Cardiac Chamber Imaging: A Comparison of Red Blood Cells Labeled with Tc-99m In Vitro and In Vivo

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A detailed comparison was performed between the quality of cardiac images obtained using red blood cells labeled in vitro and in vivo. Both methods gave cardiac images of high quality. The in vitro method resulted in subjectively superior images, better intravascular retention of injected radioactivity, and a higher left-ventricle-to-background count ratio ($p < 0.05$). The differences in image quality and left-ventricular blood-pool activity were not great, however, and the slight advantage of the in vitro method was offset by a somewhat more complicated preparative procedure. We believe that both agents are suitable for radionuclide imaging of the cardiac chambers.

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Electrocardiagraphically synchronized imaging of the cardiac chambers following administration of vascular radiotracers provides an excellent measure of left-ventricular ejection fraction (EF) and wall motion (*1–7*). In addition, the studies are easily repeated following physiologic or pharmacologic maneuvers without the need for additional injections. An ideal radiopharmaceutical for this test should be distributed solely in the vascular space and should remain there for a period exceeding the useful physical half-life of the radiolabel. Of the available agents, Tc-99m human serum albumin is most widely used at present, but this agent tends to have poor stability, which results in image deterioration with time as Tc-99m leaves the vascular space (*8*). Red blood cells (RBCs) labeled in vitro with Tc-99m are satis-

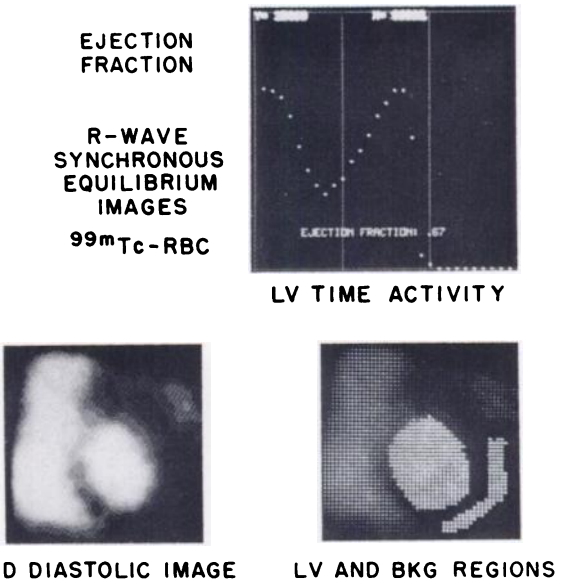

FIG. 1. Construction of left-ventricular and background regions from end-diastolic image using a light pen. The curve (top) is the background-corrected left-ventricular time-activity curve. Calculation of the ejection fraction is described in the text.

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factory in regard to distribution and stability, but require external manipulation of the patient's blood during preparation (*9*). In vivo Tc-99m labeling of the RBCs eliminates the risks of external manipulation and has been reported to produce a stable image of the vascular pool within 15 minutes of injection (*10,11*).

This report presents the results of 35 consecutive studies performed with the in vivo RBC-labeling method, along with a detailed comparison of the in vitro and in vivo methods regarding image quality, left-ventricular ejection fraction, distribution volume, and stability.

MATERIALS AND METHODS

Thirty-five consecutive patients referred for evaluation of left-ventricular function were studied. In vivo RBC labeling was performed in all, using a protocol developed by Parkey, et al. (*10*): an i.v. injection of 15 mg of stannous pyrophosphate (PPi) in 3 cc of saline was given* and was followed 30 min later by an i.v. injection of 15 mCi of [^{99m}Tc] pertechnetate. Ten patients had paired studies with both in vivo and in vitro labeled RBCs. The preparation labeled in vitro was used first for the evaluation of left-ventricular function, and 1–4 days later a repeat study was performed with labeled RBCs in vivo. The in vitro labeling was carried out as described by Richards et al. (*9*). Three of the patients were studied with both labeling techniques, blood samples being withdrawn at 1, 3, 5, 10, 15, and 30 min in order to compare stability and retention of tracer within the circulating blood.

Imaging was performed with a scintillation camera using an FWHM energy window centered at 140 keV and a high-resolution, low-energy, parallel-hole collimator. Each study consisted of at least an anterior and a left anterior oblique view of the heart. Data were recorded on Polaroid film and stored in a dedicated computer system.

