SIMULTANEOUS MEASUREMENT OF BLOOD PARAMETERS USING RADIOCHROMIUM-LABELED RED CELLS AND RADIOIRON-LABELED PLASMA

DONALD F. LOGSDON, JR., Captain, USAF, BSC, et al.

USAF School of Aerospace Medicine
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas

October 1968

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DONALD F. LOGSDON, JR., Captain, USAF, BSC
JAMES F. GREEN, Master Sergeant, USAF
GUY M. STRONG, Staff Sergeant, USAF
JOHN W. HARPER, Staff Sergeant, USAF

FOREWORD

This report was prepared in the Internal Medicine Branch, Clinical Sciences Division, under task No. 775502. The work was accomplished between January and March 1968. The paper was received for publication on 16 July 1968.

The Auto-Gamma spectrometer, model 5000, used in the study was manufactured by the Packard Instrument Company, Downers Grove, Ill.

The subjects receiving radioisotopes were informed volunteers.

This report has been reviewed and is approved.

GEORGE E. SCHAFER
Colonel, USAF, MC
Commander

ABSTRACT

Separate procedures for the measurement of erythrokinetics, ferrokinetics, and blood volume have previously been reported. The technic described in this paper measures these parameters simultaneously, thereby reducing the volume of blood and number of samples needed to be drawn, and decreasing the possibility of artifactual variations in parameters. Values obtained by this technic are in good agreement with those resulting from the separate studies.

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I. INTRODUCTION

In previous reports, standard procedures were described for measuring erythrocytes, iron metabolism, and blood volume by use of iron and chromium isotopes (1, 2). In many cases it is desirable to measure part, or all, of these parameters simultaneously, thereby reducing the total volume of blood and the number of samples needed to be drawn from the subject. Reducing the volume of blood also decreases the possibility that variations in blood parameters will be an artifact resulting from blood loss. This report presents an integrated procedure for the simultaneous measurement of blood parameters using ^{59}Fe-labeled plasma and ^{51}Cr-labeled red cells.

II. MATERIALS

Radioiron

A radioactive iron solution was prepared by dissolving ferric chloride, ^{59}Fe, in distilled water with NaOH or HCl added to adjust the pH to 7.4. The solution has a specific activity of 10 μc./mg., or 30 μc. per ml. (Iron-59 has a physical half-life of 44.5 days and emits three gamma rays, with energies of 0.19, 1.10, and 1.29 Mev.)

Radiochromium

Radioactive chromium (^{51}Cr) was used in the form of sodium chromate. As received from the supplier, it is sterile and suitable for human use. A solution was prepared with sodium bicarbonate as the buffer and with NaOH or HCl used to adjust the pH to 7.4. The chromium has a specific activity of 42.7 mc./mg., with approximately 215 μc. per ml. of solution. (Chromium-51 emits gamma rays by K-capture, 9% of which have an energy of 0.320 Mev.)

Gamma spectrometer

The chromium-51 and iron-59 activity were measured by a gamma spectrometer. The radioactivity detector consisted of a well-type NaI scintillation crystal, with dimensions of 3 by 3 inches. The spectrometer was calibrated for full-scale energy of 1 Mev at a gain setting of 40%. The instrument settings for counting the iron isotope were: coarse gain set—20% (0 to 2 Mev); fine gain, 2.3. The baseline discriminator counter was set at 500, and the upper discriminator counter at 700. These window settings count both the 1.10- and 1.29-Mev iron photopeaks. For counting the chromium-51, the discriminator settings were 144 and 164; the gain settings remained the same.

III. METHOD

Whole blood (20 ml.) was drawn into a sterile tube and 2.0 ml. of A.C.D. solution was added. The sample was centrifuged at 3,000 r.p.m. for 15 minutes to separate the plasma from the cells. Plasma (6 ml.) was placed in a sterile tube and 12 μc. of ^{59}Fe added. The solution was mixed gently and incubated at room temperature for 45 minutes. The red cells were resuspended in saline to 20 ml. and mixed; 10 ml. of this mixture were removed to a second sterile tube, and 30 μc. of chromium-51 were added. The solution was

mixed gently and incubated at room temperature for 45 minutes. The cells were then washed three times and resuspended to 10 ml. with sterile saline.

