

Measurement of Blood Volume and Red Cell Mass: Re-examination of ^{51}Cr and ^{125}I Methods

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ABSTRACT: Comparison of results of red cell mass (RCM) measurement by ^{51}Cr and ^{125}I methods in 119 patients showed virtual equivalence. Both methods have an acceptable coefficient of variation (CV) that is <5%. The ^{125}I method is simpler and much less expensive. Unrealistically narrow "normal ranges" for RCM are likely to lead to misdiagnosis of polycythemia vera. Upper normal limits of 39 mL/kg (males) and 32 mL/kg (females) are consistent with originally published data in normal persons; use of these limits as criteria would reduce the risk of misdiagnosis. No cases of "stress erythrocytosis" or Gaisbock Syndrome were encountered among the 119 cases reviewed.

Keywords: blood volume, red cell mass, ^{51}Cr , plasma volume, ^{125}I -albumin, polycythemia, erythrocytosis, hematocrit, PCV

INTRODUCTION

It is widely believed that measurement of the total volume of erythrocytic, or "red cell mass" (RCM) is essential for the diagnosis of polycythemia vera. This belief stems from the criteria adopted by the polycythemia vera study group (PVSG), that requires elevated RCM for the diagnosis of polycythemia. Originally, this was a criterion required for inclusion of patients in the study, but it has subsequently been urged as a standard requirement for diagnosis in all cases suspected of having polycythemia vera. It is, furthermore, widely believed that measurement of RCM by the ^{51}Cr -labeling method is very accurate, and that the RCM is measured independently of the venous hematocrit. It is also widely believed that measurement of RCM must be done with ^{51}Cr label. These beliefs have rarely been critically examined.

The authors review their experience with blood volume studies and the reasons they question the validity of some of these widely accepted beliefs concerning the measurement and interpretation of RCM in the diagnosis of polycythemia.

MATERIALS AND METHODS

Blood volume studies conducted at Mayo Clinic during the past 35 years are estimated to have been in aggregate approximately 10,000, with approximately 4,000 such studies having been performed since 1976. In all cases prior to 1993, the method used was that recommended by the International Committee for Standardization in Hematology (ICSH) (1-4). This method has been widely published and almost universally used in the United States and Western Europe, and therefore, will not be described in detail. Briefly, it consists of labeling erythrocytes with ^{51}Cr to

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measure RCM and radio-iodine-labeled human serum albumin to measure plasma volume. During the past two decades or more, plasma volume was measured with ^{125}I as the radionuclide; earlier ^{131}I was used. During the past 15 years, venous hematocrit was measured by Coulter Counter, rather than by centrifugation, in view of the much better linearity of the former, which may be ascribed to elimination of variability in plasma trapping (between packed erythrocytes). The studies were performed with the patient recumbent and in the non-fasting state. A single specimen of blood was obtained from the contralateral arm 15 minutes after injection of the labeled blood. The $\text{Na}_2^{51}\text{CrO}_4$ and ^{125}I -human serum albumin used in these studies were obtained from Bristol-Myers-Squibb, Inc., New Brunswick, NJ, and Mallinckrodt Corporation, St. Louis, MO, respectively.

The RCM was also calculated from the ^{125}I -albumin plasma volume and the hematocrit (Hct), based on the following relationship.

$$\text{Whole Blood Volume} = \frac{\text{Plasma volume}}{\text{“plasmacrit”}}$$

“Plasmacrit” is calculated using the venous hematocrit and the ratio of whole body hematocrit-to-venous hematocrit, a ratio designated f . f is always less than 1.0, because the venous Hct is slightly higher than the hematocrit that we might obtain if we were able to sample all the circulating blood. The ratio f is one of the most remarkable biologic constants. In published reports it has been found to be approximately 0.9 (5-7). “Plasmacrit” = $1-f$ (venous Hct.). For measuring RCM using ^{125}I -albumin, we have empirically used the value $f = 0.864$. An identical value for f was reported independently by Najean and Deschrywer (8).

Using these relationships, we calculated RCM from ^{125}I -albumin plasma volume:

$$\begin{aligned} \text{RCM} &= \text{Hct} \times \text{Whole Blood Volume from } ^{125}\text{I}\text{-albumin} \\ &= \frac{0.864 \times \text{Hct} \times \text{plasma volume}}{1 - (0.864 \times \text{Hct})} \end{aligned}$$

This equation simplifies to:

$$\text{RCM} = \frac{\text{plasma volume}}{\left[\frac{1.157}{\text{Hct}} \right] - 1}$$

In all cases, Hct as shown in these formulas was Coulter Counter “Hct,” and is expressed in decimal notation. Centrifuged hematocrit values introduce a significant non-rectilinear error.

To test the constancy of f in this series, we calculated the ratio of whole body hematocrit to venous hematocrit:

$$f = \left[\frac{\text{RCM } (^{51}\text{Cr})}{\text{RCM } (^{51}\text{Cr}) + \text{Plasma Vol. } (^{125}\text{I}\text{-albumin})} \right] \div \text{venous Hct}$$

Body weight was recorded at the time of the blood volume study, and this was used to convert volume measurements in mL to mL/kg.

No cases in this series were known to be edematous from congestive heart failure, or post-traumatic, or to have carcinoma. Therefore, the authors have not examined the alleged inconstancy of f in these conditions (9).

Data were analyzed for means, medians, standard deviations, and regression analysis was performed, using the MS-Excel program of Microsoft, Inc.

During the course of these studies, the authors discovered that blood volume studies were being done in normal, healthy persons with all normal CBC data, because of errors in the stated normal ranges for venous hemoglobin concentration, hematocrit and erythrocyte count. Therefore, for every male with Hb concentration <180 g/L and every female with Hb concentration <160 g/L in whom these studies were ordered, the primary physician was contacted and the rationale for performing blood volume studies in persons with normal CBC data was discussed. The outcome of these discussions was that in approximately half the cases, the primary physician canceled the order for blood volume studies, but in 23 cases, at the insistence of the primary physicians, the blood volume study was performed, in conventional manner using ^{51}Cr -labeled erythrocytes and ^{125}I -labeled albumin.

To estimate the attainable precision for each of these blood volume measurements we used a Monte Carlo simulation for the propagation of error through the mathematical steps used for the calculations (10). Nineteen variables are utilized in these calculations--the hematocrit measurement plus nine counts from each of the ^{125}I and ^{51}Cr counting channels. The variables represent the scintillation counts for: the background, the ^{125}I and ^{51}Cr reference material used to calculate channel cross-over in the scintillation counter, the whole blood standards (in duplicate), the plasma standards (in duplicate), the patient's whole blood specimen, and the patient's plasma specimen. The following error distributions were used for each of these variables:

Hematocrit

Gaussian distribution with mean of 0.557 and CV of 2.0% (1% from measurement error plus 1.7% biologic CV) (11).

