Recommended Methods for Measurement of Red-Cell and Plasma Volume

International Committee for Standardization in Haematology*

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1. INTRODUCTION

The International Committee for Standardization in Haematology (ICSH) Expert Panel on Diagnostic Applications of Radio-Isotopes in Haematology has previously recommended techniques for measurement of red-cell volume and plasma volume in a report that was published in 1973. Following discussions at subsequent meetings of the Panel, and taking account of comments received from various organizations and individuals, the methods have been revised. The revisions are incorporated in the following document, which has been approved by the ICSH Board for publication as an ICSH Standard (EP 8/6).

There is no practical definitive reference method available for measuring either red-cell volume or plasma volume, but the methods to be described have been designated as ICSH-selected methods on the basis of their reliability, reproducibility, and ease of operation in routine clinical use. They are (a) measurement of red-cell volume using sodium radiochromate (Cr-51) or sodium pertechnetate (Tc-99m) as a red-cell label, and (b) measurement of plasma volume using radioiodine-labeled human serum albumin as a plasma label. There is evidence that the use of a protein with molecular weight considerably greater than that of albumin will give a smaller estimate of the distribution space and probably a more accurate indication of the true plasma volume. However, in practice no suitable alternative preparations are yet available.

Concerning the limitations associated with the use of automated equipment, comments that were included in the earlier document still apply and need not be repeated here.

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2. SELECTED METHODS FOR THE MEASUREMENT OF RED-CELL (RBC) VOLUME (RCV)

2.1 General.

The following comments apply to all methods. The specimen to be labeled should be the subject's own blood. During preparation of the labeled RBC suspension for injection, all operations should be carried out by sterile techniques and all solutions, must be sterile and pyrogen free.

As muscular exercise and changes in posture may cause transient fluctuations in packed cell volume, the subject should be at rest, in a recumbent position, for 15 min before administration of the labeled RBC suspension.

The injection of the labeled RBC suspension must be entirely intravenous; it is essential to avoid extravasation.

Venous-blood specimens are mixed with solid anticoagulant. EDTA (disodium or dipotassium salt, 1.5 ± 0.25 mg/ml of blood) or heparin (10 units or 0.1 mg/ml of blood) is suitable. As excess EDTA results in alteration in cell volume and causes a falsely low packed cell volume (PCV) it is essential to use the prescribed amount of EDTA. To avoid lysis, the blood should not be subjected to excessive pressure when it is being collected.

For calculations that involve packed cell volume, the PCV, as measured, must be corrected for trapped plasma (see 4.1) to give the venous hematocrit (Hv).

Immediately before injection of labeled red cells, a sample of blood in anticoagulant should be obtained to check for any residual radioactivity from a previous study. If necessary, the radioactivity should be measured and appropriate corrections applied to subsequent samples.

2.2 Specification of sodium radiochromate (Cr-51) and sodium pertechnetate (Tc-99m)

The materials used for labeling should conform to the specifications in the second edition of the *International*

Pharmacopoeia (Supplement 1971), "Specifications for the Quality Control of Pharmaceutical Preparations," published by the World Health Organization, Geneva, 1971. This document specifies that the specific activity of sodium radiochromate (Cr-51) should not be less than 20 mCi (740 MBq)/mg of chromium at the time of use. The specific activity of sodium pertechnetate (Tc-99m) should be more than 10^6 Ci (37×10^3 TBq)/g technetium and the aluminum content of the eluate should be less than 10 μg/ml. The publication also specifies the pH, sterility, radiochemical purity, and conditions of storage.

2.3 Technique using Cr-51 and washed cells†

2.3.1 Labeling. Add 10 volumes of blood obtained by venipuncture to 1.5 volumes of NIH A ACD solution.‡ Centrifuge the suspension at approximately 1000 *g* for 5 min, then remove and discard the supernatant. If the leukocyte count is greater than 25×10^9/l and/or the platelet count is greater than 500×10^9/l the buffy coat should also be removed.

