SPLENIC SEQUESTRATION OF ^{99m}Tc-LABELED RED BLOOD CELLS

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Previous communications (*1,2*) have detailed the procedure and some of the variables involved in labeling erythrocytes with ^{99m}Tc. The objective of this study is to evaluate methods which would reduce the time necessary for firm labeling as well as to increase splenic localization. By chemical alteration it was hoped to provide a rapid, simple, and reliable procedure for splenic visualization. In this communication we describe the various methods employed for labeling and altering red blood cells as well as the results obtained with each method.

METHODS

Our basic method for labeling red blood cells with ^{99m}Tc has been described previously. It consists of adding pertechnetate in small volume to separated erythrocytes followed by reduction with stannous chloride in acid-citrate-dextrose (ACD) solution*, two washes in normal saline, and resuspension in saline.

In the patient studies to be discussed four variations of the previously published method (*1,2*) have been used to label as well as alter packed erythrocytes. In each instance, 16 ml of the patient's blood was drawn into a syringe containing 4 ml of ACD solution. The sample was centrifuged and the 4 ml of packed red blood cells were removed. The following variations of the procedure shown in Fig. 1 were carried out:

1. Excess ACD, long procedure: pertechnetate incubation for 30 min at 37°C, 0.1 mg $SnCl_2 \cdot 2H_2O$ in 0.1 ml ACD, and then 4 ml ACD added and incubated for 15 min at room temperature.
2. Excess $SnCl_2$, long procedure: pertechnetate incubation for 30 min at 37°C, 2 mg $SnCl_2 \cdot 2H_2O$ in 2 ml ACD added and incubated for 15 min at room temperature.
3. Excess ACD, short procedure: pertechnetate incubation for 5 min at room temperature, 0.1 mg $SnCl_2 \cdot 2H_2O$ in 0.1 ml ACD, and then 4 ml ACD added and incubated for 5 min at room temperature.
4. Excess $SnCl_2$, short procedure: pertechnetate incubation for 5 min at room temperature, 2 mg $SnCl_2 \cdot 2H_2O$ in 2 ml ACD for 5 min at room temperature.

Following each of the above procedures the labeled cells were washed twice which removed excess technetium and tin.

Patients were studied by a double-isotope procedure. Two aliquots of blood were drawn on each patient, the first labeled and altered with one of the above procedures using about 15 mCi ^{99m}Tc and

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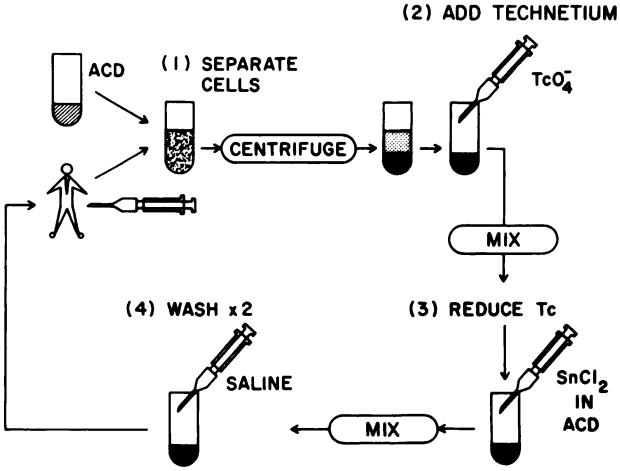

FIG. 1. General method for labeling erythrocytes with ^{99m}Tc.

* ACD: acid-citrate-dextrose (Abbott Lab.), 8 mg citric acid, 25 mg sodium citrate (anhydrous), and 12 mg dextrose/ml water.

the second labeled with about 50 μCi ^{51}Cr as sodium chromate. The chromium-labeled cells were separated from plasma, washed once with saline, and resuspended in saline prior to reinjection at the same time as the cells labeled with technetium. Aliquots were maintained for standards.

Blood samples in most patients were obtained at intervals over 3 hr, usually at 15 min, 30 min, 1 hr, 2 hr, and 3 hr after introduction of labeled cells. Urine was collected throughout the 3-hr sampling period and counted for radioactivity. Chromium-51 activity was determined several days later after the technetium activity had essentially disappeared.

"Apparent red cell volumes" were calculated at the various intervals. The 15-min ratio of ^{99m}Tc red cell volume to ^{51}Cr red cell volume was used as a measure of the stability of the technetium label relative to ^{51}Cr as well as to determine if any abnormal sequestration of unaltered red cells had occurred.

