

AIR1.941130.077c

A STANDARD METHOD FOR ^{59}Fe FERROKINETICS

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

JAMES F. GREEN, Master Sergeant, USAF

GUY M. STRONG, Staff Sergeant, USAF



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

September 1968

Qualified requesters may obtain copies of this report from DDC. Orders will be expedited if placed through the librarian or other person designated to request documents from DDC.

When U. S. Government drawings, specifications, or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

This document has been approved for public release and sale; its distribution is unlimited.

A STANDARD METHOD FOR ^{59}Fe FERROKINETICS

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

JAMES F. GREEN, Master Sergeant, USAF

GUY M. STRONG, Staff Sergeant, USAF

FOREWORD

This report was prepared in the Internal Medicine Branch under task No. 775502. The work was accomplished between January and March 1968, and the report was submitted for publication on 14 June 1968.

A Packard series 5000 Auto-Gamma spectrometer was used throughout this work.

The authors thank Staff Sergeant John Harper for his technical assistance.

This report has been reviewed and is approved.



GEORGE E. SCHAFER
Colonel, USAF, MC
Commander

ABSTRACT

A simple method for performing a ^{59}Fe ferrokinetics study has been developed, combining several procedures already in use. Methods are presented for measuring plasma iron clearance, red cell iron uptake, and the movement of iron through the hematopoietic centers. Formulas are given for calculating plasma and red cell iron turnover, hemoglobin synthesis, mean erythron life-span, and mean effective erythron hemoglobinization time.

A STANDARD METHOD FOR ^{59}Fe FERROKINETICS

I. INTRODUCTION

In previous reports, methods have been described not only for the determination of plasma iron clearance and red cell iron uptake, but also for the exterior measurement of iron taken up by the bone marrow, heart, liver, and spleen (1, 2). Pollycove and Mortimer (3) have given several formulas for calculating plasma and red cell iron turnover, hemoglobin synthesis, and the mean erythron life-span. In the present report these formulas and our methods are combined, and a comprehensive method for measuring ferrokinetics is described.

II. MATERIALS AND EQUIPMENT

Iron-59

Radioactive iron is supplied as ferric chloride- ^{59}Fe . The solution is prepared in distilled water, with NaOH or HCl added to adjust the pH to 7.4. The solution has a specific activity of 10 $\mu\text{c./mg.}$ and contains 30 $\mu\text{c./ml.}$ The ^{59}Fe has a physical half-life of 44.5 days and emits gamma rays, with energies of 0.19, 1.10, and 1.29 Mev. Of the 12 $\mu\text{c.}$ of ^{59}Fe that are used, approximately 6 $\mu\text{c.}$ are injected into the patient and approximately 6 $\mu\text{c.}$ are used as a standard.

Gamma spectrometer

The instrument used to measure the ^{59}Fe activity is a gamma spectrometer. The element which measures radioactivity is a NaI scintillation crystal, with dimensions of 3 in. x 3 in., and with a central well that is $2\frac{1}{32}$ in. x $2\frac{1}{8}$ in. Radioactivity impinging on the crystal molecules causes them to emit photons of light

which activate a photomultiplier cell that, in turn, multiplies incident photon energies to the eleventh dynode (i.e., the incident energy is raised to the eleventh power). The spectrometer has been calibrated for full-scale energy of 1 Mev, at a gain setting of 40%. In addition, the spectrometer has been calibrated for settings of: 0.5 Mev, gain equals 80%; 2 Mev, gain equals 20%; and 4 Mev, gain equals 10%. The dial settings are: coarse gain, 0 to 2; and fine gain, 2.3. The discriminator settings are set to delimit a 1% counting window. This counting window will count both the 1.10 and the 1.29 Mev photopeaks which make up 98.1% of the gamma energy emitted by ^{59}Fe .