The scintillation data were stored, in histogram form, in computer core memory using an R-wave synchronizing pulse, such that each of the sequential images represented a 40-msec segment of the cardiac cycle summed over several hundred cycles. Data collection was automatically terminated when each 40-msec image contained 100,000 counts in the central one-fourth of the image. Regions of interest were constructed from the end-diastolic image (first image after R-wave) using a light pen, as illustrated in Fig. 1, lower right. The background region was defined as a 90° crescent, 4–5 channels wide and separated from the edge of the left ventricle by 4–5 channels. Ejection fraction was calculated from the left ventricle's background-corrected time-activity curve (Fig. 1, top):

$$EF = \frac{\text{maximum counts} - \text{minimum counts}}{\text{maximum counts}}.$$

In a previous report in this journal (*12*), this method of calculating the ejection fraction has been com-

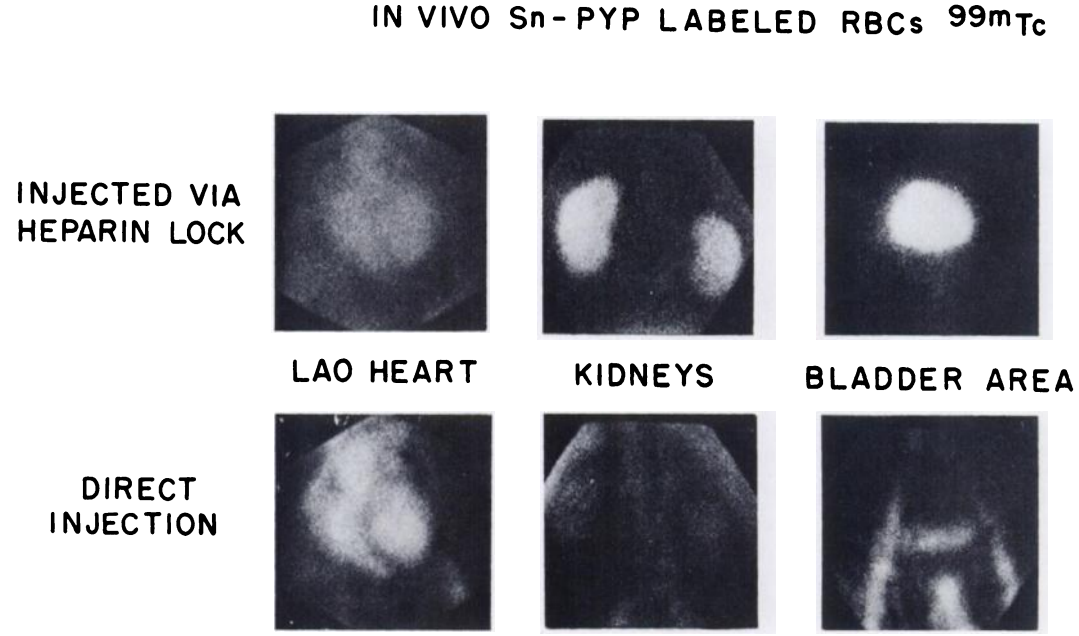

FIG. 2. Images from two studies performed with RBCs labeled in vivo (PPi) in same patient. In the upper study, the PPi and Tc-99m were injected via heparinized plastic catheter. The images below were obtained after direct injection of PPi and Tc-99m. With direct injection, activity is primarily intravascular. The study that used a heparin lock shows markedly decreased cardiac activity, with well-visualized kidneys and bladder.

pared with contrast angiographic and first-transit radionuclide techniques.

RESULTS

The first ten of the 35 patients studied with RBCs labeled in vivo gave clearly inferior findings characterized by reduced precordial activity, prominent background, and excessive tracer loss into the kidneys and bladder. These patients all had the Sn-PPi and pertechnetate administered through a heparinized catheter.† The catheter-stopcock system was flushed with heparinized saline to maintain patency. When the catheter was eliminated and the PPi and pertechnetate injected directly intravenously, the studies in the following 25 patients were all of good diagnostic quality. The importance of this modification was confirmed by comparing images in two patients studied with and without the heparinized catheter (Fig. 2).

