The Determination of Iron Absorption and Loss by Whole Body Counting

By D. C. Price, S. H. Cohn, L. R. Wasserman, P. G. Reizenstein and Eugene P. Cronkite

LACK of an accurate method for measuring absorption and excretion of iron has complicated the study of iron metabolism in man. Early knowledge of iron absorption and excretion was derived from chemical iron balance studies.1,2 When the radioactive iron isotopes Fe55 and Fe59 became available, they were utilized to determine the appearance of absorbed radioiron in circulating red cells3,4 and the fecal excretion of unabsorbed radioisotope.5,6 Determination of long-term fecal radioiron excretion7 and of Fe55 specific activity in hemoglobin,8 led to estimates of the body's daily loss of iron. These methods all remained somewhat inaccurate, however, because of difficulties in low level radioisotope counting, and necessary assumptions of radioiron distribution in various body compartments.

The recent development of whole body counters provides an accurate device for studying absorption and excretion of gamma emitting radioisotopes. It is now feasible to measure directly the body's retention of orally administered Fe59, the loss of labeled red cells from the body by hemorrhage, and the metabolic turnover of iron in the absence of significant hemorrhage.9-11 The present report describes a technic for such studies, employing a low-level whole body radiation counter, and presents data from the study of a number of patients with various hematologic disorders.

Materials and Methods

a) Instrument and Isotope

The whole body counter presented in operation at Brookhaven National Laboratory, Medical Research Center, has been described in detail elsewhere.12-14 The counter consists of an 8'' x 4'' NaI (T1) crystal detector upon which are mounted three photomultiplier tubes. The detector, suspended over an adjustable cot in a 42-ton steel room, rests on a carrier calibrated for reproducible detector distances above the patient. After linear amplification, detected pulses are analyzed with a Penco (Model PA-4) 100 channel pulse height analyzer. Each channel has an energy width of 20 Kev. The resulting spectral data
are recorded on paper tape by an automatic print-out device. Reproducibility of the counting rate with the counter is \( \pm 0.24 \) per cent for total counts in the photopeak of a point source of Cs\(^{137}\). The variation in 10-minute integrated background counts over a 30-day period is \( \pm 1.10 \) per cent. Variation in whole body counts due to changes in patient position in the cot, assessed by repeated counts of a plastic phantom containing uniformly distributed Cs\(^{137}\), is \( \pm 1.8 \) per cent. In the present studies, the detector was placed at patient-crystal distances of 0.5 and 1.0 meters. The 1-meter distance was used to try to minimize changes in counting efficiency due to early changes in body isotope distribution after oral ingestion. An understanding of these early changes in counting rate is critical for the definition of 100 per cent body activity.

The in vivo efficiency of the counter for Fe\(^{59}\) homogeneously distributed throughout the circulating blood, determined by whole body counting before and after removal of a measured quantity of labeled blood in several patients, averages 1.16 per cent at 0.5 meters and 0.39 per cent at 1 meter.

Ferrous\(^{59}\) citrate,\(^*\) of specific activity 5–10 \( \mu \)c./\( \mu \)g., was diluted with carrier ferrous citrate to a final administered dose of 1–10 \( \mu \)c. Fe\(^{59}\) in 250 \( \mu \)g. carrier iron.

b) Hematologic Assessment

Plasma iron was determined by the method of Ramsey\(^{15}\) and unsaturated iron-binding capacity (UIBC) by the iron-transferrin colorimetric technic.\(^{16}\) Bone marrow iron was estimated by the Gomori stain.\(^{17}\) Microhematocrits were done in quadruplicate. Hemoglobin was determined by the cyanmethemoglobin procedure.\(^{18}\) Red blood cell counts were performed with the Coulter Electronic Cell Counter.\(^{19}\) From the preceding, red cell indices were computed. Total blood volume was considered to be 64.0 ml./Kg. in adult females and 77.0 ml./Kg. in adult males.\(^{20}\)

c) Patient Procedure

After an overnight fast each patient was counted for 10 minutes to estimate the intrinsic background activity due to K\(^{41}\) and Cs\(^{137}\). Then 1–10 \( \mu \)c. of Fe\(^{59}\) 250 \( \mu \)g. total Fe as ferrous citrate was given orally followed by 100 cc. water, food being withheld for an additional hour thereafter. One to 2 \( \mu \)c of radioiron was used when only iron absorption was being determined and 5–10 \( \mu \)c. when long-term excretion was to be studied. Several counts of the patient were taken during the first 8–10 hours. The counting rate in each patient at 4–10 hours was used as his own 100 per cent radioisotopic activity level. At intervals of days, weeks, and months, the Fe\(^{59}\) retention was measured by a 5–to 10-minute whole body count, and subsequent radioiron loss from the body thus estimated. In the absence of any evidence for blood loss, the 16- to 21-day counting rate expressed as a percentage of the patient’s own 4- to 10-hour radioactivity was taken as the percentage absorption of radioiron. Hemolyzed samples of 3.0 cc. whole blood were taken from the patients at regular intervals. These were counted in a Packard Model 410 Auto-gamma Spectrometer and compared with the activity of a retained aliquot of the administered radioisotope made up to the same counting volume to determine the red cell incorporation of retained radioiron. Retained whole body radioactivity was plotted as an exponential function of time. When the regression appeared linear, generally after day 20, the regression coefficient was determined by the method of least squares utilizing an IBM 610 computer.