Whole blood dose

Poisson distribution with average count of 8000 proportioned to target level; times Gaussian distribution with 1% CV for pipetting error; times Gaussian distribution with 2% CV for blood resuspension error and reinjection error.

Plasma dose

Poisson distribution with average count of 8000 proportioned to target; times Gaussian distribution with 1% CV for pipetting; times Gaussian distribution with 1% CV for reinjection error.

Whole blood specimen

Poisson distribution with average count of 8000 and plasma specimen proportioned to target level; times Gaussian distribution with 1% CV for pipetting error; times Gaussian distribution with 1% CV for mixing.

These error estimations were based on the following. The current CV for automated hematocrit measurement is less than 1%. The within-person biologic variation of hematocrit was estimated at 1.7% (2). The precision of the calibrated pipettes was CV <1%. The combined

error for the resuspension and pipetting of whole blood was judged to be greater than that for the pipetting of plasma so this error was simulated at 2.0%. The scintillation counter used for these studies counts to a fixed count of 10,000. Based on the Poisson distribution, this should have a CV of 1.0%. Our experimental measurement of the day-to-day variation of these counts gave a CV of about 1.1%. Therefore, we simulated the counting error using a Poisson distribution with an average of 8000 counts. This distribution has a CV of 1.12%, as calculated from $\frac{100\sqrt{8000}}{8000}$.

The simulation was run through 10,000 cycles using a Statistical Analysis Systems (SAS) program, (12) incorporating the formulas listed for RCM (^{51}Cr), plasma volume and RCM (^{125}I -albumin). The means, standard deviations and coefficients of variation were then calculated from the simulated observations. In addition, the distribution of f was calculated using the above distributions for RCM (^{51}Cr), RCM (^{125}I -albumin) and hematocrit.

A retrospective review was made of 119 cases in which RCM and plasma volume had been performed using ^{51}Cr as a red cell label and ^{125}I -albumin as a plasma label. This consisted of two series that were combined, one of 109 consecutive cases, studied during a 1 year interval, and a smaller series of 10 cases that were included in order to augment the number of cases with markedly elevated RCM and marked splenomegaly. The majority of cases included were being investigated for presumed erythrocytosis. However, some cases of moderate anemia were included, as were a case with chronic lymphocytic leukemia with splenomegaly, compounded by tobacco abuse and chronic obstructive pulmonary disease, 2 cases of lymphoma, 1 case of hairy cell leukemia, a case of "agnogenic" myeloid metaplasia with massive splenomegaly, and a case of myeloma with anemia and high plasma concentration of IgG. There were 77 males and 42 females. Eight males were anemic, with Hct <0.40; 7 females were anemic with Hct <0.36. For males, the range of Hct was 0.31-0.69; for females the range of Hct was 0.23-

0.57. RCM values ranged from 785-5579 mL and whole blood volume 3329-9581 mL. Thus, there was a wide range in results, spanning the interval

from moderate anemia to marked erythrocytosis, permitting evaluation of the robustness of the relationships observed.

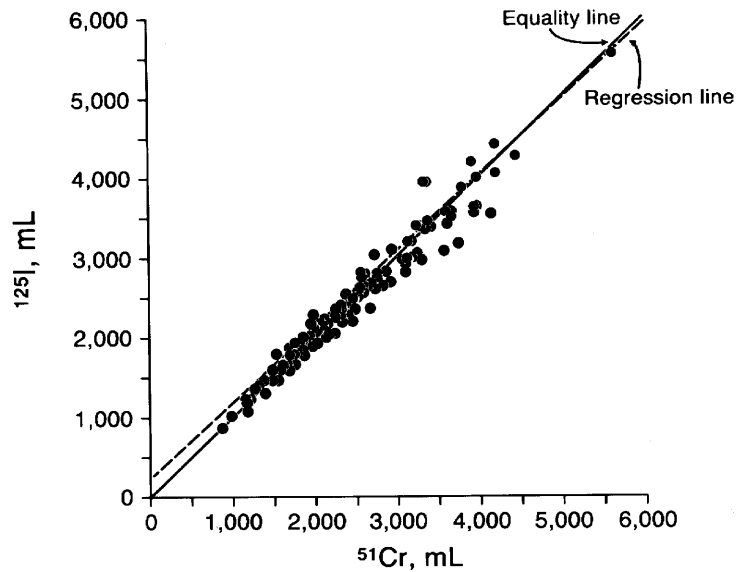


Figure 1. The correlation between results of measuring red cell mass (RCM), using ^{125}I -albumin (ordinate) vs. ^{51}Cr on abscissa, for 119 consecutive cases in which both radionuclides were used. Results are expressed in mL. The solid line represents identical results. The interrupted line is from least squares regression analysis. For the latter, $r = 0.98$, slope = 0.93, Y-intercept = 220 mL, standard error = 41.5 mL. In the range of values observed in this study, the difference between the identity line and the least squares line is negligible.

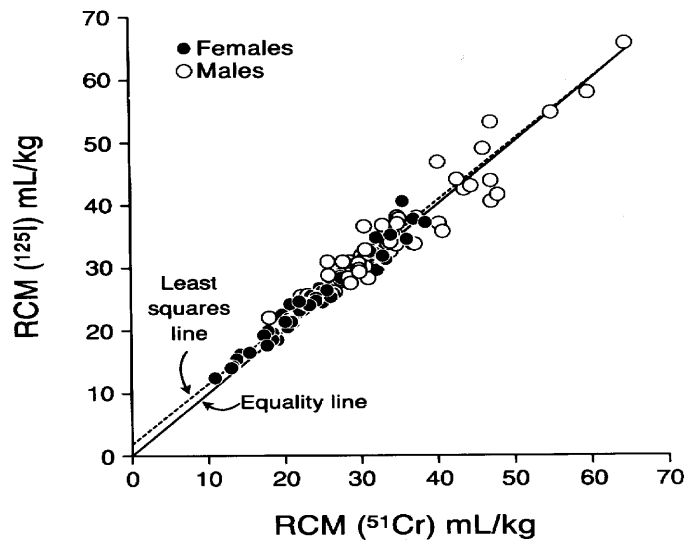


Figure 2. The same correlation as in Fig 1, expressed in mL/kg. Least squares regression analysis indicated $r = 0.97$, slope of the regression line = 0.92, Y-intercept = 2.9 mL/kg, standard error = 0.58 mL/kg.