III. PROCEDURES

A 15-ml. sample of blood is withdrawn from the patient (in heparin). The sample is centrifuged and the plasma separated. Then 12 $\mu\text{c.}$ of ^{59}Fe -ferric chloride are added to 6 ml. of this plasma which is incubated for 45 minutes at room temperature, and mixed occasionally. A 3-ml. sample of this mixture is used to prepare the ^{59}Fe standard, by diluting the 3-ml. sample to 1 liter with distilled water. A second 3-ml. sample is used as the injection dose.

The first step is to inject the labeled plasma. Exactly 10 minutes later, an 8-ml. sample of blood is drawn (in heparin). The blood is centrifuged and the plasma separated from the cells. The activity of 3 ml. of this plasma is the first value of the plasma clearance curve.

To ascertain the rest of the curve, the following steps are necessary: Draw 8 ml. of blood (in heparin) at intervals of 15, 30, 60, 120, and 180 minutes, and at 24- to 72-hour

intervals, until 10 days have elapsed. Separate the plasma from the cells. At the same time that each blood sample is collected, draw two hematocrit tubes of blood and determine the hematocrit. Correct this hematocrit value for trapped plasma by multiplying by 0.98.

Determine the activity of 3-ml. samples of plasma. Plot the values from 0 to 180 minutes vs. time in minutes to obtain the rapid component of the curve. From this curve, determine the time necessary for the initial activity to decrease to 50% (the $t_{1/2}$ for the fast-clearing component).

For the second, slower-clearing component, plot the values from 0 to 10 days vs. time. From this curve, calculate the $t_{1/2}$ of the slow-clearing component. Extrapolate this curve back to day zero to determine the intercept, or day zero activity, of the slow-clearing component.

The cell portion of the samples collected at day zero, and from 1 to 10 days after day zero, is used to determine the red cell iron uptake curve. The first step is to wash the cells three times with isotonic saline and resuspend the cells to 8 ml. with saline. Then the activity of the 8-ml. suspension is measured and the activity is divided by the hematocrit in order to calculate activity per milliliter of packed cells. The red cell mass (RCM) is calculated by multiplying the plasma volume by a ratio of the hematocrit to the plasmacrit (1-hematocrit). The activity per milliliter of packed cells is multiplied by the RCM to give the activity per total red cell volume. The activity injected is determined by multiplying the activity of 8 ml. of the ^{59}Fe standard by the dilution factor. The ratio is then calculated for the activity per total red cell volume to the activity injected. By means of these values and the following formula, the percentage of the injected activity incorporated into the red cells is calculated:

$$\frac{\text{Red cell volume} \times \text{activity/ml. cells}}{\text{Total activity injected}} \times 100 = \text{percentage injected activity incorporated}$$

If these values are plotted vs. time in days, the rate of red cell iron uptake or appearance can be determined.

Organ counting

Counting sites are outlined on the patient's body to localize the heart, liver, spleen, and sacrum. The first measurement of ^{59}Fe is made within 10 to 20 minutes after the injection of the ^{59}Fe -labeled plasma. A second measurement is made of all areas in 2 to 2.5 hours. The third measurement is made within 4 to 6 hours of the first. Additional measurements are performed at 24 to 72 hours until 10 days have elapsed. The activity measured each day is divided by the ^{59}Fe standard to give an R factor for each time (R_t). The R factor is divided by the R_0 factor (the activity at time zero divided by the standard) to give a ratio. The ratios at each time, for each tissue, are then plotted vs. time in days. This plot will show the change in ^{59}Fe concentration in each area measured during the 10-day period.

Plasma iron and hemoglobin concentration

A sample of blood is drawn immediately prior to the start of the ferrokinetic study, and the plasma iron and hemoglobin concentration is determined through standard technics.

IV. CALCULATIONS

The standard procedure was tested on a normal individual—male; 18 years old; height, 72.0 in.; and weight, 81.63 kg. The following figures and values are from this study.