Add sodium chromate (Cr-51) solution, slowly and with continuous gentle mixing, to the packed cells. The amount of Cr-51 added should be such that the patient receives not more than 0.2 μCi/kg (7.4 kBq) of body weight. If it is intended to combine the blood-volume estimation with an RBC survival study, the specific activity should be such that less than 2.0 μg of chromium is added per ml of packed red cells. The added radiochromate should be in a volume of at least 0.2 ml, being diluted in a 9 g/l (150 m*M*/l) sodium chloride solution (isotonic saline). Incubate the mixture for 15 min at 37°C. Wash the labeled cells twice in 4–5 volumes of isotonic saline. Resuspend the red cells in enough isotonic saline to allow an intravenous injection of about 10 ml (or a lesser volume if appropriate, e.g., in children), and the preparation of a standard for radioactivity measurements. When red-cell osmotic fragility is greatly increased, e.g., in cases of hereditary spherocytosis, use 12 g/l (205 m*M*/l) sodium chloride solution to wash the red cells.

2.3.2 Administration. Inject a known amount of the well-mixed labeled RBC suspension. One of the following methods should be used to determine this amount:

(a) Fill a precalibrated syringe to the mark with the labeled RBC suspension, then discard the needle used for filling the syringe, and inject the contents into a vein through another needle, preferably having inserted the needle previously into the vein. Flush it two or three times by withdrawing blood from the vein and then reinjecting it.

(b) Fill a syringe and needle with the labeled RBC suspension and weigh them. Inject the contents of the syringe without flushing it, and weigh the syringe and needle again.

(c) Weigh an empty syringe, fill it with the labeled RBC suspension and reweigh it. Inject and flush in the contents as in (a).

With any of these methods it is necessary to set aside a sufficient volume of the labeled RBC suspension for the preparation of a diluted standard solution.

(d) "Fixed-geometry method." This method depends on the prior determination of a calibration factor relating (a) the counting rate obtained with an amount of radioactivity in the injection syringe supported in a mechanical holder at a fixed distance (e.g., 40 cm) above the crystal of the well-scintillation counter to (b) the counting rate theoretically obtained with the same amount of radioactivity, in a volume equal to that of subsequent blood samples, and placed in the well of the crystal. The counting rate obtained with blood samples in the counter can thus be related directly to the corresponding counting rate due to the injected radioactivity, and the need to prepare a diluted standard solution is avoided.

Take approximately 5 ml of the labeled RBC suspension. Place the syringe in the holder above the scintillation counter and measure the counting rate. Inject the suspension. Replace the syringe in the holder and measure the residual counting rate.

2.3.3 Preparation of standard. Add an aliquot of the RBC suspension (e.g., 1 ml in method (a) above and 1 g in methods (b) and (c) above) to a volumetric flask (e.g., 100 ml) containing some 0.4 g/l NH_4OH. Fill the flask to the mark with 0.4 g/l NH_4OH. Deliver known volumes of the diluted standard thus obtained into duplicate counting tubes for radioactivity measurements. The same volume must be used for each subsequent sample.

Alternatively, deliver known small volumes (e.g., 50 μl) of the suspension directly into counting tubes containing volumes of 0.4 g/l NH_4OH equal to the volumes of the blood samples. If the fixed-geometry method is used, no standards need to be prepared.

2.3.4 Sampling. At 10 and 20 min after the injection, collect 5–10 ml of blood from a vein other than that used for injection, and add half to each of two specimen collection containers with anticoagulant. If it is suspected that mixing will be delayed (e.g., in patients with splenomegaly) another specimen should be taken at 60 min. Measure the PCV on an aliquot of each sample. Mix well and deliver known volumes (e.g., 2 or 3 ml) into counting tubes for radioactivity measurements. To achieve precision and to minimize error, pipettes of a uniform type should be used for a given series of samples. Lyse the samples in the counting tubes with saponin.

2.3.5 Radioactivity measurements. Measure the radioactivity of each sample. The coefficient of variation attributable to counting statistics should not exceed 2%.

