and counting the radioactivity in the supernate and the RBCs after centrifugation.

In vitro studies. RBCs of 31 newborn infants ages less than 24 hr, and of 11 children ages 2–7 yr, were labeled with Tc-99m. The blood samples of 15 newborns were taken from the placenta. In the other cases, blood was withdrawn before clinically indicated blood transfusion or exchange transfusion. Labeling efficiency and unbound Tc-99m after one washing was determined in each case.

In 11 placental blood samples from healthy newborn infants, binding of Tc-99m to Hb F, Hb A, and red-cell membranes was determined. For this purpose 20 ml of placental blood were collected in 5 ml ACD solution and labeled with Tc-99m using 5 ml of the dissolved kit and 20 μCi of Tc-99m. The labeled RBCs were lysed with distilled water. Hb F was quantified by means of the alkali denaturation test (*15*). This method was also used for the separation of Hb F from Hb A and the RBC membranes. One-fifth milliliter of 1.2 *N* NaOH was added to 2.8 ml of the hemolysate. Exactly 2 min later the denaturated Hb A was precipitated with 2 ml of saturated ammonium sulfate solution and the Hb F remaining in the solution was filtered. Another aliquot of hemolysate was mixed with carbon tetrachloride, incubated for 30 min at 4 °C, and centrifuged. The supernate contains the hemoglobin and only slight traces of membrane proteins. Three different hemoglobin solutions were obtained by this procedure: (a) Hb A + Hb F + RBC membranes; (b) Hb A + Hb F; and (c) Hb F. A fraction of each of the hemoglobin solutions was precipitated with freshly prepared 10% trichloracetic acid. The activities in the hemoglobin solutions and in the supernates were counted. Percent distribution of Tc-99m on Hb A, Hb F, and RBC membranes and the free activities were calculated.

Studies on patients. In five newborn infants, autologous RBCs were labeled by the described procedure. These patients were in the neonatal intensive care unit

TABLE 2. Hb F PERCENTAGE AND Tc-99m DISTRIBUTION ON COMPONENTS OF RBC

	Hb F (%)	Tc-99m distribution (%)			
		Hb A	Hb F	Membranes	Unbound
	47.8	48.2	13.5	15.3	23.0
	48.7	49.1	7.5	7.5	35.9
	53.6	34.3	13.2	20.4	32.1
	53.8	53.0	4.1	16.1	26.8
	61.0	38.5	13.4	18.4	29.7
	61.1	40.6	14.3	14.4	30.7
	62.5	49.0	11.1	6.8	33.1
	65.0	46.7	11.3	14.2	27.8
	65.7	38.0	14.7	14.8	32.5
	69.3	47.7	17.6	9.4	25.3
	71.9	50.2	10.7	13.9	25.2
$\bar{x}$	60.0	45.0	11.9	13.7	29.3
s.d.	8.1	6.1	3.7	4.3	4.0

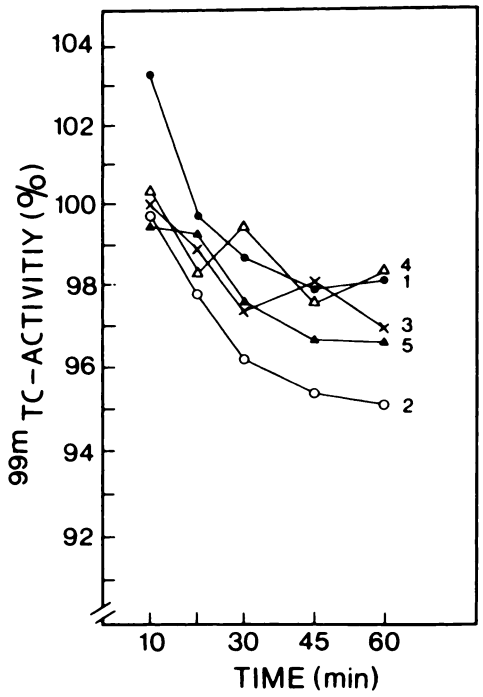

FIG. 1. Tc-99m activity of blood in five newborns as function of time after injection of Tc-99m RBCs. Activities are expressed as percentages of extrapolated zero-time values. Numbers refer to cases in Table 3.