d) Patients Studied

The first study group consisted of 14 patients with polycythemia vera, 12 of whom had previously been phlebotomized and in some cases treated with P\(^{32}\) and/or Myleran. The remaining two patients had a history of recent gastrointestinal hemorrhage. The second

\(^*\)From Abbott Laboratories, Oak Ridge, Tenn.
Figure 1.—Change in total body counting rate seen during the first 10 hours after ingestion of Fe\textsuperscript{59}, as seen in five representative patients. Note the plateau in radioactivity occurring characteristically at 4–10 hours post-ingestion.

Group of patients presented a variety of blood dyscrasias including hypochromic microcytic anemia with menorrhagia, aplastic anemia, steatorrhea, pernicious anemia, etc. Lastly, 13 patients who were hematologically normal served for comparison purposes.

RESULTS

a) General Observations

Figure 1 shows the change in whole body count as a function of time in several representative patients during the first 10 hours after ingestion of radioiron. Because a relatively stable count rate was achieved only during the 4- to 10-hour time period, for each patient the count rate at this time was arbitrarily chosen to represent his 100 per cent Fe\textsuperscript{59} activity. All counts in figure 1 were made at 1 meter. A similar pattern is obtained at the 0.5 meter patient-crystal distance.

During the first 90 days, whole body retention of orally administered Fe\textsuperscript{59} could be described by a curve appearing to represent a sum of exponential functions (fig. 2). Initially, there was a rapid fall in total body radioactivity due to fecal excretion of the unabsorbed radioiron. The rate of fall in activity during days 4-16 steadily decreased, finally appearing to resolve into a single exponential function between day 20 and day 100. Whole blood sampling to day 100 established that there was no loss of radioactivity from the labeled red cell mass. The 20- to 100-day fall in body radioactivity therefore must represent loss of radioiron from iron compartments other than circulating hemoglobin.

b) Absorption of Radioiron in Iron Deficiency

Normal absorption of radioiron with this procedure ranged from 5.7 per cent to 24.7 per cent of the tracer (15.9 ± 7.2 per cent − 1 S.D.), as determined in ten adult males and three post-menopausal females, none of whom had any abnormality of red cell production nor of iron metabolism. In contrast, iron absorption in the 14 polycythemics (table 1) varied from 20.6 per cent to 96.9 per cent (76.2 ± 22.3 per cent − 1 S.D.). Two of these patients (CD and RS) had not previously been treated and thus one might...
Fig. 2.—Changes in total body radioactivity observed in several representative patients over a period of 90 days after ingestion of Fe59.

have anticipated normal iron absorption. However, both had a history of recent extensive gastrointestinal hemorrhage. Their iron absorptions were 92.9 per cent and 69.5 per cent respectively. One patient (BU-1) had not been phlebotomized for 2 years. His radioiron absorption of 20.6 per cent was normal. Shortly thereafter, an exacerbation of his polycythemia was treated by phlebotomy of 2300 cc. over 4 months. This resulted in a marked increase in absorption of radioiron to 78.6 per cent of the tracer.

There appears to be a significant correlation between the absorption of Fe59 and the plasma iron level in the iron-deficient, polycythemic group (fig. 3). Although UIBC is significantly elevated in almost all cases, a linear correlation with iron absorption is not apparent. The red cell indices, represented by mean corpuscular hemoglobin (MCH) in figure 3, uniformly reflect iron deficiency (microcytosis, hypochromia). Some correlation with iron absorption is also suggested here. Comparison of absorption with the history of previous phlebotomies indicates that the greater the amount of blood removed—even over a period of years—the greater is the subsequent elevation of iron absorption.

c) Absorption of Radioiron in Other Hematologic Disorders

Radioiron absorption has also been studied in patients with several other hematologic disorders by the whole body counting technic. A small group of healthy young mothers with low normal or mildly anemic blood values and a chronic history of heavy menses was found to have increased absorption of iron ranging from 61 per cent to 97 per cent (75 ± 15.9 per cent — 1 S.D.) of the tracer (table 2). These patients also demonstrated other evidence of iron deficiency including microcytosis, hypochromia, depleted to
whole body counting in iron metabolism