2.3.6 Calculation of results. The total red cell volume (RCV) in ml is calculated as follows:

In method (a):

$$RCV = \frac{I}{C} = \frac{S \cdot D \cdot V_i}{C} = \frac{S \cdot D \cdot V_i \cdot H_v}{B}$$

where I = total amount of injected radioactivity (c/min)
C = concentration of radioactivity in red cells of blood sample drawn after mixing is completed (c/min per ml red cells)
S = concentration of radioactivity in diluted standard (c/min per ml)
D = dilution of diluted standard solution, i.e., final volume divided by volume of red cell suspension put into it
V_i = volume of labeled red cell suspension injected (ml)
H_v = PCV of whole blood sample corrected for trapped plasma
B = concentration of radioactivity in blood sample drawn after mixing is completed (c/min per ml blood)

In methods (b) and (c)

$$RCV = \frac{I}{C} = \frac{S \cdot R \cdot V_s}{C} = \frac{S \cdot R \cdot V_s \cdot H_v}{B}$$

where R = ratio of amount (g) of labeled red cell suspension injected to amount (g) of labeled red cell suspension added to volumetric flask in which diluted standard was prepared
V_s = volume to which standard was diluted (other symbols as for method (a) above).

Note: This general equation is valid also for method (a) if the "amounts" referred to in the definition of R are expressed in ml.

In method (d)

$$RCV = \frac{I}{C} = \frac{F(C_t - C_r)}{C} = \frac{F(C_t - C_r)H_v}{B}$$

where F = calibration factor relating counting rate of radioactivity in sample counter
C_t = net count rate of syringe before injection (c/min)
C_r = net count rate of "empty" syringe after injection (c/min)

If the blood sample obtained before injection of labeled cells indicates the presence of radioactivity from a previous study, appropriate corrections must be applied to all samples obtained after injection of the labeled cells.

2.4 Techniques using Cr-51 and ascorbic acid.

This method does not require the removal by washing of the fraction of the label that is not incorporated into red cells. However, it is necessary to determine this fraction by centrifugation of aliquots of the labeled RBC suspension and the postinjection blood samples, and by radioactivity measurements on their supernatants.

2.4.1 Labeling. The amount and specific activity of the Cr-51 added and the labeling procedure are identical with that described in paragraph 2.3.1 except that centrifugation is carried out only when the leukocyte count exceeds 25×10^9/l or the platelet count exceeds 500×10^9/l and no washing is required. Instead, 50 mg of ascorbic acid are added after 30 min.

2.4.2 Administration. Inject a known amount of the well-mixed labeled RBC suspension using methods (a), (b), or (c) of 2.3.2. Save a sufficient volume (e.g., 3 ml) of labeled suspension to prepare a whole-blood standard and (after centrifugation) a plasma standard.

2.4.3 Preparation of standards. Measure the PCV of an aliquot of the well-mixed RBC suspension. Pipette an aliquot (e.g., 1 ml) of the suspension into a volumetric flask (e.g., 100 ml) already containing some 0.4 g/l ammonia. Fill the flask to the mark with 0.4 g/l ammonia. Deliver known volumes (e.g., 2 or 3 ml) of the diluted standard thus obtained into duplicate counting tubes for radioactivity measurements (injectate standards). Centrifuge the remainder of the suspension for 10 min at about 1000 *g*. Deliver known volumes of the supernatant into duplicate counting tubes (injectate supernatants). The volumes of standard and supernatant must be the same as the volume of blood in each subsequent sample.

2.4.4 Sampling. At 10 and 20 min after the injection, take approximately 10 ml of blood from a vein other than that used for injection. If it is suspected that mixing will be delayed (e.g., in patients with splenomegaly), take another specimen at 60 min. Measure the PCV of an aliquot of each specimen. Deliver known volumes into duplicate counting tubes for radioactivity measurements, and lyse with saponin (postinjection whole blood samples). Centrifuge the remainder of each specimen for 10 min at about 1000 *g*. Deliver known amounts of the supernatants into duplicate counting tubes (postinjection plasma samples). If a preinjection blood specimen were obtained, whole-blood and plasma samples are similarly prepared for the determination of patient whole-blood and plasma radioactivity.