Plasma clearance and red cell uptake

The composite plasma iron clearance curve is presented in figure 1. The time for the activity to drop to one-half the initial value, the $t_{1/2}$, is shown for both the rapid and the slow components of the clearance curve. The upper curve depicts the rapid clearance—the lower curve, the slow component. The $t_{1/2}$ of the rapid component is 122 minutes (or

2.03 hours). The slow-component curve intercepts the activity axis at 0.0165. The $t_{1/2}$ values and intercept (C) were used to calculate factors r_1 and r_3 . This calculation was made by dividing 16.67 by the rapid component, $t_{1/2}$ in hours, to get r_1 ; and by dividing 0.693 by the slow component, $t_{1/2}$, to get r_3 . The values were: $r_1 = 8.212$, and $r_3 = 0.289$.

The red cell iron uptake curve in figure 2 shows a maximum iron incorporation of 82% by the eighth day after ^{59}Fe injection. The curve was analyzed to determine the $t_{1/5}$, the time necessary for the curve to reach 20% of the maximum incorporation. This value was: $t_{1/5} = 2.1$ days.

The plasma volume was 3,386 ml., or 41.48 ml./kg. The hematocrit (corrected) was 44.4% and the plasmacrit was 55.6%. The red cell volume was 2,671 ml., or 32.72 ml./kg. The hemoglobin value was 15.0 gm./100 ml., and the total body hemoglobin was 909 gm. The iron concentration was 147 $\mu\text{g}/100$ ml.

Formulas for ferrokinetics

Pollycove and Mortimer (3) have presented formulas for calculating iron turnover, hemoglobin synthesis, mean erythron life-span, mean effective erythron hemoglobinization time, the labile iron pool in the marrow, and the plasma iron pool. They have also presented formulas for calculating iron storage pools. Their formulas have two forms: first, when there is appreciable iron storage; and second, when there is little iron storage. Examination of figure 3 shows that there was no peak within the 10-day period in the liver curves; therefore, the formulas given are those used to calculate the parameters when there is little iron storage.

As a first step in calculating the parameters it was necessary to determine calculation factors, some of which have already been described (i.e., $r_1 = 8.212$; $r_3 = 0.289$; and $C = 0.0165$). These were used to calculate additional factors, including: factor $b = r_3 + C$ ($r_1 - r_3$), factor $c = r_3 + r_1$; and factor $e = r_1 \times r_3$. The values were: $b = 0.420$, $c = 8.501$, and $e = 2.373$. Factors b , c , and e

