Standard Techniques for the Measurement of Red-Cell and Plasma Volume

A Report by the ICSH Panel on Diagnostic Applications of Radioisotopes in Haematology

1. Introduction

The expert panel on the Diagnostic Applications of Radioisotopes in Haematology, which was established by ICSH in 1966 has, at its recent meetings in Bethesda (1970), Cadarache (1971) and Ulm (1972) discussed recommendations for the standardization of blood volume measurements. The panel comprised E.H. Belcher (I.A.E.A.), N.I. Berlin (U.S.A.), J.G. Eernisse(Netherlands), L. Garby(Sweden), H.I. Glass(U.K., Secretary), H. Heimpel (Federal Republic of Germany), M. Lee (Republic of Korea), S.M. Lewis (U.K.), P. McIntyre (U.S.A.), P.L. Mollison (U.K.), E.A. Murphy (U.S.A.), Y. Najean (France) and L. Szur (U.K., Chairman). The following document was prepared at these meetings and deals with technical and analytical aspects of red-cell and plasma volume measurements.

Estimations of blood volume are of value in those circumstances in which changes in the venous packed volume (PCV) fail to reflect accurately changes in red-cell volume (RCV) or plasma volume (PV). Thus, for example, when the PCV is increased the estimation of RCV distinguishes relative from absolute polycythaemia. In cases of absolute polycythaemia it will indicate the severity of the disease, which may be masked by plasma volume changes. In cases of gross splenomegaly, red cell pooling in the spleen and an increase in plasma volume may alter significantly the relationship between PCV and RCV. In patients who have suffered severe trauma or have undergone operations involving extensive haemorrhage, estimates of blood volume may reveal a degree of oligaemia which has not been suspected from changes in the PCV.

The volume of a closed system can be determined by using a tracer and the dilution principle. In open systems, such as the vascular system, the volume cannot be determined simply by this principle. However, if the tracer leaves the open system at a rate which is slow compared to the rate of mixing, and provided that samples are taken only after mixing has been completed, the volume can be obtained by a suitable extrapolation procedure. The volume obtained depends upon the precise extrapolation procedure used.

In most cases, RCV and PV can be estimated reproducibly using, respectively, labelled red cells or labelled proteins. Each variable can be estimated by a separate procedure and the total blood volume obtained by summation. The problems involved in estimating total blood volume in this way are discussed in detail in Section 7. The calculation of total blood volume from a measurement of either RCA or PV, although frequently done, is less reliable. When whole-body haematocrit (H^h), obtained from direct measurements of RCV and PV, is compared with venous haematocrit (H_V), i.e. PCV corrected for trapped plasma, it is usually found that in normal subjects H_B is consistently lower then H_V, the ratio H_B/H_V being approximately 0.9 when the plasma volume is estimated with albumin.

In cases of massive splenomegaly, the ratio H_B/H_V is higher than in normal subjcts and in patients with various other conditions, the ratio H_B/H_V has been reported to vary more widely than in normal subjects. Accordingly, in disease it is unsafe to deduce total blood volume from a measurement of red cell volume alone or of plasma volume alone and, as mentioned above, both must be measured if an estimate of total blood volume is required.

As a red cell label, ^{51}Cr in the form of sodium chromate is the most suitable. Other red cell labels such as ^{11}CO, ^{32}P, ^{42}K, ^{86}Rb, and $^{99}Tc^m$ have been used. All of these labels however are more rapidly eluted from the red cells in vivo than is ^{51}Cr; in addition several are not generally available. In view of the extensive prior experience with ^{32}P in the form of sodium phosphate, it is at present considered to be the label of choice when a second label is required. However, recent work suggests that $^{99}Tc^m$ in the presence of trace amounts of stannous choloride may be a suitable agent for labelling red cells for RCV estimations. The short half life of $^{99}Tc^m$ (6 hours) would have the advantages of reducing the radiation dose and facilitating serial measurements. The use of this radionuclide is now being investigated by the Panel and a recommended method will be published as a supplementary document when this study is completed.