Table 1.—Fourteen Cases of Polycythemia Vera Studied, All Previously Phlebotomized but CD and RS, Both of Whom Gave a History of Recent GI Hemorrhage (The Absorption and Loss of Fe-59 Can Be Compared with Other Parameters of Iron Deficiency)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hct. (%)</th>
<th>MCV (µl)</th>
<th>MCH (pg)</th>
<th>MCHC (%)</th>
<th>Marrow Iron</th>
<th>Serum Iron (µg%)</th>
<th>UIBC (µg%)</th>
<th>Fe-59 Absorp. (%)</th>
<th>Rate of Fe-59 Loss (%/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BE</td>
<td>45</td>
<td>86.0</td>
<td>26.1</td>
<td>30.4</td>
<td>0</td>
<td>125</td>
<td>100.0</td>
<td>80.8</td>
<td>0.024</td>
</tr>
<tr>
<td>BL</td>
<td>39</td>
<td>85.0</td>
<td>27.5</td>
<td>32.4</td>
<td>tr +</td>
<td>125</td>
<td>100.0</td>
<td>79.1</td>
<td>0.044</td>
</tr>
<tr>
<td>BU-1</td>
<td>50.2</td>
<td>100.0</td>
<td>33.2</td>
<td>33.2</td>
<td>++</td>
<td>200</td>
<td>97.6</td>
<td>87.4</td>
<td>0.012</td>
</tr>
<tr>
<td>BU-2</td>
<td>54.3</td>
<td>97.6</td>
<td>28.4</td>
<td>29.0</td>
<td></td>
<td>63</td>
<td>97.6</td>
<td>87.4</td>
<td>0.012</td>
</tr>
<tr>
<td>CD</td>
<td>59.2</td>
<td>81.3</td>
<td>26.0</td>
<td>33.0</td>
<td></td>
<td>125</td>
<td>100.0</td>
<td>87.4</td>
<td>0.012</td>
</tr>
<tr>
<td>CT</td>
<td>41</td>
<td>77.5</td>
<td>24.8</td>
<td>32.0</td>
<td>0</td>
<td>50</td>
<td>100.0</td>
<td>87.4</td>
<td>0.012</td>
</tr>
<tr>
<td>CO*</td>
<td>50</td>
<td>92.3</td>
<td>30.6</td>
<td>33.3</td>
<td>0</td>
<td>125</td>
<td>100.0</td>
<td>87.9</td>
<td>0.009</td>
</tr>
<tr>
<td>FA</td>
<td>45.2</td>
<td>87.8</td>
<td>27.7</td>
<td>31.2</td>
<td>0</td>
<td>110</td>
<td>97.6</td>
<td>81.4</td>
<td>0.023</td>
</tr>
<tr>
<td>MU</td>
<td>48.3</td>
<td>68.9</td>
<td>19</td>
<td>27.8</td>
<td>0</td>
<td>35</td>
<td>97.6</td>
<td>89.3</td>
<td>0</td>
</tr>
<tr>
<td>RS</td>
<td>70</td>
<td>87.5</td>
<td></td>
<td></td>
<td>0</td>
<td>69.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>50.5</td>
<td>66.5</td>
<td>17.9</td>
<td>27.0</td>
<td>0</td>
<td>35</td>
<td>97.6</td>
<td>96.9</td>
<td>0.029</td>
</tr>
<tr>
<td>TO</td>
<td>51.5</td>
<td>79.3</td>
<td>23.6</td>
<td>29.9</td>
<td>ft. tr.</td>
<td>90</td>
<td>97.6</td>
<td>87.7</td>
<td>0.019</td>
</tr>
<tr>
<td>TR</td>
<td>47</td>
<td>70.3</td>
<td>19.9</td>
<td>28.4</td>
<td>0</td>
<td>60</td>
<td>97.6</td>
<td>88.9</td>
<td>0.011</td>
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<tr>
<td>TS</td>
<td>45.5</td>
<td>97.1</td>
<td>32.2</td>
<td>33.3</td>
<td>ft. tr.</td>
<td>127</td>
<td>97.6</td>
<td>89.0</td>
<td>0.017</td>
</tr>
<tr>
<td>UE</td>
<td>48</td>
<td>97.7</td>
<td>28.4</td>
<td>29.0</td>
<td>tr</td>
<td>65</td>
<td>97.6</td>
<td>58.1</td>
<td></td>
</tr>
</tbody>
</table>

*Mild cirrhosis.

absent marrow iron, and 95-99 per cent incorporation of absorbed radioiron into the red cell mass.

Other patients studied are outlined in table 3. Three patients with aplastic anemia absorbed normal amounts of the tracer (5.4 – 9.0 per cent) but showed markedly depressed red cell incorporation of absorbed Fe-59 (1.2 per cent – 3.5 per cent). In two patients with malignancy, prostatic carcinoma (RB) and chronic lymphocytic leukemia (SE), there was slightly depressed absorption (4.0 per cent and 3.9 per cent) with low utilization of absorbed isotope (22.4 per cent and 44.9 per cent).