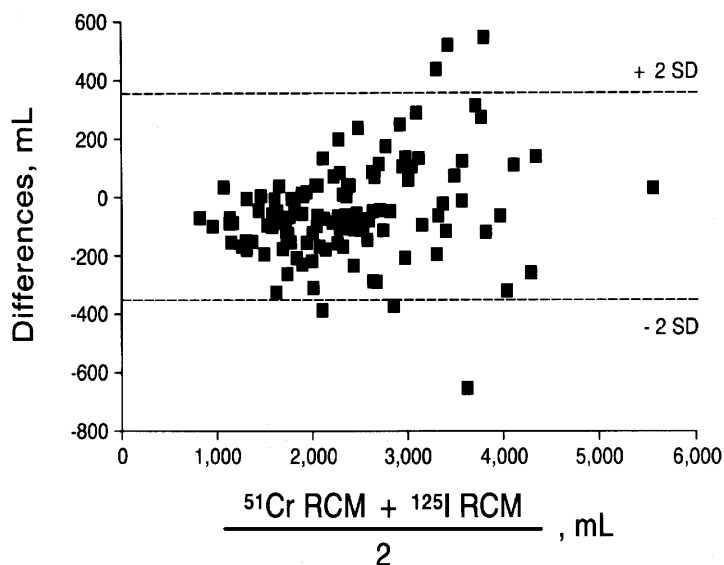


Figure 3. Scatter diagram of differences between ^{51}Cr RCM and ^{125}I RCM (ordinate) vs. mean of each pair of RCM measurements using these radionuclides. There is a slight bias of 50 mL, the ^{125}I method yielding slightly higher values overall. SD = 168 mL. Interrupted horizontal lines represent 2 SD above and below the mean difference. In this diagram, results in females are grouped toward the left, males toward the right.

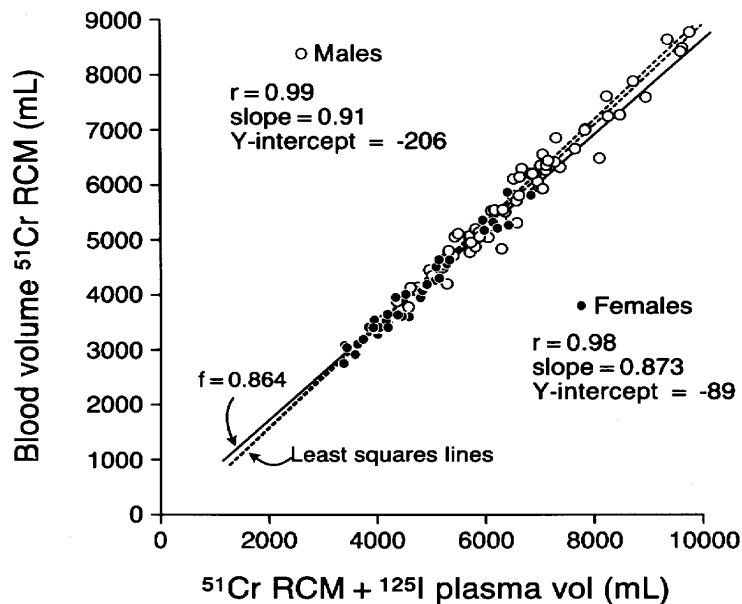


Figure 4. The correlation of whole blood volume (^{51}Cr) uncorrected for f , vs. whole blood volume obtained by adding ^{51}Cr RCM + ^{125}I plasma volume. f may be estimated from the slope of the correlation line. The slight deviations from the line $f = 0.864$ presumably reflect cumulative effect of the inherent imprecisions of a large number of variables used to estimate f . Furthermore, mean value for f in males appears to be slightly higher (0.874) than in females (0.853), although these differences are small. Throughout the wide range of blood volumes (or RCM values) in the 119 cases of this study, f showed no deviation from linearity. The 4 highest points on the lines are the 4 cases with extreme splenomegaly that are also shown in Table 4.

Table 1. Estimation of the Ratio (f) of Whole Body Hematocrit to Venous Hematocrit

	Males	Females
Mean	0.875	0.853
S.D.	0.033	0.031
S.E. of mean	0.004	0.005
C.V.	0.034	0.036
Median	0.874	0.851
Combined series mean	0.867	(Both Sexes)
From slope of Least Squares Line	0.91	0.87

RESULTS

As shown in Figures 1 and 2, an excellent agreement was observed between RCM (^{51}Cr) and RCM (^{125}I -albumin). Results are also compared in Figure 3 by plotting the differences against the mean values for RCM obtained from ^{51}Cr and ^{125}I -albumin methods (13,14). The standard deviation of the absolute differences is 168 mL. The mean and standard deviation of the differences, expressed relative to the means of each pair of data, were 0.77% and 0.15%, respectively. As would be predicted, a small number of values were either above or below the $\pm 2\text{SD}$ range of ± 336 mL. In cases with RCM < 3000 mL, mostly in anemic persons, use of ^{125}I -albumin gave results that were, on average, approximately 50 mL higher than those obtained using ^{51}Cr -labeled red cells. However, the slight overestimation of RCM by the ^{125}I -albumin method would correspond to a difference of approximately 0.6 mL/kg, and does not appear to be of any practical significance, particularly since it seems to be within the range of biological variation for either method.

Furthermore, the finding of 4 outliers beyond $\pm 2\text{SD}$, and no outliers beyond $\pm 3\text{SD}$ is consistent with the expected distribution of random "errors" for 119 pairs of measurements. When considered as a percentage of the mean of each pair of RCM values, the difference is quite small, being on average 3%. This magnitude of differences is quite close to that which should be expected for the RCM (^{51}Cr), if it could be done repeatedly,

and it therefore supports the view that results by either method are essentially equivalent.

As shown in Table 1 and Figure 4, the value for f showed little variation throughout the range of values tested. It had slightly lower value in women (mean $f = 0.853$) than in men (mean $f = 0.874$). The arbitrary use of $f = 0.864$ would result in slight overestimation of RCM (by 0.35%) in females and slight underestimation of RCM (by 0.15%) in males. These slight errors are negligible from the practical standpoint. The coefficient of variation of f in this series of 119 cases was 0.04, i.e., 4%. Results in females showed the largest deviations from 0.864 were in 4 females with $f = 0.81$. One of these was anemic (Hct 0.34) and 3 had normal Hct values.. In these 4 cases, RCM (mL/kg) values obtained by ^{51}Cr and ^{125}I respectively were 12.4, 10.9; 25.5, 22.2; 19.7, 22.6; 46.8, 40.2. The last of these was a patient with chronic lymphocytic leukemia, tobacco abuse and chronic obstructive pulmonary disease, and marked splenomegaly. Results in males showed 3 cases with significant deviations from $f = 0.864$. One of these was an anemic patient with Waldenström's macroglobulinemia ($f = 0.77$), one was a hematologically normal diabetic ($f = 0.80$) and one was a hematologic-ally normal obese male ($f = 0.80$). If the measurement of RCM had been obtained with the ^{125}I -albumin method and the assumption that $f = 0.864$, rather than with ^{51}Cr -labeled red cells, the results would not have had any consequence for diagnosis or management of any patient in this series.