Ten of the last 25 patients were studied with both in vitro and in vivo methods of labeled RBCs. Both methods gave good diagnostic information in all ten patients, although in nine of them the studies with RBCs labeled in vitro were subjectively superior because of slightly improved activity ratio between left ventricle and background (Fig. 3). A quantitative comparison of the two blood-pool agents, showed that RBCs labeled in vivo resulted in a 10%) lower left-ventricular activity (background corrected), similar background activity, and a slightly lower quotient for gross left-ventricular counts over background counts (2.3 against 2.5) (Table 1). The ejection fractions determined using the two agents were virtually identical, however (Table 1), and the correlation coefficient between the two agents was 0.98 (Fig. 4).

We desired to compare the percentages of the two tracers remaining in the circulating blood during the first 30 min after injection—the usual imaging period. For this purpose the blood volume must be known, and we estimated it by the dilution method

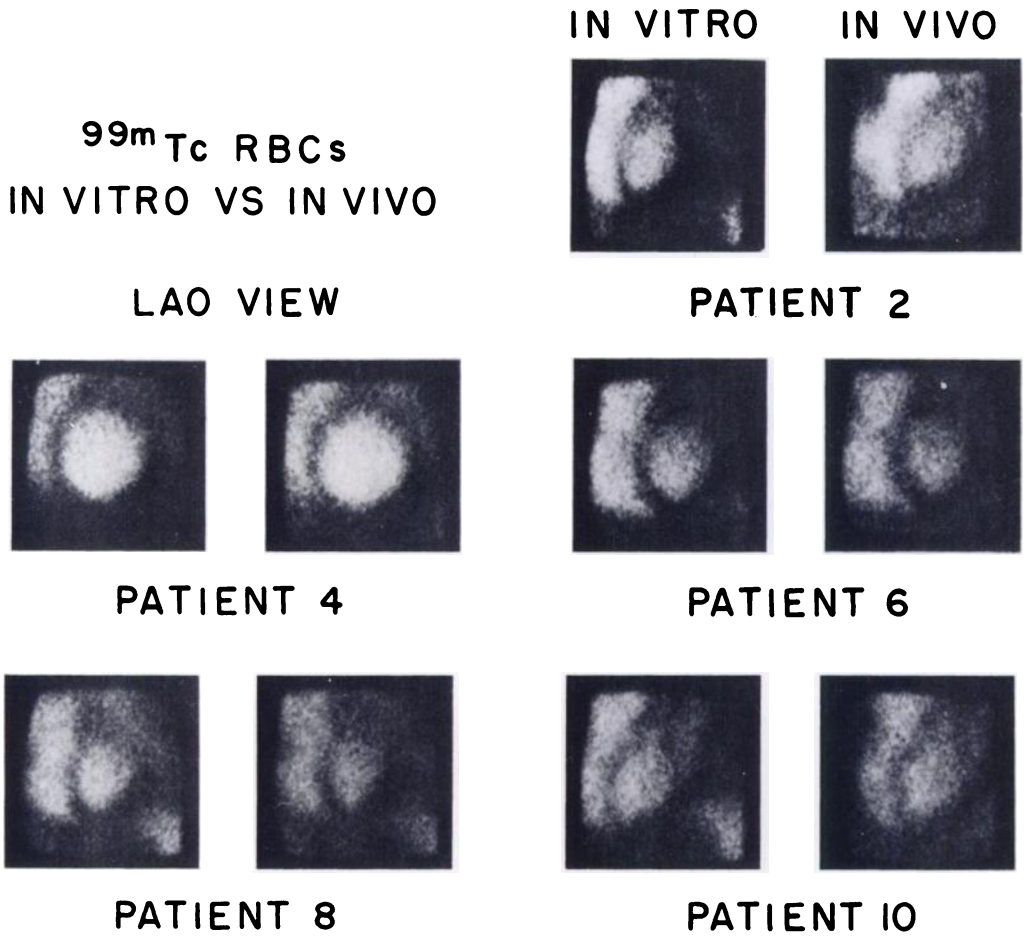

FIG. 3. Comparison of in vivo and in vitro RBC label. For each patient, the in vitro is shown on the left and the in vivo on the right. In all cases, the studies are visually quite similar.

