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EXPERIENCES WITH THE RADIOCHROMIUM METHOD FOR DETERMINATION OF RED CELL VOLUME[1]

By R. WENNESLAND, E. BROWN, J. HOPPER, JR., K. G. SCOTT, J. L. HODGES, JR. AND B. BRADLEY

From the Department of Medicine, the Cardiovascular Research Institute, the Radioactivity Research Center, and the Department of Statistics, University of California, San Francisco and Berkeley, California

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The use of radioactively labeled red cells for the determination of the red cell volume (Vrbc) has gained wide acceptance since its introduction by Hahn & Hevesy (1940). Of the procedures currently in use, the method of Sterling & Gray (1950), utilizing autogenous cells labeled with radioactive sodium chromate (Cr^{51}), meets most adequately the basic requirements for blood volume measurement by the dilution principle: *a)* that the "indicator", in this case tagged cells, can become evenly distributed in the entire blood volume within a reasonable period, and *b)* that none of the tag is lost from the circulating blood during mixing. The rate of loss of Cr^{51} from the tagged cells is so slow that the lapse in time between their injection and the estimation of their dilution in the subject's blood is not critical, as is the case with P^{32} (Sterling & Gray 1950). In subjects without hemolytic disorders, the apparent volume of distribution of cells labeled with Cr^{51} even 24 hours after injection is no more than 5 per cent greater than the volume estimated within an hour of the injection (Sterling & Gray 1950; Nomof, Hopper, Brown, Scott & Wennesland 1954; Mollison & Veall 1955).

One of the principal disadvantages of using either Cr^{51} or P^{32} is that the cells must be tagged *in vitro*, which in clinical studies means that the subject must be available at least an hour before the actual measurement. More important, however, is the possible effect on accuracy of damaging the cells by processing them *in vitro*. As will be shown, this is not an important source of error in volume determinations (Wennesland, Shepherd, Nomof, Brown, Hopper & Bradley 1957), except in patients with hemolytic tendencies. Greater care in tagging is required if the cells are to be used for studies

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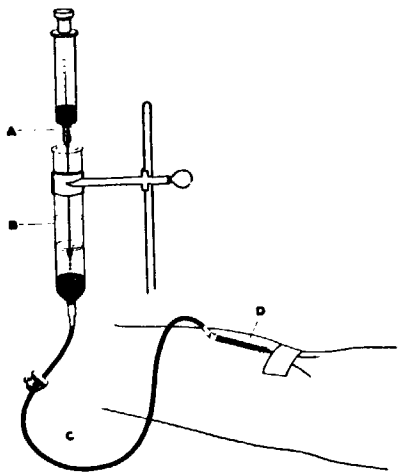

Fig. 1. Apparatus for delivering tagged cells. A = calibrated 10 ml syringe, 5⅛ inch needle; B = glass reservoir (capacities of upper and lower chambers 35 ml and 15 ml, respectively); C = disposable plastic tubing, ¼ inch diameter; D = glass adapter.

of erythrocyte survival (Hughes Jones & Mollison 1956).

The following report will describe and assess a modification of Sterling & Gray's method that we have used extensively (Nomof *et al.* 1954; Wennesland, Brown, Hopper, Hodges, Guttentag, Scott, Tucker & Bradley 1959; Rapaport, Yamauchi, Green, Brown & Hopper 1960; Brown, Hopper, Hodges, Bradley, Wennesland & Yamauchi).

METHODS

Cell tagging.

About 12 ml of the subject's blood is taken for tagging either the afternoon before or the morning of the test. In cases of severe anemia, sufficient blood is drawn to provide an equivalent quantity of cells. The blood is injected through the rubber cap of a sterile 15-ml centrifuge tube containing 2.5 ml of acid-citrate-dextrose solution.

A solution containing 0.1—1.0 μg of $NaCrO_4$[1] per μC is prepared at appropriate intervals and sterilized by autoclave. A volume not exceeding 0.5 ml containing 50—75 μC[2] is added to the blood in the centrifuge tube and mixed by gentle rotation at room temperature for 45 minutes[3]. Plasma and excess Cr^{51} are separated from the tagged cells by centrifuging and washing thrice with a volume of 0.9 per cent saline solution equal to that of the decanted plasma, at room temperature. The cells are resuspended in sufficient saline to restore the volume to that of the original blood sample and either used at once or refrigerated overnight at 4—5° C. The suspension is kept at room temperature for 45 minutes or longer before intravenous injection. Strict aseptic precautions are observed throughout.

Measuring and delivering the dose of tagged cells.

