

# DETERMINATION OF FIVE BLOOD PARAMETERS USING $^{59}\text{Fe}$

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## FOREWORD

This report was prepared in the Internal Medicine Branch under task No. 775506. The work was accomplished between November 1967 and January 1968, and the report was received for publication on 21 March 1968.

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

Staff Sergeant Guy Strong and Staff Sergeant John Harper gave technical assistance in the study.

This report has been reviewed and is approved.



GEORGE E. SCHAFFER  
Colonel, USAF, MC  
Commander

## ABSTRACT

A simplified method for determining the clearance of iron from plasma, the uptake of iron by newly formed red cells, the direct measurement of plasma volume, and the indirect measurement of blood volume and red cell mass, was achieved by modification and synthesis of existing methods. Typical values obtained were: plasma iron clearance ( $T_{1/2}$ ), 100 minutes; red cell iron incorporation, 80% to 85% within 8 days;  $^{59}\text{Fe}$  plasma volume, 41.5 ml./kg.;  $^{59}\text{Fe}$  blood volume, 72.4 ml./kg.; and  $^{59}\text{Fe}$  red cell volume, 32.7 ml./kg. The values obtained were within the limits of values previously reported as obtained by other methods. These measurements, combined with values calculated for other iron metabolic factors, provide the basis for reliable estimates of ferrokinetics and iron metabolism under normal and abnormal conditions.

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## I. INTRODUCTION

A standard procedure has been developed for measuring the clearance of iron from plasma and the uptake of iron by newly formed red cells. A direct method for determining plasma volume and indirect methods for measuring total blood volume and red cell volume were also devised. The procedures described are modifications of the methods of King and Mitchell (1), the VA Hospital, Omaha, Nebr. (2), and Abbott Laboratories (3). They provide a simple, easy-to-follow method for measuring blood parameters in ferrokinetics.

## II. MATERIALS

### Iron-59

The radioactive material used was ferric chloride  $^{59}\text{Fe}$ . A solution was prepared by use of distilled water, with NaOH or HCl added to adjust the pH to 7.4. This solution had a specific activity of 10  $\mu\text{c./mg.}$ , and contained 30  $\mu\text{c.}$  per milliliter. 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. Each test required the use of 12  $\mu\text{c.}$  of activity, with approximately 6  $\mu\text{c.}$  injected into the patient and approximately 6  $\mu\text{c.}$  used as a standard.

### Gamma spectrometer

The instrument used to measure the  $^{59}\text{Fe}$  activity was a gamma spectrometer. In this instrument, a NaI scintillation crystal, with dimensions of 3 by 3 in., and with a central well, measures radioactivity. Radioactivity impinging on the crystal molecules causes them to emit photons of light which activate a photomultiplier cell. This cell multiplies incident

photon energies to the eleventh dynode; that is, the incident energy is raised to the eleventh power. The spectrometer was calibrated for full-scale energy of 1 Mev at a gain setting of 40%. Additional settings of 0.5 Mev (gain equals 80%); 2 Mev (gain equals 20%), and 4 Mev (gain equals 10%) were used.

### Optimum counting window

The gain settings were: coarse gain, 20%; fine gain, 2.3. The discriminator settings were: baseline settings, 500; upper-level setting, 700. This window will count both the 1.10 and the 1.29 Mev photopeaks which make up 98.1% of the gamma energy emitted by  $^{59}\text{Fe}$ .

## III. METHODS

The following procedures for determining blood parameters were devised.

### Plasma volume, blood volume, red cell mass

Draw 15 ml. of blood (in heparin) and separate the plasma. Add 12  $\mu\text{c.}$  of  $^{59}\text{Fe}$  to 6.5 ml. of plasma. Mix occasionally, incubating at room temperature for 30 minutes. Withdraw 3 ml. of plasma and dilute to 1 liter with isotonic saline. Label this sample the plasma standard.

Draw up a second 3-ml. sample into a syringe; this is the injection dose. It is injected into the patient and the time recorded as the starting time. After 10 minutes draw 8 ml. of blood (in heparin). Collect two microhematocrit tubes of blood and determine the hematocrit. Correct the hematocrit for trapped plasma by multiplying by 0.98. Separate the plasma from the cells (8-ml. sample) by

centrifuging at 3,000 r.p.m. for 15 minutes. Measure the activity of 3 ml. of the plasma and 3 ml. of the plasma standard. With these values, determine the plasma volume using the following formula:

$$\frac{\text{Standard activity} \times \text{Dilution factor}}{\text{Plasma activity}} = \text{Plasma volume (PV)}$$

With the PV and the hematocrit, calculate the blood volume and red cell mass.

#### Plasma iron clearance rate

The activity of 3 ml. of the plasma collected as described above is the first value of the plasma clearance curve. For the rest of the curve, draw 8 ml. of blood (in heparin) at 15, 30, 60, 120, and 180 minutes after the first sample is drawn. Separate the plasma and measure the activity of a 3-ml. sample. Divide the activity values of each plasma sample by the activity of the initial plasma sample to convert these to percentage values. Plot the percentage values against time to obtain the plasma iron clearance curve. From this, determine the time necessary for the activity to decrease to 50%. This is the  $T_{1/2}$  (half-life) of iron clearance.