The injection dose, containing ^{51}Cr-tagged red cells and ^{59}Fe-labeled plasma, was prepared in the following manner:

With a 10-ml. syringe and needle, 5 ml. of the tagged red cell suspension were withdrawn. The needle was removed and a sterile three-way stopcock was attached to the syringe. Labeled plasma was prepared as follows: 3 ml. of radioiron and plasma were drawn into a 5-ml. syringe; the needle was removed and the syringe attached to the stopcock; the plasma was expelled into the injection syringe; the syringe was removed and the stopcock rinsed by forcing sterile NaCl through it; the stopcock was then replaced with a 20-gage needle. The labeled plasma was added to the injection syringe which was now loaded.

Standards

The remainder of the tagged red cells and labeled plasma were used to prepare standards. A quantity of 5 ml. of cells was removed and placed in a 100-ml. volumetric flask. The cells were diluted to 100 ml. with distilled water and the flask marked "^{51}Cr Standard." The plasma ^{59}Fe (3 ml.) was pipetted into a 1-liter volumetric flask and diluted to one liter with distilled water. This flask was marked "^{59}Fe Standard."

The labeled cells and labeled plasma were injected into the subject and the time recorded. After 10 minutes, a 7-ml. sample of blood (in heparin) was drawn. Two hematocrit

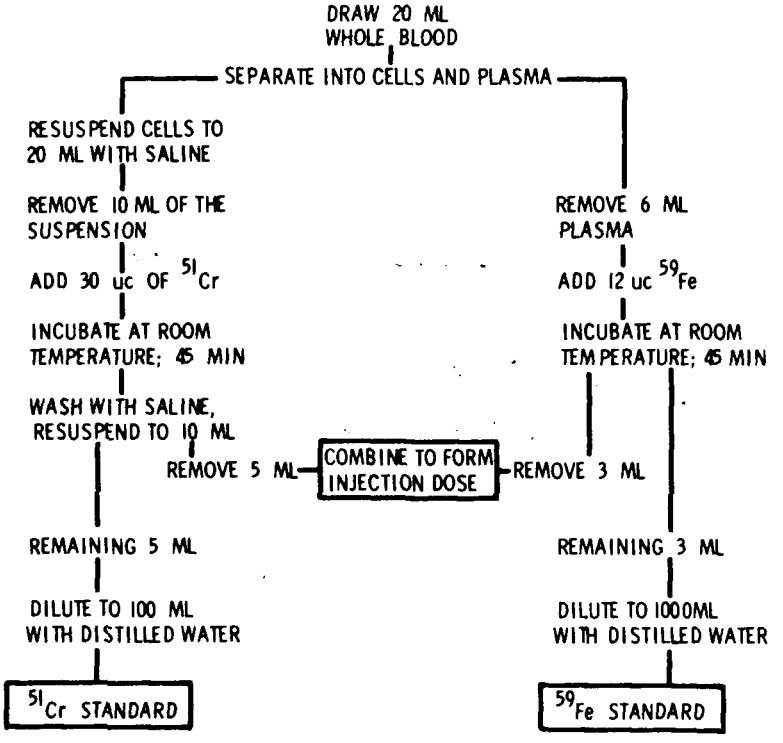

FIGURE 1

Flow chart showing the steps in preparing the injection dose and standards.

samples were collected and the hematocrit determined. The hematocrit was then corrected for trapped plasma by multiplying by 0.98. A flow chart for the preparation of injection dose and standards is shown in figure 1.

Plasma, total blood, and red cell volumes

The plasma and cells were separated by centrifugation; the cells were then resuspended to 7 ml. with saline. To find the plasma volume, ^{59}Fe activity was determined for equal volumes of the plasma and the ^{59}Fe standard. The plasma activity was then divided by the standard activity and multiplied by the dilution factor of the standard.

To find the total blood volume, equal volumes of the red cell suspension and the ^{51}Cr standard were measured for activity. The activity of the standard was divided by the activity of the red cell suspension and multiplied by the dilution factor of the standard. The plasma volume was subtracted from the total blood volume to obtain the red cell volume (RCV).

Plasma-iron clearance (rapid component, T½)

The activity of the plasma sample, collected at 10 minutes, was also used for the first value of the plasma clearance curve. Additional 7-ml. blood samples were collected (in heparin) at 15, 30, 60, 120, and 180 minutes after the initial sample was drawn. The plasma was separated at each point from the cells. The ^{59}Fe activity of 3-ml. samples of the plasma was then determined. The values were plotted versus time to derive the iron clearance curve. From this curve, the T½ for the fast component (or the time when the initial activity was reduced to 50%) could be determined.