The subject's height and weight are recorded and he reclines in a comfortably warm room for at least 30 minutes before the test. A wide-bore needle connected to a small infusion apparatus (Fig. 1) is placed in an antecubital vein. The same indwelling needle is used for delivery of tagged cells and withdrawal of samples. Enough 0.9 per cent saline is put into the apparatus so that the tubing is free of air; the lower chamber contains about 2 ml, and the upper about 10 ml. Saline is allowed to flow slowly into the vein to demonstrate the adequacy of the venipuncture before the cells are injected.

[1] Converted by the Radioactivity Research Center from $Cr^{51}Cl_3$ obtained from the Oak Ridge National Laboratory.

[2] The procedure described above is the one used at present. When we were collecting the data reported in this paper, we were using a plane scintillation detector in which only 12 per cent of the gamma disintegrations of Cr^{51} were observed as counts, so that 150—200 μC of Cr^{51} were needed for tagging.

[3] Tubes are fastened to a phonograph turntable revolving at 33⅓ r.p.m.

The sterile tagged-cell suspension is drawn from the stoppered test tube in which it was tagged into a 10-ml syringe which has been calibrated for convenient (Peters & Van Slyke 1932). A long needle (5⅛ inches) is substituted for the needle used for the transfer. Exactly 10 ml of the suspension is then delivered into the lower chamber of the infusion apparatus (see Fig. 1). The syringe and needle are washed three times with a total of 8—10 ml of saline taken from the upper reservoir. The cell suspension and washings are allowed to flow rapidly from the lower reservoir into the vein. They are followed by a small air bubble (about 0.1 ml) which helps to sweep the tubing clean. The time the air bubble enters the vein is noted as the beginning of the *in vivo* mixing period. After this, the rate of infusion is reduced to the minimum needed to assure patency of the needle (10 drops/minute or less). The unused portion of the tagged cell suspension is reserved for measurement of its radioactivity.

Sampling and counting.

Blood samples are taken 25 minutes or more after the beginning of the mixing period. Usually, two samples are taken at an interval of 5 minutes and the results averaged. To prevent dilution of the blood with saline, the infusion is discontinued 20—30 seconds before sampling. The first 2—3 ml of blood is discarded. A 5 ml sample is then taken and transferred to a test tube containing Heller's oxalate mixture (Heller & Paul 1934). Duplicate Wintrobe tubes are filled, topped with a small drop of mineral oil and centrifuged for 30 minutes at 3000 r.p.m. (distance from trunion ring to centrifuge center = 13 cm). They are read to the top of the cell column[1]. Two ml of the well-mixed sample are delivered by calibrated pipette into a test tube (12 mm internal diameter) and counted twice in a well scintillation counter[2]. Two 2 ml portions of the reserved cell suspension, diluted 1:50 with distilled water, are counted in the same manner. Countings of blood and cell suspension are alternated to minimize the influence of possible fluctuations in the sensitivity of the counter.

[1] The thickness of the buffy coat is recorded, but no correction is applied unless it exceeds 1 mm.

[2] With our present equipment, 45 per cent of the gamma rays from the Cr^{51} are observed as counts. A sufficient number of counts (4,096 or more) are observed to limit the statistical fluctuations of counting to ± 1.6 per cent.

Calculations.

$$VCr^{51}\ (ml) = \frac{A \times 50 \times V}{B}$$

where A and B are the averages of the observed counts per second per ml of the diluted cell suspension and of the blood specimens respectively, and V the volume in ml of cell suspension injected.

Cell volume (Vrbc) is the product of VCr^{51} and the average of the two hematocrit readings of the sample. At this stage it is convenient to compare the observed Vrbc with the predicted cell volume of a healthy subject of the same weight, height and sex, as established by our studies with this method in 201 healthy men (Wennesland *et al.* 1959) and 101 women (Brown *et al.*). "Predicted normal" values for Vrbc can be found by a convenient graphic method (Wennesland *et al.* 1959, Wintrobe 1961, Brown *et al.*) or by the following equations:

For men: Vrbc (ml) = 8.6 x height (cm) + 18.6 x weight (kg) − 830 (S.D. 190)

For women: Vrbc (ml) = 7.5 x height (cm) + 14.3 x weight (kg) − 603 (S.D. 134)