Table 2. Examples of Data and Results in Cases With Hb Concentration or Hct Near the Upper Limit of Normal

Sex	Wt (kg)	Hb (g/dL)	Hct L/L	RBC x10 ¹² /L	RCM ⁵¹ Cr mL/kg	RCM ¹²⁵ I mL/ kg	Plasma Vol mL/kg
M	67	17.6	0.49	5.50	23.0	24.0	32.5
M	81	16.9	0.52	7.04	33.9	32.5	38.4
M	101	16.7	0.50	6.29	29.7	28.7	38.4
M	75	17.2	0.52	5.94	29.7	31.2	37.2
M	79	18.3	0.53	5.98	30.6	30.0	36
M	93	17.3	0.51	5.54	28.9	30.1	38.4
M	93	18.1	0.52	5.70	25.6	26.2	31.9
M	65	16.8	0.45	5.39	34.8	33.6	45.9
M	77	17.2	0.52	5.22	26.7	26.1	32.3
M	65	17.3	0.51	5.51	34.2	35.4	44.4
M	87	17.1	0.51	5.75	30.9	30.1	38.8
M	89	17.6	0.53	5.71	37.1	37.7	44.3
M	107	17.2	0.51	5.54	17.7	17.6	22.4
M	86	16.9	0.50	5.87	31.4	31.8	41.2
M	79	16.8	0.49	5.31	26.4	25.8	41.2
M	75	18.0	0.52	5.70	35.8	42.0	51.2
M	70	18.0	0.53	5.78	37.2	33.8	40.0
M	79	18.3	0.53	5.48	32.1	29.6	36.0
F	56	15.4	0.46	4.85	22.2	25.5	38.3
M	69	17.3	0.52	5.49	31.5	32.6	40.3
M	83	17.1	0.52	5.93	30.2	32.0	39.6
M	100	17.0	0.50	5.52	28.6	30.7	40.1
M	90	18.0	0.51	5.78	27.7	30.9	38.6
M	74	17.3	0.51	5.56	27.5	28.4	36
M	86	16.7	0.49	5.53	29.7	30.6	41.8
F	89	14.8	0.45	5.28	20.7	24.2	36.1
M	84	17.3	0.51	5.36	36.1	34.5	43.5
M	85	18.4	0.55	6.15	23.5	25.5	27.9
F	62	15.2	0.45	4.99	23.9	25.4	39.8
Mean					29.2	29.9	38.4

Table 3. The Limits of Precision of Red Cell Mass Measurements¹

	Analytic Variability ² C.V., %	Analytic Variability ² & Biologic Variability ³ C.V., %
Red Cell Mass (⁵¹ Cr)	2.6	3.4
Red Cell Mass (¹²⁵ I)	3.4	4.7

¹In lieu of multiple replicate measurements of blood volume in normal persons or patients, these estimates of the best attainable precision were derived by Monte Carlo simulation using known levels of precision, attainable under optimal conditions by most meticulous technique and most skilled technologists, for the numerous variables included in the measurements and calculations (see text for details).

²Calculations for analytical variability include an assumption of coefficient of variation for hematocrit of 1%, on repeated measurements of hematocrit from a single specimen drawn from a single subject.

³Because hematocrit is the measurement that exhibits the largest day-to-day variation in any given subject, and is used in calculating both RCM (⁵¹Cr) and RCM (¹²⁵I), a C.V. of 1.7% was assumed for day-to-day, within-person variability of the hematocrit (biologic variability).

The measurement of RCM and plasma volume in 23 persons with normal “CBC” data was of little or no clinical utility. Of these 23 cases, 21 were found to have RCM within normal limits by any criterion. One case (female) had slightly elevated RCM by the height-weight regression formula used to define normal ranges, but she was a very small woman whose RCM was 29 mL/kg, and she would not be considered polycythemic. In another case, the RCM was considered slightly above the “normal range.” However, since the “normal range” is defined as mean $\pm 2SD$, it is expected that 0.5 cases out of every 20 will exceed the “normal range,” and the finding of one value slightly beyond the $\pm 2SD$ limit is consistent with the distribution of normal values.

In one additional case not included among the 23 described in the preceding paragraph, RCM was 40 mL/kg. His plasma volume was also increased. This patient was hematologically normal. During 6 years of followup, he has remained hematologically normal; splenomegaly has never been documented. Clearly he does not have polycythemia vera, and it is doubtful that he has erythrocytosis. It is not clear whether he

represents a “statistical outlier,” or whether the results may have been due to technical error such as unrecognized mild extravasation during injection of the labeled blood.

Table 2 provides hematological and clinical information for 29 cases with RCM near the upper limit of normal, including the cases described in the preceding paragraphs. Only one of these cases was diagnosed as having polycythemia vera, and that diagnosis was considered questionable. In this series of 119 cases, we found none that was consistent with “stress erythrocytosis,” and none was diagnosed as Gaisbock Syndrome.

The results of the Monte Carlo simulation of attainable precision of blood volume studies are shown in Table 3. These results imply 95% confidence intervals for RCM (⁵¹Cr) of approximately $\pm 7\%$, and for RCM (¹²⁵I) of approximately $\pm 9\%$.

The simulated error distributions were remarkably tight. Even when adjusted for the biologic variability of hematocrit, the CVs of both the ⁵¹Cr and the ¹²⁵I estimates of RCM were both less than 5%. Both methods provide acceptable precision.

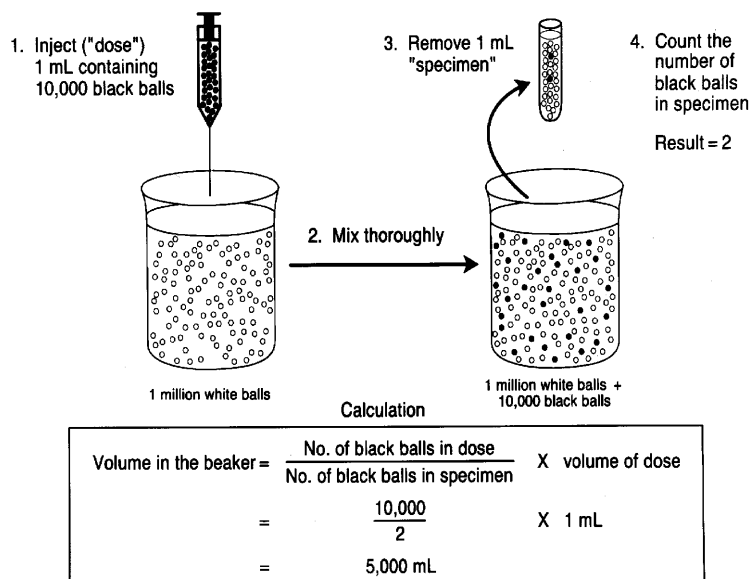


Figure 5. The dilution principle in measurement of a fluid volume such as blood volume is illustrated using a large beaker containing a million white balls (red cells) suspended in a solution of unknown volume. To this is added 1 mL of a fluid containing 10,000 black balls (^{51}Cr -labeled erythrocytes) in suspension. The contents of the beaker are thoroughly mixed, and a 1 mL sample is removed and the number of black balls is counted. Result = 2. The volume of the fluid and suspended white balls is $10,000 \div 2 = 5000$ mL. From this measurement, nothing whatever is learned about the number of white balls in the beaker. The result would be the same if there were a thousand white balls or 10 million white balls in suspension in the fluid in the beaker.