The most commonly used protein for estimation of PV is human serum albumin. There is evidence that the use of a protein with molecular weight considerably greater than that of albumin would give smaller estimates of distribution space. Such estimates are likely to be more representative of the true plasma volume. However, at present no suitable alternative preparations are widely available. One advantage of albumin is that it can be heat-treated to inactive any serum hepatitus virus which may be present. It is essential that the albumin be obtained from HB Ag—negative donors who have been repeatedly tested. Other plasma proteins, for example, purified IgM cannot be heat-treated without being seriously damaged. As a protein label radioactive iodine in the form of ^{131}I, or better ^{125}I, is suitable. $^{99}Tc^m$ and $^{113}In^m$ are potentially valuable because of the small radiation dose which they confer, but their use is still under investigation.

2. Standard Techniques for the Estimation of Red-Cell Volume

2.1. General

The following comments apply to all methods.

During preparation of the labelled red cell 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 have been at rest, in a recumbent position, for 15 minutes prior to administration of the labelled red cell suspension.

The injection of the labelled red cell suspension must be entirely intravenous and it is essential to avoid extravasation.

Venous blood samples are collected into solid anticoagulant. EDTA (disodium or dipotassium salt, 1.5 ± 0.25 mg/ml of blood) and heparin (0.1 mg/ml of blood) are suitable. As excess EDTA results in alteration in cell volume and causes a falsely low PCV it is essential to use the prescribed amount of EDTA.

For calculations which involve packed cell volume, the PCV, as measured, must be corrected for trapped plasma (see 4.1).

In the majority of cases the specimen to be labelled should be the subject's own blood. If other blood is used it is essential that it comes from a compatible donor of the ABO and Rh group and the donor must be HB Ag-negative.

2.2. Specification of Sodium Radiochromate (^{51}Cr) and Sodium Radiophosphate (^{32}P)

The materials used for labelling should conform with the specifications in the second edition of the International Pharmacopoeia (Supplement 1971) 'Specifications for the Quality Control of Pharmaceutical Preparation' published by the World Health Organisation, Geneva, 1971. This document specifies that the specific activity should not be less than 20 mCi/mg of chromium at the time of use in the case of sodium radiochromate (^{51}Cr) and not less than 1 mCi/mg of phosphate ion at the time of use in the case of sodium radiophosphate (^{32}P). It also specifies the pH, sterility, radiochemical purity and conditions of storage.

2.3. Technique using ^{51}Cr and washed cells*

2.3.1. Labelling: Add 10 volumes of blood obtained by venepuncture to 1.5 volumes of 'N.I.H. A' ACD solution** in a sterile container. A sample of blood in anticoagulant should be obtained to check for, and if necessary to measure, any residual radioactivity from a previous study. Centrifuge the suspension at approximately 1000 g for 5 min and remove and discard the supernatant plasma. If the leukocyte count is greater than $25 \times 10^9/l$ and/or the platelet count is greater than $500 \times 10^9/l$, the buffy coat should also be removed.

Add ^{51}Cr-sodium chromate solution slowly and with continuous gentle mixing to the packed red cells. The amount of ^{51}Cr added should be such that the patient receives not more than 0.2 μCi/kg of body weight. If it is intended to combine the blood volume estimation with a red-cell 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 ^{51}Cr-sodium chromate should be in a volume of at least 0.2 ml, being diluted in a 9 g/l (150 mmol/l) sodium chloride solution (isotonic saline). Incubate the mixture for 15 minutes at a temperature not less than 15°C or greater than 37°C. Wash the labelled cells twice in 4—5 volumes of isotonic saline. After the second wash there should be less than 1% of the remaining radioactivity in the supernatant liquid. If necessary a further wash should be performed. Resuspend the red cells in a sufficient volume of isotonic saline to allow an intravenous injection of about 10 ml (or a lesser volume of 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 mmol/l) sodium chloride solution to wash the red cells.

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

(a) Calibrate a syringe by weighing the amount of distilled water which is delivered when the syringe has been filled to a mark, (thus ensuring a defined volume) and the contents have been expelled into a weighed vessel. For the injection fill the syringe and the attached needle to the mark with the labelled red-cell suspension. After inserting the needle into a vein inject the contents of the syringe. Do not flush the syringe.