2.4.5 Radioactivity measurements. Measure the radioactivity of each sample. The coefficient of variation attributable to counting statistics should not exceed 2%. However, a lower statistical precision is acceptable for the plasma samples.

2.4.6 Calculation of results. The red cell volume (RCV) in ml is calculated as follows:

In method (a):

$$RCV = \frac{D \cdot V_i \cdot H_v \left[S - \frac{S_p(1 - H_i)}{D}\right]}{B - P(1 - H_v)}$$

where S_p = concentration of radioactivity in undiluted injectate supernatant (c/min per ml supernatant)

H_i = PCV of labeled red cell suspension for injection, corrected for trapped fluid

P = concentration of radioactivity in plasma of blood sample withdrawn after mixing is complete

(other symbols as in 2.3.6).

In methods (b) and (c)

$$RCV = \frac{H_v\left[S \cdot R \cdot V_s - S_p \cdot A\left(\frac{V_p}{A}\right)\right]}{B - P(1 - H_v)}$$

where A = amount (g) of labeled red cell suspension injected

$\frac{V_p}{A}$ = volume (ml) of supernatant per unit amount (g) of labeled red cell suspension injected (= $1 - H_i$ to an adequate approximation in this correction term)

(other symbols as for method (a) above and 2.3.6).

Note: This general equation is valid also for method (a) if A is expressed in ml.

If the blood sample obtained before injection of labeled cells indicates the presence of radioactivity from a previous study, appropriate corrections must be applied to all samples obtained after injection of the labeled cells.

2.5 Technique using Tc-99m.

Treatment of red cells with very small amounts of stannous ion before incubation with pertechnetate results in the subsequent binding of almost all of the added technetium, provided that essentially all of the plasma has been removed from the red cells before the addition of the stannous ion. This method also results in a more stable technetium label on the red cells, so that the rate of elution of technetium is less rapid than when technetium is added to the red cells before the stannous ion. It has been shown that optimal labeling is obtained when stannous chloride is used at a dose of about 0.015 μg stannous ion per ml of red cells (*1*). Accordingly, the following method is recommended.

2.5.1 Labeling. Take approximately 10 ml of blood into a container to which a few drops of liquid heparin have been added. Transfer exactly 5 ml of blood to another container to which 20 ml of saline have previously been added. Centrifuge the container and remove as much of the supernatant as possible to leave approximately 2 ml of packed red cells. Then add 0.015 μg of stannous ion per ml red cells. The necessary dilute solution of tin is prepared by a two-stage procedure as in the following examples.

Example 1. Weigh out 2 mg $SnCl_2 \cdot 2H_2O$ and dissolve in 10 ml saline. Take it up into a syringe and pass through a 0.22 μm Millipore filter into an empty container. Now transfer 1 ml into a 500-ml bottle of saline (as prepared for intravenous use; contents usually about 525 ml). After mixing, withdraw 0.3 ml to provide 0.03 μg stannous iron and add to the 2 ml packed red cells.

Example 2. Weigh out 2 mg $SnCl_2 \cdot 2H_2O$ in 35 ml saline. Pass this solution through a 0.22 μm Millipore filter into an empty vial. Using a tuberculin syringe, transfer 0.25 ml of the filtered solution into a sealed 250-ml bottle of saline to provide a solution containing 0.15 μg stannous ion/ml. Using a tuberculin syringe, withdraw 0.2 ml to provide 0.03 μg stannous ion to be added to the 2 ml packed red cells.

The stannous chloride may be weighed out in advance, but the time between dissolving it and adding the dilute solution to the red cells should not exceed 10 min.

Commercially available kits now provide alternative methods for pretreating the red cells, e.g., with stannous pyrophosphate or citrate, and which avoid the necessity for fresh preparation on each occasion. The acceptability of any such kits must first be checked by comparison with the recommended method, especially taking account of fractional uptake of the Tc-99m and its elution rate.

Five minutes after adding the tin, add 25 μCi (925 kBq) of freshly generated pertechnetate in approximately 0.2 ml saline to the red cells. Incubate for 5 min; then centrifuge and wash in cold saline and resuspend in a convenient volume of cold saline (e.g., approximately 25 ml to provide 20 ml suspension for injection and preparation of the standard). Reinject the patient as soon after preparation as possible, determining the amount injected as outlined in 2.3.2.