If one wants to know how many white balls are in the suspension, one may count the number of white balls in the post-mixing specimen. Then, the number of white balls in the beaker = no. of white balls/mL in the specimen X total volume in the beaker. Similarly, one can determine the volume occupied by white balls in the beaker by centrifuging an aliquot of the post-mixing specimen to obtain the "ballocrit." Then, the volume occupied by white balls in the beaker = "ballocrit" X total volume of fluid in beaker X correction for trapped fluid. The "ballocrit" substantially overestimates the space occupied by the balls because there is fluid trapped in the spaces between the balls. For uniformly packed, rigid, perfect spheres, of uniform size, the trapped fluid is 26% of the "ballocrit." Therefore, the space occupied by the balls is $0.74 \times \text{"ballocrit"} \times \text{volume in beaker}$. If, instead, rigid, uniform, perfect disks were the solids suspended, the space occupied by fluid trapped between the disks would be 9.4% of a "diskocrit", and the volume occupied by uniformly packed, rigid, perfect disks would be $0.906 \times \text{"diskocrit"} \times \text{volume in the beaker}$. Normal erythrocytes are deformable diskocytes that pack tightly on centrifugation, so that trapped fluid ("plasma trapping") is about 4% of the normal hematocrit. However, for centrifuged specimens, the proportion of trapped plasma is much less if there is anemia (and erythrocytes are normal diskocytes), and it is much greater if there is polycythemia or spherocytosis or poikilocytosis. For this reason, the centrifuged hematocrit is not a rectilinear function of the erythrocyte count; errors are inevitable if calculations are made using centrifuged hematocrit values

DISCUSSION

The implications of this study may be discussed from the standpoints of 1) widely accepted concepts concerning blood volume and RCM studies, 2) precision and accuracy of methods and 3) cost-benefit considerations.

Concepts Concerning Blood Volume Studies

It appears to be widely believed that RCM is measured directly and is independent of the venous hematocrit. However, this is not true in the method recommended by ICSH, nor in any published method using either ^{51}Cr or ^{32}P as erythrocyte label.

In all laboratories that use these methods, the labeled red cells are used to measure whole blood volume; from this and from the venous hematocrit, the RCM is calculated:

$$\text{RCM} = (\text{Venous Hct}) \times (\text{Whole Blood Volume})$$

More complex formulations of this relationship are to be found in the ICSH method, and in various other publications and textbooks. However, each of these is easily reduced to the above equation. To whatever extent there may be an error in measurement of the venous hematocrit, there will be a proportional error in the calculated RCM. In contrast, plasma volume is measured directly with ^{125}I -albumin. This measurement is independent of the venous Hct.

One might think that RCM could be determined by directly measuring whole blood volume using ^{51}Cr -labeled erythrocytes and subtracting the plasma volume that is directly measured with ^{125}I -albumin. However, RCM would then be underestimated and even, in some cases of anemia, negative results would be obtained. The reason for this paradox is that use of ^{51}Cr -labeled erythrocytes systematically underestimates whole blood volume because erythrocytes in venous blood are concentrated relative to their numbers in arterial and capillary blood. If the blood volume is measured with ^{51}Cr -labeled erythrocytes and adjusted by the factor

1.157, the reciprocal of f , excellent agreement is found with the blood volume calculated by addition of RCM (^{51}Cr) and ^{125}I -albumin plasma volume, throughout the range of RCM or blood volume from quite low to markedly elevated, as shown in Figure 4.

The measurement of RCM is arcane, complicated and time-consuming. However, it is conceptually simple. In order to clarify exactly what is done in this method, a simple diagram (Figure 5) illustrates how the total fluid volume in a large beaker, that contains a million white balls (representing erythrocytes) in suspension, can be measured by adding a known number of black balls suspended in 1 mL of fluid, mixing, sampling, and counting the number of black balls in 1 mL of the post-mixing sample.

From the ratio of the number of black balls in the "dose" injected to the number of black balls in the post-mixing "specimen" one may readily calculate the volume of fluid in the beaker. The same result would be obtained if the "black balls" were erythrocytes or molecules of albumin. This procedure alone tells nothing whatever about the number of white balls in the beaker, nor of the volume of space occupied by the white balls.

If one wishes to know the *volume of space* occupied by white balls in the beaker one needs to determine, by centrifugation, the "ballocrit" of the specimen; corrections for fluid trapped in the space between balls in the "ballocrit" are also needed, as explained in the legend for Figure 5. Then, volume of space occupied by white balls = (correction for trapped fluid) X "ballocrit" X (volume in the beaker). The same measurements and calculations are used to measure RCM. If one wishes to know the *number* of white balls in the beaker, this can be determined by counting the number of white balls in 1 mL of the "specimen." Then, the number of white balls in the beaker = (number of white balls per mL in the "specimen") X (volume in the beaker).

It is widely believed that the diagnosis of polycythemia vera requires measurement of the red cell mass. This rule was promulgated by the Polycythemia Vera Study Group without evidence to support its necessity. Furthermore, it has

placed an unreasonable burden on physicians, including hematologists and oncologists, who do not have ready access to these studies, and it adds an unreasonable expense for medical care. Review of our cases indicates that in males with hemoglobin concentration >185 g/L, Hct >0.55 and erythrocyte count $>6.5 \times 10^{12}/L$, (or females with hemoglobin conc. >165 g/L, Hct. >0.49 and erythrocyte count $>5.8 \times 10^{12}/L$) one may be confident that RCM is elevated, except in those who are dehydrated from fluid restriction or use of diuretics or in those residing at high altitudes. A few other workers have expressed similar views (15,16).

It is widely believed that there is poor correlation between RCM and hematocrit. In fact, in both anemic and normal persons, the relationship between RCM (mL/kg) and Hct is rectilinear and corresponds approximately to the relationship:

$$\text{RCM (mL/kg)} = 60 \times \text{Venous Hct (in decimal notation)}$$

There is a physiological explanation for this relationship in the homeostatic control of the total blood volume. In order to maintain constancy of the blood volume, when the RCM is lowered, by blood loss or other causes of anemia, the plasma volume increases. This control is effected in part by increased fluid reabsorption when blood pressure is lowered in arterioles due to hypovolemia, and in part through the renin-angiotensin system. This homeostatic mechanism appears to be effective in anemia, but it is inconsistent in polycythemia. Hence, in polycythemia vera, as the RCM increases, there is commonly not a compensatory reduction in plasma volume. Both the RCM and the total blood volume increase. This is illustrated in Figure 6, panel e.