(b) Calibrate a syringe by weighing it first empty and then filled to a mark (thus ensuring a defined volume) with distilled water. For the injection, fill the syringe to the mark with the labelled red-cell suspension, then discard the needle

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* For the sake of brevity, only one technique of labelling red cells with ^{51}Cr is described here. However, the citrate-wash method is equally suitable (see International Committee for Standardisation in Haematology Report, British Journal of Haematology, 21, 241, 1971; Blood, 38, 378, 1971; American Journal of Clinical Pathology, 58, 71, 1972).

**'N.T.H. A' ACD solution:

trisodium citrate dihydrate	22 g
citric acid	8 g
dextrose	25 g
water to	1 litre

used for filling the syringe and attach the syringe to a needle which has previously been inserted into a vein. Inject the contents of the syringe and flush it two or three times by withdrawing blood from the patient and then reinjecting it. This method is convenient, for example, when labelled cells have to be injected into an intravenous catheter.

(c) Fill a syringe and needle with the labelled red-cell suspension and weigh them. Inject the contents of the syringe without flushing it and weigh the syringe and needle again. The volume injected can be calculated from a knowledge of the density of the suspension, which may be derived from its haemoglobin concentration, using the formula given below.

$$\text{Volume injected(ml)} = \frac{\text{Wt. of suspension injected(g)}}{\text{Density of suspension(g/ml)}}$$

$$\text{Density of suspension} = 1.000 + \left[\frac{\text{Hb conc. of susp.(g/100 ml)}}{34} \times 0.097\right]$$

(This assumes that packed red cells have a Hb concentration of 34 g/100 ml and a density of 1.097 g/ml).

If method (a), (b) or (c) is employed it is necessary to set aside a sufficient volume of the labelled red-cell 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 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-type scintillation counter to the counting rate obtained with the same radioactivity, in a given volume 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 labelled red-cell 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 counting rate due to residual radioactivity.

2.3.3. Preparation of Standard: Pipette an aliquot (e.g. 1 ml) of the well mixed red-cell suspension into a volumetric flask (e.g. 100 ml) already containing ammonia (0.4 g/*l*). Fill the flask to the mark with 0.4 g/*l* (11.4 mmol/*l*) ammonia. Deliver known volumes of the diluted standard thus obtained into duplicate counting tubes for radioactivity measurements.

Alternatively, deliver known small volumes (e.g. 50 μl) of the suspension directly into counting tubes containing volumes of 0.4 g/*l* ammonia 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 minutes after the injection, take a 5～10 ml blood sample from a vein other than that used for injection, and divide the sample in equal volumes between two vials containing anticoagulant. Solid heparin or Na_2 or K_2-EDTA should be used as the anticoagulant. If it is suspected that mixing will be delayed (e.g. in patients with splenomegaly) another sample should be taken at 60 minutes. Measure the PCV on an aliquot of each sample. Lyse the remainder with saponin, and after further mixing deliver known volumes (e.g. 2 or 3 ml) into counting tubes for radioactivity measurements. To achieve precision and to minimize errors, pipettes of a uniform type should be used for a given series of samples. Alternatively, pre-calibrated tuberculin syringes may be used.

2.3.5. Radioactivity Measurements: Measure the radioactivity of each sample in a well-type scintillation counter or other suitable gamma-ray measurement system to a standard deviation of 2% or less.

2.3.6. Calculation of results: The red cell volme (RCV) in ml is calculated as follows. In methods (a), (b) and (c)

$$RCV = I/C = SDV/C = SDVH_V/B$$

where I = total injected radioactivity (c/min)

C = radioactivity in red cells after mixing is completed (c/min per ml red cells)

S = counting rate of standard (c/min per ml)

D = dilution of diluted standard solution, i.e. ratio of its final volume to the volume of labelled blood put into the diluted standard solution

V = volume of labelled red cell suspension injected (ml)

H_V = PCV of whole blood sample corrected for trapped plasma

B = counting rate of blood sample (c/min per ml)

In method (d)

$$RCV = I/C = F(C_t - C_r)/C = F(C_t - C_r)H_V/B$$

where F = calibration factor relating counting rate of radioactivity in syringe to counting rate of the same radioactivity in sample counter.