Red cell survival rate

The red cell–^{51}Cr activity of the initial 10-minute blood sample was also used as the initial value of the red cell survival curve. Additional 7-ml. samples of blood were drawn (in heparin) at 24-hour intervals, up to 10 days. Each day, two hematocrit tubes of blood were collected and the mean hematocrit determined. After all samples were collected, the plasma and cells were separated, the cells were resuspended to 7 ml. with saline, and the ^{51}Cr activity of the cell suspension was determined. These values were plotted versus time, in days, to form the red cell survival curve. From this curve, the red cell survival, T½, or the time neecssary for the initial activity to drop to 50%, could be determined.

Plasma-iron clearance (slow component, T½)

The ^{59}Fe activity of the plasma portion of the blood samples collected for the ^{51}Cr red cell survival curve, was measured and used to calculate the T½ of the slow component of the ^{59}Fe-plasma clearance curve.

Plasma samples (3 ml.) were measured for ^{59}Fe activity. The values were then plotted versus time in days, starting with day 1. A best-fit line was drawn through the points and extrapolated to time-zero. The extrapolated time-zero was used as the initial value of the slow-component plasma clearance curve. The time when this initial value is reduced to 50% is the T½ for the slow component.

Red cell–iron uptake

After the ^{51}Cr activity of the red cell suspension prepared for the ^{51}Cr red cell survival curve was determined, the cell suspensions were counted for ^{59}Fe activity to determine the red cell–^{59}Fe uptake curve. These values were corrected to activity per milliliter of red cells, using the corrected hematocrit value for that day. The total injected ^{59}Fe activity was determined by measuring the activity of an aliquot of the ^{59}Fe standard. Then the ^{59}Fe activity per milliliter of red cells was multiplied by the red cell volume to obtain the activity per total red cell volume. The ^{59}Fe activity per total red cell volume was divided by the total ^{59}Fe activity injected. The resulting value, multiplied by 100, was the percent injected activity incorporated. When these values were plotted versus time, the rate of red cell–iron uptake could be determined. A flow chart for the simultaneous procedure is shown in figure 2.

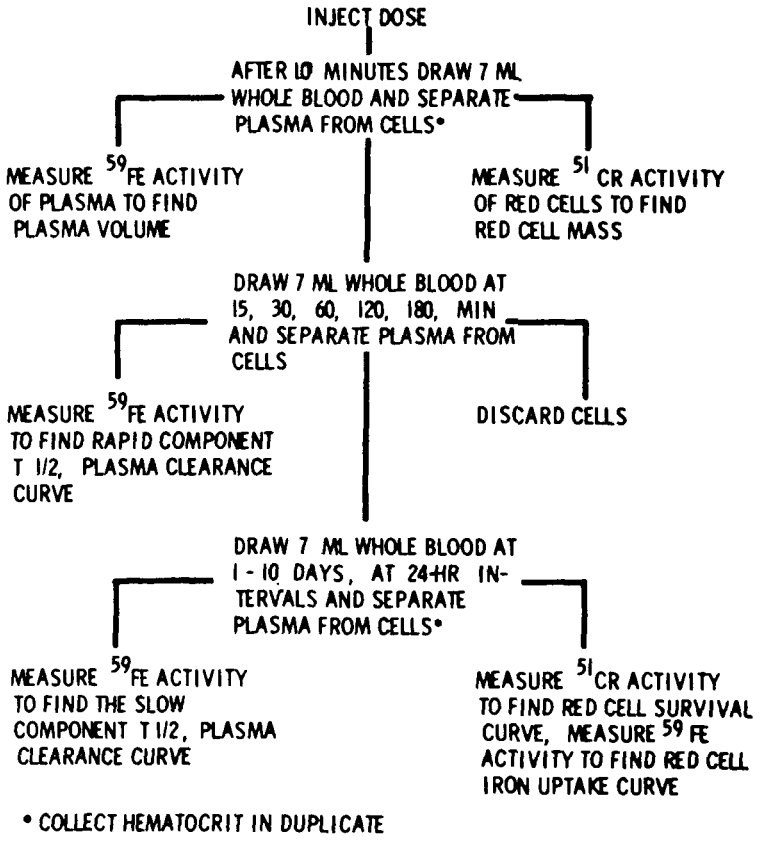

FIGURE 2

Flow chart of the simultaneous procedure.

Ferrokinetic values were calculated as described by Logsdon et al. (2). Hematopoietic center ^{59}Fe measurements were performed as described by Strong et al. (3). Plasma-iron and hemoglobin determinations were performed using standard methods. The separation of ^{59}Fe and ^{51}Cr activity in samples containing both isotopes was by the method of Harper et al. (4).