It should be noted that true cell volume is slightly smaller than Vrbc as calculated above because the cell columns of the hematocrit tubes from which the value is calculated contain 2—5 per cent of trapped plasma (Reeve 1952; Chaplin & Mollison 1952; Ebaugh, Levine & Emerson 1955; Gregersen & Rawson 1959) and the buffy coat is included in the hematocrit reading. Our prediction standards (Wennesland *et al.* 1959; Brown *et al.*) were prepared without corrections for trapped plasma because no single factor is uniformly applicable (Chaplin & Mollison 1952; Ebaugh *et al.* 1955). After observed Vrbc has been compared with the "predicted normal" for the subject, a suitable correction factor for trapped plasma may

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be used to determine the "true" Vrbc, if desired (Reeve 1952; Chaplin & Mollison 1952; Ebaugh *et al.* 1955; Gregersen & Rawson 1959). The contents of the buffy coat represent only 0.5—1.0 per cent of the cell volume in healthy people (Wintrobe 1961), and we have found the top of the cell column easier to read than the interface between red and gray layers. Therefore, we have followed the practice of Reeve (1952) in our studies of normal subjects. Hematocrit readings can be corrected appropriately when the buffy coat exceeds 1 mm.

RESULTS AND DISCUSSION

Evaluation of sources of error and comments on technique.

Errors of measuring a volume by the dilution technique can be considered in two categories. First, is the accuracy of estimating the amount of indicator added, in this case tagged cells. If the dose administered is smaller than supposed, then the volume appears larger than it really is, and vice versa. Included in this category are *a)* measurement of the volume of cells injected, *b)* the accuracy of counting the radioactivity of the tagged cell suspension, and *c)* hidden errors, such as loss of some of the tagged cells from the circulation *in vivo*, as might occur if cells were damaged by the tagging procedure and were phagocytized or otherwise removed from circulation. The second category of errors relates to the measurement of the dilution of tagged cells in the blood. Included here are *a)* the time and technique of blood sampling, and *b)* the accuracy of estimating the radioactivity of the samples. Since the measurement of radioactivity is critical to both the estimation of the dose delivered and its dilution in the blood, it will be considered first.

Errors in measurement of radioactivity. With the plane scintillation counter used during collection of the data reported here, 10 ml of the tagged cell suspension generally yielded a total of 20,000—30,000 counts/second[1], providing a good contrast between the blood specimens and the background radioactivity. The counting error averaged 1.6 per cent (Nomof *et al.* 1954). Samples and standards were placed in dishes, 42 mm in diameter, for counting. Variations in the distribution of the tagged cells in relation to crystals of the counter caused by sedimentation could be obviated either by twirling the dish just before counting or by hemolyzing the cells. We found the latter unnecessary. Thirteen duplicate samples were counted: one member of each pair was frozen and thawed, the other agitated by hand. The mean difference between the hemolyzed and agitated samples was nil, and the standard deviation of the mean of the differences was 0.18, or about 1.5 per cent of the average counts per second of the 13 hemolyzed specimens. This result also shows the approximate size of the variations that can be expected from pipetting and counting errors combined.

Errors in measuring the administered dose of tagged cells. The two most important changes we have made in the method are *a)* tagging the cells the day before instead of the day of the experiment, and *b)* using the infusion apparatus and indwelling needle for administration of tagged cells and for sampling. Tests were made to assure that

[1] With the well-type scintillation detector now being used, the 10 ml of tagged cell suspension yields 50,000—60,000 counts/second.

either innovation impairs the accuracy of the method.

1. Overnight storage of tagged cells. We prefer to study the patients before their breakfast so that their metabolic and circulatory status will be as uniform as possible. Tagging the cells in the afternoon and storing the suspension overnight is convenient, especially for tests on hospitalized patients. Table I shows that after overnight refrigeration, the supernatant saline contains less than 0.2 per cent of the radioactivity of the whole suspension. In animal experiments described elsewhere (Wennesland *et al.* 1957), we showed that although some of the tagged and refrigerated cells may be caught in the lung, liver and spleen of recipient animals, the degree of such cell loss is insufficient to affect blood volume determinations. When cells stored for 1 day were injected into dogs, the total loss of tag *in vitro* and *in vivo* was less than 0.3 per cent (Wennesland *et al.* 1957). The loss of Cr^{51} from the blood during the first 24 hours after injection of cells tagged and stored by our method averaged 5 per cent in 8 healthy subjects (Nomof *et al.* 1954). Mollison & Veall (1955) found a similar rate (6 per cent) in 16 experiments where the blood was returned immediately after tagging. Overnight storage therefore appears not to have any disadvantage, at least when dealing with normal blood[1]. Hughes Jones & Mollison (1956) believed it was unlikely that the early loss of Cr^{51} was due to the handling of the blood, because they found equal rates of loss when blood was tagged *in vitro* and *in vivo*.

