DETERMINATION OF THE CIRCULATING RED CELL VOLUME IN MAN BY RADIOACTIVE CHROMIUM [1]

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The circulating red cell volume of man and animals has been investigated heretofore by the injection of red cells labeled with the radioisotopes of iron (Fe^{55} and Fe^{59}) and phosphorus (P^{32}) (1–10).

The preparation of red cells tagged with radioactive iron necessitates the administration of radioiron for one or more weeks to volunteer donors who incorporate it into the hemoglobin of their red cells (1, 2) which must then be transfused into the experimental subject. Radioactive phosphorus may be added *in vitro* to a small volume of the experimental subject's own red cells which become labeled rapidly. This technical advantage, however, is counter-balanced by the rapid phosphate exchange *in vivo* between the red cells and the plasma, causing the radioactivity of the tagged cells to fall significantly after one to three hours (5–8).

In the present investigation a new biological tracer, radioactive chromium (Cr^{51}), with a half-life of 26.5 days, has been employed for the determination of the circulating red cell volume. When this isotope was added to erythrocytes *in vitro* as $Na_2Cr^{51}O_4$, it was taken up avidly by the red cells, which retained their radioactivity without significant loss to the plasma for periods of one day or more after injection into experimental animals (11). Since the exchange of Cr^{51} between the red cells and plasma was negligible for 24 hours, radioactive chromate appeared to be ideal for the tagging of red blood cells and the measurement of the circulating red cell volume. The application of this method to studies in dogs has been reported in a separate communication (12).

The red cell volumes of 25 normal male medical students were determined with radioactive chromium. The accuracy of the method was investigated further in hospital patients by a second determination of the red cell volume after transfusion or hemorrhage of a known volume of red cells.

EXPERIMENTAL PROCEDURE

Approximately 50 cc. of whole blood were withdrawn from the antecubital vein into a sterile heparinized syringe. The blood was centrifuged, and the supernatant plasma removed and stored in the cold. The red cells were resuspended in saline to which 40–200 microcuries of Cr^{51} as $Na_2Cr^{51}O_4$ were added (336–2800 γ chromium). The red cells were allowed to stand at room temperature for one hour in contact with the isotope, preferably with mechanical shaking, at the end of which time the cells had acquired sufficient radioactivity for use as tracers.

The red cell suspension was filtered through sterile gauze, and the red cells were then washed three times with cold sterile saline and finally resuspended in the original untreated plasma. No visible hemolysis occurred. The plasma contained less than 0.5% of the counts present in the washed red cells.

An aliquot of this reconstituted blood was taken for counting and the remainder (approximately 45 cc.) was injected intravenously from a calibrated syringe. After allowing ten minutes for mixing within the circulation, three samples of venous blood were taken at ten-minute intervals.

CALCULATIONS

The circulating red cell volume of the subject is given by the formula:

$$\text{Red cell volume in cc.} = \frac{\text{total counts in circulation}}{\text{counts per cc. packed red cells in sample}}.$$

The numerator of this fraction in the routine study is the total counts injected. This figure is the product of the counts per cc. of packed red cells, the volume of whole blood injected, and the hematocrit/100 (Table I).

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TABLE I

Protocol and calculations of red cell volume experiment

W. H., 26 year old male medical student No. 25. Wt. 66 kg. Ht. 175 cm. Surface area 1.88 sq. m.

8:50 a.m. 50 cc. of blood withdrawn from subject. Red cells tagged with $Na_2Cr^{51}O_4$ and prepared for re-injection. Aliquot removed for counting and hematocrit determination.

12:10 p.m. 40.5 cc. blood containing Cr^{51} tagged cells injected into left antecubital vein. Hematocrit of injected reconstituted blood = 51.5

$$40.5 \text{ cc.} \times \frac{51.5}{100} = 20.8 \text{ cc. red cells injected.}$$

Samples withdrawn from right antecubital vein.