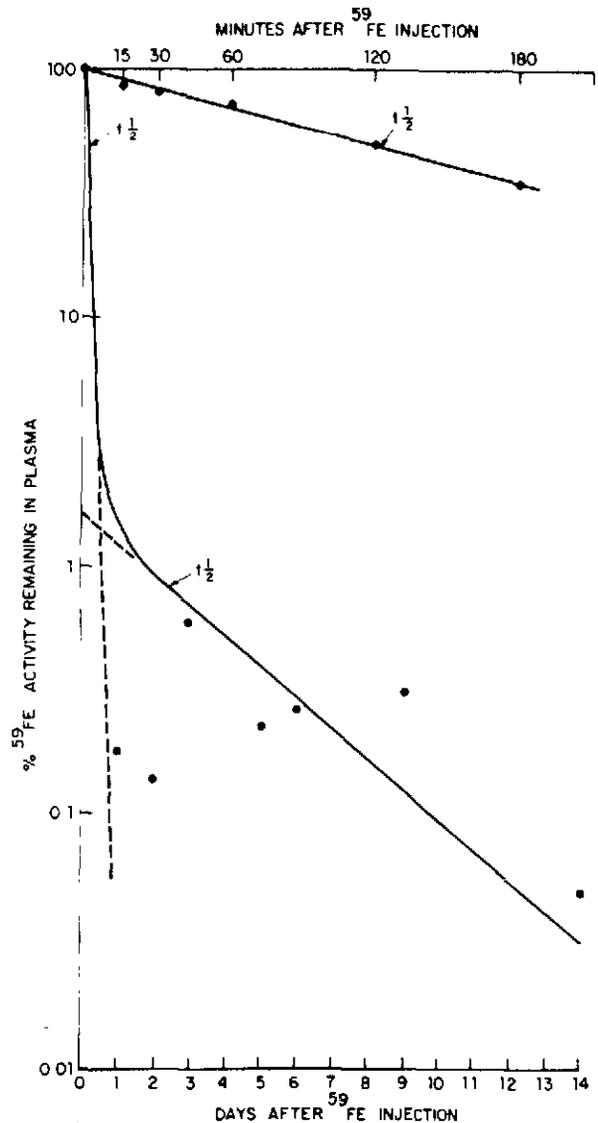


FIGURE 1

Plasma iron clearance curve showing the $t_{1/2}$ of the rapid and slow components.

were then used to calculate the rate constants M, N, P. Rate constant M was obtained by subtracting b from c; N, by dividing e by M; and P, by subtracting N from b. The rate constant values were: $M = 8.081$, $N = 0.294$, and $P = 0.126$.

The first step in calculating the labile iron pool (X_2) was to multiply the plasma volume by the iron concentration. This value, which

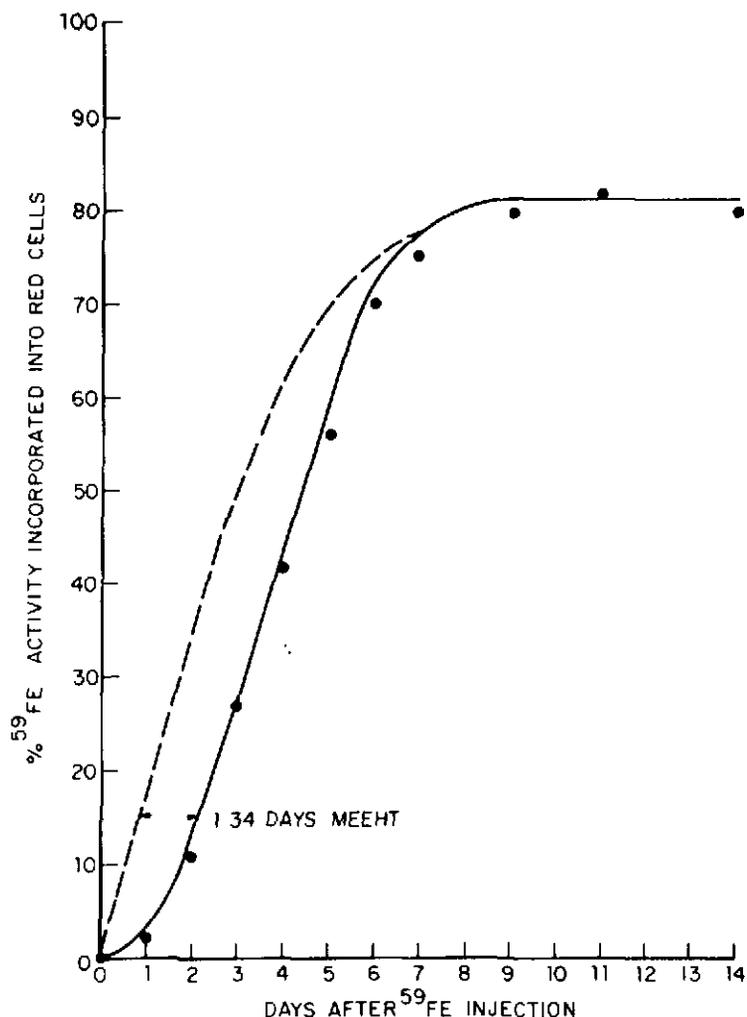


FIGURE 2

Curve showing maximal red cell iron incorporation and the mean effective erythron hemoglobinization time.

was equal to the plasma iron pool, X_1 , was multiplied by rate factor M and divided by factor b to give the labile pool, X_2 . The formulas were:

$$X_1 \text{ (mg.)} = \frac{\text{Plasma Fe } (\mu\text{g./ml.)} \times \text{Plasma volume (ml.)}}{1,000 \text{ } (\mu\text{g./mg.)}}$$

$$X_2 \text{ (mg.)} = M \times X_1 / b$$

where the value for $X_1 = 5$ mg., and for $X_2 = 95.8$ mg.

