Measurement of Splenic Function with Heat-Damaged RBCs: Effect of Heating Conditions: Concise Communication

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RBCs labeled with Cr-51 were heated in saline, and RBCs labeled with Tc-99m were heated in plasma or as packed cells. Blood clearances were then compared. Clearance for saline-heated cells was faster than for heated, packed cells, and much faster than for plasma-heated cells. RBCs heated in plasma for 20 min at 49.5°C were insufficiently damaged for measurement of spleen function, but adequate spleen images were obtained in all patients, despite half-clearance times that varied from 14 min to over 90 min.

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Recent studies of splenic macrophage function in immune-complex disorders (*1–5*) have reawakened interest in measurement of splenic function with damaged red blood cells (RBCs). Most published studies of splenic function and anatomy have been performed with labeled autologous RBCs that have been separated from plasma and damaged by heating in saline or as packed cells. When we used RBCs that had not been separated from plasma and had been heated in whole blood, we found clearance times much longer than expected. We then compared clearance produced by heating in saline with clearance produced by heating in plasma or as packed cells.

MATERIALS AND METHODS

Clearance of heat-damaged RBCs was measured in ten patients and two control patients, none of whom had any known abnormality of splenic function. In all patients, Cr-51-labeled RBCs were heated in saline. In five patients, Tc-99m RBCs were heated in plasma, and in another five patients, Tc-99m RBCs were heated as packed cells. The following procedures were used.

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1. Heating in saline: Ten ml of blood was collected in 1.5 ml of acid-citrate-dextrose (ACD) solution. The plasma was removed, the cells were washed once with saline and then suspended in saline. Fifty microcuries of sodium [Cr-51]chromate was added in a volume of 1 ml, and 30 min later the sample was heated for 20 min at 49.5°C. The cells were washed once and resuspended in saline.

2. Heating in plasma: One vial of reconstituted stannous pyrophosphate, containing 3.4 mg of stannous chloride, was administered i.v., and 20 min later 10 ml of blood was collected in 1.5 ml ACD. Ten mCi of Tc-99m pertechnetate in 1 ml saline was added to the blood specimen, and 10 min later the mix was heated at 49.5°C for 20 min.

3. Heating as packed cells: RBCs were labeled with Tc-99m as in (2) above. Before heating, the plasma was removed and the cells were washed with saline. The packed cells were then heated for 20 min at 49.5°C, then suspended again in saline.

Tests of labeling efficiency showed 94–99% efficiency of Tc-99m and Cr-51 labeling.

In the control patients, two samples of RBCs were labeled with the Cr-51 and Tc-99m respectively. After labeling, the Tc-99m-labeled cells were separated from plasma, washed with saline, and suspended in saline. The two samples of RBCs in saline were heated together.