because of severe maladaptation to extrauterine life. Blood volume measurements were clinically indicated, each indication being approved by the ethical commitee of our hospital. Informed consent was obtained from parents in each case. Equal volumes of labeled RBCs containing 0.25 μCi per kg of body weight were injected intravenously and used as standard. Plasma volume was measured simultaneously by injection of 0.2 mg Evans blue dye per kg of body weight (*16*). The thyroid gland was blocked by administration of one drop of Lugol's solution per kg of body weight 1 hr before and for 5 days after injection of the radionuclide. Five blood samples of 1.5 ml were taken at 10, 20, 30, 45, and 60 min after injection of the tracers. The blood samples were withdrawn from the umbilical artery or venous catheters before clinically indicated blood transfusion or exchange transfusion, respectively. Microhematocrit was determined in all blood samples and corrected for 2% of trapped plasma (*16*). The radioactivity of 1 ml of each blood sample was counted immediately for Tc-99m activity. The counts of each equilibration study were plotted on semilogarithmic paper and the mixing time estimated visually. The Tc-99m counts were extrapolated to zero time by the method of least squares after logarithmic tranformation (*17*). The elution rate of Tc-99m in %/hr was calculated from the counts at times zero and 60 min, as derived from the regression equation. Extrapolation to zero time and calculation of the disappearance rate of Evans blue dye were performed in the same way (*16*). The body hematocrit was calculated from red-cell mass divided by blood volume.

RESULTS

The labeling efficiency was about 90% in the RBCs from the premature and full-term infants and the children (Table 1). Less than 3% of free Tc-99m was noted after one washing.

Table 2 shows the Tc-99m distribution pattern in neonatal RBCs. Technetium-99m was associated mainly with Hb A. Markedly less activity was bound with Hb F, though the Hb F fraction was greater than 50% in most batches. About 30% of the activity in the hemolysates was unbound. Unbound Tc-99m did not change markedly during the separation procedure. The values of unbound Tc-99m in Table 2 are those measured in the supernate of the original hemolysate.

The results of the in vivo studies are shown in Fig. 1. The activities are expressed as percentages of the extrapolated zero-time values. In Patient 1, the 10-min value was omitted in calculation of the zero-time value, because mixing was delayed to 20 min. A slow rate of disappearance was noted in all patients. Elution of Tc-99m from RBCs ranged from 2.1 to 5.7% per hr (Table 3). The body-to-venous hematocrit ratio averaged 0.86 ± 0.03.

DISCUSSION

Our study has shown that Tc-99m binds preferentially

TABLE 3. BLOOD VOLUME DATA IN NEWBORN INFANTS

Case	Diagnosis	Birth weight (g)	Plasma volume (ml/kg)	Red-cell mass (ml/kg)	Body-to-venous hematocrit ratio	Tc-99m elution (%/hr)
1	RDS*	1420	57.8	31.8	0.82	2.1
2	RDS	1890	55.3	34.0	0.85	5.7
3	Sepsis	2580	54.9	34.7	0.89	3.2
4	Erythroblastosis	2800	68.4	28.3	0.85	2.1
5	Sepsis	3120	51.2	38.5	0.88	3.7

* RDS = respiratory distress syndrome (Ref. 2).

with Hb A. This confirms the results of Dewanjee (*14*), who found Tc-99m bound mainly with the beta chains of Hb A_1, which are replaced by gamma chains in Hb F. The percentage of Hb F in neonatal RBCs depends on gestational age; it is about 80% in premature infants with 30–35 wk of gestation and about 65% in full-term infants (*18*). However, our results have demonstrated that the labeling of RBCs with Tc-99m is not impaired by a high percentage of Hb F. After hemolysis of neonatal RBCs, a large fraction of Tc-99m was found unbound. It appears likely that this fraction was loosely bound within the RBCs and set free by hemolysis or addition of trichloracetic acid. Possibly the fraction of Tc-99m associated with the RBC membranes was also bound to hemoglobin attached to the membranes.

RBCs labeled with Tc-99m were found to be fairly stable in the circulation of newborn infants for the first hour. This agrees with the findings of other authors, who noted clearance rates of 3–7% during the first hour (*6–13*). The application of the double-label technique enabled us to calculate the body-to-venous hematocrit ratio (BH/VH). The mean BH/VH of 0.86 observed in the five newborn infants was lower than in healthy newborn infants (*4*), but agrees with the findings in severely sick newborns and children (*5,17*). Since marked elution of Tc-99m from RBCs would cause overestimation of red-cell mass, and thus of BH/VH, a relatively low BH/VH indicates reliability of the method.

The use of Tc-99m for the determination of the red-cell mass offers several advantages over the classical Cr-51 technique. The required radioactivity of 0.1 μCi/kg of Cr-51 results in a radiation dose of 2.5 mrad (*17*), which is markedly higher than the radiation dose of 0.5 mrad from 0.25 μCi/kg of Tc-99m (*11*). The short half-life of Tc-99m makes it suitable for repeated determinations of red-cell mass at short time intervals, which may be important in critically sick infants and children.

FOOTNOTE

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