FIGURE 1. Comparison of labeling efficiency of 99mTc-RBCs prepared with initial eluate (72-hr ingrowth) of Monday generator: ACD versus heparin (n = 5).

TABLE 1

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<tr>
<th>Effect of Carrier 99mTc on 99mTc-RBCs Labeling Yields (%) Using the BNL Kit Method* (8)</th>
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<tr>
<td>99mTc (μCi)</td>
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<td>% Labeling yield</td>
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* Standard labeling conditions, 1 ml blood and tin citrate kits (50 μg Sn4+), were used. Percent labeling yields are average of six determinations ± 1 s.d.

† Approximately 1.27 × 1018 atoms or 2.085 μg of 99mTc are produced upon the decay of 1 Ci 99Mo.

REPLY: We thank Drs. Wilson and Hung for their interest in the use and evaluation of the UltraTag™ RBC kit for labeling red blood cells (RBCs) with 99mTc. This kit (available from Mallinckrodt Medical, Inc., St. Louis, MO, since June 1991) utilizes the whole blood in vitro method developed at Brookhaven National Laboratory (BNL), first reported at the SNM Annual Meeting in June 1983 (7) and described in detail in later publications (2-4).

Wilson and Hung in their letter refer to a "potential problem" they have found concerning the use of this kit for radiolabeling RBCs. This relates to reduced labeling yields when 99mTc from a long ingrowth generator is used in conjunction with patient blood that has been collected in ACD. Presence of carrier 99mTc is by no means a problem unique to the UltraTag™ RBC kit; in fact, all 99mTc labeling kits have a range of tolerance for 99mTc and workers in the field are well aware of this problem. The issue of technetium carrier has been studied in great detail (5-7), and one has to be very mindful of this problem when "instant" technetium or 99mTc from a >24 hr ingrowth between milkings is used. The capacity of UltraTag™ RBC kit for carrier 99mTc is more than adequate to handle the eluates from all commercially available generators when Heparin is used as the anticoagulant, as shown in Table 1 (8). It will tolerate 99mTc produced from at least 200 mCi of 99Mo decay and still produce ~95% labeling yields. For example, one can use ~200 mCi of 99mTc from a fresh eluate of a 3 Ci generator that has grown-in for 72 hr before milking. If the eluate is allowed to sit 12 hr, 50 mCi 99mTc could be used. When ACD-collected blood is used, however, the levels of 99mTc that can be tolerated are substantially reduced as corroborated by Wilson and Hung. Ample quantities of 99mTc from a <24 hr ingrowth generator can nonetheless be used without any problems.

The reduced tolerance for 99mTc results from a diminished uptake of stannous tin into the RBC during the tinning process in the presence of ACD. Extensive mechanistic work done at BNL (3, and Straub RF, Srivastava SC, unpublished data) has demonstrated the negative effects on tin uptake as a function of ACD concentration (Table 2). Even though the exact reasons are not clear, it would appear that the nature of the chemical species of tin in the presence of excess citrate, and/or the adverse effects of ACD on the RBC (9), are responsible for the reduction in tin uptake. It should be noted that the kit itself contains 0.0125 mmol citrate, and the addition of 0.15 ml ACD per ml blood (widely recognized as the recommended upper limit) will increase the total citrate to 0.063 mmol if 3 ml blood are used (Fenwal ACD, formula A). This much citrate will reduce tin uptake to the extent that the tolerance for 99mTc present in longer ingrowth milkings will be reduced and labeling yields will be lower as Wilson and Hung have also noted. Given the mechanisms involved in our kit method, there is really no "optimum" ACD concentration that could overcome this situation (Table 2). It is

REFERENCES

3. Package insert of UltraTag™ RBC Kit for the preparation of technetium 99m-labeled red blood cells, R3/91, Mallinckrodt Medical, Inc., St. Louis, MO.

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preferable to use Heparin as the anticoagulant. Our results have demonstrated no adverse effects even when using much higher than recommended concentrations of Heparin (10). The observations by Porter et al. (17) do not directly relate to our particular kit system.

REFERENCES


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REPLY: Wilson and Hung point out that the package insert of the recently approved in vitro red blood cell labeling kit, UltraTag®, RBC, does not detail the amount of heparin or ACD to be used as anticoagulants when collecting the blood sample for labeling. The language in the package insert was both reviewed and approved by the FDA and reflects the variety of different forms of ACD and heparin that are commercially available. The USP XXII describes two forms of ACD, formula A and formula B, and there are at least two modified formulas of ACD commercially available. Heparin is also supplied by a number of manufacturers, both in different formulations, and over a wide range of concentrations. It would be impractical and confusing to list all of the available forms of these anticoagulants, with associated recommendations for the amount of each to be used to collect a blood sample, and thus the package insert does not do so.

In Wilson and Hung's experiments, both ACD and Heparin were diluted to 1 ml with 0.9% NaCl. The initial (0 min) labeling efficiencies (LE) with both heparin and ACD are slightly lower than expected, but at the 30-min time point they have improved to typically expected values which approach 100%. The slightly lower than expected 0 min LEs, may have resulted from the dilution of the anticoagulant with 0.9% NaCl. Expected LEs were obtained for both anticoagulants with eluates obtained from generators which had been eluted 24 hours previously (24-hr ingrowth eluates). When 72-hr ingrowth eluates were used, depressed LEs were obtained with ACD, while expected LEs were obtained with heparin.

This latter observation is of particular interest since it suggests that ACD does not perform equally to heparin in all situations. To assess this possibility, Mallinckrodt Medical, Inc. has taken a number of actions. First, all clinical studies have been thoroughly reviewed. Sixty patient studies were performed using ACD, formula A, or Mallinckrodt's Modified ACD anticoagulants at ratios of 0.15 ml per ml of blood. The average LE was above 97%, but information on the ingrowth time of the generator eluates had not been recorded. Next, an experiment was carried out wherein blood samples collected from volunteers were labeled using five varieties of commercially available ACD, at ratios of 0.17–0.18 ml per ml of blood. LE was nearly quantitative in all cases. Even holding the samples for several hours (between collection and labeling) did not produce any negative effect on LE. In another experiment, the blood samples were collected using an excess of ACD and were labeled with "mTc obtained from a 72-hr ingrowth generator. LE was excellent at the recommended levels of ACD (1), but LE was observed to decrease with increasing excess amounts of ACD. We have also confirmed the results of Wilson and Hung reported for 12-hr old technetium eluates obtained from a 72-hr ingrowth generator. Using heparin, LE was consistently greater than 97%, while LE with ACD was suboptimal (57%–72%). Mallinckrodt Medical, Inc. currently has in progress a number of additional evaluations of the effect of anticoagulant on UltraTag® RBC labeling. Further action, as warranted, will be based on these more detailed examinations. Please address any questions on the use of this product to Mallinckrodt's Technical Service Department at 1-800-325-3688.

REFERENCES


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