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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."

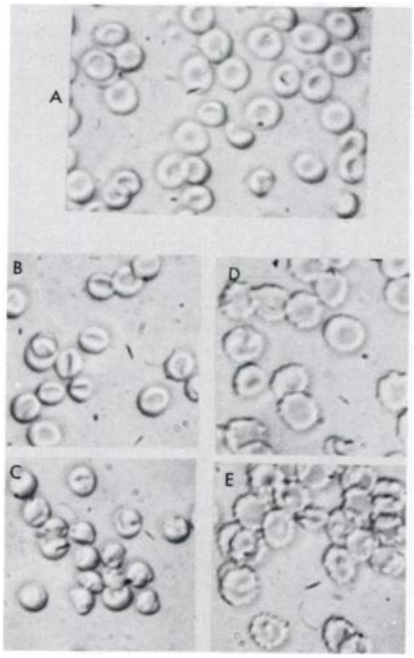

FIG. 1. Effect of EDTA on fresh red cells: RBCs suspended in physiologic saline (A); in 5% EDTA solution for 5 min (B) and 10 min (C); and in 2.5% NaCl solution for 5 min (D) and 10 min (E). (Photograph taken from suspended RBCs at 1,000× magnification.)

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.

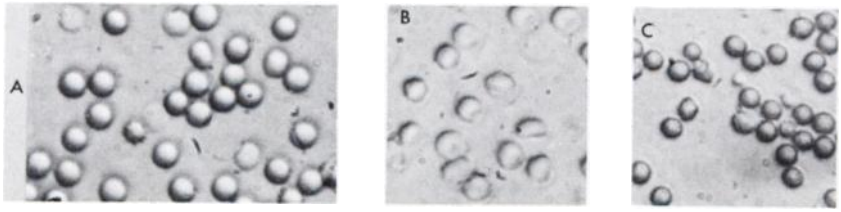

FIG. 2. Effect of EDTA on ATP-depleted red cells (spherocytes): (A) Spherocytes suspended in physiologic saline; (B) in 5% EDTA solution; and (C) in 2.5% NaCl solution. (Photograph taken from suspended RBCs at 1,000× magnification.)

Regarding the reason for the advantage of prolonged intravascular retention of the labeled RBCs, one of our clinical studies, evaluation of Raynaud's disease (*9*), requires regional blood-volume measurement sometimes 6 hr after an infusion of the labeled RBCs.

We are looking forward to seeing the "modified technique" of Gutkowski et al. which has eliminated the use of EDTA and uses less SnGh in labeling RBCs. The ACD solution we used is a commercially available (Mallinckrodt/Nuclear) sterile solution prepared for ^{51}Cr-RBC labeling.

In their letter, Gutkowski et al. suggested that suspension of RBCs before reinfusion may damage cells, whereas they do not accept the possibility that 50 mg of EDTA mixed gently into 10 ml of blood and 2 ml of ACD solution can damage red cells. In our study, RBCs were never incubated in 5% dextrose solution, but in one-third of the cases 2–3 ml of 5% dextrose was used to resuspend washed RBCs before the infusion. We did not detect any difference in the survival of RBCs suspended in 5% dextrose or in saline.

In regard to sterile pyrogen-free reagents, random samples of $SnCl_2$ dissolved in sterile ACD solution, filtered through a 0.22-μm filter, were tested for pyrogen with no instance of a positive result. When a stock solution of $SnCl_2$ in a nitrogen-filled vial is prepared, the expense becomes much less with pyrogen test than with use of SnGh.

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