	Ground red cell method	Hemolysate method
	counts per cc.	*counts per cc.*
Injected blood	43,700	598,000
10 minute sample	376	5,200
20 minute sample	396	5,300
30 minute sample	405	5,340
3 hour sample	404	5,300
24 hour sample	386	5,160
Calculations	20.8 cc. red cells injected × 43,700 counts per cc. = 910,000 counts injected.	20.8 cc. red cells injected × 598,000 counts per cc. = 12,450,000 counts injected.
	÷ 376 = 2,420 cc. ÷ 396 = 2,300 cc. 910,000 ÷ 405 = 2,250 cc. ÷ 404 = 2,250 cc. ÷ 386 = 2,360 cc.	÷ 5,200 = 2,400 cc. ÷ 5,300 = 2,350 cc. 12,450,000 ÷ 5,340 = 2,330 cc. ÷ 5,300 = 2,350 cc. ÷ 5,160 = 2,410 cc.
	Mean = 2,316 cc.	Mean = 2,368 cc.
	Standard deviation = 73.55 cc. or 3.2% of mean	Standard deviation = 34.9 cc. or 1.5% of mean

Plasma contained no significant counts.

TRANSFUSION AND HEMORRHAGE

All transfusion experiments were done with heparinized blood drawn within the hour before use. Additional venous blood samples were obtained at various intervals following the transfusion, and the activity of the circulating red cells, now diluted by the addition of the untagged blood, was determined. The circulating red cell volume was then calculated as above.

After hemorrhage, however, the radioactivity of the circulating red cells remained unaltered, since the hemorrhage represented the removal of an aliquot of blood containing an unaltered proportion of tagged and untagged red blood cells. Consequently, a second injection of tagged red cells was necessary to verify the red cell volume after hemorrhage. The same formula was employed, but in this case the numerator represented the sum of the counts of the two injections minus the counts removed by the hemorrhage.

COUNTING PROCEDURE AND PREPARATION OF SAMPLES

The blood samples, as well as the injected reconstituted blood containing chromium-tagged erythrocytes, were centrifuged in 4 cc. hematocrit tubes at approximately 2700 r.p.m. (741 *g*) for 60 minutes in the cold room. The error in hematocrit determination due to trapped plasma was assumed to be constant and was disregarded. The hematocrit readings were recorded and the plasma separated. One cc. of each plasma sample was pipetted into a weighed aluminum planchet and dried overnight in air or in an oven at 60° C. This temperature was not exceeded, since chromium may sublime at 83° C. The plasma samples were dried to constant weight in a desiccator and were counted.

The red cells were prepared for counting as *A*) dried ground red cells and as *B*) dried red cell hemolysates.

The red cell volumes of the hospital patients and the series of 25 normal volunteers were determined by the dried ground red cell method. The last 12 consecutive subjects in the series were studied by both methods simultaneously.

A. Dried Ground Red Cells

After the removal of plasma from the 4 cc. hematocrit tubes, the packed red cells were poured into unweighed planchets and dried overnight at 60° C. The dried caked

red cell mass was ground to a fine powder with a pestle in folded filter paper and counted in duplicate weighed planchets. The counts per cc. of packed red cells was obtained by computing the counts in 0.35 gm. of dried, ground red cells, since this value was found to be equivalent to 1 cc. of packed red cells (11).

The radioactivity was measured with an X-ray Geiger counter.[3] The radioactivity of the samples exceeded five times the background. The probable error of counting was less than 2%. Since Cr^{51} is a soft X-ray emitter, self absorption corrections were applied to all samples. The counts were corrected for radioactive decay.

B. Dried Red Cell Hemolysates

In preparing the hemolysates, 4 cc. hematocrit tubes were filled approximately half full with whole blood and centrifuged at approximately 2700 r.p.m. in the cold for 60 minutes. After removal of the plasma, the packed red cells were diluted to precisely four times their initial volume with distilled water as read by the calibration on the hematocrit tube, *e.g.*, from 23 to 92. The tubes were covered with Parafilm and inverted not less than ten times to insure complete hemolysis.

