SIMPLIFIED METHOD FOR SIMULTANEOUS DETERMINATIONS OF PLASMA VOLUME AND RED-CELL MASS WITH ^{125}I-LABELED ALBUMIN AND ^{51}Cr-TAGGED RED CELLS

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It has long been recognized that the body hematocrit and the large-vessel hematocrit are not the same and that this disparity introduces a systematic error into estimates of total blood volume derived by single-tracer methods. Although this does not seriously limit the practical value of such measurements for guiding replacement therapy, precise delineation of the total blood volume requires a double-tracer method in which the circulating red-cell mass and the plasma volume can be determined simultaneously and independently. This is especially important in experimental animals in which large changes in the ratio of the body hematocrit to the large-vessel hematocrit often occur, making estimates of total blood volume by a single tracer unreliable.

^{51}Cr-tagged red cells are the obvious choice for measurements of red-cell mass. But available tracers for simultaneous measurements of plasma volume have left much to be desired. The combination most commonly used is ^{51}Cr-tagged red blood cells and Evans blue dye (T-1824). ^{131}I-human serum albumin circumvents many of the vagaries of the dye method for determinations of plasma volume, but the energies of the principal gamma radiations of ^{51}Cr and ^{131}I are so close that accurate discrimination is possible only with very special spectrometric instrumentation. When ^{125}I is used in place of ^{131}I as the albumin label, the problems of isotopic discrimination by spectrometry are reduced (*1–3*). The radiation characteristics of ^{125}I, however, make possible an even simpler double-tracer method used for studies previously reported and validated in this laboratory (*4,5*).

METHOD

The principles underlying the method are illustrated schematically in Figs. 1 and 2. The relatively soft 0.027–0.032-Mev photons of ^{125}I can be blocked by a thin metal absorber that is transparent to the high-energy gamma rays of ^{51}Cr. A metal sleeve, interposed between the whole-blood samples and the scintillation crystals, prevents the radiations from the ^{125}I-albumin from influencing the assay of ^{51}Cr-RBC dilution (Fig. 1). Without the sleeve, determinations of plasma volume from the ^{125}I-albumin dilution can be made directly on the plasma obtained from the whole-blood specimens (Fig. 2).

Absorption of some of the soft ^{125}I radiations occurs within the plasma itself (self-absorption). Counting efficiency will therefore be influenced by sample geometry as well as by the material (glass or plastic) from which the specimen tube is constructed. With rigidly standardized plastic specimen tubes such as those we have used for these studies* accurate and reproducible plasma-volume measurements are readily obtained with this tracer.

An additional requirement is that the plasma contain no ^{51}Cr which would give rise to spuriously high plasma counting rates. Although one can detect and derive a subtractive correction for the contribution of contaminating ^{51}Cr to the plasma specific activity by counting the plasma specimens both without and with interposed metal sleeves, this problem is best avoided altogether by gentle handling of the blood samples to prevent hemolysis and by preparing the tagged-cell doses in such a way that all ^{51}Cr not firmly bound within the cell is removed.

Dosimetry calculations indicate that tracer doses in the ranges used (10–50 μc of ^{51}Cr and 0.5–3.0 μc of ^{125}I) do not constitute an appreciable radiation hazard. More than five of these double-tracer studies can be done on a patient without exceeding the recommended maximum permissible weekly whole-body irradiation dose (*6*).

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* From Ames-Atomium, Inc., Billerica, Massachusetts.

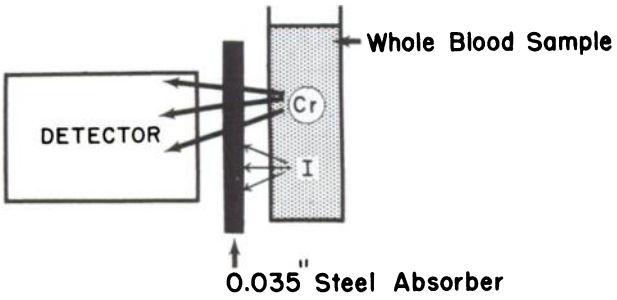

FIG. 1. Assay of ^{51}Cr in presence of ^{125}I. Soft ^{125}I radiations are blocked by metallic absorber that is transparent to relatively high-energy gamma rays of ^{51}Cr.

^{51}Cr-RBC TRACER DOSES

Twenty milliliters of freshly drawn blood from a healthy O-Rh negative donor are placed in a plastic bag containing 4 ml of special ACD solution.* This amount of blood is generally enough to prepare 10–12 doses of tagged cells. After gentle mixing of blood and anticoagulant, the bag is centrifuged at 1,500 rpm for 15 min. The supernatant plasma is drawn into a sterile 10-ml syringe and set aside. Six hundred microcuries of $Na_2{}^{51}CrO_4$ is added to the concentrated red cells, and the bag is allowed to stand with occasional gentle mixing for 15 min in a 37°C water bath. Ten milliliters of sterile saline is then mixed with the bag's contents. The bag is again centrifuged at 1,500 rpm, and the supernatant, including a small amount of red blood cells, is discarded. Second and third saline "rinses" are carried out in the same manner. About 6 ml of the original plasma is then gently mixed with the red cells, and 1.5–2.0-ml aliquots are drawn into sterile syringes which are then capped and stored at 4–10°C pending use. When they are prepared in this way, each dose syringe contains 40–50 μc of ^{51}Cr, with less than 1% contribution from plasma activity.