Patient GI, with chronic uremia secondary to hypertension, absorbed 8.5 per cent of the ingested radioiron, incorporating 45 per cent into circulating erythrocytes. His hematocrit at the time of study was 20.5 per cent, his blood urea nitrogen 33.8 mg. per cent. Patient SL (untreated, early pernicious anemia) had normal iron absorption (15.7 per cent). WE, a young man with hemochromatosis and severe pyridoxine-responsive anemia, absorbed 69.1 per cent of the ingested isotope, with small amounts later detectable in the plasma but none in the red cells. Patient TH, an interesting woman of 43 with macroglobulinuria and a concomitant chronic hemolytic anemia, absorbed much more tracer than normal (73.8 per cent) with only 64 per cent later accounted for in circulating red cells.

Of all the patients studied to date, only three have had iron absorptions significantly below the normal range. Two patients with idiopathic steatorrhea

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Fig. 3.—Absorption of Fe⁵⁹ compared with plasma iron, UIBC, red cell indices represented by mean corpuscular hemoglobin (MCH), and history of previous phlebotomies. ○ = observations in polycythemia vera; △ = observations in other cases studied.

(Ha and GU) absorbed 1.2 per cent, and 2.5 per cent of the tracer, with 67.8 per cent and 97.5 per cent incorporation respectively. Patient GU, in fact, was markedly iron deficient as a result of her iron malabsorption and possible previous gastrointestinal blood loss, as reflected in her anemia and low blood indices. The third patient (SB), a man with active rheumatoid arthritis, absorbed 0.53 per cent of the tracer. Due in part to the small amount of isotope retained, none could be detected in his circulating erythrocytes 2 weeks later.

d) Rate of Loss of Radioiron

The rate of loss of radioiron from the body, determined in many of the patients by repeated body counts over the 20 to 100-day period, is expressed in tables 1 and 3, for the purpose of comparison, as percentage of retained Fe⁵⁹ lost per day. Radioiron loss in the polycythemics ranged from 0.009 per cent to 0.044 per cent per day (0.021 ± 0.011 per cent ± 1 S.D.), the highest rate of loss occurring in the patient with the least evidence of iron deficiency (BU-1). Patients AR and VE (table 3) with aplastic anemia lost tracer iron at the rate of 0.173 per cent and 0.103 per cent per day respectively. The patient with pyridoxine-responsive anemia (WE) also lost absorbed tracer at a low rate, 0.026 per cent per day. The daily radioiron loss of 0.088 per cent in patient TH represents a loss both of nonhemoglobin radioiron and of Fe⁵⁹ tagged red blood cells in her normal menses.
Table 2.—Absorption and Red Cell Incorporation of Fe\textsuperscript{59} in Four Young, Parous Women with Slightly Depressed Blood Values and a Chronic History of Heavy Menstrual Blood Loss

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Children</th>
<th>Hgb (Gm. %)</th>
<th>Hct (%)</th>
<th>MCV (\mu l)</th>
<th>MCH %</th>
<th>MCHC (%)</th>
<th>Fe\textsuperscript{59} Absorption (%)</th>
<th>Fe\textsuperscript{59} in RCM (% of Total Absorbed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>24</td>
<td>1</td>
<td>11.3</td>
<td>36</td>
<td>79.5</td>
<td>24.9</td>
<td>31.8</td>
<td>61%</td>
<td>95.7</td>
</tr>
<tr>
<td>BR</td>
<td>39</td>
<td>3</td>
<td>8.9</td>
<td>34.5</td>
<td>72.7</td>
<td>18.8</td>
<td>25.8</td>
<td>66%</td>
<td>98.5</td>
</tr>
<tr>
<td>AU</td>
<td>35</td>
<td>2</td>
<td>11.8</td>
<td>36</td>
<td>71.4</td>
<td>23.4</td>
<td>32.8</td>
<td>75%</td>
<td>&gt; 95</td>
</tr>
<tr>
<td>SR</td>
<td>41</td>
<td>5</td>
<td>11.3</td>
<td>38</td>
<td>82.6</td>
<td>24.5</td>
<td>29.7</td>
<td>97%</td>
<td>95.7</td>
</tr>
</tbody>
</table>

Three additional patients with normal iron metabolism have been found to lose radioiron during the first 20–100 days at the rates of 0.110 per cent, 0.110 per cent and 0.182 per cent daily. Along with the tracer loss in patients AR and VE of 0.173 per cent and 0.103 per cent daily (table 3), a normal range of radioiron loss of 0.103 – 0.182 per cent per day (0.136 ± 0.039 per cent – 1 S.D.) is indicated.