IV. RESULTS

Table I illustrates typical erythrokinetic, ferrokinetic, and blood volume measurements obtained by the simultaneous procedure. The subject was an 18-year-old male, 72 inches tall, weighing 81.63 kg.

Plasma hemoglobin was 15 gm./100 ml. or 908.6 gm. total body hemoglobin. Plasma-iron concentration was 147 μg./100 ml. The hematocrit remained constant at 0.44, with a plasmacrit of 0.56.

The blood withdrawn during the 10-day procedure totaled 132 ml. Of this, about 58 ml. were red cells; 2 ml. of red cells were reinjected with the injection dose, leaving 56 ml. withdrawn. Of this, 25 ml. were removed during the first 5 hours; the remainder at the rate of about 3 ml. per day.

V. DISCUSSION

One advantage of the simultaneous procedure is that all values are measured at the

TABLE I

Erythrokinetic, ferrokinetic, and blood volume measurements obtained by the simultaneous procedure

Determination	Value
Plasma volume	3,386 ml. or 41.5 ml./kg.
Red cell volume	2,671 ml. or 25.0 ml./kg.
Total blood volume	6,057 ml. or 66.5 ml./kg.
Red cell survival, T½	24 days
Mean red cell life span	109 days
Plasma clearance, T½, rapid component	122 minutes
Plasma clearance, T½, slow component	2.4 days
Maximum red cell–iron incorporation	82%
Plasma-iron turnover	40.4 mg./day
Red cell–iron turnover	28.2 mg./day
Daily hemoglobin synthesis	8.3 gm./day (1.4 gm./liter/day)
Labile iron erythropoietic pool	95.8 mg.
Plasma-iron pool	5 mg.
Mean effective red cell hemoglobinization time	1.34 days

Subject was an 18-year-old male, 72 inches in height, weighing 81.63 kg.

same point. This means that the values can be compared directly with one another or, as a unified set of data, can be compared with other data sets obtained by the simultaneous procedure at other times, with other individuals, or under different conditions.

Another advantage of the simultaneous procedure is that it lessens the effect of blood loss on the blood parameters measured. The simultaneous procedure uses only 132 ml. of whole blood as compared to about 200 ml. required when the procedures are performed separately.

A final advantage is the reduction in the number of blood withdrawals needed. The separate procedures require 27 blood withdrawals while the simultaneous procedure requires only 16.

REFERENCES

1. Logsdon, D. F., Jr., and J. F. Green. Red cell mass, red cell survival, and total blood volume with chromium-51. SAM-TR-68-69, July 1968.
2. Logsdon, D. F., Jr., J. F. Green, and G. M. Strong. A standard method for ^{59}Fe ferrokinetics. SAM-TR-68-98, Sept. 1968.
3. Strong, G. M., D. F. Logsdon, Jr., and J. F. Green. Measuring iron metabolism in hematopoietic centers using ^{59}Fe in the presence of ^{51}Cr and ^{125}I. SAM-TR-68-92, Sept. 1968.
4. Harper, J. W., J. F. Green, and D. F. Logsdon, Jr. Simultaneous determination of ^{59}Fe, ^{51}Cr, and ^{125}I using a gamma spectrometer. SAM-TR-68-40, May 1968.

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
USAF School of Aerospace Medicine Aerospace Medical Division (AFSC) Brooks Air Force Base, Texas	Unclassified
	2b. GROUP

3. REPORT TITLE

SIMULTANEOUS MEASUREMENT OF BLOOD PARAMETERS USING RADIOCHROMIUM-LABELED RED CELLS AND RADIOIRON-LABELED PLASMA

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Jan. - Mar. 1968

5. AUTHOR(S) (First name, middle initial, last name)

Donald F. Logsdon, Jr., Captain, USAF, BSC
James F. Green, Master Sergeant, USAF
Guy M. Strong, Staff Sergeant, USAF
John W. Harper, Staff Sergeant, USAF

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
October 1968	5	4

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO.	SAM-TR-68-113
c. Task No. 775502	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	

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11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	USAF School of Aerospace Medicine Aerospace Medical Division (AFSC) Brooks Air Force Base, Texas

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DD FORM 1 NOV 65 1473

Unclassified

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Radiobiology						
Erythrokinetics						
Ferrokinetics						
Red cell survival						
Blood volume						
Chromium-51 isotope						
Iron-59 isotope						