Table I. *Loss of Radiochromium (Cr^{51}) from the Tagged Cells to the Supernatant Saline and to the Infusion Apparatus after 16—20 Hours' Storage.*

Loss of Cr^{51} to:	No. of observations	Radioactivity in % of total dose	
		Mean	S. D.
Supernatant saline	12	0.132	0.134
Infusion apparatus*			
Syringe	15	0.039	0.026
Glass bulb	10	0.010	0.002
Tubing	11	0.008	0.007

* Determined by measuring the radioactivity of washings, which were repeated until the counts observed when each portion of apparatus was placed directly in the scintillation counter did not differ significantly from the background radioactivity.

2. The infusion apparatus. The importance of a clean venipuncture and of accurate measurement of the amount of injected tag has long been recognized (Price & Longmire 1942). The radioactivity left in the tubing and glass bulb adds about 50 per cent to the very small amount left in the syringe (Table I). Variation in radioactivity remaining in the entire delivery system are quite small (Table I).

[1] *In vitro* handling of the blood, necessary in all the radioactive cell-tagging methods, may cause indeterminate errors in estimating Vrbc of patients with hemolytic tendencies unless checked by measurements of cell survival. In a large clinical experience with the method, we have had to abandon the test on rare occasions because of visible hemolysis of the tagged cell suspension. The patients usually had renal disease, and the hemolysis occurred early in the tagging procedure (overnight storage was not involved). This observation raises the possibility that *in vivo* hemolysis may be accentuated and be an important source of error in patients with hemolytic tendencies due to extracorpuscular factors.

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Table II. *Average Hematocrit and Cell Volume (Vrbc) of Blood Samples Collected 10, 20 and 30 Minutes after Injection of Tagged Cells in 27 Healthy Men.*

Time of Sampling min.	Hematocrit % cells	Vrbc l
10	44.54	2.06
20	44.41	2.06
30	44.56	2.05

Errors in collection and timing of blood samples. 1. The indwelling needle. This has the further advantage of obviating hemoconcentration resulting from repeated venipuncture and tourniquet applications. The phenomenon of "spontaneous hemodilution following venipuncture", which has been described repeatedly (Gibson & Evans 1937; Hanna & Marshall 1955) and has been observed by us under different circumstances (Brown, Hopper, Sampson & Mudrick 1958), is not found with this procedure. In 27 experiments, samples were taken 10, 20 and 30 minutes after delivering the cells. No downward trend of the hematocrit readings was observed (Table II).

Two suspicions arise about the indwelling needle and the infusion apparatus: *a)* that blood drawn for sampling might be contaminated by accumulation of Cr^{51} in the needle and vein near the site of injection and *b)* that the samples taken shortly after discontinuation of the infusion might be contaminated with saline. The following experiments were therefore done.

In 19 tests, one sample was taken from the indwelling needle in the usual way (infusion discontinued for 30 seconds and the first 2 ml of blood discarded). A second sample was taken from a distant vein, with care to avoid the effects of tourniquet stasis (blood taken no less than 30 seconds after releasing tourniquet; first 2 ml discarded). Table III shows that the Vrbc determined from blood taken from separate sites was the same although the hematocrits of blood from the freshly punctured veins averaged 1 scale division higher. To test whether the difference resulted from contamination with saline from the infusion apparatus, we compared the hematocrits of two successive 6 ml samples taken from the same needle after discontinuation of the infusion. In 15 experiments, the first sample was not significantly more dilute than the second (Table IV). Thus, it appears that the indwelling needle can be employed without fear of contamination of the samples by either saline or Cr^{51}.

It has long been known that significant hemoconcentration can occur when blood is taken during the application of tourniquets (Peters, Eisenman & Bulger 1925). When no particular care was taken to prevent stasis, *e. g.*, when blanks were drawn to determine residual radioactivity from earlier experiments, the hematocrit was sometimes drastically elevated. In one case, the elevation was as much as 7.8 scale divisions (average in 87 cases, 2.5). This artifact does not affect the determination of Vrbc since the measurement is based on the radioactivity and the hematocrit of the same sample. The derived values, Vpl and Vwb, however, can be seriously affected (Table III).