**DISCUSSION**

a) General Observations

The in vivo whole body counting of a radionuclide in a patient involves an inherent uncertainty because of internal absorption and scattering of the gamma rays. Furthermore, the counting geometry with respect to the relative position of the radioisotope to the detector in the whole body counter is reflected in the counting efficiency.\textsuperscript{21} The magnitude of this uncertainty is large with an isotope like Fe\textsuperscript{59} where the internal distribution is nonhomogeneous and not completely delineated with respect to time. Since changes in distribution, especially early after administration, produce substantial changes in the x-ray spectrum, rigorous absolute calibration is difficult. In a clinical study, absolute calibration although desirable is not necessary. It is only required to measure the patient's retained radioactivity in terms of some original, consistent 100 per cent administered value. In this study it was found that the counting rate was stable in all patients from 4–10 hours after administration and therefore this value served for each patient as his standard reference or "100 per cent dose" (fig. 1).

Other attempts at absolute calibration of the 100 per cent administered dose have been of questionable advantage. For example, one method which attempts to establish the absolute level of retained Fe\textsuperscript{59} requires i.v. injection one week after radioiron ingestion of a quantity of transferrin-bound Fe\textsuperscript{59} equal to the original oral dose.\textsuperscript{22} The ratio of the counting rate before injection to the increase in counting rate due to injected Fe\textsuperscript{59}, counted one week...
Table 3.—Absorption, Red Cell Incorporation and Total-body Loss of Tracer Fe$^{59}$ in a Variety of Hematologic Conditions Affecting Iron Metabolism

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Hct (%)</th>
<th>MCV (μm$^3$)</th>
<th>MCH (pg)</th>
<th>MCHC (%)</th>
<th>Marrow Iron (%)</th>
<th>Fe$^{59}$ Absorp'n (%)</th>
<th>Fe$^{59}$ in RCM (%)</th>
<th>Rate of Fe$^{59}$ Loss (%/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR</td>
<td>Api. An.</td>
<td>29.1</td>
<td>100</td>
<td>29.6</td>
<td>92.6</td>
<td>**</td>
<td>8.2</td>
<td>9.0</td>
<td>2.9</td>
</tr>
<tr>
<td>SC</td>
<td>Api. An.</td>
<td>16.5</td>
<td>95.3</td>
<td>34.1</td>
<td>35.7</td>
<td>++</td>
<td>9.0</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>VE</td>
<td>Api. An.</td>
<td>26.2</td>
<td>97.0</td>
<td>30.4</td>
<td>31.3</td>
<td>++</td>
<td>5.4</td>
<td>3.5</td>
<td>0.075</td>
</tr>
<tr>
<td>SB</td>
<td>Rh. Arth.</td>
<td>41.5</td>
<td>94.1</td>
<td>28.8</td>
<td>30.6</td>
<td>++</td>
<td>5.3</td>
<td>0 (0)</td>
<td>0.075</td>
</tr>
<tr>
<td>HA</td>
<td>Id. Steat.</td>
<td>35.5</td>
<td>92.5</td>
<td>30.2</td>
<td>32.7</td>
<td>++</td>
<td>1.2</td>
<td>67.8</td>
<td>0.019</td>
</tr>
<tr>
<td>GU</td>
<td>Id. Steat.</td>
<td>29.0</td>
<td>66.4</td>
<td>18.9</td>
<td>28.3</td>
<td>++</td>
<td>2.5</td>
<td>97.5</td>
<td>0.019</td>
</tr>
<tr>
<td>SE</td>
<td>G.L.</td>
<td>28.0</td>
<td>90.9</td>
<td>25.0</td>
<td>30.4</td>
<td>++</td>
<td>3.9</td>
<td>44.9</td>
<td>0.019</td>
</tr>
<tr>
<td>RB</td>
<td>Prost. CA</td>
<td>33.0</td>
<td>120.0</td>
<td>38.6</td>
<td>32.1</td>
<td>++</td>
<td>4.0</td>
<td>22.4</td>
<td>0.019</td>
</tr>
<tr>
<td>GI</td>
<td>Uremia</td>
<td>20.5</td>
<td>81.8</td>
<td>27.3</td>
<td>33.3</td>
<td>++</td>
<td>8.5</td>
<td>45.0</td>
<td>0.019</td>
</tr>
<tr>
<td>SL</td>
<td>P. A.</td>
<td>37.5</td>
<td>108.7</td>
<td>36.8</td>
<td>33.9</td>
<td>++</td>
<td>15.7</td>
<td>0.019</td>
<td>0.017</td>
</tr>
<tr>
<td>WE</td>
<td>Pyridoxine-Responsive Anemia</td>
<td>12.5</td>
<td>72.7</td>
<td>16.4</td>
<td>22.5</td>
<td>+++</td>
<td>69.1</td>
<td>0</td>
<td>0.026</td>
</tr>
<tr>
<td>TH</td>
<td>Macroglobulinemia</td>
<td>14.5</td>
<td>102.9</td>
<td>33.7</td>
<td>32.8</td>
<td>+++</td>
<td>73.8</td>
<td>64</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Subsequently, is taken to represent the Fe$^{59}$ retained after oral administration. This technic has the disadvantage of more than doubling the radiation dose to the patient. In addition, some inaccuracy will be introduced by loss of injected radioiron from the body during the first week. Finally, equilibrium of total body activity is frequently not reached by 7 days after Fe$^{59}$ injection. A time interval of 15-20 days would be much more suitable. As another possibility, the body counting rate immediately after injection, at the time of uniform plasma radioiron distribution, could be utilized for 100 per cent activity. Although this would eliminate the problem of isotope excretion, it re-introduces errors due to rapid changes in Fe$^{59}$ distribution and consequent geometry differences between absorbed and injected tracer. No single method for establishing the 100 per cent administered radioiron activity appears to resolve the problems inherent in geometry variations.