TABLE 1

	Wt. (kg)	Ejection fraction		LV-ED cps/mCi (bkg-corrected)		Bkg cps/mCi (normalized to LV area)		LV/bkg (no bkg correction)		Best study subjectively	
		In vitro	In vivo	In vitro	In vivo	In vitro	In vivo	In vitro	In vivo	In vivo	In vitro
1. RAS	50	.08	.09	122	136	42	44	3.9	4.1		+
2. RED	57	.43	.39	35	23	34	26	2.0	1.9	+	
3. HOM	58	.69	.67	32	29	27	35	2.2	1.8	+	
4. JCB	93	.17	.16	76	67	36	38	3.1	2.8	+	
5. CMK	98	.55	.59	19	15	11	13	2.7	2.2	+	
6. CWK	86	.61	.65	32	26	40	30	1.8	1.9	+	
7. WAD	—	.17	.18	81	67	48	43	2.7	2.6	+	
8. JHD	80	.57	.52	27	22	30	27	1.9	1.8	+	
9. CGN	68	.32	.40	63	60	44	54	2.4	2.1	+	
10. CBW	52	.64	.63	45	35	47	41	2.0	1.9	+	
Mean		.42	.43	53	48*	36	35	2.5	2.3†		

* $p = .06$.
† $p = .04$.

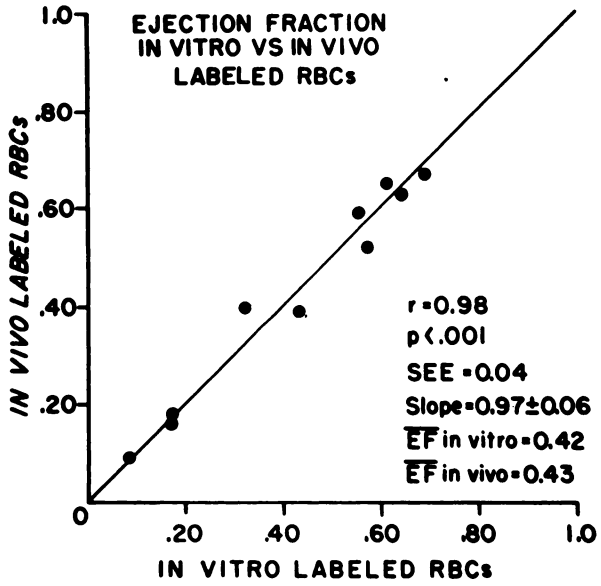

FIG. 4. Comparison of left-ventricular ejection fractions, determined with RBCs labeled in vivo vs. in vitro. Results are essentially identical.

$$\text{Blood-pool volume} = \text{dose volume} \times \frac{\text{cpm/cc in blood sample}}{\text{cpm/cc in dose}},$$

assuming that blood-pool volume would be indicated by the greatest circulating concentration of the RBCs labeled in vitro. This was found to occur at about 3 min after injection (*13*), and the retention of administered activity calculated from this volume was taken as 100%. In each of three patients, the volume thus calculated was used for both preparations, and the derived retention curves are shown in Fig. 5. For the RBCs labeled in vitro, the retention averaged 97% during the first 30 min. Those labeled in vivo, on the other hand, required 3–10 min for mixing, and retention averaged 76% for the remainder of the 30 min.

DISCUSSION

The direct comparison of the in vivo and in vitro methods of labeling red blood cells shows that the in vitro method provides a slightly superior intravascular agent in terms of image quality and left-ventricular end-diastolic counts in relation to background. However, the estimates of left-ventricular ejection fraction over a wide range of values (0.08–0.69) were essentially the same with the two agents. This indicates that the slight difference in percentage of total injected activity retained in the blood (95%+ for in vitro; 75%+ for in vivo) does not significantly alter a quantitative assessment of left-ventricular function. Additionally, in all ten cases in which the agents were directly compared, the images with both agents were satisfactory for visual assessment of ventricular wall motion.