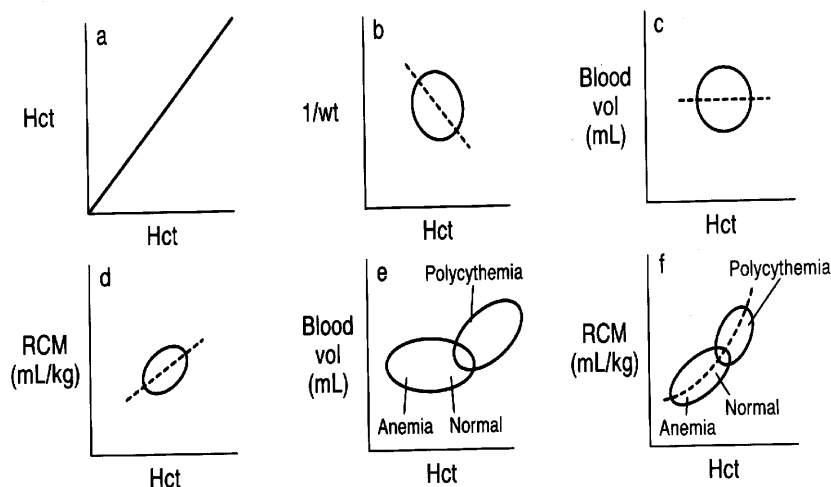


Figure 6. An illustration of the way in which misleading graphs result from plotting on the ordinate a compound variable which contains the same factor (Hct) as is plotted on the abscissa. For RCM expressed as mL/kg, the component variables are Hct, the reciprocal of body weight in kg, and whole blood volume (mL). Six panels analyze the effect of each of these three component variables on the plot of RCM (mL/kg) vs. Hct. The effect of each of the three component variables is shown in panels a-c. Description is given in the text. Authors, editors and readers should be wary of the effect of plotting against x any variable that contains x as a factor (e.g. Hct times Blood Volume on the ordinate vs. Hct on the abscissa). In several published articles in which there were graphs of RCM (mL/kg) vs. Hct for anemic, normal and polycythemic cases, an exponential relationship was apparent. As shown in panel f, however, this relationship (represented by the interrupted line) can best be explained as an artifact of the way in which the data were plotted. [Panels a-d are based on 246 normal males (from same sources as table 2); panels e and f are composites based on these 246 normal males and the authors' series of 119 cases that included anemic, normal and polycythemic patients.]

If all data are plotted, for anemic, normal and polycythemic persons, there appears to be an exponential relationship, RCM increasing more rapidly than Hct (7,17). However, published graphs of this relationship overlook the fact that venous hematocrit is a factor on both the ordinate and the abscissa, since $RCM \text{ (mL/kg)} = \text{Blood Volume} \times \text{Hct} \times 1/\text{wt}$. The effect of combining these 3 variables on the ordinate is shown in Figure 6 by plotting each separately against Hct on the abscissa. Panel a shows the plot of Hct vs. Hct, a straight line with slope of 1.0. Panel b shows the relationship of 1/body weight on the ordinate vs. Hct on the abscissa for 246 normal adult males. The graph portrays an ellipse with the long axis having a slope of approximately -1.0. This reflects the fact that there is a slight tendency for heavier people to have higher hematocrits (18). Panel c shows the plot of whole blood volume in mL vs. Hct. The appearance is that of an ellipse that is almost a circle (no correlation whatever), with a long axis that has a slope of 0. Panel d shows the plot of RCM in mL/kg vs. Hct. The wide ellipse of panel c has now been rotated counter-clockwise because the relationships of Hct vs. Hct (panel a) and Hct vs. 1/wt (panel b) have been imposed on the ellipse of panel c. The adjustment for differences in body weight results in less scatter, so the ellipse is smaller. Panel e illustrates the phenomenon actually observed when whole blood volume data are plotted against Hct for anemic and normal persons (leftward broad, lower ellipse) and for persons with an expanded blood volume, as in polycythemia vera (upper, rightward ellipse), some of whom have normal Hct, some elevated Hct. If the long axes of the two ellipses are rotated 45° counter-clockwise by multiplying whole blood volume x Hct to obtain RCM in mL/kg, the resulting graph (panel f) would closely simulate published graphs that plot RCM in mL/kg (on the ordinate) vs. Hct (on the abscissa). A graph of RCM (mL/kg) vs. Hct cannot be understood without examining the component variables. Furthermore, the traditional graphing of RCM (mL/kg) vs. Hct obscures the fact that there are two distinct populations, i.e., nonpolycythemic and polycythemic, and that in

polycythemic states there is characteristically an increase in *total blood volume*. Illustrations of these relationships might be more informative were blood volume (mL/kg), rather than RCM, plotted vs. Hct.

The ⁵¹Cr “Gold Standard”

Since the introduction of the use of ⁵¹Cr-labeled red cells as a method for measurement of RCM, this method has been considered essentially infallible, and any alternative method that does not give identical results has been denigrated. No allowance has been made for the possibility of random error, technician error, analytical precision, etc., in the ⁵¹Cr method. Yet, we are aware of only one published study of the precision (reproducibility on repeated measurements) of this method (18). It is, of course, difficult to do repeated measurements in any person because 1) a very large number of venipunctures would be required, 2) reproducibility would be compromised by escalation of “background” radioactivity in blood specimens from each injection of labeled red cells, and 3) there are concerns about the accumulated in vivo dose of ⁵¹Cr that would result from repeated measurements of RCM. However, lack of information concerning the analytical precision of this method means that results must be accepted on faith alone. Furthermore, those who have observed discrepancies between results with the ⁵¹Cr method and alternative methods have failed to show whether the discrepancies were due to errors principally in the ⁵¹Cr method, principally in the alternative method, or in both methods.

Precision of Methods

There have been few studies of precision or “accuracy” of estimation of RCM (⁵¹Cr). Sterling and Gray, (19) and Gray and Frank, (20) compared ⁵¹Cr count rates of venous blood before and after removal or transfusion of known amounts of blood, in several normal persons, and concluded that the observed values were approximately ±3% of the predicted values.