#### Red cell uptake curve

Wash the red cell portion of the 8 ml. of whole blood drawn at 10 minutes postinjection three times with saline. Resuspend to 5 ml. with saline, measure the activity, and using the hematocrit value, calculate the activity per milliliter of packed cells. This is the first sample of the red cell uptake curve.

After 24 hours, and at intervals of 24 to 72 hours up to 10 days, draw 5-ml. samples of blood (in heparin) and separate the plasma from the cells. Collect two hematocrit tubes of blood and determine the hematocrit. Wash the cells three times with saline and resuspend to 5 ml. with saline. Measure the activity of the 5-ml. suspension and divide by the hematocrit to calculate activity per milliliter of packed cells. The red cell mass (RCM) is calculated by multiplying the PV by a ratio of the

hematocrit to 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 5 ml. of the standard by the dilution factor. The ratio of the activity per total red cell volume to the activity injected is then calculated. Using these values and the following formula, the percent of the injected activity incorporated into the red cells is calculated:

$$\frac{\text{Red cell volume} \times \text{Activity per milliliter of cells}}{\text{Total activity injected}} \times$$

$$100 = \text{Percent injected activity incorporated}$$

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

## IV. RESULTS

#### Plasma volume, blood volume, and red cell volume

Normal blood values are: Total blood volume, 51.0 to 81.2  $\pm$  7.3 ml./kg.; plasma volume, 27.6 to 52.0  $\pm$  6.1 ml./kg.; and red cell volume, 20.4 to 31.2  $\pm$  2.7 ml./kg. (4). Typical values obtained by use of  $^{59}\text{Fe}$  were: total volume, 74.2 ml./kg.; plasma volume 41.5 ml./kg.; and red cell volume, 32.7 ml./kg.

#### Plasma iron clearance

A normal  $T_{1/2}$  for iron plasma clearance is from 80 to 120 minutes. The lower curve of figure 1 shows a typical plasma iron curve. The point on the semilogarithmic curve where the activity drops to 50% of the initial activity was used to determine the  $T_{1/2}$  value, which in this case was 100 minutes.

#### Red cell iron uptake

The upper curve of figure 1 illustrates the red cell iron uptake. After an initial rapid removal of iron, the plasma level becomes constant. Then the iron removed from the plasma is incorporated into newly formed red cells and begins to appear in the circulating red cells. In 8 days, the curve of the  $^{59}\text{Fe}$  incorporation reaches a plateau of about 80% to 85%.

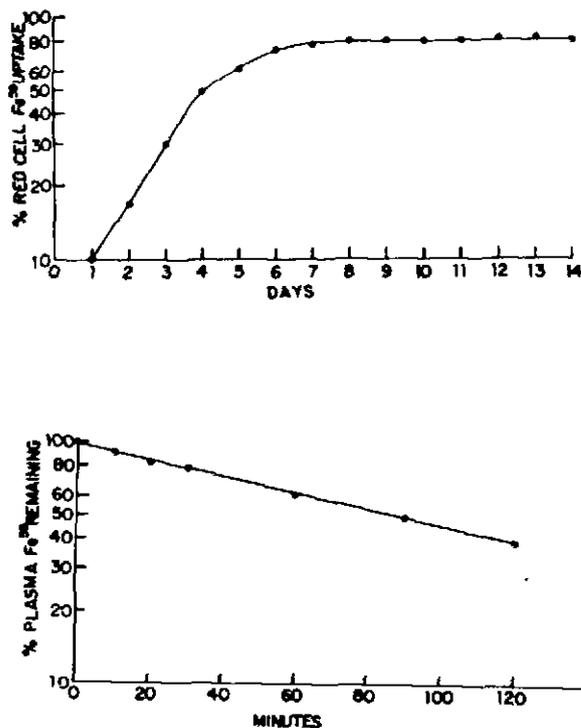


FIGURE 1

The upper curve shows the change in percent of  $^{59}\text{Fe}$  uptake by days after injection of  $^{59}\text{Fe}$ -labeled plasma. The lower curve shows percent of  $^{59}\text{Fe}$  activity remaining in the plasma by minutes after injection of  $^{59}\text{Fe}$ -labeled plasma.

## V. DISCUSSION

These methods provide a standard, reliable procedure for determining blood parameters. Values obtained were within the limits of values reported in the literature. Huff et al. (5) present several formulas for calculating other iron metabolic factors. First, the plasma iron rate is determined by dividing the natural logarithm of 2 (0.693) by the  $T_{1/2}$  in hours. The turnover rate of plasma iron can then be determined from the product of the iron rate, the plasma volume, and the iron concentration (micrograms of iron per milliliter of plasma). This product is multiplied by 24, to obtain the milligrams of iron entering and leaving the plasma per day. The turnover rate of plasma iron can then be used to calculate the turnover rate of red cell iron. The plasma turnover is multiplied by the percent of iron incorporated within 8 to 10 days, to give the red cell turnover in milligrams per day.

The relative concentration of  $^{59}\text{Fe}$  in the hematopoietic centers can be determined using a photomultiplier probe and ratemeter system (6). The areas normally measured include the liver, spleen, and sacrum. These measurements, combined with the values calculated above, provide the basis for reliable estimates of ferrokinetics and iron metabolism under normal and abnormal conditions.

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