C_t = counting rate of filled syringe before injection (c/min)

C_r = counting rate of 'empty' syringe after injection (c/min)

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

2.4. Techniques using ^{51}Cr and ascorbic acid

This method does not require the removal by washing of the fraction of the label which is not incorporated into red cells. However, it is necessary to determine this fraction by centrifugation of aliquots of the labelled red-cell suspension and the post-injection blood samples and radioactivity measurements on their supernatants.

2.4.1. Labelling The amount and specific activity of ^{51}Cr added and the labelling procedure are identical with that described in paragraph 2.3.1. except that centrifugation is carried out only when the leukocyte count exceeds $25\times10^9/l$ or the platelet count exceeds $500\times10^9/l$, and no washing is required. Instead, 50 mg of ascorbic acid are added after 30 minutes to the tube containing the labelled red-cell suspension and the tube is inverted gently several times. If is then allowed to stand for 3 minutes.

2.4.2. Administration: Inject a known amount of the well mixed labelled red-cell suspension. Save a sufficient volume (e.g. 3 ml) of labelled suspension to prepare a whole blood standard a plasma standard.

2.4.3. Preparation of standard: Measure the PCV of an aliquot of the well mixed red-cell suspension. Pipette an aliquot (e.g. 1 ml) of the suspension into a volumetric flask (e.g. 100 ml) already containing 0.4 g/l ammonia. Fill the flask to the mark with 0.04 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 1,000 g. Deliver known volumes of the supernatant into duplicate counting tubes (standard supernatants).

2.4.4. Sampling: At 10 and 20 minutes 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 sample at 60 minutes. Measure the PCV of an aliquot of each sample. Diliver known volumes into duplicate counting tubes for radioactivity measurements, and lyse with saponin (post-inection whole blood samples). Centrifuge the remainder of the samples

for 10 minutes at about 1,000 g. Deliver known amounts of the supernatants into duplicate counting tubes (post-injection plasma samples). If a pre-injection blood sample was 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 in a well-type scintillation counter or other suitable gamma-ray measurement system to a standard deviation of 2%. A low 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:

$$RCV = DVH_v[S_i - S_s(1-H_i)]/[B - P(1-H_v)]$$

where D=dilution of diluted standard solution, i.e. ratio of its final volume to the volume of labelled blood put into the diluted standard solution

V=volume of labelled red-cell suspension injected (ml)

H_v=PCV of sample corrected for trapped plasma

S_i=counting rate of injectate standard (c/min per ml)

S_s=counting rate of standard supernatant (c/min per ml)

H_i=PCV of labelled red-cell suspension corrected for trapped fluid

B=counting rate of blood sample (c/min per ml)

P=counting rate of plasma sample (c/min per ml)

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

2.5. Technique using ^{32}P

2.5.1. Labelling: The stock of ^{32}P-sodium phosphate solution should be diluted in citrate phosohate dextrose solution* partitioned into ampoules, each containing approximately 5 μCi of ^{32}P, and autoclaved. Draw 2 or 3 drops of sterile heparin solution (at least 5,000 i.u./ml) into a syringe and then obtain about 10 ml blood by venepuncture. A sample of uncoagulated blood should be obtained at the same time to check for and, if necessary, to measure any residual radioactivity. Transfer the heparinised blood to a sterile container. Centrifuge for at least 5 min at about 1,000 g. Discard the supernatant plasma and as much as possible of the buffy coat. Add 15~20 ml citrate-phosphate-dextrose solution. Mix. Centrfuge for 5 min at about 1,000 g. Discard the supernatant. Add 0.05 μCi ^{32}P/kg body weight, to the packed red-cell suspension. Mix. Incubate at 37°C for 30 minutes. Wash the red cells twice in 15~20 ml isotonic saline at 0~4°C and then make up in isotonic saline in an adequate volume for the subsequent procedure. Keep the container in an ice-bath until the time of injection.