2.5.2 Preparation of standard. As described in 2.3.3.

2.5.3 Sampling. At 10 min after injection, collect blood from a vein other than that used for injection and add half to each of two specimen containers with anticoagulant. Measure the PCV on an aliquot of each sample. Dispense an appropriate volume into counting tubes as described in 2.3.4. Where delayed mixing is suspected (see 2.3.4), another sample should be collected at 30 min and similarly treated.

2.5.4 Radioactivity measurements. Because of the short half-life of Tc-99m, radioactivity has to be measured on the day of the test. The coefficient of variation attributable to counting statistics should not exceed 2%.

2.5.5 Calculation of results. Measurement is normally based on the 10-min sample. The RCV is calculated using the formulas given in 2.3.6.

Although up to 2% of the radioactivity is eluted from the red cells in 30 min, it is not considered necessary to apply a correction factor to measurements calculated from the 10-min and 30-min samples; however, as the elution increases significantly with time, this method is not suitable for sampling at 60 min. If in cases where some delayed mixing is suspected, there is a significant difference between measurements at 10 and 30 min, the 30-min measurement is likely to be more reliable and should be used for calculating the red-cell volume.

2.5.6 Simultaneous measurement of Cr-51 and Tc-99m. If Tc-99m radioactivity has to be determined with Cr-51 radioactivity already present, the total counts in the Tc-99m channel may be corrected for the contributing Cr-51 radioactivity by means of a Cr-51 standard counted separately in both channels. Tc-99m counts in the Cr-51 channel can be neglected when the appropriate channels are used.

3. SELECTED METHOD FOR THE MEASUREMENT OF PLASMA VOLUME (PV)

3.1 Specifications of radioiodine-labeled human serum albumin.

The materials used for injection should conform with the specifications in the second edition of the *International Pharmacopoeia* (Supplement 1971) "Specifications for the Quality Control of Pharmaceutical Preparations" published by the World Health Organization, Geneva, 1971. In addition, not more than 2% of the radioactivity should be in the form of free iodine at the time of use. The protein concentration should be about 20 g/l. To guard against the risk of serum hepatitis, Hb_sAg-negative donors must be used as a source of albumin, although note that serum hepatitis virus is probably destroyed by heating for 10 hr at 60°C.

3.2 Administration.

Collect approximately 20 ml of blood into a heparinized syringe by venipuncture; transfer the blood to a sterile container and centrifuge at about 1000 *g* for 5–10 min. Transfer a known amount (e.g., 2 or 3 ml) of the supernatant plasma into a counting tube to check for—and, if necessary, to measure—any residual radioactivity. Transfer approximately 7 ml of the plasma to a second container and add 0.05 μCi (1.8 kBq)/kg body weight of radioiodine-labeled human serum albumin. Alternatively, the radioiodine-labeled human serum albumin may be added to a solution of 50 g/l human serum albumin. Mix thoroughly. Inject a known amount (e.g., 5 ml) of the solution intravenously using one of the methods described in Section 2.3.2. Start a stop watch at the midpoint of the injection. Set aside the remainder of the solution for preparation of a standard for subsequent radioactivity measurement. If it is desired to block thyroidal uptake of radioiodine by administration of stable iodine (see Section 8) a suitable dosage is 20–40 mg iodine/day (i.e., potassium iodide 60 mg/day) commencing 1–2 days before injection of labeled protein and continuing for 2–4 wk.

3.3 Preparation of Standards.

Pipette an aliquot (e.g., 1 ml) of the labeled plasma or labeled human serum albumin into a volumetric flask (e.g., 100 ml) containing isotonic saline to which a small volume of alcohol has been added. Fill the flask to the mark with isotonic saline. Deliver known volumes of diluted standard thus obtained into duplicate counting tubes for radioactivity measurements. Alternatively, deliver known small volumes (e.g., 50 μl) of the labeled plasma or albumin solutions directly into counting tubes containing isotonic saline and alcohol equal to the volume of blood sample. If the fixed geometry method is used, no standards need be prepared.