Table III. *Results of Measurements on Blood Samples Collected from an Indwelling Needle and from a Newly Placed Needle in a Distant Vein in 32 Experiments.*

	Specimen Collected from:				Difference	
	Indwelling Needle		Newly Placed Needle			
	Mean	S. D.	Mean	S. D.	Mean	S.D.
Hematocrit % cells	45.30	3.40	46.40	3.30	1.10	0.7
Vrbc, liters	2.08	0.30	2.08	0.31	0.00	0.0
Vpl, liters	2.52	0.41	2.42	0.37	0.10	0.0

2. Time and number of samples. In direct determinations of Vpl based on a single sample, 10 minutes is usually considered an adequate mixing time (Noble & Gregersen, 1946). However, three recent studies involving the use of rapid multiple sampling have shown that fluctuations in the concentration of tagged cells or radioactive iodinated albumin may continue for longer than 10 minutes, and even as long as 25 minutes, after injection of tags, even in healthy subjects (Pritchard, Moir & MacIntyre 1955; Funkhauser 1957; Tuckman, Finnerty & Buchholz 1959). In 27 experiments in which samples were taken 10, 20 and 30 minutes after injection of the tagged cells, mean Vrbc was the same at each sampling time (Table II). The variation in radioactivity between the 3 samples in a single experiment in this series, however, was significantly higher than in a succeeding 103 experiments in which samples were taken at 25, 30 and 35 minutes (S.D. of a single reading = 52 ml in the first 27, and 36 ml in the succeeding 103 experiments). As long as at least 10 minutes are allowed for mixing and the subject has no circulatory disorder, errors due to premature sampling not be larger than 1—2 per cent (Noble & Gregersen 1946). We prefer to wait 25 minutes or longer for the reasons outlined above and because there is no problem with loss of tag when using Cr^{51}.

Table IV. *Hematocrits of Two Successive 6 ml Samples of Blood Taken from Indwelling Needle after Discontinuing the Saline Infusion in 15 Experiments.*

	Hematocrit, % cells		
	First sample	Second sample	Difference
Mean	42.2	42.3	+0.08
Range	38.0–48.0	38.0–48.1	−0.3– +0.8
S. D.	3.3	3.4	0.27

Three samples were taken at 5-minute intervals in 103 experiments on healthy men (Wennesland *et al.*, 1959). Analysis of the differences between individual results showed, for Vrbc,

standard deviation of a single value = 36 ml,

standard error of mean of 3 values = 21 ml.

These volumes are small in comparison with the standard deviations of the mean predicted cell volumes for men and women, 190 and 134 ml, respectively (Wennesland *et al.* 1959, Brown *et al.*). The S.D. of a single value, 36 ml, represents less than half of the mean difference between results of repeated measurements on individual subjects, 80 ml (Table VI). Thus, the result obtained from a single sample is accurate enough for most clinical work; we take 2 samples, primarily as a safeguard against loss or breakage, and average the results.

Mixing may be delayed to an important degree in a number of pathological conditions, and premature sampling gives spur-

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Table V. *Cell Volume (Vrbc) at Normal Body Temperature and as Found by Injecting a Second Dose of Tagged Cells after Rectal Temperature Had Fallen to 27-28°C. Two Patients Subjected to Hypothermia for Surgical Operation.*

Subject	Rectal Temp. °C	Mixing Time min.	Vrbc l	Hematocrit % cells
[redacted]				
First dose of cells	38.0 37.8	55 65	1.60 1.61	41.2 41.0
Second dose	27.4 26.9 26.7	16 32 52	1.34 1.42 1.44	46.6 46.6 46.9
[redacted]				
First dose of cells	37.7 37.7	51 59	2.95 2.95	47.1 46.4
Second dose	27.0 26.7 26.2	15 25 62	2.68 2.61 2.73	50.6 50.6 48.2

iously low values (Nylin & Hedlund 1947; Brown, Hopper, Sampson & Mudrick 1951; Reilly, French, Lau, Scott & White 1954; Pritchard *et al.* 1955). In cases of congestive heart failure we take samples at intervals of 15 minutes for one hour or longer. The resultant "mixing curves" are nearly always flat by the end of half an hour. Caution is required in interpreting results from tests made during shock or hypothermia because there is evidence that portions of the vascular tree may never be reached by the tagged cells in these conditions (Rodbard, Saiki, Malin & Young 1951; Prentice, Olney, Artz & Howard 1954). Thus, the mixing curve may be flat, but the observed volume of distribution represents the circulating, rather than the total, cell volume. Observations on two patients subjected to hypothermia before surgical procedures (Table V) illustrate this point. Vrbc was measured in the usual way before induction of anesthesia and hypothermia. A second dose of tagged cells was given after rectal temperature had fallen about 10° C. Vrbc appeared to be 7—10 per cent lower during hypothermia than just prior to its induction. Similar results were obtained in 3 of 4 splenectomized dogs studied during experimental hypothermia.

