

DIAGNOSTIC NUCLEAR MEDICINE

Acid-Citrate-Dextrose Compared with Heparin in the Preparation of In Vivo/In Vitro Technetium-99m Red Blood Cells

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Red blood cells labeled in vivo/in vitro with Tc-99m (Tc-99m RBC) were prepared in a series of 21 patients and two normal volunteers. In each subject both heparin and acid-citrate-dextrose (ACD) solutions were used to label tandem blood samples. The immediate preinjection binding efficiency (BE) was then determined. In each of the 23 studies, the ACD preparation yielded superior BE. The average BE was 93.47% (± 3.78) with ACD and 87.23% (± 4.29) with heparin. With the ACD method the effect of carrier Tc-99 may be as great as a 24% reduction in BE observed when initial eluates from long-ingrowth-time generators were used. Improved image quality with minimal renal and urinary-bladder activity results with ACD labeling. It is concluded that the use of ACD results in superior RBC labeling with less nontarget activity relative to heparin and is preferred over heparin for preparing in vivo/in vitro Tc-99m RBC.

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Red blood cells labeled with Tc-99m are a valuable radioagent with a wide range of clinical applications including: (a) anatomical and functional assessment of the cardiovascular system (1-4); (b) splenic imaging (5-6); (c) estimation of red-cell mass (7); and most recently, (d) detection of gastrointestinal hemorrhage (GI bleeding) (8-10).

In the past decade, numerous methods for the preparation of Tc-99m RBC have been reported. The three basic methods used are: (a) in vitro (11-12), (b) in vivo (13,14), and, (c) combined in vivo/in vitro (15-17). The latter approach seeks to combine the superior binding efficiencies (BE) achieved by in vitro methods with the convenience of in vivo labeling and has become widely used to image suspected GI bleeders. The routine use of a labeling method that consistently yields the highest achievable preinjection BE should aid in maximizing the sensitivity and specificity of this procedure.

Having occasionally observed the profound deleterious effect of heparin on the BE of Tc-99m RBC prepared in vitro, as well as a distracting degree of renal and urinary-bladder activity on our initial studies of in vivo/in vitro labeling for GI bleeding, we abandoned the use of heparin in favor of acid-citrate-dextrose (ACD). We undertook the following study to document the superiority of ACD for this procedure.

MATERIALS AND METHODS

Patient population. In vivo/in vitro Tc-99m RBC labeling was performed on two normal volunteers and 21 patients referred to our department for either a gated cardiac study (15 patients) or a study of GI bleeding (six patients).

Red-cell labeling. The entire contents of a commercial kit* containing 1 mg stannous chloride, 10 mg sodium pyrophosphate, and 30 mg sodium trimetaphosphate was reconstituted with 0.9% sodium chloride for injection and administered by antecubital i.v. injection. One milliliter of ACD† was withdrawn into a 12-ml syringe. A second 12-ml syringe was "heparinized" by rinsing with 1 ml

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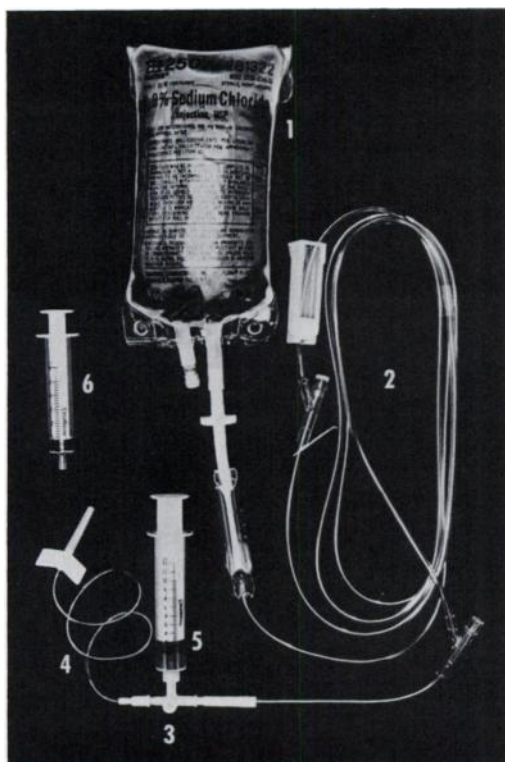


FIG. 1. Apparatus for in vivo/in vitro labeling: (1) 0.9% NaCl for injection, (2) solution administration set, (3) 3-way stopcock, (4) 19 gauge infusion set, (5) 12-ml labeling syringe containing 1 ml ACD and pertechnetate, (6) 6-ml syringe used for clearing infusion line and stopcock.

