ideally suited for a routine user interested perhaps only in the clinical application rather than the counting method.

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Radiolabeling of Red Blood Cells

We have read with interest the article by Ryo and associates (*1*) and wish to commend the authors on their undertaking. Nevertheless, we are not in complete agreement with some of the methods and results presented in the article.

Their binding efficiencies employing the stannous glucoheptonate (SnGh) method (Method B[1]) for labeling RBCs with ^{99m}Tc (*2*) are not in agreement with our earlier published results, nor with our current work employing a modified, abbreviated method. The modified procedure requires only 7% of the reported amount of SnGh (equivalent to 4 μg of stannous ion and 14 mg of sodium glucoheptonate), eliminates EDTA, and requires only one saline wash. This reduces the preparation time without altering the binding efficiency or biologic behavior of the technetium-tagged RBCs. By either of our own labeling methods, binding efficiencies for human RBCs are greater than 95%, compared to the 90.1% average reported by Ryo et al.

It is difficult to understand the proposed advantage cited by these authors for a prolonged intravascular retention of technetium-labeled RBCs beyond 2 hr, since all imaging procedures can be completed easily within that time span. However, in order to reconfirm our results and form a basis for comparison with the authors' blood-disappearance studies by Method B, RBC-disappearance studies with ^{99m}Tc and ^{51}Cr were performed in two additional dogs. For one animal the technetium labeling was performed according to our published Method B, and in the second animal the RBCs were labeled by the abbreviated method above. All injected cells had a binding efficiency greater than 98%. Blood-disappearance results are shown in Table 1. In all whole-blood samples, more than 95% of the technetium or chromium radioactivity was associated with the RBCs. Chromium-51-RBC counts, expressed as a percent of the 15-min sample, indicate no sample less than 90% for the times shown. Comparing these results with those reported by Ryo et al. in their Tables 1 and 2 (*1*), technetium-tagged RBCs prepared in our laboratory by Method B show an intravascular retention in dogs more prolonged than those prepared by the authors when they employed the same method. In fact, our results indicate a comparable if not "superior" biologic behavior for our technetium-labeled RBCs over those prepared by the authors' Method A. The reason for the discrepancy between the results is not fully understood. However, the authors do not indicate the binding efficiency of the technetium-labeled RBCs reinfused into the dogs, nor do they fully explain the broad range in biologic half-life (5–35 hr) obtained in patients when employing technetium-tagged RBCs labeled by Method A.

TABLE 1. WHOLE-BLOOD ^{99m}Tc RADIOACTIVITY IN DOGS AFTER INJECTION OF ^{99m}Tc-LABELED AUTOLOGOUS RED BLOOD CELLS

Time after injection (hr)	SnGh method (Method B)	Modified SnGh method
½	97%	97%
1	92%	97%
2	88%	91%
24	65%	67%

Technetium-99m radioactivity present in the whole-blood sample is expressed as a percentage of the radioactivity in the 15-min sample.

We feel that technical details may be critical. Ryo et al. imply that the SnGh RBC labeling technique (Method B) was employed unmodified; yet the Unitag system, which was used, has not been commercially available since January 2, 1974. We now employ a plastic syringe method for labeling RBCs, with minimal agitation. When other labeling procedures are employed, errors could result from:

1. Incompatibilities among the reagents, red blood cells, and the labeling container.
2. Insufficient capacity of the labeling vessel to contain the 20 ml of saline for adequate washing of the RBCs. This would result in inefficient removal of the excess stannous ion and lead to poor binding efficiency.
3. Differences in ACD formulation that would result in changes in pH or dextrose concentrations, which could be damaging to the RBCs.
4. Excessive agitation.

In addition to this, we noted that at times the authors employed 5% dextrose solutions as the suspending agent for the labeled RBCs. It is well known that 5% dextrose is incompatible with RBCs, causing aggregation of cells. Moreover, RBCs incubated in 5% dextrose solutions attain a high intracellular concentration of dextrose and these cells lyse on reinjection (*3*). These factors may play a role in the shortened and irregular biologic behavior of the authors' labeled RBCs.

Observations relating to this work are: (A) poor reproducibility and low labeling yields (not seen with Method B in our laboratory); (B) poor reproducibility of in vivo data (not seen with Method B in our laboratory); and (C) Method A is time-consuming, requires excessive handling of radioactive washes, and reagents are not commercially available in sterile pyrogen-free form.

All factors considered, the authors' claim that their method "appears to be the best for labeling RBCs. . . ." seems somewhat premature.

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Reply

We thank Gutkowski et al. for their comments on our recent report (*1*), and we appreciate their thoughtful questions and their efforts in rechecking their work. In order to make the article as concise as possible we had to omit some information that could be considered nonessential. But the elimination of "background information" can lead to a misunderstanding by the readers. Our publication lacks some detailed information, and this, we believe, caused some of the questions raised by Gutkowski et al. Likewise their article (*2*), which is a concise communication, lacks some details that caused some misinterpretation on our part.