We recommend that these tracer doses be used within 12 hr after preparation. When older doses are used, spuriously high values for red-cell mass result. The effect of dose age on these determinations is illustrated in Fig. 3 from studies in an 18-year-old woman in whom the red-cell mass was reasonably assumed to be constant during the 48-hr period in which the observations were made. The progressive rise in red-cell mass (+5% at 24 hr, +13% at 48 hr) constitutes an appreciable error. This rise is attributable primarily to impaired viability of the tagged cells during storage because the *in vitro* elution rate of chromium from the erythrocytes is approximately 1% per day (*7*).

* Unitag bag, Abbott Laboratories.

^{125}I-ALBUMIN TRACER DOSES

^{125}I-labeled human serum albumin is available in high specific activity. Individual tracer doses are prepared by dilution with 1% human serum albumin in saline solution so that each syringe contains approximately 3.0 μc in 1.0–1.5 ml. Glass syringes are recommended for this purpose because effects of prolonged storage in plastic containers are uncertain. In contrast to ^{51}Cr-RBC this tracer has a long useful shelf-life. Doses prepared from refrigerated stock solutions as old as 8 months have yielded the same values for plasma volume as fresh doses of ^{131}I-albumin.

TECHNIQUE FOR DOUBLE-TRACER STUDIES

Although many types of scintillation-detection systems can be used for these studies, our experience has been with the Volemetron (*4,5,8*) with a readout of the scaler memory incorporated in the device. This makes it possible to measure separately the strengths of the ^{51}Cr-RBC and ^{125}I-albumin doses injected into the circulation (D_r and D_p, respectively) with corrections for background and residual syringe activity. With the instrument reset to a known scaler loading (D_o) observed readings for blood volume from dilution of tagged cells and labeled albumin can then be converted to actual values by the factors D_r/D_o and D_p/D_o, respectively.†

In each instance a baseline ("premix") blood sample should be obtained. At the desired interval(s) following injection of both tracers, a "postmix" blood sample is drawn from a vein distant from the site of injection. Determinations of the blood volume from tagged-cell dilution (BV_r) are made on whole blood in large plastic specimen tubes (about 8 ml). Measurements of plasma volume are then made with small plastic specimen tubes (about 2 ml) containing plasma from the blood samples.

† This procedure is simplified by the setable readout feature of the more recent model of the Volemetron (BV-3) which also incorporates an "Isotope Selection Switch" for ^{51}Cr, ^{131}I, or ^{125}I.

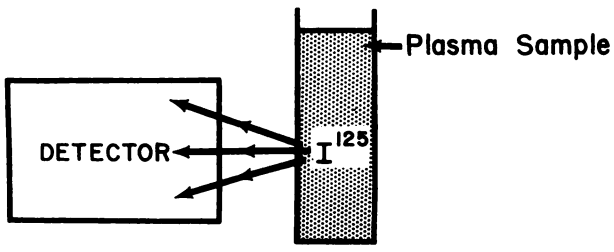

FIG. 2. Assay of ^{125}I. Without interposed absorber, determination of plasma volume is made by measuring ^{125}I-albumin dilution in plasma sample.

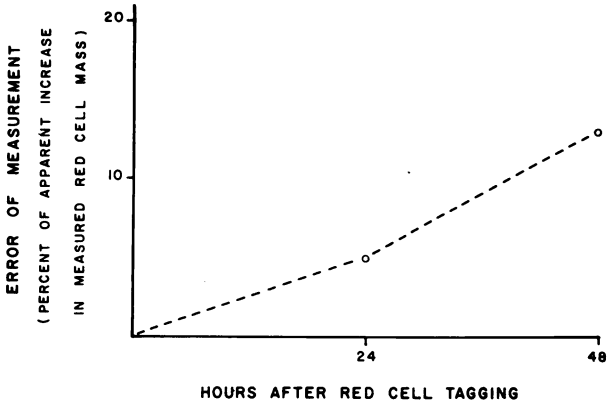

FIG. 3. Effect of dose age on red-cell mass measured with ^{51}Cr-tagged, O-neg red cells

Hematocrits are determined on the whole-blood samples by centrifugation at 3,000 rpm for 30 min in a clinical centrifuge and multiplied by 0.96 to correct for plasma trapping. This corrected hematocrit is used for the subsequent calculations.

As indicated above, the plasma volume (PV) is measured directly. The circulating red-cell mass (RCM) is calculated by multiplying BV_r by the corrected hematocrit. The whole-body hematocrit is calculated by dividing RCM by the true blood volume (RCM + PV).

SUMMARY

A simplified method is described for simultaneously determining red-cell mass and plasma volume with ^{51}Cr-tagged erythrocytes and ^{125}I-labeled albumin. A metallic absorber that is opaque to the soft radiations of ^{125}I permits differential assays of these isotopes with generally available scintillation-detection equipment.

ACKNOWLEDGMENT

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