The observed advantages of the in vitro preparation are offset by a somewhat more complicated preparative procedure, which requires drawing the patient's blood and about 30 min of sterile manipulation to produce a labeled product. Another disadvantage is the possibility of injecting contaminated or heterologous blood back into the patient. The in vivo technique, on the other hand, also requires two needle punctures and separate periods of incubation, the first to ensure saturation of the red blood cells

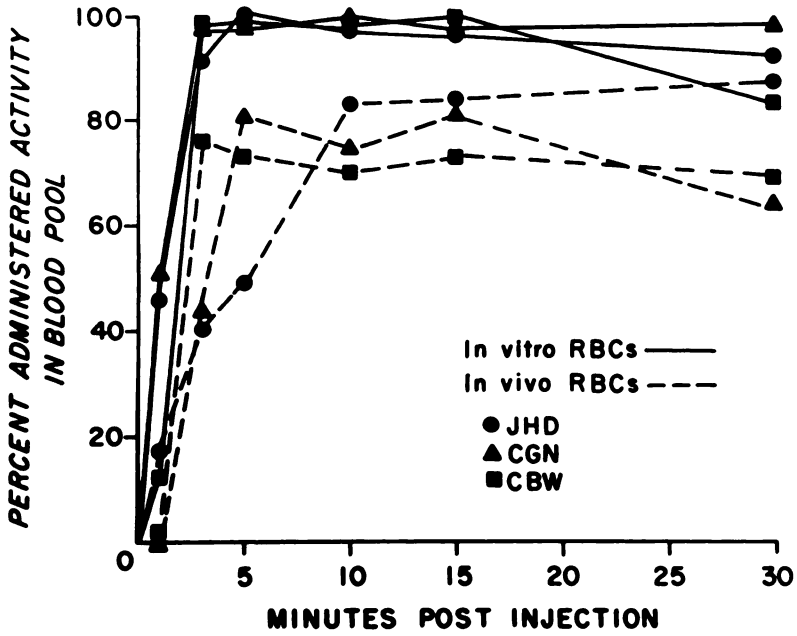

FIG. 5. Comparison of percentage of injected activity in blood pool from 0–30 min with RBCs in vitro or in vivo. The highest value of the in vitro-labeled cells was normalized to 100%. During the usual imaging period (5–30 min after injection), a greater percentage of the total injected activity remained in blood pool with cells labeled in vitro.

with Sn-PPi, and the second period to obtain binding of pertechnetate to the RBCs. The total preparation time from initial patient contact to the start of cardiac imaging is about the same for the two techniques, typically 30–45 min.

The body distribution of the small percentage of activity that does not stay within the blood pool appears to be somewhat different for the two agents. With RBCs labeled in vitro, the spleen concentrates the radioactivity. For the in vivo preparation, unbound radioactivity is cleared by the kidneys and is excreted. Both of these processes are very rapid, and the amount of radioactivity in the plasma is a small part (<5%) of the intravascular radioactivity. Neither preparation has appreciable free pertechnetate, and thyroid and stomach uptake are negligible.

The importance of the injection technique to the quality of in vivo RBC labeling has not been previously reported. We were surprised to find that injection through a heparinized catheter consistently resulted in poor-quality studies, with diminished cardiac-chamber activity and increased renal uptake and excretion. We do not know the mechanism for this effect, but by injecting the Sn-PPi and pertechnetate directly into a peripheral vein, studies of high diagnostic quality were reliably achieved.

CONCLUSION

In summary, in vitro (Brookhaven kit) and in vivo (Sn-PPi) methods of RBC labeling both give cardiac imaging studies high diagnostic quality. The RBCs labeled in vitro had a significantly greater retention in the blood pool, with correspondingly higher left-ventricular end-diastolic counting rate and better image quality. The in vivo RBC method, however, is more convenient and gives virtually identical results for quantitative measurement of left-ventricular ejection fraction and qualitative assessment of wall motion. We believe that both agents are suitable for radionuclide imaging of the cardiac chambers.

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FOOTNOTES

* Mallinckrodt TechneScan® PYP.

† The catheter consisted of a Model No. 4535-08 short Teflon Abbocath-T (3.2 cm long), connected to a three-way plastic stopcock (Pharmaseal Model K-75).

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