Two-tenths cc. aliquots of the hemolysates were pipetted into aluminum planchets with 0.2 cc. pipettes. Eight-tenths cc. distilled water was added to the planchets immediately and the hemolysates were dried in the air overnight or under an ordinary lamp for five hours and then placed in a desiccator before counting.

When diluted in 1 cc. of distilled water, the hemolyzed red cells dried to a smooth film with no significant cracking. Although the films were thicker at the border of the planchets because of adhesion to the walls, they appeared quite reproducible on inspection and agreed within 3% on counting. This technique circumvented the difficulty of counting the dried hemolysate of 1 cc. of packed red cells which was unsatisfactory because of the cracking of the film on drying.

The self absorption of a series of hemolysates prepared in an identical manner was found to be satisfactorily uniform with a standard deviation from the mean of 1%. Therefore, the absorption correction was considered uniform and was omitted from the calculations.

This method of preparing the blood samples is rapid and simple. It eliminates the necessity of weighing planchets, grinding dried red cells, and correcting for self absorption and weight.

The hemolysates were counted satisfactorily on the Geiger counter if a sufficient tagging dose of $Na_2Cr^{51}O_4$ was added to the red cells (preferably 100 to 200 microcuries).

The dried red cell hemolysates in this communication were counted on the continuous flow windowless proportional counter developed by Charles V. Robinson (13). This instrument has a high counting efficiency and permits a counting rate approximately ten times higher than that obtained with the Geiger counter with only a two-fold increase in background.

[3] North American Phillips, tube type 62017—Amperex

DOSAGE

The total dosage of radioactivity need not exceed 0.1 rep, which is well within the tolerance limits for human use. From the disintegration scheme of H. Bradt and his associates (14), the administration of 0.4 microcurie of Cr^{51} per kilogram body weight approximates a total dose of 0.1 rep (15), on the conservative assumptions that there is no excretion and that radiation is localized in the red cells.[4] The amount of chromium injected into a subject per kilogram body weight varied from 6 γ to 48 γ depending upon the specific activity of the sample. For a single red cell volume determination the total dose of chromium did not exceed 1 mg., and was usually less than 0.5 mg. This is well below the toxicity level. This isotope has been approved for human use by the Isotopes Division of the Atomic Energy Commission.

RESULTS

Repeated determinations of the circulating red cell volume computed from the blood samples obtained routinely 10, 20, and 30 minutes after the injection of the tagged erythrocytes were reproducible in each of the 25 normal volunteers with a standard deviation of less than 5%. Measurements of the red cell volume were still in agreement within this range after 24 hours.

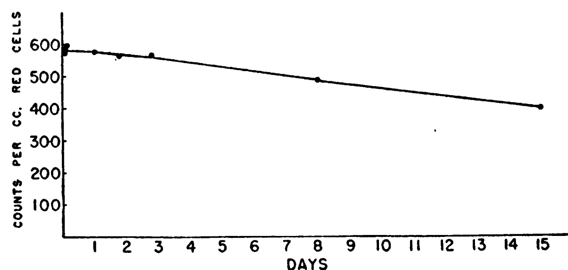

Fig. 1

Counts per cc. red cells in the ordinate signifies counts per minute.

[4] The formula from Marinelli, Quimby, and Hine (16) for beta-emitters and very soft X-ray emitters was used for the calculation of radiation dosage.

Total dosage in rep = 88 ETC

where E = average energy per disintegration in million electron volts (Mev). For Cr^{51} this constant is 4.92×10^{-3} Mev.

T = half-life of isotope in days. For Cr^{51} this constant is 26.5 days.

C = concentration in microcuries per gram of tissue.