Reproducibility and relative magnitude of component sources of error.

In three experiments, subjects were given a second dose of tagged cells immediately after the first. Differences between first and second Vrbc were 0, 90 and 50 ml, or 0, 4.5 and 3.4 per cent.

Seventeen healthy men were subjected to second or third tests after intervals of 3—31 weeks in order to avoid using the larger amounts of radioactivity needed for immediately sequential measurements (Table VI). The mean of 20 differences was 77 ml of cells, or 3.9 per cent (range, 10—190 ml; 0.6—8.8 per cent). These results define the outer limits of experimental error or beyond, since they are affected not only by all the components of the method but by possible changes within subjects. Differences of the same magnitude, however, have been found by others who repeated measurements at shorter intervals. Those measuring VCr^{51} reported differences of 0.4—10.0 per cent (Reilly *et al.* 1954), 4 per cent (Eisenberg 1954) and 2—8 per cent (Walser, Duffy & Griffith 1956). The experience with P^{32} has been similar. Differences between paired measurements were 0.1—6.2 per cent of cells (Chaplin 1954), 3.5—7.5 per cent of cells

Table VI. *Repeated measurements of cell and whole blood volume in 17 healthy men at 3 to 31 week intervals.*

Subject	Interval between measurements	Hematocrit * %	Cells			Whole Blood		
			Found l	Difference l	Change %	Found l	Difference l	Change %
[redacted]	5	47.2 47.0	2.44 2.25	0.19	7.8	5.18 4.79	0.39	7.5
	4 23	43.4 43.3 40.1	1.86 1.81 1.97	0.05 0.16	2.7 8.8	4.30 4.19 4.90	0.11 0.71	2.6 16.9
	7 22 4	40.8 40.6 42.1 42.4	1.67 1.62 1.66 1.65	0.05 0.04 0.01	3.0 2.4 0.6	4.10 4.00 3.95 3.89	0.10 0.05 0.06	2.4 1.3 1.5
	5	48.9 49.5	1.80 1.81	0.01	0.6	3.67 3.66	0.01	0.3
	31	48.8 48.0	1.81 1.76	0.05	2.8	3.71 3.67	0.04	1.1
	13	48.4 48.1	1.93 2.02	0.09	4.2	4.00 4.20	0.20	5.0
	24	45.2 45.6	2.30 2.43	0.13	5.7	5.09 5.33	0.24	4.7
	6	45.5 45.1	2.13 2.04	0.09	4.2	4.69 4.53	0.16	3.4
	12	40.2 38.0	1.83 1.93	0.10	5.5	4.56 5.07	0.51	11.2
	10	40.7 43.8	1.63 1.62	0.01	0.6	4.00 3.69	0.31	7.7
	13	44.3 44.8	1.78 1.86	0.08	4.5	4.02 4.16	0.14	3.5
	13	46.9 45.5	2.14 2.03	0.11	5.1	4.55 4.46	0.09	2.0
	9	44.9 45.2	1.82 1.88	0.06	3.3	4.04 4.17	0.13	3.2
	9	46.7 44.2	2.19 2.28	0.09	4.1	4.69 5.16	0.47	10.0
	7	44.7 46.3	2.29 2.27	0.02	0.9	5.12 4.89	0.23	4.5
	10	46.0 47.2	1.82 1.75	0.07	3.9	3.94 3.69	0.25	6.4
	3	44.3 43.5	1.92 2.05	0.13	6.8	4.34 4.71	0.37	8.5
Mean				0.08 0.05	3.9 2.3		0.23 0.18	5.2 4.1

* Uncorrected for trapped plasma and read to the top of the cell column.

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	26.7	52	1.44	46.9
[redacted]				
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	37.7	59	2.95	46.4
Second dose	27.0	15	2.68	50.6
	26.7	25	2.61	50.6
	26.2	62	2.73	48.2

iously low values (Nylin & Hedlund 1947; Brown, Hopper, Sampson & Mudrick 1951; Reilly, French, Lau, Scott & White 1954; Pritchard *et al.* 1955). In cases of congestive heart failure we take samples at intervals of 15 minutes for one hour or longer. The resultant "mixing curves" are nearly always flat by the end of half an hour. Caution is required in interpreting results from tests made during shock or hypothermia because there is evidence that portions of the vascular tree may never be reached by the tagged cells in these conditions (Rodbard, Saiki, Malin & Young 1951; Prentice, Olney, Artz & Howard 1954). Thus, the mixing curve may be flat, but the observed volume of distribution represents the circulating, rather than the total, cell volume. Observations on two patients subjected to hypothermia before surgical procedures (Table V) illustrate this point. Vrbc was measured in the usual way before induction of anesthesia and hypothermia. A second dose of tagged cells was given after rectal temperature had fallen about 10° C. Vrbc appeared to be 7—10 per cent lower during hypothermia than just prior to its induction. Similar results were obtained in 3 of 4 splenectomized dogs studied during experimental hypothermia.