We had no intention of implying that the SnGh RBC labeling technique was employed "unmodified." One of our objectives was to compare the stability of RBCs labeled by incubation of cells with tin ion alone, with addition of SnGh and EDTA under the same conditions. From their letter and from further correspondence with them, we have realized that the technique described in Gutkowski and Dworkin's article (*2*) was in part misinterpreted by us. We regarded Steps 1 to 4 of the SnGh method (*2*) (10 ml blood + 2 ml ACD + 2 ml ACD + SnGh solution + 1 ml, 5% EDTA) as the mixing phase and Steps 5 and 6 (add 10 ml saline and centrifuge) as the washing phase. Thus, red cells were exposed to EDTA at 4.16 mg/ml concentrations longer in our study (5 min) than what the authors had intended by the phrase "mix gently."

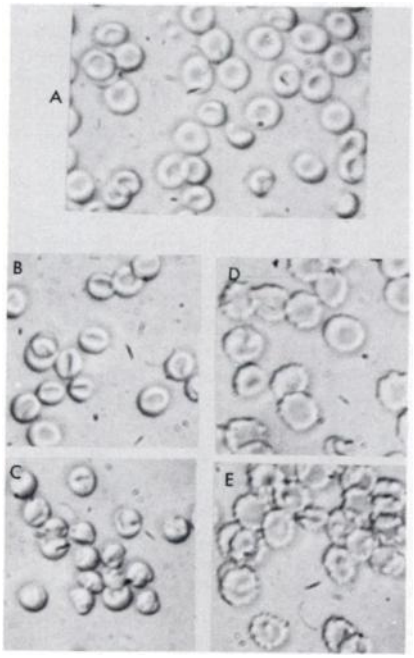

FIG. 1. Effect of EDTA on fresh red cells: RBCs suspended in physiologic saline (A); in 5% EDTA solution for 5 min (B) and 10 min (C); and in 2.5% NaCl solution for 5 min (D) and 10 min (E). (Photograph taken from suspended RBCs at 1,000× magnification.)

The inferior labeling efficiency that resulted in our study can be attributed mainly to two factors, the first being the effect of EDTA. More than 2.3 mg of EDTA mixed in 1 ml of blood causes distortion and shrinkage of RBC (*3*), and 5 mg/ml EDTA can cause a 10% change in hematocrit value (*4*). Depletion of extracellular Ca ion by EDTA is known to affect RBC membrane permeability to potassium ion (*5*). Introduction of EDTA into the cell causes a marked change in red cell shape and elasticity of the membrane, thus showing the critical role of calcium ion on the cell membrane (*6,7*).

Since direct effect of EDTA on fresh RBCs has not been well demonstrated, a simple in vitro experiment was carried out in our laboratory. Fresh RBCs were exposed to a high concentration of EDTA solution. It is well known that RBC becomes flatter with a "strawberry surface" when exposed to hypertonic solution (Figs. 1D and 1E). When RBCs were exposed to high concentrations of EDTA, however, stomatocytes were formed in 5 min and the majority of cells acquired a walnut appearance after 10 min (Figs. 1B and 1C). When ATP-depleted spherocytes were exposed to a high concentration of EDTA, the cells became flatter and larger in diameter, whereas hypertonic saline caused them to become smaller, with a granular surface (Figs. 2B and 2C). These are extreme examples of the EDTA effect on RBC, indicating that EDTA in the medium can change the RBC membrane properties significantly. The mechanism of stomatocyte formation in high concentrations of EDTA is unknown. One may presume, however, that contractile proteins of the cell membrane are damaged by the EDTA since these proteins regulate red cell shape and permeability (*8*).

In our opinion, EDTA, even at a lower concentration such as 2–3 mg/ml, changes the membrane properties, and EDTA at the concentration we used is at least partly responsible for the rapid disappearance of labeled RBCs from the circulation.

The second factor was the Vacutainer glass tube (B–D, No. 4705) and the rotator (Mallinckrodt/Nuclear, 12 rpm) that we used for ^{99m}Tc labeling of RBCs. The glass tube is sterilized by irradiation and has an interior coating with silicone. However, after 10–20 min of incubation on the rotator or in a shaking water bath, a recognizable hemolysis was noted in many samples. This hemolysis is probably another major cause of inferior labeling efficiency and marked variations in in vivo stability in our study.

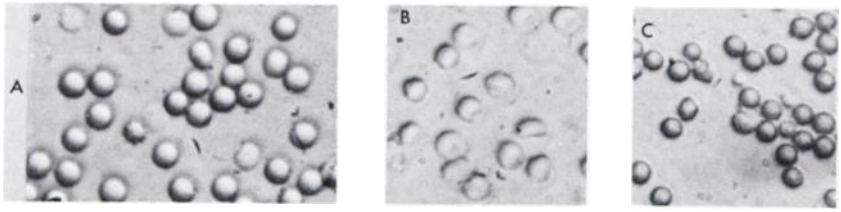

FIG. 2. Effect of EDTA on ATP-depleted red cells (spherocytes): (A) Spherocytes suspended in physiologic saline; (B) in 5% EDTA solution; and (C) in 2.5% NaCl solution. (Photograph taken from suspended RBCs at 1,000× magnification.)