Beutler and West labeled erythrocytes simultaneously with both ^{51}Cr and $^{99\text{m}}\text{Tc}$, and observed nearly identical values for RCM calculated using each of these radionuclides in the same specimens (21). However, such limited studies excluded most of the variables that would be included in a proper study of reproducibility. Wennesland, et al. repeatedly measured RCM by ^{51}Cr method over successive days or weeks in 15 normal males and found CV of 3% (18). Nomof et al. (22) compared red cell mass measured by ^{51}Cr and by CO, two entirely independent methods, in 35 persons, including 6 healthy adults and 29 patients hospitalized with various diseases, in which RCM ranged from 1000 mL to 4000 mL, (Hct 0.28-0.64), i.e. ranging from moderate anemia to moderate erythrocytosis. They observed that the relationship was rectilinear with a slope of 1.16, the ^{51}Cr method systematically giving lower results. Re-examination of their data and their graph correlating RCM measured by these two unrelated methods indicates a relationship similar to that which we show in Figures 1 and 2, although with somewhat greater scatter in their data, particularly in cases with high RCM, $r = 0.95$, and standard error 225 mL.

The observations of Nomof et al., and those which we here report, suggest that neither perfect correlation, nor perfect identity of results, is likely to be attainable when RCM measurement is compared by two different methods; the slight observed scatter of data may reasonably be attributed to the large number of variables comprising each method.

Since it has proven impractical to measure analytical precision directly, we have estimated the limit of precision, i.e., the best level of precision that can reasonably be expected with the most meticulous technique, performed by experienced technologists (Table 3). This requires reasonable assumptions as to the precision of a large number of component variables. The 19 variables included in the Monte Carlo simulation all have variances that are known or can be verified in the laboratory. For example, we have verified that the pipettes used, that are certified to have CV $\pm 1\%$, provide this level of precision even

when used with blood, a more viscous fluid than water.

In laboratories in which blood volume studies are not performed frequently, or technologists are not experienced in these methods, precision may be less than that which we have estimated. Then, the much simpler ^{125}I method, that requires fewer steps, fewer venipunctures, and has fewer variables, may prove to be more reliable than the extremely complicated ^{51}Cr method.

In addition to the above considerations, extravasations are more likely to occur with either the ^{51}Cr method or the combined radionuclide method than with the ^{125}I method, since it is more difficult to be sure that a needle has been properly introduced into a vein when the syringe contains blood to be injected than when it contains a clear solution of ^{125}I -albumin. Because of this difficulty in the injection of labeled blood, and because small extravasations are sometimes overlooked, this variable may be larger in practice than we have estimated.

Between-person variability. For apparently healthy adult males in the series of 337 published data discussed below, the standard deviation of the ^{51}Cr RCM was 3.4 mL/kg, providing a coefficient of variation of 12%. If a Gaussian distribution were assumed, approximately 95% of normal results would occur within the range mean $\pm 24\%$ of the mean.

Constancy of the value of f . As noted above, f showed acceptable constancy in the cases reviewed here. This is not a new observation; it merely corroborates the observations of Chaplin, Mollison and Vetter (5) and of Muldowney and Flanagan, (6) made decades ago. Of note is that patients with extreme splenomegaly did not have unusual deviation in the value of f (Table 4). It has been asserted that f is not reliable in persons with high concentrations of immunoglobulins in plasma, as in multiple myeloma. One case of myeloma was included, in which plasma IgG was increased (53 g/L), but f was 0.871. The need to do RCM measurement in patients with myeloma must be rare.

Mollison has pointed out that in cases with marked splenomegaly, the in vivo mixing of

labeled erythrocytes is not complete until 40 minutes (23). In his graph demonstrating delayed mixing, that appeared in several successive editions of *Blood Transfusion in Clinical Medicine*, in a case with splenomegaly (960 g spleen), the mixing of ^{51}Cr -labeled erythrocytes was >90% complete at 15 minutes, the time we arbitrarily selected for performing our blood volume studies. Examination of the equation defining f indicates that this ratio is independent of completeness of mixing: the value would be the same if mixing were 50%, 90% or 100% complete, so long as the rates of mixing of the labeled erythrocytes and labeled albumin in the whole body blood pool are the same. Since we

have not observed any deviation of f in persons with mild to extreme splenomegaly, it appears that the mixing rates must be the same for the two labels. A slower mixing rate in persons with marked splenomegaly might have resulted in a slight underestimation of the total blood volume and RCM. If our data for patients with marked splenomegaly were re-interpreted with Mollison's estimate of the mixing rate in a patient with marked splenomegaly, the RCM and whole blood values would be increased by a factor of about 1.11, but in none of the cases we studied would this small increase have been of any clinical consequence.

Table 4. Cases with Extreme Splenomegaly

Case No.	Diagnosis	RCM		Blood Vol (mL)	RCM (mL/kg)	Hct % (L/L)	RBC ($\times 10^{12}/\text{L}$)	f
		^{51}Cr mL	^{125}I					
1	P. vera	3909	3637	9190	47.1	0.47	6.51	0.922
2	P. vera	3880	4246	8797	49.1	0.53	7.60	0.846
3	Myeloid metapl.	3940	4004	9429	64.6	0.49	7.60	0.877
4	P. vera	3636	3512	8573	44.5	0.48	5.97	0.903
mean difference, ^{51}Cr RCM vs. ^{125}I RCM = 8.5 mL						mean f =		0.877

Note: RCM in mL/kg was calculated using ^{51}Cr RCM in mL and body weight. Blood volume in mL was calculated by adding ^{51}Cr RCM to ^{125}I -albumin plasma volume. f is the ratio of body Hct to venous Hct, a ratio used in calculating RCM from ^{125}I plasma volume. The ratio that we systematically used was 0.864, which is within 1.5% of the mean value for f in this subset of 4 cases. The mean value of f in these 4 cases is identical with that which we calculated from data of 77 males, i.e., 0.877. The cases tabulated above indicate that the value was satisfactory even in those with extreme splenomegaly. The 4 cases also demonstrate that if one wants to know what the total blood volume (and the RCM) is in patients with massive splenomegaly, one can't tell from the venous hematocrit. In each of these 4 cases there must have been at least 3 or 4 liters of blood in the spleen.

These 4 cases are represented in figs. 1-4 by the points farthest to the right.

One of the authors (VFF) (24) previously reported low values for f in patients with polycythemia vera and marked splenomegaly, an observation that is discordant with the observations here reported. Part of the explanation may be that in previous studies, Hct was determined by centrifugation, a method that is known to be non-rectilinear with the erythrocyte count because of increased plasma trapping when hematocrit is high and decreased plasma trapping when hematocrit is low. This may account for the lower values of f reported in earlier studies of patients with marked splenomegaly in contrast with the values found in this study. However, we cannot discount such observations. Any attempt to interpret such anomalies must consider the following relationship that has been used to estimate f , both in this study, and in those reported by others.

$$f = \frac{\text{Blood Volume } (^{51}\text{Cr})}{\text{Hct} \times \text{Blood Volume } (^{51}\text{Cr}) + \text{Plasma Volume } (^{125}\text{I}\text{-albumin})}$$

An anomalous low value for f might be due to: (1) underestimation of total blood volume by the ^{51}Cr method, or (2) overestimation of plasma volume by ^{125}I -albumin method, or (3) overestimation of venous hematocrit by centrifugation method when there is polycythemia or marked poikilocytosis.