(1000 units) of heparin sodium, and the excess heparin was expelled. Twenty millicuries (740 MBq) of pertechnetate ($Tc-99m$) was then added to each syringe. Freshly eluted pertechnetate, obtained from either a 2.25-Ci (83.25 GBq) or a 3.3-Ci (122.1 GBq) generator with ingrowth times of 4–24 hr was used in 20 studies. In three others, the initial eluate of the 3.3-Ci generator was used in order to ascertain the effect of carrier $Tc-99m$ on the BE. In four normal volunteers, the effect of ingrowth time on the BE of the ACD method was evaluated using initial eluates from different generator manufacturers.

The apparatus used is shown in Fig. 1. After injecting the stannous pyrophosphate, a 19 gauge infusion set was inserted in the contralateral antecubital vein, and the remaining i.v. apparatus was connected. An i.v. bag and solution-administration set were used in lieu of a large saline-filled flush syringe to minimize the potential for loss of patency secondary to clot formation. At thirty minutes the infusion line and stopcock were cleared by withdrawing and discarding 5 ml of whole blood, using the 6-ml syringe. Immediately, 5 ml of whole blood was withdrawn into each of the two previously prepared 12-ml labeling syringes. The shielded syringes were gently rotated to mix, and allowed to incubate at room temperature for 10 min. Samples were then removed for

quality control, as described below. The labeled red cells were then reinjected via the 3-way stopcock, alternating the ACD or heparin preparations in each successive patient. The infusion set and 3-way stopcock were then flushed by opening the saline administration set. The i.v. apparatus for blood labeling was then removed and imaging commenced.

Imaging. A large-field Anger camera equipped with low-energy, all-purpose parallel hole collimator, with a 25% window centered on 140 keV, was used for the entire study. Fifteen patients, who were referred to the department for MUGA studies, had a single 750,000-count anterior abdominal image in the supine position at 30 min after the injection of the labeled cells. In the six patients suspected of GI bleeding, 750,000-count anterior abdominal images were obtained in the supine position, at 5-min intervals, until 30 min after injection. If no abnormalities were seen on the initial study, delayed images were recorded at 3 and 24 hr.

Urinary excretion. Urinary excretion was quantitated in two patients for each labeling method. Complete urine samples were collected following administration of the labeled cells, and excreted activity was measured at 60 min and 24 hr.

Image quality. Fifteen 30-min anterior abdominal images (eight ACD, seven heparin) were blindly evaluated by four board-certified nuclear medicine physicians. Urinary activity was rated on a scale of 1–10 with 1 indicating negligible activity and 10 indicating a high degree of activity. For reference, the highest degree of urinary activity present in the 15 images was arbitrarily set at 10. In addition, the nonurinary parailiac background activity was subjectively rated on a similar scale of 1–10.

Quality control. A minor modification of the method proposed by Callahan et al. (15) was used to assess the immediate preinjection BE for each preparation. After the 10-min incubation period, an aliquot of the ACD or heparin preparation was injected into an entire vial of stannous DTPA that had been previously reconstituted with 1 ml of normal saline.

The resulting $Tc-99m$ RBC/DTPA mixture was allowed to incubate for an additional 10 min. Aliquots

TABLE 1. BINDING EFFICIENCIES OF $Tc-99m$ RBC PREPARED IN VIVO/IN VITRO: ACD VS. HEPARIN

Method	Binding efficiency % BE (mean \pm 1 s.d.)*
ACD	93.47% \pm 3.78
Heparin	87.23% \pm 4.29

* Statistical analysis by two-tailed paired T-test $p < 0.001$, $N = 20$.

TABLE 2. EFFECT OF CARRIER Tc-99m* ON THE BINDING EFFICIENCY OF Tc-99m RBC PREPARED IN VIVO/IN VITRO: ACD VS. HEPARIN

Method	Binding efficiency % BE (mean % and range)
ACD	69.37% (65.7–71.8)
Heparin	63.90% (60.5–66.1)

* Initial eluate of 3.3 Ci (122.1 GBq) fission generator with approximate 96-hr ingrowth.

containing 1.0–2.5 mCi (37.0–92.5 MBq) were transferred to test tubes containing 10 ml of normal saline, then centrifuged for 7 min at 450 g. Unbound or weakly bound technetium is strongly chelated by the DTPA and remains in the supernate. The spun tubes were placed in a dose calibrator to record the total sample activity. The supernate was then carefully removed and the RBC-bound activity determined. The BE was calculated by dividing the RBC-bound activity by the activity of the total sample.

RESULTS

Labeling efficiency: 4-to-24-hr generator ingrowth. The paired patient data for ACD compared with heparin are shown in Table 1. In each of the 20 cases, superior labeling is observed with the ACD method: its average preinjection binding was 6.2% greater (93.47% compared with 87.23%). The maximum individual difference observed was 12.6%, the minimum 1.2%.