Plasma samples were also counted at the above-stated intervals and the total percentage of administered radioactivity in the plasma was calculated by multiplying percent administered dose ^{99m}Tc per milliliter by plasma volume determined with ^{51}Cr. The amount in the spleen was estimated by noting the percent increase in apparent ^{99m}Tc blood volume from 15 min to 3 hr and subtracting from this the cumulative percent excreted in the urine in 3 hr and the percent in the plasma at this time. Scintiphotos over the head, chest, and abdomen were obtained to exclude any locus of radioactivity concentration other than in the spleen and blood pool. Complete accounting for radioactivity in urine, plasma, and red cells was considered to be more quantitative than monitoring radioactivity over the spleen.

All patients but two had chronic lymphatic leukemia. One patient (EDi) had Hodgkin's disease and another (AB) was hypertensive. Two of the patients were studied by two different methods.

RESULTS

A small portion of the injected technetium was not firmly labeled to the red cells as shown by the presence of about 6% of the administered ^{99m}Tc in the plasma at 15 min. Even the addition of a third saline wash in one study failed to remove this activity. The form of the technetium was not pertechnetate as it failed to concentrate in the thyroid, salivary glands, or stomach. It appeared to be excreted in the urine since the cumulative urinary excretion showed an inverse relationship to plasma levels of activity similar to the behavior of chelates (Fig. 2). Gel filtration of the urine confirmed that the ^{99m}Tc present was not in the form of pertechnetate.

None of the methods had a clear-cut advantage over the others (Table 1). Concerning the labeling aspect of the procedure, labeling yields tended to be slightly lower for the excess ACD short procedure method and slightly higher for the excess $SnCl_2$ short procedure method.

Concerning the altering aspect of the procedure, only one patient (CW) had a very high splenic uptake. In his study performed with excess $SnCl_2$ at room temperature, over 26% of the technetium-labeled (altered) cells was calculated to be in the spleen at 3 hr. However, he had a positive Coomb's

TABLE 1. LABELING YIELD AND DISTRIBUTION OF ^{99m}Tc-RED BLOOD CELLS IN THE SPLEEN AND BLOOD

Method	Patient	Labeling yield (%)	^{99m}Tc activity in spleen (%)		Ratio		
			2 hr	3 hr	(^{99m}Tc-blood vol, 15 min)/(^{51}Cr-blood vol, 15 min)	(^{99m}Tc-blood vol, 3 hr)/(^{99m}Tc-blood vol, 15 min)	(^{51}Cr-blood vol, 3 hr)/(^{51}Cr-blood vol, 15 min)
Excess ACD—37°C, long procedure	CS	63	2.5	11.6	1.01	1.19	1.03
	HC	54			1.00	1.05*	0.99*
	EC	69			0.97	1.13†	1.04†
Excess $SnCl_2$—37°C, long procedure	AB	62	5.1	8.9	1.08	1.16	1.05
	ED	65			0.90	1.14*	1.12*
Excess ACD—room temperature, short procedure	FK	41			1.02	1.07	0.94
	ED	65	8.8	12.2	1.07	1.21	1.05
	CS	34	2.3	8.9	1.06	1.18	1.00
Excess $SnCl_2$—room temperature, short procedure	CW	76	14.9	26.6	1.02	1.34	1.19
	VN	68	4.7	12.9	1.02	1.21	1.02
	HC	75	11.3	16.4	1.01	1.24	1.08

* 1-hr determination.
† 2-hr determination.

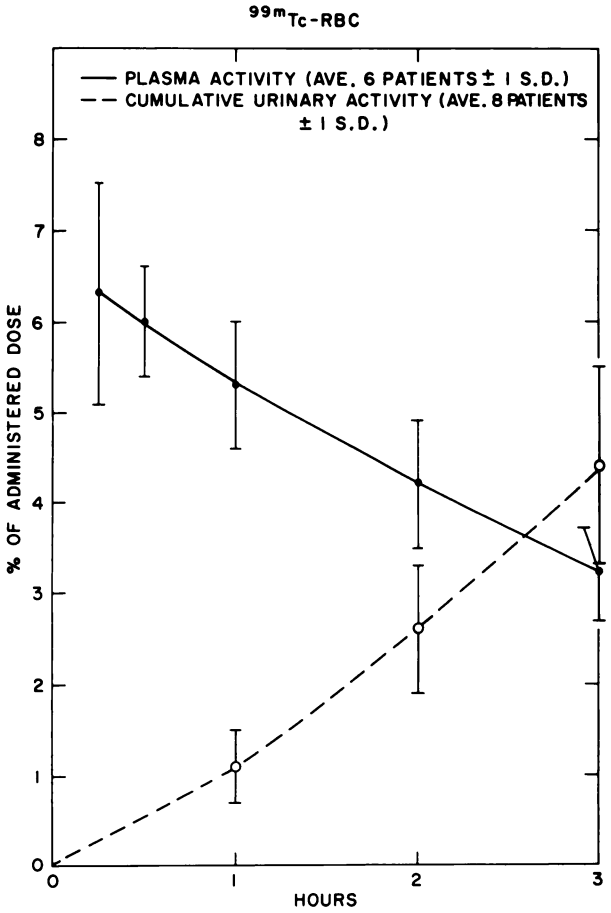

FIG. 2. Cumulative urinary radioactivity excretion and plasma radioactivity levels over 3-hr period following administration of ^{99m}Tc-labeled erythrocytes.