In contrast with results of this study, Fudenberg et al. (25) consistently found $f > 1.0$ in patients with extreme splenomegaly. They used $^{51}\text{Cr}^{+3}$ as a plasma volume label, a method that provides estimates of RCM that are systematically higher than reported in other studies (20).

The excellent agreement that these studies show between RCM measured with ^{51}Cr and RCM measured with ^{125}I -albumin, throughout a very wide range of values, raises the question why some previous studies have not also shown equivalency. Possible explanations include the following. 1) Most of the earlier studies were performed using ^{131}I -albumin. The energy peaks of ^{131}I (MeV = 0.360) and ^{51}Cr (MeV = 0.323) are not widely separated, and this increases the level of imprecision in the count rates for each of these

radionuclides in the presence of the other; in contrast, the energy peaks of ^{125}I (MeV = 0.035) and ^{51}Cr are quite well separated. 2) ^{125}I -human serum albumin that is presently in use may be more stable in vivo than was the ^{131}I -albumin that was used 25-30 years ago. 3) For a procedure as complex as the measurement of RCM, that is infrequently performed in most laboratories, anomalous results may have represented unrecognized technical variability.

Nielsen and Rodbro (26) reported a similar study in 1989. Our study differs in that 1) our series spanned a much wider range of RCM values, from <750 mL to >5500 mL; 2) in spite of this, we observed much less scatter than they did when differences were plotted against means; 3) f showed less variability in our study, and no values in excess of 0.96 were observed, even in cases with extreme splenomegaly. Further, we consider that there were two critical fallacies in their interpretation of their results: 1) whereas they referred to RCM obtained with radiochromium-labeled erythrocytes as E_m (measured erythrocyte mass) and RCM obtained using ^{125}I -albumin as E_c (calculated erythrocyte mass), in fact, both of these variables are calculated; neither is measured directly; 2) it was illogical to assume that one of these estimates of RCM is inherently more accurate than the other.

Normal Ranges (Reference Intervals)

Because it is neither practical nor possible for each laboratory to define normal ranges for RCM independently, every laboratory relies upon published normal ranges for the interpretation of results. Widely accepted upper limits for the normal range are 36 mL/kg in adult males and 32 mL/kg in adult females. These values are appropriate for persons who are not overweight. Various alternatives have been proposed to correct for obesity, including a calculation from the patient's height cubed, and a number of regression equations based on height and weight. We have found the regression equations of Retzlaff et al. (27,28) easy and practical to use for estimation of normal value ranges based on height and weight.

Since these equations were adopted to predict normal ranges, rather than expressing blood volume or RCM in mL/kg, “stress erythrocytosis” has virtually disappeared among Mayo Clinic patients. It should be recognized, however, that the predictions of normal ranges are imprecise for those who are either very heavy or very light because few such persons have been included in normal range studies. Even for those who are neither markedly obese nor undernourished, the issue must be addressed as to how wide the “normal range” intervals should be. To re-evaluate this, we have reviewed all accessible

published data in which were tabulated RCM by ⁵¹Cr method, venous hematocrit and body weight in normal, healthy adults, that is, all data that are sufficient to permit comparison and re-evaluation (18,29-33). None of our data are included in this review of published normal value data, and we have not knowingly excluded any published series. There were negligible differences between these studies; therefore, they are combined and analyzed in Table 5. If, through inadvertence, the authors have not included some published data, it is unlikely that any such omission would affect the values shown in Table 4, since *n* is quite large.

Table 5. Published Normal Values for ⁵¹Cr RCM in 477 Healthy Adults, mL/kg (12,27-31)

	N	Mean	SD	Range (all)
Males	337	28.5	3.4	20.0 - 39.7
Females	140	24.9	2.5	17.2 - 32.3

Table 6. Cost Comparison for RCM Using ⁵¹Cr or ¹²⁵I Methods

	Tech Time (\$)	Reagents (\$)*	Total (\$)
⁵¹ Cr	69	223	292
¹²⁵ I-albumin	29	85	114

* Estimated costs in June 1996

For 337 adult males, the mean value for RCM was 28.5 mL/kg, S.D. 3.4 mL/kg; range of all observed values was 20.0 - 39.7 mL/kg. For adult males, approximately 95% of normal values would fall within the range 22 - 35 mL/kg. One person out of every 40 would be expected to have RCM >35 mL/K. If this mean ±2SD range had been applied to the 337 healthy males that comprised the original normal value studies, 10 would have been declared “polycythemic.” (If the widely accepted “upper limit” of 36 mL/kg had

been applied to the original normal value series, 4 normal persons would have been declared “polycythemic.”) If the mean ±2SD rule were applied to a community of 1,000,000 persons, 25,000 would be declared “polycythemic.” But polycythemia vera is a rare disorder, with an estimated prevalence of not more than 20 per million persons. For persons with RCM >35 mL/kg, the odds are about 1,000 to 1 that they do not have polycythemia vera.

The arbitrary application of “normal range”

criteria based on mean $\pm 2SD$ leads to extensive, inappropriate laboratory testing and misdiagnosis of erythrocytosis or polycythemia vera in normal, healthy persons. (The authors are aware of such errors that have occurred, leading to inappropriate treatment with hydroxyurea or ^{32}P).

More realistic criteria are needed. It is recommended that 39 mL/kg be adopted as a more realistic "upper limit" for the RCM in non-obese adult males, since this is just below the uppermost value that was empirically observed in the 337 normal, healthy males that comprised the original normal value study data. This proposed criterion approximates the normal mean + 3SD. There would still be some cases misdiagnosed as having erythrocytosis and subjected to inappropriate additional laboratory testing and treatment, but the number of such cases would be substantially reduced as compared with current experience. The doctrine "*primum non nocere*" is appropriate to the interpretation of laboratory results. Furthermore, the promulgation of "normal ranges" that are unrealistically narrow does not enhance the credibility of laboratories that perform these studies.

Economic Considerations

The combined measurement of RCM using a ^{51}Cr label, and plasma volume using a ^{125}I -albumin label, is an unusually complicated and time-consuming laboratory procedure. Furthermore, the price of the radionuclides is high and is increasing. Table 6 shows the estimated cost of performing the combined procedure using both ^{51}Cr and ^{125}I as compared with measuring RCM and plasma volume using only ^{125}I -albumin.

Since we have found no particular advantage to use of ^{51}Cr , but much greater cost for this radionuclide and for technologists' time, in 1993 we discontinued use of ^{51}Cr for RCM measurement at Mayo Clinic, with unanimous approval of hematologists and other clinicians. During the subsequent 3 years, we have encountered no problems that resulted from this decision.

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