2.5.2. Administration: Inject a known amount (e.g. 10~20 ml) of the labelled red-cell suspension using one of the methods (a), (b) or (c) described in section 2.3.2. Start a stop-watch at the mid-point of the injection. Set aside the remainder of the suspension for preparation of a standard for subsequent radioactivity measurement counting.

2.5.3. Preparation of standard: Prepare a 1 in 100 dilution of the labelled red-cell suspension in 0.4 g/l, ammonia for use as a standard in radioactivity measurements.

2.5.4. Sampling: At 10, 20 and 30 minutes after the injection take an appropriate amount of blood from a vein other than that used for the injection, and divide the samples equally between

*trisodium citrate (dihydric) 30 g
sodium dihydrogen phosphate (dihydric) 0.15 g
dextrose (anydrous) 2 g
water to 1 litre

two containers. If it is suspected that mixing will be delayed (e.g. in patients with splenomegaly) sample should be taken instead 30, 45 and 60 minutes. The additional samples are required because of the significant loss of ^{32}P from the label in this time. Solid heparin or EDTA may be used as anticoagulant. Measure the PCV on an aliquot of each sample. Lyse the remainder with saponin and mix well, preferably on a mechanical rotary mixer for 5 minutes.

2.5.5. Radioactivity measurements: Measure the radioactivity of each sample in either a liquid Geiger-Müller (G-M) counter or a planchet G-M counter to a standard deviation of 2% or less.

2.5.6 Calculation of results: The red-cell colume (RCV) in ml is calculated as follows:

$$RCV = SDVH_v/B_o$$

where S=counting rate of standard (c/min ml)

D=dilution of diluted standard solution, i.e. ratio of its final volume to the volume of labelled blood put into the the diluted standard solution

V=volume of labelled red-cell suspension injected (ml)

B_o=counting rate of blood sample corrected to zero time (c/min ml)

H_v=corrected PCV of blood sample

When several blood samples are taken, B_o is determined by linear extrapolation on semi-logarithmic graph paper. When only a single 10 min sample is taken, the mean net counting rate is multiplied by 1.015 to correct for the loss of label during the 10 min period.

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

3. Standard Techniques for the Estimation of Plasma Volume

Most of the general comments in (2.1) above apply equally to plasma volume estimations.

3.1. Specification of Radioiodine-labelled 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 Organisation, 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 Ag-negative donors must be used as a source of albumin although it may be noted that serum hepatitus virus is probably destroyed by heating for 10 hours at 60°C.

3.2. Administration

Obtain approximately 20 ml blood into a heparinized syringe by venepuncture, transfer the blood to a sterile container and centrifuge at about 1,000 g for 5~10 mins. 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 sterile container and add 0.05 μCi/kg body weight of radioiodine-labelled human serum albumin. Alternatively, the radioiodine-labelled 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.

If it is desired to block thyroidal uptake of radioiodine by administration of stable iodine(see section 9) a suitable dosage is 20~40 mg iodine/day(e.g. potassium iodide 60mg/d) commencing 1~2 days before injection of labelled protein and continuing for 2~4 weeks.

3. 3. Sampling

At 10, 20 and 30 min collect 5 ml of blood from a vein other than that used for injection. Solid heparin or EDTA may be used as an anticoagulant. The multiple sampling technique is to be preferred and is recommended. If in exceptional circumstances multiple sampling is impracticable, a single sample may be taken at 10 min. It is then necessary to assume an empirical factor to correct for the loss of labelled albumin from the plasma during the 10 min period (see 3. 5). The error involved in this procedure is likely to be significant when radioiodine-labelled albumin is lost at a faster than average rate or when mixing is delayed.

3. 4. Preparation of Standard and Sample; Radioactivity Measurements

From each specimen, separate plasma by centrifugation and transfer a known amount (e.g. 2 or 3 ml) into a counting tube. Prepare standards (unless fixed geometry method is used) from the injection mixture as described in section 2. 2. 3. above. The diluent, however, should be isotonic saline to which a small amount of detergent has been added. Measure the radioactivity of the standard and of the samples in a well-type scintillation counter or other suitable gamma-ray measurement system to a standard deviation of 2% or less.