3.4 Sampling.

At 10, 20, and 30 min collect 5 ml of blood from a vein other than that used for injection into specimen collection containers with anticoagulant. Centrifuge the samples to separate plasma.

3.5 Radioactivity measurements.

Measure the radioactivity of the standard and of the samples in a well scintillation counter or other suitable gamma counting system. The coefficient of variation attributable to counting statistics should not exceed 2%.

3.6 Calculation of results.

The plasma volume in ml is calculated as follows:

$$PV = \frac{S \cdot D \cdot V}{P_0}$$

where S = concentration of radioactivity in standard (c/min per ml)
D = dilution of diluted standard solution
V = volume of radioiodine-labeled albumin solution injected (ml)
P_0 = concentration of radioactivity in plasma sample (c/min per ml) corrected to zero time by extrapolation of three samples to the y axis after plotting the concentration of radioactivity in each against time on semilog paper.

If the amount of labeled solution injected is estimated by weight or if the fixed geometry method is used, plasma volume may be calculated using formulas similar to those given in 2.3.6 for methods b, c, and d. When only a single 10 min blood sample is taken the loss of radioactivity at this time is, as a rule, small and it is not considered necessary to apply a correction factor to measurements calculated from this sample. It is more reliable, however, to have three samples as above.

4. MEASUREMENT OF PACKED RED CELL VOLUME (PCV)

In the context of blood-volume estimation, PCV should preferably be measured by a direct method, by centrifuging in a capillary tube. Results may differ to some extent from those obtained by electronic counters, in which PCV is computed from MCV or other cell-size measurements. PCV obtained by an electronic counter is acceptable provided that the counter has recently been calibrated by reference preparations to give results for PCV comparable to those obtained by centrifugation. In published reports on blood volume, the method used for measuring PCV must be stated.

In order to reduce random errors in the PCV, the following points should be noted.

(a) The blood should be well mixed.

(b) Dry heparin or EDTA should be used as an anticoagulant. Excess EDTA causes alteration in cell volume.

(c) The measurement must be carried out within 6 hr of collecting the blood, which should be stored at 4°C until required for measurement.

(d) Errors due to trapped plasma are considered below.

4.1 Micromethod.

This method requires the use of capillary glass tubing of uniform bore, capable of sealing with a flat internal base when properly heat-sealed. Tubes conforming to national standards are suitable. The centrifuge should be capable of a constant minimal centrifugal force of 10,000 *g* at the sealed tip of the tube.

Plasma trapping of the order of 2% occurs when the blood is centrifuged for 5 min at 10,000 *g*, and a correction factor for this should be applied. When the PCV is more than 0.5, centrifugation should be continued for a further 5 min and a correction factor of 3% should then be subtracted.

4.2 Variation of plasma trapping in red-cell diseases.

In spherocytosis, thalassaemia, and some other red-cell disorders, plasma trapping may be slightly increased. In sickle-cell anemia the trapping is greater (5% or more) and it is essential to oxygenate the blood before a determination is made. Even then, a high level of trapped plasma may occur giving a misleading reading of PCV. In such cases hematocrit computed by an electronic counter may reflect the true PCV more accurately than centrifugation.

5. SEQUENTIAL BLOOD-VOLUME ESTIMATIONS

Sequential blood-volume estimations pose the problem that residual radioactivity may be present in subsequent blood samples and may contribute to their counting rate. It is necessary to select a nuclide with an effective half-life such that the residual radioactivity is reduced between one estimation and the next to 1% or less of the preceding radioactivity. The residual radioactivity may then be neglected. Accordingly, Tc-99m should be used for sequential measurements at short intervals. It is sometimes possible to use a different tracer for each estimation, selecting each so that the later measurements can be made independently of the earlier ones in mixed samples.