TABLE II

Circulating red cell volume of 25 normal male medical students determined by radioactive chromium

Subject	Wt.	Ht.	Surface area	Circulating red cell volume		
				Total	cc. per kg. body weight	L per sq. m. surface area
	kg.	*cm.*	*sq. m.*	*cc.*		
R. C.	72.0	182	1.93	2790	38.7	1.45
D. W.	75.0	185	1.97	2402	32.0	1.22
B. K.	75.0	175	1.91	2378	31.7	1.25
L. T.	70.5	175	1.85	1940	27.5	1.05
G. R.	96.3	183	2.18	2520	26.2	1.16
J. W.	76.0	185	1.99	2239	29.5	1.12
F. H.	79.6	188	2.05	2717	34.2	1.33
D. G.	83.4	190	2.10	2548	30.6	1.21
A. S.	77.3	173	1.92	2723	35.2	1.42
N. N.	63.7	183	1.84	2080	32.6	1.13
W. H.	63.7	183	1.84	2032	31.9	1.10
P. S.	66.0	183	1.86	2297	34.8	1.24
A. K.	75.0	188	2.01	2397	32.0	1.19
H. M.	75.0	183	1.97	2660	35.5	1.35
G. E.	79.6	180	2.00	2193	27.8	1.10
W. T.	63.6	170	1.74	1887	29.7	1.08
H. K.	84.0	183	2.06	2257	26.9	1.10
J. K.	86.5	183	2.09	2637	30.6	1.26
D. W.	70.5	168	1.81	1792	25.4	0.99
D. E.	80.5	188	2.07	2850	35.4	1.38
J. L.	68.3	173	1.81	2136	31.2	1.18
D. Z.	70.5	173	1.84	2153	30.5	1.17
A. H.	68.6	175	1.83	2563	37.4	1.40
J. P.	70.5	185	1.92	2257	32.1	1.18
W. H.	66.0	175	1.88	2316	35.2	1.23
Mean				2351	31.8	1.21
Standard deviation				290	3.5	0.12
Standard error of mean				58	0.7	0.02
Radioactive iron determinations (Gibson and associates [2])				2208	29.7	1.15

When tagged erythrocytes were injected into the circulation, they retained their radioactivity without significant loss to the plasma for 24 hours. There was a gradual decline in red cell activity thereafter (Figure 1).

The consistency of the erythrocyte radioactivity, and consequently the reproducibility of the red cell volume measurements over a period of 24 hours are demonstrated in Table I. There were no significant counts in the plasma and only 1.9% of the injected counts appeared in the 24 hour urine collection.

The data obtained by the ground red cell and hemolysate methods were in close agreement (Table I).

The mean circulating red cell volume of 25 normal male students was 2351 cc. as determined by the radioactive chromium method compared to 2208 cc. obtained by Gibson and his colleagues (2) with radioactive iron (Table II). When calculated on the basis of weight and surface area, the mean red cell volume was 31.8 cc. per kg. and 1.21 L per sq. m., which was slightly higher than the values of 29.7 cc. per kg. and 1.15 L per sq. m. reported with the radioactive iron method (Table II).

The accuracy of the method was verified by a second Cr^{51} determination of the red cell volume after the transfusion or hemorrhage of a known volume of red cells. The results agreed with the calculated values within 3% (Table III). A simi-

TABLE III

Red cell volume determination by Cr^{51} before and after transfusion or hemorrhage

Patient	Sex	Weight	Initial hematocrit	Diagnosis	Initial red cell volume as determined by Cr^{51} method	Measured transfusion or hemorrhage	Calculated final red cell volume	Final red cell volume as determined by Cr^{51} method	Difference	Per cent difference
		kg.								
J. M.	♂	71	38	Laennec's cirrhosis	2,430 cc.	+265 cc.	2,695 cc.	2,700 cc.	− 5 cc.	−0.2
J. L.	♂	64	30	Bronchogenic carcinoma	1,275 cc.	+238 cc.	1,513 cc.	1,530 cc.	−17 cc.	−1.1
C. R.	♂	88	36	Laennec's cirrhosis	2,265 cc.	+254 cc.	2,519 cc.	2,543 cc.	−24 cc.	−0.9
C. T.	♀	66	67	Polycythemia vera	2,993 cc.	−286 cc.	2,707 cc.	2,636 cc.	+71 cc.	+2.7
R. Z.	♀	54	53	Polycythemia vera	2,427 cc.	−245 cc.	2,182 cc.	2,190 cc.	− 8 cc.	−0.4

lar accuracy has been observed in the dog experiments reported previously (12).