3. 5. Calculation of Results

The plasma volume (PV) in ml is calculated as follows

$$PV = SDV/P_0$$

where S=counting rate of standard (c/min/ml)

D=dilution of diluted standard solution

V=volume of radioiodine-labelled albumin solution injected (ml)

P_0=counting rate of plasma sample corrected to zero time (c/min/ml)

When only a single 10 min blood sample is taken the mean net counting rate is multiplied by 1. 015 to correct for the loss of label during the 10 min period. If the pre-injection plasma sample indicates the presence of residual radioactivity appropriate corrections must be applied to the post-injection sample counting rates.

4. Measurement of Packed Cell Volume

PCV should be measured only by a direct method by centrifuging in a Wintrobe tube ('macromethod') or a capillary tube ('micromethod'). In order to reduce the errors in the PCV the following possible sources of error should be noted.

(a) The blood must be well mixed in order to oxygenate the red cells.

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

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

(d) Errors due to trapped plasma are considered for each method separately.

4. 1. Macromethod

The extent of trapping of plasma within the packed cells depends on the centrifugal force and time of centrifugation and on the magnitude of the PCV. When the centrifugal force is approximately 1, 500 g a correction chart can be prepared

from the following data:

Time of Centrifugation	PCV	Trapping (% of PCV)
30 min	0.75	5.3
	0.20	2.5
55 min	0.75	3.2
	0.20	1.8

4.2. 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.

The trapping is smaller than with the macromethod. Nonetheless, trapping of the order of 2.0% occurs when blood is centrifuged for 5 minutes at 10,000 g and a correction factor of 0.98 should be applied. When the PCV is more than 0.5, centrifugation should be continued for a further 5 minutes and a correction factor of 3% should then be applied.

4.3. Variations of plasma trapping in red cell diseases

In spherocytosis, thalassaemia and in some other red-cell disorders plasma trapping may be slightly increased. In sickle cell anaemia the trapping is greater (5% or more) and it is essential to oxygenate the blood before a determination is made.

5. Automated Equipment for the Estimation of Blood Volume

The fixed geometry method of measuring red-cell volume and/or plasma volume is a valid method which does not require the preparation of standards. The principles of this method have been detailed in section 2.3.3. Several manufacturers have used this principle to build blood volume measuring equipment which automatically calculates the volume from the radioactivity injected and from the radioactivity of a post-injection whole blood or plasma sample. Some of these instruments are unsatisfactory for use whenever combined measurements are needed (e.g. determination of plasma volume with radioiodine-labelled human serum albumin and red-cell volume with ^{51}Cr) or when there is any possibility of significant plasma radioactivity being present when using labelled red cells (e.g. with the ascorbic acid method or ^{51}Cr labelling). Some of the newer models have attempted to overcome these limitations. However, this has been done at the cost of making the instrument more complicated and difficult to use. Another relative disadvantage of the automatic instruments is that they are usually designed for use with ^{125}I and ^{131}I-labelled human serum albumin in precalibrated syringes which are relatively expensive. Furthermore some instruments do not allow flexibility in the amount of tracer to be administered. It has also been demonstrated that the proteins tend to settle in the syringe with prolonged storage and this may alter the geometrical exposure of the tracer to the detector. Moreover, the fraction of free radioiodine increases substantially with storage in these prepacked syringes. In view of these factors there seems to be little advantage in using automated equipment which is available at the present time.

6. 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 the counting rate of such samples. In repeated estimations with the same tracer, it may therefore be necessary to take a blood sample before each

injection of tracer to estimate the counting rate due to residual radioactivity. The presence of residual radioactivity increases the statistical counting errors inherent in any single estimation and it may be necessary in sequential estimations to increase progressively the sample counting time or, if permissible, the amount of tracer injected. Table 1 gives an indication of the increases needed at various levels of residual radioactivity in order to maintain statistical counting errors at the level of a single isolated estimation.