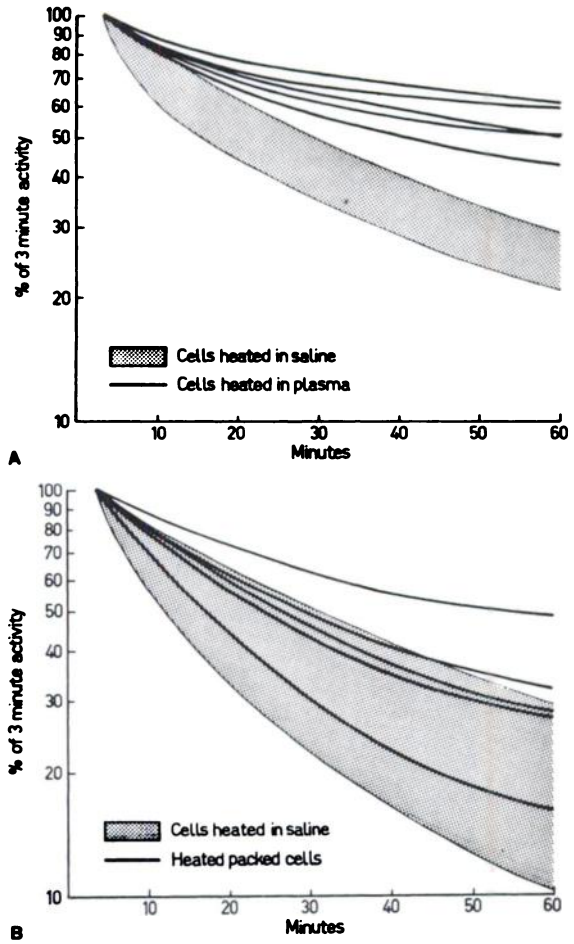

FIG. 1. Blood-clearance curves obtained with heat damaged RBCs in ten patients. A: RBCs damaged in saline and those damaged in plasma; B: RBCs damaged in saline and as packed cells. Range of clearance curves obtained with RBCs in saline is shown in shaded areas. RBCs heated in plasma were cleared much more slowly than those heated in saline. RBCs heated as packed cells were also cleared more slowly, but difference was less marked.

In all patients, the Cr-51- and Tc-99m-labeled cells were injected together and blood samples were taken at intervals from 3 min to 90 min after injection. The samples were assayed for Tc-99m activity the next day and for Cr-51 activity 2 days later. Time-activity curves were generated as described by Marsh et al. (*6*), and the half-clearance time was taken as the time when activity had fallen to half of the 3-min value. One to two hours after injection, scintiphotos of the spleen, liver, and precordium were made, using Tc-99m energy settings.

In all patients, RBC structure was evaluated by light microscopy. RBCs and platelets were counted in an automated cell counter, and histogram displays of RBC and platelet volumes were obtained.

RESULTS

Figure 1 shows comparisons of Tc-99m and Cr-51 blood-clearance curves, and Table 1 shows the half-clearance times. RBCs heated in plasma were cleared much more slowly than those heated in saline, with no overlap of clearance times. RBCs heated as packed cells were also cleared more slowly than those heated in saline, but the difference was less marked. There was no difference in clearance times between Tc-99m-labeled and Cr-51-labeled cells when both were heated in saline, indicating that differences in clearance were not due to differences in labeling procedures.

Good quality images of the spleen were obtained in all 12 patients, despite wide variation in half-clearance times, which ranged from 14 min to over 90 min. Some blood-pool activity was seen in the two patients with the slowest clearances, and liver activity was seen in three patients. However, this was visible only on high-intensity displays and was not significant on standard scintiphotos. Representative images, obtained in control patient RR and in patient PG, whose half-clearance times were 25 min and over 90 min, respectively, are shown in Fig. 2.

Microscopic examination showed spherocyte formation and "budding," with formation of microspherocytes and fragmented forms, and mean RBC volume decreased in all patients. RBC and platelet counts, together with histogram plots of platelet volume, showed the appearance of small particles, similar in size to platelets, after heating. These particles were seen in all patients and probably represented red-cell fragments. In all patients, the fragments were more numerous in the saline-heated samples, but there was no correlation be-

TABLE 1. HALF-CLEARANCE TIMES OF HEAT-DAMAGED RBCs

Patient	Heated in saline	Heated in plasma	Patient	Heated in saline	Heated as packed cells
1	14 min	>90 min	LF	12 min	20 min
2	20 min	39 min	CL	29 min	48 min
3	25 min	>90 min	JS	21 min	25 min
4	17 min	64 min	KD	28 min	34 min
5	13 min	63 min	TB	9 min	14 min