The initial rapid fall in whole body radioactivity shown in the curves in figure 2 represents fecal excretion of unabsorbed tracer, and its slope varies widely with the individual. In the absence of any blood loss, the 20 to 100-day component represents the rate of loss of Fe$^{59}$ from the nonhemoglobin compartments (i.e., labile pool, myoglobin, respiratory enzymes and stores). Its slope therefore depends upon the red cell uptake of absorbed Fe$^{59}$, the body's efficiency of conservation of metabolically active iron, and the size of the labile pool of iron available for excretion. Expressed as percentage of retained Fe$^{59}$ lost per day, the rate of tracer loss can be translated into absolute excretion of iron only by accurate measurement of the iron compartment available for excretion, or by determining specific activity of excreted iron in the feces.

The intermediate, 4- to 16-day component of the curve of whole body radioactivity is not easily explained. It could represent changes in isotope-crystal
geometry as the Fe$^{59}$ gradually enters mature red cells and is released into general circulation. Since maximum circulating activity is reached by 8–10 days, however, this could not be the principal explanation. It is more likely that there is a continuing low-level loss of radioiron from the body over many days, probably from the G.I. tract. We have found, as have others, negligible amounts of activity in the urine during this period, and little is discovered in sweat. Recent autoradiographic studies of rat intestinal mucosa after oral Fe$^{59}$ indicate that a significant portion of the tracer is retained in mucosal cells for many days after ingestion, and is lost apparently only with death and sloughing of the cells. This transitory mucosal retention of iron provides a possible mechanism of delayed iron absorption, which in our studies has influenced as much as 9 per cent of the ingested tracer. In the iron deficient patients this delayed loss of radio-iron was markedly diminished or absent. Thus, the iron retained in mucosal cells appears to be available for further absorption.

**Radioiron Absorption**

The existence of moderate to severe iron deficiency in phlebotomized polycythemics is an expected finding. Thus, this is an excellent group to assess the effectiveness of whole body counting in the measurement of radioiron absorption and loss, and to establish the rate of Fe$^{59}$ loss in iron deficiency. Although a good correlation exists between the absorption of tracer radioiron and both the plasma iron level and the extent of blood loss by previous phlebotomy (fig. 2), the UIBC and the red cell indices do not relate so well to iron absorption. Consistent cytologic depletion of stainable iron in the bone marrow has again been noted to be an early indication of iron depletion. However, we believe that increased absorption of radioiron will precede histologically demonstrable iron depletion.

It is interesting to note the degree of iron deficiency present in four heavily menstruating, parous women (table 2). Menstruating women have a greater iron loss than men or post-menopausal women, and consequently a greater iron requirement. These absorption studies suggest the severity of iron depletion to which menstrual blood loss and past pregnancies may lead. With almost 100 per cent isotope incorporation into circulating red cells in such iron deficient patients, any fall in body radioactivity would quantitatively reflect the amount of blood lost during a single menstrual period. Study of the quantitation of menstrual blood loss by this method is under way and will be reported in the future.

Normal absorption of Fe$^{59}$ with decreased red cell incorporation in the three patients with aplastic anemia (table 3) is in itself sufficient explanation for the increased iron stores found, although repeated transfusions add a further burden of iron for the body to eliminate. The presence of normal absorption with hematocrits of 16.5 per cent–20.1 per cent suggests that anemia is not a predominant factor in the regulation of iron absorption.

Low absorption of tracer Fe$^{59}$ in idiopathic steatorrhea (patients HA and GU—1.2 per cent and 2.5 per cent) has been noted by others. In patient GU this malabsorption of iron resulted in a marked iron deficiency,
which responded characteristically to parenteral iron alone. Depressed absorption of radioiron in rheumatoid arthritis (SB—0.53 per cent) may in some way relate to the abnormal protein metabolism in that disease. Although depressed iron absorption and utilization have been noted in uremia, patient GI absorbed normal amounts of iron (8.5 per cent) with only a moderate decrease in utilization (45 per cent). In patient TH, the presence of hemolytic anemia, normal menstrual blood loss and macroglobulinemia make adequate interpretation of her increased iron absorption (73.8 per cent) virtually impossible. Her menstrual blood loss was not excessive, however, and in itself probably does not explain the high tracer absorption.