Table 1. Increased sample counting times or increased amounts of injected tracer needed in repeated blood volume estimations with same tracer to maintain statistical counting errors at level for single isolated estimation

'Base line' counting rate due to residual radioactivity, expressed as fraction of net sample counting rate in single isolated estimation	Quantity by which sample counting time must be multiplied if amount of tracer kept constant*	Quantity by which amount of tracer must be multiplied if sample counting time kept constant*
0	1.0	1.0
0.5	1.7	1.4
1.0	2.4	1.7
1.5	3.1	2.0
2.0	3.9	2.2

*Net sample counting rate in single isolated estimation assumed to be 5 times background counting rate.

If the intervals between the estimations are sufficiently long, it may be possible to select a tracer with an effective half life such that the residual radioactivity is reduced between one estimation and the next to 1% or less of the original activity. The residual radioactivity may then be neglected. It is likely that $^{99}Tc^{m}$ will be suitable as a red cell-label (see introduction) and this will be of particular value when sequential measurements are required. It may be possible to use a different tracer for each estimation, selecting each so that the later ones can be measured independenty of the earlier ones in mixed samples, e.g. ^{125}I-labelled albumin followed by ^{131}I-labelled albumin.

7. Estimation of Total Blood Volume as the Sum of Red Cell-Volume and Plasma Volume

Total blood volume is often derived by summing simultaneous estimates of red-cell volume and plasma volume 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 leukaemia the volume of circulating leukocytes may constitute a substantial fraction of the total circulating blood cells. The total blood volume will than be underestimated, if the sum of the red-cell volume and the plasma volumes is used to estimate blood volume. The simultaneous estimation of red-cell and plasma volume requires two different tracers, which can be measured independently of each other in appropriate samples. The two tracers may be mixed prior to injection or injected sequentially through the same needle.

The choice of tracer for such simultaneous estimations is governed by the need to measure each independently. Whilst a plasma sample virtually free from the red-cell tracer can readily be obtained it may be difficult to obtain a red-cell sample completely free from the plasma tracer. It is therefore desirable to choose as the red-cell tracer one which can be measured independently in the presence of the plasma tracer. The red-cell volume estimation can then be based on a measurement of a sample of whole blood. The combination of ^{125}I-labelled human serum albumin and ^{51}Cr-labelled red cells is almost ideal in this respect, since the ^{125}I can be measured in a plasma sample by means of a well-type scintillation counter and the ^{51}Cr can be measured in a whole-

blood sample by means of the same counter used under conditions in which it is completely insensitive to ^{125}I. Other combinations are possible (see Table 2).

Table 2. Combinations of tracers for simultaneous plasma and red-cell volume estimations

Tracers		Comments
Plasma	Red cells	
^{125}I-albumin	^{32}P	^{125}I measured in plasma by well-type scintillation counter. ^{32}P measured in whole blood by G-M counter. Small correction needed for contribution of ^{125}I to counting rate of whole blood sample.
^{125}I-albumin	^{51}Cr	^{125}I measured in plasma by well-type scintillation counter. ^{51}Cr measured in whole blood by same counter.
^{125}I-ablumin	$^{99}Tc^{m}$	^{125}I measured in plasma by well-type scintillation counter. $^{99}Tc^{m}$ measured in whole blood by same counter.
^{131}I-albumin	^{32}P	^{131}I measured in plasma by well-type scintillation counter. $^{99}Tc^{m}$ measured in whole blood by end-window G-M counter with absorber or thick-walled G-M counter. Small correction needed for contribution of ^{131}I to counting rate of whole blood sample.
^{131}I-albumin	^{51}Cr	^{131}I measured in plasma by well-type scintillation counter. ^{51}Cr measured in whole by same counter. Large correction needed for contribution of ^{131}I to counting rate of whole blood sample; this may be based on PCV or on measurement on 0.64 MeV gamma rays.
^{131}I-albumin	^{99}Tc·	^{131}I measured in plasma by well-type scintillation counter. $^{99}Tc^{m}$ measured in whole blood by same counter. Correction needed for contribution of ^{131}I to counting rate of whole blood sample.