Labeling efficiency: 96-hr ingrowth. Table 2 shows the binding efficiencies that resulted from the use of the initial eluate of a 3.3-Ci generator with an ingrowth time of approximately 96 hr. The deleterious effect of the Tc-99m carrier results in a decrease in BE of ~24%, whether with the ACD (93.5% compared with 69.4%) and heparin (87.2% compared with 63.9%). Again in

TABLE 3. EFFECT OF GENERATOR INGROWTH TIME ON THE BINDING EFFICIENCY OF Tc-99m RBC PREPARED IN VIVO/IN VITRO BY THE ACD METHOD

Generator size	Approximate ingrowth time†	Initial eluate BE	Fresh eluate BE
3.31 Ci*	96 hr	69.3%	93.5% (N = 3)
3.0 Ci*	66 hr	83.0%	98.8% (N = 2)
2.25 Ci†	22 hr	97.3%	97.9% (N = 2)

* Weekend generator.

† Midweek generator.

‡ Per manufacturer.

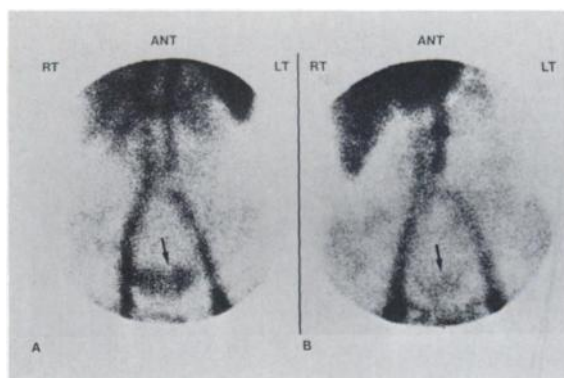


FIG. 2. Normal studies (MUGA patients). 30-min anterior abdominal 750-k count images. A. Heparin method. B. ACD method. Decreased urinary bladder activity (arrows) is demonstrated with the ACD method.

each case, however, the ACD is superior to heparin with an average difference of 5.5%.

Labeling efficiency: Effect of ingrowth time. The initial eluates from three fission Tc-99m generators from different manufacturers were used to determine the degree of labeling interference with the ACD method. The results are shown in Table 3 and, as expected, indicate that the length of ingrowth time is directly related to the degree of interference observed.

Image quality. The ACD images were judged superior to the heparin images with respect to urinary activity. The combined average score for the ACD images was 1.75 compared with 7.1 for the heparin (highest degree of urinary bladder activity set at 10).

The average nonurinary parailiac background activity was essentially similar for both methods with ACD receiving a 3.75 rating and heparin a 3.32. Great variation was present, however, among the four observers despite the similar averages.

Imaging. Anterior abdominal images comparing the two methods are shown in Fig. 2. The ACD image was judged superior with respect to extravascular accumulation of activity, particularly in the kidneys and urinary bladder. By comparison, in the image obtained following administration of a heparin-anticoagulated preparation (A), renal and bladder activity are prominent.

Figure 3 shows the 5- and 30-min anterior abdominal images of a 90-yr-old male following administration of 20 mCi (740 MBq) Tc-99m RBC prepared by the ACD method. The patient came to the emergency room earlier in the day with a chief complaint of having passed bright-red blood per rectum. Admitting laboratory studies were unremarkable except for the CBC, which revealed a hemoglobin of 12.1 g/dl with normal indices. The patient had a previous history of lower GI bleeding requiring multiple transfusions, but no bleeding site could be determined by routine radiographic procedures, and it was felt that arteriography was not warranted.

Shortly after the current admission, the radionuclide

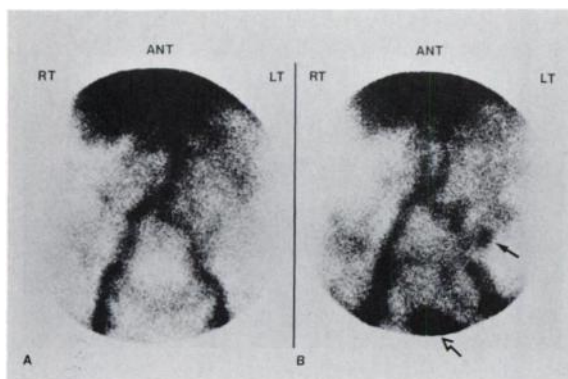


FIG. 3. Positive study for lower-intestinal bleeder in a 90-yr-old male. A: 5-min anterior abdominal 750-k count image (note photon-deficient area of urinary bladder). B: 30-min image demonstrates activity in transverse and descending colon with pooling in sigmoid colon (solid arrow), and penile blood pool (open arrow).

study was performed. The scan was positive for lower GI bleeding, most likely from the distal transverse or proximal descending colon. The 5-min image shows excellent blood-pool concentration of the tracer, with a photon-deficient area clearly delineating the urinary bladder. The 30-min image demonstrates a lower-GI bleeder with minimal accumulation of urinary bladder radioactivity. The preinjection BE of the radiopharmaceutical was 93.9%.