Comparison of the red cell volume determinations with radioactive chromium by the ground red cell and the hemolysate methods in 12 consecutive normal volunteers revealed a mean difference of 1.1%. The differences were both positive and negative, indicating no systematic discrepancy between the two methods.

DISCUSSION

The Cr^{51} method of determining the circulating red cell volume combines the accuracy of the radioiron technique with the simplicity of the radiophosphorus method. The radioiron technique offers the advantage of labeling the erythrocyte with a component which is an integral part of the cell. The necessity, however, of administering the radioactive iron to volunteer donors for longer than a week and the difficulties of obtaining compatibility between the tagged donor red cells and the subject's plasma present technical obstacles.

The chromium method shares with the P^{32} technique the decided advantage that a small sample of the subject's own blood may be tagged rapidly *in vitro* and then re-injected. In contrast to P^{32} tagging, however, the Cr^{51} tagged cells retained their activity without significant loss to the plasma for approximately 24 hours after injection.

Measurement of the red cell volume ten minutes after the injection of the Cr^{51} labeled erythrocytes agreed within 3% to 5% with the red cell volume determined 24 hours later. After three days, the red cell activity decreased by approximately 15% compared to a similar fall after three hours when P^{32} tagged red cells are used (5–8). The constancy of red cell tagging with Cr^{51} should prove of value in clinical studies where the red cell volumes may be followed throughout the course of a hospital day or where significant fluctuations in the circulating red cell volume are anticipated.

If an unknown amount of blood has been lost after the initial red cell volume determination, a second injection of tagged erythrocytes is necessary. The red cell volume may then be calculated by dividing the total counts in the second injection by the difference in counts per cc. packed red cells between the samples obtained before and after the second injection (2).

The low radioactivity dosage (total dose of 0.1 rep or less) permits repeated use of the Cr^{51} method on the same individual. The amount of chromium injected for a single red cell volume experiment does not exceed 1 mg. and is usually less than 0.5 mg., which may be administered repeatedly with safety.

The accuracy of the Cr^{51} method, as verified by hemorrhage and transfusion experiments with measured volumes of blood, was within 3% to 5%, which compares favorably with other isotope methods (1, 2, 5–10).

Determinations of the circulating red cell volume by radioactive chromium and radioiron on populations of normal male students at the Harvard Medical School at different times agreed within 5% to 6%.

SUMMARY

1. A new biological tracer, radioactive chromium (Cr^{51}) with a half-life of 26.5 days, has been employed for the determination of the circulating red cell volume in man.

2. A small aliquot of the subject's erythrocytes was tagged rapidly *in vitro* with radioactive sodium chromate and was re-injected within a few hours.

3. After the tagged cells had mixed in the circulation, the radioactivity of the subject's erythrocytes was determined.

4. The circulating red cell volume was calculated by the isotope dilution principle.

5. The radioactivity of the circulating red blood cells remained constant for approximately 24 hours without significant loss to the plasma.

6. Repeated measurements of the red cell volume were reproducible within 5% for approximately 24 hours.

7. The accuracy of the method was within 5% and was verified by hemorrhage and transfusion experiments with measured volumes of blood.

8. The circulating red cell volumes of 25 normal male medical students were determined:

Mean circulating red cell volume .... 2351 cc.
Mean red cell volume per kilogram body weight 31.8 cc.
Mean red cell volume per square meter surface area .... 1.21 liters

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