8. Presentation and Analysis of Estimates of Red-Cell Volume and Plasma Volume

The interpretation of an estimate of red-cell volume, plasma volume or blood volume depends upon a comparison between the observed value and either another value previously obtained in the same subject or the value which would be found in the same subject in health.

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

The commonest 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 practicable as a routine procedures 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.

Predictions of blood volume from various formulae based on height and weight or some derivatives or combinations of these measurements are slightly better than those based on body weight alone. However, the 95% confidence limits of the best predictions for an individual taken at random are of the order of $\pm 15\%$.

Because predictions of blood volume based on relatively complicated formulae are only slightly better, in most subjects, then those based on weight alone it is recommended that for routine purposes i^{+} should be regarded as adequate to express blood volume results (except in infants in the first few week of life) as ml/kg body weight. Nevertheless, slightly better predictions will be obtained by using one of the formulae referred to above and one of these should be used when the most accurate prediction is required.

Normal values for red-cell volume 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.

Recent estimates of plasma volume in normal subjects with radioiodine-labelled albumin have given an average value of about 40 ml/kg both in men and women. The mean values for plasma volume in different published series vary more widely then those for red-cell velume, due probably to the fact that plasma volume is labile and varies, for example, with posture and also due possibly to differences in the quality of the labelled albumin used for estimation. For these reasons it is not possible to give well-based confidence limits for normal plasma volume.

Total blood volume (BV) is often calculated form the total circulating RCV and corrected PCV, using the formula:

$BV=RCV/(0.9H_v)$

Blood volume is also sometimes calculated from PV as estimated with labelled albumin and corrected PCV using the formula:

$BV=PV/(1-0.9H_v)$

Since the figure of 0.9 for the ratio HB/H_v does not apply in some patients, deduction of blood volume from the red-cell volume or plasma volume alone is liable to significant error.

9. 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. The Table shows the dose in normal subjects 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 per μCi of administered radioactivity. It should be emphasized that 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 radioisotope labels in the body are lacking. Data for a given procedure in normal subjects could be very different in pathological conditions. The data do not therefore give more than an approximate indication of the radiation dose to the patient in

Table 3. Radiation dose to the patient in red-cell and plasma volume estimations

Estimation	Label or Tracer	Critical Organ	Fraction administered radioactivity reaching critical organ	Mean radiation dose to patient per μCi administered (mrad)
Red cell volume	^{32}P	Bone	0.5	51.1[a]
	^{51}Cr	Spleen	0.2	3.87[b]
	$^{99}Tc^{m}$	Blood	1.0	0.067[b]
Plasma volume	^{125}I-albumin	Blood	1.0	[c]
	^{125}I-albumin	Thyroid	0.3	[d]
	^{131}I-albumin	Blood	1.0	[c]
	^{131}I-albumin	Thyroid	0.3	[d]

a: Intravenous injection of labelled cells.
b: Intravenous injection of labelled compatible cells with normal survival.
c: Intravenous injection of labelled protein/Thyroidal/metabolized normally uptake of radioiodine blocked.
d: Intravenous injection of labelled protein/Thyroidal/metabolized normally uptake of radioiodine not blocked.

the various procedures.

When ^{32}P-labelled red cells are injected the label is quite rapidly lost from the cells in the circulation. About 50% of the label thus lost is incorporated into bone which may be taken as the critical organ. When ^{51}Cr-labelled cells are injected ^{51}Cr is slowly eluted from the cells in the circulation. The labelled cells eventually undergo destruction in the reticulo-endothelial tissues, from which the deposited radioactivity is again slowly eluted. In these circumstances the critical organ may be taken as the spleen. When $^{99}Tc^{m}$ labelled red cells are cells are injected the radiation dose to the various organes is 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-labelled 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 labelled 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 iodide pool the label is then almost entirely excreted in the urine and the critical organ may be taken as the blood. The second is that in which thyroidal uptake is not blocked. A significant proportion of the label, assumed here to amount to 30%, then accumlates in the thyroid, which becomes the critical organ and receives a relatively high radiation dose.