jnm/ CONCISE COMMUNICATIONS

MEASUREMENT OF SPLENIC RED CELL VOLUME AND VISUALIZATION OF THE SPLEEN WITH ^{99m}Tc

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A method has been developed for direct in vivo measurement of the splenic red cell volume by radioisotope scanning after administration of red cells labeled with ^{99m}Tc. For more precise delineation of the spleen, the same isotope can be used as a label for red cells which are first heat-damaged. Measurements have been carried out in five normal controls and in 11 patients. Technetium-99m has a short but convenient half-life, is readily available, and its radiation is especially suitable for visualization. It is thus likely to replace other radionuclides for direct measurements of splenic red cell volume.

An enlarged spleen may cause anemia as a result of red cell pooling, and it has been reported that up to two thirds of the total red cell mass may be sequestered in the spleen in massive splenomegaly. In clarifying the role of the spleen in anemia, it may be important to assess its size and to measure the volume of blood contained in it. An approximate estimate of splenic red cell volume can be obtained from total red cell volume measurements using values obtained at intervals after injection of ^{51}Cr-labeled cells (*1*) or by the difference in radioactivity over the spleen before and after mixing has occurred in vivo (*2*). More recently, direct measurement of splenic red cell mass has been obtained by quantitative scanning after administration of red cells labeled with ^{11}C-carbon monoxide (^{11}CO) (*3–5*). With this technique, the actual area of the spleen may need to be delineated by a subsequent scan as spleen and heart images may overlap. This can be done with red cells labeled with ^{81}Rb and damaged by heat or by mercurihydroxypropane (MHP) to ensure selective uptake by the spleen. As this method requires the cyclotron-produced isotopes ^{11}C and ^{81}Rb, its use is restricted.

A method therefore has been developed which is, in principle, similar to the one described with ^{11}CO and ^{81}Rb, but uses the radioisotope ^{99m}Tc as a label for both phases of the test.

METHOD

The red cells were labeled with ^{99m}Tc by a modification of the technique of Eckelman, et al (*6*) and Korubin, et al (*7*). Approximately 15 ml of venous blood were collected onto ACD and centrifuged at 1,500 G for 5 min. After separating the plasma, the cells were incubated for 5 min at room temperature with 5 mCi of ^{99m}Tc. A volume of a freshly prepared 1% solution of $SnCl_2$ in saline was added (approximately 20 μg/ml red cells) and the mixture allowed to stand for a further 5 min. The cells were then washed twice with 9 gm/liter NaCl and resuspended in plasma. One milliliter of this suspension was retained as a standard. The radioactivity of a measured volume was then counted, and this volume was injected. Blood specimens were collected at 10 and 20 min and a third specimen at 30 min when necessary, and the total red cell volume was estimated. After at least 30 min, the splenic area was scanned using a dual-detector scanner with two focusing collimators at a speed of 100 cm/min. The patient thickness in the region of the spleen was measured. Data were simultaneously recorded on magnetic tape in computer-compatible form.

Subsequently, to delineate the spleen, approximately 15 ml blood in ACD were withdrawn, an aliquot of which was used to estimate blood radioactivity while the remainder was centrifuged at 1,500 G for 5 min. Five milliliter packed red cells were heat-damaged for exactly 20 min at 49.5°C. The cells were then labeled as before, washed twice with 9 gm/liter NaCl, resuspended with an equal volume

Received Jan. 17, 1973; revision accepted May 3, 1973.

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of saline, and injected. Blood specimens were taken at 3, 10, 20, 30, 45, and 60 min, and the half-time of disappearance of damaged cells from the circulation (T_{50}) was calculated. At the end of an hour or later, the area over the spleen was scanned as before.

The scan data were processed by the method described previously using ^{11}CO (*4*) with one modification. The geometric mean of the detector outputs was used instead of the arithmetic mean. This was done to obtain uniform sensitivity of response with depth with the lower energy gamma ray emitted by ^{99m}Tc.

RESULTS

Five healthy volunteers and 11 patients were investigated. The spleen was not palpable in any of the normal volunteers.

The data are summarized in Table 1. Five normals and 11 patients were studied. The percentage of the total red cell volume in the spleen ranged from 1.6 to 7.1% with a mean of 3.9% in the healthy subjects. One volunteer (No. 3) had a splenic red cell volume of 162 ml, and it will be noted that the surface area of his spleen, as calculated from the scan, was significantly larger than those of the other normal subjects. Eight patients who suffered from myelo- or lymphoproliferative disorders had splenic red cell volumes ranging from 97 to 538 ml, with a mean of 271 ml. In five of these (Patients 6, 7, 10, and 13) and in Patient 16, who had hemaglobin H disease, the sequestration of red cells in the spleen probably contributed significantly to anemia. One patient (No. 14), was being investigated for malabsorption; the association of malabsorption with steatorrhea and celiac disease with concomitant splenic atrophy is not uncommon. This patient, however, had a normal spleen scan and a splenic red cell volume of 44 ml, which was within the range obtained in our controls. In general, the data correlated well with the findings reported by Pettit, et al (*4*).