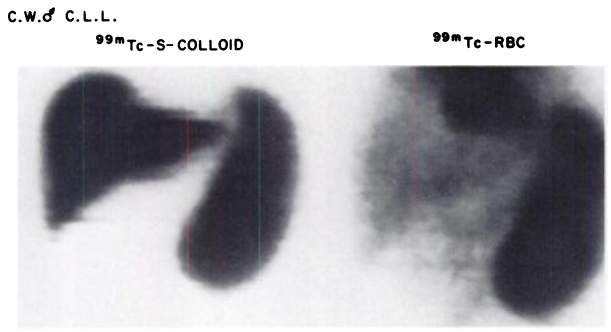

FIG. 3. Comparison of rectilinear scans performed with ^{99m}Tc-sulfur colloid (left) and ^{99m}Tc-labeled erythrocytes (right) in same patient. With labeled red blood cells most of activity is in heart blood pool and spleen. Liver can be identified by virtue of its blood pool.

titre with a very large spleen and the apparent red cell volume determined with unaltered ^{51}Cr-labeled red cells increased by 19% from 15 min to 3 hr. The total proportion of the injected dose which concentrated in the spleen was comparable to amounts reported by Mayer, et al (*3*) but generally less than with the heat treatment method (*4*). However, in every case the spleen was readily visualized in the scintigraphs or scans. Figure 3 shows the differences in scans performed with technetium-sulfur colloid and technetium-labeled altered red cells in the same patient.

DISCUSSION

Two basic methods have been used to visualize the spleen: (A) reticuloendothelial phagocytosis of radiocolloids (*5,6*), and (B) alteration of labeled red blood cells by sensitization (*7*), heat damage (*8*), and chemical damage (*9,10*). Labeling of the reticuloendothelial system with radiocolloids lacks specificity although it has the advantage of simplicity. Sensitization methods are difficult to employ and are generally unsatisfactory. The experience with heat-treated cells has been inconsistent and possibly introduces a slight risk of enhancement of bacterial growth. It has been the hope that a chemical method would be rapid, consistent, and safe.

Red cell labeling and damage with bromomercuri-hydroxypropane is no longer performed in this country. One disadvantage is localization of free mercury in the kidneys. Another method of red cell damage is the use of excess ACD (*4*) with the labeled red cells. We have also demonstrated an increase in red cell fragility when exposed to concentrations of $SnCl_2$ in excess of that required to reduce ^{99m}Tc (*2*).

In other studies of localization in the spleen, estimates of the administered dose in the spleen were made on the basis of disappearance of the label from the circulating red cells (*3,4*). We are not aware of any corrections being made for radioactivity in urine and plasma. In our data there was approximately 8% of administered activity accounted for at 3 hr by cumulative urinary excretion and plasma levels. This is equal to about 75% of the portion of the administered dose calculated to be in the spleen.

In this study we have shown few differences among the several methods we employed. With all methods a considerable portion of the labeled and altered cells remained within the vascular pool. The shorter procedures, performed at room temperature without incubation, were as good as those performed at 37° for longer periods. With the shortened method the entire procedure could be performed in 35–45 min. Percentage labeling was relatively consistent and no evidence of unreduced technetium was evident in the scintiphotos.

We have previously noted (*11*) that about 3% of the injected activity was released in the plasma when a labeling procedure was used. When attempting to damage the cells for splenic imaging a greater amount of ^{99m}Tc is found in the plasma at 15 min

after administration of labeled cells. This most likely occurs in vivo since the cells were washed twice with saline prior to resuspension in saline for injection. With the attempt to damage the cells chemically a greater amount, about 6½%, was found in the plasma. The addition of a third wash did not reduce this amount. The plasma radioactivity was cleared by the kidneys without being converted back to pertechnetate indicating the presence of a technetium chelate.

The chemical alteration method, although not giving the high splenic uptake obtained by heat-treated cells, has the advantage of simple, rapid preparation which still produces satisfactory splenic visualization.

CONCLUSIONS

The use of excess ACD or excess $SnCl_2$ to alter the physiological behavior of labeled red blood cells, either with incubation at 37°C or by a shorter, room-temperature procedure was studied. No advantage to the longer procedures was evident. The final concentration of radioactivity in the spleen was determined by the change in apparent red cell volume by dilution of the label corrected for plasma activity and urinary excretion. The final amount in the spleen at 3 hr was not so great as determined for heat-treated cells by other authors but was sufficient to give good splenic images.

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