The plasma iron turnover, determined by multiplying the plasma pool value X_1 by the

rate factor M , was 40.4 mg./day. The red cell turnover was calculated by multiplying X_2 by N . This value was 28.2 mg./day. Because 1 gm. of hemoglobin will contain 3.4 mg. of iron, the daily hemoglobin synthesis can be determined from the red cell turnover value. The turnover value was divided by 3.4 to determine the daily hemoglobin synthesis: 8.3 gm. hemoglobin/day, or 1.4 gm./liter blood per day.

Mean erythron life-span

The first step was to calculate the total body hemoglobin. This value, 908.6, was then

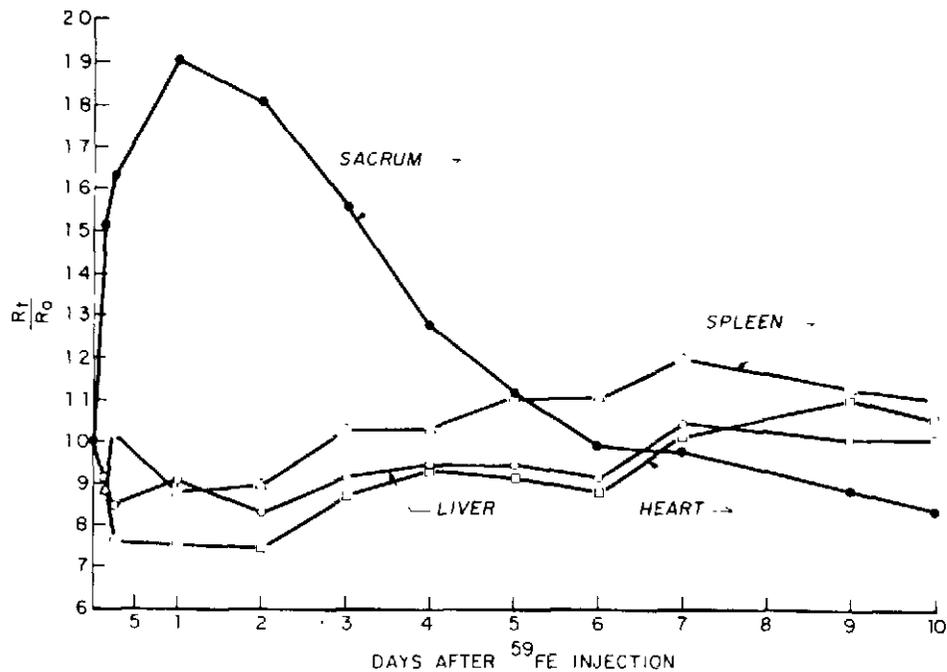


FIGURE 3

Change in organ ⁵⁹Fe concentration with time.

divided by the daily hemoglobin synthesis value to give a mean erythron life-span of 109 days. The assumption necessary for this calculation was that the red cell volume remain constant throughout the test period. Changes in the red cell volume would have required additional calculations.

Mean effective erythron hemoglobinization time (MEEHT)

This value is the time interval between the incorporation of iron into the marrow erythrons and its appearance in circulating erythrocytes. The first step in calculating this parameter was to calculate the $t_{1/2}$ of the red cell iron incorporation curve. This value was 2.1 days. The next step was to calculate, according to Pollycove and Mortimer (3), the " $t_{1/2}$ (F)." This was calculated by dividing 0.223 by rate factor N. This value, 0.76, was then subtracted from the $t_{1/2}$. The MEEHT was 1.34.