Reproducibility and relative magnitude of component sources of error.

In three experiments, subjects were given a second dose of tagged cells immediately after the first. Differences between first and second Vrbc were 0, 90 and 50 ml, or 0, 4.5 and 3.4 per cent.

Seventeen healthy men were subjected to second or third tests after intervals of 3—31 weeks in order to avoid using the larger amounts of radioactivity needed for immediately sequential measurements (Table VI). The mean of 20 differences was 77 ml of cells, or 3.9 per cent (range, 10—190 ml; 0.6—8.8 per cent). These results define the outer limits of experimental error or beyond, since they are affected not only by all the components of the method but by possible changes within subjects. Differences of the same magnitude, however, have been found by others who repeated measurements at shorter intervals. Those measuring VCr^{51} reported differences of 0.4—10.0 per cent (Reilly *et al.* 1954), 4 per cent (Eisenberg 1954) and 2—8 per cent (Walser, Duffy & Griffith 1956). The experience with P^{32} has been similar. Differences between paired measurements were 0.1—6.2 per cent of cells (Chaplin 1954), 3.5—7.5 per cent of cells

Table VI. *Repeated measurements of cell and whole blood volume in 17 healthy men at 3 to 31 week intervals.*

Subject	Interval between measurements	Hematocrit* %	Cells			Whole Blood		
			Found l	Difference l	Change %	Found l	Difference l	Change %
[redacted]	5	47.2 47.0	2.44 2.25	0.19	7.8	5.18 4.79	0.39	7.5
	4 23	43.4 43.3 40.1	1.86 1.81 1.97	0.05 0.16	2.7 8.8	4.30 4.19 4.90	0.11 0.71	2.6 16.9
	7 22 4	40.8 40.6 42.1 42.4	1.67 1.62 1.66 1.65	0.05 0.04 0.01	3.0 2.4 0.6	4.10 4.00 3.95 3.89	0.10 0.05 0.06	2.4 1.3 1.5
	5	48.9 49.5	1.80 1.81	0.01	0.6	3.67 3.66	0.01	0.3
	31	48.8 48.0	1.81 1.76	0.05	2.8	3.71 3.67	0.04	1.1
	13	48.4 48.1	1.93 2.02	0.09	4.2	4.00 4.20	0.20	5.0
	24	45.2 45.6	2.30 2.43	0.13	5.7	5.09 5.33	0.24	4.7
	6	45.5 45.1	2.13 2.04	0.09	4.2	4.69 4.53	0.16	3.4
	12	40.2 38.0	1.83 1.93	0.10	5.5	4.56 5.07	0.51	11.2
	10	40.7 43.8	1.63 1.62	0.01	0.6	4.00 3.69	0.31	7.7
	13	44.3 44.8	1.78 1.86	0.08	4.5	4.02 4.16	0.14	3.5
	13	46.9 45.5	2.14 2.03	0.11	5.1	4.55 4.46	0.09	2.0
	9	44.9 45.2	1.82 1.88	0.06	3.3	4.04 4.17	0.13	3.2
	9	46.7 44.2	2.19 2.28	0.09	4.1	4.69 5.16	0.47	10.0
	7	44.7 46.3	2.29 2.27	0.02	0.9	5.12 4.89	0.23	4.5
	10	46.0 47.2	1.82 1.75	0.07	3.9	3.94 3.69	0.25	6.4
[redacted]	3	44.3 43.5	1.92 2.05	0.13	6.8	4.34 4.71	0.37	8.5
Mean				0.08	3.9		0.23	5.2
[redacted]				0.05	2.3		0.18	4.1

* Uncorrected for trapped plasma and read to the top of the cell column.

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(Samet, Fritts, Fishman & Cournand 1957) and 5.7 per cent of blood (Berlin, Hyde, Parsons & Lawrence 1952).

The data presented earlier make it clear that with our procedure used in healthy subjects and animals, errors in Vrbc due to the *in vitro* handling of blood and to the technique of administering the cells and of sampling are very small, totaling well under 1 per cent (Tables I, III and IV). With an average counting error of 1.6 per cent (Nomof *et al.* 1954), the error arising from estimating radioactivity of the average of two blood specimens, compared to that of the tagged cell suspension, is less than 2 per cent. At least 2 per cent of the average test-retest variation remains to be explained.