6. ESTIMATION OF TOTAL BLOOD VOLUME AS THE SUM OF RCV AND PV

Total blood volume is derived by summing simultaneous estimates of RCV and PV on the assumption that the volume of red cells is virtually the same as that of the total circulating blood cells. However, in some conditions, such as leukemia, the volume of circulating leukocytes may constitute a substantial fraction of the total circulating blood cells. The total blood volume will then be underestimated if the sum of the RCV and PV is used to estimate blood volume. The simultaneous estimation of RCV and PV requires two different tracers that can be measured independently of each other in appropriate samples. The two tracers may be mixed before injection or injected sequentially through the same needle. The choice of tracer for such simultaneous estimation is governed by the need to measure each independently. The decision as to which combination to use depends upon the ease of separation of the principal emissions or the relative decay rates, which might allow measurements of radioactivity of one nuclide after that of the other has fallen to a negligible level (see Table 1).

Total blood volume is often calculated from either circulating RCV or PV and corrected PCV (H_v) using the formula:

$$BV = \frac{RCV}{0.9H_v} \text{ or } \frac{PV}{1 - 0.9H_v}.$$

The figure 0.9 represents a mean of the ratio of whole-body hematocrit (H_b) to venous hematocrit (H_v). However, there is a wide deviation from this mean, both in normal subjects and in patients with various conditions—notably in cases of splenomegaly.

7. PRESENTATION AND ANALYSIS OF ESTIMATED RCV AND PV

The interpretation of an estimate of RCV or total blood volume depends upon a comparison between the observed value and either another value previously ob-

TABLE 1. PRINCIPAL PHOTON EMISSIONS AND HALF-LIVES OF RADIONUCLIDES USED FOR SIMULTANEOUS PLASMA AND RED-CELL VOLUME ESTIMATIONS

Plasma tracers			Red-cell tracers		
Isotope	Principal photon energy (MeV)	Half-life (days)	Isotope	Principal photon energy (MeV)	Half-life
I-125 albumin	~0.03	60	Cr-51	0.320	27.8 days
I-111 albumin	0.365	8.05	Tc-99m	0.140	6 hr

tained in the same subject or the value that would be found in the same subject in health.

In practice no method is available for predicting accurately the blood volume of a given normal individual, so that only fairly large deviations from normal values can be established.

The most common method of presenting blood-volume estimates is in terms of body weight (ml/kg). This method is theoretically unsatisfactory because the relation between blood volume and body weight varies according to body composition; for example, in obese subjects blood volume tends to be low in relation to body weight.

Blood volume is more closely correlated with lean body mass than with body weight, but the determination of lean body mass is not practical as a routine procedure, and, in any case, predictions of blood volume based on this variable do not appear to be greatly superior to those based on body weight, except in very thin or very fat subjects.

In significantly obese patients, the value should be expressed on the basis of the estimated ideal or lean body mass for the given patient based on sex and height. In the seriously ill individual who previously had a body weight within the broad lines of normal but who recently has had severe weight loss, these results should be expressed on the basis of his/her body weight before the onset of the recent illness.

Normal values for RCV in adult males are usually taken as 30 ml/kg (95% confidence limits about 25–35 ml/kg) and for adult females as 25 ml/kg (95% confidence limits about 20–30 ml/kg). These values apply only to subjects living at sea level, and even then may not apply to all populations.

TABLE 2. FORMULAS FOR OBTAINING BLOOD-VOLUME REFERENCE VALUES FROM BODY SURFACE AREAS (S)

Red cell volume	
Men	$RCV(2) = 1100 \cdot S$
	$RCV(3) = 1486 \cdot S^2 - 4106 \cdot S + 4514$
	$RCV(4) = 1550 \cdot S - 890$
Women	$RCV(2) = 840 \cdot S$
	$RCV(4) = 1167 \cdot S - 479$
Plasma volume	
Men	$PV(2) = 1630 \cdot S$
	$PV(3) = 995e^{0.6085 \cdot S}$
	$PV(4) = 1580 \cdot S - 520$
Women	$PV(2) = 1410 \cdot S$
	$Pv(3) = 1278 \cdot S^{1.289}$

Estimates of PV in normal subjects made with radioiodine-labeled albumin, have given an average value of about 40 ml/kg in both men and women. The mean values for PV in different published series vary more widely than those for RCV probably because PV is labile and varies, for example, with posture, and also possibly because of differences in the quality of the labeled albumin used for the estimation. For these reasons it is not possible to give well-based confidence limits for normal PV.