Patient WE, characteristic of the pyridoxine-responsive anemias in his microcytic hypochromic morphology, totally saturated iron-binding capacity, markedly increased iron stores and good hematologic response to pyridoxine, had greatly increased absorption of Fe59 (69.1 per cent) with no red cell incorporation, and yet a low rate of Fe59 loss (0.026 per cent/day) comparable to that of iron deficiency. The basis for the increased iron stores in this unusual disease is thus evident, but the defect in control of iron absorption and excretion remains obscure. Patient WE will be reported in greater detail subsequently.

c) Rate of Loss of Radioiron

Expressed as percentage of retained radioiron lost per day over days 20–100, the rate of tracer loss in patients AR, VE, and the three normals (0.103 per cent–0.182 per cent per day) falls in the same range of normal iron loss as that observed in a single patient by Bonnett et al. using a simple body counter (0.14 per cent/day). Dubach, Moore and Callender found considerably lower fecal excretion of radioiron, 0.008 per cent–0.015 per cent daily. Finch, following Fe59 loss from circulating hemoglobin over 54 months, and assuming uniform mixing of the radioiron during that time, estimated the rate of loss of radioiron from the body to be 8.3 per cent per year in men, 10.8 per year in non-menstruating women, and 20.8 per cent per year in actively menstruating women. This would represent a daily radioiron loss of 0.022 per cent, 0.030 per cent, and 0.055 per cent respectively, in the same order of magnitude as that found by Dubach et al.

It must be emphasized that during the life span of the initially labeled red cell population (day 0–120), the rate of loss of radioiron from the body determined by whole body counting cannot be interpreted as a simple exponential function:

\[ R_t = R_0 e^{-\lambda t} \]

where \( R_0 \) = extrapolated retention of Fe59 at day 0, \( R_t \) = retention of Fe59 at day \( t \), and \( \lambda \) = rate constant expressed as fractional loss per day. This interpretation presumes loss of Fe59 from a uniformly mixed, labile total body compartment, whereas it is well established that under normal conditions 65–85 per cent of tracer iron is taken up in the fixed compartment of circulating red cells, consequently becoming unavailable for continuous excretion. Such a kinetic system therefore would require the mathematical model:

\[ R_t = U + R_0 e^{-\lambda t} \]
where \( U \) is the proportionate red cell uptake of \( \text{Fe}^{59} \), and \( t \) is less than 120 days. Even this model may be an oversimplification since some radioiron will be fixed in myoglobin for a finite period, and some will become incorporated into relatively fixed iron stores.

Although these difficulties in mathematical analysis could be obviated by following body activity through many red cell life spans, thus eliminating the role of the long-lived, fixed compartment of circulating hemoglobin, this is unfortunately not possible with \( \text{Fe}^{59} \), whose physical half-life is 45.1 days. The interpretation of observed total body loss of radioiron thus will remain a complex study of a multicompartmental system.

Since the present study did not include accurate determination of red cell incorporation of radioiron, it is impossible to assess the observed excretion data in terms of iron loss from the nonhemoglobin compartment. Only in those patients with negligible incorporation of radioiron into red cells—as occurs in aplastic anemia (AR and VE) and in patients with complete absence of erythropoietic maturation (patient WE)—does the measured rate of loss of \( \text{Fe}^{59} \) from the body represent loss from the nonhemoglobin fraction during days 20–100. If patients with aplastic anemia excrete iron from the nonhemoglobin compartment at a normal rate, the values obtained in aplastic anemia of 0.173 per cent (AR) and 0.103 per cent (VE) loss of radioiron per day will approximate the normal rate of loss of nonhemoglobin iron. The low rate of \( \text{Fe}^{59} \) loss in pyridoxine-responsive anemia (VE—0.026 per cent per day) may reflect decreased excretion of iron from the body, but could also represent loss of normal quantities of iron from a greatly increased pool of available iron. Similarly, the rate of loss of radioiron in the iron deficient polycythemics (0–0.044 per cent per day) may not indicate a specific body mechanism for conserving iron, but rather normal loss of iron from a greatly reduced pool of iron stores, combined with almost 100 per cent isotope incorporation into circulating red cells. In fact, if one assumes 95 per cent incorporation of radioiron into red cells in these patients, the observed rate of radioiron loss from the body then becomes 0.2 per cent–0.7 per cent nonhemoglobin iron lost per day.

d) Conclusion

In conclusion, whole body counting has been found to provide an excellent method for the assessment of absorption and loss of \( \text{Fe}^{59} \) in man, obviating many inaccuracies of previous technics. The method permits accurate iron studies using 1 \( \mu \text{c} \) of \( \text{Fe}^{59} \) or less, thus reducing radiation exposure of the patient to a minimum. Although long-term studies are limited by the 45.1 day half-life of \( \text{Fe}^{59} \), adequate activity for accurate counting still remains after many months with an initial administration of 10 \( \mu \text{c} \). The high degree of accuracy of the technic makes it desirable now to repeat many of the earlier studies in iron metabolism, and in addition opens up great potential for a clearer understanding of the long-term body turnover of iron.