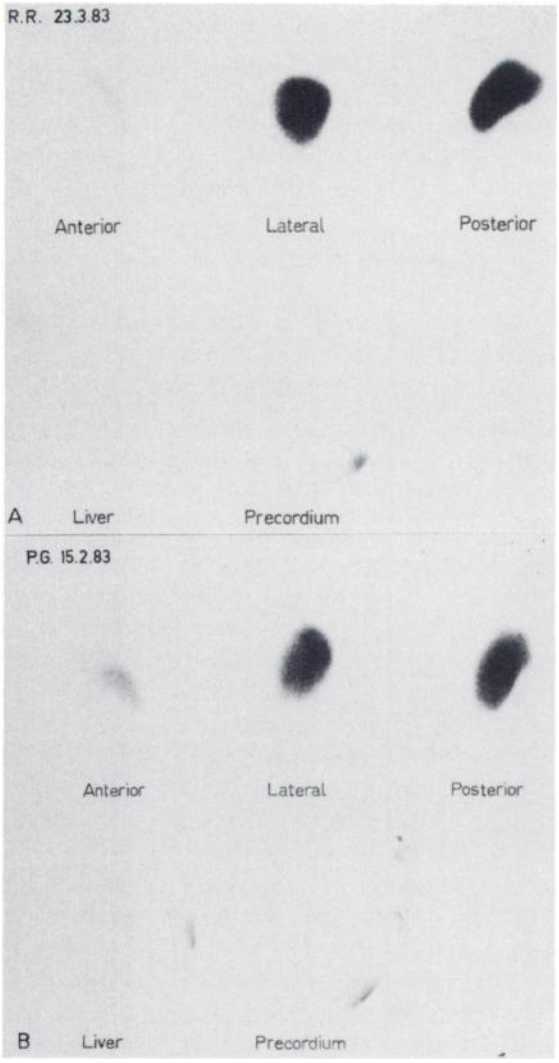

FIG. 2. Spleen scintiphotos, taken 1–2 hr after injection of heat-damaged RBCs. A: RBC half-clearance time 25 min (control patient RR); B: RBC half-clearance time over 90 min (patient PG). Images (300,000 counts) were obtained in posterior view, and other views were taken for same time. Note absence of visible blood-pool or liver activity.

tween the fragment count and half-clearance time from patient to patient.

DISCUSSION

The results show that RBCs heated in plasma are less damaged than those heated in saline or heated as packed cells. This protective effect of plasma was first suggested in 1948 by Ham et al. (*7*), who found that heating RBCs in saline produced a greater increase in osmotic fragility than heating in plasma. The difference in heat sensitivity also explains the findings of Armas et al. (*8*), who first described the use of RBCs heated in whole blood for spleen imaging. They found that time of heating at 49.5°C had to be increased from 15 min to 35 min, whereas shorter times had been sufficient in earlier published studies. Most previous studies had been performed with RBCs heated in saline for 15 min (*9–11*), or with packed RBCs heated for 20 min (*6,12,13*), and this difference in heating times could also be explained by the difference in heat sensitivity, since comparable degrees of damage could be expected by these two methods.

This effect is particularly important in the measurement of RBC clearance, which is an established test of splenic macrophage function. The medium in which the RBCs are heated is as important as the time and temperature of heating. Heating in plasma at 49.5°C for 20 min produces clearance times that are too slow and too variable for the measurement of splenic function. Heating as packed cells or in saline produces faster and more uniform clearance times, and heating as packed cells allows comparison of results with previously published studies. However, these labeling and heating procedures are lengthier and require repeated washing of RBCs. Heating cells in plasma for a longer time, as described in the imaging studies of Armas et al. (*8*), is simpler and may prove to be the best method, but its use for splenic function studies still requires evaluation in a control group.

The use of damaged RBCs to measure splenic function requires a constant degree of RBC damage and a means of demonstrating the degree of damage, to eliminate differences in the RBCs as a cause of delayed clearance. We attempted to correlate the fragment counts and clearance times obtained with the two samples of heated cells in each patient, but found no consistent relationship between these factors. Elkon et al. (*14*) measured changes in RBC plasticity, volume, and clearance produced by different durations of heating, and found that changes in plasticity correlated with changes in clearance times. Reduced plasticity is probably the most important factor in RBC clearance; it is simple to measure and may provide a usable means of standardizing heat damage.

For spleen scintigraphy, precise control of the degree of RBC damage is less important. Adequate images of the spleen were obtained with RBCs heated in plasma for 20 min in all patients in this study, despite half-clearance times over 90 min in two. Other studies have also reported adequate images of the spleen over wide ranges of RBC damage (*9–11,15,16*) and comparable images of the spleen can be obtained with RBCs heated in saline, in plasma, and as packed cells. The procedure described by Armas et al. (*8*) requires the least manipulation of the cells and offers advantages of simplicity and sterility.

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