The S.D. of the hematocrit of a single blood specimen, measured in duplicate, was 0.27 scale divisions in the 103 experiments where 3 samples were taken. The experience is about the same as described by Wintrobe (1934), from whose data it appears that the maximum expected variation of the test as performed *in vitro* is 0.86 per cent. Our practice of averaging the duplicated hematocrits of 25- and 30-minute samples lowers this source of error. An indeterminate error in calculating Vrbc from the radioactivity of whole blood relates to the quantity of plasma trapped in the cell column. With normal blood and uniform procedures of anticoagulation and centrifugation, this quantity might be expected to represent a constant fraction of the cell column. However, from the difficulties encountered in measuring this fraction (Chaplin & Mollison 1952; Ebaugh *et al.* 1955; Furth 1956; Gregersen & Rawson 1959), this expectation may not be justified. Such error could conceivably amount to 2 per cent or more. An error, probably not exceeding one per cent, arises from assuming constancy of the fraction of the cell column occupied by white cells and platelets, or from errors in estimating the thickness of the buffy coat (Reeve 1952; Wintrobe 1961).

Vpl and Vwb are derived values and uncertain to the extent that the observed hematocrit of blood taken from a large vein or artery differs from "true" body hematocrit (Chaplin, Mollison & Vetter 1953; Gregersen & Rawson 1959). Tourniquet stasis or dilution of samples with saline or anticoagulant solutions can seriously affect Vwb and Vpl without affecting Vrbc. In repeated determinations (Table VI) we found that Vwb was less constant than Vrbc. This can probably be accounted for by the lability of Vpl and its dependence on body water and cardiovascular phenomena. Because the ratio of body hematocrit to large vessel hematocrit is affected in several clinical conditions where blood volume is an important variable (Brown, Hopper & Wennesland 1957), our prediction standards for healthy men and women were prepared without applying corrections for this discrepancy. If VCr^{51} is thus considered to be Vwb, and Vpl = Vwb — Vrbc, observed values can be compared to the values derived from the prediction charts and equations (Wennesland *et al.* 1959; Brown *et al.*).

"True" Vwb averages about 110 per cent of VCr^{51}, although the relationship is not constant (Chaplin *et al.* 1953; Samet *et al.* 1957). Failure to correct for the body hematocrit: venous hematocrit ratio, or the assumption of a ratio not applicable under the circumstances of the study, will lead to errors in estimating "true" Vwb from VCr^{51} and hematocrit, amounting to as much as 10 per cent in healthy subjects at rest, 20 per cent in cases of congestive heart failure (Samet *et al.* 1957; Brown *et al.* 1957) and 50 per cent with massive splenomegaly (Fudenberg, Baldini, Mahoney & Dameshek 1961). Birkeland (1960) has pointed out that when blood volume is calculated from the hematocrit and a measurement of only cell or plasma volume, errors due to mistaken assumptions about the body hematocrit: venous hematocrit ratio will be greater when the hematocrit is high than when it is low. In many clinical situations it is desirable to make separate measurements of cell and plasma volumes.

The major sources of error are *a)* the determination of radioactivity of blood specimens and tagged cell suspension, and *b)* determination of the centrifuged hematocrit, particularly with respect to the percentage of trapped plasma in the cell column. Failure to measure accurately the volume of tagged cell suspension delivered to the subject, a serious potential source of error, proved to be relatively unimportant with the technique used. Errors relating to the collection and handling of blood for hematocrit determinations and in the prediction of the "body hematocrit: venous hematrocrit ratio can materially affect the estimation of blood and plasma volumes, but not cell volume.

SUMMARY

We have described in detail a modification of Sterling and Gray's Cr^{51} method for blood volume determination with which we have had considerable experience. The dose of Cr^{51} needed for tagging 12 ml of the patient's blood need not exceed 50—75 μC if a well-type scintillation counter is used. The tagged cell suspension is usually stored overnight so that the test can be done conveniently before breakfast. The cells are delivered from a small infusion apparatus through an indwelling needle which is also used for sampling.

The over-all error of the measurement of cell volume, as shown by repeating the test after intervals of 3 to 31 weeks, averages 3.9 per cent. This compares favorably with results obtained with other modifications of the Cr^{51} method and with P^{32}, even though the long time interval between tests in this study allowed the possibility of within-subject changes of cell volume.

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