V. RESULTS

The formulas of Pollycove and Mortimer (3) give the following estimate: a plasma iron turnover of 40.4 mg. Fe per day, or 40.4 mg. Fe leaving the plasma per day; a red cell iron turnover of 28.2 mg. Fe per day, or 28.2 mg. Fe used in hemoglobin synthesis per day. In other words, the daily feedback of iron (12.2 mg.), from the labile erythropoietic pool (95.8 mg.) to the plasma pool (5 mg.), is approximately one-third of the amount of iron (40.4 mg.) daily entering the labile erythropoietic pool from the plasma.

Hemoglobin synthesis per day is 8.3 gm., or 1.4 gm. per liter per day. The mean erythrocyte life span is 109 days and the mean effective erythrocyte hemoglobinization time is 1.34 days. With a total body hemoglobin value of 908.6 gm., the hemoglobin synthesis per day is 0.91% of the circulating hemoglobin.

VI. DISCUSSION

The formulas of Pollycove and Mortimer (3) are extensions of the formulas of Huff et al. (4), who proposed a method for calculating hemoglobin synthesis in which the plasma iron turnover was multiplied by the maximal fraction incorporated into new red cells. This gave the milligrams of iron needed to form new red cells per day, or the daily hemoglobin synthesis rate. On the basis of a mean erythron life-span of 100 to 120 days, however, the daily hemoglobin synthesis rate should not exceed 0.75% to 1.00% of the total body hemoglobin.

Values found by using the formulas of Huff et al. were 1.2% to 2.00%. Pollycove and Mortimer (3) prepared a model of ferrokinetics in which the flow of iron between compartments was analyzed. From this they devised rate constants for use in calculating ferrokinetics. In the present study, the daily hemoglobin synthesis using the formulas of Pollycove and Mortimer was found to be within expected limits. The methods described here, combined with their formulas (3), provide accurate measurement of the various ferrokinetic pools and rate factors for iron movement between them.

REFERENCES

1. Logsdon, D. F., Jr., and J. F. Green. Determination of five blood parameters using ^{59}Fe . SAM-TR-68-56, June 1968.
2. Strong, G. M., J. F. Green, and D. F. Logsdon, Jr. Measuring iron metabolism in hematopoietic centers using ^{59}Fe in the presence of ^{51}Cr and ^{125}I . SAM-TR-68-92, Sept. 1968.
3. Pollycove, M., and R. Mortimer. The quantitative determination of iron kinetics and hemoglobin synthesis in human subjects. *J. Clin. Invest.* 40:753 (1961).
4. Huff, R. L., C. A. Tobias, and J. H. Lawrence. A test for red cell production. *Acta Haemat.* 7:129 (1952).

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) USAF School of Aerospace Medicine Aerospace Medical Division (AFSC) Brooks Air Force Base, Texas		2a. REPORT SECURITY CLASSIFICATION Unclassified
		2b. GROUP
3 REPORT TITLE A STANDARD METHOD FOR ⁵⁹ FE FERROKINETICS		
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		
6 REPORT DATE September 1968	7a. TOTAL NO. OF PAGES 6	7b. NO. OF REFS 4
8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S) SAM-TR-68-98	
b. PROJECT NO.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	
c. Task No. 775502		
d.		
10 DISTRIBUTION STATEMENT This document has been approved for public release and sale; its distribution is unlimited.		
11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY USAF School of Aerospace Medicine Aerospace Medical Division (AFSC) Brooks Air Force Base, Texas	
13 ABSTRACT A simple method for performing a ⁵⁹ Fe ferrokinetics study has been developed, combining several procedures already in use. Methods are presented for measuring plasma iron clearance, red cell iron uptake, and the movement of iron through the hematopoietic centers. Formulas are given for calculating plasma and red cell iron turnover, hemoglobin synthesis, mean erythron life-span, and mean effective erythron hemoglobinization time.		

14

KEY WORDS

LINK A

LINK B

LINK C

ROLE

WT

ROLE

WT

ROLE

WT

Internal medicine
Radiobiology
Ferrokinetics
Iron-59
Plasma iron turnover
Red cell iron turnover
Hemoglobin synthesis