Urinary excretion. The 60-min urinary excretion expression as percent of injected dose (% ID) was 0.6% and 0.01% ID for the two ACD patients and 1.5% and 3.6% ID for the two heparin patients. The 24-hr figures for the ACD patients were 14.3% and 15.6% ID, whereas in the heparin patients 26.4% and 30.7% ID was excreted.

DISCUSSION

Accurate evaluation of a suspected GI bleeder depends upon the delivery of the radioagent to an actively bleeding site, and the ability to differentiate the extravasated tracer from nontarget extravascular activity.

Due to its short plasma half-time, the use of Tc-99m sulfur colloid is effectively restricted to patients who are actively bleeding at the time of injection (8). Moreover, upper GI hemorrhage may be masked by intense hepatosplenic uptake. Tc-99m RBC have been advocated to overcome the limitations of sulfur colloid. Studies in rabbits with in vitro-labeled In-111 RBC have demonstrated the ability to reveal a bleed as small as 2 ml (1% of blood volume) (18). However, if in vivo labeling with pertechnetate is performed, this potentially improved sensitivity would be compromised by gastric secretion of the injected pertechnetate, as well as by the renal component. In general, with in vitro-prepared Tc-99m RBC, this initial extravascular distribution of pertechnetate is minimized, but the additional tagging manipulations involved limit the acceptability of this method.

Combined in vivo/in vitro RBC labeling can provide a nearly ideal physiologic intravascular tracer, provided the cells are not damaged and the immediate preinjection radiochemical purity approaches 95%. If it is assumed that the remaining 5% is free pertechnetate, and that, with in vivo labeling, 85% of the pertechnetate is RBC-bound, then with the ACD in vivo/in vitro method less than 1% free pertechnetate would initially be available for extravascular distribution. Moreover, the use of nasogastric suction (13) or cimetidine (10) would be unnecessary.

Heparin has been successfully labeled with Tc-99m by stannous reduction and has been shown to accumulate in damaged coronary arteries and myocardium in dogs (19). However, the biodistribution of Tc-99m heparin is primarily hepatic and renal. It has also been shown that 20–60% of S-35-labeled heparin will appear in the urine (20). In addition, inferior scan quality as well as increased renal and urinary activity have been reported for in vivo-labeled RBC when heparinized catheters were used for the administration of Sn PPI and pertechnetate (21). Previously published in vivo/in vitro methods have used heparin either diluted in an i.v. solution (15) or, as we have chosen for comparison with ACD, by heparinization of the labeling syringe (8). In this study the anticoagulant and pertechnetate were mixed together in the syringe before withdrawal of blood for labeling. This was done to avoid the need to transfer radioactivity in the imaging room or have the syringe returned to the nuclear pharmacy for this step. In the method described by Callahan et al. (15) the activity is added following mixing of the blood and anticoagulant. The use of a fixed amount of heparin was intentionally avoided in this study, as it was felt that in practice the act of “heparinizing” a syringe would result in a wide range of residual heparin. We did determine that approximately 100 ± 16 units were delivered by performing five pseudoheparinizations with saline and a known concentration of pertechnetate. ACD is the preferred anticoagulant for the preparation of Cr-51 RBC (22), and we feel it should be used exclusively for the preparation of in vitro and in vivo/in vitro Tc-99m RBC.

It is possible that in the presence of hematuria, excessive urinary-bladder activity may be observed with ACD prepared in vivo/in vitro RBC. We have encountered one such case in this series in a patient whose urinalysis revealed 3+ heme and 6–10 RBC per high-power field.

The use of a Sn-DTPA solution for quality control was originally used to characterize the binding kinetics and minimum incubation time necessary to perform the modified in vivo labeling technique (15). With the 10-min incubation time used in this study, it was originally assumed that labeling would be complete and firm and therefore that the Sn-DTPA would be unnecessary. For quality control, however, five determinations comparing

DTPA and saline only revealed an average BE for the DTPA procedure of $95.12\% \pm 1.85\%$ and $98.5\% \pm 1.59\%$ for saline. It was therefore decided to use the Sn-DTPA quality control procedure.

Finally, it is recommended that initial generator eluates be avoided in Tc-99m RBC labeling due to the inability to achieve an acceptable preinjection BE.

FOOTNOTES

* Pyrolite, New England Nuclear Corp., N. Billerica, MA.

† Anticoagulant citrate dextrose solution USP (ACD Formula-A), Fenwall Corp., Deerfield, IL.

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