Summary

A technic for the study of radioiron absorption and loss is described employing an NaI (T1) crystal-detector whole body counter and 1–10 \( \mu \text{c} \) \( \text{Fe}^{59} \).
in 250 µg. elemental iron. Changes in whole body Fe<sup>56</sup> activity during the first few hours and the next 90–100 days after oral ingestion are described and their significance discussed. Normal absorption with this technic ranges from 5.7–24.7 per cent of the administered tracer. In 14 patients with polycythemia vera, 12 previously phlebotomized and 2 with a recent history of gastrointestinal hemorrhage, iron deficiency as evidenced by increased iron absorption (20.6 per cent–96.9 per cent) correlates well with the extent of preceding phlebotomy, and relatively well with the plasma iron at the time of study. Although other parameters reflect iron deficiency, none correlate well with the absorption of radioiron. Next to increased iron absorption, depletion of iron stores in the marrow seems to be the earliest evidence of iron deficiency.

Iron absorption and erythrocyte incorporation of radioiron was also studied in several other hematologic disorders, including four heavily menstruating women, three cases of aplastic anemia, and a small number of other conditions. The findings are described and discussed.

Radioiron loss in three normal patients was 0.110 per cent, 0.110 per cent, and 0.182 per cent daily, and in two patients with aplastic anemia 0.103 per cent and 0.173 per cent daily, defining the normal range of tracer loss over days 20–100. Radioiron loss in the polycythemics ranged from 0–0.044 per cent daily. An unusual case of pyridoxine-responsive anemia with increased absorption of radioiron (69.1 per cent), but no red cell incorporation, lost only 0.026 per cent/day. Some problems in the interpretation of such data are discussed.

The results demonstrate the effectiveness of the technic of whole body counting in the study of various aspects of iron metabolism.

**Summario in Interlingua**

Es describite un technica pro le studio del absorption e perdita de radioferro, empleante un contator a corpore total con detector de crystallo de NaI (T1) e 1 a 10 µC de Fe<sup>56</sup> in 250 µg de ferro elemental. Alterationes in le activitate de Fe<sup>56</sup> pro le corpore total durante le prime horas e le subsequente 90 a 100 dies post le ingestion oral es describite, e lor significacion es discutite. Con le uso de iste technica le valor normal del absorption es inter 5.7 e 24.7 pro cento del administrate traciator. In 14 patientes con polycythemia vera (12 previemente phlebotomisate e 2 con recente antecedentes de hemorrhagia gastrointestinal), le deficiencia de ferro evidentiata per un augmento del absorption de ferro (20.6 a 96.9 pro cento) es ben correlationate con le extension del phlebotomia e relativamente ben con le ferro del plasma al tempore del studio. Ben que altere parametros reflecte deficiencia de ferro, nulle mostra un bon correlation con le absorption de radioferro. Post le augmentate absorption de ferro, le plus precoce evidentia de deficiencia de ferro pare esser le depletion del reservas de ferro in le medulla.

Absorption de ferro e incorporation erythrocytic de radioferro esseva etiam studiate in plure altere disordines hematologic, incluse le casos de quatro fortemente menstruante feminas, de tres subjectos con anemia aplastic, e de
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micro numeros de patientes con altere conditiones. Le resultatos de iste studios es describite e discutite.

Le perdita de radioferro in tres subjectos normal esseva 0,110, 0,110, e 0,182 pro cento per die e in duo patientes con anemia aplastic 0,103 e 0,173 pro cento per die. Isto defini le normal variationes del perdita de traciator pro le dies 20 a 100. Le perdita de radioferro in le patientes con polycythemia variava inter 0 e 0,044 pro cento per die. Un caso inusual esseva illo de un paciente con anemia respondente a pyridoxina. In iste caso le absorption de radioferro esseva augmentate (69,1 pro cento). Nulle incorporation in erythrocytos esseva detegite, e le perdita per die esseva solmente 0,026 pro cento. Certe problemas que se presenta in le interpretation de tal datos es discutite.

Le resultatos demonstra le efficacia del technica del contation a corpore total in le studio de varie aspectos del metabolismo de ferro.

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The Determination of Iron Absorption and Loss by Whole Body Counting

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