Predictions of blood volume based on height and weight, or on some derivation from or combination of these measurements (e.g., surface area), are slightly more reliable than those based on body weight alone, especially in obese subjects. None of the prediction formulas gives 95% confidence limits better than ±10%, but their advantage is that they allow an individual subject's

TABLE 3. RADIATION DOSES TO PATIENT IN RED-CELL AND PLASMA VOLUME ESTIMATIONS

Estimation	Label or tracer	Organ (critical organ in bold type)	Fraction of administered radioactivity reaching organ	Mean radiation dose to organ per μCi administered (mrad)*
Red-cell volume	Cr-51	**Spleen**	0.2	3.9†
	Tc-99m	**Blood**	1.0	0.067†
	Tc-99m	Thyroid	0.002	0.04†
Plasma volume	I-125 albumin	Blood	1.0	2.6‡
	I-125 albumin	**Thyroid**	0.2	750.0‖
	I-131 albumin	Blood	1.0	7.0‡
	I-131 albumin	**Thyroid**	0.06	300.0‖

* 1 μCi = 37 kBq; 1 mrad = 10 μGy.

† Intravenous injection of labeled compatible cells, with normal survival.

‡ Intravenous injection of labeled protein; metabolized normally; thyroid uptake of radioiodine blocked.

‖ Intravenous injection of labeled protein; metabolized normally; thyroid uptake of radioiodine not blocked.

observed value to be interpreted by comparison with the appropriate reference value. Some of the formulas that have been proposed using surface area, and that appear to be fairly reliable, are given in Table 2.

8. RADIATION DOSE TO THE PATIENT IN RED CELL AND PLASMA VOLUME ESTIMATIONS

The radiation doses to a 70-kg patient in the procedures described above are shown in Table 3. This shows the doses in normal subjects, per μCi of administered radioactivity, to the critical organ or tissue—i.e., the organ or tissue of the body most likely to receive the highest radiation dose as a consequence of the procedure in question. The calculations on which these data are based involve certain assumptions. The circulating blood cannot be represented in terms of a simple physical model for purposes of dose calculations. Detailed quantitative data concerning the distribution and fate of the different radioactive labels in the body are lacking. Data for a given procedure in normal subjects could be very different in disease conditions. The data, therefore, do not give more than an approximate indication of the radiation dose to the patient for the various procedures.

When Cr-51-labeled cells are injected, Cr-51 is slowly eluted from the cells in the circulation. The labeled cells eventually undergo destruction in the reticuloendothelial tissues, from which the deposited radioactivity is again slowly eluted. In these circumstances, the critical organ may be taken as the spleen. When Tc-99m-labeled red cells are injected, the radiation doses to the various organs and tissues are determined mainly by the rapid physical decay of the label in the circulation. In these circumstances the critical organ may be taken as the blood.

When a radioiodine-labeled protein is injected, the tracer at first mixes with the intravascular protein pool and later becomes distributed between that pool and the extravascular protein pool. With subsequent metabolic degradation of the labeled protein, the label is released, in the form of inorganic iodide, to the exchangeable iodide pool. Two situations are considered. The first of these is that in which thyroidal uptake of radioiodine is blocked by administration of stable iodine. After its release to the exchangeable iodine pool, the label is then almost entirely excreted in the urine, and the critical organ may be taken as the blood. The second case is that in which thyroidal uptake is not blocked. A significant proportion of the label, assumed here to amount to 20%, then accumulates in the thyroid, which becomes the critical organ and receives a relatively high radiation dose.

FOOTNOTES

† For the sake of brevity, only one technique of labeling red cells with Cr-51 is described here. However, the "citrate-wash" method is equally suitable (see ICSH, 1971; 1972).

‡ NIH A ACD solution: trisodium citrate dihydrate, 22 g; citric acid (monohydrate), 8 g; dextrose (hydrous), 25 g; water to 1 liter.

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