DISCUSSION

Variable results were obtained with earlier attempts to use ^{99m}Tc as a red cell label (*8*). However, the method described here is reliable and causes no obvious damage to the cells. The use of ^{99m}Tc-labeled red cells for the estimation of splenic red cell volume or of heat-damaged cells labeled with ^{99m}Tc has not been described previously. Splenic visualization has been achieved in the past with ^{99m}Tc when the cells were damaged with considerably larger amounts of stannous chloride (*9*).

The values obtained in normal subjects for splenic red cell volume are consistent with previous reports when ^{11}CO and ^{81}Rb have been used. However, it is not known whether this will apply to all situations, e.g. patients with congenital hemolytic anemia, in whom the effects of ^{99m}Tc and $SnCl_2$ on red cells have not yet been studied. The rate of clearance of damaged cells is a useful index of splenic function: values obtained by the present method were very close to those obtained when ^{81}Rb was used in the same subjects. Technetium-99m has a number of advantages. It gives a low radiation dose, has a short but convenient half-life, and is readily available. The gamma-ray emission (140 keV) is par-

TABLE 1. MEASUREMENTS OF SPLENIC SIZE AND SPLENIC RED CELL VOLUME

Patient No.	Diagnosis	Hb (gm/100 ml)	Size of spleen on scan: Maximum diameter (cm)	Size of spleen on scan: Area (cm^2)	Body red cell volume (ml)	Fraction of red cell volume in spleen (%)	Splenic red cell volume (ml)
1	Normal	15.1	10.5	48	1,960	4.1	80
2	Normal	15.2	9.8	56	1,610	4.9	62
3	Normal	15.2	13.8	79	2,150	7.1	162
4	Normal	15.6	9.7	57	2,420	1.6	36
5	Normal	15.1	9.8	47	1,800	2.9	52
6	Chronic lymphocytic leukemia	10.2	20.6	195	1,480	18.7	277
7	Lymphosarcoma	11.0	20.8	169	1,440	37.4	538
8	Lymphosarcoma	12.7	15.0	78	2,250	6.2	129
9	Thrombocythemia	11.1	18.3	157	1,210	25.2	304
10	Thrombocythemia	12.3	14.0	72	1,700	12.1	216
11	Myelosclerosis	16.7	21.1	173	3,470	11.1	385
12	Myelosclerosis	9.6	12.0	69	1,140	8.5	97
13	Chronic granulocytic leukemia	10.3	23.9	209	1,670	12.9	216
14	Malabsorption	11.9	11.0	69	1,530	4.4	67
15	Hepatosplenomegaly	8.0	19.0	123	1,370	11.0	151
16	Hemoglobin H disease	10.9	19.8	126	1,290	23.4	302

ticularly suitable for imaging. With heat-damaged cells, a high concentration in the spleen can be obtained, and there is a low background radioactivity which provides improved visualization compared with other radionuclides. When estimating splenic red cell volume, ^{11}C has the disadvantage, in the case of patients with splenomegaly, of a very short half-life (20 min) since mixing in vivo may be delayed.

In methods previously described for spleen scanning when either ^{51}Cr or ^{81}Rb has been used as a red cell label, the cells have been physically damaged by heating at 49.5°C for 20 min (*10*) or chemically with MHP (*11*). The usual technique is to damage the cells after labeling as a last manipulation before administration. When this procedure was tried with ^{99m}Tc as the label, marked lysis of red cells occurred. However, when red cells were first damaged at 49.5°C for 20 min and then labeled, no lysis was observed and labeling efficiency was about 50%. When these cells were deliberately lysed in hypotonic solution, the ^{99m}Tc was found to be bound to the red cell ghosts. It is interesting to note that in our present study, the cells were cleared from the circulation in normal subjects at the same rate as ^{51}Cr or ^{81}Rb-labeled cells damaged by heating.

Because of these advantages, it seems likely that ^{99m}Tc will replace the other techniques as a method of choice for splenic visualization and estimation of splenic red cell volume.

ACKNOWLEDGMENTS

We thank Denise Stafford, Susan Moss, and Margaret Boylan for valuable technical assistance.

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